The Cereal Rusts

VOLUME I

ORIGINS, SPECIFICITY, STRUCTURE, AND PHYSIOLOGY

Edited by William R. Bushnell and Alan P Roelfs

Cereal Rust Laboratory
Agricultural Research Service
U.S. Department of Agriculture
University of Minnesota
St. Paul, Minnesota

ACADEMIC PRESS, INC.
(Harcourt Brace Jovanovich, Publishers)
Orlando San Diego San Francisco New York London Toronto Montreal Sydney Tokyo Sao Paulo

ACADEMIC PRESS, INC.
Orlando, Florida 32887

United Kingdom Edition published by
ACADEMIC PRESS, INC. (LONDON) LTD.
24/28 Oval Road, London NW1 7DX

Library of Congress Cataloging in Publication Data

Main entry under title:

The Cereal rusts.

Includes index.
Contents: v. 1. Origins, specificity, structure, and physiology.
1. Cereal rusts—Collected works. I. Bushnell, William R. (William Rodgers) II. Roelfs, Alan P. SB741.R8C47 1984 633.1′049425 83-15035 ISBN 0-12-148401-7 (alk. paper)

PRINTED IN THE UNITED STATES OF AMERICA

84 85 86 87 9 8 7 6 5 4 3 2 1

To the memory of the pioneers who developed the techniques and concepts that have made economical control of cereal rusts possible

CONTRIBUTORS TO THIS VOLUME

Y. Anikster
W. R. Bushnell
J. Chong
Richard D. Durbin
J. V. Groth
D. E. Harder
R. Heitefuss
William Q. Loegering
Vladimir Macko
J. Manisterski
Kurt Mendgem
A. P. Roelfs
R. Rohringer
J. B. Rowell
D. B. (). Savile
J. F. Schafer
A. Segal
Richard C. Staples
I. Wahl
P. G. Williams

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Part II. Specificity

Contributors

Numbers in parentheses indicate the pages on which the authors' contributions begin.

Y. **Anikster** (39, 115), Faculty of Life Sciences, Department of Botany, Tel Aviv University, Tel Aviv 69978, Israel

W. R. Bushnell (3, 477), Cereal Rust Laboratory, Agricultural Research Service, U.S. Department of Agriculture, University of Minnesota, St. Paul, Minnesota 55108

J. Chong (431), Agriculture Canada Research Station, Winnipeg, Manitoba R3T 2M9, Canada

Richard D. Durbin (509), Agricultural Research Service, U.S. Department of Agriculture, and Department of Plant Pathology, University of Wisconsin, Madison, Wisconsin 53706

J. V. Groth (231), Department of Plant Pathology, University of Minnesota, St. Paul, Minnesota 55108

D. E. Harder (333, 431), Agriculture Canada Research Station, Winnipeg, Manitoba R3T 2M9, Canada

R. **Heitefuss** (193), Institut fur Pflanzenpathologie und Pflanzenschutz, 3400 Gottingen-Weende, Federal Republic of Germany **William Q. Loegering** (165), Department of Plant Pathology, University of Missouri at Columbia, Columbia, Missouri 65211 **Vladimir Macko** (255), Boyce Thompson Institute for Plant Research, Cornell University, Ithaca, New York 14853

J. Manisterski (39), Faculty of Life Science, Tel Aviv University, Tel Aviv 69978, Israel

Kurt Mendgen (375), Fakultät für Biologie, Lehrstuhl Phytopathologie, Universität Konstanz, D-7750 Konstanz, Federal Republic of Germany A. P. **Roelfs** (3, 131), Cereal Rust Laboratory, Agricultural Research Service, U.S. Department of Agriculture, University of Minnesota, St. Paul, Minnesota 55108

R. Rohringer (193), Agriculture Canada Research Station, Winnipeg, Manitoba R3T 2M9, Canada

J. B. Rowell (291), Cereal Rust Laboratory, Department of Plant Pathology, University of Minnesota, St. Paul, Minnesota 55108 **D. B. O. Savile** (79), Biosystematics Research Institute, Agriculture Canada, Ottawa, Ontario K1A 0C6, Canada

J. F. **Schafer** (3), Cereal Rust Laboratory, Agricultural Research Service, U.S. Department of Agriculture, University of Minnesota, St. Paul, Minnesota 55108

A. **Segal** (39), Faculty of Life Sciences, Tel Aviv University, Tel Aviv 69978, Israel

Richard C. Staples (255), Boyce Thompson Institute for Plant Research, Cornell University, Ithaca, New York 14853

I. Wahl (39), Faculty of Life Sciences, Tel Aviv University, Tel Aviv 69978, Israel

P. G. Williams (399), New South Wales Department of Agriculture, Biological and Chemical Research Institute, Rydalmere, New South Wales 2116, Australia

Preface

The cereal rusts are potentially serious disease threats to cereal crops and have caused widespread losses in wheat, oats, barley, and related crops. To assist in the worldwide effort to control the cereal rusts, this two-volume treatise brings together in a single reference source the accumulated knowledge of the cereal rusts. Not since K. Starr Chester's "The Cereal Rusts," published in 1946, have any of these diseases been treated comprehensively in a single work. In the interval since then, research on these historically devastating diseases has proliferated, leading to new principles concerning the nature of the diseases and new strategies for their control. Contributing to this new knowledge have been biochemists, cytologists, geneticists, physiologists, taxonomists, and epidemiologists, as well as pathologists. The work of these diverse specialists as applied to cereal rusts forms the basis of these volumes.

The two volumes will serve the needs not only of cereal rust investigators who have found it increasingly difficult to assimilate the world's cereal rust literature, but also of plant pathologists generally, as a reference source for teaching, extension, and research. Many of the principles of plant pathology have been developed from studies of cereal rusts. Agronomists and other agriculturalists concerned with cereal crop production or world food supplies will also find in these volumes useful summaries and evaluations of past work as well as projections for the future by many of the leading workers in the field.

Periodically, severe and chronically damaging cereal rust epidemics have plagued mankind since the dawn of agriculture. Consequently, cereal rust diseases were among the first to receive intensive investigation as the science of plant pathology emerged in the late 1800s and early 1900s. The rust fungi were soon found to be "shifty enemies" (as E. C. Stakman put it) with a persistent ability to evolve new virulences that could overcome newly

introduced resistant cereal cultivars. Periodic major rust epidemics persisted into the 1950s and continue to be a threat today.

However, a new science of disease stabilization and management is now emerging which utilizes improved understanding of the complexities of rust disease to slow the evolution of dangerous new virulences, to retard epidemics, and to minimize losses. The knowledge on which these new strategies are based is presented in these volumes. Their contents reflect the great diversity and extent of cereal rust knowledge, including results of research at the level of molecules, cells, plants, fields, and even epidemics sweeping across continents. In total, the cereal rusts have received more investigation than any other like-sized group of plant diseases.

Contributors to these volumes were asked to provide historical perspectives, give current trends, and project future problems and needs. They were encouraged to emphasize areas of special personal interest and to present their own unique perspectives to their assigned topics. The resulting varied treatments provide a rich compilation of the complex, challenging science of cereal rusts.

Volume I presents the historical, evolutionary, taxonomic, structural, genetic, and physiological characteristics of cereal rust fungi and the diseases they cause in cereal crops. A section on origins treats the pioneering contributions of early scientists to knowledge of cereal rusts, the evolution of cereal rusts, and the taxonomy of cereal rust fungi. A section on specificity includes *formae speciales*, race specificity, pathogen-host genetics, histology and molecular biology of host-parasite specificity, and the genetics of rust fungus populations as reflected by virulence frequency. A section on structure and physiology includes germination of urediospores and differentiation of infection structures, infection under artificial conditions, ultrastructure of hyphae and urediospores, development and physiology of teliospores, obligate parasitism and axenic culture of rust fungi, structure and physiology of haustoria, structural and physiological alterations in susceptible hosts, and effects of rust on plant development in relation to nutrient translocation.

Volume II is devoted to individual cereal rust diseases, their distribution, epidemiology, and control. Each of the major cereal rusts is presented in an individual chapter. Epidemiology of cereal rusts is described in chapters on Australia and New Zealand, Europe, India, and North America. A chapter is devoted to modeling and simulation of epidemics. Chapters on methods of rust control include use of chemicals and deployment of resistances to rust.

In these volumes, the reader will find several inconsistencies in terminology and concepts reflecting differences in viewpoint among authors. Thus, plant pathologists usually have used the term “rust” for disease, whereas others with a more mycological orientation have used “rust” to designate the fungus. Similarly, the taxonomist and plant pathologist use different methods for classifying cereal rust fungi. The taxonomist, using mainly morphological

characters, defines subspecies and varieties; the plant pathologist, using host range, defines *formae speciales*. Each system serves useful purposes, but the results are sometimes in conflict. The two viewpoints are presented in Chapters 3 (Savile) and 4 (Anikster) of Volume I.

The terminology chosen by the editors for various spore-bearing structures in the rust life cycle follows widely accepted usage in North America. However, the reader will find both "uredium" and "uredinium" used for the repeating stage on cereal hosts, and the corresponding terms "urediospore" and "urediniospore" for spores produced. No consensus was reached among our authors on these terms, reflecting the lack of consensus generally among rust workers. Though favoring "uredium," we leave this impasse to be resolved by urediniologists. The interested reader will find a brief discussion of the uredium-uredinium controversy in the section on J. C. Arthur in Chapter 1, Volume I.

Given the large number of cereal rust workers, the selection of authors for these volumes involved difficult choices. We thank the authors who willingly and capably contributed chapters and extend thanks to our many colleagues who reviewed chapters and provided advice and encouragement during this project. Special thanks are given to T. Kommedahl and C. J. Eide for their helpful reviews, to Gail Bullis and Brenda Anderson who provided excellent secretarial assistance, and to Colleen Curran who patiently proofread most manuscripts and provided essential logistical support.

W. R. Bushnell
A. P. Roelfs

PART I

Origins

1

Contributions of Early Scientists to Knowledge of Cereal Rusts

J. F. Schafer
A. P. Roelfs
W. R. Bushnell
Cereal Rust Laboratory, Agricultural Research Service, U.S. Department of Agriculture, University of Minnesota, St. Paul, Minnesota

I. Introduction

Cereal rusts have no doubt been present and evolving during domestication of cereal crops as a major segment of agriculture. Kislev (1982) reported archaeological evidence of *Puccinia graminis* on wheat lemma fragments dated at 1400-1200 B.C. Savile and Urban (1982) reviewed the evolution of cereal rusts relative to human-guided evolution of cereal crops. Ancient observations of cereal diseases and attempts to relate them to specific modern diseases such as rusts, smuts, and mildews were reviewed by Arthur (1929) and Chester (1946). The history of these diseases thus fades into antiquity. However, recognizable references are found in the oldest literature, much of which was

summarized by Chester (1946). Arthur (1929) referred to biblical sources and to Grecian and Roman writings. This chapter starts with the recognition of "rust" as a parasitic fungus in 1767.

Recent advancements in understanding are based on preceding work. Thus it is useful to include a history of major contributors who provided the foundation for the current understanding of cereal rusts. We proceed through the intellectual leaders and breakthroughs of the late 1800s and early 1900s and end with those who worked into the mid-1900s with emphasis on researchers who are now deceased. More recent history is found in specific chapters in both volumes of this treatise. Any account of history is not totally objective. Decisions must be made concerning whom and what to cover. Often a person's location had an influence on the impact of the work. Certain works were more widely read and cited because of language, availability, or audience, possibly to the exclusion of other useful but less widely recognized works.

In terms of scholarly contributions, the early detailed descriptions of cereal rusts are those of Fontana (1767) and Targioni Tozzetti (1767), both at Florence, Italy, but published independently. These writings were translated into English and published by the American Phy-topathological Society as "Phytopathological Classics."

II. Description and Taxonomy of Cereal Rust Fungi

A. G. TARGIONI TOZZETTI (1712-1783)

The contribution of Giovanni Targioni Tozzetti (1767) is encompassed in a chapter on diseases of plants, which is the fifth and last part of a much broader volume addressed "for the relief of the poor" but largely meteorological and agricultural in nature. Goídàních (1943) described Targioni Tozzetti's "Alimurgia" chapter as "the first treatise on plant pathology, in the sense in which today we would speak of such publications." Targioni Tozzetti described rust as "the terrible scourge … to which the fields of the greater part of the temperate zone of the Northern Hemisphere have been subjected … even from distant ages. …" He further proposed that rust "merits the most serious attention of naturalists for the purpose of investigating the causes of it, and proposing, if it should be possible, some remedy." The year before his publication (1766) was one of severe rust attack "in which the rust was universal over the whole of Italy, and in all the different levels and exposures of its territory." He stated it to be "certain that the rust is a very ancient scourge of the greater part of the country, cultivated by mankind in the old world," and he referred to the scriptures and the writings of Theophrastus in Greece, and Varro, Horace, Virgil, and Pliny in Italy. "The ancient writers … were ignorant

of the true nature of the rust, but nevertheless noted punctiliously some phenomena and effects."

He found rust to be "little groups or masses … situated under the cuticle of wheat." The original tiny spots were reported to amplify and swell speedily, separating cuticle from parenchyma, producing a blister. The rust then becomes visible as a very fine powder. As first appearing, it is a bright yellow, soon becoming orange, and finally after days, becoming black. He found that rust increased as it generated anew on successive days; however, a "bunch … does not grow, nor distend more than to its final fullness, which it has attained to cause the cuticle to split." The rust was microscopically examined, and the change from orange, rounded bodies, obtained from young masses, to black, more oblong and pointed bodies, being attached by little stalks, was observed. He reported that each "knot of rust … is an internal, very tiny, parasitic plant." He concluded that all of the rusts that he had enumerated formed "a section or family of microscopical parasitic intercutaneous plants."

B. F. FONTANA (1730-1805)

Felice Fontana's contribution was an independent small pamphlet (1767), also motivated by the cereal rust epidemic of 1766. He stated that "On the 10th of June of last year, I discovered that the rust, which had devastated the lands of Tuscany, is a grove of plant parasites that nourish themselves at the expense of the grain." After discussing and discarding the contemporary theories on the nature of the rust, he described "ovules" that were a dark reddish yellow and "nails" with large rounded heads and the appearance of fungi. These two kinds of rust were described in considerable detail from both visual and microscopic observation and carefully illustrated. He discounted the presence of active movement as by an animal and suspected "that the nails are very minute plants that nourish themselves at the expense of the grain." Each form occurred alone, such that the "eggs" always came from reddish spots, and the "nails" from black-rust spots. He concluded that there were two kinds of rust on the grain crop, although the two also occurred together. On extensive, difficult examination, he occasionally observed very delicate stems connected to the "eggs," and concluded that they likewise were plants.

Having established, to his satisfaction, that grain rusts were masses of incalculable numbers of small parasitic plants, Fontana explained their devastating effect on grain yield, due to their absorption of nutritive materials from leaf and stalk tissue. He related severity of damage to the time of attack. He made a plea for additional research on the nature of the disease and on the nature and "economy" of the parasitic plants causing it, because such information would facilitate discovery of control measures.

C. C. H. PERSOON (1755-1837)

Following the major advances in plant taxonomy by Linnaeus from 1735 through 1753 (Reed, 1942), Christiaan Hendrick Persoon (1794), an independent Dutch researcher, made the first significant effort at classification of fungi. With advances in use of the microscope, the major fungal groups as now known could be recognized. Within his classification of 77 genera of fungi, he established three genera of rusts: *Aecidium, Uredo,* and *Puccinia. Puccinia* was taken from earlier work of Micheli (Arthur, 1929) but in a different usage. The genera *Aecidium* and *Uredo* are now designated as "form-genera" and are applied only to aecial or uredial stages for which the full life cycle is not known. Arthur (1929) credited Persoon as the first to recognize the rusts as a distinct group. Thus Persoon provided the first binomial epithets to rusts recognized as such. With complex life cycle relationships not yet established, he gave separate designations for telial, uredial, and aecial states of heteroecious forms such as the cereal rusts, with *Puccinia graminis* Pers., *Uredo linearis*Pers., and *Aecidium berberidis* Pers. being the species names given to the respective forms of the stem rust fungus. The latter names are now relegated as synonyms for the telial designation. Persoon (1801) is the authoritative landmark for Uredinales, Ustilaginales, and Gasteromycetes, and the beginning of accepted nomenclature for the rust fungi and these other groups.

D. L. R. TULASNE (1815-1885)

Louis Rene Tulasne was a French mycologist who collaborated with his brother Charles, the illustrator of their works. His major classification of the rusts (1854b) was published one year after de Bary's famous "Die Brandpilze," and provided the basis for all subsequent taxonomic treatments of the rust fungi (Arthur, 1929). He categorized the rusts into five groups: Aecidnei, Melampsorei, Phragmidiaceae, Pucciniei, and Cronartiei. The morphology and development of these fungi were studied more thoroughly than before. Tulasne and Tulasne (1847) illustrated and correctly interpreted germination of teliospores and illustrated germ pores on both urediospores and teliospores. Tulasne (1853) was the first to suggest that the rust fungi were basidiomycetous in nature, a concept subsequently further developed by Brefeld. The Tulasne brothers greatly expanded the knowledge of structure of fungi, which provided the basis for subsequent studies of life histories. Tulasne (1854a) himself showed that uredia and telia on wheat stems arise from the same mycelia and are not two distinct species of fungi (Craigie, 1931).

E. J. C. ARTHUR (1850-1942)

Joseph Charles Arthur was the leading American uredinologist, publishing from 1882 until 1936, with many of his publications after his official retirement in 1915. He studied life cycles, relationships, classification, and distribution, and was influential in the development of the rules of botanical nomenclature. Brief reviews of his life and works were published by Kern (1942) and Cummins (1978); both were former students, and the latter was his successor for many years at Purdue University. Arthur described many new species of rust fungi, studied host relations through experimental inoculations, and established life histories of many species of rust fungi. Beginning in 1905 and culminating in his "Manual" in 1934, he modified and updated classification of the Uredinales. His 11 parts of the *North American Flora* on the Uredinales, between 1907 and 1927, were the backbone of this effort (Kern, 1942). His valuable contributions on structures and their importance and on life cycles were often rejected because of objections to his nomenclatural proposals. He abandoned some of his earlier nomenclatural positions when writing his "Manual" near the end of his career (Cummins, 1978). "The Plant Rusts" and "Manual," his two major books, are addressed in Section VIII,I.

Arthur also initiated an extensive herbarium of plant rust specimens, located at Purdue University, and now known as the Arthur Herbarium. Baxter and Kern (1962) credited the original nucleus to collections by Arthur when a student. This herbarium, one of the finest in the world, contains over 100,000 specimens of rust fungi (J. Hennen and J. McCain, personal communication).

Arthur (1905) introduced the terms "pycnium," "aecium," "uredinium," and "telium" to designate the principal spore-bearing structures found in rusts. The corresponding spore forms were designated "pycniospores," "aeciospores," "urediniospores," and "teliospores." These terms avoided confusion with names of form-genera—as for example, the term "uredospore," which had been widely used—and gained wide acceptance in North America. Later, Arthur (1932) changed "uredinium" to "uredium," and he introduced the corresponding term "urediospore." Arthur also emphasized that spore forms in rusts should be designated in relation to the preceding form in the life cycle and not only by morphology. Savile (1968) found this to be unworkable for some rusts, leading him to reject "uredium" in favor of a return to "uredinium," which he also found to be etymologically more appropriate. Although controversies over terminology in rust continue (Cummins, 1978), Arthur's terms—in either the 1905 or 1932 version—predominate in North America.

F. J. ERIKSSON (1848-1931)

Jakob Eriksson (1894), a Swedish pathologist, reported that individual cereal rust fungal species were not homogeneous in their host ranges. This

provided for a further “taxonomic” separation within species based on host specificity. This concept was further developed in a major publication on the cereal rusts (Eriksson and Henning, 1896; see Section VII,C). These pathogenically specialized taxa within species were designated *formae speciales.* In a series of publications from 1894 through 1908, Eriksson studied this phenomenon extensively. His work stimulated further studies, and others including Klebahn, Schroeter, Hitchcock and Carleton, Rostrup, and Magnus also soon contributed to the development of this knowledge (Arthur, 1929).

G. E. C. STAKMAN (1885-1979)

Elvin Charles Stakman became a graduate student at the University of Minnesota in 1909 (Christensen, 1979). By this time, Eriksson and others had divided *Puccinia graminis* into *formae speciales,* based on their ability to parasitize various host species. The stability of these was questioned by Ward (1903), who believed that when *Puccinia dispersa* was avirulent on a *Bromus* species, virulence could be derived by culturing the rust on a host taxonomically between the resistant species and the common host of the rust. This bridging-host concept suggested that the *forma specialis* might not be a valid taxonomic division. E. M. Freeman, who had studied with Ward, was Stakman's advisor, and he started Stakman testing the bridging host concept with *P. graminis.* Of 15 wheat cultivars included in the study, not all were susceptible to *P. graminis* f. sp.*tritici* (Stakman, 1914). The work was expanded to a wider host range, and pathogen cultures were obtained from many locations in the United States (Stakman and Piemeisel, 1917b). This widespread sampling led to detection of different phenotypes for pathogenicity within *formae speciales,* and different phenotypes for resistance within host species. Twelve separate forms of *P. graminis* f. sp. *tritici* were found in the United States (Stakman *et al.,* 1919). These studies showed, contrary to the bridging-host theory, that *P. graminis* comprised many stable forms (Stakman *et al.,* 1918). Each form (physiologic race) was a constant identifiable group of individuals within a *forma specialis* of *P. graminis,* based on infection types produced when inoculated to a selected group of hosts. Thus physiological race became a second-level taxon determined by physiological rather than morphological characters. The presence of these forms explained the differences in resistances shown by host cultivars in different locations. The concept placed breeding for host resistance on a firm basis, as consistent results could be obtained when the same pathogen race was used or was present in the natural population. The work on physiologic specialization was continued, with the first key to races published by Stakman and Levine (1922). This key set forth the 12 differential hosts that became the international set for wheat stem rust. It was last updated in 1962 (Stakman *et al.,* 1962) and is still widely used (Roelfs, Chapter 5, this volume). These studies made a tremendous impact

throughout all of plant pathology. Variants were rapidly found in other pathogens, and breeding for rust resistance accelerated.

III. Life Cycles and Cytology of Cereal Rust Fungi

A. A. DE BARY (1831-1888)

When Anton de Bary (1853), at age 22, wrote his famous monograph on the "Brandpilze," he regarded the uredial and telial stages of rusts as two distinct fungal species, living communally as a mixture of mycelia within pustules. The "aecidial" forms on alternate hosts were also thought to be independent species, although de Bary recognized that rust in grain occurred near barberry "… whether it be because of this in and of itself or because of the Aecidium growing on its leaves."

In successive university positions at Tubingen, Freiburg, Halle, and Strasbourg, de Bary continued to apply his remarkable abilities to observe, experiment with, and interpret plant diseases, publishing his principal works on cereal rusts in 1866 and 1867. He recognized, as had Tulasne (1854a), that uredia and telia were successive stages of a single fungus, and he showed by inoculation experiments that the "aecidial" stages on dicotyledonous species were alternate stages of the cereal rusts. He studied *Puccinia graminis, P. coronata,* and *P. straminis (recondita)* on their grass hosts and their respective alternate hosts, Berberis, Rhamnus, and Anchusa. He described germination of urediospores, penetration of gramineous hosts through the stomata, and subsequent development of uredia. de Bary further described germination of teliospores and production of basidiospores. In turn, he described germination of basidiospores on the alternate hosts, development of appressoria, direct penetration of host epidermal cells, and formation of pycnia and aecia. He described pycniospores and later (1884) conjectured that they were sexual fertilizing bodies such that aecia were the product of the presumed fertilization, a concept proven much later by Craigie (Section III,D). de Bary (1867) also showed that aeciospores produce germ tubes that produce appressoria over stomata, then enter the stomata and produce uredia on gramineous hosts. Thus he established the complete succession of spores in macrocyclic rusts as understood today. de Bary introduced the term *heteroiceous* (heterocisch) for rusts that require more than one host to complete their life cycle.

B. H. M. WARD (1854-1906)

Harry Marshall Ward, working at Cambridge University, and once a student of de Bary, undertook studies of *Puccinia dispersa* on *Bromus* spp., including a

careful cytological investigation of infection by urediospores (Ward, 1904). He was particularly interested in formation of haustoria, because he believed that Eriksson had mistaken haustoria for "*corpuscules speciaux,"* a form in which Eriksson thought the rust fungus emerged from an invisible "mycoplasm" stage. Ward provided an extensive set of clear drawings showing appressoria, penetration pegs, substomatal vesicles, infection hyphae, haustoria at several stages of development, and "runner" hyphae beginning to ramify in host tissue. The number and location of nuclei were shown for each type of fungal cell.

Ward (1905) extended his studies to wheat infected with *P. glumarum (striiformis),* including development on an immune cultivar obtained from Biffen. He described "death changes" in the immune host, what Stakman (1915) later termed "hypersensitiveness." Ward surmised that host cell death resulted from either starvation or poisoning and that "… the hyphae attack the cells too vigorously at the outset." Resistance responses of nonhost species to several rust fungi, including *P. graminis* and *P. glumarum (striiformis),* were described cytologically by Ward's student, C. M. Gibson (1904), who showed that the fungi usually were able to enter stomata, although several failures to enter were recorded.

C. V. G. TRANSHEL (1868-1942)

Vladimir Genrikhovich Transhel was influenced by M. S. Voronin, 30 years his senior and regarded as the founder of Russian mycology, who in turn had been a student of de Bary. Most of Transhel's career was at Leningrad (St. Petersburg), where he described and curated mycological collections. He also wrote theoretical compositions including "Rust Fungi in their Relation to the Systematics of Vascular Plants" (1927), "'Fischer's Law' and 'Transhel's Method' in Rust Fungi" (1934a), and "Alternate Hosts of Grain Rusts and their Distribution in the USSR" (1934b). He summarized 50 years of his studies in "Conspectus" (1939), his most comprehensive work (Section VII,K). Like Arthur in the United States, he established a major herbarium of rust fungi, which is actively maintained in the Institute of Botany in Leningrad.

Transhel is best known for his life-cycle studies, which he continued from earlier research by Voronin. He proved the relationships of numerous heteroecious rusts and determined host specialization of many species. Of particular interest is Transhel's method or law, proposed in 1904 (Kuprevich and Transhel, 1957). This is a method of prediction stating that on finding morphological similarity between the telial stages of a presumed heteroecious rust on one host and a microcyclic species on an unrelated host, the aecial host of the heteroecious rust will likely be the same as, or related to, the host of the microform. He explained this relationship on an evolutionary basis; that is, although aecia and uredia are lost from the life cycle, basidiospores can infect only the previous aecial host; thus for a microcyclic species to complete its life

cycle and survive without aecia, telia are now required on the previous aecial host. This “law” led to the concept of correlated species and later the broadening of its definition by Cummins (1959) to include endocyclic species.

In Transhel's correlative taxonomic studies with rusts and hosts, he reported that primitive Uredinales parasitize ferns, whereas more highly differentiated rust species attack advanced plant families such as Leguminosae and Rosaceae. He also found that rusts may distinguish groups of hosts more readily than taxonomists, thus assisting in taxonomic studies of higher plants (Transhel, 1936). Transhel's ideas in relation to evolution of cereal rusts are discussed further in Wahl, Chapter 2, this volume.

D. J. H. CRAIGIE (1887-)

After years of speculation by uredinologists about sex in the rust fungi and the function of pycnia and pycniospores, John Hubert Craigie (1927a,b), at the Canadian Dominion Rust Research Laboratory, found that pycnia are in fact sexual structures and that the rust fungi studied are heterothallic but not dioecious. With cytological support from subsequent workers (Andrus, 1931; Allen, 1930), his research showed that pycniospores are functional male gametes. [Harder (Chapter 11, this volume) describes the sexual function of pycniospores.]

The cytological basis for Craigie's discovery had been set by Sappin-Trouffy (1896), whose studies under Dangeard's direction showed that pycniospores and the hyphae from which they originate are uninucleate, but that aeciospores and urediospores are binucleate, as are the cells of immature teliospores (Craigie, 1931). Sappin-Trouffy showed that conjugate nuclei fuse during maturation of teliospores and that two divisions, one a reduction, occur in the basidium (Arthur, 1929). This was considered a sexual process. Blackman (1904) demonstrated the origin of the binucleate condition at the base of the aecium, but the source of the nuclei involved in dikaryotization was not known.

Craigie's research solved the final enigma of sex in the rusts and ended years of controversy revolving around this gap in understanding rust life cycles. It opened the vista for subsequent cytological and genetic studies. A more thorough treatise was published later (Craigie, 1931), with an extensive literature review on the problem and the history of previous developments as well as a comprehensive presentation of the details of his experiments. Green *et al.* (1980) provided a biography.

E. R. F. ALLEN (1879-1963)

Ruth F. Allen, United States Department of Agriculture and University of California, published two series of cytological investigations of cereal rusts.

The first described development of infection structures and uredia in cereal hosts; the second showed stages of infection and development of pycnia and aecia in alternate hosts. These studies were characterized by excellent comprehensive descriptions, presented in both words and drawings.

Allen's work on uredial development was directed largely toward comparisons of resistant and susceptible wheats (e.g., Allen, 1924, 1926, 1927). For *Puccinia graminis* and *P. triticina* (*recondita*), fungal development was followed through formation of appressoria, sub-stomatal vesicles, infection hyphae, haustoria, and intercellular hyphae. The number of nuclei per fungal cell was clearly documented through each developmental stage. Responses of chloroplasts, nuclei, and cytoplasm in host cells were shown in both susceptible and resistant hosts. The collapse and death of host cells as part of the resistance response was shown. These descriptions remain the basic reference work for cereal rust cytologists and physiologists, especially those concerned with compatibility and incompatibility.

Following Craigie's discovery of heterothallism in rusts in 1927, Allen (e.g., 1930, 1932, 1934) used her cytological expertise to describe development of pycnia and aecia in cereal rusts, including work with *P. graminis, P. triticina (recondita), P. coronata,* and *P. sorghi.* She described the development of germ tubes and appressoria from basidio-spores, the penetration of the leaf, the development of hyphae within the leaf, and the formation of pycnia and aecia. She showed pycniospores fused with paraphyses of the pycnium, provided evidence that pycniospores fuse with “receptive hyphae” emerging from the leaf surface, and that pycniospores could germinate and invade leaf tissues, the latter two phenomena disputed by Buller (Section VII,H).

F. M. NEWTON (1887-1971)

Margaret w and A. M. Brown at the Canadian Dominion Rust Research Laboratory to pioneer genetic studies on *Puccinia graminis* in the period immediately following Craigie's discovery of heterothallism and the function of pycnia. This research group monitored the occurrence of races of the fungus as a practical follow-up of Stakman's earlier research. They found occasional color mutants. Subsequently, they pursued the sexual cycle of the fungus and studied the genetics of uredial pathogenicity and spore color following hybridization on the barberry (Newton *et al.,* 1930a,b). This effort produced knowledge on inheritance of pathogenicity, as described by race designation, along with contemporary studies in the United States (Stakman *et al*, 1930) and Australia (Wa-terhouse, 1929a). It provided the genetic information to parallel the cytological research pursued concurrently by Allen (1930). When combined with host plant genetics, this was a stepping stone to the classical interorganismal genetics of Flor (1946) in the next decade (Section VIII).

A major treatise on pathological specialization, race distribution, and hybridization of *Puccinia graminis* f. sp. *tritici* was published by Newton and Johnson (1932). They pointed out that as long as the rust fungus remained in the uredial stage, permanent changes in pathogenicity were rarely encountered. In contrast, on completing the life cycle on barberry, races were found usually to be heterozygous for pathogenicity, segregating and recombining into new races. For the most part, hybridizing followed Mendelian laws, although some cytoplasmic influences were evident. Similar studies were continued for some years by this research group and particularly by Johnson following Newton's retirement in 1945.

IV. Epidemiology of Cereal Rusts

A. E. C. STAKMAN (1885-1979)

In addition to his studies on pathogenic specialization (Section II,G), Stakman became interested in sources of inoculum for the vast epidemics of stem rust in North America in 1904 and again in 1916. With the finding of the second biologic form of *P. graminis* f. sp. *tritici* in 1916 (Stakman and Piemeisel, 1917a), there were detectable markers in the pathogen. This assisted in the identification of sources of inoculum (Stakman and Hoerner, 1918). Starting in 1917, Stakman and co-workers began a study of the epidemiology of wheat stem rust. Methods of conducting field surveys, collecting samples, trapping spores, and following spore movement were developed and spread worldwide by Stakman, his co-workers, and students. These studies continued for 36 years under his guidance.

Studies elsewhere indicated the involvement of barberry as a source of inoculum. Although others had major roles (H. L. Bolley deserves special mention), Stakman personally led a campaign to eradicate susceptible *Berberis* and *Mahonia* species as a source of inoculum of *P. graminis*. This campaign involved administrative, scientific, and publicity phases. Stakman *et al.* (1934) utilized Canadian, Australian, and American research on sexuality and variation to support barberry eradication. The effects of this program have been summarized by Roelfs (1982).

Studies were also initiated on movement of urediospores from the southern United States and Mexico. Spore traps were designed and exposed from aircraft and ground stations (Stakman *et al.*, 1923). Urediospores were abundant at altitudes up to 3333 m and were present in trace amounts to 5000 m. Spore numbers trapped were related to ground disease severities. The results of 30 year's study were summarized by Stakman and Harrar (1957).

Additionally, extensive annual field surveys were conducted to monitor disease severity and prevalence and to obtain collections for identification of physiological races (Stakman *et al.*, 1929). This information was used in selection of parents for breeding resistant cultivars (Harrar *et al.*, 1944). The effect of temperature on epidemics was analyzed (Stakman and Lambert, 1928). Histories were developed for individual annual epidemics (e.g., Stakman *et al.*, 1925; Stakman, 1935) and comparisons made between years (Stakman and Harrar, 1957). The history of development and spread of important pathogenic races was studied (Stakman and Cassell, 1938; Stakman, 1950). These long-term studies revealed relationships linking severity of stem rust epidemics with dates of disease onset (Hamilton and Stakman, 1967). These studies became the largest collection of epidemiological information obtained on a single plant disease.

B. K. C. MEHTA (1892-1950)

Karamchand Mehta became interested in cereal rust epidemiology at Cambridge in the early 1920s (Mehta, 1923). He shortly initiated experiments on the annual recurrence of rusts on wheat and barley in India (Mehta, 1933). During the next decade, Mehta determined that *Berberis* and *Thalictrum* spp. occurring in the hills of India did not have a significant role in the life cycle of rust in India (Mehta, 1929, 1933, 1940). He further found that intense summer heat (>38°C daily maximum lasting for weeks) destroyed rust in the plains where wheat is grown (Mehta, 1929). Mehta used extensive field surveys throughout India and Nepal to determine that rusts survive the summers in the Himalaya mountains of the north and the Nilgiri and Pulney hills in southern India (Mehta, 1929). Inoculum had to be blown from these areas to the wheat-growing regions (Mehta, 1952). Mehta's contributions were reviewed by Prasada (1950). The “green revolution” has created a renewed interest in epidemiology of the cereal rusts in India (see Nagarajan and Joshi, Chapter 12, Vol. II).

C. K. S. CHESTER (1906-1969)

Kenneth Starr Chester was an active researcher on cereal rust epidemiology in Oklahoma from 1937-1948, a relatively short period. His influence on epidemiology, however, was significant. He summarized great volumes of previous work including much from eastern Europe (Chester, 1946; Chester *et ah*, 1951). The conclusions presented by Chester were not always those widely held by his contemporaries, but by 1960 his work was widely respected and cited. His pleas for crop loss measurements (Chester, 1950) are only now receiving attention.

Chester's research involved the development of the “critical month” theory (Chester, 1946). This theory states that final severity of wheat leaf rust on fall-sown wheat is primarily determined during the spring 30-day period in which the daily mean temperature average is approximately 10°C (Chester, 1942, 1943, 1944). In Oklahoma this is the month of March, but it would be earlier farther south and later northward. In contrast, little correlation was found between the amount of leaf rust that developed in the fall and that occurring in the later spring. Little pathogen increase occurred until mean temperatures reached 10°C. The temperature and moisture during the 30-day period (critical month) immediately after winter when the normal mean temperature was 10°C were the most vital to disease development. Following this period little correlation was found between weather and final disease severity, because the daily mean temperatures were generally in the range that allowed pathogen development, and the interyear variation in weather did not significantly affect rust development. Thus the interyear variation of this later period was of no forecasting value. The rust prediction system developed by Chester was used successfully in Oklahoma for more than 30 years.

V. Resistance to Cereal Rusts

A. R. H. BIFFEN (1874-1949)

The rediscovery in 1901 of Mendel's laws of segregation and independent assortment of genes stimulated genetic evaluation of plant disease resistance data. The first report was by Rowland Harry Biffen (1905) at Cambridge. He crossed the stripe rust susceptible wheat cultivar Red King with the resistant Rivet. His F1 plants were susceptible, and in the F2 generation he found 195 infected plants and 64 rust-free plants—fulfilling a 3:1 prediction of Mendelian genetics, with sus- ceptibility being dominant. (Observations earlier in disease development gave three categories in a 1:2:1 ratio, indicating incomplete dominance.) Resistance and susceptibility were also independent of other plant characters (Biffen, 1907).

The discovery of discrete, heritable differences created doubts about the bridging-host theory of Ward (1903), which held that a pathogen could gradually adapt to a resistant host by passing through taxonomically intermediate hosts. Within a few years many more examples of Mendelian inheritance were found. However, the confusion resulting from the occurrence of physiologic races of pathogens, polygenic inheritance, varying effects of the environment on disease, and the bridging-host theory resulted in a continuing debate for several years before agreement was reached that Mendel's genetic principles were applicable to resistance against cereal rusts.

Biffen (1931) further reported that when resistance is intermediate and not clearly defined, the distinct classification of progenies becomes impracticable. He concluded that the details of inheritance are not so important, the significant result being that resistant lines are obtained, following segregation, in a stable form in later generations.

B. H. K. HAYES (1884-1972)

Three sources of resistance to cereal rusts have been of such great long-term value that they deserve mention. Perhaps other sources of resistance may subsequently be added to this list. Initial wheat breeding in Minnesota for stem rust resistance, led by Herbert Kendall Hayes, supplied a 1914 cross between Marquis hard red spring wheat and resistant Iumillo durum (Hayes *et al.*, 1920). Marquillo was selected in 1918 as a resistant hard red spring wheat and distributed in 1928. This cultivar did not become important, but in the next breeding cycle a sib selection was crossed with a Marquis/Kanred derivative in 1921. This cross was made to study the genetics of resistance when the parents were known to possess different types of resistance (Hayes *et al.*, 1925). Thatcher wheat was selected from this "double cross" in 1925 and released in 1934 (Hayes*et al.*, 1936). Although not yet in widespread use, Thatcher was spectacularly effective against the 1935 stem rust epidemic and provided valuable protection in 1937. Thatcher has *Sr5, 9g, 12, 16,* and at least two additional recessive genes, a combination that continues to provide useful resistance 55 years after the release of Marquillo (Nazareno and Roelfs, 1981).

Thatcher was an important parent in subsequent North American cultivars, Mida, Rushmore, Pembina, Justin, Chris, Era, Fortuna, Manitou, Waldron, Olaf, Sinton, Neepawa, and Marshall among many others, essentially all of the North American hard red spring wheats for many years. In the CIMMYT (Centro Internacional de Mejoramiento de Maiz y Trigo) program, Thatcher occurs in the pedigrees of Ciano 67, Yaqui 48, Penjamo 62, and through these lines in many others. The Australian cultivars Eagle, Gatcher, Mersey, Summit, Tarsa, and Zenith all have a Thatcher parentage.

C. E. S. MCFADDEN (1891-1956)

In 1915, shortly after the first Iumillo durum cross, Edgar S. McFadden found an emmer wheat to be resistant to stem rust. This emmer (designated Yaroslav) was also crossed to Marquis. A selection subsequently named Hope was made in 1923 on the McFadden farm near Webster, South Dakota (McFadden, 1925), and increased and released in 1927 (McFadden, 1930). Like Marquillo, Hope was not itself a successful cultivar. However, Hope and a sib selection H-44 were probably the widest used sources of stem rust resistance in the world. Hope possesses *Sr2, 7b, 9d,* and *17.* The adult plant resistance *Sr2* is

still effective worldwide except under special circumstances at high inoculum densities (Sunderwirth and Roelfs, 1980; Roelfs, Chapter 1, Vol. II). Hope was back-crossed to Thatcher in Minnesota, and a selection with combined resistance named Newthatch was also widely used as a parent (Ausemus *et ah*, 1944). Important Hope and H-44 derivatives include Rival, Regent, Pilot, Mida, Rushmore, Selkirk, Renown, Pembina, Justin, Centurk, Scout, Genwari, Hopps, and numerous others. The present North American hard red spring wheats largely include a parentage of Thatcher and/or Hope or H-44, combined with additional genes for stem rust resistance.

D. I. BECKMAN (1896-1971)

In 1925, at the Veranopolis Experiment Station in Brazil, a cross was made by Iwar Beckman between two local wheat cultivars, Polysu and Alfredo Chaves 6121. These two locally grown cultivars had survived through the many diseases and soil problems common in Rio Grande de Sul. Lines from this hybrid were taken by Beckman to San Luis Gonzaga in 1926 and then to Bage in 1929. During a stripe rust epidemic, plants of this cross were unaffected. Beckman named several selections from this cross in 1934: Frondoso, Fronteira, and Surpreza.

To add earlier maturity he crossed Fronteira with Mentana, resulting in the cultivar Frontana. Unpublished reports do not indicate leaf rust resistance to be a major selection factor; however, resistance evidently was transferred from Alfredo Chaves 6121 to its offspring (Beckman, 1954). These cultivars, particularly Frontana, have been a major source of leaf rust resistance. *Lr13* from Frontana is utilized worldwide and is known to be an important part of the leaf rust resistance of the cultivars Chris, Era, and Columbus. Surpreza is a parent of Redcoat, the North American soft red winter wheat, and Frondoso of the Atlas cultivars.

E. W. L. WATERHOUSE (1887-1969)

Walter Lawry Waterhouse initiated research on cereal rusts in Australia in 1919 (Watson and Frankel, 1972). He started by collecting urediospores from as many sources as possible (Waterhouse, 1929b). This led to an interest in variation for virulence in the cereal rust pathogens. He established that passage of *Puccinia graminis* through barberry resulted in variation among aeciospores (Waterhouse, 1929a). He showed that within races of *P. trticina* (*recondita*), identified on the international differential series, additional variation could be identified using other sources of resistance (Waterhouse, 1929b). This demonstrated that races were not necessarily homogeneous units but "packages" of similar pathogenic characteristics as determined by a designated host series. Waterhouse also documented the effect of temperature on race

determinations (Waterhouse, 1929b). His early experiences with variation in the pathogen populations provided the background that allowed Waterhouse and associates later to determine the effect of resistant cultivars on the frequency and distribution of combinations of virulence (Waterhouse, 1935; Watson and Waterhouse, 1949). He very early moved into breeding for resistance (Waterhouse, 1930). During the period of his activity, Australian cultivars progressed from very susceptible, to those possessing single gene resistance that was overcome in time, to cultivars with several genes for resistance that have remained effective (Watson and Waterhouse, 1949).

VI. Physiology of Cereal Rusts

Changes in host plant physiology as a consequence of rust in cereals were reviewed by Chester (1946), who emphasized that rust increases transpiration of cereals, adding to stress under conditions of limited water supply. From available studies of respiration, photosynthesis, and related processes in rusted cereals, Chester concluded that "… the effect of reduced photosynthesis is increased by its association with accelerated rates of respiration… . Meanwhile, the economical utilization of the products of photosynthesis is impaired by the disruption of normal translocation and amylase activities." These conclusions remain valid today (Bushnell, Chapter 15, this volume; Durbin, Chapter 16, this volume).

A. G. GASSNER (1881-1955)

As part of their comprehensive program to develop methods of rust control, Gustav Gassner and colleagues at the Braunschweig Technische Hochschule investigated the mineral nutrition of cereals in relation to rust development. They found that high levels of nitrogen favored rust development, whereas high levels of potassium tended to reduce it (Gassner and Hassebrauk, 1931, 1933). Rust development was correlated with capacity of leaves to assimilate $C0_2$ (Gassner and Goeze, 1932). Increasing the concentration of $C0_2$ in air to above normal concentrations enhanced $C0_2$ assimilation and in turn stimulated rust development (Gassner and Straib, 1929). Gassner and Franke (1938) determined the amount of protein and soluble nitrogen in leaf and stripe rusted wheat, showing that disease usually retarded loss of nitrogen compounds from wheat leaves. Gassner also showed that rust development varied with leaf position on adult cereal plants and with the age of leaves and plants (Gassner, 1932; Gassner and Kirchhoff, 1934).

B. S. DICKINSON (1898-)

Sydney Dickinson (e.g., 1949, 1971, 1977), Cambridge University, pioneered the study of cereal rust fungi on artificial membranes, showing that membrane surfaces stimulated formation of infection structures by germinating spores. He manufactured membranes from nitrocellulose and other materials, using carefully prescribed formulations to obtain the most effective membranes. Several developmental phenomena were observed, including zigzag growth of germ tubes and differentiation of structures resembling appressoria, substomatal vesicles, infection hyphae, and haustoria. This work clearly implicated the effect of leaf surfaces on fungal differentiation and led to ongoing efforts to understand the mechanisms involved (Staples and Macko, Chapter 9, this volume). Furthermore, Dickinson (1949) demonstrated that infection structures must be produced before leaf-colonizing hyphae can grow, a finding instrumental in the culture of *Puccinia graminis* on artificial media (Williams, Chapter 13, this volume).

C. P. J. ALLEN (1914-1976)

Paul James Allen (e.g., 1953, 1954, 1959), University of Wisconsin, wrote a series of influential reviews on the physiological aspects of plant disease. He had earlier determined rates of respiration and photosynthesis in powdery mildew of wheat and was interested in the causes of metabolic change in host tissues. His theory that a toxin uncoupled phosphorylation from respiration (Allen, 1953) stimulated research into the causes of respiratory change in powdery mildews and rusts. Later, Allen (1966) acknowledged that the toxin hypothesis was untenable and that the respiratory changes were akin to "wound" or "developed" respiration involving extensive cellular adjustment and new protein synthesis.

Allen also contributed to our understanding of germination of urediospores and differentiation of infection structures. He demonstrated that urediospores contained a self-inhibitor of germination, and that substances in spore extracts could induce differentiation of infection structures (Allen, 1955, 1957, 1976). This provided the foundation for work by others on spore and germling physiology (Staples and Macko, Chapter 9, this volume).

VII. Books and A Newsletter of Special Significance

Books of special importance to the cereal rusts are listed in Table I. Several of these, of course, are broader in scope than the cereal rusts. As evidenced by the citations in the present volumes, there is a further vast literature on cereal

and other rust fungi. If, in addition to listing books, one chose to include comprehensive bulletins, or publications on local flora, the list would include many more entries from all over the world, in most written languages. Authors of several more general texts not listed here have used one or more of the cereal rusts as a vehicle in discussing epidemiology, genetics of pathogens, disease resistance, or other relevant topics. Except for the more recent listings, not considered “historical” at this time, the publications in Table I and their authors are reviewed here.

Table I

Selected Books and a Newsletter of Major Worldwide Importance on the Cereal or Plants Rusts

Author(s)	Date	Title
de Bary, A.	1884	Comparative Morphology and Biology of the Fungi, Mycetozoa, and Bacteria
Plowright, C. B.	1889	A Monograph of the British Uredineae and Ustilagineae with an Account of their Biology Including the Methods of Observing the Germination of their Spores and of their Experimental Culture
Eriksson, J., and Henning, E. J.	1896	Die Getreideroste. Ihre Geschichte und Nature sowie Massregeln gegen Dieselben
Klebahn, H.	1904	Die wirtswechselnden Rostpilze. Versuch einer Gesamtdarstellung ihrer biologischen Verhältnisse
McAlpine, D.	1906	The Rusts of Australia. Their Structure, Nature, and Classification
Yachevski, A. A.	1909	(Rusts of Grain Crops in Russia)
Grove, W. B.	1913	The British Rust Fungi (Uredinales). Their Biology and Classification
Buller, A. H. R.	1924	Researches on Fungi, Vol. III
	1950	Researches on Fungi, Vol. VII
Arthur, J. C.	1929	The Plant Rusts (Uredinales)
	1934	Manual of the Rusts in United States and Canada
Lehmann, E., Kummer, H., and Dannenmann, H.	1937	Der Schwarzrost, seine Geschichte, seine Biologie und seine Bekämpfung in Verbindung mit der Berberitzenfrage
Transhel, V. G.	1939	(Conspectus of the Rust Fungi of the USSR)
Naumov, N. A.	1939	(The Rusts of Grain Crops in the USSR)
Chester, K. S.	1946	The Nature and Prevention of the Cereal Rusts as Exemplified in the Leaf Rust of Wheat
Vallega, J., Cenoz, H. P., and Tessi, J. L.	1956–1967	Robigo (A Newsletter)
Cummins, G. B.	1971	The Rust Fungi of Cereals, Grasses, and Bamboos
Kvostova, V. V., and Shumnyi, V. K.	1978	(Resistance of Wheat to Leaf Rust. Genetic Diversity of the Fungus and the Host Plant)
Littlefield, L. J., and Heath, M. C.	1979	Ultrastructure of Rust Fungi
Littlefield, L. J.	1981	Biology of the Plant Rusts, An Introduction
Scott, K. J., and Chakravorty, A. K., eds.	1982	The Rust Fungi

A. DE BARY, A. (1884) COMPARATIVE MORPHOLOGY AND BIOLOGY OF THE FUNGI, MYCETOZOA, AND BACTERIA

de Bary (Section III,A) brought together the mycological knowledge of his time in this book, perhaps the single most important mycological publication of all time. It was translated into English in 1887. Included among his comprehensive descriptions of structure and development were the cereal rust fungi, principally from his own studies of 1866-1867.

de Bary discussed the nature of parasitism and introduced the term "obligate parasites" for organisms such as rusts "… to which a parasitic life is indispensable for the attainment of their full development" including "strictly obligate parasites … which as far as we know, live only as parasites. …" He wisely cautioned that the definition "… hold(s) good in the natural, and … the spontaneous course of things… . Artificial conditions may in some cases be established which may result for example, in the development of a spontaneously and strictly parasitic fungus in a way not parasitic. …" Thus he anticipated culture of rusts on artificial media but believed this should not alter their classification as obligate parasites (see Williams, Chapter 13, this volume).

B. PLOWRIGHT, C. B. (1889) A MONOGRAPH OF THE BRITISH UREDINEAE AND USTILAGINEAE

Building on the work of Tulasne, de Bary, and others, Charles Bagge Plowright (1849-1910) presented descriptions and life histories of the known British rust and smut fungi. He discussed major structural and developmental features of urediospores, teliospores, pycniospores, aeciospores, and mycelia, including figures of germinating urediospores and teliospores of cereal rusts. He described the methods he used to study spore germination and to inoculate plants without contamination by unwanted rust spores. He reviewed the many reports indicating that barberry was associated with cereal rust and included the complete text of "The Barberry Law of Massachusetts" of 1764. He discussed the available evidence regarding the role of pycniospores and described their apparent budding, concluding incorrectly that they more likely functioned as conidia than as sexual organs.

C. ERIKSSON, J., AND HENNING, E. J. (1896) DIE GETREIDEROSTE

Two years following his exposition of *formae speciales* within the cereal rust fungi, Jakob Eriksson (Section II,F) joined with Ernst Johan Henning in this major reference work on cereal rusts. This book reviewed history, etiology, infection processes, and geography of occurrence of six cereal rust species, and included fine colored illustrations. Possibly the main long-term contribution

was the detailing of *formae speciales* within the context of a thorough presentation on cereal rusts. They proposed four *formae speciales* within *P. graminis,* two within *P. dispersa* (*recondita*), and three within *P. glumarum (striiformis).* They referenced a wide range of foreign authors including Arthur, Bolley, Cobb, de Bary, Dietel, and McAlpine, as well as many of Eriksson's own previous publications. Later on, Eriksson authored several other books including a general plant pathology text in German, the second edition of which was translated into English (1930). Cereal rusts accounted for 30 pages of this text.

D. KLEBAHN, H. (1904) DIE WIRTWECHSELNDEN ROSTPILZE

The earliest publication of Henrich Klebahn on rusts concerned blister rust of pine. His interests in rust fungi, however, gradually focused on heteroecious species and their biology, culminating 17 years later in his book "The Rust Fungi with Alternating Hosts, a Presentation of their Biological Relations." The general portion of the book comprises a discussion of the concepts of heteroecism, mycoplasma, specialization, and sexuality, as well as of environmental requirements for spore distribution, spore germination, and infection. In the specialized section of the book Klebahn discussed the cereal rusts and their nearest relatives. Included were *Puccinia graminis, P. dispersa* (now *P. recondita* f. sp. *secalis* and f. sp. *tritici),* and near relatives (including *P. hordei), P. glumarum {P. striiformis), P. coronata,* and many other rusts attacking both Gramineae and nongrass hosts. For *P. graminis,* Klebahn reviewed the relationship of barberry to stem rust. Otherwise, he provided a listing of the associated aecial, uredial, and telial hosts as given in the literature for each rust. This was the major presentation of heteroecism in the rust fungi of its day.

E. MCALPINE, D. (1906) THE RUSTS OF AUSTRALIA

Daniel McAlpine (1849-1932) was contemporary with Eriksson, Plowright, Arthur, and Klebahn. He provided the Australian arm of the worldwide expansion of knowledge of plant rusts in the late nineteenth and early twentieth centuries. His primary contribution was the well-known "Rusts of Australia." This was the third of five major books that McAlpine authored, and it followed numerous of his previous research papers of which he cited 36.

His book is divided into two parts: a general discussion and description of the rust fungi, followed by a systematic arrangement of all of the rust species known to occur in Australia. At the end of the first part, a chapter is included on rust in wheat in Australia. A unique observation is that barberry had not been infected by *Puccinia graminis* in Australia either naturally or artificially, although teliospores were readily germinated. He included discussion and

speculation on the epidemiology and control of stem rust of wheat. He concluded that the only effective means of control were to cultivate the most rust-resisting plants, to choose early-maturing cultivars, and to sow early. Like Eriksson and Henning's text of a decade earlier, McAlpine included illustrative color plates as well as photomicrographs of spores.

F. YACHEVSKI, A. A. (1909) (RUSTS OF GRAIN CROPS IN RUSSIA)

Artur Arturovich Yachevski (1863-1932) was a mycologist at the Leningrad institute where Voronin, Transhel, and Naumov also worked. He was prominent early in forest pathology, having written the first large handbook on that topic in Russia in 1897. His "Fundamentals of Mycology," a large general text, appeared in 1933 after his death. However, he wrote a series of articles on various plant rusts and a book entitled "Rusts of Grain Crops in Russia" (1909). This volume contained information on disease distribution, damage caused, environmental conditions favorable for disease development, disease control, and a list of the important rust fungi in Russia. The principal pathogens covered were *Puccinia graminis, P. dispersa* (*recondita*), *P. triticina (recondita*), *P. glumarum* (*striiformis*), *P. simplex (hordei), P. coronifera (coronata),*and *P. maydis.* Yachevski was particularly reputed for his opinion that the selection of resistant cultivars was the basic method for controlling rust.

G. GROVE, W. B. (1913) THE BRITISH RUST FUNGI (UREDINALES)

This text by William Bywater Grove (1848-1938) was published 24 years after Plowright's monograph, with the author's purpose to update knowledge of the Uredinales in a major British text. He pointed out that great progress in elucidating the biology of rust fungi had been made in the intervening years. He addressed life histories, sexuality, specialization, and classification. The general part is followed by a systematic portion that includes species found in Britain. With Biffen's studies on genetics of resistance now available and the Mendelian nature of resistance determined, Grove made a strong case for the likelihood of the pycniospores being male gametes in his discussion of sexuality. He pointed out that because of the minute specialization of the rusts, a host "variety may be immune to one rust while susceptible to another, or may even be immune in one country but susceptible to the same rust in a different climate."

H. BULLER, A. H. R. (1924, 1950) RESEARCHES ON FUNGI, VOLS. III AND VII

A. H. Reginald Buller (1874-1944), at the University of Manitoba, devoted two major parts of his seven-volume "Researches on Fungi" to rust fungi. In Volume III (1924), he described basidiospore and aeciospore discharge in *P. graminis.* He documented the timing of events in basidiospore growth and

discharge, including the formation of a water droplet at the base of the basidiospore prior to discharge, and the distance traveled by discharged spores. He speculated about mechanisms of discharge and compared discharge phenomena in rusts and Hymenomycetes, pointing out many similarities. In Volume VII (1950), published posthumously, Buller presented what was then known of the sexual process in the Uredinales. Here, he reviewed historical aspects of pycnial development and function. He described the fusion between pycniospores and the flexuous hyphae of the pycnium, showing pycniospores of *P. coronata* fused to flexuous hyphae, and he defended the flexuous hypha as the only structure that can fuse with pycniospores. Proto-aecia were described in detail, including their transition into aecia following dikaryotization. He marshaled the available evidence that fungal nuclei migrate through septal pores, deducing that cells at the base of preformed proto-aecia are dikaryotized by nuclei migrating from flexuous hyphae.

Buller speculated about the role of insects in movement of pycniospores from one pycnium to another. As had Plowright (1889) earlier, he took special interest in the content and probable function of pycnial nectar as an insect attractant. He recounted (1950) how he demonstrated to Craigie that flies move from pycnium to pycnium, which led to Craigie's discovery of heterothallism in rusts.

I. ARTHUR, J. C. (1929) THE PLANT RUSTS (UREDINALES); (1934) MANUAL OF THE RUSTS IN UNITED STATES AND CANADA

In addition to innumerable research reports throughout his long lifetime (Section II,E), Arthur's most widely recognized contributions are two major books. “The Plant Rusts” (1929) is a treatise on the overall biology of this group of fungi, in collaboration with six of his former students and associates, and “Manual of the Rusts in United States and Canada” (1934), an exhaustive taxonomic presentation. The latter has for many decades been the primary authoritative source for taxonomic and nomenclatural treatment of the rust fungi in North America. “The Plant Rusts” is the most comprehensive presentation of the biology of the rust fungi with discussion and integration of prior literature. More recent texts have served to supplement and update rather than to replace it. Arthur's manual is so comprehensive that Cummins (1962), who illustrated the original edition in 1934, chose to have it reprinted in 1962 without revision, adding only a 24-page supplement.

J. LEHMANN, E., KUMMER, H., AND DANNENMANN, H. (1937) DER SCHWARZROST

This book summarized most of the worldwide literature on *P. graminis* available in 1937 and is the only widely circulated monograph on this important plant pathogen. It was an important source of information for readers of German for many years. Lehmann and Kummer had previously published jointly and independently a series of articles on barberry distribution, effect of barberry on stem rust occurrence, and control of stem rust through barberry eradication in 1934 and 1935 (Lehmann *et al.*, 1937). Their book had an extensive content with emphasis on the history of understanding of the disease and its biology, relation to barberry, resistance and pathogenicity, epidemiology, distribution, losses, and control. There was a thorough review of literature except for that from Russia, represented only by English and German language articles. This text and Chester's subsequent book (1946) on leaf rust have been the only book-length works devoted to individual rusts of cereals, although several excellent pamphlet-length treatises are available.

K. TRANSHEL, V. G. (1939) (CONSPECTUS OF THE RUST FUNGI OF THE USSR)

This review by Transhel (Section III,C) summarized a lifetime of rust research. Kuprevich (Kuprevich and Transhel, 1957) stressed the great importance of this publication in the study of rust fungi in the Soviet Union. It provided an exhaustive list of rust fungi with critical notes, keys, hosts, areas of occurrence, and fungal and host indices. It also included a presentation of the morphology, taxonomy, geographical distribution, and biology of the rust fungi. Much of this information was abbreviated and updated under the authorship of Kuprevich and Transhel (1957), 15 years after the death of Transhel, and was subsequently translated into English. It provides a historical review of investigations of the rust fungi in the Soviet Union. Like the earlier texts of McAlpine (1906), Grove (1913), and Arthur (1929, 1934), it has an extensive general discussion of the biology of rust fungi plus a detailed systematic part, providing the Russian counterpart to these other major texts on rust fungi.

L. NAUMOV, N. A. (1939) (THE RUSTS OF GRAIN CROPS IN THE USSR)

Nikolai Aleksandrovich Naumov (1888–1959) was a mycologist in the research institute at Leningrad that was the professional home of A. A. Yachevski, V. G. Transhel, and many other important Russian mycologists and plant pathologists. Like his associates, he published numerous articles on the mycoflora of Russia. He was intensively involved in the epidemiology of cereal rusts, with several titles relating environmental conditions to rust development.

During the 1920s and 1930s numerous articles by various authors on cereal rusts were published in Russia. In 1939 Naumov brought this information together in a monograph on "The Rusts of Grain Crops in the USSR." This appears to be the first such compilation since the similar title by Yachevski 30 years earlier, and was published the same year as Transhel's more broadly oriented "Conspectus." The book covered current information on biology, ecology, physiological specialization, losses incurred, and control measures. Foreign literature was reviewed, and much of the information was from outside the Soviet Union. Included among the pathogens discussed were *P. graminis*, *P. triticina* (*recondita*), *P. glumarum* (*striiformis*), *P. dispersa* (*recondita*), *P. coronifera* (*coronata*), and *P. anomala* (*hordei*).

M. CHESTER, K. S. (1946) THE NATURE AND PREVENTION OF THE CEREAL RUSTS

Chester was head of the Department of Plant Pathology at the present Oklahoma State University when he conducted his research on leaf rust of wheat (Section IV,C). This interest provided the impetus for a worldwide search of the literature on the cereal rusts. He used this information and his considerable language and writing abilities to compile a monograph on leaf rust of wheat (1946). This book comprehensively presented the current state of knowledge, to which Chester frequently added his opinions, which often differed from conventional views. Time has generally proven Chester right. A major contribution was the broad review for the first time in an English-language text of the Russian literature. After leaving Oklahoma for Battelle Memorial Institute, Chester also coauthored a summary report on stem rust of wheat (Chester *et al.*,1951).

N. VALLEGA, J. (1956-1967) ROBIGO (A NEWSLETTER)

Jose Vallega (1909-1978), like Chester, was a relatively recent contributor to cereal rust research. His importance in the context of this section was the development of an international publication "Robigo," which compiled "cereal rust news from everybody to everybody." Vallega began his research on races of cereal rusts in 1934 at the Instituto de Fitotechnia de Santa Cataline of the University of La Plata in Argentina (Eide, 1978). He later became interested in breeding for resistance and then in genetics of resistance (Villar, 1979). These experiences led him to establish the international Robigo for exchange of information on the cereal rusts. This newsletter was published from 1956 to 1967, initially with Vallega as responsible editor assisted by Hugo P. Cenoz and Juan L. Tessi. He relinquished this position to his assistants in 1960 to become honorary editor following his move to the Food and Agriculture Organization (FAO) in Rome. He proposed Robigo in 1956 because an investigator confined to a country or region could not efficiently defend crops

against a pathogen that had many cultivated and noncultivated hosts worldwide. Therefore, Vallega felt the need for an international work team, with Robigo as its permanent round table to be a place to exchange informally and continually results and ideas even of a preliminary nature. Robigo ended with its nineteenth issue in 1967 following the death of its editor, Hugo P. Cenoz. Today, the Cereal Rusts Bulletin (1973-), published by the European and Mediterranean Cereal Rusts Foundation, and the Proceedings of the European and Mediterranean Cereal Rusts Conferences (every fourth year) have largely become this forum.

VIII. Epilogue—H. H. Flor (1900–)

The classical studies of Harold Henry Flor (1946, 1971) on the genetic interaction of flax and the flax rust fungus (*Melampsora lini)* provide a capstone to the historic contributions outlined in this chapter. Flor's research, of course, was not on a cereal rust, but had its heritage in the earlier studies on cereal rusts and, in turn, provided new vision for further cereal rust research. His “gene-for-gene” theory (see Loegering, Chapter 6, this volume), has been confirmed for the cereal rusts and is the basis for virtually all current research on them.

Flor, a student of E. C. Stakman, worked for the United States Department of Agriculture at North Dakota State University. He worked mostly alone, coming to his theory with great insight only after diligent genetic work.

We view Flor's contribution as the threshold between the early contributors discussed in this chapter and those who came later. His theory set the stage for much that was to come and continues as the dominant force in understanding the cereal rusts.

Acknowledgment

We thank David H. Casper for translating several Russian papers into English.

References

Allen, P. J. (1953). Toxins and tissue respiration. *Phytopathology* 43, 221-229.

Allen, P. J. (1954). Physiological aspects of fungus diseases of plants. *Annu. Rev. Plant Physiol.* 5, 225-248.

Allen, P. J. (1955). The role of a self-inhibitor in the germination of rust uredospores. *Phytopathology* 45, 259-266.
Allen, P. J. (1957). Properties of a volatile fraction from uredospores of *Puccinia graminis* var. *tritici* affecting their germination and development. I. Biological activity. *Plant Physiol.* 32, 385-389.
Allen, P. J. (1959). Physiology and biochemistry of defense. *In* "Plant Pathology: An Advanced Treatise" (J. G. Horsfall and A. E. Dimond, eds.), Vol. 1, pp. 435-467. Academic Press, New York.
Allen, P. J. (1966). The role of the host in the development of disease symptoms—a review. *Phytopathology* 56, 255-260.
Allen, P. J. (1976). Control of spore germination and infection structure formation in the fungi. *Encycl. Plant Physiol., New Ser.* 4, 51-85.
Allen, R. F. (1924). Cytological studies of infection of Baart, Kanred, and Mindum wheats by *Puccinia graminis tritici* forms **III** and XIX. *J. Agric. Res. (Washington, D.C.)* 26, 571-604.
Allen, R. F. (1926). Cytological studies of forms 9, 21, and 27 of *Puccinia graminis tritici.* on Khapli emmer. *J. Agric. Res. (Washington, D.C.)* 32, 701-725.
Allen, R. F. (1927). A cytological study of orange leaf rust, *Puccinia triticina* physiologic form 11, on Malakoff wheat. *J. Agric. Res. (Washington, D.C.)* 34, 697-714.
Allen, R. F. (1930). A cytological study of heterothallism in *Puccinia graminis. J. Agric. Res. (Washington, D.C.)* 40, 585-614.
Allen, R. F. (1932). A cytological study of heterothallism in *Puccinia coronata. J. Agric. Res. (Washington. D.C.)* 45, 513-541.
Allen, R. F. (1934). A cytological study of heterothallism in *Puccinia sorghi. J. Agric. Res. (Washington, D.C.)* 49, 1047-1068.
Andrus, C. F. (1931). The mechanism of sex in *Uromyces appendiculatus* and *U. vignae. J. Agric. Res. (Washington. D.C.)* 42, 559-587.
Arthur, J. C. (1905). Terminology of the spore structures in the Uredinales. *Bot. Gaz. (Chicago)* 39, 219-222.
Arthur, J. C. (1929). "The Plant Rusts (Uredinales)." Wiley, New York.
Arthur, J. C. (1932). Terminologie der Uredinales *Ber. Dtsch. Bot. Ges.* **50A,** 24-27.
Arthur, J. C. (1934). "Manual of the Rusts in United States and Canada" (reprinted with a supplement by G. B. Cummins, Hafner, New York, 1962).
Ausemus, E. R., Stakman, E. C, Hanson, E. W., Geddes, W. F., and Merrit, P. P. (1944). Newthatch wheat. *Minn., Agric. Exp. Stn., Tech. Bull.* **166.**
Baxter, J. W., and Kern, F. D. (1962). History of the Arthur herbarium at Purdue University. *Proc. Indiana Acad. Sci.* 71, 228-232. Beckman, I. (1954). Sobre o cultivo e melhoramento do trigo (*Triticum vulgare* Vill.) no sul do Brasil. *Agron. Sulriograndense Porto Alegre* 1, 64-72.
Biffin, R. H. (1905). Mendel's law of inheritance and wheat breeding. *J. Agric. Sci.* 1, 4-48.
Biffen, R. H. (1907). Studies in the inheritance of disease-resistance. *J. Agric. Sci.* 2, 109-128.
Biffen, R. H. (1931). The cereal rusts and their control. *Trans. Br. Mycol. Soc.* 16, 19-37.
Blackman, V. H. (1904). On the fertilization, alternation of generations, and general cytology of the Uredineae. *Ann. Bot. (London)* 18, 323–373.
Buller, A. H. R. (1924). "Researches on Fungi," Vol. III. Longmans, Green, New York.
Buller, A. H. R. (1950). "Researches on Fungi," Vol. VII. University of Toronto, Toronto.
Chester, K. S. (1942). A suggested basis for the prediction of wheat leaf rust epiphytotics. *Plant Dis. Rep.* 26, 213-217.

Chester, K. S. (1943). The decisive influence of late winter weather on wheat leaf rust epiphytotics. *Plant Dis. Rep., Suppl.* **143,** 133-144.
Chester, K. S. (1944). Low incidence of wheat leaf rust associated with unfavorable late winter weather and antagonism of *Septoria tritici. Plant Dis. Rep.* 28, 280-287.
Chester, K. S. (1946). "The Nature and Prevention of the Cereal Rusts as Exemplified in the Leaf Rust of Wheat." Chronica Botanica, Waltham, Massachusetts.
Chester, K. S. (1950). Plant disease losses: Their appraisal and interpretation. *Plant Dis. Rep., Suppl.* **193,** 190-362.
Chester, K. S., Gilbert, F. A., Haye, R. E., and Newton, N. (1951). "Cereal Rusts: Epidemiology, Losses, and Control," Battelle Mem. Inst., Columbus, Ohio.
Christensen, C. M. (1979). Elvin Charles Stakman, 1885-1979. *Phytopathology* 69, 195.
Craigie, I. H. (1927a). Experiments on sex in rust fungi. *Nature {London)* **120,** 116-117.
Craigie, J. H. (1927b). Discovery of the function of the pycnia of the rust fungi. *Nature (London)* **120,** 765-767.
Craigie, J. H. (1931). An experimental investigation of sex in the rust fungi. *Phytopathology* **21,** 1001-1040.
Cummins, G. B. (1959). "Illustrated Genera of Rust Fungi." Burgess, Minneapolis, Minnesota.
Cummins, G. B. (1962). Supplement to Arthur's Manual of the rusts in United States and Canada. *In* "Manual of the Rusts in United States and Canada" (by J. C. Arthur), pp. 1A-24A. Hafner, New York.
Cummins, G. B. (1971). "The Rust Fungi of Cereals, Grasses and Bamboos." Springer-Verlag, Berlin and New York.
Cummins, G. B. (1978). J. C. Arthur: The man and his work. *Annu. Rev. Phytopathol.* 16, 19-30.
de Bary, A. (1853). "Investigations of the Brand Fungi and the Diseases of Plants Caused by them with reference to Grain and Other Useful Plants" (translated from German by R. M. S. Heffner, D. C. Arny, and J. D. Moore, edited by D. C. Arny, J. D. Moore, and R. N. Schwebke. Phytopathol. Classics No. 11. Am. Phytopathol. Soc, St. Paul, Minnesota, 1969).
de Bary, A. (1866). Neue Untersuchungen über die Uredineen, insbesondere die Entwicklung der *Puccinia graminis* und den Zusammenhang derselben mit *Aecidium Berberidis. Monatsber. K. Preuss. Akad. Wiss. Berlin* pp. 15-50.
de Bary, A. (1867). Neue Untersuchungen iiber Uredineen. *Monatsber. K. Preuss. Akad. Wiss. Berlin* pp. 205-215.
de Bary, A. (1884). "Comparative Morphology and Biology of the Fungi, Mycetozoa and Bacteria" [translated from German by H. E. F. Garnsey, revised by I. B. Balfour, Oxford Univ. Press (Clarendon), London and New York, 1887].
Dickinson, S. (1949). Studies in the physiology of obligate parasitism. II. The behavior of the germ-tubes of certain rusts in contact with various membranes *Ann. Bot. (London)* **13,** 219-236.
Dickinson, S. (1971). Studies in the physiology of obligate parasitism. VIII. An Analysis of fungal responses to thigmotropic stimuli. *Phytopathol. Z.* 70, 62-70.
Dickinson, S. (1977). Studies on the physiology of obligate parasitism. X. Induction of responses to a thigmotropic stimulus. *Phytopathol. Z.* 89, 97-115.
Eide, C. J. (1978). Jose Vallega 1909-1978. *Phytopathology* 68, 1109.
Eriksson, J. (1894). Ueber die specialisirung des Parasitismus bei den Getreiderostpilzen. *Ber. Dtsch. Bot. Ges.* 12, 292-331.
Eriksson, J. (1930). "Fungous Diseases of Plants," 2nd ed. (translated from German by W. Goodwin). Bailliere, London.

Eriksson, J., and Henning, E. (1896). “Die Getreideroste. Ihre Geschichte und Natur sowie Massregeln gegen Dieselben.” Verlag von P. A. Norstedt & Soner, Stockholm.

Flor, H. H. (1946). Genetics of pathogenicity in *Melampsora lini. J. Agric. Res. {Washington, D.C.)* 73, 335-357.

Flor, H. H. (1971). Current status of the gene-for-gene concept. *Annu. Rev. Phytopathol.* 9, 275-296.

Fontana, F. (1767). “Observations on the Rust of Grain” (translated from Italian by P. P. Pirone, Phytopathol. Classics No. 2. Am. Phytopathol. Soc, St. Paul, Minnesota, 1932).

Gassner, G. (1932). Uber Verschiebungen der Rostresistenz wahrend der Entwicklung der Getreidepflanzen. *Phytopathol. Z.* 4, 549-596.

Gassner, G., and Franke, W. (1938). Untersuchungen iiber den Stickstoffhaushalt rostinfizierter Getreideblatter. Ein Beitrag zum Problem der Teleutosporenbildung. *Phytopathol. Z.* 11, 517-570.

Gassner, G., and Goeze, G. (1932). Uber den Einfluss der Kaliernahrung auf die Assimilationsgrosse von Weizenblattern. *Ber. Dtsch. Bot. Ges.* **50A,** 412-482.

Gassner, G., and Hassebrauk, K. (1931). Untersuchungen über die Beziehungen zwischen Mineralsalzernahrung und Verhalten der Getreidepflanzen gegen Rost. *Phytopathol. Z.* 3, 535-617.

Gassner, G., and Hassebrauk, K. (1933). Uber die Beeinflussung der Rostanfalligkeit durch Eintauchen geimpfter Blatter in Losungen von Mineralsalzen und anderen Stoffen. *Phytopathol. Z.* 5, 323-342.

Gassner, G., and Kirchhoff, H. (1934). Einige vergleichende Versuche iiber Verschiebungen der Rostresistenz in Abhangigkeit vom Entwicklungszustand der Getreidepflanzen. *Phytopathol. Z.* 7, 43-52.

Gassner, G., and Straib, W. (1929). Untersuchungen iiber die Abhangigkeit des Infektionsverhaltens der Getreiderostpilze vom Kohlensauregehalt der Luft. *Phytopathol. Z.* 1, 1-30.

Gibson, C. M. (1904). Notes of infection experiments with various Uredineae. *New Phytol.* 3, 184-191.

Goidanich, G. (1943). Life and works of Giovanni Targioni Tozzetti. *R. Accad. Ital., Stud. Doc.* 12, 7-11 (translated from Italian by L. R. Tehon in Phytopathol. Classics No. 9, pp. *vii-xi.* Am. Phytopathol. Soc, St. Paul, Minnesota, 1952).

Green, G. J., Johnson, T., and Conners, I. L. (1980). Pioneer leaders in plant pathology: J. H. Craigie. *Annu. Rev. Phytopathol.* 18, 19-25.

Grove, W. B. (1913). “The British Rust Fungi (Uredinales). Their Biology and Classification.” Cambridge Univ. Press, London and New York.

Hamilton, L. M., and Stakman, E. C. (1967). Time of stem rust appearance on wheat in the western Mississippi basin in relation to the development of epidemics from 1921-1962. *Phytopathology* 57, 609-614.

Harrar, J. G., Loegering, W. Q., and Stakman, E. C. (1944). Relation of physiologic races of *Puccinia graminis tritici* to wheat improvement in southern Mexico. *Phytopathology* 34, 1002 (abstr.).

Hayes, H. K., Parker, J. H., and Kurtzweil, C. (1920). Genetics of rust resistance in crosses of varieties of *Triticum vulgare* with varieties of *T. Durum* and *T. dicoccum. J. Agric. Res. (Washington, D.C.)* 19, 523-542.

Hayes, H. K., Stakman, E. C, and Aamodt, O. S. (1925). Inheritance in wheat of resistance to black stem rust. *Phytopathology* 15, 371-387.

Hayes, H. K., Ausemus, E. R.. Stakman, E. C, Bailey, C. H., Wilson, H. K., Bamberg, R.

H., Markley, M. C, Crim, R. F., and Levine, M. N. (1936). Thatcher wheat. *Stn. Bull.— Minn., Agric. Exp. Stn.* 325.

Kern, F. D. (1942). Joseph Charles Arthur 1850-1942. *Phytopathology* 32, 833-844.
Kislev, M. E. (1982). Stem rust of wheat 3300 years old found in Israel. *Science* 216, 993-994.
Klebahn, H. (1904). "Die wirtswechselnden Rostpilze. Versuch einer Gesamtdarstellung ihrer Biologischen Verhaltnisse." Borntraeger, Berlin.
Kuprevich, V. F., and Transhel, V. G. (1957). Rust fungi. No. 1. Family Melampsoraceae. Vol. IV. Fungi (1). *In* "Cryptogamic Plants of the USSR" (V. P. Savich, ed.) (translated from Russian by E. Rabinovitz, Israel Program for Scientific Translations, Jerusalem, 1970).
Kvostova, V. V., and Shumnyi, V. K. (1978). "Resistance of Wheat to Leaf Rust: Genetic Diversity of the Fungus and the Host Plant" (in Russian). Nauka, Sibirskoe, Novosibirsk (partial translation by D. H. Casper).
Lehmann, E., Kummer, H., and Dannenmann, H. (1937). "Der Schwarzrost seine Geschichte, seine Biologie und seine Bekampfung in Verbindung mit der Berberitzenfrage." J. F.
Lehmanns Verlag, Munich and Berlin. Littlefield, L. J. (1981). "Biology of the Plant Rusts. An Introduction." Iowa State Univ. Press, Ames.
Littlefield, L. J., and Heath, M. C. (1979). "Ultrastructure of Rust Fungi." Academic Press, New York.
McAlpine, D. (1906). "The Rusts of Australia. Their Structure, Nature, and Classification." Government Printer, Melbourne.
McFadden, E. S. (1925). "Synthetic" rust proof bread wheats. *Dak. Farm.* 45, 102.
McFadden, E. S. (1930). A sucessful transfer of emmer characters to *vulgare* wheat. J. *Am. Soc. Agron.* 22, 1020-1034.
Mehta, K. C. (1923). Observations and experiments on cereal rusts in the neighborhood of Cambridge, with special reference to their annual recurrence. *Trans. Br. Mycol. Soc.* 8, 142-176.
Mehta, K. C. (1929). Annual recurrence of rusts on wheat in India. (Presidential Address) *Proc. Indian Sci. Congr., 16,* pp. 199-223.
Mehta, K. C. (1933). Rusts of wheat and barley in India. A study of their annual recurrence, life-histories and physiologic forms. *Indian J. Agric. Sci.* 3, 939-962.
Mehta, K. C. (1940). "Further Studies on Cereal Rusts in India. Part I," Sci. Monogr. No. 14. Published by Manager of Publications, Delhi.
Mehta, K. C. (1952). "Further Studies on Cereal Rusts in India. Part II," Sci. Monogr. No. 18. Published by Manager of Publications, Delhi.
Naumov, N. A. (1939). "The Rusts of Cereals in the USSR. A Monographical Study." Selkhosgiz State Printing Office, Moscow and Leningrad (translated from Russian by D. H. Casper).
Nazareno, N. R. X., and Roelfs, A. P. (1981). Adult plant resistance of Thatcher wheat to stem rust. *Phytopathology* 71, 181-185.
Newton, M., and Johnson, T. (1932). Specialization and hybridization of wheat stem rust, *Puccinia graminis tritici*, in Canada. *Can., Dep. Agric, Bull.* 160.
Newton, M., Johnson, T., and Brown, A. M. (1930a). A preliminary study on the hybridization of physiologic forms of *Puccinia graminis tritici. Sci. Agric. (Ottawa)* 10, 721-731.
Newton, M., Johnson, T., and Brown, A. M. (1930b). A study of the inheritance of spore colour and pathogenicity in crosses between physiologic forms of *Puccinia graminis iritici. Sci. Agric. (Ottawa)* 10, 775-798.
Pcrsoon, C. H. (1794). Neuer Versuch einer systematischen Eintheilung der Schwamme. *Roemer's Neues Mag. Bot.* 1, 63-128.

Persoon, C. H. (1801). “Synopsis Methodica Fungorum.” Gottingen (Johnson Reprint Corp., New York, 1952).
Plowright, C. B. (1889). “A Monograph of the British Uredineae and Ustilagineae,”Kegan Paul, Trench, London.
Prasada, R. (1950). Karamchand Mehta, 1892–1950. *Indian Phytopathol.* 3, 1-3.
Reed, H. S. (1942). “A Short History of the Plant Sciences.” Chronica Botanica, Waltham, Massachusetts.
Roelfs, A. P. (1982). Effects of barberry eradication on stem rust in the United States. *Plant Dis.* 66, 177-181.
Sappin-Trouffy, P. (1896). Recherches histologiques sur la famille des Uredinees. *Botaniste* 5, 59-244.
Savile, D. B. O. (1968). The case against “uredium.” *Mycologia* 60, 459-464.
Savile, D. B. O., and Urban, Z. (1982). Evolution and ecology of *Puccinia graminis. Preslia* 54,97-104.
Scott, K. J., and Chakravorty, A. K., eds. (1982). “The Rust Fungi.” Academic Press, New York.
Stakman, E. C. (1914). A study in cereal rusts: Physiological races. Stn. *Bull.—Minn., Agric. Exp. Stn.* 138.
Stakman, E. C. (1915). Relation between *Puccinia graminis* and plants highly resistant to its attack. J. *Agric. Res. (Washington. D.C.)* 4, 193-200.
Stakman, E. C. (1935). The 1935 black stem rust epidemic. *Northwest. Miller* **184,** 62.
Stakman, E. C. (1950). History, prevalence, and distribution of stem rust race 15B. *Rep. Int. Wheat Rust Conf.. 1950* p. 1.
Stakman, E. C., and Cassell, R. C. (1938). The increase and importance of race 56 of *Puccinia graminis tritici. Phytopathology* 28, 20.
Stakman, E. C, and Harrar, J. G. (1957). “Principles of Plant Pathology.” Ronald Press, New York.
Stakman, E. C, and Hoerner, G. R. (1918). The occurrence of *Puccinia graminis triticicompacti* in the southern United States. *Phytopathology* 8, 141-149.
Stakman, E. C, and Lambert, E. B. (1928). The relation of temperature during the growing season in the spring wheat area of the United States to the occurrence of stem rust epidemics. *Phytopathology* **18,** 369-374.
Stakman, E. C, and Levine, M. N. (1922). The determination of biologic forms of *Puccinia graminis* on *Triticum* spp. *Stn. Bull.—Minn., Agric. Exp. Stn.* 8.
Stakman, E. C., and Piemeisel, F. J. (1917a). A new strain of *Puccinia graminis. Phytopathology 7,* 73 (abstr.).
Stakman, E. C, and Piemeisel, F. J. (1917b). Biologic forms of *Puccinia graminis* on cereals and grasses. J. *Agric. Res. (Washington, D.C.)* 10, 429-495.
Stakman, E. C, Piemeisel, F. J., and Levine, M. N. (1918). Plasticity of biologic forms of *Puccinia graminis. J. Agric. Res. (Washington, D.C.)* 15, 221-250.
Stakman, E. C, Levine, M. N., and Leach, J. G. (1919). New biologic forms of *Puccinia graminia. f. Agric. Res. (Washington, D.C.)* 16, 103-105.
Stakman, E. C., Henry, A. W., Curran, G. C, and Christopher, W. N. (1923). Spores in the upper air. J. *Agric. Res. (Washington, D.C.)* 24, 599-606.
Stakman, E. C, Lambert, E. B., and Christensen, J. J. (1925). Summary of the epidemiology situation for the season of 1925. How much black stem rust developed in 1925, and why? *U. S. Dep. Agric. B.P.I. Off. Cereal Invest. Cereal Courier* 17, 359-365.
Stakman, E. C, Levine, M. N., and Wallace, J. M. (1929). The value of physiologic form surveys in the study of the epidemiology of stem rust. *Phytopathology* 19, 951-959.

Stakman, E. C, Levine, M. N., and Cotter, R. U. (1930). Origin of physiologic forms of *Puccinia graminis* through hybridization and mutation. *Sci. Agric. (Ottawa)* 10, 707-720.

Stakman, E. C., Levine, M. N., Cotter, R. U., and Hines, L. (1934). Relation of barberry to the origin and persistance of physiologic forms of *Puccinia graminis. J. Agric. Res. (Washington, D.C.)* 48, 953-969.

Stakman, E. C, Stewart, D. M., and Loegering, W. Q. (1962). Identification of physiologic races of *Puccinia graminis* var. *tritici.* U.S. *Agric. Res. Serv., ARS* **E617.**

Sunderwirth, S. D., and Roelfs, A. P. (1980). Greenhouse evaluation of adult plant resistance of *Sr2* to wheat stem rust. *Phytopathology* 70, 634-637. Targioni Tozzetti, G. (1767). "True Nature, Causes and Sad Effects of the Rust, the Bunt, the Smut, and other Maladies of Wheat and of Oats in the Field" (translated from Italian by L. R. Tehon, Phytopathol. Classics No. 9. Am. Phytopathol. Soc, St. Paul, Minnesota, 1952).

Transhel, V. G. (1927). "Rust Fungi in their Relation to the Systematics of Vascular Plants" (in Russian), pp. 282-291. I. P. Borodinv.

Transhel, V. G. (1934a). "Fischer's law" and "Transhel's method" in rust fungi (in Russian). *Sov. Bot.* 1, 85-90.

Transhel, V. G. (1934b). Alternate hosts of grain rusts and their distribution in the USSR (in Russian). *Tr. Zashch. Rast., Ser. 2,* 5, 1-40.

Transhel, V. G. (1936). Rust fungi as indicators of their host's kinship in connection with the phylogenesis of rust fungi (in Russian). *Sov. Bot.* 6, 133-144.

Transhel, V. G. (1939). "Conspectus of the Rust Fungi of the USSR" (in Russian). Leningrad.

Tulasne, L. R. (1853). Note sur la germination des spores des Uredinees. C. *R. Hebd. Seances Acad. Sci.* 36, 1093-1095 (translation in *Ann. Mag. Nat. Hist. (2)* 12, 221-223 (1853)].

Tulasne, L. R. (1854a). Sur le Dimorphisme des Urédinées. C. *R. Hebd. Seances Acad. Sci.* 38, 761-765.

Tulasne, L. R. (1854b). Second memoire sur les Urédinées et les Ustilaginées. *Ann. Sci. Nat., Bot. Biol. Veg. (*4) 2, 77-196.

Tulasne. L. R., and Tulasne, C. (1847). Mémoire sur les Ustilaginees comparees aux
Urédinées. *Ann. Sci. Nat., Bot. Biol. Veg.* (3] 7, 12-127.

Vallega, J., Cenoz, H. P., and Tessi, J. L., eds. (1956-1967). "Robigo," Vols. 1-19 Instituto de Fitotecnia, Castelar, Argentina.

Villar, A. D. (1979). Jose Vallega 1909-1978. *Bol. Genet. (Engl. Ed.)* 10, 1-4.

Ward, H. M. (1903). Further observations on the brown rust of the bromes, *Puccinia dispersa* (Erikss.) and its adaptive parasitism. *Ann. Mycol.* 1, 132-151.

Ward, H. M. (1904). On the histology of *Uredo dispersa,* Eriks., and the "mycoplasm" hypothesis. *Philos. Trans R. Soc. London, Ser. B* **196,** 29-46.

Ward, H. M. (1905). Recent researches on the parasitism of fungi. *Ann. Bot. (London)* 19, 1-54.

Waterhouse, W. L. (1929a). A preliminary account of the origin of two new Australian physiologic forms of *Puccinia graminis tritici. Proc. Linn. Soc. N.S.W.* 54, 96-106.

Waterhouse, W. L. (1929b). Australian rust studies. I. *Proc. Linn. Soc. N.S.W.* 54, 615-680.

Waterhouse, W. L. (1930). Australian rust studies. III. Initial results of breeding for rust resistance. *Proc. Linn. Soc. N.S.W.* 55, 596-636.

Waterhouse, W. L. (1935). Australian rust studies. V. On the occurrence of a new physiologic form of wheat stem rust in New South Wales. *Proc. Linn. Soc. N.S.W.* 60, 71-72.

Watson, I. A., and Frankel, O. H. (1972). Walter Lawry Waterhouse. *Rec. Aust. Acad. Sci.* 2.
Watson, I. A., and Waterhouse, W. L. (1949). Australian rust studies. VII. *Proc. Linn. Soc. N.S.W.* 74, 113-131.
Yachevski, A. A. (1909). "Rusts of Grain Crops in Russia" (in Russian). Dep. Zemled. Tr. Buro Mikol. Fitopatol. Sib., St. Petersburg, Russia (partially translated from Russian by D. H. Casper).

2

Evolution at the Center of Origin

I. Wahl
Y. Anikster
J. Manisterski
A. Segal
Faculty of Life Sciences, Tel Aviv University, Tel Aviv, Israel

I. Introduction

Rust fungi as obligate parasites have coexisted and coevolved hand in hand with their hosts as components of a system much influenced by ecologic conditions. The concept of host—parasite coevolution first formulated by Dietel (1904) implies that either constituent of this association has decisively influenced its counterpart. This reciprocal impact is reflected in a multiplicity of morphologic and biologic traits (Anikster and Wahl, 1979). Dietel's idea has spawned into various fields of science, such as taxonomy, cytology, genetics, and plant protection.

The main purpose of this chapter is to elucidate some problems of rust diseases and their evolution at the centers of their origin. Special interest is focused on the evolution of different forms and levels of host defense correlated with the evolution of virulence in a broad spectrum of ecosystems undisturbed by humans. The analysis not only deals with the protection of individual plants but also embraces the nature of defense systems of plant populations in unmanaged ecosystems, their constituents, and patterns of their interaction and cohesion. Browning (1974) labeled this phenomenon "protection of indigenousness," and Schmidt (1978), "ecosystem disease resistance." It is akin to the "evolutionary stable strategy" concept (Robinson, 1980).

The graminicolous plants are characterized at their centers of origin by extreme genetic variability and heterozygosity ascribed by Zhukovsky (1961) and also to the high rate of open pollination even in selfpollinated plants. On the basis of his own studies and Bodenheimer's entomological research in Israel, Vavilov (1939) advanced the hypothesis that the evolution of fungus parasites and insect pests parallels that of their hosts. Maximum diversity coincides with that of the host and its indigenous wild relatives. Their interaction and reciprocal selection pressure has resulted in "balanced polymorphism" (Mode, 1958), as expressed by a multitude of protection types in the hosts, matched by broad virulence specialization of their parasites. Nelson (1978) postulated that "coepicenters, geographic areas in which both host and parasite have evolved, most accurately depict the story of evolution of genes for virulence and resistance." In such areas "the long process of coevolution resulted in the ultimate accumulation of many resistance and virulence genes." In Flor's opinion (1971), "the primary gene center [of host–parasite coevolution] has been and probably will continue to be the plant breeder's principal source of both vertical and horizontal resistance." Coons (1953) contended that common experience has "shaped our thinking and forced recognition of the first and fundamental principle of breeding for disease

resistance, namely, that where host and parasite are long associated, then in the evolutionary process resistance forms are developed by natural selection."

Obviously, there seems to be a broad consensus that protection from disease should be sought in the centers of host–parasite coevolution and introduced from those regions (Leppik, 1970). D'Oliveira (1940, 1951, 1960a) provided ample evidence that epicenters of origin of cereal rusts are situated in the geographic regions where the centers of origin and genetic diversification of the main and alternate hosts coincide. In such regions teliospores are produced abundantly and germinate readily at least in some of the fungus strains. "The absence of an aecidial host from regions where a given rust exists, seems only to indicate that the sporophytic host, as well as the rust, have been introduced recently" (D'Oliveira, 1951).

II. Evolution of Cereal Rust Diseases

A. PLANT DEFENSE AND PARASITE VIRULENCE AT THE ORIGIN CENTERS

The problem of wheat and wheat rusts origin and coevolution was discussed by Leppik (1961, 1965, 1970), Vavilov (1939), and Zhukovsky (1959, 1961, 1964, 1965). According to Leppik (1970), the rust resistance of wheat, a classic case, demonstrates most convincingly the importance of gene center of hosts and sources of disease resistance. Zhukovsky (1959) was of the same opinion. He emphasized that "wild relatives of cultivated plants usually do not possess absolute immunity. This can be considered as a rule." Their defense is based on "field resistance" and tolerance. Ordinarily, the fungus parasitizes only certain plant parts, causing some necrosis and reduced sporulation. Thus the survival of the host and the parasite lasts over millennia. In their common center of origin, hosts and their parasite undergo parallel evolution resulting in development of a plethora of new protection types and fungus strains with increased virulence.

B. CENTERS OF WHEAT ORIGIN

Zhukovsky (1959, 1961) postulated that the home of wheat is Transcaucasia, the central and western parts of Asia Minor, the eastern Mediterranean areas, and the western part of Iran. These regions abound in endemic wild and cultivated wheat and store the variation potential of the genera *Triticum, Aegilops*, and *Secale*. Vavilov (1939) maintained that in the mentioned regions are the world's richest concentration of wild relatives of small grains. Zhukovsky (1965) reported that "Transcaucasia (Georgia, Azerbaijan, and Armenia) is a primary gene centre of speciation of the genus *Triticum.*"

In the hilly steppes of Asia Minor is commonly found the diploid einkorn wheat *T. boeoticum* Boiss. emend. Schiem. (Fig. 3 in Harlan and Zohary, 1966), with two races: a small one-seeded race, often called *T. aegilopoides*, and a large two-seeded race, frequently designed *T. thaoudar* (Harlan and Zohary, 1966). The cultivated relative of this species is *T. monococcum* L. Both *T. aegilopoides* and *T. monococcum*possess resistance to stem rust and leaf rust (Zhukovsky, 1959). Also *T. thaoudar* contains rust resistant plants (Zhukovsky, 1964). Gerechter-Amitai *et al.* (1971) transferred stem rust resistance from *T. aegilopoides*collected in Turkey to susceptible *T. durum* cultivars. The F3 and F4 progenies displayed low reaction resistance to a broad array of stem rust races. The resistance was apparently controlled by a single dominant gene, or by a set of linked factors.

Of the tetraploid wheats endemic in the geographic regions concerned, two are of particular interest: T. *timopheevi* Zhuk., a half-weed, half-cultivated wheat of Soviet Georgia and Armenia, which is often used in breeding as a source of disease resistance (Vavilov, 1939), and T. *persicum* Vav. ex Zhuk. (T. *carthlicum* Nevski), a cultivated species from Gruziya, highly resistant to rusts and powdery mildew (Vavilov, 1939; Zhukovsky, 1965; Leppik, 1970).

The hexaploid T. *zhukovskyi* Men. et Er. is a spontaneous hybrid between *T. timopheevi* and *T. monococcum*, combining resistance genes of its parents (Leppik, 1970).

Description of the aforementioned *Triticum* species and information concerning their genomic formulas and geographic distribution are presented by Feldman and Sears (1981), Harlan and Zohary (1966), Peterson (1965), and Zohary (1971, 1973).

C. WHEAT RUSTS IN THE CENTERS OF *Triticum* ORIGIN

Zhukovsky (1961) and Vavilov (1939) ascertained that the epicenters of wheat are also the homeland of the most destructive wheat rust parasites, *Puccinia recondita, P. striiformis*, and *P. graminis*. The uredia and telia of *P. graminis* capable of developing on wheat, parasitize many genera of Gramineae (Zhukovsky, 1961). Protracted observations have proved that all wheats of the Caucasus rust to various degrees of severity. Even the ordinarily rust-resistant species T. *timopheevi, T. carthlicum, T. zhukovskyi*, and *T. monococcum* are protected by field resistance and not by absolute immunity (Zhukovsky, 1959, 1961, 1964). Reactions of T. *persicum (T. carthlicum)* to stem rust, leaf rust, and stripe rust range from field resistance to susceptibility (Zhukovsky, 1961).

D. ORIGIN AND EVOLUTION OF *Puccinia graminis*

The problem of origin and evolution of wheat stem rust is of great theoretical and practical importance (Anikster and Wahl, 1979). Klebahn (1904) considered barberry as the primary source of *Puccinia graminis*Pers. Leppik (1961, 1965, 1970) postulated that the stem rust fungus has originated in central Asia and East Africa on its aecial *Berberis* host. Both *Berberis* spp. and grasses belonging mainly to the subfamily Festucoideae that harbor the sporophytic stage of *P. graminis* f. sp. *tritici*, have coexisted with the parasite for a sufficiently long time to enable the evolution to the full heteroecious cycle of the fungus. "It is not a mere coincidence that the assumed aboriginal area of stem rust matches well with the terrain where all gene-centers of cultivated Festucoideae are located" [Figs. 1 and 2 of Leppik (1965). The same figures appear in Leppik (1961), and partly in Leppik (1970).]

Notably, the sporophytic generation of the fungus inhabits 70-80 genera of grasses with several hundred species, whereas the gametophytic generation is limited to the two closely related genera *Berberis* and*Mahonia*. This pattern of uneven biologic specialization suggests that barberry was the source host and grasses the secondary host of *P. graminis* f. sp. *tritici*. From the center the parasitic fungus migrated westward and eastward in association with the alternate host. However, the worldwide distribution of the fungus is a result of the everexpanding cultivation of wheat (Leppik, 1965; Fig. 3 of Leppik, 1970). In many regions the parasite has become independent of the aecial host and even innocuous on it (Anikster and Wahl, 1979).

1. Taxonomy of Puccinia graminis *on Wheat and Related Grasses*

Puccinia graminis is a complex "mammoth" (Gaumann, 1959) species, and numerous attempts have been made to subdivide it into simpler taxa. Urban (1968, 1969, 1980) adopted a "phytocenotic"–phylogenetic approach for taxonomic classification of the fungus. He separated the European stem rust into two subspecies (see also Savile, Chapter 3, this volume), one of them compatible mainly with wild grasses was designated as *P. graminis* Pers. ssp. *graminicola* Urban. This subspecies alternates with *Berberis* and ordinarily does not attack cultivated cereals in nature.

In Czechoslovakia, subspecies *graminicola* has evolved by repeated cycling annually between grasses and *Berberis* (Urban, 1961). The other subspecies that parasitizes cultivated cereals and is independent of*Berberis*, was designated *P. graminis* Pers. ssp. *graminis* (with no combined authority). The two subspecies are cross-incompatible. These data gained primarily in Czechoslovakia question the importance of *Berberis*and wild grasses in the epidemics of stem rust in cultivated cereals and indicate the need for reinvestigation of this problem (Urban, 1968).

Urban attributed the evolution of the two subspecies to phylogenetic processes interlaced with the progress of wheat cultivation. He accepted Leppik's hypothesis that the stem rust organism and *Berberis* have their common center of origin in central Asia where it coincides with the area of wheat and barley origin. The ancestral stem rust forms have parasitized wild grasses, including *T. boeoticum, T. dicoccoides,* and T.*timopheevi.* About 8000 to 9000 years ago, wheat cultivation commenced including the ancient cultivated wheats that belong to the diploid species T. *monococcum,* the tetraploid T. *dicoccum,* and the hexaploid T.*spelta.* They were compatible with *P. graminis* ssp. *graminicola.* With the immense geographic expansion of grain crops, the plants adopted new morphologic and physiologic traits. Conditions were created for urediospore dissemination over huge areas, and the significance of the *Berberis* as an alternate host has gradually diminished. Hence, *P. graminis* ssp. *graminis* adapted to cereal crops is of secondary origin, and its predominance has been favored by our management of agroecosystems. Azbukina's studies (1971) in the far eastern region of the Soviet Union corroborated Urban's classification of *P. graminis.* Urban (1969) is aware of the possible inappropriateness of such a subdivision of stem rust in Transcaucasia, Asia Minor, the Mediterranean regions, and other centers of wheat evolution where the fungus is virulent on wild wheats and their relatives considered to be resistant in other geographic areas, as well as on cultivated wheat. It obviously possesses parasitic affinity to both subspecies, *graminicola* and *graminis,* and may pose a hazard to wheat crops in other parts of the world resistant to local races, if transported there by long-distance wind dissemination. Therefore, utilization of urediospore inoculum from primary and secondary evolutionary centers for screening of stem rust resistance was recommended (Urban, 1980). A similar conclusion was drawn by the senior author (Wahl, 1958), and experience gained in the ensuing years has fully justified it.

2. *Interrelationship between Stem Rust on Wheat and Grasses in Israel*

Studies pursued over many years in Israel have shown that stem rusts on wheat crops and on native wild grasses are closely interrelated in their parasitic traits and epidemic development (Gerechter-Amitai and Wahl, 1966; Gerechter-Amitai, 1973). The same parasitic races predominante on wheat and grasses. For example, race 14 of P. *graminis* f. sp. *tritici,* which ranked first in the frequency of occurrence on wheat at the time of investigation, was also the most common on grasses and had the widest host range during the entire period of studies. It was isolated in nature from 26 grass species of 8 genera. Race 21 rated second in both host groups and was identified on 18 species of 6 genera. Stem rust on native grasses is represented by the following four *formae speciales: P. graminis* f. sp. *avenae, P. graminis* f. sp. *tritici, P. graminis* f.

sp. *secalis*,and P. *graminis* f. sp. *lolii*. Nearly all hosts are in the subfamily Festucoideae.

Some grasses carried more than one *forma specialis* of stem rust, and a single plant of *Poa sinaica* Steud. was compatible with all four *formae speciales*, allowing urediospore production of each of them. The broad spectrum of wheat stem rust hosts among native grasses is assumed to be an outcome of prolonged host-parasite coevolution involving the dikaryotic stage of the fungus, in the absence of the alternate host in Israel. Notably, samples of wheat stem rust 3300 years old were found in Israel (Kislev, 1982).

Common hosts for different genetic entities enable somatic hybridization of the fungus and may play an important role in increasing the range of parasitic variation. Wild grasses in Israel are of paramount significance in the development of stem rust epidemics on wheat crops. The occurrence of the same parasitic races in grain fields and in grasses in their neighborhood indicates "a large-scale exchange of inoculum between the two host groups" (Gerechter-Amitai, 1973). The wild grasses can play a very essential role in trapping urediospores, building up of inoculum, and disseminating it to cereal fields. Obviously, races in Israel combine parasitic attributes of both subspecies, P. *graminis* ssp. *graminicola* and P. *graminis* ssp. *graminis*. Their coevolution with the host was much different from that of wheat stem rust in Czechoslovakia.

Savile and Urban (1982) and Savile (see Chapter 3, this volume) discussed the possibility of nuclear exchange between hyphae of the two stem rust subspecies on a common host, and evidence of such hybridization has presumably been found (Savile, Chapter 3, this volume).

3. *Stem Rust Reaction of Indigenous Wild Wheat and* Aegilops *Species*

The tetraploid wild emmer *Triticum dicoccoides* Korn., discovered in Israel by Aaronson in 1906, is one of the ancestors of cultivated wheat (Feldman and Sears, 1981). This species, recognized by Aaronson as a source of rust resistance, has its center of distribution and diversity in Israel, southwestern Syria, and southeastern Lebanon. Hybrids between *T. dicoccoides* and *T. durum*, as well as highly introgressed *T. dicoccoides*populations exist in the semisteppe hills of eastern Galilee (Zohary and Brick, 1961). This wild emmer has shown resistance to many races of wheat stem rust and leaf rust in the United States (Anikster and Wahl, 1979).

Gerechter-Amitai and Wahl (1966) tested collections of *T. dicoccoides* procured from 31 widespread locations and artificially inoculated at the seedling stage with races 14 and 21 of P. *graminis* f. sp. *tritici*.About 100 of the 5700 seedlings tested had low reaction to both races at moderate temperatures, whereas the others were susceptible. At high temperatures only a very few seedlings were resistant to either race. In field trials none of the plants

was highly resistant when exposed to artificially induced epidemics of races 14 and 21, which occurred commonly. Presumably they are protected by other defense mechanisms like slow rusting, escaping, and tolerance. It should be noted that wheat stem rust outbreaks in Israel start from the end of April to the beginning of May, when T. *dicoccoides* is already well advanced in age. Hence the host-parasite coexistence is in that case of brief duration, and there may be little selection pressure for high-level resistance.

In addition, five indigenous species of *Aegilops,* closely related to *Triticum* or even integrated with it (Feldman and Sears, 1981), were screened for resistance to *P. graminis* f. sp. *tritici,* namely, *Ae. bicornis*(Forsk.) J. et S., *Ae. ligustica* Coss., *Ae. longissima* Schw. et Muschl., *Ae. sharonensis* Eig, and *Ae. speltoides* Tausch. Nearly all collections of the five *Aegilops* species, although mostly heterogeneous in their stem rust reaction, contained at least some seedling resistance to one of the tested races. In all except *Ae. bicornis,* supplemental inoculations revealed numerous seedlings with resistance to all race composites used for inoculation. Fifteen *Aegilops* selections with high seedling resistance produced progenies with adult plant resistance in naturally inoculated nurseries maintained across the country.

Gerechter-Amitai and Loegering (1977) screened bulk and single-plant accessions of *Ae. longissima* and *Ae. sharonensis* from Israel for reaction to *P. graminis* f. sp. *tritici* in the United States. Forty-four lines from these collections and 23 *Triticum* sp. lines monogenic for different *Sr* genes were inoculated with 20 fungus cultures from the United States. The analysis of results indicated the presence in *Aegilops* plants of 12 to 15 genes for low reaction. Some of these genes do not appear to be available in cultivated wheat.

Vavilov (1939), in a most interesting analysis of evolution of resistance to stem rust in cultivated and wild wheats, concluded that in arid environments *Triticum* populations lack resistance to this parasite. He maintained that dry air and high temperature usually interfere with rust development on wheat and do not favor natural or artificial selection. For these reasons wheat stem rust resistance is absent in southwestern Asia.

4. Wheat Stem Rust on Hordeum spontaneum

Hordeum spontaneum harbors the wheat stem rust parasite in nature, but severe disease flare-ups have never been recorded. Z. K. Gerechter-Amitai and I. Wahl (unpublished) have ascertained in field trials that some plants samples often form heterogenic reaction patterns fitting the phenomenon of “regional resistance,” as described by Goulden *et al.* (1930). The term “regional resistance” denotes the “tendency in the mature stage of a plant to rust more heavily in certain regions than in the others, particularly above the nodes and on the culms between the uppermost leaf and head.” Browning (1974)

considered regional resistance as a means of keeping the disease in balance. This type of defense is in accord with Zhukovsky's description (1959) of the "field resistance" symptoms prevalent in plants in the centers of their origin.

In numerous *H. spontaneum* plants, older tillers bore uredia of the susceptible type, whereas the younger ones harbored pustules characteristic of low reactions.

Slow rusting associated with retarded disease progress and low infection severity was observed on 20% of plants. Some accessions were resistant and others susceptible throughout the test.

5. *Oat Stem Rust in Israel: Parasite Specialization and Host Protection*

The oat stem rust fungus *Puccinia graminis* Pers. f. sp. *avenae* Eriks. et E. Henn. annually attacks wild native grasses and cultivated oats in Israel, forming only uredia and telia. The alternate host is absent. Disease incidence is severe under favorable conditions. The oat stem rust season is distinctly shorter than that of crown rust, being very brief in arid regions, where the host practically escapes the disease. At higher elevations the duration of host-parasite coexistence is prolonged by a few weeks. Both the crown rust and stem rust organisms can be tided over the rainless summer on volunteer oats or wild plants that preserve viability at scattered sites with sufficient soil moisture. The signifi- cance of these perpetuation foci in the development of stem rust epidemics is not clear.

For decades races 72 (= 6F) and 8 were the most prevalent. In recent years race 7 has become common. These races embrace a broader spectrum of virulence genes. The host range of *P. graminis* f. sp. *avenae*in native grasses is broad. It parasitizes plants of 107 species belonging to 44 genera. Significantly, a single isolate of race 2 produced uredio-spores on 80 species of native grasses (Gerechter-Amitai, 1973).

In *Avena sterilis* L., indigenous to the Mediterranean region and the putative progenitor of cultivated oats (*Avena sativa* L.), resistance of low-reaction type to stem rust operating over the whole life of the plant is very rare (Sztejnberg and Wahl, 1976). J. Manisterski (unpublished) found that numerous accessions harbored uredia of both susceptible and resistance classes on the same stems, sheaths, or blades of the flag leaf and other leaves. The patterns of their distribution on the host resembled the phenomenon of "regional resistance."

Protection of the slow-rusting type against stem rust was discovered in *A. sterilis* by Murphy (Sztejnberg and Wahl, 1976). It is manifested in reduced infectability of the host and diminished spread of the disease, while uredia denote susceptible reactions. Slow-rusting resistance was proven to be stable and effective against many races. Zillinsky and Murphy (1967) found in *A. sterilis* that plants that "exhibited a resistant reaction to stem rust prior to heading, may be susceptible at later stages of maturity. This type of resistance,

however, provides considerable protection among species which remain in the juvenile stage for extended periods of their natural habitat."

Distribution of defense components in *A. sterilis* varies with the region. In locations situated below sea level, plants rusted severely but ripened early, thus avoiding the damaging impact of the disease. In communities originating from the central coastal plain, the protection system is composed of 25% of slow rusters, which had infection severity of less than 40% even at the milk-early dough stage. Notably, slow rusters, late rusters, and some of the moderately fast rusters harbor uredia with necrotic lesions interspersed with uredia of susceptible class. The symptoms of late rusters are those of Luke *et al.* (1975).

The brief coexistence of *A. sterilis* with *P. graminis* f. sp. *avenae* during the season apparently prevents severe disease damage. "The *A. sterilis* stem rust–environmental system in Israel seems to be less balanced and coordinated than the system involving crown rust. The difference is reflected in the evolution of defense mechanisms against the two rust organisms" (Segal *et al.*, 1980). The most salient distinc- tion is manifested in the common occurrence of low reaction in defense systems against crown rust, and its insignificance in stem rust.

E. LEAF RUST OF WHEAT

1. Pathways of Evolution

Leaf rust of wheat caused by *Puccinia recondita* Rob. ex Desm. f. sp. *tritici* Eriks. has become an extremely serious disease worldwide and accounts for the greatest loss among cereal rusts over the long term.

Evolution of various lineages of the fungus with alternate hosts in the Ranunculaceae and Boraginaceae was discussed by Anikster and Wahl (1979). One physiologic group completes the life cycle on *Thalictrum*,another group alternates with plants of the Boraginaceae family, and the Siberian group alternates with *Isopyrum fumarioides* L. Congeniality among the lineages was found to exist only when the gametophytic and sporophytic hosts belong to the same center of origin. Markova and Urban (1977) postulated that the recent form of wheat leaf rust represents at least in some countries an autonomous species and has no alternate host. They surmised that the recent form descended from an ancestral progenitor the that has its origin in the evolutionary centers of hexaploid wheats (Near East, the Mediterranean region). "Originally it was heteroecious (species of *Thalictrum* and other genera) and plurivorous," that is, parasitic on a broad variety of hosts. Conceivably, evolution of wheat leaf rust in other geographic regions may have proceeded along different pathways.

2. Studies in Israel

This research is in preliminary stage and deals mainly with disease incidence on *Triticum dicoccoides,* one of the cultivated wheat progenitors. Populations of the species at some locations are seriously stricken by *P. recondita* f. sp. *tritici.*

a. Parasite Specialization. Fungus isolates sampled from *T. dicoccoides* across the country were inoculated to seedlings of wheat cultivars carrying genes for low reaction, *Lrl, Lr2a, Lr2c, Lr3a, Lr3b, Lr3c, Lr9, LrlO, Lrl7, Lrl8, Lrl9, Lr21, Lr23, Lr24.* The results (unpublished) revealed considerable diversity in parasitic traits. Accessions with genes *Lr24* and *Lrl9,* respectively, were highly resistant. This was also true to a lesser degree of seedlings with gene *Lr2a.* Seed- lings endowed with gene *Lr9* were often moderately susceptible, carrying infection type 3.

b. Types of Protection in Triticum dicoccoides. In wheat breeding, genes for low reaction (Browder, 1980) and slow-rusting resistance (also referred to as "partial resistance"; Parlevliet, 1979a) are utilized. We have intended to identify the defense types evolved in undisturbed stands of *T. dicoccoides.* Therefore, single heads were sampled with the transect method from plants in natural habitats at about 1 -m inter vals, regardless of their reaction to the disease. Seeds were planted in 1- m rows in field nurseries in the order of the disposition of their parents in nature. Border rows sown to a universally susceptible cultivar were periodically inoculated with leaf rust cultures collected across the country. In these reconstructed stands of T. *dicoccoides,* a small number of accessions showed low reaction with infection ranging from 1 to 2+. Most plants displayed symptoms of slow rusting or later rusting. Severely rusted plants were always present. Presumably some of them have been protected by tolerance.

c. Search for the Alternate Host. So far, alternate hosts of *P. recondita* f. sp. *tritici* have not been found in Israel. Studies by Chabelska (1938) in this country demonstrated that the leaf rust fungus, *Puccinia aegilopis* Maire, which in nature inhabits *Aegilops variabilis* Eig [*Ae. peregrina* (Hack.) Eig], alternates with *Anchusa strigosa* Labill. Y. Anikster (unpublished) inoculated leaf rust teliospores produced, respectively, on cultivated *T. durum* wheat, *T. aestivum* wheat, *T dicoc coides, Ae. variabilis, Ae. longissima,* and *Ae. sharonensis* to plants of the following Boraginaceae species: *Anchusa azurea* Mill., *A. strigosa, A. hybrida*Ten., *Echium angustifolium* Mill., and *E. judaicum* Lac- aita. Aeciospores were formed on all listed Boraginaceae species inocu lated with teliospores from *Aegilops* plants, but not with teliospores from the *Triticum*plants. These aeciospores inoculated to the *Aegilops* source hosts elicited formation of urediospores. Obviously, leaf rusts of T. *durum* wheat, T. *aestivum* wheat, and *T. dicoccoides* differ from leaf rust on *Aegilops,*even

though the genus *Aegilops* is considered to be a part of the genus *Triticum* (Feldman and Sears, 1981).

F. WHEAT STRIPE RUST

1. Evolution

The disease is caused by *Puccinia striiformis* West, (see chapter by Stubbs in Vol. II). Its center of origin coincides with that of wheat (Zhukovsky, 1965). In Hassebrauk's opinion (1965, pp. 6-7) wild grasses were ancestral hosts of the fungus, from which it expanded to cereals. This hypothesis is substantiated by observations in countries where the rust only recently became established on cereal crops. For example, the disease appeared in North and South America first on wild grasses and later on wheat, barley, and rye. *P. striiformis* putatively originated from a heteroecious progenitor (Hassebrauk, 1965, p. 8).

2. Wheat Stripe Rust in Israel

The disease appears sporadically on wheat cultivars and on wild grasses belonging to *Triticum dicoccoides, Aegilops, Hordeum,* and other genera. Some isolates of the fungus have a host range embracing over 40 species of 17 genera (Z. K. Gerechter-Amitai, unpublished). Most of the isolates studied by Gerechter-Amitai were virulent on seedlings of Lee C.I. 1248 and Compair. Both accessions are resistant to the pathogen in some countries. The cultivar Compair possesses the resistance gene *Yr8* derived from *Aegilops comosa* Sibth. et Sm. Lee is characterized by susceptibility to race group 20A, common in the Mediterranean region (E. Fuchs, personal communication). Zadoks (1965) suggested that the rise of stripe rust importance in the Mediterranean region may be due to a better adaptation of some of the fungus biotypes to higher temperatures than the others. Z. K. Gerechter-Amitai (unpublished) proved that stripe rust inoculum isolated from *Aegilops kotschyi* Boiss. and *H. spontaneum* in the arid southern region with average annual rainfall of below 100 mm was virulent on wheat.

Gerechter-Amitai (1982) found in T. *dicoccoides* diverse types of resistance. Accessions from 44 locations were resistant to stripe rust cultures from India, Pakistan, Kenya, Egypt, Tunisia, Chile, North America, and western Europe.

The susceptibility of T. *dicoccoides* and of diverse *Aegilops* species in Israel supports the hypothesis that wild grasses were ancestral hosts of the fungus (Hassebrauk, 1965). The coevolution of *P. striiformis* and wild grasses in Israel

apparently has resulted in the presence of numerous genes for resistance in them, as exemplified by T. *dicoccoides.*

G. CROWN RUST OF OATS

1. Crown Rust of Oats in Israel

Israel is located in the center of origin and genetic diversification of several oat species that are annually attacked there by *Puccinia coro- nata* Cda. f. sp. *avenae* F. et L. The inoculum is present throughout the year. The disease gains momentum in March, culminates in April, and declines sharply in May. Of special interest is the disease incidence on *Avena sterilis.*

2. Life Cycle

The fungus is heteroecious and alternates with *Rhamnus* species (see chapter by Simons in Vol. II). In Israel *R. palaestina* Boiss. is indigenous and a common element of the Mediterranean vegetation. It functions as the alternate host of *P. coronata* f. sp. *avenae* in the Galilee, Samaria, Mt. Carmel, and the Judean Mountains. Aecia are formed throughout the winter. Because *Rhamnus* shrubs sprout from December to April, they develop hospitable substrate for the parasite for several months. In natural stands in various parts of the country, teliospore germination proceeds from the onset of the rainy season (late November to early December) until April. Therefore, production of basidiospores takes place over several months and coincides with the development of young tissue in *R. palaestina.*

The prevalence and long-lasting coexistence of the *Avena-P. coro-nata-Rhamnus* system prompted our studies on the effect of the coevolution of the components in this system on the following biologic traits: (i) parasitic specialization of fungus, (2) evolution of different types and levels of protection against crown rust, and (3) evolution of protection systems against the rust in natural, undisturbed populations of *A. sterilis.*

3. Parasitic Specialization of Puccinia coronata f. *sp.* avenae

About 100 parasitic races of the fungus were identified in samples of countrywide origin. Some races were discovered first in Israel, including the very dangerous race 264 that combines a very broad range of virulence genes, race 270, and races virulent on Santa Fe but avirulent on Landhafer. The prediction made by the senior author (I. W.) in 1958 that some of the virulent races found in Israel are likely to appear also in other oat-cultivatine regions, was soon confirmed (Wahl, 1959). Studies by Wahl *et al.* (1960) revealed

distinct similarity in the composition of race populations in oat species and *R. palaestina.* For example, the "Landhafer races" group 263-264-276-277, the race group 202-203, and races 286 and 270 have appeared in the same order of prevalence on the main and alternate hosts. At the same time the "Victoria races" group 216-217, which is rare in oats, was absent in the aecial material. *Rhamnus alaternus* L., which serves as alternate host in Israel, also harbors aecia of *P. coronata* f. sp. *avenae* in Portugal and seems to be an important source of crown rust inoculum there (D'Oliveira, 1940). According to Santiago (1968), variation of this fungus in Portugal is most probably associated with the infections occurring in the alternate host, which is widely spread throughout the country. The alternate host contributes to the diversification of the parasitism spectra of race populations in crown rust in Israel. Wahl *et al.* (1960) obtained only one race in eight collections from oats, but one in three from buckthorn and more than one race from a single aecial cup. Oat crown rust races selfed by Dinoor (1967) were heterozygous, entailing more variability of the fungus. Browning and Frey (1969) suggested that in the dikaryotic rust fungi, heterozygosity provides survival advantage to the organism. Eshed (1978) concluded that heterozygosity of pathogenicity is common in the *formae speciales* of *P. coronata.* Despite the continuous production of new races, the composition of race populations has remained stable over nearly three decades of race surveys. For example, the very versatile race 276 has predominated annually throughout Israel for many years. The same is true to a lesser degree also of races 202 and 264. This stability is attributable to the permanence in the composition of wild oats and other compatible native grasses, undisturbed by human interference, because oat cultivation is very limited. *Avena sterilis* and *A. barbata* Pott., which are very congenial hosts for *P. coronata* f. sp. *avenae,* represent most important constituents of the country's herbaceous vegetation. Additionally, prevalent races possess preferential survivability as expressed in their adaptation to a broad spectrum of ecologic conditions and prolific sporulation in such environments (Brodny, 1980).

4. Defense against Crown Rust

Vavilov (1939) reported good resistance to crown rust in Mediterranean oats. Studies in Israel, the United States, Puerto Rico, Canada, and elsewhere (Wahl, 1970) have proved that *A. sterilis* populations are abundant, diversified reservoirs of new and readily usable genes for low reaction resistance to *P. coronata* f. sp. *avenae.* Murphy *et al.* (1967) postulated that "a natural balance … appears to have been established between *A. sterilis,* crown rust, and *R. palaestina,* in which *A. sterilis,* although infected, produces seed of good quality." Various types and levels of resistance and tolerance "have apparently resulted from natural selection under conditions of regular and heavy crown rust infection and a relatively high level of outcrossing in *A. sterilis"* (Murphy *et al,* 1967).

The following components of defense have been identified in *A.* sterilis: (*a)* conventional resistance associated with low reaction, *(b)* slow rusting, (c) tolerance, and (*d)* escape (avoidance).

a. Low Reaction Resistance. Numerous accessions of *A. sterilis* are endowed with resistance of this type to at least 14 races of *P. coronata* f. sp. *avenae* embracing a broad spectrum of virulence (Brod- ny *et al.,* 1976). Simons *et al.* (1978) provided a list of 25 genes for resistance to oat crown rust derived from *A. sterilis,* mainly of Israel origin. Zillinsky and Murphy (1967) found crown rust resistance in *A. sterilis*sampled in Italy, Morocco, Algeria, Tunisia, and Tripolitania. Harder (1980) reported in Canada that genes extracted from *A. sterilis* "generally proved a high level of resistance to *P. coronata…* . Further studies on wild *A. sterilis* accessions are expected to reveal additional resistance genes for use in breeding programs." Studies in Israel (Wahl, 1970) have ascertained that distribution of sources for low reaction to crown rust is countrywide and falls in natural populations in distinct geographic patterns that have remained unchanged over many years. The accumulation of resistance is conditioned by ecologic conditions favoring intense selection pressure on *A. sterilis.* Segal's investigations (1981) brought out that the evolution of resistance is much influenced by the prevailing races of the parasite. "Intermediate" resistance asso ciated with infection types 2-3 is rather common but sometimes more sensitive to rise of temperature than resistance expressed by infection types 0; -1. Plant acessions vary in the spectrum of resistance, some of them being effective to several races, whereas others offer protection to a single race only. Segal (1981) also reported that in some entries, resistance operates throughout the whole life of the plant, whereas in others it is limited to certain growth stages of the host.

b. Slow-Rusting Resistance. This type of protection is featured by low receptivity, retarded disease progress, and reduced sporulation, whereas uredia are mostly of infection type 3, denoting susceptible reactions. Slow rusting is of overriding importance across the country. Ordinarily, it is more common than the low-reaction type of resistance, being less influenced than the latter one by race composition but more sensitive to fluctuations of climatic factors. In slow-rusting accessions, infection severity in field trials was within the range of 5 to 20%, whereas in fast rusters it amounted to 70 to 80%.

c. Tolerance. In identifying the tolerance form of protection, we adopted Simons' definition (1969): "A tolerant variety shows signs and symptoms similar to those on a susceptible variety, but it is damaged less by infection than is the susceptible variety." Wahl (1958) reported that in Israel, "despite severe rust infection the wild oats do not seem to suffer from the disease, they show excellent tolerance to both rusts." On the examined plants, infection severity ranged from 50 to 80%, and reactions to the disease belonged to the susceptible and very susceptible class. Simons (1972) found that some progenies of crosses between *A. sativa*cultivars susceptible to crown rust and tolerant *A.*

sterilis accessions "were as much as 15% higher than the cultivated parent in kernel weight response and 20% higher in yield response."

d. Escape. Disease escape is the ability of an otherwise susceptible plant to avoid damaging disease stress because it grows in places and times unsuitable for the parasite. This form of protection is particularly useful in natural ecosystems (Nelson, 1973). In arid regions, *A. sterilis* is either completely free of crown rust, because adverse climatic conditions avert the disease, or the plant escapes infection for long periods thanks to early ripening.

Littlefield (1981) has stressed the importance of the search for genes for crown rust resistance in Israel as follows: "… the great genetic diversity in oats in Israel has provided sustained resistance to crown rust. The magnitude and diversity of rust resistance genes, both of race-specific and race-non-specific nature, in that center of origin of oats, have provided an invaluable contribution to plant pathologists and breeders alike |p. 94]."

5. *Integration of Defense Components against Crown Rust in* A. sterilis

Studies on natural defense systems were pursued by inoculating parasite cultures of countrywide origin to plant populations reconstructed in test plots (Segal *et al*, 1982). The reconstruction was achieved by sampling single panicles from plants at 1-m intervals, regardless of the disease performance on the parental plant. In the ensuing season seeds were sown in the nursery in the order of disposition of their parents in natural habitats. The nursery was periodically inoculated with crown rust cultures isolated countrywide. Infection type and infection severity were recorded several times during the season. The composition of defense systems against crown rust in *A. sterilis* vary distinctly with the locations. They comprise the aforementioned four types of protection in different proportions. The profiles of their interaction at a given site are stable (Segal *et al*,1980). For instance, in the Plateau Menashe, prevalence of low-reaction resistance has remained unchanged during 17 years of study. Details concerning the most common protection systems were reported by Segal *et al*. (1980) and are shown in Fig. 1. They are characterized by the high percentage of plants with infection type 3 and low to moderate infection severity, whereas in about 30% of the plants infection type 2 is associated with infection severity of 5 to 30%.

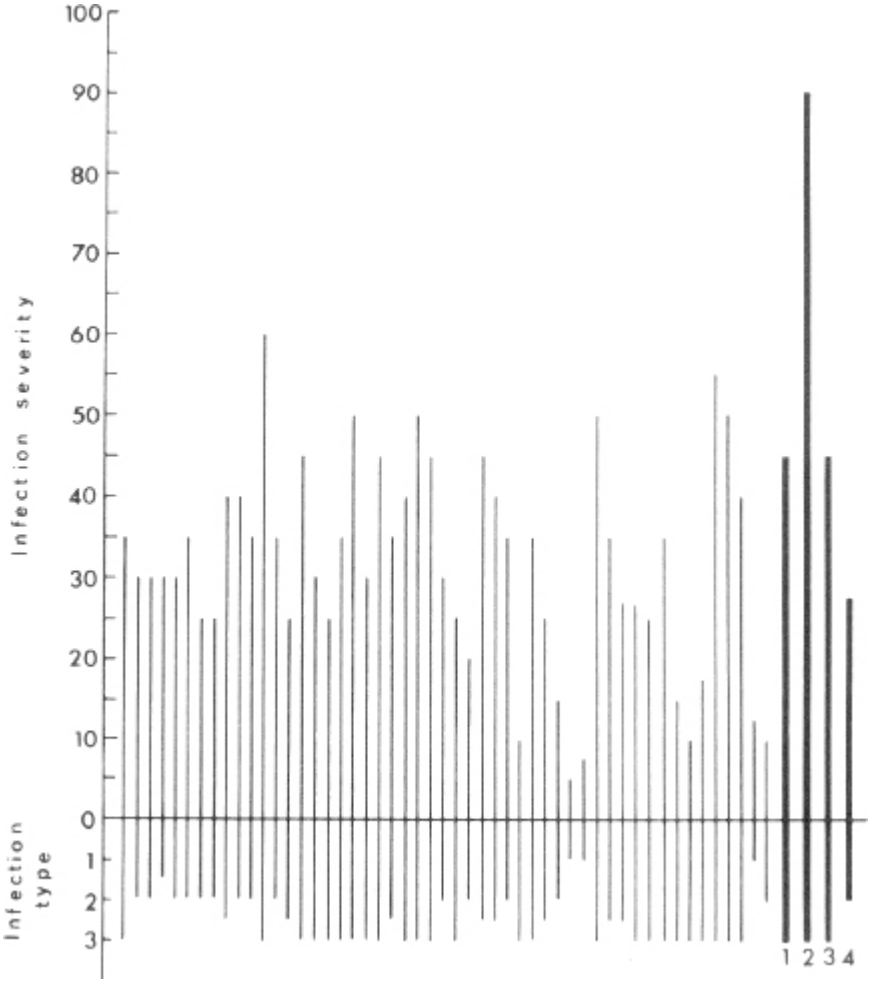

Fig. 1. Representative defense system against *Puccinia coronata* f. sp. *avenae* in transect samples ot *Avena sterilis,* as compared with crown **rust** performance of slow-rusting cultivars Red Rustproof (1) and **New** Nortex (3), fast-rusting cultivar Markton (2), and cultivar Lodi (4), which is moderately resistant—moderately susceptible to crown rust at **the adult** stage. All accessions at the flowering-early milk age. [From Segal el *al.* (1980), reproduced with permission from "Plant Disease: **An** Advanced Treatise" **(J. G. Horsfall** and **E. B. Cowling, eds.|, Vol.** 5, pp. 75-102. Academic Press].

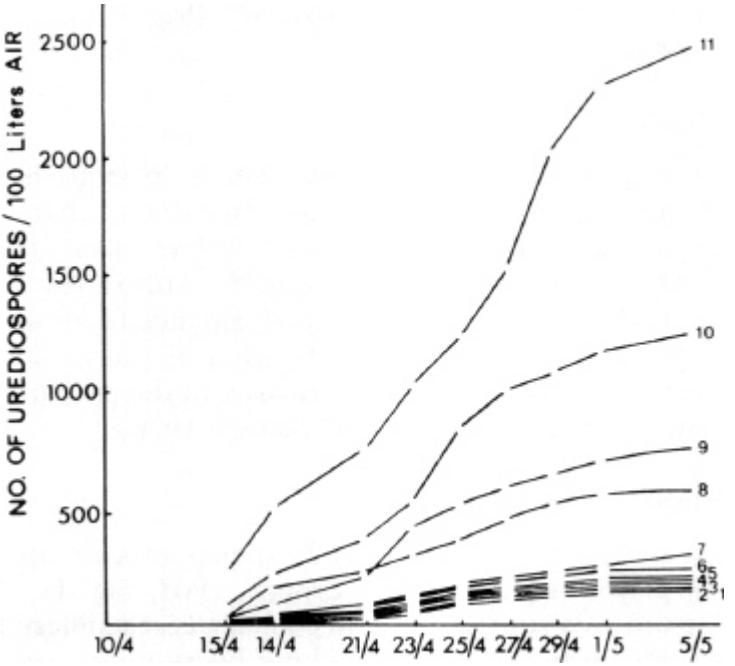

Fig. 2. Cumulative urediospore production of *Puccinia coronata* f. sp. *avenae* on the "standard" susceptible cultivar Markton (11); two recurrent hosts of the Iowa Multilines, Clintford + C649 (9) and C237-IV-89 (10); three Iowa Multiline cultivars (1, 3, 5); and five transect-reconstructed populations of *Avena sterilis* (2, 4, 6, 7, 8). Field tests, Bet Dagan, 1980. |From Segal *et al.*, (1982), reproduced with permission from "Resistance to Diseases and Pests in Forest Trees" (M. H. Hcybroek, B. R. Stephan, and K. von Weissenberg, eds.), pp. 361-370. PUDOC Centre Agric. Publishing & Documentation, Wageningen].

In extensive field trials, the composition of crown rust populations and their urediospore production was studied on (1) natural populations of *A. sterilis,* (2) reconstructed *A. sterilis* populations, (3) Iowa Oat Multilines and their recurrent parents, and (4) the standard susceptible cultivar Markton (Segal *et al.,* 1982). The results have ascertained that although the ubiquitous race group 276-264 prevailed in all populations, its concentration was diluted on *A. sterilis* stands and the multilines. The seasonal urediospore production was lower in both the reconstructed *A. sterilis* populations and Iowa Multilines than on the recurrent hosts and Markton (Fig. 2). Notably, protection against crown rust in *A. sterilis* is conditioned by both slow rusting and low reaction, whereas in the Iowa Multilines defense is based mainly on low-reaction resistance (A. Segal, J. Manisterski, J. A. Browining, G. Fischbeck, and I. Wahl, unpublished). "*A. sterilis* populations con- stitute to some extent 'natural multilines'" (Segal *et ah,* 1982) in indigenous ecosystems.

6. Host Range

The host range of *P. coronata* f. sp. *avenae* in Israel is very broad (Eshed and Dinoor, 1981). Eshed (1978) demonstrated that some plants serve as common hosts to two to seven *formae speciales* of *P. coronata.* Such hosts are conceivably suitable substrates for somatic hybridization between varieties. The performance of *formae speciales* and their hybrids seems to be "a reflection of the evolution of the host-parasite relationships started way back in the past and still going on at present in natural ecosystems" (Eshed, 1978).

7. Evolution Tendencies

Rhamnus is the putative primary host of *Puccinia coronata* (Klebahn, 1904, p. 180 and Table VI: Leppik, 1967, Fig. 1). The fungus "radiated" from this source to the secondary Festucoideae hosts. The wide range of alternative graminicolous hosts of *P. coronata* f. sp. *avenae* in Israel attests to the antiquity of the fungus in the region (Wahl and Anikster, 1982). Another piece of evidence supporting this hypothesis is the countrywide prevalence of the microcyclic *Puccinia mesnieriana* Tnüm. on *Rhamnus.* This microform most probably descended from *P. coronata* in a process of protracted regressive evolution. Observations of many years have revealed that *P. mesnieriana* is distinctly more common in Israel than aecia of P. *coronata* f. sp. *avenae.* Apparently the evolutionary process has reached a phase marked by decline of the gametophytic stage of the progenitor and upsurge of its short-cycled progeny. The semiarid environment favors evolution of short-cycled rusts that are adapted for survival under adverse conditions (Anikster and Wahl, 1979). Aecia of P. *coronata* f. sp. *avenae* and telia of P. *mesnieriana* are occasionally found even on the same leaf.

H. LEAF RUST OF BARLEY

1. Life Cycle of Puccinia hordei *Otth.*

The taxonomy, biology, cytology, and evolutionary trends of *Uromyces* species causing leaf rust of barley were discussed elsewhere (Anikster and Wahl, 1979). Here we deal mainly with leaf rust caused by P.*hordei.* For a general treatment of this disease, see Clifford (Vol. II). *P. hordei* alternates in nature with *Ornithogalum* species (Fig. 3). Tranzschel (1939) reported that the presence of the alternate host in Crimea intensifies the disease on cultivated barley. Telia on the main host are profusely formed where *Ornithogalum* plants are present, and scarce in the central Soviet Union, where the alternate host was not found (Tranzschel, 1939). Similar preferential

selection pressure of alternate hosts favoring the evolution of fungus strains developing abundant telia has also been observed in other heteroecious rust organisms (de Bary, 1879, p. 784; Klebahn, 1904, pp. 47-48; Wahl and Anikster, 1982).

The coevolution of the *Hordeum-P. hordei-Ornithogalum* system (Fig. 3) was one of the important topics of D'Oliveira's fundamental studies (1960a,b) on the evolution of rust fungi in the geographic regions where the centers of origin an genetic diversification of the main and alternate hosts overlap. He demonstrated that *P. hordei* from *H. spontaneum* is incompatible with *Ornithogalum* species in the primary centers of their diversification in Africa, south of the Sahara, where no native species of *Hordeum* are known to occur. In contrast, of all 33 species tested that belong to the secondary center of *Ornithogalum* diversification—the Mediterranean, Irano-Turanian, and Saharo-Sin-dian regions—only one, *Ornithogalum arabicum*, was not compatible with the rust. These regions cover either part of the center of origin and distribution of *H. spontaneum*, or they correspond to regions where barley is cultivated since prehistoric times (D'Oliveira, 1960b).

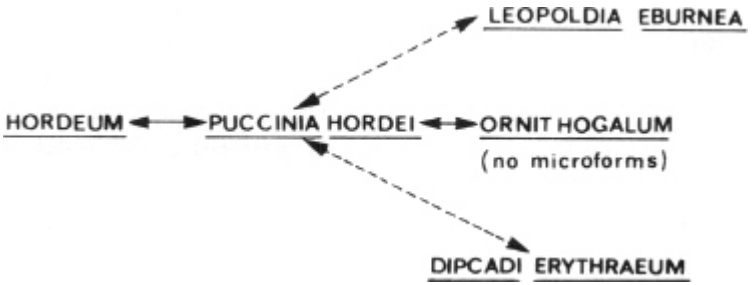

Fig. 3. Life cycle of *Puccinia hordei* in Israel. The fungus cycles in nature between the main *Hordeum* host and alternate *Ornithogalum* host (solid lines). The heteroecious process integrates parallel coevolution of the double complex: (1) *Hordeum*—rust fungus (sporophytic stage), and (2) *Ornithogalum*—rust fungus (gametophytic stage). This co-evolution has taken place in geographic regions where the centers of origin and genetic diversification of the main and alternate hosts coincide (Oliveira, 1960b). *P. hordei* also cycles between *Hordeum* and *Dipcadi erythraeum*, or *Hordeum* and *Leopoldia eburnea*, artificially inoculated in the greenhouse or naturally inoculated on transplanting these alternate hosts to humid locations (dashed lines). Microforms correlated with *P. hordei* have never been found.

D'Oliveira postulated, "It seems as if, in that common ground, the rust and the hosts for its sporophytic and gametophytic stages have gone through a parallel process of evolution and adaptation" (1960b). He envisioned "the possible existence of aecidial hosts belonging to genera other than *Ornithogalum* and *Dipcadi*, in different genocenters of … *Hordeum* congenial to this rust and to nearly related species, in

particular*Uromyces iranesis* Vienn.-Bourg." Studies in Israel have fully substantiated D'Oliveira's hypothesis. Anikster (1982) proved that *Dipcadi erythraeum* Webb et Bert, and *Leopoldia eburnea* Eig et Feinbr. are also potential alternate hosts of *P. hordei.*

2. Life Cycle in Israel

Israel is located in the center of origin and genetic diversification of *Hordeum spontaneum* C. Koch. Populations of this species are of countrywide distribution and represent a wide range of morphologic and physiologic variation. In addition, the following species belong to the native *Hordeum* flora, *H. bulbosum* L. (tetraploid type, $2n = 28$), and *H. murinum* L. The *Hordeum* center of diversification coincides with that of the genus *Ornithogalum,* consisting of O. *narbonense* L., O. *brachystachys* C. Koch, O. *divergens* Bor., *O. eigii* Feinbr., O. *lanceolatum* Lab., O. *montanum* Cyr., and *O. trichophyllum* Boiss. et Heldr. The*Ornithogalum* flora coexists in many areas with *Hordeum* plants, and particularly with *H. spontaneum* and *H. bulbosum.*

The listed *Hordeum* species are annually attacked by *P. hordei.* Parasitically the fungus is confined to the source host species, except that reciprocal inoculations with leaf rust of *H. spontaneum* and *H. vulgare* L. were successful. For these reasons, *P. hordei* was subdivided into three *formae speciales* (Y. Anikster, unpublished), namely, *P. hordei* Otth f. sp. *spontanei* Anikst. (also virulent on cultivated barley); *P. hordei* Otth f. sp. *bulbosi* Anikst., and *P. hordei* Otth f. sp. *murini* Anikst. The latter *forma specialis* supersedes *P. hordei* f. sp. *murini* Buchw., which should not be considered as an autonomous species (Anikster and Wahl, 1979). Notably, according to D'Oliveira (1960a), "amongst hundreds of seedlings of *H. bulbosum* tested in Portugal, and belonging to several accessions from different regions, only one plant proved to be congenial to some of our physiologic races of this rust." These results and data obtained from Iran prompted D'Oliveira to suggest that *H. bulbosum* harbors a different *forma specialis* of leaf rust. Studies in Israel are in accord with this supposition. In contrast, Tranzschel (1939) maintained that *P. hordei* hibernates in many parts of the Soviet Union in the uredial stage on wild *H. bulbosum,* and the alternate host was of little significance for the dissemination of the fungus.

The aforementioned three *formae speciales* of *P. hordei* are compatible with *Ornithogalum* plants being less specialized on the alternate host than on the main one (Anikster, 1982). For example, they all are capable of completing the life cycle on O. *brachystachys, O. eigii,* and O. *trichophyllum.* However, aeciospores from naturally inoculated O. *lanceolatum* and O. *montanum* studied thus far were infectious only on*H. bulbosum.*

The alternate *Ornithogalum* hosts are important in Israel in the perpetuation of *P. hordei* over the year. Barley plants desiccate at the beginning of the rainless season at the end of May and beginning of June. Dormant teliospores in barley stubble retain viability during the summer and start to germinate at the onset of the ensuing season in November, liberating basidiospores that infect the foliage of *Ornithogalum* plants that emerge at the same time. Aeciospores thus formed infect seedlings of the native wild and cultivated barley cultivars. Presumably the coordinated sequence of development of different stages of the life cycle of the fungus and its hosts, and the adaptability to diverse environmental conditions are an outcome of a prolonged host-parasite coevolution (Anikster and Wahl, 1979).

Germinating teliospores of *P. hordei* from *H. spontaneum, H. bulbosum,* and *H. murinum* induce aeciospore formation on *Dipcadi erythraeum* and *Leopoldia eburnea* (Fig. 3). Both species are restricted in distribution to the desert areas of Israel, which are practically free of *P. hordei.* However, accessions of these two species emerging from bulbs planted by us in relatively humid regions became infected by the parasite as a result of natural inoculation (Anikster, 1982).

3. Evolution of Parasitism

In some cereal rusts, such as wheat stem rust and oat crown rust, the alternate host plays an important role in evolution of parasitism (Anikster and Wahl, 1979). In view of the significance of *Ornithogalum* plants in the life cycle of *P. hordei* in Israel, the parasitic specializations of cultures of aecial origin and uredial origin were compared. The cultures involved were sampled in nature countrywide over 4 years in five regions from*Ornithogalum brachystachys, O. eigii,* and O. *narbonense,* and from *H. spontaneum*. The experiments led to the following results:

1. Rust populations on the main and alternate host were similar in their parasitic features. For instance, 592 of 615 uredial cultures iso- lated from *H. spontaneum,* and 348 of 447 cultures of aecial origin, had 17 virulence patterns in common. The term *virulence pattern* denotes a combination of resistant and susceptible reactions induced by a specific rust culture on components of a set of differential cultivars at the seedling stage (Simons *et al.,* 1979).

2. The sexual stage contributes to the diversification of the spectrum of parasitism of *P. hordei.* This conclusion is adduced from the following data: (1) Rust populations on the alternate host included five virulence patterns that were not identified in the inoculum from the main host, whereas only a single pattern was confined to uredial populations derived from *H. spontaneum;* (2) uredial populations isolated from *H. spontaneum*adjacent to a functional alternate host were parasitically more diverse than those originated from the main host distant from *Ornithogalum* plants (Y. Anikster, unpublished); (3) cultures virulent on

barley cultivars endowed with the genes of resistance *Pa7* were obtained first from an alternate host and only thereafter from *H. spontaneum* (Golan *et al.*, 1978).

3. Some virulence patterns have predominated in Israel annually on both the alternate and main hosts during the 4 years of studies in all five regions implicated in the research. In contrast, Simons *et al.* (1979) could not find virulence patterns common to 1975 and 1976 aecial isolates of *Puccinia coronata* that were collected in Minnesota buckthorn-oat nursery that had been perpetuated since 1958. The difference is most likely attributable to the fact that in Israel the *H. spontan-eum-P. hordei-Ornithogalum* association has evolved for millennia as a part of a natural ecosystem and reached a stage of balance. This does not seem to be the case in the human-managed oats–P. *coronata–buckthorn association in Minnesota, which is of recent origin.*

4. Rust populations on *H. spontaneum* and *Ornithogalum* have comprised cultures rendering ineffective all known genes for leaf rust resistance, including genes Pa_3, Pa_7 and Pa_9.

4. Evolution of Defense against Puccinia hordei

Over 10,000 accessions of *Hordeum spontaneum* were screened for low-reaction seedling resistance, by inoculating them with composite cultures of *P. hordei.* About 10% of the accessions showed low-reaction resistance except to the strain(s) virulent on Cebada Capa. The geographic distribution of resistance sources shows a distinct pattern, the sources being concentrated in areas where climatic conditions favor severe disease incidence and strong selection pressure (i.e., the Upper Galilee, Esdraelon Valley, and Judean Foòthills) (Fig. 4). Practically no resistance of low-reaction type was detected in arid regions

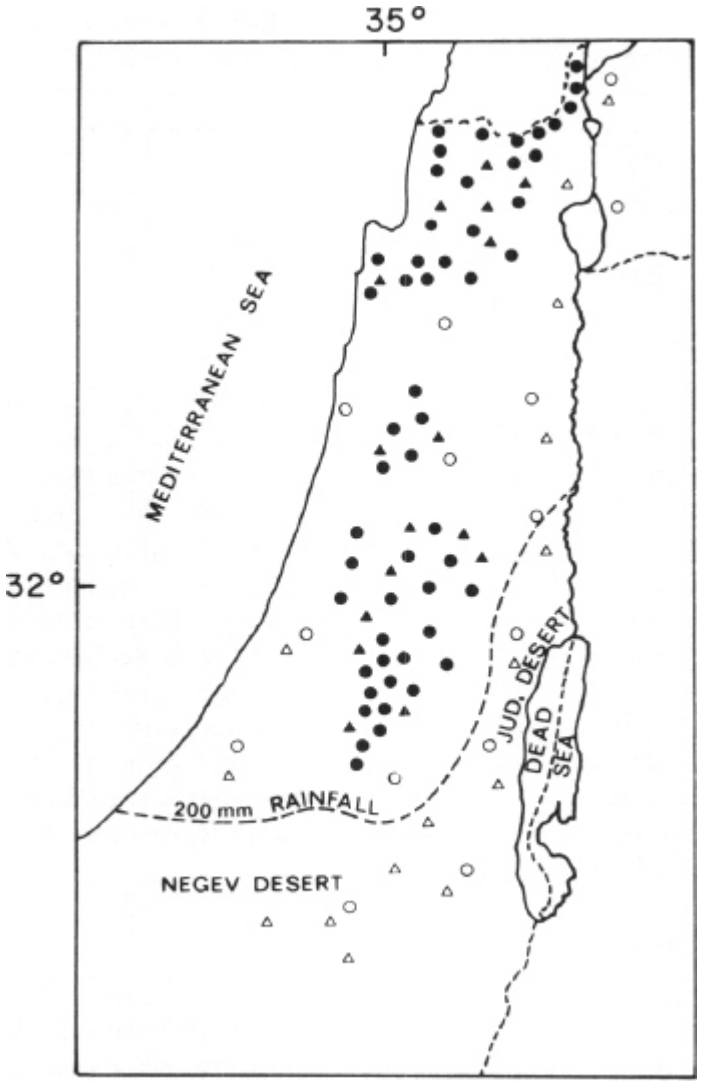

Fig. 4. Effect of *Puccinia hordei* incidence on evolution of low-reaction type resistance in populations of *Hordeum spontaneum*. Resistant barley accessions (▲) were found in geographic regions favorable for disease development, and particularly near rusted *Ornithogalum plants* (●). *Susceptible accessions of H. spontaneum* (A) were prevalent in the Negev desert and the Judean desert with annual rainfall less than 200 mm, where *Ornithogalum* plants (O) do not rust. Aridity prevents *P. hordei* development and selection for rust resistance in *H. spontaneum* (Y. Anikster, J. G. Moseman, and I. Wahl, unpublished).

(Fig. 4). Resistance appears to be especially common in the vicinity of rusting *Ornithogalum* plants. G. Fischbeck (unpublished) found that *H. spontaneum* selections that are resistant to powdery mildew in Israel display resistance to this disease, leaf rust, and stripe rust in Europe as well. Moseman *et al.* (1980) reported high resistance to *P. hordei* in *H. spontaneum* selections from Israel, which was frequently combined with resistance to other diseases in the United States. The minimum number of genes conferring resistance in the five *H. spontaneum* accessions to culture 57.19 of *P. hordei* was one, two, or three, depending on the accession.

Y. Anikster's recent studies (unpublished) revealed that *H. spontaneum* in Israel is a rich pool of resistance to leaf rust of the slowrusting and late-rusting type.

5. Reaction of Ornithogalum *Plants*

About 8000 accessions of the native *Ornithogalum* species were inoculated with 300 different cultures of *P. hordei*, and about 18,650 successful infections were obtained. Only very few hypersensitive reactions were elicited. They were limited to the foliage of *O. nar-bonense.* No hypersensitive reactions were found on artificially inoculated foliage or bulbs or other *Ornithoealum* species. Presumably, other types of defense protect *Ornithogalum* plants from the rust organism. Cross sections through the fungus-bearing scale tissue stained in cotton blue solution reveal formation of intracellular hyphae invading the host cells by filamentous proliferations (Lumbroso *et al.*, 1977). Such haustoria-like structures, described by Harder and Chong (Chapter 14, this volume), differ distinctly in structure and shape from haustoria produced in the cereal host, and occur in a number of alternate hosts of heteroecious rusts.

6. Puccinia hordei-Uromyces *Relationship*

Taxonomic, physiologic, and cytologic problems in *Uromyces* species on barley in Israel were elaborated by Anikster and Wahl (1979). They postulated that all local barley *Uromyces* species and their shortcycled derivatives on Liliaceae are phylogenetically correlated with *P. hordei* in the sense of Arthur *et al.* (1929, pp. 100-101) or Cummins (1959, p. 9). Notably, although barley *Uromyces* species are correlated with a number of microcyclic species formed on their Liliaceae alternate hosts (Viennot-Bourgin, 1969), microforms associated with *P. hordei* were never found. Figure 5 shows the correlation of macrocylic *Uromyces*organisms with their microcyclic derivatives. All implicated macrocylic and microcylic taxa of *Uromyces* and *Puccinia are* compatible with *Leopoldia eburnea.* The development of short-cycled descendants attests to the more ancient origin of barley *Uromyces* rusts. *P.*

hordei evidently has not yet reached the stage of simplification in the retrogressive process of evolution, and is presumably younger than its *Uromyces* counterparts.

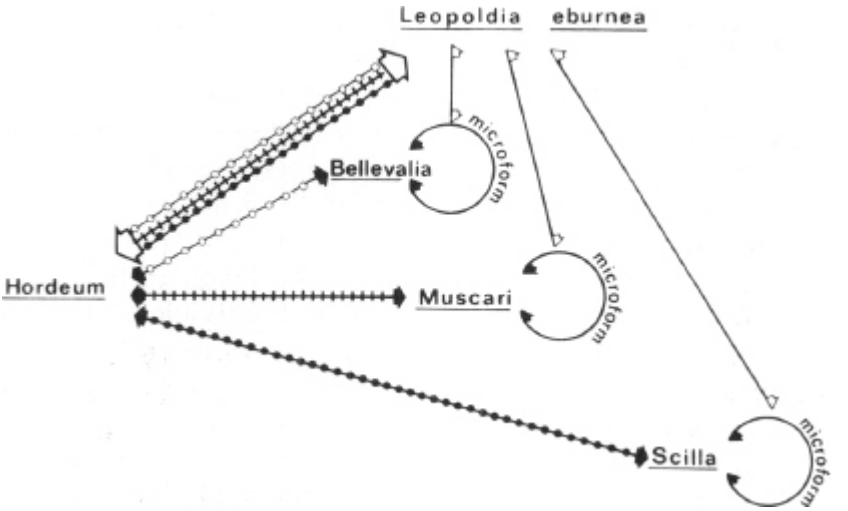

Fig. 5. Heteroecious *Uromyces* species cycling in nature between the *Hordeum* main ' hosts and alternate hosts belonging, respectively, to the genera *Bellevalia* (), Muscari (), or *Scilla* (). Each rust species is compatible only with one of the alternate hosts, but all rusts alternate with *Leopoldia eburnea* in artificial inoculation trials (). *Bellevalia, Muscari,* and *Scilla* alternate hosts harbor in nature microforms confined to the source host and phylogenetically correlated with the respective heteroecious rust species. Each of the microforms is compatible in artificial inoculation trials with *L. eburnea* (). The latter species is a common host for all heteroecious *Uromyces* rusts on *Hordeum,* their microforms, and for *Puccinia hordei.* Each rust preserves on *L. eburnea* its parasitic specificity with respect to the source hosts (Y. Anikster, unpublished).

I. DEFENSE OF MAIZE AGAINST RUSTS

The problems of maize protection against the two rust fungi, *Puccinia polysora* Underw. and *P. sorghi* Schw., in the center of evolution of the crop were admirably analyzed by Borlaug (1972). Herein are condensed some of his findings and ideas.

Maize apparently originated in the highlands of Mexico, Guatemala, and perhaps Peru, long before the beginning of recorded history. Its wild forms were used for food about 7000 years ago, whereas cultivation was extensive in some areas about 5000 years ago.

Originally, maize was cultivated as an open-pollinated crop throughout Mexico, Central America, and the highlands of northern South America. Open-

pollinated cultivars remain the basis of maize cultivation in most of Latin America, Africa, and Asia.

Two rust species attack maize in Latin America: *P. sorghi*, which is common at higher elevations and lower temperatures, and *P. polysora*, which predominates at higher temperatures. "Although one or the other of these rusts is commonly found infecting nearly every plant of maize throughout its natural range in Mexico, Central America, and northern South America, infection seldom occurs in sufficient intensity to cause appreciable damage, except rarely and locally," where the equilibrium existing between hosts and pathogens is upset. Borlaug concluded that a host-parasite balance conditioned by general resistance "*is established on the basis of both latitude and elevational environments, resulting in harmonious survival of host and pathogen with little damage being done to either"* (Borlaug's italics).

Kim and Brewbaker (1977) reported the effectiveness of general resistance in maize to *P. sorghi* in Hawaii. According to Hooker (1973), general (nonspecific) resistance to *P. sorghi* is common in American maize and "is believed to be the major reason why *P. sorghi* fails to develop in destructive proportions in the U.S.A." Van der Plank (1968) maintained that resistance to *P. polysora* is general ("horizontal") or at •least mostly general. Resistance to both rusts in maize can also be accounted for by the genetic heterogeneity of an open-pollinated crop.

III. Concluding Remarks

A. CENTERS OF COEVOLUTION

Nelson (1979) described host-pathogenic fungi coevolution as "a unique and spectacular biological saga." The saga envelops a multiplicity of fundamental problems, and some of them were elaborated elsewhere (Anikster and Wahl, 1979). This chapter deals mainly with the plant pathological aspects.

There seems to be a general consensus that protection against disease and parasite virulence should be sought and studied in the centers of host-obligate parasite origin. In the case of heteroecious rusts, the studies should be pursued in the common centers of origin of the main and alternate hosts. In such centers, genes of plant defense and fungus virulence are stockpiled. There, the host and parasite have reached a state of balanced polymorphism as a result of reciprocal natural selection, associated with their prolonged coexistence. Recognition of mechanisms regulating the equilibrium provides information that is most useful for managing disease and its control in agroecosystems. Theoretical aspects of such equilibria are discussed by Growth (Chapter 8, this volume).

In the foregoing sections, summaries have been made of the basic attributes of host-parasite coevolution and balance, in three different regions: (1) the Caucasus and its neighboring areas, (2) the Mediterranean regions (Portugal and Israel), and (3) Latin America. In the first two regions, studies dealt with small-grain rusts, whereas in Latin America, maize rusts were investigated. Significantly, conclusions gleaned from the three remote regions have a common pivotal base. Zhukovsky (1964, pp. 89-92) presented a comprehensive picture of the host-parasite coevolution process in the Caucasus. He found that the most promising sources of defense against diseases can be selected in the home of the host-parasite systems. Both components in the couplet vary continuously and reach dynamic balance, implicating a multiplicity of protection types and virulence forms. The latter are often new and agressive. In those regions is located the center of origin of *Puccinia graminis, P. recondita,* and *P. striiformis.* The spectra of fungus races and biotypes are more heterogenic in Transcaucasia than elsewhere in the Soviet Union.

B. DEFENSE TYPES AND THEIR INTEGRATION IN POPULATIONS

Plant communities in the Caucasus and the neighboring areas are conglomerates of moderate resistance, "field resistance," tolerance, and moderate to high susceptibility. They are devoid of absolute immunity. This is true even of the most resistant *Triticum* species, such as T. *persicum* (T. *carthlicum)* and T. *zhukovskyi.*

Studies in Israel reveal a similar situation. The most common form of protection is slow rusting, with symptoms resembling Zhukovsky's "field resistance." It was further proven that the protection systems comprise, in varying proportions, resistance of low- to intermediate-reaction type, slow rusting, tolerance, and escape. Genetic diversity, in itself, is not a safeguard against epidemics. Effective diversity needs to be "ordered" and "patterned" (Dinus, 1974), or molded by selection pressure of the parasite under specific environmental conditions. Segal's research (1981) has ascertained that "population resistance" (Browning *et ah,* 1979) inherent in indigenousness, which effectively sheltered *A. sterilis* populations from crown rust in northern Israel, was less satisfactory when the populations were maintained in the central coastal plain. Populations from arid regions, where crown rust incidence is very mild and selection pressure inconsequential, develop rust rather seriously in the central coastal plain. Similarly, Borlaug (1972) stressed that "if one moves open-pollinated maize lowland varieties, into higher elevations, they will rust severely." By the same token, when high-elevation maize varieties are sown in the tropical lowlands, they become seriously infected. Each environment requires a suitable set of genes to mollify disease incidence by damping excessive perturbations.

Studies in Israel have also demonstrated that lush stands of *A. sterilis* are permanently exposed to the *P. coronata* f. sp. *avenae* race group 276-264, which comes close to the conceptual "superrace" as far as hexaploid oats are concerned. These stands do not appear to suffer visibly from the disease. Obviously, the protection associated with indigenousness buffers *A. sterilis* against this race group. This situation seems to dispel the lingering fears of potential hazards that may arise with the increased prevalence of "superraces." The findings in Israel are in agreement with Borlaug's reports on maize rusts, which infect nearly every plant of the crop throughout its natural range. Yet the infection is seldom intense enough to cause appreciable damage. The dilemma presumably starts when humans disturb the natural balance in agroecosystems. "Man domesticated species of small grains, took them from their centers of origin, improved them agronomically, always narrowing their genetic base… . Man-guided evolution of the pathogen, boom-and-bust years with the host, the vicious circle of small grain 'improvement,' and low marks of specific resistance as means of disease control, were the all-too-frequent results" (Browning *et al*, 1979).

C. STABILIZING SELECTION

Van der Plank (1963) introduced the concept of stabilizing selection. He stated that "we take it as axiomatic that simple races are the fittest to survive on simple varieties." Parlevliet (1981) discussed the merits and demerits of the concept and concluded, "although SS [stabilizing selection] sensu van der Plank seems to be an empty concept in crop pathosystems, it need not to be so in wild pathosystems." Studies on crown rust and stem rust of *A. sterilis* do not attest to the applicability of van der Plank's "axiom" to wild pathosystems. Research conducted in Israel for over 30 years has demonstrated a continued and countrywide prevalence of crown rust race 276 and oat stem rust race 72. Both races incorporate many "unnecessary" virulence genes that do not seem to impair the parasitic fitness of the fungi. Brodny's studies on race 276 of *P. coionata* f. sp.*avenae* (1980) demonstrate its adaptability to a wide range of ecologic conditions, high urediospore productivity, and strong infectivity. These findings support Leonard's contention (1977) that "Thus, unnecessary genes for virulence can attain high frequency in a population if they are introduced in a genotype of superior fitness."

D. PLURIVORITY VERSUS PARASITIC SPECIALIZATION

In agroecosystems "obligate plant pathogens … exhibit a great deal of specificity and can grow only on certain varieties of the host" (Sequeira, 1979). Hence, considerable interest if focused on pathogen specificity on the species and cultivar level (Heath, 1981). Specificity has a decisive impact on the

achievements of conventional breeding for disease resistance and is responsible for the ephemerality of the attained protection.

Results of studies in natural ecosystems reveal an entirely different picture (Browning, 1979). Gerechter-Amitai's research on *Puccinia graminis* (1973), as well as investigations on *P. coronata* (Eshed and Dinoor, 1981) and on barley powdery mildew (Eshed and Wahl, 1970), show that fungi characterized by strict specificity in agroecosystems possess a wide host range in natural ecosystems. Eshed and Wahl (1970) postulated that the wide host range among indigenous grasses is at least partly accounted for by the major trends in the phylogeny of Gramineae. According to Stebbins (1956), "most of the common species of grasses … contain in varying proportions, gene combinations derived from two, three, four or more separate and sometimes widely diverging ancestors." Conceivably wild grasses, as a result of their genetic interrelationships, are less specialized in their rust reaction than cultivated cereals.

Savile (1979) attempted to explain the wide host range of *P. coronata* f. sp. *avenae* in Israel by claiming that "*Puccinia coronata* is an atypical species" and by alluding that "we recognize many genera of festucoid grasses more for their possession of handy key characters than for their genetic diversity." The first explanation is hardly plausible, because *P. graminis* f. sp. *avenae* and the barley powdery mildew fungus, too, have similarly broad host ranges. In our opinion, Savile's second explanation is more convincing and agrees with Stebbins' Gramineae phylogeny concept, which helps in understanding the wide host range of the mentioned parasites on grasses.

Congeniality in host–parasite associations at the centers of their origin becomes obvious also on the intraspecific level of the host. In our studies, interactions of *H. spontaneum* with indigenous powdery mildew cultures, and of *A. sterilis* with native crown rust isolates, were more compatible than in the case of infection with alien cultures (Segal *et al.*, 1980). Incompatible coexistence is characterized by low reaction and symptoms of hypersensitivity. This may explain the success in various countries in selecting *H. spontaneum* and *A. sterilis* from Israel for low-reaction resistance to barley powdery mildew and oat crown rust, respectively. In these tests, fungus cultures originating in the countries to which the hosts were introduced were used for inoculation. Plants in Israel exhibiting compatible reaction are most likely protected by mechanisms other than low reaction.

Besides, in dealing with the parasitic specialization of modern rust fungi, we have to consider the fact that ancestral rusts were putatively plurivorous (pleophagous), that is, parasitic on a variety of taxonomically remote hosts. Fischer (1898, p. 115) asserted that Uredineae were originally "omni- or plurivorous." Dietel (1899, p. 117) speculated that rust fungi in ancient times were plurivorous. Their specialization was very inconspicuous, and some rust species inhabited a number of plant families. Also Klebahn (1904, pp. 163-165,

179-180) favored the idea of plurivority (pleophagy) in ancestral rusts. He contended that the origin of heteroecious rust organisms should be sought mainly on the alternate host, from which the fungus migrated to numerous species and families. On the latter hosts the fungus became diversified and attained advanced specialization. The concept of evolution of parasitic specialization from unspecialized forms is shared by Ellingboe (1976), Keen (1982), Nelson (1979), and Parlevliet (1979b). Ellingboe (1976) envisioned that specific interactions associated with incompatibility were superimposed upon a "basic compatibility" between host and parasite. In the case of many graminicolous rusts, the fungus expanded from the alternate host to grasses. According to Johnson *et al.* (1967), "most authorities assume that long before cereals came into existence, the rusts were present on grasses ancestral to cereals and that the rusts adapted to cereals as they came into being." On cereal cultivars, which are genetically well-delimited entities, specialization in the host- parasite interaction made great progress. Hence, the transition of hosts to nonhosts on various taxonomic levels may not be less important than the reverse process.

In Israel small grains occupy a limited acreage. Still, their wild ancestors and relatives are ubiquitous and prolific, and they rust annually. These rusts are mostly at the grass-host stage of evolution. The outlined phylogenetic approach may contribute to a better understanding of the plurivorous behavior of rusts on indigenous grasses in the centers of their origin and genetic diversification. The monokaryotic phase in heteroecious rusts is distinctly less specialized than the dikaryotic one (Green, 1971; Wahl and Anikster, 1982) and has thus preserved the attributes of its progenitors. "The relatively unspecialized growth habit of pycnial and aecial mycelia … may explain the wide host range of some pycnial and aecial rusts," in contrast to the extreme parasitic specialization of uredial and telial rusts (Rijkenberg and Truter, 1973).

Elucidation and exploitation of factors conditioning host-parasite interaction at the "basic compatibility" stage of evolution (*sensu* Ellingboe, 1976) is expected to stabilize disease resistance since this association does not seem to be influenced by specialization.

E. PROSPECTS

As put by Dunin (1959), "production of disease resistant crops is not a very difficult problem. The most difficult objective to attain is to insure durable resistance to cultivars in mass production." This is the crux of the problem. According to Kilpatrick (1975), average longevity of conventional resistance to wheat rusts throughout the world was less than 10 years. Borlaug (1978) emphasized that "stable resistance to the three rusts remains the first objective of the wheat scientist."

The studies reported here on the evolution of rust disease in the centers of their host origin in the Caucasus, Israel, and Latin America show that in these regions indigenous wild and cultivated cereals attain a state of balanced coexistence with the rust fungi. The disease cannot be obviated, but it can be tamed and kept within constraints. The regulatory mechanisms stem from the fact that the protection systems in these plant communities consist of various types and levels of defense elements, often including conventional resistance. Their integration patterns are molded by reciprocal host–parasite selection pressure decisively influenced by environmental conditions. This is the essence of Browning's concept (1974) of "protection of indigenousness." The importance of extrapolating this concept to agroecosystems was emphasized (Browning *et al.*, 1982).

Corollary research on rust virulence proves that some strains can be extremely virulent and approach the conceptual "superrace." Yet their destructiveness is buffered by the dynamic balance among the different protection components and the patterns of their integration and cohesion in defense systems. Studies on parasitism may furnish a deeper insight and broader view on their virulence potential and a preview of what can be expected in other regions (Wahl, 1958).

It is postulated that information ferreted out from studies on host–parasite coevolution in their centers of origin can be used to make "shifty" enemies at least partly less shifty and to surmount the "stubborn biological barrier to rapid progress in increasing and insuring future food supplies" (Stakman, 1968).

Acknowledgments

This chapter is dedicated to the shining memory of Esther Wahl.

We are grateful to Drs. J. A. Browning, G. Fischbeck, J. G. Moseman, and G. Viennot-Bourgin for their most valuable cooperation over the years. Dr. Browning was the pioneering and leading collaborator in studies on "protection of indigenousness." Drs. Z. M. Azbukina, F. Mlodzianowski, D. B. O. Savile, and Z. Urban kindly provided important literature and valuable information.

Our studies were supported by the United States Department of Agriculture, under PL 480, and by the United States-Israel Binational Science Foundation (BSF), Jerusalem, Israel.

References

Anikster, Y. (1982). Alternate hosts of *Puccinia hordei. Phytopathology* 72, 733-735.

Anikster, Y., and Wahl, I. (1979). Coevolution of the rust fungi on Graminae and Liliaceae and their hosts. *Annu. Rev. Phytopathol.* 17, 367-403.

Arthur, J. C. *et al.* (1929). "The Plant Rusts (Uredinales)." Wiley, New York.

Azbukina, Z. M. (1971). Rust fungi producing aecia on Berberidaceae species in the Far East. *Mikol. Fitopatol.* 5(5), 420-425 (in Russian).

Borlaug, N. E. (1972). A cereal breeder and ex-forester's evaluation of the progress and problems involved in breeding the rust resistant forest trees: Moderator's summary. *In* "Biology of Rust Resistance in Forest Trees," Mis. Publ. No. 1221. pp. 615-642. U.S. Dept. Agric. For. Serv. Washington, D.C.

Borlaug, N. E. (1978). Stable resistance comes first. *In* "CIMMYT Review 1978," p. 56. Centro Internacional de Mejoramiento de Maiz y Trigo, Mexico.

Brodny, U. (1980). Studies on the nature of mechanisms determining the composition of physiological races populations of *Puccinia coronata* Cda. var. *avenae* Fraser & Ledingham on *Avena sterilis* L. in Israel. Ph.D. Thesis, Tel Aviv University, Tel Aviv (in Hebrew, with English summary).

Brodny, U., Briggle, L. W., and Wahl, I. (1976). Reaction of U.S. crown rust resistant oat selections and Israeli *Avena sterilis* selections to *Puccinia coronata* var. *avenae. Plant Dis. Rep.* 60, 902-906.

Browder, L. E. (1980). A compendium of information about named genes for low reaction to *Puccinia recondita* in wheat. *Crop Sci.* 20, 775-779.

Browning, J. A. (1974). Relevance of knowledge about natural ecosystems to development of pest management programs for agroecosystems. *Proc. Am. Phytopathol. Soc.* 1, 191-199.

Browning, J. A. (1979). Genetic protective mechanisms of plant-pathogen populations: Their coevolution and use in breeding for resistance. *In* "Biology and Breeding for Resistance" (M. K. Harris, ed.), pp. 52-75. Texas A&M University, College Station.

Browning, J. A., and Frey, K. J. (1969). Multiline cultivars as a means of disease control. *Annu. Rev. Phytopathol.* 7, 355-382.

Browning, J. A., Frey, K. J., McDaniel, M. E., Simons, M. D., and Wahl, I. (1979). The biologic of using multilines to buffer pathogen populations and prevent loss. *Indian J. Genet. Plant Breed.* 39, 3-9.

Browning, J. A., Manisterski, J., Segal, A., Fischbeck, G., and Wahl, I. (1982). Extrapolation of genetic and epidemiologic concepts from indigenous ecosystems to agroecosystems. *In* "Resistance to Diseases and Pests in Forest Trees" (H. M. Heybroek, B. R. Stephan, and K. von Weissenberg, eds.), pp. 371-386. Pudoc Cent. Agric. Publ. & Doc, Wageningen.

Chabelska, H. (1938). Life-cycle of the rust on *Anchusa strigosa Lab. Palest. J. Bot., Jerusalem Ser.* 1, 101-103.

Coons, G. H. (1953). Breeding for resistance to disease. *In* "Plant Diseases," The Year-book of Agriculture 1953, pp. 174-192. U.S. Dept. Agric, Washington, D.C.

Cummins, G. B. (1959). "Illustrated Genera of Rust Fungi." Burgess, Minneapolis, Minnesota.

de Bary, A. (1879). *Aecidium abietinum. Bot. Centralbl.* 37(49), 777-789.

Dietel, P. (1899). Waren die Rostpilze in fruheren Zeiten plurivor? *Bot. Centralbl.* 79, 81-85, 113-117.

Dietel, P. (1904). Betrachtungen iiber die Verteilung der Uredineen auf ihren Nahrpflanzen. *Zentralbl. Bakteriol., Parasitenkd. Infektionskr., Abt. 2, Naturwiss.: Allg. Landwirtsch. Tech. Mikrobiol.* 12, 218-234.

Dinoor, A. (1967). The role of cultivated and wild plants in the life cycle of *Puccinia coronata* Cda. var. *avenae* F. & L. and the disease cycle of oat crown rust in Israel. Ph.D. Thesis, Hebrew University, Jerusalem (in Hebrew, with English summary).

Dinus, R. J. (1974). Knowledge about natural ecosystems as a guide to disease control in managed forests. *Proc. Am. Phytopathol. Soc.* 1, 184-190.

D'Oliveira, B. (1940). Notas sobre a produção da fase aecidica de algumas ferrugens dos cereais em Portugal. *Rev. Agron.* 28(2), 201-208 (in Portugese, with English summary).

D'Oliveira, B. (1951). The centers of origin of cereals and the study of their rusts. *Agron. Lusit.* 13(3), 221-226.

D'Oliveira, B. (1960a). Ideas concerning the evolution and distribution of cereal rusts. *Port. Acta Biol. Ser. A* 6(2), 111-124.

D'Oliveira, B. (1960b). Host range of the aecidial stage of *Puccinia hordei* Otth. *Malhoramento* 13, 161-188.

Dunin, M. S. (1959). Preface to the Russian edition of the book by E. C. Stakman and J. G. Harrar. "Principles of Plant Pathology." Innostrannoi Literatury, Moscow.

Ellingboe, A. H. (1976). Genetics of host-parasite interactions. *Encycl. Plant Physiol., New Ser.* 4, 761-778.

Eshed, N. (1978). The genetical basis of the pathogenicity of *Puccinia coronata* Cda. in Israel. Ph.D. Thesis, Hebrew University, Jerusalem (in Hebrew, with English summary).

Eshed, N., and Dinoor, A. (1981). Genetics of pathogenicity in *Puccinia coronata:* The host range among grasses. *Phytopathology* **71**, 156-163.

Eshed, N., and Wahl, I. (1970). Host ranges and interrelations of *Erysiphe graminis hordei, E. graminis tritici,* and *E. graminis avenae. Phytopathology* 60, 628-634.

Feldman, M., and Sears, E. R. (1981). The wild gene resources of wheat. *Sci. Am.* 244, 102-112.

Fischer, E. (1898). Entwicklungsgeschichtliche Untersuchungen iiber Rostpilze. 1. Uber Beziehungen zwischen Uredineen, welche alle Sporenformen besitzen und solchen von reduziertem Entwicklungsgang. *Beitr. Kryptogamenflora Schweiz* 1(1), 109-121.

Flor, H. H. (1971). Current status of the gene-for-gene concept. *Annu. Rev. Phytopathol.* 9, 275-296.

Gaumann, E. (1959). Die Rostpilze Mitteleuropas. *Beitr. Kryptogamenflora Schweiz* **12,** 7-1407.

Gerechter-Amitai, Z. K. (1973). Stem rust, *Puccinia graminis* Pers. on cultivated and wild grasses in Israel. Ph.D. Thesis, Hebrew University, Jerusalem (in Hebrew, with English summary).

Gerechter-Amitai, Z. K. (1982). Major and minor genes for resistance to stripe rust in wild emmer wheat, *Triticum dicoccoides* Korn. p. B/4. *In* "Gifrid Workshop on Wheat and Barley Breeding and Cultivation under Semi-arid and Arid Conditions," pp.

Gerechter-Amitai, Z. K., and Wahl, I. (1966). Wheat stem rust on wild grasses in Israel. Role of wild grasses in the development of the parasite and in breeding for resistance. *In* "Cereal Rust Conference," pp. 207-217. Plant Breed. Inst., Cambridge, England.

Gerechter-Amitai, Z. K., and Loegering, W. Q. (1977). Genes for low reaction to *Puccinia graminis tritici* in *Aegilops* and *Triticum. Crop Sci.* 17, 830-832.

Gerechter-Amitai, Z. K., Wahl, I., Vardi, A., and Zohary, D. (1971). Transfer of stem rust seedling resistance from wild diploid einkorn to tetraploid *durum* wheat by means of a triploid hybrid bridge. *Euphytica* 20, 281-285.

Golan, T., Anikster, Y., Moseman, J. G., and Wahl, I. (1978). A new virulent strain of *Puccinia hordei. Euphytica* 27, 185-189.

Goulden, C. H., Newton, M., and Brown, A. M. (1930). The reaction of wheat varieties at two stages of maturity to sixteen physiologic forms of *Puccinia graminis tritici*. *Sci. Agric.* **11,** 9-25.

Green, G. J. (1971). Hybridization between *Puccinia graminis tritici* and *Puccinia graminis secalis* and its evolutionary implications. Can. J. Bot. 49, 2089-2095.

Harder, D. E. (1980). Virulence and distribution of *Puccinia coronata avenae* in Canada in 1979. *Can J. Plant Pathol.* 2, 249-252.

Harlan, J. R., and Zohary, D. (1966). Distribution of wild wheats and barley. *Science* **153,** 1074-1080.

Hassebrauk, K. (1965). Nomenklatur, geographische Verbreitung und Wirtsbereich des Gelbrostes, *Puccinia striiformis* West. *Mitt. Biol. Bundesanst. Land-Forstwirtsch., Berlin-Dahlem* **116.**

Heath, M. C. (1981). A generalized concept of host-parasite specificity. *Phytopathology* 71, 1121-1123.

Hooker, A. L. (1973). Maize. *In* "Breeding Plants for Disease Resistance" (R. R. Nelson, ed.), pp. 132-154. Pennsylvania State Univ. Press, University Park.

Johnson, T., Green, G. J., and Samborski, D. J. (1967). The world situation of the cereal rusts. *Annu. Rev. Phytopathol.* 5, 183-200.

Keen, N. T. (1982). Specific recognition in gene-for-gene host–parasite systems. *Adv. Plant Pathol.* 1, 35-82.

Kilpatrick, R. A. (1975). New wheat cultivars and longevity of rust resistance, 1971-75. *U.S., Agric. Res. Serv., Northeast. Reg. [Rep.] ARS-NE* 64.

Kim, S. K., and Brewbaker, J. L. (1977). Inheritance of general resistance in maize to *Puccinia sorghi* Schw. *Crop Sci.* 17, 456-461.

Kislev, M. E. (1982). Stem rust of wheat 3300 years old found in Israel. *Science* **216,** 993-994.

Klebahn, H. (1904). "Die wirtswechselnden Rostpilze." Borntraeger, Berlin.

Leonard, K.J. (1977). Selection pressures and plant pathogens. *Ann. N.Y. Acad. Sci.* 287, 207-222.

Leppik, E. E. (1961). Some viewpoints on the phylogeny of rust fungi IV. Stem rust genealogy. *Mycologia* 53, 378-405.

Leppik, E. E. (1965). A pathologist's viewpoint on plant exploration and introduction. *U.S., Agric. Res. Serv., Plant Introd. Invest.* 1, 1-5.

Leppik, E. E. (1967). Some viewpoints on the phylogeny of rust fungi. VI. Biogenic radiation. *Mycologia* 59, 568-579.

Leppik, E. E. (1970). Gene centers of plants as sources of disease resistance. *Annu. Rev. Phytopathol.* 8, 323-344.

Littlefield, L. J. (1981). "Biology of the Plant Rusts." Iowa State Univ. Press, Ames.

Luke, H. H., Barnett, R. D., and Chapman, W. H. (1975). Types of horizontal resistance of oats to crown rust. *Plant Dis. Rep.* 59, 332-334.

Lumbroso, E., Anikster, Y., Moseman, J. G., and Wahl, I. (1977). Completion of life cycles of *Puccinia hordei and Uromyces scillarum* on detached leaves of their hosts. *Phytopathology* 67, 941-944.

Markova, J., and Urban, Z. (1977). To the knowledge of the brown rust of couch grass in Bohemia and Moravia. 2. *Česka Mykol.* 31, 72-80 (in Czech, with English summary).

Mode, C. J. (1958). A mathematical model for the coevolution of obligate parasites and their hosts. *Evolution* 12, 158-165.

Moseman, J. G., Baenziger, P. S., and Kilpatrick, R. A. (1980). *Hordeum spontaneum*—an overlooked source of disease resistance. *Proc.—Eur. Mediterr. Cereal Rusts Conf., 5th, 1980* pp. 91-94. Bari and Rome, Italy.

Murphy, H. C., Wahl, I., Dinoor, A., Miller, J. D., Morey, D. D., Luke, H. H., Sechler, D., and Reyes, L. (1967). Resistance to crown rust and soilborne mosaic virus in *Avena sterilis*. *Plant Dis. Rep.* 51, 120-124.

Nelson, R. R. (1973). The meaning of disease resistance in plants. *In* "Breeding Plants for Disease Resistance" (R. R. Nelson, ed.), pp. 13–25. Pennsylvania State Univ. Press, University Park.

Nelson, R. R. (1978). Genetics of horizontal resistance to plant diseases. *Annu. Rev. Plant Pathol.* 16, 359-378.

Nelson, R. R. (1979). Some thoughts on the coevolution of plant pathogenic fungi and their hosts. *In* "Host-Parasite Interfaces" (B. B. Nickol, ed.); pp. 17-25. Academic Press, New York.

Parlevliet, J. E. (1979a). Components of resistance that reduce the rate of epidemic development. *Annu. Rev. Phytopathol.* 17, 203-222.

Parlevliet, J. E. (1979b). The co-evolution of host-parasite systems. *Symb. Bot. Ups.* 22(4), 39-45.

Parlevliet, J. E. (1981). Stabilizing selection in crop pathosystems: An empty concept or a reality? *Euphytica* 30, 259-269.

Peterson, R. F. (1965). "Wheat." Leonard Hill Books, London.

Rijkenberg, F. H. J., and Truter, S.J. (1973). Haustoria and intracellular hyphae in the rusts. *Phytopathology* 63, 281-286.

Robinson, R. A. (1980). New concepts in breeding for disease resistance. *Annu. Rev. Phytopathol.* 18, 189-210.

Santiago, J. C. (1968). Physiologic specialization of the oat crown rust fungus in Portugal. *Cereal Rusts Conf., 1968* pp. 89-91. Oeiras, Portugal.

Savile, D. B. O. (1979). Fungi as aids to plant taxonomy: Methodology and principles. *Symb. Bot. Ups.* 22(4), 135-145.

Savile, D. B. O., and Urban, Z. (1982). Evolution and ecology of *Puccinia graminis. Preslia* 54, 97-104.

Schmidt, R. A. (1978). Diseases in forest ecosystems: The importance of functional diversity. *In* "Plant Disease: An Advanced Treatise" (J. G. Horsfall and E. B. Cowling, eds.), Vol. 2, pp. 287-315. Academic Press, New York.

Segal, A. (1981). Elements of resistance against *Puccinia coronata* Cda. var. *avenae* F. & L. and their integration in the defense structures of *Avena sterilis* L. populations in natural ecosystems undisturbed by man in Israel. Ph.D. Thesis, Tel Aviv University Tel Aviv (in Hebrew, with English summary).

Segal, A., Manisterski, J., Fischbeck, G., and Wahl, I. (1980). How plant populations defend themselves in natural ecosystems. *In* "Plant Disease: An Advanced Treatise" (J. G. Horsfall and E. B. Cowling, eds.), Vol. 5, pp. 75-102. Academic Press, New York.

Segal, A., Manisterski, J., Browning, J. A., Fischbeck, G., and Wahl, I. (1982). Balance in indigenous plant populations. *In* "Resistance to Diseases and Pests in Forest Trees" (H. M. Heybroek, B. R., Stephan, and K. von Weissenberg, eds.), pp. 361-370. Pudoc Cent. Agric. Publ. & Doc, Wageningen.

Sequeira, L. (1979). Recognition between plant hosts and parasites. *In* "Host-Parasite Interfaces" (B. B. Nickol, ed.); pp. 71-84. Academic Press, New York.

Simons, M. D. (1969). Heritability of crown rust tolerance in oats. *Phytopathology* 59, 1329-1333.

Simons, M. D. (1972). Crown rust tolerance of *Avena sativa*—type oats derived from *Avena sterilis. Phytopathology* 62, 1444-1446.

Simons, M. D., Martens, J. W., McKenzie, R. I. H., Nishiyama, I., Sadanaga, K., Sebesta, J., and Thomas, H. (1978). Oats: A standardized system of nomenclature for genes and

chromosomes and catalog of genes governing characters. *U.S. Dep. Agric., Agric. Handb.* **509.**

Simons, M. D., Rothman, P. G., and Michel, L. J. (1979). Pathogenicity of *Puccinia coronata* from buckthorn and from oats adjacent to and distant from buckthorn. *Phytopathology* 69, 156-158.

Stakman, E. C. (1968). What are the prospects for permanent control of cereal rusts? *Cereal Rusts Conf., 1968* pp. 217-230. Oeiras, Portugal.

Stebbins, G. L. (1956). Cytogenetics and evolution in the grass family. *Am. J. Bot.* 43, 890-905.

Sztejnberg, A., and Wahl, I. (1976). Mechanisms and stability of slow stem rusting resistance in *Avena sterilis. Phytopathology* 66, 74-80.

Tranzschel, W. A. (1939). Sovremiennoe sostaianie znanii po biologii rshavchin khlebnykh zlakov (Present status of knowledge about the biology of cereal rusts). *In* "Rshavchina Zernovykh Kultur" (Rusts of Ceral Crops) (N. A. Naumov and A. K. Zubareva, eds.), pp. 21-28. Selkhosgiz, Moscow.

Urban, Z. (1961). Zur Schwarzrostfrage in Bohmen. Z. *Pflanzenkd.* 27, 67-68.

Urban, Z. (1968). Zum Artbegriff bei Rostpilzen. *In* "Das Art-und Rassenproblem bei Pilzen," Int. Symp. Werringerode am Harz (1967), pp. 19-25.

Urban, Z. (1969). Taxonomie der parasitischen Pilze und die Widerstandsfahigkeit der Kulturpflanzen. *Česka Mykol.* 23, 236-242.

Urban, Z. (1980). Rust ecology and phytocenology as aids in rust taxonomy. *Rep. Tottori Mycol. Inst.* 18, 269-273.

Van der Plank, J. E. (1963). "Plant Diseases: Epidemics and Control." Academic Press, New York.

Van der Plank, J. E. (1968). "Disease Resistance in Plants." Academic Press, New York.

Vavilov, N. I. (1939). Selekstia ustoichovykh sortov kak osnovnoi metod borby s rshavchinoi (Selection of resistant varieties as a basic method for rust control). *In* "Rshavchina Zernovykh Kultur" (Rusts of Cereal Crops) (N. Z. Naumov and A. K. Zubareva, eds.), pp. 3-20. Selkhozgyz, Moscow.

Viennot-Bourgin, G. (1969). Mission phytopathologique en Iran en 1968. *Ann. Phytopathol.* 1, 5-36.

Wahl, I. (1958). Studies on crown rust and stem rust on oats in Israel. *Bull. Res. Counc. Isr., Sect. D* 6, 145-166.

Wahl, I. (1959). Physiologic races of oat crown rust identified in Israel in 1956-59. *Bull. Res. Counc. Isr., Sect. D* 8, 25-30.

Wahl, I. (1970). Prevalence and geographic distribution of resistance to crown rust in *Avena sterilis. Phytopathology* 60, 746-749.

Wahl, I., and Anikster, Y. (1982). Role of the alternate host in evolution of some cereal rusts. *Garcia de Orta, Ser. Estud. Agron.* (Volume dedicated to Professor B. D'Oliveira) (in press).

Wahl, I., Dinoor, A., Halperin, J., Schreiter, S., (1960). The effect of *Rhamnus palaestina* on the origin and persistence of oat crown rust races. *Phytopathology* 50, 562-567.

Zadoks, J. C. (1965). Epidemiology of wheat rusts in Europe. *FAO Plant Prot. Bull.* 13, 97-108.

Zhukovsky, P. M. (1959). Interrelation between host and parasite in their origin and beyond it. *Vestn. S-kh. Nauki (Moscow)* 4(6), 25-34 (in Russian, with English summary).

Zhukovsky, P. M. (1961). Grundlagen der Introduktion der Pflanzen auf Resistenz gegen Krankheiten. *Zuechter* 31, 248-253.

Zhukovsky, P. M. (1964). "Kulturnye Rasteniia i ikh Sorodichi" (Cultivated Plants and their Wild Relatives), 2nd ed. Publ. Kolos, Leningrad.

Zhukovsky, P. M. (1965). Main gene centers of cultivated plants and their wild relatives within the territory of the U.S.S.R. *Euphytica* 14, 177-188.

Zillinsky, F. J., and Murphy, H. C. (1967). Wild oat species as sources of disease resistance for the improvement of cultivated oats. *Plant Dis. Rep.* 51, 391-395.

Zohary, D. (1971). Origin of south-west Asiatic cereals: Wheats barley, oats and rye. *In* "Plant Life of South-West Asia" (P. H. Davis *et al.*, eds.), pp. 235-263. Botanical Society of Edinburgh.

Zohary, D. (1973). The origin of cultivated cereals and pulses in the Near East. *Chromosomes Today* 4, 307-320.

Zohary, D., and Brick, Z. (1961). *Triticum dicoccoides* in Israel: Notes on its distribution, ecology and natural hybridization. *Wheat Inf. Serv.* 13, 6-8.

3

Taxonomy of the Cereal Rust Fungi

D. B. O. Savile

Biosystematics Research Institute, Agriculture Canada, Ottawa, Ontario, Canada

I. Introduction

The grass rusts are numerous and taxonomically complex. For simplicity I treat, and key, those of cereals by natural host groups: (1) the temperate cereals, which are festucoid grasses in either Triticeae [wheat (*Triticum*), barley (*Hordeum vulgare*), and rye (*Secale cereale*)] or Aveneae [oats (*Avena sativa*)], (2) maize or corn (*Zea mays*) in Maydeae, (3) sorghum (*Sorghum bicolor*) in Andropogoneae, and (4) sugarcane (*Saccharum officinarum*) in the group Saccharininae of Pilger (1940) of Andropogoneae subtribe Saccharinae.

This is legitimate, for most cereal and other grass rusts have restricted host ranges, and the chance of their going to widely unrelated genera in more than trace amounts is remote. We must note, however, that Festucoideae is very closely knit, although sharply distinct from other subfamilies. Many rusts and other parasites attack genera in several festucoid tribes (examples in Savile, 1979). Probably genetic diversity in Festucoideae is less than morphological diversity suggests.

Urban (1967) greatly clarified the stem rust complex, *Puccinia graminis* Pers. *s. lat.*, as we will see (Section IV,B). There have been reports (Cummins, 1971) of *P. graminis* on nonfestucoid grasses, some surely based on trace infections on nonfestucoids associated with rusted festucoids, but some erroneous. The report of *Echinochloa crus-galli* as a host is based on rusted leaves of *Elymus* collected with a healthy inforescence of *Echinochloa muricata* (Savile, 1981). Reports of *P. graminis* on *Oryza* in southern Europe, summarized by Diehl (1944), which later vanished from the literature, probably stem from occurrence of a rusted festucoid grass in rice fields.

My descriptions are based largely on material in the National Mycological Herbarium of Canada (DAOM), which is especially strong in north temperate material, supplemented heavily by loans from the Arthur Herbarium at Purdue University (PUR) for predominantly tropical rusts, and small numbers from Naturhistoriska Riksmuseum Stockholm (S) and University of Michigan (MICH), together covering approximately the known range for each rust. Specimens examined in detail range from ~370 for the three *P. graminis* taxa (including wild grasses) to 17 for *P. melanocephala*, 13 for *P. kuehnii*, 9 for *P. rufipes*, and 5 for *P. miscanthi* (total ~950).

II. Methods of Study

A. INSPECTING SPECIMENS AND MAKING MOUNTS

My survey of cereal rusts has shown various mixed infections not noted by their collectors. Such errors are misleading in assessing disease resistance. Making mounts under the stereomicroscope at x30 to x 40 in a focused light beam helps to reveal mixtures or misdeterminations by clarifying the color and structure of the sori. It also helps in avoidance of immature or overmature sori. If unrediniospores adhere together in a crust, they are usually overrun by bacteria and will not clear well (Section II,B,4).

I make mounts in lactophenol. A very small drop is put in the center of the slide and a liberal streak applied on each side. Spores are put only in the small drop. When the cover is applied and the slide warmed nearly to boiling point, full turgor and clearing are quickly achieved, and most spores remain in the middle of the mount. Measurements and colors in my descriptions are based on this readily reproducible method. Lactophenol mounts finally deteriorate but stay unchanged for many weeks.

It may be difficult to show the full range of teliospore sizes from a single specimen of such species as *Puccinia graminis,* in which the spores have firm pedicels and are tightly packed in the sorus. Eventually most spores of this species are either clavate with long pedicels or fusoid with short pedicels. The fusoid spores fill the spaces between the pedicels and lower cells of the clavate spores (Fig. 1). Thus the spore shapes are partly prescribed by mutual pressure before the walls are thickened. In young telia, and those in which teliospores replace urediniospores, there is little crowding, and extremes of spore dimensions and pedicel length are seldom reached. Try to make mounts from central parts of large and erumpent sori. By using a finely pointed diagonal scalpel to cut successive slices through the base of the sorus we maximize the chance of securing teliospores with intact pedicels (confirmed by attached remnants of meristematic basal cells). This technique ensures mounts with ample paraphyses and basal stroma in species having them. Freezing microtome sections are occasionally helpful, but the method is too slow for routine use if many specimens are to be examined; and scrape mounts are still needed to secure the full range of spore sizes. Large groups of teliospores should be loosened with the scalpel tip; then they are fanned out for study by pressing on the coverslip.

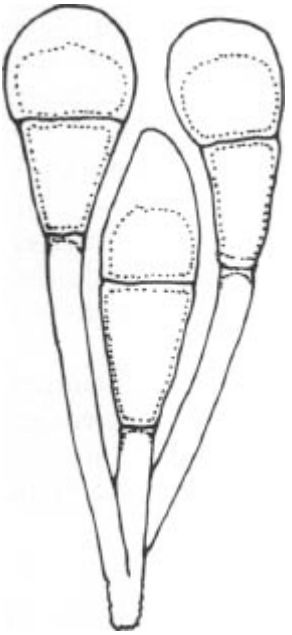

Fig. 1. Filling of space by teliospores of *Puccinia graminis.*

B. MICROSCOPIC STUDY

1. General

All pycnia of true Pucciniaceae (Savile, 1976) are flask-shaped with peripheral paraphyses. Accordingly, they are not described in this chapter.

Although the 4-mm objective is adequate for measuring large spores or long pedicels, the x 1OO oil-immersion lens, at x 800 or more, is needed for details of wall thickness, laminations, and sculpturing. All but the largest spores are measured fastest and most accurately under oil immersion. I routinely locate spore groups under the x 10 objective and then swing directly to the oil-immersion lens. Using Carl Zeiss phase-contrast optics I leave the oil-immersion phase ring continuously in position and locate spores effectively by dark field, which speeds examination.

Precise recording of sizes of aeciospore verrucae and urediniospore echinulae requires high-contrast phase optics. I measure spores with x8 eyepieces and the magnification changer at 1.0, but swing it to 2.0 (i.e., x 1600) to measure verrucae, echinulae, and thin walls. This method allowed detailed study of over 900 mounts in the months available.

In recording measurements, the common range is in open figures, occasional extremes are in single parentheses, and rare extremes in double parentheses. This symbolism, now commonly used in systematic studies, simplifies the assessment of an unknown specimen.

2. Aeciospore Sculpturing

Aeciospores of grass rusts were shown (Savile, 1973) to be of five types (Fig. 2). In type 1 the walls are uniformly finely verrucose; in type 2 they are bizonate with finely and coarsely verrucose belts; in type 3 the walls are as in type 2 except for small plugs (extra large warts surrounded by an appreciable clear area or tonsure); in type 4 there are a few large, readily detaching plugs and several small ones; and in type 5 there are a few large plugs but few or no small ones. The spore type is constant within species, intermediates are few, and it is often a good taxonomic character. Thus it distinguishes some members of the *Puccinia recondita*complex (Section IV,C). These rusts have type 1, 2, or 3 spores (Savile, 1973, pp. 231-232; but in that paper two names were misapplied: for *P. recondita* read *P. persistens,* and for *P. dispersa* read *P. recondita).*See also *P. sorghi* (Section V,B). Under optimal conditions verrucae are fully resolved down to ~0.2 ~μ m diameter; but finer ones, probably ~0.1 μm diameter, are indistinct; and even finer ones register as even pale gray, in contrast with the bright tonsures.

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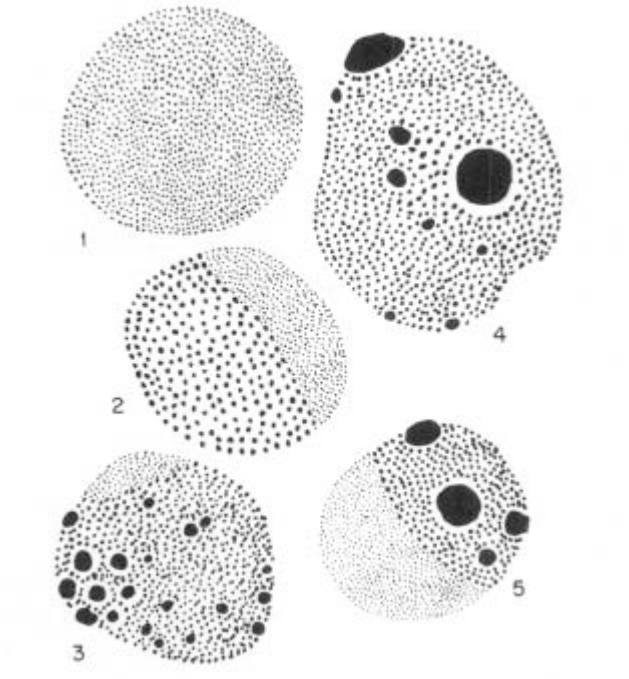


Fig. 2. Aeciospores types (1) to (5).

3. *Urediniospore Echinulation*

The sizes of echinulae are occasionally distinctive, and they should be measured. Terms such as “finely echinulate” are too subjective to be meaningful. Heights of echinulae are usually difficult to measure, in that their apices are often invisible in side view, and in surface view they often lie obliquely; but the basal diameters can be measured closely by focusing down from the tips and stopping just before the base goes out of focus. In broadly conical echinulae the rim is thin and gives a pale but still sharp image. In most populations of *Puccinia graminis*, echinulae at the spore equator are scarcely half the diameter of those near the ends.

Note that I use “uredinium” and “urediniospore,” because “uredium” and “urediospore,” sometimes used in North America, are etymologically incorrect and abandon morphology in favor of supposedly immutable state sequence (Savile, 1968).

4. Urediniospore Germ Pores

Chemically defined germ pores are present in all pucciniaceous urediniospores; but the refractive index of the pore material is close to that of the wall, and pores are usually obscure unless *(a)* the wall is appreciably pigmented, *(b)* there is an appreciable annular wall thickening round the inside of the pore (the internal ring), or (c) there is an appreciable pale blister-like cap over the pore. Pore caps help to make pores visible in lateral view, and an internal ring enhances its image in all views, but without wall pigment a full pore count is usually difficult. Observation of pores is impeded by inadequate clearing of spores. Spores in good condition, fresh or dried by approved botanical methods, usually clear promptly in warmed lactophenol. Old spores, often overrun by saprophytes, often contain refractive globules that give an out-of-focus bright spot simulating a pore. Very obscure pores are usually revealed by a Congo red technique (Urban, 1963), but it is time-consuming.

III. Species Concepts in Rusts

Between the pioneer phase of rust study some 150 years ago, when every new host or host pair observation was regarded (sometimes correctly) as a new species, and widespread lumping of recent decades, a rational compromise must be sought. Part of the trouble with an ultra-broad species concept is that a description compiled to incorporate all its populations is a psychological trap suggesting to the uninformed user that all components are identical, not only in morphology but also in physiology, ecology, and etiology; whereas usually no single population approaches the total range of characters. Arthur (1907-1931) presented a detailed treatment of the rusts, marred mainly by the unrealistic adoption of genera based on life cycles; but in his manual (1934), presumably under limits of time and manuscript length, he seriously oversimplified the taxonomy of many species complexes, including various grass rusts, and some valid species were not even included in synonymy. We cannot arbitrarily rule on the amount of morphological distinction that denotes a species, for there is no indication that easy distinction to the human eye has ever been a factor in rust evolution. I examplified this problem (Savile, 1965) in a group of sedge rusts. The morphological distinction between two sympatric but genetically isolated species was no more than between one of them and its geographically separate subspecies.

An outstanding example of damage caused by too wide a species concept was the long insistence that dwarf bunt of wheat (caused by *Tilletia controversa*) is identical with common bunt (due to *T. caries* and *T. foetida*), whereas it is very distinct in morphology, ecology, and, naturally, genetics of

resistance. Failure to distinguish beteen subtly distinct but genetically isolated pathogens in our nomenclatural system amounts to sweeping our problems under the rug and does a gross disservice to agricultural science. It is satisfying to workers who are not taxonomists to be able to put a binomial on every specimen, but to insist that the species concept must be wide enough to make that end simple is to turn back the clock. It is wiser to get help from a taxonomist even if some delay in naming specimens results. A narrower species based on real and consistent distinctions warns users that they are dealing with separate fungi.

We shall see (Section IV,B) that the action of Urban (1967) in naming three distinguishable entities in *Puccinia graminis* is fully justified: It clarifies our understanding of the complex, and it confirms that some hosts may take more than one of these rusts (Section IV,B,5). The partial splitting of the *P. recondita* complex is similarly justified, for we find that, correlated with host differences, we have small but constant differences in all spore states; moreover, the evidence is that these rusts are genetically isolated.

The use of *formae speciales* in rust nomenclature is contentious. Article 4 of the Botanical Code has long sanctioned the term for taxa that are distinct from a physiological standpoint but scarcely or not at all from a morphological standpoint. Setting them up without a diagnosis was sanctioned because of this stated lack of morphological distinction. If there is morphological distinction, obviously they are invalid because they do not comply with article 4. Oat and barley stem rusts can legitimately be called f. spp. of *P. graminis* ssp. *graminis* var. *stakmanii*, but wheat stem rust (*P. graminis* ssp. *graminis* var. *graminis*) is clearly distinct in morphology and cannot be considered a mere f. sp. of the oat and barley rusts.

IV. Rusts of Temperate (Festucoid) Cereals

A. INTRODUCTION AND PRIMARY KEY

As noted in Section I, the genera and tribes of Festucoideae are closely interrelated but relatively isolated from other grasses. This unity and isolation are reflected in their rusts and other pathogens (Savile, 1979, pp. 467–472). There are various rust groups on festucoids, but we are concerned here with only two, distinguished as follows:

a. Urediniospores with generally 3–5 equatorial germ pores; telia without fused paraphyses or stroma, erumpent and soon naked……Stem Rust, *Puccinia graminis* (Section IV,B)

a. Urediniospores with several scattered germ pores; telia with brown fused paraphyses and stroma often enclosing spore groups, usually sunken, long covered by adherent epidermis……Leaf Rusts, *Puccinia* spp. (Section IV,C)

B. STEM RUST (*Puccinia graminis*)

1. Evolution and Morphological Variation

Puccinia graminis Pers. was divided by Urban (1967) into three morphologically distinct units with largely separate host ranges. The cereal rusts fall into *P. graminis* ssp. *graminis,* whereas most noncereal grasses (in several tribes) usually take small-spored ssp. *graminicola* Urban. The cereal rusts were further divided into two varieties. The lectotype of *P. graminis* being Persoon's specimen on wheat (Jørstad, 1958; Hylander*et al.,* 1953; Cummins, 1971), wheat stem rust, with the largest spores, becomes *P. graminis* Pers. ssp. *graminis* var. *graminis* (with no combining authority); its principal hosts are *Aegilops, Elymus,* and *Triticum,*but it may attack at least *Agropyron, Secale,* and *Hordeum,* probably usually when wheat is heavily rusted (Conners, 1967, p. 135).

Stem rust of barley, rye, and oats, *P. graminis* ssp. *graminis* var. *stakmanii* Guyot *et al.,* is mainly distinguished from var. *graminis* by smaller urediniospores; other statistical rather than absolute distinctions reinforce spore size. Uredinispore germ pores are mainly four or five in both varieties, but three pores are very rare and six occasional in var. *graminis,* whereas three are occasional and six very rare in var. *stakmanii.*Maximum teliospore lengths and widths and pedicel lengths tend to be greater in var. *graminis,* but variation, partly due to differing maturity of sampled sori (Section II,A) limits use of these characters.

Urban's clarification (in English) of *P. graminis* was a notable advance, which, despite its adoption by Cummins (1971), has been unaccountably ignored by North American pathologists. His segregates show how many hosts may take more than one rust (summarized in Section IV,B,5). We cannot afford to deprive geneticists and breeders of this insight into the behavior of this complex species. His revision is widely accepted in Europe.

Detailed study of over 360 specimens fully confirms the validity and value of Urban's segregates. Rarely a specimen on wheat has unusually short urediniospores (~26–32 μm long), but in compensation the widths are above average, indicating normal spore volume, and the pores are mostly four to six, confirming assignment to var. *graminis.*

Macroscopic symptoms may often reflect growth conditions, but it is notable that var. *graminis* on barley usually has longer and more fully open uredinia (as on wheat) than does var. *stakmanii.*

Although *P. graminis* ssp. *graminicola* does not attack cereals, we cannot completely ignore it. It certainly supplied much of the germ plasm of both varieties of ssp. *graminis.* More importantly, both ssp. *graminis*varieties

occasionally attack grasses that are normal hosts of ssp. *graminicola.* Such occurrences may allow gene exchange with ssp. *graminicola* (Section IV,B,5).

There may be doubt whether a specimen with urediniospores smaller than typical for var. *stakmanii* is ssp. *graminicola.* Usually urediniospore pore numbers or teliospore sizes allow a firm assignment, but urediniospore echinulation may also help. In all specimens of var. *graminis* and var. *stakmanii* the echinulae are about half the diameter on the spore equator that they are on the ends (see descriptions in Section IV,B,2–4). On some grass genera (Section IV,B,4) the echinulae on spores of ssp. *graminicola* are scarcely wider at the ends than at the equator, which gives us another distinction. Unfortunately on other genera the echinulae are nearly as in var. *stakmanii,* suggesting that these *graminicola* biotypes contain *stakmanii* genes.

Tajimi (1975–1979) demonstrates the wide adaptations of some *P. graminis* biotypes. Most conspicuously four of his *"formae speciales"* are fully compatible with *Dactylis glomerata,* throwing doubt on the reality of the *forma specialis* concept. How do we determine the identity of f. sp. *dactylidis?* (See also Section IV,B,5.)

The evolution of *P. graminis* is discussed by Savile and Urban (1982). *P. graminis* ssp. *graminicola* is evidently close to ancestral *P. graminis* of Eurasian grasslands. The complex makeup of contemporary mesic European grasslands, in which several widely adapted grasses coexist indefinitely, allows frequent transfer of spores between such grasses, and this genetic swamping has prevented individual strains from speciating. Selection of allopolyploids of *Triticum* and *Avena* as cereals, near the start of agriculture, allowed growth in one plant of rusts adapted to the individual parental diploids. Hyphal fusion and nuclear exchange allowed recombination and perhaps polyploidy in the new parasite strains.

I give here a general description of *P. graminis,* to save duplication in the descriptions of its components, which stress detailed dimensions.

Puccinia graminis Pers. (*Syn. Meth. Fung.,* p. 228. 1801) *sensu lato.*

Pycnia and aecia on *Berberis,* notably *B. vulgaris* and allied species, or rarely *Mahonia.* Pycnia amphigenous. Aecia mainly hypophyllous, in groups, cupulate (or cylindric in stable moisture conditions), peridium white. Aeciospores 15–22 (–24.5) × (12–)14–17(–19) µm, with walls 0.5–0.8 µm exluding verrucae at sides, 3.5–7 µm at apex, hyaline; verrucae in bizonate pattern: (*a*) very fine and close-set, partly below limit of resolution, and seen as gray blur, ~0.2–0.3 µm high, ?<0.1–0.2 µm diam., <0.5 µm between centers; or (b) ~0.5 µm high, (0.2–)0.3–0.6 µm diam., ~0.8–1.0 µm spacing, with several small (usually retained) plugs ~1.0–1.3 µm diam., and few large, freely shed plugs (1.5–)1.8–2.5(–3.7) µm diam. (type 4 spores, Fig. 2). Uredinia and telia on many festucoid grasses, mainly on sheaths and culms of maturing

plants, but amphigenous on leaves of young cereal plants and of mown or otherwise persistently vegetative plants. Uredinia from short elliptical, and often covered by torn epidermis, to linear and promptly naked, generally deep golden brown, without paraphyses. Urediniospores short-ellipsoid to long-ellipsoid or subcylindrical, walls thinnest between equatorial pore belt and ends, outer layer light yellow to yellow-brown when mature (subhyaline in pore belt), usually darkest near apex, thin inner layer chestnut; echinulae finest on pore belt and coarsest near ends (except some populations of ssp. *graminicola*); germ pores generally 3–5, approximately equatorial, or slightly irregular if 5 or more, usually distinct, slight to moderate internal ring, slight hyaline cap to 1.3 µm high. Telia generally linear, early naked, strongly erumpent, without paraphyses or appreciable stroma; shining individual spores easily seen under stereomicroscope. One-celled teliospores occasional, especially at edges of young sori; two-celled spores fusoid with subrostrate apex or clavate with rounded apex, usually slightly constricted at septum, occasionally with l–3(–4) delicate shallow longitudinal ridges; walls ~1.0–2.0 µm min. in lower cell and subhyaline to yellow-brown, to 4.0–12(–15) µm at apex and yellow-brown to chestnut (often with thin deep chestnut layer ~1–2 µm out from lumen); dimple of upper germ pore central to moderately offset, that of lower cell at septum when visible; hilum generally orange-brown; pedicel firm, light to deep yellow throughout if short but often centrally subhyaline if long, about twice as long on clavate as on fusoid spores (Fig. 1).

So few available aecial specimens are firmly linked to telial hosts that I cannot say whether the three segregates differ in aeciospore size. The description just given applies to the whole species.

The segregates of *Puccinia graminis* are separable as follows:

a. II spores ((23–))(25–)26–38(–>42) µm long, pores ((3–))4–5(–6)((–7)); III spores ((32–))34–75(–81) × 14.5–25(–26.5)((–28.5)) µm……ssp. *graminis* var. *graminis.*

a. II spores (20–)22–33(–35) µm long, pores (3–)4–5((–6)); III spores (30–)32–69(–77) × 14–24(–25)((–27)) µm…….ssp. *graminis* var. *stakmanii*

a. II spores (18–)20–29(–31)((–33)) µm long, pores (2–)3–4(–5); III spores (28–)29–63(–68) × (12–)13–23(–24) µm……ssp. *graminicola*

2. Wheat Stem Rust

Puccinia graminis Pers. (*Syn. Meth. Fung.*, 228. 1801) ssp. *graminis* var. *graminis.* [*Puccinia graminis* Pers. var. *tritici* Guyot, Massenot & Saccas, *Ann. Ec. Nat. Agric. Grignon, Ser. 3* **5**, 145 (1946), *nom. subnud.*]

The lectotype of *Puccinia graminis* being on wheat (Section IV,B,1), the rules of nomenclature require the epithet just given for the type segregate. My

description is based on many specimens on *Triticum aestivum* and smaller numbers on *Aegilops*, *Agropyron*, *Elymus*, *Hordeum*, and *Secale*.

Urediniospores ((23–))(25–)26–38(–42)((–51)) × 14.5–20(–22)((–23)) µm; wall 1.5–2.0 µm min. between pores and ends, (2.0–)2.2–2.8(–3.0) µm at ends, echinulae 0.2–0.5(–0.6) µm min. diam. on pore belt, grading to 0.7–1.2(–l.5) µm diam. near ends; germ pores ((3–))4–5(–6)((–7)), equatorial to slightly irregular (especially if 6 or 7), slight or moderate internal ring, cap slight or rarely to 1.0 µm high. Small one-celled teliospores rare, two-celled spores ((32–))34–65(–75)((–81)) × (13–) 14.5–24.5(–26.5)((–28.5)) µm; wall 0.8–2.2 µm min., (5–)6–12 µm at apex of clavate spores or to 15(–17) µm in fusoid spores; pedicel (17–)20–40 µm long on fusoid or 38–83(–90) µm on clavate spores.

3. Stem Rust of Barley, Rye, and Oats

Puccinia graminis Pers. ssp. *graminis* var. *stakmanii* Guyot, Massenot & Saccas ex Urban [*Česka Mykol.* **21**, 14 (1967)].

[*Puccinia graminis* Pers. var. *stakmanii* Guyot, Massenot & Saccas, *Ann. Ec. Nat. Agric. Grignon, Ser. 3* 5, 145 (1946), *nom. subnud.*]

Lectotype: On *Avena sativa* L., France: Les Clayes-sous-Bois, 31 July 1942, A. L. Guyot (Herb. Ecole nat. d'Agriculture de Grignon).

This description is based on many specimens on *Avena sativa*, *Agropyron* (*sensu. lato*), and *Hordeum vulgare*, and fewer on *Avena* spp., *Hordeum* spp., *Secale cereale*, *Dactylis glomerata*, and *Elymus dahuricus.* Urediniospores (20–)22–33(–35) × (13–)14–19(–21) µm; wall (1.2–)1.5–2.0(–2.2) µm min. between pores and ends, 2.0–2.8(– 3.0) µm at ends; echinulae (0.2–)0.3–0.5(–0.6) µm min. diam. on pore belt grading to 0.7–1.3(–1.5)((–1.7)) µm near ends; germ pores (3–)4–5((–6)), ± equatorial, slight (to moderate) internal ring, cap occasionally to 1.0(–1.3) µm high. Small one-celled teliospores rare, two-celled spores (30–)32–69(–77) × 14–24(–25)((–27)) µm; wall (0.8–)1.0–1.8(–2.2) µm min., (3–)4.5–9 µm at apex of clavate or to 13(–15) µm in fusoid spores; pedicel ((10–))15–40(–45) µm long on fusoid or 35–72(–80)((–90)) µm on clavate spores. I find no morphological distinction between specimens on *Avena* and *Hordeum.*

4. Stem Rust of Forage Grasses

Puccinia graminis Pers. ssp. *graminicola* Urban [*Česka Mykol.* **21**, 14 (1967)].

Holotype: On *Dactylis glomerata* L., Bohemia: Vyšenské kopce near Českýy. Krumlov, 13 July 1960, Z. Urban (PRC).

Urban (1967, pp. 14–15) gives an extensive synonymy.

This description is based on specimens on (*a*) *Arrhenatherum elatius*, *Agropyron* spp., *Agrostis* spp., *Brachypodium pinnatum*, and *Dactylis glomerata;* and (*b*) *Lolium perenne*, *Poa* spp., and *Phleum pratense.* In group

(*a*), including eight specimens on the type host, urediniospore echinulae are nearly uniform over the spore surface or vary erratically, but in (*b*) they vary from fine on the pore belt to coarse at the ends as in ssp. *graminis*. Urediniospores (18–)20–29(–31)((–33)) × 13–19(–20) µm; wall 1.2–1.8 µm min. each side of pore belt to 1.7–2.5 µm max. at ends, colors as in species but inner layer often too thin to be distinct; echinulae either 0.3–0.6 µm diam. on pore belt and 0.5–0.7 µm diam. at ends or varying randomly without zonation (group *a*), or (nil–0.2–)0.3–0.6(–0.7) µm diam. on pore belt and grading to 0.6–1.0(–1.3) µm diam. at ends (group b); germ pores (2–)3–4((–5)), equatorial, slight to moderate internal ring, slight cap to 1.0 µm high. Teliospores rarely one-celled, two-celled spores (28–)29–63(–68) × (12–)13–23(–24) µm; wall 0.7–1.8(–2.0) µm min., (3.5–)4.5–12(–13.5) µm at apex; pedicel 16–35 µm long on fusoid and 35–70(–75) µm on clavate spores.

5. *Notes on Host Range*

Mycologists and pathologists are familiar with the phenomenon of heavy infection of a compatible host causing trace infection on an adjacent normally resistant host. Such infections are not self-perpetuating and should not be recorded in host lists without qualifying comments.

In *Puccinia graminis* we see a different situation in which, given adequate inoculum, a "wrong" host may be heavily infected and bear large, freely sporulating sori. In an incomplete survey of *P. graminis* I have seen the following examples.

Agropyron sensu lato mainly harbors ssp. *graminis* var. *stakmanii*, which I have on *A. dasystachyum, junceum, repens, scabrum, smithii, spicatum*, and *trachycaulum* vars., but *A. smithii* and *trachycaulum*accept ssp. *graminis* var. *graminis*, and *A. repens* has been found infected by ssp. *graminicola* near Ottawa, Canada. Specimens on *A. caninum* (Finland) and *A. intermedium* (Moravia) are also ssp. *graminicola*.Thus the genus harbors all three stem rusts, and three species harbor two.

In North America *Hordeum vulgare*, the widespread *H. jubatum*, and the western *H. brachyantherum* normally take ssp. *graminis* var. *stakmanii*, but I have seen six specimens of *H. vulgare* and four of *H. jubatum* freely infected by ssp. *graminis* var. *graminis*. This finding seems to explain the statement by Conners (1967, p. 135) that, when stem rust is heavy on wheat in Manitoba and Saskatchewan, it is also prevalent on barley. Most *H. vulgare* specimens were correctly identified under the stereomicroscope by the large, elongate, and fully naked uredinia resembling those on wheat. This phenomenon may be commonest in major wheat-growing regions. Two European specimens on *H. secalinum* are freely infected by ssp. *graminicola*. Thus *Hordeum*, like *Agropyron*, may accept all three stem rusts.

Elymus usually takes ssp. *graminis* var. *graminis.* Of two specimens on *E. dahuricus,* grown in western Canada, one is var. *graminis* but the second is var. *stakmanii.*

Dactylis glomerata, type host of ssp. *graminicola,* may take ssp. *graminis* var. *stakmanii* freely. All six available specimens are from experimental stations, one in Michigan and five in Canada. I deduce that all the*Dactylis* plantings were close to rusted cereal plantings.

So many aberrant rust associations found in a survey of less than 400 specimens suggest that this phenomenon is relatively common. Although ssp. *graminicola* seems never to attack cereals, we cannot ignore it. When cereal rusts attack normal hosts of ssp. *graminicola,* mixed infections must occasionally occur. Hyphal fusions and nuclear exchanges between genetically distinct mycelia (Savile, 1964) may introduce*graminicola* genes into a cereal rust gene pool. I have seen one specimen that suggests such a hybridization. A collection on *Elymus triticoides* from Morden, Manitoba, has urediniospores in the size range of*graminis* var. *graminis* (26–38 × 15–20 µm), but with (2–)3–4(–5) germ pores as in ssp. *graminicola.*

Burdon *et al.* (1981) have recently indicated that a rust virulent on *Agropyron scabrum* in Australia is a somatic hybrid between *P. graminis* ssp. *graminis* var. *stakmanii* and
var. *graminis,* from *Secale* and*Triticum,* respectively. Their finding confirms what the taxonomic data lead us to expect.

C. LEAF RUSTS

1. General Discussion and Key

The five rusts that concern us are all members of the large *Puccinia recondita* lineage (Savile, 1971, p. 543; 1979, pp. 470, 490). This clearly natural group comprises some 50 grass rusts and various autoecious derivatives on aecial host genera in Liliaceae, Ranunculaceae, and Hydrophyllaceae. The graminicolous rusts are somewhat scattered in Cummins (1971), because he keyed major groups on presence or absence of uredinial paraphyses. (See descriptions in Sections VI and VII.) As shown earlier (Savile, 1976, p. 173), paraphyses have been dropped in some rust groups, probably because they were ineffective against increasingly powerful mycophagous animals. Thus closely related rusts may differ in this character. Some populations of *Puccinia coronata* produce abundant paraphyses, but in *P. coronata* var. *avenae* they are very rare or apparently absent. (A weakly developed paraphysis may resemble a urediniospore pedicel.)

Rusts in this lineage share these characters: urediniospores with several scattered pores; telia sunken and long covered by epidermis, and more or less

divided into locules by fused brown paraphyses and stroma; teliospores with only moderate apical thickening; teliospore pedicels usually less than 20 µm long and light to dark yellow; and teliospores without germ pores.

It would be difficult to key this entire complex morphologically, but the five cereal rusts are consistently separable, although occasional uredinial specimens need careful study. When telia are also present, separation is easier.

The relationship of the other rusts to *P. coronata* is further shown by their teliospores occasionally bearing a few shallow protuberances (hereinafter called "bumps") at the apex, approaching the condition in some *P. coronata* populations.

Because uredinial and telial distinctions show little correlation in these closely related species, separate keys are given here. Workers not very familiar with these rusts may find the uredinial key difficult, and its optimal use demands good phase-contrast optics. Until these researchers gain experience, they should rely largely on telial characters and host identity. The host, as part of the rust's environment, is a valid character, but we cannot put full reliance on it because rusts do occasionally go to "wrong" hosts. A cardinal rule of taxonomy is to avoid undue reliance on single characters.

Key to Uredinia (II)

a. II spore pores invisible to moderately obscure, no or very slight internal ring……*b.*

b. II sori randomly dispersed; spore wall usually with thin brown inner layer; echinulae 0.4–0.6(–0.8) µm diam. and (1.0–)1.3–2.7(–3.0) µm spacing; pores ~7–9(–12); on *Avena* (aecia on *Rhamnus*)……*P. coronata* var.*avenae*

b. II sori usually on pale stripes; spore wall with ± hyaline inner layer; echinulae (0.2–)0.3–0.5(–0.6) µm diam. and (0.8–)1.0–2.3 µm spacing; pores ~7–13(–15); mainly on Triticeae (aecia unknown)……*P. striiformis*

a. II spore pores usually distinct; internal ring slight to moderately strong…….c.

c. II spores generally 21–27 µm wide; on *Secale* (aecia on Boraginaceae). ……*P. recondita*

c. II spores ~18–25 µm wide…….*d.*

d. Echinulae (0.3–)0.5–0.8(–1.0) µm diam.; pores (6–)7–11(–13); pore caps to 1.0 µm high; on *Hordeum* (aecia on *Ornithogalum*)……*P. hordei*

d. Echinulae 0.4–0.6 µm diam.; pores (6–)7–9(–10); no appreciable pore caps, mainly on *Triticum* (aecia on *Thalietrum*)……*P. triticina*

Key to Telia (*III*)

a. Stroma light, locules incomplete. Maximum III spore length (excluding appendages) ~70–80 µm……*b.*

b. III spore apices with usually 2–10 conspicuous digitate appendages ~3–16 µm long; on *Avena*……*P. coronata* var. *avenae*

b. III spore apices with at most 1–4 shallow bumps ~1.0 µm high; on *Secale*……*P. recondita*

a. Stroma heavy, locules usually complete. Maximum III spore length ~60–62 µm. c.

c. III spores ~14–27 µm wide, shape very variable……*d.*

d. III mainly on leaf stripes; one-celled spores only occasional; on *Aegilops, Agropyron, Elymus, Hordeum* spp. (rarely *vulgare*), *Triticum*… P. *striiformis*

d. III scattered; one-celled spores occasionally predominant; on *Hordeum* esp. *vulgare*……*P. hordei*

c. III spores (10–)11–20(–22) µm wide, generally subcylindrical; on *Triticum*……*P. triticina*

2. Oat Crown Rust (Puccinia coronata *var.* avenae)

Oat crown rust, indigenous to Eurasia and mainly on *Avena* spp. and *Rhamnus cathartica*, is now nearly worldwide. In North America, introduced *R. cathartica* is the main aecial host in the East, but *R. alnifolia*can take oat crown rust as well as at least one native rust (of *Calamagrostis*); and *R. cathartica* occasionally takes what seems to be the *Calamagrostis* rust. These rusts have distinct aeciospores: uniformly finely verrucose (type 1) in var. *avenae*, and bizonate (type 2) in the native rust. (Aecia of some other rusts, including the *Agropyron* rust mentioned later, remain to be studied.)

My description of the uredinia and telia is based on many specimens on *Avena sativa* (*sensu* Baum, 1977), and smaller numbers on *A. brevis, fatua, longiglumis, nuda, sterilis,* and *strigosa.* All this material is very uniform. The only aberrant *Avena* specimen seen is one on *A. barbata* from Puerto Varas, Chile, with abundant uredinial paraphyses. (This rust surely spread from a native grass to this adventive species.) Without a full study of *P. coronata sensu lato*, I cannot say to what extent the *Avena* rust occurs on other genera. Urban (1967) showed that var. *avenae* embraces at least two forms: f. sp. *avenae* occurring on *Avena*(Aveneae) and *Lamarkia* (Festuceae), and f. sp. *graminicola* being specific to *Arrhenatherum* (Aveneae). Clearly, host relationships do not correlate fully with host taxonomy.

In artificial inoculation of seedlings, *P. coronata* var. *avenae* infects many festucoid grasses (Eshed and Dinoor, 1981), but probably few except *Avena* are natural hosts on which the rust persists.

Based on cultures from aecia received from eastern Canada, Peturson (1954) described *P. coronata* f. sp. *secalis*. In his cultures it went more freely to *Agropyron* spp. and *Hordeum jubatum* than to *Secale.*Later, we repeatedly found *P. coronata* on *Agropyron repens* in Ontario associated with spent aecia on *Rhamnus cathartica*, a rust clearly introduced from Europe on *A. repens.* I know of no confirmed field occurrence of *P. coronata* on *Secale* in North America. However, an exceptional occurrence in the Soviet Union on winter rye, barley, and wheat, of what was identified as *P. rangiferina*, was reported by Azbukina (1956) and was stated to have originated from *Hordeum* (*Critesion*) *jubatum.* This may have been the same as our *Agropyron* rust. *P. rangiferina*, described from Japan

on *Calamagrostis*, has strongly digitate and long slender spores; its range is uncertain.

Puccinia coronata Corda [(*Icon. Fung.* **1**, 6 (1837)] var. *avenae* Fraser & Led. [*Sci. Agric.* **13**, 322 (1933)]

Puccinia coronata var. *coronata*, on *Calamagrostis* sp. (as *Luzula albida*), has substantially shorter teliospore appendages than var. *avenae*.

Pycnia and aecia on *Rhamnus*. Pycnia mainly adaxial. Aecia mainly abaxial, cupulate, with firm white peridium. Aeciospores 18–25 × (13.5–(14.5–19 µm; wall 0.7–1.2 µm excluding verrucae, hyaline; verrucae ~0.5 µm high, 0.2–0.3 µm diam. and evenly dispersed at ~0.6–0.8 µm between centers (type 1 spores). Uredinia and telia on *Avena*. Uredinia adaxial to amphigenous (or on sheath), most advanced adaxially, small to moderately large, generally long covered by epidermis, light yellow when open; hyaline clavate paraphyses very rare. Urediniospores (20–)22–29(–32) × 17–23(–25) µm, wall 1.0–1.8 µm, hyaline to pale yellow sometimes with thin brownish inner layer; echinulae uniformly 0.4–0.6(–0.8) µm diam. and (1.0–)1.3–2.7(–3.0) µm between centers; germ pores invisible to moderately obscure; apparently 7–9(–12), scattered, no appreciable internal ring or cap. Telia abaxial to amphigenous (or on sheath), largest and most abundant abaxially; usually plumbeous from persistent epidermis, but epidermis may slough off showing black spores duller than in *P. graminis*. Stroma and fused paraphyses usually weakly developed, often yellow rather than orange-brown; paraphyses (35–)40–55(–60) µm long. Two-celled teliospores (excluding appendages) (32–)38–70(–77)((-84)) × 13–23 µm, not (or slightly) constricted, usually subcylindrical to gradually clavate, occasionally abruptly clavate above, rarely ellipsoid, occasionally with l-3(–4) delicate longitudinal ridges; wall 0.6–1.0 µm min. in lower cell and hyaline to pale yellow-brown, 2.0–6.0(–7.0) µm at rounded apex and yellow-brown to light chestnut; apex with (1–)2–10(–11) erect to divergent usually digitate appendages (1–)3–16(–22) µm long, 2–4.5 µm diam. near base and 1.2–3.0 µm above, occasionally branched, concolorous with spore wall below but usually paler above; no germ pores; hilum (deep yellow to) orange-brown; pedicel light to deep yellow, seen intact 13–22 µm long, basal. One-celled spores few (to many), (22–)25–40(–48) × 12–19 µm, appendages few.

3. Barley Leaf Rust (Puccinia hordei)

The widespread *Puccinia hordei* forms small sori. It is best known on cultivated barley, but occurs on several wild species of *Hordeum*, both in Europe and as introduced elsewhere. Aecia are on *Ornithogalum* in Europe, but even there overwintering seems to be mostly by uredinia on winter barley or perennial *Hordeum* spp. I have no record of aecia in eastern North America

where *Ornithogalum* is planted and naturalized. See also *P. striiformis* (Section IV,C,5).

Puccinia hordei Otth [*Mitt. Naturf. Ges. Bern, 1870*, p. 114 (1871)].
[P. *hordei* Fckl. *Jahrb. Nassau. Ver. Naturkd.* **27–28:** 16 (1873)]
[Uromyces hordei Niels. *Ugeskr. Landm. IV 9*(*1*), 567 (1875)].
[*P. anomala* Rostr. in Thuem. *Flora*(*Jena*)**61**, 92 (1878)].

Pycnia and aecia on *Ornithogalum* in Europe. Pycnia amphigenous. Aecia amphigenous, grouped around pycnia. Aeciospores, in one specimen from Öland distr., Sweden (s), 25–29.5 × 21–24.5 µm; wall 1.0–1.5 µm excluding verrucae, hyaline; verrucae ç0.5–0.8 µm high, either 0.2–0.4 µm diam. and crowded, or 0.4–0.8 µm diam. and more widely spaced, larger ones usually predominant, occasionally bizonate but small warts usually in small groups among large ones (± type 2 spores). Uredinia and telia on *Hordeum* spp., especially *H. vulgare.* Uredinia amphigenous but often heavier and earlier adaxially, small, usually soon naked, bright yellow to brownish yellow, aparaphysate. Urediniospores 23–30(–33) × 18–25 µm; wall 1.0–1.7(–2.0) µm, with pale (or medium) yellow outer and thin yellow-brown inner layer; echinulae (0.3–)0.5–0.8(–1.0) µm diam. and (1.0–)1.2–2.2(–2.5) µm between centers; germ pores scattered (6–)7–11(–13), usually distinct, slight to moderate internal ring, small cap rarely to 1.0 µm high. Telia amphigenous but larger and more abundant abaxially, occasionally on sheaths; usually small, firm, plumbeous from persistent epidermis, often raised on blade but flat on sheath. Stroma and fused paraphyses heavy and orange-brown to chestnut; paraphyses 60–70(–80) µm long; stroma above and below spores combines with paraphyses to form complete locules. Two-celled teliospores 33–62 × 16.5–27 µm, slightly constricted, clavate, ellipsoid or irregular, 1–3 delicate ridges common in some collections; wall 1.0–1.8 µm min. in lower cell, (subhyaline to) light yellow-brown; 2.5–8 µm at rounded to truncate apex, yellow-brown to chestnut, if truncate often with few bumps to ~1.0 µm high; no germ pores; hilum deep yellow to orange; pedicel hyaline to pale yellow except deeper yellow at spore, 12–16(–21) µm long, ± basal. One-celled teliospores rare to 95%, (21–)25–49 × 14.5–23 µm.

I am reminded belatedly of the occurrence of *Uromyces turcomanicus* (Cummins, 1971, p. 441) on barley in southwestern Asia. It is clearly one of the more advanced members of the *Puccinia recondita* lineage, with diasporic teliospores and with aecia on *Bellevalia* and *Muscari* (Liliaceae, Scilleae). It has not been available for study. *U. fragilipes* (Cummins, 1971, p. 462), also on *Hordeum* spp., is closely related and with similar range.

4. Rye Leaf Rust (Puccinia recondita)

The name *Puccinia recondita* recently has been applied to various related grass rusts with aecia recorded on such diverse plants as *Impatiens,*

Ornithogalum, a few Boraginaceae and Hydrophyllaceae, and numerous Ranunculaceae. In addition to small but consistent uredinial and telial distinctions, the complex involves three separate aeciospore types: with evenly finely verrucose walls, bizonately finely and coarsely verrucose walls, and bizonate walls with various small plugs in the coarse zone (types 1, 2, and 3 of Savile, 1973). The suppression of these differences amounts to what in Roman law was termed *suppressio veri et suggestio falsi.* Inevitably biological distinctions correlate with those of morphology. Such lumping suggests a spurious biological uniformity. As noted in Section III, oversimplifying a complex sweeps our problems under the rug: Ease in applying a binomial is given at the cost of making it meaningless.

Puccinia recondita seems to be confined to rye, but some other rusts occasionally go to rye. See *P. striiformis* (Section IV,C,5), *P. triticina* (Section IV,C,6, where it and *P. recondita* are contrasted), and note in Section IV,C,2.

Puccinia recondita Rob. ex Desm. [*Bull. Soc. Bot. Fr.* **4**, 798 (1857)] *sensu stricto.* [*P. dispersa* Erikss. &. Henn., Z. *Pflanzenkr. Gallenkd.* **4**,17 (1894), f. sp. *secalis* Erikss. et Henn., *ibid.* p. 259].

Pycnia and aecia on *Anchusa, Lithospermum,* and *Lycopsis* (Boraginaceae) in Europe, apparently not confirmed in North America, although some hosts are widely established. Pycnia amphigenous. Aecia mainly abaxial or caulicolous. Aeciospores 18–24(–25.5) × 16–22) µm; wall 0.6–1.0 µm exluding verrucae. hyaline; verrucae 0.3–0.6 µm high, bizonately ~0.1–0.2(–0.3) µm and 0.3–0.8(–1.0) µm diam. (type 2 spores). Uredinia and telia confirmed only on *Secale cereale,* but possibly some perennial Triticeae serve as reservoir hosts. Uredinia adaxial, amphigenous (or on sheaths), most advanced adaxially, medium sized, from early naked to long covered and spores sifting through slit in epidermis, light or medium yellow-brown, aparaphysate. Urediniospores (22–)25–29(–32)((-35)) × (19–)21–27(–29) µm; wall (0.8–)1.0–1.7 µm, outer layer light yellow, inner light to dark brown; echinulae 0.5–0.8(–1.0) µm diam., 1.3–3.0 µm between centers; germ pores scattered, (6–)7–9(–10), usually clear, (slight to) moderate (to strong) internal ring, cap occasionally to 1.0 µm high. Telia abaxial or on sheath, plumbeous when covered but scanty stroma may allow epidermis to split and peel off (sorus then black); stroma and fused paraphyses usually weak and often yellow rather than orange-brown; paraphyses ~55–75(–90) µm long; incomplete locules allow easy removal of spores. Teliospores typically two-celled, but few one-celled spores in many collections and three-celled ones in a few; two-celled spores (30–)35–78(–85) × (11.5–)13–22(–24) µm, (not to) slightly (to moderately) constricted, generally subcylindrical except few short and wide teratological spores, occasionally with 1–3 delicate longitudinal ridges ç1.0–1.5 µm wide; wall 0.5–0.8(–1.0) µm min. in lower cell, hyaline to pale yellow; 2.5–6.5(–7.5) µm at apex, yellow-brown to chestnut, conical-rounded to truncate (then occasionally with 1–3 shallow bumps to ~ 1.0 µm high); no germ pores; hilum orange; pedicel generally

orange at spore and yellow below, hyaline in mid part if long, rarely to 22 µm long (minimum doubtful because commonly broken).

5. *Stripe Rust of Wheat and Other Triticeae* (Puccinia striiformis)

Although stripe rust usually merits its name, from the sori being confined to chlorotic strips, sori may be scattered on seedling or emergent leaves. Moreover, other leaf rusts may occur on leaves bearing stripes not caused by the rust. Occasional mixed infections are an added complication. *Puccinia striiformis* seems to occur mainly in and near montane regions, where uredinia hibernate readily under heavy snow, or where they may aestivate when lowlands are too dry to support the rust and too hot to keep hosts susceptible, but in mesic Europe it occurs more generally (see Stubbs, Vol. II).

Technically, this rust is *P. striiformis* var. *striiformis,* because Manners (1960) described a small-spored rust of *Dactylis glomerata* as var. *dactylidis,* but it may be a distinct species. The relationships of it and other rusts of Festuceae assigned to *P. striiformis* (Cummins, 1971) need further study.

Although specimens on other grasses, notably *Bromus* (Festuceae), are not convincingly distinct, my description is based on specimens on Triticeae: *Aegilops, Agropyron, Elymordeum, Elymus, Hordeum,* and*Triticum.* An odd population in south-central Alberta has substantial numbers of three- or four-celled spores, with the extra cells often from transverse divisions, so that the spores are three-lobed or square in side view. The specimens are on *Agropyron smithii, A. trachycaulum, Hordeum jubatum,* and *Triticum aestivum;* thus these perennial grasses are effective sources of rust on wheat and barley, which agrees with observations by Baĭmataeva (1980). In North America barley is infected mainly in montane regions.

Puccinia striiformis Westend. *[Bull. R. Acad. Belg., C1. Sci.* **21**, 235 (1854)] var. *striiformis.* [*P. glumarum* Erikss. & Henn., Z. *Pflanzenkr. Gallenkd.* **4**, 197 (1894)].

Pycnia and aecia unknown, probably rarely functional. Uredinia and telia commonly on *Triticum* and other Triticeae. (Genetic relationships of rusts on other grasses are uncertain.) Uredinia small, often crowded, tardily naked, pale to bright yellow when fresh (paling as cytoplasmic pigment fades), occasionally with few thin-walled paraphyses, mainly adaxial, on narrow chlorotic streaks on older leaves but often scattered on young leaves. Urediniospores 26–30(–33) × (16–) 18–24.5(–26.5) µm; wall (0.8–)1.0–1.8 µm, hyaline (to subhyaline), often visibly bilaminate but usually no pigment in inner layer; echinulae (0.2–)0.3–0.5(–0.6) µm diam. and (0.8–)1.0–2.3 µm between centers; germ pores

often obscure, scattered, apparently 7–13(–15), generally very slight internal ring and no appreciable cap. Telia mainly abaxial or on sheaths, covered by persistent epidermis, plumbeous, elongate, with light to moderately heavy orange-brown stroma, orange-brown fused paraphyses ~50–70 µm long generally abundant and dividing sorus into locules. Teliospores occasionally one-celled (28–34 × 11–15.5 µm) or irregularly three- to four-celled, but typically two-celled and 30–60(–65) × (13–)14–27(–30)((-33)) µm, usually slightly constricted, irregularly clavate or fusoid, rarely subcylindrical, often with 1–3 faint longitudinal ridges; wall 0.6–1.0 µm min. in lower cell and subhyaline; 2.5–7.5 µm at apex and yellow-brown, if apex subtruncate often with few bumps to 1.0(–1.5) µm high, but rarely 1–3 sub-digitate appendages to 4.0 µm high; no germ pores; hilum orange-brown; pedicel pale to dark yellow, rarely to 16 µm long.

6. *Wheat Leaf Rust* (Puccinia triticina)

Wheat leaf rust has aecia on *Thalictrum* (Ranunculaceae) in Europe, but it does not ordinarily attack North American species. *Th. orientale* in the mountains of western North America harbors at least one related rust probably mainly from freely associated *Agropyron trachycaulum.* We have two aecial specimens from inoculations made long ago at Winnipeg: One on *Thalictrum glaucum,* definitely from wheat straw spread on the plot, has conspicuously bizonate (type 2) aeciospores and is presumably typical *P. triticina;* the other, on *Th. aquilegiifolium,* lacks detailed information, has irregular rather than fully bizonate spores, and is possibly from a native grass. My description covers the observed extremes. Aecia of rusts on other Ranunculaceae seem to have uniformly type 1 spores.

In material on hand I have found *Puccinia triticina* mainly on *Triticum* but rarely on *Aegilops* and *Secale.* Careful searches may reveal other hosts.

Puccinia triticina Erikss. [*Ann. Sci. Nat., Bot. Biol. Veg.* [8] **9**, 270 (1899)]
[*P. dispersa* Erikss. & Henn., f. sp. *tritici* Erikss. & Henn., Z. *Pflanzenkr. Gallenkd.* **4**, 259 (1894)]
[*Puccinia persistents* Plowr. Monogr. Br. Ured. Ustil. 180. 1889, ssp. *persistens* var. *triticina* (Erikss.) Urban &. Marková, *Česka Mykol.* **31**, 77 (1977)].

Pycnia and aecia on *Thalictrum.* Pycnia adaxial or amphigenous. Aecia mainly abaxial with firm white peridium. Aeciospores 18.5–27 × (12–)13.5–20) µm; wall 0.7–1.2 µm excluding verrucae, hyaline; verrucae ç0.4–0.6 µm high, either mostly 0.2–0.3 µm diam. but occasionally to 0.6 µm, or distinctly bizonate with about half surface having warts 0.5–0.8 µm diam. (±type 2 spores). Uredinia and telia mainly on *Triticum*,occasionally on *Aegilops* and *Secale.* Uredinia adaxial to amphigenous, opening earlier

adaxially, small to moderately large, usually soon naked and light yellow to medium yellow-brown, occasionally in interveinal rows but not on chlorotic strips, aparaphysate. Urediniospores (20–)22–30(–33) × (18–)19–24.5(–26) µm; wall ~1.0–1.5 µm with light yellow outer and thin yellow-brown inner layer; echinulae 0.4–0.6 µm diam. and ((1.0–))(1.3–) 1.5–2.8 µm between centers; germ pores scattered, (6–)7–9(–10), usually distinct, (slight to) moderate (to strong) internal ring, no appreciable cap. Telia amphigenous, abaxial or on sheaths, often moderately large and nearly as wide as long, long-covered, plumbeous, often considerably raised; moderately heavy orange-brown stroma above and below spores, adherent to epidermis and to fused orange-brown paraphyses to 75 µm long, often forming complete locules. Teliospores normally two-celled, (29–)32–60(–62) × (10–)11–20(–22) µm, not (to slightly) constricted, subcylindrical to weakly clavate, often with 1–3 faint longitudinal ridges; wall 0.5–0.8(–1.0) µm min. and hyaline to pale yellow in lower cell; apex 2.5–6 µm, yellow-brown to chestnut, subcorncal, rounded or truncate (and occasionally with few bumps to 1.0 µm high); no germ pores; hilum orange-brown; pedicel orange-brown to pale yellow, rarely to 16 µm long.

Marková (1976) and Marková and Urban (1977), although concurring that it is fully distinct from *Puccinia recondita,* find P. *triticina* to be close to the *P. persistens* complex on Eurasian *Agropyron sensu lato.*It seems to be practically confined to *Triticum,* probably spreading to hexaploid wheat thousands of years ago and now essentially genetically isolated on it (Marková and Urban, 1977). This relationship is one that can be solved only in Eurasia. However, for our purpose and in view of its isolation from the *Agropyron* rusts, it seems preferable to treat *P. triticina* as a species.

To recapitulate: I treat P. *triticina* as specifically distinct from P. *recondita* because it has an unrelated aecial host and a distinct, although related, telial host; it has slightly different aeciospores, appreciably different urediniospores, and markedly different telial sori and teliospores; it shows no intergradation but behaves as, and is, a genetically distinct species. The inexact use of the name P. *recondita* is further aggravating because we see articles in which neither the title nor the abstract state that the disease in question is one of wheat rather than rye. Although *P. triticina* has been widely accepted in Europe, I believe that Conners (1967), with his wide grasp of the European literature, may have been the first to recognize it in North America.

*V. Rusts of Maize (*Zea mays*)*

A. DISCUSSION AND KEY

The three *Zea* rusts are inevitably of New World origin, like their hosts *Zea, Euchlaena,* and *Tripsacum;* and *Physopella zeae* is still only neotropical.

Puccinia sorghi was widespread in Europe by 1906 (Hecke, 1906) and soon became nearly coextensive with maize. *P. polysora* reached Africa during World War II and then spread rapidly (see Hooker, Vol. II).

Puccinia sorghi and *P. polysora* have been freely confused, partly because *P. polysora* was described from *Tripsacum* and was not recognized as a rust of *Zea* until the work of Cummins (1941). Underwood's description of *P. polysora* was inadequate and misleading. The urediniospores were described as scarcely echinulate, although the echinulae are coarser than in *P. sorghi.* Even Arthur (1934), long after the publication of *P. polysora,* gave incomplete urediniospore dimensions and misdescribed the telia as linear. Despite Cummins's work, confusion continues, as shown by specimens on hand. I accordingly give separate uredinial and telial keys to the three rusts and very detailed descriptions of *P. polysora* and *P. sorghi.* The telia are completely different, but telia are scarce in many tropical regions, and only detailed study of urediniospores will allow positive determination of a few atypical specimens (e.g., *P. polysora* with short urediniospores and only four germ pores).

Modern maize is essentially a man-made and man-dispersed species. Whatever its precise origin (still debated), it cannot have been important before domestication. Certainly, the three maize rusts did not evolve de novo in the few thousand years of its cultivation. Significantly, they all occur on *Euchlaena mexicana,* or teosinte, a weedy annual grass that hybridizes freely with *Zea.* This grass was probably a primary host for all three rusts. Until maize became a widespread crop, most infection may have originated on teosinte, but we must remember that *P. polysora* also attacks *Tripsacum* spp. With maize now a major world crop, wild hosts may be less important, but they may yield new biotypes.

Key to Uredinia (*II*)

a. II spores whitish to light yellow in mass; spore wall ± hyaline; germ pores usually invisible……*Physopella zeae*

a. II spores deep golden brown in mass; spore walls pale to deep yellow-brown; pores usually distinct……*b.*

b. II spores usually globoid, 30(–33) µm max. length; pores 3–4((–5)] with distinct internal ring; hilum rugulose……*Puccinia sorghi*

b. II spores usually ellipsoid (35–)37–40(–44) µm max. length; pores (3–)4–6(–7), slight or no internal ring, hilum smooth……*Puccinia polysora*

Key to Telia (*III*)

a. III long covered by epidermis, plumbeous, spore walls 1.5–4 µm at apex……*b.*

b. III often grouped in ellipse around single II, moderately erumpent; spores one-celled, sessile in columns of generally 2–3……*Physopella zeae*

b. III not usually associated with II, composed of several small loosely grouped spore locules, not appreciably erumpent; spores one- to two-celled, with short pedicels……*Puccinia polysoia*

a. III soon naked, strongly erumpent, spores black in mass; spore walls 3.5–8 µm at apex……*Puccinia sorghi*

B. *Puccinia sorghi*

Puccinia sorghi Schw. [*Trans. Am. Philos. Soc.* [2] **4**, 295 (1832)]
[*Puccinia maydis* Béreng. *Atti Sci. Ital.* **6**, 475 (1845)].
[*Puccinia zeae* Béreng. in Klotsch, *Herb. Viv. Mycol., Suppl.* No. 18 (1851)].

Pycnia, aecia on *Oxalis;* pycnia amphigenous, aecia mainly abaxial, cupulate. Aeciospores 17–22.5 × 16–20 µm; wall 0.6–0.8 µm excluding verrucae, hyaline; verrucae ç0.3–0.5 µm high, 0.2–0.5 µm diam., 0.4–0.8 (–1.0) µm between centers, uniform (type 1 spores); germ pores invisible. Uredinia amphigenous to mainly adaxial, long covered by split epidermis, spore mass golden brown. Urediniospores (22–)23–31(–33) × (20–)21–28 µm, globose to short-ellipsoid; wall 1.3–2.3 µm min., with light yellow to medium yellow-brown outer and deep yellow-brown to chestnut inner layer, 2.0–3.3 µm and paler at base; hilum ~5–8 µm diam., always verruculose with verrucae ~0.2–0.3 µm diam. and 0.5–0.7 µm between centers; echinulae to 1.0 µm high, 0.3–0.6 µm diam., 1.0–2.5(–2.8) µm between centers; germ pores 3 or 3–4((–5)), usually nearly equatorial, occasionally irregular or one near apex, (slight to) moderate (to strong) internal ring and usually small cap occasionally 1.0(–1.5) µm high. Telia amphigenous or mainly adaxial, usually linear, strongly erumpent, soon naked and spore mass black. One-celled teliospores none to few; two-celled spores (27–)30–48(–53) × 15–27(–29) µm, (not to) slightly (to moderately) constricted, ellipsoid to obovoid or broadly clavate; wall 1.0–2.3 µ min. in lower cell, 3.0–8.5 µm at apex, smooth or rarely with 1–2 inconspicuous ridges, outer layer medium to deep yellow-brown, thin chestnut middle layer, and thin inmost pale layer (often scarcely distinguishable from cytoplasm); upper germ pore with conspicuous nearly central dimple, lower inconspicuous but apparently at septum; pedicel persistent, 18–95((–120)) µm long, yellow at both ends, but subhyaline in mid part if long, basal to slightly (or moderately) offset.

Arthur and Bisby (1918) showed that the only (thus lectotype) Schweinitz specimen consists of many pieces of *Zea mays* leaves. It was labeled *Puccinia*

sorghi, to which "& Zeae" was later added. Presumably Schweinitz thought at first that he had the rust on *Sorghum*, and later thought that he had it on *Zea* also. Despite the early confusion in host identity, *P. sorghi* was validly published and the name holds, but it never attacks *Sorghum.*

The aeciospores of *Puccinia andropogonis* var. *oxalidis*, also on Ox*alis* spp., are nearly the same size as those of *P. sorghi*, but they are type 2–3 rather than type 1.

C. *Puccinia polysora*

Puccinia polysora Underw. *[Bull. Torrey Bot. Club* **24**, 86 (1897).

Pycnia and aecia unknown. Uredinia amphigenous or adaxial, usually long covered by split epidermis, spore mass deep golden brown. Urediniospores 26–40(–44) × (17–)19–28(–33) µm, usually distinctly ellipsoid; wall 1.0–1.8(–2.2) µm min., slightly more at pores and 2.0–2.7 µm at base, outer layer pale yellow to light yellow-brown, inner thin and deep yellow-brown to chestnut; hilum ~3.5–5.5 µm diam., smooth; echinulae ~0.8–1.5 µm high, 0.6–0.8(–1.0) µm diam., 1.8–3.5(–4.3) µm between centers; germ pores (3–)4–6(–7), nearly equatorial if 4 or 5 but irregular or at least one near apex if 6 or 7, very slight or no internal ring or cap. Telia amphigenous or initially adaxial by growth from uredinia, usually composed of several nearly separate sunken spore locules, plumbeous through persistent epidermis; locules without appreciable bounding tissue or paraphyses. One-celled teliospores occasional to abundant; two-celled spores 30–49(–51) × 18–30 µm, usually moderately constricted, both cells globose if spores uncrowded but usually irregular through pressure in locules, often with 1–4 fine longitudinal to nearly transverse ridges; wall 0.8–1.5 µm min. near pedicel, 1.5–3.5(–4.0) µm max. opposite pedicel (i.e., both sides of septum in diorchidoid spores), usually light chestnut; no germ pores; pedicel delicate, hyaline to yellow on *Zea* (yellow-brown on *Tripsacum*), usually broken in mounts but seen intact 8–38 µm long, basal to strongly offset or at septum (diorchidoid).

Purseglove (1972) stated the aecia to be on *Oxalis*, presumably through confusion with *P. sorghi.* The dark pedicels on *Tripsacum* probably merely indicate a freer source of melanin than in *Zea.* An unusual biotype, with somewhat flattened urediniospores and two pores on each face, is known to me from Cuba, Jamaica, North Borneo, and the Philippines (see Hooker, Vol. II).

D. *Physopella zeae*

Physopella zeae (Mains) Cumm. &. Ramachar [*Mycologia* **50**, 743 (1958)].
[*Angiopsora zeae* Mains, *Mycologia* **30**, 42 (1938)].

Pycnia and aecia unknown. Uredinia mainly adaxial, but sparingly abaxial in some collections, usually small and nearly round but conspicuously elongate on young and vigorous leaves, ringed by steeply erumpent epidermis often with some adherent mycelium, underlain by shallowly cupulate brown stroma, but without peridium, paraphyses, or overlying stroma. Urediniospores whitish or cream in mass (dry), (20–)21–32.5 × (15.5–)16.5–21.5 µm; wall 0.8–2.0 µm, ± hyaline, unilaminate; echinulae ~1.0–1.5 µm high, 0.6–1.0 µm diam., (1.3–(1.8–3.5 µm between centers; germ pores generally invisible, but small flat (or slightly convex) areas on inner wall surface suggest several, possibly 6–8, scattered; pedicels ephemeral and not seen in mature sori, but scrapings from young sori yield thin-walled apical parts of pedicels attached to immature spores. Telia amphigenous, somewhat raised, gray-brown through dry epidermis but purplish brown when wetted, often grouped in ellipse or rectangle round single uredinium. Deep red-brown stroma underlies sorus and curves up slightly at margin to meet epidermis, but does not overtop the spores, although epidermal cells may contain some brown hyphae. Teliospores in free columns of 1–2 spores at margin and 2–3(–4) toward center of sorus, weakly united within columns but separating easily, 17–42(–48) × (9–(10–19 µm, cylindrical to ovoid with ends truncate to tapered; wall 1.0–1.7 µm at sides, 1.5–3.5(–4.5) µm at apex, subhyaline to light yellow-brown especially at apex; germ pores 1–2(–3), usually at or slightly below edge of apical thickening.

The teliospore walls have been described as golden brown to chestnut, and may appear dark in thick sections, but in thin sections and isolated spores they are always pale. The apparent color is mainly due to brown light scattered from the basal stroma. Teliospore pores are best seen in separate spores secured by scarifying a soaked sorus with a scalpel tip, mounting macerated groups, and pressing on the coverslip. Such isolated spores also show that, as spores elongate in the column, their tapering ends occasionally press past each other (as in a welder's scarfed joint). Such mounts simplify obtaining full spore lengths.

Mains (1.c.) described the urediniospores as sessile, before the ephemeral nature of the pedicels of Pucciniastraceae and some Melampsoraceae was widely appreciated. There has probably never been a rust with sessile urediniospores (Savile, 1976, p. 149).

My description is based on specimens on *Zea mays,* including the type (MICH). Cummins (1971) recorded it also on *Euchlaena mexicana* and *E. perennis. Euchlaena* is probably the ancestral host (Section V,A). This rust is commonest in the Caribbean region but known also from Peru.

VI. Rusts of Sorghum *Species*

Puccinia purpurea Cke. *[Grevillae* 5, 15 (1876)].
[*Uredo sorghi* Pass., *Comm. Soc. Critt. Ital.* **2**, 449 (1867), non *P. sorghi* Schw.].
[*Uredo sorghi* Fuckel, *Bot. Z.* **21**, 27 (1871)].
[*P. sanguinea* Diet. ex Atkinson, *Bull. Cornell Univ.* **3**, 19 (1897)].
[Uredo sorghi-halepensis Pat., *Bull. Soc. Mycol. Fr.* **19**, 253 (1903)].
[*P. prunicolor* H. Syd., P. Syd. & Butl., *Ann. Mycol.* 4, 435 (1906)].
[*P. sorghi-halepensis* Speg., *An. Mus. Nac. Buenos Aires* **31**, 386 (1922)].

Sorghum spp. are rich in anthocyanins. As epithets given to this rust indicate, the pigment is freely taken up by walls of paraphyses, urediniospores, and teliospores. I have tried to give the normal colors in my description (see also Section VII).

Pycnia and aecia not definitely known. Uredinia mainly abaxial, occasionally amphigenous or adaxial, rarely light on culms or heavy and adaxial on old loose sheaths; deep brown, pulverulent, ± naked, in pigmented spots. Paraphyses abundant, curved, clavate (to subcapitate), 45–110 µm long, 3–5 µm wide, and thin-walled at base, (11–) 13–22(–25) µm wide at apex with walls (2–)3–8(–10) µm, hyaline to yellowish. Urediniospores (27–)28–41(–44) × 20–30(–31) µm, ellipsoid, obovoid, or rarely globose; wall 1.5–2.0 µm at sides, generally to ç2.5 µm at apex and base, with yellow-brown outer and thin chestnut inner layer; echinulae ~0.8–1.2 µm high, (0.5–)0.6–0.8 µm dram., (1.3–)1.5–2.5(–2.8) µm between centers; germ pores (4–)5–9(–10)((-12)), often ± equatorial if 4–6 but scattered to nearly two-ranked if >6, moderate to strong internal ring and cap rarely to 1.3 µm high. Telia usually displacing uredinia, black, naked, strongly erumpent in age. Paraphyses abundant at least in telia derived from uredinia. One-celled teliospores very rare; two-celled spores (33–)35- 52(–56)((-61)) × (20–)23–32(–34) µm, usually slightly constricted, often both cells rounded and equal in width; wall (2.0–)2.5–3.5 µm min. in lower cell, (3.0–)3.5–6.5(–7.5) µm max. at apex, with deep yellow outer and chestnut inner layers; upper germ pore shown by very shallow nearly central dimple, that of lower apparently at septum but usually obscure; pedicel thin-walled but persistent, hyaline, or yellowish at spore, seen intact (22–)28–125(–138) µm long, basal to slightly (moderately) offset.

LeRoux and Dickson (1957) indicated *Oxalis* to be an aecial host. As Cummins (1971) noted, they gave no morphological details and deposited no specimens. As *Oxalis* spp. in Wisconsin harbor aecia of both P.*sorghi* (Section V,B) and a race of *P. andropogonis*, we cannot, without details, discount the chance of their results being due to contamination.

Puccinia purpurea is apparently confined to both sections of *Sorghum* subgen. *Sorghum: Arundinacea*, nonrhizomatous annuals,

including *S. bicolor sensu lato,* the cultivated sorghums; and *Halepensia,*rhizomatous perennials, including the aggressively weedy *S. halepense,* or Johnson grass. In summarizing measurements I treated specimens on *Arundinacea* (*S. arundinacea* var. *sudanense* and, mainly, *S. bicolor*)separately from those on *Halepensia* (*S. × almum* and *S. halepense*). The ranges of variation in paraphyses; urediniospore size, pore position, and number; and teliospore sizes agree closely in both groups. The one apparent difference is that maximum teliospore pedicel lengths tend to be higher on *S. halepense.* This difference probably reflects the availability of vigorous, fully mature telial specimens on *S. halepense.* On the annual *S. bicolor,* leaf senescence must often stop growth before telia are fully developed. The morphological agreement suggests that many biotypes of the rust attack both *S. bicolor* and *S. halepense,* and that *S. halepense* is a source of inoculum for *S. bicolor.*

Three other rusts are recorded on *Sorghum* alone or with other genera of Andropogoneae (see Cummins, 1971, for details), but involving only other subgenera. *Puccinia jaagii* Boed., described on *S. plumosum*(subgen. *Stiposorghum*)from Java, was assigned by Cummins to synonymy with P. *levis* var. *panici-sanguinalis;* it is quite unrelated to P. *purpurea. P. nakanishikii* Diet., reported on *S. nitidum* (subgen. *Para-sorghum*)and other Andropogoneae, is probably somewhat related to P. *purpurea. Uredo geniculata* Cumm. is known sparingly on S. *nitidum* in the southwestern Pacific. It seems to be a very small-spored relative of *P. purpurea.*

VII. Rusts of Sugarcane

A. DISCUSSION AND KEY

Two rusts are recorded on cultivated cane (*Saccharum officinarum*): *Puccinia kuehnii* and *P. melanocephala,* but at least two others occur on other species of *Saccharum s. str.,* and several more if we include*Erianthus* in *Saccharum* following recent practice. Any rusts of *Erianthus, Imperata, Miscanthus,* and *Sclerostachya* deserve consideration, but space restricts me to *P. miscanthi* and *P. rufipes. S. officinarum* is a complex species, probably in part with *Erianthus* genes, and these other rusts might attack some cultivars. Indeed the main natural host of *P. melanocephala* is *S.* (*Erianthus*) *rufipilum.* The four rusts of*Saccharum s. str. are* separable thus:

a. Paraphyses always rare or absent, head <15 µm diam., thin-walled; teliospores very rare, 10–18 µm wide……*P. kuehnii*

a. Paraphyses usually abundant, head ~10–20(–25) µm diam., thick-walled; teliospores abundant, ~ 16–25 µm diam……*b.*

b. Wall of paraphysis head not occluding over half lumen; teliospore pedicels ~8–22 µm long……c.

b. Wall of paraphysis head often occluding almost all lumen; teliospore pedicels often 30–100 µm……*P. rufipes*

c. Teliospores (27–)29–53(–56) µm long……*P. melanocephala*

c. Teliospores (27–)33–72(–77) µm long……*P. miscanthi*

Walls of paraphyses and spores of these rusts often absorb host anthocyanin. I have tried to give normal wall colors only.

B. *Puccinia kuehnii*

Puccinia kuehnii Butler [*Ann. Mycol.* **12**,82 (1914)].

(*Uredo kuehnii* (Krueger) Wakk. & Went, Die Ziekten van het Suekerviet Java, Lieden, p. 144. 1898).

[*Uredo ravennae* Maire, *Bull. Soc. Nat. Hist. Afr. Nord*, 8,153 (1917)].

Aecia unknown. Uredinia amphigenous on *Saccharum arundinaceum* and *Sclerostachya fusca*, mainly abaxial on *Saccharum officinarum* and *spontaneum*, light to dark yellow-brown. Paraphyses rare or none, thin-walled, cylindrical to clavate, 20–40 µm long, 10–15 µm diam. above. Urediniospores 27–44(–47)((-50)) × (17–)19–27(–28) µm; wall 1.3–2.0 µm on sides, (1.7–)2.2–6.5(–8) µm at apex, outer layer light yellow to light yellow-brown, thin inner yellow-brown to light chestnut; echinulae 0.6–1.0 µm diam., (2.0–)2.3–4.5(–5.0) µm between centers; hilum with conspicuous downwardly pointing rim; germ pores ((3–))4–5(–6)((-7)), equatorial or ± scattered if >5, usually clear in mature spores, slight or no internal ring or cap. Telia very rare, apparently known only from Type (Bassein, Burma, on *S. spontaneum*), arising in uredinia or small separate sori. Teliospores (teste Butler) 25–40 × 10–18 µm, oblong-clavate, apex rounded or truncate, not or slightly constricted; wall pale yellow, scarcely thickened above; pedicel hyaline, very short.

Although, as Butler suggested, the spores may have been immature, after failing to find any teliospores in 13 specimens (5 on *S. spontaneum*), I suspect telia are undergoing elimination. The description of *Uredo ravennae*, on *S.* [*Erianthus*] *ravennae* in the Mediterranean region, fits the description just given, and I regard it as synonymous. Specimens with telia on *S. spontaneum* from near Delhi in PUR and DAOM are *P. rufipes*. This minor cane rust is further separable from P. *melanocephala* by coarser urediniospore echinulae, distinct hilar ring, and frequent apical thickening. It is not known in the New World.

C. *Puccinia melanocephala*

Puccinia melanocephala H. & P. Syd. [Syd. & Butl., *Ann. Mycol*. 5,500 (1907)].
[*Puccinia erianthi* Padw. & Khan, *Imp. Mycol. Inst. Kew, Mycol. Pap*. **10**, 32–33 (1944)].

Aecia unknown. Uredinia and telia abaxial (to amphigenous). Uredinia deep yellow-brown, naked. Paraphyses abundant, 35–80(–90) µm long, 3.5–5 µm diam. at base, wall 0.7–1.5 µm; clavate to capitate apex (9–)11–25.5(–27)((-29)) µm diam., wall 2.5–9 µm max., hyaline to light yellow, not occluding lumen. Urediniospores (25–)27–38(–41)((-46)) × (17–)18–26(–28)((-30)) µm; wall uniformly 1.5–2.0 µm, outer layer light yellow to yellow-brown below or dark yellow-brown above, inner (≃= outer) yellow-brown below to chestnut above; echinulae (0.3–)0.4–0.6(–0.7) µm diam., (1.2–)1.5–2.2(–2.5) µm between centers; hilum without distinct rim, usually bordered by close echnulae; germ pores ((3–))4–5(–6)((-7)), equatorial to slightly superequatorial (± scattered if 6 or 7), moderate to strong internal ring, slight cap rarely to 1.0 µm high. Telia usually abundant and conspicuous on older leaves, blackish brown, moderately pulvinate. Teliospores ((27–))(29–)31–53 (-56)((-58)) × 16–23(–24.5) µm, slightly constricted, clavate (to subcylindrical or irregular); wall 0.7–1.5 µmmin. below and light yellow to light yellow-brown, 2.5–5.5(–6.5) µm at apex and yellow-brown to light chestnut with usually thin deep chestnut inner layer; upper pore shown by shallow nearly central dimple, lower invisible; pedicel orange-brown, often broken short in mounts, seen intact 10–22 µm long, basal (or rarely sublateral on short spores).

The host of *P. melanocephala* was given as *Arundinaria* (Bambusoideae). Consequently, Cummins (1953) accepted the name P. *erianthi.* J. R. Reeder later determined an inflorescence in the type to be*Erianthus* (*Saccharum*) *ravennae* (Cummins, 1971). *P. erianthi* is unquestionably synonymous. My description is based on eight specimens on *S. rufipilum* (India), three on *S. officinaium* (India), and six on *S. officinaium* (neotropical). Summaries from the three groups gave almost identical measurement ranges. Some specimens seemed to differ because they were too sparse for ample sampling. Dr. Daniel Martinéz sent me a large collection from Veracruz, Mexico, from which I made many liberal mounts. The range of measurements from this collection virtually equaled that for the species. Thus the species is inherently rather than geographically variable; the New World outbreaks may have stemmed from one introduction.

D. MINOR *Saccharum* RUSTS

Puccinia rufipes Diet. [*Bot. Jahrb.* **32**, 48 (1902)].
[*Puccinia stichosora* Diet., *Bot. Jahrb.* **37**, 100 (1905)].

Aecia on *Thunbergia* (not seen). Aeciospores stated to be 19–28 × 16–25 µm; wall thin, hyaline, finely verrucose. Uredinia and telia amphigenous. Paraphyses from scarce (? nil) to abundant, 25–62(–67) µm long, delicate stalk 3–5 µm diam. with very thin wall; head usually capitate (8–)10–19(–21) µm diam., wall 2–11 µm max., hyaline to dull yellow, often occluding 80–95% of lumen. Uredinia deep yellow-brown, naked. Typical urediniospores (23–)25–35 × 18–25 µm; wall 1.5–2.5 µm at sides, 1.8–3.0 µm at apex, thin outer layer light to dark yellow-brown (or chestnut at apex), slightly thicker chestnut inner layer occasionally almost black at apex; echinulae 0.4–0.6 µm diam., (1.0–)1.3–2.3 µm between centers; hilum often obscure, without evident rim; germ pores (3–)4–5(–6), ± equatorial, slight to strong internal ring, slight cap <1.0 µm high. Apparently amphisporic urediniospores (scarcely intergrading) 28–40(–43) × 21–29 µm; wall 2.0–2.3 µm at sides, 5.5–10 µm at apex; echinulae 0.5–0.7 µm diam., 1.7–3.3 µm between centers. Telia promptly naked, black, slightly to strongly erumpent. Teliospores 24–41(–46) × (15–(17–25(–28) µm, slightly or not constricted, clavate to ellipsoid (globoid to irregular if pedicel lateral); wall 1.2–2.0(–2.5) µm min. in lower cell, 2.5–5.5 µm at apex, light yellow-brown to light chestnut, usually with thin deep chestnut inner layer, smooth or occasionally faintly reticulate with 0.2 µm diam. bars forming 0.5–0.7 µm diam. meshes; slight ± central dimple under cap and asymmetrically thickened septum indicate central and septal pore positions; pedicel variable, (10–)20–30 µm long in young, (20–)40–122 µm in mature sori, yellow-brown to orange-brown if short but often subhyaline except ends if long, basal to moderately offset (or at septum), fragile and easily broken in making mounts.

My description is from nine specimens on *Imperata cylindrica* vars. (Japan, Okinawa, Taiwan, Philippines, Natal), and two on *Saccharum spontaneum* (India). Amphispores predominated in the Natal collection and were occasional in specimens from Japan and Philippines. Reticulate spores (few to many) in *Imperata* specimens from Japan, Okinawa, and Natal, and one *S. spontaneum* specimen from India (near Delhi) indicate the teliospores to be incipient diaspores (Savile, 1976, p. 160).

Puccinia miscanthi Miura (Fl. Manchuria & E. Mongolia, part 3: 302. 1928).

Aecia on *Plantago* (not seen); aeciospores described as (20–)22–27(–29) × (17–)20–24 µm. Uredinia and telia abaxial on *Miscanthus*, amphigenous on *Saccharum*. Paraphyses scarce to abundant, 33–70(–78) µm long, stalk 3–8 µm diam. with wall 0.5–2.0 µm; apex ± capitate, 10–23 µm diam. with wall 2.5–9 µm, hyaline to brownish yellow, not occluding lumen. Urediniospores 27–36(–38) × 20–27 µm; wall 1.5–2.0 µm (or to 2.5 µm at apex), outer layer yellow or light yellow-brown, inner yellow-brown to chestnut; echinulae 0.5–0.7 µm diam., 1.5–2.3 µm between centers; hilum without distinct rim; germ pores (3–)4–5, equatorial, slight (to strong) internal ring, very slight cap.

Teliospores (27–)33–72(–77) × (15–)17–25(–27) µm, slightly constricted, usually long-clavate; wall 0.7–1.2 µm min. and yellow or pale yellow-brown, 5–9 µm at apex and yellow-brown grading inward to chestnut (often thin dark chestnut inmost layer); upper pore often shown by shallow ± central dimple; pedicel orange-brown, 8–16 µm long, basal. Seen on *Miscanthus japonicus, M. sinensis, Saccharum narenga, S.* sp., eastern Asia.

Other species attacking *Erianthus* (inter alia) are *P. daniloi, P. erianthicola, P. erythropus,* and *P. microspora,* described in Cummins (1971). *P. pugiensis* (unavailable) is perhaps a long-spored variant of *P.rufipes,* which occurs on its host (*S. spontaneum*). *Uredo ravennae* is apparently *P. kuehnii* (q.v.).

Acknowledgments

I thank the curators of MICH, S, and especially PUR for the loan of specimens, and Dr. Daniel Martinéz for a bulk collection of *Puccinia melanocephala.* Dr. B. R. Baum guided me in relationships of *Avena* and Triticeae. Dr. Zdeněk Urban reviewed the manuscript in great detail.

References

Arthur, J. C. (1907–1931). Uredinales. *North Am. Flora* 7(2–13), 83–969.

Arthur, J. C. (1934). "Manual of the Rusts in United States and Canada." Purdue Res. Found., Lafayette, Indiana.

Arthur, J. C., and Bisby, G. R. (1918). An annotated translation of the part of Schweinitz's two papers giving the rusts of North America. *Proc. Am. Philos. Soc.* **57**, 173–292.

Azbukina, Z. M. (1956). Specialization of rust fungi on cultivated cereals of the littoral region in the uredo stage. *Not. Syst. Sect. Crypt. Inst. Bot. Acad. Sci., U.S.S.R.* **11**, 150–161 [Abstract in *Rev. Appl. Mycol.* **36**, 91 (1957)).

Balmataeva, B. K. (1980). Variability of wheat yellow rust and closely related rust populations on wild Gramineae in the Alma-Ata district. *Izv. Akad. Nauk Kaz. SSR, Ser. Biol.* **6**, 73–79 [Abstract in *Rev. Plant Pathol.* **60**, 401 (1981)).

Baum, B. R. (1977). "Oats: Wild and Cultivated. A Monograph of the Genus *Avena* L. (Poaceae)," Monogr. 14, Canada Dept. of Agriculture, Ottawa.

Burdon, J. J., Marshall, D. R., and Knight, N. H. (1981). Isozyme analysis indicates that a virulent cereal rust pathogen is a somatic hybrid. *Nature* (*London*)**293**, 565–566.

Conners, I. L. (1967). "An Annotated Index of Plant Diseases in Canada," Publ. 1251. Research Branch, Canada Dept. of Agriculture, Ottawa.

Cummins, G. B. (1941). Identity and distribution of three rusts of corn. *Phytopathology* **31**, 856–857.

Cummins, G. B. (1953). The species of *Puccinia* parasitic on the Andropogoneae. *Uredineana* **4**, 5–90.

Cummins, G. B. (1971). "The Rust Fungi of Cereals, Grasses and Bamboos." Springer-Verlag, Berlin and New York.
Diehl, W. W. (1944). Bibliography and nomenclature of *Puccinia oryzae. Phytopathology* **34**, 441–442.
Eshed, N., and Dinoor, A. (1981). Genetics of pathogenicity of *Puccinia coronata:* The host range among grasses. *Phytopathology* **71**, 156–163.
Hecke, L. (1906). Infectionsversuche mit *Puccinia maydis* Béreng. *Ann. Mycol.* **4**, 418–420.
Hylander, N., Jørstad, I., and Nannfeldt, J. A. (1953). Enumeratio Uredinearum Scandinavicarum. *Opera Bot.* **1**(1), 1–102.
Jørstad, I. (1958). The genera *Aecidium, Uredo* and *Puccinia* of Persoon. *Blumea* **9**, 1–20.
LeRoux, P. M., and Dickson, J. G. (1957). Physiology, specialization, and genetics of *Puccinia sorghi* on corn and *Puccinia purpurea* on sorghum. *Phytopathology* **47**, 101–107.
Manners, J. G. (1960). *Puccinia striiformis* Westend. var. *dactylidis* var. nov. *Trans. Br. Mycol. Soc.* **43**, 65–68.
Marková, J. (1976). To the knowledge of the brown rust of couch grass in Bohemia and Moravia. 1. *Ceska Mycol.* **30**, 90–105.
Marková, J., and Urban, Z. (1977). To the knowledge of the brown rust of couch grass in Bohemia and Moravia. 2. *Česka Mykol.* **31**, 72–80.
Peturson, B. (1954). The relative prevalence of specialized forms of *Puccinia coronata* that occur on *Rhamnus cathartica* in Canada. *Can. J. Bot.* **32**, 40–47.
Pilger, R. (1940). "Gramineae III in Die Natürlichen Pflanzenfamilien," Vol. 14e. Engelmann, Leipzig.
Purseglove, J. W. (1972). "Tropical Crops, Monocotyledons 1." Longmans, Green, New York.
Savile, D. B. O. (1964). Geographic variation and gene flow in *Puccinia cruciferarum. Mycologia* **56**, 240–248.
Savile, D. B. O. (1965). *Puccinia karelica* and species delimitation in the Uredinales. *Can. J. Bot.* **43**, 231–238.
Savile, D. B. O. (1968). The case against "uredium." *Mycologia* **60**, 459–464.
Savile, D. B. O. (1971). Co-ordinated studies of parasitic fungi and flowering plants. *Nat. Can.* 98, 535–552.
Savile, D. B. O. (1973). Aeciospore types in *Puccinia* and *Uromyces* attacking Cyperaceae, Juncaceae and Poaceae. *Rep. Tottori Mycol. Inst.* **10**, 225–241.
Savile, D. B. O. (1976). Evolution of the rust fungi (Uredinales) as reflected by their ecological problems. *Evol. Biol.* **9**, 137–207.
Savile, D. B. O. (1979). Fungi as aids in higher plant classification. *Bot. Rev.* **45**, 377–503.
Savile, D. B. O. (1981). The supposed rust of *Echinochloa. Mycologia* 73, 1007–1008.
Savile, D. B. O., and Urban, Z. (1982). Ecology and evolution of *Puccinia graminis. Preslia* 54, 97–104.
Tajimi, A. (1975). A phylogenetic study on the host plant of *Puccinia graminis* f. sp. *lolii. Bull. Natl. Grassl. Res. Inst.* 7, 81–98.
Tajimi, A. (1976). On the host plant of timothy rust, *Puccinia graminis* f. sp. *phleipratensis. Bull. Natl. Grassl. Res. Inst.* **9**, 25–40.
Tajimi, A. (1977). On the host plant of stem rust of orchard-grass, *Puccinia graminis* f. sp. *dactylidis. Bull. Natl. Grassl. Res. Inst.* **11**, 106–119.
Tajimi, A. (1978). On the host plant of wheat stem rust, *Puccinia graminis* f. sp. *tritici. Bull. Natl. Grassl. Res. Inst.* 13, 79–84.
Tajimi, A. (1979). On the host plant of stem rust of bluegrass, *Puccinia graminis* f. sp. *poae. Bull. Natl. Grassl. Res. Inst.* 15, 88–102.

Urban, Z. (1963). A new method for observing urediospore germ-pores and its use in the taxonomy of graminicolous rust species. *Česka Mykol.* **17**, 193–194.
Urban, Z. (1967). The taxonomy of some European graminicolous rusts. *Česka Mykol.* **21**, 12–16.

PART II

Specificity

4

The *Formae Speciales*

Y. Anikster
Department of Botany, Tel Aviv University, Tel Aviv, Israel

I. Definition and Historical Background

Within the taxonomic unit that constitutes a botanical species of a pathogenic fungus and that is defined mainly by its morphological characters, we can distinguish subunits that we call *formae speciales* (singular *forma specialis*). These subunits are defined mainly by their fitness to a host species or group of host species. As the criterion for the definition of the *formae speciales,* we examine the differences of the host range at the level of species and above—genera, tribes, or even subfamilies of the hosts.

Within a *forma specialis* can be found "physiological forms." These forms, generally called "races," differ in their ability of attacking varieties of a single species.

Dating back to the end of the previous century, plant pathologists recognized differences in host range of isolates within a species of rust fungi. It was realized that although belonging to one species, various isolates could attack one crop and do no damage to another, and further that various isolates could pass from wild grasses to some cultivated cereals and not to others.

Although a *forma specialis* is named according to the genus it attacks (either the most common one or the one on which it was discovered first), the pathogenicity of the form is not necessarily restricted to the genus, the tribe, or even the subfamily to which this genus belongs (Robinson, 1969, 1976).

The first to use the term *forma specialis*[1] for rust fungi was Eriksson (1894) in Sweden. In a work that encompassed the years 1890–1897, Eriksson defined six *formae speciales* of stem rust—*Puccinia graminis* Pers., five *formae speciales* of *P. glumarum,* and four *formae speciales* of *P. recondita.* He classified the crown rust fungi into two species depending on their ability to produce aecia on various species of *Rhamnus.*

Eriksson noted at this early date that some *formae speciales* may have more than one genus as a host. He found no morphological differences between various *formae speciales* of the same rust species. "We have not succeeded in discovering, … any distinguishing differences in the outer appearance, such as the size, color or distribution of the pustules, the shape and size of the spores, etc. However, there is a difference between them with regard to their inner nature that is of no little practical interest" (Eriksson, 1898).

II. Host Range

Some cereal rusts enjoy a very wide host range that includes dozens of genera and hundreds of species for each species of these rusts. *Puccinia coronata* has, according to Simons (1970), 290 host species belonging to 72 genera. Eshed (1978) and Eshed and Dinoor (1981) found, in the Israeli flora, 11 additional species belonging to nine new genera that may serve as hosts to this fungus. Gäumann (1959) formulated a list of 365 species belonging to 54 genera that may serve as hosts for *P. graminis.* According to Cummins (1971), the number of host genera for this rust species is 70. Gerechter-Amitai (1973), in Israel, expanded the existing number of hosts for this species by an additional 45 species belonging to nine new genera.

In contrast to this situation, there are other cereal rust fungi, such as *Puccinia hordei* (barley leaf rust), *Puccinia sorghi* and *Physopella zeae* (corn rusts), and *Puccinia kuehnii* (sugarcane rust), that have very few hosts, mostly a few species belonging to one or two related genera. However, this difference may be partially artificial, because there is a positive correlation between the intensive work on rusts of major agronomical importance and our

knowledge of their host range. We can expect to find additional *formae speciales* in cereal rusts not yet under intensive research, and by expanding the geographical regions in which the research is taking place.

Some *formae speciales* are of worldwide distribution, such as forms that attack cultivated species (e.g., *P. graminis* f. sp. *tritici* and f. sp. *avenae*). In contrast, some forms have a limited distribution, either because the distribution of the host (or hosts) is limited, as in the case of *Uromyces iranensis* (Viennot-Bourgin, 1969), or for other reasons, as in *P. coronata* f. sp. *secalis* (Peturson, 1954), *P. graminis* f. sp.*festucae granatensidis* (Guyot, 1961).

In analyzing the host range of a *forma specialis,* we must take into account the variability of the host, whether the plants have been naturally infected or artificially inoculated, and the host age.

A. THE VARIABILITY OF THE HOST

Among uncultivated wild species and genera, many of which are cross-pollinators (especially if we compare results from regions all over the world), we have to expect high degrees of variability within each species in many characters, including acceptance of and fitness to the rust organisms. Eshed and Dinoor (1980) found segregation in response of various grass seedlings to infection with pure cultures of *Puccinia coronata* (single urediospore cultures), even when those grasses (from a given species) originated from one plant. They found that *Festuca arundinacea* and *Lolium multiflorum* were susceptible to both *formae speciales* of *P. coronata*—f. sp. *lolii* and f. sp. *festucae.* In contrast, Wilkins *et al.* (1974) based their work on the assumption that each one of these grass species is susceptible only to its *forma specialis* and strictly resistant to the other one. This partially explains the differences in the information we have from different sources on the host range of species and *formae speciales* of various rusts.

B. NATURALLY INFECTED OR ARTIFICIALLY INOCULATED PLANTS

The host range of a *forma specialis* on artificially inoculated plants grown in a greenhouse or growth chamber is much wider than in plants naturally infected in their natural habitat. Even in outdoor nursery experiments, we will generally find a wider host range (compared to naturally infected plants) when we grow, in close proximity, plants of species and genera that never grow in the same region or in the same season in nature. Gerechter-Amitai (1973) found, for *P. graminis* f. sp. *tritici,* 78 species belonging to 34 genera that may be hosts with artificial inoculation. Only 28 species belonging to eight genera served as hosts in nature. For *P. graminis* f. sp. *secalis* he found only 3 species

that served as natural hosts, compared to 39 species that were susceptible to this form in artificial inoculation. *P. coronata* f. sp. *festucae* was found to have a tremendously large host range when artificially inoculated in Israel—75 species belonging to 41 genera. In nature, however, this rust is rare even on *Festuca arundinacea* itself.

C. HOST AGE

There are differences in the response of species and genera to various *formae speciales* depending on their age growth stage, especially when we compare seedling and adult stages. Because of technical limitations, most research has been done on the seedling stage. Only a few works deal with comparisons of the response of seedling and adult stages to rust attack of various *formae speciales.* Eshed and Dinoor (1980), using two isolates of P. *coronata* (f. sp. *alopecuri* and f. sp. *phalaridis*), infected 106 species of grasses in the seedling and the adult stages. They found that for most species, the responses in both stages were the same or nearly the same, but in some host species they found differences. *Avena longiglumis* remained susceptible in the adult stage to f. sp. *alopecuri* but became resistant to the *phalaridis* form, whereas *Poa axilis* remained susceptible to the *phalaridis* form and became resistant to the *alopecuri* form. Of course, these are results for artificial inoculation, and comparisons of adult and seedling stages in natural habitats may give different results. Environmental factors, such as temperature, light intensity, and length of day may be of great importance to the response of various species in various stages to rust attack (Roberts and Koo, 1954).

The host range of various *formae speciales* differs from one geographical region to another and from one *forma specialis* to another.

For example, *P. hordei* f. sp. *bulbosi,* in Israel, has only one species as a host—*Hordeum bulbosum* (Y. Anikster, unpublished). In the same region, *P. graminis* f. sp. *tritici* has 78 host species, and *P. graminis* f. sp. *avenae* has 107 host species. The numbers reported from the United Kingdom, United States, and Canada on the host range of various *formae speciales* of *P. graminis* are much lower (Batts, 1951; Fischer and Levine, 1941; Guyot, 1958, 1961; Massenot, 1961; Sydow, 1904). It is surprising to find so wide a host range for *P. graminis* in Israel, because the alternate host for *P. graminis* (*Berberis* spp.) does not exist in the area, so we would expect a much narrower host range for the various *formae speciales* of *P. graminis.*

In some geographical regions we may find a similarity in the host range of some *formae speciales,* such as those for *P. coronata* f. sp. *lolii* and f. sp. *festucae* in Europe (Brown, 1937; Mühle, 1959). In Israel, Eshed and Dinoor (1981) found an extraordinarily wide host range for some *formae speciales* of *P. coronata* (using for each *forma specialis* a single urediospore

line). They infected, with *P. coronata* f. sp. *festucae,*75 species belonging to 41 genera from five different tribes in the subfamily Festucoideae. *P. coronata* f. sp. *hold* could infect 76 species belonging to 38 genera in five tribes. The narrowest pattern they found was for*P. coronata* f. sp. *arrhenatheri;* this form attached 13 species belonging to 12 genera in two different tribes. Urban (1961), being aware of this situation, writes, "In nature, a physiological form is a product of the definite historical and actual conditions relating to a particular place. Therefore, it is not possible to find two absolutely identical physiological forms with the same host range in different localities."

III. The Alternate Host

Most of the rust fungi that attack cereals belonging to the subfamily Festucoideae are heteroecious (excepting *P. striiformis,* a rust species for which the sexual stage is unknown and may no longer exist). Investigators generally have given taxonomic significance to the alternate host.

Eriksson (1894) described stem rust on *Phleum pratense* as a separate species—*Puccinia phlei-pratensis*—and did not include it as a *forma specialis* belonging to *Puccinia graminis,* because he could not prove its connection to *Berberis* (although it was very close to *P. graminis*). This connection has now been proven, and now it is named *P. graminis* f. sp. *phlei-pratensis* (Guyot, 1961; Wilson and Henderson, 1966). Eriksson also classified the crown rust fungi into two species, depending on their ability to produce aecia on various species of *Rhamnus,* the alternate host for these fungi: *P. coronata,* with five *formae speciales* producing their aecial stage on *Rhamnus frangula,* and *P. coronifera,* with six *formae speciales* that alternate on *Rhamnus cathartica.* It was later shown (Dietz, 1926; Melhus *et al.,* 1922) that there is no justification for this separation, and today all are *formae speciales* of *P. coronata.* In the species *Uromyces hordeastri* (Anikster and Wahl, 1979), the main host is *Hordeum* spp.; the alternate hosts are from the Liliaceae family. Some *formae speciales* of this rust species differ, not in their main host—*Hordeum bulbosum*—but in their alternate hosts. So, *U. hordeastri* f. sp. *bulbosi bellevaliae flexuosae* has its aecial stage on *Bellevalia flexuosa,* and *U. hordeastri* f. sp. *bulbosi scillae autumnalidis* has its aecial stage on *Scilla autumnalis.* A similar situation is found within the complex species of *Puccinia recondita.* Some *formae speciales* of this species differ in their alternate host only. On *Agropyron repens* there exist three *formae speciales* of this species: f. sp. *echii agrophyrina,* f. sp. *agropyrina,* and f. sp. *persistens* (Wilson and Henderson, 1966). In the case of *P. recondita* f. sp. *tritici* we have, according to D'Oliveira and Samborski (1966), two separate and different organisms: one that uses as its alternate host *Anchuza* spp. from

the Boraginaceae, and the commoner one that produces its aecia on *Thalictrum* spp. and other species from the Ranunculaceae. Both rusts are included as one *forma specialis,* f. sp. *tritici.* In *Puccinia hordei,* parallel to the differences in the main host species, we find in different *formae speciales* differences in the host range of the gametophytic stage on *Ornithogalum* spp. (Y. Anikster, unpublished). All naturally infected plants belonging to the species *Ornithogalum montanum,* and *O. lanceolatum* bear pycnia and aecia of the f. sp. *bulbosi* only.

IV. Crossings and Hybrids

A fundamental step in any research on the relations between related organisms is an attempt to cross the organisms and to examine the descendants in the F_1 and F_2 generations.

In rust fungi this may be very difficult, because in many rust species inducing germination of the teliospores (especially the teliospores that have been produced in greenhouses and growth chambers) is very difficult.This barrier prevents systematic crosses, and as a result we have very few works on crosses between *formae speciales,* even on the most important rust fungi, such as *P. graminis* f. sp. *tritici* and *P. graminis* f. sp.*avenae.*

Stakman *et al.* (1930) were the first to cross *formae speciales* of cereal rusts. They succeeded in crossing *P. graminis* f. sp. *tritici* (they proved by selfing that the *tritici* isolate they used was pathogenetically homozygous on wheat) with *P. graminis* f. sp. *agrostidis.* They obtained aecia only by transferring nectar from f. sp. *agrostidis* pycnia, to f. sp. *tritici* pycnia; in the reciprocal transfer they did not get any aecia, exactly the same result as Johnson *et al.* (1932). The F_1 hybrids attacked wheat only, but not the other parent—*Agrostis alba.* On wheat, they obtained eight different races, all of them different from the parent obtained from wheat. Three of these races were new to science. All of the F_1 hybrids showed very low infection types on wheat differentials. Johnson *et al.* (1932), making the same cross, had the same results in the response on wheat, but they succeeded in infecting *A. alba*—which gave a resistant response.

In crossing *P. graminis* f. sp. *tritici* and f. sp. *secalis,* Stakman *et al.* (1930) found that most of the hybrids behaved like the *tritici* parent, few were similar to the rye parent, and two hybrid lines were intermediate between the rye and the wheat forms, giving rise to a highly resistant reaction on rye, a moderately susceptible reaction on barley, and a fully susceptible reaction on wheat.

Johnson (1949) had similar results with the same cross on wheat, rye, and barley. According to Levine and Cotter (1931) and Levine *et al.* (1934), the hybrids of their crosses between *P. graminis* f. sp. *secalis*and f.

sp. *tritici* belong to a new synthetic *forma specialis: P. graminis* f. sp. *hordei.* One of these hybrids attacked barley only; another attacked wheat, barley, and rye. Green (1971), also crossing *P. graminis* f. sp.*tritici* and f. sp. *secalis,* found that both the wheat and rye parents were highly to moderately resistant to the F_1 hybrids. Testing the F_2 population of this hybrid, he found the same pathogenic types as he found in the F_1population and concluded, "The progeny of crosses between wheat stem rust and rye stem rust have less virulence on rye than the rye stem rust parent and less virulence on wheat than on the wheat stem rust parent."

Johnson and Newton (1933) are the only ones to succeed in crossing *P. graminis* f. sp. *tritici* and f. sp. *avenae.* The hybrid inherited from its *tritici* parent the ability to attack some wheats and *Agropyron*(although its virulence on all of the hosts was very low). Johnson, in a later work (1949), made crosses in all possible combinations between the forms *tritici, secalis, agrostidis,* and *poae,* of *P. graminis.* As a rule, he found that the hybrids had a wider host range than each of the parents but that the hybrids were less virulent. The crosses in one direction were sometimes much more successful than in the reciprocal direction. He found also that in some crosses he received a higher percentage of interfertility, as in the pairs *tritici-secalis* and *agrostidis-poae,* than in other pairings. It seems that members of such "pairs" are genetically closer.

Shifman (1958) crossed some rust species belonging to the *Puccinia recondita* group, using as an alternate host *Thalictrum leptopyrum.* He crossed *P. triticina* (which attacks *Agropyron repens* and *Elymus arvensis*)with *P. agropyrina,* and also crossed *P. alternans* with *P. triticina* and P. elymi with *P. agiopyrina.* He concluded that these rust species should be counted as *formae speciales* of *P. persistens.*

Crossing various *formae speciales* of *P. hordei,* Y. Anikster (unpublished data) obtained some hybrids that could slightly infect both parents. Eshed (1978) and N. Eshed and A. Dinoor (unpublished data), in their comprehensive work on *formae speciales* of *P. coronata,* have different results as to the degree of virulence of the hybrid lines. They crossed three forms, *phalaridis, avenae,* and *alopecuri,* in all possible combinations. They grew the hybrids on common hosts,[2] using mainly *Vulpia membranacea* and *Pholiurus incurvus,* so they had the possibility of propagating hybrids that could not attack either parent. In the F_1population they obtained some lines that were more virulent than either of their parents. These were more virulent both in the type of reaction and in the ability of the hybrid to attack more plants of susceptible species than its parents did (if only one plant of a species was attacked by a rust, this species was considered susceptible). Some of these hybrids also had a wider host range than did their parents. They tested 52 progenies of the F_2 generation of the cross f. sp. *avenae* × f. sp. *phalaridis.* All of these lines formed high (susceptible) reaction types on at least one of the parent species.

All of these 52 F_2 lines were very virulent on *Bryza maxima* and *Aegilops bicornis*. Some of the F_2 lines attacked species that neither the parents nor the F_1 lines attacked, such as *Phalaris brachystachis*. They obtained some hybrid lines whose host range was entirely different from their parents' and could be described as new *formae speciales* of *P. coronata.*

V. Common Hosts and Somatic Hybridization

In his pioneering studies, Eriksson (1898) noted that each form of rust species has its host range and that "—rye and barley—can be infected by (with rust from) *Triticum repens, T. caninum* and several other grasses. In the same manner, oats may be infected with black rust from *Dactylis glomerata, Alopecurus pratensis* and several other grasses."

We now know that the host range of many *formae speciales* is much wider than it was imagined by Eriksson, and that in many cases there is overlap of the host range of two or more *formae speciales.* Thus many species of grasses may serve as hosts for more than one form of rust (Batts, 1951; Cagas, 1978; Dietz and Clokey, 1924; Eriksson, 1894; Fischer and Levine, 1941; Guyot and Massenot, 1952; Hassebrauk, 1936, 1962; Simons, 1970; Wilson and Henderson, 1966). Gerechter-Amitai(1973) found in nature 12 species that were hosts to two *formae speciales* of *P. graminis* and two species (of the genus *Lolium*)that were hosts to three *formae speciales* of *P. graminis.* By artificial inoculation, he found 20 species that were susceptible to the four *formae speciales* of *P. graminis* (f. sp. *tritici, secalis, avenae, lolii*)he used for inoculation. Eshed (1978) and Eshed and Dinoor (1981) found in artificial inoculation 11 species that were susceptible to all eight forms of *P. coronata* tested, and 14 more species that were susceptible to seven forms. In such a situation, we could expect to have natural somatic hybridization between related *formae speciales* on some of the common hosts. Nevertheless, it seems that the *forma specialis* is a constant biological unit, and we cannot usually find in nature any form that is a result of somatic hybridization.

In Australia, however (Watson and Luig, 1959, 1962; Luig and Watson, 1972, 1976), we have proof that natural somatic hybridization occurs and repeats itself constantly year after year. It occurs between *P. graminis* f. sp. *tritici* and f. sp. *secalis;* the common host is *Agropyron scabrum.* Watson and Luig (1962) have succeeded in artificial somatic hybridization between the same *formae speciales* on *A. scabrum,* as have Bridgmon and Wilcoxson (1959) with barley as a common host.

It is important to note that both *P. graminis* f. sp. *tritici* and *P. graminis* f. sp. *secalis* are of worldwide distribution and have a common host—the cultivated barley—many of whose varieties are susceptible to both*formae*

speciales, but we do not have any information on hybridization of these forms on the cultivated barleys or on any *Hordeum* species (except on *Hordeum lepurinum* from Australia).

According to Luig and Watson (1972), there is a danger that by somatic hybridization we will obtain a race (or races) of *P. graminis* f. sp. *tritici* that will have the gene (or genes) to attack sources of resistance transferred to wheat from rye or from other grasses.

VI. Morphological Differences between Formae Speciales

As Johnson indicates (1968), morphology, both gross and microscopic, has traditionally been the main criterion of classification. Several workers have attempted to find morphological differences between *formae speciales* of rust species.

Levine (1923) found differences with statistical significance by comparing the mean dimensions of urediospores, teliospores, and aeciospores of five *formae speciales* of *P. graminis* (all had been collected in one small region). Waterhouse (1951) in Australia indicated that spores of *P. graminis* f. sp. *lolii* were small in size, when compared to other *formae speciales.* Batts (1951), in England, indicated that the urediospores of*P. graminis* f. sp. *agrostidis* were small. Peturson (1954) described *P. coronata* f. sp. *secalis,* saying that it is easily distinguished by the dark color of its urediospores.

Guyot *et al.* (1945–1946), Urban (1966a, 1967), and Savile in a comprehensive work (Chapter 3, this volume) suggest a taxonomic classification of the cereal rusts based on morphological characters. This classification may be very good for the samples it is based on, but it may not fit other samples. Thus, for example, a sample of *P. coronata* found by Urban (1966b) in Iraq on *Avena fatua* was described as P.*coronata* f. sp. *avenae,* but the number of germ pores in the urediospores and the existence of paraphyses in the uredia do not fit Savile's taxonomy.

Furthermore, *formae speciales* known to be closely related by crossing experiments, such as P. *graminis* f. sp. *tritici* and f. sp. *secalis* are, according to Savile, of two different varieties (var. *graminis* and var.*stakmanii*), whereas the much less related *forma specialis,* f. sp. *avenae,* is in the same variety.

VII. Evolution

We accept Leppik's (1953, 1967) concept of biogenic radiation, that a rust radiates from an alternate host to many different main hosts.

According to Green (1971), the *formae speciales* of *P. graminis* (and, of course, it can be the same with other rust species) have evolved from a rust species that attacked an alternate host (*Berberis* spp. in the case of *P. graminis*)and certain gramineous hosts, mainly of the subspecies Festucoideae. They appear to have evolved through gene recombination that increased virulence on certain of these gramineous hosts, probably at the expense of virulence on others. Consequently, some hybrids between *formae speciales* could be expected to resemble the ancestral type more closely than the specialized form of today. We agree with Green (1971) and Johnson (1949) that there is no immediate danger as a result of natural (or artificial) crosses between *formae speciales* of a rust species. Such crosses are important, so that we may learn about their genetics, and about relations between *formae speciales,* but they do not have the potential of producing a future "super *forma specialis."*

Of interest is the fact that although the alternate hosts serve as common hosts for various *formae speciales* of cereal rusts, we rarely hear (Massenot, 1961) about natural crossings between *formae speciales,* and it seems that there are some kinds of barriers to prevent this type of crossing.

However, if a hybrid is created, either by sexual or by somatic recombination, it apparently cannot compete with its parents' forms that fit better to most hosts in the surroundings.

VIII. Discussion and Conclusions

The great importance of certain species of cereal rusts affects and directs our research in many disciplines concerning these organisms, and necessitates the establishment of a good and effective system for classifying and identifying the subunits of these species. It is essential because of the many variations to be found within the species.

We have to know whether uredial pustules found on a wild grass are capable of infecting nearby cultivated fields, in which case the wild grass is being used by the rust organism as a host for overwintering or oversummering, and as a source for primary infection, or whether this rust attacks the wild grass (or grasses) only (Anikster and Wahl, 1979; Dinoor, 1967; Gerechter-Amitai, 1973; Guyot, 1958; Guyot *et al.*, 1957; Hassebrauk, 1962; Joshi and Lele, 1964; Malençon, 1961, 1963; Peturson, 1949b; Santiago, 1961; Sibilia, 1952; Skorda, 1962–1963;

Stakman and Piemeisel, 1917; Stakman and Harrar, 1957; Thorpe and Ogilvie, 1961; de Urries, 1962–1963; Vallega, 1947; Waterhouse, 1929; Watson and Luig, 1959).

The attempt to base the taxonomy of the subunits of the cereal rust species on morphological differences (see Savile, Chapter 3, this volume) has not always been successful, or useful for some purposes. Morphological differences may be a sufficient means of identification in one region and sorely lacking in others. The morphological similarity and overlap in the range of dimension of spores belonging to different subunits makes the use of morphology for identification and classification of the subunits of cereal rust species very difficult.

Conversely, the use of *formae speciales* as a major means of classification and identification is limited because of their unclear host range.

Because various investigators of different regions have supplied us with varying information regarding the host range of *formae speciales*, it has been claimed that the host range of the *formae speciales* is not constant and hence not reliable (Hassebrauk, 1962; Urban, 1961). In our opinion the *formae speciales* is a dependable means of classification. The great differences in host range are due mainly to the use of grasses with different genetic backgrounds. It is essential to use an international set of differentials of grass species, whose genetic properties are known. Of course, we could find a variety of host ranges among *forma specialis*samples taken from different places (Eshed and Dinoor, 1980), but these differences notwithstanding, it can be shown that a portion of the host range in every case is a constant for a *forma specialis.* It is this factor that makes identification possible. The basic work that remains to be done in order to clarify the genetic background of the *forma specialis* must consist of inducing teliospore germination, crossing the *formae speciales* of each rust species, and examining the F_1 and F_2 descendants.

This process will enable us to identify the genetic background of the *formae speciales* and the degree of relationship between its various forms.

References

Anikster, Y., and Wahl, I. (1979). Coevolution of the rust fungi on *Gramineae* and *Liliaceae* and their hosts. *Annu. Rev. Phytopathol.* **17**, 367–403.

Batts, C. C. V. (1951). Physiologic specialization of *Puccinia graminis* Pers. in south-east Scotland. *Trans. Br. Mycol. Soc.* **34**, 533–538.

Bridgmon, G. H., and Wilcoxson, R. D. (1959). New races from mixture of urediospores of varieties of *Puccinia graminis. Phytopathology* **49**, 428–429.

Brown, M. R. (1937). Study of crown rust, *Puccinia coronata* Corda. in Great Britain. *Ann. Appl. Biol.* **24**, 504–527.

Cagas, B. (1978). The host range of crown rust. *Ceska Mykol.* **32**, 174–181.

Cummins, G. B. (1971). "The Rust Fungi of Cereals, Grasses and Bamboos." Springer-Verlag, Berlin and New York.

de Urries, M. J. (1962–1963). La plantes del genero *Bromus* y del ciclo de *P. graminis tritici* en Espana. *Boll. Stn. Patol. Rome* [3] *Veg.*, **20**, 157–159.

Dietz, S.M. (1926). The alternate hosts of crown rust *Puccinia coronata* Corda. *J. Agric. Res.* (*Washington, D.C.*) **33**, 953–970.

Dietz, S. M., and Clokey, I. W. (1924). *Achyrodes aureum* (L.) Kuntze, a host for many rusts. *Phytopathology* **14**, 36–37 (abstr.).

Dinoor, A. (1967). The role of cultivated and wild plants in the life cycle of *Puccinia coronata* Corda. var. *Avenae* F. &. L. and in the disease cycle of crown rust in Israel. Ph.D. Thesis, Hebrew University, Jerusalem.

D'Oliveira, B., and Samborski, D. J. (1966). Aecial stage of *Puccinia recondita* on *Ranunculaceae* and *Boraginaceae* in Portugal. *Cereal Rust Conf., 1964* pp. 133–150.

Eriksson, J. (1894). Ueber die Specialisierung des Parasitismus bei den Getreiderostpilzen. *Ber. Dtsch. Bot. Ges.* **12**, 292–331.

Eriksson, J. (1898). A general review of the principal results of Swedish research into grain rust. *Bot. Gaz.* (*Chicago*) **25**, 26–38.

Eshed, N. (1978). The genetical basis of the pathogenicity of *Puccinia coronata* Corda. in Israel. Ph.D. Thesis, Hebrew University, Jerusalem.

Eshed, N., and Dinoor, A. (1980). Genetics of pathogenicity in *Puccinia coronata:* Pathogenic specialization at the host genus level. *Phytopathology* **70**, 1042–1046.

Eshed, N., and Dinoor, A. (1981). Genetics of pathogenicity in *Puccinia coronata:* The host range among grasses. *Phytopathology* **71**, 156–163.

Fischer, G. W., and Levine, M. N. (1941). Summary of the recorded data on the reaction of wild and cultivated grasses to stem rust (*Puccinia graminis*), leaf rust (*Puccinia rubigo-vera*), stripe rust (*Puccinia glumarum*), and crown rust (*Puccinia coronata*)in the United States and Canada. *Plant Dis. Rep., Suppl.* **130**.

Gäumann, E. (1959). Die Rostpilze Mitteleuropas. *Beitr. Kryptogamenflora Schweiz* 12, 7–1407.

Gerechter-Amitai, Z. K. (1973). Stem rust, *Puccinia graminis* Pers. On cultivated and wild grasses in Israel. Ph.D. Thesis, Hebrew University, Jerusalem.

Green, G. J. (1971). Hybridization between *Puccinia graminis tritici* and *Puccinia graminis secalis* and its evolutionary implication. *Can. J. Bot.* **49**, 2089–2095.

Guyot, L. (1958). Rôle des graminées spontanées dans l'épidémiologie de la rouille noire des céréales en Europe et Afrique septentrionalc. Colloq. Eur. Rouille Noire des Céréales, *1st, 1958.*

Guyot, L. (1961). Distribution des formes spéciales de *Puccinia graminis* dans les massifs montagneux de l'Europe du Sud-Ouest et de l'Afrique du Nord. *Coloq. Eur. Roya Negra Cereal. 2nd, 1961.*

Guyot, L., and Massenot, M. (1952). Dix ans d'expérimentation sur la rouille noire des céréales 1941–1950. *Ann. Inst. Natl. Rech. Agron.* 3, 351–391.

Guyot, L., Massenot, M., and Saccas, A. (1945–1946). Considérations morphologiques et biologiques sur l'espèce *Puccinia graminis* Pers. sensu latu. *Ann. Ec. Natl. Agric.*
Grignon 5, 82–146.

Guyot, L., Malençon, G., and Massenot, M. (1957). De l'existence d'un foyer montagnard de rouille noire du blé (*Puccinia graminis tritici*)sur certaines graminées indigénes du Moyen et du Haut Atlas Marocains. C. *R. Seances Acad. Agric. Fr.* **43**, 213–224.

Hassebrauk, K. (1936). Die Ergebnisse der Getreiderostforschung der letzten 10 Jahre. *Forschungsdienst* **2**, 503–581.

Hassebrauk, K. (1962). Uredinales. *In* "Handbuch der Pflanzenkrankheiten" (H. Richter, ed.), Vol. 3. pp. 2–275. Parey, Berlin.

Johnson, T. (19491. Intervarietal crosses in *Puccinia graminis. Can. J. Res., Sect. C* **27**, 45–65.

Johnson, T. (1968). Host specialization as a taxonomic criterion. *In* "The Fungi: an Advanced Treatise" (G. C. Ainsworth and A. S. Sussman, eds.), Vol. 3, pp. 543–556. Academic Press, New York.

Johnson, T., and Newton, M. (1933). Hybridization between *Puccinia graminis tritici* and *Puccinia graminis avenae. World's Grain Exhibition and Conf., Can., Proc.* **2**, 219–223.

Johnson, T., Newton, M., and Brown, A. M. (1932). Hybridization of *Puccinia graminis tritici* with *Puccinia graminis secalis* and *Puccinia graminis agrostidis. Sci. Agric.* (*Washington, D.C.*) **13**, 141–153.

Joshi, L. M., and Lele, V. C. (1964]. Role of *Vulpia myuros* and *Briza minor* in the perpetuation of black rust of oats in the Nilgiri hills. *Indian Phytopathol.* **17**, 245–248.

Leppik, E. (1953). Some viewpoints in the phylogeny of rust fungi. I. Conifer rusts. *Mycologia* **51**, 512–528.
Leppik, E. (1967). Some viewpoints on the phylogeny of rust fungi. IV. Biogenic radiation. *Mycologia* 59, 568–579.
Levine, M. N. (1923). A statistical study of the comparative morphology of biologic forms of *Puccinia graminis. J. Agric. Res.* (*Washington, D.C.*)24, 539–571.
Levine, M. N., and Cotter, R. U. (1931). A synthetic production of *Puccinia graminis hordei* F. and J. *Phytopathology* **21**, 107 (abstr.).
Levine, M. N., Cotter, R. U., and Stakman, E. C. (1934). The production of an apparently new variety of *Puccinia graminis* by hybridization on barberry. *Phytopathology* 24, 13–14 (abstr.).
Luig, N. H., and Watson, I. A. (1972). The role of wild and cultivated grasses in the hybridization of formae speciales of *Puccinia graminis. Aust. J. Biol. Sci.* **25**, 335–342.
Luig, N. H., and Watson, I. A. (1976). Strains of *Puccinia graminis* virulent on wheat plants carrying gene Sr 27 derived from imperial rye. *Phytopathology* 66, 664–666.
Malençon, G. (1961). L'évolution periodique de la rouille noire du blé au Maroc. *Coloq. Eur. Roya Negra Cereal., 2nd.* Ed. S. Salazar. *1961.*
Malençon, G. (1963). Le role de *Berberis hispanica* du Moyen et du Haut Atlas dans le cycle évolutif de la rouille noire du blé au Maroc. *Boll. Stn. Patol. Veg., Rome* **20**[3], 123–132.
Massenot, M. (1961). Expérimentation réalisée au Laboratoire de Botanique de l'Ecole Nationale d'Agriculture de Grignon â partir d'ecidies de *Puccinia graminis* prelevés sur *Berberis vulgaris* en 1959 et 1960. *Coloq. Eur. Roya Negra Cereal. 2nd, 1961.*
Melhus, I. E., Dietz, S. M., and Willey, F. (1922). Alternate hosts and biologic specialization of crown rust in America. *Res. Bull.—Iowa, Agric. Home Econ. Exp. Stn.* **72**, 207–236.
Mühle, E. (1959). Zur Frage des Resistenzverhaltens der Grässer gegenüber bakteriellen und pilzlichen Krankheitserregern. *Biol. Zentralbl.* 78, 622–630.
Peturson, B. (1949a). The occurrence of *Puccinia coronata* on *Rhamnus frangula* in Canada. *Phytopathology* **39**, 142–148.
Peturson, B. (1949b). Epidemiology of rust in western Canada as influenced by the introduction of stem rust resistant varieties. *Sci. Agric.* (*Washington, D.C.*) **29**, 230–236.
Peturson, B. (1954). The relative prevalence of specialized forms of *Puccinia coronata* that occur on *Rhamnus cathartica*, in Canada. *Can J. Bot.* 32, 40–47.
Roberts, B. S., and Koo, F. K. S. (1954). Infection of rye by *Puccinia graminis avenae. Phytopathology* **44**, 504.
Robinson, R. A. (1969). Disease resistance terminology. *Rev. Appl. Mycol.* **48**, 593–606.
Robinson, R. A. (1976). "Plant Pathosystems," Adv. Ser. Agric. Sci., Vol. III. Springer-Verlag, Berlin and New York.
Santiago, J. C. (1961). Epidemiology of the wheat stem rust fungus in Portugal in 1958 and 1960. *Coloq. Euro. Roya Negra Cereal. 2nd, 1961.*
Shifman, J. A. (1958). Methods and results of hybridization of cereal brown rust forms. *Tr. Vses. Nauchno-Issled. Inst. Zashch. Rast.* **10**, 137–152 [Abstract *Rev. Appl. Mycol.* **38**, 198 (1959)].
Sibilia, C. (1952). *In* "Report on the Meeting of Experts on Scientific Problems of Cereal Rusts," UNESCO, Tech. Note 408, pp. 3–8.
Simons, M. D. (1970). Crown rust of oats and grasses. *Am. Phytopathol. Soc. Monogr.* **5** 1–47.
Skorda, E. (1962–1963). Factors influencing stem rust race distribution and persistence in Greece. *Boll. Stn. Patol. Veg., Rome* **20**[3], 143–145.
Stafleu, F. A., Bonner, C. E. B., McVaugh, R., Meikle, R. D., Rollins, R. C., Ross, R., Schopf, J. M., Schulze, G. M., DeVilmorin, R., and Voss, E. G. (1972). "International Code of Botanical Nomenclature." Int. Assoc. Plant Taxon., Utrecht.
Stakman, E. C., and Harrar, S. G. (1957). "Principles of Plant Pathology." Ronald Press, New York.
Stakman, E. C., and Piemeisel, F. S. (1917). Biologic forms of *Puccinia graminis* on cereals and grasses. *J. Agric. Res.* (*Washington, D.C.*) **9**, 429–495.
Stakman, E. C., Levine, M. N., and Cotter, R. U. (1930). Origin of Physiologic forms of *Puccinia graminis* through hybridization and mutation. *Sci. Agric.* (*Ottawa*) **10**, 707–720.
Sydow, P., and H. (1904)."Monographia Uredinearum," Vol.I. Fratres Berntraeger Lipsiae.
Thorpe, I. G., and Ogilvie, L. (1961). Black stem rust of wheat: *Puccinia graminis* var. *tritici* Eriks et Henn. Investigations in England 1955–1960. *Coloq. Eur. Roya Negra Cereal. 2nd, 1961.*

Urban, Z. (1961). Inoculation experiments with the stem rust *Puccinia graminis* in Bohemia (Czechoslovakia). *Bull. Res. Counc. Isr., Sect. D* **10**, 294–301.
Urban, Z. (1966a). On the natural evolutionary groups in the genera *Puccinia* and *Uromyces. Rev. Roum. Biol., Ser. Bot.* **11**, 247–253.
Urban, Z. (1966b). Uredinales collected in Iraq. *Uredineana* **6**, 5–58.
Urban, Z. (1967). The Taxonomy of some European graminicolous rusts. *Ceska Mykol.* 21, 12–16.
Vallega, J. (1947). Reacion de algunas especies espontaneas de *Hordeum* con respecto a las royas que afectan el trigo. *Rev. Invest. Agric.* **1**, 52–62.
Viennot-Bourgin, G. (1969). Mission phytopathologique en Iran en 1968. *Ann. Phytopathol.* **1**(1), 5–36.
Waterhouse, W. L. (1929). Australian rust studies. I. *Proc. Linn. Soc. N.S.W.* 54, 615–680.
Waterhouse, W. L. (1951). Australian rust studies. VIII. *Puccinia graminis lolii*, an undescribed rust of *Lolium* spp. and other grasses in Australia. *Proc. Linn. Soc. N.S.W.* 76, 57–64.
Watson, I. A., and Luig, N. H. (1959). Somatic hybridization between *Puccinia graminis* var. *tritici* and *Puccinia graminis* var. *secalis. Proc. Linn. Soc. N.S.W.* **84**, 207–208.
Watson, I. A., and Luig, N. H. (1962). Selecting for virulence on wheat while inbreeding *Puccinia graminis* var. *secalis. Proc. Linn. Soc. N.S.W.* **87**, 39–44.
Wilkins, P. W., Carr, A. J. H., and Lewis, E. J. (1974). Resistance of *Lolium multifloirum/Festuca arundinacea* hybrids to some diseases of their parent species. *Euphytica* **23**, 315–320.
Wilson, M., and Henderson, D. M. (1966). "British Rust Fungi." Cambridge Univ. Press, London and New York.

[1]Some investigators have used the term *"varietas"* (var.) instead of *"forma specialis."* The International Code of Botanical Nomenclature (Stafleu *et al.*, 1972) permits the use of *"formae speciales"* within species for taxa that are characterized by their adaptation to different hosts and that are characterized "scarcely or not at all from a morphological standpoint." The term *"varietas"* (var.) should be used only when morphological characters can be used to distinguish among the taxa involved.

[2]Acommon host for two or more *formae speciales* is a host species that can be infected by those forms. In many cases a hybrid between two *formae speciales* cannot infect either of the parents, but may infect a common host. This is the only possible way to propagate some F_1 cultures and later on to obtain the F_2 generation. For example, the hybrid of *P. coronata* f. sp. *avenae* and f. sp. *phalaridis* cannot infect either *Avena* or *Phalaris*, but is compatible to *Vulpia membranaceae* and*Pholiuros incurvus.*

5

Race Specificity and Methods of Study

A. P. Roelfs

Cereal Rust Laboratory, Agricultural Research Service, U. S. Department of Agriculture, University of Minnesota, St. Paul, Minnesota

I. Introduction

As pointed out in the previous chapter, within most species of the cereal rust fungi, there are a number of *formae speciales*. These *formae speciales* in turn are composed of many biotypes that differ in several characteristics but primarily in their virulence on host cultivars. A biotype is defined as a population of individuals of the same genotype; thus, theoretically, the progeny of an aeciospore or urediospore would constitute a pathogen biotype. However, current technology permits only the identification of the pathogen phenotype expressed by a limited number of genes. Thus the biotype remains a useful concept but often has been incorrectly applied in practice to different cultures of the same race because they had a similar avirulence/virulence phenotype.

The avirulence/virulence pattern of a culture is determined by inoculating a selected group of host plants of differing genotypes for rust resistance. A group of biotypes with a similar avirulence/virulence pattern on a selected group of host plants is considered a physiologic race. The race thus is a taxon below the *forma specialis* level, which is distinguished by physiologic differences (pathogenic differences in hostpathogen interactions) rather than morphologic differences. The physiologic differences are shown as differing avirulence/virulence patterns when the differential host series is independently inoculated with different cultures. Thus a race could be a single biotype but is more likely to be a group of similar biotypes that can be distinguished from other phenotypes with a reasonable amount of effort and certainty by differences in their virulence patterns on a selected differential series. The avirulence/virulence phenotype is determined from the disease infection types. Therefore, even though two cultures of a single race result in the production of

the same phenotypes (avirulence/virulence pattern), they may not be the same biotype (genotype) even for pathogenicity, as the infection type may be due to either a homozygous or heterozygous pathogen genotype for virulence.

A. DIFFERENTIAL SERIES

A selected group of host lines has been designated a differential host series or set for many cereal rusts. Thus the 12 host cultivars chosen for the race differential series for wheat stem rust (Stakman *et al.*, 1962) have become known as the international, standard, or sometimes as the Stakman differentials. Other differential sets were used by other workers, which necessitates the designation of the differential set used. In some cases, additional differential hosts were included with the international differential series, and they often became known as supplemental differentials. Unfortunately, sometimes this resulted in physiologic races so described, to be considered as subraces (based on less important differences) rather than as subdivisions of a standard race (that were as important as the original divisions) (Stakman *et al.*, 1962) as intended.

B. INFECTION TYPES

The use of the infection type as a measurement of disease had been developed and was used by Stakman and co-workers at Minnesota by 1919 (Hoerner, 1919). The characterizations of the infection types have been described in slightly different ways during the past 65 years. However, those developed by Stakman and co-workers for the wheat stem rust system have been adapted to most of the cereal rusts. A major exception is stripe rust, *P. striiformis*, which results in a systemic infection. The modification of the original system currently in use at the Cereal Rust Laboratory is shown in Table I. Two variations of the mesothetic reaction class (X infection type) have been recognized since 1919. The Y infection type was added for wheat leaf rust by Johnston (1963) and the Z infection type for common corn rust by Van Dyke and Hooker (1969). These infection types have also been observed with *Puccinia graminis* Pers. f. sp. *tritici* on wheat and barley.

Other systems of classifying infection types have been developed but offer little advantage for use in most race specificity studies. Under carefully controlled environmental conditions and inoculum densities, the system of Browder and Young (1975), which considers the size of the sporulating area and lesion independently on a 0 to 9 basis, should be considered. This system is very advantageous in genetic studies, but its precision often is superfluous for race surveys with host lines possessing a “single gene” for disease resistance. Generally the distinction needed in race surveys is between the high-infection

types (e.g., between pathogen genotype *P_* and host *hh* and pathogen type *pp* and hosts *HH* and *hh*) and the low-infection types (e.g., between pathogen genotype *P_* and host *HH*). This distinction is adequate for a host–pathogen system that follows a gene-for-gene relationship. Although some aspects for gene-for-gene theory in relation to race identification are discussed in Section IV, the full impact of gene-for-gene theory on cereal rust studies is beyond the scope of this chapter. However, the gene-for-gene theory proposed by Flor has resulted in a better understanding of the significance of infection types (see Loegering, Chapter 6, this volume). A major change has been that if the high-infection type is 4 (e.g., pathogen genotype *pp* and host *hh*), then any lower infection type indicates a level of resistance.

Table I

Description of Infection Types Used in Physiologic Specialization Studies of the Cereal Rusts at the Cereal Rust Laboratory[a]

Host response (class)[b]	Disease: Infection type[c]		Disease: Symptoms
Immune (Res)	0	low	No uredia or other macroscopic sign of infection
Nearly immune (Res)	;	low	No uredia, but hypersensitive necrotic or chlorotic flecks of varying size present
Very resistant (Res)	1	low	Small uredia surrounded by a necrosis
Moderately resistant (Res)	2	low	Small to medium uredia often surrounded by chlorosis or necrosis; green island may be surrounded by chlorotic or necrotic border
Heterogeneous (Mes)	X	low	Random distribution of variable-sized uredia on single leaf with a pure culture
Heterogeneous (Mes)	Y	low	Ordered distribution of variable-sized uredia, with larger uredia at leaf tip
Heterogeneous (Mes)	Z	low	Ordered distribution of variable-sized uredia, with larger uredia at leaf base
Moderately susceptible (Sus)	3	low	Medium-sized uredia that may be associated with chlorosis or rarely necrosis
Susceptible (Sus)	4	high	Large uredia without chlorosis or necrosis

[a] After Roelfs and McVey (1979); Stakman *et al.* (1962).

[b] Res, Resistant; Mes, mesothetic; Sus, susceptible.

[c] The infection types are often refined by modifying characters as follows: =, uredia at the lower size limit for the infection type; –, uredia somewhat smaller than normal for the infection type; +, uredia somewhat larger than normal for the infection type; + +, uredia at the upper size limit for the infection type; C, more chlorosis than normal for the infection type; and N, more necrosis than normal for the infection type. Discrete infection types on a single leaf when

infected with a single biotype are separated by a comma (e.g., 4,; or 2=,2+ or 1, 3C). A range of variation between infection types is recorded by indicating the range, with the most prevalent infection type listed first (e.g., 23 or; 1C or 31N).

II. Why Study Race Specificity?

This question is answered differently depending on the interest of the respondent. Thus race surveys may vary in operation depending on their established goals. The race concept has been important in enabling the development of useful resistance to the small-grain cereal rusts in North America and Australia. Apparently, the race concept is the most useful in asexually reproducing pathogens that are obligate parasites or function only as parasites. The usefulness of a race concept decreases as frequency of virulence changes increases as a result of sexual or parasexual recombinations, or mutation. Thus experience has shown the race concept is most useful in *P. graminis* and the least so for *P. striiformis* among the wheat rusts.

A. DETECTION OF NEW VIRULENCES

Originally, most race surveys were designed to detect new virulent pathogen phenotypes before they increased to economically important levels (Simons and Michel, 1959). Cultures that resulted in a susceptible host response when previously cultures had produced a mesothetic or resistant host response were considered to be potentially threatening. Such cultures were therefore used to test commercial cultivars and breeding lines. The detection of new virulent phenotypes remains a major goal of most race surveys. However, by using host differential lines with a “single gene” for resistance, it is now possible to detect changes in virulence on a gene for resistance and know if that results in a virulence combination that is capable of overcoming the combinations of resistance in commercially grown cultivars or advanced breeding material. Most new combinations of virulence that we have detected are avirulent on the commercial cultivars and have little economic potential for causing crop losses.

B. SOURCE OF CULTURES

Race surveys historically have been the source of cultures used in testing host lines in genetic and plant breeding studies. The advances made in culture storage techniques (Rowell, chapter 10, this volume) greatly improved the precision of these studies by making it possible to use the same culture over a

period of years without the risk of loss or contamination. This reduced the need to obtain large numbers of cultures for this purpose annually. The race survey, however, remains the source of nearly all new combinations of pathogen virulence. With the development of "single-gene" differential host lines, it is now possible to search for particular pathogen avirulence/virulence phenotypes. These cultures are extremely useful in host genotype postulation. A postulation of the host genotype for specific resistance is possible by infecting the host with a selected group of cultures of known virulence phenotype (McVey and Roelfs, 1975). The most efficient method is to use cultures that are identical except for the pathogenicity of a single host gene pair. These differences exist in a very low frequency in asexually reproducing populations. Cultures that differ in pathogenicity only on a single host gene pair are common in a sexually reproducing population; however, it becomes necessary to use a very large number of differential hosts to avoid missing other differences in pathogenicity on other host genes for resistance that were previously unknown. These undetected host genes could result in incorrect host gene postulation.

C. DISTRIBUTION OF PATHOGEN VIRULENCES

Race surveys provide data for mapping the distribution and frequency of races, thereby providing the necessary information for selection of sources of host resistance, or in establishing a host gene deployment system. This information in the past has not always been as effectively used as was possible, but renewed interest may enhance its value. That certain races of wheat leaf rust were principally present in the same area, year after year, was reported by Chester (1946). Such patterns still exist, and generally the reasons are unclear (Roelfs, 1974). Host resistance, pathogen adaptability, environment differences, and geographic isolation may all be factors affecting the pathogen distribution patterns observed. The leaf rust resistance provided by the resistance gene *(Lr9)* was adequate in Indiana for 10 years before 1982, whereas the same cultivars were seriously rusted in the states bordering the Gulf of Mexico.

D. EPIDEMIOLOGY STUDIES

The race surveys have an important role in determining the source of disease inoculum. The rusts are obligate parasites and have no macroscopic differences among cultures in the field of a given species. So the epidemiologist studying naturally occurring populations is limited to following the disease occurrence. The only suitable marker available for distinguishing between individuals of a given *forma specialis* or species is virulence. Thus in determining spore movement, the marker used is race (see Roelfs' epidemiology chapter in Vol. II).

Although sources of inoculum cannot be determined by race frequency data alone, with adequate sample size some possible sources often can be eliminated (Rowell and Roelfs, 1971) by a comparison of the races present. A new race in an area indicates (1) an input of exogenous inoculum (Luig, 1977), (2) a mutation for virulence or avi-rulence in an existing race (Stakman *et al.*, 1930), (3) sexual or parasexual recombinations (Newton *et al.*, 1930), or (4) the detection of a race previously below the detection threshold. These sources of variation can often be distinguished.

With exogenous inocolum the "new" race should be identical with one in the source area. A mutation for avirulence/virulence should result in a race identical in all but one characteristic with a race previously present. Sexual or parasexual recombination results in a race that varies from existing races by possessing combinations of characters of the putative parents and would likely differ from races in adjacent source areas. However, in the United States differences in virulence are of limited use for wheat stem rust, as a single race makes up 50% of the population, and one oat stem rust race currently makes up nearly 95% of the isolates annually. The use of races to distinguish between sources of inoculum generally provides negative information; that is, the inoculum could not have come from an area, but currently virulence is the only marker adequately studied to use. In recent years, a group of wheat stem rust races of pathogen genotypes that probably are from a series of single mutations (clusters) from a source culture have been studied. The race 15 "cluster" has not been found in southern Texas or Mexico (Roelfs *et al.*, 1978). However, it has been the predominant cluster in the central and northern Great Plains. Thus we have postulated that a source of overwintering inoculum exists outside of Mexico. Over several years these types of data indicate that the principal source of inoculum for the race 15 cluster is northern Texas and southern Oklahoma in most years. The inoculum of the race 29-32, 113, 11, and 151-32 clusters probably originates farther south in Texas and Mexico. The distribution of pathogen virulence can also be compared with the distribution of host genes for resistance. The effects of the alternate host(s) and wild grasses that can be hosts must be considered; however, little is known about their resistance. Host resistance exerts selection pressure on the pathogen, which can result in a shift in the virulence in the pathogen population. This has been documented in the case of oat stem rust (Stewart and Roberts, 1970). For wheat stem rust in the United States, pathogen distribution appears to be controlled more by isolation, or pathogen adaptability and aggressiveness, than by virulence. Another example of the effect of isolation is given in detail by Luig in Vol. II of this treatise.

E. PATHOGEN STABILITY

In early years (1918-1925) the races of wheat stem rust changed rapidly from year to year (Roelfs and Groth, 1980). However, currently there is, for wheat stem rust at least, an underlying stability. The degree of stability is apparent only by studying race survey data over a period of years. Green (1975) explained that most of the variation in race 15 of wheat stem rust in Canada was by single gene changes. Roelfs and Groth (1980) broadened this approach to show that in the Great Plains of the United States, clusters of races (closely related genotypes) existed that differed only by a few gene pairs for virulence/avirulence (0–2 genes) and that between these clusters there were more gene pair differences (4–10), when pathogenicity to 16 host resistance genes was studied. They suggested that the variation within the cluster was similar, but that the differences between clusters increased if the number of host resistance genes was increased to all the designated ones (approximately 40) for wheat stem rust resistance. The within-cluster differences in combinations of virulences were interpreted as evidence for single mutations from an existing genotype within the cluster. The distances between clusters were taken to indicate a lack of sexual or parasexual recombination between members of the different clusters. Examination of a sexually reproducing population showed neither clusters of genotypes nor spaces between groups of genotypes. The combinations of virulence observed closely fitted a Poisson distribution based on random gene association.

Because the wheat stem rust population in Canada and the United States is currently relatively stable, host cultivars with combinations of resistance genes that match the pathogen virulence midway between clusters should have a long period of usefulness. However, little is known about these clusters of virulence. Virulence and avirulence for a few *Sr* genes are found in almost every cluster (i.e., *Sr8),* whereas virulence or avirulence for other *Sr* genes occur only in a single cluster (i.e., *Sr9e, Sr15, Sr30).* The latter combinations of resistance may be of more value in breeding for resistance. Some clusters, such as the race 15 or 113 clusters, have many members that differ by a single gene, whereas other clusters seem to be composed of only a couple of phenotypes (i.e., race 56). Clusters like race 15 and 56 have had a history of being important and resulted in epidemics in the United States and Canada (Stakman and Harrar, 1957), whereas others like race 11, race 32—151, and race 113 never have, although they have occurred during the same years and often apparently have had the necessary genes for virulence. Different genotypes of the race 15 cluster have predominated for the past 20 years in North America, and several have been able to incite at least local epidemics. Possibly certain clusters have accumulated combinations of genes for aggressiveness that remain in the asexual reproducing population, even though some changes have occurred in

virulence patterns. This accumulation of genes for aggressiveness probably has been the result of many mutations and selection for over 30 years. If adaptability is a multigenically inherited character, such genes might accumulate and remain in an asexually reproducing cluster. A mutation for virulence at a locus formerly avi-rulent in the race 15 cluster (this cluster is well adapted) would likely result in an adapted new race, whereas the same mutation for virulence in another cluster that never had indicted an epidemic might be expected to be similar in adaptation to other races in the cluster.

Another use of the historical approach to race studies is the study of shifts in virulence frequencies. In the race 15 cluster in the 1950s a high proportion of the population was virulent on *Sr17;* however, by 1974 this had decreased to a few percent (Roelfs and McVey, 1975), and by 1980, it had increased again to 31% (Roelfs *et al.,* 1982). No reason is apparent for this shift in virulence. In other clusters during this period, virulence on *Sr17* always or never occurred.

F. DISSEMINATION OF INFORMATION

Because cultures of a *forma specialis* of the cereal rusts differ in their capacity to cause disease on cultivars of a host species, the portion of the pathogen population under discussion must be specified. If only a few host genotypes exist it is easy to describe the pathogen phenotypes as virulent or avirulent on a particular host. This can also be done by specifying the virulence/avirulence phenotype of the culture. However, when 10 or more gene pairs are involved, a long and difficult symbol is required for identification. In most of the cereal rust fungi there are relatively large numbers of pathogen phenotypes and host genotypes, making some system of coding groups of similar pathogen phenotypes necessary. These groups of phenotypes are races and are designated by numbers, letters, or a combination of both. After the codes are used for several years they become well known and very useful; however, with the advent of new pathogen phenotypes and cultivars the system nomenclature has to be expanded. These problems and their possible solutions are discussed in Section **IV.**

III. History of Race Specificity

In the second decade of this century, E. C. Stakman at Minnesota started his studies in an effort to control wheat stem rust. The early research at Minnesota involved the testing of Marshall Ward's theory of bridging hosts. This involved studying changes in pathogenicity of cultures of stem rust that were serially passed from a susceptible to a moderately resistant to a highly resistant host.

The bridging-host theory held that through this process virulence would be gained. In 1916, during the course of this study, differences were found in the ability of two cultures of *Puccinia graminis* f. sp. *tritici* to attack two cultivars of wheat (Stakman and Piemeisel, 1917). The two variants were initially designated as strains, then as biological forms, and finally as races. Races subsequently were identified by differences in infection types produced on a set of host cultivars differing in resistance. This was followed by a search for races in many plant pathogens. The earliest reports of races in the cereal rusts are shown in Table II. The 1920s became the decade of the race (Stakman, 1929; Stakman *et al*, 1935).

Most previous taxonomic work had been based on morphological differences; thus it was natural to seek morphological differences between races. Levine (1928) made many measurements on spore width and length; and although differences existed, ranges overlapped between races. Levine concluded that although there was some morphological basis for distinguishing races, they were most adequately identified by their parasitic behavior. Morphological differences certainly would now be unrealistic to use with 343 races described on the international differentials. Hartley and Williams (1971) reported differences in infection structures formed by different races on an artificial medium, but this was not confirmed in our studies (A. Roelfs and L. Martell, unpublished). Burdon *et al.* (1982) have reported different isozymes present in sporelings of different races. Through these differences, evidence was gained to support the proposed evolution of wheat stem rust in Australia (see the chapter by Luig in Vol. II of this treatise). These studies may have far-reaching effects in future studies of evolution and diversity of the cereal rusts. The isozymes represent markers that are inherited but relatively unaffected by the selective influences of host resistances. Many questions concerning origin, genetic interchange, and diversity may be answered in the future. However, at this time, isozyme markers must be considered a new technique with many possible uses. A direct association may not necessarily exist between isozymes and virulence.

Table II

Table II

Initial Studies of Physiologic Specialization in the Cereal Rust Diseases Caused by *Puccinia* spp.

Host	Pathogen (*Puccinia*)	Number of: Host differentials	Number of: Pathogen races	Date published	Author(s)
Triticum spp.	*graminis* f. sp. *tritici*	—	—	1917	Stakman and Piemeisel
Triticum spp.	*graminis* f. sp. *tritici*	12	—	1922	Stakman and Levine
Avena spp.	*coronata* f. sp. *avenae*	2	4	1919	Hoerner
Avena spp.	*graminis* f. sp. *avenae*	3	5	1923	Stakman *et al.*
Triticum spp.	*recondita* f. sp. *tritici*	7	12	1926	Mains and Jackson[a]
Secalis	*recondita* f. sp. *secalis*	1	2	1926	Mains[b]
Hordeum	*hordei*	2	2	1926	Mains
Zea	*sorghi*	3	4	1926	Mains[c]
Triticum	*striiformis*	6	4	1930	Allison and Isenbeck
Secalis	*graminis* f. sp. *secalis*	5	3	1932	Cotter and Levine[b]

[a]Earlier abstracts by Mains and Jackson (1921, 1923).
[b]Earlier abstract by Levine ane Stakman (1923), with three differential hosts.
[c]Also an abstract by Stakman and Christensen (1926).

Infection types are not always a perfect measurement of the host or pathogen genotype (Luig and Rajaram, 1972). Infection types are affected by temperature, light, host nutrition, humidity, infection density, and plant age. Chester (1946) reviewed much of the literature on this subject. Some of the differences in infection types were due to very large variations in experimental methods. In the case of wheat stem rust this variation can be greatly reduced by standardizing experimental conditions. Some host-pathogen interactions are very sensitive to temperature and light, but temperatures of 18° to 22°C and a 12-hour day length with 10,000 lux of fluorescent light were generally adequate even for the most sensitive interactions with wheat stem rust. It has also been noted that the host genetic background affects some *Sr* genes, and some backgrounds result in more stable and recognizable infection types than others (Roelfs and McVey, 1979).

The development of the gene-for-gene theory by Flor was gradually refined by many workers, resulting in the relationship between the host, disease, and pathogen (see Loegering, Chapter 6, Fig. 4, this volume). For the cereal rusts, the definitive phenotypes are usually low-infection types; the nondefinitive are usually high-infection types. As indicated in the figure in Chapter 6, the infection type is a property of the interaction between host and parasite (of the aegricorpus), and a low-infection type (a definitive phenotype) can be used to determine both the host resistance and pathogen virulence phenotypes. The low-infection type occurs only when the pathogen is avirulent *[PP* or *Pp)* with respect to the corresponding host gene pair (*HH or Hh).* With rye, the self-sterility of the host has hampered the use of homozygous host lines.

The gene-for-gene relationship of Fig. 4 in Chapter 6 of this volume can be represented in the more familiar square, Table IIIA. For the purposes of discussion, the low- and high-infection types were indicated by their numerical value. Although these infection types are not actual data, they represent general experience. The possible hostpathogen combinations are represented in Table IIIA in a gene-for-gene system involving the interaction of one host and one

pathogen gene. These types of data are typical in genetic studies when crossing is done with both the pathogen and host. In the small number of cases studied, incomplete dominance exists with both heterozygous host and pathogen genotypes (Loegering, Chapter 6, this volume). In studying pathogen races, only homozygous host genotypes are usually used, reducing the combinations as shown in Table IIIB. The race is often based on differences between the high- and low-infection types, and no distinction is normally made between the two low-, and four high-infection types.

Table III

Theoretical Scheme Showing Infection Types Resulting from Gene-for-Gene Host–Pathogen Relationships[a,b]

A

Host	Pathogen *PP*	*Pp*	*pp*
HH	0	1	4
Hh	1	1+	4
hh	4	4	4

B

Host	Pathogen *PP*	*Pp*	*pp*
HH	0	1	4
hh	4	4	4

C

Host	Pathogen *PPQQ*	*PPqq*	*ppQQ*	*ppqq*
HHTT	0	0	2	4
HHtt	0	0	4	4
hhTT	2	4	2	4
hhtt	4	4	4	4

D

Host	Pathogen *PPQQ*	*PPQq*	*PPqq*	*PpQQ*	*PpQq*	*Ppqq*	*ppQQ*	*ppQq*	*ppqq*
HHTT	0	0	0	1	1	1	2	2+	4
HHtt	0	0	0	1	1	1	4	4	4
hhTT	2	2+	4	2	2+	4	2	2+	4
hhtt	4	4	4	4	4	4	4	4	

[a]Low-infection types are based on limited data; some variation may also occur in the high-infection types.

[b]Resistance (*H* and *T*) and avirulence (*P* and *Q*) are shown as dominant characters, which most frequently is the case. Part **A** of table shows the interaction between single host–gene pairs. **B**, as in **A**, except heterozygous gene pair in host is omitted. **C**, interaction between two host–pathogen gene pairs, heterozygous gene pairs omitted, demonstrating effect of epistasis. **D**, as in **C**, except heterozygous gene pairs in the pathogen are included, demonstrating effects of incomplete dominance.

Most commercial host cultivars possess several genes for rust resistance, and Table IIIC is a theoretical representation of a two-gene system of a gene-for-gene relationship, with the five possible heterozygous host and five heterozygous pathogen genotypes omitted. In this example, infection type 0 results when the *HHPP* hostpathogen genotype is expressed, and infection type 2 results when the *TTQQ* genotype is expressed. In the presence of both *HHPP* and *TTQQ*, the lower of the two infection types (infection type 0), is expressed. The gene pair that results in the lowest infection type is generally expressed in cereal rusts, although exceptions may occur (Loegering, Chapter 6, this volume). The dominance of a gene pair over a nonallelic gene pair is termed *epistasis.*

The combined effect of incomplete dominance and epistasis results in a wide range of low-infection types, especially as in the case illustrated in Table HID when the two corresponding hostpathogen gene pairs result in infection types that are considerably different. This range of low-infection types frequently is seen with a differential host possessing two resistance genes that are both ineffective against a portion of the pathogen population. Marquis, one of the original standard differential cultivars for wheat stem rust, has five genes for resistance to stem rust (Roelfs and McVey, 1979). Assuming Marquis was homozygous for resistance at the five loci and all possible pathogen genotypes exist, then 242 different host—pathogen gene pairs could result in a low-infection type. Such complex host resistance would result in almost continuous variation from the lowest to the highest low-infection type, making it impossible to classify them accurately in the trichotomous key Stakman used for the standard differentials. Distinction of the same cultures for avirulence/virulence (P _/pp) on the five "single-gene" host lines for these *Sr* genes is not a major problem.

A race classification based on adult rather than seedling host response has been proposed several times. Although such a classification was not done on a large scale, it may have some value in field studies and with those resistances expressed only in adult plants (see chapter by Zadoks in Vol. II of this treatise). It would seem to be limited by many of the same factors limiting seedling evaluation. Additionally, if adult plant evaluation were used in the field—where it would be most useful—temperature, race mixtures, and inoculum density would be difficult to control. Currently with wheat stem rust, only *Sr2* is a single-gene adult plant resistance that cannot be adequately detected in seedling plants. For wheat leaf rust, host genes *Lrl2*, *Lrl3*, *Lr22a*, and *Lr22b* condition adult plant resistances, as do *Pg11* and *Pgl2* for oat stem rust. Currently, information is incomplete on the effect of plant growth stage, plant age, inoculum density (Roelfs *et al.*, 1972), temperature, light, and perhaps host nutrition on the response of adult plants to the rust. The host response with *Sr2* is not only in size of lesion (infection type) but also the location of lesions and number of lesions (Sunderwirth and Roelfs, 1980). The

latent period (period between inoculation and sporulation) for *P. graminis* f. sp. *tritici* and P. *hordei* is about 7 days for seedlings maintained at 18°C, but it is twice as long for plants inoculated after heading for both wheat stem rust (Sunderwirth and Roelfs, 1980) and barley leaf rust (Andres, 1982). Perhaps this increased length of latent period will be another factor to evaluate in adult plant responses. The disadvantages of using adult plants in the glasshouse as differentials as opposed to seedlings are the need for more space (at least 10 times), a longer time (at least 5 times), more inoculum, and the resulting problem in monitoring and maintaining plants free of other diseases and insects for a longer time.

Bjorkmann (1960) and several others have proposed using detached leaves for identification of races of the cereal rusts. This technique allows a great reduction in space required for growing infected differential series. Disadvantages have been some variation in infection types resulting from the detached leaf culture, and the unique facilities required.

The development of methodology and techniques to handle large numbers of both host and pathogen genotypes has greatly improved race specificity studies. These advances include use of special equipment for planting and inoculating (Browder, 1971), and use of cyclone separators, long-term spore storage, and oil as a spore carrier (reviewed in detail by Rowell, Chapter 10, this volume). These techniques at the Cereal Rust Laboratory permit two people to do all the activities associated with 20 new collections, making 60 single uredia isolates, and taking notes on 60 differential series daily. Thus it is possible to determine the races in a collection within 40 days after it arrives at the laboratory.

IV. Race Nomenclature

Initially, most of the cereal rusts were grouped into races based on an internationally used set of differential hosts. Because of local differences in pathogen virulence and host resistances, investigators gradually adopated local sets of differential hosts that better reflected their needs. Although this change was important in making local progress, it gradually reduced the possibility of international communication. In an effort to improve the international understanding of the diversity and evolution of pathogen phenotypes, the members of the First International Congress of Plant Pathology established a worldwide survey of pathogen virulence. This survey again demonstrated the advantage of international communications. The current use of hosts with "single-gene" resistance could allow international exchange of data and make possible meaningful comparisons of pathogen populations. In fact, exchanges among Australian, Canadian, and United States scientists have already

facilitated our understanding of *P. graminis.* This exchange of data has occurred despite the use of different types of race nomenclature and some different differential hosts. These successes certainly would increase if even a basic set could be evaluated worldwide annually.

A. HISTORY

The debate on the type of nomenclature to use for identifying groups of rust biotypes has been continuous since Waterhouse (1929) found that the cultivar Thew (now known to possess *Lr20)* would subdivide the international races of wheat leaf rust. The original systems were all closed systems; that is, there was no provision for adding additional differential hosts to the series. The problem of variation with international races was solved in many ways. In wheat stem rust, supplemental differential cultivars were chosen but without international acceptance. In crown rust, new sets of differential host cultivars were chosen (Fleischmann and Baker, 1971; Simons and Murphy, 1955). Wheat leaf rust races were reduced in number by Basile (1957) by establishment of the unified numeration (UN) scheme, which eliminated the three differentials that were the most sensitive to changes in environmental conditions. This system was later modified further in North America by the use of a system of supplemental differentials (Loegering *et al.,* 1959, 1961; Young and Browder, 1965). There has been a need to add differential hosts as new resistances are found and used or as the pathogen population gains virulence on previously "universally" resistant host genotypes. Previously differential hosts that become universally susceptible or nearly so to the evolving pathogen population may be advantageously omitted. Thus is is probably unrealistic to assume that race surveys can use an international differential set for more than 10 years. However, continual changing of differential sets also leads to confusion and restricts communication as well as historical points of reference. In oat stem rust, three differential sets have been used since 1970 in the United States (a modification of Stakman *et al.,* 1923; Stewart and Roberts, 1970; Martens *et al.,* 1979).

B. RACE NOMENCLATURE SYSTEMS

No agreement exists on ways to name races or not to name them; however, in general they can be classified into a small number of similar systems. Some workers prefer only a listing of selected virulent and avirulent combinations (Browder *et al.,* 1980). However, as more genes are included the listing becomes longer and longer, or else much information is omitted that may be valuable in understanding pathogen populations. Race nomenclature is merely a means to simplify communication of information. Long designations are

expensive to publish and difficult to communicate accurately, and they may be understood only by those who use them regularly. Some of the systems of race nomenclature in current use are shown in Table IV. Most of the current systems are based on differential series with host lines having a single known gene for disease resistance. The comparisons are based on a low- and high-infection type per host-pathogen gene pair. For comparison purposes, the system used by Stakman and Levine (1922) is included, and because this system had 12 differential hosts, all the values were calculated on that basis. Most of the early systems (Table II) had two classes of low and one class of high-infection type, and thus were trichotomous. Infection types from 0 (immune) to 2 were classified as resistance host response, and the X infection type was classified as a mesothetic host response. Infection types 3 and 4 indicated that the host had a susceptible response. The use of a trichotomous key with 12 host differentials would result in 531,411 instead of the 4096 races obtained with a dichotomous or high-low system.

C. OPEN-ENDED SYSTEMS

The original systems for race identification were all closed; that is, no provision was made for the inclusion of new differential hosts. An ideal system of race nomenclature would consist of a short code, easily obtained, and open-ended so additional differential hosts could be added without greatly changing the nomenclature. In order to obtain a usable system, compromises normally are made. Because the coding in the modified Potato–*Phytophthora infestans* system only indicates the susceptible hosts, it is impossible to determine what differential hosts were evaluated (Table V). Thus the code is the same when a host is resistant or not evaluated. For example, in Table V, culture 96, races 1, 2, 3, 4, 8, and 9 indicate that hosts 1, 2, 3, 4, 8, and 9 were susceptible, and that if tested, hosts 5, 6, and 7 were resistant. Further, it is impossible without a list of differential hosts to know if hosts 10 through 12 were tested, were resistant, or were some combination of the two (Table V). In articles about physiologic race surveys a host list is usu-sally provided that eliminates the confusion; however, in articles lacking a list there is no way to tell which differentials were resistant or not tested. Some systems have solved this problem by indicating a year with the race or by designating the differential host set by a code, making it possible to find the pathogen phenotype. Of the open-ended systems examined, the binomial (Fleischmann and Baker, 1971), decanary (Habgood, 1970), and octal notation (Gilmour, 1973) seem to offer the most advantages; however, all may be too complicated for those who work with the system only occasionally. Addition of a new host differential that is susceptible can change the race designation considerably; that is, for the decanary and binomial systems, races 0 and 1 (host R) become 2 and 3 (hosts

S,R), and likewise races 3 and 4 (hosts S,S) change to races 7 and 8 (hosts S,S,S), respectively. In the octal notation race 0 becomes 2 and race 3 becomes 7; however, adding an additional differential that is susceptible retains the last digit thus, hosts S,S,S,S is race 17 and hosts S,S,S,S,S, is race 37.

Table IV

Comparison of Race Classification Systems Assuming a Low- and a High-Infection Type for Each of 12 Host–Pathogen Pairs Using Hosts with "Single-Gene" Resistance (Total of 4096 Pathogen Races)

Differential system[a]	Type of system	Type of key	Race assignment	Unique nomenclature combinations	Mean no. of characters/code[b]
Stakman	Closed	Trichotomous	Chronologic	531,411[c]	5.8
Dichotomous	Closed	Dichotomous	Chronologic	4096	3.7
Virulence formula	Open	None	None	4096	27.0[d]
Coded virulence formula	Semiopen[e]	Virulence list	Chronologic	4096	3.7
Modified potato–*Phytophora infestans*	Open	Host list	Preassigned	4096	12.2[f]
Binomial and decanary	Open	Mathematical	Preassigned	4096	3.7
Octal notation	Open	Mathematical	Preassigned	4096	3.8
Coded sets	Semiopen[g]	Dichotomous	Preassigned	$16^{[h]}$	3.0

[a]References: Stakman *et al.* (1962), Loegering *et al.* (1959), Samborski (1968), Green (1965), Watson and Luig (1961), Fleischmann and Baker (1971), Habgood (1970), Gilmour (1973), and Roelfs and McVey (1972).
[b]Assuming all races are found, the number of digits or letters that describes the pathogen phenotype.
[c]Based on 3 host reaction classes for 12 hosts (3^{12}).
[d]A character code per pathogen gene separated by a space, comma, or slash.
[e]Addition of differential host requires new code or retesting of type cultures.
[f]Susceptible hosts are listed separated by a space.
[g]Sets of differential hosts can be added; less than a set require a partial virulence formula.
[h]Based on 3 sets of 4 host differentials, 16 combinations per set; thus 16^3, or 4096 phenotypes, are described.

D. RACE KEYS

Most of the systems, except for the virulence formula and modified *potato–Phytophthora infestans* system, require some sort of key or mathematical device for assigning race codes. The modified po*tato–Phytophthora infestans* system, however, requires a listing of the hosts (see also wheat and rye stem rusts chapter by Roelfs in Vol. II). The easiest and quickest systems for assigning races are the simple mathematical and short dichotomous keys. Ideally, the key should be simple enough for the daily user to learn and for others to use within a few minutes. The race codes can be assigned in many ways, but a preassigned number (the race designation is determined before the race is actually found) eliminates a delay in communication until a new key is issued, a major fault of chronological keys. With 12 differential hosts and a dichotomous system, the number of possible unique combinations becomes large—4096 (2^{12}, a number of alternatives to the power of the number of differential hosts).

Table V

Examples of Race Designations Using the Various Systems of Race Nomenclature[a]

Culture	Response on differential hosts											
	1	2	3	4	5	6	7	8	9	10	11	12
1	S	S	S	S	S	S	S	S	S	S	R	R
40	S	S	S	S	S	S	S	S	S	S	S	R
63	S	S	R	S	S	S	S	S	S	S	R	R
75	S	S	R	S	X	X	X	S	S	S	R	R
96	S	S	S	S	R	R	R	S	S	R	R	R

Type of system	Race designation for culture				
	1	40	63	75	96
Stakman	11	15	17	29	56
Dichotomous	11	15	17	17	56
Virulence formula	1,2,3,4,5,6,7, 8,9,10/11,12	1,2,3,4,5,6,7, 8,9,10,11/12	1,2,4,5,6,7,8,9, 10/3,11,12	1,2,4,8,9,10/ 3,5,6,7,11,12	1,2,3,4,8,9/ 5,6,7,10,11,12
Coded virulence formula	C12	C-9	C-1	C-1	C-17
Modified potato–*Phytophthora infestans*	1,2,3,4,5, 6,7,8,9,10	1,2,3,4,5,6, 7,8,9,10,11	1,2,4,5,6, 7,8,9,10	1,2,4,8,9,10	1,2,3,4,8,9
Bionomial	4093	4095	3581	3357	3865
Decanary	4092	4094	3580	3356	3864
Octal notation	7774	7776	6774	6434	7430
Coded sets	TTQ	TTS	RTQ	RCQ	TCL

[a]Hypothetical data were chosen to demonstrate differences between systems.

A coding system shorter than a virulence formula can be developed by placing the sets of differentials in subsets (e.g., set of 4) and then using a repeating system for additional sets (Roelfs *et al.*, 1982). For each set of 4 differential hosts, there are 16 unique combinations (2^4) of high- and low-infection types. These 16 combinations were arranged in a dichotomous system from all low- to all high-infection types. Each combination was assigned an English letter code in alphabetic order using consonants only (B through T). Thus the combination of 4 low-infection types on the first subset of differentials is coded B, and of 4 low-infection types on the second set of differentials is also coded B. Thus the 4096 unique pathogen phenotypes in a 12-differential system can be divided into 3 sets of 4 differentials each and be described by means of 16 codes repeated three times. The biggest disadvantage of the system is that it is only semi-open-ended. Its utility is the shortness of the race notation—which in the studied systems varied from 3.0 in the coded sets to 24.0 with a virulence/avirulence formula (Tables IV and V)—and its ease in coding and decoding (see also Roelfs' chapter on wheat and rye stem rusts in Vol. **II).**

V. Source of Collections

The usefulness of a race survey depends on the source of samples. Ideally, the collections should be made on a stratified random basis, but currently this is not done. Thus an effort has been made to increase the number of samples to compensate for some nonrandomness in sampling. In many cases quality of

sampling could replace quantity of collections if quality collections could be defined and obtained at a cost within the economic limit. Currently, the number of samples is usually correlated with the ability to detect races occurring at a low frequency. The lower the frequency at which a hazardous culture is initially detected, generally the longer the time available for finding new sources of resistance and developing them into cultivars, or for initiating other control strategies. With organisms that have as high a reproduction rate as the cereal rusts, no number of samples that can be handled, even with current technology, will result in all pathogen phenotypes being detected. Thus, even in years when over 2600 isolates from 900 collections from the United States were studied, some physiological races were still detected only once (Roelfs and McVey, 1975). Other races were detected only once in several years, so either the same mutation has recurred in otherwise the same pathogen phenotype, or, more likely, the race has existed undetected.

A. SAMPLES FROM COMMERCIAL FIELDS AND WILD HOSTS AT PEAK DEVELOPMENT

The most important and most nearly randomly obtained cultures studied are those made in commercial fields at the height of rust disease development. These collections would be improved if they were collected on a purely random basis. Random sampling was attempted in the Dakotas and in northeastern Montana one year with wheat stem rust, but such a survey was very expensive, and no rust was found even though using traditional methods rust was found in trace amounts. The expense involved locating the random points and getting to them, as most sites were not near roads. Furthermore, experienced personnel in field surveys gain a sense of where to look for rust, as its location in the field and on a plant varies with the environmental conditions. In the year the random survey was done, stem rust was limited to field edges in low-lying areas of late-planted fields. The field edges were not considered in the random survey, and the majority of the fields were upland, thus reducing the likelihood of finding the pathogen in the random survey. Therefore, the Cereal Rust Laboratory has continued with the traditional survey. In the United States, this survey involves approximately six trips over 25,000 km (15,000 miles), with stops at the first small-grain field after each 32 km (20 miles) on the car odometer. The routes are prechosen through the major cereal-producing areas where rust has historically been a problem. To these collections are added collections made by other cooperators, which may or may not be taken on a systematic basis, but the collections are from commercial fields or wild hosts. These samples provide the basis of our data on pathogen phenotype frequency and distribution. Because of host resistance, however, such data indicate only the extent of the disease spread but give no clue to viable pathogen spore dispersal. Because most

urediospores are generally produced in the last 10 days of the epidemic, races occurring primarily early in the season, races originating outside the area arriving late in the season, mutants occurring on a previously susceptible cultivar, and nonagressive phenotypes can be missed totally in these samples.

B. SAMPLES FROM COMMERCIAL FIELDS AND WILD HOSTS EARLY IN THE SEASON

Collections are also made early in the season from the first uredia observed. The early collections from the overwintering area and from the early exogenous inoculum in the northern areas are used in epidemiologic studies on disease movement. The early-season surveys are conducted in the same manner, and because of the limited number of total collections for the purpose of studying pathogen distribution and frequency, data from these collections are included with the other data from commercial fields.

Generally, the pathogen phenotype found initially in an area is the most common race at host maturity. This is because the short time between initial infection and host senescence is sufficient to permit only a few pathogen generations. Exceptions normally occur when a previously resistant host cultivar, which is planted on a significant part of the area, is highly susceptible to a race that appears late in the season.

C. SAMPLES FROM NURSERIES AND PLOTS

Samples of rust are also taken from uninoculated nurseries and trap plots. Although this inoculum is part of the natural rust population, the selection pressure created by the combinations of host resistance, or lack of resistance that exists in such nurseries often affects the frequencies of the races found. In the case of oat stem rust in the United States, race NA-27 predominates in the production fields, and race NA-16 is seldom found except on wild oats *(Avena fatua* L.) and on nursery lines without *Pg2* and *Pg4* (Roelfs *et al.,* 1980). These two host genes nearly eliminate NA-16 from commercial fields.

In nurseries, unique combinations of host resistance may exist on which a virulent but generally nonaggressive race may increase without competition from its avirulent aggressive competitors and thus may be detected. The real value of trap plots is when unique host genotypes are used that can detect virulent pathogen phenotypes, perhaps before they become a major part of the pathogen population. However, with asexual reproduction most pathogen variation is due to mutation; thus new races would occur at random. Because most spores are produced in commercial fields, most mutations would occur there, but they could multiply selectively on appropriate host genotypes in nurseries. For the detection of unique pathogen phenotypes and the estimations

of viable inoculum movement, we have maintained 50-75 trap plots in the eastern two-thirds of the United States for the past 10 years. Data from these plots are included in the annual race survey reports but are kept separately from collections made in commercial fields and on wild hosts. Otherwise, cultures with many virulence or avirulence genes, and cultures with little aggressiveness could be overemphasized. This is especially true in those years when the number of collections is low because of a sparsity of rust, as in 1980 when, of the 54 collections of wheat stem rust that were made, 45 (83%) of them were from nurseries (Roelfs *et al.,* 1982).

D. SAMPLES FROM THE ALTERNATE HOST

Samples from the alternate host can also be very important in areas where it is a significant source of inoculum. A problem arises because of the high number of pathogen phenotypes produced (Roelfs and Groth, 1980). Roberts *et al.* (1966) used a screening nursery of cereal hosts of varying genotypes adjacent to a barberry hedge to aid in selecting pathogen phenotypes of a potential threat to crop production. Caution must be used, as most cultures from sexual recombination commonly have avirulences for previously undetected host resistances. Some of these cultures are unusual in being avirulent on *SrLC, Srl6, SrMcN,* while being virulent on *Sr6, Sr10, Sr17,* etc. Thus the differential host can be resistant because of a previously undetected resistance gene. Because teliospores and basidospores travel only short distances, unique local populations may occur near the alternate host. This means that more samples are required per unit of land area. In isolated areas where the alternate host is not an important source of inoculum for cereal crops, rust collections can still serve as an important source of new pathogen phenotypes that are useful in genetic studies of the host.

E. SAMPLES FROM INOCULATED NURSERIES

Data from collections made in inoculated nurseries should not be included in reports on pathogen frequency or distribution. Our procedure has normally been to exclude frequency data of any collections made within 2 to 10 km of a known inoculated nursery depending on its size. The collections, however, provide valuable information on frequency of races in nurseries and thus aid in decisions on host resistances. Some information is also obtained about survival of races in composite inoculum after several generations of uredial increase.

VI. Importance of Type Cultures

With the advent of vacuum drying, liquid nitrogen, and ultra-low refrigeration storage of urediospores (Rowell, Chapter 10, this volume), cultures can be maintained indefinitely with relative ease. Thus unique pathogen cultures can be preserved and used as a type for physiologic races. In early physiologic specialization studies, most comparisons were between data on a previously studied race and the currently available isolates of that race. It was impossible to determine whether the differences or similarities in infection types were due to differences in environmental conditions, host resistance, or pathogen virulence. The latter, of course, is the only reasonable basis for race designations. It is now possible with better storage methods to compare cultures directly, to provide the much-needed historical continuity. These stored cultures are also useful in verifying that differential host lines have remained genetically identical for resistance to that pathogen. We have had several experiences in which the least effective of two or more genes for stem rust resistance was lost from the population over a period of years despite normal procedures for maintaining seed purity. Stored cultures assure that the same culture can be used throughout a breeding, genetic, or other type of study.

VII. Single Uredium Isolates

The understanding of race distribution and the ease in classification of infection types can be improved by the use of single uredium isolates. Bulk collections of urediospores used to inoculate differential hosts often result in a mixture or range of infection types on a single differential host. This can mask some low-infection types completely, such as the infection type 0 obtained with *Sr5* or *Sr36 (Tt1)* in wheat stem rust (Roelfs and McVey, 1979). This can also result in the missing of an X infection type or misclassifying a mixture of infection types as an X infection type. Single uredium isolates eliminate this problem. Stakman *et al.* (1962) outlined the procedure of making separations when bulk collections were used to inoculate differential series. Although processing a bulk collection is initially faster than making single uredium isolates first, the separations generally required subsequently when using bulk collections often lengthen that process considerably. Separations are also difficult to handle in a determination of race frequency. Separations consist of isolating one or more single uredia from about 1000 that occur on a differential set. The progeny of each uredium is then inoculated to a separate differential series.

However, the frequency at which it occurred in the field population is then impossible to estimate. Contamination may also occur in race survey cultures

because of the large volumes of materials handled. These contaminants are often isolated when making such separations. The initial use of single uredium isolates reduces the contamination problem and solves the problem of estimating frequencies. The number of isolates needed per collection may vary depending on the variation in the pathogen population, although three or four seem adequate (Roelfs and Johnston, 1966). Single uredia are easily obtained by inoculating maleic hydrazide-treated host plants (see Rowell, Chapter 10, this volume). Six to ten plants are planted in clumps at the four corners of a 7 × 7 cm pot. When uredia erupt, four isolated uredia on separate plants are saved, and remaining diseased tissue is clipped out. The plants are reincubated to germinate loose urediospores and then placed in isolation cages for 24 to 48 hr, after which cyclone separators are used to collect rust separately from three single uredia. Each uredium furnishes adequate inoculum to inoculate as many as six to eight seedlings each of up to 24 cultivars in the case of stem rust. For leaf rust of wheat and barley and crown rust of oats, the single uredium isolates usually are increased on a susceptible host before inoculating differential hosts, which lengthens the time for identification by 2 weeks.

VIII. Selection of Differential Hosts

The differential hosts chosen determine the usefulness of race data. The original differential hosts were all those discovered that resulted in a different host response pattern when infected with the limited number of cultures available. Using every differential host known is no longer desirable for most of the cereal rust pathogens, as thousands of resistant host lines are known. We have comprehensively studied 45 different "single-gene" host resistances (Roelfs and McVey, 1979) and now are investigating 12 more for wheat stem rust. Thus some selection of differential hosts must be made even when "single genes" are being used. In selecting differential cultivars, investigators give varying weight to different factors. Six factors that merit important consideration are discussed in the following paragraphs.

A. HISTORICAL BASE

A historical base is important. Not all races are detected annually; therefore, several years of data are needed to build a working base. In the case of wheat stem rust, most of the original host differentials had a multiple-gene basis for their resistance. However, an examination of the historical data for the United States showed that *Sr5*, *Sr7b*, *Sr9d*, *Sr9e*, and *Sr21* were the resistances on which separation of races had been done throughout the surveys. These resistance genes were those for which the differences between low- and high-

infection types were clearly distinguishable and that were stable over a range of environmental conditions. Thus with the current asexual population of *P. graminis* in the Great Plains, it is possible to predict the international race by knowing the response of *Sr5, Sr7b, Sr9d,* and *Sr9e.* The response of *Sr21* is unnecessary, as a low-infection type is obtained only when *Sr9d* is also low. This combination of genes does not work with a sexually reproducing population or one in which other genes in the standard differential host series were important in determining race differences as they exist outside the Western Hemisphere.

B. RESISTANCE USED IN COMMERCIAL CULTIVARS

Genes that are used or that are under consideration for use in commercial cultivars often are valuable differential hosts. In some areas of the world, race-specific resistances may exist in other native, cultivated, or escaped hosts and may also be important to consider. Differential hosts used should have a differential reaction; that is, they should not be susceptible or resistant to all cultures evaluated from the population.

C. STABILITY OF THE DISEASE INFECTION TYPE

Ideally, hosts chosen as differentials must not be severely affected by the range of temperatures, light, and inoculum density likely to occur during the race survey. Even though *Sr6* is sensitive to small changes in temperature, we have successfully used this host gene. It is stable enough to use in the greenhouse when the temperature fluctuates with a daily minimum of less than 21° or 22°C (Roelfs and McVey, 1979). Further, when differential hosts are examined daily with similar pathogen genotypes, an investigator becomes accustomed to the gradual changes in infection types caused by changes in temperature and light (e.g., a change with*Sr6* from a 0; to 3 ⁺ C over a period of a month. Additionally, with "single-gene" host lines, the high-infection type on the background line without *Sr6* is consistently 44+. Some other lines (i.e., *Sr7a* and *Srl5)* are so sensitive to environmental conditions that they are of little use in a race survey (Roelfs and McVey, 1979).

D. USEFULNESS OF INFORMATION

The host differentials must also provide information useful to the purpose of the survey. In the United States, for example, *Sr12* occurs in many of the host cultivars grown in the spring wheat area, but it provides resistance only against one race that is already distinguished by the infection types produced on other more stable host lines. This example is similar to the one of *Sr21* given in

Section VIII,A; however, *Sr21* does not occur in the commercial cultivars grown in the United States. This does not necessarily indicate that these host genes or the virulences on them are linked; it merely reflects that in the asexual population we are sampling, all cultures avirulent on *Sr9d* are also avirulent on *Sr12* and *Sr21*. This association should remain until a mutation for virulence occurs at one of the loci or until sexual or parasexual recombination occurs.

E. "SINGLE-GENE" DIFFERENTIALS

Although it is impossible to be sure a host line has a single gene for rust resistance, it is possible to establish lines that have only one gene that is effective against the pathogen population available for study. These "single-gene" lines have many advantages as differential hosts, the chief one being that the pathogen phenotype is clearly measured. With multiple-gene differential hosts, some interactions are masked because of epistasis, and specific low-infection types may be difficult to distinguish because of the large number resulting from the many gene-for-gene interactions as described earlier (Table HID).

A "single-gene" line has a smaller range of low-infection types, and thus environmental effects are of less importance. Often when a multi-gene differential is evaluated, epistasis and similar low-infection types produced by different host genes prevent a complete determination of the effect of each individual host gene. Thus a low-infection type on the tested multigene differential may not help in predicting the host response of other untested hosts. The use of "single-gene" differential host lines often enables one to predict the infection type of host lines with a known genotype. For example, the cultivar Selkirk has *Sr6, Sr7b, Sr9d, Sr17,* and *Sr23*and could be susceptible to a given culture, whereas Bowie with *Sr6* and *Sr8* was resistant, and vice versa. However, if the "single-gene" differentials *Sr6* and *Sr8* are susceptible, then Bowie inoculated with the same culture will also be susceptible; if a culture inoculated to *Sr6* results in a fleck, then Bowie will result in a fleck; if *Sr8* results in a type 2 and *Sr6* a 4, then Bowie will be a fleck. A note of caution, however; other genes in the host can modify the infection type expression and in rare cases can completely change it (Dyck and Samborski, 1982; Kerber and Green, 1980).

F. SEED AVAILABILITY

When a host line with a new resistance is found, insufficient seed is generally available to use it in the race survey. However, a line with limited seed can be used as a supplemental differential for a part of the pathogen population, by being tested only against selected cultures. Some of the currently

available “single-gene” host lines are very difficult to grow in some areas of the world because of a lack of adaptation, and thus inadequate seed stocks continue to be a major problem.

IX. “Universal” Resistance Series

Most race surveys in time identify a group of cultivars, lines, or “single-gene” lines that are resistant to all the cultures evaluated (Loegering *et al,* 1959, 1961). In a few cases, these lines are treated as differential hosts, although they obviously are not. They are more effectively evaluated in a special “universally” resistant series. This series is primarily used for detecting new virulence or combinations of virulence. It is not needed to evaluate frequency or distribution in the main survey. Thus the use of bulk collections instead of single uredium isolates is more efficient. The bulk used is composed of a portion of the urediospores from each collection received from a particular area or cultivar over a period of days. The infection density on the susceptible check (low, medium, or high) is noted, and the low-infection types for each line are compared against the expected range of low-infection types. A high-infection type on a resistant line normally indicates a new gene or combination of genes for pathogen virulence. A higher than expected low-infection type, if stable, usually indicates a virulence for the previously expressed resistance gene, but avirulence on another host gene previously not expressed as a result of epistasis. The cultures producing a higher low-infection type and/or high-infection type are isolated and (re)evaluated on the differential and “universal” resistance series. Off-type plants (those without the desired resistance genes) can be a problem in this series; however, if the high-er(er) infection types are on a single plant it is almost always an off-type plant. This kind of series is a powerful tool for detecting new virulences although probably not as effective as planting large trap nurseries of resistance genotypes in scattered locations.

X. Prospects

A long-range emphasis needs to be placed on methodology of field sampling. Current sampling methods have many biases of unknown effect. In comparison to the large numbers of pathogen individuals, even the largest survey samples only a minute part of the population. Major gains in sample quality are not expected in the immediate future. The possible and probable sources of inoculum for most major cereal-producing areas should be delineated. This would allow early sampling of the population and an early

forecast of the pathogen phenotype perhaps before the crop is planted in some areas.

Additional markers (other than virulence markers) should be available for use in studying pathogen populations in the future. These additional markers may permit more conclusive studies of changes in the pathogen population. Studies on mutation rates of individual pathogen virulence genes from asexually reproducing populations will permit a better basis for selecting resistance sources for use in commercial cultivars.

"Single-gene" lines will be developed for many more resistances. These resistance genes will be placed in genetic backgrounds that result in more stability in the resulting infection types with a wide range of cultures and environments. Studies of interorganismal genetics will clarify the differences among infection types formed by all the combinations of host—pathogen gene pairs. Interactions between pairs of host and pairs of pathogen genes will be studied. These category IV interactions (see Loegering, Chapter 6, this volume) may open a whole new field of understanding of host-pathogen relations and result in major changes in the way host-pathogen interactions are viewed in race surveys. New nomenclature systems may be required to express these interactions.

References

Allison, C. C, and Isenbeck, K. (1930). Biologische spezialisierung von *Puccinia glumarum tritici,* Erikss, und Henn. *Phytopathol. Z.* 2, 87-98.

Andres, M. W. (1982). Latent period and slow rusting in the *(Hordeum vulgare L.-Puc- cinia hoidei* Otth.) host-parasite system. Ph.D. Thesis, University of Minnesota, St. Paul.

Basile, R. (1957). A diagnostic key for the identification of physiological races of *Puccinia rubigo-vera tritici.* Grouped according to a Unified Numeration scheme. *Plant Dis. Rep.* **41,** 508-511.

Bjorkmann, I. (1960). Experiments with cereal rusts on detached leaves in benzimidazole. *Bot. Not.* **113,** 82-86.

Browder, L. E. (1971). Pathogenic specialization in Cereal Rust fungi, especially *Puccinia recondita* f. sp. *tritici:* Concepts, methods of study, and application. *U.S., Dep. Agric, Tech. Bull.* **1432,** 1-51.

Browder, L. E., and Young, H. C, Jr. (1975). Further development of an infection-type coding system for the cereal rusts. *Plant Dis. Rep.* 59, 964-965.

Browder, L. E., Lyon, F. L., and Eversmeyer, M. G. (1980). Races, pathogenicity phenotypes, and type cultures of plant pathogens. *Phytopathology* 70, 581-583.

Burdon, J. J., Marshall, D. R., Luig, N. H., and Gow, D. J. S. (1982). Isozyme studies on the origin and evolution of *Puccinia graminis* f. sp. *tritici* in Australia. *Aust. J. Biol. Sci.*35, 231-238.

Chester, K. S. (1946). "The Nature and Prevention of the Cereal Rusts as Exemplified in the Leaf Rust of Wheat." Chronica Botanica, Waltham, Massachusetts.

Cotter, R. U., and Levine, M. N. (1932). Physiologic specialization in *Puccinia graminis* secalis. J. Agric. Res. (Washington, D.C.) 45, 297-315.

Dyck, P. L., and Samborski, D. f. (1982). The inheritance of resistance to *Puccinia recondita* in a group of common wheat cultivars. *Can. J. Genet. Cytol.* 24, 273-283.

Fleischmann, G., and Baker, R. f. (1971). Oat crown rust race differentiation: Replacement of the standard differential varieties with a new set of single resistance gene lines derived from *Avena stehlis. Can. J. Bot.* 49, 1433-1437.

Gilmour, J. (1973). Octal notation for designating physiologic races of plant pathogens. Nature (London) **242,** 620.

Green, G. J. (1965). Stem rust of wheat, rye and barley in Canada in 1964. *Can. Plant Dis. Surv.* 45, 23-29.

Green, G. J. (1975). Virulence changes in *Puccinia graminis i.* sp. *tritici* in Canada. *Can. J. Bot.* 53, 1377-1386.

Habgood, R. M. (1970). Designation of physiological races of plant pathogens. *Nature (London)* 227, 1268-1269.

Hartley, M. J., and Williams, P. G. (1971). Morphological and cultural difference between races of *Puccinia graminis t.* sp. *tiitici* in axenic culture. *Trans. Br. Mycol. Soc.* 57, 137-144.

Hoerner, G. H. (1919). Biologic forms of *Puccinia coronata* on oats. *Phytopathology* 9,309-314.

Johnston, C. O. (1963). The "Y" type infection in the leaf rust of wheat. *Robigo* 15, 1-2.

Kerber, E. R., and Green, G. J. (1980). Suppression of stem rust resistance in the hexaploid wheat CV. Canthatch by chromosome 7DL. *Can. /. Bot.* 58, 1347-1350.

Levine, M. N. (1928). Biometrical studies on the variation of physiologic forms of *Puccinia graminis tritici* and the effects of ecological factors on the susceptibility of wheat varieties. *Phytopathology* 18, 7-123.

Levine, M. N., and Stakman, E. C. (1923). Biologic specialization of *Puccinia graminis secalis. Phytopathology* 13, 35 (abstr.).

Loegering, W. Q., Johnston, C. O., Samborski, D. J., Caldwell, R. M., Schafer, J. F., and Young, H. C. (1959). A proposed modification of the system of wheat leaf rust identification and nomenclature. *Plant Dis. Rep.* 43, 613-615.

Loegering, W. Q., Johnston, C. O., Samborski, D. J., Caldwell, R. M., Schafer, J. F., and Young, H. C. (1961). The North American 1961 set of supplemental differential wheat varieties for leaf rust race identification. *Plant Dis. Rep.* 45, 444-446.

Luig, N. H. (1977). The establishment and success of exotic strains of *Puccinia graministritici* in Australia. *Proc. Ecol. Soc. Aust.* 10, 89-96.

Luig, N. H., and Rajaram, S. (1972). The effect of temperature and genetic background on host gene expression and interaction to *Puccinia graminis tritici. Phytopathology* 62, 1171-1174.

McVey, D. V., and Roelfs, A. P. (1975). Postulation of genes for stem rust resistance in the entries of the 4th International Winter Wheat Performance Nursery. *Crop Sci.* 15, 335-337.

Mains, E. B. (1926). Rye resistance to leaf rust, stem rust, and powdery mildew. *Agric. Res.* 32, 201-221.

Mains, E. B., and Jackson, H. S. (1921). Two strains of *Puccinia triticina* on wheat in the United States. *Phytopathology* 11, 40 (abstr.).

Mains, E. B., and Jackson, H. S. (1923). Strains of the leaf rust of wheat, *Puccinia triticina,* in the United States. *Phytopathology* 13, 36 (abstr.).

Mains, E. B., and Jackson, H. S. (1926). Physiologic specialization in the leaf rust of wheat, *Puccinia triticina,* Erikss. *Phytopathology* 16, 89-120.

Martens, J. W., Roelfs, A. P., McKenzie, R. I. H., Rothman, P. G., Stuthman, D. D., and Brown, P. D. (1979). System of nomenclature for races of *Puccinia graminis* f. sp.*avenae. Phytopathology* 69, 293-294.

Newton, M., Johnson, T., and Brown, A. M. (1930). A preliminary study on the hybridization of physiologic forms of *Puccinia graminis tritici. Sci. Agric. (Ottawa)* 10,721-731.

Roberts, B. J., Eckess, E. D., and Romig, R. W. (1966). A new and virulent race of oat stem rust, race 6AFH. *Plant Dis. Rep.* 50, 570-571.

Roelfs, A. P. (1974). Evidence for two populations of wheat stem and leaf rust in the U.S.A. *Plant Dis. Rep.* 58, 806-809.

Roelfs, A. P., and Groth, J. V. (1980). A comparison of virulence phenotypes in wheat stem rust populations reproducing sexually and asexually. *Phytopathology* 70,855-862.

Roelfs, A. P., and Johnston, C. O. (1966). Comparison of methods of analysis of field collections of *Puccinia recondita* f. sp. *tritici. Phytopathology* 56, 1143-1148.

Roelfs, A. P., and McVey, D. V. (1972). Wheat stem rust races in the Yacqui Valley of Mexico during 1972. *Plant Dis. Rep.* 56, 1038-1039.

Roelfs, A. P., and McVey, D. V. (1975). Races of *Puccinia graminis* f. sp. *tritici* in the U.S.A. during 1974. *Plant Dis. Rep.* 59, 681-685.

Roelfs, A. P., and McVey, D. V. (1979). Low infection types produced by *Puccinia graminis i.* sp. *tritici* and wheat lines with designated genes for resistance. *Phytopathology* 69, 722-730.
Roelfs, A. P., McVey, D. V., Long, D. L., and Rowell, J. B. (1972). Natural rust epidemics in wheat nurseries as affected by inoculum density. *Plant Dis. Rep.* 56, 410-414.
Roelfs, A. P., Casper, D. H., and Long, D. L. (1978). Races of *Puccinia graminis* f. sp. *tritici* in the U.S.A. during 1978. *Plant Dis. Rep.* 63, 701-704.
Roelfs, A. P., Long, D. L., and Casper, D. H. (1980). Races of *Puccinia graminis* f. sp. *avenae* in the United States during 1979. *Plant Dis. Rep.* 64, 947-949.
Roelfs, A. P., Long, D. L., and Casper, D. H. (1982). Races of *Puccinia graminis* f. sp. *tritici* in the United States and Mexico in 1980. *Plant Dis. Rep.* 66, 205-207.
Rowell, I. B., and Roelfs, A. P. (1971). Evidence for an unrecognized source of overwintering wheat stem rust in the United States. *Plant Dis. Rep.* 55, 990-992.
Samborski, D. J. (1968). Leaf rust in Canada in 1967. *Can. Plant Dis. Surv.* 48, 6-8.
Simons, M. D., and Michel, L. f. (1959). A comparison of different methods used in conducting surveys of races of the crown rust fungus. *Plant Dis. Rep.* 43, 466-469.
Simons, M. D., and Murphy, H. C. (1955). A comparison of certain combinations of oat varieties as crown rust differentials. *U.S., Dep. Agric, Tech. Bull.* 1112, 1-22.
Stakman, E. C. (1929). Physiologic specialization in pathogenic fungi. *Proc. Int. Congr. Plant Sci., 1926* Vol. 2, pp. 1312-1330.
Stakman, E. C, and Christensen, J. J. (1926). Physiologic specialization of *Ustilago zeae* and *Puccinia sorghi* and their relation to corn improvement. *Phytopathology* 16, 84 (abstr.).
Stakman, E. C., and Harrar, J. G. (1957). "Principles of Plant Pathology." Ronald Press, New York.
Stakman, E. C., and Levine, M. N. (1922). The determination of biologic forms of *Puccinia graminis* on *Triticum* spp. *Minn., Agric. Exp. Stn, Tech. Bull.* 8, 1-10.
Stakman, E. C., and Piemeisel, F. f. (1917). A new strain of *Puccinia graminis. Phytopathology* 7, 73 (abstr.).
Stakman, E. C., Levine, M. N., and Bailey, D. L. (1923). Biologic forms of *Puccinia graminis* on varieties of *Avena* spp. *Agric. Res.* 24, 1013-1018.
Stakman, E. C., Levine, M. N., and Cotter, R. U. (1930). Origin of physiological forms of *Puccinia graminis* through hybridization and mutation. *Sci. Agric. (Ottawa)* 10, 707-720.
Stakman, E. C., Levine, M. N., Christensen, J. J., and Isenbeck, K. (1935). Dec Bestimmung physiologischer rassen pflanzenpathogener pilze. *Nova Acta Leopold.* 3, 281-336.
Stakman, E. C., Stewart, D. M., and Loegering, W. Q. (1962). Identification of physiologic races of *Puccinia graminis* var. *tritici. U.S., Agric. Res. Serv.* E-617 (rev.), 1-53.
Stewart, D. M., and Roberts, B. J. (1970). Identifying races of *Puccinia graminis* f. sp. *avenae.* A. modified international system. *U.S., Dept. Agric, Tech. Bull.* 1416, 1-23.
Sunderwirth, S. D., and Roelfs, A. P. (1980). Greenhouse evaluation of adult plant resistance of *Sr2* to wheat stem rust. *Phytopathology* 70, 634-637.
Van Dyke, C. G., and Hooker, A. L. (1969). The Z reaction in corn to *Puccinia sorghi. Phytopathology* 59, 33-36.
Waterhouse, W. L. 1929. Australian rust studies. I. *Proc. Linn. Soc. N.SW.* 54, 615-680.
Watson, I. A., and Luig, N. H. (1961). Leaf rust on wheat in Australia: A systematic scheme for the classification of strains. *Proc. Linn. Soc. N. S. W.* 86, 241-250.
Young, H. C., Jr., and Browder, L. E. (1965). The North American 1965 set of supplemental differential wheat varieties for leaf rust race identification. *Plant Dis. Rep.* 49, 308-311.

6

Genetics of the Pathogen—Host Association

William Q. Loegering

Department of Plant Pathology, University of Missouri at Columbia, Columbia, Missouri

I. Introduction

This treatise deals with the cereal rust diseases, not the cereal rust fungi or their cereal hosts alone. For this reason this chapter considers the interorganismal genetics of the association of the cereal rust pathogens and their hosts rather than their intraorganismal genetics. The inheritance of reaction in the host and of pathogenicity in the pathogen have been studied extensively. These studies have shown that inheritance is usually simple with low reaction and low pathogenicity usually dominant, whereas high reaction and high pathogenicity are usually recessive. It is common to find allelism for

reaction; however, allelism for pathogenicity has seldom been observed. There are numerous reports of interactions among genes for reaction and a few for pathogenicity. These reports were either wrong or the definitive studies to demonstrate their validity were not made. The observed interactions could just as well be at the interorganismal level and perhaps are. Interorganismal genetics, the genetics of symbiosis, has its foundations in Flor's gene-for-gene concept (1971). Pathogen-host associations are considered to be symbiotic. The aegricorpus (Loegering, 1966) is the result of such a symbiosis and is defined as the living manifestation of the genetic interactions in and between pathogen and host. In the cereal rusts, the infection type (IT) is the phenotype of the aegricorpus, not that of the pathogen or host. Thus the central concept of interorganismal genetics is that the genotypes are of the symbionts, but the phenotype is of the symbiosis.

II. The Origin of the Gene-for-Gene Concept

H. H. Flor (1946, 1947) pioneered the study of interorganismal genetics using flax rust *[Melampsora lini* (Pers.) Lev.-Linum *usitatissimum* L.) as his model. Based on these studies he developed the gene-for-gene concept (Flor, 1971). In his initial studies he dealt with a gene pair in the pathogen corresponding to a gene pair in the host—the corresponding gene pairs (CGP). It should be noted that CGP is plural. The expression “gene-for-gene” is used to designate a concept and should never be translated literally.

To demonstrate the gene-for-gene relationship the ideal model would involve two symbionts in which classical diploid inheritance occurs and that can be propagated as clones. This would make it possible to observe the phenotype of all possible combinations of the F2 individuals of both symbionts. This has never been done, and no such combination of symbionts is available at present. Of the models worked with, the one that best approximates the ideal is flax rust. *M. lini* has diploid inheritance, and uredial cultures are clones. It is genetically stable and is easily maintained. Flax is self-pollinated and has normal diploid inheritance but is difficult to propagate as a clone. Sequential inoculation of a given plant with different cultures of the pathogen, however, is relatively easy to accomplish. Flor found that the IT was consistent as the plant aged. Often this is not true for the hosts of the cereal rust fungi. It must be remembered that Flor did not set out to demonstrate the gene-for-gene relationship. He developed it as a result of the characteristics of his model, the creative analysis of his data, and the development and use of special methods.

Flor demonstrated more than 25 gene-for-gene relationships in flax rust. Data (Flor, 1946, 1947) for two will be used for illustration (Tables I-VIII). The

symbolization he used for genes and phenotypes will be used in the first tables to illustrate some of the problems encountered with the presentation of the data.

Table I

Inheritance of Pathogenicity in a Cross of Cultures of Race 22 and 24 of *Melampsora lini* When the F2 Population of Cultures Was Used to Inoculate the Ottawa 770B

	Cultures[b]				
Cultivar	Race 24	Race 22	F_1	F_2	
Ottawa 770B	I	S	I	I	S
				101	32
				Ratio 3:1	
				$\chi^2 = 0.06$; $p = .95–.99$	

[a]Data from Flor (1946).
[b]I, Immune; S, susceptible.

He crossed a culture of race 24 with one of race 22 of *M. lini* and used the F2 progeny to inoculate the flax cultivar Ottawa 770B (Table I). The cultivar was immune (I) with race 24 (IT 0) and susceptible (S) with race 22 (IT 4). Segregation was 3:1 I:S, indicating that recombination for a single gene pair in the pathogen occurred and immunity was dominant. This gene was designated *AlAl.* He then crossed the Ottawa 770B and Bombay flax cultivars and inoculated the F2 progeny with the culture of race 24 (Table II). Ottawa 770B was immune (I = IT 0), and Bombay was susceptible (S = IT 4). Segregation was 3:1 I:S, indicating that recombination for a single gene pair occurred in the host and immunity was dominant. This gene was designated *LL*. Because Ottawa 770B and race 24 were common to both studies, the data are combined in Table III with the genotypes added and the ITs placed in parentheses after Flor's I-S symbolization.

Table II

Inheritance of Reaction in a Cross of Ottawa 770B and Bombay Flax Cultivars When Inoculated with a Culture of Race 24 of *Melampsora Iini*[a]

Cultivars	Culture race 24[b]
Ottawa	I
Bombay	S
F_1	I
	Ratio 3:1
F_2	I 142
	S 52

$X^2 = 0.32$, $p = .50\text{-}95$

[a]Data from Flor (1947).

[b]I, Immune; S, susceptible.

Table III
Tables I and II Combined with Genotypes and Infection Types[a] Added[b]

Cultivar	Genotypes	Cultures[c]		
		Race 24 *AlAl*[d]	F_1 *Alal*[d]	Race 22 *alal*[d]
Ottawa 770B	*LL*	I (0)	I (0)	S (4)
F_1	*Ll*	I (0)		
Bombay	*ll*	S (4)		

[a]Genotypes and infection types from Flor (1946, 1947].

[b]F2 data has been omitted.

[c]I, Immune; S, susceptible.

[d]Infection types are in parentheses.

As part of the experiment just described, Flor inoculated Bombay and the F2 plants with the culture of race 22 by using sequential inoculations and inoculated Bombay with each of the F2 cultures at the time he inoculated Ottawa 770B. Bombay with race 22 was immune (I = IT 0), and Ottawa 770B was susceptible (S = IT 4). The segregation in both organisms was 3:1 I:S (Tables IV and V), although exactly the same number of individuals were not found in each class as in the work with

Table IV

Inheritance of Pathogenicity in a Cross of Cultures of Race 22 and 24 of *Melampsora lini* When the F2 Population of Cultures Was Used to Inoculate the Bombay Cultivar of Flax[a]

	Cultures[b]				
Cultivar	Race 24	Race 22	F_1	F_2	
Bombay	S	I	I	I	S
				105	28
				Ratio 3:1	
				$\chi^2 = 1.10$; $p = .50–.95$	

[a]Data from Flor (1946).

[b]1, Immune; S, susceptible.

Table V

Inheritance of Reaction in a Cross of Ottawa 770B and Bombay Flax Cultivars When Inoculated with a Culture of Race 22 of *Melampsora lini*[a]

Cultivars	Culture race 22[b]
Ottawa 770B	S
Bombay	I
F_1	I
	Ratio 3:1
F_2	I 153
	S 41
	$X^2 = 1.54$, $p = .20$-$.50$

[a]Data from Flor (1947).

[b]I, Immune; S, susceptible.

Ottawa 770B. Because Bombay and race 22 were common to both tests, the data are combined in Table VI. Following this report of Flor's data may be somewhat confusing, because in using I and S he sometimes meant immunity and susceptibility of the host, and sometimes he used I and S as symbols for IT. He evidently was aware of the problem, because in his 1959 review the footnote to Table 2, which reports data on the results of inoculating the F2 plant populations, reads: “I = immune; S = susceptible,” whereas in Table 3, which reports the result of using the F2 cultures to inoculate the two cultivars, the footnote reads: “I = immune (avirulent); S = susceptible (virulent).” The confusion arises from utilizing host-oriented genetic concepts in presenting the data and failure to recognize that the IT is not the phenotype of either symbiont but of the aegricorpus. To produce the IT 0, a particular genotype must be present in both organisms, whereas IT 4 is the result of the alternate genotype

in at least one of the organisms. To avoid the potential confusion of the I-S symbolization, the actual phenotypes (ITs) will be used hereafter.

Table VI

Tables IV and V Combined with Genotypes and Infection Types Added[a,b]

Cultivar	Genotypes	Cultures[c] Race 24 *anan*[d]	F_1 *Anan*[d]	Race 22 *AnAn*[d]
Ottawa 770B	*nn*	S (4)		
F_1	*Nn*	I (0)		
Bombay	*NN*	I (0)	I (0)	S (4)

[a]Genotypes and infection types from Flor (1946, 1947).
[b]F_2 data has been omitted.
[c]I, Immune; S, susceptible.
[d]Infection types are in parentheses.

The two studies just reported showed that there were two genes segregating independently both in the F2 population of the cross of Ottawa 770B and Bombay flax and of the cross of cultures of races 22 and 24 of*M. lini.* Actually, the two studies were part of a single experiment in which the same F2 populations were used. Ottawa 770B, Bombay, and their F2 were inoculated with the cultures of race 22, race 24, and their F2. Thus the two sets of data can be combined. Table VII is made up of the data much as presented by Flor (1959). This demonstrates that the two genes in each of the organisms were inherited independently. Table VIII presents the same data in another manner based on what has been learned since Flor published the data in 1946 and 1947.

Table VIII

Infection Types Observed by Flor for All Possible Genotype Combinations of Two Independent Genes for Reaction (*I* and *N)* and for Pathogenicity (*Al* and An) in *Melampsora lini*

Host genotypes	Ratios	Pathogen genotypes *Al_An_* 9	*Al_anan* 3	*alalAn_* 3	*alalanan* 1
*L_N_*	9	0	0	0	4
L_nn	3	0	0	4	4
*llN_*	3	0	4	0	4
llnn	1	4	4	4	4

It is clear in Table VIII that IT 0 developed only when either or both *Al__ /L__* or *An__/N__* came together, but in no other combinations. *Al__* and L__ corresponded, and *An__* and *N__* corresponded; thus there are two sets of corresponding gene pairs. This correspondence of dominant genes giving low-infection type was the origin of the expression "gene-for-gene." Later, the expression came to refer to the gene pairs. It is important to understand that the phenotype (IT) is not a genetic character of the host or the pathogen, but is the result of the genotype of both host and pathogen.

From the accumulation of data such as those in Tables VII and VIII came the concept of gene-for-gene. Based on these data there emerged several general principles concerning the genetics of symbiosis.

Table VII

Relationship between the Segregation of the F_2 of the Cross of Ottawa 770B and Bombay Flax Cultivars, and of Cultures of Race 22 and 24 of *Melampsora lini*[a]

		Cultures[b]							
						F_2			
Cultivars	Genotypes	Race 24 *AlAlanan*	Race 22 *alalAnAn*			*Al__An__*	*Al__anan*	*alalAn__*	*alalanan*
Ottawa 770B	*LLnn*	0[I]	4[S]			0	0	4	4
Bombay	*llNN*	4[S]	0[I]			0	4	0	4
				O[c]		78	23	27	5
					C[c]	9	3	3	1
							p = .5–.7		
	L__N__	0	0	110	9				
	L__nn	0	4	32	3				
F_2	*llN__*	4	0	43	3				
	llnn	4	4	9	1				
				p = .3–.5					

[a]Data from Tables I–VI combined. Adapted from Flor (1959).
[b]0 and 4 are ITs; I, immune; S, susceptible.
[c]O, Observed plants and cultures; C, calculated ratios.

1. The phenotype (IT) is of the aegricorpus, not of host or pathogen; but the genotypes are of the symbionts.
2. The difference between a gene and the organism that has the gene became clear from Flor's work. While a cultivar or culture may have genes for "resistance" and "avirulence," it may be "susceptible" or "virulent," respectively. Thus we are alerted to be careful in how we use such terms as resistance, susceptibility, avirulence, and virulence.
3. In Flor's work, although he discussed race 22, race 24, and so on, he was very careful always to use the same culture of these races and understood why it was important. A race is an unofficial taxon and often is made up of many genotypes. In genetic studies the pathogen unit is the culture, not the race.

Present concepts concerning interorganismal genetics will certainly become modified with time and additional principles developed.

III. The Gene-for-Gene Model

A model (Fig. 1) has been developed as a generalization of the gene-for-gene concept. [This model is sometimes erronelusly referred to as the "quadratic check"; however, this term was originally published by Rowell *et ah* (1963) as the name for a suggested experimental design for biochemical studies.] The symbols *P* and *H* are assigned to the gene pair in pathogen and host, respectively. The model is derived from the phenotypes of the nine possible combinations of the homo- and hetero-zygotes of the two symbionts as follows:

	PP	*Pp*	*pp*
HH	0	0	4
Hh	0	0	4
hh	4	4	4

This is then reduced to the idealized gene-for-gene model (Fig. 1). The combination of genotypes *PP/HH, PP/Hh,/PpHH,* and *PpHh* all give IT 0 and are grouped in the upper left-hand corner of the model; *pp/HH*and *pp/Hh* give IT 4 and are grouped in the upper right-hand corner; *PP/hh* and *Pp/hh* give IT 4 and are grouped in the lower left-hand corner; and *pp/hh,* which also gives IT 4, is placed in the lower right-hand corner. Thus the four-way model deals with nine genotype combinations and, in addition, represents a genetic system more complex than it appears. For clarification, the model requires considerable explanation, definition of several new terms, and introduction of a set of symbols.

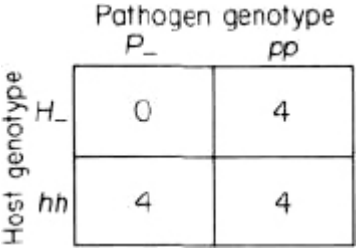


Fig. 1. The idealized gene-for-gene model for a single set of corresponding gene pairs for the cereal rusts.

IV. Categories of Genetic Interaction that Control Disease Development

A. DISEASE VERSUS AEGRICORPUS

Traditionally, "disease" is defined as a process and/or a condition of the host and may include the idea of cause. In actual usage the disease and pathogen are often considered synonymous. The result is that "pests" include weeds, insects, and diseases instead of weeds, insects, and pathogens. A third concept of disease is found in models such as Fig. 2A where disease results from the interaction of pathogen-host-environment. These variable concepts of disease make the word too imprecise to be useful in interorganismal genetics.

The "aegricorpus" results from the interactions among genes in pathogen and host. Environment, which includes the genetic background of each symbiont as well as external factors, may affect the interaction by acting on the pathogen, host, and aegricorpus independently or in combination (Fig. 2B). We know, for example, that temperature has a large effect on the phenotype of the *Sr6* CGP for low IT, resulting in variation from IT 0;1 at 20°C to IT 4 at 24°C. We do not know, however, if the effect is on the pathogen, host, aegricorpus, or combinations of them. Because environment does have an effect on the final phenotypic expression of CGPs, it will be considered a constant in the following discussion.

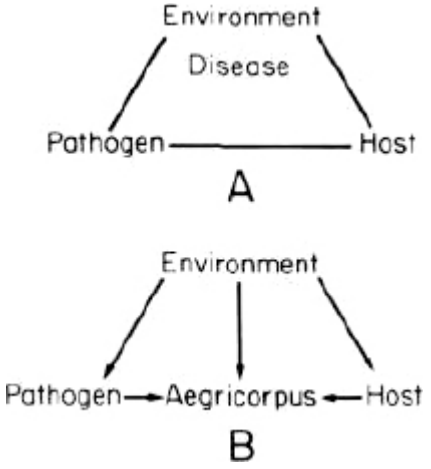


Fig. 2. Comparison of the disease (A) and aegricorpus (B) models.

B. CATEGORIES OF GENETIC INTERACTIONS

Four categories (Fig. 3) of genetic interactions may occur in symbioses. Two of these occur at the intraorganismal level: category I involves the interaction between alleles at a single locus in a single organism, which results in dominance and recessiveness; category II involves the interaction among the genotypes at two or more loci in a single organism, which results in epistasis in all its forms. It will be assumed that the reader is familiar with these kinds of interactions. In interorganismal genetics, two parallel categories of genetic interaction have been shown but at a different level of biological activity. Category III is the interaction within one set of CGP as seen in the idealized gene-for-gene model (Fig. 1). The pathogen and host genotypes are parallel to the dominant and recessive alleles of Category I. The category IV interaction is among two or more sets of CGP. Each set of CGP is parallel to the loci in category II interactions.

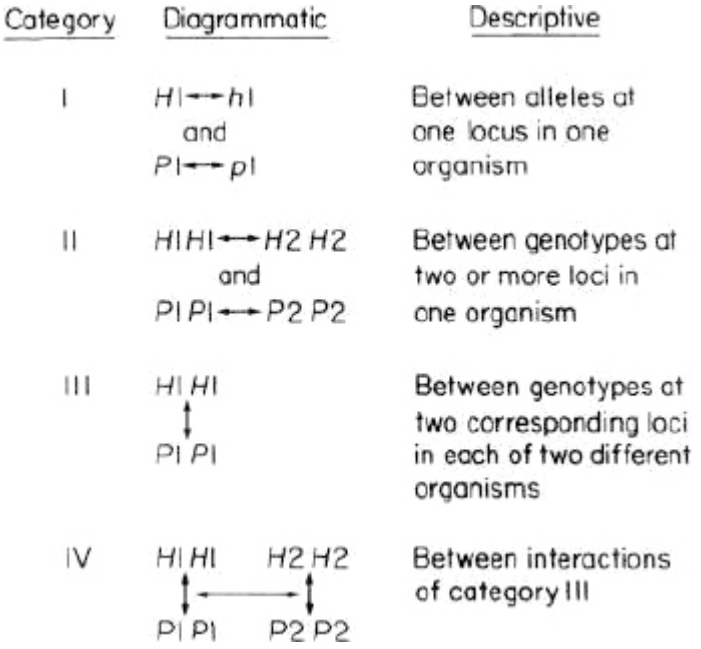


Fig. 3. Categories of genetic interaction that may be found in a host-pathogen association. *H, h, P,* and *p* indicate alleles for reaction of the host and pathogenicity of the pathogen, respectively. Numbers indicate loci. Adapted from Loegering and Powers (1962).

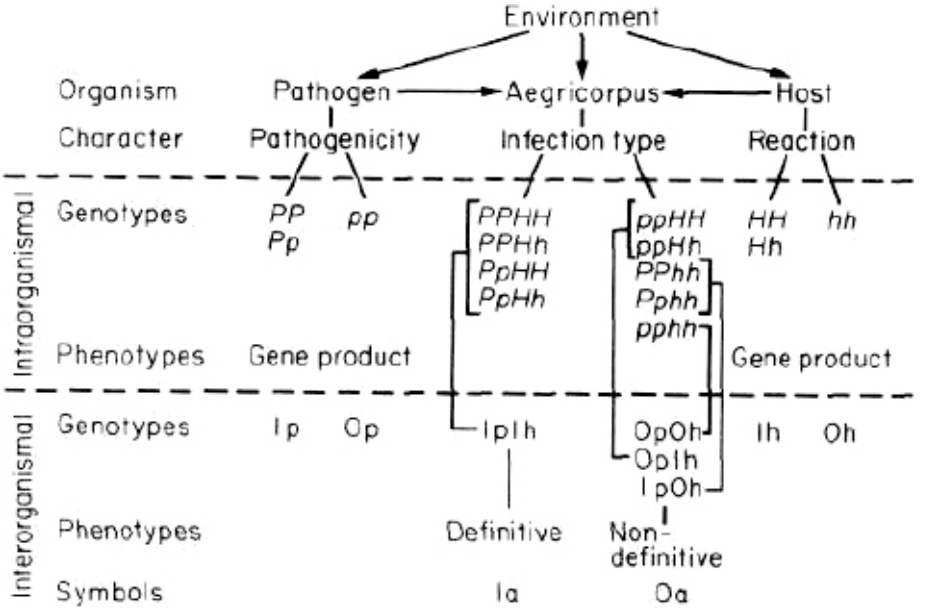


Fig. 4. The category III interaction of interorganismal genetics representing a set of corresponding gene pairs typical of the cereal rusts.

1. The Category III Genetic Interaction

Figure 4 is an expanded version of the gene-for-gene model (Fig. 1) and shows the complexity of the category III interaction. As presented, it represents what has been found for most, but not all, known CGP in the cereal rusts, but it can be adapted to all symbiotic associations.

a. The Organisms. The pathogen and host together produce the aegricorpus, and all three are organisms (Fig. 4). Certainly, the aegricorpus is not an organism in the same sense as the pathogen and host but exists at a different level of biological activity. We recognize the various cereal rusts macroscopically not only by signs (color of the massed urediospores) and symptoms (chlorosis and/or necrosis), but also by the shape and location of the pustules, which are neither signs or symptoms. Thus the aegricorpus has an identity of its own, is organized, and is living (sometimes the life span is very short). It is in this sense that the aegricorpus is considered an organism.

b. The Characters. A genetic character deals with the variations in phenotype resulting from the presence of more than one allele at a locus. For example, seed color in wheat is a character that is variable in its expression as a

result of various combinations of alleles at three loci. In the cereal rusts the characters include pathogenicity of the pathogen, reaction of the host, and the IT of the aegricorpus (Fig. 4). None of these indicates what the phenotype is. Pathogenicity is used in the sense that Nelson *et al.* (1970) defined it, "as the ability of an intity to incite disease on given members of a host species." Reaction is a poor term, because it suggests that the host reacts and the pathogen attacks. This is a decidedly anthropocentric point of view and is not what the word is meant to convey, but is used with respect to the host in the same way as pathogenicity is considered a character of the pathogen. Infection type is used in its traditional sense. In the cereal rusts ITs are generally identified by codes for descriptions of their appearance. In many other diseases such codes are not available; only codes for the amount and/or incidence of disease are available. It is important that we do not use the latter codes in the same way we use infection type in studies of interorganismal genetics.

c. Intraorganismal Genotypes. The intraorganismal genotypes of the pathogen and host are taken from Fig. 1. The genotypes of the aegricorpus are the nine possible combinations of the homo- and heterozygous genotypes of pathogen and host (Fig. 4).

d. Intraorganismal Phenotypes. As pointed out previously, only the aegricorpus has a phenotype as measured by infection type. The pathogen and host have phenotypes (Fig. 4) measured only by specific gene products (or elaborations of them) or absence of such products (Loegering and Sears, 1981). It is important to distinguish these two concepts, because biochemical research is often based on IT as a measure of one of the organisms in the symbiosis. Furthermore, biochemical characteristics are ascribed to that organism, when in actuality the IT is the result of the biochemistry of both pathogen and host and the biochemical interactions taking place in the aegricorpus. The aegricorpus itself has no genetic control of the latter, even though substances might be isolated from the association that do not occur in either host or pathogen. The host and pathogen, however, do not have phenotypes as measured by IT.

e. Interorganismal Genotypes. As the shift is made from intra- to interorganismal genetics in Fig. 4, a new set of symbols and terminology is presented. The need for these will become apparent in discussion of the category IV interaction, of postulating pathogen and host genotypes, and of general resistance. Although there is a similarity between the concepts of category I and III genetic interactions, they are not the same. In Fig. 1 we see that the four combinations of *P__/H__* give the same result; thus a single symbol can be used for *P__* and another for *H__*—, and two additional symbols are needed for *pp and hh,* a total of four symbols that can be combined in four

ways. This suggests the use of the "truth" tables of Boolean algebra. Two of the possible 16 truth tables of Boolean algebra fit the category III interaction:

A	B
1 + 1 = 1	0 + 0=1
1 + 0 = 0	0+1=0
0 + 1 = 0	1 + 0 = 0
0 + 0 = 0	1 + 1=0

These "truth" tables with their plus and equals signs are used in computer programming and should not be confused with mathematical functions, where 1 + 1 = 2. In Boolean algebra, where 1 + 1 = 1, we mean that item 1 combined with a second item 1 results in a 1 for something else. I chose tabulation A partly at random and partly because it avoids confusion with ideas of "resistance," "virulence," and so on.

In Fig. 4 this "truth" table is put to use. We can have a lp or Op genotype in the pathogen culture for a particular CGP and corresponding lh or Oh genotype in the host cultivar. Note that the lp represents both *PP*and *Pp,* and the Op represents *pp*. Likewise, lh and Oh are used to represent the host genotypes. It is very important to recognize that the 1–0 symbolization represents the genotypes of the two symbionts. These symbols are *not* the intraorganismal genotypes. Thus in using the 1-0 symbolization we do not need to know in a diploid whether the symbols represent the homozygous dominant, the heterozygote, or the homozygous recessive.

f. Interorganismal Phenotypes. At the interorganismal level the pathogen and host do not have phenotypes. The phenotype of the aegricorpus is either *definitive* or *nondefinitive* (Fig. 4). The use of "definitive" was suggested by Wheeler (1975). The definitive phenotype results when both symbionts have the 1 genotype and the non-definitive when either or both symbionts have the 0 genotype. These phenotypes are symbolized as la (definitive) or 0a (nondefinitive), where a is aegricorpus. With these symbols we can change Fig. 1 to represent any category III genetic interaction for any symbiotic association (Fig. 5). (The p and h symbols can be changed to fit the particular association being studied.)

This symbolization adequately symbolizes the cereal rusts because, in so far as we know, la is always a "low" IT. It also avoids consideration of dominance and recessiveness in the associated organisms. If a cultivar is inoculated with a culture and the result is "low" IT, we know that the genotype for at least one CGP is l p/ l h, although we have no information as to dominance and recessiveness in the individual symbionts. If the result is "high" IT, all we know is that at least one of the symbionts does not have the definitive genotype for each of the CGP. If information on the dominance or recessiveness of the lp or lh genotype is desired, then it becomes necessary to do an intra-organismal

genetic study. One organism must be held constant while the other is crossed to a 0 genotype and the segregating population studied by inoculation of or with the constant member of the association.

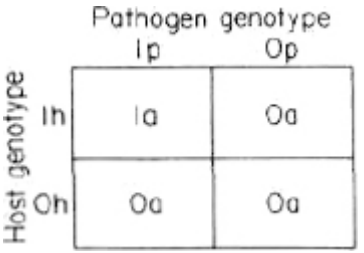


Fig. 5. A generalized model for a single set of corresponding gene pairs in any symbiotic association.

g. The 1–0 *Symbolization Can Be Used for All Symbiosis.* The 1–0 symbols can be utilized for representation of the genetics of all symbiotic associations and is used to represent alleles and genotypes at the intraorganismal level as well as genotypes and phenotypes at the interorganismal level. At the intraorganismal level *P* and *H are* lp and lh alleles, whereas *p* and *h* are Op and Oh alleles. They are shown in Fig. 4 as dominant and recessive by conventional symbolization; that is, capital letters indicate dominant alleles. At the interorganismal level, the lp genotype represents the dominant genotypes of the pathogen, *PP* and *Pp,*whereas the Op genotype represents the recessive *pp* genotype. Likewise, the lh genotype represents the dominant genotypes of the host, *HH* and *Hh,* whereas the Oh genotype represents the recessive *hh* genotype. This representation is typical for nearly all CGP in the cereal rusts. However, there are exceptions, as for example the *Sr17* host gene, where the lh allele is recessive; thus lhsr17 represents the recessive host genotype, and the dominant genotypes are represented by *Ohsr17.* Likewise, the lp allele at the *Vwb* locus in *M. lini* is recessive, and the homozygous recessive is the lp genotype and the dominant genotypes are represented by Op. The 1–0 representation therefore is not dependent on the dominance or recessiveness of the category I interaction of Mendelian genetics.

In Fig. 4 both symbionts are shown as diploid. In the cereal rusts this is usual, although haploid basidiospores produce haploid pycnia on the alternate host. In the powdery mildews of cereals, the fungus growing in association with the cereal host is haploid. Here the lp genotype represents one allele and Op the other allele. The idea that 1 and 0 represent genotypes but are not the genotypes can be extended to include heterokaryotic genotypes and even cytoplasmic factors.

In Fig. 4 there is no indication what the definitive and nondefinitive phenotypes are. Because this treatise deals with the cereal rust, it perhaps is

assumed that the nondefinitive phenotype (Oa) is IT 4 (“susceptibility”), while the definitive phenotype (la) is less than IT 4 (“resistance”). At present we know of no exceptions to this in the cereal rusts; however, in diseases such as Victoria blight of oats the reverse is true, thus definitive and nondefinitive should not and cannot be used as synonyms for “resistance” and “susceptibility.” The la phenotype results only when the l p and l h genotypes occur in the two associated symbionts and in no way indicate what the phenotype is. In summary, the 1–0 symbolization of interorganismal genetics is a representation of alleles, genotypes, and phenotypes without indicating dominance at the intraorganismal level or the appearance of the resulting phenotype of the aegricorpus. The representation is useful for postulating genotypes from IT data (see Section V,B), to design experiments in biochemistry and genetics, and in modifying our philosophical approach to the study of plant pathology.

2. The Category IV Genetic Interaction

The preceding conceptual discussion of the category III genetic interactions and the symbolization introduced permit discussion of the category IV interactions, which are what the geneticist and plant breeder must always deal with. Each set of CGP occur at multiple loci in the genomes of the symbionts. These genomes have two kinds of genes: (1) intraorganismal, which control the characters of each symbiont such as awns, type of panicle, color of spores, and cell wall constituents, and (2) interorganismal, which control the symbiosis. It is highly probable that some genes in one or both symbionts have pleotropic effects and are involved at both the intra- and interorganismal levels.

Category IV involves the interactions between and among CGP. For example, in stem rust of wheat, at least 33 loci for reaction have been clearly identified in the host with seven additional lh alleles at two of these loci. Because allelism rarely occurs in *Puccinia graminis tritici*, there are as many as 40 loci in the pathogen corresponding to the loci and alleles in the host. It has been suggested on the basis of hypothetical genetic studies that there are two to three times this number of loci in host and pathogen—a highly polygenic system that can be illustrated using four sets of CGP. Each set of CGP is identified by a number following the interorganismal symbol to represent the respective loci (e.g., lpl/Ohl, 0p2/lh2). Because each set of CGP can occur in four combinations, four sets can occur as 16 different formulas. Four of these are illustrated and discussed next. In these illustrations it is assumed that IT 4 is the nondefinitive (0a) phenotype, whereas any lower IT is a definitive (la) phenotype.

Pathogen genotype	$\frac{\text{1p1 0p2 1p3 0p4}}{\text{0h1 1h2 0h3 0h4}}$	= IT 4 = 0a (1)
Host genotype		
Category III phenotypes	IT4 IT4 IT4 IT4	
Category III phenotype symbol	0a1 0a2 0a3 0a4	

In Eq. (1) each or the CGP has a genotype that gives the 0a phenotype even though the pathogen has two definitive genotypes (lpl and lp3), whereas their corresponding host genotypes are nondefinitive (Ohl and 0h3). Likewise the host has one definitive genotype (lh2), but the pathogen has the corresponding nondefinitive genotype (0p2). The total result of the genotypes of the four CGP is IT 4 = 0a. Note that the presence of definitive genotypes in one or the other organism does not affect the Category IV phenotype.

Pathogen genotype	$\frac{\text{1p1 0p2 1p3 0p4}}{\text{1h1 1h2 0h3 0h4}}$	= IT 2 = 1a1 (2)
Host genotype		
Category III phenotype	IT2 IT4 IT4 IT4	
Category III phenotype symbol	1a1 0a2 0a3 0a4	

In Eq. (2) one change has been made from Eq. (1). The genotype for locus 1 in the host has been changed from Ohl to l h l. The definitive l p l / l h l gives IT 2, whereas the other three CGP again result in IT 4. Obviously, both phenotypes cannot be expressed. In all work with cereal rusts done to date the definitive phenotype—IT 2 in Eq. (2)—is expressed and thus is "epistatic" to the nondefinitive category III phenotypes.

Pathogen genotype	$\frac{\text{1p1 1p2 1p3 0p4}}{\text{1h1 1h2 0h3 0h4}}$	= IT 1 = 1a2 (3)
Host genotype		
Category III phenotype	IT2 IT1 IT4 IT4	
Category III phenotype symbol	1a1 1a2 0a3 0a4	

In Eq. (3) one change has been made from Eq. (2). The genotype for locus 2 in the pathogen has been changed from 0p2 to lp2. the definitive Ip2/lh2 gives IT 1, whereas the other three CGP have the same phenotypes as in Eq. (2). It is obvious that all three phenotypes cannot be expressed. What has been found is that the lowest definitive phe-notype (IT 1) in Eq. (3) is usually expressed.

Pathogen genotype	$\frac{\text{1p1 1p2 1p3 0p4}}{\text{1h1 1h2 1h3 0h4}}$	= IT 0; = 1a2+3 (4)
Host genotype		
Category III phenotype	IT2 IT1 IT3 IT4	
Category III phenotype symbol	1a1 1a2 1a3 0a4	

In Eq. (4) one change has been made from Eq. (3). The genotype for locus 3 in the host has been changed from 0h3 to lh3. This illustrates complementary interaction between two sets of CGP. In Eq. (4) both the host and pathogen have definitive genotypes at loci 2 and 3, and there is an interaction that results

in IT 0; instead of IT 1 as would be expected on the basis of Eq. (3). Because 0; is the lowest phenotype, it is expressed. If we change Eq. (4) to

$$\frac{1p1\ \ 1p2\ \ 1p3\ \ 0p4}{1h1\ \ 1h2\ \ 1h3\ \ 0h4} = \text{IT } 0; = 1a2{+}3$$

we may assume the interaction involves only CGP 2 and *3*. If additional studies are made and it is found that

$$\frac{1p2\ \ 1p3}{0h2\ \ 1h3} = \text{IT } 3 \text{ and } \frac{1p2\ \ 1p3}{1h2\ \ 0p3} = \text{IT } 1$$

then the interaction is not in the pathogen, and if

$$\frac{0p2\ \ 1p3}{1h2\ \ 1h3} = \text{IT } 3 \text{ and } \frac{1p2\ \ 0p3}{1h2\ \ 1h3} = \text{IT } 1$$

then the interaction is not in the host.

Although this does not prove that the interaction is occurring in the aegricorpus, this hypothesis should be considered seriously. There are numerous reports of interaction between genes for low reaction and a few for low pathogenicity, but none of these has been adequately demonstrated because the necessary complex studies have not been made. More likely, the reported interactions are not category II (intraorganismal) but category IV interactions (interorganismal).

The concept that there are interactions occurring between and among CGP is indispensable when considering the cereal rust diseases, because “gene-for-gene” is a polygenic system, not an oligogenic one as commonly stated in the literature. The latter idea has led to much of the misunderstanding concerning the biology of the pathogen-host

system. In experimental designs and in discussion of the gene-for-gene concept, the assumption that there is only one gene for “resistance” or “virulence” in the association being studied is nearly always false. Person (1959) demonstrated this in his model using five CGP. It is time that biologists recognize that in the cereal rusts (and many other diseases) we do not deal with an oligogenic system, even though it is easy to follow one gene at a time. It is true that a cultivar may not be damaged because it has a lh genotype at one locus and the pathogen population is homogeneous for a corresponding lp genotype. This is a pragmatic and useful point of view, but it fails miserably when used to develop biological hypotheses to be tested.

C. INTERORGANISMAL GENETIC INTERACTIONS ARE COMPLEX

The complexities of interorganismal genetics are illustrated in Fig. 6 using two CGP. In the category I interaction there may be dominance, incomplete dominance, or recessiveness. Although there are no documented instances of category II interactions, this does not mean they do not occur, and possibly reports of inhibitors are of this category. The category III interaction is relatively simple, because genotypes are represented without specifying what is dominant or if there is category II epistasis. The category IV interaction, which is characteristic of the cereal rusts, is extremely complex, involving "epistasis" of several kinds and possibly at two levels. Yet it is the category IV interaction that we see expressed in the cereal rusts.

D. INCOMPLETE DOMINANCE

The occurrence of incomplete dominance in category I causes additional complexities and may lead to misinterpretations of genetic data. The data of Samborski (1963) illustrate this. He worked with CGP lr9.

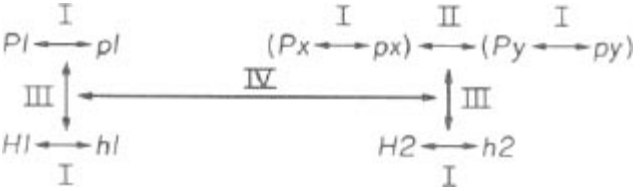


Fig. 6. The genetic interactions of symbiosis. *P, p,* and *H, h* are generalized gene symbols for pathogen and host, respectively. *1, 2, x,* and y are loci, and I—IV are the categories of genetic interaction.

The *lhlr9* allele was transferred from *Aegilops umbellulata* Zhuk. to hexaploid wheat by Sears (1956), and the breeding line was named "Transfer." Over a period of several years this cultivar was essentially immune to all cultures of *Puccinia recondita* Rob. ex. Desm.; however, Samborski (1963) observed IT 1+ on "Transfer" in plots at Winnipeg. A culture was established on the cultivar "Thatcher," which lacks the lhir9 genotype, and telia were produced. The *S1* gave three kinds of cultures, which on "Transfer" gave ITs 0;, 1+, and 4, respectively, indicating that the original culture was heterozygous for pir9, and incomplete dominance occurred. Samborski retained cultures that appeared to be homozygous for the partially dominant lpir9 allele and the recessive 0pir9 allele. He next crossed "Transfer" and "Thatcher" and produced an adequate number of F1 (heterozygous) seeds. He then inoculated the two cultivars and their F1 with the three cultures of *P. recondita* and obtained the following results (Samborski, 1963):

Host genotype	Pathogen genotype					
	PP		*Pp*		*pp*	
RR (Transfer)	0	(R)	1+	(R)	4	(S)
Rr F_1	0	(R)	3	(S)	4	(S)
rr (Thatcher)	4	(S)	4	(S)	4	(S)

With the homozygous lpir9 culture he found that the lhir9 allele was clearly dominant and resulted in IT 0 in both the homozygous and heterozygous condition. With the heterozygous culture he obtained IT 1+ on "Transfer," IT 3 on the F1, and IT 4 on "Thatcher." This indicated that the *lhlr9* allele was incompletely dominant or, if we use the concepts of race identification in which IT 3 and 4 = susceptibility, then the *lhlr9* allele was recessive. This latter interpretation is shown in parentheses above using R and S as phenotypic symbols. These data suggest a "reversal of dominance," and several studies with other rusts have been interpreted in this manner. Very likely, the true interpretation is that two cultures, one homozygous and the other heterozygous, were used in those studies.

E. GENE SYMBOLS

The intraorganismal gene symbols for the cereal rusts have been assigned by three methods. In the wheat rusts the common name of the disease has been the basis; for example, *sr*, stem rust; *h*, leaf rust;

and *yr*, yellow rust (stripe rust). In the oat rusts the Latin name of the pathogen has been the basis, for example, *pc* = *Puccinia coronata.* Flor assigned letters to the loci in the host and then used the allele designation to indicate the corresponding gene in the pathogen. Uniformity would be desirable but is not necessary; however, the use of a symbol based on the disease name is more logical, because both the pathogen and host produce the disease and the same designation can be used for both species.

Mcintosh (1973) has recommended that for the wheat rusts the convention of upper and lower case initial letters should not be used to indicate dominance and recessiveness. This will avoid confusion that can arise from several sources. Not all lh (or lp) genotypes represent dominant genotypes; for example, *hsr11* is dominant for the lh genotype, whereas *hsrl7* is dominant for the Oh genotype. Perhaps more important is that dominance and recessiveness become meaningless in cases where allelism occurs. If a cultivar homozygous for *lhsr9a* is crossed with one homozygous for l*hsr9b* and the segregating population inoculated with a culture of the genotype *Ipsr9a0psr9b*, the *sr9a* allele is dominant and the *sr9b* allele recessive. If, however, the segregating population is inoculated with a culture

of the genotype *0psr9alpsr9b* the reverse is true. Thus the apparent dominance and recessiveness exhibited in the host is due not to the host alleles, but to the genotype of the pathogen. Furthermore, there are no *proven* cases of alleles for the Oh genotype. This first became apparent from the work of Kerr (1960), who showed that the recessive Oh allele in Bison flax at the *1* locus was in reality a dominant lh allele *[L9)* when certain Australian *M. lini* cultures were used to inoculate Bison. Loegering and Sears (1981) clearly demonstrated that absence of a locus resulted in the Oh genotype for the *sr6, sr8, sr9a,* and *srl 1* loci in wheat. In addition, they showed that even when the lh alleles were present, the la phenotype could be changed to 0a by changing the pathogen genotype or in the case of *sr6* by changing the temperature.

When conducting studies at the intraorganismal level, the conventional symbolization may be used by specifying what the definitive and nondefinitive phenotypes are and then holding one member of the symbiosis contant.

V. Applications of Interorganismal Genetics

Over the past 40 years concepts of interorganismal genetics have evolved as a result of the discovery of the gene-for-gene relationship.

As a consequence, there has been a change in how we think of the pathogen—host association. We can view the aegricorpus as a natural and normal symbiotic association instead of an unnatural and abnormal disease. Such a viewpoint should and can change our view of plant pathology from its present pragmatism to a more fundamental understanding of the biology of the pathogen—host symbiosis.

There are many possible applications of interorganismal genetics with respect to cereal rusts. Only two will be discussed: (1) deriving hypothetical genotypes of pathogen and host from IT data and (2) developing a theoretical genetic basis for general resistance.

A. HYPOTHETICAL GENOTYPES BASED ON IT DATA

Computers are useful tools for development of hypotheses regarding the pathogenicity genotypes of pathogen cultures and reaction genotypes of host cultivars without making crosses. Such hypotheses are useful in developing experimental designs for basic studies in genetics and biochemistry, and as a basis for breeding programs. In the past breeders and pathologists have intuitively used the principles when they have transferred a “new” gene for “resistance” to new commercial cultivars. Unfortunately, their viewpoint that the resulting resistance was determined by the host gene alone has been

adopted by geneticists, pathologists, physiologists, and biochemists. As a result a basic understanding of the pathogen—host association has eluded us.

To develop hypothetical genotypes of pathogen and host from IT data, we use the principles of interorganismal genetics. In the cereal rusts we know that l p / l h = la. This category III formula can be reversed, and we find that la = l p / l h . Thus if we inoculate a cultivar with a culture and obtain a la phenotype, we know that in at least one set of CGP the pathogen has a lp genotype and the host the corresponding lh genotype. On the other hand, if we find a Oa phenotype we know that if the pathogen has any lp genotypes, the host has the corresponding Oh genotypes, or if the host has lh genotypes, then the pathogen has the corresponding Op genotypes. Thus we would not know the genetic reason for the Oa phenotype. In day-to-day laboratory investigation we actually work in this manner. For example, when we inoculate two cultivars with two cultures the results are obtained as infection types:

		Culture X	Culture Y
Cultivar	A	0;	4
	B	4	4

Initially, we knew nothing about the genotypes of the cultures or cultivars, but the IT 0; tells us that for one set of CGP, culture X has the lp genotype and cultivar A the corresponding lh genotype. Thus we can insert this information into the box.

			Culture X lp	Culture Y
Cultivar	A	lh	0;	4
	B		4	4

We now know the genotype for culture X and cultivar A with regard to one set of CGP. If cultivar A has the *lh* genotype but when inoculated with culture Y we obtain the 0a phenotype, we know the culture must have the corresponding *0p* genotype. The same reasoning can be used with culture X and cultivar B. Thus we can fill in the genotypes as follows:

			Culture X 1p	Culture Y 0p
Cultivar	A	1h	0;	4
	B	0h	4	4

To use this in computerized studies we need to put the diagram in its final form as follows:

			Culture X 1p	Culture Y 0p
Cultivar	A	1h	1a	0a
	B	0h	0a	0a

In the explanation of Flor's work we started with known genotypes and derived phenotypes. Here we start with known phenotypes and derived genotypes. To understand that this shift has been made is fundamental to deriving pathogen and host genotypes from IT data.

There are seven basic patterns (Fig. 7) of results from inoculating two cultivars with two cultures, although there may be as many as four variations in each pattern (Loegering and Burton, 1974). For example, in Fig. 7A, the la could be found in any one of the four corners of the diagram. This results in a change in the hypothetical genotypes for the cultures and cultivars but does not change the principle. The three additional variations of Fig. 7A are as follows:

	1p	0p
0h	0a	0a
1h	1a	0a

	0p	1p
0h	0a	0a
1h	0a	1a

	0p	1p
1h	0a	1a
0h	0a	0a

In Fig. 7B using the same logic we can identify two sets of CGP. Of the seven basic patterns only Fig. 7A and B permit assigning genotypes to both cultures and both cultivars. The other five patterns leave at least one of the genotypes as an unknown. Figure 7C and D leave the genotype for one culture and cultivar, respectively, as unknown. Because there is at least one set of CGP, we arbitrarily assign lp/lh to one of the la phenotypes. The second la phenotype could be due to the same CGP or due to a second set. Thus one la phenotype is of unknown genotype, and one culture in Fig. 7C and one cultivar in Fig. 7D are of unknown genotype. Figure 7E is made up of Fig. 7B, C, and D. It is like Fig. 7B because there are two la phenotypes on a diagonal opposite a 0a

phenotype, demonstrating that two CGP are present. This diagonal is extremely useful in examining large data sets visually. The la phenotype in the corner opposite the 0a phenotype makes Fig. 7E a combination of Fig. 7C and D, and the same unknown genotypes of these two configurations are present. Figure 7F and G are the last two configurations and give little or no useful information regarding genotypes but are important for this reason.

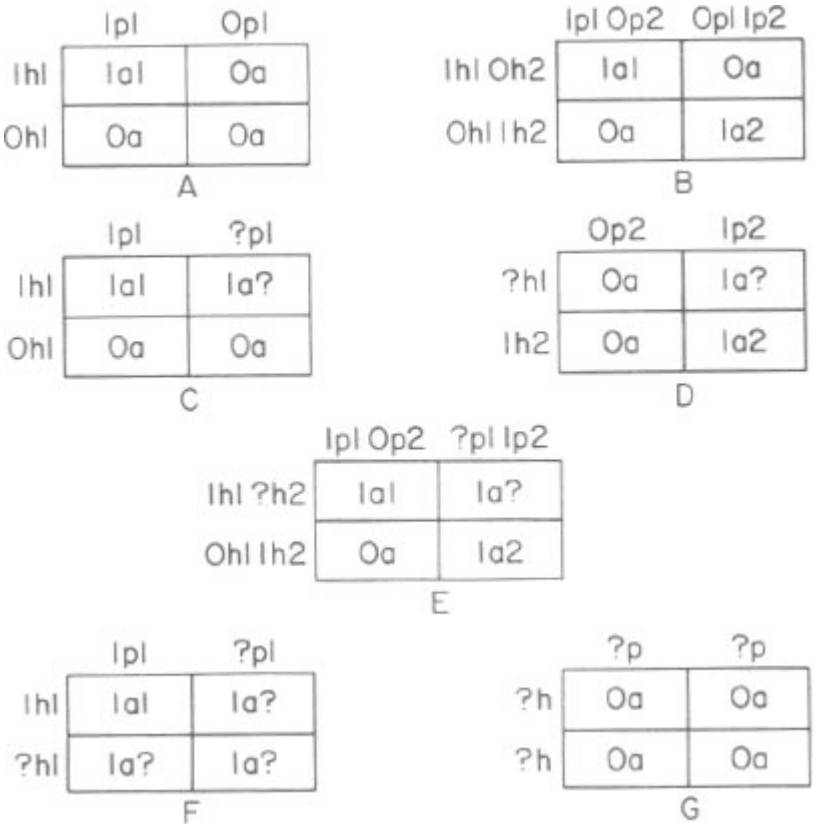


Fig. 7. The seven basic "box arrangements" resulting from inoculating two host cultivars (sides) with two pathogen cultures (tops), lp, Op, lh, and Oh represent definitive (1) and nondefinitive (0] genotypes of pathogen (p| and host (h); la and 0a represent the definitive (1) and nondefinitive |0) phenotypes of the aegricorpus (a); 1 and 2 following la or 0a represent corresponding gene pairs and the definitive phenotype. The question mark has two meanings: la? means that a la phenotype was observed, but it is uncertain what the lp/lh genotype is; ?pl means that it is not certain whether the genotype is lpl or 0pl. Adapted from Loegering and Burton (1974).

These seven basic boxes are used in computer analysis of a data set made up of the infection types from inoculation of a group of cultivars with a group of

cultures. Three kinds of information can be obtained: (1) grouping cultivars and/or cultures that in a data set have the same patterns, (2) comparing cultures and/or cultivars of unknown genotype with ones of known genotype, and (3) postulating genotypes of the cultivars and/or cultures in the data set. The methods (Loegering *et al.*, 1971; Loegering and Burton, 1974) of doing these operations will not be detailed here. There is some variation in the procedures as developed by these authors and that developed by Browder and Eversmeyer (1980) and as applied by Roelfs *et al.* (1982). This variation is based on the degree of confidence the respective workers have in their ability to distinguish variations in the la phenotypes; however, the basic principles are the same.

In the method of Loegering *et al.* (1971; Loegering and Burton, 1974), all data are classified as la or 0a (L and H in their original publications); thus there are only two classes, whereas Browder and Eversmeyer (1980) and Roelfs *et al.* (1982) use each different IT, and whenever a diagonal of two ITs occurs opposite a higher IT, those on the diagnoal indicate two different CGP. The valid criticism made of the Browder and Eversmeyer method by Knott and Johnson (1981), that the final results of the computer analysis are dependent on the initial arrangement of the data set, is also true for the method of Loegering *et al.* (1971). It must be remembered, however, that the results obtained by either method only develop hypotheses to be tested and not proof.

B. GENERAL RESISTANCE

For centuries farmers observed that some individual plants were less damaged by diseases than other individuals. These less damaged plants

were propagated, and sometimes the farmers were successful in avoiding some of the damage from certain diseases. Evidence for this is found in the winter wheats grown in the south-central United States. Even in severe outbreaks of leaf rust, many of the wheats return an acceptable yield. These wheats originated from collections made in farmers' fields in southwest Asia where they had been grown for centuries.

In 1905, Biffin found that resistance to *Puccinia striiformis* West was controlled by a single gene. The "monogene" concept of resistance in plants to diseases was born, and over half a century of breeding for disease resistance was based on the lodestone of "monogene" resistance. Then, suddenly, what farmers had known for centuries was rediscovered but was now called "horizontal resistance." Attempts to define horizontal resistance have not been successful, and many other names have been applied to the phenomenon. Of these, "general resistance," as defined by Caldwell (1968) as being "durable," has some validity and is used in this chapter. Considering our present understanding of the phenomenon, the definition used by Loegering (1972),

"that 'non-specificity' can only be defined as a host-pathogen relationship for which specificity has not been demonstrated," is useful.

At present it seems likely that much of what we call general resistance is due to specificity. The first indication that this might be true was published by Slezinsky and Ellingboe (1969). They worked with powdery mildew of wheat and studied the transfer of ^{35}S from the host to the pathogen using the quadratic-check experimental design. The relative amount of ^{35}S per conidium was low for l p / l h, high for Op/Oh and lp/0h, but intermediate for 0p/lh, indicating that the 0p/lh genotype was physiologically different from the lp/0h and 0p/0h genotypes, even though the phenotypes (ITs) did not appear to differ. This raised the question whether or not careful measurements would reveal differences in disease development. That such differences do occur was shown by Martin and Ellingboe (1976) using powdery mildew of wheat. They found that 0p*pm4*/lh*pm4*compared with Ip*pm4*/0h*pm4* and 0p*pm4*/0h*pm4* showed reduced infection efficiency and longer generation time, even though the final phenotype was IT 4.

In stem rust of wheat Rowell (1981) showed for the *Srtt 1* CGP that 0p/lh had a strong effect on the 0a phenotype. Skovmand *et al.* (1978), however, could not demonstrate differences between 0p/lh and Op/Oh for the *Sr5, Sr6, Sr7b,* and *Si11* CGP. Thus it appears that for some CGP the 0p/lh is not equal to the lp/0h and 0p/0h genotypes, whereas for other CGP they are.

Applying interorganismal genetics to these observations, it can be shown how general resistance theoretically could be due to specificity using the following assumed information for three CGP designated as X, Y, and Z. The la phenotype for each of the three CGP is IT 0; and for the 0a phenotypes, IT 4. We then measure the latent period, spore production, and infection efficiency of the nine possible 0a phenotypes. By comparing these we find that 0p/lh for *Srx* has a latent period of one extra day, for *Sry* 20% fewer spores, and for *Srz* an infection efficiency of 75%. Let us also assume that each of these reduces the rate of increase by 3%. Such a decrease would be difficult to determine by simple observation, but if all three occur together as the additive effects of a category IV interaction perhaps would result in a reduction in rate of increase greater than 3%. This would still be difficult to measure under field conditions. Very likely, however, the effect would be more than additive, in that it would take longer to produce fewer spores and fewer spores would produce infections. Thus reduction in rate of increase might be great enough to be observed in the field and might be mistaken for "general resistance."

$$\frac{0px\ 0py\ 0pz}{1hx\ 1hy\ 1hz} = 0a$$

There are two features of such a system that can explain the ideas of durability and polygenic inheritance, which are often used in defining the

phenomenon of general resistance. Durability is built into the system, because if the pathogen acquires the lp genotype at any of the three loci through hybridization, mutation, or parasexualisim, the result would be IT 0:. Because any of the l p / l h genotypes would result in this phenotype, a culture with a lp genotype at one of the loci would not survive, thus the Op genotype is maintained. In nature a host that changed from lh to 0h at any of the three loci would have the potential of being more heavily damaged and thus would have a reduced survival capability. Thus there would be a tendency for the 0p/lh system to be self-perpetuating in nature. When using IT as the measure of resistance in a breeding program, one would be apt to lose the host lh genotype combinations associated with general resistance without being aware of doing so.

The origin of the idea that general resistance is polygenic and a character of the host should be apparent. Crosses of the 0px0py0pz culture with a lpxlpylpz culture and using the lhxlhylhz cultivar as a tester would result in segregation based on IT. However, if the cultivar was crossed with a 0hx0hy0hz cultivar and inoculated with the 0px0py0pz culture there would be a range of disease development in terms of rate of increase. The distribution of the segregating progeny would be dependent on the variation of the hypothetical 3% reduction of each pair of CGP as well as on what effect heterozygosity would have on disease increase.

When general resistance results from the 0p/lh genotype, it differs genetically from the l p / l h genotype, as demonstrated by Johnson and Taylor (1976), where reduced sporulation was the result of a l p / lh genotype. The definitive lp*sr13*/lh*sr13* gives IT 3-, which also results in reduced sporulation. There seems to be no reason that some morphological characters of the host could not result in reduced disease. General resistance due to an accumulation of 0p/lh genotypes in the pathogen-host association and/or to morphological characters of the host would likely be durable, whereas that due to l p / l h genotypes perhaps would not because a change from lp to Op would have survival value for the fungus.

References

Biffin, R. H. (1905). Mendel's laws of inheritance and wheat breeding. *J. Agric. Sci.* **1,** 4–48.

Browder, L. E., and Eversmeyer, M. G. (1980). Sorting of *Puccinia recondita: Triticum* infection-type data sets toward the gene-for-gene model. *Phytopathology* **70,** 666-670.

Caldwell, R. M. (1968). Breeding for general and or specific plant disease resistance. Proc. *Wheat Genet. Symp., 3rd. 1968* pp. 227-238.

Flor, H. H. (1946). Genetics of pathogenicity in *Melampsora lini. J. Agric. Res. (Washington, D.C.)* **73,** 335-357.

Flor, H. H. (1947). Inheritance of reaction to rust in flax. *J. Agric. Res. (Washington, D.C.)* **74,** 241-262.

Flor, H. H. (1959). Genetic controls of host-parasite interactions in rust diseases. *In* "Plant Pathology: Problems and Progress, 1908-1957" (C. S. Holton *et al.*, eds.), pp. 137-144. Univ. of Wisconsin Press, Madison.

Flor, H. H. (1971). Current status of the gene-for-gene concept. *Annu. Rev. Phytopathol*. **9,** 275-296.

Johnson, R., and Taylor, A. J. (1976). Spore yield of pathogens in investigations of the race-specificity of host resistance. *Annu. Rev. Phytopathol.* **14,** 97-119.

Kerr, H. B. (1960). The inheritance of resistance of *Linum usitatissimum* to the Australian *Melampsora lini* race complex. *Proc. Linn. Soc. N.S.W.* **85,** 273-321.

Knott, D. R., and Johnson, R. (1981). The sorting and analysis of infection types from *Triticum aestivum/Puccinia recondita* interactions. *Phytopathology* **71,** 1010-1012.

Loegering, W. Q. (1966). The relationship between host and pathogen in stem rust of wheat. *Hereditas, Suppl.* **2,** 167-177.

Loegering, W. Q. (1972). Specificity in plant disease. *In* "Biology of Rust Resistance in Forest Trees," Misc. Publ. 1221, pp. 29-37. Forest Service, Washington, D.C.

Loegering, W. Q., and Burton, C. H. (1974). Computer-generated hypothetical genotypes for reaction and pathogenicity of wheat cultivars and cultures of *Puccinia graminis tritici. Phytopathology* **64,** 1380-1384.

Loegering, W. Q., and Powers, H. R., Jr. (1962). Inheritance of pathogenicity in a cross of physiologic races 111 and 36 of *Puccinia graminis* f. sp. *tritici. Phytopathology* **52,** 547–554.

Loegering, W. Q., and Sears, E. R. (1981). Genetic control of disease expression in stem rust of wheat. *Phytopathology* **71,** 425-428.

Loegering, W. Q., Mcintosh, R. A., and Burton, C. H. (1971). Computer analysis of disease data to derive hypothetical genotypes for reaction of host varieties to pathogens. *Can. J. Geneti. Cytol*. **13,** 742-748.

Mcintosh, R. A. (1973). A catalogue of gene symbols for wheat. *Proc. Int. Wheat Genet. Symp. 4th, 1973* pp. 893-937.

Martin, T. J., and Ellingboe, A. H. (1976). Differences between compatible parasite/host genotypes involving the *Pm4* locus of wheat and the corresponding genes in *Erysiphe graminis* f. sp. *tritici. Phytopathology* **66,** 1435-1438.

Nelson, R. R., McKenzie, D. R., and Scheifele, G. L. (1970). Interaction of genes for pathogenicity and virulence in *Trichomelasphaeria turcica* with different numbers of genes for vertical resistance in *Zea mays. Phytopathology* **60,** 1250-1254.

Person, C. (1959). Gene-for-gene relationships in host:parasite systems. *Can. J. Bot*. **37,** 1101-1130.

Roelfs, A. P., Baker, F. D., and McVey, D. V. (1982). An interactive computer-based system for comparing cultures of *Puccinia graminis* and postulating *Sr* genotypes in wheat. *Phytopathology* **72,** 597-600.

Rowell, J. B. (1981). The relationship between slow rusting and a specific resistance gene for wheat stem rust. *Phytopathology* **71,** 1184-1186.

Rowell, J. B., Loegering, W. Q., and Powers, H. R., Jr. (1963). Genetic model for physiologic studies of mechanisms governing development of infection type in wheat stem rust. *Phytopathology* **53,** 932-937.

Samborski, D. J. (1963). A mutation in *Puccinia recondita* Rob. ex. Desm. f. sp. *tricici* to virulence on Transfer, Chinese Spring x *Aegilops umhellulata* Zhuk. Can. J. Bot. **41,** 475-479.

Sears, E. R. (1956). Genetics in plant breeding. *Brookhaven Symp. Biol*. 9, 1-22.

Skovmand, B., Roelfs, A. P., and Wilcoxon, R. D. (1978). The relationship between slow-rusting and genes specific for stem rust resistance in wheat. *Phytopathology* **68,** 491-499.

Slezinski, R. S., and Ellinboe, A. H. (1969). The effects of various parasite/host genotypes on ^{35}S transfer from wheat to *Erysiphe graminis* f. sp. *tritici. Phytopathology* **59,** 1050 (abstr.).

Wheeler, H. (1975). "Plant Pathogenesis." Springer-Verlag, Berlin and New York.

7

Histology and Molecular Biology of Host—Parasite Specificity

R. Rohringer

Agriculture Canada Research Station, Winnipeg, Manitoba, Canada

R. Heitefuss

Institut fur Pflanzenpathologie und Pflanzenschutz, Göttingen–Weende, Federal Republic of Gennany

I. Introduction

Analysis and explanation of host-parasite specificity in cereal rusts requires an approach from genetical, histological, and biochemical points of view. Although considerable progress has been made in recent years, the high standards of molecular biology and its combination with ultrahistology and genetics have rarely been achieved in investigations of cereal rust systems. However, considerable impetus has come from these fields, and this has stimulated new ideas and experimental approaches that were made possible by progress in methodology.

A. IMPACT OF GENETICS

Understanding and interpretation of host-parasite genetics was strongly influenced by the classical gene-for-gene concept (Flor, 1956; cf. Flor, 1971), as discussed in detail in Chapter 6 by W. Q. Loegering in this volume. On the basis of this concept, Rowell *et al.* (1963) proposed use of a quadratic check consisting of two host and two pathogen lines differing in one gene each as an experimental set for investigating gene-for-gene specificity. This has stimulated a considerable number of investigations to correlate physiological and biochemical differences with compatibility or incompatibility in the host-parasite interaction (cf. Ellingboe, 1976, 1981). The introduction of near-isogenic lines, differing in specific genes for resistance, has further improved the chances to identify the reactions responsible for compatibility and incompatibility (Loegering and Harmon, 1969; Daly, 1972).

The genetic data so far suggest that for a number of host–pathogen systems where the gene-for-gene hypothesis applies, the specific interaction of genes or gene products is associated with incompatibility and not compatibility. On the basis of several pieces of evidence, the conclusion has been drawn that incompatibility is the active process requiring a gene product for resistance produced by the host and a gene product for avirulence produced by the pathogen (Ellingboe, 1976, 1981, 1982). In contrast, the suggestion of Daly (1972) that "induced susceptibility" may be the active process implies, in its simplest form, the existence of a specific interaction between products of genes for susceptibility and products of genes for virulence. As Heath (1981a) has pointed out, the experimental data available so far supply some support for, but no clear evidence against, this latter hypothesis.

B. LEVELS OF SPECIFICITY

Attention has been drawn to different levels of specificity, a concept that is of relevance to the molecular biology of host-parasite interactions (Heath,

1980, 1981a,b). This approach distinguishes “nonhost resistance” from “cultivar resistance.” Heath, in accordance with Ward and Stoessl (1976), defines nonhost resistance as a more general type of one or more defense reactions nonspecifically triggered by a microorganism on a plant species that normally cannot be considered to be a host for it. This nonhost resistance is apparently effective in protecting plants against the overwhelming majority of microorganisms capable of using higher plants as a substrate.

Ellingboe (1976) presented, and Heath (1981b) further discussed the hypothesis that host specificity may primarily be determined by a “basic compatibility” between host and pathogen. Ellingboe (1976) approached this from an evolutionary viewpoint and proposed that host and parasite interacting over a period of time would be expected finally to exchange low and high molecular weight compounds and possibly even organelles, thereby achieving a state of harmony or coexistence, which he termed “basic compatibility.” According to Heath (1981a,b), this state can be reached only if the pathogen has not triggered, or has overcome, the nonspecific defense reactions of the “non-host resistance,” that is, if an essential metabolic relationship or “induced susceptibility” has been established (Fig. 1).

Bushnell and Rowell (1981) and Heath (1982) have presented a hypothesis to explain, on a molecular level, the relationship between “basic compatibility” and “cultivar resistance.” In this, it is postulated that specific host receptors recognize and fit fungus-derived suppressors, and that this recognition renders inoperable the defense mechanism triggered in response to fungus-derived nonspecific elicitors. This then would establish a “basic compatibility” state. If the host acquires a gene specifying cultivar resistance, altering the receptor site for the suppressor, the latter no longer “fits,” and elicitor action can take place (cultivar resistance), until the parasite population, through mutation and selection pressure, acquires the corresponding virulence gene. This, in turn, would restore the suppressor—receptor fit, so that the elicitor would not longer be effective (cultivar susceptibility). This hypothesis separates the problem of specificity into different but interconnected levels and assigns specificity to fungus-derived suppressors. This is in contrast to the conventional view according to which elicitors (of phytoalexin synthesis), also derived from the fungus, may carry specificity. It would be most important to know the molecular events that determine the different levels of specificity and which amount of information and structural difference must be present in a molecule to be recognized within this system. As a first step in efforts to answer these questions, it is important to determine which structural components of host and parasite are involved in these specific interactions and at what stage in the interaction they are operative.

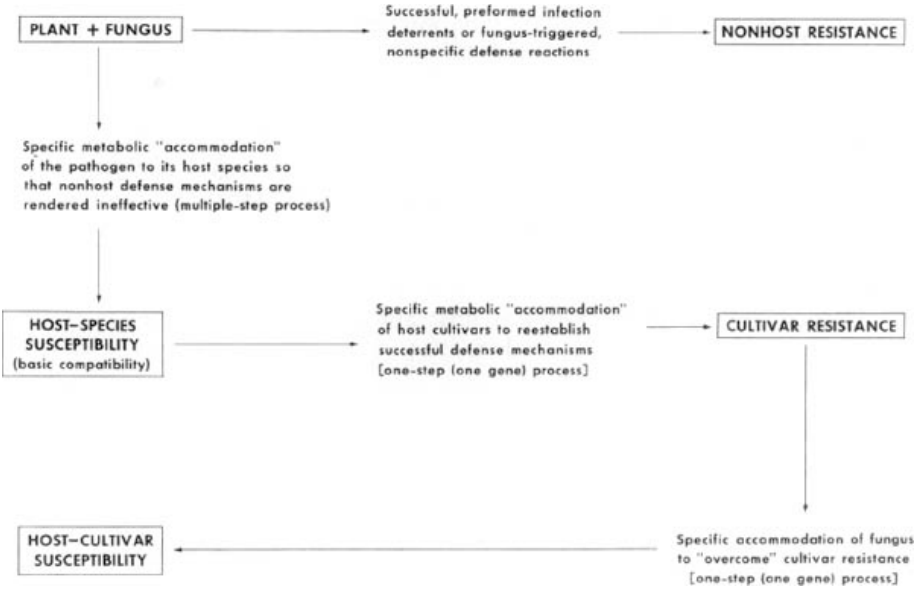


Fig. 1. Postulated events leading to species and cultivar specificity of a fungal pathogen (redrawn from Heath, 1981b).

II. Histology

A. GENERAL REMARKS

Histological studies on cereal rusts have experienced a kind of renaissance in recent years, probably for three main reasons: (1) to determine the phenomena that should be investigated biochemically in order to clarify the molecular mechanism specifying resistance in a particular interaction, |2) to compare the histological events in hosts that are better defined genetically than those available earlier, and (3) to take advantage of recent technological advances in histology and histochemistry.

1. Variability among Incompatible Interactions

Much of the histological work at the light microscope level on rust-infected cereals was undertaken to help explain the basis of host cultivar specificity, but optimistic expectations of earlier days, which often implied that observations made on one particular incompatible interaction may be typical for many, have not been fulfilled. Rothman (1960) distinguished at least three different types of

incompatible interactions between eight cultivars of oats and *Puccinia coronata*. In a large histological survey of cultivars and single-gene lines of wheat infected with stem rust of wheat, Brown *et al.* (1966) and Ogle and Brown (1971) found that there was no consistent relationship between leaf area colonized and leaf area exhibiting necrosis. A more recent example of the differences in the histopathology of incompatible interactions was provided by Mendgen (1978), who reported on the *Uromyces phaseoli–french* bean system. This diversity on a cellular level demonstrated quite clearly that it is difficult to generalize about resistance mechanisms operating in incompatible interactions in one particular host–parasite system, let alone in different combinations involving another host (Heath, 1976).

In addition to the effect of major genes for resistance, the genetic background in which they operate has been shown to modify the histologically observable events in certain gene interactions (Brown *et al.*, 1966; Rohringer *et al.*, 1979). On the surface, this view may appear to be rather pessimistic, but it probably will be very helpful to further research in this area if we expect to find more differences than similarities in incompatible interactions. In a purely pragmatic sense, the great variability existing among incompatible interactions presents a problem when attempts are made to classify them into histopathologi-cal types. Without knowledge of the mechanisms involved, any classification is bound to be artificial. The grouping used in Section II,B-E recognizes the salient features of tissue necrosis and inhibition of the pathogen as a function of time after inoculation. Table I gives an overview of incompatibility features of interactions discussed in this chapter. For further details regarding classification of infection types, see Chapter 5 on race specificity by A. P. Roelfs in this volume.

2. Detection of Hypersensitive Cell Necrosis

Necrosis is best defined in morphological terms because little is known about the functional impairment of the cell as a part of the necrotization process. The onset of necrosis can be defined morphologically as the stage at which the first symptoms of structural disorganization become visible with the electron microscope. Typically, disorganization of subcellular structures follows a different pattern in host cells and in cells of the parasite (Harder *et al*, 1979b). A totally necrotic cell is electron-dense, and its structure is disorganized to such an extent that individual organelles are no longer recognizable. By light microscopy, necrotic cells stain differently and more intensely with Trypan Blue, and necrotic host cells, after fixation and removal of chlorophyll, display a characteristic type of autofluorescence that can readily be distinguished from the weak autofluorescence exhibited by normal cells.

Table I

Some Incompatibility Features of Cereal-Rust Combinations

Cereal–rust combination and effective gene for resistance/gene for avirulence	Infection type[a]	First evidence (hr after inoculation) of	
		Inhibition of fungal growth	Host cell necrosis or collapse
Wheat–*Puccinia graminis* f. sp. *tritici*[b]			
Sr6/P6	1+	≤24	≤24
Sr5/P5	0	≤24	≤24
Sr8/P8	1+	72	60
Sr22/P22	2	96	72
Wheat–*Puccinia recondita*[c] *Lr20/P20*	;	48	36[e]
Oats cv. Shokan I–*Puccinia coronata* race 226[d]	0	20	28

[a]For those interactions in which expression of incompatibility is temperature dependent (*Sr6/P6, Lr20/P20*), the infection type listed is that observed at the nonpermissive (lower) temperature.
[b]Rohringer *et al.* (1979).
[c]Jones and Deverall (1977a).
[d]Tani and Yamamoto (1979).
[e]First physiological changes detected 20 hr after inoculation.

The fluorescence technique for detection of necrotic host cells was pioneered for rust-infected tissue by Marte and Montalbini (1972), who had shown that cellular autofluorescence of a certain type is correlated with conventional staining properties characteristic for necrotic bean cells. Recent unpublished observations have shown that this type of autofluorescence in hypersensitively reacting wheat leaf cells emanates largely from the cell content as well as the cell wall (Figs. 2–4). The identity of the autofluorescing material is not known; treatment with alkali, that would be expected to remove ester-bound phenolic acids, caused little loss of fluorescence intensity, but delignification with chlorine dioxide reduced fluorescence of cell walls to negligible levels (Beardmore *et al.*, 1983). That this type of wheat cell autofluores-cence is not specific to incompatible interactions between host and pathogen was demonstrated by treatment of healthy leaves with di-ethylaminoethyldextran (DEAE-dextran), which induced necrosis in cells that then showed the same type of autofluorescence and similar ultrastructural changes as could be observed in hypersensitively reading cells (Harder *et al.*, 1979b).

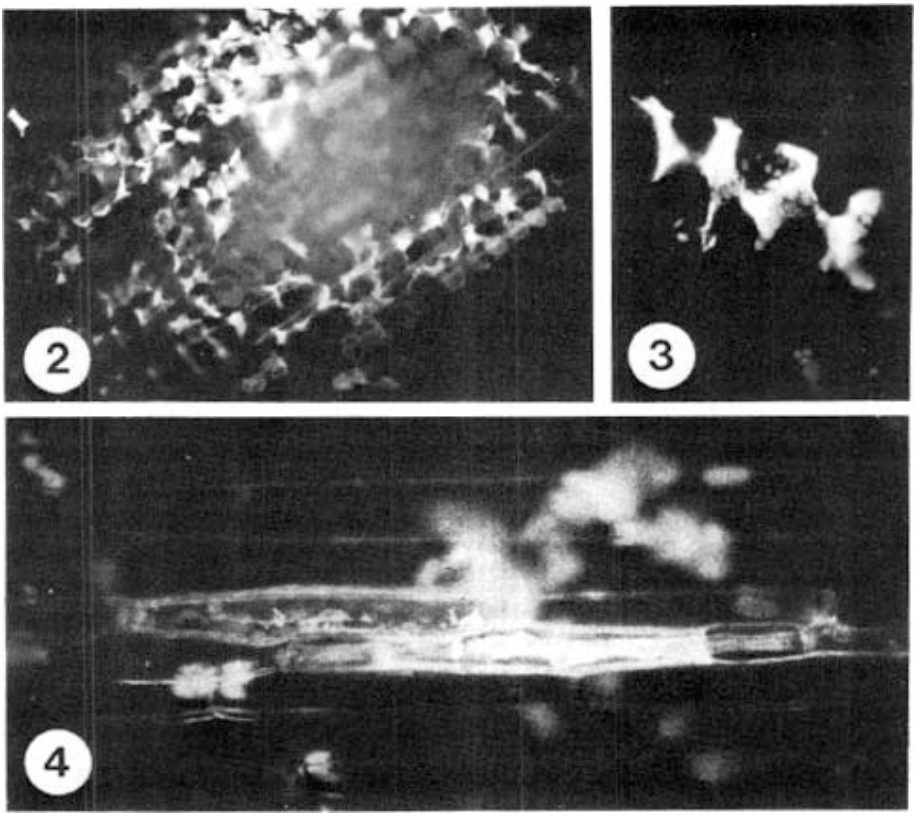


Fig. 2. Fluorescence photomicrograph of autofluorescing wheat leaf cells from incompatible interactions with stem rust of wheat. A fungal colony is shown, isolated by macerating enzymes from an infected leaf and surrounded by necrotic and collapsed mesophyll cells *(Sr6/P6* interactions; W. K. Kim, unpublished).

Fig. 3. Fluorescence photomicrograph as in Fig. 2. A single collapsed mesophyll cell is shown, isolated by macerating enzymes from infected tissue *(Sr6/P6* interaction; W. K. Kim, unpublished).

Fig. 4. Fluorescence photomicrograph as in Fig. 2. Wall and content of epidermal cell are seen to autofluoresce after interaction with avirulent fungus; specimen was frozen and thawed to collapse protoplast |cv. Feldkrone-race 32; K. Achenbach-Blasberg, unpublished).

After staining with the fluorochrome calcofluor (Polysciences, Inc.), fungal structures display a different type of fluorescence and can be visualized side by side with the autofluorescing host cells in the same field of view (Rohringer *et al.*, 1977). In its latest form (Ruck *et al.* 1981), this technique can also visualize haustoria in invaded host cells. It yields basically the same information as older

methods using conventional stains such as Trypan Blue, but it is much more convenient and less time consuming, permitting rapid observations of large numbers of interactions that are desirable for statistical data treatment.

3. Measurement of Fungal Growth

None of the available methods is ideal for an accurate determination of fungal growth. Chemical assays for characteristic fungal constituents such as glucosamine (Mayama *et al.*, 1975a,b) yield an overall estimate of fungal development in the leaf but are not sensitive enough for the initial stages in the interaction when there is little fungal tissue, and chemical analyses of whole leaves are not suitable for observations on a colony basis. Light microscopy is a fairly reliable method for estimating the growth of rust as long as the colonies are relatively small. Once colonies have developed to include more than about 10 haustorium mother cells per colony, an accurate estimation of colony size becomes very cumbersome at best. At still later stages of colony development, when there are hundreds of haustorium mother cells per colony, colony development has been measured by determining linear growth, a method that ignores the irregular, complex, three-dimensional structure of colonies.

B. EARLY INHIBITION OF FUNGAL GROWTH

In some incompatible interactions between cereals and their rusts, the development of the parasite is arrested or severely inhibited very early after inoculation. In some of these cases host cell necrosis apparently precedes inhibition of the fungus, as for example in the resistance response of wheat to stem rust of wheat conditioned by the *Sr5* gene. In other cases, exemplified by the interaction between oat cv. Shokan I and race 226 of *Puccinia coronata*, inhibition of the fungus is observed some hours before host cell collapse. These two examples will be discussed in this section.

The interaction between wheat and *Puccinia graminis* f. sp. *tritici* involving the *Sr5* and *PS* genes in host and parasite, respectively, conditions a so-called immune reaction (Table I) where no lesions can be detected with the unaided eye (0 infection type). Colony development in this interaction is very restricted. Generally, only one or two haus-torium mother cells are produced per colony and there is no further colony growth 24 hr after formation of appressoria, although the genetic background of the host can influence the expression of the *Sr5* gene and may permit some further development of the fungus. The *Sr5/P5* system is the only stem rust-wheat interaction so far investigated in which the gene for resistance is expressed in both mesophyll and epidermal cells (Rohringer *et al.*, 1979; Harder *et al.*, 1979a): Invaded epidermal cells autofluoresced, although, unlike invaded mesophyll cells, they did not collapse.

Epidermal cell reaction may be important in limiting the growth of avirulent rust colonies in this gene interaction.

In their study of the *Sr5/P5* system, Rohringer *et al.* (1979) had concluded that fungal growth was inhibited before host cell necrosis occurred, because some colonies not associated with host cell necrosis were smaller than those in the compatible *sr5/P5* interaction 24 and 48 hr after inoculation. However, rust colonies show great variability in size (Skipp and Samborski, 1974), and the necrosis-free colonies in the incompatible interaction selected for this comparison may have represented the "slow growers" from among the colonies of the total population.

Evidence for a causal relationship between host cell necrosis and inhibition of fungal growth in the *Sr5/P5* system comes from recent histochemical work on lignification of the affected host cells using the phloroglucinol/HCl reagent (R. Tiburzy and H.-J. Reisener, personal communication). Lignification, first detected 24 hr after inoculation, took place during haustorial development inhibiting growth of the haustorial body. The lignified host cells were necrotic; development of haustoria in the genotypically incompatible system (*Sr5/P5*) was similar to that in the genotypically compatible system *(sr5/P5)* when leaves were subjected to treatments that inhibited lignification (low ambient temperature, or infiltration of leaves with water or with an aqueous solution of *p*-mercuribenzoate). Histochemical studies by Beardmore *et al* (1983) extended earlier work (Rohringer *et al.*, 1967; Fuchs *et al.*, 1967) on accumulation of phenolic compounds in the incompatible interaction conditioned by the *Sr6* gene for resistance; in incompatible interactions conditioned by *Sr5* and *Sr6* genes, material exhibiting properties of lignin accumulated in walls of necrotic host cells, and this was accompanied by deposition of lesser amounts of alkali-soluble phenolic compounds, presumably bound esters.

Rust colony-associated necrosis of the host tissue is not necessarily a determinant of incompatibility in interactions where fungal growth is inhibited early after infection. Although host cell necrosis is very prominent in the interaction between oats (cv. Shokan I) and *P. coro-nata* race 226 (infection type 0), it occurs later than inhibition of fungal growth (Table I). The sequence of events in this system is summarized schematically in Fig. 5. Hyphal growth was slower than in the susceptible host already at 20 hr after inoculation, that is, prior to formation of haustoria and 8 hr before host cell collapse was observed in the incompatible system (Onoe *et al.*, 1976). The first ultrastructural symptoms of incompatibility were an increase in the number of Golgi vesicles and the occurrence of electron-dense material in these vesicles and in the host cell envelope. These changes were apparent 12 hr after inoculation. From this, one can infer that the collapse of host cells took approximately 16 hr after cytopathological symptoms were first evident. These

symptoms were first detected at the same time or shortly after recognition of the avirulent parasite took place.

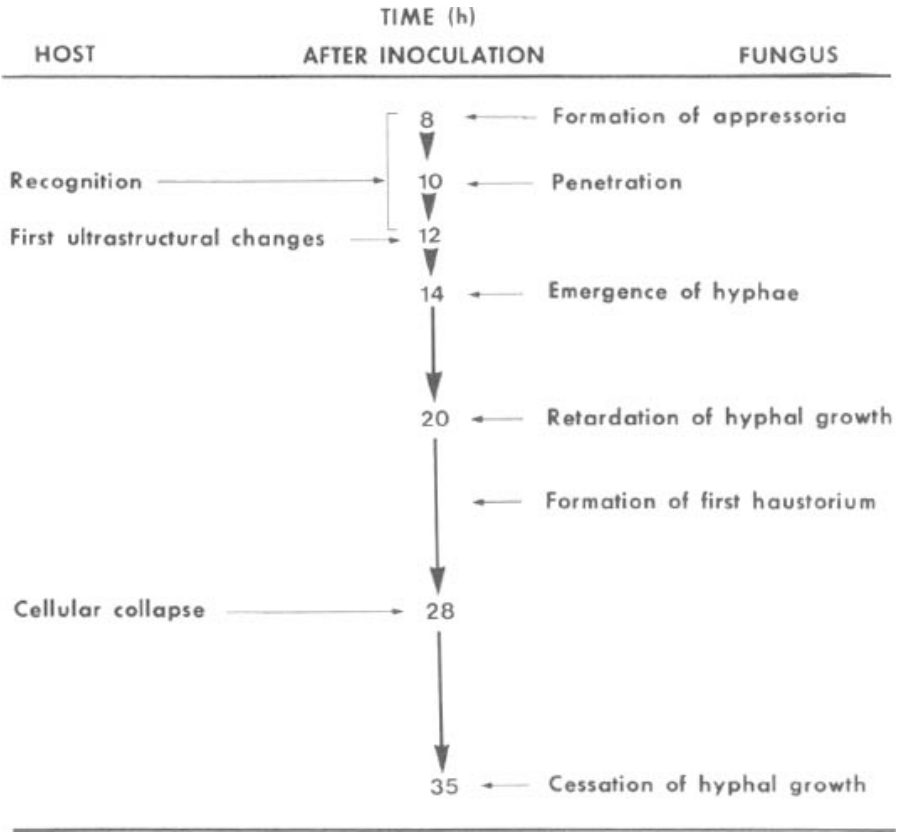


Fig. 5. Schematic representation of events in the incompatible interaction between oat leaves (cv. Shokan I) and crown rust of oats (race 226) (after Tani and Yarnamoto, 1979).

To determine the time of recognition, Tani *et al.* (1975a,b) used an ingenious method combining heat treatment and successive inoculation with two races: Oat leaves were inoculated with an avirulent race, then heat-treated at different times to kill the fungus, and finally inoculated on the reverse side with a virulent race. The development of this second, virulent race was then compared with that occurring in leaves that were not preinoculated. The results indicated that initiation of the incompatible reaction occurred between 8 and 12 hr after inoculation. After that time, heat treatment of the avirulent race had no effect on development of the virulent race, indicating that the significant events determining resistance or susceptibility had taken place approximately 10 hr before inhibition of fungal development was first detected. When cordycepin,

blasticidin S, or puromycin was supplied to the host-parasite system during the determinative phase, development of the genotypically incompatible race was stimulated and became identical to that of the compatible race, suggesting that activation of RNA and protein synthesis is required for the expression of resistance in this system (Tani and Yamamoto, 1979).

Evidently, important events determining the outcome of this host-parasite interaction occur in the intercellular space of the leaves prior to haustorial penetration. It would be of interest to know whether the product of the gene for resistance is present in the intercellular space of leaves before infection, or whether it is first synthesized in response to the presence of the fungus. Either one of these two possibilities would be compatible with the facts known so far.

C. INDETERMINANT HYPERSENSITIVITY

In many incompatible cereal–rust interactions the fungus does not appear to be inhibited before host cell necrosis is observed and continues to grow slowly as the incompatible host tissue becomes necrotic. This indeterminant hypersensitivity may be operating in the incompatible interaction of wheat and stem rust of wheat specified by the *Sr6* gene (Table I). Using the autofluorescence technique and the temperature sensitivity of the expression of the *Sr6* gene for stem rust resistance, Mayama *et al.* (1975a) reported that there were no significant differences in the number of autofluorescing sites between the incompatible response of Sr6-containing plants at 20°C (infection type 1 +) and the compatible response of these plants at 26°C (infection type 3+). They concluded that the hypersensitive response of host cells was not a determinant of resistance in the *Sr6/P6* system.

The opposite conclusion was reached by Skipp and Samborski (1974) and by Samborski *et al.* (1977) using the Trypan Blue and autofluores-cence technique, respectively, to identify necrotic cells. That hypersensitive necrosis at the nonpermissive temperature was correlated with the occurrence of a characteristic autofluorescence of host cells was confirmed (Beardmore *et al.*, 1983). In the Canadian studies, the fluorochrome calcofluor was used for the first time in this type of work to visualize fungal structures. This made it possible to exclude those necrotic host cells that were not in contact with the fungus and had evidently undergone necrosis for reasons unrelated to infection [noninfected, apparently healthy leaves often contain necrotic cells, especially near the apex and margin of the leaf (Samborski *et al.*, 1977)]. This work showed that there was a correlation between inhibition of fungal growth and incidence of necrosis in the colony-associated host cells in the incompatible *Sr6/P6* interaction. At all temperatures permitting the expression of incompatibility, host cell necrosis kept up with the slowly advancing rust mycelium. Necrosis-free colonies were observed only at very early stages in the

interaction, and these were of the same size as colonies in the compatible (*sr6/P6)* combination, indicating that incompatibility was not expressed prior to host cell necrosis.

The temperature sensitivity of the system provided an opportunity for further insight into the dynamics of this system: If maintained at the higher temperature, the genotypically incompatible host tissue contained few, if any, necrotic cells associated with colonies of the avirulent fungus, but when such plants were transferred to the lower, nonpermissive temperature, a ring of necrotic host cells developed around the established fungal colonies. This doughnut-shaped ring had the same inside dimensions as the colonies measured before the temperature shift (Fig. 6), indicating that necrosis occurred largely in those host cells in which haustorium development took place after the temperature had been lowered. Conversely, host cells in which haustorium development occurred at the higher, permissive temperature did not become necrotic after the temperature was lowered. During the formation of haustoria the plasmalemma of the invaded host cells is invaginated.

The temperature shift experiments therefore led to the conclusion that invagination of the host cell is the critical step in the *Sr6/P6* interaction when resistance or susceptibility is expressed at the cellular level. Subsequent ultrastructural work on this system (Harder *et al.,* 1979b) strengthened this view: Genotypically incompatible mesophyll cells that were invaded at the higher temperature did not develop any fine-structural changes attributable to incompatibility after they had been transferred to the lower temperature that normally permits the expression of incompatibility. In host tissue that appeared to be invaded after this temperature change, one of the earliest observable ultrastructural symptoms of incompatibility was a more electron-dense and often perforated invaginated host plasmalemma (Figs. 7 and 8). At this stage of the interaction, the cell contents of both haustorium and the invaded host cell appeared to be quite “normal” ultrastruc-turally. At a more advanced stage in the haustorium-host cell interaction, when the contents of the haustorium had already become more electron-dense, observation of near-adjacent ultrathin sections showed that the plasmalemma perforations can easily be missed (compare Figs. 9 and 10). In the incompatible *Sr6/P6* interaction, host cell necrosis was not always accompanied by haustorial necrosis or vice versa (Figs. 11 and 12), although they usually occurred together.

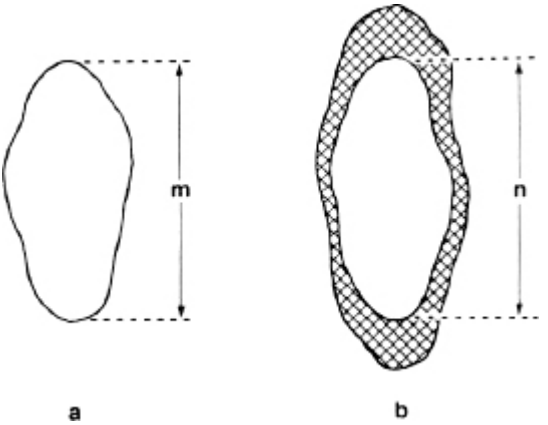


Fig. 6. Schematic representation of the "necrotic ring effect" in the temperature-dependent *Sr6/P6* interaction between wheat and stem rust of wheat. This effect is observed when the genotypically avirulent race is grown initially at 25°C (compatible) and then transferred to 19°C (incompatible), (a) A colony grown to size *m* after 2 days at the higher temperature. (b) Necrotic ring (internal diameter *n)* of host cells after a further 2 days at 19°C; $m = n$, indicating that necrosis occurred largely in cells that were invaded after the transfer to 19°C, when the Sr6 gene was reactivated (drawn from data by Samborski *et al.*, 1977).

That host cell necrosis in the hypersensitive reaction conditioned by the *Sr6* gene does not result from the death of the avirulent fungus was also concluded from experiments using the antimetabolites ethionine and polyoxin D (Kim *et al.*, 1977). Ethionine is a powerful inhibitor of stem rust of wheat. Polyoxin D is an inhibitor of chitin synthetase and is toxic to fungi containing chitin as a wall constituent. Both antimetabolites, used at concentrations that inhibited fungal growth but were not phytotoxic, inhibited the production of host cell necrosis in the incompatible system. It is not known whether the fungus was killed by these treatments.

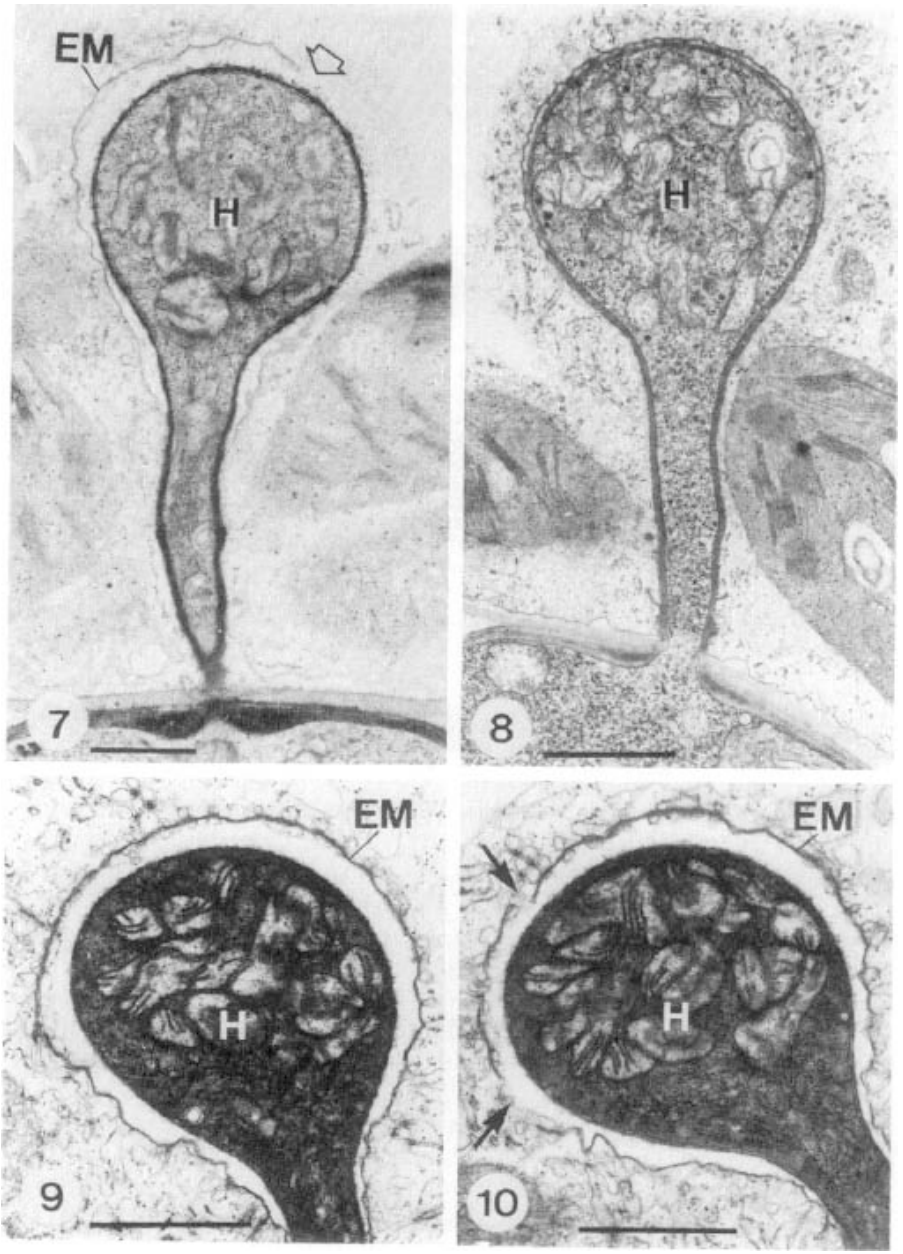


Fig. 7. Electron photomicrograph of *a* young haustorium (H) of wheat stem rust in the Sr6/*P6* interaction (incompatible); the extrahaustorial membrane (EM) is discontinuous

(open arrow) around a portion of the haustorial body (x13,700; bar 1 μm; Harder *et al.*, 1979b).

Fig. 8. Electron photomicrograph of a typical young haustorium of wheat stem rust in **the** compatible (*sr6/P6*) interaction, here illustrated for comparison (x17,100; bar 1μm, Harder *et al.*, 1978).

Fig. 9. Electron photomicrograph of a young haustorium of wheat stem rust in a wheat mesophyll cell with *Sr5* resistance. The haustorium is uniformly electron-dense. In this section, the extrahaustorial membrane (EM) is continuous (x20,600; bar 1 μm; Harder *et al.*, 1979b).

Fig. 10. Electron photomicrograph of near-adjacent section of the haustorium in Fig. 9. The apparently intact extrahaustorial membrane (EM) shown in Fig. 9 is here shown to be extensively fragmented (arrows) (x20,600; bar 1μm; Harder *et al.*, 1979b).

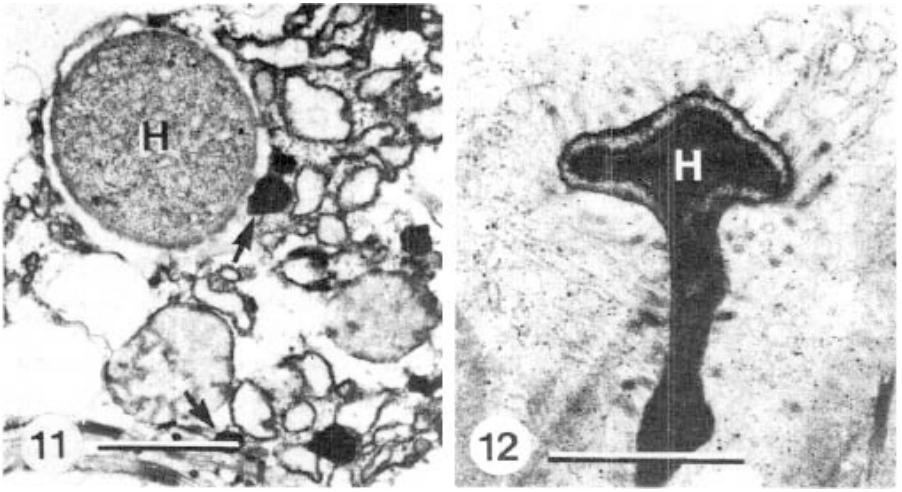


Fig. 11. Electron photomicrograph of an apparently "normal" haustorium (H) in the *Sr6/P6* interaction (incompatible) between wheat and stem rust of wheat. The electron-dense deposits (arrows) indicate the onset of host cell necrosis (x18,200; bar 1 μm; Harder *et al.*, 1979b).

Fig. 12. A totally necrotic and collapsed haustorium (H) in an apparently "normal" mesophyll cell in the *Sr6/P6* interaction between wheat and stem rust of wheat (x24,900; bar 1 μm, Harder *et al.*, 1979b).

In the incompatible interaction between stem rust of wheat and wheat leaves containing the *Sr6* gene for resistance, products from a necrotic haustorium or from a necrotic host cell do not appear to be responsible for necrosis in the other participant of the interaction, although both interacting cells eventually

become necrotic. It is not known if necrosis of a cell of one of the participants affects the physiological competence of the other, or what the biochemical reactions are in the transition from an invaded but ultrastructurally "normal" cell to a cell in which cytopathological changes are evident. To investigate these processes further, microautoradiography may be used. Using this technique on the incompatible *Sr6/P6* system, Manocha (1975) showed that [^{3}H]leucine is not incorporated into the extrahaustorial matrix. Incorporation of this precursor into haustoria ceased at 12 days after inoculation in the susceptible host and 4 days in the resistant host, at about the same times when a conspicuous extrahaustorial matrix ("sheath") was observed around haustoria in either interaction. It is not known whether the extrahaustorial matrix acts as a "barrier" to metabolite (leucine) transfer (at late stages in the compatible interaction and at earlier stages in the incompatible interaction), or whether the decrease of precursor incorporation reflects a decreased biosynthetic competence in either system, but at different times after inoculation.

In the incompatible *Sr6/P6* interaction, autofluorescence was seen in invaded mesophyll but not in invaded epidermal cells, indicating that these two cell types responded differently to infection (Rohringer *et al.*,1979). Electron microscopy confirmed (Harder *et al.,* 1979a) that the *Sr6* gene was expressed in mesophyll cells but not in epidermal cells. This difference in reactivity is of interest, because in up to 40% of the infection sites in the *Sr6/P6* interaction the first haustorium is formed in an epidermal cell (Skipp *et al.,* 1974). Evidently, these colonies get a better start than those in which mesophyll cells are first invaded, possibly accounting for rapid growth of some colonies in the incompatible host (Rohringer *et al.,* 1979).

D. DIFFUSIBLE FUNGAL DETERMINANT OF HYPERSENSITIVITY ("RUST TOXIN")

Typically, hypersensitive reactions are associated with necrosis of host cells at the infection site. In most such interactions, cell necrosis is strictly localized; that is, cell collapse is not usually observed in advance of the fungal mycelium. The concept of a diffusible "rust toxin" has been used to explain why chlorotic areas surrounding incompatible interactions between cv. Khapli and race 56 of wheat stem rust can be displaced from the infection site through application of an electrical field (Olien, 1957). Silverman (1960) extracted and purified a substance from another incompatible combination of wheat and stem rust of wheat. The phytotoxic substance produced chlorosis in nonin-fected wheat leaves much like that produced after infection. Unfortunately, these earlier investigations appear not to have been followed up, and it is not clear whether these substances originated from the rust or if they were products of the affected host cells.

However, in recent years, Jones and Deverall (1977a,b, 1978) have shown evidence for a diffusible substance originating from races of *Puccinia recondita* avirulent on wheat containing the *Lr20* gene for leaf rust resistance. It is possible that this substance is the product of the avirulence gene *P20*. If this is correct, elucidation of the resistance mechanism would be facilitated, because at least one of the interacting determinants is present in soluble form in the interaction and thus more accessible to isolation and purification.

In this system (Table I), changes in host cells leading to necrosis preceded detectable changes in the fungus by at least 18–20 hr: Host protoplasts at the infection site responded differently to Trypan Blue at about 28 hr after inoculation when the formation of the first haustoria was nearly complete. The host protoplasts collapsed at about 36 hr after inoculation, whereas the first inhibition of mycelial growth was detected another 12 hr later. Expression of the *Lr20* gene is sensitive to ambient temperature conditioning infection type; 1 or; at 20.5°C. At 30.5°C the genotypically incompatible system was phenotypically completely compatible. Experiments involving transfer of inoculated plants from 20.5° to 30.5°C confirmed that the first observable effects of the *Lr20* gene on fungal growth occurred at 48 hr after inoculation (Jones and Deverall, 1977a). Transfer of the genotypically incompatible system from the higher to the lower temperature caused the collapse of host protoplasts in cells surrounding fungal colonies, but not that of host cells invaded at the higher temperature. Evidence for a toxic substance produced by the avirulent race was obtained by using a heat treatment that prevented further rust growth: After the heat shock, extensive host tissue necrosis occurred around avirulent colonies but not around virulent colonies, when the heat treatment was followed within 15 hr by transfer from 30.5° to 20.5°C. Evidently, a "toxin" was made by the avirulent mycelium at 30.5°C, and host cells responded to this substance at the lower temperature where the *Lr20* gene is effective. The width of the area affected in the host implied that the toxic substance is diffusible (Jones and Deverall, 1977b). The results of further experiments (Jones and Deverall, 1978), involving leaf transplants, are in agreement with the idea that the "toxin" is diffusible and *Lr20*-gene-specific.

E. DELAYED EXPRESSION OF INCOMPATIBILITY

In contrast to all previous examples where rust development was inhibited very early in incompatible systems, the gene interactions described in the following are characterized by late inhibition of fungal growth.

An example of this group is the resistant reaction of wheat against stem rust specified by genes *Sr8* or *Sr22*, conditioning infection types 1+ or 2, respectively (Table 1). In incompatible interactions specified by these genes, necrotic host cells first appeared in significant numbers 60 or 72 hr,

respectively, after inoculation when rust colonies were of considerable size (Rohringer *et al.*, 1979). Although inhibition of colony growth was detected 12-24 hr later, many colonies at that time were still not associated with necrotic host cells. For this reason alone, host cell necrosis probably was not a determining factor in inhibiting fungal growth. In fact, the linear growth of necrosis-free colonies in the genotypically incompatible system *(Sr8/P8)* was only about 75% of that in the genotypically compatible system *(sr8/P8)* 72 hr after inoculation, showing that inhibition of fungal growth occurred before host cell necrosis was evident.

Perhaps the first significant effect of the *Sr8* gene was an inhibition of the growth of "runner hyphae," that is, hyphae that are free of haustorium mother cells and that spread rapidly into the host tissue from the perimeter of established colonies at a time when these contained several dozen or hundreds of haustorium mother cells. This was shown by measuring the distance in many colonies from the ap-pressorium to the apex of the longest runner hyphae and to the furthest removed haustorium mother cell (Fig. 13). At 72 and 84 hr after inoculation, linear growth, as measured by the distance to the furthest removed haustorium mother cell, was the same in incompatible and compatible interactions, while growth of runner hyphae was significantly inhibited in the incompatible interaction. In the *Sr22/P22* (incompatible) interaction, inhibition of fungal growth was first detected 96 hr after formation of appressoria. The late inhibition of fungal growth can be interpreted as delayed expression of the *P8* and *P22* genes for avirulence. It is not known why avirulence genes *P8 and P22*may not be expressed until very late in the host-parasite interaction. Perhaps the products specified by these genes are produced only in runner hyphae, or they may be present in young colonies, but at that time they may not be "accessible" to the host so that recognition of the avirulent parasite cannot occur. Alternatively, late expression of incompatibility in this system may be due to a delay in the expression of the gene for resistance, perhaps in response to products of the fungus formed only in advanced stages of colony development.

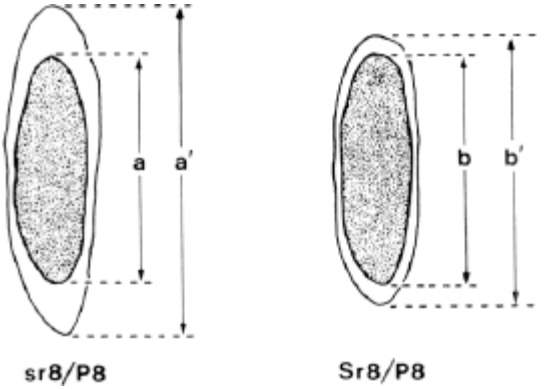


Fig. 13. Schematic representation illustrating delayed expression of incompatibility in stem rust of wheat. Wheat leaves near-isogenic with respect to the *Sr8* gene for re-sistence were inoculated with a race containing the *P8*gene for avirulence; gross colony structure was observed 72 hr after inoculation. The portion of colonies containing haustorium mother cells (stippled areas) is the same in both compatible (*sr8/P8*) and incompatible (*Sr8/P8*) interactions *(a = b),* but growth of "runner hyphae" in the incompatible interaction is much less compared to that in the compatible interaction (*b' < a'*). Host cell necrosis (at 72 hr, three to four necrotic host cells per colony) does not appear to be an important factor in limiting fungal growth in the *Sr8/P8* interaction (drawn from data by Rohringer *et al,* 1979).

Another example of late inhibition of fungal growth is the incompatible interaction between oats cv. ML-4 and isolate P-7-2 of *Puccinia coronata* (Prusky *et al.,* 1980). Dead haustoria were seen in many non-necrotic host cells as well as in some host cells that were necrotic on the fourth day after inoculation, but colony growth was not inhibited until the sixth day after inoculation. When infected leaves were treated with heat or with the fungicide oxycarboxin (2,3-dihydro-5-carboxy-anilido-6-methyl-l,3-oxathiin 4,4-dioxide), hyphal growth stopped almost immediately and haustoria necrosed. Death of host cells occurred later. Evidently, in this system, haustorial cell death precedes host cell necrosis.

III. Molecular Biology

A. GENERAL REMARKS

Nonhost resistance is species-specific; that is, it prevents, by physical or chemical means, a nonhost plant from being parasitized by a microorganism pathogenic to other plant species. Basic compatibility, and to an even higher level cultivar resistance, require highly specific interactions of host and

parasite. For cereal rusts, Flor's gene-for-gene concept can be applied, and the specificity encountered here can best be explained by assuming that the interacting molecules of host and parasite must have a relatively high information content and are therefore presumably of high molecular weight. Furthermore, at least one of the two types of interacting macromolecules is likely to be surface-bound and present in or on structures in the host-parasite interface that come into contact during pathogenesis when incompatibility is expressed.

A number of candidate compounds known or assumed to be involved in specific recognition phenomena in other biological systems are presently under discussion and investigation in several host-parasite systems. Albersheim and Anderson-Prouty (1975) have drawn attention to cell surface recognition phenomena mediated through the interaction of carbohydrate-containing macromolecules and proteins, such as apparently operate in recognition of sexual mating types in yeast, and in host recognition by *Rhizobium* species. The recognition in pollen-stigma interactions—that is, the incompatibility response that prevents pollen tube development beyond the probe tube stage— also seems to involve genotype-specific glycoproteins (Ferrari *et al.*, 1981). Lectins are involved in these systems (Sharon, 1977; Stacey *et al.*, 1980; Bauer, 1981). That they may play a role in host-pathogen specificity has been discussed in detail by Albersheim and Anderson-Prouty (1975), Callow (1977), Etzler (1981), and others. Although specific *in vitro* binding of certain lectins to fungal surfaces and subsequent inhibition of fungal development have been observed, no information is available on their physiological role in host-pathogen systems *in vivo*. So far, a molecular mechanism explaining gene-for-gene specificity has not been demonstrated in any cereal-rust system.

B. PROTEINS AND MACROMOLECULAR GLYCOSUBSTANCES

The role of glycoproteins has been investigated and discussed largely in those host–parasite systems in which phytoalexins apparently contribute to the expression of resistance, for example, in the *Phytophthora megasperma*–soybean system. Albersheim and co-workers reported that low molecular weight β-(l→3)-glucans released from the fungal wall were nonspecific elicitors of phytoalexin synthesis in soybeans (for reviews, see Albersheim and Valent, 1978; Bailey and Mansfield, 1982). Glycoproteins isolated from compatible races of *P. megasperma* specifically inhibited the action of the nonspecific elicitors (Ziegler and Pontzen, 1982). In contrast, Keen and colleagues reported that glycoproteins, present on the cell surface of *P. megasperma* or in the culture filtrate, may function as race-specific elicitors of phytoalexin synthesis in this system (Keen and Legrand, 1980; Keen, 1982).

Glucomannans from walls of this fungus were identified as race-specific elicitors (Keen *et al.,* 1983); these carbohydrates can be released from the fungal wall through the action of β-l,3-endoglucanases present in soybean tissue (Keen and Yoshikawa, 1983). In the *Phytophthora infestans*–potato system, phytoalexin synthesis can be nonspecifically elicited by high molecular weight carbohydrate wall components of the fungus, as well as by eicosapentanoic and arachidonic acids (cf. Kuć, 1982). In this interaction, specificity seems to reside in the ability of compatible races to suppress the hypersensitive response including phytoalexin synthesis; but the specific repressors of the fungus seem to be water-soluble glucans (Doke *et al.,* 1980) and not glycoproteins.

There are only two known systems involving production of phy-toalexins in rust-infected cereals: several antifungal substances produced in the incompatible interaction between wheat cv. Little Joss and *Puccinia striiformis* race 104E137 (Cartwright and Russell, 1980), and the avenalumins synthesized in oats cv. Shokan I after infection with an incompatible race of *P. coronata* (Mayama *et al.,* 1981a,b,c, 1982a,b). It is not known whether the resistance response (infection type 0) of cv. Shokan I oats to the incompatible race of crown rust can be elicited (specifically or nonspecifically) by polysaccharides or glycoproteins from the fungus, whether a specific suppression of this reaction is possible, and whether phytoalexins generally are involved in the resistance response of cereals against rust fungi. In rust diseases of legumes, for example, *Uromyces phaseoli* on *Phaseolus vulgaris,* phytoalexins occur in the incompatibility response, and carbohydrate elicitors have been shown to stimulate nonspecifically their synthesis (Hoppe *et al.,* 1980; cf. Bailey and Mansfield, 1982). It is tempting to speculate whether β-lectins of the host may be involved in the mechanism of elicitation of phytoalexin synthesis by fungus-derived β-glucans (Clarke *et al.,* 1979). The β-lectins have been detected in most higher plants of 104 families tested (Jermyn and Yeow, 1975) and are concentrated in the intercellular spaces. Although they show no sugar specificity, they all have an affinity toward β-D-glycopyranosyl linkages and thus can be expected to bind β-glucans. Their extraordinary evolutionary stability may be explained if they possess a function in a general, nonspecific defense mechanism of higher plants against microorganisms that excrete or contain β-glycans in their cells.

If incompatibility instead of compatibility requires the recognition of the invading parasite by the resistant host in gene-for-gene specificity, it is possible that information-containing glycoproteins at the host-parasite interface may be responsible for the expression of incompatibility. On the other hand, specific suppressors (glycoproteins or other compounds) could prevent the incompatible response against cereal rusts and thereby induce compatibility. At the level determining "basic compatibility," surface-bound, information-carrying

glycoproteins may serve as a means for the parasite to recognize the host by reacting with appropriate receptor sites in the latter, or vice versa.

Some of the earlier investigations in plant pathology were partly influenced by phenomena in vertebrates where "immunity" can be achieved through formation of specific antibodies, primarily proteins (Chester, 1933; cf. Fuchs, 1976). The term *immune* is still used in describing highly resistant reaction types, or in connection with acquired resistance of plants brought about by preinoculation with the same or closely related pathogens. However, the assumption that specific antigen-antibody reactions involving high molecular weight proteins are responsible for these phenomena in plants is not supported by the evidence. Novel proteins were observed in virus-infected, sys-temically resistant leaves or following injection of leaves with poly-acrylic or salicylic acids that induce resistance, but the function of these proteins apparently is to limit multiplication or spread of viruses in the hypersensitive reaction (cf. Van Loon, 1982; Gianinazzi, 1982).

New proteins also occur in fungus-infected, resistant-reacting plants, as in *Phaseolus vulgaris* inoculated with an incompatible race of *U. phaseoli* (G. Wolf, unpublished). In addition, such proteins are observed after elicitation of the resistance response by an unspecific glucan elicitor preparation (H. H. Hoppe and G. Wolf, unpublished). In oat leaves inoculated with compatible or incompatible races of *Puc-cinia coronata*(specifying infection types 4 and 0, respectively), no differences in isotope incorporation into soluble proteins were detected, but a possibly "new" protein of host origin was found in extracts from the incompatible interaction (Yamamoto *et al.*, 1975, 1976). In later experiments the same group demonstrated an enhanced synthesis of RNA and appearance of six additional minor proteins in the incompatible but not in the compatible reaction very early after inoculation. Tani and Yamamoto (1979) proposed that activation of mRNA and protein synthesis by the plant is required for expression of resistance but not for establishing susceptibility. On the other hand, the results of Barna *et al.* (1978) do not support the idea that serologically or electrophoretically determined new proteins are involved in resistance of wheat to stem rust of wheat.

Evidently, new proteins may or may not occur rather early during pathogenesis of rust diseases. They may function as enzymes or structural proteins accompanying the resistance or susceptibility response of the host. However, they cannot be regarded as determinants of specificity.

C. NUCLEIC ACIDS

Although we know on which particular locus of a chromosome a certain resistance gene may be located, we do not know how it is transcribed. From studies mainly with prokaryotes the mechanism of transcription and translation

of the genetic information is now fairly well understood. Progress has been made also with eukaryotic systems. However, studies with diseased plants are especially complicated, as they involve two separate but not independent organisms. Several extensive reviews deal with the subject of transcription and translation in diseased plants (Samborski *et al.,* 1978), with the role of RNA in host-parasite specificity (Chakravorty and Shaw, 1977a,b), and with nucleic acids in host-parasite interactions (Heitefuss and Wolf, 1976). Major changes in nucleic acid concentration and synthesis have been observed in different host-parasite systems (Chakravorty and Shaw, 1971), including cereals and rust. Some of these as they relate to the metabolic alterations in the infected host are discussed by W. R. Bushnell in Chapter 15 of this volume. Here, only those that may relate to specificity will be discussed.

Gene expression at the level of transcription is controlled in eukaryotic cells by chromatin-associated histones and nonhistone protein. The former appear to be involved in nonspecific repression of transcription, whereas nonhistone proteins and possibly chromosomal RNA appear to effect histone displacement and gene derepression. In earlier work, some differences were reported regarding nuclear DNA- bound histones in rust-infected susceptible and resistant wheat, but these differences have not been correlated with gene derepression (Bhattacharya *et al,* 1968).

Transcription of the genetic information encoded in DNA requires the action of RNA polymerase. RNA polymerase I synthesizes ribo-somal RNA precursor, RNA polymerase II synthesizes messenger RNA precursors, and RNA polymerase III synthesizes low molecular weight ribosomal RNA and transfer RNA precursors. Differential stimulation of specific polymerases early in the host-parasite interaction may contribute to the specificity of the interactions. So far only a few reports are available in which alterations of polymerase activity and their properties have been observed. The results, published in two abstracts (Flynn *et al.,* 1976; Scott *et al.,* 1976), are discussed in two reviews (Chakravorty and Shaw, 1977a,b); RNA polymerases I and II have been isolated from an Australian wheat cultivar susceptible to *P. graminis* f. sp. *tritici.* Substantial changes in template activity of both polymerases occurred during the initial 4 days after inoculation. A significant increase in activity was observed for polymerase I only. Differences in template activity between polymerases I and II obtained from the fungus grown in axenic culture suggested a change in host enzymes in the inoculated leaves. However, no comparable results were available for compatible or incompatible combinations. Furthermore, the question needs to be resolved whether RNA polymerase is indeed involved in the expression of specific gene-for-gene interactions or merely in that of basic compatibility between host and parasite.

Translation of mRNA takes place at the ribosomes, resulting in the synthesis of new polypeptide chains and proteins. New enzyme proteins can be detected by assaying their activity. A more direct approach is to detect

translation products by means of tracer studies or with high-resolution chromatography, isoelectric focusing, and/or electrophoresis. Cell-free systems containing isolated polysomes may be used in this approach.

Von Broembsen and Hadwiger (1972) studied six gene-for-gene interactions between flax and *Melampsora lini* with respect to changes in synthesis of soluble protein 6-18 hr after inoculation. By means of a double-labeling technique, a net increase in certain protein fractions was found in four incompatible combinations, whereas the protein synthesis remained constant or decreased in two compatible combinations. Separations on Sephadex G-200 did not permit isolation of single proteins, although distinctive patterns for each gene interaction were recognized. These results are similar to those obtained by Tani and Yamamoto (1979) (see earlier) in their study of the *Puccinia coronata*—oat system *in vivo.*

With an *in vitro* protein synthesis system, Pure *et al.* (1979) showed that polysomes from infected leaves produce different polypeptides than those from healthy leaves and that these changes involve, at least in part, cytoplasmic mRNA (Pure *et al.,* 1980). Unfortunately, these studies have not been extended to compare compatible and incompatible combinations. Therefore, conclusions with regard to host-parasite specificity at the cultivar level cannot be drawn.

Gene expression is further regulated at the posttranscriptional level by several enzymes that modify precursor RNA into biologically functional RNA molecules. Of these enzymes, ribonuclease has been investigated extensively for different host-parasite combinations including mildew (Chakravorty and Scott, 1979) and rust on cereals. In earlier studies with stem rust on wheat (Rohringer *et al.,* 1961), a considerable increase in RNase activity with quantitative differences during early and late stages of pathogenesis have been noted. Later studies of Shaw's group with wheat stem rust (Chakravorty *et al.,* 1974a) and flax rust (Chakravorty *et al.,* 1974b,c) concentrated on qualitative changes in ribonucleases that could be found in the infected host with respect to substrate specificity, pH response, thermal stability, or *K*m and Vmax. The observed differences in enzyme properties in rust-infected flax were attributed to complementation between enzyme subunits produced by the host and by the parasite. However, this hypothesis could not be confirmed (Sutton and Shaw, 1982). The relative amounts of both enzymes changed markedly during infection, but their properties were the same in extracts from both resistant and susceptible, or from healthy and infected plants. The observed qualitative differences in RNase following infection can therefore be attributed to changes in the relative amount of the different isozymes during pathogenesis (Sutton and Shaw, 1982). Similar observations have been reported earlier for ribonucleases of wheat after infection with *P. graminis* f. sp. *tritici* (Sachse *et al.,* 1971).

Stimulation of RNase may be a rather unspecific response of plants to different stress conditions, and the quantitative and qualitative changes after

inoculation of resistant or susceptible cultivars may be interpreted as biochemical symptoms not directly related to or involved in the specificity of host—parasite interactions and cultivar resistance or susceptibility. They may be functionally related to the increase in host ribosomal RNA as observed in several host—parasite combinations at the beginning of fungal sporulation (cf. Heitefuss and Wolf, 1976).

IV. Present Trends, New Technology

A. HISTOLOGY AS AN AID TO BIOCHEMICAL WORK ON HOST-PARASITE SPECIFICITY

The evidence shows that incompatible interactions between cereals and their rusts can differ greatly, not only regarding their macroscopi-cal phenotype, but also when they are examined at the cellular level. Generalizations about incompatibility may be justified only if thorough histological observations have shown similar features in interactions that are to be compared.

The significance of necrosis in hypersensitive reactions is far from clear. Necrosis has received much attention, no doubt in part because it is irreversible and so readily detectable. The general statement, that necrosis is a determinant of incompatibility, is no longer tenable, although it may be true in certain interactions, such as the *Sr5/P5* system in stem rust of wheat. Even here, it is difficult to interpret the morphological evidence, because so little is known regarding the mechanism leading to collapse of cells in incompatible host-parasite interactions.

Histological observations are uniquely suited to determine the stage in the interaction when recognition is likely to occur, and the structures of host and parasite that are likely to be involved in this gene-specific event. This in turn can yield valuable clues for timing of sample collection in biochemical studies that are intended for detection and eventual purification of the products of the interacting genes.

Histochemical methods applicable to ultrastructural work have been used to determine the macromolecular composition of structures at the host-parasite interface. Wall structures containing glycosub-stances and protein have been partially characterized, among others, in the downy mildew-pea system (Hickey and Coffey, 1978), and in crown rust of oats and stem rust of wheat (Chong *et al.,* 1981). Ultra-structural localization of enzymes, particularly that of glycosyltrans-ferases (Klohs *et al.,* 1978), would be of great interest to workers in this field. Immunocytochemical methods can be used to determine the location of certain macromolecules in the tissue, once these have been obtained in pure form and are available for raising the appropriate antiserum. The

method employing protein A labeled with colloidal gold (Roth *et al.*, 1978) is a useful tool for such studies at the ultrastructural level, especially when combined with low-temperature dehydration (-18°C) and embedding (-30°C) techniques that tend to preserve the antigenicity of endogenous protein (Carlemalm *et al.*, 1980; Roth *et al.*, 1981). Treatment of ultrathin sections with the appropriate immunoglobulin and with protein A gold conjugate may reveal which structures at the host–parasite interface contain the macromolecules of interest. Alternatively, biotinylated immunoglobin may be used as a probe and detected using avidin labeled with an electron-dense marker (Skutelsky and Bayer, 1979).

For all morphological and histochemical studies of fine structure, improvements in procedures for tissue fixation are vitally important (Ingram, 1982). Many of the published electron photomicrographs probably contain artifactual distortions or alterations of membranes. Freeze-substitution is much superior to other methods of fixation to preserve membrane structure. It has been used successfully on *Fusarium* cultures grown on slides or cellulose membranes (Howard and Aist, 1979; Howard, 1981), but it is still very difficult to apply to thicker tissues such as cereal leaves. Dehydration is entirely avoided during freeze-etching, a technique uniquely suited for morphological studies of membrane surfaces. In conjunction with filipin treatment, it has been used to study the extrahaustorial membrane in bean–abean rust interactions (Harder and Mendgen, 1982). A major problem in work with freeze-etching and freeze-substitution techniques is to obtain artifact-free freezing of the tissue without the use of chemical fixatives or intracellular cryoprotectants.

B. BIOCHEMICAL APPROACHES

Research on biochemical symptomatology, prominent during the last two decades, is still being pursued along with renewed emphasis on histology, including the use of histochemical methods. In general, interest in low molecular weight metabolites (such as sugars, amino acids, or phenolic acids) has waned, probably because many of the biochemical symptoms involving these compounds are likely to be secondary to the interactions concerned with specificity. However, some low molecular weight compounds deserve increased attention, even if they play a role only in “late,” nonspecific reactions leading to cell death or inhibition of fungal growth. For example, the discovery of the avenalumins (see Section III,B) is of interest and will probably stimulate further research in this area, because the role of phytoalexins in the Gramineae, particularly after infection by rusts, has not been well established.

The biochemical interaction determining gene-for-gene specificity must involve macromolecules, because only these can provide the information content necessary for recognition. Efforts to detect the products of

the corresponding genes, or the macromolecules that are involved in the recognition event between cereals and their rusts, have not yet met with success. The approaches described in this section may be productive for detection and eventual isolation of the macromolecules of interest. If the recognition event in cereal–rust interactions involves macromolecules at the cell surface as determinants of incompatibility, isolated host cells or isolated host protoplasts (living or fixed) might be used to detect macromolecular fungal constituents assumed to exhibit gene specificity. This approach assumes that interacting molecules from host and parasite possess an affinity for each other strong enough to result in "binding" of the fungal constituents to the surface of the isolated host cells or protoplasts. In work on the *Phytophthora infestans*-potato system, this approach has already been applied successfully. It showed that the potato cell plasmalemma is the organelle that likely contains the sites for recognition of fungal wall components (Doke and Tomiyama, 1980a), and it demonstrated that fungal glucans suppress the elicitation of hypersensitivity caused by these wall components (Doke and Tomiyama, 1980b).

There are many potential difficulties in work with isolated protoplasts. When macerating enzymes are used for protoplast isolation, enzymes may alter, or remove from the cell surface, the very components that are of interest. Further difficulties may arise when fixed host protoplasts are bound to an inert support and used for affinity chromatography of the putative products of the genes for avirulence. In such an approach a very complex system (host protoplasts) would be used to fractionate a perhaps equally complex system (e.g., fungal wall extractives). However, this type of cell column chromatography has been used successfully (Sela and Edelman, 1977) for the purification of immunoglobulins specific for cell surface glycoproteins, and it may, in principle, also be useful in work on host-parasite specificity.

If the product of a certain gene for resistance is suspected to be part of the host plasmalemma, purification of the plasmalemma and subsequent solubilization of bound proteins from such preparations may be attempted as a first step in comparing samples from near-isogenic host lines. Plasmalemma-enriched fractions have been obtained from roots and etiolated leaves of cereals (cf. Quail, 1979), but none from green tissue, as the presence of chloroplasts is a major complicating factor. A possible compromise is the preparation of crude membrane material (Strobel, 1973), but plasmalemma is only a portion of the membranes isolated. Redistribution of proteins or protein subunits may be a major difficulty in any of these procedures.

If the molecules of interest can be assumed to be present in the intercellular space of cereal leaves, "intercellular washing fluid" may be prepared to serve as the starting material. Such a procedure would exclude most components present in the cytosol and in subcellular organelles, and it would avoid many difficulties normally encountered in fractionating extremely complex mixtures. A modification of the technique described by Hagborg (1970) would allow

recovery of small amounts of fluid from cut ends of injected, attached leaves. A procedure more suited for obtaining larger amounts of "intercellular washing fluid" (Soding, 1941; H. Lehman-Danzinger and G. Wolf, unpublished) employs infiltration of detached leaves and subsequent gentle centrifugation for recovery of the fluid. A similar technique (Rath-mell and Sequeira, 1974) was used by Mayama *et al.* (1982a) to recover phytoalexins from the intercellular spaces of oat leaves and by deWit and Spikman (1982) to isolate race and cultivar-specific elicitors from tomato leaves infected with *Cladosporium fulvum*. "Intercellular washing fluid" from barley leaves has been shown to contain numerous proteins; addition of small amounts of detergent to the solution used for infiltration yielded additional proteins, apparently without disrupting the barley plasmalemma (Rohringer *et al.*, 1983).

Advances in separation of complex protein mixtures (O'Farrell, 1975) by two-dimensional isoelectric focusing—gel electrophoresis have made it more realistic to search for the substances that convey specificity to the interaction between host and parasite. Potentially, this method can resolve thousands of polypeptides in a mixture, **but** problems of "streaking" still plague the analysis of total leaf proteins. Fewer obvious difficulties are encountered in the analysis of fungal proteins, although care must be taken here also to guard against autolytic protein degradation prior to isoelectric focusing. Two-dimensional techniques of this type have been used to determine the polypeptide content of fungal spores. Several cultures of barley mildew (Gabriel and Ellingboe, 1982; Torp, 1982) and stem rust of wheat (Howes *et al.*, 1982) could be distinguished on that basis. In stem rust of wheat more than 290 polypeptides were detected, and isolates of several races differed in their polypeptide content (Howes *et al.*, 1982).

Membrane-bound proteins usually possess lipophilic regions and are frequently glycosylated. Use of appropriate affinity systems (e.g., lectins or detergents immobilized on carriers suitable for column chromatography) can greatly facilitate their purification and isolation. Affinity chromatography may offer possibilities for an even more selective procedure to isolate determinants of a host–parasite interaction: Once gene-specific macromolecules have been isolated from one participant of the interaction, they may be bound to an inert support and used for the isolation of the corresponding gene-specific molecules from the other participant, provided that these two types of molecules have some affinity for each other. Assuming that a binding affinity exists between the interacting macromolecules of host and parasite, these macromolecules may be detected using a potentially very sensitive *in vitro* system in which the components of one of the partners are separated in acrylamide gels, blotted (Gershoni and Palade, 1983) onto cellulose nitrate membranes, and exposed to a biotinylated (Bayer *et al.*, 1979) preparation from the other partner, to be subsequently visualized on the membranes with avidin-peroxidase conjugate. For preparative purposes, a recovery system is available making use of the

easily reversible binding between avidin and 2-iminobiotin (Orr, 1981). Crossed affinoelectrophoresis (Owen *et al*, 1977) is another method that may be useful in the search for proteins in the host that may have gene-specific binding affinity with proteins in the parasite.

An important aspect of the work on specificity-conferring constituents is the need to demonstrate biological activity. The simplest approach is to measure the growth of the avirulent fungus in genotypically compatible host tissue that has been treated with a preparation from genotypically incompatible leaves suspected to confer incompatibility. This technique could be used in systems where inhibition of the fungus occurs in the absence of a hypersensitive reaction of the host. In systems where phytoalexin production has been shown to occur, synthesis of these compounds can be used as a measure of biological activity of the isolated macromolecules. This approach was widely used to demonstrate the occurrence of elicitors (e.g., from *Uromyces phaseoli;* Hoppe *et al*, 1980) generally believed to be nonspecific, but it was also useful in systems that involve both elicitors and specific suppressors of phytoalexin production (Garas *et al.*, 1979; Ziegler and Pontzen, 1982). In the host, hypothetical receptors for fungus-derived elicitors or suppressors may possibly be visualized after conjugating these substances with electron-dense markers (cf. Rohringer *et al.*, 1982). Possible binding to host tissue in ultrathin sections may be observable with the electron microscope, and appropriate controls could be used to determine if such binding is genespecific. Although further improvements in many potentially useful techniques can be anticipated for the near future, it still sounds Utopian to expect that gene amplification by DNA cloning may be available as a technique to produce larger amounts of the products of the genes for resistance and avirulence. However, the mapping of resistance genes and well-known "marker" genes (e.g., for wheat germ gliadins) has progressed to the point where at least one of the prerequisites for this technique in wheat appears to be fulfilled.

References

Albersheim, P., and Anderson-Prouty, A. J. (1975). Carbohydrates, proteins, cell surfaces, and the biochemistry of pathogenesis. *Annu. Rev. Plant Physiol.* **26,** 31-52.

Albersheim, P., and Valent, B. S. (1978). Host-pathogen interactions in plants. J. *Cell Biol.* **78,** 627-643.

Bailey, J. A., and Mansfield, J. W., eds. (1982). "Phytoalexins." Blackie, Glasgow.

Barna, B-, Balazs, E., and Kiraly, Z. (1978). Common antigens and rust disease. *Phytopathol. Z.* **93,** 336-341.

Bauer, W. D. (1981). Infection of legumes by rhizobia. *Annu. Rev. Plant Physiol.* **32,** 407-449.

Bayer, E. A., Skutelski, E., and Wilchek, M. (1979). The avidin-biotin complex in affinity cytochemistry. *In* "Methods in Enzymology" (D. B. McCormick and L. D. Wright, eds.), Vol. 62, pp. 308-315. Academic Press, New York.

Beardmore, J., Ride, J. P., and Granger, J. W. (1983). Cellular lingnification as a factor in the hypersensitive resistance of wheat to stem rust. *Physiol. Plant Pathol.* **22,** 209-220.

Bhattacharya, P. K., Shaw, M., and Naylor, J. M. (1968). The physiology of host-parasite/relations. XIX. Further observations on nucleoprotein changes in wheat leaf nuclei during rust infection. *Can. J. Bot.* **46,** 11-16.

Brown, J. F., Shipton, W. A., and White, N. H. (1966). The relationship between hypersensitive tissue and resistance in wheat seedlings infected with *Puccinia graminis tritici. Ann. Appl. Biol.* **58,** 279-290.

Bushnell, W. R., and Rowell, J. B. (1981). Suppressors of defense reactions: A model for roles in specificity. *Phytopathology* **71,** 1012-1014.

Callow, J. A. (1977). Recognition, resistance, and the role of plant lectins in host-parasite interactions. *Adv. Bot. Res.* **4,** 1-49.

Carlemalm, E., Villiger, W., and Acetarin, J. D. (1980). Advances in specimen preparation for electron microscopy. I. Novel low-temperature embedding resins and a reformulated vestopal. *Experientia* **36,** 740.

Cartwright, D. W., and Russell, G. E. (1980). Possible involvement of phytoalexins in durable resistance of winter wheat to yellow rust. *Trans. Br. Mycol. Soc.* **76,** 323-325.

Chakravorty, A. K., and Scott, K. J. (1979). Changes in two barley leaf ribonuclease fractions during infection by the powdery mildew fungus. *Physiol. Plant Pathol.* **14,** 95-97.

Chakravorty, A. K., and Shaw, M. (1971). Changes in the transcription pattern of flax cotyledons after inoculation with flax rust. *Biochem. J.* **123,** 551-557.

Chakravorty, A. K., and Shaw, M. (1977a). The role of RNA in host-parasite specificity. *Annu. Rev. Phytopathol.* **15,** 135-151.

Chakravorty, A. K., and Shaw, M. (1977b). A possible molecular basis for obligate host pathogen interactions. *Biol. Rev. Cambridge Philos. Soc.* **52,** 147-179.

Chakravorty, A. K., Shaw, M., and Scrubb, L. A. (1974a). Ribonuclease activity of wheat leaves and rust infection. *Nature (London)* **247,** 577-580.

Chakravorty, A. K., Shaw, M., and Scrubb, L. A. (1974b). Changes in ribonuclease activity during rust infection. I. Characterization of multiple molecular forms of ribonuclease from flax rust grown in host-free media. *Physiol. Plant Pathol.* **4,** 313-334.

Chakravorty, A. K., Shaw, M., and Scrubb, L. A. (1974c). Changes in ribonuclease activity during rust infection. II. Purification and properties of ribonuclease from healthy and infected flax cotyledons. *Physiol. Plant Pathol.* **4,** 335-358.

Chester, K. S. (1933). The problem of acquired physiological immunity in plants. *Q. Rev. Biol.* **8,** 129-154, 275-324.

Chong, J., Harder, D. E., and Rohringer, R. (1981). Ontogeny of mono- and dikaryotic rust haustoria: Cytochemical and ultrastructural studies. *Phytopathology* 71, 975–983.

Clarke, A. E., Anderson, R. L., and Stone, B. A. (1979). Form and function of arabinogalac-tans and arabinogalactan-proteins. *Phytochemistry* **18,** 521-540.

Daly, J. M. (1972). The use of near-isogenic lines in biochemical studies of the resistance of wheat to stem rust. *Phytopathology* **62,** 392-400.

deWit, P. J. G. M., and Spikman, G. (1982). Evidence for the occurrence of race and cultivar-specific elicitors of necrosis in intercellular fluids of compatible interactions of *Cladosporium fulvum* and tomato. *Physiol. Plant Pathol.* **21,** 1-11.

Doke, N., and Tomiyama, K. (1980a). Effect of hyphal wall components from *Phytophthora infestans* on protoplasts of potato tuber tissues. *Physiol. Plant Pathol.* **16,** 169-176.

Doke, N., and Tomiyama, K. (1980b). Suppression of the hypersensitive response of potato tuber protoplasts to hyphal wall components by water soluble glucans isolated from *Phytophthora infestans. Physiol. Plant Pathol.* **16,** 177-186.

Doke, N., Garas, N. A., and Kuc, J. (1980). Effect on host hypersensitivity of suppressors released during the germination of *Phytophthora infestans* cystospores. *Phytopathology* **70,** 35-39.

Ellingboe, A. H. (1976). Genetics of host-parasite interactions. *Encycl. Plant Physiol. New Ser.* **4,** 761-778.

Ellingboe, A. H. (1981). Changing concepts in host-pathogen genetics. *Annu. Rev. Phytopathol.* **19,** 125-143.

Ellingboe, A. H. (1982). Genetical aspects of active defence. *NATO Adv. Study Inst. Ser. Ser. A* **37,** 179-192.

Etzler, M. E. (1981). Are lectins involved in plant-fungus interactions? *Phytopathology* **71,** 744-746.

Ferrari, T. E., Lee, S. S., and Wallace, D. H. (1981). Biochemistry and physiology of recognition in pollen-stigma interactions. *Phytopathology* **71,** 752-755.

Flor, H. H. (1956). The complementary genetic systems in flax and flax rust. *Adv. Genet.* **8,** 29-54.

Flor, H. H. (1971). Current status of the gene-for-gene concept. *Annu. Rev. Phytopathol.* **9,** 275-296.

Flynn, J. G., Chakravorty, A. K., and Scott, K. J. (1976). Changes in the transcription patterns of wheat leaves during the early stages of infection by *Puccinia graminis tritici. Proc. Aust. Biochem. Soc.* **9,** 44.

Fuchs, A., Rohringer, R., and Samborski, D. J. (1967). Metabolism of aromatic compounds in healthy and rust-infected primary leaves of wheat. II. Studies with L-phenylalanine-U-^{14}C, L-tyrosine-U-^{14}C, and ferulate-U-^{14}C. *Can. J. Bot.* **45,** 2137-2153.

Fuchs, W. H. (1976). History of physiological plant pathology. *Encycl. Plant Physiol., New Ser.* **4,** 1-26.

Gabriel, D. W., and Ellingboe, A. H. (1982). Polypeptide mapping by two-dimensional electrophoresis and pathogenic variation in field isolates and induced mutants of *Erysiphe graminis* f. sp. *tritici. Phytopathology* **72,** 1496-1499.

Garas, N. A., Doke, N., and Kuc, J. (1979). Suppression of the hypersensitive reaction in potato tubers by mycelial components from *Phytophthora infestans. Physiol. Plant Pathol.* **15,** 117-126.

Gershoni, J. M., and Palade, G. E. (1983). Protein blotting: Principles and applications. *Anal. Biochem.* **131,** 1-15.

Gianinazzi, S. (1982). Antiviral agents and inducers of virus resistance: Analogies with interferon. *Nato Adv. Study Inst. Ser., Ser. A* **37,** 275-298.

Hagborg, W. A. F. (1970). A device for injecting solutions and suspensions into thin leaves of plants. *Can. J. Bot.* **48,** 1135-1136.

Harder, D. E., and Mendgen, K. (1982). Filipin-sterol complexes in bean rust and oat crown rust-fungal/plant interactions: Freeze-etch electron microscopy. *Protoplasma* **112,** 46-54.

Harder, D. E., Rohringer, R., Samborski, D. J., Kim, W. K., and Chong, J. (1978). Electron microscopy of susceptible and resistant near-isogenic *[sr6/Sr6)* lines of wheat infected by *Puccinia graminis tritici*. I. The host-pathogen interface in the compatible *[sr6/P6]* interaction. *Can. J. Bot.* **56,** 2955-2966.

Harder, D. E., Rohringer, R., Samborski, D. J., Rimmer, S. R., Kim, W. K., and Chong, J. (1979a). Electron microscopy of susceptible and resistant near-isogenic (*sr6/Sr6)* lines of wheat infected by *Puccinia graminis tritici*. II. Expression of incompatibility in mesophyll and epidermal cells and the effect of temperature on host-parasite interactions in these cells. *Can. J. Bot.* **57,** 2617-2625.

Harder, D. E., Samborski, D. J., Rohringer, R., Rimmer, S. R., Kim, W. K., and Chong, J. (1979b). Electron microscopy of susceptible and resistant near-isogenic (*sr6/Sr6)* lines of wheat infected by *Puccinia graminis tritici*. III. Ultrastructure of incompatible interactions. *Can. J. Bot.* **57,** 2626-2634.

Heath, M. C. (1976). Hypersensitivity, the cause or the consequence of rust resistance? *Phytopathology* **66,** 935-936.

Heath, M. C. (1980). Reactions of nonsuscepts to fungal pathogens. *Annu. Rev. Phytopathol.* **18,** 211-236.

Heath, M. C. (1981a). Resistance of plants to rust infection. *Phytopathology* **71,** 971-974.

Heath, M. C. (1981b). A generalized concept of host-parasite specificity. *Phytopathology* **71,** 1121-1123.

Heath, M. C. (1982). The absence of active defense mechanisms in compatible hostpathogen interactions. *NATO Adv. Study Inst. Ser., Ser. A* **37,** 143-156.

Heitefuss, R., and Wolf, G. (1976). Nucleic acids in host-parasite interactions. *Encycl. Plant Pathol, New Ser.* **4,** 480-508.

Hickey, E. L., and Coffey, M. D. (1978). A cytochemical investigation of the host-parasite interface in *Pisum sativum* infected by the downy mildew fungus *Peronospora pisi. Protoplasma* **97,** 201-220.

Hoppe, H. H., Humme, B., and Heitefuss, R. (1980). Elicitor induced accumulation of phytoalexins in healthy and rust infected leaves of *Phaseolus vulgaris. Phytopathol. Z.* **97,** 85-88.

Howard, R.). (1981). Ultrastructural analysis of hyphal tip cell growth in fungi: Spitzenkorper, cytoskeleton and endomembranes after freeze-substitution. J. *Cell Sci.* **48,** 89-103.

Howard, R. J., and Aist, J. R. (1979). Hyphal tip cell ultrastructure of the fungus

Fusarium: Improved preservation by freeze-substitution. *J. Ultrastruct. Res.* **66,**
Howes, N. K., Kim, W. K., and Rohringer, R. (1982). Detergent-soluble polypeptides extracted from urediospores of 4 physiologic raees of *Pvccima graminis f.* sp. *tritici. Physiol. Plant Pathol.* **21,** 361-366.
Ingram, D. S. (1982). A structural view of active defense. *NATO Adv. Study Inst. Ser., A* **37,** 19-38.
Jermyn, M. A., and Yeow, Y. M. (1975). A class of lectins present in the tissues of seed plants. *Aust. J. Plant Physiol.* **2,** 501-531.
Jones, D. R., and Deverall, B. J. (1977a). The effect of the *Lr20* resistance gene in wheat on the development of leaf rust, *Puccinia recondita. Physiol. Plant Pathol.* **10,** 27S-284.
Jones, D. R., and Deverall, B. J. (1977b). Experimental manipulation of the hypersensitive ponse associated with the expression of the *Lr20* gene in wheat following infection by leaf rust, *Puccinia recondita. Physiol. Plant Pathol* **10,** 285-290.
Jones, D. R., and Deverall, B. J. (1978). The use of leaf transplants to study the cause of hypersensitivity to leaf rust, *Puccinia recondita,* in wheat carrying the *Lr20* gene. *Physiol. Plant Pathol.* **12,** 311-319.
Keen, aN. T. (1982). Mechanisms conferring specific recognition in gene-for-gene plant-parasite systems. *NATO Adv. Study Inst. Ser.. Ser. A* **37,** 67-84.
Keen, N. T., and Legrand, M. (1980). Surface glycoproteins: Evidence that they mayfunction as the race-specific phytoalexin elicitors of *Phytophthora megasperma f.* sp. *glycinea. Physiol. Plant Pathol.* **17,** 175-192.
Keen, N. T., and Yoshikawa, M. (1983). B-l,3-Endoglucanase from soybean releases elicitor-active carbohydrates from fungus cell walls. *Plant Physiol.* **71,** 460-465.
Keen, N. T., Yoshikawa, M., and Wang, M. C. (1983). Phytoalexin elicitor activity of carhohydrates from *Phytophthora megasperma* f. sp. *glycinea* and other sources. *Plant Physiol.* **71,** 466-471.
Kim, W. K., Rohringer, R., Samborski, D. J., and Howes, N. K. (1977). Effect of blasticidin S, ethionine, and polyoxin D on stem rust development and host-cell necrosis in wheat isogenic for gene Sr6. *Can. J. Bot.* **55,** 568-573.
Klohs, W. D., Goff, C. W., and Bernacki, R. J. (1978). Glycosyltransferases in plant and animal tissues. Effects of fixation and lead on enzyme activity. *J. Histochezn. hem.* **26,** 772-781.
Kuc,J. (1982). Phytoalexins from the Solanaceae. *In* "Phytoalexins" (J. A. Bailey and). W. Mansfield, eds.), pp. 81-105.
Blackie, Glasgow. Kuck, K. H, Tiburzy, R., Hännssler, G., and Reisener, H.-J. (1981). Visualization of rust haustoria in wheat leaves by using fluorochromes. *Physiol. Plant Pathol.* **19,** 439-441.
Loegering, W. Q., and Harmon, D. L. (1969). Wheat lines near isogenic for reaction to *Puccinia graminis tritici. Phytopathology* **59,** 456-459.
Manocha, M. S. (1975). Autoradiography and fine-structure of host-parasite interface in temperature-sensitive combinations of wheat stem rust. *Phytopathol. Z.* **82,** 207-215.
Marte, M., and Montalbini, P. (1972). Microfluorescenza in foglie di fagiolo suscettibile e resistente alia ruggine. *Phytopathol. Z.* **75,** 59-73.
Mayama, S., Daly, J. M., and Rehfeld, D. W. (1975a). Hypersensitive response of nearisogenic wheat carrying the temperature-sensitive *Sr6* allele for resistance to stem rust. *Physiol. Plant Pathol.* **7,** 35-47.
Mayama, S., Rehfeld, D. W., and Daly, J. M. (1975b). The effect of detachment on the development of rust disease and the hypersensitive response of wheat leaves infected with *Puccinia graminis tritici. Phytopathology* **65,** 1139-1142.
Mayama, S., Tani, T., Matsuura, Y., Ueno, T., and Fukami, H. (1981a). The production of phytoalexins by oat in response to crown rust, *Puccinia coronata* f. sp. *avenae. Physiol. Plant Pathol.* **19,** 217-226.
Mayama, S., Tani, T., Matsuura, Y., Ueno, T., Hirabayashi, K., Fukami, H., Mizuno, Y., and Irie, H. (1981b). Biologically active substances related to the induced resistance in oat crown rust disease. J. *Chem. Soc. Jpn.* **5,** 695-704.
Mayama, S., Tani, T., Ueno, T., Hirabayashi, K., Nakashima, T., Fukami, K., Mizuno, Y., and Irie, H. (1981c). Isolation and structure elucidation of genuine oat phytoalexin, avenalumin. I. *Tetrahedron Lett.* **22,** 2103-2106.
Mayama, S., Matsuura, Y., Iida, H., and Tani, T. (1982a). The role of avenalumin in the resistance of oat to crown rust, *Puccinia coronata* f. sp. *avenae. Physiol. Plant Pathol.* **20,** 189-199.

Mayama, S., Hayashi, S., Yamamoto, R., Tani, T., Ueno, T., and Fukami, H. (1982b). Effects of elevated temperature and α-aminooxyacetate on the accumulation of avenalumins in oat leaves infected with *Puccinia coronata* f. sp. *avenae. Physiol.Plant Pathol.* **20,** 305-312.

Mendgen, K. (1978). Der Infektionsverlauf von *Uromyces phaseoli* bei anfalligen und resistenten Bohncnsortcn. *Phytopathol. Z.* **93,** 295-313.

O'Farrell, P. H. (1975). High resolution two-dimensional electrophoresis of proteins. *J. Biol. Chem.* **250,** 4007-4021.

Ogle, H. J., and Brown, J. F. (1971). Quantitative studies of the postpenetration phase of infection by *Puccinia graminis tritici. Ann. Appl. Biol.* **67,** 309-319.

Olien, C. R. (1957). Electrophoretic displacement of the necrotic area from the region of mycelium development in Khapli emmer wheat infected with race 56 of *Puccinia*
graminis var. *tritici*. *Phytopathology* **47,** 26 (abstr.).

Onoe, T., Tani, T., and Naito, N. (1976). Changes in the fine structure of Shokan I oat leaves at the early stage of infection with incompatible race 226 of crown rusi
fungus. *Ann. Phytopathol. Soc. Jpn.* **42,** 481-488.

Orr, G. A. (1981). The use of 2-iminobiotin-avidin interaction for the selective retrieval of labeled plasma membrane components. *J. Biol. Chem.* **256,** 761-766.

Owen, P., Oppenheim, J. D., Nachbar, M. S., and Kessler, R. E. (1977). The use of lectins in the quantitation and analysis of macromolecules by affinoelectrophoresis. *Anal. Biochem.* **80,** 446-457.

Prusky, D., Dinoor, A., and Jacoby, B. (1980). The sequence of death of haustoria and host cells during the hypersensitive reaction of oat to crown rust. *Physiol. Plant Pathol* **17,** 33-40.

Pure, G. A., Chakravorty, A. K., and Scott, K. J. (1979). Cell-free translation of messenger RNA isolated from healthy and rust-infected wheat leaves. *Physiol. Plant Pathol.* **15,** 201-209.

Pure, G. A., Chakravorty, A. K., and Scott, K. J. (1980). Changes in wheat leaf polysomal messenger RNA populations during the early stages of rust infection. *Plant Physiol.* **66,** 520-524.

Quail, P. H. (1979). Plant cell fractionation. *Annu. Rev. Plant Physiol.* **30,** 425-484.

Rathmell, W. G., and Sequeira, L. (1974). Soluble peroxidase in fluid from intercellular spaces of tabacco leaves. *Plant Physiol.* **53,** 317-318. Rohringer, R., Samborski, D. J., and Person, C. O. (1961). Ribonuclease activity in rusted wheat leaves. *Can. J. Bot.* **39,** 775-784. Rohringer, R., Fuchs, A., Lunderstadt, J., and Samborski, D. J. (1967). Metabolism of aromatic compounds in healthy and rust-infected primary leaves of wheat. I. Studies with ^{14}C02, quinate-U-^{14}C, and shikimate-U-^{14}C as precursors. *Can. J. Bot.* **45,** 863-889.

Rohnnger, R., Kim, W. K., Samborski, D. J., and Howes, N. K. (1977). Calcofluor: An optical brightener for fluorescence microscopy of fungal plant parasites in leaves. *Phytopathology* **67,** 808-810.

Rohringer, R., Kim, W. K., and Samborski, D. J. (1979). A histological study of interactions between avirulent races of stem rust and wheat containing resistance genes *Sr5, Sr6, Sr8,* or *Sr22. Can. J. Bot.* **57,** 324-331.

Rohringer, R., Chong, J., Kim, W. K., and Harder, D. E. (1982). Cytochemistry of the host/parasite interface and the use of gold markers in cereal/rust interactions. *NATO Adv. Study Inst. Ser., Ser. A* **37,** 349-350.

Rohringer, R., Ebrahim-Nesbat, F., and Wolf, G. (1983). Proteins in intercellular washing fluid from leaves of barley *[Hordeum vulgare* L.). *J. Exp. Bot.* (in press).

Roth, J., Bendayan, M., and Orci, L. (1978). Ultrastructural localization of intracellular antigens by the use of protein A—gold complex. *J. Histochem. Cytochem.* **26,** 1074-1081.

Roth, J., Bendayan, M., Carlemalm, E., Villiger, W., and Garaviro, M. (1981). The enhancement of structural preservation and immunocytochemical staining in low temperature embedded pancreatic tissue. *J. Histochem. Cytochem.* **29,** 663-671.

Rothman, P. G. (1960). Host-parasite interactions of eight varieties of oats infected with race 202 of *Puccinia coionata* var. *avenae. Phytopathology* 50, 914-918.

Rowell, J. B., Loegering, W. Q., and Powers, H. R. (1963). Genetic model for physiological studies of mechanisms governing development of infection type in wheat stem rust. *Phytopathology* **53,** 932-937.

Sachse, B., Wolf, G., and Fuchs, W. H. (1971). Nucleinsaure abbauende Enzyme in Blättern von *Triticum aestivum* nach Infektion mit *Puccinia graminis tritici. Acta Phytopathol. Acad. Sci. Hung.* **6,** 39-49.

Samborski, D. J., Kim, W. K, Rohringer, R., Howes, N. K., and Baker, R. J. (1977). Histological studies on host-cell necrosis conditioned by the *Sr6* gene for resistance in wheat to stem rust. *Can. J. Bot.* **55,** 1445-1452.

Samborski, D. J., Rohringer, R., and Kim, W. K. (1978). Transcription and translation in diseased plants. *In* "Plant Disease" (J. G. Horsfall and E. B. Cowling, eds.), Vol. 3, pp. 375-390. Academic Press, New York.

Scott, K. J., Chakravorty, A. K., and Flynn, J. G. (1976). Biochemical aspects of gene expression in wheat and barley after infection with rust and mildew fungi. *Aust. Plant Pathol. Soc. Newsl.* **5,** Suppl., 9.

Sela, B.-A., and Edelman, G. M. (1977). Isolation by cell-column chromatography of immunoglobulins specific for cell surface carbohydrates. *J. Exp. Med.* **145,** 443-449.

Sharon, N. (1977). Lectins. *Sci. Am.* **236,** 108-119.

Silverman, W. (1960). A toxin extracted from Marquis wheat infected by race 38 of the stem rust fungus. *Phytopathology* **50,** 130-136.

Skipp, R. A., and Samborski, D. J. (1974). The effect of the *Sr6* gene for host resistance on histological events during the development of stem rust in near-isogenic wheat lines. *Can. J. Bot.* **52,** 1107-1115.

Skipp, R. A., Harder, D. E., and Samborski, D. J. (1974). Electron microscopy studies on infection of resistant (5r6 gene) and susceptible near-isogenic wheat lines by *Puccinia graminis* f. sp. *tritici. Can. J. Bot.* **52,** 2615-2620.

Skutelski, E., and Bayer, E. A. (1979. The ultrastructural localization of cell surface glycoconjugates: Affinity cytochemistry via the avidin-biotin complex. *Biol. Cell.* **36,** 237-252.

Soding, H. (1941). Uber den Nachweis einer aus dem Interzellularraum von *E*scheveriablattern auswaschbaren bakteriziden Substanz. *Ber. Dtsch. Bot. Ges.* **59,** 458-466.

Stacey, G., Paau, A. S., and Brill, W. J. (1980). Host recognition in the *Rhizobium-soy*bean symbiosis. *Plant Physiol.* **66,** 609-614.

Strobel, G. A. (1973). The helminthosporoside-binding protein of sugarcane. *J. Biol. Chem.* **248,** 1321-1328.

Sutton, B. C. S., and Shaw, M. (1982). Changes in two ribonuclease isozymes during rust infection of flax cotyledons. *Plant Physiol.* **69,** 205-209.

Tani, T., and Yamamoto, H. (1979). RNA and protein synthesis and enzyme changes during infection. *In* "Recognition and Specificity in Plant Host-Parasite Interaction" (J. M. Daly and I. Uritani, eds.), pp. 273-287. Univ. Park Press, Baltimore, Maryland.

Tani, T., Yamamoto, H., Onoe, T., and Naito, N. (1975a). Initiation of resistance and host cell collapse in the hypersensitive reaction of oat leaves against *Puccinia coro-nata avenae. Physiol. Plant Pathol.* **7,** 231-242.

Tani, T., Ouchi, S., Onoe, T., and Naito, N. (1975b). Irreversible recognition demonstrated in the hypersensitive response of oat leaves against the crown rust fungus. *Phytopathology* **65,** 1190-1193.

Torp, J. (1982). The barley/powdery mildew interaction: Characterization of fungal cultures by two-dimensional gel electrophoresis. *NATO Adv. Study Inst. Ser., Ser. A* **37,** 361-362.

Van Loon, L. C. (1982). Regulation of changes in proteins and enzymes associated with active defence against virus infection. *NATO Adv. Study Inst. Ser., Ser. A* **37,** 247-273.

von Broembsen, S. L., and Hadwiger, L. A. (1972). Characterization of disease resistance responses in certain gene-for-gene interactions between flax and *Melampsora lini. Physiol. Plant Pathol.* **2,** 207-215.

Ward, E. W. B., and Stoessl, A. (1976). On the question of "elicitors" or "inducers" in incompatible interactions between plants and fungal pathogens. *Phytopathology* **66,** 940-941.

Yamamoto, H., Tani, T., and Naito, N. (1975). Changes in protein contents of oat leaves during the resistant reaction against *Puccinia coronata avenae. Phytopathol. Z.* **82,** 138-145.

Yamamoto, H., Tani, T., and Hokin, H. (1976). Protein synthesis linked with resistance of oat leaves to crown rust fungus. *Ann. Phytopathol. Soc. Jpn.* **42,** 583-590.

Ziegler, E., and Pontzen, R. (1982). Specific inhibition of glucan-elicited glyceollin accumulation in soybeans by an extracellular mannan-glycoprotein of *Phytophthora megasperma* f. sp. *glycinea. Physiol. Plant Pathol.* **20,** 321-331.

PART III

Structure and Physiology

A. The Rust Fungus
   Chapters 9 through 13
B. The Host-Parasite Interface
   Chapter 14
C. The Rusted Host
   Chapters 15 and 16

8

Virulence Frequency Dynamics of Cereal Rust Fungi

J. V. Groth
Department of Plant Pathology, University of Minnesota, St. Paul, Minnesota

I. Introduction

Virulence frequency dynamics denotes the phenomenon of change in frequency of virulence in rust fungus populations usually due to manipulation of the population of host plants by humans. In order to describe adequately changes in frequency of virulence, quantitative descriptions are necessary. Unfortunately, except in a few instances, neither careful measurement nor theoretical treatment of the dynamics of virulence has been attempted. The purpose of this chapter is therefore to suggest a framework of theoretical considerations and limitations, based largely on elementary developments in quantitative ecology and population genetics, and, wherever possible, to cite data and examples from plant pathology. The subject is in its infancy and lends itself to diverse speculation and a priori argument.

Changes in virulence frequency are influenced by many selective forces.[1] Some of these forces, in some circumstances, can be

accounted for simply; most probably cannot. Although it would be desirable to account for all important forces and their interactions, this is quite unrealistic considering our present lack of understanding and our in ability to measure individual forces, and thereby to verify the accuracy with which the forces have been described. Simplifying assumptions are necessary to examine the role of each force in affecting change in virulence frequency. The danger in this approach is in oversimplifying the case to where it is unrealistic and has no predictive value.

As with models of population growth of plant pathogens that have been developed by plant epidemiologists, models describing virulence frequency changes will have to be verified (Teng, 1981). Unlike population growth models, however, neither conception nor verification of such models can occur in small experimental plots. As Johnson (1979) pointed out, the durability of resistance (the lack of which is usually due to virulence frequency changes) can only be noted retrospectively, after the resistance has had full-scale use. This implies that our endeavor to understand the dynamics of virulence changes is not analogous to attempts by epidemiologists to understand population dynamics. Verification will be a slow process carried out under largely uncontrolled conditions. Our goals must remain modest if there is to be any chance of their being met. As a first (and modest) goal, this chapter will explore the dynamics of change in virulence that is simply inherited and race-specific—behaving more or less according to the gene-for-gene relationship (Person, 1959).

Such genes are important but are not the sole determinants of fitness of obligate parasites. Background polygenes play a role as well, and

these will be considered at the end of the chapter. That such polygenes largely determine the amount of, and variation in, aggressiveness of isolates is an assumption that underlies nearly all of the following discussions. For clarity, "virulence" in this chapter refers to specific, simply inherited changes of large magnitude. "Aggressiveness" refers to more subtle effects that result in changes in pathogen fitness and ability to cause disease. These two phenomena are usually but not always distinct.

II. Virulence Dynamics Curve

In discussing the dynamics of change in virulence, it will be convenient to refer to an idealized curve (Fig. 1) that shows changes in virulence frequency over time, in a manner quite analogous to what has been done with disease progress during the course of an epidemic (Van der Plank, 1963). The curve is based on a number of documented examples of virulence (usually as race) shifts. The time frame is longer than is normally considered for disease

progress curves, being figured in years or even decades, rather than the usual single season. The complete cycle of increase followed by decrease of virulence frequency is considered as was first treated by Person (1967). Race-specific, high-level virulence is commonly determined by a single recessive allele (Person and Ebba, 1975). The discussion about Fig. 1 will assume this, but most of the arguments and forces involved would not be qualitatively different were dominance of virulence assumed. The analysis of this curve is based on population genetics theory, and hence

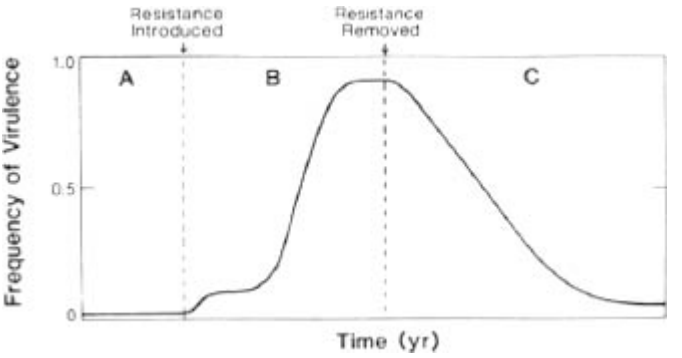


Fig. 1. Idealized curve of changes in frequency of specific virulence in a plant pathogen population before (A), during (B), and after (C) incorporation of corresponding resistance in the host.

it addresses genie changes. Because the idealized curve is based on phenotype, monogenic virulence whose alleles are in Hardy-Weinberg equilibrium must be assumed throughout. Three stages in Fig. 1 are delineated: The initial gene frequency of the virulence before resistance is added, the rapid increase in virulence to some maximum frequency, and the decrease in virulence once resistance is removed resulting in a new equilibrium gene frequency. These will be discussed separately.

A. INITIAL VIRULENCE FREQUENCY

1. Equilibrium Virulence Gene Frequency

Mutation is the source of new virulence genes. In large populations such as those of most or all of the rust fungi, mutation can be thought of as a recurrent rather than a unique event (Maynard Smith, 1968). The equilibrium frequency of a gene is determined by two forces: mutation and selection. In order for the intial frequency of phenotypes that are virulent with reference to a particular resistance to be low enough that resistance would have been selected for use,

these two forces must oppose one another in determining the equilibrium frequency. In other words, the fitness of phenotypes possessing the virulence must be slightly lower, on average, than that of phenotypes lacking it. In such cases, most mutations are toward virulence, because avirulence predominates. If the mean fitness of virulent phenotypes is greater than that of avirulent phenotypes, selection and mutation will together cause the virulence frequency to increase until it reaches an unacceptably high level before the matching resistance has been incorporated into the host. Examples of such "excess" virulence occur (Mac Key and Mattson, 1972; Martens and McKenzie, 1973). They are interesting because they show that not all specific, high-level resistance is valuable before it is incorporated into widely grown cultivars. Beyond this, such virulence will not be considered further, because corresponding resistance is unlikely to be used by plant breeders, nor would the virulence dynamics follow the pattern of Fig. 1.

What expectations might we have about the frequency of virulence in the "normal" case where specific virulence is initially rare? The equilibrium frequency is, first, likely to be higher than the rate of mutation toward virulence (Li, 1955; Person *et al.*, 1976). If fitness of the specifically avirulent phenotypes on hosts lacking the corresponding resistance is defined as 1, and that of the virulent phenotypes as 1 - s (where *s* is the selection coefficient), equilibrium frequency will only equal the mutation rate in the case where the new, recessive virulence gene is essentially lethal (s = 1) in the homozygous state. For dikaryotic rust fungi that regularly undergo sexual recombination, the balance between mutation and selection is approximately described by

$$q = (\mu/S)^{1/2} \quad (1)$$

where q is the equilibrium virulence gene frequency, μ is the pergeneration rate of mutation toward virulence, and s is the selection coefficient, per generation (including both sexual and asexual selection), against the virulent homozygous recessive (Falconer, 1960). It must be pointed out that the recessiveness of the effect of excess virulence on fitness is an unproven assumption based only on the possibility that the recessive virulence alleles import (pleiotropically, perhaps) a fitness reduction that is also recessive. If this is not true, the next best assumption is that there is no dominance in the effect of virulence alleles on fitness. The equilibrium relationship that approximates this case is

$$q=2\mu/S \quad (2)$$

The difference from Eq. (1) is that the heterozygote has a fitness that is exactly intermediate between the two homozygotes rather than being the same as the homozygous dominant. Both Eqs. (1) and (2) begin with the assumption of Hardy-Weinberg equilibrium (Falconer, 1960). When compared with Eq. (1), Eq. (2) shows that for a given value of *S*, the equilibrium frequency will be

closer to the mutation rate. There are no data to enable even an approximation of either the size of s or which equation best applies.

If sexual reproduction is rare or absent, neither those two nor any other equations derived from Hardy–Weinberg expectations in Falconer (1960) will apply (K. J. Leonard, personal communication). In such a case, relative rates of mutation toward virulence, back-mutation toward avirulence, and selection operating on relatively fixed genotypes are forces that interact to determine equilibrium gene and genotype frequencies. Numerous relationships seem possible between these forces because of our lack of knowledge of them. In some of the possibilities, equilibrium virulence gene frequencies will again be higher than simple mutation rates. Virulence frequency usually will not be so simple a function of virulence gene frequency, as it is when sexual reproduction is common. Genotype fitness becomes more critical, so that genotype frequency replaces gene frequency as a parameter. Population genetics theory has historically been centered around

sexually reproducing, diploid organisms, and has not been as long or thoroughly applied to either clonally or alternating clonally and sexually reproducing organisms.

2. *The Magnitude of* s *(the Selection Coefficient against Virulence)*

Equations (1) and (2) are only as accurate as the independent variables that comprise them. Of the two forces that determine equilibrium frequency, *s* is the more poorly known. We currently have no way of accurately measuring small values of *s* directly. Nor do we dare, as we do with mutation rates, extrapolate from other organisms or other kinds of genetic markers. Selection coefficients do not have a logical, definable biochemical basis as do (to be somewhat simplistic) mutation rates, and the effect of unnecessary virulence genes on fitness has never been studied carefully enough to allow confident estimation of these effects or understanding of their basis. They may be the result of linkage or pleiotropy, overdominance, or the chance occurrence of the allele for virulence finding itself in more poorly adapted isolates than the population in general. Because we have not measured *s*, we can only speculate about the relationship between the mutation rate and equilibrium frequency by inserting into Eqs. (1) or (2) some reasonable values. For example, if fitness effects of excess virulence are recessive [Eq. (1)], an *s* of 0.01 will give an equilibrium genotype frequency (q^2) in a dikaryotic rust that is 100 times μ, and a gene frequency (q) that is 10 times the square root of μ (e.g., 10,000-fold greater than μ if $\mu = 10^{-6}$). Similar exercises with larger values of s or using Eq. (2) will yield less spectacular discrepancies between μ and *q*.

Selection coefficients against isolates possessing unnecessary virulence are likely to be much smaller than those operating against avirulent isolates when the corresponding resistance has been incorporated into the host. (This fact will

also be mentioned in Section II,C,1, in conjunction with the rate of decrease in virulence frequency following the removal of resistance.) Most specific resistance involves rather drastic and obvious curtailment of pathogen reproduction, whereas the loss of fitness of rust phenotypes that has been attributed to excess virulence (or to other causes), if it can be directly measured at all, requires more precise measurement techniques. These include measurement of urediospore yields (Johnson and Taylor, 1976; Clifford and Clothier, 1974) and "competition" studies in which differential urediospore reproduction results in replacement of one isolate by another (Loegering, 1951; Katsuya and Green, 1967; Leonard, 1977a; Martens, 1973). These methods, as well as related methods of measuring fitness of fungus pathogens (MacKenzie, 1978; Nass *et al.,* 1981), have not been shown to be capable of accurately characterizing fitness in its entirety. The methods all suffer from measuring fitness of only a portion of the life cycle of the pathogen, and precise studies usually must be done under a narrow range of environmental conditions. Many of the methods are accompanied by large or cumulative experimental errors (Groth and Barrett, 1980). In short, the accurate measurement of fitness is a very difficult undertaking. R. C. Lewontin (1974, p. 236), speaking of this problem in general, states: "To the present moment no one has succeeded in measuring with any accuracy the net fitness of genotypes for any locus in any species in any environent in nature." Subtle fitness differences will be measured with more difficulty than will large differences.

Wolfe (1971) and Dekker (1976), dealing with very similar problems of measuring fitness of fungicide-tolerant forms of fungal pathogens, warn against using laboratory fitness measurements in describing field phenomena. Wolfe states that there is no reason to believe that isolates of a plant pathogen possessing additional fungicide tolerance will be drastically reduced in fitness in the absence of the fungicide. The population of tolerant isolates may exhibit a reduced mean fitness as compared with the original population because of the elimination of genetic heterogeneity, with its accompanying adaptability. Fitness measurements on individual isolates may therefore give negative or misleading results, because the isolates do not adequately represent the popula-

The problem is complicated by the fact that the differences in fitness between virulent and avirulent isolates are of undetermined size, and may be quite small, or may be larger than one might expect (Leonard, 1977b), considering the similar appearance of disease reactions involved. A graphic illustration of our inability to detect differences in mostly compatible reactions readily is given by Johnson and Taylor (1976). They show that within the higher infection-type classes in stripe rust of wheat, differences in urediospore production on individual seedlings were large, indicating that our ability to resolve fitness differences visually in compatible hostparasite combinations is poor.

3. The Magnitude of μ (the Rate of Mutation toward Virulence)

Mutation rates toward virulence have been measured, but they have not often been expressed in absolute terms that are comparable between studies (Watson, 1957, Zimmer *et al.*, 1963; Luig, 1979). One might assume, as Day (1974) did, that mutation rates for virulence are comparable to those determined for various markers in *Neurospora*, around 10^{-8}. In many instances, however, the inheritance of virulence is not known, so that Eqs. (1) and (2) will not describe the equilibrium frequency accurately. In at least one study (Zimmer *et al*, 1963), the rates of mutation toward virulence in three single isolates of *Puccinia coronata* were more than 10^{-4}. Assuming that Day is correct in his estimate of 10^{-8}, the range in rates of mutation toward virulence is roughly the same as that found for tolerance to fungicides in various fungi (Bartels-Schooley and MacNeill, 1971; Timmer *et al*, 1970). The nature of genetic control of fungicide tolerance is also not well understood, however (Dekker, 1976). Again, as with measurement of *s*, extrapolation to field populations of laboratory data on mutation rates, usually of a single isolate of the fungus, is not meaningful, because in the field selection must be considered and because a single isolate cannot be considered representative of a field population. Mutation rates calculated from laboratory studies of single isolates should not be used as estimates of initial frequency of genes for virulence or tolerance in field populations, as has been done in mathematical modeling (Day, 1974, p. 180; Skylakakis, 1982).

B. RAPID INCREASE IN FREQUENCY OF VIRULENCE

1. Do Plateaus Exist prior to Rapid Increase in Frequency!

Figure 1 illustrates a phenomenon that is seen in several cases in which the change in frequency of virulent races of rust fungi have been plotted over time as new resistances are incorporated into the host population. For a period of time preceding the rapid and relatively steady increases in the frequency of virulence, a plateau is seen (Johnson, 1953; Steward and Roberts, 1970; Roelfs *et al*, 1978). These plateaus are sometimes level and are at low but easily detectable frequencies, usually around .05 or higher. By its very nature, a cumulative plot of change of a gene or genotype frequency will be S-shaped, when a uniform selection pressure is applied (Falconer, 1960, p. 33). However, some of the plateaus appear to be more abrupt initially than they might be if this were their only basis. These plateaus are also more extended in time than the leveling at the other end of the period of rapid increase that might be expected from the theoretical shape of the curve.

The accuracy with which race surveys measure early, low-virulence frequencies has been questioned (Browder, 1966; Van der Plank, 1968, p. 97). Race surveys are designed to detect, as early as possible, specific virulences more than to provide unbiased estimates of their frequencies. Certain virulences can be considered more dangerous because of the proposed or early actual use of the corresponding resistance in the host. These virulences are usually overrepresented in the samples, as Browder (1966) clearly showed by comparing frequencies obtained in surveys with those obtained from the same population of stem rust using more representative sampling techniques. For this reason the plateaus may be explained on the basis that once a virulence has been identified as important, sensitive methods of screening, in the form of selective "trap" cultivars, are used to ensure that even trace amounts of the virulence in the population are detected. However, such screening cultivars can be used to obtain frequency estimates by also obtaining estimates of total population on susceptible cultivars in "mobile nurseries" (Eyal *et al.*, 1973), in which plants can be exposed to airborne inoculum for a specified period of time.

Although the plateaus just discussed may be artifacts, there is no reason to think that there cannot be an extended lag period during which the virulent isolates cannot increase in frequency at a rapid rate. This may especially be the case with the rust species that lack sexual recombination. As Roelfs and Groth (1980) have shown, the kind of variation one such population exhibited was very restricted. If variation in background genes is as limited as that in virulence genes for such populations, it would mean that total adaptation via the accumulation of genes for aggressiveness (or via the occurrence of mutations toward virulence in progressively better adapted backgrounds) in isolates possessing the necessary specific virulence would occur only slowly, even under intense selection pressure. This would appear as a slowly rising plateau.

2. The Period of Rapid Increase in Virulence Frequency

When resistance is incorporated widely into a crop, the pathogen's reproduction is reduced, because phenotypes that lack corresponding virulence are either unable to reproduce or reproduce only very poorly. It is probably safe to say that even when resistance is only partial, the magnitude of fitness reduction for those pathogen phenotypes that are affected by the resistance can be large (Johnson and Taylor, 1976; and as discussed in Section II,A,2). In mixture studies with *Uromyces phaseoli* (Pers.) Wint. var. *typica* Arth., we found, on two different bean cultivars that two virulent (but noticeably different) isolates had 3.7-fold fitness differences (corresponding to an s of 0.73). Spore yield differences in two trials on one of these cultivars averaged 7.6-fold [$s = 0.87$). Fitness differences are all measured from rates of replacement of collection of spores in discrete- or single-generation tests.

Continuous-generation tests should result in even greater rates of replacement or spore yield differences, because differences in reproduction each generation would be compounded, as will be shown in Eq. (4) versus Eq. (5).

The rate of replacement of a less virulent phenotype by one that is more virulent, once a new resistance has been introduced, is represented by the slope of the line in section B of Fig. 1. A number of things will influence this rate (Wolfe, 1973); some have been partially measured, and others are known only intuitively. The most obvious factor influencing this rate is the magnitude of the fitness difference between the new, favored phenotype(s) and those that compose the original population. Because the difference between the two is likely to be large, some of the methods of measuring either major components of vegetative or complete fitness are applicable here and have been used by many workers (see Section II,A,2). Experimental errors associated with the most precise of these methods should be small enough to allow fitness of individual phenotypes to be measured in specific environments. Extrapolation of these measurements to field environments, however, has been sometimes unsuccessful, even when the same isolates were used (Katsuya and Green, 1967; Martens, 1973).

Assuming that fitness of new and old phenotypes can be measured and accurately accounted for, predictions about the rate of replacement are presently still not possible. As indicated by their parameter α, Barrett and Wolfe (1979) have defined the effect of rate of reproduction, which is expressed in α, on change in phenotype frequency. In the present context, this means that the rate of replacement of avirulent by virulent phenotypes of rust fungi will vary directly with the rate of reproduction of the pathogen, so that replacement can be expected to occur more rapidly in favorable (explosive) disease years or locations. Modeling of this relationship is still in a theoretical stage. This may also help to explain why diseases that increase and spread more slowly, such as smut fungi (Holton, 1967), soil pathogens (Van der Plank, 1968), and slowly spreading airborne pathogens (Clifford, 1975), tend to overcome resistance in the host more slowly than do more rapidly reproducing and/or widely disseminated fungi such as rusts and powdery mildews.

Speed and final extent of incorporation of a new resistance into the crop in an epidemiological area will also directly affect the rate of increase of a new, matching virulence. Use of the resistance in only a portion of the crop will result in reduced selection pressure in favor of virulence, as compared with use of resistance in the entire crop. Because of widespread interest in partial resistance (see chapter by Parlevliet in Vol. II), it is useful to compare total, incompletely incorporated resistance with the use of partial resistance throughout the crop. With reference to selection against avirulent phenotypes, the affect of incompletely used resistance will be identical to the effect of using partial resistance if, in both cases, the resistant and susceptible portions of the crop are randomly attacked by virulent and avirulent phenotypes of the

pathogen, that is, if there is truly random association of the two organisms. If m = the proportion of susceptible acreage, t = the reduction in fitness due to high-level resistance, and s = the reduction in fitness due to partial resistance, overall fitness reduction in the case of incomplete use of high-level resistance and complete use of partial resistance, respectively, is

$$w = 1(m) + (1 - t)(1 - m) = 1(1 - s)$$

or

$$s = t(1 - m) \tag{3}$$

When t = 1 (resistance allows no reproduction), s = 1 – *m.*

If there is not random association, selection for the virulent phenotypes will proceed more rapidly in the local areas where the resistance is concentrated. At the limit of this picture, local and locally adapted (in the case of large, physically heterogeneous epidemiological areas) subpopulations of the pathogen may develop whose frequency of virulence is directly proportional to the intensity of use of the resistance in the region. The maintenance of these locally adapted subpopulations will depend on the degree of movement of propagules within the epidemiological area. Generally, the less movement that occurs, the higher the local virulence frequency that will be attained.

Many cereal rusts are sufficiently mobile within their epidemiological areas that local areas of high virulence frequency may not be allowed to develop. Examples of locally adapted races or phenotypes of rusts can be found, however (Browning and Bustamante, 1973; Roelfs, 1974; Brown, 1975), indicating that random association is not occurring over the entire geographical host range in many instances. Most disturbing is that even stem rust of wheat in central North America seems to have locally adapted subpopulations despite being one of the best documented examples of a highly mobile pathogen (Stakman and Harrar, 1957). The degree of local adaptation must eventually be quantitatively measured over the entire geographical host range if we are to understand and predict the durability of resistance, whether it be partial or complete. No serious attempts have been made to measure or even account for this variable. It may not be simply accounted for once it is characterized, however, because two distinct (and opposing) effects obtain from lack of random association. Local inbreeding subpopulations result in local equilibrium virulence frequencies, but they also result in a reduction in the effective population size, so that the assumption of a large population may not be valid.

Models incorporating equilibria between migration and selection as predominant forces may be applicable in such cases. The island model of Wright (cf. Crow and Kimura, 1970, pp. 267–268) is one possibility. The base population within which mutation, selection, inbreeding, or random drift operate then becomes something smaller than the entire population but larger than the local population because of migration. All that can be presently stated

is that, given that randomness of association is a determinant variable, the more randomly associated the host and pathogen populations are, the more durable (and desirable) will be partial resistance as compared with the incomplete use of high-level resistance. The proposed, intelligent regional deployment of resistance to stem rust of wheat (Frey *et al,* 1973) is a case in point. The effectiveness and durability of this example of the approach depends on knowledge of seasonal movement of the pathogen from region to region. Coupled with a degree of selection against unnecessary virulence (if such selection can be clearly proven), such an approach should provide greater durability than a more serendipitous deployment of genes in the same block areas and frequencies.

The best example of random nonregional deployment of genes in an incomplete manner is the advocated use of multilines where to a given phenotype of the pathogen, high-level resistance randomly occurs in some host plants but not in others. The theory of multilines has been reviewed recently (see chapter by Mundt and Browning in Vol. II; Leonard and Czochor, 1980) and will not be covered here. In the present context, it should be noted that multilines will depend for their durability on the interaction of the reductions in fitness due to several to many excess virulences in the pathogen, as most simplistically presented in a deterministic model (Groth, 1976). Multilines, if used exclusively in a crop, also represent the most random of all possible incomplete uses of single genes. If pathogen phenotypes possessing matching virulence are indeed less fit on the host component lacking the resistance, they should ensure greater stability than equally frequent but less random use of the resistance (as blocks, for instance, ranging in size from whole fields to regions). Because we are concerned in this chapter with virulence dynamics of single genes, these considerations will not be dealt with further.

Disregarding Barrett and Wolfe's (1979) α for the present, because I have argued that in the case of more explosive diseases, it may not be an important force (Groth, 1978), algebraic approximations of rate of virulence shift have been given in the literature for cases where pathogen reproduction is asexual (Leonard, 1977a). The logic used is the same as that so carefully presented by Van der Plank (1968) for approximating rate of disease increase. There are some notable differences, however. Because the incorporation of a new resistance in the host puts existing nonadapted phenotypes at a fitness disadvantage that can reach zero fitness, it is necessary to define the average fitness w of these phenotypes in the range $0 < w < 1$; hence $w = 1 - s$, where s is the selection coefficient. The mean fitness of pathogen phenotypes virulent on the new resistance (we have not had methods of looking closely enough to know whether one or more than one phenotype is involved in overcoming resistance) is then automatically defined as 1. (The alternative to this, defining the fitness of nonadapted phenotypes as 1 and of those virulent on the new resistance as $1 + s$, can result in the unreasonable and mathematically

unmanageable case of defining the higher fitness as infinite, if resistance is total.) Leonard (1977a) has derived the following relationship between rate of frequency change and fitness, assuming discrete, nonoverlapping generations

$$\frac{p_n}{q_n} = \frac{p_0}{q_0}(1 - s)^n \qquad (4)$$

where p is the frequency of phenotypes that are not adapted to the new resistance, $q(= 1 - p)$ is the frequency of those that are, and n is the number of generations. This relationship has been modified for cases where reproduction is continuous rather than discrete, by combining the exponential growth formula (Van der Plank, 1968) and the relationship between the aforementioned two uses of the selection coefficient (Groth and Barrett, 1980), giving

$$\frac{p_n}{q_n} = \frac{p_0}{q_0}\left(e^{\left(\frac{-sn}{1-s}\right)}\right) \qquad (5)$$

In general, the relationship in Eq. (5) gives a faster rate of replacement than does the relationship in Eq. (4) for the same values of s and n (n continuing to be expressed as generation equivalents). The discrepancy between the two relationships is greater, however, when the fitness difference between virulent and avirulent phenotypes is higher.

C. DECREASE OF VIRULENCE FREQUENCY AND FINAL EQUALIBRIUM

1. Selection as the Rate-Determining Force

Irrespective of whether the frequency of virulent phenotypes actually reaches fixation (effectively 100% of the population), the losses in crop yield due to the disease eventually become unacceptable, and the advantage gained earlier by incorporating the resistance is lost. The frequency of the resistance now begins to decline as cultivars containing it are replaced by others. Subsequently, the frequency of virulence should also begin to decline because of two forces: (1) the selective disadvantage due to the (now excess) virulence and (2) the selective advantage of "new" virulent phenotypes due to incorporation into the host of another resistance gene.

Van der Plank (1968, Chapter 4) has described the first of these forces, calling it "stabilizing selection." He states that the rate with which virulence decreases after the resistance has been removed determines the magnitude of the fitness disadvantage accompanying the now excess virulence, and this in turn is a measure of the "strength" of a resistance gene. "Weak" resistance genes are overcome by phenotypes that are nearly or fully as fit as phenotypes lacking the matching virulence. In this case, the virulence frequency should

decline slowly or, in extreme cases, not at all. No decline is only likely when a resistance gene is used in a different geographical area than its origin, because excess virulence that does not reduce fitness is likely to occur at relatively high initial frequency in the pathogen population, and locally, corresponding resistance to such virulence would probably never be used, because the virulent phenotypes would be quickly detected in breeders' nurseries. Given that some excess virulence imparts a fitness reduction, the logic of the argument just advanced is sound. However, it fails to consider the second important force as well as other factors determining the rate of virulence decline, and it therefore is of questionable value experimentally.

The second force to be considered is that of newly introduced resistance displacing that which has been overcome. A change in frequency can be looked at from two sides. Van der Plank chose to consider only the drop in frequency against an implied stable, passive background of other phenotypes, with the result that rate of decline of the virulence appeared to be primarily a function of its fitness in the absence of its corresponding resistance in the host. The other side of this change that must be considered when resistance genes are being incorporated in rapid succession is the resultant increase in fitness of some of the complementary phenotypes that serve equally to define the frequency of the "old" virulence. This phenomenon can be called "displacement."

Displacement of phenotypes adapted to the "old" resistance by those that are adapted to the "new" resistance is likely to be the dominant force determining rate of decline in frequency of the former virulence, if the new resistance directly replaces the old without a period of time during which neither resistance is being used. The rapid and uninterrupted use of strings of resistance genes is a common practice where rust is a chronic problem in cereal crops (Stakman and Harrar, 1957). If the effect of such displacement is not accounted for, attempts to determine the "strength" of a resistance gene by measuring the rate of decrease in frequency of corresponding virulence once the resistance has been withdrawn must be considered invalid. In the Eureka wheat *(Sr6* resistance) stem rust example presented by Van der Plank (1968, p. 67), the fact that the rate of virulence decline was only slightly less than was the rate of prior virulence increase (see next paragraph) suggests that forces other than the putative fitness reduction due to excess virulence (stabilizing selection) may have been largely responsible for the decline. The most likely of these was the displacement of the old phenotype by some newly favored phenotype(s). In fact, the resistance gene *Sr11* was included in Australian cultivars that quicly replaced Eureka (Watson and Luig, 1963).

Displacement should not affect the rate of decline of virulence frequency of genes for virulence that are independent of one another, because the rise in frequency of one gene should have no effect on the frequency of the other, in such cases. In asexual populations of rust fungi, however, it is a mistake to

assume independence of virulence genes either from one another or from their backgrounds. Roelfs and Groth (1980) have shown that in such a population, virulences are not randomly distributed among isolates, and there may be only a small number of virulence phenotypes. Virulence to overcome the new resistance may arise through mutation in any background. Those backgrounds that are best adapted to the environment and to the new host cultivar are most likely to predominate, and they may or may not contain the old virulence. In such a population, the occurrence of reduced fitness associated with excess virulence could influence but not solely determine the rate of decline of the old virulence (Leonard, 1977b). One might expect that the backgrounds of the formerly predominant phenotypes would be likely to contain (via mutation) the new virulence as well, because they are obviously well adapted to their environment and would allow the frequency of the new virulence to increase quickly. Two unknown factors may counteract this: (1) the influence of a change in host background, which usually accompanies the introduction of new resistance and which might make predominant pathogen backgrounds less well adapted, and (2) possible specific interactions between virulence genes and pathogen background genes, resulting in adapted gene complexes that cannot be readily broken up or changed (Clegg*et al,* 1972; Lewontin, 1974).

Assuming displacement is not operating in a particular case, the rate of decline of virulence will be directly proportional to the mean fitness difference between the virulent (with respect to the resistance now withdrawn) and avirulent phenotypes. What expectations might we have about the size of this fitness difference? In absolute terms we have no idea whatsoever, especially in that we do not know the basis for assuming that excess virulence is accompanied by reduced fitness. We can, however, compare the expectations for rate of virulence frequency decline with that of prior virulence frequency. When the frequency of virulence is increasing, the avirulent phenotypes are severely curtailed in their reproduction, even in cases where resistance is less than complete (Johnson and Taylor, 1976). When resistance has been removed, however, reproduction of competing phenotypes, although subtly different, is similar enough that all of them are categorized as virulent. Therefore, in the absence of displacement, the rate of virulence frequency decline is usually going to be less than the rate of virulence frequency increase, as illustrated in Fig. 1.

Finally, as in the case of increase of virulence frequency, the decrease will be retarded if resistance is not removed all at once. Removal of the resistant cultivar corresponds in this case to a gradual rather than an abrupt relaxation of selection pressure against avirulent phenotypes.

2. *The Final Equilibrium Frequency of Virulence after Removal of Resistance*

The same two forces—mutation opposing selection—should operate to bring the frequency of virulence to a level similar to that existing prior to the use of the corresponding resistance. But we know from studies of tolerance to fungicides (Wicks, 1976; Ruppel *et al*, 1980; Shabi and Katan, 1980) and race surveys of rust fungi that the frequency of the tolerant or virulent isolates often does not return to as low a level as before, and in some cases, does not diminish at all.

Race 15B of stem rust is a good example of virulence that has not diminished but has remained predominant in the Great Plains, even though it has possessed no advantageous combination of virulence since 1953 (A. P. Roelfs, personal communication). This suggests that the genes for tolerance or virulence per se were not responsible for whatever slight fitness reduction kept them at low frequencies initially. A likely alternative is that in the period when selection pressure allowed tolerant isolates to predominante, the population of such isolates became sufficiently large to allow more fit tolerant or virulent isolates to evolve through the slow accumulation of genes that enhance fitness. A gradual improvement in specific interactions between the gene for tolerance or virulence and the background genes cannot be ruled out here. This process would undoubtedly be accelerated by sexual or parasexual recombination (Muller, 1932), but Maynard Smith (1968) has demonstrated the likelihood that in very large populations, mutation without recombination can generate a random distribution of at least a few genes. The size of the population of tolerant or virulent individuals appears, then, to be the most critical variable.

Another less easily understood force that might affect large versus small populations differently is that of random drift. Because this is a stochastic force whose direction of effect is not predictable, it must suffice to say that random drift could be operating to keep the equilibrium frequency low initially, but not operating at all once the population of tolerant or virulent isolates becomes large, and continuing not to operate unless or until selection diminished the absolute size of the population of tolerant or virulent isolates.

The final frequency of virulence will be the same as or higher than its initial frequency was, but not lower. Mutation rate toward that virulence should not have changed. Selection against the unnecessary virulence will, if anything, be less, if one believes that genes interact to give a balanced and harmonious whole, as the balance theory of selection predicts (Lewontin, 1974). Once virulence has become widespread in a large rust population, it has a greater chance of being brought into a more nearly ideal background than when the virulence was at a low level. Removal of the corresponding resistance lifts directional selection for virulence, and the frequency should move to some

lower level. Several things will determine the final frequency. An obvious and often neglected one is the presence of hosts that continue to favor the virulence, in the form of residual plantings of the resistant cultivars or, perhaps, alternative host species. Host plants with cryptic resistance, unrecognized for several reasons, influence upward the initial virulence frequency also, but in the beginning there can be no residual resistance in cases where "new" resistance is being bred into the crop.

One other interesting point in the dynamics of host resistance frequency can be noted. As the resistant cultivar is being replaced by the susceptible one, a point is reached where the frequency of resistant and susceptible cultivars allows the virulent and avirulent isolates to reproduce equally well. Given that there is random association, if s is the average fitness reduction of avirulent phenotypes on the resistant cultivar that is at frequency p, k is the average fitness reduction of virulent phenotypes on all cultivars [Leonard's (1977b) cost of virulence], and the fitness of avirulent phenotypes on the susceptible cultivar—which is at frequency 1 -p—is defined as 1, then reproduction on virulent and avirulent phenotypes is equal at which is the frequency of resistant cultivars at which virulent and avirulent phenotypes are reproducing identically. In words, if fitness of virulent phenotypes is only slightly less than that of avirulent phenotypes, the presence of only a small proportion of the resistant cultivar will maintain the competitive ability of the virulent phenotypes. This is a variation of a similar relationship of Person *et al.* (1976), where the fitness of the virulent race on the resistant cultivar was defined as 1.0. The picture is complicated considerably when local populations develop, because the influence of migration between local regions has to be accounted for. If there is no migration between regions, the cultivar proportions locally become important in determining the composition of the pathogen population.

$$p(1 - k) + (1 - p)(1 - k) = p(1 - s) + 1 - p(1)$$

or

$$p = k/s \qquad (6)$$

III. Polygenic Nature of Fitness

By now it has become obvious that it is impossible to treat this topic by considering only genes for virulence, because reproductive ability or fitness is determined by the whole genome, not simply by the complement of virulence genes possessed by an isolate. The replacement of avirulent phenotypes of a rust pathogen by virulent ones is the result of a discrepancy in reproductive ability between the two groups. Examples of rust phenotypes that possessed

necessary virulence but were unable to predominante because they lacked aggressiveness (i.e., high fitness) are widespread (Roane *et al.*, I960; Clifford, 1975, Roelfs and Rothman 1976).

Fitness of individual isolates within races is likely to vary with time. The lack of a sexual stage in some rust fungi reduces the amount of genetic diversity (Groth and Roelfs, 1982) and the rate—and perhaps extent—of adaptation of the population to its environment. Unfortunately, this is about all that can be said right now. Only recently have attempts been made to use markers other than virulence to characterize diversity (Burdon*et al.*, 1982).

It is unwise to believe that a rust race lacks genetic diversity or, as a consequence of this thinking, that it cannot change over time. The race concept is useful, but it has led us to avoid or ignore the fact that a race is defined by only a few phenotypic traits. One explicit danger in this thinking that Van der Plank (1975, p. 1650) and Brown (1975) both caution about is our tendency to consider individual single-spore isolates as representatives of whole-race subpopulations. It is clear that rust fungi can adapt to resistance gradually, presumably through polygenic changes (Clifford and Clothier, 1974). Some of these changes appear to be specific ones involving a reversal of cultivar resistance ranking at a subtle level. However, Johnson and Taylor (1976) have pointed out that small adaptations to specific cultivars need not involve rank reversal at all. Adaptation by pathogens through small, possibly additive, genetic change is a subject that can only be studied using biometrical methods. Gene number, gene interaction, the inheritance of fitness, and other central questions cannot be answered with assurance using such methods (Falconer, 1960). In fact the detection of isolate–cultivar interactions that are due to specific gene-for-gene recognition may not be possible if several or many genes, each with small effect, govern resistance and aggressiveness (Parlevliet and Zadoks, 1977). This presumably is true only if strong, divergent selection has not been operating on the variants of host and parasite that would be subject of such investigations. If our understanding of virulence dynamics as discussed in this chapter is incomplete, it is largely because precise data and theoretical interpretation of polygenic phenomenon are wanting. We are no different in this respect from other applied biologists. It is to be hoped that we will be able to devote more effort to the study of all facets of host–parasite population genetics in the future.

References

Barrett, J. A., and Wolfe, M. S. (1979). Multilines and super-races—a reply. *Phytopathology* 69, 1535–1537.

Bartels-Schooley, J., and MacNeill, B. (1971). A comparison of the modes of action of three benzimidazoles. *Phytopathology* **61**, 816–189.

Browder, L. E. (1966). A rapid method of assaying pathogenic potential of populations of |*Puccinia graminis tritici*. *Plant Dis. Rep.* **50**, 673–676.
Brown, J. F. (1975). Factors affecting the relative ability of strains of fungal pathogens to survive in populations. *J. Aust. Inst. Agric. Sci.* **41**, 3–11.
Browning, J. A., and Bustamante, E. (1973). Evidence for environmental races of *Puccinia graminis* f. sp. *avenae*. *Abstr. Pap., Int. Congr. Plant Pathol., 2nd, 1973* Abstr. No. 717.
Burdon, J. J., Marshall, D. R., Luig, N. H., and Gow, D. J. S. (1982). Isozyme studies on the origin and evolution of *Puccinia graminis* f. sp. *tritici* in Australia. *Aust. J. Biol. Sci.* **35**, 231–238.
Clegg, M. T., and Allard, R. W., and Kahler, A. L. (1972). Is the gene the unit of selection? Evidence from two experimental plant populations. *Proc. Natl. Acad. Sci. U.S.A.* **69**, 2474-2478.
Clifford, B. C. (1975). Stable resistance to cereal diseases: Problems and progress. *Rep.— Welsh Plant Breed. Stn. (Aberystwyth, Wales)* pp. 107–113.
Clifford, B. C., and Clothier, R. B. (1974). Physiologic specialization of *Puccinia hordei* on barley hosts with non-hypersensitive resistance. *Trans. Br. Mycol. Soc*. 63, 421–430.
Crow, J. F., and Kimura, M. (1970). "An Introduction to Population Genetics Theory." |Harper & Row, New York.
Day, P. R. (1974). "Genetics of Host-Parasite Interaction." Freeman, San Francisco, California.
Dekker, J. (1976). Acquired resistance to fungicides. *Annu. Rev. Phytopathol*. 14, 405-428.
Eyal, Z., Yurman, R., Moseman, J. G., and Wahl, I. (1973). Use of mobile nurseries in pathogenicity studies of *Erysiphe graminis* f. sp. *hordei* on *Hordeum spontaneum. Phytopathology* 63, 1330–1394.
Falconer, D. S. (1960). "Introduction to Quantitative Genetics." Oliver & Boyd, London and New York.
Flor, H. H. (1958). Mutation to wider virulence in *Melampsora lini. Phytopathology* 48, 297-301.
Frey, K. J., Browning, J. A., and Simons, M. D. (1973). Management of host resistance genes to control diseases. *Z. Pflanzenkr. Pflantzenschutz* **80**, 160–180.
Groth, J. V. (1976). Multilines and "super-races": A simple model. *Phytopathology* **66**, 937–939.
Groth, J. V. (1978). Rebuttal to "multilines and super-races—a reply." *Phytopathology* **68**, 1538–1539.
Groth, J. V., and Barrett, J. A. (1980). Estimating parasitic fitness. A reply. *Phytopathology* **70**, 840–842.
Groth, J. V., and Roelfs, A. P. (1982). Effect of sexual and asexual reproduction on race abundance in cereal rust fungus populations. *Phytopathology* **72**, 1503–1507.
Holton, C. S. (1967). Distribution and prevalence of virulence for Victoria oats in *Ustilago avenae. Plant Dis. Rep.* **51**, 846–849.
Johnson, R. (1979). The concept of durable resistance. *Phytopathology* **69**, 198–199.
Johnson, R., and Taylor, A. J. (1976). Spore yield of pathogens in investigations of the race specificity of host resistance. *Annu. Rev. Phytopathol.* **14**, 97–119.
Johnson, T. (1953). Variations in the rusts of cereals. *Biol. Rev. Cambirdge Philos. Soc*. **28**, 105–157.
Katsuya, K., and Green, G. J. (1967). Reproductive potentials of races 15B and 56 of wheat stem rust. *Can. J. Bot.* **45**, 1077-1091. Leonard, K. J. (1977a). Virulence, temperature optima, and competitive abilities of isolines of Race T and O of *Bipolaris tnaydis. Phytopathology* **67**, 1273–1279.
Leonard, K. J. (1977b). Selection pressures and plant pathogens. *Ann. N.Y. Acad. Sci.* **287**,
207–222.
Leonard, K. J., and Czochor, R. J. (1980). Theory of genetic interactions among populations of plants and their pathogens. *Annu. Rev. Phytopathol.* **18**, 237–258.
Lewontin, R. C. (1974). "The Genetic Basis of Evolutionary Change." Columbia Univ. Press, New York.
Li, C. C. (1955). "Population Genetics." Univ. of Chicago Press, Chicago, Illinois.
Loegering, W. Q. (1951). Survival of races of wheat stem rust in mixtures. *Phytopathology* **41**, 56–65.
Luig, N. H. (1979). Mutation studies in *Puccinia graminis tritici. Proc. Int. Wheat Genet. Sympo., 5th, 1978* pp. 533–539.
MacKenzie, D. R. (1978). Estimating parasitic fitness. *Phytopathology* **68**, 9–13.
Mac Key, J., and Mattson, B. (1972). Rasspecifik resistensforadling mot skandinavisk havresvartrost. *Sver. Utsadesfoeren. Tidskr*. **82**, 186–203.
Martens, J. W. (1973). Competitive ability of oat stem rust races in mixtures. *Can. J. Bot*. **51**, 2233–2236.

Martens, J. W., and McKenzie, R. I. H. (1973). Resistance and virulence in the *Avena: Puccinia coronata* host-parasite system in Kenya, Ethiopia. *Can. J. Bot.* **51**, 711–714.
Maynard Smith, J. (1968). Evolution in sexual and asexual populations. *Am. Nat.* **102,** 469–473.
Muller, H. J. (1932). Some genetic aspects of sex. *Am. Nat.* 8, 118–138.
Nass, H. A., Pederson, W. L., MacKenzie, D. R., and Nelson, R. R. (1981). The residual
effects of some "defeated" powdery mildew resistance genes in isolines of winter wheat. *Phytopathology* **71**, 1315–1318.
Parlevliet, J. E., and Zadoks, J. C. (1977). The integrated concept of disease resistance; a new view including horizontal and vertical resistance in plants. *Euphytica* **26**,5–21.
Person, C. (1959). Gene-for-gene relationship in host-parasite systems. *Can. J. Bot.* **37**, 1101–1130.
Person, C. (1967). Genetic aspects of parasitism. *Can. J. Bot.* **45**, 1193–1204.
Person, C, and Ebba, T. (1975). Genetics of fungal pathogens. *Genetics, Suppl.* 79, 397–408.
Person, C., Groth, J. V., and Mylyk, O. M. (1976). Genetic change in host-parasite populations. *Annu. Rev. Phytopathol.* **14**, 177–188.
Roane, C. W., Stakman, E. C., Loegering, W. Q., Stewart, D. M., and Watson, W. M. (1960). Survival of physiologic races of *Puccinia graminis var. tritici* on wheat near barberry bushes. *Phytopathology* **50**, 40–44.
Roelfs, A. P. (1974). Evidence for two populations of wheat stem rust in the U.S.A. *Plant Dis. Rep.* **58**, 806–809.
Roelfs, A. P., and Groth, J. V. (1980). A comparison of virulence phenotypes in wheat stem rust populations reproducing sexually and asexually. *Phytopathology* **70**, 855–862.
Roelfs, A. P., and Rothman, P. G. (1976). Races of *Puccinia graminis* f. sp. *avenae* in the U.S.A. during 1975. *Plant Dis. Rep.* **60**, 703–706.
Roelfs, A. P., Casper, D. H., and Long, D. L. (1978). Races of *Puccinia graminis f.* sp. *avenae* in the United States during 1977. *Plant Dis. Rep.* **62**, 600-604.
Ruppel, E. G., Jenkins, A. D., and Burtch, L. M. (1980). Persistance of benomyl tolerant
strains of *Cercospora beticola* in the absence of Benomyl. *Phytopathology* 70, 25–26.
Shabi, E., and Katan, T. (1980). Fitness of *Venturia pinna* isolates resistant to benzimidizole fungicides. *Phytopathology* **70**, 1172–1174.
Skylakakis, G. (1982). Epidemiological factors affecting the rate of selection of biocide-resistant genotypes of plant pathogenic fungi. *Phytopathology* **72**, *271–273.*
Stakman, E. C., and Harrar, J. G. (1957). "Principles of Plant Pathology." Ronald Press, New York.
Stewart, D. M., and Roberts, B. J. (1970). Identifying races of *Puccinia graminis* f. sp. *avenae*—A modified international system. *U.S. Dept. Agric, Tech. Bull.* **1416,**1-23. Teng, P. S. (1981). Validation of computer models of plant disease epidemics: A review of philosophy and methodology. *Z. Pflanzenkr. Pflanzenschutz* **88**, 49–63.
Timmer, L. W., Castro, T., Erwin, D. C., Beiser, W. L., and Zentmeyer, G. A. (1970).
Genetic evidence for zygotic meiosis in *Phytophthoia capsici. Am. J. Bot.* **57**, 1211–1218.
Van der Plank, J. E. (1963). "Plant Disease: Epidemics and Control." Academic Press, New York.
Van der Plank, J. E. (1968). "Disease Resistance in Plants." Academic Press, New York.
Van der Plank, J. E. (1975). "Principles of Plant Infection." Academic Press, New York.
Watson, I. A. (1957). Mutation for pathogenicity in *Puccinia graminis* var. *tritici. Phytopathology* **47**, 507–509.
Watson, I. A., and Luig, N. H. (1963). The classification of *Puccinia graminis* var. *tritici* in relation to breeding resistant varieties. *Proc. Linn. Soc. N. S. W.* **88**, 235–258.
Wicks, T. (1976). Persistance of benomyl tolerance in *Verturia inaequalis. Plant Dis. Rep.* **60**, 818–819.
Wolfe, M. S. (1971). Fungicides and the fungus population problem. *Proc. Bri. Insectic. Fungic. Conf.* **3**, 724–734.
Wolfe, M. S. (1973). Changes and diversity in populations of fungal pathogens. *Ann. Appl. Biol.* **75**, 123–149.
Zimmer, D. E., Schaefer, J.F., and Patterson, F. L. (1963). Mutations for virulence in *Puccinia coronata. Phytopathology* **53**, 171–176.

[1]Definitions of terms used in this chapter—*Selective force:* Any force that influences reproduction of rust fungus phenotypes. Some forces operate specifically on some phe-notypes; others influence all of them equally. *Virulence frequency:*The proportion of isolates in a rust fungus population that exhibits a relatively high-infection type on a particular host line separated from low-infection types by an easily

recognized discontinuity. Virulence frequency is equated in this chapter with virulence genotype frequency, even though evidence for single genes is often only circumstantial or lacking. *Virulence gene:* A Mendelian factor conditioning a high-infection type on a particular host line. *Random drift:* The fluctuation of genotype frequencies in small populations due to small sample size in natural processes, such as migration, mutation, selection, or mating. The magnitude but not the direction of random drift can be estimated. *Migration:* Ingress and egress of genotypes between populations that are mostly isolated from one another, as by host preference or geographical barriers. *Mutation rate:* The proportion of alleles at a single genetic locus showing recognizable changes per generation. This precise definition is used in this chapter even though it is only assumed that observed changes in pathogen virulence are mutational events or that they are occurring at a single locus.

9

Germination of Urediospores and Differentiation of Infection Structures

Richard C. Staples
Vladimir Macko
Boyce Thompson Institute for Plant Research, Cornell University, Ithaca, New York

I. Introduction

Urediospores of the rust fungi imbibe water, swell, and germinate to form a germ tube. When colonizing living plants, the germ tubes of the wheat stem rust fungus *[Puccinia graminis* f. sp. *tritici* Eriks, and E. Henn.) invade their

hosts via the stomata by developing a set of infection structures (Fig. 2). The structures are developed when the tip of the germ tube encounters a stoma, and parasitism occurs when a haustorium is produced within a host cell within the leaf.

The first part of this chapter deals with the effect of endogenous and exogenous factors on that phase of urediospore germination that precedes appressorium formation, that is, events and factors closely associated with urediospore dormancy and its reversal leading to germination. The second part emphasizes what is known of how rust urediospores and their germlings recognize and respond to the environment of the host surface leading to differentiation of infection structures.

Long-cycle rusts have five spore types of which the urediospore is one, but very little research has appeared that deals with germination and penetration by the other types of spores. Our earlier reviews have dealt with some aspects of dormancy and recognition in the rusts and other fungi (Staples and Macko, 1980; Macko, 1981; Macko *et al*, 1976; Staples and Huang, 1982). However, some important extensions of our knowledge of recognition in the rusts have occurred since then, and a resynthesis of our concepts of fungal sensing is very timely.

Several aspects of urediospore germling differentiation not covered here are discussed in another review (Wynn and Staples, 1981), especially Wynn's research on waxless mutants. In addition, Littlefield and Heath (1979) have thoroughly reviewed the ultrastructure of rust development.

Urediospores of the wheat stem rust fungus appear to produce infection structures primarily in response to stimulation by chemicals and heat rather than by contact stimuli as with germlings of the bean rust fungus (cf. Staples and Macko, 1980). However, urediospores of the bean rust fungus *[Uromyces phaseoli* (Pers.) Wint.] have now been shown to respond to chemicals and heat as well (Staples and Hoch, 1982), and studies on the physiology of a diverse range of urediospore species may prove to be more efficient for developing an understanding of how germlings develop infection structures than studies on cereal rusts alone. Hence, literature pertinent to infection structure development that deals with fungi other than the cereal rusts is also included in this chapter.

II. The Process of Germination

A. A HISTORIAL PERSPECTIVE

In the past, the most interesting aspect of urediospore physiology was the search for a “lesion” that results in an apparent inability of the rust urediospore to grow beyond the germ tube stage of development, even when provided with

an endogenous nutrient supply. However, such a lesion was not found; moreover, it was established that germinating urediospores possess all the basic machinery of a eukaryotic cell. A new era in the study of obligate parasitism started when it was discovered that wheat stem rust could be cultured axenically starting from aseptic urediospores placed directly on fairly simple agar medium (Williams *et al.*, 1966; see also Williams, Chapter 13, this volume). Now the emphasis has shifted toward an understanding of how the infection structures including the haustorium are differentiated, and matters that deal with fungal responses to resistant and susceptible host plants.

B. EXTERNAL FACTORS THAT CONTROL GERMINATION

1. Light

Germination of *Puccinia graminis* f. sp. *tritici* urediospores is inhibited by continuous irradiation (Givan and Bromfield, 1964,ab; Sharp *et al,* 1958; Lucas *et al.,* 1975). High-intensity white light, in excess of 10,000 lux, completely inhibits urediospore germination, although after 24 hr continuous irradiation there is a partial recovery of germination (Knights and Lucas, 1980). Light was found to affect germination prior to emergence of the germ tube. Because this stage coincides with pore plug dissolution (see p. 261), an event reversibly inhibited by the native self inhibitor methyl *cis*-ferulate, there may be a link between the self-inhibitor and the photocontrol mechanism. Washing the spores relieves both photoinhibition (Chang *et al.,* 1974) and self-inhibition (Hess *et al.,* 1975), and Knights and Lucas (1980) have suggested that the self-inhibitor may have a role in how light inhibits germination.

Negative phototropism in urediospores of several species of rust fungi occurs in response to light through oriented growth of the germ tube (Gettkandt, 1954). The germ tube apparently acts as a cylindrical lens, and light is focused on the wall farthest from the source of illumination. Because light in this case inhibits growth, the germ tube bends away from the light, resulting in a negative phototropism. Maximal effective wavelengths for negative phototropism of *P. graminis* f. sp. *tritici* germ tubes were in the vicinity of 400 nm (Chang and Calpouzos, 1973).

Phytochrome control of several physiological responses in higher green plants is well established. Because a number of fungal photoresponses are mediated by red and far-red light, perhaps a phytochrome type of system is operative in fungi as well. Reports have now appeared indicating that germination of *P. graminis* f. sp. *tritici* may be affected by a photoreversible pigment system similar to phytochrome (Calpouzos and Chang, 1971; Lucas *et al.*, 1975; Schneider and Murray, 1979).

2. Temperature

The urediospores of rust fungi are short-lived, and so they are maintained by periodic infections of host plants. Therefore, preservation of these spores in a viable condition is important in plant-breeding programs directed against these pathogens, as well as for physiological and biochemical studies (see Rowell, Chapter 10 this volume). Procedures developed to prolong the viability of rust urediospores have been developed using liquid nitrogen for storage (Bromfield, 1964; Flor, 1954; Loegering *et al.,* 1966), but spore germinability after freezing critically depends on the method used to revive the spores (Bromfield, 1964). Thus, when air-dry urediospores of *P. graminis* f. sp. *tritici* are cooled in liquid nitrogen to – 196°C, germination is markedly reduced. Loss of germinability can be reduced by a heat shock (Loegering *et al,* 1961) or vapor-phase hydration (Bromfield, 1964), which suggests that freezing induces a reversible cold dormancy in urediospores of this species. Finally, it was demonstrated that there are no obvious ultrastructural differences between cold-treated and untreated urediospores, but that profound ultrastructural changes do develop during rehydration of cold-dormant spores in water (Sussman *et al.,* 1969). From this and subsequent physiological studies (Maheshwari and Sussman, 1971), it was concluded that cold dormancy is a condition of supersensitivity of frozen spores to rapid hydration by liquid water. Wetting spores results in irreversible damage to them unless the spores are first warmed briefly. Such a gentle treatment with heat apparently protects permeable membranes during hydration of the spores. For more on rehydration injury, see Rowell, Chapter 10, this volume.

3. Ions

High concentrations of large air ions inhibit the germination of urediospores of *Puccinia striiformis* (Sharp, 1967, 1972). Germlings are most sensitive during the first hour of germination. Combustion nuclei from automobile exhaust can be an important source of large air ions, including lead. The sensitivity of urediospores of other rust fungi to air ions has not been reported.

4. Effect of Microorganisms

Bacteria have been shown to inhibit the *in vitro* growth of certain plant pathogenic fungi and to reduce disease *in vivo.* Levine *et al.* (1936) suggested that the bacteria affecting *P. graminis* were in the genus*Bacillus.* French *et al.* (1964) reported that the bacteria affecting *P. graminis* were *Bacillus megaterium,* whereas Morgan (1963) found that it was the *Bacillus pumilus* that adversely affected three cereal rusts. McBride (1969) reported that *B. cereus, B.*

mycoides, and an unidentified *Bacillus* sp. were components of leaf surface microflora of the Douglas fir *[Pseudotsuga menziensii)* in Canada. Mixtures of nutrient broth cultures of these organisms controlled the rust *Melampsora medusae* on Douglas fir in greenhouse experiments.

Finally, *Bacillus cereus* has been shown to be associated with urediospores of *Puccinia allii* Rud. on the leaves of leek, *Allium porrum* (Doherty and Preece, 1978). Living cells of *B. cereus* completely inhibited the germination of the urediospores of *P. allii* on agar. The effective agent passed through cellophane.

Most workers were apparently unaware of the potency of *Bacillus*– fungus interactions until they were observed accidentally. The usual habitat of *B. cereus* is the soil. On this basis Doherty and Preece (1978) feel that a systematic and thorough study of the distribution, and nature, of the antagonistic interactions between members of the genus *Bacillus* and fungi infecting the leaves of plants is now long overdue.

C. INTERNAL FACTORS THAT CONTROL GERMINATION

1. Metabolism

Carbohydrate and lipid metabolism was studied by Daly *et al.* (1967). During germination and germ tube extension of *P. graminis* f. sp. *tritici* urediospores, there is a rapid utilization of palmitic, oleic, linoleic, and 9,10-epoxyoctadecanoic acids. These authors concluded that the germination process in rust urediospores is not based solely on the utilization of lipids, but that of carbohydrates as well.

Phospholipids in *Uromyces phaseoli* urediospores are extensively degraded at a very early stage of germination and then later are resynthesized (Langenbach and Knoche, 1971a,b). These changes in phospholipid levels may reflect a degradation and resynthesis of membranes. Alternatively, the changes may result from the very rapid early pace of synthesis, which eventually slows as the germ tube reaches maturity.

Germinating urediospores apparently have a limited capacity for synthesis of proteins, but ribosomes isolated from bean rusts and wheat rust urediospores are fully functional (Staples *et al*, 1972). Studies on bean rust urediospores, whose endogenous substrates have been labeled with carbon-14, showed that a 25% increase in protein content occurs after 1.5 hr of germination (Trocha and Daly, 1970).

A detailed study of the composition of the cell walls from germinating bean rust spores showed that glycoprotein is present. Carbohydrates of the urediospore and germ tube cell walls were identified as glucose and

glucomannan; germ tube walls in addition contained a chitin-like polymer (Trocha and Daly, 1974; Trocha *et al*, 1974; Wynn and Gajdusek, 1969).

In summary, numerous studies have shown that rust urediospores have a limited synthetic capacity during germination. The main function of the catabolism of lipids and carbohydrates during germination no doubt is to provide starting materials for several synthetic processes, especially cell wall formation and the production of new cell membranes.

2. Self-Inhibitors of Germination

Spores of a wide range of fungi contain self-inhibitors of germination (Allen, 1976; Macko, 1981). Germination of spores that contain self-inhibitors is reversibly inhibited by the chemicals within them. These endogenous chemicals are released when the spores are floated on water, and the water extracts contain the inhibitory principle. During germination, spores that contain self-inhibitors exhibit the well-known "crowding effect," the phenomenon in which spores crowded together on a surface germinate poorly or not at all. There are other types of dormant spores that contain endogenous germination inhibitors that are not readily soluble in water, and there are spores that possess permeability barriers, so that the inhibitor is not released when the spore is floated (Trione, 1980). Such spores do not exhibit a crowding effect. Nevertheless, there is sufficient inhibitor within each spore to cause dormancy. Teliospores of the dwarf bunt fungus, *Tilletia controversa*, are an example of the latter type (Trione, 1980).

The ecological advantage of self-inhibition is probably to prevent germination in the sorus and to prevent rapid germination of all spores at the same time. The numerous reports of self-inhibition have been summarized in reviews by Allen (1976), Macko *et al.* (1976), and Macko (1981). More detailed studies of the crowding effect have been carried out, particularly with the rust fungi after Allen (1955) and Yarwood (1954, 1956) showed that a chemical inhibitory principle is involved.

The definitive evidence supporting the existence of self-inhibitors was developed with urediospores of wheat stem rust and bean rust fungi (Allen and Dunkle, 1971; Bell and Daly, 1961). Since then, the self-inhibition phenomenon in fungal spores has been reported for a wide range of taxonomic groups comprising more than 50 fungal species (Allen, 1976).

Some of the active substances have been isolated and characterized, and the chemical identity of a few inhibitors is now established. The self-inhibitor of bean, sunflower, corn, snapdragon, peanut, and stripe rust urediospores is methyl cis-3,4-dimethoxycinnamate, whereas that of wheat stem rust urediospores is methyl cis-4-hydroxy-3-methoxycinnamate (methyl cis-ferulate) (Foudin and Macko, 1974; Macko *et al*, 1970, 1971a,b, 1972, 1977). The inhibitory concentration of the dimethoxycinnamate compound ranges

from a few picograms per milliliter (10^{-11} *M*) for peanut rust urediospores to a few nanograms per milliliter (10^{-8} *M*) for bean rust.

Wall material in the region of the germ pore plug is digested as germination progresses in rust urediospores (Fig. 1). The phrase *germ pore plug* refers to a predetermined point of germ tube emergence, actually a region of spore wall of different composition. Following a study of the mode of action of methyl *cis*-ferulate, Hess *et al.* (1975) proposed that the self-inhibitor blocks dissolution of the germ pore plug. In such a scheme, removal of the inhibitor at any time prior to disappearance of the plug material leads to reversal of the inhibition of the postulated hydrolytic enzyme and to an ensuing digestion of the plug. However, aerobic conditions are required for digestion of the plug, and the inhibitor may not act directly on the digestive enzymes or their substrates (Allen, 1976).

Hess *et al.* (unpublished data of S. L. Hess, P. J. Allen, and H. H. Lester) demonstrated that the germ pore plug material is composed of mannoprotein. This mannoprotein represented 0.3% of the spore wall, an appropriate range to be expected if the released material was exclusively from the pore region. Hydrolytic enzymes, including pronase, chitosanase, ß-glucanase, and zymolase were ineffective in removing electron-dense material in the region of the pore, and no dissolution of the pore plug occurred when isolated walls were incubated in the presence of a spore homogenate prepared during the initial stage of dissolution.

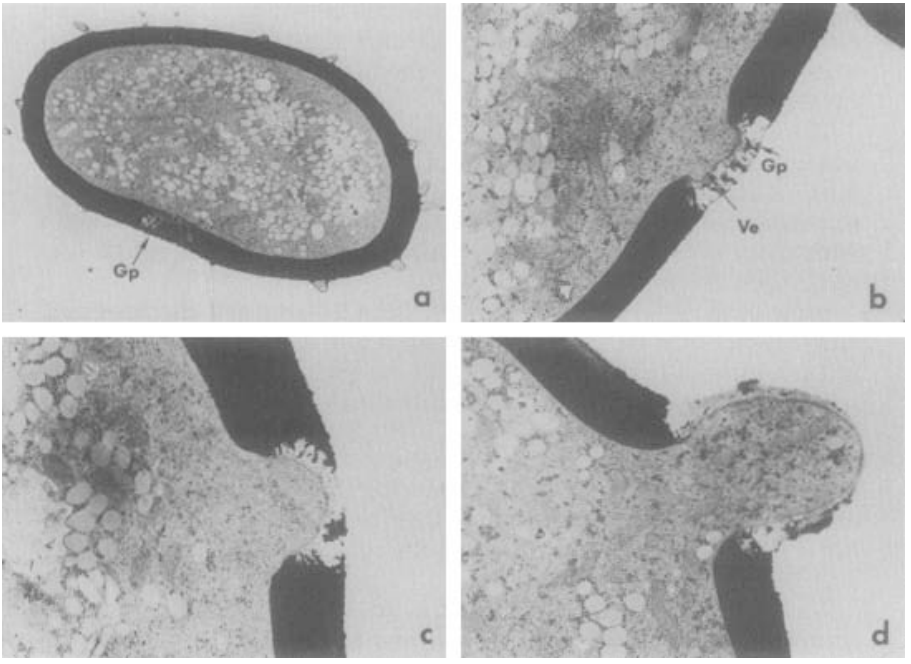


Fig. 1. Electron micrographs of thin sections of bean rust urediospores, showing especially the germ pore region, (a) Ungerminated spore. Arrow indicates region of germ pore (Gp) in wall. The long dimension of urediospore is approximately 25 μ.m. (b) Urediospore germinated 15 min. Germ pore (Gp) now readily visible in opaque wall. Vesicles (Ve) in tip zone of the developing germ tube marked by arrow, (c) Urediospore germinated 45 min. Germ pore now nearly ruptured, (d) Urediospore germinated 60 min. Germ pore now just protruding from spore. At this point, the self-inhibitor no longer arrests elongation of the germ tube.

Despite the appeal of the concept of germ pore plug digestion as a mechanism controlling germination, an alternative process should also be considered—that of germ tube growth. The earliest germ tube growth coincides with the initiation of germ pore plug digestion, and signs of new wall formation occur at the apex of the developing germ tube shortly after germination is initiated. Cytoplasmic vesicles are arranged in the tip zone of the developing hypha (Fig. lb), in a manner similar to that described by Grove and Bracker (1970) for other fungi. These vesicles are evident within a few minutes of the start of germination. Thus the self-inhibitor may actually function to block early growth of the germ tube while it is still within the confines of the spore wall, and dissolution of the germ pore plug may simply be a coordinate activity.

This early growth of the germ tube is not sensitive to the presence of inhibitors of protein or RNA synthesis (Dunkle *et al.,* 1969). For example, cycloheximide at 50 ppm did not affect the first 30 min of germ tube growth, but it effectively blocked protein synthesis (Hess *et al.,* 1975). The processes of early growth of the germ tube and dissolution of the germ pore plug material have not yet been experimentally separated. Consequently, it cannot be said with certainty which of these is the primary process affected by self-inhibitor action.

3. Germination Stimulants

Germination-promoting substances are present in fungal spores in addition to germination inhibitors. Nonanal was identified as an endogenous germination stimulant from urediospores of *P. graminis* f. sp. *tritici*(French and Weintraub, 1957). Subsequently the same compound was isolated from *P. coronata, P. sorghi, P. recondita, P. striiformis, P. helianthi,* and *Uromyces phaseoli* (Rines *et al.,* 1974). A different germination stimulant, 6-methyl-5-hepten-2-one, was found in *P. graminis* f. sp. *tritici* and *P. striiformis.* In screening experiments, some 23 species of rust, smut, and *Penicillium* fungi have been stimulated by nonanal and many other chemicals known previously as components of natural flavors and fragrances (French *et al.,* 1978).

Some of the stimulants (ß-ionone and cinnamaldehyde) are structural analogs of the endogenous germination inhibitors, but very little information is available about their modes of action. Stimulants overcome the self-inhibition

that prevails in dense populations of spores without reacting with the self-inhibitor molecule (Macko *et al.*, 1976).

III. Germling Differentiation

A. MORPHOLOGY

In the cereal rusts, as in many other rusts, germ tubes of urediospores penetrate through the stomata. On reaching a stoma (Fig. 2), growth of the germ tube ceases and an appressorium is produced over the stomatal aperture. A short peg then pushes through the stomatal opening, and the vesicle is formed in the substomatal cavity. Infection hyphae elongate from the vesicle and form haustoria. This series of new cells, which are specialized in form and function, are called infection structures. Their formation is accompanied by nuclear division (Dickinson, 1949b; Maheshwari *et al.*, 1967).

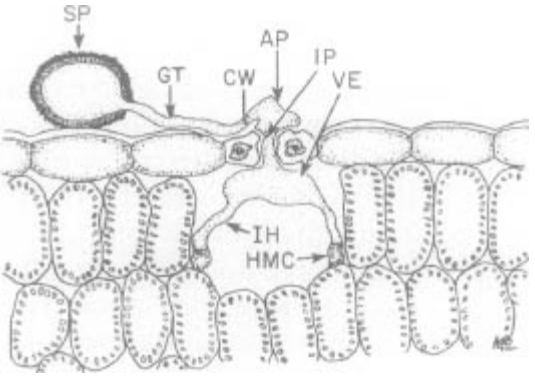


Fig. 2. Diagram of infection structures produced by the bean rust fungus on the surface of a leaf. Only those structures seen on collodion membranes are shown, and haustoria are omitted. AP, appressorium; GT, germ tube; CW, cross wall; IH, infection hyphae; IP, infection peg; HMC, haustorial mother cell; SP, urediospore; VE, vesicle. (After Staples and Huang, 1982.)

Before penetration, wheat stem rust urediospore germ tubes become oriented on leaves and usually grow across leaves at right angles to veins until they reach a stoma (Johnson, 1934). Orientation phenomena are not limited to wheat rust urediospore germ tubes, however, and the phenomenon is exhibited by a wide range of other rusts (Maheshwari and Hildebrandt, 1967; Staples and Macko, 1980). Electron microscopic studies show a regular lattice of wax crystals that covers the cuticle of wheat leaves. Germ tubes develop parallel to the short axis of the leaf on contact with the lattice (Lewis and Day, 1972). This

is interpreted as a thigmotropic response of the germ tube to the lattice, which serves as an orientation mechanism maximizing the probability that a germ tube will contact a stoma.

That the stimulus received from a membrane is derived only from the topographical features of the surface and not from any chemical properties of the oil or surface waxes was shown clearly by Wynn (1976). He used plastics to make copies of leaf cuticles in such a way that surface waxes were entirely eliminated. Appressoria were found to be induced over images of the stomata, and Wynn demonstrated that it was the protruding lip of the stomatal guard cells that induced formation of the infection structures.

B. HISTORICAL REVIEW

Historically, the concept of "appressorium" had included those structures produced by fungi, such as the rust or anthracnose fungi, which have a distinct morphology including separation of the germ tube or hypha by a septum (Emmett and Parbery, 1975). Such appressoria clearly are only one component of the infection structures, which include a peg, and in compound infection structures, vesicles, hyphae, and haustoria as well. Other types of appressoria, in which a delimiting septum is absent, usually have included all structures that have the capacity to adhere to a host surface together with the ability to penetrate the host. As Emmett and Parbery (1975) point out, such structures are both appressoria and infection structures.

Frank (1883) introduced the term a*ppressorium,* but he believed that it was an adhesive disk. In this chapter, restricted as it is to the rust fungi, we will use the term appressorium in the formal sense: It is the first infection structure that appears on the germling, and it is separated from the germ tube by a septum.

De Bary (1866, 1867) was one of the earliest workers to make careful microscopical studies of grain rusts. In 1865–1866, he saw the germination of the urediospores, migration of the protoplast with the germ tube tip, and—in the case of aeciospores—the formation of the appressoria on the stomata. Ward (1903, 1904, 1905), in his accounts of *Puccinia dispersa* Eriks. on bromes, figured and described with great detail the substomatal vesicle, the hyphae growing from it, and the development of haustoria.

Probably the best understanding of the development of rust fungi from a modern point of view was provided by Ruth F. Allen (1923a,b, 1926), who studied the development of *P. graminis* f. sp. *tritici* and *P. triticina* on susceptible wheat plants in order to follow up some previous work by E. B. Mains. She described the complete process of infection structure development, and her articles are carefully illustrated with detailed drawings. Several of these observations are important today, including the fact that nuclear division occurs at the time when the appressorium develops. Another important feature of the

leaf rust–wheat complex observed by Allen (1926) was that the time of entrance appeared to be conditioned by daily stomatal movements.

Ruth Allen also described nuclear behavior in the leaf rust fungus as the infection structure develops.

> As already noted, the spore has two nuclei, the appressorium four or more, the substomatal vesicle commonly eight, and the infecting hypha, after forming the first haustorium mother cell, often contain six. In the further development, one or two of these nuclei usually are left behind close to the substomatal vesicle, and the others divide and their progeny become distributed to the branches. Early hyphae of the young fungus have a somewhat irregular nuclear content.

Multinucleate cells in the first hyphae of the uredial mycelium of *Puccinia triticina* were first observed by Pole-Evans (1907). He observed them at a similar stage in *P. phleipratensis, P. glumarum, P. dispersa, P. simplex, P. coronifera,* and *P. sorghi;* however, Allen (1926) regarded them as cases of delayed septation. In general, then, it was clear before the end of the first half of this century that in the uredial generations of rusts exemplified by *P. triticina,* the urediospore has two nuclei, the appressorium usually four, and the substomatal vesicle commonly eight.

In the year following Ruth Allen's studies, workers began to examine what we now recognize as tropic responses. In 1934, Johnson reported on the directional growth of germ tubes of *P. graminis* f. sp. *tritici.* By 1949, Dickinson (1949a,b,c) began to reveal his studies on the development of a remarkable collodion membrane system. This invention, which consisted essentially of a thin film of collodion with paraffin oil added before it was set, made possible all of the more modern studies on the cell biology of infection structures. In a series of articles, Dickinson (1949a,b, 1969, 1970, 1971, 1972) showed that the morphology of the germ tube elongating on membranes of nitrocellulose, polystyrene, and poly(methyl methacrylate) depends on the frequency and height of the ridges of these membranes. In response to these membranes, spores produced germ tubes that were unbranched or zigzag, or were differentiated. The thigmotropic response resulted from contact of the germ tube with a repetitive series of changes in thickness in plastic membranes or with parallel ridges in nitrocellulose.

Thus by the 1960s an adequate understanding of infection structure development and some tools for its study had become available. These tools were uncomplicated, and they avoided confusion with host responses. The often remarkable research that built upon this knowledge and technique will be the subject of the remainder of this chapter.

C. THE CONCEPT OF RESPONSE SEQUENCE

Although it was clear from Sidney Dickinson's ingenious experiments that responses to a source of stimulation are involved in development of the

infection structures, appreciation that more than one stimulus is involved in stomatal recognition has been relatively recent (Wynn and Staples, 1981). In fact, five responses have been postulated to be involved in the preinfection sequence of stomate-entering rust fungi, and in independent work, Wynn demonstrated that if any of the responses were to fail, infection of the host was reduced or prevented (Wynn and Staples, 1981).

The first of these responses, germ tube adherence, is more difficult to describe than to understand. Although observed repeatedly for rust fungi (Allen, 1923a, 1926; Lewis and Day, 1972; Wynn, 1976), it was not until Wynn systematically utilized waxless mutants of corn that germ tube behavior was observed suggesting that unless there was adherence to the substrate, the germ tube did not recognize a second following stimulus by responding with germ tube orientation (Fig. 3). The fact that germ tubes orient at right angles to surface ridges might be a useful experimental indicator that the germ tube has adhered sufficiently so that the surface is physiologically effective. The phenomenon for several wheat rusts has been reported many times (Wynn and Staples, 1981) and was beautifully illustrated for the bean rust fungus by Pring (1980).

With proper germ tube adherence and orientation, all of the infection structures appear in a well-ordered sequence when the germ tube tip strikes the lip of the stomatal guard cell. This response was designated “appressorium formation” (Wynn, 1976). However, additional information other than that for appressorium development must also be conveyed, because the orientation of peg growth to accomplish penetration between the guard cells is determined by the way in which the appressorium lies over the stomatal opening. Where waxless mutants have provided an insufficient footing, the infection peg may emerge with the wrong orientation, so that the infection structures develop aberrantly (Fig. 3). This latter response is called “directional peg emergence” by Wynn and Staples (1981).

The final infection structure to emerge on membranes (where the host is absent) commonly is the haustorial mother cell, because with the bean rust fungus the substomatal vesicle and infection hyphae appear in order once the appressorium has been initiated. Apart from the host, the haustorial mother cells are formed at low frequency (Maheshwari *et al.*, 1967). Both Pring (1980) and Heath (1981) have now shown that contact of haustorial mother cells with a wall of the host (usually a mesophyll cell) actually induces the haustorial apparatus (penetration peg and haustorium) to form, and Wynn and Staples (1981) have proposed that this response, *haustorial induction*, be recognized as the last in a sequence required for host colonization.

Previously these responses were called tropisms (Wynn and Staples, 1981). *Tropisms* are bending responses of cylindrical organs in response to external stimuli (Fuller and Tippo, 1949). Except for germ tube orientation, most of these responses are not true tropisms, but they do involve

differentiation, the production of the infection structures. Hence in place of thigmotropism, we propose that a new term,*thigmodifferentation,* be used for "appressorium formation" in response to a surface topography. We believe that germ tube orientation is a "contact tropism." Chemically stimulated differentiation might be termed*chemodifferentiation.*

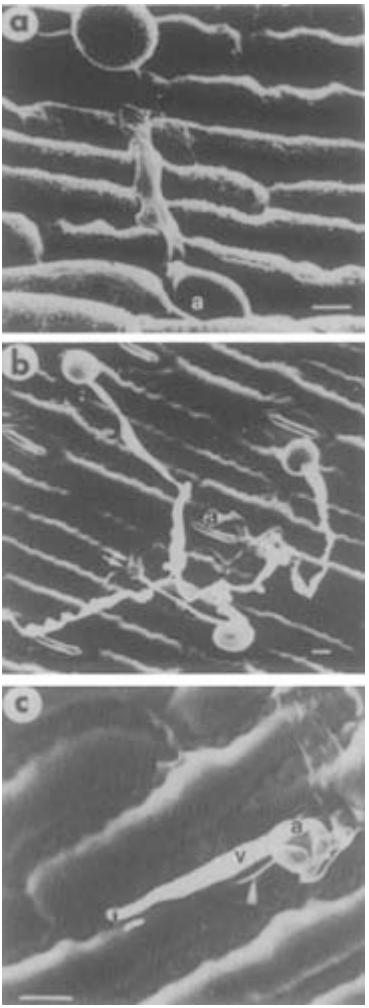


Fig. 3. Scanning electron micrographs of normal preinfection development after 6 hr germination and tropic mistakes after 24 hr germination of two rust fungi on normal and waxless leaves. a, Appressorium; i, infection hypha; v, vesicle. (a) Normal directional growth and appressorium formation over stomate (obscured) by *Puccinia sorghi* on corn. (b) Failure of germ tube adherence and directional growth by *P. sorghi* on waxless corn. The germ tubes that appear white (single arrow) grew up in the air from the spores and then later

fell down on the surface, where they appear transparent (double arrows); the appressorium did not form over the stomate. (c) Failure of directional infection peg emergence from appressorium (over stomate, arrow) by *P. sorghi* on waxless corn. Vesicle and infection hypha grew on the surface. (Bars, 10 µm.) (After Wynn and Staples, 1981.)

D. THE SURFACE INDEPENDENCE OF THE WHEAT STEM RUST FUNGUS

Urediospore germlings of the wheat stem rust fungus (*P. graminis* f. sp. *tritici*) do not develop appressoria on collodion membranes (Macko *et al.*, 1976), yet this fungus consistently forms appressoria away from the host or artificial membranes when induced by temperature shock or by chemical means (Allen, 1957; Maheshwari *et al.*, 1967; Macko *et al.*, 1978). Spores floating on aqueous solutions produce infection structures in response to heat shock or to distillates from urediospore extracts (Maheshwari *et al.*, 1967; Dunkle and Allen, 1971). The latter finding suggested to P. J. Allen that a volatile chemical agent is present that initiates infection structure formation. This chemical inducer of appressoria has now been identified as 2-propenal (acrolein) by Macko *et al.* (1978), who induced formation of infection structures with it while the spores were germinating on a water surface.

In nature, a germling usually forms appressoria only over the stomatal pore, and the responsiveness of wheat stem rust germlings to acrolein and their failure to differentiate on plastic replicas (W. K. Wynn, personal communication), suggest that recognition of the stomatal guard cells involves a localized stimulus of some kind, in addition to a chemical stimulus. Somehow, the germ tubes of this fungus must receive specific information in order to recognize the guard cells, and this may be solely physical. Wheat stem rust urediospore germlings do form appressoria in response to scratches on such membranes as polyethylene sheets (Rowell and Olien, 1957; Staples *et al.*, 1983b).

The concept that infection structures are induced by a chemical environment around the stoma has received impetus from a series of articles by Grambow and associates (Grambow and Reisener, 1976; Grambow, 1977, 1978; Grambow and Grambow, 1978; Grambow and Riedel, 1977). These authors demonstrated that volatile leaf constituents stimulate formation of infection structures, and they have suggested that certain compounds leached from the guard cell walls provide the biochemical environment needed for appressorium development. These may be required for vesicle development (Staples *et al.*, 1983b).

E. THE SURFACE DEPENDENCE OF THE BEAN RUST FUNGUS

For the bean rust fungus (*Uromyces phaseoli*), differentiation appears to depend on the topography of the stomatal guard cell and not on its chemical

properties. Wynn (1976) demonstrated that templates of the leaf surface made of liquid silicone rubber induced urediospore germlings to develop appressoria over the images of the stoma where the germ tubes had encountered the guard cells.

Originally, it was thought that bean rust urediospores require a surface with a particular topography for differentiation, and will not form infection structures on either water or agar. This is shown by the responsiveness of spore germlings to Dickinson's collodion membranes (Dickinson, 1974), or to scratches on polyethylene sheets (Wynn, 1976), in contrast to the complete lack of differentiation on smooth membranes or on water or agar. However, bean rust urediospore germlings will differentiate on these passive surfaces if potassium ions are present (Staples *et al.,* 1983a) or if reduced nucleotides are used (Staples *et al.,* 1982). These chemicals apparently bypass the need for a membrane to start differentiation. With bean rust urediospores, then, the ability to perceive and to respond to surface topography is an additional part of their physiology. At least four other rust fungi appear to share the property of responsiveness to membranes, including *P. helianthi, P. antirrhini, P. arachidis,* and *P. sorghi.* Urediospores of all of these rusts contain methyl *cis*-3,4-dimethoxycinnamate as the self-inhibitor of germination (Macko *et al.,* 1976).

F. EPIDERMAL PENETRATING RUSTS

Basidiospore germlings penetrate alternate hosts directly, but urediospores of several rusts also penetrate the host surface directly rather than through the stomata. These are *Puccinia psidii, Ravenelia humphreyana, Physopella zeae,* and the soybean rust fungus, *Phakopsora pachyrhizi* (Bonde *et al.,* 1976). In a detailed discussion, these authors described the penetration process by these fungi.

Puccinia psidii, a pathogen of rose apple (*Syzygium jambos*), produces an appressorium at the tip of a short germ tube, and a narrow penetration peg develops from it that penetrates the leaf surface between epidermal cells. *Ravenelia humphreyana,* on *Caesalpinia pulcherrima,* produces an appressorium that often is sessile to the urediospore. A penetration peg develops from the appressorium that penetrates a leaf epidermal cell, and the fungus body enlarges within the cell to form a “vesicular haustorium.” This fungus subsequently colonizes adjacent epidermal and mesophyll cells by formation of intracellular mycelium (cf. Bonde*et al.,* 1976).

The histology of soybean penetration by *P. pachyrhizi* was studied first by Kitani and Inoue in Japan (1960), and more recently by Keogh (1974) and Keogh *et al.* (1980) in Australia, and by Bromfield's group in the United States (Bonde *et al.,* 1976). Urediospores of the soybean rust fungus germinate within 2 hr to produce a germ tube (Bonde *et al.,* 1976). Clearly defined appressoria

develop shortly thereafter, and are separated from the germ tube by a cross wall. Some appressoria are sessile to the spore (Bonde *et al.*, 1976). Penetration then occurs directly through the epidermis, and a "transepidermal vesicle" is produced. The inner wall of the epidermis is breached, and the "primary hypha" grows into the mesophyll (Keogh, 1974).

Kitani and Inoue (1960) have demonstrated that urediospores of the soybean rust fungus germinated on a glass slide develop appressoria. This suggests that these germlings produce an appressoria in response to contact with a hard surface reminiscent of that required by several of the anthracnose fungi, which also penetrate their hosts directly (Staples *et al.*, 1976).

G. CELL BIOLOGY OF GERMLING DIFFERENTIATION

1. The Microfibrillar Network

The nature of the initial activation provided to the bean rust fungus by the membrane surface seems to involve sensing. For example, as described earlier, Wynn (Wynn and Staples, 1981) found that germ tubes of a number of rust species germinated on waxless corn leaves were disoriented, and they failed to recognize stomatal guard cells when contact was made by the germ tubes. Even the orientation mechanism was disabled, and the germ tubes grew away from the leaf surface. It would seem that the surface on which spores germinate somehow activates a germ tube signal receptor. Staples and Hoch (1982) suggested that the postulated sensing mechanism may involve elements of the microfibrillar network of the spore to carry appropriate signals from the germ tube tip to the nucleus.

It probably is not too surprising that the urediospore germling has a microfibrillar (cytoskeletal) network (Heath and Heath, 1978; Herr and Heath, 1982), because it has been found in most cells that have been examined (Porter, 1966). Eukaryotic cells contain three basic fibrillar structures: microfilaments, microtubules, and an intermediate structure. These structures are thought to be intimately involved in the maintenance of cell shape, cell movement, organelle movement, and other important cellular functions.

2. Microtubules and Organelle Movements

Division, locomotion, and changes of the eukaryotic cell shape are accompanied by the processes of assembly and disassembly of microtubules and other cytoskeletal structures. In urediospores, too, microtubules have been implicated in the movements of various organelles. Working with urediospore germlings of the cowpea rust fungus (*Uromyces phaseoli* var. *vignae*), Heath and Heath (1978) demonstrated by direct visual observations and time-lapse

films that differentiating infection structures exhibited three types of organelle movements. One was a general movement of cytoplasm and organelles into developing portions of the fungus. During this movement, the nuclei and mitochondria maintained characteristic positions with remarkable constancy (Fig. 4). A second type was a relatively slow, erratic movement of various organelles such that they became displaced relative to one another and to the growing fungal tip. Finally, usually spherical bodies, including lipid particles, were involved in erratic, rapid saltations.

Serial section ultrastructural analyses of glutaraldehyde-fixed material showed that microtubules were typically oriented parallel to the direction of cytoplasmic migration. Heath and Heath presented statistical evidence for an association of microtubules with mitochondria but not with microbodies or lipid droplets.

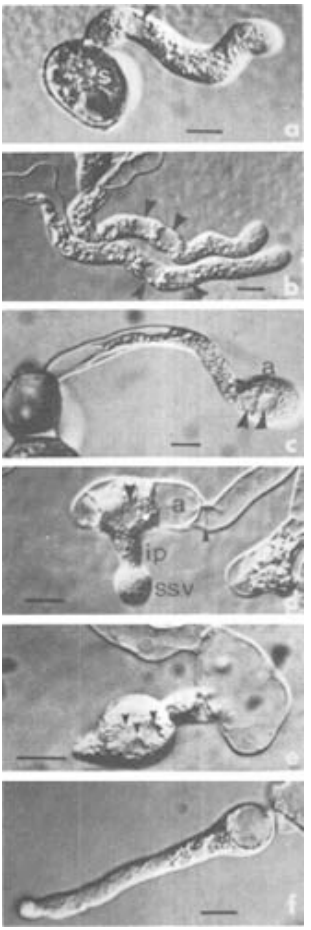


Fig. 4. Microtubules and organelle movements in the cowpea rust fungus *Uromyces phaseoli* var. *vignae*. (a) Young germ tube developing from a urediospore (s). Approximately half the cytoplasm has left the spore, and the first nucleus (arrow) has also emerged. The second nucleus was in the exit pore of the spore (x687; bar, 10 μm). (b) Two long germ tubes showing the location of the pairs of nuclei (arrows) in approximately the center of the migrating cytoplasmic mass. Note the "empty" vacuolate region behind the cytoplasm in each tube (x536; bar, 10 μm). (c) A short germ tube that is forming an appressorium (a), into which the cytoplasm and contained nuclei (arrow) have already moved (x571; bar, 10 μm). (d) A later stage of development than that shown in Fig. 3. The cytoplasm is migrating from the appressorium (a), through the infection peg (ip), into the developing substomatal vesicle (ssv). Note the cross wall (small arrow) between the germ tube and appressorium and the nucleus (one of four, arrow) still in the appressorium (x770; bar, 10 μm). (e) Nearly mature substomatal vesicle showing three of the four nuclei (arrows) and an infection hypha beginning to develop on its distal end (x934; bar, 10 μm). (f) Mature infection hypha that developed from the now vacuolate substomatal vesicle (x702; bar, 10 μm). (After Heath and Heath, 1978.)

Further evidence that microtubules are indeed involved in organelle positioning during germination of cowpea rust urediospores was provided by Herr and Heath (1982). These workers used several anti-microtubule drugs in their study and found that nocodazole, Oncovin, and griseofulvin elicited striking changes in the relative positions of mitochondria, nuclei, and vacuoles, as well as inhibiting the saltatory movements of lipid bodies. The germ tubes continued to grow in a reasonably normal fashion. In contrast, the antimicrotubule drugs colchicine, Colcemid, and isopropyl *n*-phenylcarbamate (IPM) had no marked or consistent effect, possibly a result of poor penetration.

Ultrathin serial sections of glutaraldehyde-fixed material showed that Oncovin caused a general disappearance of microtubules, whereas a normal distribution was seen in Colcemid-treated germ tubes. These complementary ultrastructural studies suggested again that microtubules are involved in the positioning of cytoplasmic components in the rust germ tube, perhaps in conjunction with a more complex cytoskeletal (microfibrillar) system.

3. Microfilaments, Microtubules, and the Spitzenkörper

The massive cluster of vesicles at the growing tip of hyphae and germ tubes of several nonrust fungi is increasingly identified with the Spitzenkörper (Howard and Aist, 1977, 1979, 1980; Hoch and Howard, 1980). Under phase-contrast light optics the position of this vesicle cluster has been used as an indicator of the direction of future cell expansion, because a slight change in the position of the Spitzenkörper within the apical dome results in a subsequent change in direction of growth (Grove, 1978). Howard has suggested that the existence of the Spitzenkörper, which exhibits movement en masse of the vesicle cluster, may be attributable to a network of microfilaments (Howard, 1981). Microtubules were distributed among the component vesicles and were

envisioned as having a role in maintaining the supply of vesicles to the Spitzenkörper from their point of origin at the dictyosomes (Howard and Aist, 1980; Hoch and Howard, 1980).

Howard (1981) suggested that cytoplasmic microtubules mediate long-distance intracellular transport of cell wall precursors in hyphal tip cells. He ascribed to microtubules the function of maintenance of internal organization and cell polarization, the loss of which would interfere with efficient transport phenomena.

Microfilaments may have a role in secretion, because they appeared to be associated with vesicles in the hyphae. Such an activity was correlated with the inhibition of hyphal tip growth by cytochalasins A and B, which inhibit elongation of the actin-containing microfilaments in the ascomycetes (Sweigard *et al.*, 1979; Howard, 1981). One mechanism by which the microfilaments may control secretion as suggested by Howard (1981) was control of the fusion between secretory vesicles and the plasma membrane at a specific site.

The germ tube tip of the bean rust fungus also has a cluster of vesicles (Heath and Heath, 1978). These early studies were carried out with glutaraldehyde fixation, which tends to destroy the cytoplasmic microtubules at the hyphal tip (Howard and Aist, 1980), so the organization of the nondifferentiated germ tube tip was reexamined using a freeze-substitution protocol (Hoch and Staples, 1983). A concentration of apical vesicles exists in the region of the Spitzenkörper (Fig. 5). Immediately proximal to this region is a concentration of mitochondria. Numerous microtubules occur in the tip region, and most of them are oriented parallel to the long axis of the hypha.

The apices of differentiating germ tubes germinated on a polycarbonate membrane do not contain Spitzenkörper or discrete concentrations of apical vesicles (Fig. 6). Instead, most apical vesicles are distributed more or less uniformly around the periphery of the expanding appressorium initial. Microtubules are likewise distributed around the periphery. However, Hoch and Staples have repeatedly observed a greater concentration of randomly oriented microtubules in the developing appressorium near the appressorium–substrate interface (Fig. 6). Apical vesicles, filasomes, and microfilaments are also dispersed in this region. Thus with differentiation (development of the appressorium), the Spitzenkörper appears to be dispersed into a spherical locus of wall-building foci.

4. Recognition of External Stimuli

As pointed out earlier, bean rust urediospores germinating on a water surface or a passive membrane are unresponsive unless stimulated by the potassium ion, certain reduced nucleotides (Staples *et al.*, 1983a), or such physical manipulations as heat shock (Staples and Hoch, 1982). Bean rust

urediospores differentiate in the absence of these stimuli only when germinated on a suitable membrane surface (Wynn, 1976), and it seemed possible to us that urediospores have an extra mechanism for utilizing the topography of surfaces as an aid to host colonization.

Staples and Hoch (1982) have demonstrated that nuclear division and some infection structures would develop in germlings of bean rust urediospores stimulated by certain antitubulin drugs (demecolicine, griseofulvin, nocodazole), and by several antimicrofilament agents (cytochalasins C, E). Stimulation was also provided by sonication and by heat shock, treatments that can disrupt microtubules. To be effective, the treatments had to be applied while the urediospores were germinated on a membrane surface. Taken together, the data suggest that the microtubule-microfilament (microfibrillar) system somehow represses nuclear division in urediospores until released by suitable stimulators such as heat shock or an inductive membrane topography. The precise mechanisms are unknown.

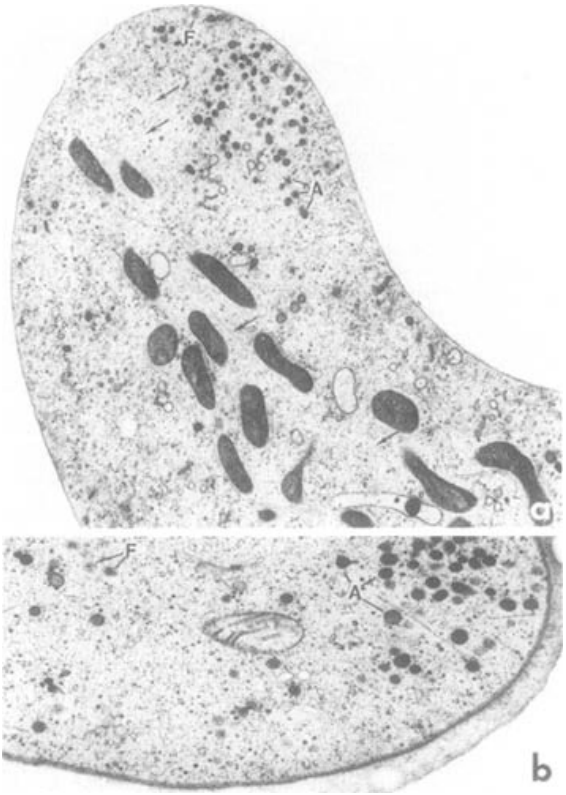


Fig. 5. Electron micrographs (a and b) of noninduced urediospore germ tube apices at 6 hr on polyethylene membranes. Apical vesicles (A), filasomes (F),

and microtubules (arrows) are readily observed in the apical region. Both micrographs represent longitudinal sections somewhat tangential to the median axis [(a) x 19,900; (b) x26,000]. (After Hoch and Staples, 1983.)

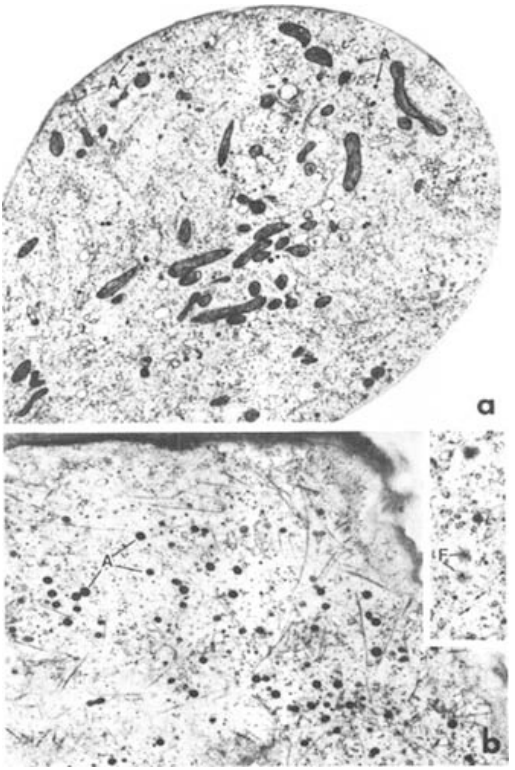


Fig. 6. Developing appressoria of *Uromyces phaseoli* at 6 hr on polycarbonate membranes. (a) Apical vesicles (A) are distributed peripherally about the expanding germ tube apex (x16,900). (b and inset) Numerous microtubules, randomly oriented, in the developing appressorium near the cell–substrate interface. Apical vesicles (A), filasomes (F), and filaments (small arrows in inset) are distributed in this area. |(a) x20,100; inset magnification in (b), x29,400). (Unpublished micrographs by H. Hoch.)

5. *Some Consequences of Nuclear Activation during Differentiation*

a. DNA Synthesis. One of the earliest new biosynthetic events to occur is the synthesis of nuclear DNA (Staples, 1974). It is important to remember that the energy and carbon for this synthesis, carried out as it is before the host is invaded, is obtained by conversion of stored lipids apparently through the

mediation of the microbodies that are present in the infection structures (Mendgen, 1973). These matters have been reviewed by Reisener (1976).

Urediospores begin the synthesis of nuclear DNA sometime after the second hour of germination, about the time when the nuclei begin to divide. Until then, DNA synthesis is entirely confined to the mitochondria, and replication of nuclear DNA does not occur.

The relationship between DNA synthesis and differentiation was examined by using metabolic inhibitors (Staples *et al.*, 1975). Ac-tinomycin D inhibits synthesis of DNA (Staples *et al.*, 1975), and no earlier biosynthetic event has been found that is associated with formation of infection structures.

In agreement with this, it was found that cordycepin also inhibits nuclear activity but blocks only substomatal vesicle development, not appearance of appressoria. This drug inhibits nuclear division but not synthesis of DNA (Staples *et al.*, 1975), and differentiating spores exposed to it develop apparently normal appressoria and vesicle initials. However, the vesicles do not elongate, and neither nuclear division nor nuclear migration occurs. Although nuclear division need not precede formation of appressoria, apparently replication of DNA is required.

b. DNA Polymerase. Rust urediospores contain mitochondrial and nuclear DNA polymerases, and these were purified from bean rust urediospores (Yaniv and Staples, 1978; Staples and Yaniv, 1978). Theenzymes were readily soluble when extracted from resting spores; however, only small quantities of DNA polymerase have been obtained from differentiated spores, apparently because the nuclear enzyme becomes bound with the onset of replication (R. C. Staples, unpublished information).

The molecular weight of the nuclear DNA polymerase is about 175,000. The optimum pH for enzyme activity was found to be 7.5, and the reaction requires the simultaneous presence of all four deoxynucleotide triphosphates. A DNA template and magnesium ions also are required, and best activity is obtained if the template is activated. The nuclear enzyme is inhibited by *N*-ethylmaleimide and aphidicolin, so the enzyme is a DNA α-polymerase (Huberman, 1981). The molecular weight of the mitochondrial enzyme is 150,000, and it has an iso-electric point of 5.4. Good activity is obtained when the enzyme is primed with poly(dA-dT)$_{10}$. This enzyme is inhibited by *N*-ethyl-maleimide, too.

The ability to extract these enzymes from urediospores implies that synthesis of DNA, one of the earliest events required for appressorium formation, is repressed in the spores even though they have the full complement of DNA polymerases needed for its synthesis. It is not yet known how nuclear DNA polymerase is activated when the spores are induced to differentiate, but the studies of Staples and Hoch (1982) reviewed earlier suggest that activation

of nuclear DNA polymerase is mediated by the microfibrillar network (cytoskeleton) of the spore.

c. Protein Synthesis. The synthesis of proteins and the proteinsynthetic apparatus have been extensively studied in rust fungi, especially in germinating urediospores (Staples and Yaniv, 1976); however, there have been few comparable studies during formation of infection structures (Staples *et al.,* 1975). Infection structure formation by the wheat stem rust fungus is generally blocked by inhibitors of protein synthesis and RNA synthesis (Dunkle and Allen, 1971). The protein content of bean rust urediospores increases about 20 to 30% during the first 3 hr of germination (Trocha and Daly, 1970), and G. Wolf (personal communication) has demonstrated that the spores readily synthesize a number of soluble proteins during germination. However, few studies have been extended to include the period of infection structure development when ribosome activity increases threefold (Yaniv and Staples, 1974).

During studies of gene activity in differentiating urediospores, Huang and Staples (1981) examined the bean rust germling for the synthesis of special proteins. Spores were pulse-labeled with [^{35}S]methionine for consecutive intervals of 1.5 hr (Fig. 7). Spores induced to differentiate develop appressoria beginning at about 3 hr, whereas substomatal vesicles develop by 6 hr. Many proteins are synthesized during differentiation, but at least two new proteins (MW 18,500 and 24,000) appear during the earliest period of differentiation (Fig. 7). Labeling of an additional protein (MW 23,000) becomes apparent later when formation of the vesicles begins. Labeling experiments showed that processing of heavier proteins to form the new lighter proteins is not involved, in agreement with the fact that the proteins were labeled for equal lengths of time during the period of differentiation.

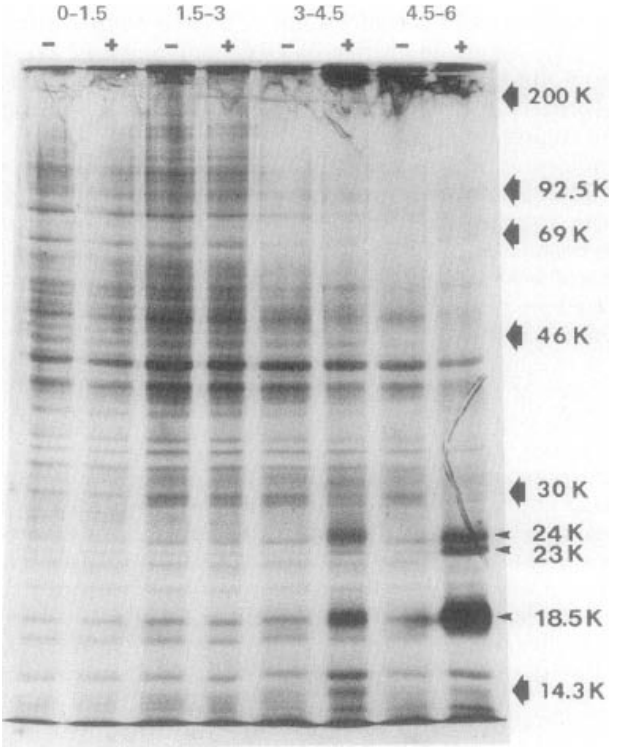


Fig. 7. Comparison of proteins synthesized by differentiated and nondifferentiated germlings of bean rust urediospores. Differentiated (+) and nondifferentiated (–) spores were pulse-labeled with [^{35}S]methionine for four consecutive 1.5-hr intervals after spore imbibition. Proteins were then extracted and separated by gel electrophoresis (SDS– PAGE). The gels were stained with coomassie blue, then autoradiographed. The molecular weights were obtained by co-running with ^{14}C-methylated protein mixture. The same amount of radioactivity of hot TCA-insoluble proteins was used for each track. The comparisons were 0–1.5 hr for spore rehydration and swelling, 1.5–3.0 hr for germ tube extrusion, 3.0–4.5 hr for initial differentiation (appressoria beginning to form], and 4.5–6.0 hr for completion of differentiation (appressoria and vesicles]. Molecular weights of stage-specific proteins synthesized were 18,500, 23,000, and 24,000 (shown as 18.5 K, 23 K, and 24 K, respectively. (After Huang and Staples, 1981.)

It is clear from these studies that urediospores synthesize a wide range of proteins. Spores committed to develop infection structures shift their synthetic program somewhat, and a small number of new proteins are produced that either are different from those present in germinating spores, or their synthesis is accelerated.

d. Messenger RNA. Urediospores contain a complete system for synthesis of proteins (Yaniv and Staples, 1974). These components include the ribosomes, mRNA, tRNA, and the enzymes required for translation. Of the components studied quantitatively, tRNA and ribosomal RNA are synthesized in small amounts throughout gerruination and differentiation. Polyribosomes are present that actively incorporate amino acids in a cell-free system, and the template activity of the mRNA fraction increases sharply between the fourth and the sixth hour in spores that form infection structures.

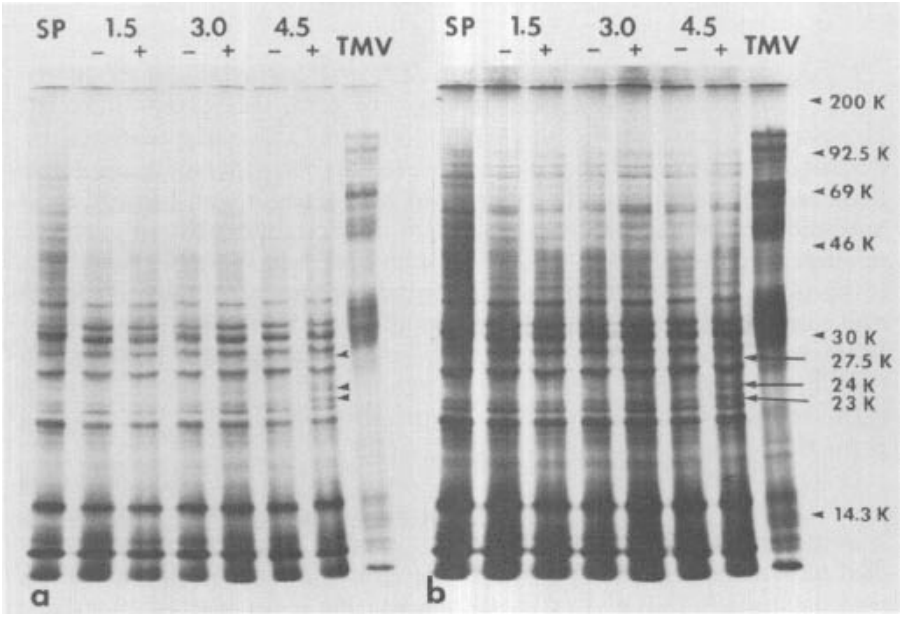


Fig. 8. Gel electrophoresis (SDS–PAGE) of *in vitro* translation products of poly-A$^+$ RNA. PolyA$^+$ RNA isolated from both differentiated (+) and nondifferentiated (–) spores germinated 0, 1.5, 3, and 4.5 hr after imbibition was translated by using a cell-free wheat germ S23 system. Tobacco mosaic virus-RNA was also translated as a reference standard. Radiolabeled TCA-insoluble proteins (30,000 cpm) were pipetted onto each track of the SDS–PAGE gels. Gels were treated with En^{3}Hance and fluorographed for 2 days (a) and 6 days (b), respectively. (After Huang and Staples, 1981.)

Huang and Staples (1981) have studied the control of the differentiation-related proteins using a cell-free system prepared from wheat germ. Total RNA, polyA$^+$RNA, and polyA$^-$RNA extracted from spores served as the template in the assay system. The protein products of translation, analyzed by Polyacrylamide gel electrophoresis (Fig. 8), clearly show that newly transcribed polyA$^+$RNA is responsible for the synthesis of at least two of the stage-specific proteins (MW 23,000 and 24,000). Transcription of polyA$^+$ mRNA apparently

is the important process that controls appearance of the differentiation-specific proteins.

IV. Some Reflections

It has been known at least since Sidney Dickinson's work in the 1940s (Dickinson, 1949a) that urediospore germ tubes respond to the physical structure of the underlying surface. The early workers, especially Ruth Allen (1926), have reported on the propensity for germlings to differentiate at the ridges and grooves of a leaf, but the tools and scientific orientation necessary for understanding the importance of such responses to the fungus simply were not available then. The importance of the surface on which urediospores germinate, although often observed, was finally appreciated when Lewis and Day (1972), following observations made initially by Maheshwari and Hildebrandt (1967), provided their incisive interpretation that the directed growth guided by the venations of a leaf improved the statistical chance that a germ tube would make a stomatal contact.

Later, Wynn (1976) demonstrated that the differentiation stimulus provided by the stomatal guard cell is entirely physical, at least for the bean rust fungus, and the suspicion grew that investigators really were dealing with a series of special responses or tropisms. The proposal has now been made that germ tube differentiation is orchestrated by these to provide a critically necessary sequence of germ tube development (Wynn and Staples, 1981). The sequence of recognition must occur, or germlings fail to sense stomatal contact.

The earliest cellular change after initiation of germling differentiation of the bean rust fungus apparently results from a series of fairly rapid metabolic reactions. DNA replication, transcription, and translation, nuclear division, and reorganization of the apical vesicles in the germ tube tip occur almost together and signal the beginning of construction of the appressorium. The sequence in which these changes occur has not yet been satisfactorily resolved because of technical difficulties, but the earliest occurrence probably is DNA replication (Staples *et al.,* 1975; Huang and Staples, 1981). Whatever the proper sequence proves to be, differentiation involves activation of the germ tube nuclei (Staples and Huang, 1982), which suggests that there is a mechanism in the germ tube for activation of the nucleus in response to the stimulus of physical contact. Wheat stem rust germlings, which respond to chemical stimulation (Macko *et al.,* 1978), must also have a similar mechanism, because heat shock stimulates both fungi to differentiate (Maheshwari *et al.,* 1967; Staples and Hoch, 1982).

It has now been proposed that there is a sensing mechanism in the germ tube that involves parts of the complex microfibrillar (cytoskeletal) network of microtubules and microfilaments (Staples and Hoch, 1982). When these early

conjectures have been properly researched, we will have learned much about how cells adapt to survive in the natural environment. The strategy that germlings use to fit their pattern of development to the morphology of their host obviously has survival value by maximizing the opportunity to colonize with minimum effort. As these fungal responses sometimes fail (Wynn and Staples, 1981), knowledge about why the occasional failure occurs may lead to a basis for exploiting them for control of disease.

Acknowledgments

We thank W. K. Wynn and H. C. Hoch for use of their micrographs, S. L. Hess for selected use of his data prior to publication, and F. B. Herr and M. C. Heath for permission to read their manuscript prior to its publication. We thank P. G. Williams, D. E. Harder, and H. C. Hoch for critically reading the manuscript, and Joanne Martin for expert secretarial aid. This chapter is affectionately dedicated to G. L. McNew, formerly managing director of Boyce Thompson Institute, who made it possible for us to work on the arcane science of urediospore physiology. Research of the authors reported here was supported in part by grants from the U.S. Department of Agriculture.

References

Allen, P. J. (1955). The role of a self-inhibitor in the germination of rust urediospores. *Phytopathology* **45**, 259–266.

Allen, P. J. (1957). Properties of a volatile fraction from urediospores of *Puccinia graminis* var. *tritici* affecting their germination and development. I. Biological activity. *Plant Physiol.* **32**, 385–389.

Allen, P. J. (1976). Control of spore germination and infection structure formation in the fungi. *Encycl. Plant Physiol. New Ser.* **4,** 51–85.

Allen, P. J., and Dunkle, L. D. (1971). Natural activators and inhibitors of spore germination. *In* "Morphological and Biochemical Events in Plant-Parasite Interaction" (S. Akai and S. Ouchi, eds.), pp. 23–58. Phytopathol. Soc. Jpn., Tokyo.

Allen, R. F. (1923a). A cytological study of infection of Baart and Kanred wheats by *Puccinia graminis tritici. J. Agric. Res.* (*Washington, D.C.*) **23**, 131–152.

Allen, R. F. (1923b). Cytological studies of infection of Baart, Kanred, and Mindum wheats by *Puccinia graminis tritici* forms III and XIX. *J. Agric. Res.* (*Washington, D.C.*) **26**, 571–604.

Allen, R. F. (1926). A cytological study of *Puccinia triticina* physiologic form 11 on Little Club wheat. *J. Agric. Res. (Washington, D.C.)* **33**, 201–222.

Bell, A. A., and Daly, J. M. (1962). Assay and partial purification of self-inhibitors of germination from urediospores of the bean rust fungus. *Phytopathology* **52,** 261–266.

Bonde, M. R., Melching, J. S., and Bromfield, K. R. (1976). Histology of the susceptpathogen relationship between *Glycine max* and *Phakopsora pachyrhizi*, the cause of soybean rust. *Phytopathology* **66**, 1290–1294.

Bromfield, K. R. (1964). Cold-induced dormancy and its reversal in urediospores of *Puccinia graminis* var. *tritici. Phytopathology* **54**, 68–74.

Calpouzos, L., and Chang, H. (1971). Fungus spore germination inhibited by blue and far red radiation. *Plant Physiol.* **47**, 729–730.

Chang, H.-S., and Calpouzos, L. (1973). Phototropism of the uredospore germ tubes of *Puccinia graminis tritici. Bot. Bull. Acad. Sin.* **14**, 35–40.

Chang, H.-S., Wilcoxson, R. D., and Calpouzos, L. (1974). Inhibition of urediospore germination by light partially relieved by soaking spores in water. *Phytopathology* **64**, 158

Daly, J. M., Knoche, H. W., and Wiese, M. V. (1967). Carbohydrate and lipid metabolism during germination of urediospores of *Puccinia graminis tritici. Plant Physiol.* **42**, 1633–1642.

De Bary, A. (1866). Neue Untersuchungen über die Uredineen, insbesondere die Entwicklung der *Puccinia graminis* und den Zusammenhang derselben mit *Aecidium Berberidis. Monatsber. K. Preuss. Akad. Wiss. Berlin* pp. 15–50.

De Bary, A. (1867). Neue Untersuchungen über Uredineen. *Monatsber. K. Preuss. Akad. Wiss. Berlin* pp. 205–215.

Dickinson, S. (1949a). Studies in the physiology of obligate parasitism. I. The stimuli determining the direction of growth of the germ-tubes of rust and mildew spores. Ann. *Bot.* (*London*) [N.S.] **13**, 89–104.

Dickinson, S. (1949b). Studies in the physiology of obligate parasitism. II. The behavior of the germ tubes of certain rusts in contact with various membranes. *Ann. Bot. (London)* [N.S.] **13**, 219–236.

Dickinson, S. (1949c). Studies in the physiology of obligate parasitism. IV. The formation on membranes of haustoria by rust hyphae and powdery mildew germ-tubes. *Ann. Bot. (London)* [N.S.] **13**, 345–353.

Dickinson, S. (1969). Studies in the physiology of obligate parasitism. VI. Directed growth. *Phytopathol. Z.* **66**, 38–49.

Dickinson, S. (1970). Studies in the physiology of obligate parasitism. VII. The effect of a curved thigmotropic stimulus. *Phytopathol. Z.* **69**, 115–124.

9. Germination of Urediospores and Differentiation of Infection Structures

Dickinson, S. (1971). Studies in the physiology of obligate parasitism. VIII. An analysis of fungal responses to thigmotropic stimuli. *Phytopathol. Z.* **70**, 62–70.

Dickinson, S. (1972). Studies in the physiology of obligate parasitism. IX. The measurement of a thigmotropic stimulus. *Phytopathol. Z.* **73**, 347–358.

Dickinson, S. (1974). The production of nitrocellulose membranes as used in the study of thigmotropism. *Physiol. Plant Pathol.* **4**, 373–377.

Doherty, M. A., and Preece, T. F. (1978). *Bacillus cereus* prevents germination of urediospores of *Puccinia allii* and the development of rust disease of leek, *Allium porrum*, in controlled environments. *Physiol. Plant Pathol.* **12,**123–132.

Dunkle, L. D., and Allen, P. J. (1971). Infection structure differentiation by wheat stem rust urediospores in suspension. *Phytopathology* **61,** 649–652.

Dunkle, L. D., Maheshwari, R., and Allen, P. J. (1969). Infection structures from rust urediospores: Effect of RNA and protein synthesis inhibitors. *Science* **163,** 481**–**482.

Emmett, R. W., and Parbery, D. G. (1975). Appressoria. *Annu. Rev. Phytopathol.* **13,** 147–167.

Flor, H. (1954). Longevity of urediospores of flax rust. *Phytopathology* **44,** 469–471.

Foudin, A. S., and Macko, V. (1074). Identification of the self-inhibitor and some germination characteristics of peanut rust urediospores. *Phytopathology* **64,** 990–993.
Frank, B. (1883). Ueber einige neue und weniger bekannte Pflanzenkrankheiten. *Ber. Dtsch. Bot. Ges.* **1,** 29–34.
French, R. C., and Weintraub, R. L. (1957). Pelargonaldehyde as an endogenous germination stimulator of wheat rust spores. *Arch. Biochem. Biophys.* **72,** 235–237.
French, R. C., Novotny, J. F., and Searles, R. B. (1964). Properties of bacteria isolated from wheat stem rust spores. *Phytopathology* **54,** 970–973.
French, R. C, Long, R. K., Latterell, F. M., Graham, C. L., Smoot, J. J., and Shaw, P. E. (1978). Effect of nonanal, citral, and citrus oils on germination of conidia of *Penicillium digitatum* and *Penicillium italicum. Phytopathology* **68,** 877–882.
Fuller, H. J., and Tippo, O. (1949). "College Botany." Holt, New York.
Gettkandt, G. (1954). Zur Kenntnis des Phototropismus der Keimmyzelien einiger Parasitischer Pilze. *Wiss. Z. Univ. Halle-Wittenberg, Math.-Naturwiss.* **3,** 691–710.
Givan, C. V., and Bromfield, K. R. (1964a). Light inhibition of urediospore germination in *Puccinia recondita. Phytopathology* **54,** 116–117.
Givan, C. V., and Bromfield, K. R. (1964b). Light inhibition of urediospore germination in *Puccinia graminis* var. *tritici. Phytopathology* **54,** 382–384.
Grambow, H. J. (1977). The influence of volatile leaf constituents on the *in vitro* differentiation and growth of *Puccinia graminis* f. sp. *tritici. Z. Pflanzenphysiol.* **85,** 361–372.
Grambow, H. J. (1978). The effect of nordihydroguajaretic acid on the development of the wheat rust fungus. *Z. Pflanzenphysiol.* **88,** 369–372.
Grambow, H. J., and Grambow, G. E. (1978). The involvement of epicuticular and cell wall phenols of the host plant in the *in vitro* development of *Puccinia graminis* f. sp. *tritici. Z. Pflanzenphysiol.* **90**, 1–9.
Grambow, H. J., and Reisener, H. J. (1976). Differenzierung und Wachstum von *Puccinia graminis* f. sp. *tritici* in vitro. *Ber. Dtsch. Bot. Ges.* **89,** 555–561.
Grambow, H. J., and Riedel, S. (1977). The effect of morphogenically active factors from host and nonhost plants on the *in vitro* differentiation of infection structures of *Puccinia graminis* f. sp. *tritici. Physiol. Plant Pathol.* **11,**213–224.
Grove, S. N. (1978). The cytology of hyphal tip growth. *Filamentous Fungi* **3,** 28–50.
Grove, S. N., and Bracker, C. E. (1970). Protoplasmic organization of hyphal tips among fungi: Vesicles and Spitzenkörper. *J. Bacteriol.* **104,** 989–1009.
Heath, I. B., and Heath, M. C. (1978). Microtubules and organelle movements in the rust fungus *Uromyces phaseoli* var. *vignae. Cytobiologie* **16,** 393–411.
Heath, M. C. (1981). Nonhost resistance. *In* "Plant Disease Control: Resistance and Susceptibility" (R. C. Staples and G. H. Toenniessen, eds.), pp. 201–217. Wiley, New York.
Herr, F. B., and Heath, M. C. (1982). The effects of antimicrotubule agents on organelle positioning in the cowpea rust fungus, *Uromyces phaseoli* var. *vignae. Exp. Mycol.* **6,** 15–24.
Hess, S. L., Allen, P. J., Nelson, D., and Lester, H. (1975). Mode of action of methyl cisferulate, the self-inhibitor of stem rust urediospore germination. *Physiol. Plant Pathol.* **5,** 107–112.
Hoch, H. C., and Howard, R. J. (1980). Ultrastructure of freeze-substituted hyphae of the basidiomycete *Laetisaria arvalis. Protoplasma* **103,** 281–297.
Hoch, H. C, and Staples, R. C. (1983). Ultrastructural organization of the non-differentiated urediospore germling of *Uromyces phaseoli. Mycologia* (in press).

Howard, R. J. (1981). Ultrastructural analysis of hyphal tip cell growth in fungi: Spitzenkörper, cytoskeleton and endomembranes after freeze-substitution. *J. Cell Sci.* **48,** 89–103.

Howard, R. J., and Aist, J. R. (1977). Effects of MBC on hyphal tip organization, growth, and mitosis of *Fusarium acuminatum,* and their antagonism by D_2O. *Protoplasma* **92,** 195–210.

Howard, R. J., and Aist, J. R. (1979). Hyphal tip cell ultrastructure of the fungus *Fusarium:* Improved preservation by freeze-substitution. *J. Ultrastruct. Res.* **66,** 224–234.

Howard, R. J., and Aist, J. R. (1980). Cytoplasmic microtubules and fungal morphogenesis: Ultrastructural effects of methyl benzimidazole-2-ylcarbamate determined by freeze-substitution of hyphal tip cells. *J. Cell Biol.* **87,** 55–64.

Huang, B.-F., and Staples, R. C. (1981). Synthesis of proteins during differentiation of the bean rust fungus. *Exp. Mycol.* **6,** 7–14.

Huberman, J. A. (1981). New views of the biochemistry of eucaryotic DNA replication revealed by aphidicolin, an unusual inhibitor of DNA polymerase α. *Cell* **23,** 647–648.

Johnson, T. (1934). A tropic response in germ tubes of urediospores of *Puccinia graminis tritici*. *Phytopathology* **24,** 80–82.

Keogh, R. C. (1974). Studies on *Phakopsora pachyrhizi* Syd.: The causal agent of soybean rust. M.S. Thesis, University of Sydney, Australia.

Keogh, R. C., Deverall, B. J., and McLeod, S. (1980). Comparison of histological and physiological responses to *Phakopsora pachyrhizi* in resistant and susceptible soybean. *Trans. Br. Mycol. Soc.* **74,** 329–333.

Kitani, K., and Inoue, Y. (1960). "Studies on the Soybean Rust and Its Control Measure," Part I. Shekoku Agric. Exp. Stn., Zentsuji, Japan.

Knights, I. K., and Lucas, J. A. (1980). Photosensitivity of *Puccinia graminis* f.sp. *tritici* urediniospores *in vitro* and on the leaf surface. *Trans. Br. Mycol. Soc.* **74,** 543–549.

Lagenbach, R. J., and Knoche, H. W. (1971a). Phospholipids in urediospores of *Uromyces phaseoli.* I. Identification and localization. *Plant Physiol.* **48,** 728–734.

Lagenbach, R. J., and Knoche, H. W. (1971b). Phospholipids in the urediospores of *Uromyces phaseoli*. II. Metabolism during germination. *Plant Physiol.* **48,** 735–739.

Levine, M. N., Bamberg, R. H., and Atkinson, R. E. (1936). Microorganisms antibiotic or pathogenic to cereal rusts. *Phytopathology* **26,** 99–100 (abstr.).

Lewis, B. G., and Day, J. R. (1972). Behaviour of urediospore germ-tubes of *Puccinia*

9. Germination of Urediospores and Differentiation of Infection Structures

graminis tritici in relation to the fine structure of wheat leaf surfaces. *Trans. Br. Mycol. Soc.* **58,** 139–145.

Littlefield, L. J., and Heath, M. C. (1979). "Ultrastructure of Rust Fungi." Academic Press, New York.

Loegering, W. Q., McKinney, H. H., Harmon, D. L., and Clark, W. A. (1961). A long term experiment for preservation of urediospores of *Puccinia graminis tritici* in liquid nitrogen. *Plant Dis. Rep.* **45,** 384–385.

Loegering, W. Q., Harmon, D. L., and Clark, W. A. (1966). Storage of urediospores of *Puccinia graminis tritici* in liquid nitrogen. *Plant Dis. Rep.* **50,** 502–506.

Lucas, J. A., Kendrick, R. E., and Givan, C. V. (1975). Photocontrol of fungal spore germination. *Plant Physiol.* **56,** 847–849.

McBride, R. P. (1969). A microbiological control of *Melampsora medusae. Can. J. Bot.* **47,** 711–715.

Macko, V. (1981). Inhibitors and stimulants of spore germination and infection structure formation in fungi. *In* "The Fungal Spore: Morphogenetic Controls" (G. Turian and H. R. Hohl, eds.), pp. 565–584. Academic Press, New York.

Macko, V., Staples, R. C., Gershon, H., and Renwick, J. A. A. (1970). Self-inhibitor of bean rust urediospores: Methyl 3,4-dimethoxycinnamate. *Science* **170,** 539–540.

Macko, V., Staples, R. C., Allen, P. J., and Renwick, J. A. A. (1971a). Identification of the germination self-inhibitor from wheat stem rust urediospores. *Science* **173,** 835–836.

Macko, V., Staples, R. C., and Renwick, J. A. A. (1971b). Germination self-inhibitor of sunflower and snapdragon rust urediospores. *Phytopathology* **61,** 902.

Macko, V., Staples, R. C., Renwick, J. A. A., and Pirone, J. (1972). Germination self-inhibitors of rust urediospores. *Physiol. Plant Pathol.* **2,** 347–355.

Macko, V., Staples, R. C., Yaniv, Z., and Granados, R. R. (1976). Self-inhibitors of fungal spore germination. *In* "The Fungal Spore" (D. J. Weber and W. M. Hess, eds.), pp. 73–100. Wiley, New York.

Macko, V., Trione, E. J., and Young, S. A. (1977). Identification of the germination self-inhibitor from urediospores of *Puccinia striiformis. Phytopathology* **67,** 1473–1474.

Macko, V., Renwick, J. A. A., and Rissler, J. F. (1978). Acrolein induces differentiation of infection structures in the wheat stem rust fungus. *Science* **199,** 442–43.

Maheshwari, R., and Hildebrandt, A. C. (1967). Directional growth of the urediospore germ tubes and stomatal penetration. *Nature (London)* **214,** 1145–146.

Maheshwari, R., and Sussman, A. S. (1971). The nature of cold-induced dormancy in urediospores of *Puccinia graminis tritici. Plant Physiol.* **47,** 289–95.

Maheshwari, R., Hildebrandt, A. C., and Allen, P. J. (1967). The cytology of infection structure development in urediospore germ tubes of *Uromyces phaseoli* var. *typica* (Pers.) Wint. *Can. J. Bot.* **45,** 447–450.

Mendgen, K. (1973). Microbodies (Glyoxysomes) in infection structures of *Uromyces phaseoli. Protoplasma* **78,** 477–482.

Morgan, F. L. (1963). Infection inhibition and germ-tube lysis of three cereal rusts by *Bacillus pumilus. Phytopathology* **53,** 1346–1348.

Pole Evans, I. B. (1907). The cereal rusts. I. The development of their uredo mycelia. *Ann. Bot. (London)* **21,** 441–466.

Porter, K. R. (1966). Cytoplasmic microtubules and their functions. *In* "Principles of Biomolecular Organization" (G. E. W. Wolstenholme and M. O'Connor, eds.), pp. 308–345. Little, Brown, Boston, Massachusetts.

Pring, R. J. (1980). A fine-structural study of the infection of leaves of *Phaseolus vulgaris* by urediospores of *Uromyces phaseoli. Physiol. Plant Pathol.* **17,** 269–276.

Reisener, H. J. (1976). Lipid metabolism of fungal spores during sporogenesis and germination. *In* "The Fungal Spore" (D. J. Weber and W. M. Hess, eds.), pp. 165–185. Wiley, New York.

Rines, H. W., French, R. C, and Daasch, L. W. (1974). Nonanal and 6-methyl-5-hepten-2-one: Endogenous germination stimulators of urediospores of *Puccinia graminis* var. *tritici* and other rusts. J. *Agric. Food Chem.* 22, 96-100.

Rowell, J. B., and Olien, C. R. (1957). Controlled inoculation of wheat seedlings with urediospores of *Puccinia graminis* var. *tritici. Phytopathology* 47, 650-655.

Schneider, M. J., and Murray, B. J. (1979). Phytochrome mediation of urediospore germination in the fungus *Puccinia graminis. Photochem. Photobiol* 29, 1051-1052.

Sharp, E. L. (1967). Atmospheric ions and germination of urediospores of *Puccinia striiformis. Science* **156,** 1359-1360.

Sharp, E. L. (1972). Relation of air ions to air pollution and some biological effects. *Environ. Pollut.* 3, 227-239.

Sharp, E. L., Schmitt, C. G., Staley, J. M., and Kingsolver, C. H. (1958). Some critical factors involved in establishment of *Puccinia graminis* var. *tritici. Phytopathology* 48, 469-474.

Staples, R. C. (1974). Synthesis of DNA during differentiation of bean rust urediospores. *Physiol. Plant Pathol.* 4,415-424.

Staples, R. C, and Hoch, H. C. (1982). A possible role for microtubules and microfilaments in the induction of nuclear division in bean rust urediospore germlings. *Exp. Mycol.* 6, 293-302.

Staples, R. C, and Huang, B.-F. (1981). Gene activation during differentiation of the rusts and anthracnose fungi. *In* "The Fungal Spore: Morphogenetic Controls" (G. Turian and H. R. Hohl, eds.), pp. 335-353. Academic Press, New York.

Staples, R. C, and Macko, V. (1980). Formation of infection structures as a recognition response in fungi. Exp. *Mycol.* 4, 2-16.

Staples, R. C, and Yaniv, Z. (1976). Protein and nucleic acid metabolism during germination. *Encycl. Plant Physiol. New Ser.* 4, 86-103.

Staples, R. C, and Yaniv, Z. (1978). Mitochondrial DNA polymerase from germinated bean rust urediospores. *Exp. Mycol.* 2, 290-294.

Staples, R. C, Yaniv, Z., and Bushnell, W. R. (1972). Spore germination and ribosomal activity in the rust fungi. I. Comparison of a bean rust fungus and a culturable wheat rust fungus. *Physiol. Plant Pathol.* 2, 27-35.

Staples, R. C, App, A. A., and Ricci, P. (1975). DNA synthesis and nuclear division during formation of infection structures by bean rust urediospore germlings. *Arch. Microbiol.* **104,** 123-127.

Staples, R. C, Laccetti, L., and Yaniv, Z. (1976). Appressorium formation and nuclear division in *Colletotrichum truncatum. Arch. Microbiol.* **109,** 75-84.

Staples, R. C, Grambow, H. J., and Hoch, H. C. (1982). Potassium ion induces bean rust germlings to develop infection structures. *Phytopathology* 72, 957.

Staples, R. C, Grambow, H. J., and Hoch, H. C. (1983a). Potassium ion induces rust fungi to develop infection structures. *Exp. Mycol.* 7, 40-46.

Staples, R. C, Grambow, H. J., and Hoch, H. C. (1983b). Contact stimuli induce wheat stem rust urediospores to differentiate appressoria but not vesicles. *Phytopathology* 73, 1436-1439.

Sussman, A. S., Lowry, R. J., Durkee, T. L., and Maheshwari, R. (1969). Ultrastructural studies of cold-dormant and germinating urediospores of *Puccinia graminis var. tritici. Can. J. Bot.* 47, 2073-2078.

Sweigard, J. A., Grove, S. N., and Smucker, A. A. (1979). Cytochalasin as a tool for investigating the mechanism of apical growth in fungi. *J. Cell Biol.* 83, 307a.

Trione, E. I. (1981) J. Natural regulators of fungal development. *In* "Plant Disease Control: Resistance and Susceptibility" (R. C. Staples and G. H. Toenniessen, cds.|, pp. 85–102. Wiley, New York.

Trocha, P., and Daly, J. M. (1970). Protein and ribonucleic acid synthesis during germination of urediospores. *Plant Physiol.* 46, 520-526.

Trocha, P., and Daly, |. M. (1974). Cell walls of germinating urediospores. II. Carbohydrate polymers. *Plant Physiol.* 53, 527-532.

Trocha, P., Daly, J. M., and Lagenbach, R. *J.* (1974). Cell walls of germinating urediospores. I. Amino acid and carbohydrate constituents. *Plant Physiol.* 53, 519-526.

Ward, H. M. (1903). Further observations on the brown rust of the bromes, *Puccinia dispersa* (Erikss.) and its adaptive parasitism. *Ann. Mycol.* 1, 132-151.

Ward, H. M. (1904). On the histology of *Uredo dispersa* Erikss. and the "Mycoplasm" hypothesis. *Philos. Trans. R. Soc. London. Ser. B.* 196, 29-46.

Ward, H. M. (1905). Recent researches on the parasitism of fungi. *Ann. Bot.(London)* 19, 1-54.

Williams, P. G., Scott, K. J., and Kuhl, J. L. (1966). Vegetative growth of *Puccinia graminis* f.sp. *tritici in vitro*. *Phytopathology* 56, 1418-1419.

Wynn, W. K. (1976). Appressorium formation over stomates by the bean rust fungus: Response to a surface contact stimulus. *Phytopathology* 66, 136-146.

Wynn, W. K., and Gajdusek, C. (1969). Metabolism of glucomannan-protein during germination of bean rust spores. *Contrib. Boyce Thompson Inst.* 24, 123-128.

Wynn, W. K., and Staples, R. C. (1981). Tropisms of fungi in host recognition. *In* "Plant Disease Control: Resistance and Susceptibility" (R. C. Staples and G. A. Toenniessen, eds.), pp. 45–69. Wiley, New York.

Yaniv, Z., and Staples, R. C. (1974). Ribosomal activity in urediospores germinated on membranes. *Phytopathology* 64, 1111-1114.

Yaniv, Z., and Staples, R. C. (1978). DNA polymerase from nongcrminated bean rust urediospores. *Exp. Mycol.* 2, 279-289.

Yarwood, C. E. (1954). Mechanism of acquired immunity to a plant rust. *Proc. Natl. Acad. Sci. U.S.A.* 40, 374-377.

Yarwood, C. E. (1956). Simultaneous self-stimulation and self-inhibition of urediospore germination. *Mycologia* 48, 20-24.

10

Controlled Infection by *Puccinia graminis* f. sp. *tritici* under Artificial Conditions

J. B. Rowell

Cereal Rust Laboratory, Department of Plant Pathology, University of Minnesota, St. Paul, Minnesota

I. Introduction

Most investigations of the cereal rusts require dependable methods for producing plant infection under artificial conditions. During natural epidemics, the explosive spread of rust infection on cereals suggests that infection occurs easily on a susceptible host. In my experience with *Puccinia graminis* E. Henn., however, reproducible control of the amount of infection has been difficult to achieve. This chapter will summarize my observations and insights acquired during 26 years at the Cereal Rust Laboratory into the infection process initiated by urediospores of the wheat stem rust pathogen.

II. Production of Inoculum

The dependable production of pure, viable inoculum is influenced by the host genotype and environmental conditions. The host cultivar, cultural conditions, isolation procedures to prevent contamination, and method of harvesting spores can affect the quantity and quality of inoculum produced.

A. HOST SELECTION AND STATUS

Obviously, a suitable host cultivar for inoculum production must be susceptible to the rust cultures under investigation. The ideal cultivar for increasing rust would be a universal suscept to all genotypes of the rust pathogen. By definition, a universal suscept for a pathogen has no specific genes for resistance to that pathogen. At the Cereal Rust Laboratory, however, a universal suscept for wheat stem rust has not been found. The wheat cultivars Little Club, Baart, McNair 701, and W2691 are susceptible to most genotypes of the wheat stem rust fungus and frequently are used to propagate this rust

pathogen. The soft white wheat cultivars Little Club and Baart possess a specific gene for resistance, *SrLc,* for which the corresponding gene for avirulence occurs with a low frequency in the North American population of J. *graminis i.* sp. *tritici* (Roelfs and McVey, 1979). The soft red wheat cultivar McNair 701 is used at the Cereal Rust Laboratory as the increase host for propagating rust from collections of wheat stem rust in the annual race survey. Several cultures of*P. graminis* f. sp. *tritici* isolated from aecia on barberry, however, are avirulent on McNair 701, and these indicate the presence of a resistance gene, *SrMcN* (Roelfs and McVey, 1979). Watson and Luig (1963) tried to develop a universal suscept by selection of suitable progenies from the cross Little Club//Gabo*3/ Charter. The selected line, W2691, has been susceptible to all North American isolates of *P. graminis* f. sp. *tritici*tested. It is resistant, however, to certain cultures of *P. graminis* f. sp. *secalis* that can attack some wheat cultivars (Roelfs and McVey, 1979). Thus W2691 would not be a satisfactory host for increasing field collections of rusted wheat infected by such genotypes. Thus cultivars that appear to be universally susceptible are likely to possess unrevealed resistances simply because of the lack of a test with rust cultures that possess the appropriate gene for avirulence.

Good yields of urediospores are produced by erect, long-lived, sturdy seedling leaves, whereas spores are difficult to harvest from recumbent, elongated, or flaccid leaves. The growth habit of seedling leaves is influenced by genotype and environment. Under a single set of environmental conditions, the length, width, and duration of the erect status of the first seedling leaf varies among wheat cultivars. Furthermore, some wheats have a genetic trait for premature senescence of seedling leaves exposed to stress from heat, drought, or high numbers of infections. Regulation of environmental conditions can alter the growth habit of the first leaf in wheat seedlings. Low temperatures (15°-20°C) and long light periods of high intensity favor the development of short, broad, sturdy leaves, whereas high temperatures (25°—30°C) and short light periods of low intensity favor the development of elongated, slender, weak leaves.

Another important consideration in selecting a host cultivar for propagating rust is a source of vigorous, healthy seed. Often, a cultivar highly susceptible to the rust pathogen is unavailable commercially, and rust workers must produce their own seed. Thus the selected cultivar should be agronomically adapted to the growing conditions where it will be increased. Furthermore, it is important that the seed be free from seedborne pathogens that cause seed rots, damping off, and seedling blights. These diseases debilitate or kill the host plants, and spores of the unwanted pathogen may contaminate the harvested rust spores.

B. ENVIRONMENTAL CONDITIONS

The prevailing environmental conditions largely determine the longevity and productivity of increase cultures of rust. Uredial infections of *P. graminis* grow indeterminately as long as vigorous chlorenchyma cells are accessible to the parasitic hyphae. Thus the longevity of an increase culture is dependent on that of the host leaves. First leaves of wheat live about 30 to 35 days after emergence at 18° to 21°C with 12 hr of sunlight per day in a glasshouse. Primary uredia produce spores continuously for about 10 days, with maximum production from 4 to 8 days after the uredium erupts through the host epidermis. Secondary uredia appear around the senescing primary uredium and produce additional spores as long as the host leaf remains photosynthetically active. Prabhu and Wallin (1971) found that spores were released for 16 days from a single uredium on a seedling leaf at 24°C with a 12-hr light period of 8600 lux.

Under my conditions, 18°-21°C was the most suitable temperature range for producing inoculum of the wheat stem rust pathogen. Sporulation is greater at 24° and 28°C than at 18°C (Prabhu and Wallin, 1971), but high temperatures shorten the longevity of the first leaf and favor the development of other pathogens. Similarly, moderate moisture conditions prolong inoculum production. When atmospheric and soil moisture is excessive, guttation drops form and wet leaf surfaces, favoring infection by contaminants including rust. Continuous exposure to such conditions causes some urediospores to germinate within uredia, which reduces inoculum quality. High temperatures and humidities often occur in small, enclosed isolation chambers used in glasshouses to protect rust cultures from contamination, and these conditions can kill the spores *in situ.*Filtered ventilation can prevent excessively high temperatures and humidity (Emge *et al.,* 1970). Inoculum production is curtailed by drought, which unduly stresses infected plants because of high transpiration rates through ruptures in the epidermis.

Duration and intensity of light are essential factors in the vigor and longevity of infected leaves and the production of inoculum. In Minnesota during winter, day length is too short and the intensity too low in glasshouses to support adequate spore production by wheat stem rust. About 8 hr of supplementary light from fluorescent lamps in open frames without reflectors mounted 10–15 cm above the tips of the wheat seedlings are used to supplement and compensate for the inadequate natural light. This provides about 10,000 lux of supplemental light at the leaf tips.

Maleic hydrazide is used routinely at the Cereal Rust Laboratory to control the growth of wheat and oat seedlings used to propagate stem rust in the glasshouse. This compound, applied to the soil surface when coleoptiles emerge, permits the first foliar leaf to develop and remain erect, suppresses growth of secondary leaves, and prolongs sporulation. Under our conditions the

effective dose in 50 ml of water added to the soil in a 10-cm plastic pot is 5 and 10 mg in winter and summer, respectively.

C. PURITY

Perpetuating a pure culture of cereal rusts through successive transfer generations in the presence of other rust cultures is difficult. Urediospores become airborne so readily that precautions are needed to prevent the deposition of contaminating spores on the host before inoculation, during the inoculation procedures, and throughout incubation and sporulation of the culture. Single uredial transfers aid in maintaining a pure culture of rust. At the Cereal Rust Laboratory we select a vigorous plant with a single, well-developed isolated uredium near a leaf base, and we remove the leaf blade above the selected uredium and other infected plants in the pot. The isolated plant and uredium are washed to remove spores, and the plant is exposed to dew for a 10- to 12-hr dark period. This dew period renders the remaining spores inocuous by inducing their germination. After the dew period, the infected plant is held in an isolation booth for 48 hr while the uredium produces a new crop of spores. These spores are collected and used as inoculum for the next transfer generation.

Single-spore isolation is the surest procedure for establishing a pure rust culture. Urediospores adhere poorly to glass, moderately well to animal hairs, and strongly to plant cuticle. If dry spores are dusted lightly on a glass slide, individual spores can be located under a stereoscopic microscope at about x60 and picked up manually on the tip of a short animal hair that has been cemented to a handle. Hairs that taper to a fine point such as guard hairs from a straight-haired dog are best. The hair is inspected microscopically to ensure that only a single urediospore is present. Then the spore is touched to a leaf surface while observing microscopically to be sure that it adheres to the cuticle when the hair is removed. Depositing the spore near an India ink mark on the leaf conveniently locates the site where the infection should appear. Acetone or 95% ethanol removes unwanted spores from the hair.

After exposure to a suitable dew period (Section VII) for infection and incubation in a rust-free environment for 4 days, the plants are inspected daily for the appearance of the fleck stage of infection. Infected plants are transferred to individual isolation chambers to permit the uredium to develop as a source of a pure line. Often with *P. graminis.* infection success is less than 10%, so 30 to 40 single-spore isolations are needed to ensure the development of at least two uredia. Inoculum from each uredium is increased and tested individually against appropriate differential cultivars to establish the identity of the pure cultures. After a pure culture is established, propagation in glass or plastic isolation chambers in a glasshouse will maintain purity.

Airborne, foliar pathogens also are troublesome contaminants, and *Erysiphe graminis* DC. is the most common one in cereal rust cultures. Sulfur controls powdery mildew readily, but it cannot be applied directly to plants that will be inoculated or used as sources of rust inoculum, because of its fungicidal action on the rust spores. Vapor from sulfur placed on steam pipes controls the spread of powdery mildew and can inhibit active infections with no apparent effect on rust development or urediospore viability. In glasshouses heated with forced air, the sulfur is placed in glass dishes on strategically located electric hot plates. Care must be taken to heat the sulfur sufficiently for sublimation and not oxidation (96°-119°C), because sulfur dioxide can injure plants. Another effective fungicide is ethirimol (Millstem), a systemic fungicide specifically effective against *E. graminis.* When applied as 50 ml of a 40-ppm solution to the soil surface in a 10-cm pot at planting, it prevents the development of mildew for 4 to 5 weeks in cultures of *P. graminis* f. sp. *tritici* or *P. recondita* Rob. ex Desm., without any apparent effect on the rust pathogens (Rowell, 1972). A useful method for inactivating oidia of *E. graminis* present in urediospore inoculum is to disperse the inoculum in an oil carrier (Section IV,C), because oidia in oil do not infect susceptible hosts.

Helminthosporium sativum is another common foliar pathogen of small grains that can contaminate rust cultures and can affect urediospore germination, host penetration, and the development of infections (Stewart and Hill, 1965). Because *H. sativum* is seedborne, this contaminant can be avoided by using only clean, healthy seed. A contaminated rust culture can be purified of this fungus by the singleuredial transfer method.

Occasionally, *Darluca filum* (Biv.-Bern.) Cast. occurs as a hyper-parasite of rust fungi. Use of spores from a single, healthy uredium for transfer inoculum eliminates this contaminant.

P. recondita is a refractory contaminant of cultures of *P. graminis* f. sp. *triitici.* Once established in a culture, it will predominate in one or two transfer generations under artificial conditions, because a greater percentage of its urediospores successfully infect the host than those of the stem rust pathogen. Most leaf rust contamination of cultures of *P. graminis* f. sp. *tritici* can be avoided by using a host resistant to the former and susceptible to the latter pathogen such as McNair 701, a winter wheat cultivar that possesses the resistance conditioned by *LR9.* Use of triazbutyl (Indar), a fungicide specifically effective only against *P. recondita,*also prevents leaf rust contamination of wheat stem rust cultures. This fungicide is a good protectant but a weak eradicant, so it is most effective when plants are treated prior to infection (Rowell, 1972; see chapter by Rowell, Vol. II). Application of 50 ml of a 2-ppm solution to the surface of the soil in a 10-cm pot at least 2 days before plants are inoculated prevents leaf rust.

D. HARVEST

When a rust culture is sporulating abundantly, the spores can be collected about every other day. Again, precautions against contamination must be observed. The collecting equipment and the operator should be clean and free of any viable spores. Spores should be collected as early as feasible in the workday as soon as the infected plants are dry and free of any guttation moisture. Cyclone collectors (Tervet *et al.*, 1951) have been designed in a variety of sizes from microcollectors that harvest the spores from a single uredium (Browder, 1971), to macrocollectors that will collect gram quantities of spores from massed infected plants. These units are easily sterilized between collections and are the best means of collecting spores free from contamination. Masses of spores are easily dislodged from uredia when the leaves are shaken over an open collection dish, but this procedure also releases many airborne spores, which can contaminate other cultures in the work area. Harvested spores often contain bits of leaf tissues, aphids, and soil particles that can be removed by passage through a 60-mesh soil sieve.

III. Storage of Inoculum

A. FACTORS AFFECTING UREDIOSPORE LONGEVITY

The individual rust urediospore is a vegetative spore without a true dormancy. It remains in the resting state only in the absence of conditions favorable for germination. Although germination inhibitors produced naturally by the cereal rusts are sufficiently concentrated to inhibit germination in masses of urediospores as in uredia, these substances appear to have no effect on dispersed spores in contact with water, presumably resulting from dilution below the effective dose (Tollenaar and Houston, 1966). At room temperatures and moderate relative humidities, spores of *P. graminis* remain viable for about 4 to 6 weeks. Vacuum drying or freezing at ultralow temperatures are dependable methods for long-term storage of viable urediospores. Storage conditions, however, affect urediospore form, moisture content, germinability, semipermeability, and respiration.

1. Morphological Effects

Germination can be a misleading indicator of urediospore viability after storage, because the percentage of urediospores that will germinate varies with the method used (see Section IV,D). In my experience, placing urediospores in an oil drop on water has been the most reliable germination test, because it

minimizes rehydration injury (see Section I V,D,4). Dried urediospores of *P. graminis* invariably are shriveled. The spore is flattened along the long axis and has a pronounced indentation at right angles to this axis across the flattened surface over the equatorial pores. The wall and protoplast of such dried spores rehydrate at differential rates on direct contact with water (Rowell, 1956), and this differential rehydration can injure the spore.

The differential hydration of wall and protoplast is readily observed with a microscope by watching the structural changes in dried spores on a glass slide as water is allowed to flow under the coverslip. The wall immediately expands to its original shape on contact with water, but the protoplast at first remains contracted in a granular central mass separated from the wall, and then most of them gradually swell to fill the wall volume in 20 to 60 min. When water is brought into contact with a drop of oil containing dispersed urediospores under a coverslip, the spores initially remain shriveled and the oil has no observable effect. Gradually over 20 to 60 min, the wall and protoplast of spores in the oil drop expand simultaneously to the original shape, and rarely is a spore observed in which the protoplast remains contracted. In this type of experiment, urediospores germinate poorly under glass coverslips, but germination does occur if the observations are made on dried urediospores covered with a small square of 0.013-mm polyethylene film, which is permeable to oxygen and carbon dioxide. Under the film, only a few of the expanded spores in direct contact with water germinate, whereas most of the expanded spores in the oil droplet germinate. Thus the oil apparently reduces injury by preventing the sudden separation of the wall from the plasmalemma.

2. Moisture Changes

The moisture content of freshly collected spores will vary with the environmental conditions. Moisture content of urediospores cannot be determined precisely, because a constant weight is not attained in a drying oven. After a large weight loss in the first 24 hr, weight continues to decrease slowly with further drying. Conventional determination of spore moisture indicated that water content was linearly proportional (from about 4 to 20% water) to atmospheric relative humidity (from 0 to 80% RH), but abruptly increased to about 30% water at 92% RH (J. B. Rowell, unpublished data).

Urediospore germinability varies with time and humidity during storage. Dispersed spores stored at 20% RH have had the best overall survival and least rehydration injury (Table I). Differential rates of germination on water and in oil on water agar (Table I) show that rehydration injury is most severe at 0% RH, but unaccountably a similar, unexplained injury occurs after prolonged storage at 66% and higher RH (Table I). Spores stored en masse respond to atmostpheric humidity and lose germination at slower rates than dispersed spores.

Table I

Effect of Time and Relative Humidity (RH) during Storage on Germinability of Dispersed Urediospores of *Puccinia graminis* f. sp. *tritici* [a,b]

Relative humidity (%)	Germination medium	Germination after indicated days of storage (%)		
		3	7	10
0	Water	22	10	0
20	Water	78	69	61
66	Water	85	15	4
84	Water	87	28	3
92	Water	93	55	0
0	Oil on agar	73	18	32
20	Oil on agar	82	70	63
66	Oil on agar	100	91	82
84	Oil on agar	97	69	12
92	Oil on agar	97	75	0

[a]J. B. Rowell, unpublished data.

[b]Dispersed spores on small squares of polyethylene film were exposed to indicated RH in sealed chambers, and germinability was determined by inverting the squares so the spores were in contact **with** distilled water or oil on 1% water agar in darkness at 18°C.

3. Semipermeability Changes

Sudden wetting of dried spores with water results in enhanced leakage of cell electrolytes into the ambient water, and the amount of leakage is greater for spores stored at 0% RH than at higher humidities (Table II). Again, spores stored at 0% RH have lower germination rates when placed on water than when germinated in oil. These observations suggest that the sudden rehydration of dried urediospores irreversibly disrupts the semipermeability of the plasmalemma and thereby prevents germination. Spores stored at high humidity (90% RH) had low germination rates on both water and in oil and no increase in electrolyte loss (Table II), indicating that loss of membrane semipermeability was not the cause of reduced ability to germinate.

4. Respiratory Changes

Patterns of respiration indicate how spore activity varies when spores are stored at different relative humidities (RH; Fig. 1). Respiratory activity is high for spores stored in 90% RH, but germinability at this RH declines rapidly after 10 days storage. The loss of germinability apparently is due to the loss of

energy reserves, because 34% of the spores failing to germinate on water agar after 22 days storage germinated on glucose agar. About one-third of the respiration of spores stored 13 days at 90% RH was judged from experiments with heat-killed spores to be due to contaminating microorganisms, which are evident as traces of fungal mycelia after 20 days storage. Thus storage in 90% humidity supports active metabolism in the spores, which increases moisture content by the accumulation of metabolic water and reduces longevity by exhausting metabolic reserves. Spores stored at 80% RH (Fig. 1) have a steady, low respiratory rate for 27 days. Spore germination at this humidity started to decline after 17 days of storage and was 23% at 27 days. Germination of these spores is not increased on 2% glucose, which suggests that the nature of the loss in germinability at 80% RH is not simply from depletion of nutrients. Spores stored at 50% RH have had no detectable levels of respiration, and more than 90% of the spores have germinated after 27 days.

Table II

Effect of Relative Humidity during Storage on Leakage of Electrolytes from Urediospores of *Puccinia graminis* f. sp. *Iritici*

Relative humidity (%)	Storage for 7 days			Storage for 14 days		
	Resistance (1000 Ω)	Germination (%)		Resistance (1000 Ω)	Germination (%)	
		In water	In oil		In water	In oil
0	70.7	39	89	73.8	10	85
20	100.5	61	92	105.0	72	81
50	98.6	71	89	82.0	28	78
90	101.0	54	87	97.0	0	16

[a]J. B. Rowell, unpublished data.

[b]Electrolyte leakage determined by the resistance of the filtrate after I hr extraction of 50 mg of urediospores in 100 ml double glass-distilled water Duplicate 50-mg spore lots were sampled to determine germination in direct contact with water on polyethylene film and in oil on 1% agar.

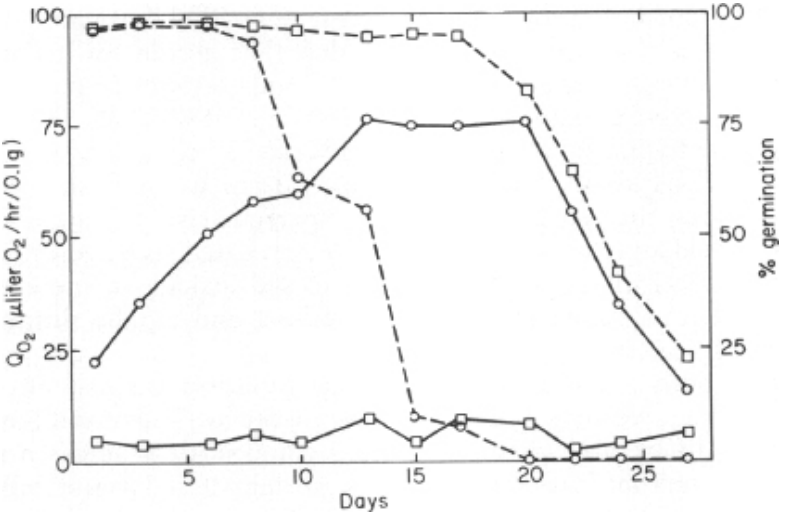


Fig. 1. Effect of relative humidity during storage on respiration and germinability of masses of dry urediospores of *Puccinia graminis* f. sp. *tritici* (J. B. Rowell, unpublished data). (O—O), Respiration at 90% RH; (O- -O), germination in oil on water agar at 90% RH; (□—□), respiration at 80% RH; (□- -□), germination in oil on water agar at 80% RH.

5. *Poststorage Effects*

Dry urediospores of *P. graminis* have a wide temperature tolerance. These spores reportedly survived exposure for up to 10 hr at 60°C (Hwang, 1942). Subfreezing temperatures were once considered lethal to urediospores. The discovery (Loegering *et al.*, 1961) that dry urediospores survive cryogenic freezing at - 196°C when the retrieved spores are thawed for a few minutes at 37°C indicated that ultracold temperatureswere not lethal but induced a heat-reversible dormancy. Subsequent studies (Bromfield, 1964) revealed that germinability was restored in spores frozen at a range of temperatures from –1.1° to –196°C by exposures to either a "heat shock" at 40°C for 5 min or to vapor-phase hydration for 16 to 24 hr in a sealed container over water at 20°C. Subsequently, Maheshwari and Sussman (1971) showed that frozen spores thawed at 20°C were supersensitive to liquid water, which caused an irreversible injury that prevented spore germination. This hydration injury to the spore increased leakage of metabolites, suppressed incorporation of isotopic carbon, and rapidly diminished respiratory activity.

Clearly, both dehydration and freezing condition urediospores to injury by sudden wetting with water. The report by Grieve and Povey in 1981 that differential freezing reverses osmotic flow across a membrane suggests that frozen urediospores are dehydrated osmotically to a state comparable to that of

desiccated spores. The mechanism by which sudden wetting with water affects the plasmalemma to disrupt cell permeability remains unclear.

It is evident that water relations during storage profoundly affect urediospore germinability. Excessive drying irreversibly injures spores, and excessive moisture vapor fosters respiration that exhausts metabolite reserves or other essential components required for germination. Spore germinability is also reduced by abrupt changes in spore moisture when spores are retrieved from storage.

B. METHODS OF STORAGE

No special procedures are necessary for urediospores kept for short periods of time. Storage in a covered container at room temperature and humidity are satisfactory when freshly harvested spores will be used within a few days. Under these conditions, however, germinability will decline within a week or so. Freshly harvested spores stored in a sealed container at 4°C may be germinable from several months to a year. Under refrigeration, urediospores of *Puccinia coronata* and P'. *recondite* appear to survive better than those of *P. gram-inis.* At the Cereal Rust Laboratory, freshly collected urediospores from the leaf and stem rusts of wheat and oats are stored temporarily in open tubes at 4°C over a solution of 82.4 g KOH/100 ml water with a vapor pressure that gives about 20% RH. This storage is satisfactory for routine inoculations within a few weeks after collection without any conditioning treatment of the spores (see Section IV,A). Storage of urediospores for extended periods of time in inoculation oil is unsatisfactory at either room or refrigeration temperatures, because the spores settle to the bottom of the container and provide a source of sufficient nutrient and moisture to support the growth of saprophytic microorganisms.

Urediospores can be preserved for extended periods of time either by drying and storing the spores in a vacuum or by freezing and storing the spores at very low temperatures. The vacuum-drying method is simpler and less expensive but is less dependable than storage in liquid nitrogen cryostats for periods of 5 or more years.

The vacuum-drying method (Sharp and Smith, 1957) was developed as a modification of the lyophilization technique used to preserve bacteria and some fungi. The vacuum-drying method differs from lyophilization procedures in that the spores are not frozen in a hygroscopic embedding medium but are dried en masse and sealed in tubes under vacuum. Spores preserved by this method are not inert (Wynn *et al,* 1966), and preservation results from the suppression of biochemical changes by the reduced availability of water and oxygen.

A vacuum-drying system consists of a manifold to hold lyophil tubes connected to a moisture trap and evacuated by an oil vacuum pump capable of lowering pressure to about 0.1 mm Hg. Either a cold trap chilled by dry C02 in

acetone, or a chemical desiccant trap containing anhydrous CaS04 or silica gel is suitable for a moisture trap. For use, the opened tubes are placed in a sealed container over water for 16 to 24 hr to allow gradual rehydration of spores and prevent injury on direct contact with water. Loss in germination can occur during the drying of the spores, and germination gradually declines with time in storage. Vacuum-dried spores survive better in tubes stored at 4°C than at room temperatures. Survivability can vary for spores of a given rust culture that are produced at different times in a glasshouse. The major difficulty of this method is determining when spores have reached the optimal moisture content for maximum preservation. Too much desiccation is lethal, and insufficient desiccation shortens storage life of urediospores. Generally, spores dried for 2 to 3 hr in a vacuum of 10 to 250 µm Hg with a moisture trap will be sufficiently dry (about 2% moisture) for extended storage. Small quantities (10-50 mg) of spores, however, will dry faster than large quantities (500 mg).

The most reliable method for long-term preservation of urediospores is to store them in sealed tubes in a liquid nitrogen (LN) cryostat (Loegering *et al.*, 1961). Cultures stored at the Cereal Rust Laboratory during 1963 in a LN cryostat gave high rates of germination and infection in 1981. A readily available source of LN is needed, because the cryostats require refilling with LN at regular intervals to maintain the temperature in the range of –160 to –193°C. Dry spores are placed in small tubes made from 5- to 7-mm-diameter borosilicate tubing; the tubes are then sealed with a high-temperature gas torch and are placed in the LN cryostat for freezing and storage. Heat-resistant borosilicate glass tubes are required to withstand the temperature stress of freezing and thawing. Several sealed tubes are mounted on a "cane" placed in the cryostat. Tubes must be sealed completely to prevent entry of LN, which could cause an explosion during thawing. Leaks in sealed tubes can be detected by external gas bubbles that appear when the tube is immersed in a 40°-45°C water bath or by penetration of a dye solution into tubes when they are immersed in the dye at 4°C. At the Cereal Rust Laboratory, canes removed from LN are immediately inserted into a 5-cm cast iron pipe to contain any explosive shattering of the tubes due to LN leakage. Occasionally, tubes have exploded on opening the tubes several days after removal from LN, presumably because of gaseous nitrogen trapped under pressure in the tube. Thus operators should use protective eye and face shields as well as gloves whenever handling retrieved tubes.

Because frozen spores thawed at room temperature germinate poorly when directly wetted with water, a conditioning treatment on retrieval from LN is used to restore good germination and infectivity. Exposing the retrieved spores to heat by immersing the sealed vial in a water bath at 45°C for several minutes or rehydrating the spores at 80-90% relative humidity for 16 to 24 hr restores germinability and infectivity. Once spores are frozen rapidly by immersion in LN, the vials do not have to be immersed continuously in LN, because the low

temperature of the gaseous nitrogen in the cryostat preserves spores. Furthermore, spores in sealed vials remain viable after repeated cycles of freezing and thawing. Because rapid frosting of canister and tube surfaces on removal from the cryostat obliterates labels, a system for storing culture tubes by location in the cryostat ensures retrieval of a given culture (Leath *et al.*, 1966). (A small paper label inside each tube identifies the culture.)

The disadvantages of LN storage are the expense and the hazardous properties of LN. The N2 emitted continuously from the cryostats can displace oxygen in unventilated, confined spaces and become an asphyxiation hazard. Furthermore, LN is extremely cold, and it, as well as canisters, canes, and tubes removed from the cryostat, can instantaneously cause severe freezing injury on contact with skin. Operators of LN refrigerators should be instructed in safe handling techniques and be provided with approved protective equipment.

Unpublished studies by M. Prescott (personal communication) demonstrated that urediospores stored at -55°C in ultralow-temperature freezers and heat treated at 45°C for several minutes after retrieval from storage gave nearly as good survival as spores in LN. Bulk storage of urediospores in such freezers is convenient for accumulating large amounts of inoculum. I have successfully stored 0.5 g of spores sealed in 10- by 15-cm flat polyethylene bags at -45°C for 2 years with negligible loss of germinability or infectivity. In my method, the freshly collected spores are held at 50% RH **for** 2 days (to dry the spores partially) and then placed in the polyethylene bag, which is heat sealed about 3 cm below the opening. A dated label identifying the culture is inserted in the remaining pocket and held in place by a second heat seal. Initially, the bags are placed flat on the bottom of the freezer for several hours to freeze the spore mass rapidly, and the bags are subsequently bundled together for storage. On retrieval the bag is immersed in a water bath at 45°C for 5 min to restore germinability. Commercially available plastic tubes for cryogenic storage are also satisfactory for bulk storage of spores.

A variation of the ultralow-refrigeration method was developed to allow quick storage of rust isolates from annual race surveys for future studies. Several infected leaves with numerous sporulating uredia of an isolate are placed in a 5- by 15-cm labeled glassine bag. The bags are placed in a vacuum desiccator containing anhydrous CaS04, and the **air** is evacuated to 12 to 15 mm Hg. After several hours, depending on the number of samples in the desiccator, the leaves become visibly dry and shriveled. Then the bags are removed from the desiccator and immediately placed flat on the bottom of the freezer. Several hours later the refrigerator is opened, and the bags are quickly transferred to a storage box inside the refrigerator. When a collection is retrieved for use, the glassine bag is inserted into a polyethylene bag for protection against wetting the sample, and the bags are suspended in a water bath at 45°C for 10 min. After this heat treatment, the glassine bag is placed in a sealed chamber over 23.5% KOH (giving 80% RH) at room temperature for

at least 4 hr of hydration before the spores are used as **inoculum.** Germination percentage of spores stored by this method is less than that of samples collected from the same source material and stored under LN; however, more than 50% of the spores germinate after 1 year of storage, which is adequate for routine inoculations.

IV. Preparation of Inoculum

A. CONDITIONING TREATMENTS

Freshly harvested urediospores do not require conditioning before use as inoculum, and neither do spores held for several days at room temperature and relative humidities between 50 and 80%. In my experience with controlled inoculations, freshly harvested spores were more dependable than stored spores for reproducible control of the infection per unit of spores from experiment to experiment.

Spores stored by either vacuum drying or freezing require heat and/or hydration treatments to obtain maximum effectiveness as inoculum. The advantages of heat treatment of stored urediospores are the brevity of the treatment period and the broad range of effective temperatures. The effective range is 36° to 60°C, and treatments at 40°C have been equally effective for exposure times of 0.25 to 40 min. Apparently, the critical aspect for heat treatment is to make sure that the entire spore mass is heated to the effective temperature. Furthermore, the spores can be thawed at room temperature and subsequently reactivated by a heat treatment (Loegering and Harmon, 1962; Bromfield, 1964), after which retrieved spores have about the same longevity as fresh spores (Bromfield, 1964).

Reactivation of vacuum-dried or frozen spores by vapor-phase hydration is as effective as heat treatment, but it takes longer (Bromfield, 1964). Hydration over pure water in a closed container at room temperatures for 16 to 24 hr is generally effective. The duration of an effective hydration treatment is determined by the time required for moisture equilibration, which is governed by the surface area and volume of the spore mass, the volume of air in the container, and the exposed surface area of water. I prefer to use a 23.5% KOH as the source of water vapor, which gives 80% RH at 20°C, to avoid moisture condensation from chance drops in temperature.

B. MEASURING INOCULA

A convenient measure of inocula is by weight. The number of urediospores per unit weight, however, can vary with its moisture content or by presence of

contaminants. Spore number per unit weight often is determined by using a hemacytometer. I dilute spores in a 1:1 mixture of light inoculating oil and light petrolatum, and I use a 5-mm platinum loop to place a uniform drop on the grid surface. An average number of 450,000 spores/mg was found in repeated trials with freshly harvested spores from infected plants grown in the glasshouse under good light and temperature conditions. During the poor light conditions of winter, however, the average number of freshly harvested spores was 620,000 per mg.

The number of urediospores in inoculum preparations can be determined with a nephelometer if a mixture of 2 ml of inoculation oil with 5 ml of carbon tetrachloride is used to suspend the spores. This mixture has a density of 1.35 that will hold spores in suspension adequately for a nephelometric determination (the density of urediospores is approximately 1.35). The procedure is as follows: pipet 2 ml of urediospores suspended in light inoculation oil into a nephelos cuvet; add 5 ml of CC14 and mix it thoroughly; determine the number of nephelos units in a Coleman nephelometer in comparison to Coleman nephelos standard 21 expanded by a factor of 3; subtract the number of nephelos units for a sporeless blank of the oil-CCl4 mixture; and multiply the remainder by 3794 to obtain the number of spores per milliliter in the initial inoculum suspension in oil.

C. CARRIERS

Most inoculations with urediospores require dilution of the inoculum in a carrier for efficient, uniform dispersal of the spores. Dry spores are generally mixed in a ratio of 1:4 with dry carriers such as talc, diatomaceous earth, or Lycopodium powder for dusting applications. Large-scale inoculations in the field with the dry carriers can be applied with commercially available hand-operated or mechanized backpack dusters. Small-scale inoculations in the glasshouse often use a simple “puff” duster constructed with a large test tube, a two-hole stopper in which two pieces of glass tubing bent at right angles are inserted, and a rubber atomizing bulb attached to one tube. Dust inoculation is inefficient for depositing spores on target plants, is subject to large losses from drift, and contaminates the work area with large numbers of airborne spores.

Water serves as a carrier of urediospores for hypodermic inoculations and occasionally for spray applications to foliage. Spores conditioned initially by vapor-phase hydration should be used to avoid the injury induced by suddenly wetting dry spores with water. Because the hydrophobic urediospore surface resists wetting and dispersal in water, surfactants are often used to prepare spore suspensions. However, I have not found a satisfactory surfactant for use with urediospores. I have tested several, including Tween 20 and Aerosol OT, which have been used by some workers to suspend spores in water. At 100 ppm, they failed to disperse 20 mg of urediospores completely in 5 ml and

reduced the percentage of spore germination as well as the length of germ tubes. At 10 ppm, germination was normal in many surfactants, but spore wetting and dispersal were negligible.

Urediospores can be suspended satisfactorily in water by first preparing a paste of spores. A spore mass is placed in a small beaker; one drop of water is then added and kneaded into the spore mass with the rounded end of a glass rod. This process is repeated by adding one drop of water at a time until the moistened spore mass has the pasty consistency of a heavy cream. At this point the bottom of the beaker is placed in the bath of an ultrasonic cleaner for about 1 min, and the remaining volume of water required for the final suspension is added during the sonication. This step degasses the spore surfaces, which improves spore wettability and yields almost complete dispersal of the spores in water. Good but incomplete suspensions leaving a film of unwetted spores on the water surface are obtained if the sonication is omitted. Spores in suspensions prepared by this method germinate normally.

Urediospores are highly lipophilic and are readily wetted and suspended by oils and other nonpolar fluids. Some highly refined, non-phytotoxic petroleum oils make excellent carriers for rust inoculum (Rowell and Hayden, 1956; Rowell and Olien, 1957). These oils are commercial products synthesized catalytically from selected petroleum fractions. They are mixtures of various saturated hydrocarbon compounds in proportions that will vary with the source of the crude petroleum. The oils I have found to be most effective have physical characteristics similar to Isopar M (Exxon Co., United States) as follows: ASTM initial boiling point 207°C, final boiling point 260°C, specific gravity 0.78, and flash point 77°C. Two types of oils, isoparaffinic and naphthenic, have been satisfactory carriers. Isoparaffinic oils such as Isopar, Mobilsol 100 (Mobil Oil Co.,), and Soltrol 170 (Phillips Petroleum Co.) are alkane hydrocarbons, which have a saturated linear chain or branched structure. A naphthenic oil such as Odorless Insecticide Base Oil W-4 (American Mineral Spirits Co.) consists of cycloalkane hydrocarbons, which have saturated ring structures. These oils have not differed significantly from each other in comparative controlled inoculations in the number of spores deposited on foliage, the percentages of germination and host penetration, and the numbers of infections. All of these oils have extremely low phytotoxicity and are used also as diluents for insecticides. These oils are most successful as carriers when spray deposits are light and invisible. They are moderately volatile, however, and visible deposits should be allowed to evaporate before exposing inoculated plants to dew. These are volatile hydrocarbon solvents classed as combustible liquids and should be kept away from heat, sparks, and open flame. Operators also should avoid prolonged skin contact or breathing oil vapors.

In addition to industrial oils, I have tested several purified hydrocarbon compounds as carriers of urediospores (Table III). In general, the higher the boiling point of the compound, the greater the amount of infection.

Germination of urediospores from each suspension dispersed in an oil drop on water agar, however, did not differ significantly among the compounds, with the exception of cycloheptane, in which spore germination and germ tube length were reduced appreciably. In other tests, plants were inoculated with spores carried in some of these compounds at two concentrations and examined for pathogen development (Table IV). The major difference observed between the carrier oils was in number of spores deposited on the leaves. The higher the boiling point of the carrier oil, the greater the number of spores deposited per square centimeter of leaf surface, which mostly accounts for the greater number of infections observed.

Table III

Effect of Some Hydrocarbon Fluids as Inoculation Carriers of Urediospores of *Puccinia graminis* f. sp. *tritici*[a,b]

Hydrocarbon compound	Empirical formula	Boiling point (°C at 760 mm Hg)	Infections per leaf
Alkanes			
n-Heptane	C_7H_{16}	98.4	4.7
Trimethylpentane	C_8H_{18}	99.3	5.2
Octane	C_8H_{18}	125.6	5.7
Nonane	C_9H_{20}	150.7	10.0
n-Decane	$C_{10}H_{22}$	174.0	13.0
Dodecane	$C_{12}H_{26}$	214.5	18.7
Soltrol 170	—	196–261	25.9
Cycloalkanes (naphthenes)			
Cyclohexane	C_6H_{12}	80.7	3.7
Cycloheptane	C_7H_{14}	118.1	4.0
Cyclooctane	C_8H_{16}	148.0	7.9
Odorless Insecticide Base Oil W-4	—	196–246	16.0

[a] J. B. Rowell, unpublished data.

[b] Infection data from Little Club wheat seedlings inoculated by atomization of 0.2 ml of a suspension containing 0.5 mg of spores/ml.

A variety of inert, synthetic fluids are effective carriers of urediospores. Trichlorotrifluoroethane (Freon 113, E. I. Dupont De Nemours & Co.), boiling point 47.6°C, is an effective carrier (Miller, 1965). Spores float in this dense fluid (1.57 g/cm^3), and spore germination declines after several hours' exposure. This fluid has a low viscosity (0.66 centipoise) and boiling point, which results in rapid flow through spray nozzles. In comparative tests as carriers of urediospores, trichlorotrifluoroethane deposited fewer spores, had

more spore aggregates, and gave fewer infections than light mineral oils. Percentages of spore germination and appressorial formation were similar in both carriers.

Table IV

Effect on Spore Deposition, Germination, Appressorium Formation, and Infection of Selected Hydrocarbon Fluids as Carriers of Urediospores of *Puccinia graminis* f. sp. tritici[a,b]

Hydrocarbon compound	Spores/cm²[c]		Germination[c] (%)	Appressoria/cm²[c]		Infection per leaf[d]	
	No.	% of control		No.	% of control	No.	% of control
Heptane	122	18	77.3	38	16	4.5	17
Nonane	168	25	87.6	51	21	12.3	47
n-Decane	467	70	73.8	132	54	13.3	51
Dodecane	667	101	66.9	203	83	19.2	74
Control (Soltrol 170)	663	—	81.2	245	—	26.0	—

[a] J. B. Rowell, unpublished data.

'Data from Little Club wheat seedlings inoculated by atomization of 0.2 ml of a urediospore suspension. 'On leaves inoculated with 20 mg urediospores/ml. [d]On leaves inoculated with 0.5 mg urediospores/ml.

Several perfluorochemicals, (FC-43 and FC-77, Fluorinert electronic liquids, 3M Co.), although more costly than mineral oils, are also effective carriers (Bushnell and Rowell, 1967). These fluids have remarkably low toxicity and a high capacity to dissolve oxygen and carbon dioxide. Composed mostly of perfluorotributylamine, FC-43 has excellent spray characteristics (viscosity 2.8 centipoise at 25°C), depositing spores at rates comparable to light mineral oils, but more spore aggregates are present. The liquid deposited on plant surfaces evaporates slowly (vapor pressure 0.3 mm Hg at 25°C; boiling point 170°C). Spores applied in sprays with this carrier germinated, formed appressoria, and infected plants at frequencies similar to that for light mineral oils. Dispersed spores rise to the surface of this dense fluid (1.88 g/cm³ at 25°C). FC-77, a mixture of perfluorinated 8-carbon compounds is too volatile (vapor pressure 30 mm Hg at 25°C; boiling point 99°-107°C) for usage in controlled sprays but is useful for topical applications of suspended urediospores directly to leaf surfaces with a cotton swab. These fluids should be effective carriers for hypodermic inoculations.

D. GRMINATION TESTS

Ideally, a test for estimating the inoculum potential of a urediospore sample should evaluate the capacity for completion of the infection process, which includes germ tube initiation, germ tube growth, and germ tube differentiation of infection structures. Generally, investigators only test the percentage of spores that produce germ tubes, and even those tests are subject to errors dependent on the method employed. For instance, the percentage of germination obtained on water agar at 21°C for a sample of urediospores of *P. graminis* f. sp. *tritici* was 27% for spores smeared across the agar surface, 73% for spores dusted onto the surface, and 99% for spores dispersed in a drop of oil and placed on the surface. These different results are due to differing rates of spore hydration, and the extent of the variation between methods is affected by the moisture content and age of the spores (see Section III,A). In addition, the outcome of a germination test is affected by temperature, light, substrate surface, water source, and air purity.

1. Temperature and Light

Temperature is not a major limiting factor for the initiation of germination because of the relatively wide range of optimum temperatures, 15°-23°C (Burrage, 1970). Light temporarily inhibits the *in vitro* initiation of germination of spores hydrated after storage (Givan and Bromfield, 1964), but appears to have little effect on freshly harvested spores (Burrage, 1970).

2. The Substrate Surface

The nature of the substrate surface is crucial to the successful determination of germination rates, because the surface can alter the distribution and orientation of spores, the way germ tubes are initiated, and the subsequent pattern of germ tube growth. Ideally, the substrate surface should induce behavior similar to that on the host surface.

Urediospores landing on the host generally are oriented with the flattened long axis of the spore contiguous to the hydrophobic cuticle. Swellings appear at all pores when dew is deposited, and the germ tube initiates from a pore swelling in contact with the cuticle. The tube grows closely appressed to the cuticle and becomes oriented at right angles to the long axis of the host surface. Aborted short branches may form at right angles to the tube when the tip encounters surface discontinuities such as the wall boundaries between epidermal cells, sclerenchyma ridges, or stomata. Appressoria generally form when the germ tube encounters the slight depression between closed stomatal guard cells. Lewis and Day (1972) have shown that the cuticular crystal lattice

governed germ tube development (see Staples and Macko, Chapter 9, this volume).

Urediospores landing on aqueous surfaces behave atypically as a result of the hydrophobic surfaces of the spore wall. On water, the spores quickly gather into a mass at the bottom of the meniscus. On water agar the spore often makes minimal contact with the surface, and the germ tube develops from a pore opposite to the point of contact. The tube elongates into the air until the germ tube mass rolls the spore over and the tube falls to the substrate surface. Subsequent germ tube growth may be above, on, or below the substrate. Occasionally, the germ tube protoplast will extrude from the tip of a germ tube, and the germling dies. Extrusion is induced in 60 to 80% of the germ tubes grown on 1% Noble's agar (Difco Co.) in distilled water by initiating spore germination in the dark at 18°C for 3 hr followed by transferring the germlings to 30°C. This temperature regimen is similar to that used to induce the formation of infection structures (Maheshwari *et al.*, 1967).

Urediospores deposited on hydrophobic plastics orient flat against the surface. When dew is deposited on smooth polystyrene (Burrage, 1969), the germ tube initiates from a pore adjacent to and grows appressed to the surface. These tubes branch dichotomously at regular intervals, but only one branch continues to grow and the other aborts. These germ tubes do not form appressoria. Spores in oil drops on water agar behave similarly. Germ tube growth, development, and appressorium formation on polyethylene film etched with many parallel scratches by fine aluminum oxide sandpaper resembles that on host surfaces (Rowell, 1967), and this method may be a useful test of the potential infectivity of inoculum.

3. Water

Water quality is a major source of error in tests of urediospore germinability. Urediospore germination is inhibited by a wide variety of organic and inorganic substances that contaminate water. For many years the tap water in my laboratory came from deep wells and had 200 ppm hardness. Spores germinated poorly, if at all, in this water. A single distillation of this water in a metal still improved the water quality for urediospore germination but did not support as good germination as water from a second glass distillation. Distillation does not necessarily assure water of sufficient purity, because inhibitory inorganic and organic substances can be carried over to the distillate in tiny droplets formed from bursting bubbles during vigorous boiling.

Substances toxic to urediospore germination also may be released or dissolved from the walls of storage or experimental containers. Urediospore germination is inhibited in soft glass containers cleaned by dichromate–sulfuric acid solution followed by five rinses in distilled water. This toxicity can be eliminated by soaking the glassware in 0.05 M phosphate buffer in distilled

water overnight and rinsing five times in double-distilled water. I have found that various commercial humidifiers introduce inhibitory substances, especially those made of copper or brass, when used as a source of finely atomized mists for urediospore germination and infection. Tests of standing water from a humidifer's copper reservoir by my assay (see chapter by Rowell, Vol. II) indicated that toxicity to germinating urediospores equaled 19.9 ppm Cu^{2+}.

Water purified by deionization in mixed-bed, ion-exchange columns used intermittently is toxic to urediospore germination, apparently because of microbial growth on the resins. Similar microbial growth and toxicity may occur in plastic piping used for distilled water distribution systems.

4. An Assay Method

In my experience, the most reliable method to test the rate of urediospore germination has been to disperse spores in a drop of oil on water agar. The details of the procedure are as follows: Urediospores other than freshly harvested spores are conditioned by hydration in a sealed container at either 50 or 80% RH for at least a day before the test. The test substrate consists of 1% Noble's agar (Difco Co.) in double glass-distilled water. The agar is poured into sterile Petri dishes 24 hr before the test to permit drying of the surface water film and to prevent spreading of the oil drop. A 1:1 mixture of light inoculation oil such as Isopar M and light petrolatum U.S.P. is used as the carrier for the spores. Spores are handled in an atmosphere >50% RH to prevent artifacts caused by rehydration injury. A 5-mm-diameter platinum loop is used to place individual drops of the germination oil on the agar surface. A similar drop of germination oil is placed on the surface of a clean glass slide, and an extremely small mass of spores, about one half to one-eighth the size of a pinhead, is dispersed in this drop. One drop of the suspended spores is transferred with a 3-mm-diameter platinum loop to each oil drop on the agar. It is important to touch the platinum loop lightly to the top surface of the oil drop and not smear the oil drop across the agar surface. The inoculated plates are incubated in the dark at 18°C for 16 hr, and the number of germinated spores per 100 in each drop are counted. Although the percentage of germ tube initiation can be determined after 4 hr of incubation, the longer incubation time permits and evaluation of the vigor and normalcy of germ tube growth and development.

The germ tubes from freshly harvested urediospores in an oil drop on water agar grow in a sympodial pattern similar to that observed on plastic surfaces (Burrage, 1969). Occasionally, a germ tube will penetrate and grow into the agar substratum, but I have never observed a germ tube that penetrated the oil-air interface. Components of the light isoparaffinic oils used as inoculum carriers gradually oxidize during prolonged storage, and in such oils germ tubes grow atypically straight and unbranched with a tapered tip. Such oils should not be used for quantitative studies but are satisfactory for routine inoculations.

V. Preparation of Host

Plants of uniform size and development are required for experiments concerned with rust infection under artificial conditions. Temperature, light, and moisture before and after inoculation affect both plant development and stem rust infection.

A. PREDISPOSITIONING FACTORS

A satisfactory standard procedure for growing uniform wheat seedlings for controlled inoculation is as follows: Two seeds are sown in a mixture of soil, sand, and peat at each point of a pentagon around the inner edge of a 6.5-cm-diameter clay pot and covered with 1 cm of soil mixture. After watering, the pots are placed on a bed of sand heated to 25°C in the glasshouse. When the first coleoptiles become visible, the pots are transferred to watering trays in a growth chamber programmed for 18° ± 1.5°C and a 14-hr photoperiod from cool white fluorescent lamps that deliver 16,000 lux of light at the tips of fully emerged first foliar leaves. Seven days after planting, the seedlings have erect first foliar leaves about 10 cm long with the ligule present at the base of the blade. The five most uniform leaves in each pot are then selected for inoculation, and the remainder are removed.

Germination of wheat seed initiated in warm soil is more rapid and uniform than in cool soil. Continuous exposure to either high or low soil temperatures, however, results in either long, narrow, recumbent or short, broad, upright leaves, respectively. The transfer of plants to the cool environment when the tips of the coleoptiles emerge from the soil gives rapid development of upright, uniform, moderate-sized leaves suitable for inoculation.

The intensity, quality, and duration of light during the growth of seedlings prior to inoculation affects the amount of infection produced on a susceptible host by a unit of inoculum. During the short, cloudy days of winter in Minnesota, plants grown in the glasshouse under natural light prior to inoculation will have only about 10% of the infection that is produced by the same inoculum on plants grown with natural light supplemented by 8 to 12 hr of 10,000 lux from fluorescent lights as described in Section II,B. Sharp *et al.* (1958) also noted variation in infection success in seedlings exposed to different preinoculation light intensities. Daly (1964) compared the effect of light from fluorescent lamps alone to the light from fluorescent plus incandescent lamps in pre- and postinoculation environments, using a 13-hr photoperiod. His data suggest that preinoculation plant growth under fluorescent plus incandescent light was more favorable for infection than fluorescent light alone, but that the incandescent component was deleterious for infection after inoculation.

Soil moisture also influences infection. Waterlogged soil inhibits seedling growth and favors root and seedling blights, and drought stress prior to inoculation also renders the growing plants less receptive to infection. I have tested short periods of drought stress imposed at various stages in the growth of test plants prior to inoculation by withholding water from replicated pots until incipient wilt symptoms were evident, at which time the pots were watered and the plants recovered. Drought stress at any time during the 3 days prior to inoculation markedly reduced infection. Burrage (1970) found that drought stress imposed immediately before inoculation diminishes infection success with increases in the time that water was withheld.

B. HOST CONFIGURATION

The form and growth habit of the plant organs are also variables that affect rust inoculations. The first foliar leaf of the wheat seedling initially grows upright and generally has a right-hand twist. When seeds are planted vertically with the embryo down and the groove facing the center of the pot, the seedlings emerge with the first foliar leaf facing the pot rim. The junction between blade and sheath appears about a week after planting, and the blade ultimately folds to a recumbent position at this junction. The length:width ratio of the lamina of the first foliar leaf varies with environmental conditions and differs among wheats. Winter bread wheats and durums often have longer and narrower blades than spring bread wheats. Stomata are present on both leaf surfaces but generally are more numerous on the adaxial surface, and the number per square centimeter varies slightly with growing conditions. The longitudinal curling of wheat leaf blades under drought stress is controlled by rows of bulliform cells on the adaxial surface parallel to the long axis of the blade. The transverse topography of the adaxial leaf surface is more uneven than the abaxial surface, which hinders direct microscopic observations on spore germination and appressorial development.

The numerous tall, limber tillers of adult wheat plants are difficult to inoculate uniformly. The tillering stage commences after the third foliar leaf has emerged about 3 to 4 weeks after planting. The ultimate number of tillers produced is dependent on plant genotype and environmental conditions. Winter wheats require vernalization (exposure to temperatures below 7°C for about 6 weeks) to make the transition from the tillering to the shooting stage. A 1-hr interruption of the dark period with incandescent light during the tillering stage stimulates the formation of floral initials and shifts development from the tillering to the shooting stage. Careful control of light regimens often will limit development to two or three tillers. The tillers mature at slightly different rates.

The adult plant generally has six internodes, the first two of which are short and usually below ground. The fully developed tiller usually has four large foliar leaves at the heading stage. Plants are often inoculated at this stage, when

most of the stem surface including the peduncle is covered by the leaf sheaths, and the plants, about two-thirds of their final height, are comparatively sturdy. As the peduncle elongates and the head weight increases from the developing grain, the tillers become top-heavy and readily break if unsupported. Wheat cultivars vary widely in rate of development, and infection frequencies per unit of inoculum may vary with growth stage (Rowell and McVey, 1979). Hence, comparative studies of infection frequencies on diverse cultivars under artificial conditions require different planting dates to synchronize the stage of host development at inoculation.

VI. Procedure of Inoculation

Innumerable methods have been devised to inoculate cereal plants with urediospores of the cereal rust pathogens (Browder, **1971).** Carriers for inoculations are discussed in Section IV,C. I limit my discussion here to procedures used for quantitative control of deposited inoculum.

A. DUSTING

A variety of settling towers have been developed for quantitative deposition of dry urediospores on cereal leaves. This method requires the reproducible generation of a uniform dust cloud of spores in the chamber. The settling tower of Eyal *et al.* (1968) is representative of this inoculation method. The tower consists of an upper cloud chamber separated by a shutter from a lower settling chamber where the exposed leaf blades are taped to the surface of a disk. A quantity of spores are explosively ejected into the upper chamber from tubing connected to a modified (Crosman) $C0_2$ pistol (Lange *et al.,* 1958). The shutter is used to regulate the deposition period and to exclude spore clumps that settle from the cloud more rapidly than individual spores. The chamber should be grounded, because electrostatic charges may induce irregularities in the spore cloud and spore deposition. A major disadvantage of settling towers for inoculation is the considerable potential for contaminating the surrounding work area and the difficulty of cleaning all components of adhering spores between successive inoculations. The exposure of only one surface of a single leaf per plant in this settling chamber may be a disadvantage for epidemiology studies.

Melching (1967) devised a turntable to inoculate adult cereals with rust pathogens in a large settling tower. The plant pots are mounted on a series of small plates that are arranged symmetrically near the periphery of a large turntable. As the turntable makes one revolution in one direction, the plates rotate the plants once in the opposite direction. A spore cloud is ejected by

modified C02 pistol into the large cylindrical tower, and spores are deposited by impinging rather than settling onto the host surfaces. Theoretically, rotation of the plant as it moves through the spore cloud exposes all host surfaces to inoculum. My observations on adult plants inoculated by this method, however, have indicated that spores are deposited heavily on one side of the leaf blade and sparsely on the opposite side. This inequity in spore deposi-tion apparently results from the asymmetrical orientation as leaf blades flap about as a result of effects of centripetal force and air resistance on the rotating plant during its circular path around the chamber. Furthermore, more spores are deposited on the upper than the lower plant parts presumably because of the creation of a gradient in the descending spore cloud as the plant tops sweep through it. In modifications of this procedure, a mist is generated in the chamber by atomizing spores carried in oil (Politowski and Browning, 1975) or an inert perfluorinated fluid (Mortensen *et al.*, 1979).

B. SPRAYING

Spraying is a convenient means of inoculating cereal plants with urediospores. However, pressure spray nozzles are unsatisfactory for inoculation because of the wide range in droplet size and the tendency of the small orifice to clog. Venturi atomizers, in which the spore suspension is broken into fine droplets by the rapid passage of air across the end of the fluid delivery tube, are generally used. This type of sprayer produces a solid cone of spray droplets with a pattern of spray deposition that is most dense in the center and diminishes progressively toward the periphery. Furthermore, the velocity of the spore droplets and spores decelerates rapidly with distance from the sprayer, so that they soon have insufficient inertia to penetrate the boundary layer and deposit on plant surfaces. I have used a spray chamber with a moving column of air to increase the uniformity of droplet velocity and pattern (Rowell and Olien, 1957). Air drawn around the atomizer carries the spray across plants rotating on a turntable, passes through the rear opening, and is exhausted outside of the work area. The effective spray pattern at 38 cm from the atomizer is 16 cm in diameter. This method was rapid and useful for inoculating seedling plants, but the small cross-sectional area of effective spore deposition limits the utility for inoculating adult plants.

Commercially available mechanical backpack mist blowers are readily adapted to inoculate field plants uniformly (Rowell and McVey, 1979). A small orifice is required for the fluid delivery nozzle to adapt the sprayer for applying low volumes of oil. Application rates equivalent to about 6 liters/ha can be achieved. Frequent agitation is needed to maintain a uniform suspension of spores; therefore, a plastic bottle is mounted on the spray wand as the inoculum reservoir to shorten the fluid delivery tube. Periodically during spray

application, the spray wand is raised vertically to shut off the spray, drain the delivery tube, and agitate the spore suspension.

VII. Requirements for the Infection Process

Successful artificial conditions for infection by the cereal rust pathogens should duplicate as nearly as possible the favorable natural conditions of temperature, light, moisture, and atmospheric purity. For most of the cereal rust pathogens, the entire infection process is completed in dew and darkness at a single optimum temperature. The infection process of *P. graminis,* however, is adapted to a diurnal pattern of temperature and light.

A. PHYSICAL FACTORS

Spores of *P. graminis* germinate and appressoria form on the moist leaf surface during darkness within the optimal temperature range of 15° to 24°C (Sharp *et al,* 1958). If plants are then left under these conditions, only about 1% of the appressoria produce penetration pegs and substomatal vesicles. Most appressoria remain quiescent if left in dark, moist dew conditions or if dried slowly in dim light at a slightly lower temperature than that used during appressorial formation (Rowell *et al.,* 1958). Appressoria produce penetration structures if plants are kept wet with dew and the temperature is increased to 30°C either in darkness or with light greater than 5400 lux (Sharp *et al.,* 1958). Penetration also occurs from appressoria on plants dried slowly at about 3° to 5°C below that during the dew period temperature if the plants are then transferred to natural light at about 30°C in the glasshouse (Rowell *et al,* 1958).

With *P. graminis,* infection rates under artificial conditions have never approached the expected maximum. Spore germination rates of 98 to 100% are possible in an oil drop on water agar, appressoria are produced consistently on plants by 50 to 60% of germinating spores under optimum conditions, and nearly all appressoria can penetrate plants under favorable field conditions. Thus infection rates of at least 50% would seem to be attainable. In practice, maximum infection rates of only 15 to 25% have been attainable. Germination on leaf surfaces of urediospores freshly collected from mature uredia generally is 10-20% less than that in an oil drop on water agar. This loss in germinability is not due to self-inhibitors, which do not inhibit germination at densities less than 7000 spores/cm^2 (Tollenaar and Houston, 1966), but may be due to the materials found by Woodbury and Stahmann (1970) to be associated with urediospores of *P. graminis* that form films on water surfaces and oxidize to form products inhibitory to spore germination. It is also possible that nongerminating spores on the leaf surface failed to have contact with the free

moisture that is essential for germ tube growth, because dew does not wet the entire leaf surface (Burrage, 1969). Although rates of appressorial formation under artificial conditions are comparable to those under natural conditions, the best rates of penetration achieved in trials, however, range between 30 and 40% of the appressoria produced. Thus either my artificial environments were suboptimal, or other factors were unfavorable for the completion of the penetration process.

The role of elevated temperatures and light in the completion of the infection process has not been resolved completely. As described by Staples and Macko (Chapter 9, this volume), infection structures have been induced to differentiate *in vitro* by exposure to 30°C without light (Maheshwari *et al.,* 1967), by a volatile substance present in steam distillates of urediospore extracts (Allen, 1957) thought to be acrolein (2-propenal) (Macko *et al.,* 1978), and by phenols extracted from epicuticular wax and cell walls combined with volatile fractions from wheat leaves (Grambow and Riedel, 1977). However, the appressoria induced by physical and chemical stimulation of germlings on aqueous media or polar membranes *in vitro* do not mimic exactly the structures formed on the wheat leaf. These methods of stimulating infection structures *in vitro* induce the formation of a roughly globular appressorium that immediately proceeds without interruption to produce the penetration structures. On the wheat leaf, however, the appressoria produced when a germ tube encounters stomata are elongated at right angles to the germ tube; they are tapered at the ends (roughly cigar-shaped) and are quiescent until stimulated to differentiate the penetration structures by exposure to light and elevated temperatures. When the regimen used *in vitro* to induce the formation of infection structures was used *in vivo* in dark dew chambers by first initiating spore germination on wheat leaves for a short period at 18°C and then transferring plants to 30°C, the penetration structures were differentiated on the external leaf surface (Sharp *et al.,* 1958). Thus the appressoria stimulated to form *in vitro* appear to be transitory, atypical structures formed in response to stimuli primarily required for the development of the penetration structures.

In contrast to the *in vitro* development of infection structures, *in vivo* development is enhanced by exposure to light alter the appressoria are fully developed in the dark (Sharp *et al.,* 1958; Rowell *et al.,* 1958; Yirgou and Caldwell, 1968). Reduced penetration and infection of inoculated plants incubated in dark dew chambers does not appear to be due to physical exclusion by closed stomates. With P*uccinia recondita,*exclusion of penetration structures by closed stomates results in deformation of appressoria and the formation of penetration structures on external host surfaces (Romig and Caldwell, 1964). With *P. graminis,* I have never observed distorted appressoria and rarely have seen penetration structures produced externally. Furthermore, Yirgou and Caldwell (1968) found that most stomates remain closed in normal and high light under appressoria and that most penetrated stomates were closed.

They showed that high $C0_2$ concentrations inhibited penetration, which suggests that penetration is enhanced when C02 levels in the leaf are reduced by high photosynthetic activity in the light and inhibited by high respiratory C02 in the dark.

B. ATMOSPHERIC PURITY

Air pollutants can also reduce infection success of urediospore inoculum. Tobacco smoke inhibits the rate of germination and of germ tube growth (Melching *et al.*, 1974). Exposure of wheat seedlings to 03 prior to inoculation reduced the frequency of infection structures (Heagle and Key, 1973). Exposure to 0.1 ppm S02 either before or after inoculation seemed to reduce infection on Thatcher wheat, but infection on Prelude wheat was unaffected (Laurence *et al.*, 1979). The effect of the pollutant on the infection process during the dew period was not studied. Sharp (1972) found that urediospores of *Puccinia sthiformis* germinated poorly in his laboratory during periods of high automotive traffic nearby when an atmospheric inversion existed, and the atmospheric concentration of large air ions and lead increased. Exposure to the pollutant during the first hour of spore hydration in the dew environment was the crucial time for inhibition.

C. DEW CHAMBER OPERATION

Programmable dew chambers were developed at the Cereal Rust Laboratory to provide a reproducible diurnal environment with dark and light phases at different temperatures for dependable incubation of seedling plants inoculated with *P. graminis.* The dew compartment is 1.52 m wide by 1.37 m long by 0.2 m high. Hot water at 32°C can be circulated in a pan beneath the plants, and coolant at 7°C can be circulated through copper coils in the top of the chamber. Cool white fluorescent lamps mounted on 19-cm centers beneath the cooling coils provide about 10,000 lux of light at the tips of the seedling leaves. The incubation cycle is started by placing inoculated seedlings in the chamber and cooling the system to 18°C for about 2 hr. Then warm water at 32°C is circulated in the bottom pan, and vapor from this warm surface condenses on the cool plant surfaces. After about 16 hr of incubation in the dark at 18°C, the light cycle begins, and air temperature rises gradually to a maximum of 29°C. After at least 3 hr of incubation in light, the plants are allowed to dry slowly before transfer to the glasshouse to prevent adverse stress on transfer from a very moist to a very dry environment. Microscopic examination of heavily inoculated leaf surfaces indicate that penetration occurs from about 10 to 15% of the appressoria in this chamber.

I have achieved penetration from 30 to 40% of the appressoria in chambers made from polyethylene sheeting and placed under cool white fluorescent

lamps in a chamber with programmable light and temperature. Light intensity at the tips of seedling leaves was about 16,000 lux. Free moisture was deposited and maintained on leaves with a fog generated from double glass-distilled water by a DeVilbis nebulizer. Air temperature was 18°C in the chamber during an initial 12-hr dark period, followed by 27°C during a 4-hr light cycle.

VIII. Environment during Incubation

Vegetative growth of the stem rust fungus after penetration of the host appears most vigorous under the environmental conditions most favorable for host development. During June in Minnesota, when daily sunshine is about 16 hr and mean temperature is about 21°C, initial sporulation generally appears 6-7 days after artificial inoculation of wheat in the field if conditions are favorable for infection on the date of inoculation. When warmer conditions prevail (i.e., mean temperatures of about 26°C), initial sporulation appears within 5 days. Similar rapid development is observed on artificially inoculated wheat in the glasshouse with high-intensity light conditions and temperatures of 27° to 30°C. Generally, the number of infections that develop from uniform inoculation of a susceptible host with a virulent race is not affected by different conditions during incubation.

IX. Techniques for Measuring Infection

Investigators commonly measure rust infection by counting numbers of uredia per unit area of host surface or by estimating the severity of infection, which is an estimate of the percentage of the host plant infected by rust. Such data are useful for disease surveys and applied research on control of rust by genetic or chemical means, but alone they often are not adequate for comparative studies on the host-pathogen interaction. Ideally, quantitative data are needed on all phases of the infection process from spore deposition to sporulation, to account for the fate of all deposited spores and to adequately assess the reasons for differential rates of infection.

A. INOCULUM DOSAGE

Although each viable urediospore has the potential to infect the host, the preceding section illustrated that many factors affect the efficiency of infection. Hence, it is often useful to determine the number of infections per unit of inoculum.

Spore deposits on leaf blades can be examined on the leaf surface with bright incident light from a microscope epicondenser; however, this is laborious because of the uneven field of focus. A convenient but indirect means of determining inoculum rates is to trap spores during inoculation on 0.025-mm polyethylene strips wrapped around a 5-mm-diameter glass rod (Knutson, 1972). The 10-mm by 20-mm strips are dipped in a 1:8 solution of Dow Corning 200 Silicone Fluid in benzene and are dried on paper toweling in a dust-free chamber. The strip is then wrapped around the glass rod, which is then exposed during inoculation. The silicone oil acts as an adhesive to hold the strip in place as well as for trapping impacted spores. Strips are then removed by forceps and mounted on glass slides for microscopic examination. Although the method cannot be used as a precise measure of the spore deposit on plant surfaces because of differences in surface topography and geometry, it is useful for calibrating a method of inoculation, for determining the standard error between repetitive inoculations, and for determining the variation in spore number between inocula from different sources. Furthermore, the strips can be exposed with the inoculated plants during the dew period to determine the rate of germination of inoculum.

B. PREPENETRATION DEVELOPMENT

Observations on the prepenetration phases of the infection process are readily made by spraying the host surface with an alcoholic solution of acid fuchsin and cotton blue (Andersen and Rowell, 1962). The stain should be freshly mixed for best results. The mixture is made from stock solutions as follows: 0.3 ml 2% acid fuchsin in ethyl alcohol, 0.3 ml 2% cotton blue in ethyl alcohol, 4.0 ml 1.24% acetic acid, 18.0 ml 95% ethyl alcohol, **and** 1 drop (0.05 ml) Tween 20 (polyethylene sorbitan monolaurate). The stain is applied with a Paasche H "3 in 1" Airbrush until the plant surface is coated evenly with fine droplets. The stain can be applied either to the intact plant or to a leaf segment mounted on a microscope slide by adhesive tape. The stained tissue is examined either in bright light from an epicondenser for thick, opaque tissues or from a substage condenser for thin tissues. Fungal protoplasts stain red, and walls stain blue. The extent of germ tube development, appressorial formation, and protoplast migration is readily apparent. This procedure is known not to disturb ungerminated spores on the host surface and can be used to determine spore deposition on leaf blades. The empty appressorium from which the protoplast has migrated into the substomatal vesicle indicates that the stomate has been penetrated. Some appressoria are observed to be partially empty, which presumably indicates that the penetration process was incomplete.

C. POSTPENETRATION DEVELOPMENT

Postpenetration development is observed readily in segments of seedling leaves by the method for fluorescence microscopy of Roh-ringer *et al.* (1977). In this method, infected tissue segments fixed in dilute lactophenol in ethanol are stained with an optical brightener (Calcofluor, American Cyanamid Co., Wayne, New Jersey) and then examined under incident ultraviolet light. The extent of pathogen development in the infection process from appressorium through the penetration peg, substomatal vesicle, infection hyphae, haustorial mother cells, secondary hyphae, sporophytic hyphae, and spore initials can be clearly traced. Mature urediospores do not fluoresce. Although haustoria are not visible in the preprations by this method, Kuck *et al.* (1981) have successfully stained haustoria by a modified procedure. Hypersensitively collapsed and necrotic host cells have a distinctive yellow-green fluorescence. Each necrotic cell is evident as a linear group of two to four fluorescing disks, each representing one of the two to four connected lobes of typical mesophyll cells. One can determine by this method the extent of development of each penetrant, and whether infection was established or the stage in the infection process at which development ceased (Rowell, 1981). Unfortunately, this method is ineffective for leaf blades from adult plants, presumably because of the absorption of UV light by the heavy cuticle. Penetrant development in such adult tissues can be observed if the tissue fixed in lactophenol is cleared for several hours in saturated chloral hydrate and washed with water before further processing. The effective time for treating adult tissue in chloral hydrate varies with tissue age and cuticle thickness.

The method of McBride (1936) of clearing whole leaf segments with saturated chloral hydrate solution and staining with acid fuchsin is useful for observing haustoria (Leath and Rowell, 1966). The time required for clearing by this method can be reduced to about 24 hr by incubating samples at 60°C. Optimal clearing is critical for successful application of this method. Undercleared host tissue stains heavily with acid fuchsin, which obscures the fungal structures, whereas over-cleared host tissue becomes very fragile and the fungal structures will not retain stain. Adult tissues do not clear readily by this method.

Growth of the rust fungus can be measured inside living leaves, although the method is laborious. With bright transmitted light at about X400, hyphal tips at colony borders can be seen just beneath the epidermis in wet mounts (0.1% Tween 20) of seedling leaves. Apochromatic objective lenses are required with this method. Bushnell (1970) found a relatively constant growth rate of 1.1 mm/day in a compatible host-pathogen combination by repeatedly measuring the elongation of 8 to 10 colonies from first appearance of fleck to leaf senescence. Although the mature stem rust infection has a characteristic diamond shape, early colonial growth is highly variable in size and shape

(Rowell, 1981). Some penetrants produce two infection hyphae, one at each end of the vesicle, but most penetrants produce only a single hypha. Growth of the infection hyphae and the initial secondary hyphae generally, but not invariably, is parallel to the long axis of the leaf. Thus early growth of the stem rust fungus in the host often is measured as the maximum extension of secondary hyphae into the host.

Although this measure may not accurately reflect the increase in mass of fungal tissue, it provides some perspective on the early rate of development of the rust pathogen in the host. In a study (Rowell, 1981) of postpenetration development of stem rust, my measurements of the maximum extension of secondary hyphae indicated that initial growth was very slow, about 58 µm/day, and the lag phase of growth extended for about 100 hr from inoculation (Fig. 2). Mayama *et al.* (1975) also found a prolonged lag phase in measuring growth of *P. graminis* by the glucosamine content of the infected susceptible host. In my study of 15 colonies/cm^2, the growth rate appeared to become constant after 113 hr at about 360 µm/day, whereas Bushnell's measurements (1970) at lower rates of infection indicated that hyphal extension in a mature uredium was about 550 µm/day. Growth rates under crowded conditions are probably suppressed by intercolony competition for nutrients.

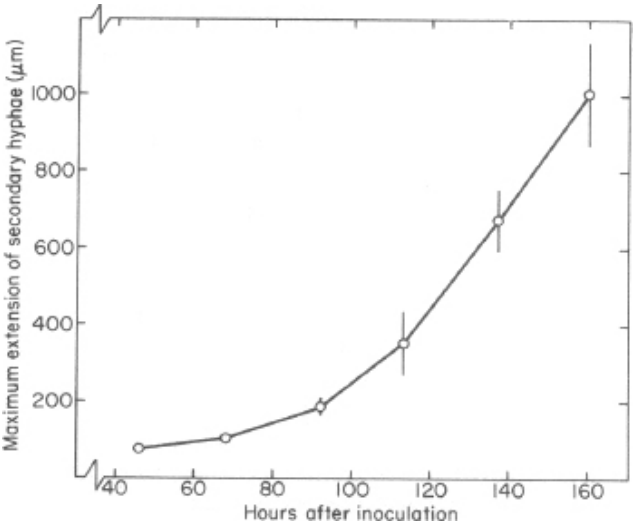


Fig. 2. Rate of spread of secondary hyphae of race 15B-TLM of *Puccinia graminis* f. sp. *tntici* into susceptible Baart wheat (J. B. Rowell, unpublished data). Vertical bars indicate one standard deviation on each side of means.

D. FREQUENCY OF UREDIA

Measuring rust infection in terms of number of uredia per unit area of the host requires reasonably accurate measurements of the area of host tissues. Ideally, the host tissue should be measured just prior to inoculation to eliminate errors due to subsequent elongation of leaves and stems.

Area measurements of host-tissues can be made indirectly by taping individual blades and stems on glass plates and preparing images on Ozalid paper (Higgins and Schreiber, 1954), or by reproducing the image on a photocopying machine. The surface area of the image is calculated from either weight or planimeter measurements. Unfortunately, this method destroys the host plant; therefore, a duplicate set of plants is required for measurement if the surface area is to be determined at the time of inoculation.

Equipment is available that directly measures leaf area. I have worked with the Model LI 3000 Portable Area Meter (Lambda Instruments Corp., Lincoln, Nebraska), which can measure rapidly the leaf area of intact wheat plants. The area measurements of a wheat seedling leaf with this instrument are reproducible within an error□≤5%, and the variation found between means of replicates each consisting of 24 leaves was ±3.8%. Contact with the scanning head before inoculation has had no effect on subsequent number of infections.

The number of uredia per square centimeter is not linearly proportional to the amount of inoculum at all dose levels, especially at higher levels (Fig. 3). In my experience with numerous inoculations of susceptible cultivars, the number of infections has been directly proportional to the amount of inoculum only up to about 10 uredia/cm^2 (~20-30 uredia per leaf); thereafter, the rate of increase in number of uredia declined. The decrease in efficiency of infection presumably results from competition and overgrowth between closely adjacent colonies. This presumption has not been tested, because the apparent decline in rate of infection occurred at colony densities too low for histological studies of spore deposition and the infection process. In my experimental work requiring controlled inoculation for comparison of treatments by the number of uredia produced, I used rates that yielded 10-20 uredia per leaf.

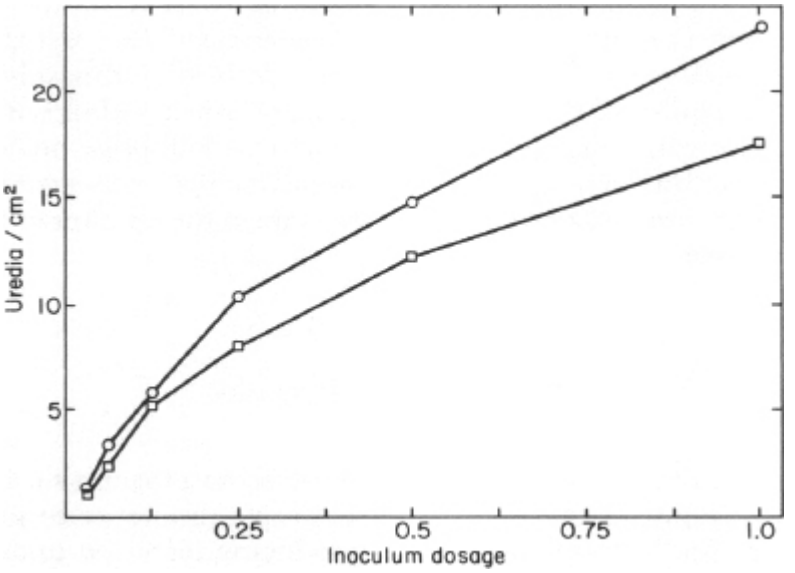


Fig. 3. Relation of inoculum dosage of race 15B-TLM of *Puccinia graminis* f. sp. *tritici* to the number of uredia produced on susceptible wheat cultivars Purdue 5481C1 (O) and Baart (□). [Inoculum dosage 1.0 = 4.25 mg of urediospores/ml in spray inoculum applied by a standard procedure (Rowell and Olien, 1957).] (J. B. Rowell, unpublished data.)

E. DISEASE SEVERITY ESTIMATES

When the number of uredia exceeds about 100 per leaf or plant, counting uredia on many samples is an extremely time-consuming method of data collection. Infection frequency under these conditions generally is estimated by using modified Cobb scales (Peterson *et al.*, 1948) or similar scales. The Cobb scale is based on the observation that the uredial area is about one-third of the total infected area. Thus at 100% severity only about one-third of the plant surface is occupied by uredia, but the host tissue is completely infected. The scale consists of a series of idealized diagrams that depict the frequencies of uredia for a series of percentages of disease severity. In practice, an investigator matches the frequency of uredia on the host with the appropriate diagram to determine the percentage severity. Counts of the number of uredia in each diagram yield a mean frequency of 0.35 uredia/cm^2 for 1% disease severity. The total surface area of blades, sheaths, and stems of a typical culm is about 150 cm^2; therefore 53 uredia would be equivalent to 1% severity (with leaf rust, which usually infects only leaf blades, 20 uredia would be equivalent to 1% severity for a typical plant with 56 cm^2 of leaf blade area). These frequencies are higher than that of 10 uredia per culm for 1% severity derived by Kingsolver *et al.* (1959) from counts of the number of uredia on wheat culms

with different levels of estimated severity. For practical purposes, however, 10 uredia per culm for 1% severity is a satisfactory conversion factor because of the progressive changes with time in the amount of vulnerable host tissue.

X. Concluding Remarks

Success in the cooperative USDA—Minnesota program for breeding hard red spring wheat cultivars with enduring resistance to stem rust emerged as proficiency improved in producing infection under artificial conditions. When I started working with wheat stem rust in 1955, investigations on this disease in our glasshouses were suspended from late November until late February because of the difficulty of obtaining suitable levels of infection under the poor light conditions of winter. Stem rust cultures were maintained by periodic transfers on susceptible hosts, which severely limited the size of culture collections. Enormous amounts of labor were used to initiate and develop severe epidemics in breeding nurseries. Rust spreader rows of susceptible cultivars for these nurseries were planted early in the growing season 4-6 weeks before test lines were planted, and repeatedly inoculated to assure abundant inoculum and heavy rust infection. This atypical late cultivation of the test wheats distorted the evaluation of the temperature-sensitive resistance conditioned by *Sr6*,which was considered ineffective under Minnesota growing conditions, a concern that was refuted by the performance of released cultivars such as Selkirk that possessed this resistance.

The efficiency and productivity of stem rust investigations were greatly increased by the progress made in developing long-term storage of rust spores, efficient inoculation techniques, improved dew chambers, effective environmental growth chambers, and adequate supplemental light in glasshouses. These improvements in methodology enabled large numbers of breeding lines to be tested intensively with numerous, pathogenically diverse rust cultures in the glasshouse as well as in the field. The increased effectiveness of the tests is evident in the endurance of the resistant hard red spring wheat cultivars released by the Minnesota program since 1955, none of which has succumbed to stem rust. This program also produced about 80% of the wheat lines identified as elite germ plasm for stem rust resistance in tests in the Uniform and International Spring Wheat Rust Nurseries. These achievements testify to the importance of the improved techniques now available for working with wheat stem rust.

References

Allen, P. J. (1957). Properties of a volatile traction from urediosporcs of *Puccinia graminis* var. *trilici* affecting their germination and development. I. Biological activity. *Plant Physiol.* **32,** 385-389.

Andersen, A. S., and Rowell, I. B. (1962). Duration of protective activity in wheat seedlings of various compounds against stem rust. *Phytopathology* 52, 909-913.

Bromfield, K. R. (1964). Cold-induced dormancy and its reversal in urediosporcs of *Puccinia graminis* var *tritici. Phytopathology* 54, 68-74.

Browder, L. E. (1971). Pathogenic specialization in cereal rust fungi, especially *Puccinia recondita f.* sp. *tritici:* Concepts, methods of study, and application. *U.S.. Dep. Agric. Tech. Bull.* **1432,** 1-51.

Burrage, S. W. (1969). Dew and the growth of the urediospore germ tube of *Puccinia graminis* on the wheat leaf. *Ann. Biol.* 64, 495-501.

Burrage, S. W. (1970). Environmental factors influencing the infection of wheat hy P***uc*** *cinia graminis. Ann. Appl. Biol.* 66, 429-440.

Bushnell, W. R. (1970). Patterns in growth, oxygen uptake, and nitrogen content of single colonies of wheat stem rust on wheat leaves. *Phytopathology* 60, 92-99.

Bushnell, W. R., and Rowell, J. B. (1967). Flurochcmical liquid as a carrier for spores of *Erysiphe graminis* and P*uccinia graminis. Plant Dis. Rep.* 51, 447-448.

Daly, J. M. (1964). Pre- and postinoculation effects of light quality on infection intensity of stem rust of wheat. *Phytopathology* 54, 1342-1345.

Emge, R. C, Melching, I. S., and Kingsolver, C. H. (1970). A portable chamber for the growth and isolation of infected plants. *Plant Dis. Rep.* 54, 130-131.

Eyal, Z., Clifford, B. C, and Caldwell, R. M. (1968). A settling tower for quantitative inoculation of leaf blades of mature small grain plants with urediospores. *Phytopathology* 58, 530-531.

Givan, C. V., and Bromfield, K. R. (1964). Light inhibition of urediospore germination in Puccinia graminis var. *tritici. Phytopathology* 54, 382-384.

Grambow, H. J., and Riedel, S. (1977). The effect of morphugenically active factors from host and nonhost plants on the in viro development of *Puccinia gramint f. sp. tritici. Physiol. Plant Pathol.* 11, 213-224.

Grieve, P. W., and Povey, M. J. W. (1981). Evidence for the osmotic regulation dehydration theory of freeze damage. /. *Sci. Food Agric.* 32, 96-98. Heagle, A. S., and Key, L. W. (1973). Effect of ozone on the wheat stem rust fungus.*Phytopathology* 63, 397-400.

Higgins, C. G., and Schreiber, H. A. (1954). Simple method of measuring opaque objects. *Science* **120,** 76. Hwang, L. (1942). The effect of light and temperature on the viability of urediospores of certain cereal rusts.*Phytopatliology* 32, 699-711.

Kingsolver, C. H., Schmitt, C. G., Peet, C. E., and Bromfield, K. R. (1959). Relation of quantity of inoculum and growth stage of wheat and rye at infection to yield reduction by stem rust. *Plant Dis. Rep.* 43, 855-862.

Knutson, D. M. (1972). Cylindrical rods: More efficient spore samplers *Plant Dis, Rep.* 56, 719-720.

Kuck, K. H., Triburzy, R., Hanssler, G., and Rcisener, H. J. (1981). Visualization of rust haustoria in wheat leaves by fluorochromes. *Physiol. Plant Pathol.* 19, 439-441.

Langc, C. T., Kingsolver, C. H., Mitchell, I. E,, and Cherry, E. (1958). Determination of the effects of different temperatures on uredial infection with *Puccinia graminis* var. tritici. *Phytopathology* 48, 658-660.

Laurence, J. A., Weinstein, L. H., McCune, D. C, and Aluisio, A. L. (1979). Effects of sulfur dioxide on southern com leaf blight of maize and stem rust of wheat. *Plant Dis. Rep.* 63, 975-978.

Leath, K. T., and Rowell, J. B. (1966). Histological study of the resistance of *Zea mays* to *I'uccinia graminis. Phytopathology* 56, 1305-1309.

Leath, K. T., Romig, R. W., and Rowell, J. B. (1966). A system for storing rust spores in liquid nitrogen. *Phytopatliology* 56, 570.

Lewis, B. G., and Day, (. R. (1972). Behavior of urediospore germ tubes of *Puccinia graminis tritici* in relation to the fine structure of wheat leaf surfaces. *Trans. Br. Mycol. Soc.* 58, 139-145.

Loegering, W. Q., and Harmon, D. L. |1962|. Effect of thawing temperature on uredio spores of *Puccinia graminis* f, sp. *tritici* frozen in liquid nitrogen. *Plant Dis. Rep.* 46, 299-302.

Loegering, W. Q., McKinney, H. H., Harmon, D. L., and Clark, W. A. (1961). A long term experiment for preservation of urediospores of *Puccinia graminis tritici* in liquid nitrogen. *Plant Dis. Rep.* 45, 384-385.

McBride, M. C. (1936). A method of demonstrating rust hyphae and haustoria in unsectioned leaf tissue. *Am.* /. *Bot.* 23, 686-688.

Macko, V., Rcnwick, *]*. A. A., and Rissler, J. F. (1978). Acrolein induces differentiation of infection structures in the wheat stem rust fungus. *Science* 199, 442-443.

Maheshwari, R., and Sussman, A. S. (1971|. The nature of cold-induced dormancy in urediospores of *Puccinia graminis tritici. Plant Physiol.* 47, 289-295.

Maheshwari, R., Allen, P. J., and Hildebrandt, A. C. (1967). Physical and chemical factors controlling the development of infection structures from urcdiospore germ tubes of rust fungi. *Phytopathology* 57, 855-862.

Mayama, S., Rehfeld, D. W., and Daly, J. M. (1975). A comparison of the development of *Puccinia graminis tritici* in resistant and susceptible wheat based on glucosamine content. *Physiol. Plant Pathol.* 7, 243-257.

Melching. J. S. (1967). Improved deposition of airborne urediospores of *Puccinia graminis* and *P. striiformis* on glass slides and on wheat leaves by use of a turntable. *Phytopathology* 57, 647 |abstr.).

Melching, J. S., Stanton, J. R., and Koogle, D. L. (1974). Deleterious effects of tobacco smoke on germination and infectivity of spores of *Puccinia graminis tritici* and on germination of spores of *Puccinia striiformis. Pyricularia oryzae* and an *Alternari'a* species. *Phytopathology* 64, 1143-1147.

Miller, W. E. (1965). Freon-113 as a dispersal medium for urediospores of *Puccinia graminis* var *tritici. Plant Dis. Rep.* 49, 268.

Mortensen, K., Green, G. J., and Atkinson, J. (1979). A method for uniform infection of seedling and adult cereal plants by *Puccinia graminis* f. sp. *tritici. Phytopathology* 69, 420-423.

Peterson, R. F., Campbell, A. B., and Hannah, A. E. (1948). A diagrammatic scale for estimating rust intensity on leaves and stems of cereals. *Can. J. Res., Sect. C* 26, 496-500.

Politowski, K., and Browning, J. A. (1975). Effect of temperature, light, and dew duration on relative numbers of infection structures of *Puccinia coronata avenae. Phytopathology* 65, 1400-1404.

Prabhu, A. S., and Wallin, J. R. (1971). Influence of temperature and light on spore production of *Puccinia graminis tritici. Phytopathology* 61, 120-121.

Roelfs, A. P., and McVey, D. V. (1979). Low infection types produced by *Puccinia graminis* f. sp. *tritici* and wheat lines with designated genes for resistance. *Phytopathology* 69, 722-730.

Rohringer, R,, Kim, W. K., Samborski, D. J., and Howes, N. K. (1977). Calcofluor: An optical brightener for fluorescence microscopy of fungal plant parasites in leaves. *Phytopathology* 67, 808-810.

Romig, R. W., and Caldwell, R. M. (1964). Stomatal exclusion of *Puccinia recondita* by wheat peduncles and sheaths. *Phytopathology* 54, 214—218.

Rowell, J. B. (1956). Rehydration injury of dried urediospores of *Puccinia graminis* var. *tritici. Phytopathology* 46, 25 (abstr.).

Rowell,). B. (1967). Infection process and physiologic specialization of *Puccinia graminis* f. sp. *tritici. In* "Sourcebook of Laboratory Exercises in Plant Pathology" (Sourcebook Committee of the American Phytopathological Society. A. Kelman, Chairman), pp. 73-77. Freeman, San Francisco, California.

Rowell, J. B. (1972). Fungicidal management of pathogen populations. /. *Environ. Qual.* 1, 216-220.

Rowell, J. B. |1981). Relation of postpenetration events in Idaed 59 wheat seedlings to low reccptibility to infection by *Puccinia graminis* f. sp. *Intici. Phytopathology 71,* 732-736.

Rowell, J. B., and Hayden, E. B. (1956). Mineral oils as carriers of urediospoxes of the wheat stem rust fundus for inoculating field grown wheat. *Phytopathology* 46, 267-268.

Rowell, J. B., and McVey, D. V. (1979). A method of field evaluation of wheats for low receptivity to infection by *Puccinia graminis* f. sp. *tritici. Phytopathology* 69, 405-409.

Rowell, I. B., and Olien, C. R. (1957). Controlled inoculation of wheat seedlings with urcdiospores of *Puccinia graminis* var *tritici. Phytopathology* 47, 650-655.

Rowell, J. B., Olien, C. R., and Wilcoxson, R. D. (1958). Effect of certain environmental conditions on infection of wheat by *Puccinia graminis. Phytopathology* 48, 371-377.

Sharp, E. L. (1972). Relation of air ions to air pollution and some biological eft *Environ. Pollut.* 3, 227-239. Sharp, E. L., and Smith, F. G. (1957). Further study of the preservation of *Puccinia* uredospores. *Phytopathology* 47, 423-429.

Sharp, E. L., Schmitt, C. G., Staley, J. M., and Kingsolvcr, C. H. (1958). Some critical factors involved in establishment of *Puccinia graminis* var. *tritici. Phytopathology* 48, 469-474.

Stewart, D M., and Hill, H. J. (1965). An extradf produced by *Helmmihosporium sorokinianum* toxic to *Puccinia graminis* var. tritici. *Plant Dis, Rep.* 49, 280-2×3.

Tervet, 1. W., Rawson, A. *J.,* Cherry, E., and Saxon, R. B. (1951). A method of collecting icroscopic particles. *Phytopathology* 41, 282-285.

Tollenaar, H., and Houston, B. R. (1966). *In vitro* germination of urcdiospores of *Puccinia graminis* and *P. striiformis* at low spore densities. *Phytopathology* 56, 1036-10.

Watson, 1. A., and Luig, N. H. (1963). The classification of *Puccinia grammis* var *tritici* in relation to breeding resistant varieties *Proc. linn. Soc. N.S.W.* 88, 235-258.

Woodbury, W., and Stahmann, M. A. (1970). Role of surface films in the germination of rust urcdiospores. *Can. J. Bot.* 48, 499-511.

Wynn, W. K., Staples, R. C, Strouse, B., and Gajdusek, C. |1966|. Physiology of urcdiospores during storage. *Contrib. Boyce Thompson Inst.* 23, 229-242.

Virgou, D., and Caldwell, R. M. (1968). Stomatal penetration of wheat seedlings by stem and leaf rusts in relation to effects of carbon dioxide, light, and stomatal aperture. *Phytopathology* 58, 500-507.

11

Developmental Ultrastructure of Hyphae and Spores

D. E. Harder
Agriculture Canada Research Station, Winnipeg, Manitoba, Canada

I. Introduction

Much of the ultrastructural work in the cereal rusts has its foundations in the excellent light microscope work of earlier times, particularly that of Allen (1923, 1928, 1932a,b, 1933a,b, 1934), Rice (1927), and Ruttle and Fraser (1927). Since the pioneering electron microscopic work in the rusts by Ehrlich and Ehrlich (1961, 1962, 1963), Moore (1963a,b), and Moore and McAlear (1961), there have been steady improvements in the processing of tissue, and a great number of details have emerged. A review by Littlefield and Heath (1979) has provided a comprehensive view of structure in the rusts. However, work since 1979 in ultrastructural cytochemistry has revealed additional details. In this chapter the basic fungal structures as they pertain to the cereal rusts will be reviewed and supplemented with cytochemical data where possible, with emphasis on research on *Puccinia coronata* or *P. graminis* f. sp. *tritici* conducted in our own laboratory. The following section deals primarily with dikaryotic parasitic growth. With a few possible exceptions, structural features of hyphae in axenic growth do not differ substantially from those in parasitic growth. Also, there is little in the literature to indicate that the hyphal protoplasts of the various rusts in either the dikaryotic or monokaryotic state are substantially different.

II. Intercellular Hyphae

Following infection structure development and formation of the primary haustorium, intercellular hyphal growth begins with branching of the infection hypha proximal to the primary haustorium mother cell (Fig.1). Figure 1 is a generalized illustration of an approximately 60-hr-old rust fungal colony in a cereal leaf. Growth and branching of the hyphae continue, with haustorium formation, until the mycelium has extensively ramified through an area of leaf tissue. Initial colony growth tends to occur to one side of the substomatal vesicle, thus the colonies are often somewhat asymmetric with respect to the point of infection.

A. CYTOPLASMIC CONTENTS

The constituents of the mycelial protoplasts and their appearance vary depending on the physiologic state of the cell. Figure 2 is representative of a young hyphal cell near the edge of an advancing parasitic colony. The cytoplasm is typically dense with closely packed ribosomes, endoplasmic reticulum (ER), mitochondria, vacuoles (some with electron-dense inclusions),

multivesicular bodies, and storage products either as lipid or glycogen. The majority of the ribosomes occur free in the cytoplasm, although where ER occurs, ribosomes may also be attached to it. The hyphal tip cell apex, despite the importance of this region in fungal growth, has not been studied in detail in any of the cereal rusts. However, the structure of this zone appears to be characteristic in a wide range of fungi. [See Howard (1981) for an analysis of the hyphal tip in *Fusarium acuminatum* using freeze-substitution, a method that appears to result in improved structural preservation of this fragile zone.] The most characteristic feature of the hyphal apex in the fungi is a zone generally free of organelles but containing an accumulation of apical vesicles, which are associated with hyphal tip growth. In the rusts these have been illustrated in hyphae of *Melampsora lini* (Coffey, 1975) and in germ tubes of *Uromyces phaseoli* var. *vignae* (Littlefield and Heath, 1979) and *Gym-nosporangium juniperi-virginianae* (Mims, 1977). A possibly unique type of apical body in penetrating haustoria of *P. coronata* or *P. gra-minis* f. sp. *tritici* is discussed by Harder and Chong in Chapter 14 of this volume, Section IV,D,1.

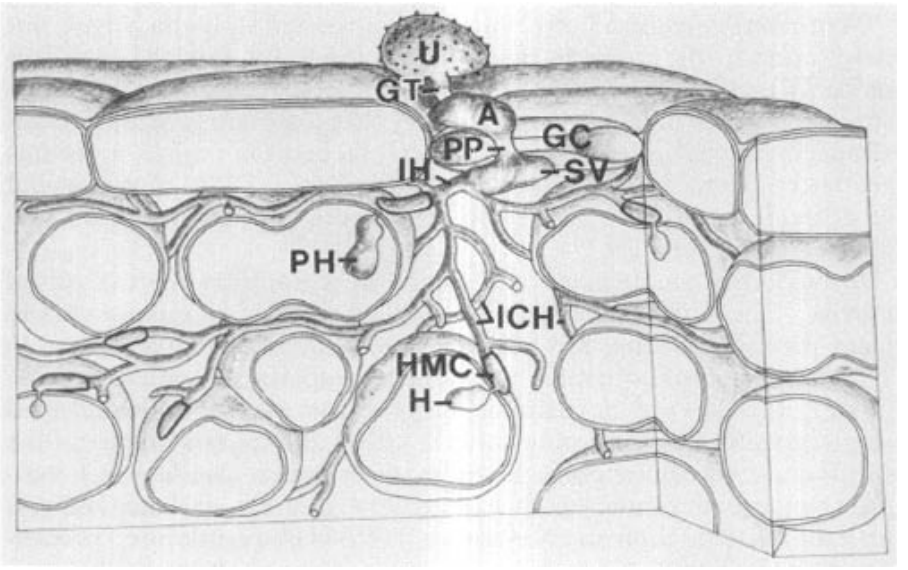


Fig. 1. A diagrammatic representation of a young rust fungal colony, about 60 hr old, in a cereal leaf. The sequence of development is germination of the urediospore (U), formation of an appressorium (A) from the germ tube (GT) over a stomate, penetration past the guard cells (GC) via a penetration peg (PP), formation of a substomatal vesicle (SV), growth of an infection hypha (IH), formation of the primary haustorium (PH), then branching and growth of intercellular hyphae (ICH), and formation of additional haustoria (H). The haustorium mother cells (HMC) are indicated in bold outline. (Drawn by Dr. J. Chong.)

Membranes organized into an easily recognized Golgi body have not been found in the rusts. In most septate fungi, functional Golgi sites probably exist mainly as single cisternae (Beckett *et al.*, 1974). It was suggested (Littlefield and Heath, 1979) that a similar single-cisternal arrangement applies to the rusts. The Golgi bodies in other organisms are derived from the ER (Morre and Mollenhauer, 1974); thus it would be difficult to differentiate regions of smooth ER that may have specialized Golgi function.

Microbodies are typically found in the cytoplasm of rust fungal hyphae. The distribution, morphology, and function of microbodies in plant pathogenic fungi have been reviewed by Maxwell *et al.* (1977). The term *microbody* refers to small cytoplasmic bodies, approximately 0.2-1.5 μm in diameter (Maxwell *et al.*, 1977), bound by a single membrane. They may contain either amorphous or crystalline substances, which are concentrations of enzymes. Catalase is a common constituent of microbodies of higher organisms, and the DAB test for catalase (Frederick and Newcomb, 1969) is frequently used to identify microbodies.

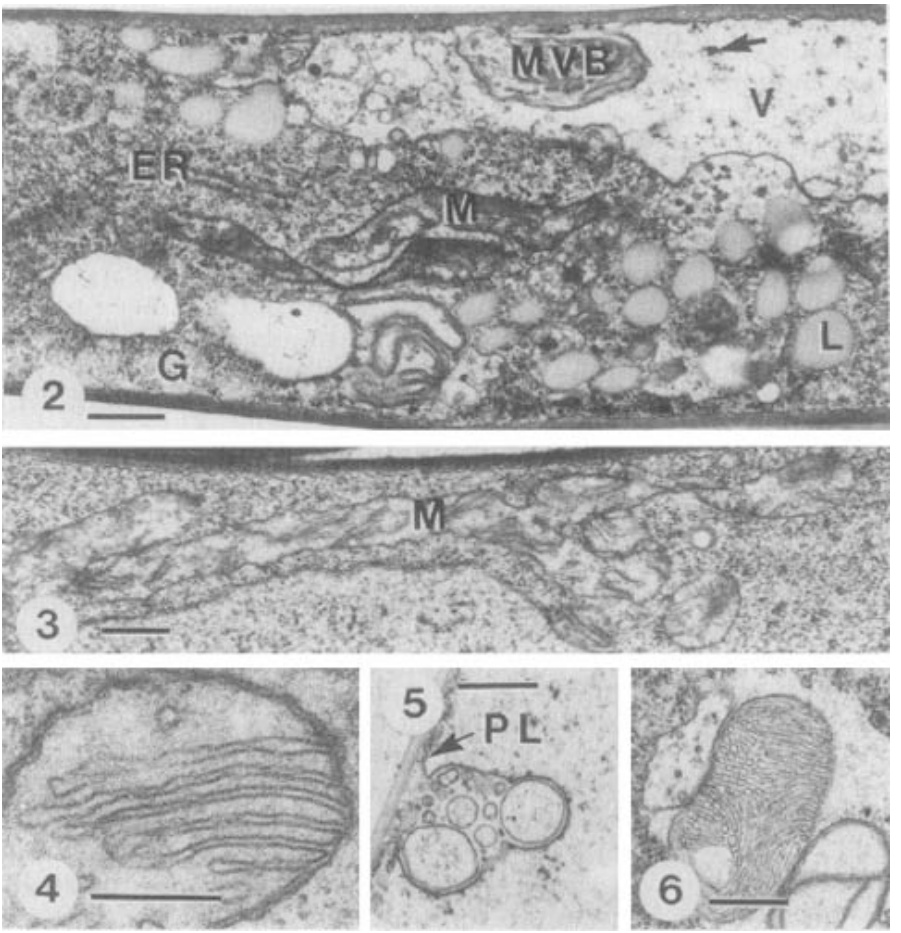
In the rusts, microbodies of either amorphous or crystalline contents may be found (Fig. 16). They are most frequently associated with septa, although they may also be found elsewhere in the cytoplasm. Although they conform to a morphological definition of microbodies, tests for catalase in the crystal-containing microbodies of *Puccinia helianthi* (Coffey *et al*, 1972) or amorphous-content microbodies of *U. phaseoli* (Mendgen, 1973) have been negative.

The mitochondria in the intercellular hyphal cells are irregularly shaped, generally filiform, and may be lobed or branched (Fig. 3). Where series of sections are available to trace the conformation of mitochondria, many of those that appear as single bodies in individual sections are actually lobes or branches of much larger structures. This type of mitochondrial structure is reminiscent of the single giant, branched mitochondrion per cell of the yeast *Saccharomyces cere-visiae* (Hoffmann and Avers, 1973). However, series of sections through the cells show that there are at least several mitochondria per cell in the rusts.

The cristae normally occur as an invagination of the inner membrane of the mitochondrial envelope and are typically arranged in parallel platelike stacks (Fig.4), which is characteristic of most higher fungi. This arrangement of cristae has been consistent in all growth phases of the cereal rusts examined. The matrix of the mitochondria is variable in electron density and may contain mitochondrial ribosomes. Coffey *et al.* (1972) reported considerable variation with respect to density of the matrix, amorphous inclusions, and conformation of cristae of mitochondria of *P. helianthi* or *M. lini*, depending on location (haustorial or hyphal) or whether growth was parasitic or axenic. These variations have not been noted with consistency in any of the cereal rusts except for conformational changes in the haustorium mother cells (see Harder and Chong, Chapter 14, this volume).

Microtubules are common components of rust fungal cytoplasm. They are most frequently encountered where there appears to be movement of organelles, such as locations where there is hyphal branching or where there are pseudosepta (see Section II,C,2) around which organelles are in an apparent stage of migration (D. E. Harder, unpublished). In an analysis of organelle movement in *U. phaseoli* var. *vignae,* I. B. Heath and Heath (1978) indicated that microtubules were most commonly associated with nuclei and mitochondria, and that the microtubules were involved in the control of the position of these organelles. The latter conclusion was strengthened through experiments using antimicrotubule agents (Herr and Heath, 1982). In *U. phaseoli* var. *vignae,* most of the microtubules in the hyphae were located in the peripheral region of the cytoplasm, were oriented in the direction of cytoplasm movement, were usually less than 2 μm long (although some were up to 8 μm long), and were probably anchored to microfilaments in the cytoplasm. Although a similar detailed analysis has not been conducted in any of the cereal rusts, observations of *P. coronata* and *P. graminis* f. sp. *tritici* (D. E. Harder, unpublished) indicate similar associations with organelles and orientation of microtubules. For a more detailed discussion of fungal microtubules see Staples and Macko, Chapter 9, this volume.

Various configurations and aggregations of membrane-bound vesicles are frequently encountered in hyphal cells. These conform to the definition of a lomasome (Moore and McAlear. 1961), where the vesicles occur between the fungal wall and plasmalemma (Fig. 5). However, no function has been ascribed to these structures, and they have gained little prominence in the recent literature. Perhaps they are stress-related artifacts of preparation procedures. A second type of multivesicular body occurs within the cytoplasm, and these are usually an aggregation of tubules or vesicles within a membrane-bound body (Fig. 6), or they consist of concentric rings of membrane that resemble myelin figures. Again, no function for these bodies is known, and their existence in living cells is also not certain. However, similar membrane configurations have frequently been found near the base of haustorial bodies (J. Chong and D. E. Harder, unpublished). Coffey *et al.* (1972) considered similar bodies in *M. lini* to be artifacts, but they have also been seen in freeze-substitution preparations (D. E. Harder and K. Mendgen, unpublished), thus they may have a functional role, perhaps in the synthesis of plasma membranes. A third type of multivesicular body is a group of small vesicles enclosed within a large vesicle. These are most frequently found in apparently physiologically active cytoplasm, and they aggregate in particular near the poles of mitotic nuclei (see Fig. 12). No specific function for these bodies is known, although they resemble the multivesicular bodies in *Mucor rouxii,* which were reported to resemble chitosomes (Bracker *et al.,* 1976).



Figs. 2-6. Some cytoplasmic components of intercellular hyphae of *Puccinia coronata*. ER, Endoplasmic reticulum; G, glycogen; L, lipid; M, mitochondrion; MVB, multivesicular body; PL, plasmalemma; V, vacuole. (All figures are from D. E. Harder, unpublished.) Fig. 2. A hyphal cell from near the colony edge, which is representative of the appearance of the protoplast of this type of active, growing cell. The arrow points to electron-dense (probably polyphosphate) granules, (x 17,300; bar, 0.60 ?µm). Fig. 3. An elongated, branched, and lobed mitochondrion (x 18,500; bar, 0.50 ?µm). Fig. 4. A parallel array of platelike mitochondrial cristae (x8000; bar, 0.20 µm). Fig. 5. A multivesicular body located between the plasmalemma and hyphal cell wall, defined as a lomasome (x25,000; bar, 0.40 µm). Fig. 6. A multivesicular body located within the cytoplasm (x22,100; bar, 0.45 µm).

Large vacuoles are the most prominent in older cells of the mycelium, which presumably reflects the loss of synthetic activity of these cells. However,

in the cytoplasm of young active cells there are also smaller vacuoles that frequently contain electron-dense inclusions (see Fig. 2). In haustoria or haustorium mother cells of *P. coronata,* similar inclusions were concluded to be composed mainly of polyphosphate (Chong, 1981). However, Heath and Heath (1979) found no phosphate in similar-appearing inclusions in "vacuole precursor vesicles" in infection structures of *U. phaseoli* var. *vignae,* although possibly the phosphate was extracted during processing. The latter vesicles were considered (Heath and Heath, 1979) to be involved in one of two pathways leading to vacuole formation.

B. NUCLEI AND NUCLEAR DIVISION

1. Interphase Nuclei

a. Morphology. The dikaryotic hyphal cells of the cereal rusts usually contain two roughly oval-shaped nuclei of somewhat variable diameter. In the intercellular hyphae the nuclei exist in the "expanded" form (*sensu*Savile, 1939). The chromatin is typically dispersed and is not visible in the electron microscope. Each nucleus contains a prominent nucleolus.

There are exceptions to the normal oval-shaped nuclei. In some axenically cultured (Fig. 7) and parasitic hyphal cells, elongated dumbbell-shaped nuclei may be found. These show no evidence of mitosis and are frequently associated with pseudosepta (see Section II,C). Observations of multinuclear cells in the hyphae of the rust fungi may in part be due to the halves of the dumbbell forms appearing as discrete nuclei in the light or electron microscope.

b. Nucleoli. In physiologically active cells the nucleoli are promient, occupying up to 60% of the nuclear volume (Harder, 1976a). In parasitic growth of those cereal rusts examined, the nucleoli are structurally typical of those of most eukaryotic organisms. The nucleolar matrix is composed of fibrillar and granular regions, interspersed with lacunar spaces. The lacunae appear as meandering channels through the nucleolus (Harder, 1976a), and these are continuous with the nucleoplasm and a larger central lacunar space. In axenic culture the nucleoli are typically more compact and lack the clear differentiation of fibrillar and granular regions. The granular component is generally more prominent (also see Manocha, 1971). The granularity of the nucleolus generally reflects synthetic activity (Smetana and Busch, 1974), which may be of relatively greater importance in axenic culture than in parasitic growth.

c.*The Nuclear Envelope.* The nuclear envelope in the rusts is consistent with that of other eukaryotic organisms; it is composed of a double membrane, the outer of which is continuous in places with the ER (*P. coronata;* D. E. Harder, unpublished) and contains nuclear pores. The freeze-etch preparation in Fig. 8

shows the nuclear pores of *P. coronata* to be complex structures, with strands of material radiating from a central granule to the pore boundary. In cross section the poresmeasure about 65 to 75 nm in diameter. The nuclear pore structure in the rusts appears to be consistent with the model of pore structure proposed for higher plants (Gunning and Steer, 1975).

d. Nucleus-Associated Organelle. A characteristic body, referred to mainly as the nucleus-associated organelle (NAO) (Girbardt and Hadrich, 1975) or spindle-pole body (SPB) (Aist and Williams, 1972), is associated with the rust fungal nuclei. Structurally this body is relatively consistent throughout the Uredinales and is a constant feature of nuclei at all growth stages of the rust fungi. Although NAO and SPB are used synonymously in the current literature, the use of either term has functional implications. The only known function of this body is as a microtubule organizer during nuclear division (McLaughlin, 1981), although Heath (1981) has argued for other possible functions. Until the latter aspect is resolved, either the SPB or NAO designation is equally valid. The term NAO, although not necessarily favored over SPB, will be used here to remain consistent with recent reviews (Heath, 1978, 1981; Littlefield and Heath, 1979).

An interpretation of the structure of the NAO of *P. coronata* is illustrated in Fig. 10 [for illustrations of other rusts see Heath and Heath (1976) and O'Donnell and McLaughlin (1981c)]. A longitudinal perpendicular section through a NAO of *P. coronata* is shown in Fig. 9.

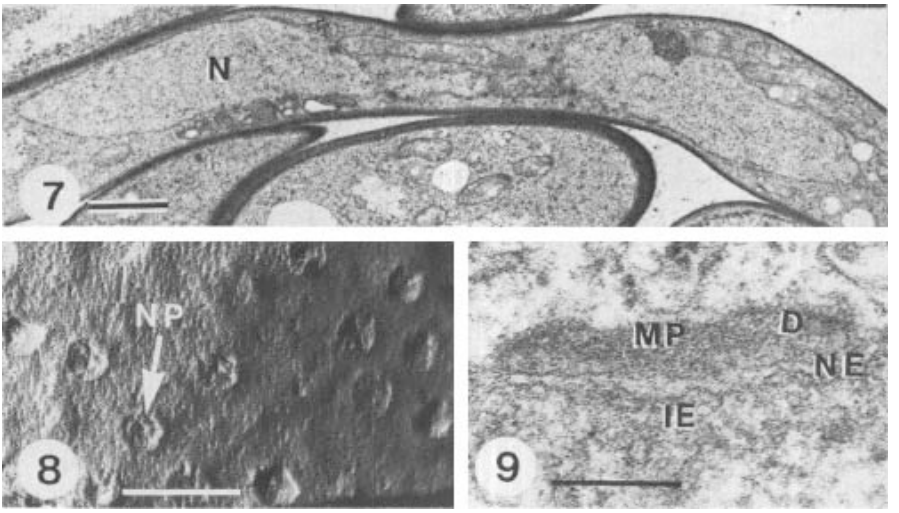


Fig. 7. A nonmitotic, dumbbell-shaped nucleus (N) in an axenically cultured hypha of *Puccinia giaminis i.* sp. *tritici* (x9200; bar, 1.10 μm). (From Harder, 1976b. Reproduced by permission of the National Research Council of Canada.) **Fig.** 8. A freeze-etch replica of a nuclear envelope of *Puccinia coronata.* A central granule is evident in most of the nuclear

poles (NP), and the granules are joined to the pore margins by threadlike processes (x70,000; bar, 0.21 μm). (From D. E. Harder, unpublished.) **Fig.** 9. An interphase nucleus-associated organelle (NAO) of *Puccinia coronata.* A disk (D) lies at an inclined angle on either side of a middle piece (MP). An associated bilayered intranuclear element (IE) subtends the NAO inside the nuclear envelope (NE) (x85,500; bar, 0.20 (Am). (From Harder, 1976a. Reproduced with permission of the National Research Council of Canada.)

Fig. 10. A diagrammatic interpretation of the side (a) and top (b) views of the interphase nucleus-associated organelle of *Puccinia coronata.* D, Disk; MP, middle piece; IE, intranuclear element; NE, nuclear envelope. The broken line across the pore in the NE indicates that this pore may or may not be present.

The NAO characteristically lies in a depression of the nuclear envelope. An apparent pore in the nuclear envelope, located centrally underneath the NAO, has been observed in several *Puccinia* spp. (Harder, 1976a; Wright *et al.*, 1978), but this has not been seen in other studies (Heath and Heath, 1976; O'Donnell and McLaughlin, 1981c). This apparent pore may be due to sensitivity of this region of the nuclear envelope to tissue-processing procedures, or it may represent a particular stage of the nuclear cycle. The interphase NAO basically consists of two roughly circular, probably several-layered disks lying at an inclined angle on a middle piece. The tapered ends of the middle piece are inserted into the layers of the disks. The disks consist of an electron-dense upper layer and one or more diffuse lower layers. The disk layers become more distinct during mitosis (Fig. 12). A bilayered hemispherical structure subtending the NAO occurs in the nucleoplasm. This structure, designated the intranuclear element (McLaughlin, 1981), consists of an amorphous region immediately inside the nuclear envelope, subtended by a zone of loosely organized strands of material. In several instances a thread-like connection has been observed to extend from the latter zone to the nucleolus (Harder, 1976a). A function for the intranuclear element has not been established.

2. *Mitosis*

In the cereal rusts, mitosis has been studied ultrastructurally in only two species *(P. coronata,* Harder, 1976a,b; *P. striiformis,* Wright *et al.,* 1978), thus

the picture of mitosis in these rusts is sketchy. Heath (1978, 1980) has reviewed mitosis in fungi, including the Uredinales.

As a prelude to mitosis in the rusts just mentioned, the nucleus becomes deformed and variable portions of the nucleolus, along with some of the nucleoplasm, are ejected into the cytoplasm (Harder, 1976a; Wright*et al.*, 1978). Similar conclusions were reached for other rusts through light microscopy (Craigie, 1959; Saville, 1939). However, Heath and Heath (1976) indicated that in *U. phaseoli* var. *vignae,* nucleolar ejection was later, beginning during anaphase and completed by telophase. The timing of nucleolar ejection may be variable. Figure 11 illustrates a nucleus of *P. coronata* in metaphase, and serial sections had shown the adjacent nucleolus to be completely detached from the parent nucleus. Nucleoli in a stage of ejection, but still attached to the nucleus, have been found no later than early metaphase (D. E. Harder, unpublished). Thus in*P. coronata,* nucleolar ejection appears to be completed by metaphase. Regardless of the timing of nucleolar ejection, the portion of the nucleus involved in mitosis is smaller than the normal interphase nucleus, and the reduction in size appears to be brought about largely by expulsion of the nucleolus, along with variable amounts of nucleoplasm.

The onset of mitosis is indicated by separation of the disks of the NAO, these disks becoming positioned at the poles of the mitotic spindle (*U. phaseoli* var. *vignae,* Heath and Heath, 1976). Division of the NAO has not been traced in the cereal rusts, although it presumably is similar to that in *U. phaseoli* var. *vignae.* In *P. coronata* the mitotic polar disks are enlarged and the disk layers are more distinct as compared to their interphase state. During metaphase, a number of multivesicular bodies aggregate in the cytoplasm adjacent to either mitotic pole (Fig. 12). Despite the consistency of the occurrence of these bodies (see also Heath and Heath, 1976; Wright *et al,* 1978; O'Donnell and McLaughlin, 1981a,b,c), no clear function for them has been established (see also Section II,A).

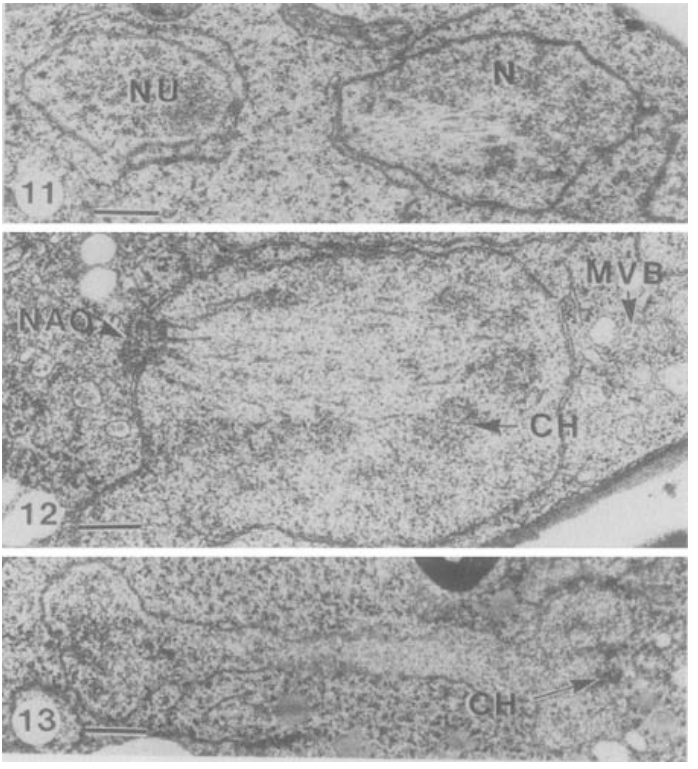
The mitotic spindle of the rusts contains both chromosomal and pole-to-pole tubules (most clearly documented for *U. phaseoli* var. *vignae*, Heath and Heath, 1976). During metaphase the chromosomes become somewhat condensed, but they are not sharply contrasted in the electron microscope. There is no condensation of chromosomes into a metaphase plate; rather, they become arranged around the periphery of the spindle (Fig. 12). In the cereal rusts clearly identifiable kinetochores have not yet been found. However, the chromosomes are presumably attached to the spindle microtubules at a kinetochore-equivalent region. At telophase, longer, straighter tubules can be seen that pass from the NAO into the constricted portion of the nucleus (Harder, 1976b). These are the pole-to-pole tubules, which probably function to push the halves of the dividing nucleus apart.

Telophase is marked by elongation of the nucleus and constriction in the middle to assume a dumbbell form (Fig. 13). The chromatin has aggregated at

the poles around the periphery of the spindle. At all stages of mitosis until telophase, the nuclear envelope remains intact. At late telophase the central portion of the nuclear envelope together with the spindle tubules appear to disintegrate to allow formation of the daughter nuclei.

C. CELL WALLS AND SEPTA

The hyphal walls of the rusts appear to be bilayered (Littlefield and Heath, 1979), although this is not apparent in most preparations for electron microscopy. Differentiation of layers within the wall is most evident at the point of septation (see Fig. 16), where the electron-translucent middle septal lamella ends in a discrete line along the outer layer of the periclinal wall and an inner wall layer is continuous with the septal wall layers. At times a third but less discrete outer layer is evident, which has been interpreted as a covering, with possibly protective or other functions (see Littlefield and Heath, 1979). This layer is also continuous around the haustorium mother cell and apparently is involved in adhesion of the haustorium mother cell to the host wall. This material is removed after protease treatment (see Harder and Chong, Chapter 14, this volume, Section IV,E), indicating a proteinaceous content.



Figs. 11-13. Mitosis in *Puccinia coronata.* (From Harder, 1976b. Reproduced with permission of the National Research Council of Canada.) Fig. 11. A nucleolus (NU) that has completely separated from the metaphase nucleus (N) (x20,600; bar, 0.50 |xm). Fig. 12. A metaphase nucleus. The chromosomes (CH) are arranged around the periphery of the spindle, the tubules of which originate in the nucleus-associated organelle (NAO). Note the multivesicular bodies (MVB) in the cytoplasm adjacent to either mitotic pole (x27,500; bar, 0.36 μm). Fig. 13. A dumbbell-shaped telophase nucleus. The chromatin (CH) has aggregated at either pole, and the nuclear envelope appears to be partially disorganized in the middle of the constricted portion of the nucleus (x 14,700; bar, 0.68 μm).

The hyphae of the rusts are compartmentalized by cross walls, or septa. Two major types of septa have been identified in the rusts; the typical hyphal septa that normally form following conjugate nuclear division, and more unusual "pseudosepta." These are discussed in turn in the following subsections.

1. Typical Septa

The formation and structure of septa is shown diagrammatically in Fig. 14. The first indication of septum formation is an invagination of the plasmalemma (the septal initial), with an electron-lucent zone appearing within the invagination and extending partially into the inner wall layer (Fig. 14a). Septal growth continues by centripetal invagination of the plasmalemma, accompanied by deposition of wall material within the invagination (Fig. 14b). Following invagination of the plasmalemma, the wall material condenses and two electron-dense lamellae form within the invagination. These lamellae are continuous with the inner layer of the periclinal wall and are separated along the length of the septum by an electron-lucent zone, to form two independent walls (Figs. 14c, 17). Near the centers of perforate septa the walls taper to a point to form the periphery of the central pore.

The pores of mature septa in the Uredinales normally have associated with them a characteristic structure, the *septal pore apparatus* (see Coffey *et al*, 1972; Harder, 1976b; Heath, 1975). The pore apparatus consists of a membranous diaphragm that bounds both sides of the pore and contacts the plasmalemma somewhat beyond the apices of the septal walls to form a pulley-like shape (Figs. 14d, 15). On either side of the pore there is usually an organelle-free hemispheric zone of diffuse material (Figs. 14d, 16). Microbodies containing either crystalline inclusions or amorphous material (Figs. 14d, 16) occur around the periphery of the diffuse zone. The pores are often occluded with an electron-dense substance (Fig. 16). Where the occlusion occurs, it acquires the pulley shape of the membranous diaphragm around the pore.

In the intercellular hyphae of *P. coronata* the diameters of the septal pores vary, ranging from 23 to 66 nm. However, in the haustorium mother cell

septum the pore is much smaller, about 9.5 nm in diameter (Fig. 18). Smaller diameter pores are also found in the septa delimiting the base of spores (see Sections V,B and VI).

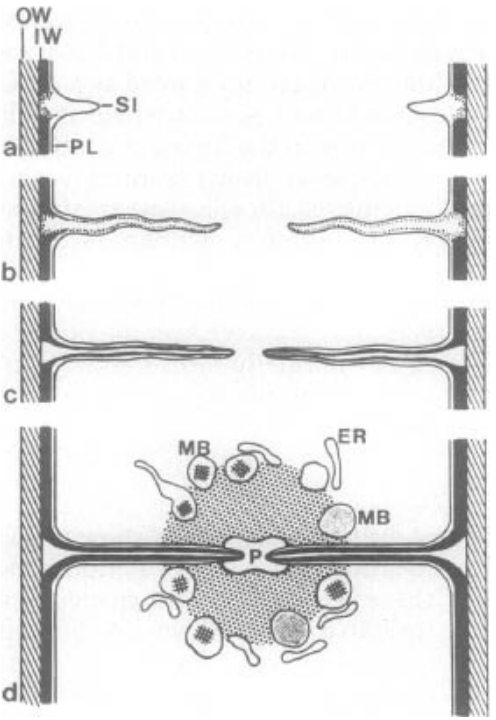


Fig. 14. A diagrammatic representation of the formation of a typical septum in a rust fungus. (a) Initially the plasmalemma (PL) invaginates to form a septal initial (SI), and the inner layer of the hyphal wall (IW) dissolves at the SI. The outer wall layer (OW) remains intact. (b) The septum then grows inward centripetally, and wall material is deposited within the invaginated plasmalemma. (c) The septum has completed inward growth, leaving a central pore. The wall material has formed two electron-dense lamellae that are continuous with the inner layer of the longitudinal hyphal wall. (d) A mature septum with septal pore apparatus. A diaphragm, which in cross section is pulley-shaped, surrounds the central pore (P). A zone of amorphous material occurs on either side of the pore, which in turn is surrounded by microbodies (MB) that contain either crystalline (crosshatched) or amorphous (dotted) material. The microbodies are probably derived from the endoplasmic reticulum (ER). (Drawn by Dr. J. Chong.)

2. *Pseudosepta*

The pseudosepta (Ehrlich *et al.*, 1968) are characterized by the occurrence of all layers of the longitudinal wall across the septum (see Fig. 20), an acentric pore of variable diameter, and absence of a pore apparatus. They have also been

designated as partial septa (Littlefield and Bracker, 1971a) or infolded-wall septa (Rijkenberg and Truter, 1975). Figure 19 shows several hyphal cells near the base of a teliosorus of *P. coronata* to be partially compartmentalized by pseudosepta.

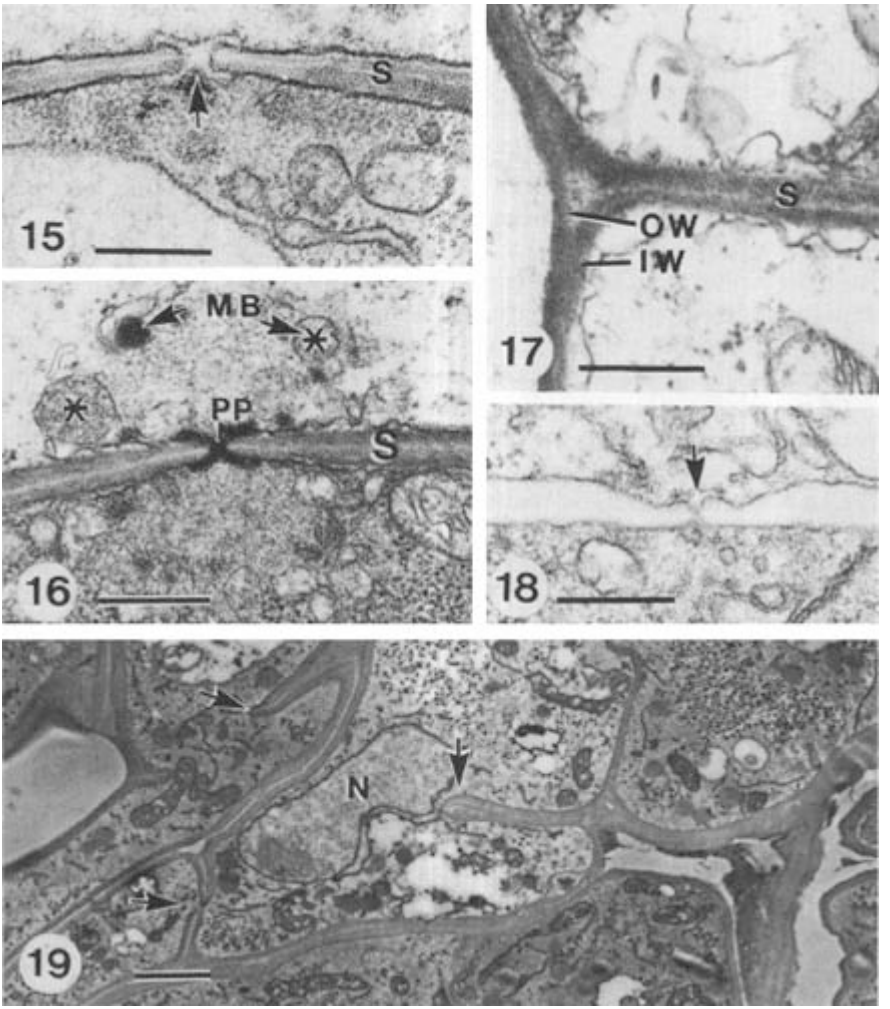


Fig. 15. A nonoccluded septal pore (arrow) in a hyphal septum (S) of *Puccinia coronata*. Note the pulley-shaped diaphragm that surrounds the pore (x 60,000; bar, 0.25 µm). (From Chong, 1981.) **Fig.** 16. A hyphal septum (S) of *Puccinia coronata* with septal pore apparatus. In this septum the central pore is occluded with a pore plug (PP). Note the diffuse amorphous zone on either side of the pore and the microbodies |MB) with either crystalline (densely staining) or amorphous (asterisk) contents (x33,600; bar, 0.45 µm). (From D. E. Harder, unpublished.) **Fig.** 17. Part of a mature hyphal septum (S) of

axenic *Puccinia graminis* f. sp. *tritici*. Note the continuity of the septal lamellae with the inner layer (IW) of the hyphal wall. The outer wall layer (OW) is continuous across the end of the septum (x32,000; bar, 0.50 μm). (From D. E. Harder, unpublished.) **Fig.** 18. A septal pore (arrow) in a haustorium mother cell septum. The pore is smaller than those in intercellular hyphae, and there is no pore apparatus (x52,900; bar, 0.28 μm). (From Chong, 1981.) **Fig.** 19. A number of cells of *Puccinia coionata* near the base of a telial sorus, which are compartmentalized by pseudosepta (arrows). Note the nucleus (N) associated with one of the septa (X8200; bar, 1.20 μm). (From D. E. Harder, unpublished.)

The pseudosepta could arise either through an infolding of the longitudinal hyphal wall or through dissolution of a section of the walls of anastomosing hyphae. Structurally it is not easily determined if the ends of the septa are the result of growth or degradation. Figure 20 illustrates the latter two possible modes of development of these septa.

Of particular interest is the possibility of anastomosing hyphae. The pseudosepta are especially prominent in the layer of multinucleate cells near the aecial base of *P. sorghi* (Rijkenberg and Truter, 1975), and are also common in the closely packed pseudoparenchymatous cells at the bases of sori. These conditions would provide the greatest opportunity for hyphal anastomoses. The pseudosepta also occur, but to a lesser extent, in hyphae of axenic growth or in hyphae near the leading edge of colonies (D. E. Harder, unpublished). Frequently associated with pseudosepta are nuclei in an apparent stage of migration (Fig. 19). These nuclei are usually in an elongated dumbbell form and may have two nucleoli (D. E. Harder, unpublished). These configurations suggest hyphal anastomoses and possible nuclear fusions. A suggested mechanism for providing genetic variation in the rusts is somatic recombination. Although this concept has not gained very wide experimental support, there are a number of reports in the literature that indicate the operation of such a mechanism. The previous observations may provide a structural basis for somatic recombination.

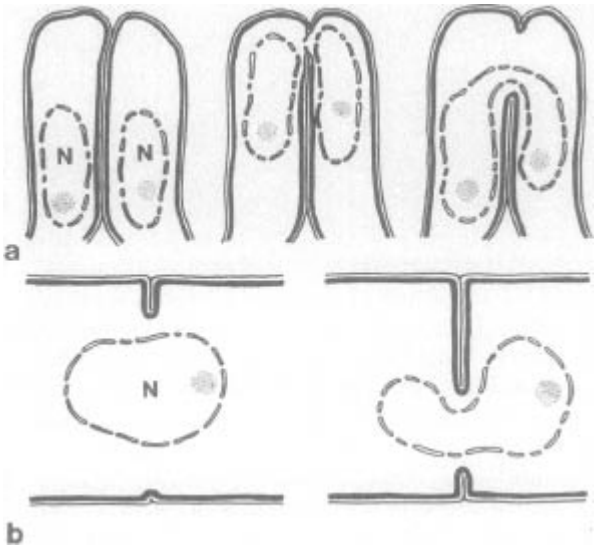


Fig. 20. Illustration of two possible modes of pseudoseptum formation. (a) The common wall between two anastomosing hyphae partially dissolves, allowing mixing of the cell contents of both hyphal cells and possible fusion of nuclei (N). (b) Asymmetric invagination of the longitudinal walls of a hyphal cell to form a pseudoseptum. (Drawn by Dr. J. Chong.)

III. Pycnia

The pycnium is the structure in which the gametes of the rust fungi are produced. Except for *Puccinia striiformis*, *P. kuehnii*, and *P. melanocephala*, in which the sexual stage is unknown, the cereal rust discussed in these volumes are macrocyclic heteroecious fungi, in that their sexual stage occurs on an alternate host. Following teliospore germination and meiosis, infection of an alternate host by haploid basidiospores results in the establishment of a monokaryotic hyphal colony, from which the pycnia arise. The monokaryotic colonies appear essentially similar to the dikaryotic colonies except for a lesser preponderance of haustoria relative to hyphal growth in the monokaryotic state (see Harder and Chong, Chapter 14, this volume, Section VII).

A. MORPHOLOGY

Hiratsuka and Cummins (1963) differentiated 11 types of pycnial structures in the rusts. Of these, the cereal rusts are represented by their type 4: a subepidermal, determinate structure having a strongly convex hymenium (i.e., flask-shaped) and bounded by well-developed periphyses. An individual

pustule derived from a single basidiospore may contain a number of pycnia *{P. graminis* f. sp. *tritici*, Craigie, 1927b). Multi-pycnial infections have also been observed for *P. coronata* (D. E. Harder, unpublished). The morphology of a typical pycnium is illustrated in Fig. 21.

B. CELL TYPES

Lining the base of the pycnium is a closely packed layer of *pseudoparenchymatous* (= hymenial) cells. These cells give rise to the *pycniosporophores, paraphyses* (= periphyses, or sterile hyphae), and probably the *flexuous* (i.e., receptive) *hyphae*. The flexuous hyphae are not illustrated in Fig. 21 because of insufficient structural information. The pycniosporophores produce the *pycniospores.* Interspersed among the pycniosporophores are sterile cells of indeterminate origin or fate. These cells are marked by dense protoplasts and variously shaped electron-lucent inclusions; see Section V,A for a further description of this type of cell.

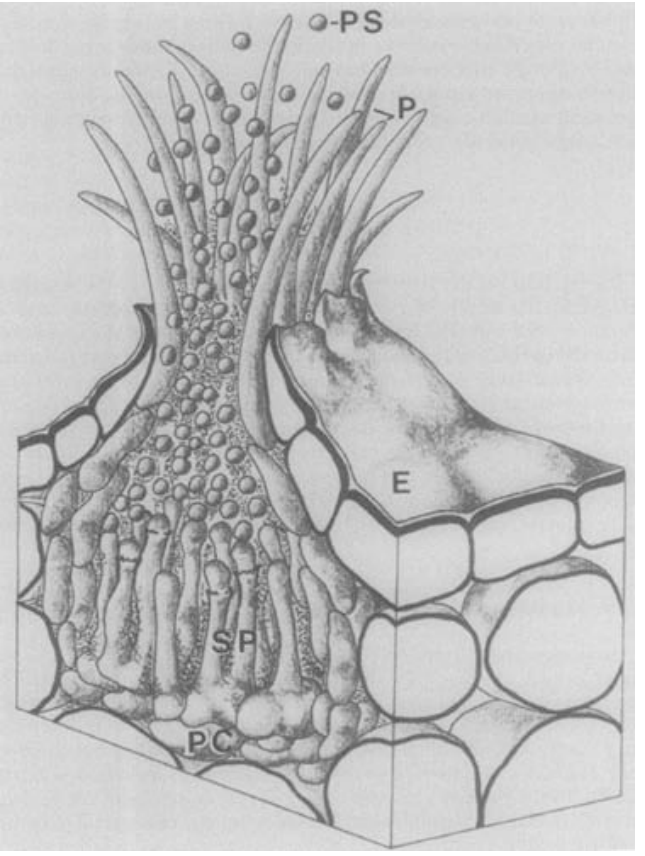


Fig. 21. A diagrammatic representation of a pycnium of *Puccinia coronata*. A layer of pseudoparenchymatous cells (PC) lines the base of the pycnium, from which are derived the pycniosporophores (SP) and the paraphyses (P). The pycniospores (PS) are produced by the pycniosporophores. E, Host epidermis. (Drawn by Dr. J. Chong.)

Each mononucleate pseudoparenchymatous cell may give rise to one or more pycniosporophores. The pycniosporophores in turn may be branched, forming a candelabra-like structure. The pycniosporophores are uninucleate and elongated, forming a palisade of closely packed, somewhat intertwined cells near the base of the pycnium. There are no unusual features that distinguish the protoplasts of these cells from most other hyphal cells of the monokaryotic thallus. The paraphyses are elongated robust cells that arise from the pseudoparenchymatous cells at the sides of the pycnial cavity. In a mature pycnium the paraphyses flare upward and outward, surrounding an opening in the pycnium, the *ostiole*. In the early stages of pycnium formation the paraphyses are aggregated into a somewhat pointed structure (Buller, 1950; Gold *et al.*, 1979) that apparently functions to rupture the host epidermis. Ultrastructurally the protoplasts of the paraphyses are much like other cells of the pycnium, except that the nuclei are usually somewhat elongated and microtubules are very prominent adjacent to and parallel with the walls. The pyenial paraphyses of *P. coronata* do not contain any of the unusual inclusions that occur in the uredial paraphyses of the same fungus (see Section V,A).

There are as yet no transmission electron microscopic illustrations of the flexuous hyphae in the rusts, although they have been shown by scanning electron microscopy in pycnia of *P. recondita* (Gold *et al*, 1979),*M. lini* (Gold and Littlefield, 1979), and *Gymnosporangium clavipes* (Kozar and Netolitzky, 1975). In the *Puccinia* spp. the flexuous hyphae occur intermixed among the paraphyses; approximately 20 such hyphae per pycnium were reported to occur in *P. graminis* (Buller, 1950). The flexuous hyphae can be distinguished from the paraphyses by being less erect and less pointed at their apices.

C. PYCNIOSPORE ONTOGENY

Among the cereal rusts, pycniospore formation has been described ultrastructurally for *P. sorghi* (Rijkenberg and Truter, 1974a) and *P. coronata* (Harder and Chong, 1978), and in several noncereal rusts byCodron (1981), Mims, *et al.* (1976), and Metzler (1981). Figure 22 is a diagrammatic summarization of pycniospore formation in *P. coronata*, and may be referred to in the following description of pycniospore development.

The pycniospores are produced successively in chains from the pycniosporophores; a single pycniosporophore gives rise to a large number of pycniospores. An understanding of the mechanism of pycniospore formation is contingent on understanding the cell wall relationships during spore formation.

The pycniosporophore walls are composed of two layers: a broad outer layer and a relatively narrower inner layer. The formation of the first pycniospore is marked by a swelling of the pycniosporophore apex. Both wall layers extend around the swelling apex. The formation of successive spores essentially recapitulates the first spore in that a complete bilayered wall is synthesized to envelop each forming spore. During the swelling process, mitosis occurs, after which one nucleus migrates into the pycniospore and the other remains in the pycniosporophore. Following nuclear migration, septa-tion occurs (Fig. 22a) to delimit the pycniospore. During septation only the inner layer of the periclinal wall is initially disrupted. At maturity the septum is composed of two bilayered walls separated by an electron-lucent lamella (Fig. 22b). The septal wall layers are continuous with the respective inner and outer wall layers of the pycniosporophore and immature pycniospore. Pycniospore secession then occurs by rupture of the outer layer of the periclinal wall (Fig. 22c). The rupture of this wall initially leaves a remnant, often seen as a basal frill, on the young pycniospore. The outer wall layer of the immature pycniospore than gradually becomes thinner (Fig. 22c) and is replaced by the thickening inner layer, until at maturity the inner layer comprises the bulk of the pycniospore wall (Fig. 22c). In Fig. 22d the process begun in Fig. 22a, involving the same pycniosporophore, is repeated. The secession of pycniospores leaves pronounced wall remnants (annular scars) on the pycniosporophore walls. Each succeeding pycniospore is formed by extension of the bilayered pycniosporophore wall from inside the base of the most recent annular scar. The walls of all succeeding pycniospores originate at about the same locus on the pycniosporophore. In this way, repeated pycniospore secession leads to a buildup of a series of concentric annular scars to form a thickened collar at the pycniosporophore apex.

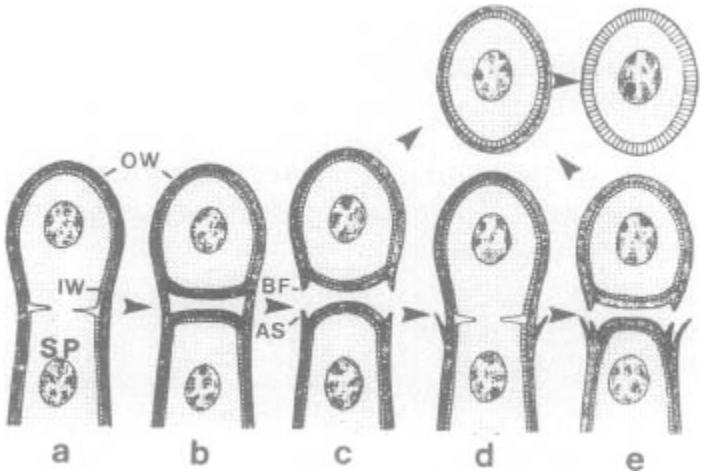


Fig. 22. A diagrammatic interpretation of pycniospore formation in *Puccinia coronata*. The sequence of the development of the first spore is from a to c, and repeat spore formation from d to e. (a) After nuclear division and swelling of the pycniosporophore (SP) apex, septum formation has begun by invagination of the plasmalemma and breakdown of the inner wall layer (IW) within the invagination. (b) Both inner and outer wall (OW) layers have grown across the septum, but the outer layer of the periclinal wall is intact. (c) Spore release occurs by rupture of the outer wall layer, leaving a basal frill (BF) on the immature spore and an annular scar (AS) on the pycniosporophore. (d) and (e) Repeat stages of (a) and (b), respectively, showing formation of the second spore from the pycniosporophore and showing the beginning of accumulation of annular scars, at the same vertical level, to form a collar at the pycniosporophore apex. Maturation of the pycniospore is marked by thickening of the inner wall layer and disappearance of the outer wall layer [above (d) and (e)].

IV. Aecia

The aecium is the fruiting structure that produces the aeciospores following dikaryotization and reinitiates the dikaryotic life cycle phase. The aecia typically form on the undersides of the leaves shortly after pycnium development. A single aecial pustule consists of multiple aecia.

A. MORPHOLOGY

Five major morphological types of aecia in the rusts have been defined (Cummins, 1959). The aecia of all of the cereal rusts are typified by the "aecidoid" type. This type of aecium is a somewhat cylindrical to trumpet-shaped structure bounded by a single layer of peridial cells, and originates subepidermally. The aeciospores are produced within the confines of the peridium. The structure of the aecium is reconstructed in the drawing in Fig. 23.

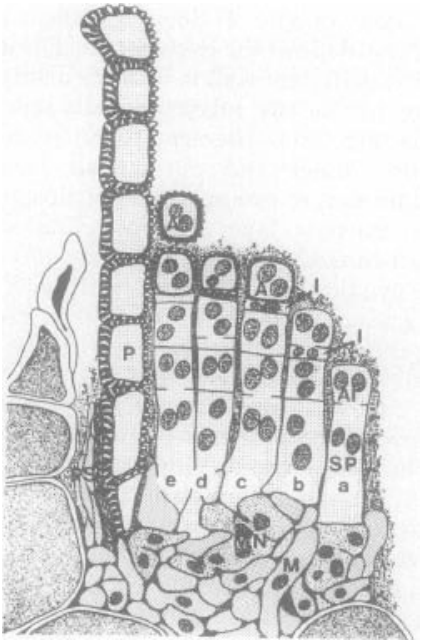


Fig. 23. Diagrammatic representation of a cereal rust aecium. At the base of the aecium are monokaryotic (M) and multinuclear fusion cells (MN). The latter cells give rise to the dikaryotic aeciosporophores (SP) and peridial (P) cells. Outside the peridium is an aggregation of crushed prosenchymatous cells (PS). The sequence of aeciospore formation is shown from a to e. (a) Division of the aeciosporophore to form an aeciospore initial (AI). (b) Formation of additional aeciospore initials and division of the first aeciospore initial to form an immature aeciospore (A) and an intercalary cell (I), (c) Formation of more aeciospore initials, and secondary wall and ornament formation in the aeciospore. (The number of aeciospore initials formed is probably variable.) (d) Maturation of the aeciospore and disorganization of the intercalary cell. (e) Release of the aeciospore and continued aeciospore formation.

B. CELL TYPES

The following descriptions of cell types in aecia are from ultrastructural studies of P. *sorghi* (Rijkenberg and Truter, 1974b, 1975), *P. recondite* (Gold *et al.*, 1979), *P. graminis* f. sp. *tritici* (Holm and Tibel, 1974), and *P. coronata* (D. E. Harder, unpublished).

The sides of the aecium are bounded by a cylinder consisting of a single layer of dikaryotic *peridial cells.* These cells are characterized by a unique wall structure (Fig. 24). The walls to the outside of the aecium are considerably thicker than the inward-facing walls. The walls are highly differentiated into a uniformly electron-dense portion and electron-lucent, branched, dagger-shaped processes. In the outward-facing wall the processes extend from near the

plasmalemma through the wall to the outer surface, but not extending beyond it. In the inner wall facing the aecial cavity, the primary wall materials surrounding the processes disintegrate, partially exposing them. These then form irregularly spaced and shaped ornaments, or clavae (Gold *et al.*, 1979), over the surface of this wall.

The differential thickness of the peridial walls has been suggested (Littlefield and Heath, 1979; Savile, 1954) as a possible mechanism of opening and closing the aecium in response to changes in humidity. The thin inner wall may respond more rapidly to changes in humidity; during periods of low humidity the inner wall would contract to close the aecium, and vice versa during periods of high humidity.

Near the base of the peridium but external to it are a number of fungal cells in various stages of disintegration and compaction. These cells, designated *prosenchymatous* cells in aecia of *P. sorghi* (Rijkenberg and Truter, 1974b), have no apparent function in the aecial complex.

Rijkenberg and Truter (1974b) differentiated three types of cells in the closely packed, intertwining stroma of hyphae at the aecial base. These were (1) uninucleate, often vacuolate cells, (2) multinucleate *fusion cells,* and (3) degenerate cells with large vacuoles and granular inclusions. The origin or fate of the latter cells was not followed, and they may be degenerating uninucleate or other cells; thus they may not be a distinct cell type. A fourth cell type, the binucleate *sporophores,* arise from the multinucleate cells at the base of the aecium. The sporophores give rise to *intercalary* (= *disjunctor;* Holm and Tibell, 1974) cells and the*aeciospore.*

C. DIKARYOTIZATION

The dikaryotization process in the rusts, despite its importance in the life cycle, is poorly understood. Virtually all of the information available has been obtained from light microscopy (for reviews, see Lamb, 1935; Buller, 1950). The initial phase of dikaryotization, the fusion of (+) and (—) mating types, occurs in the pycnium, but the formation of dikaryotic cells apparently occurs first at the base of the aecium.

The light microscopic studies as reviewed by Buller (1950) have shown several variations in the fusion of (+) and (-) mating types. Of those pertinent to the cereal rusts, the following variants have been documented:*(a)* fusion of (+) and (–) basidiospore-derived hyphae *(P. graminis* f. sp. *tritici,* Craigie, 1927a,b), and *(b)* fusion of (+) or (–) pycniospores, respectively, with (–) or (+) flexuous hyphae (*P. graminis* f. sp. *tritici*,Craigie, 1927a,b, 1933). In addition, Cotter (1960) and Garrett and Wilcoxson (1960) demonstrated (c) the fusion of aeciospore or urediospore germ tubes with flexuous hyphae of *P. graminis* f. sp. *tritici.* Although variants (*a*) and (c) just described could conceivably add to

the pool of nuclei for later reassortment in the aecium, they probably are not significant in nature.

The fusion of pycniospores with flexuous hyphae is well documented by light microscopy. In most of the rusts studied, a fusion tube of variable length or diameter forms between the pycniospore and the flexuous hypha. The only electron micrograph available is a scanning micrograph of apparent fusion in *Melampsora lini* (Littlefield and Heath, 1979). In *P. graminis* f. sp. *tritici,* the fusion tube is reduced to a slightly raised papilla on the flexuous hypha, through which the passage of the pycniospore nucleus was observed (Savile, 1939). In the cereal rusts studied by Buller (1950), fusion could occur at any point along the flexuous hypha. In the latter work tropisms between the flexuous hyphae and pycniospores, which induced branching or bending of the former to the pycniospore, were indicated. Although Buller (1950) indicated that in the main only one fusion occurs between a flexuous hypha and a pycniospore, each pycnium contains of a number of flexuous hyphae; thus multiple fusions can occur within a single pycnial sorus.

The stages of the dikaryotization process following fusion are the least well understood. One criterion used by Craigie and Green (1962) to trace the fate of the pycniospore nucleus of *P. graminis* f. sp. *tritici* was that the latter nuclei are in a compact "unexpanded" form, whereas those of the haploid thallus are "expanded." Using this criterion, the pycniospore nuclei were traced to cells of the protoaecium, where they required about 20 to 25 hr to arrive. How the nuclei arrive at the protoaecium and details of their postarrival fate are not known. Craigie and Green (1962) indicated that the pycniospore nuclei do not undergo mitosis during their migration, although Rijkenberg and Truter (1975) indicated that these nuclei underwent mitosis soon after arrival. The cells at the base of the aecium are mainly either uni- or multinucleate, and arise by cell fusion and/or nuclear division (Rijkenberg and Truter, 1975). Allen (1934) showed multinucleate cells in the protoaecium of *P. sorghi,* this condition presumably occurring before the arrival of a pycniospore nucleus. The next known development is that the dikaryotic primary aeciosporophores arise from the multinucleate fusion cells (Rijkenberg and Truter, 1974b). It was suggested by the latter authors that perhaps the injection of a pycniospore nucleus into a multinucleate cell is necessary to begin the final phase of dikaryotization. Each multinucleate cell gives rise to several sporophores, thus an assortment of compatible mating-type nuclei must occur at this stage to form the stable dikaryon. However, no details of this process are known.

D. AECIOSPORE FORMATION

1. Ontogeny

The formation of successive aeciospores from the sporophores is shown diagrammatically in Fig. 23. This diagram is reconstructed from *P. recondita* (Gold *et al.*, 1979), *P. sorghi* (Rijkenberg and Truter, 1974b, 1975), and *P. graminis* f. sp. *tritici* (Holm and Tibell, 1974). The primary aeciosporophore may divide to form secondary aeciospores (not shown in Fig. 23). Following mitotic nuclear divisions, the two daughter nuclei migrate to the distal end of the sporophore, followed by septation to form an aeciospore initial. In P. *graminis* f. sp. *tritici* (Holm and Tibell, 1974), several aeciospore initials are cut off to form a chain of these cells. The aeciospore initials, beginning first with the uppermost one, undergo a further division to form the aeciospore and a usually wedge-shaped intercalary cell. In this way continuous chains of aeciospores are produced. The intercalary cells then disintegrate to release the mature aeciospores.

2. Aeciospore Ornamentation

The aeciospores of all of the cereal rusts are covered with ornamental processes (see Fig. 25). Littlefield and Heath (1979) differentiated the two most common types of ornaments as either coglike or annulate knobs. The coglike knobs are somewhat cylindrical and flattened at the apex, whereas the annulate knobs appear as irregular stacks of disks. All of the cereal rusts that have been studied were judged to have the coglike ornaments. However, these two types of ornaments may not be distinctly different but may represent variations in the degree of differentiation of the individual disks. Both immature and mature (Fig. 26) aecioscope ornaments of P. *coronata* show lateral striations that correspond to irregularities in their sides, indicating a stacked-disk arrangement. The aecial ornaments of the cereal rusts, although previously defined as coglike, are probably built up by the stacking of disks.

With two known exceptions, the aeciospores of the cereal rusts are covered with only the coglike processes. The exceptions are *P. giaminis* f. sp. *tritici* (Fig. 25) (Holm *et al.*, 1970) and *P. poarum* (Henderson *et al.*, 1972), which in addition have large refractile granules interspersed among the coglike processes.

The process of aeciospore wall ornament development is interpreted diagrammatically in Fig. 27. The ornaments begin to form within the primary wall of the immature aeciospore shortly after intercalary cell formation. They first appear as electron-lucent areas against the plasmalemma and extend into the wall. The primary wall continues to thicken, and at the same time the

ornaments grow outward, presumably by periodic addition of new material at their bases against the plasmalemma. After the ornaments have attained their full extension the primary wall begins to disintegrate. Subsequently, a secondary wall forms that intervenes between the ornaments and the plasmalemma. The secondary wall continues to thicken, and at the same time the primary wall dissolves away from around the ornaments, leaving them exposed and attached to the surface of the secondary **wall.**

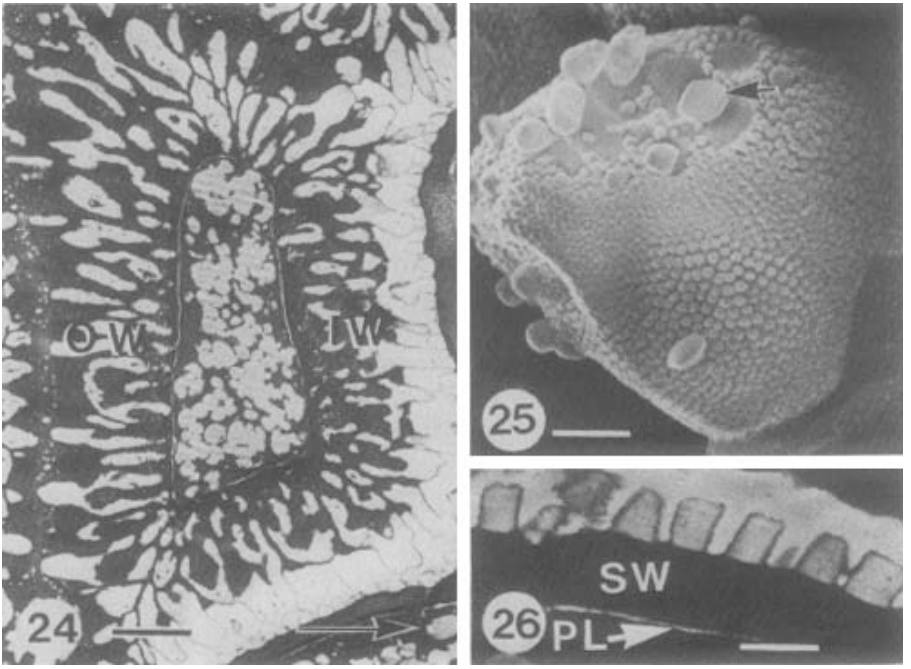


Fig. 24. An aecial peridial cell of *Puccinia coronata.* The cell wall (OW) facing the outside of the aecium is thicker than the wall (IW) facing the aecial cavity (arrow). The wall consists of an electron-dense matrix through which occur electron-lucent, branched processes. The processes extend beyond the surface of only the inward-facing wall (x4200; bar, 2.40 μm). (From D. E. Harder, unpublished.) **Fig.** 25. A scanning electron micrograph of a mature aeciospore of *Puccinia giaminis* f. sp. *tritici.* Note the small coglike ornaments and the larger refractile granules (arrow) (x3000; bar, 3.30 μm). (From Holm *et al.,* 1970. Reproduced with permission of the editor, *Svensk Botanisk Tidskrift.)* **Fig.** 26. Wall ornaments located on the surface of the secondary wall (SW) of a mature aeciospore of *Puccinia coronata.* PL, Plasmalemma. This micrograph was overexposed to reveal the probable stacked-disk construction of the ornaments (x 12,500; bar, 0.80 μm). (From D. E. Harder, unpublished.)

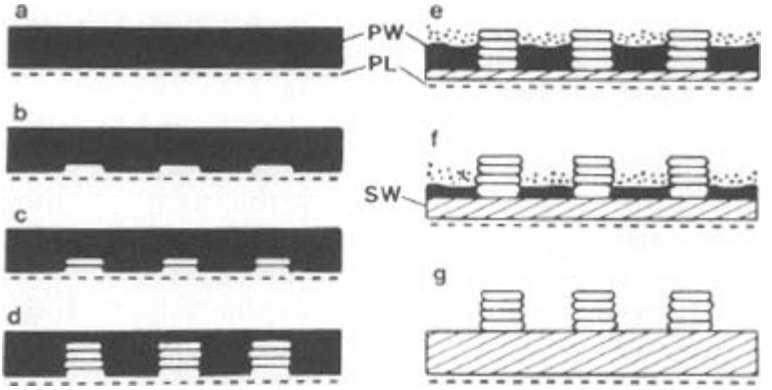


Fig. 27. Diagrammatic representation of ornament formation in *Puccinia* spp. (a) Primary wall (PW) with plasmalemma (PL), (b) First disk formed in the primary wall against the plasmalemma. (c) and (d) Formation of successive disks until the mature size of the ornament is attained, (e)-(g) Dissolution of the primary wall and formation and thickening of the secondary wall (SW). At maturity, the ornaments rest on the surface of the secondary wall. (Drawn by Dr. J. Chong.)

V. Uredia

A. MORPHOLOGY AND CELL TYPES

The uredia of the cereal rusts are not bound by a defined layer of cells, thus they are morphologically not discrete. The first phase of uredium development is marked by an aggregation of fungal cells in an intercellular space underneath the epidermis. These cells, the *sporogenous* (i.e., basal) cells, become closely packed and form the base of the uredium. The sporogenous cells are somewhat elongated hyphalike cells, enlarged at the spore-forming end. The protoplasts of these cells are characteristic of those of the intercellular hyphae. The sporogenous cells give rise to the *pedicels* (i.e., stalk cells) and *mediospores*. The uredia of some rusts contain accessory cells such as *paraphyses* and/or sterile interstitial cells. Of the cereal rusts, only the uredia of *P. coronata* contain paraphyses.

Figure 28 is a scanning micrograph through a uredium of *P. coronata* showing the urediospores, paraphyses, and pedicels or interstitial cells. The paraphyses tend to predominate at the margin of the uredium, although they also may occur within the uredium. The paraphyses in Fig. 28 appear somewhat collapsed, probably largely because of dehydration during processing. The paraphyses, along with the interstitial cells, contain unusual inclusions in their cytoplasm. Figure 29 is a longitudinal section through a

young paraphysis cell of P. *coronata,* which contains irregularly shaped electron-lucent inclusions. In contrast to an earlier conclusion (Harder, 1976c), similar inclusions have subsequently been found in uredia of *P. recondita, P. graminis* f. sp. *tritic,* and P. *graminis* f. sp. *avenae* (Fig. 30), which are not paraphysate. Cells with similar inclusions are also found in pycnia of P. *coronata* (D. E. Harder, unpublished) and *Gymnosporangium juniperi-virgin-ianae* (Mims *et al.,* 1976), buffer cells in telia of the latter fungus (Mims, 1977), and various cells in uredia of *Melampsora lini* (Hassan and Littlefield, 1979). The common factor in all of the cells with these inclusions is that they are sterile cells in the various fruiting bodies of the rusts. In the case of buffer cells or paraphyses, they perhaps add mechanical support. However, the composition of the inclusions is not known, and they also do not occur in the pycnial paraphyses of P. *coronata* (D. E. Harder, unpublished). Many of the cells in which they are found appear to have no traceable function; they are isolated cells occurring interspersed in the sporogenous tissue, and they are frequently degenerative. Mature uredia of P. *coronata* contain numerous cells of this type. The inclusions in these cells continue to grow; they coalesce (Fig. 31), and eventually the cells collapse. These peculiar cells appear to be of wide occurrence in the fruiting tissue of the rusts; in the case of the nonparaphysate uredia of the cereal rusts, they may represent aborted paraphysis-type cells. A similar situation also could apply to P. *coronata,* except that some of these cells, particularly at the uredial margins, develop into paraphyses.

B. UREDIOSPORE ONTOGENY

Urediospores have been defined morphologically as always borne singly on pedicels that arise from successive new growing points on a sporogenous cell (Kunholtz-Lordat, 1943). This definition essentially describes the mode of urediospore formation in most rust fungi, including *Puccinia.* The succession of urediospores from a sporogenous cell defines them as sympoduloconidia (Hughes, 1970). The successive formation of urediospores from a sporogenous cell is illustrated in Fig. 32. Of the cereal rusts, urediospore formation has been studied ultrastructurally in *P. coronata* (Harder, 1976c), *P. sorghi* (Rijkenberg, 1975), and *Physopella zeae* (Heath and Bonde, 1983).

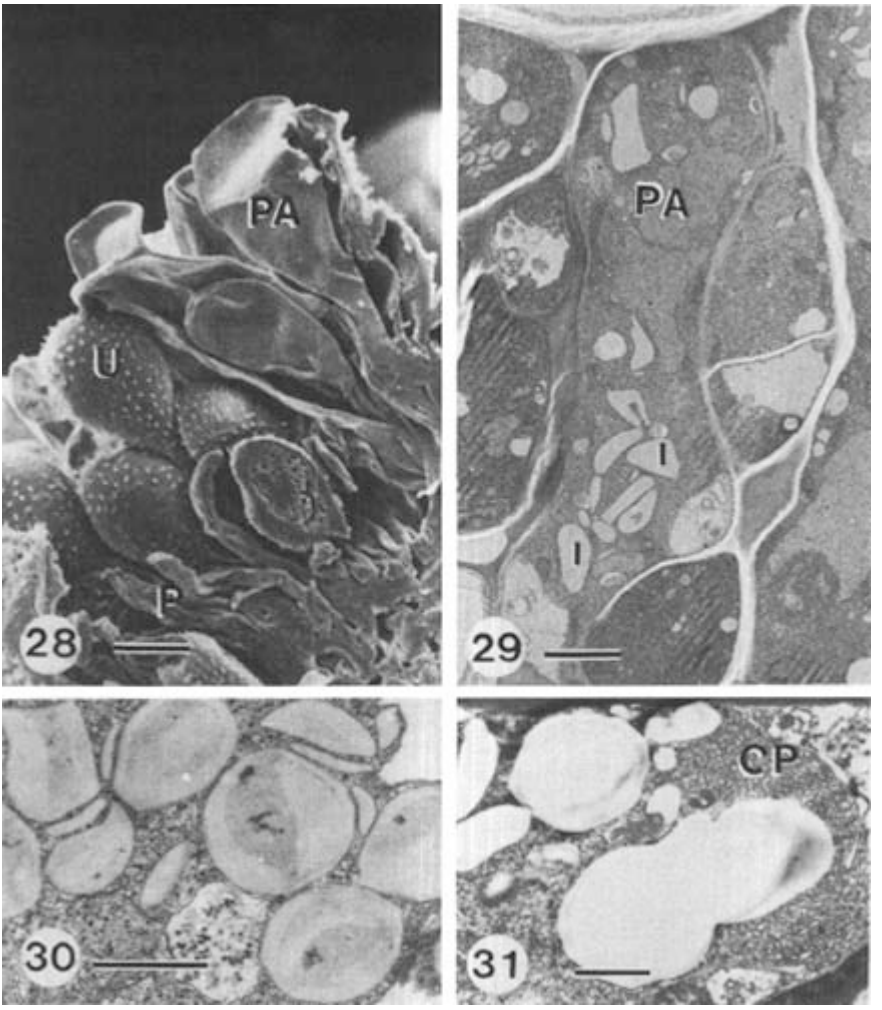


Fig. 28. A scanning electron micrograph near the margin of *Puccinia coronata.* Note the paraphyses (PA), urediospores (U), and smaller cells (P), which are either pedicels after the release of urediospores or interstitial cells (x1000; bar, 10.0 μm). (From Tak-ahashi and Furuta, 1973. Reproduced with permission from Dr. N. Hiratsuka, The Tottori Mycological Institute.) **Fig.** 29. A young paraphysis cell (PA) in a uredium of *Puccinia coronata.* Note the irregularly shaped inclusions (I) in this cell (x4200; bar, 2.40 μm). Figs. 30 **and** 31. Inclusions similar to those in Fig. 29, in interstitial cells in uredia of (Fig, 30) *Puccinia graminis* f. sp. *avenae* and (Fig. 31' *P. coronata.* The cytoplasmic membranes in these cells frequently form a finely membranous network or may appear as a "crochet pattern" network (CP, Fig. 31) (Fig. 30: X30,000; bar, 0.50 μm. Fig. 31: x13,300; bar, 0.75 urn). (From D. E. Harder, unpublished.)

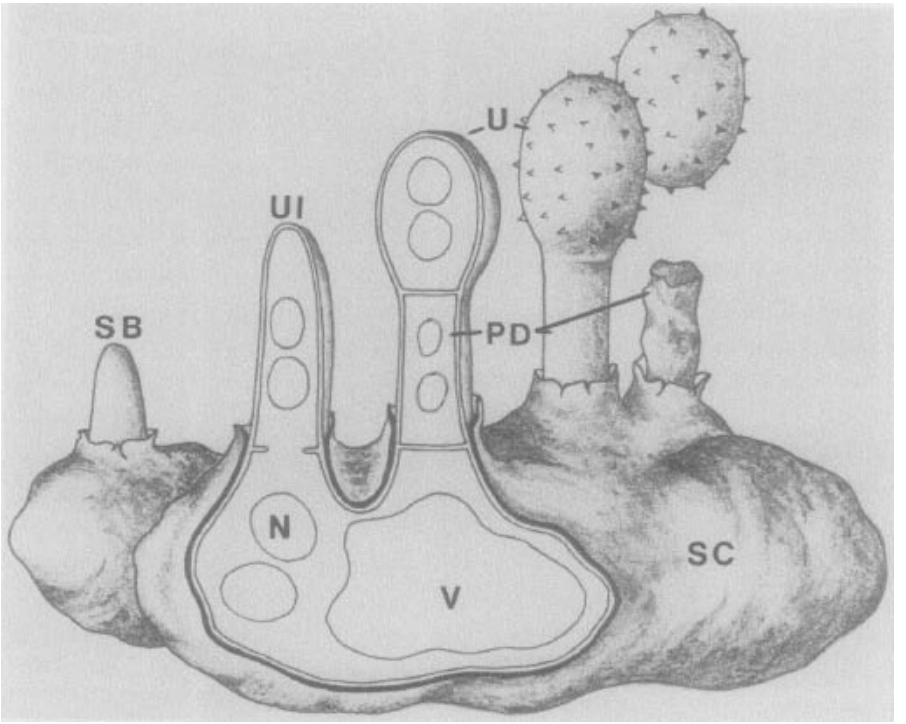
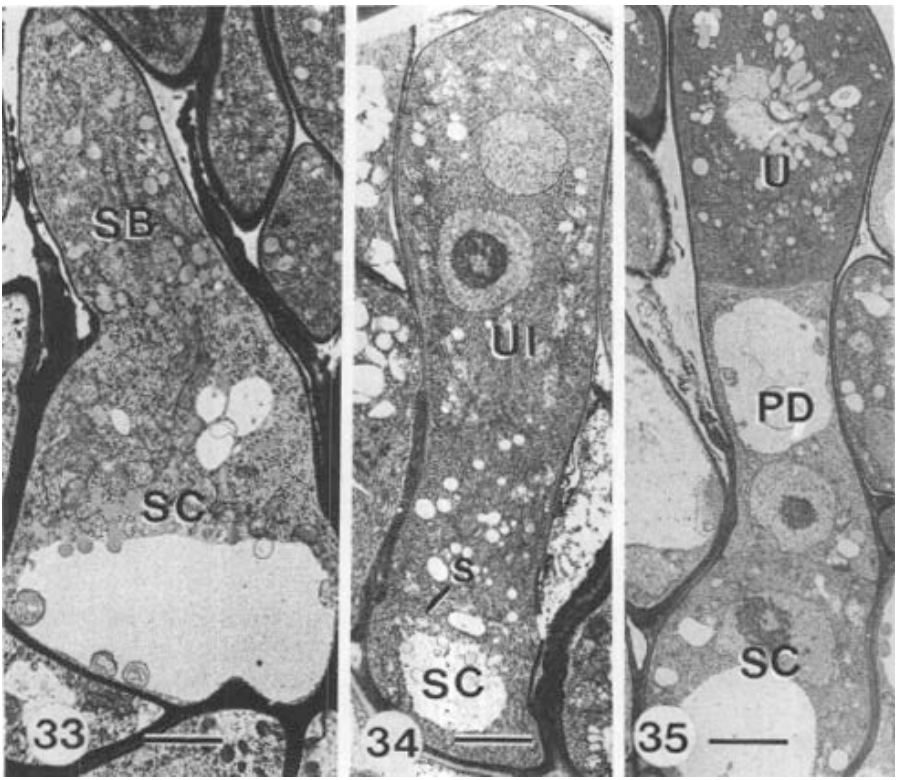


Fig. 32. A diagrammatic partially cutaway illustration of urediospore formation in *Puccinia coronata.* The urediospores (U) are produced successively from new growing points on a sporogenous cell (SC). Shown is the successive formation of a spore bud (SB), urediospore initial (UI), pedicel (PD), and urediospore. N, Nucleus; V, vacuole. (Drawn by Dr. J. Chong.)

Urediospore formation is initiated by the outgrowth of a *spore bud* from the swollen end of a sporogenous cell (Fig. 33). The spore bud is formed by evagination of the inner wall layer through a rupture in the outer wall layer of the sporogenous cell. The relatively thin wall of the spore bud is continuous with the inner layer of the sporogenous cell. Conjugate nuclear division then occurs, the spore bud elongates, and septation occurs to delineate the *urediospore initial* from the sporogenous cell (Fig. 34). During continued growth of the urediospore initial, the number of lipid droplets in this cell increases. A second nuclear division then occurs, followed by nuclear migration and septation to partition the *pedicel* and immature *urediospore* (Fig. 35).

The nuclei in the pedicels remain smaller than those in sporogenous cells or intercellular hyphae (Harder, 1976c). These correspond to the “unexpanded” nuclei in pedicels of *Uromyces fabae* (Savile, 1939). The smaller size of these

nuclei appears to be brought about by their failure to grow to normal ("expanded") size rather than by expulsion of part of the nucleus as during mitosis.



Figs. 33-35. Urediospore formation in *Puccinia coronala*. (Figs. 33 and 35 from Harder, 1976c. Reproduced by permission of the National Research Council of Canada. Fig. 34 from D. E. Harder, unpublished.) Fig. 33. A spore bud (SB) in a stage emerging from a sporogenous cell (SC) by outgrowth of the inner wall layer of the SC (X5100; bar, 2.0 μm). Fig. 34. The stage next to that in Fig. 33; septum (S) formation has begun, to divide the spore bud from the sporogenous cell (SC), to form the urediospore initial (UI) (x4100; bar, 2.40 μm). Fig. 35. The urediospore initial has divided to form the pedicel (PD) and immature urediospore (U) (x3500; bar, 2.90 μm).

The young urediospores rapidly grow to mature size, accompanied by increased density of the cytoplasm, disappearance of vacuoles, increased accumulation of lipid droplets, wall thickening, and spine development. The septal wall separating the urediospore and pedicel is thickened only on the urediospore side of the septum. On the pedicel side, the septal wall remains approximately as thick as that in the intercellular hyphae. A channel extends

through the thickened portion of the cross wall to a septal pore (*P. graminis* f. sp. *tritici*, Ehrlich and Ehrlich, 1969; also see an equivalent channel and pore in a teliospore, Fig. 41). The septal pores at the spore bases appear to be smaller than those in intercellular hyphal septa, although insufficient sections have been examined to obtain reliable measurements. These pores also do not possess a septal pore apparatus.

C. UREDIOSPORE MORPHOLOGY

1. Protoplasts

The urediospore protoplasts are dense and contain most of the usual cellular constituents, that is, nuclei, mitochondria, endoplasmic reticulum, vesicles, ribosomes, and storage material. The mitochondria are more rounded and more compact than those in intercellular hyphae. The urediospores are typically packed with lipid droplets, which is their major storage product. Glycogen has been reported to occur in *P. graminis* f. sp.*tritici* (Ehrlich and Ehrlich, 1969) and P. *recondita* (Salako, 1981), but it was not found in urediospores of *P. coronata* (Harder, 1976c).

There are conflicting reports concerning the absence or presence of nucleoli in nuclei of mature urediospores or in germ tubes (see M. C. Heath and Heath, 1978, and references). Nucleoli were found in all growth phases, including mature urediospores, of *Uromyces phaseoli* var. *vignae* (M. C. Heath and Heath, 1978). In *P. coronata* the nucleolus in the most mature urediospore in which it could be found was a fibrillar ring-shaped structure with a large central lacuna (D. E. Harder, unpublished). A similar configuration was found in a mature urediospore of *P. graminis* f. sp. *tritici* (Mitchell and Shaw, 1969) or in a germ tube of *U. phaseoli* var. *vignae* (M. C. Heath and Heath, 1978). This type of nucleolus has been associated with presumed decrease in nucleolar function (Smetana and Busch, 1974). M. C. Heath and Heath (1978) attributed the inability to find nucleoli, or reports of their reduced size in mature urediospores, as possibly due to insufficient numbers of sections of any one sample being examined. One further problem is that little is known about the effects of the conventional processing procedures on the protoplasts of mature urediospores. The thick walls and dense protoplasts of these spores make structural preservation by chemical means very difficult. The nucleoli that have been shown in mature urediospores appear to exist in a modified fibrillar form. There may be variation in levels of preservation or contrasting by various workers, contributing to the inconsistency in the literature.

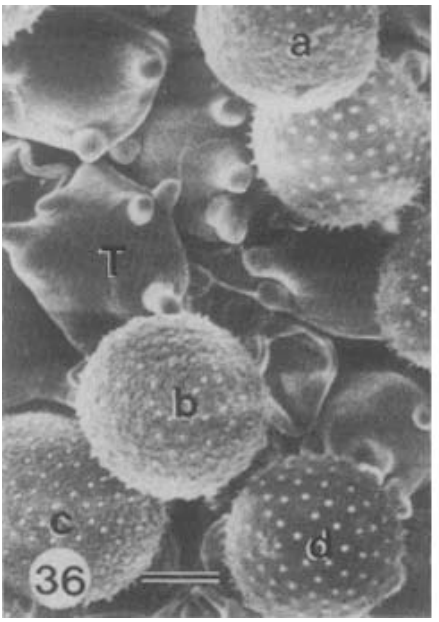
2. *Walls and Ornaments*

The mature urediospore walls consist entirely of secondary wall material, the primary walls having dispersed during spore maturation (see later and Fig. 38). The walls of hydrated spores are ~ 1.0-1.5 nm thick and consist of several layers. Earlier reports (Ehrlich and Ehrlich, 1969; Thomas and Isaac, 1967; Williams and Ledingham, 1964) had indicated a three-layered wall: a thin pellicle-like outer layer, a relatively narow middle layer, and a broad inner layer. However, the resolution of wall layers appears to depend on the processing methods used. With freeze-etch *(Melampsora lini,* Littlefield and Bracker, 1971b) or several histochemical treatments *(P. graminis* f. sp. *tritici,* Rohringer *et al,* 1984), the broad inner zone may be resolved into at least two layers, indicating a four-layered urediospore wall.

The urediospores of the *Puccinia* spp. are echinulate, with minor variations in surface morphology among some species (Brown and Brotzman, 1979). The spines are normally slightly bent at the tip and are located on the surface of the spore wall, surrounded by a somewhat raised annulus (Fig. 37).

Spine development in *P. graminis* f. sp. *tritici* was first described by Thomas and Isaac (1967). A correlative scanning and transmission electron microscope study of *P. spargenoides* (Amerson and Van Dyke, 1978) has provided the most comprehensive view of spine development in the rusts. With minor variations, the ontogeny of urediospore spines appears essentially similar in most of the rust fungi. Figure 36 is a scanning electron micrograph showing urediospores in several stages of development (labeled a-d, from youngest to oldest). Spine development is illustrated diagrammatically in Fig. 38. Spine initials first become evident at about the time that secondary wall formation occurs along the pedicel—urediospore septum; they appear as an electron-lucent area just beneath the primary wall (Fig. 38a). There appears to be a concentration of endoplasmic reticulum around the inner periphery of the spore at this stage *(P. coronata;* D. E. Harder, unpublished). Most reports indicate the persistence of endoplasmic reticulum at the base of developing spines. As the spine begins to lengthen, some primary wall material is deposited toward the base of the spine, but the wall disperses at the tip of the spine (Fig. 38b). Subsequently, secondary wall material is formed that invaginates into the spore around the base of the spine (Fig. 38c). Further development is marked by thickening and straightening of the secondary wall and disintegration of the primary wall radially from the spine, until the spine is fully exposed on the surface of mature spore wall (Fig. 38d-f). The pellicle remains intact during this process, and eventually covers the mature spine. In scanning micrographs the surfaces of immature urediospores are wrinkled (evident in Fig. 36b), which is likely due to the partial disintegration of the primary wall. The dissolution of

the primary wall leaves polygonal ridges between the spines of *P. coronata* (Corlett, 1970), some evidence of which remains in Fig. 37.



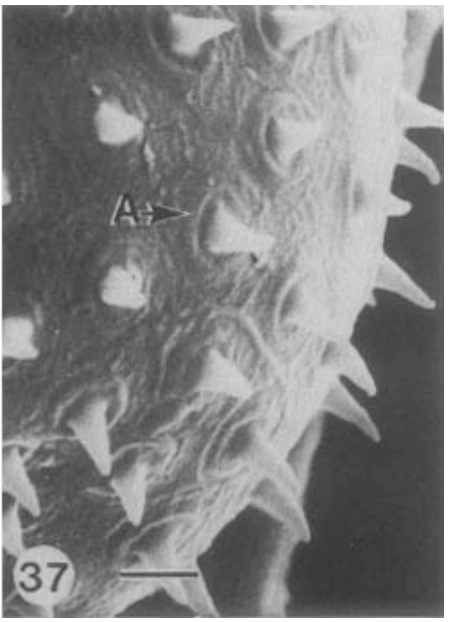


Fig. 36. A scanning electron micrograph of part of a mature uredium of *Puccinia coronata*. Various stages in sequence of spine emergence may be seen in spores a-d. Teliospores (T) are developing in this uredium (x 1300; bar, 7.70 am). (From Takahashi and Furuta, 1973. Reproduced with permission by Dr. N. Hiratsuka, The Tottori Mycological Institute.) **Fig.** 37. A scanning electron micrograph of a mature urediospore of *Puccinia coronata*. The spines are surrounded by a raised annular ring (A) (x 10,000; bar, 1.0 μm). (From Takahashi *et al.*, 1978. Reproduced by permission from Dr. R. Kawashima, Agricultural Research Center, Japan.)

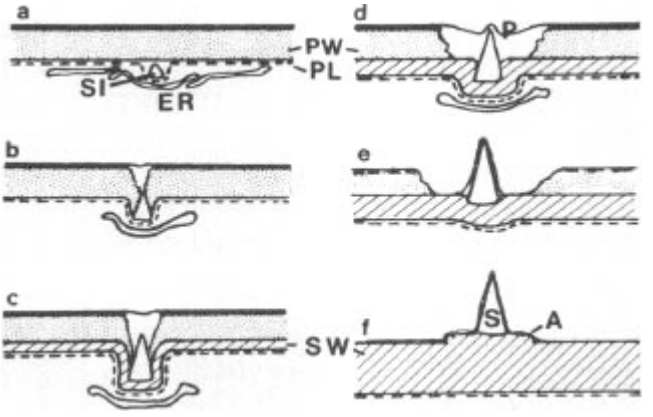


Fig. 38. Diagrammatic illustration of the successive stages of urediospore spine (S) formation. (a) Formation of a spine initial (SI) between the plasmalemma (PL) and primary spore wall (PW). Endoplasmic reticulum (ER) is prominent in this region. The primary wall consists of a relatively broad inner layer and a thin electron-dense pellicle-like outer layer, (b) Lengthening of spine, dissolution of the primary wall at the spine apex, and some growth of the primary wall toward the base of the spine. A pellicle (P) remains intact across the dissolved portion of the primary wall at this and all subsequent stages. (c) and (d) Further growth of the spine, continued dissolution of the primary wall radially from the spine, and development of a secondary wall (SW) layer. The thickening of the secondary wall layer pushes the spine through the opening in the primary wall. (e) The spine has attained its mature length, the primary wall has nearly dissolved away, and the secondary wall continues to thicken. (f) A mature spine (S) on a somewhat raised annular ring (A) on the surface of the secondary wall. The layers of the secondary wall are not shown in this diagram. The pellicle remains continuous around the spine.

VI. Teliospore Ontogeny

Details of teliospores and their structure are covered in Chapter 12 by Mendgen, in this volume. Hence, in this chapter only teliospore ontogeny will be described. The ultrastructure of teliospore development in several *Puccinia* spp. has been studied by Bennett *et al*. (1978), Harder (1977), and Mims and Thurston (1979).

At various stages in the development of infection the teliospores begin to form alongside the urediospores in the urediosorus. The following description of teliospore ontogeny is from observations of *P. coronata*(Harder, 1977). The teliospore-bud stage is indistinguishable from the comparable urediospore stage. The succeeding stages of teliospore initial, pedicel, and primary spore cell (single teliospore cell stage) formation are also comparable to urediospore formation (comparable stages of teliospore and urediospore formation are respectively illustrated in Figs. 39 and 35). The main feature that distinguishes a teliospore at this stage is thickening of the spore wall at the distal end of the primary spore cell (Fig. 39) and an accumulation of glycogen in the sporogenous and spore tissue. There is relatively much less glycogen in the uredial tissue of *P. coronata,* but it is not known if a comparable distribution occurs in other rusts.

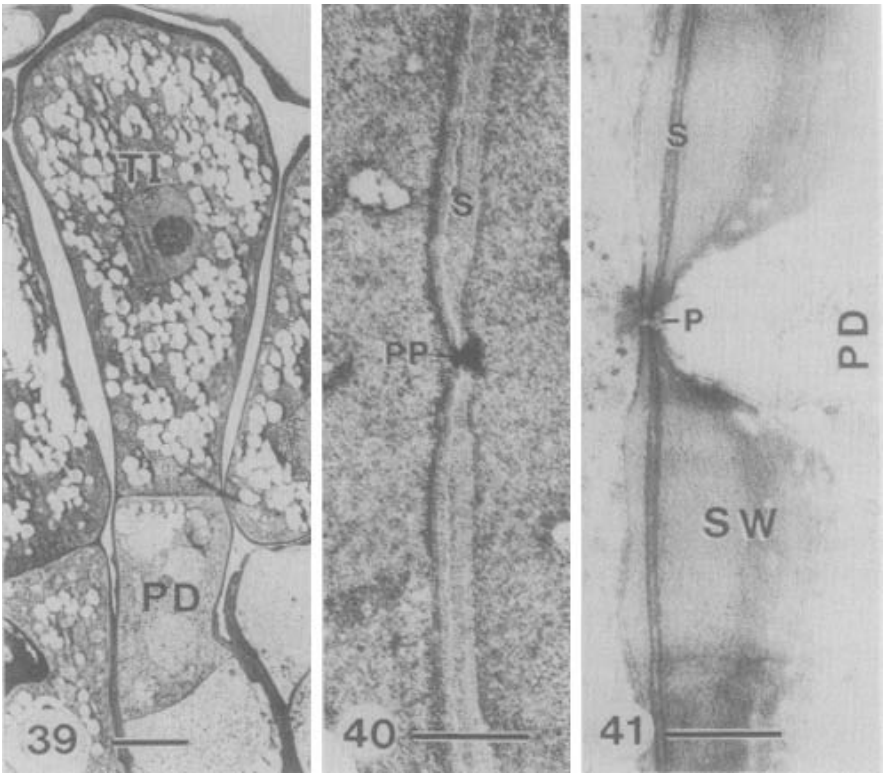


Fig. 39. A stage of teliospore formation of *Puccinia coronata* comparable to that of urediospore formation in Fig. 35. Shown are the pedicel (PD) and teliospore initial (TI). The teliospore initial will undergo one further division to form the two-celled teliospore (x3400; bar, 2.90 μm). (From Harder, 1977. Reproduced with permission of Academic Press, New York.) **Fig.** 40. The septum (S) dividing the two cells of a nearly mature teliospore of *Puccinia coronata.* A pore plue (PP) occludes the septal pore, and (x27,500; bar, 0.55 μm). (From D. E. Harder, unpublished.) **Fig.** 41. The septum (S) and wall between the teliospore and pedicel (PD) in *Puccinia coronata.* Secondary wall (SW) formation has occurred mainly on the pedicel side of the septum. This septum has a small pore (P) at the base of a channel in the secondary wall, which at this stage appears partially occluded (x3500; bar, 0.43 μm). (From D. E. Harder, unpublished.)

Further teliospore development is marked by elongation of the primary spore cell, conjugate nuclear division, and septation to form the final two-celled structure of the teliospore. The septum dividing the two cells is perforate with an electron-dense occlusion in the pore (Fig. 40). There is no septal pore apparatus surrounding these pores. At this stage the wall has not appreciably thickened at the proximal end, but has thickened further at the distal end to form the “crown” for which *P. coronata* is named. Wall thickening at the base

of the spore first occurs along the pedicel-teliospore cross wall. This thickening occurs first as patches along the septum, which coalesce and thicken until mature-wall thickness is attained. There is considerable thickening of the cross wall before there is much thickening of the wall of the lower part of the spore. The cross-wall thickening occurs predominantly on the pedicel side, which distinguishes it sharply from the urediospore-pedicel cross wall, where the thickening occurs only on the spore side. The cross wall at the base of the teliospore is perforate, with a channel extending through the thick secondary wall (Fig. 41). In the latter figure there is a moderately electron-dense, somewhat diffuse occlusion in the pore.

One of the major unknowns of teliospore formation is the precise timing and mode of diploidization. Mature teliospores are highly resistant to ultrastructural processing procedures, thus little is known of their detailed structure. Beyond a certain stage of maturation, membranes appear to be poorly preserved (Harder, 1977). As the teliospores approach maturity, the nuclei of each pair in both cells of the teliospore become closely appressed to one another and appear ready for fusion, but because of poor preservation of membranes, actual fusion has not been observed with certainty.

Note Added in Proof

Recently published information on the tropical rust fungus *Physopella zeae* (Heath and Bonde, 1983) has shown that several successive urediospores are produced from the same site on a sporogenous cell.

References

Aist, J. R., and Williams, P. H. (1972). Ultrastructure and time course of mitosis in the fungus *Fusarium oxysporum. J. Cell Sci.* 55, 368-389. Allen, R. F. (1923). A cytological study of infection of Baart and Kanred wheats by*Puccinia graminis tritici. J. Agric. Res.* (*Washington, D.C.*) 23, 131-152.

Allen, R. F. (1928). A cytological study of *Puccinia glumarum* on *Bromus marginatus* and *Triticum vulgare. J. Agric. Res.* (*Washington, D.C.*) **36**, 487-513.

Allen, R. F. (1932a). A cytological study of heterothallism in *Puccinia triticina. f. Agric. Res. (Washington, D.C.)* 44, 733-754.

Allen, R. F. (1932b). A cytological study of heterothallism in *Puccinia coronata. J. Agric. Res. {Washington, D.C.)* 45, 513-541.

Allen, R. F. (1933a). Further cytological studies of heterothallism in *Puccinia graminis. f. Agric. Res. {Washington, D.C.)* 47, 1-16.

Allen, R. F. (1933b). The spermatia of corn rust, *Puccinia sorghi. Phytopathology* 23, 923-925.

Allen, R. F. (1934). A cytological study of heterothallism in *Puccinia sorghi. J. Agric. Res. (Washington, D.C.)* 49, 1047-1068.

Amerson, H. V., and Van Dyke, C. G. (1978). The ontogeny of echinulation in uredio-spores of *Puccinia sparganioides. Exp. Mycol.* 2, 41-50.

Beckett, A., Heath, I. B., and McLaughlin, D. J. (1974). "An Atlas of Fungal Ultrastructure." Longmans, Green, New York.

Bennett, A. P., Henderson, D. M., and Prentice, H. T. (1978). The teliospores *Puccinia smyrnii* (Uredinales). *Grana* 17, 17-27.

Bracker, C. E., Ruiz-Herrera, J., and Bartnicki-Garcia, S. (1976). Structure and transformation of chitin synthetase particles (chitosomes) during microfibril synthesis *in vitro*. *Proc. Natl. Acad. Sci. U.S.A.* 73, 4570-4574.

Brown, M. F., and Brotzman, H. G. (1977). "Phytopathogenic Fungi: A Scanning Stereoscopic Survey," Ext. Publ. University of Missouri, Columbia.

Buller, A. H. R. (1950). "Researches on Fungi," Vol. VII. Univ. of Toronto Press, Toronto.

Chong, J. (1981). Ontogeny of mono- and dikaryotic haustoria of *Puccinia coronata avenae:* Ultrastructure, cytochemistry and electron probe x-ray analysis. Ph.D. Thesis, Dept. of Botany, University of Manitoba, Winnipeg.

Codron, D. (1981). Etude ultrastructurale des spermagonies et de la spermatogénèse chez quelques Uredinales. *Cryptog. Mycol. 2,* 163-187.

Coffey, M. D. (1975). Obligate parasites of higher plants, particularly rust fungi. *Symp. Soc. Exp. Biol.* 29, 297-323.

Coffey. M. D., Palevitz, B. A., and Allen, P. J. (1972). The fine structure of two rust fungi, *Puccinia helianthi* and *Melampsora lini. Can. J. Bot.* 50, 231-240.

Corlett, M. (1970). Surface structure of the urediniospores of *Puccinia coronata* f. sp. *avenae. Can. J. Bot.* 48, 2159-2161.

Cotter, R. V. (1960). Fertilization of pycnia with urediospores and aeciospores in *Puccinia graminis. Phytopathology* 50, 507-508.

Craigie, J. H. (1927a). Experiments in sex in rust fungi. *Nature* (*London*) **120,** 116-117.

Craigie, J. H. (1927b). Discovery of the function of the pycnia of the rust fungi. *Nature (London)* **120,** 765-767.

Craigie, J. H. (1933). Union of pycniospores and the haploid hyphae in *Puccinia helianthi* Schw. *Nature (London)* 131, 25.

Craigie, J. H. (1959). Nuclear behavior in the diploidization of haploid infections of *Puccinia helinathi. Can. J. Bot.* 37, 843-855.

Craigie, J. H., and Green, G. J. (1962). Nuclear behavior leading to conjugate association in haploid infections of *Puccinia graminis. Can. J. Bot.* **40**, 163-178.

Cummins, G. B. (1959). "Illustrated Genera of Rust Fungi." Burgess, Minneapolis, Minnesota.

Ehrlich, H. G., and Ehrlich, M. A. (1961). Electron microscopy of the host-parasite relationships in stem rust of wheat. *Am. }. Bot.* 48, 538 (abstr.).

Ehrlich, H. G., and Ehrlich, M. A. (1962). Fine structure of *Puccinia graminis tritici* in resistant and susceptible host varieties. *Am. J. Bot.* **49**, 665 (abstr.).

Ehrlich, H. G., and Ehrlich, M. A. (1963). Electron microscopy of the host-parasite relationships in stem rust of wheat. *Am. J. Bot.* **50**, 123-30.

Ehrlich, M. A., and Ehrlich, H. G. (1969). Urediospore development in *Puccinia graminis. Can. J. Bot.* 47, 2061-2064.

Ehrlich, M. A., Ehrlich, H. G., and Schafer, J. F. (1968). Septal pores in the *Heterobasidiomycetidae, Puccinia graminis* and *P. recondita. Am. J. Bot.* **55**, 1020-1027.

Frederick, S. A., and Newcomb, E. H. (1969). Cytochemical localization of catalase in leaf microbodies (peroxisomes). *J. Cell Biol.* **43**, 343—353.

Garrett, W. N., and Wilcoxson, R. D. (1960). Fertilization of pycnia with urediospores in *Puccinia graminis* var. *tritici. Phytopathology* **50**, 636 (abstr.).

Girbardt, M., and Hadrich, H. (1975). Ultrastruktur des Pilzenkernes. III. Des Kern—assozierten Organelles (NAO = "KCE"). Z. *Allg. Mikrobiol.* **15**, 157-167.
Gold, R. E., and Littlefield, L. J. (1979). Ultrastructure of the telial, pycnial and aecial stages of *Melampsora lini. Can. J. Bot.* **57**, 629-638.
Gold, R. E., Littlefield, L. J., and Statler, G. D. (1979). Ultrastructure of the pycnial and aecial stages of *Puccinia recondita. Can. J. Bot.* **57**, 74-86.
Gunning, B. E. S., and Steer, M. W. (1975). "Ultrastructure and the Biology of Plant Cells." Arnold, London.
Harder, D. E. (1976a). Mitosis and cell division in some cereal rust fungi. I. Fine structure of the interphase and premitotic nuclei. *Can. J. Bot.* **54**, 981-994.
Harder, D. E. (1976b). Mitosis and cell division in some cereal rust fungi. II. The processes of mitosis and cytokinesis. *Can. J. Bot.* 54, 995-1009.
Harder, D. E. (1976c). Electron microscopy of urediospore formation in *Puccinia coronata avenae. Physiol. Plant Pathol.* 10, 21-28.
Harder, D. E. (1977). Electron microscopy of teliospore formation in *Puccinia coronataavenae. Physiol. Plant Pathol.* 10, 21-28.
Harder, D. E., and Chong, J. (1978). Ultrastructure of spermatium ontogeny in *Puccinia coronata avenae. Can. J. Bot.* **50**, 395-403.
Hassan, Z. M., and Littlefield, L. J. (1979). Ontogeny of the uredium of *Melampsora lini.* Can. *J. Bot.* **57**, 639-649.
Heath, I. B. (1978). Experimental studies of mitosis in the fungi. *In* "Nuclear Division in the Fungi" (I. B. Heath, ed.), pp. 89-176. Academic Press, New York.
Heath, I. B. (1980). Fungal mitosis, the significance of variations on a theme. *Mycologia* **72**, 229-250.
Heath, I. B. (1981). Nucleus-associated organelles in fungi. *Int. Rev. Cytol.* 69, 191-221.
Heath, I. B., and Heath, M. C. (1976). Ultrastructure of mitosis in the cowpea rust fungus *Uromyces phaseoli* var. *vignae. J. Cell Biol.* **70**, 592-607.
Heath, I. B., and Heath, M. C. (1978). Microtubules and organelle movements in the rust fungus *Uromyces phaseoli* var. *vignae. Cytobiologie* **16**,393-411.
Heath, I. B., and Heath, M. C. (1979). Structural studies of the development of infection structures of cowpea rust, *Uromyces phaseoli* var. *vignae.* II. Vacuoles *Can. j. Bot.* **57**, 1830-1837.
Heath, M. C. (1975). Structure and function of fungal septa. *Proc. Int. Congr. Inst. Assoc. Microbiol. Soc. 1st, 1974* Vol. 2, pp. 117-124.
Heath, M. C., and Bonde, M. R. (1983). Ultrastructural observations of the rust fungus *Physopella zeae* in *Zea mays. Can. J. Bot.* **61**, 2231-2242.
Heath, M. C., and Heath, I. B. (1978). Structural studies of the development of infection structures of cowpea rust, *Uromyces phaseoli* var. *vignae.* I. Nucleoli and nuclei. *Can. J. Bot.* **56**, 648-661.
Henderson, D. M., Prentice, H. T., and Eudall, R. (1972). The morphology of fungal spores. III. The aecidiospores of *Puccinia poarum. Pollen et Spores* **14**, 17-24.
Herr, F. B., and Heath, M. C. (1982). The effects of antimicrotubule agents on organelle positioning in the cowpea rust fungus, *Uromyces phaseoli* var. *vignae. Exp. Mycol.* **6**, 15-24.
Hiratsuka, Y., and Cummins, G. B. (1963). Morphology of the spermagonia of the rust fungi. *Mycologia* **55**, 487-507.
Hoffman, H.-P., and Avers, C. J. (1973). Mitochondrion of yeast: Ultrastructural evidence for one giant, branched organelle per cell. *Science* 181, 749-751.
Holm, L., and Tibell, L. (1974). Studies on the fine structure of aeciospores. III. Aeciospore ontogeny in *Puccinia graminis. Sven. Bot. Tidskr.* 68, 136-152.

Holm, L., Dunbar, A., and von Hofsten, A. (1970). Studies on the fine structure of aeciospores. II. *Sven. Bot. Tidskr.* 64, 380-382.

Howard, R. J. (1981). Ultrastructural analysis of hyphal tip cell growth in fungi: Spizenkorper, cytoskeleton and endomembranes after freeze-substitution. *J. Cell Sci.* **48**, 89-103.

Hughes, S. J. (1970). Ontogeny of spore forms in Uredinales. *Can. J. Bot.* 48, 2147-2157. Kozar, F., and Netolitzky, H. J. (1975). Ultrastructure and cytology of pycnia, aecia, and aeciospores of *Gymnosporangium clavipes. Can. J. Bot.* 53, *972-977.*

Kuhnholtz-Lordat, G. (1943). Essai de biologie des spores der diplonte chez less Uredinees. *Bull. Soc. Mycol. Fr.* **59**, 78-156.

Lamb, I. N. (1935). The initiation of the dikaryophase in *Puccinia phragmitis* (Schum.) Korn. *Ann. Bot.* (*London*) **49**, 403-438.

Littlefield, L. J., and Bracker, C. E. (1971a). Ultrastructure of septa in *Melampsora lini. Trans. Br. Mycol. Soc.* 56, 181-188.

Littlefield, L. J., and Bracker, C. E. (1971b). Ultrastructure and development of urediospore ornamentation in *Melampsora lini. Can. J. Bot.* 49, 2067-2073.

Littlefield, L. *}.,* and Heath, M. C. (1979). "Ultrastructure of Rust Fungi." Academic Press, New York.

McLaughlin, D. J. (1981). The spindle pole body and postmeiotic mitosis in *Auricularia fuscosuccinea. Can. J. Bot.* **59**, 1196-1206.

Manocha, M. S. (1971). Presence of nucleolus in flax rust axenic culture. *Phytopathol. Z.* 71, 113-118.

Maxwell, D. P., Arementrout, V. N., and Graves, L. B., Jr. (1977). Microbodies in plant pathogenic fungi. *Annu. Rev. Phytopathol.* 15, 119-134.

Mendgen, K. (1973). Microbodies (glyoxysomes) in infection structures of *Uromyces phaseoli. Protoplasma* 78, 477-482.

Metzler, B. (1981). Pyknidialstruktur und Pyknosporgenese bei *Gymnosporangium fuscum* DC. *Z. Mykol.* **47**, 271-280.

Mims, C. W. (1977). Ultrastructure of teliospore formation in the cedar-apple rust fungus *Gymnosporangium juniperi-virginianae. Can. J. Bot.* 55, 2319-2329.

Mims, C. W., and Thurston, E. L. (1979). Ultrastructure of teliospore formation in the rust fungus *Puccinia podophylli. Can. J. Bot.* 57, 2533-2538.

Mims, C. W., Seabury, F., and Thurston, E. L. (1976). An ultrastructural study of spermatium formation in the rust fungus *Gymnosporangium juniper-virginianae. Am. J. Bot.* 63, 997-1002.

Mitchell, S., and Shaw, M. (1969). The nucleolus of wheat stem rust urediospores. *Can. J. Bot.* 47, 1887-1889.

Moore, R. T. (1963a). Fine structure of mycota. X. Thallus formation in *Puccinia podophylli. Mycologia* 55, 633-642.

Moore, R. T. (1963b). Fine structure of mycota. XI. Occurrence of the Golgi dictyosome in the Heterobasidiomycete *Puccinia podophylli. J. Bacteriol.* 86, 866-871.

Moore, R. T., and McAlear, *J.* H. (1961). Fine structure of mycota. 8. On the aecidial stage of *Uromyces caladii. Phytopathol. Z.* **42**, 297-304.

Morre, D. J., and Mollenhauer, H. H. (1974). The endomembrane concept: A functional integration of endoplasmic reticulum and Golgi apparatus. *In* "Dynamic Aspects of Plant Ultrastructure" (A. W. Roland, ed.), pp. 87-137. McGraw-Hill, New York.

O'Donnell, K. L., and McLaughlin, D. J. (1981a). Ultrastructure of meiosis in the hollyhock rust fungus, *Puccinia malvacearum.* I. Prophase I—prometaphase I. *Pro-toplasma* **108,** 225-244.

O'Donnell, K. L., and McLaughlin, D. J. (1981b). Ultrastructure of meiosis in the hollyhock rust fungus, *Puccinia maivacearum.* II. Metaphase I—telophase I. *Protoplasma* **108,** 245-263.

O'Donnell, K. L., and McLaughlin, D. J. (1981c). Ultrastructure of meiosis in the hollyhock rust fungus, *Puccinia maivacearum.* III. Interphase I—interphase II. *Protoplasma* **108,** 265-288.

Rice, M. A. (1927). The haustoria of certain rusts and the relationship between the host and pathogen. *Bull. Torrey Bot. Club* **54**, 63-153.

Rijkenberg, F. H. J. (1975). The uredial stage of maize rust. *Proc.—Electron Microsc. Soc. South Afr.* **5**, 35-36.

Rijkenberg, F. H. J., and Truter, S. J. (1974a). The ultrastructure of sporogenesis in the pycnial stage of *Puccinia sorghi. Mycologia* **66,** 319-326.

Rijkenberg, F. H. J., and Truter, S. J. (1974b). The ultrastructure of the *Puccinia sorghi* aecial stage. *Protoplasma* **81**, 231-245.

Rijkenberg, F. H. J., and Truter, S. J. (1975). Cell fusion in the aecium of *Puccinia sorghi*. *Protoplasma* **83**, 233-246.

Rohringer, R., Harder, D. E., and Chong, J. (1984). Ultrastructure and cytochemistry of the walls of urediosporelings of *Puccinia graminis tritici* and P. *coronata.* In preparation.

Ruttle, M. L., and Fraser, W. P. (1927). A cytological study of *Puccinia coronata* Cda. on Banner and Cowra 35 oats. *Univ. Calif., Berkeley, Publ. Bot.* **14**,21-72.

Salako, E. A. (1981). The ultrastructure of the urediospore of *Puccinia recondita. Mycopathologia* **76**, 3-12.

Savile, D. B. O. (1939). Nuclear structure and behavior in species of the Uredinales. *Am. J. Bot.* **26**, 585-609.

Savile, D. B. O. (1954). Cellular mechanics, taxonomy and evolution in the Uredinales and Ustilaginales. *Mycologia* **46**, 736-761.

Smetana, K., and Busch, H. (1974). The nucleolus and nucleolar DNA. *In* "The Cell Nucleus" (H. Busch, ed.). Vol. 1, pp. 73—147. Academic Press, New York.

Takahashi, H., and Furuta, T. (.1973). Observation on surface structure and behavior of *Puccinia coronata* Corda var. *avenae* Fraser et Leding on the host plant by scanning electron microscopy. *Rep. Tottori Mycol. Inst.* **10**, 253-272.

Takahashi, H., Yamada, M., and Furuta, T. (1978). Studies on surface fine structure of some rust fungi, *J. Cent. Agric. Exp. Stn.* 27, 93-155.

Thomas, P. L., and Isaac, P. K. (1967). The development of echinulation in urediospores of wheat stem rust. *Can. J. Bot.* **45**, 287-289.

Williams, P. G., and Ledingham, G. A. (1964). Fine structure of wheat stem rust urediospores. *Can. J. Bot.* **42**, 1503-1508.

Wright, R. G., Lennard, J. H., and Denham, D. (1978). Mitosis in *Puccinia striiformis.* II. Electron microscopy. *Trans. Br. Mycol. Soc.* **70**, 229-237.

12

Development and Physiology of Teliospores

Kurt Mendgen
Fakultät für Biologie, Lehrstuhl Fhytopathologie,
Universitat Konstanz, Konstanz, Federal Republic of Germany

I. Introduction

In moderate climates generally during autumn, the dikaryotic mycelium that forms uredia with urediospores (see Harder, Chapter 11, this volume) begins to differentiate the overwintering spore form of the rusts, the teliospores. Teliospores were first described as fungal spores by the Tulasne brothers (Tulasne and Tulasne, 1847), who called them "perfect," or true spores. De Bary (1865) proposed for these true spores the term "Teleutosporen," because it means spores that appear at the end of the development of the species and may be applied to all homologous organs of rusts ("… Teleutosporen … bedeutet Sporen, welche am Ende der Entwicklung der Species auftreten und darum fur

die homologen Organe *sämmtlicher* Uredineen anwendbar sind"). Later, Arthur (see Arthur, 1929, 1932) defined the term *teliospore,* because they are the spores that are formed in the telia, the last fruiting structure of the rusts. More recently, teliospores have been defined as the basidia-producing spores of the rust fungi (Hiratsuka, 1973).

The teliospores are important for overwintering of most cereal rusts, and they are largely responsible for the formation of new physiologic races of the rusts (see Anikster and Wahl, 1979). Form and ornamentation of teliospores help to categorize the rusts. For a morphological description of the respective cereal rust teliospores, the reader is referred to the manuals of Cummins (1971), Gaumann (1959), and Urban (1969). The older, more general information on teliospores is summarized by Arthur (1929, 1934), Lehmann *et al.* (1937), Cummins (1959), and Hassebrauk (1962) in their descriptions of the different species. This chapter gathers more recent information on general characteristics of teliospores of cereal rust fungi. Where the information on important aspects is missing for cereal rusts, results from other rusts are included.

II. Morphology and Ontogeny of Teliospore and Basidiospore Formation

A. TELIOSPORE MORPHOLOGY AND ONTOGENY

Following urediospore production, teliospores are formed as a second type of spore in the uredium. The uredium is thus transformed into the telium or telial sorus. The telium may be open or covered by the epidermis of the host plant; see individual descriptions by Cummins (1971) or Gaumann (1959). The teliospores develop as pedicellate spores (Hughes, 1970; Harder, Chapter 11, this volume), which are produced from sporogenous cells (Fig. 1). The sporogenous cells are also responsible for urediospore formation (see Harder, Chapter 11, this volume). When a uredium has differentiated into a telium, the sporogenous cell of*Puccinia coronata* divides to form a remnant sporogenous cell (Figs. 1B and 2), a pedicel, and, in case of a telium, a primary teliospore cell (Harder, 1977). After nuclear division and subsequent development of a septum, the two-celled teliospore forms with two nuclei per cell (Fig. 1C,D). A heavy cap of wall material forms the crown at the terminal end of the outer cell (Fig. 3). Maturation of the teliospore is accompanied by an increase of cytoplasmic density, disappearance of vacuoles, and an accumulation of lipid droplets and glycogen-like material (Fig. 4). Densely staining secondary wall material forms, and a complex multilayered (as many as six layers) wall structure develops. Finally, the two nuclei within each cell become closely appressed and nuclear fusion occurs (Fig. 1E). The process of fusion has not

been observed ultrastructurally, because fixation of spores appears to be very difficult at that stage (Harder, 1977). Sometimes nuclear fusion is delayed in teliospores of *P. sorghi* and may happen during overwintering (Pavgi, 1975).

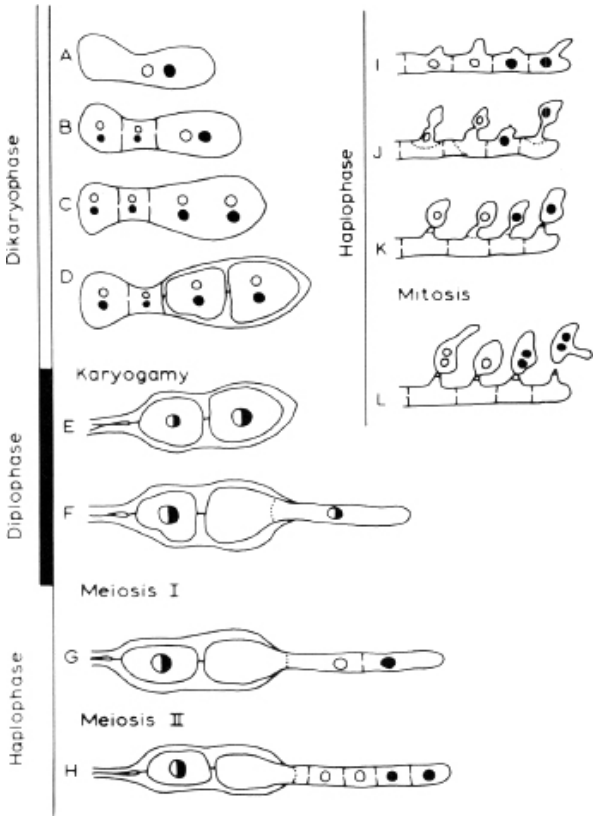


Fig. 1. Schematic sequence of teliospore and basidiospore formation. (A) Sporogenous cell. (B) Remnant sporogenous cell, pedicel, and primary teliospore cell. (C) Mitosis in the primary teliospore cell. (D) Septum formation results in the two-celled teliospore. (E) Nuclear fusion and maturation of the teliospore. |F) Germ tube emergence. (G) First meiotic division. (H) Second meiotic division. (I) Development of sterigma. (I) Vesicles with cytoplasm and nucleus develop at the end of the sterigma. (K) The basidiospores form. (L) Mitosis in the basidiospores and subsequent germination.

Teliospore formation appears to be similar in the different rusts studied ultrastructuraily, such as *Uromyces appendiculatus* (Müller *et*

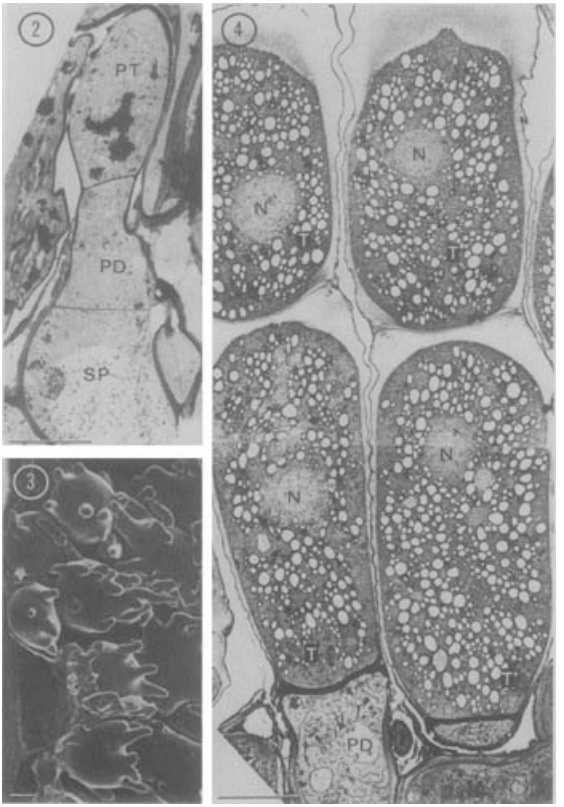


Fig. 2. Transmission electron micrograph (TEM) showing a sporogenous cell of *Puccinia coronata* that has divided to form the primary teliospore cell |PT), the pedicel (PD), and the remnant sporogenous cell (SP) (x3780; bar, 5 μm) (courtesy D. E. Harder, Winnipeg). **Fig.** 3. Scanning electron micrograph (SEM) of surface view of a telial sorus, showing the cap or crown of teliospores of *P. coronata*. (x11OO; bar, 5 μm). (From Takahashi and Furuta, 1973, with permission.) **Fig.** 4. Cross section of a mature teliosorus of *P. coronata,* showing the two teliospore cells (T) and the adhering pedicel (PD). Nuclear fusion has occurred; N, nucleus (TEM, x3700; bar, 5 μm) (courtesy D. E. Harder, Winnipeg).

al., 1974), *Gymnosporangium juniperi-virginianae* (Mims *et al.,* 1975; Mims, 1977b), *P. podophylli* (Mims and Thurston, 1979), *P. smyrnii* (Bennell *et al.,* 1978), and *Tranzschelia* (Bennell and Henderson, 1978). The substructure of the spore wall is somewhat different, however. The results are difficult to interpret, because staining characteristics of the wall differ very much with spore age and fixation (Mims and Thurston, 1979; Rijkenberg, 1977).

P. coronata has an apical or lateral germ pore that is indicated by a marked reduction in cell wall thickness (Harder, 1977). In *P. sorghi*, the wall becomes thickest at the apex of the spore, and a blunt, peglike projection of cytoplasm into the thickening apical wall marks the site of the germ pore (Rijkenberg, 1977) (Fig. 5).

Teliospore ornamentation is extremely variable, and a classification of the ornaments based on electron microscopy is offered by von Bran-denburger and Schwinn (1971) and Littlefield and Heath (1979). Wartlike ornaments of *P. smyrnii* appear initially, formed beneath the primary teliospore wall, and later are extruded to the surface of the spore (Bennell *et al.*, 1978). The digitate processes that extend from the apex of *P. coronata* teliospores (Fig. 3) represent extensive wall thickenings (Harder, 1977). In *P. podophylli* (Mims and Thurston, 1979), the cytoplasm initially extends to the tip of the many thin-walled spines. Later, the cytoplasm disappears as a consequence of thickenings of the spore wall.

Most cereal rust teliospores have been described only by light microscopy (Cummins, 1971; Gaumann, 1959; Guyot, 1938, 1951, 1956; Ullrich, 1977; Urban, 1969). Morphology may be race-specific, as observed in *P. sorghi* (Pavgi, 1969), or differ after growth on different host grasses (Arthaud, 1969). Teliospores may be one- to five-celled (Ka-pooria, 1973) in *P. penniseti*. One-celled teliospores (mesospores) may comprise as much as 80% of the total found in *P. hordei* Otth. (Gaumann, 1959) and 2-17% of the total found in *P. striiformis* (Gaumann, 1959). In some isolates of *P. hordei* and *P. recondita*, 1% of teliospores may be three-celled (Y. Anikster, personal communication). Even more variability is observed when teliospores of *P. graminis* are formed in artificial culture (Rajendren, 1972).

B. TELIOSPORE GERMINATION AND BASIDIOSPORE FORMATION

Teliospore germination and basidiospore formation have been reviewed by Gaumann (1926), Arthur (1929), Hassebrauk (1962), and more recently by Petersen (1974) and Littlefield and Heath (1979). The important aspects are described here, including results from *P. sorghi* (Pavgi, 1975) and work on noncereal rusts (Kohno *et al.*, 1974, 1975; Mims, 1981; Mims *et al.*, 1976; O'Donnell and McLaughlin, 1981a-d). In most cereal rusts, teliospores are dormant and germinate only after some months exposure to outdoor conditions (see next section). After hydration and before germ tube emergence, vesicles were observed in the cytoplasm of *Uromyces phaseoli* teliospores (Gold and Mendgen, 1981a). Fusion and reduction in size of lipid droplets, appearance of electron-translucent regions in the cytoplasm, and an enlargement of the fused nucleus were observed in *Gymnosporangium clavipes* (Mims, 1981). The pedicel of *P. sorghi* swells and disintegrates (Pavgi, 1975). In *G. clavipes*, the outer portion of the germ pore region disintegrates while the inner portion is

pushed out and filled by the emerging germ tube (Mims, 1981). This germ tube or promycelium emerging from the teliospore is called a metabasidium, because meiosis occurs within this structure (for nomenclature, see Littlefield and Heath, 1979; Petersen, 1974; see also Wells and Wells, 1982). In *P. sorghi*, the metabasidium grows out of the germ pore of the apical cell alone or of both cells simultaneously (Fig. 6). The germ tube wall appears to be continuous with an inner layer of the teliospore wall (Kohno *et al.*, 1975; Mims *et al.*, 1975; Mims, 1981). The entire protoplast usually migrates with the diploid nucleus into the metabasidium, and a septum is laid down at the base of the metabasidium in *P. sorghi*. Sometimes, a portion of the cytoplasm remains behind in the spore. The nucleus expands during interphase, and meiotic division proceeds rapidly under optimal moisture and aeration (Fig. 1F-H).

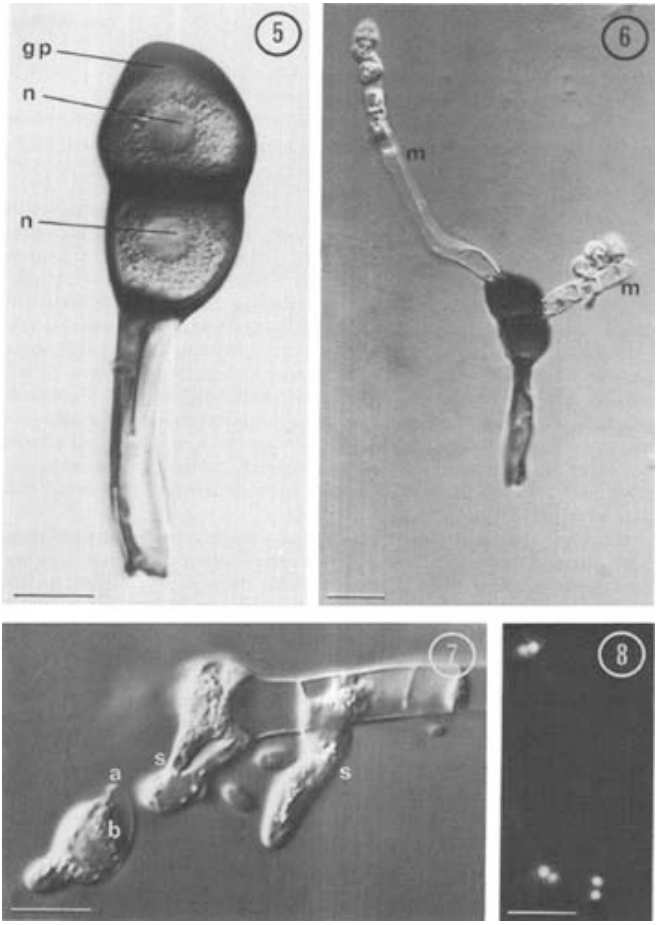


Fig. 5. Teliospore of *P. sorghi* showing nuclei with nucleoli (n) and the germ pore (gp) of the upper teliospore cell. The lateral germ pore of the lower teliospore cell cannot be recognized (xl400; bar, 10 μm) (interference contrast, K. Mendgen, unpublished). Fig. 6. Germinated teliospore of *P. sorghi* with two metabasidia (m) (xlOOO; bar, 10 μm) (interference contrast, K. Mendgen, unpublished). Fig. 7. Metabasidium of *P. sorghi.* b, Basidiospore; a, apiculus; s, sterigmata. (xl400; bar, 10 μm) (interference contrast, K. Mendgen, unpublished). Fig. 8. Basidiospores of *P. hordei* (host: *Hordeum vulgare)* showing the two nuclei in each spore by fluorescence microscopy (~x 1200; bar, 10 μm) (courtesy Y. Anikster, Tel Aviv).

A fine-structural study of the meiosis of cereal rusts is still missing. In *Gymnosporangium,* synaptonemal complexes, which indicate meiotic chromosome pairing (~prophase I), have been detected shortly after karyogamy, before the telial sorus is opened (Mims, *1977b,* 1981). This would indicate that meiosis can begin before teliospores reach maturity and is then interrupted or delayed until teliospore germination. In *P. malvaceaium,* a short-cycled rust, O'Donnell and McLaughlin (1981a-d) observed the spindle pole bodies, responsible for spindle formation, when the metabasidium reached about 80% of its maximum length (Fig. 9). The fully developed spindle shows in Fig. 10 (~metaphase I). The regrouping of the chromosomes at the cell poles shows in Fig. 11 (~telophase I). After this first nuclear division, called meiosis I, a septum forms between these two nuclei, and meiosis II proceeds (Fig. 12). Interestingly, the nuclei undergo a coordinated migration and rotation during meiosis. Differences in the development and form of the spindle pole body potentially provide important information on phylogenetic relationships among rusts (see O'Donnell and McLaughlin, 1981c).

The septa are formed centripetally and contain a narrow central pore with dense material (Fig. 13). After meiosis II and formation of another two septa, sterigma (Figs. II and 7) develop from small papillae. At the apex of the sterigma, a small vesicle with cytoplasm and nucleus emerges (Fig. 1J). The vesicles or basidiospores enlarge and are delicately supported by the sterigmatal ends (Figs. 1K and 7). Sterigma and basidiospore formation resembles those in various basidiomycetes (Lit-tlefield and Heath, 1979; Minis, 1981) and has been studied ultrastruc-turally for *G. clavipes:* The basidiospore is delimited from the sterigma by a septum at the base of the basidiospore and a septum in the neck of the sterigma (Mims, 1981).

The basidiospore in *P. giaminis* is either uninucleate (Craigie and Green, 1962) or, after a subsequent mitotic division, binucleate. Examples for rusts with binucleate basidiospores are *P. giaminis* f. sp. *tritici, P. giaminis* f. sp. *avenae, P. giaminis* f. sp. *secalis, P. hordei* (Fig. 8), *P. recondita, P. striifoimis* (Y. Anikster, personal communication), *P.*

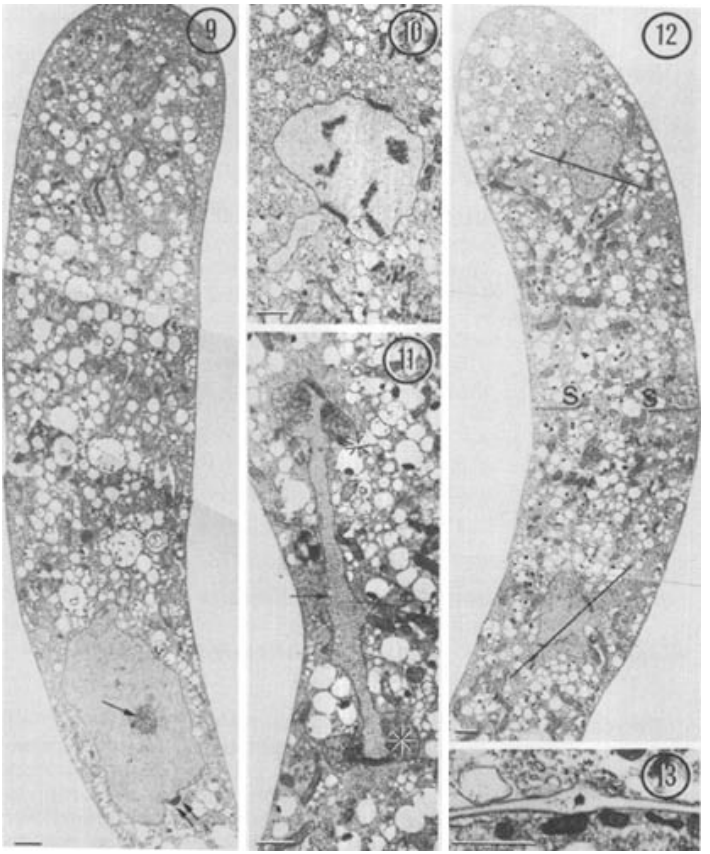


Fig. 9. Cross section through a metabasidium of *P. malvaceaium* showing a nucleus during prophase I with a dispersing nucleolus (arrow) and an extranuclear, duplicated spindle pole body (double arrow) (x4300; bar, 1 μm). (From O'Donnell and McLaughlin, 1981a, **with** permission.) **Fig.** 10. A metabasidium of *P. malvacearum* with an oblique early-metaphase spindle during meiosis I (x5500; bar, 1 μm). (From O'Donnell and McLaughlin, 1981b, with permission.) **Fig.** 11. A metabasidium at late telophase during meiosis I, showing beginning disruption of nuclear envelope (arrow) in the narrow interzonal region and the chromosomes at the cell poles (asterisks) (x6300; bar, 1 μm). (From O'Donnell and McLaughlin, 1981b, with permission.) **Fig.** 12. Early meiosis II (metaphase) showing nuclei with spindle axes (lines). The median septum (S) is still incomplete (x3400; bar, 1 μm). (From O'Donnell and McLaughlin, 1981c, with permission.) **Fig.** 13. Median section through a septal pore of *P. malvacearum* at the end of meiosis II (interphase II), containing electron-dense material and separated from the adjacent basidial compartments by a wall layer (x 13,700; bar, 1 μm) (courtesy D. J. McLaughlin, St. Paul).

malvacearum (Allen, 1933), and many other rusts (Kapooria, 1968; Kulkarni, 1958; Pavgi, 1975; Mims, 1977a; Duncan and Galbraith, 1972;

Kohno *et al.*, 1977). In *P. horiana,* 31.5% spores were tetranucle-ate (Kohno *et al.*, 1974). There are conflicting reports on the number of chromosomes (three to five) in the different rusts (Kapooria, 1968; Pavgi *et al.*, 1960).

In *P. sorghi,* many abnormalities from "normal" basidiospore formation in long-cycled rusts were observed by Pavgi (1975). Sometimes, inhibition of septum formation resulted in promycelial cells with two nuclei. From such abnormalities, a production of basidiospores with two compatible nuclei seems possible, and Pavgi (1975) suggests that they may produce aecia without the need of a transfer of spermatia from compatible pycnia, a conclusion also drawn from experiments with *Uromyces* spp. (Anikster *et al.*, 1980). Thus both long- and short-cycled rusts can produce metabasidia with only two basidiospores, each containing nuclei of both mating types and therefore with the ability to produce aecia directly without first forming pycnia (e.g., *Uromyces viennot-bourgonii, U. christensii, U. hordeatri,* Y. Anikster, personal communication).

III. Physiology of Teliospores

A. FACTORS THAT INDUCE TELIOSPORE FORMATION UNDER NATURAL CONDITIONS

The teliospores of the macrocyclic cereal rusts are generally formed late in the season. The physiological background of teliospore formation remains obscure. Gassner and Franke (1938) cite many arguments against the assumption that the depletion of nutrients in older leaves induces teliospore formation. Benada (1966) tries to correlate the lower pH of the leaf sap with teliospore formation late in the season. However, teliospores are also produced by *P. recondita* on young plants (Jackson and Young, 1967). There are many examples showing that teliospore formation correlates with the cultivar—race combination used. Examples are *P. recondita* (Freitas, 1972; Waters, 1928; Takahashi *et al.*, 1965; Jackson and Young, 1967), *P. graminis* f. sp. *tritici, P. coro-nata* (Rothman, 1974; Prasada, 1948; Sebesta and Bartos, 1966; Simons, 1954; Lisovii and Yabukova, 1973; Pillai *et al.*, 1978; Hassebrauk, 1962; Takahashi *et al.*, 1973; Zimmer and Schafer, 1960), and *P. hordei* (Joshi, 1965). An oat line that induces early formation of teliospores after inoculation by *P. coronata* does not show the same effect with *P. graminis* f. sp. *avenae* (Rothman. 1974). For such studies, contaminant-free telia of single physiologic race isolates of oat crown rust were obtained by injecting urediospores between the leaf sheath and culm at the base of each internode (Fleischmann, 1964).

Early teliospore formation by cereal rusts can be induced by infection of rusted leaves with *Cephalosporium acremonium* (Hassebrauk, 1936) or with *Aphanocladium album* (Biali *et al,* 1972). This is also possible in leaf culture (Lesovoi *et al,* 1980) and axenic culture (Yaniv *et al,* 1979) of rusts. Extraction of *A. album* cultures with ethyl acetate or chloroform yielded an extract that reliably induced teliospore formation of *P. graminis, P. sorghi,* and *P. dispersa* (Forrer, 1977). A similar effect was observed after simultaneous infection of cereals with *P. recondita* f. sp. *triticina* and *Septoria nodorum* (Van der Wal, 1970).

B. ARTIFICIAL INDUCTION OF TELIOSPORE GERMINATION

Teliospores of *P. heterospora* germinate readily in less than 8 hr at 26° to 28°C without any resting period and remain viable for less than 10 days (Kotwal, 1970). Teliospores of *P. graminis* f. sp. *tritici* do not germinate readily and may remain viable at least 6 years under laboratory conditions (Johnson, 1941). Nearly all life durations in between have been reported (Lehmann *et al.,* 1937). Most (Kühn, 1858; Tulasne and Tulasne, 1847) but not all *(P. glumarum,* Gaumann, 1959; *P. purpurea,* Prasada, 1948) teliospores of the cereal rusts are dormant. *P. sorghi* and *P. graminis* f. sp. *avenae* are somewhat intermediate (or nonuniform), in that some spores germinate at once, the others only after a period of some months (Godoy and Bruny, 1952; Hingorani, 1952; Neuhaus, 1966). For some rust fungi, such as *P. glumarum* (*=striiformis),* there are conflicting reports (Gaumann, 1959; Prasada, 1948). Following the definition of Allen (1965) and Sussman and Halvorson (1966), the dormancy of teliospores is constitutional, because it is an innate property of the spore that requires an activation process to be broken and is not a consequence of the presence of inhibitory environmental factors.

Several methods have been proposed to induce germination of dormant teliospores. In some cases, a prerequisite for any germination at all is that the teliospores be produced below 15° to 18°C (Joshi, 1965; Hennessy and Sackston, 1970). It also should be noted that reports appear very often in conflict, and a method adequate for one rust species or race of a species may not work with others (Chin *et al.,* 1965). A representative selection of methods recommended mainly for cereal rusts is presented here:

1. Storage of spores or leaves with telia (De Bary, 1863; Kiihn, 1858) outside, under humid conditions (Eriksson and Henning, 1896; Klebahn, 1916; Lambert, 1929). Storage under dry conditions was very often unfavorable (Schilberszky, 1930; Ward, 1888).
2. Freezing and thawing the spores (Johnson, 1930; McAlpine, 1906)
3. Wetting and drying the spores (Dinoor, 1962; Hooker and Yar-wood, 1966; Klebahn, 1914; Lumbroso *et al,* 1977)
4. Treatment of spores with X rays (Line, 1963) and heat (Gold and Mendgen, 1981a,b; Maneval, 1927)

5. Treatment of teliospores of *P. graminis* with citric acid (Thiel and Weiss, 1920), buffers (Maneval, 1927), and other acids (Sibilia, 1930); treatment of teliospores of *P. caithami* with volatile poly-acetylenes from safflower (Binder *et al,* 1977; Klisiewicz, 1972, 1973) and treatment of *U. appendiculatus* teliospores with unknown volatile substances from bean (Gold and Mendgen, 1981b)
6. Keeping fresh spores on agar and waiting until they germinate— some always will (Groth and Mogen, 1978; Maneval, 1927)
7. Exposing spores to light regimens (Neuhaus, 1969)

Unfortunately, very few quantitative data exist, making it impossible to compare the different methods. Two ways might be recommended for a beginner: (1) storage outside under winter conditions and (2) washing fresh spores or pieces of rusted leaves with distilled water at 13° to 16°C and transferring them onto 4% agar (with 40 ppm chloramphenicol). Either method will induce at least some (~0.0001%) germination (Y. Anikster and I. Wahl, personal communication). With bean rust *{U. appendiculatus),* germination of teliospores was observed on water agar after outside storage during winter, freezing and thawing cycles of fresh spores, heat treatment of fresh-dried spores (5 or 10 days at 40°C; R. E. Gold and K. Mendgen, unpublished), heat treatment of fresh spores on agar (4 days at 31.6°C), or treatment of fresh spores with volatile substances from bean germlings (Gold and Mendgen, 1981a,b). However, germination varied considerably with the bean rust isolate used.

To improve our understanding of teliospore dormancy, some quantitative data from Gold and Mendgen (1981b) on teliospore germination of *U. appendiculatus* var. *appendiculatus (= U. phaseoli) are* described here. If fresh teliospores are stored at 4°C in a refrigerator, and samples of these are tested periodically on agar at 18°C, germination starts after a dormancy period of about 6 months (Fig. 14). This dormancy period can be interrupted with any of the methods previously described. With refrigerator-stored teliospores or after an activation such as with volatile substances from the host plant, we observed a preemergence lag of about 4 to 5 days at 18°C before the germ tube emerged (Fig. 15). The formation of the metabasidium with the basi-diospores and basidiospore discharge takes only 4-6 hr at 18°C (R. E. Gold, personal communication). The data should not be generalized before other rusts are examined, because very few time course studies on teliospore germination have been performed (e.g., Spaulding and Rathbun-Gravatt, 1926). Dietel (1911, 1912b, 1915, 1921) reported that, depending on the resting period, teliospores of *P. graminis* take from 2.5 to 30 hr before they germinate. Lambert (1929) mentions a preemergence lag of 3 days before teliospore germination of this fungus begins. A very broad temperature range for germination (15°-22°C) is also reported (Lehmann *et al,* 1937). Overwintered teliospores of *P. sorghi* begin to germinate at 17°C after 48 hr on agar. After the preemergence lag, basidiospore formation is finished within the following 6 to 12 hr at 17°C (K. Mendgen, unpublished). During the time of teliospore germination and

basidiospore formation, high humidity conditions are needed. However, teliospores should not be covered with water as observed for *P. graminis* (Blackman, 1903).

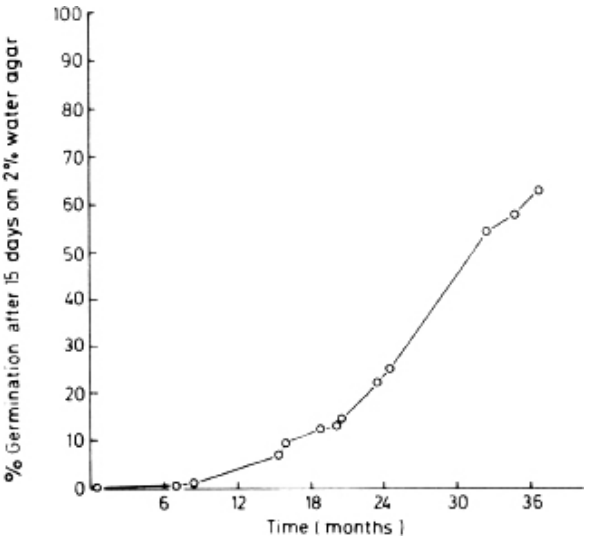


Fig. 14. Germination of samples of teliospores during a 3-year storage period at 4°C. (*Uromyces appendiculatus* var. *appendiculatus*, modified after Gold and Mendgen, 1981b.)

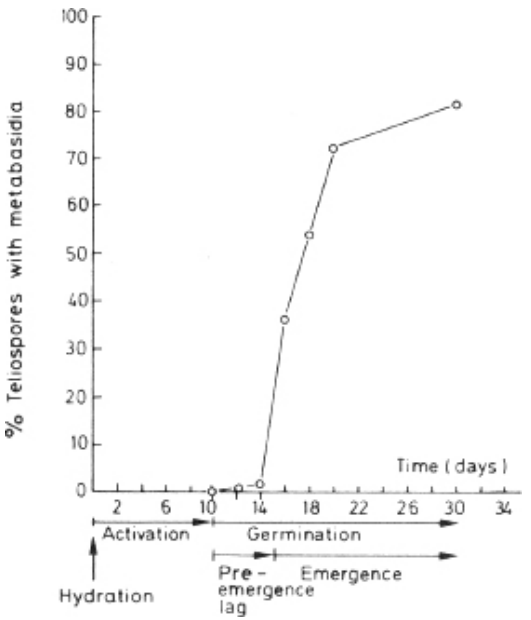


Fig. 15. Germination of fresh teliospores of *Uromyces appendiculatus* after activation with volatile substances from the host plant in a closed chamber and subsequent incubation on agar at 18°C. The preemergence lag was also observed when 3-year-old teliospores were used, which germinate readily without activation. (Modified after Gold and Mendgen, 1981b.)

Teliospore germination is influenced by light regimes very often (Carter and Banyer, 1964; Pady and Kramer, 1971; Pearson *et al,* 1977; Van Arsdel, 1967). Teliospores of *U. appendiculatus* germinate and release their basidiospores ~7 ± 0.7 hr after a light-off signal (Fig. 16). A daily exposure to 1000 lux for 0.5 hr was sufficient for the induction of the germination process. Thus under a day-night regimen, the teliospores have, after appropriate activation, the lag of about 4 to 5 days and then a rhythmic basidiospore discharge during the following 4 to 6 nights (Gold and Mendgen, 198lb; Gold and Mendgen, 1983a,b). In the cereal rusts, a recent study of light influence exists only for *P. sorghi* (Neuhaus, 1969). The teliospores of this rust needed only 1000 lux during 1 min for germination. Studies with *P. graminis* (Maneval, 1927; Lambert, 1929) did not find an influence of a day-night regimen. More studies are needed to elucidate this question in the cereal rusts.

A mechanism that would explain constitutive dormancy is known neither for rusts nor for other fungi. Harder (1977) discusses wall qualities as one factor with respect to dormancy in the crown rust fungus. In other fungi with similar qualities, the roles of compartmen-talization within the spore (Mandels, 1981), membrane features (see Turian and Hohl, 1981), and catalytic activities of mitochondria (Wenzler and Brambl, 1981) have been discussed.

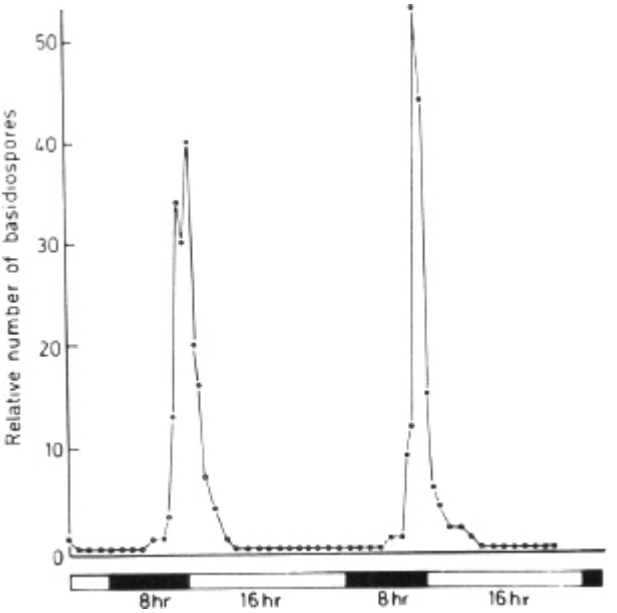


Fig. 16. Basidiospore discharge during a regimen of 8 hr darkness (dark segment of bar) and 16 hr light (light segment of bar) (1000 lux, 18°C). The teliospores had been stored at 4°C for 2 years. Basidiospore discharge began in the fourth or fifth dark period during the indicated light regimen. (Modified after Gold and Mendgen, 1981b.)

IV. Germination and Penetration of Basidiospores

The basidiospores of the rusts in general do not seem to have special morphological characteristics (Littlefield and Heath, 1979). Basidiospores of rusts (e.g., *G. juniperi-virginianae*, Mims, 1977a) have a prominent apiculus, an appendage by which the spore was attached to the sterigma (Fig. 7). The basidiospores of different *Puccinia* species are catapulted from their sterigma as far as 0.6 to 1 mm (Y. Anikster, personal communication; Buller, 1924; Dietel, 1912a; Lambert, 1929). Dietel (1912a,b) calculates from such measurements a catapulting speed of 8 cm/sec. Once airborne, the basidiospores may be transported by the wind at high air humidity over a distance of about 5 m (Yamada *et al.*, 1973). Basidiospores germinate under such high-humidity conditions without delay (De Bary, 1865; 1866; Waterhouse, 1921), with a short, delicate germ tube. It may act as a sterigma by producing secondary basidiospores (De Bary, 1866) or lead to formation of an appressorium-like structure on the host leaf (De Bary, 1865, 1866; Waterhouse, 1921).

The basidiospores of *P. graminis* (Waterhouse, 1921) and *G. fuscum* (Metzler, 1982) need less then 20 hr at 20°C to penetrate the epidermis.

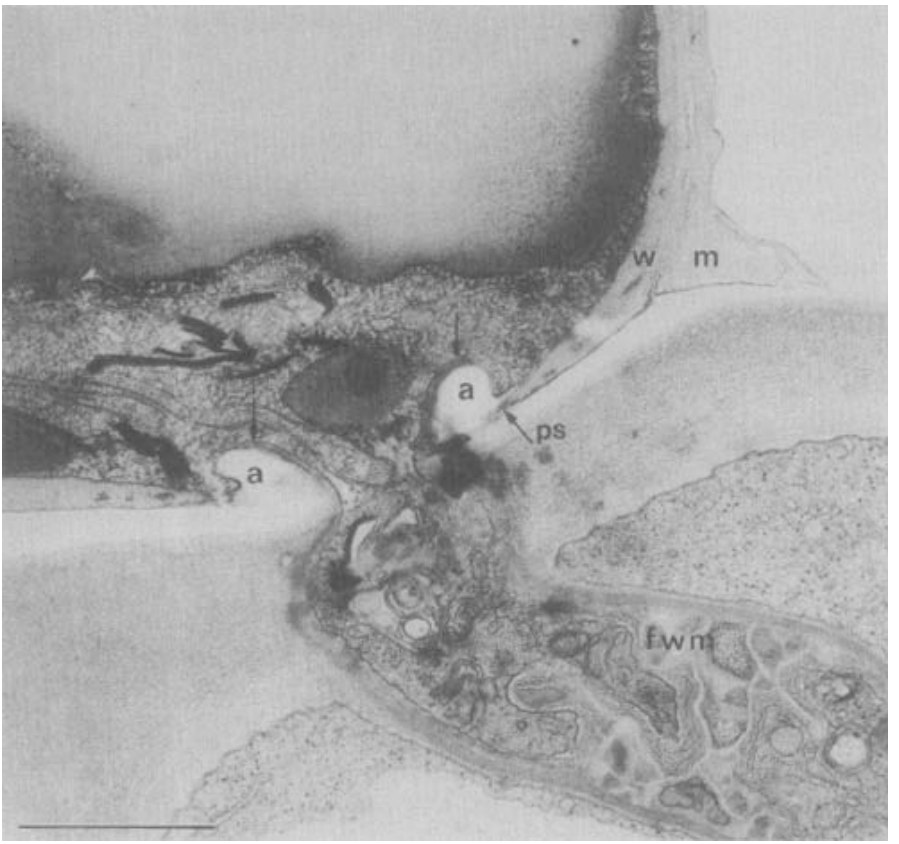


Fig. 17. Cross section at the penetration site through the basidiospore germ tube of *Gymnosporangium fuscum* on a pear leaf. The wall (w) of the basidiospore germ tube (appressorium) is covered with mucilage (m) and thins out at the penetration site (ps). A new wall (arrows) is laid down around an "appressorial ring" (a), thus delimiting the penetration peg. At this late stage of infection, the penetration peg is occluded with fungal wall material (fwm). (TEM, ×25,520; bar, 1 µm). (From Metzler, 1982, with permission.)

During germination of *Gymnosporangium*, large lipid bodies can be observed in the cytoplasm that seem to be degraded gradually. Numerous vesicles are present in the spore near the germ tube and in the germ tube (Mims, 1977a). The germ tube and the appressorium of *P. graminis* basidiospores are covered with a mucilage layer (Waterhouse, 1921; Novotelnova, 1935). The germ tube wall formed by the basidiospore is continuous with a newly formed inner wall layer in the basidiospore.

The appressorium of *G. fuscum* formed on contact with the host is not separated by a septum from the germ tube (Metzler, 1982). As with *P. graminis,* the appressorium is surrounded by a mucilage that may stick to the host surface (Fig. 17). The appressorium wall thins out at the penetration site and differentiates an inner ring (appressorial ring) before penetration of the host cell (Fig. 17). The function of the appressorial ring is still unknown. The penetration peg itself is formed by a new inner wall layer near the appressorial ring (Metzler, 1982) and is not continuous with the appressorial wall. After successful penetration, the penetration peg is occluded by fungal wall material (Fig. 17). This wall material seems to separate the protoplast in the growing hyphae from the empty appressorium outside on the epidermis. For *P. graminis,* only a light microscope description of the infection process by basidiospores exists (Waterhouse, 1921). Melander and Craigie (1927) observed that the progressive increase in resistance of very young to old leaves of *Berberis vulgaris* to basidiospore infection of *P. graminis* is positively correlated with increased thickness of the cuticle of epidermal cells and increased resistance to mechanical puncture. However, physiological reasons for differences in susceptibility have not been excluded.

The intercellular and intracellular structures subsequently formed by the monokaryotic rust fungus are described by Harder in Chapter 11 of this volume.

V. Conclusions

After their first description by Tulasne and Tulasne (1847), the teliospores of the rust fungi have been studied in many details. Most articles deal with teliospore morphology and infectivity of basidiospores (Cummins, 1971; Gäumann, 1959; Hassebrauk, 1962). Some describe the metabasidium and basidiospore formation. Ultrastructural studies are restricted to very few rust species, mainly *P. malvacearum* and*Gymnosporangium* spp. There is a lack of ultrastructural studies on the cereal rust teliospores, their germination, and the basidiospore infection process. Studies on the physiology of teliospore dormancy and teliospore germination are still only beginning. Experiments with the cereal rusts similar to the studies on other rusts as described in this chapter are urgently needed, because teliospore behavior plays an important role in the perpetuation of the disease from one season to the next.

Acknowledgment

I thank Y. Anikster, W. R. Bushnell, and D. J. McLaughlin for reviewing the manuscript.

References

Allen, P. J. (1965). Metabolic aspects of spore germination in fungi. *Annu. Rev. Phytopathol.* **3**, 313–342.

Allen, R. F. (1933). A cytological study of the teliospores, promycelia, and sporidia in *Puccinia malvacearum. Phytopathology* **23**, 572–586.

Anikster, Y., and Wahl, I. (1979). Coevolution of the rust fungi on *Giamineae* and *Liliaceae* and their hosts. *Annu. Rev. Phytopathol.* **17**, 367–403.

Anikster, Y., Moseman, J. G., and Wahl, I. (1980). Development of basidia and basidiospores in *Uromyces* species on wild barley and *Liliaceae* in Israel. *Trans. Br. Mycol. Soc.* **75**, 377–382.

Arthaud, J. (1969). Contribution à l'étude des anomalies sporales chez le *Puccinia graminis. C. R. Hebd. Seances Acad. Sci., Ser. D* **268**, 1502–1504.

Arthur, J. C. (1929). "The Plant Rusts (*Uredinales*)." Wiley, New York.

Arthur, J. C. (1932). Terminologie der *Uredinales. Ber. Dtsch. Bot. Ges.*, **50A,** 24–27.

Arthur, J. C. (1934). "Manual of the Rusts in United States and Canada." Purdue Res. Found., Lafayette, Indiana.

Benada, J. (1966). The occurrence of telia of rusts and of cleistothecia of powdery mildew on cereals and an attempt to find a factor conditioning it. *Zentralbl. Bakteriol., Parasitenkd., Infektionskr. Hyg., Abt. 2, Naturwiss.: Aug., Landwirtsch. Tech. Mikrobiol.* **120**, 427–433.

Bennell, A. P., and Henderson, D. M. (1978). Urediniospore and teliospore development in *Tranzschelia* (*Uredinales*). *Trans. Br. Mycol. Soc.* **71**, 271–278.

Bennell, A. P., Henderson, D. M., and Prentice, H. T. (1978). The teliospores of *Puccinia smyrnii* (*Uredinales*). *Grana Palynol.* **17**, 17–27.

Biali, M., Dinoor, A., Eshed, N., and Kenneth, R. (1972). *Aphanocladium album*, a fungus inducing teliospore production in rusts. *Ann. Appl. Biol.* **72**, 37–42.

Binder, R. G., Klisiewicz, J. M., and Waiss, A. C., Jr. (1977). Stimulation of germination of *Puccinia carthami* teliospores by polyacetylenes from safflower. *Phytopathology* **67**, 472–474.

Blackman, V. H. (1903). On the conditions of teleutospore germination and of sporidia formation in the *Uredineae. New Phytol.* **2**, 10–14.

Buller, A. H. R. (1924). "Researches on Fungi," Vol. III. Longmans, Green, New York.

Carter, M. V., and Banyer, R. J. (1964). Periodicity of basidiospore release in *Puccinia malvacearum. Aust. J. Biol. Sci.* **17**, 801–802.

Chin, K. J., Chang, K. C., and Hsuen, L. H. (1965). On the teliospore germination of *Puccinia graminis var. tritici. Acta Phytophylacica Sin.* **4**, 201–206.

Craigie, J. H., and Green, G. J. (1962). Nuclear behavior leading to conjugate association in haploid infections of *Puccinia graminis. Can. J. Bot.* **40**, 163–178.

Cummins, G. B. (1959). “Illustrated Genera of Rust Fungi.” Burgess, Minneapolis, Minnesota.

Cummins, G. B. (1971). “The Rust Fungi of Cereals, Grasses and Bamboos.” Springer-Verlag, Berlin and New York.

De Bary, A. (1863). Recherches sur le développement de quelches champignons parasites. *Ann. Sci. Nat., Bot. Biol. Veg.* (*4*)**20**, 5–148.

De Bary, A. (1865). Neue Untersuchungen über die *Uredineen,* insbesondere die Entwicklung der *Puccinia graminis* und den Zusammenhang derselben mit *Aecidium berberidis. Monatsber. K. Preuss. Akad. Wiss. Berlin* pp. 15–50.

De Bary, A. (1866). Neue Untersuchungen über *Uredineen. Monatsber. K. Preuss. Akad. Berlin Wiss.* pp. 205–215.

Dietel, P. (1911). Versuche über die Keimungsbedingungen der Teleutosporen einiger *Uredineen.* I. *Zentralbl. Bakteriol., Parasitenkd., Infektionskr. Hyg.,* Abt. *2, Naturwiss.: Allg., Landwirtsch. Tech. Mikrobiol.* **31**, 95–106.

Dietel, P. (1912a). Über die Abschleuderung der Sporidien bei den *Uredineen. Mycol. Centralbl.* **1**, 355–359.

Dietel, P. (1912b). Versuche über die Keimungsbedingungen der Teleutosporen einiger *Uredineen.* II. *Zentralbl. Bakteriol., Parasitenkd., Infektionskr. Hyg.,* Abt. *2, Naturwiss.: Allg. Landwirtsch. Tech. Mikrobiol.* **35**, 272–285.

Dietel, P. (1915). Versuche über die Keimungsbedingungen der Teleutosporen einiger *Uredineen.* III. *Zentralbl. Bakteriol., Parasitenkd., Infektionskr. Hyg.,* Abt. *2, Naturwiss.: Allg. Landwirtsch. Tech. Mikrobiol.* **42**, 698–705.

Dietel, P. (1921). Versuche über die Keimungsbedingungen der Teleutosporen einiger *Uredineen.* IV. *Zentralbl. Bakteriol., Parasitenkd., Infektionskr. Hyg.,* Abt. *2, Naturwiss.: Allg. Landwirtsch. Tech. Mikrobiol.* **54**, 215–219.

Dinoor, A. (1962). Note on germination of crown rust teliospores and infection of Buckthorn. *Can. J. Plant Sci.* **42**(3), 537–538.

Duncan, E. G., and Galbraith, M. H. (1972). Post-meiotic events in the Homobasidiomycetidac. *Trans. Br. Mycol. Soc.* **58**, 387–392.

Eriksson, J., and Henning, E. (1896). “Die Getreidroste, ihre Geschichte, Natur, sowie Massregeln gegen dieselben.” Norstedt, Stockholm.

Fleischmann, G. (1964). Production of telia of pure races of oat crown rust for genetic studies of virulence. *Can. J. Bot.* **42**, 1457–1458.

Forrer, H. R. (1977). The influence of metabolites of the mycoparasite *Aphanocladium album* on teliospore formation of rusts. *Phytopathol. Z.* **88**, 306–311.

Freitas, A. P. C. e (1972). Teliospores of *Puccinia recondita Rob.* on wheat seedlings. *Actas Congr. Uniao Fitopatol. Mediterr., 3rd, 1972* pp. 127–132.

Gassner, G., and Franke, W. (1938). Untersuchungen über den Stickstoffhaushalt rostinfizierter Getreideblätter. *Phytopathol. Z.* **11**, 517–570.

Gäumann, E. (1926). “Vergleichende Morphologie der Pilze.” Fischer, Jena.

Gäumann, E. (1959). Die Rostpilze Mitteleuropas. *Beitr. Kryptogamenflora Schweiz* **12**, 1–1407.

Godoy, E. F., and Bruny, O. (1952). Ciclo evolution de las royas del Lino (*Melampsora lini*)y del Maiz (*Puccinia sorghi*)en la Argentina. *Rev. Argent. Agron.* **10**, 21–34.

Gold, R. E., and Mendgen, K. (1981a). Induction of teliospore germination of bean rust, *Uromyces phaseoli var. typica. Phytopathology* **71**, 219–220.

Gold, R. E., and Mendgen, K. (1981b). Induktion der Teleutosporenkeimung beim Bohnenrost (*Uromyces appendiculatus var. appendiculatus*). *Mitt. Biol. Bundesanst. Land- Forstwirtsch., Berlin-Dahlem,* **203**, 318–319.

Gold, R. E., and Mendgen, K. (1983a). Activation of teliospore germination in *Uromyces appendiculatus* var. *appendiculatus.* I. The influence of aging and temperature. *Phytopathol. Z.*, in press.

Gold, R. E., and Mendgen, K. (1983b). Activation of teliospore germination in *Uromyces appendiculatus* var. *appendiculatus.* II. The influence of light and host volatiles. *Phytopathol. Z.*, in press.

Gottlieb, D. (1978). "The Germination of Fungus Spores." Meadowfield Press, Durham, England.

Groth, J. V., and Mogen, B. D. (1978). Completing the life cycle of *Uromyces phaseoli var. typica* on bean plants. *Phytopathology* **68**, 1674–1677.

Guyot, A. L. (1938). Les Urédinées, Tome I: Genre Uromyces. *Encycl. Mycol.* **8**, 1–335.

Guyot, A. L. (1951). Les Urédinées, Tome II: Genre Uromyces. *Encycl. Mycol.* **15**, 1–647.

Guyot, A. L. (1956). Les Urédinées, Tome III: Genre Uromyces. *Encycl. Mycol.* **29**, 1–647.

Harder, D. E. (1977). Electron microscopy of teliospore formation in *Puccinia coronata avenae. Physiol. Plant Pathol.* **10**, 21–28.

Hassebrauk, K. (1936). Pilzliche Parasiten der Getreideroste. *Phytopathol. Z.* **9**, 513–516.

Hassebrauk, K. (1962). *"Uredinales"* (Rostpilze). *In* "Handbuch der Pflanzenkrankheiten. *Basidiomycetes"* (H. Richter, ed.), 6th ed., Vol. III, pp. 2–275. Parey, Berlin

Hennessy, C. M. R., and Sackston, W. E. (1970). Studies on sunflower rust. V. Culture of *Puccinia helianthi* throughout its complete life cycle on detached leaves of sunflower (*Helianthus annuus*). *Can. J. Bot.* **48**, 1811–1813.

Hingorani, M. K. (1952). Factors affecting the survival ability of certain physiologic races of *Puccinia graminis avenae Erikss. & Henn. Phytopathology* **42**, 526–531.

Hiratsuka, Y. (1973). The nuclear cycle and the terminology of spore states in Uredinales. *Mycologia* **65**, 432–443.

Hooker, A. L., and Yarwood, C. E. (1966). Culture of *Puccinia sorghi* on detached leaves of corn and *Oxalis corniculata. Phytopathology* **56**, 536–539.

Hughes, S. J. (1970). Ontogeny of spore forms in *Uredinales. Can. J. Bot.* **48**, 2147–2157.

Jackson, A. O., and Young, H. C. (1967). Teliospore formation by *Puccinia recondita f. sp. tritici* on seedling wheat plants. *Phytopathology* **57**, 793–794.

Johnson, T. (1930). The germination of wheat stem rust teliospores. *Can. Dep. Agric. Rep. Dom. Bot.* pp. 51–52.

Johnson, T. (1941). Longevity of teliospores of *Puccinia graminis* under laboratory conditions. *Phytopathology* **31**, 197–198.

Joshi, L. M. (1965). Studies on *Puccinia hordei.* II. Formation and germination of teleutospores. *Indian Phytopathol.* **18**, 356–359.

Kapooria, R. G. (1968). Cytological studies of the germinating teliospores and basidiospores of *Puccinia penniseti. Neth. J. Plant Pathol.* **74**, 2–7.

Kapooria, R. G. (1973). Morphological variability in teliospores of *Puccinia penniseti. J. Gen. Microbiol.* **77**, 443–446.

Klebahn, H. (1914). Kulturversuche mit Rostpilzen. XV. Bericht. Z. *Pflanzenkr.* **24**, 1–32.

Klebahn, H. (1916). Kulturversuche mit Rostpilzen. XVI. Bericht. Z. *Pflanzenki. Gallenkd.* **26**, 257–277.

Klisiewicz, J. M. (1972). Effect of host plant materials and temperature on germination of teliospores of *Puccinia carthami. Phytopathology* **62**, 436–438.

Klisiewicz, J. M. (1973). Effect of volatile substances from safflower on germination of teliospores of *Puccinia carthami. Phytopathology* **63**, 795.

Kohno, M., Nishimura, T., Ishizaki, H., and Kunoh, H. (1974). Cytological studies on rust fungi. I. Teliospore germination and nuclear behaviors in chrysanthemum white rust (*Puccinia horiana P. Hennings*). *Bull. Fac. Agric., Mie Univ.* **47**, 1–9.

Kohno, M., Nishimura, T., Ishizaki, H., and Kunoh, H. (1975). Cytological studies on rust fungi. III. Nuclear behaviors during the process from teliospore stage through sporidial stage in two short-cycled rusts, *Kuehneola japonica* and *Puccinia horiana. Bull. Fac. Agric., Mie Univ.* **49**, 21–29.

Kohno, M., Nishimura, T., Ishizaki, H., and Kunoh, H. (1977). Cytological studies on rust fungi. VII. The nuclear behavior of *Gymnosporangium asiaticurn Miyabe et Yamada* during the stages from teliospore germination through sporidium germination. *Trans. Mycol. Soc. Jpn.* **18**, 211–219.

Kotwal, S. N. (1970). The mode of germination of teliospores of *Puccinia heterospora*. *Curr. Sci.* **39**, 285–286.

Kühn, J. (1858). "Die Krankheiten der Kulturgewächse, ihre Ursachen und ihre Verhütung". Bosselmann, Berlin.

Kulkarni, U. K. (1958). Studies in the development and cytology of *Puccinia penniseti Zimm. Trans. Br. Mycol. Soc.* **41**, 65–73.

Lambert, E. B. (1929). The relation of weather to the development of stem rust in the Mississippi valley. *Phytopathology* **19**, 1–71.

Lehmann, E., Kummer, H., and Dannenmann, H. (1937). "Der Schwarzrost, seine Geschichte, seine Biologie und seine Bekämpfung in Verbindung mit der Berberitzenfrage." Lehmann, München and Berlin.

Lesovoi, M. P., Panteleev, V. K., and Parkhomenko, N. A. (1980). Metod uskorennogo polucheniya teleitospor *Puccinia triticina Eriks.* v. izolirovannoi kul'ture list'ev pshenitsy. *Mikol. Fitopatol.* **14**, 79–81.

Line, R. F. (1963). Factors affecting the germination of spores of *Puccinia graminis* var. *tritici. Diss. Abstr.* **24**, 23.

Lisovii, M. P., and Yabukova, I. V. (1973). Formuvannya teleitospor zbudnika buroi irzhi pshenitsi v. umovakh izol'ovanoi listkovoi kul'turi. *Zakhist Rosl.* **18**, 76–78.

Littlefield, L. J., and Heath, M. C. (1979). "Ultrastructure of Rust Fungi." Academic Press, New York.

Lumbroso, E., Anikster, Y., Moseman, J. G., and Wahl, I. (1977). Completion of life cycles of *Puccinia hordei and Uromyces scillarum* on detached leaves of their hosts. *Phytopathology* **67**, 941–944.

McAlpine, D. (1906). "The Rusts of Australia." Brain, Melbourne.

Mandels, G. R. (1981). Compartimentation of metabolic systems in the regulation of dormancy in fungus spores. *Exp. Mycol.* **5**, 278–291

Maneval, W. E. (1927). Further germination tests with teliospores of rusts. *Phytopathology* **17**, 491–493.

Melander, L. W., and Craigie, J. H. (1927). Nature of resistance of *Berberis spp.* to *Puccinia graminis. Phytopathology* **17**, 95–114.

Metzler, B. (1982). Studien über *Heterobasidiomyceten* (23): Basidiosporenkeimung und Infektionsvorgang beim Birnengitterrost. *Phytopathol. Z.* **103**, 126–138.

Mims, C. W. (1977a). Finc structure of basidiospore of the cedar-apple rust fungus *Gymnosporangium juniperi-virginianae. Can. J. Bot.* **55**, 1057–1063.

Mims, C. W. (1977b). Ultrastructure of teliospore formation in the cedar-apple rust fungus *Gymnosporangium juniperi-virginianae. Can. J. Bot.* **55**, 2319–2329.

Mims, C. W. (1981). Ultrastructure of teliospore germination and basidiospore formation in the rust fungus *Gymnosporangium clavipes. Can. J. Bot.* **59**, 1041–1049.

Mims, C. W., and Thurston, E. L. (1979). Ultrastructure of teliospore formation in the rust fungus *Puccinia podophylli. Can. J. Bot.* **57**, 2533–2538.
Mims, C. W., Seabury, F., and Thurston, E. L. (1975). Fine structure of teliospores of the cedar-apple rust *Gymnosporangium juniperi-virginianae. Can. J. Bot.* **53**, 544–552.
Mims, C. W., Seabury, F., and Thurston, E. L. (1976). An ultrastructural study of spermatium formation in the rust fungus *Gymnosporangium juniperi-virginianae. Am. J. Bot.* **63**, 997–1002.
Müller, L. Y., Rijkenberg, F. H. J., and Truter, S. J. (1974). A preliminary ultrastructural study on the *Uromyces appendiculatus* teliospore stage. *Phytophylactica* **6**, 123–128.
Neuhaus, W. (1966). Über die Bedeutung des Zwischenwirtes von *Puccinia sorghi Schw. Arch. Pflanzenschutz* **2**, 147–154.
Neuhaus, W. (1969). Zur Teleutosporenkeimung von *Puccinia sorghi Schw. Zentralbl. Bakteriol., Parasitenkd., Infektionskr. Hyg., Abt. 2, Naturwiss.: Allg., Landwirtsch. Tech. Mikrobiol.* **123**, 266–271.
Novotelnova, N. S. (1935). Some observations on the germination of the teleutospores and basidiospores of *P. gr. f. sp. avenae* and of the uredospores of *P. triticina. Plant Prot.* (*Leningrad*)**4**, 98–106.
O'Donnell, K. L., and McLaughlin, D. J. (1981a). Ultrastructure of meiosis in the hollyhock rust fungus, *Puccinia malvacearum.* I. Prophase I—prometaphase I. *Protoplasma* **108**, 225–244.
O'Donnell, K. L., and McLaughlin, D. J. (1981b). Ultrastructure of meiosis in the hollyhock rust fungus, *Puccinia malvacearum.* II. Metaphase I-telophase I. *Protoplasma* **108**, 245–263.
O'Donnell, K. L., and McLaughlin, D. J. (1981c). Ultrastructure of meiosis in the hollyhock rust fungus, *Puccinia malvacearum.* III. Interphase I-interphase II. *Protoplasma* **108,** 264.
O'Donnell, K. L., and McLaughlin, D. J. (1981d). Promycelium development in the rust *Puccinia malvacearum. Mycol. Soc. Am. Newsl.* **32,** 43.
Pady, S. M., and Kramer, C. L. (1971). Basidiospore discharge in *Gymnosporangium. Phytopathology* **61**, 951–953.
Pavgi, M. S. (1969). Morphology and genetics of pathogenicity of *Puccinia sorghi Schw. Mycopathol. Mycol. Appl.* **38**, 175–187.
Pavgi, M. S. (1975). Teliospore germination and cytological aberrations in *Puccinia sorghi Schw. Cytologia* **40**, 227–235.
Pavgi, M. S., Cooper, D. C., and Dickson, J. G. (1960). Cytology of *Puccinia sorghi. Mycologia* **52**, 608–620.
Pearson, R. C., Aldwinckle, H. S., and Seem R. C. (1977). Teliospore germination and basidiospore formation in *Gymnosporangium juniperi-virginianae:* A regression model of temperature and time effects. *Can. J. Bot.* **55**, 2832–2837.
Petersen, R. H. (1974). The rust fungus life cycle. *Bot. Rev.* **40**, 453–513.
Pillai, P. K., Wilcoxson, R. D., and Raychaudhuri, S. P. (1978). Production of teliospores by some races of *Puccinia graminis f. sp. tritici* in detached wheat and barley leaves. *Mycopathologia* **64**, 101–103.
Prasada, R. (1948). Studies on the formation and germination of teliospores of rusts. I. *Indian Phytopathol.* **2**, 119–126.
Rajendren, R. B. (1972). Cytology of *Puccinia graminis f. sp. tritici* on an artificial medium. *Mycologia* **64**, 591–598.
Rijkenberg, F. H. J. (1977). The telial stage of *Puccinia sorghi. Proc. Int. Mycol. Congr., 2nd, 1977,* pp. 1–563.

Rothman, P. G. (1974). Induction of early teliospore formation in oat rusts. *Plant Dis. Rep.* **58**, 467–468.
Schilberszky, K. (1930). Der Berberitzenstrauch und die Schwarzrostfrage. *Phytopathol. Z.* **2**, 615–637.
Sebesta, J., and Bartos, P. (1966). Race and varietal specificity of teleutospore formation in wheat stem rust. *Ochr. Rost.*, [N.S.] **2**, 91–98.
Sibilia, C. (1930). Ricerche sulle ruggini dei cereali. 2. La germinazione delle teleutospore di *P. gr.* e *P. triticina. Boll. Stn. Patol. Veg., Rome* **10**, 164–190.
Simons, M. D. (1954). The relationship of temperature and stage of growth to the crown rust reaction of certain varieties of oats. *Phytopathology* **44**(5), 221–223.
Spaulding, P., and Rathbun-Gravatt, A., Jr. (1926). The influence of physical factors on the viability of sporidia of *Cronartium ribicola Fischer. J. Agric. Res.* (*Washington, D.C.*)**33**, 397–433.
Sussman, A. S., and Halvorson, H. O. (1966). "Spores, Their Dormancy and Germination." Harper, New York.
Takahashi, H., and Furuta, T. (1973). Observation on surface structure and behavior of *Puccinia coronata* Corda var. *avenae* Fraser et Leding, on the host plant by scanning electron microscopy. *Rep. Tottori Mycol. Inst.* **10**, 253–272.
Takahashi, K., Yamada, M., and Takahashi, H. (1965). Teleutospore formation of leaf rust, *Puccinia recondita f. sp. tritici* on young seedlings of wheat. 1. Isolate-variety relation and some characters of teleutospores. *Ann. Phytopathol. Soc. Jpn.* **30**, 54–61.
Takahashi, K., Tanaka, T., Yamada, M., and Takahashi, H. (1973). On the relationship between physiologic races and alternate hosts of wheat stem rust, *Puccinia graminis Pers. f. sp. tritici* in Japan. *Rep. Tottori Mycol. Inst.* **10**, 273–282.
Thiel, A. F., and Weiss, F. (1920). The effect of citric acid on the germination of the teliospores of *P. gr. tr. Phytopathology* **10**, 448–452.
Tulasne, L. R., and Tulasne, C. (1847). Mémoire sur les *Ustilaginées* comparées aux *Urédinées. Ann. Sci. Nat., Bot. Biol. Veg.* **3**, 12–126.
Turian, H., and Hohl, H. R., eds. (1981). "The Fungal Spore." Academic Press, New York. Ullrich, J. (1977). Die mitteleuropäischen Rostpilze der Futter- und Rasengräser. *Mitt. Biol. Bundesanst. Land- Forstwirtsch., Berlin-Dahlem* **175**, 1–71.
Urban, Z. (1969). Die Grasrostpilze Mitteleuropas mit besonderer Berücksichtigung der Tschechoslowakei. *Rozpr. Cesk. Akad. Ved., Rada Mat. Prir. Ved.* **79**, 3–104.
Van Arsdel, E. P. (1967). The nocturnal diffusion and transport of spores. *Phytopathology* **57**, 1221–1229.
Van der Wal, A. F. (1970). Interaction between *Puccinia recondita* f. sp. *triticina* and *Septoria nodorum* on wheat, and its effects on yield. *Neth. J. Plant Pathol.* **76**, 261–263.
von Brandenburger, W., and Schwinn, F. J. (1971). Über Oberflächenfeinstrukturen von Rostsporen. Eine rasterelektronenmikroskopische Untersuchung. *Arch. Mikrobiol.* **78**, 158–165.
Ward, H. M. (1888). Illustrations of the structure and life-history of *P. gr.*, the fungus causing the rust of wheat. *Ann. Bot.* (*London*)**2**, 217–222.
Waterhouse, W. L. (1921). Studies in the physiology of parasitism. VII. Infection of *Berberis vulgaris* by sporidia of *P. gr. Ann. Bot.* (*London*)**35**, 557–564.
Waters, C. W. (1928). The control of teliospore and urediniospore formation by experimental methods. *Phytopathology* **18**, 157–213.
Wells, K., and Wells, E. K., eds. (1982). "Basidium and Basidiocarp." Springer–Verlag, New York/Heidelberg.

Wenzler, H., and Brambl, R. (1981). Mitochondrial biogenesis during fungal spore germination. *J. Biol. Chem.* **14**, 7166–7172.

Yamada, M., Takahashi, K., Takahashi, H., and Tanaka, T. (1973). Studies on alternate host, *Thalictrum thunbergii* DC., as an origin of physiologic races of wheat leaf rust, *Puccinia recondita* Roberge ex Desm. f. sp. *tritici* in Japan. *Rep. Tottori Mycol. Inst.* **10**, 283–302.

Yaniv, Z., Kenneth, R. G., and Miura, J. (1979). Teliospore formation in *Puccinia graminis* f. sp. *tritici* grown in axenic culture, induced by the fungus *Aphanocladium album. Physiol. Plant Pathol.* **14**, 153–156.

Zimmer, D. E., and Schafer, J. F. (1960). Variability of telial formation of *Puccinia coronata. Proc. Indiana Acad. Sci.* **70**, 91–95.

13

Obligate Parasitism and Axenic Culture

P. G. Williams

New South Wales Department of Agriculture, Biological and Chemical Research Institute, Rydalmere, New South Wales, Australia

I. Introduction

It was to be expected that the successful axenic culture of *Puccinia graminis* f. sp. *tritici* (Williams *et al.*, 1966, 1967) would provoke research on the axenic culture of rust pathogens of other cereal and noncereal hosts. The first decade produced about 40 articles and 5 reviews (Scott and Maclean, 1969; Scott, 1972, 1976; Wolf, 1974; Williams, 1975c). Since 1976, activity has declined steadily, and at the present rate it will take at least two decades for the literature to double in size.

It is not hard to explain why the axenic culture of rust fungi has been a bit of a 7-day wonder. Any unusual finding is bound to attract a certain amount of short-lived, faddish interest. It is also true that research priorities change. What

seemed a question of vital importance 30 or 40 years ago finds itself a long way down the list today. The low level of current activity is also attributable to the limited effectiveness of present methods. Some mycologists will have been disappointed when, having followed the recommended techniques carefully, the strains of rust of particular interest to them failed to grow. Others will have been deterred from trying by the mounting evidence that the genetic identity of the inoculum and the mycelia that it forms cannot be assumed.

It is time to look critically at the present methods for culturing rust fungi and to seek possible ways to improve them; it is also time to confront the fact that available media are so far from optimal for the growth of normal mycelia that genetically aberrant forms are encouraged to develop.

The Editors' invitation to write this chapter came 6 years after I had given up work on rust fungi, so I hesitated before accepting. On reflection, I saw that my distance from rust fungi could be an advantage rather than a handicap. Also, the chapter presents an opportunity to tackle some unanswered historical questions with an ex-participant's benefit of hindsight.

The chapter consists of a section (Section II) on the meaning of obligate parasitism and two sections on historical and technical themes. Section III,A proposes reasons the axenic culture of cereal rust fungi remained a mystery for so long and why K. J. Scott's project succeeded where others failed. Section III,B gives an account of the successful investigation at the University of Sydney. Section IV,A,B covers possible ways of improving present methods of axenic culture and the problems raised by the occurrence of genetically variant mycelia in axenic cultures. The chapter is thus decidedly selective. For a detailed account of axenic cultures of cereal and other rust fungi, especially their nutrition and metabolism, readers are directed to the review by D. J. Maclean (1982).

This chapter is confined to studies of axenic cultures started from urediospores. Rust colonies in leaves have been used successfully as inoculum for culturing several cereal rust fungi (Lu *et al.*, 1964; Ingram and Tommerup, 1973; Ando *et al.*, 1979). This type of starting material promises distinct advantages over urediospores of greater reliability and higher yields of colonies. A direct comparison of the two forms of inoculum is an important topic for future study.

II. Obligate Parasitism

I am uncomfortable with the conjunction of the terms *obligate parasitism* and *axenic culture.* I believe, with others (Thrower, 1966; Shaw, 1967; Lewis, 1973), that obligate parasitism and obligate parasite are

degraded expressions whose meaning is ambiguous. Therefore, they no longer have a place in the language of science.

Anton De Bary (1887) coined the term *obligate parasite* to describe a fungus for which a parasitic phase is an essential part of its life cycle. As well as I am able to determine, from Ward (1901) through Arthur (1929) to Brown (1936), there was steadfast adherence to De Bary's definition based on ecological relationships. But during the late 1930s, obligate parasite came also to denote a fungus that had not been grown in artificial culture. By the early 1940s, this metonymy had become so widespread that Ainsworth and Bisby gave two definitions of obligate parasite in the first edition of their "Dictionary of the Fungi" (1943). Twenty years later, the usefulness of the term obligate parasite had become completely eroded. For Gäumann (1950) and Yarwood (1956), the two meanings were even linked by a mysterious kind of "logic":

Gäumann/If a pathogen can feed only on living matter and hence cannot be cultured on an artificial medium in the laboratory, the botanist describes it as an obligate parasite.

Yarwood/When one organism can grow only by securing its food from continued association with another living organism, the condition is termed obligate parasitism. Obligate parasites, therefore, are those organisms which cannot be cultured on non-living substrata.

Brian (1966) and Shaw (1967) recognized that using obligate parasite as a term denoting nonculturability created a muddle, but neither was able to offer an effective solution. Thrower (1966) made a step forward by proposing to revive the words *biotroph* and *necrotroph* (Link, 1933) to define modes of parasitism that implied different nutritional peculiarities. Then, in 1973, D. H. Lewis published a thoughtful critique of the situation. He abandoned culturability as a basis for classifying fungi and proposed separate schemes based on ecological and nutritional behavior. From these schemes he arranged the fungi into five groups. It is not appropriate to consider the whole classification here. The rust fungi fall into Lewis' Group 5, "Ecologically Obligately Symbiotic Biotrophs," or Obligate Biotrophs for short. This is the term I will use in this chapter.

Given the ambiguity of obligate parasitism, what does the title of this chapter mean? In the De Baryan sense it means that the subject of the chapter is what axenic cultures have revealed about why cereal rust fungi are obliged to live as intercellular parasitic symbionts. If that were the case, this chapter would be short and sweet. First, nutritional investigations have not uncovered any "essential" nutrient that could only be provided by a living cereal host plant. Work with axenic cultures of *P. graminis* f. sp. *tritici* has shown that the fungus is heterotrophic for reduced sulfur (Kuhl *et al.*, 1971; Howes and Scott, 1973). Because this "metabolic lesion" also occurs in the water molds, which are obligate saprotrophs (Cantino, 1950), it does not help explain the obligate biotrophy of the rust fungi.

The growth of saprophytic rust mycelia depends on inoculum density (Kuhl *et al.*, 1971) due, perhaps, to a permeability defect causing leakage of metabolites into the medium (Scott and Maclean, 1969). If parasitic mycelia are similarly flawed, and some observations agree with the idea (Dickinson, 1955; Williams and Shaw, 1968; Williams, 1975c), this may be a clue to the obligatory need of these parasites for an intercellular life-style.

I assumed that the editors intended the metonymic sense of obligate parasitism in the title of this chapter. Therefore, the chapter is about how cereal rust fungi were, for a long time, "difficult or impossible to cultivate on an artificial medium," and how most of them still are!

III. Historical Overview

In the following section I have set two complementary objectives. The first is to consider the reasons the axenic culture of *P. graminis* f. sp. *tritici* did not happen until the second half of the twentieth century. The second objective is to explain why it *did* happen when and where it did.

A. CONCEPTUAL HANDICAPS

The axenic culture of cereal rust fungi remained an apparently difficult matter for many decades because of two enduring misconceptions about the germination of a urediospore. One related to the nature of the germ tube, the other to its growth rate. Each false idea shaped the way in which culture experiments were conducted. Eventually, different ideas about germ tube function were adopted, the culture experiments were conducted with different expectations, and success followed.

1. Mycelial Primordium or Promycelium?

It will surprise many, as it did me, to learn that the teaching of Anton De Bary was responsible for a widespread and persistent misconception about the nature of a urediospore germ tube (De Bary, 1887, pp. 109–115). He distinguished two kinds of fungal spore germination, sprout or yeastlike germination and tube germination. In tube germination, the spore grows out at one or more places into a tubular process, the germ tube, which "is of the nature of a fungal hypha." In cases where it is nourished and develops directly into a mycelium, the germ tube is "the primordium of the mycelium." In other cases, the tube ceases growth, abstricts some smaller spores, and dies. Here, the product of germination is referred to as a "promycelium."

We will never know why De Bary chose the germination of a urediospore and of a teliospore of *P. graminis* to illustrate these elegant concepts. He even included drawings (in his Fig. 55) from Julius Sachs' textbook. By choosing a urediospore germ tube as an example of a mycelial primordium, De Bary placed the urediospores of *Puccinia* in the same category as the conidia of molds such as *Penicillium* and *Aspergillus*. Thus he determined what three generations of observers expected to see when a rust fungus began to grow in axenic culture: The germ tubes would branch and form mycelia. It was an unfortunate choice because, as discussed below, it was wrong: A urediospore germ tube is a promycelium, not a mycelial primordium. Before considering why De Bary's choice of an example was a mistake, it is important to examine how his teaching determined the way in which axenic culture experiments with urediospores were carried out.

The classical rust culture experiment consisted of sowing urediospores on a medium containing the nutrient being tested. After incubation for about 1 day (the reason that the incubation was rarely longer is discussed below), germ tube growth was compared with that from spores on a control medium lacking the test substance. It was expected, according to De Bary's concept of the urediospore germ tube as a mycelial primordium, that if the test substance supplied the essential nutrient(s) the germ tubes would branch and form mycelia, or at least grow a little longer.

Since mycelia were never seen and the only branches formed were the result of sympodial growth, giving a germ tube a "stagshorn" appearance, the length of germ tubes was measured to detect growth stimulation. To make it easier to measure individual germ tubes, the urediospores were seeded thinly. We now know that the growth of rust mycelia in axenic culture is favored by a heavy seeding density. The emphasis on measuring germ tube length therefore handicapped attempts to culture rust fungi for many years. Conversely, adopting other means of detecting growth-stimulating activity abolished the need to sow the inoculum thinly. This proved to be a significant factor in the success of the investigation at the University of Sydney.

De Bary's concept of the germ tube as a mycelial primordium was not challenged until after World War II. S. Dickinson's work with several cereal rust fungi supported the view that the differentiation of an infection structure is an obligatory path of development for a urediospore germ tube. Details of the infection process in several cereal rust fungi had been known for many years (Pole Evans, 1907; Allen, 1923), but there was no understanding of the significance of the various steps in the process. Dickinson observed (1949, 1955) that there are great differences in morphology, physiology, and cytology between germ tubes and intercellular mycelia, indicating that the formation of the latter from the former involves a profound change. Dickinson's experiments showed that a germ tube is incapable of forming intercellular mycelia until it has been induced to form an infection structure. This finding was confirmed by

Chakravarti (1966). The formation of an infection structure by a urediospore germ tube is now seen to be a natural and indispensable function. The urediospore germ tube is therefore homologous with the tube produced by a germinating teliospore; both function as a promycelium. The true mycelial primordium of the dikaryophase is a tube that grows from a substomatal vesicle, that is, an infection hypha.

Dickinson's writings influenced the conduct of the Sydney rust culture project in significant ways. For example, his view that a urediospore germ tube is a promycelium and that physical aspects of the environment are important in stimulating its differentiation into an infection structure was implicit in the design of many of the early culture experiments (p. 410). One of Dickinson's observations formed the basis of the criteria for detecting growth stimulation by test media. Dickinson believed that germlings that only completed one of the first steps in differentiation, nuclear division, had received a suboptimal stimulus. The Sydney group took the view that such germlings might be cases of incomplete development of a germ tube as a mycelial primordium. The group argued that if that were the case, the provision of suitable nutrients should cause the formation of a septum between the daughter nuclei, followed by normal, monopodial branching. The formation of septa and true (monopodial), rather than stagshorn (sympodial) branches, became the criteria for detecting growth-stimulating activity.

When germ tube length was abandoned as a measure of growth, it became a practice to crowd an inoculum into a small area. This made it easier to mount a seeded zone in lactophenol cotton blue and scan it for septa and branches in germ tubes or infection hyphae. Inadvertently, the new technique also made it more likely that an inoculum would produce saprophytic mycelia.

I now regard the formation of mycelia from a germ tube as the result of abnormal germling development (Williams, 1971). Also, I suspect that many of the septa formed in such a germ tube will be found to be adventitious, that is, poreless septa formed in relation to the movement of protoplasm, in contrast to primary septa, which have a pore and are formed in relation to mitosis (Talbot, 1971). It is ironic that the criteria for growth stimulation that played an important part in the success of the investigation embodied a false idea about the nature of a urediospore germ tube.

2. Guesswork about Growth Rates

The second enduring misconception that delayed the axenic culture of cereal rust fungi was that urediospore germ tubes grow at essentially the same rate as intercellular rust mycelia. Like most rust workers, I suspect, I had never given much thought to such a question and would have agreed with Yarwood's assertion (1956) that the maximum germ tube growth rates of downy mildew and rust fungi “are as high as occur on the living host.”

For about 3 hr after its emergence, a urediospore germ tube grows rapidly at 70 to 100 μm/hr. Then the rate declines steadily until, by about the twentieth hour after seeding, extension growth virtually stops (Stock, 1931). Because germination and germ tube growth require only water, it is apparent that these processes take place at the expense of endogenous reserves. Accordingly, the declining rate of growth was interpreted as the result of the depletion of a store of an essential substance. It was expected that on a medium that contained the essential substance, the initial high growth rate would be maintained or its decline abated. It followed from this reasoning that growth-stimulating activity ought to be detectable after no more than 1 day of incubation. On the basis of these expectations, media were evaluated by measuring germ tube length (discussed in Section III,A,1), and inocula were collected without regard to asepsis. Two exceptional studies used aseptic precautions that allowed cultures to be incubated for more than 1 day. Mains (1917) kept his cultures for 4 days and recorded that sugars prolonged the life of germlings. Fuchs and Gaertner (1958) incubated their cultures for 10 days and became the first to observe that sulfhydryl compounds promote the formation of saprophytic hyphae of the wheat stem rust fungus.

In 1963, few rust workers would have considered that the accepted interpretation of germ tube growth kinetics was incorrect. I was alerted to this possibility on a visit to the Cereal Rust Laboratory in St. Paul in that year. J. B. Rowell put it to me then that, compared to other fungi, the rust fungi grow very slowly. For me, this was a brand-new fact about the rust fungi. Moreover, it was a fact that provoked new lines of thought about the problem of axenic culture.

Table I

Linear Growth Rate of Some Fungi

Organism	Growth rate (mm/day)
In vitro	
Neurospora crassa[a]	75
Rhizopus nigricans[a]	60
Fusarium oxysporum[a]	5
In vivo	
Sclerotinia fructigena[b]	7.2
Botrytis cinerea[b]	4.8
Penicillium expansum[b]	3.1
Melampsora lini[c]	0.16–0.46
Puccinia graminis f. sp. *tritici*[c]	0.07–0.22

[a] Cochrane (1958).

[b] Cole and Wood (1961).

[c] P. G. Williams and M. Shaw (unpublished data), parasitic growth from callus (*Melampsora*)or within leaves (*Puccinia*).

When I got back to M. Shaw's laboratory where I was working at the time, I checked out Rowell's observation. Data from various sources confirmed that he was correct (Table I). The intercellular mycelia of rust fungi grow at a rate that is one order of magnitude less than the mycelia of facultative necrotrophs like *Sclerotinia, Botrytis,* and *Penicillium,* and two orders of magnitude below that of obligate saprotrophs such as*Neurospora* and *Rhizopus.*

It was immediately apparent that, contrary to belief, the initial burst of growth by a germ tube is at least 10 times faster than the normal rate at which intercellular rust mycelia colonize a leaf. What a stunning insight! Perhaps the axenic culture of a rust fungus was achievable after all. If the rust fungi naturally grew slowly and if, when they first grew in an axenic culture, they grew at a fraction of their normal rate, it might be necessary to incubate a culture for months before growth was evident. In that case, it was clearly time to abandon length measurements and adopt more sensitive techniques of growth assessment. During the last months in Shaw's laboratory I made some exploratory experiments with *Melampsora lini,* but it was not until I returned to Australia in 1965 that an opportunity arose to put the new ideas properly to the test.

B. THE UNIVERSITY OF SYDNEY PROJECT

The background to the axenic culture of *P. graminis* f. sp. *tritici* illustrates the important role that the personality, interests, and relationships of the principal characters have in the making of an unusual scientific discovery. It also highlights the fact that scientists not directly taking part in the work, the supporting personae in the action so to speak, make a vital contribution to its progress. This section describes the background and relationships of the people directly and indirectly involved in K. J. Scott's rust culture project. It then gives a personal account of the course of the investigation and its aftermath.

1. The People

a. Principals. K. J. Scott graduated in Agricultural Science from the University of Sydney in 1955, specializing in plant pathology and biochemistry. He completed a master's degree in the Department of Biochemistry with Adele Millerd on the biochemistry of host-parasite relations in powdery mildew of barley and obtained his doctorate in G. Krotkov's laboratory at Queen's University, Kingston, Ontario, with a thesis on

biochemical changes associated with crown gall disease. During postdoctoral study in Australia and the United States, he resumed his interest in the barley mildew system. When he returned to the Department of Biochemistry at the University of Sydney as a staff member, Scott was no stranger to the head of the department, J. L. Still, whose part in the story is explained below.

On joining the department, Scott took over the supervision of a graduate student, Joy L. Kuhl, who had begun her master's degree under his predecessor. She was working on a problem in plant cell culture and had developed an expertise in plant tissue culture techniques. This suited her very well to work on the axenic culture project that Scott was planning. When she completed her degree Joy Kuhl became a graduate assistant in Scott's laboratory on a research grant that he had obtained in the meantime from the Wheat Industry Research Council (W.I.R.C).

I graduated from the same School of Agriculture as K. J. Scott but one year ahead of him. We shared a common interest in biotrophic fungi that we owe to the enthusiasm and sparkling teaching of N. H. White. It was also White who guided me into my first professional job as a potato pathologist in the Tasmanian Department of Agriculture. From Tasmania, I went to North America where I spent 7 years becoming a rust physiologist—3 years studying for a doctorate in botany and biochemistry with P. J. Allen at the University of Wisconsin, Madison, followed by 4 years of postdoctoral research in Saskatoon. The first 2 years were spent with G. A. Ledingham at the National Research Council's Prairie Regional Laboratory, the second with M. Shaw in the Department of Biology in the University of Saskatchewan. Those years also provided opportunities to visit other cereal rust labs in the region, in Winnipeg, Manitoba, and St. Paul, Minnesota.

My association with K. J. Scott's axenic culture project resulted from a chance meeting with him shortly after I returned to Australia from Canada in January 1965. Over lunch, Scott told me of his research grant from the W.I.R.C. to grow the stem rust fungus in artificial culture. His main idea was to attempt to culture *P. graminis* f. sp. *tritici* using the tissue culture approach that V. M. Cutter (1959) had apparently used successfully for the cedar-apple rust fungus, *Gymnosporangium junipeiae-virginianum.* I spoke about my conviction that the traditional experiment with urediospores needed to be repeated with different expectations. Scott invited me to join him, and within a couple of weeks I had a temporary academic post in the Department of Biochemistry.

D. J. Maclean trained originally as a pharmacist but returned to the university to obtain a degree in biochemistry. Here he met K. J. Scott, who persuaded him to spend his final year of undergraduate studies working on the axenic culture project that, in January 1966, was making exciting headway. Maclean obtained his degree with Honors and continued research on axenic culture for his doctorate.

b. Supporting Personae. N. H. White, professor of plant pathology, played two seminal roles in the rust culture project. As I have already mentioned, he taught Scott and me about plant pathology and passed on to us his own curiosity about rust and mildew diseases. He must also be credited with raising his friend J. L. Still's awareness of the problem of the axenic culture of obligate biotrophs such as the rust fungi.

J. L. Still, professor of biochemistry, had a background in bacterial nutrition. This made him especially receptive to the challenge presented by the axenic culture of *P. graminis* f. sp. *tritici.* He supported Scott's application to the W.I.R.C. for funds and kept a close interest in the progress of the work. Still often walked through the laboratory on his way to or from a teaching hour and would stop and chat about the research. On one occasion in July 1965 he inquired if we had tested yeast extract yet. We had not, but I took this advice. It proved to be the turning point of the investigation (p. 410).

Another close associate of N. H. White, I. A. Watson, also had a decisive role in the success of Scott's project. Watson was professor of genetics and plant breeding at the University of Sydney. His support was important in winning the W.I.R.C.'s approval of funds for the axenic culture project. Watson contributed directly to the success of the project by choosing the now celebrated Culture No. 334, race 126-Anz-6,7[1] from the many accessions in the university's Collection of Strains. Watson selected this isolate, which proved so much easier to grow than many others, not at random but for sound genetic reasons. This race had first appeared in Western Australia in 1926. It quickly grew to dominate the Australian stem rust population and influenced wheat breeding for many years (see chapter by Luig, Vol. II; Watson, 1981). Successful axenic culture would offer new approaches to studying the genetics of No. 334.

W. R. Bushnell and I formed a longstanding friendship as graduate students of P. J. Allen at the University of Wisconsin in the late 1950s. When Scott's project succeeded, Bushnell was among the first to hear about it and shortly found himself involved in providing independent confirmation of the artificial culture of *P. graminis.*

2. The Story

Of all Australian universities, the University of Sydney was the most appropriate for an effort to culture the wheat stem rust fungus. First, this university has been a leading center for cereal breeding and rust research since 1921 through the work of W. L. Waterhouse and I. A. Watson. Second, the university stands on the site of the first stem rust epidemic recorded in the former colony in 1795 (White, 1981).

The project began in 1963 when Scott took up his appointment in J. L. Still's Department of Biochemistry and began planning his research activities.

By the time I joined the group, he had obtained funds from the W.I.R.C., and Joy Kuhl was already getting some callus-like growth from wheat hypocotyls.

My active participation, according to my laboratory notebook, dates from St. Valentine's Day, February 14, 1965. The first task was fairly straightforward: to work out a way of producing urediospores under aseptic conditions. In the method adopted, infected leaves with unopened urediospori were surface-disinfected and placed on sterile nutrient in tubes. Uncontaminated urediospores could be collected from the uredia a few days later. By the end of March, regular batches of urediospores were being produced by this procedure, and axenic culture experiments by the direct method of seeding media with urediospores got under way.

The design of many early tests took into account Dickinson's observations (1949) on the sensitivity of rust germlings to contact with surfaces. Urediospores were sown not only directly on the surface of the agar test media but also on different supports placed on the agar (e.g., filter paper, cellulose powder, and leaves of wheat, broad-leafed grasses, and banana), which had been extracted in hot ethanol and boiling water. The possibility that growth in axenic culture was suppressed by the formation of autoinhibitors (and this may be the case, see p. 419) prompted many experiments. Various adsorbents, such as charcoal, serum albumin, and glass microbeads, were tested. Another experiment involved flowing nutrient in a descending chromatography arrangement; in another, urediospores were incubated in an electric field!

Other lines of work were pursued in the early stages of the project. These included experiments on the tissue culture of wheat and barberry aimed at setting up dual cultures of rust fungus and host. Preparations were made to monitor protein and nucleic acid synthesis using autoradiographic techniques, with the idea of detecting sub-morphological growth stimulation or delayed death of urediospore germlings. These programs received less and less effort and were finally abandoned as the direct culture experiments progressed.

By May, nutrient media were being prepared with Czapek's mixture of sucrose and minerals as a base. The persistence of the carotene pigmentation in germlings indicated that they remained alive longer on media containing this mixture than on plain agar (cf. Mains, 1917). In early August my notebook records the first use of a basal medium containing Czapek's mixture and a pinch (0.1 g/liter) of yeast extract, a suggestion of J. L. Still (p. 408). Soon afterward, the first germlings were found with septa and “true” branches. They were very rare and occurred unpredictably from one inoculum to another. Nonetheless, this kind of development in a germling was unique, and I had a feeling we were on the right track.

Further experiments showed that germlings with septa and branches were more frequent at higher yeast extract concentrations up to about 1.5 g/liter. At the higher concentrations, rare germlings formed a knot of short, intensely

branched hyphae. In late November, work began on isolating the active principle(s) in yeast extract.

A set of media were prepared containing fractions obtained from yeast extract using ion-exchange resins. On December 10, the media were seeded with urediospores and placed in a reconditioned refrigerator at 8°C to keep them going through Christmas and New Year, the peak summer holiday season. Five weeks later, on January 13, I found small white tufts of rust mycelia on several plates—and not a soul in the building to share the excitement!

Experiments in the next few months confirmed that heavy seedings of urediospores incubated for 3 to 4 weeks under cool, moist conditions on Czapek's medium plus yeast extract would produce mycelia of *P. graminis* f. sp. *tritici* with reasonable consistency. A brief report of these observations was sent to *Nature,* but it was promptly returned with a curt rejection slip. Puzzled and dismayed, we trimmed the text a little, deleted one illustration, and sent it to *Phytopathology*, where it was accepted as it stood (Williams *et al.*, 1966). A year or two later, the editor of *Nature,* J. Maddox, wrote requesting a reprint and regretting that he had been advised that the paper was "more exclusively within plant pathology than the general readership of *Nature* would require. …"

Events in early 1966 moved fast. D. J. Maclean joined the group and began by reading widely in the literature on fungal nutrition. Articles by Fries (1955) and Sedlmayr *et al.* (1961) on the growth requirements of saprophytic basidiomycetes gave him the idea to test peptone as a supplement to, or a substitute for, yeast extract. In my view, it was not an appropriate time for branching out in new directions, but Maclean went ahead. He soon showed that growth on media containing peptone was more vigorous and reliable than on media with yeast extract only. Moreover, adding peptone to the sucrose–minerals–yeast extract medium brought a new development. One morning Joy Kuhl found several cultures in which the edges of the mat of white mycelia had rolled inward, exposing a yellow-orange undersurface. A scraping from the orange zone showed masses of urediospores. The fungus was sporulating!

The events that followed might have been predicted: Teliospores were found on ageing stromata, tests of infectivity showed that mycelia grown on media containing peptone could cause rust infections on wheat leaves; mycelia renewed growth, albeit rather slowly, when pieces of a culture were transferred to fresh medium. A second short article in *Phytopathology* (Williams *et al.*, 1967) placed the question beyond doubt that the wheat stem rust fungus had at last been cultivated on an artificial medium.

W. R. Bushnell, not without some initial difficulty, because the American isolates he tested initially did not grow, made an independent confirmation that the Australian race 126-Anz-6,7 could be grown in axenic culture (Bushnell, 1968). The Sydney group was grateful, although Bushnell's article gave temporary support to skeptics who believed the Australian fungus was a freak (Trocha and Daly, 1970).

3. Epilog

The apparent importance of knowing how to grow cereal rust fungi in axenic culture was at its highest in the 1940s. For many cereal rust workers of that era, axenic culture held the key to such fundamental questions as heteroecism, obligate biotrophy, physiological specialization, and the mechanism of disease resistance (Chester, 1946). Since then, rust control through breeding for resistance has been widely successful, and new priorities have emerged. The news in 1967 of the successful axenic culture of *P. graminis* f. sp. *tiitici* was therefore greeted by a new breed of rust workers for whom this discovery was not "the philosopher's stone." The event was a generation too late. W. L. Waterhouse spoke for old rust hands when he wrote in a letter of congratulation, "It is a wonderful achievement and opens up completely new approaches to the age-long problem of wheat stem rust. I regard it as an epoch-making event."

As a recognized historian of mycology, G. C. Ainsworth, director of the Commonwealth Mycological Institute, Kew, took a broad historian's view. His remark on a visit to the Department of Biochemistry in 1968 was offered as a compliment. "The artificial culture of the wheat stem rust fungus," he said, "has all the marks of an important breakthrough in science; it was done by obscure people, in an obscure place and was rejected for publication by *Nature."* Scott, who was not an obscure scientist, was justly insulted.

4. Postscript

The University of Sydney rust culture outfit broke up in 1967–1968. At the end of 1967, Kuhl left and is now a forensic biologist in the Sydney Coroner's Court. In the following year, K. J. Scott moved to the University of Queensland. There, he swiftly gained promotion to a chair of biochemistry, and in this post he continues his research on rust and powdery mildew diseases. D. J. Maclean went with Scott and completed his doctorate at the University of Queensland. After postdoctoral experience in England, he returned to the same university, where he now teaches biochemistry.

When Scott and Maclean went to Queensland I crossed the campus to the Faculty of Agriculture, where I studied rust culture on contract to the W.I.R.C. Since 1977 I have been at my present location investigatingvesicular–arbuscular mycorrhizal fungi for the Australian Meat Research Committee and the Australian Wool Corporation.

IV. Problems

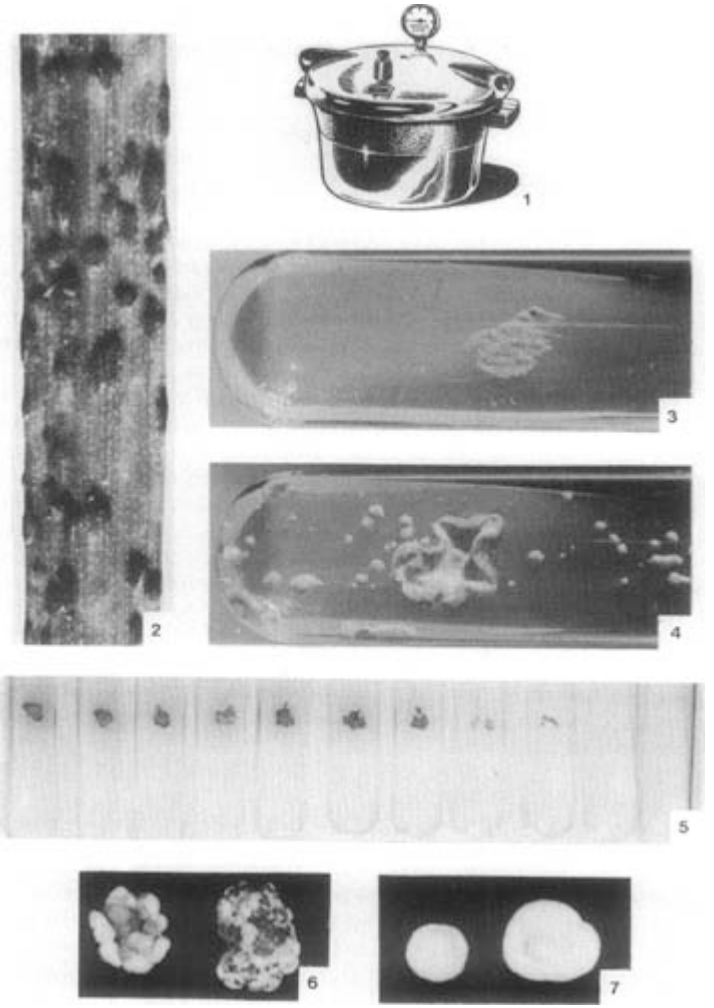
A. THE NEED FOR BETTER METHODS

Figures 1–7 outline the basic steps in setting up an axenic culture of a cereal rust fungus from urediospores and illustrates the types of cultures that may be obtained. The technique of inoculation is very simple, and a rust worker can choose from a variety of easily prepared nutrient media. Regrettably, however, no set of directions can be given that will ensure success. This essay addresses this problem.

The methods devised so far for culturing cereal rust fungi on artificial media provide only marginally suitable conditions for full saprophytic development of the fungi and their sustained growth in subculture. The methods have succeeded with less than half the strains tested. As a result, axenic cultures of rust fungi have become more of a scientific curiosity than a useful tool. Saprophytic mycelia will remain novelties until the methods for growing them are improved to allow the culture of any chosen isolate. This section first discusses the several variations that have been made empirically to the original procedures and have resulted in more vigorous or consistent growth of easily cultured lines or in the growth of previously uncultured lines. Then, I discuss three phenomena that determine the result of an inoculation and that need to be understood if the techniques are to be improved in a rational way.

1. Empirical Modifications

a. Inoculum Density. In early work, the most vigorous growth of rust mycelia occurred in zones of high inoculum density (Williams *et al.*, 1966; Bushnell, 1968). It seems currently to be generally recognized that in testing the culturability of an isolate, the inoculum must be applied thickly. Bushnell (1976) obtained more consistent growth of an isolate of *P. graminis* f. sp. *tritici* race 17 by increasing the volumetric ratio of inoculum to medium. However, many isolates of this species have never produced saprophytic mycelia, even in the most heavily seeded cultures (Wong and Willetts, 1970; Bushnell and Stewart, 1971; Hartley and Williams, 1971a).



Figs. 1–7. Axenic culture of a cereal rust fungus from urediospores. Example: *P. graminis* f. sp. *tritici.* First, prepare slants or dishes of agar growth medium following one, or better still several, of the many published recipes. For best results, sterilize the medium in a pressure cooker (Fig. 1) rather than an autoclave. Next, produce urediospores on seedling leaves of a susceptible cultivar (Fig. 2). Raise the inoculum on plants in pots and accept 5–15% contamination of axenic cultures, or take the trouble to prepare aseptic leaf cultures (Williams *et al.*, 1966) and enjoy a low risk of contamination. Inoculate cultures by spreading spores heavily in a discrete zone about 15 mm in diameter. Incubate under cool, moist conditions for 10 to 20 days or, if there is still no sign of growth, 1 to 2 months (but watch out for variant mycelia). Unsuccessful inoculations look like the culture in Fig. 3, which consists of a mat of collapsed germ tubes. Figure 4 illustrates a successful inoculation. The likelihood of a successful result is unpredictable. Eleven inoculations of an easily

cultured isolate (No. 334, race 126-Anz-6,7) made on March 27, 1969, are shown in Fig. 5. Two failed, seven formed mycelia that turned brown, and two produced a thick, dark stroma. Pieces of a vigorous primary culture like that in Fig. 4 transferred to fresh medium may continue to grow. Subcultures mostly retain the dark, compact form shown in Fig. 6. Variant mycelia that grow as white fluffy colonies (Fig. 7) may arise during long-term maintenance of subcultures or prolonged incubation of cultures that originally produced few mycelia. [(Fig. 2, ×2.5; Figs. 3 and 4, × 1.5; Fig. 5, ×0.5; Figs. 6 and 7, × 1.5) (Fig. 1, Courtesy of Namco Industries, Victoria, Australia; Fig. 2, D. J. S. Gow, unpublished; Figs. 3–5, P. G. Williams, unpublished; Figs. 6 and 7, adapted from Bushnell and Bosacker, 1982)].

b. Natural Products. There have been several reports of significant benefits as the result of changing the amounts and kinds of natural products in the original yeast extract and peptone medium of Williams *et al.*(1967). Bushnell's experiments (Bushnell, 1976) with two isolate sof *P. graminis* f. sp. *tritici,* which he had earlier (Bushnell and Stewart, 1971) found difficult to grow on that medium, are a good example. An isolate of race 38 that died after growing poorly through five subcultures on the medium of Williams *et al.* grew well on a medium enriched in peptone and having yeast extract replaced by casein hydrolysate; on the same medium, an isolate of race 17 that had previously only produced sparse mycelia and had not grown at all in subculture grew more consistently and could be subcultured indefinitely (Bushnell, 1976).

Kuck (1979) obtained promising growth of an isolate of race 34 of *P. graminis* f. sp. *tritici* on a chemically defined medium whose composition was based in part on the free amino acid composition of wheat leaves. Very sparse (about 10 per square millimeter) seedings of urediospores gave rise to tiny (0.5–1 mm), fertile uredia on a medium containing various sugars, vitamins, and mineral salts, together with the amino acids. On a medium in which the composition of the mixture mimicked the free amino acids in rusted wheat leaves, the colonies remained vegetative.

c. Diverse Supplements. A number of substances have been reported to stimulate the growth of cereal rust fungi when added to yeast extract and peptone media. Kuhl *et al.* (1971) found that pectin, gelatin, sodium citrate, and bovine serum albumin (BSA) increased the growth of particular isolates of *P. graminis* f. sp. *tritici* in early experiments at the University of Sydney. They also supplemented media with one or another of these substances to obtain mycelia of *P. graminis* f. sp. *avenae, P. graminis* f. sp. *secalis, P. recondita* f. sp. *tritici,* and *P. coronata.* However, none of these substances has been studied systematically and, with the exception of BSA, their growth-promoting action has not been widely confirmed (Wong and Willetts, 1970; Bushnell, 1976; Coffey *et al.,* 1969).

Grambow *et al.* (1977) obtained substantial growth stimulation by the addition of bisindolylmethane to a medium containing Czapek's minerals, glucose, and casein hydrolysate. Further experiments suggested that the

enhancement is the result of an increased formation of infection structures (Grambow and Muller, 1978).

Amending media with compounds specifically aimed at preventing the formation of oxidized phenols has been investigated by H. J. Willetts. Ascorbic acid and glutathione, mixed in the agar or applied weekly in filter paper strips, reduced the intensity of brown discoloration in cultures but did not improve the growth of an easily cultured isolate of *P. graminis* f. sp. *tritici* (Wong and Willetts, 1970). A less rapidly oxidized reducing agent, dithiothreitol, was very effective in preventing the buildup of brown pigment but gave only slight growth stimulation (Fry and Willetts, 1974).

Activated charcoal is a common adsorbent but has not been widely tested. Ingram and Tommerup (1973) reported that its addition to the agar sometimes improved the survival of aging colonies of *P. coronata* f. sp.*avenae* and *P. recondita* f. sp. *tritici.*

d. Coculture. Some difficult strains of the wheat stem rust fungus have been grown by coculture with another vigorous isolate of the same species. Hartley and Williams (1971b) observed that an isolate of *P. graminis* f. sp. *tritici* race 21-Anz-1,2,3,7 exerted a specific stimulatory effect in mixtures with isolates of other races. Visible colonies of two isolates, 126-Anz-1,6,7 and 126-Anz-1,4,6,7, grew for the first time when cultured together with 21-Anz-1,2,3,7.

2. Basic Phenomena

Three phenomena determine the outcome of an inoculation: endoregulation, autostimulation, and autoinhibition. Examples of each can be found in other fungi, but the operation of all three in the rust fungi makes these fungi difficult to culture.

a. Endoregulation. Endoregulation plays an important part in the early development of rust fungi both in nature and in axenic culture. This is because spore germination and germ tube differentiation are to a large extent controlled by endogenous factors (see Staples and Macko, Chapter 9, this volume). Through these two processes, the rust organism is transformed from a resting state to a vegetative state. The morphological and cytological steps in the transformation were first described in detail by Ruth Allen (1923). They involve the construction of a system of tubes and chambers (the germ tube and infection structure) together with coordinated mitoses, movements of nuclei and cytoplasm, and the formation of adventitious septa (p. 405). The final event in the sequence is the inauguration of the first vegetative cell, the so-called haustorium mother cell, which is delimited by a primary septum.

Brown (1971) and Ogle and Brown (1971) made quantitative studies of infection in stem rust of wheat. Their results showed that a proportion of germlings of *P. graminis* f. sp. *tiitici* were unsuccessful in establishing an infection in wheat tissue as a result of failure at one stage or another in the

differentiation of an infection structure. According to their observations, the genotype and the provenance of an inoculum determine how many of the germlings complete the differentiation sequence. The physical and chemical stimuli that trigger the processes leading to the initiation of intercellular mycelia are treated by Staples and Macko in Chapter 9, this volume.

The genotype (Hartley and Williams, 1971a) and provenance (Bushnell, 1976; Jones, 1974; Kuck, 1979; Williams, 1971, 1976) of an inoculum also affect the initiation of mycelia in axenic culture. This agrees with the idea that the completion of normal germ tube differentiation is also important for fungal development *in vitro*.

Individual germlings vary in ability to establish colonies on nutrient agar (Kuhl *et al.*, 1971). Some germlings form an infection structure, and a proportion of these produce a colony. Mycelia also arise from germlings that have not developed a complete or normal infection structure. The mycelia arising from these germlings (as seen in thinly seeded cultures) may have one nucleus instead of two nuclei per cell (Williams, 1971; Grambow and Muller, 1978). The colonies composed of uninucleate mycelia are thought to be haploid (Williams and Hartley, 1971).

The operation of endogenously regulated processes at the early stages of an axenic culture introduces a measure of uncertainty about how many germlings will establish mycelia and how many mycelia will be genetically normal. The relation between the conditions in which urediospores are produced and their subsequent performance as inocula needs to be investigated. Until procedures are available for producing urediospores of a uniform high quality, mycelia of established rust colonies in leaves may be the preferred starting material for axenic cultures.

b. Autostimulation. As noted earlier, the best growth of saprophytic mycelia occurs in zones of high inoculum density. The growth of adjacent mycelia depends on a mutually stimulatory interaction transmitted through the agar (Kuhl *et al.*, 1971).

Population-dependent growth is uncommon in fungi. The development of *Phytophthora infestans* from zoospores on a semisynthetic medium is sensitive to population density (Clarke, 1966). Darling and McArdle (1954) described a mutant of *Aspergillus amstelodami* that grew poorly in thin seedings on Sabouraud and Czapek-Dox agar. The media could be "conditioned" by first germinating a heavy inoculum of *Aspergillus*on a disk of cellophane and then removing the disk. Colonies grew from single spores placed on the "conditioned" agar. High cell density is critical for growth in the amoebal parasite causing malaria (Moulder, 1962), in human cell cultures (Eagle and Pietz, 1962), and in suspensions of plant cells and protoplasts (Cocking, 1972).

The mechanism of the mutual stimulation between adjacent rust mycelia is incompletely understood. Howes and Scott's experiments (1973) with isotopic

sulfur indicated that the interaction can be attributed in part to the loss of certain amino acids and peptides to the external medium. This agrees with an earlier speculation of Scott and Maclean (1969) that the rust fungi resemble human cell cultures. Mammalian cell lines have a membrane defect causing the loss to the medium of numerous amino acids that they can synthesize but not retain inside the cell in sufficient quantity for growth (Eagle and Pietz, 1962). The fact that effective media for axenic culture of rust fungi are rich in amino acids (Bushnell and Rajendren, 1970; Foudin and Wynn, 1972; Kuck, 1979) is consistent with the possibility that rust mycelia, too, have a permeability defect allowing the leakage of these compounds. The possibility that saprophytic mycelia also leak other substances in addition to amino acids needs to be investigated. "Preconditioned" media—that is, media supplemented with appropriate amounts of all leaked metabolites—may support the growth of many more stains than can be grown at present.

c. Autoinhibition. Observations suggest that mycelial growth in axenic culture is subject to autoinhibition. Numbers of authors have recorded that inoculations with strains that usually grow well sometimes failed. At first, white aerial mycelia grew strongly, but after a time the mycelia became discolored, matted together, and collapsed. The medium became discolored by a brown pigment—a process often associated with "staling" in other fungi (Hawker, 1950). The early death and discoloration of mycelia is a regular occurrence in the development of strains with weak ability to grow in axenic culture (Green, 1976; Bushnell and Stewart, 1971; Hartly and Williams, 1971a). The reason for this behavior may be that mycelia of weak strains succumb to an antagonistic process generated by their own growth. Experiments with mixtures of vigorous and weak strains agree with this possibility.

Hartley and Williams (1971b) studied three strains of *P. graminis* f. sp. *tritici* grown singly and in mixed culture (details on p. 416). One was a vigorous strain that readily formed visible mycelia when grown by itself. The other strains were weak, and their sparse mycelia grew for only 3–4 days in lightly or heavily seeded cultures. The inoculation of the vigorous strain and either of the weak strains together in a lightly seeded culture resulted in a growth interaction. The outcome of the interaction depended on the proportion of each strain present: When the weak strain represented about 20% of the mixture, the vigorous strain grew normally and appeared to stimulate the weak strain, which formed visible colonies for the first time. When the content of the weak strain was increased to more than 50%, mycelia of both strains grew for 8 to 10 days and died, and a brown pigment formed in the agar. In this case, the autoinhibitory processes of the weak strain were apparently sufficiently intense to counteract the stimulatory action of the vigorous strain.

Growth of compact mycelia (Fig. 6) in subcultures seems also to be sensitive to autoinhibition, particularly in the early transfers. Bushnell and

Stewart (1971) and Bushnell (1976), who used low temperatures and enriched media, were more successful than Williams *et al.* (1967) in maintaining compact mycelia in subculture.

There is no clear evidence of the nature of the autoinhibition. As already mentioned (Section IV,A,1,c), efforts to prevent the formation of oxidized phenols in axenic culture have not resulted in notably better growth. The beneficial effect of charcoal (Ingram and Tommerup, 1973) and serum albumin (Kuhl *et al.*, 1971) may be due to the absorption of autoinLibitors. The stimulation of growth by serum albumin is augmented by treating it to remove fatty acids (Kuhl *et al.*, 1971), compounds that are known to inhibit the growth and membrane function of fungi (Lode and Pedersen, 1970).

On present evidence, it seems likely that autoinhibitors of growth are not preformed but are generated during growth. If so, superior media for culturing rust fungi might be designed by eliminating those media constituents from which the autoinhibitors are synthesized.

d. Dynamics of Axenic Cultures. I will conclude with an attempt at integration, to put forward my view of how an axenic culture works. The success or failure of inoculating an artificial medium with urediospores appears to depend on a complex interaction of several factors, some of which are mutually incompatible.

The first element of an axenic culture is the inoculum. Its genotype and history determine, in the first instance, how many mycelia are initiated and how many of them are normal for the dikaryophase. The density of the inoculum is the next determinant, because it influences how many of the mycelial initials will proceed to grow into colonies. The number of germlings per milliliter sets the balance between auto-inhibitory and autostimulatory processes. The relative importance of each of these is primarily under genetic control. Hence, an "easily cultured" strain initiates many normal mycelia that can mutually support one another on present media at a relatively low population density and that only autoinhibit one another weakly.

The artificial medium has many roles to play. First, its constituents cooperate to make a more or less effective signal to trigger germ tube differentiation, resulting in the initiation of mycelia. Second, it provides an energy source, a supply of reduced nitrogen, and reduced sulfur, together with sundry vitamins and mineral elements, as needed by most heterotrophs. Next, the medium provides a physical milieu and a set of metabolites in particular relative concentrations that compensate for the leakage of metabolic intermediates from inside the mycelia. Finally, the medium contains also some growth-limiting substances. These may be present from the beginning, having been introduced in the components of the medium. Alternatively, they may be generated during heat sterilization (see Kuck, 1979), or they may accrue after inoculation, being leached from the urediospores or synthesized from nutrients by the mycelia themselves.

I think it is unlikely that axenic culture of rust fungi from urediospores will ever be a simple matter. Established rust colonies in leaves look like much more promising material for starting cultures. However, improved methods for use with urediospores giving more consistent results with a wider range of strains will be developed when we achieve a deeper understanding of the many interacting processes in axenic cultures.

B. GENETIC STATUS OF MYCELIA

Inevitably, the successful culture of *P. graminis* f. sp. *tritici* aroused suspicions that "such cultures arise from mutants for saprophytic growth rather than from the normal fungus populations occurring in nature" (Trocha and Daly, 1970). Investigations with the wheat stem rust fungus show that although mycelia in axenic cultures may be genetically identical, as far as can be determined, with the disease-causing fungus, cultures may also contain aberrant mycelia that are unknown in the dikaryophase (Williams, 1975c). My purpose here is to review what is known about abnormal mycelia in axenic cultures and to draw the attention of the unwary to the possibility that the genotype that one puts into an axenic culture may not be the same as the genotype that one gets out.

1. Normal Mycelia

a. Primary Cultures. The mycelia of cereal rust fungi in their cereal host plant are dimorphic. Intercellular mycelia proliferate from the substomatal cavity, first as long, sparingly branched hyphae. After this phase of primary colonization, growth assumes an intensive form with the development of short, highly branched hyphae around the site of the substomatal vesicle. These hyphae subsequently give rise to a compact stroma that forms a uredium and later a telium. The development of mycelia in axenic cultures inoculated with urediospores broadly follows the same course in light (Hartley and Williams, 1971a) and heavy (Williams *et al.*, 1967; Bushnell, 1968) seedings.

Hartley and Williams (1971a) showed that the mycelia in some axenic cultures, at least, are genotypically identical in virulence to the mycelia of the organism as its parasitic form. They inoculated wheat with mycelia from axenic cultures of several races of *P. graminis* f. sp. *tritici* and demonstrated that the cultured mycelia had the same virulence on host differentials as the parent race. The mycelial colonies used in these tests arose from germlings that had produced an infection structure and were therefore presumed to be binucleate and haploid (Williams, 1971). The genotype of binucleate mycelia formed from germlings that have differentiated abnormally (Grambow and Muller, 1978) has not been investigated.

b. Subcultures. Transferring compact mycelia (Fig. 4) formed in a primary culture to fresh medium may lead to renewed growth (Bushnell and Stewart, 1971; Bushnell, 1976) that is also firm and is initially lighter in color (Fig. 6). The new growth is composed of cells that are mostly binucleate (Rajendren, 1972; Maclean and Scott, 1970) and may produce small numbers of urediospores and teliospores. Attempts to infect wheat with compact subcultures have mostly been unsuccessful (Bushnell and Stewart, 1971). Successful infections with some compact cultures have been obtained in early generations, but the cultures appeared to become slowly less pathogenic (W. R. Bushnell, unpublished). This behavior is in line with the view that the growth of *P. graminis* is determinate, in contrast to the indeterminate growth of *P. striiformis,* for example.

2. Variant Mycelia

First Scott and Maclean, then others, discovered that axenic cultures also contain mycelia referred to as variants that differ in one or more respects from those described above. Variant mycelia have been obtained in axenic cultures of *P. coronata* (Jones, 1974) and *P. recondita* f. sp. *tritici* (K. Katsuya, unpublished), but most is known about the variants of *P. graminis* f. sp. *tritici.*

a. Primary Cultures. Thinly seeded cultures of *P. graminis* f. sp. *tritici* contain two types of colonies, normal and variant (Williams, 1971; Williams and Hartley, 1971). The normal type of colony is globose and fast-growing, and arises from germlings that have undergone apparently normal differentiation. It is composed of coarse hyphae whose cells contain two nuclei. These are assumed to be haploid and complementary. Such colonies are able to infect wheat.

The variant type of colony is irregular in shape and slow-growing, and develops from germlings in which differentiation has been abnormal. Its hyphae are narrow and highly branched, and are composed of cells with one, presumably haploid, nucleus. These colonies do not continue to grow in subculture. The ability of this type of colony to infect wheat or barberry has not been investigated. The frequency of haploid variants in thinly seeded cultures is variable. In heavily seeded cultures haploid variants may not persist; mycelia of complementary mating type would be expected to anastomose and produce dikaryotic mycelia whose growth is favored on present media.

b. Subcultures. Scott and Maclean (1969) originally discovered variant colonies in flask cultures that had produced little vegetative growth immediately after seeding. The cultures were put aside until about 1 month after seeding, when many tiny white colonies were observed floating among the necrotic germlings. On transfer to fresh medium, the colonies grew vigorously and could be subcultured indefinitely as fluffy white mycelia (Fig. 7). The hyphal cells contained one nucleus that was at first believed to be haploid

(Maclean and Scott, 1970; Maclean *et al.*, 1971). Few of the variants could infect wheat. During long-term maintenance of cultures, new lines arose as sectors with different cultural characters. One new variant was composed of binucleate cells (Maclean, 1974).

Williams and Hartley (1971) reported isolating variants from subcultures of primary mycelia. The variants closely resembled those described by Maclean and Scott (1970). They were easily subcultured as fluffy white mycelia and were uninucleate. None of these variants was able to infect wheat. The nuclei were believed to be diploid.

The difference of opinion about the genetic status of the variants was based in part on counts of mitotic chromosomes. These counts are notoriously unreliable in fungi. The question was tentatively resolved by studies of spores formed on wheat by a pathogenic variant, V1C. Measurements of cell and nuclear size in urediospores and teliospores (Maclean *et al.*, 1974; Williams, 1975a), the relative DNA content of urediospore nuclei measured by cytofluorometry (Williams and Mendgen, 1975), and the relative sensitivity of urediospore nuclei to inac-tivation by ultraviolet light (Williams, 1975b) indicated diploidy. It was assumed that the spore nuclei were genetically identical to the hyphal nuclei of V1C. The possibility that this assumption is incorrect is discussed below.

Variant mycelia have also been isolated from subcultures of Canadian (Green, 1976) and American (Bushnell and Bosacker, 1982) strains of *P. graminis* f. sp. *tiitici.* Green subcultured from what he described as a white mycelial sector in an 8-day-old primary culture of race 11 yellow. The line grew at variable rates as fluffy colonies in seven subcultures over about 12 months. In the eighth transfer it showed some instability: Two cultures died, two grew like the parent, and two grew as gray and brown, stromatic mycelia resembling “normal” subcultures. The mycelia of the white fluffy parent were binucleate: Their volume was estimated from electron micrographs to be half the volume of a uninucleate, supposedly diploid line obtained from the Australian Culture No. 334, race 126-Anz-6,7. The nuclear condition of the stromatic culture is unknown.

Bushnell and Bosacker (1982) reported a study of normal and variant mycelia of American collections of *P. graminis* f. sp. *tritici* grown in subculture for 2 to 13 years. Variants derived from Australian culture No. 334 race 126-Anz-6,7 were included for comparison. Nuclear volume and number were determined systematically in 1976 and were checked again in 1981. Mycelia were either fluffy or compact, uninucleate or binucleate. Three sizes of nuclei were found. These were related by volume in the ratio 1:2:4, which was interpreted as haploid, diploid, and tetraploid. Bushnell and Bosacker's findings are summarized in Table II.

Bushnell and Bosacker suggested that if the relationship between colony type and total genome were generally true, it would present a convenient way

of assessing ploidy of nuclei in axenic cultures: Compact cultures have a diploid total genome; fluffy cultures have a tetraploid total genome. However, the fluffy variants of Maclean *et al.* (1971) and Williams and Hartley (1971) seemed to be an exception; as mentioned previously, evidence from spores formed on wheat by the variant V1C pointed to diploidy. Yet Bushnell and Bosacker's measurements of hyphal nuclei indicated that V1C (referred to in their article as Pgt 126-Anz 6,7–1969) is tetraploid.

Two explanations can be suggested for this contradiction. The first one accepts that the relationship between colony type and total genome is not generally true. The V1C variant was diploid in 1971 when it was used to infect wheat for genetic (Green *et al.*, 1978) and microscopic (Williams, 1975a) studies. It became tetraploid some time between then and 1976, when Bushnell and Bosacker measured its hyphal nuclei. The change may have been caused by its storage in liquid nitrogen in October 1972 or its retrieval in February 1976.

An alternative explanation proposes that colony type is a generally valid indicator of total genome in axenic cultures of *P. graminis* f. sp. *tritici* (Table II). The uninucleate, fluffy variant V1C has always been tetraploid. When it is inoculated to wheat, it breaks down to form uninucleate diploid infections. The media currently available for axenic culture are selective for tetraploids. Hence, when Maclean *et al.* (1971) inoculated an axenic culture with urediospores taken from a diploid infection caused by the presumed tetraploid variant V1, they obtained a uninucleate, fluffy tetraploid, V1C.

Table II

Possible Relation between Colony Type, Nuclear Condition, and Total Genotype of Axenic Mycelia[a]

Colony type	Nuclei per cell	Nuclear ploidy	Total genotype
Compact (?)[b]	1	*n*	*n*
Compact	2	*n*	2*n*
Compact	1	2*n*	2*n*
Fluffy	2	2*n*	4*n*
Fluffy	1	4*n*	4*n*

[a]Modified from Bushnell and Bosacker (1982).

[b]Growth beyond microscopic colonies has not been achieved.

I prefer the second argument because it unifies a number of apparently discrepant observations. Also, the instability of variant uredial cultures in early generations on wheat (Green *et al.*, 1978) and the general lack of pathogenicity of fluffy cultures is, I find, more understandable if these variants are tetraploid rather than diploid.

V. Conclusions

The historical background to the axenic culture of cereal rust fungi in general and *P. graminis* f. sp. *tiitici* in particular instructs in several ways about the advancement of knowledge. Another point of general significance is that, as often happens, contemporaneous research in widely separated laboratories converged, as it were, on the solution of the problem. I am thinking particularly of Fuchs and Gaertner's experiments (1958) at Göttingen. Their work came close to success but was eventually abandoned because of the variable activity of different samples of egg yolk and latent bacterial contamination (Gaertner and Fuchs, 1960). Naito and co-workers (Naito and Matsuka, 1965) at Kagawa University was another group that was "getting warm." In the early 1960s they were producing infection structures and infection hyphae of many species of rust fungi on nutrient media containing peptone. At the same time, in Peking, Lu and collaborators (1964) were demonstrating that colonies of the leaf rust fungus in leaf segments remained alive on a maltose–peptone–salts agar for 40 to 50 days, long after the host cells were shown to have died. Mycelia of the rust fungus were reported to have extended from within leaf segments onto the surrounding medium. With a little further work the first successful culture of a cereal rust fungus might have happened in Göttingen, Kagawa, or Peking. However, the German studies were halted by technical problems and those in China by the political upheavals of the time (W. R. Bushnell, personal communication). The right combination of circumstances finally came together a few years later, and the event occurred in Sydney, Australia.

My final task here is to suggest priorities for future research, as I see them. At the top of the list I set two problems of equal urgency. The first is to obtain sound evidence of the nuclear ploidy of the various kinds of uni- and binucleate mycelia now recognized in primary and subcultures. I also recommend an urgent study of the kinds and amounts of substances leaking from mycelia in axenic culture; I would begin with normal (binucleate haploid) mycelia. This information is essential for the immediate improvement of culture media. It will allow the composition of a medium to be tailored, by preconditioning, to the specific needs of the mycelia whose growth it is to support.

After these come several problems of lesser rank. Can the present methods of culture be made more reliable by understanding how the provenance of an inoculum affects its performance? Can chemical regulators of germ tube differentiation be useful in this (Grambow *et al.*, 1977; Macko *et al.*, 1978)? Are strains that are difficult or impossible to culture from urediospores easier or possible to culture from colonies in leaves? What characters distinguish an easily cultured strain from a difficult one? These are questions that can be answered with existing knowledge.

A challenge of a slightly higher degree of difficulty is the development of a method for the sustained growth of the apparently haploid mycelia occurring in thinly seeded cultures of the wheat stem rust fungus. This would provide a base for more ambitious studies such as sexuality and heteroecism. It would also enable a direct test of my colleague Jennifer Hartley's theory that a dikaryon may harbor many genotypes within a single phenotype (Hartley and Williams, 1971c). Of course, the development of methods for axenic culture of haploids will truly signal a new era in which the techniques of genetic analysis for molds will become accessible for rust studies. I can see no shortage of challenging and worthwhile projects in this field and commend them to the attention of future mycologists.

Acknowledgments

I am indebted to my wife Maria for her advice and forbearance. I am also grateful to R. L. Parkin for painstaking criticism of the manuscript and to M. Hill and D. J. S. Gow for help with the illustrations. The chapter was written with the financial support of the Australian Meat Research Committee.

References

Ainsworth, G. C., and Bisby, G. R. (1943). "A Dictionary of the Fungi." Imperial Mycological Institute, Kew, Surrey.

Allen, R. F. (1923). A cytological study of infection of Baart and Kanred wheats by *Puccinia graminis tritici. J. Agric. Res.* (*Washington, D.C.*)**23**, 131–152.

Ando, K., Sato, S., and Katsuya, K. (1979). Mycelial types and pathogenicity of *Puccinia recondita* f. sp. *tritici* grown on artificial media. *Ann. Phytopathol. Soc. Jpn.* **45**, 660–667.

Arthur, J. C. (1929). "The Plant Rusts (Uredinales)." Wiley, New York.

Brian, P. W. (1966). Obligate parasitism in fungi. *Proc. R. Soc. London, Ser. B* **168**, 101–118.

Brown, J. F. (1971). Histological studies of the factors affecting infection of wheat by the stem rust fungus. *Proc. Cereal Rusts Conf., Oeiras, Portugal 1968* pp. 129–131.

Brown, W. (1936). The physiology of host-parasite relations. *Bot. Rev.* **2**, 236–281.

Bushnell, W. R. (1968). In vitro development of an Australian isolate of *Puccinia graminis* f. sp. *tritici. Phytopathology* **58**, 526–527.

Bushnell, W. R. (1976). Growth of races 38 and 17, *Puccinia graminis* f. sp. *tritici*, on artificial media. *Can. J. Bot.* **54**, 1490–1498.

Bushnell, W. R., and Bosacker, P. L. (1982). Nuclear volume and number in long term *in vitro* cultures of *Puccinia graminis. Can. J. Bot.* **60**, 1827–1836.

Bushnell, W. R., and Rajendren, R. B. (1970). Casein hydrolysates and peptones for artificial culture of *Puccinia graminis* f. sp. *tritici. Phytopathology* 60, 1287 (abstr.).

Bushnell, W. R., and Stewart, D. M. (1971). Development of American isolates of *Puccinia graminis* f. sp. *tritici* on an artificial medium. *Phytopathology* **61**, 376–379.

Cantino, E. C. (1950). Nutrition and phylogeny in the water molds. *Q. Rev. Biol.* **25**, 269–277.

Chakravarti, B. P. (1966). Attempts to alter infection processes and aggressiveness of *Puccinia graminis* var. *tritici. Phytopathology* **56**, 223–229.

Chester, K. S. (1946). "The Nature and Prevention of the Cereal Rusts as Exemplified in the Leaf Rust of Wheat." Chronica Botanica, Waltham, Massachusetts.

Clarke, D. D. (1966). Factors affecting the development of single zoospore colonies of *Phytophthora infestans. Trans. Br. Mycol. Soc.* **49**, 177–184.

Cochrane, V. W. (1958). "Physiology of Fungi." Wiley, New York.

Cocking, E. C. (1972). Plant cell protoplasts—isolation and development. *Annu. Rev. Plant Physiol.* **23**, 29–50.

Coffey, M. D., Bose, A., and Shaw, M. (1969). *In vitro* growth of gelatin suspensions of uredospores of *Puccinia graminis* f. sp. *tritici. Can. J. Bot.* **47**, 1291–1293.

Cole, M., and Wood, R. K. S. (1961). Types of rot, rate of rotting and analysis of pectic substances in apples rotted by fungi. *Ann. Bot.* (*London*)[N.S.] **25**, 417–434.

Cutter, V. M. (1959). Studies on the isolation and growth of plant rusts in host tissue cultures and upon synthetic media. I. *Gymnosporangium. Mycologia* **51**, 248–295.

Darling, W. M., and McArdle, M. (1954). Effect of inoculum dilution on spore germination and sporeling growth in a mutant strain of *Aspergillus amstelodami. Trans. Br. Mycol. Soc.* **42**, 235–242.

Dickinson, S. (1949). Studies in the physiology of obligate parasitism. II. The behaviour of certain rusts in contact with various membranes. *Ann. Bot.* (*London*)[N.S] **13**, 219–236.

Dickinson, S. (1955). Studies in the physiology of obligate parasitism. V. Further differences between the uredospore germ-tubes and leaf hyphae of *Puccinia triticina. Ann. Bot.* (*London*)[N.S.] **19**, 161–171.

De Bary, A. (1887). "Comparative Morphology and Biology of the Fungi, Mycetozoa and Bacteria." Oxford Univ. Press (Clarendon), London and New York.

Eagle, H., and Pietz, P. K. (1962). The population-dependent requirement by cultured mammalian cells for metabolites which they can synthesize. *J. Exp. Med.* **116**, 29–43.

Foudin, A. S., and Wynn, W. K. (1972). Growth of *Puccinia graminis* f. sp. *tritici* on a defined medium. *Phytopathology* **62**, 1032–1040.

Fries, N. (1955). Studies on the physiology of *Coprinus.* I. Growth substances, nitrogen and carbon requirements. *Sven. Bot. Tidskr.* **49**, 475–535.

Fry, D. C., and Willetts, H. J. (1974). Stimulation of growth of wheat stem rust in axenic culture by dithiothreitol (DTT). *Trans. Br. Mycol. Soc.* **62**, 198–202.

Fuchs, W. H., and Gaertner, A. (1958). Untersuchungen zur Keimungsphysiologie des Schwartzrostes *Puccinia graminis tritici* (Pers.) Erickss. u. Henn. *Arch. Mikrobiol.* **28**, 303–309.

Gaertner, A., and Fuchs, W. H. (1960). Weiteres zur Keimungsphysiologie von *Puccinia graminis tritici* (Pers.) Erikss. u. Henn. *Arch. Mikrobiol.* **35**, 377–378.

Gäumann, E. (1950). "Principles of Plant Infection" (transl. by W. B. Brierley). Crosby, Lockwood, London.

Grambow, H. J., and Muller, D. (1978). Nuclear condition, types of hyphal development from differentiating and non-differentiating uredosporelings and effect of 3, 3'-bisindolemethane on *Puccinia graminis* f. sp.*tritici in vitro. Can. J. Bot.* **56**, 736–741.

Grambow, H. J., Garden, G., Dallacker, F., and Lehmann, A. (1977). 3, 3'-Bis-indolylmethane: a growth regulator for *Puccinia graminis* f. sp. *tritici. Z. Pflanzenphysiol.* **82**, 62–67.

Green, G. J. (1976). Axenic culture of *Puccinia* species collected in Canada. *Can. J. Bot.* **54**, 1198–1205.

Green, G. J., Williams, P. G., and Maclean, D. J. (1978). Variability of uredial cultures of *Puccinia graminis tritici* derived from "vegetative" axenic mycelium. *Can. J. Bot.* **56**, 855–861.

Hartley, M. J., and Williams, P. G. (1971a). Morphological and cultural differences between races of *Puccinia graminis* f. sp. *tritici* in axenic culture. *Trans. Br. Mycol. Soc.* **51**, 137–144.

Hartley, M. J., and Williams, P. G. (1971b). Interactions between strains of *Puccinia graminis* f. sp. *tritici* in axenic culture. *Trans. Br. Mycol. Soc.* **57**, 129–136.

Hartley, M. J., and Williams, P. G. (1971c). Genotypic variation within a phenotype as a possible basis for somatic hybridization in rust fungi. *Can. J. Bot.* **49**, 1085–1087.

Hawker, L. E. (1950). "Physiology of Fungi." Univ. of London Press, London.

Howes, N. K., and Scott, K. J. (1973). Sulphur metabolism of *Puccinia graminis* f. sp. *tritici* in axenic culture. *J. Gen. Microbiol.* **76**, 345–354.

Ingram, D. S., and Tommerup, I. C. (1973). The study of obligate parasites in vitro. *In* "Fungal Pathogenicity and the Plant's Response" (R. J. W. Byrde and C. V. Cutting, eds.), pp. 121–136. Academic Press, London.

Jones, D. R. (1974). Axenic culture of *Puccinia coronata* var. *avenae* from urediospores. *Trans. Br. Mycol. Soc.* **63**, 593–594.

Kuck, K. H. (1979). Uber die infektionsbedingten Veränderungen der Aminosäuren und Fettsäuren in mit *Puccinia graminis* f. sp. *tritici*, Rasse 32, infizierten Weizcnblättern und die *in vitro*-Sporulation des Pilzes. Doctoral Dissertation, Rheinisch-Westfälische Technische Hochschule, Aachen.

Kuhl, J. L., Maclean, D. J., Scott, K. J., and Williams, P. G. (1971). The axenic culture of *Puccinia* species from uredospores. *Can. J. Bot.* **49**, 201–209.

Lewis, D. H. (1973). Concepts of fungal nutrition and the origin of biotrophy. *Biol. Rev. Cambridge Philos. Soc.* **48**, 261–278.

Link, G. K. K. (1933). Etiological phytopathology. *Phytopathology* **23**, 843–862.

Lode, A., and Pedersen, T. A. (1970). Fatty acid induced leakage of organic compounds from *Boletus variegatus. Physiol. Plant.* **23**, 715–727.

Lu, S.-Y., Ts'ai, M. Y., Yiao, H. C., Wang, T. S., Chen, M. J., Chen, Y. C., and Yu, H. M. (1964). Studies on the physiology of rust parasitism with special reference to *Puccinia triticina. Acta Phytopathol. Sin.* **3**, 237–248.

Macko, V., Renwick, J. A. A., and Rissler, J. F. (1978). Acrolein induces differentiation of infection structures in the wheat stem rust fungus. *Science* **199**, 442–443.

Maclean, D. J. (1974). Cultural and nutritional studies on variant strains of the wheat stem rust fungus. *Trans. Br. Mycol. Soc.* **62**, 333–349.

Maclean, D. J. (1982). Axenic culture of rust fungi. *In* "The Rust Fungi" (K. J. Scott and A. K. Chakravorty, eds.), pp. 37–120. Academic Press, London.

Maclean, D. J., and Scott, K. J. (1970). Variant forms of saprophytic mycelium grown from uredospores of *Puccinia graminis* f. sp. *tritici. J. Gen. Microbiol.* **64**, 19–27.

Maclean, D. J., Scott, K. J., and Tommerup, I. C. (1971). A uninucleate, wheat-infecting strain of the stem rust fungus isolated from axenic cultures. *J. Gen. Microbiol.* **65**, 339–342.

Maclean, D. J., Tommerup, I. C., and Scott, K. J. (1974). Genetic status of monokaryotic variants of the wheat stem rust fungus isolated from axenic culture. *J. Gen. Microbiol.* **84**, 364–378.

Mains, E. B. (1917). The relation of some rusts to the physiology of their hosts. *Am. J. Bot.* **4**, 179–220.

Moulder, J. W. (1962). "The Biochemistry of Intra-cellular Parasitism." Univ. of Chicago Press, Chicago, Illinois.

Naito, N., and Matsuka, N. (1965). Infection-type structures produced on artificial media by spores of rust fungi. II. *Tech. Bull. Fac. Agric., Kagawa Univ.* **17**, 8–16.

Ogle, H. J., and Brown, J. F. (1971). Some factors affecting the relative ability of two strains of *Puccinia graminis tritici* to survive when mixed. *Ann. Appl. Biol.* **67**, 157–168.

Pole Evans, I. B. (1907). The cereal rusts. I. The development of their Uredo mycelia. *Arm. Bot.* (*London*) **21**, 441–446.

Rajendren, R. B. (1972). Cytology of *Puccinia graminis* f. sp. *tritici* on an artificial medium. *Mycologia* **64**, 591–598.

Scott, K. J. (1972). Obligate parasitism by phytopathogenic fungi. *Biol. Rev. Cambridge Philos. Soc.* **47**, 537–572.

Scott, K. J. (1976). Growth of biotrophic parasites in axenic culture. *In* "Physiological Plant Pathology" (R. Heitefuss and P. H. Williams, eds.), pp. 719–742. Springer-Verlag, Berlin and New York.

Scott, K. J., and Maclean, D. J. (1969). Culturing of rust fungi. *Annu. Rev. Phytopathol.* ***7***, 123–146.

Sedlmayr, M., Beneke, E. S., and Stevens, J. A. (1961). Physiological studies on *Calvatia* species. I. Vitamin requirements. *Mycologia* **53**, 98–108.

Shaw, M. (1967). Cell biological aspects of host-parasite relations of obligate fungal parasites. *Can. J. Bot.* **45**, 1205–1220.

Stock, F. (1931). Untersuchungen über Keimung und Keimschlauchwachstum der Uredosporen einiger Getreideroste. *Phytopathol. Z.* **3**, 231–238.

Talbot, P. H. B. (1971). "Principles of Fungal Taxonomy." Macmillan, London.

Thrower, L. B. (1966). Terminology for plant parasites. *Phytopathol. Z.* **56**, 258–259.

Trocha, P., and Daly, J. M. (1970). Protein and ribonucleic acid synthesis during germination of uredospores. *Plant Physiol.* **46**, 520–526.

Ward, H. M. (1901). The nutrition of fungi. *Trans. Br. Mycol. Soc.* **1**, 124–142.

Watson, I. A. (1981). Wheat and its rust parasites in Australia. *In* "Wheat Science—Today and Tomorrow". (L. T. Evans and W. J. Peacock, eds.), pp. 129–147. Cambridge Univ. Press, Cambridge.

White, N. H. (1981). A history of plant pathology in Australia. *In* "Plants and Man in Australia" (D. J. Carr and S. G. M. Carr, eds.), pp. 42–95. Academic Press, Sydney.

Williams, P. G. (1971). A new perspective of the axenic culture of *Puccinia graminis* f. sp. *tritici* from uredospores. *Phytopathology* **61**, 994–1002.

Williams, P. G. (1975a). Evidence for diploidy of a monokaryotic strain of *Puccinia graminis* f. sp. *tritici. Trans. Br. Mycol. Soc.* **64**, 15–22.

Williams, P. G. (1975b). UV light inactivation kinetics of wheat stem rust uredospores. *Aust. Plant Pathol. Soc. Newsl.* **4**, 21–22.

Williams, P. G. (1975c). The characteristics of rust fungi in axenic culture. *In* "Advances in Mycology and Plant Pathology" (S. P. Raychaudhuri, A. Varma, K. S. Bhargava, and B. S. Mehrotra, eds.), pp. 67–82. Professor R. N. Tandon's Birthday Celebration Committee, New Delhi.

Williams, P. G. (1976). Development of *Puccinia striiformis* West, on nutrient agar. *Arch. Microbiol.* **110**, 173–175.

Williams, P. G., and Hartley, M. J. (1971). Occurrence of diploid lines of *Puccinia graminis tritici* in axenic culture. *Nature* (*London*), *New Biol.* **229**, 181–182.

Williams, P. G., and Mendgen, K. W. (1975). Cytofluorometry of DNA in uredospores of *Puccinia graminis* f. sp. *tritici. Trans. Br. Mycol. Soc.* **64**, 23–28.

Williams, P. G., and Shaw, M. (1968). Metabolism of glucose-^{14}C, pyruvate-^{14}C, and mannitol-^{14}C by *Melampsora lini.* I. Uptake. *Can. J. Bot.* **46**, 435–440.

Williams, P. G., Scott, K. J., and Kuhl, J. L. (1966). Vegetative growth of *Puccinia graminis* f. sp. *tritici in vitro*. *Phytopathology* **56**, 1418–1419.

Williams, P. G., Scott, K. J., Kuhl, J. L., and Maclean, D. J. (1967). Sporulation and pathogenicity of *Puccinia graminis* f. sp. *tritici* grown on an artificial medium. *Phytopathology* **57**, 326–327.

Wolf, F. T. (1974). The cultivation of rust fungi upon artificial media. *Can. J. Bot.* **52**, 767–772.

Wong, A. L., and Willetts, H. J. (1970). Observations on growth of selected Australian races of wheat stem rust in axenic culture. *Trans. Br. Mycol. Soc.* **55**, 231–238.

Yarwood, C. E. (1956). Obligate parasitism. *Annu. Rev. Plant Physiol.* **7**, 115–141.

[1]Culture No. 334 has the revised designation, race 126-Anz-5,6,7,11.

14

Structure and Physiology of Haustoria

D. E. Harder
J. Chong
Agriculture Canada Research Station, Winnipeg, Manitoba, Canada

I. Introduction

Haustoria in the fungi were first mentioned by De Bary (1863), and the first detailed description for the rusts was provided by Ward (1882). Bushnell (1972) defined a fungal haustorium as "a specialized organ which is formed inside a living host cell as a branch of an extracellular (or intercellular) hypha or thallus, which terminates in that host cell, and which probably has a role in the interchange of substances between host and fungus." The absorption of nutrients has generally been considered its main function, although Rice (1927) already questioned this concept. Since that time, work with the electron microscope has largely elucidated haustorial structure. However, for the rusts, direct evidence for the nutrient absorption role of haustoria is still lacking.

The morphology of haustoria and their relationships with their hosts have recently been thoroughly reviewed (Bracker and Littlefield, 1973; Littlefield and Heath, 1979). The physiology and possible function(s) of haustoria have been reviewed by Bushnell (1972). The basic structure of haustoria in dikaryotic rust infections is relatively uniform. Rather than making another comprehensive review on this topic, in this chapter we will detail the structure of two cereal rust fungi, *Puccinia graminis* f. sp. *tritici* and *P. coronata* on the basis of our own published or unpublished material. Research results obtained since the recent reviews will be emphasized, and where applicable, enhancement or modifications of earlier interpretations will be made.

II. Methodology and Interpretation

In the literature a variety of ultrastructural descriptions may pertain to given biological structures. These discrepancies may be due to variations in the stage of development of a structural component or to the methods used in preparing tissues for electron microscopy. To assess more reliably the occurrence and relationship of structures, a variety of processing methods should be employed. Many of the descriptions used in this chapter are the result of specific procedures to reveal particular components. The various methods used to elucidate the structure and composition of the parts of the haustorial apparatus are outlined, with their interpretations, as follows:

1. *Conventional processing* (Glt/OsO_4–UA/PbC). Tissue fixation is with glutaraldehyde (Glt) and osmium tetroxide (OsO_4) followed by staining with uranyl acetate/lead citrate (UA/PbC). Variations of the procedure may involve omitting the OsO_4 fixation or omitting the PbC stain.

2. *Periodic acid-thiocarbohydrazide-silver proteinate* (*PA–TCH–SP*) *staining* (Thiery, 1967). When used with proper controls, this method is specific for polysaccharides with vicinal hydroxyl groups (glycogen and starch are common examples).

3. *Periodic acid–chromate–phosphotungstate* (*PACP*) (Roland *et al.*, 1972). This stain enhances the electron density of the plant cell plasmalemma.

4. *Subtractive methods used in conjunction with conventional or specific stains.* Specific cellular components may be removed by enzymatic (e.g., protease or cellulase) digestion or lipid solvent extraction. The presence or absence of these components then is tested for by various staining procedures.

5. *Lectin* (*WGL, Con A*)*–colloidal gold markers.* A wide variety of plant lectins bind with more or less specificity to particular cell components. The lectins may be conjugated with colloidal gold particles; then the lectins, which bind to cell components, can be detected in the electron microscope. Wheat germ lectin (WGL) has been used to detect chitin (Horisberger and Rosset, 1977) in fungal material and concanavalin A (Con A) to assay for α-linked glucose or mannose in polysaccharides (Horisberger and Vonlanthan, 1977), which results in relatively nonspecific detection of a variety of carbohydrates.

6. *Energy-dispersive X-ray* (*EDX*) *analysis.* This method is useful to detect mineral elements (atomic number of 11 or higher in the periodic table) where they occur in sufficient concentration in cellular components.

7. *Freeze-etch.* Rapidly frozen specimens are fractured and etched to reveal details of membrane topography, composition, or organization. One of the major advantages is that tissues undergo a minimum of chemical alteration and are not extracted. If freezing damage can be avoided, this method is a reliable indicator of structure with a minimum of artifacts. Freeze-etch may be combined with histochemistry, as in the detection of membrane sterols in the host–pathogen interaction with the polyene antibiotic filipin (Harder and Mendgen, 1982).

The interpretation of results using the various methods just described may be quite subjective. The subtractive methods such as enzyme digestion or solvent extraction depend on the absence or reduction of staining intensity as compared to untreated controls. If controls are rigorously applied and observations are made repeatedly, conclusions regarding the likelihood of the existence and location of a chemical component may be made.

III. Terminology and Definitions

Considerable inconsistency exists in the literature in designating component parts of the haustorial apparatus. In this chapter the terminology as outlined by

Bushnell (1972) and modified by Littlefield and Heath (1979) will be followed. The term haustorium itself has been the subject of controversy in reference to dikaryotic and monokaryotic infections. In monokaryotic infections the haustoria morphologically differ substantially from those in dikaryotic infections, and more closely resemble mycelial hyphae. In this chapter the terms D- and M-haustoria as used by Littlefield and Heath (1979) are used to designate the intracellular structures of dikaryotic or monokaryotic infections, respectively. Detailed comparisons between the D- and M-haustoria will be made. (See *1* in Note Added in Proof.)

The terms used, their definitions, and abbreviations for D-haustoria (Fig. 1) are as follows:

1. *Collar:* an irregularly occurring deposition of material between the host plasmalemma and host cell wall at the penetration site (Figs. 38–41). The collar may extend around the haustorial neck up to the base of the haustorial body. The collar is not a part of the haustorial apparatus.
2. *Extrahaustorial matrix* (*EH matrix*): a region of varying dimensions and density that occurs between the haustorial body wall and the EH membrane.
3. *Extrahaustorial membrane* (*EH membrane*): an extension of the host plasmalemma that surrounds the entire intracellular haustorium.
4. *Haustorial body:* the irregularly shaped bulk of the haustorium that begins where the neck expands at its distal end.
5. *Haustorial body wall:* the fungal cell wall enclosing the haustorial body.
6. *Haustorial neck:* the constricted portion of the haustorium originating inside the host cell wall and extending to the base of the haustorial body.
7. *Haustorium initial:* the postpenetration finger-like projection into the host cell. After swelling at its distal end to form the haustorial body, it becomes the haustorial neck (Figs. 2, 15, and 19).
8. *Haustorium mother cell* (*HMC*): a slightly swollen, terminal cell of an intercellular hypha that attaches to a host cell and gives rise to the haustorium. It is found only in dikaryotic infections.
9. *HMC septum:* the septum that delimits the HMC from the penultimate hyphal cell (Fig. 2).
10. *Neck ring:* an electron-dense portion of the neck wall that occurs approximately midway along the haustorial neck. Where the neck ring is composed of more than one “ring,” the composite is the neck ring, and each portion is designated as a band.

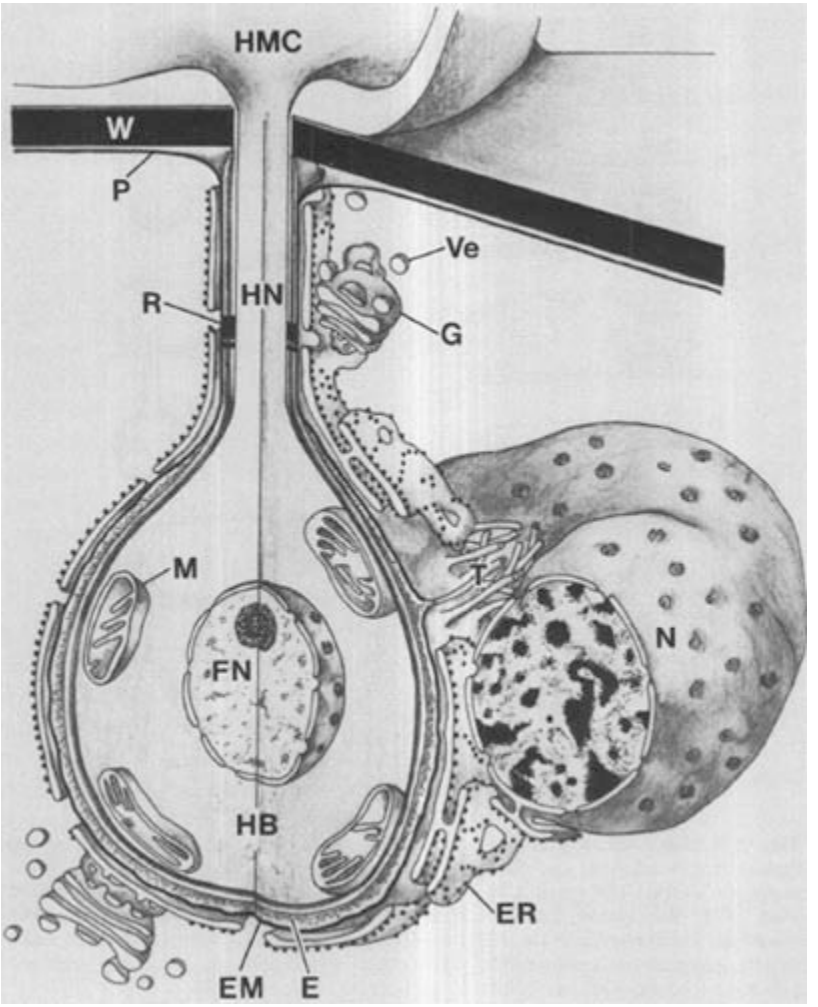


Fig. 1. Diagram of an invaded host cell cut open at the site of penetration to show the three-dimensional structure of a mature D-haustorium of *P. coronata*, and its association with the host cell organelles involved. The structures are not drawn to scale, and some are illustrated by only a few examples (e.g., Golgi bodies, vesicles, ribosomes). E, Extrahaustorial (EH) matrix; EM, extrahaustorial (EH) membrane; ER, endoplasmic reticulum; FN, fungal nucleus; G, Golgi body; HB, haustorial body; HMC, haustorial mother cell; HN, haustorial neck; M, mitochondrion; N, host nucleus; P, plasmalemma; R, neck ring; T, tubule complex; Ve, vesicle; W, host cell wall.

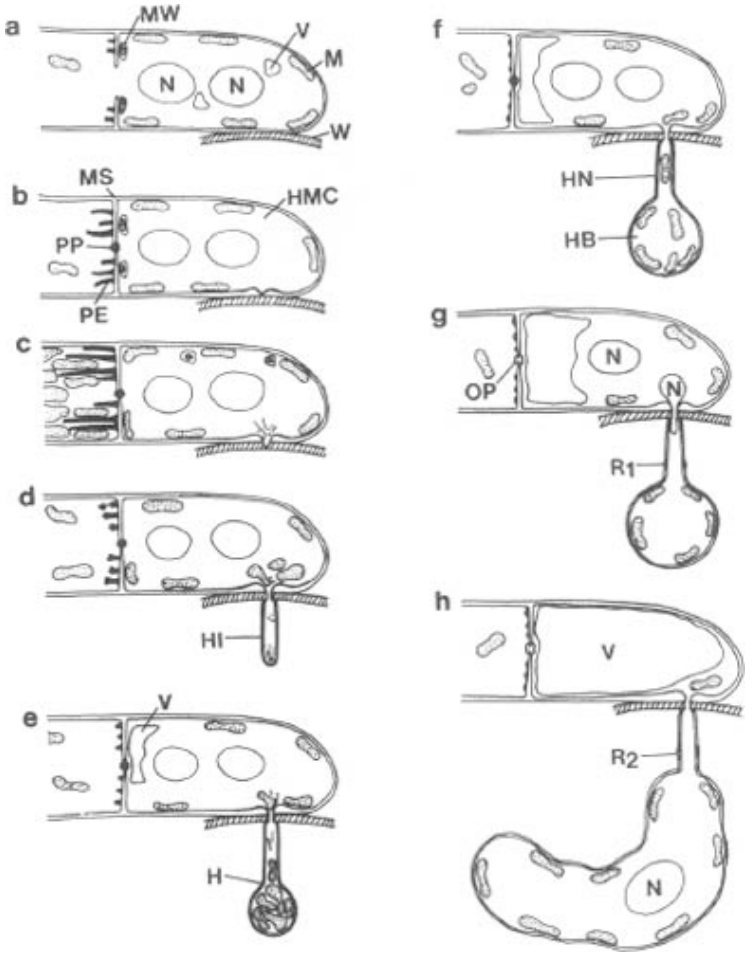


Fig. 2. A diagrammatic, chronological (a–h) representation of the events of D-haustorium development and the correlated state of the septal pore apparatus of the haustorium mother cell septum. H, Haustorium; HB, haustorial body; HI, haustorium initial; HMC, haustorium mother cell; HN, haustorial neck; M, mitochondrion; MS, haustorium mother cell septum; MW, membranous whorl; N, nucleus; OP, open septal pore; PE, plasmalemma elaboration; PP, plugged septal pore; R_1, neck ring with one band; R_2, neck ring with two bands; V, vacuole; W, host cell wall.

11. *Neck wall:* the fungal cell wall extending along the haustoial neck.

12. *Penetration peg:* the narrowest portion of the haustorium that passes through the host cell wall (Fig. 11).

Similar terms to those above are applied to the M-haustorium where applicable (see Fig. 37).

IV. Dikaryotic Haustoria

Figure 1 is an interpretation of a mature haustorium of *P. coronata* and is intended as a reference for haustorial structure in this fungus and its relation to the host cell. However, rust haustoria undergo numerous structural changes during their formation, therefore any one description of a haustorium is valid only for the point in its development when it was sampled. Thus the following discussion traces the structure of the haustorium from differentiation of the HMC through to maturity. The various stages of haustorium formation are illustrated in the drawing in Fig. 2.

A. HAUSTORIUM MOTHER CELL (HMC) DIFFERENTIATION

The induction of HMC differentiation is discussed in Staples and Macko (Chapter 9, this volume). In this section we will deal with specialized morphological features of the HMCs. Although the cytoplasmic contents of the HMCs do not differ from those of intercellular hyphal cells, the mitochondria undergo a change in conformation and distribution. In both *P. coronata* (Fig. 3) and *P. graminis* f. sp. *tritici* (Chong, 1981), the mitochondria are uniformly distributed around the periphery of the HMC protoplasts, are compact, and have a flattened apparently ovoid form, oriented with the flat face parallel to the HMC wall. Compare this conformation to the randomly distributed, irregular filiform mitochondria in the intercellular hyphal cells (see Harder, Chapter 11, this volume, Section II,A,4). The nuclei in the HMCs also are more compact and more regularly oval-shaped (Fig. 3) than those in the intercellular hyphae. Compact nuclei were also reported in HMCs of *Uromyces fabae* (Savile, 1939) and *U. phaseoli* var. *vignae* (Heath and Heath, 1978).

The HMCs and hyphal cells of both *P. graminis* f. sp. *tritici* and *P. coronata* can be clearly differentiated on the basis of their walls and septa (Chong, 1981). After UA/PbC (Fig. 4) or PA–TCH–SP staining, the HMC walls are thicker and have more layers than do the hyphal walls. Of the layers of the hyphal walls that are continuous with the outer layers of the HMC walls, the lightly staining outermost layer is probably not a rigid structural part of the wall, but a mucilaginous coating substance (Fig. 4). This layer also apparently serves to affix the HMC to the host cell wall. It is susceptible to protease digestion, and after treatment with protease, the HMC becomes detached from the host cell (see Fig. 24). The HMC septa are also composed of more layers

than the hyphal septa. The wall layer that is adjacent to the fungal plasmalemma on the HMC side of the septum is continuous around the rest of the HMC (Fig. 4) and is presumably a new layer formed during HMC differentiation. After protease treatment, much of the UA/PbC stainability of the hyphal wall of *P. graminis* f. sp. *tritici* is removed (Fig. 5), but the PA–TCH–SP staining is unaffected. In contrast, the HMC wall is less affected by the treatment. In *P. coronata,* both the hyphal and HMC walls are more resistant to protease (Chong *et al.,* 1981). In tests for WGL binding in *P. graminis* f. sp. *tritici* (R. Rohringer and J. Chong, unpublished), WGL was observed to bind to all layers of the HMC and hyphal walls, demonstrating the presence of chitin throughout these walls. With Con A, however, there was binding to the hyphal walls and the adjoining outer HMC wall layers, but not to the inner HMC wall layers, suggesting that the various layers of the HMC walls differ in composition. Those layers that have no Con A-receptor sites continue across the HMC septum, distinguishing this septum from hyphal septa. The HMC thus exhibits a greater degree of specialization than do the hyphal cells. However, it is not yet entirely clear at what point the differentiation of the HMC takes place. Wynn and Staples (1981) argued that contact between the fungus and mesophyll cell wall is necessary for the induction of haustorium differentiation. Indeed, there is evidence that for *P. coronata,* the inward-facing epidermal cell walls (facing the mesophyll), but not the outward-facing epidermal cell walls, stimulate haustorium formation (Mendgen, 1982). From the following section (IV,B), it is evident that the HMC septum is already specialized at the time of its formation. It is probable that metabolic changes in the nascent HMC begin at wall contact but prior to septation. During this time new wall layers are added, and some of the organelles undergo conformational changes.

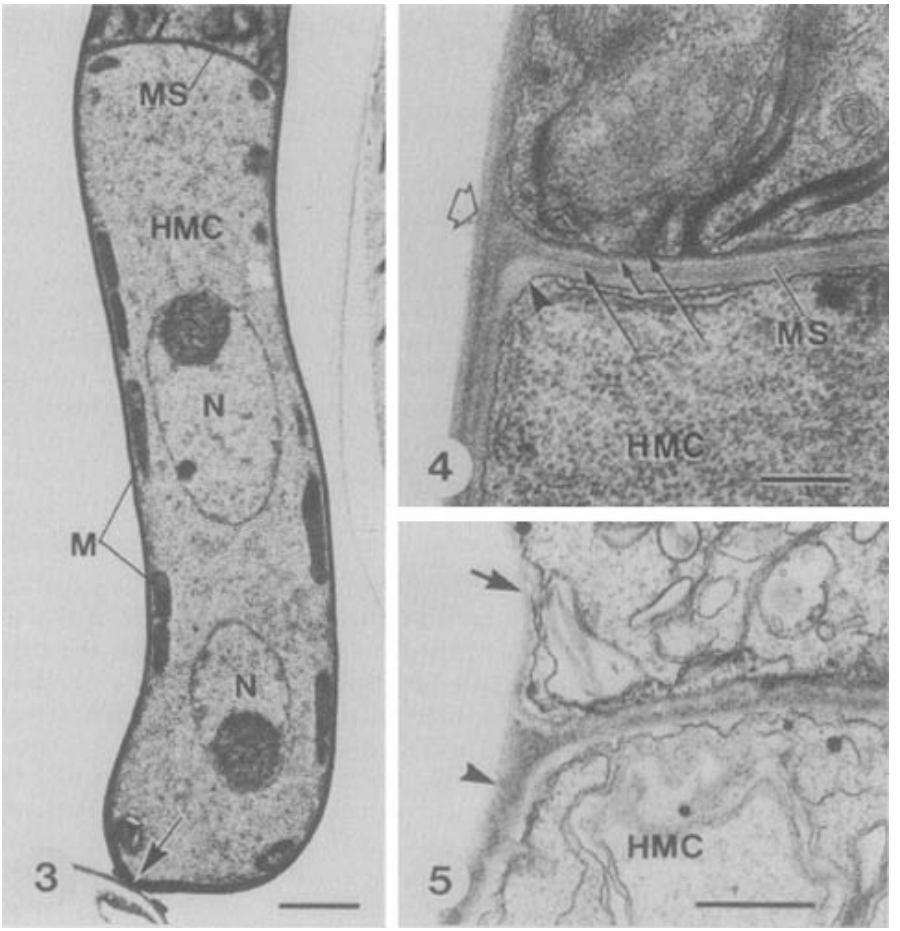


Fig. 3. A section taken from one of a series of sections of a young haustorium mother cell (HMC) of *Puccinia coronata*. Host wall penetration had begun (arrow), but the haustorium had not yet formed. Mitochondria (M) are densely stained and are located around the periphery of the cell adjacent to the plasmalemma. The nuclei (N) are ovoid and compact. MS, Haustorium mother cell septum (Glt/OsO_4; UA/PbC) (× 10,300; bar, 1 µm). (From Chong, 1981.) **Fig. 4.** A section showing part of a haustorium mother cell (HMC) of *Puccinia coronata.* The HMC wall is multilayered and is thicker than the hyphal wall, which has only two layers. These two layers are continuous with the outer layers of the HMC wall. The HMC septum (MS) is composed of four layers. The two electron-opaque layers (long arrows) are continuous with the periclinal wall (open arrow). A third, more lightly stained lamella (short arrow) separates the two electron-opaque layers and ends at the periclinal wall. The fourth lightly stained layer (arrowhead) is continuous around the rest of the HMC (Glt/OsO_4; UA/PbC) (×44,300; bar, 0.25 µm). (From Chong, 1981.) **Fig. 5.** Part of a young haustorium mother cell (HMC) of *Puccinia graminis* f. sp. *tritici* after protease treatment. The hyphal wall (arrow) is almost completely extracted, but the HMC wall

(arrowhead) is less affected. The HMC wall lacks its usual layered appearance (Glt-protease-OsO_4; UA/PbC) (×30,400; bar, 0.5 μm). (From R. Rohringer and J. Chong, unpublished.)

B. A TRANSFER APPARATUS ASSOCIATED WITH THE HMC SEPTUM

Membranous elaborations have been observed to occur on the hyphal side of the HMC septum of a number of rusts during the early stages of host cell penetration (Chong *et al.*, 1981; Heath and Heath, 1975; Reynolds, 1975). These elaborations occur as long "protrusions" originating from the plasmalemma at the HMC septum. They begin to form during HMC septum formation (Chong, 1981) (see Fig. 2), and in *P. coronata* attain a length of about 4.2 μm. When the elaborations have attained their full length, they are associated with a marked aggregation of mitochondria (Fig. 6). In conventionally processed material, the elaborations are bound on either side by a membrane and contain an electron-dense material (Fig. 6, inset). The bounding membranes of the elaborations are continuous with the plasmalemma across the septum (Fig. 7). From these views and from serial sections we have deduced that each "protrusion" is an elongated flattened cisterna (a modification of the tubular protrusion as described by Heath and Heath, 1975), closed at the end distal to the HMC septum, containing a somewhat electron-dense matrix and a more electrondense core. The elaborations are often interconnected (Chong, 1981), thus forming a large labyrinth-like complex. See Fig. 9 for an interpretation of this complex.

The complex is reminiscent of the wall–membrane elaborations of transfer cells, which facilitate the intercellular movement of substances. These basically involve "surface area amplification" (Gunning, 1977). Heath and Heath (1975) suggested that the occurrence of the septal elaborations during early haustorium formation provided additional membrane area to facilitate energy-requiring rapid transport of materials across the HMC septum.

Although there is no direct evidence for the function of this apparatus, the available information (see also Section IV,H,4) strongly suggests that transfer is a major function, hence we propose the term *septal transfer apparatus.* The term *elaboration* will be retained to designate each of the elongated membranous components of the apparatus.

The growth and retraction of the septal transfer apparatus in both *P. graminis* f. sp. *tritici* and *P. coronata* was closely correlated with stages of haustorium formation (Chong *et al.*, 1981), as summarized in Fig. 2. At first, the elaborations occurred on incompletely formed HMC septa, and varied in length on different septa. At this stage or slightly later, other membrane formations in the form of small whorls of very short duration appeared on the HMC side of the septum (Fig. 8). These stained similarly to fungal plasmalemma after PACP treatment, and may be derivatives of plasmalemma-type membrane.

The septal transfer apparatus attains its maximum size during host wall penetration, then decreases in length during subsequent growth of the haustorial neck. During retraction, the elaborations become angular in outline and later remain as an irregular ridge across the HMC septum. The angular appearance during retraction has been interpreted (Chong *et al.*, 1981) as due to a rapid loss of their contents. The septal transfer apparatus is thus a transient structure, persisting only until the time of haustorial neck formation.

Cytochemical tests of the septal transfer apparatus have indicated that the matrix of the elaborations contains some polysaccharide, un-saturated lipids, and a large amount of protein (Chong *et al.*, 1981). By using selective extraction and specific staining it was concluded that the polysaccharide, lipid, and protein components may exist as a complex glycolipoprotein (Chong et *al.*, 1981).

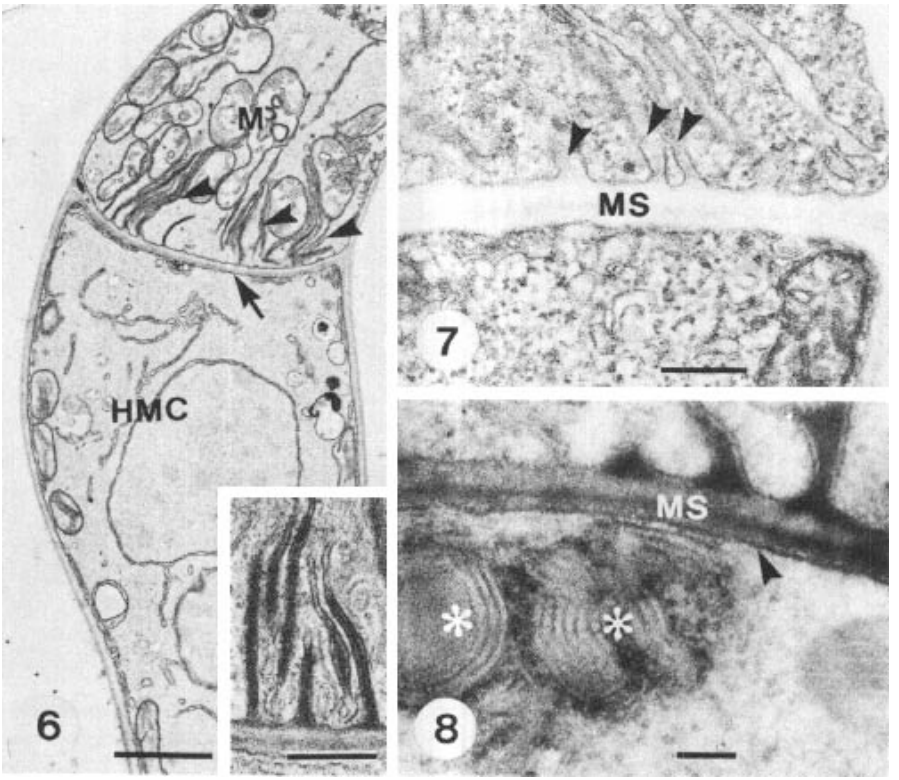


Fig. 6. A haustorium mother cell (HMC) of *Puccinia coronata* sectioned longitudinally to show the fungal plasmalemma elaborations (arrowheads) and associated mitochondria (M) on the hyphal side of the HMC septum (arrow) (Glt/OsO_4). This section was partially oxidized with periodic acid, then stained with TCH-SP (× 12,600; bar, 1 μm). (J. Chong, unpublished.) Inset: Each elaboration is bound by a membrane and contains an electron-

dense matrix and a more electron-dense core (Glt/OsO_4; UA/PbC) (×45,000; bar, 0.25 µm). (From Chong *et al.*, 1981.) **Fig. 7.** Continuity of the bounding membranes of the elaborations (arrowheads) with the plasmalemma across the haustorium mother cell septum (MS) in *Puccinia coronata.* This is clearly demonstrated after treatment with acetone to extract the septal wall material (Glt-acetone-OsO_4; UA/PbC) (×44,300; bar, 0.25 µm). (From Chong, 1981.)**Fig. 8.** Whorls of membrane (asterisks) stained in a manner characteristic of the plasmalemma (arrowhead), found on the haustorial mother cell side of the septum (MS) in *Puccinia coronata* (Glt/OsO_4; PACP) (×74,300; bar, 0.1 µm). (From Chong, 1981.)

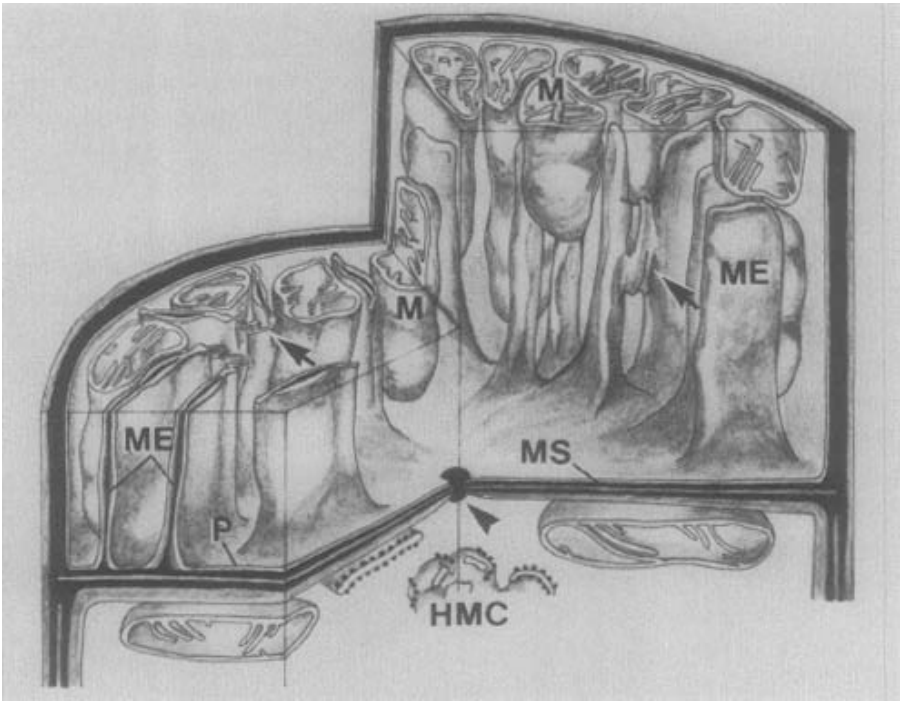


Fig. 9. Diagram of the haustorium mother cell (HMC) septal region cut open to give a three-dimensional interpretation of the principal components: membrane elaborations (ME) (= septal transfer apparatus), plasmalemma (P), HMC septum (MS), and mitochondria (M). For clarity they are not drawn to scale, and some are illustrated by only a few examples (e.g., membrane elaborations and mitochondria). Note the interconnection (arrows) among the membrane elaborations to form a large labyrinth-like complex. The HMC septal pore (arrowhead) is plugged at this stage.

C. HOST CELL PENETRATION

The host cell penetration phase begins with the formation of a penetration peg within an area of contact of the HMC with a host cell. At the site of host cell penetration the HMC wall thickens to assume a convex lens-like shape.

This thickening of the HMC wall appears to be universal in all rusts so far examined, and appears to result from the deposition of new wall material (Chong, 1981; Littlefield and Heath, 1979). The penetration peg develops at the center of this thickened region. In the penetration zone the cytoplasm of the HMC is characterized by the presence of electron-dense granules, membranous whorls, and microtubules (Fig. 10), indicating intense cytoplasmic activity.

The ultrastructural evidence indicates that host wall penetration is mainly a wall dissolution process (Littlefield and Heath, 1979). The act of host wall penetration is difficult to determine by electron microscopy because the process occurs rapidly, and a set of serial sections are required to determine unambiguously the level of penetration. One such set of serial sections obtained for *P. coronata* (Chong *et al.*, 1981) demonstrated that a halo occurred in the host wall in advance of the penetration peg after PA–TCH–SP staining (Fig. 11). This indicates that wall polysaccharides are being dissolved or modified in advance of the penetration peg.

One of the problem areas in the literature has been the evaluation of the occurrence of fungal wall material through the penetration zone. In conventionally processed material there is a very thin layer of fungal wall material in the penetration zone with *P. coronata* and a thicker layer with *P. graminis* f. sp. *tritici* (Chong, 1981). However, better resolution has been obtained by varying the preparative procedures. With *P. graminis* f. sp. *tritici*, material from both the middle and inner wall layers of the HMC wall could be seen in the penetration zone, respectively, after PACP (Chong *et al.*, 1981) and PA–TCH–SP (Fig. 12) staining. Treatment with gold-conjugated Con A, which differentiates host wall and fungal wall material, also showed fungal wall material in the penetration zone (R. Rohringer and J. Chong, unpublished). Our observations have indicated that material from the middle layer of the HMC wall intermingles with host wall material in the penetration zone.

Invasion by *P. coronata* or *P. graminis* f. sp. *tritici* results in the host wall immediately around the penetration site becoming resistant to cell wall-macerating enzymes (*P. graminis* f. sp. *tritici*, Fig. 13). The nature of this host wall modification is not known. The pore in the HMC septum apparently closes and opens during penetration and subsequent haustorial growth. Heath and Heath (1975) first observed that during penetration by *U. phaseoli* var. *vignae*, the HMC septal pore was plugged with a dense material, which persisted until the haustorial body had grown to just beyond its globose form. The pore then lost the dense "plug" and remained open during the mature haustorial phase. A similar sequence of "plugged" and "unplugged" states was found for *P. coronata* and *P. graminis* f. sp. *tritici* (Chong, 1981). This correlation is illustrated for *P. coronata* in Fig. 2. This sequence would appear to restrict the flow of materials out of the HMC during haustorium formation,

then allow the reverse passage of materials after the haustorium has begun to mature.

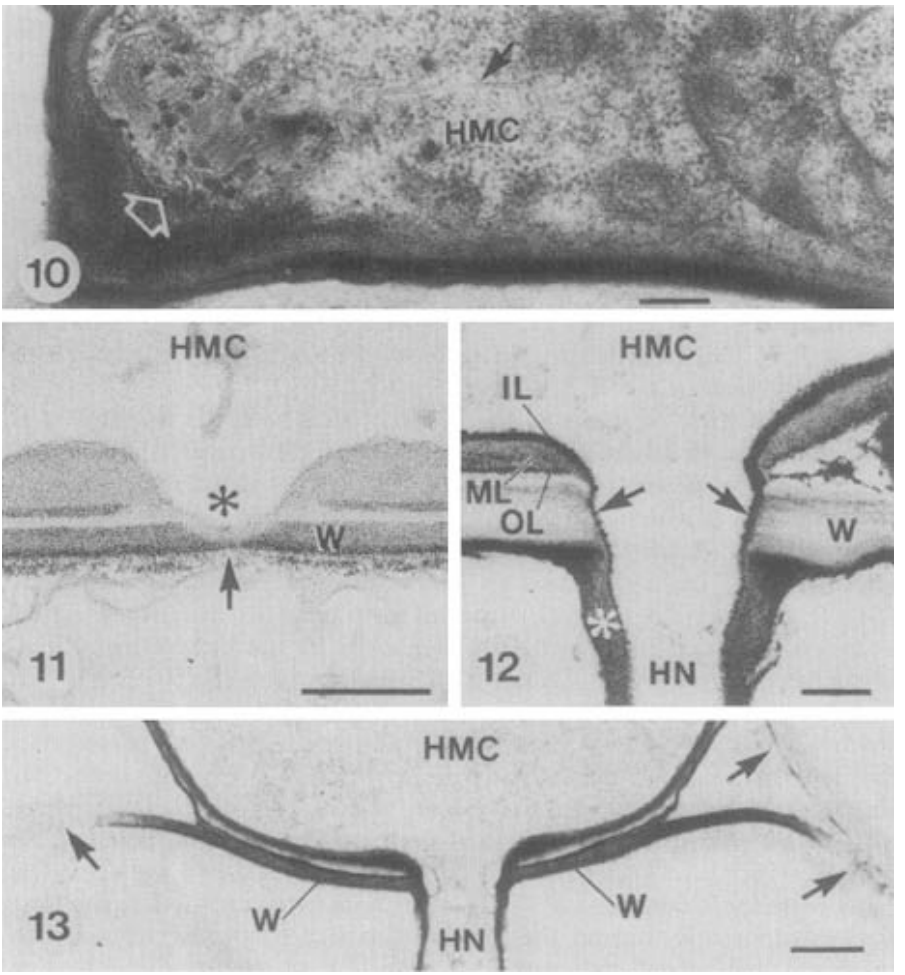


Fig. 10. A nonmedian section of a young haustorium mother cell (HMC) of *Puccinia coronata* to show a microtubule (arrow), membranous materials, and electron-dense granules in the HMC cytoplasm at the site of host penetration (open arrow) (Glt/OsO_4; UA/PbC) (×35,700; bar, 0.25 µm). (From Chong, 1981.) **Fig. 11**. A median section from a series of closely adjacent sections to show a penetration peg (asterisk) formed from a young haustorium mother cell (HMC) of *Puccinia coronata*. There is a halo (arrow) in the host wall (W) in advance of the penetration peg (Glt/OsO_4; PA–TCH–SP) (×65,000; bar, 0.25 µm). (From Chong *et al.*, 1981.) **Fig. 12**. A near-median section through the penetration region in *Puccinia graminis* f. sp. *tritici.* The PA–TCH–SP staining shows a distinct fungal wall layer (arrows) through the penetration region, which is continuous with the inner layer (IL) of the haustorium mother cell (HMC) wall, and with the haustorial neck (HN) wall (asterisk).

ML and OL, Middle and outer layers of HMC wall, respectively; W, host cell wall (Glt; PA–TCH–SP) (×35,700; bar, 0.25 μm). (From R. Rohringer and J. Chong, unpublished.) **Fig. 13**. A median section through the penetration region in *Puccinia graminis* f. sp. *tritici*. The portion of the host cell wall (W) around the penetration region is resistant to macerating enzymes, whereas the rest (arrows) of the host cell wall is largely extracted. HMC, Haustorial mother cell; HN, haustorial neck (macerating enzymes-Glt/OsO_4; UA/PbC) (×17,900; bar, 0.5 μm). (From R. Rohringer and J. Chong, unpublished.)

D. POSTPENETRATION GROWTH OF THE HAUSTORIUM

1. The Haustorial Neck

The early postpenetration growth of the haustorium occurs as a tubular finger-like projection into the host cell (Figs. 14 and 15). This projection is referred to as the haustorium initial and later becomes the haustorial neck. Electron-dense granules, some of which are membrane- bound, or amorphous materials occur in the cytoplasm, but no other organelles are present. The cytoplasm is continuous with that of the HMC, which still contains all of the organelles. The electron-dense granules are probably similar to those that aggregate at the penetration site (see Fig. 10). Littlefield and Heath (1979) noted that similar granules in *Melampsora lini* may be involved in the secretion of host wall-degrading enzymes, and that they did not resemble the apical vesicles that are typical of hyphal tip cells. The latter interpretation may be valid, but the occurrence of similar bodies in the cytoplasm of the haustorium initial suggests another role. In histochemical tests the matrix of the transfer apparatus reacted similarly to the haustorial neck wall in *P. coronata* (Chong, 1981; Chong *et al.*, 1981), indicating a similar composition (see Section IV,E on the histochemistry of the neck wall). It is possible that the matrix of the transfer apparatus is used directly in the synthesis of the neck wall, and the electron-dense granules may represent a unique type of “apical vesicle” that is involved in the transfer of this material.

2. Haustorium Expansion Phase

After the haustorium initial has grown to a length of about 4 μm, the haustorial body begins to form (Fig. 16). Haustorial bodies at this stage are packed with mitochondria, which apparently migrate from the HMC. At this stage, the neck wall of *P. coronata* is seen to be composed of two moderately stained layers separated by a middle more electron-opaque layer (Fig. 17). These layers appear to merge at the base of the haustorial body to form a single-layered body wall (Fig. 18). In contrast, the neck wall of *P. graminis* f. sp. *tritici* was seen to consist of only one densely staining layer in similarly processed material.

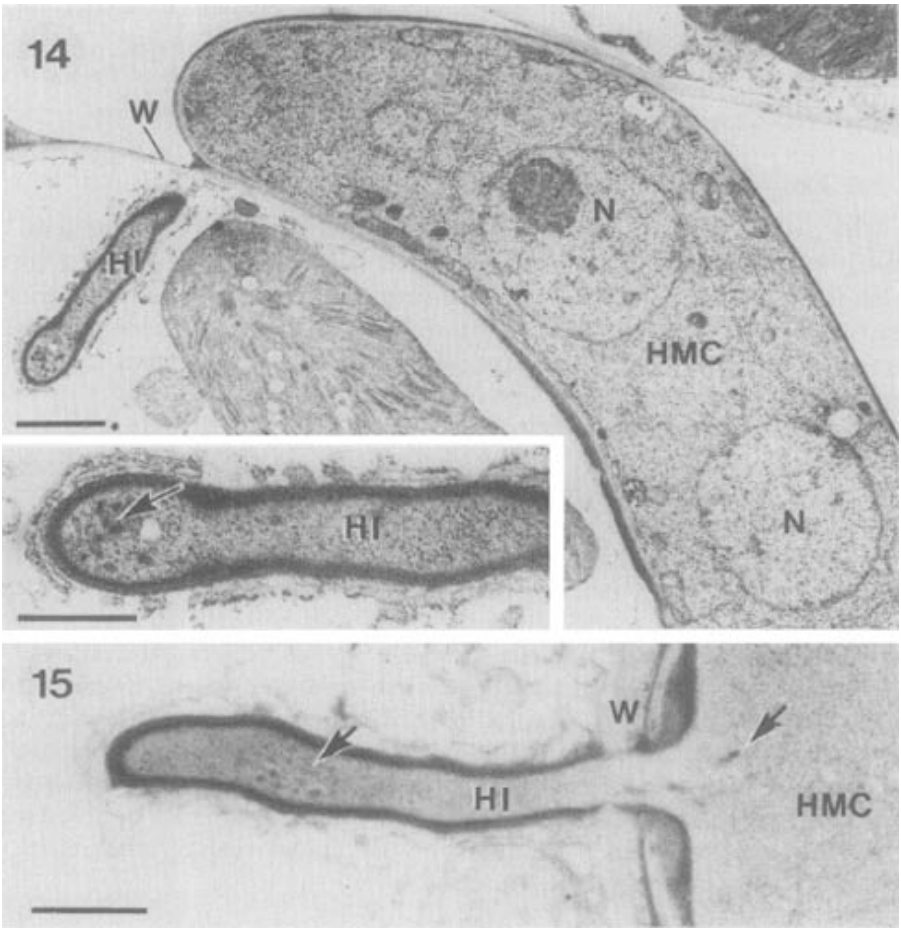


Fig. 14. A haustorium initial (HI) of *Puccinia coronata* consisting of a tubular fingerlike projection about 2.6 µm long, extending into the host cell. Adjacent sections did not reveal the presence of a haustorial body. HMC, Haustorial mother cell; N, nucleus; W, host cell wall. Inset: Higher magnification of the haustorium initial to show the presence of electron-dense granules (arrow) in the cytoplasm. The wall of the haustorium initial is seen as one densely staining layer (Glt/OsO_4; UA/PbC) (Fig. 14, × 11,100; bar, 1 µm. Inset, ×30,400; bar, 0.5 µm). (From Chong, 1981.) **Fig. 15**. A haustorium initial (HI) of *Puccinia graminis* f. sp. *tritici* consisting of a tubular finger-like projection. Note the electron-dense granules (arrows) in the fungal cytoplasm. HMC, Haustorial mother cell; W, host cell wall (Glt/OsO_4; PACP) (×28,600; bar, 0.5 µm). (From Chong, 1981.)

The EH membrane lies closely against the wall along the length of the neck, then becomes separated from the wall near the base of the haustorial body of either *P. coronata* or *P. graminis* f. sp. *tritici*. Near this point a variable

somewhat electron-lucent (after UA/PbC staining) area, the EH matrix (see Section IV,H,2), intervenes between the EH membrane and the body wall.

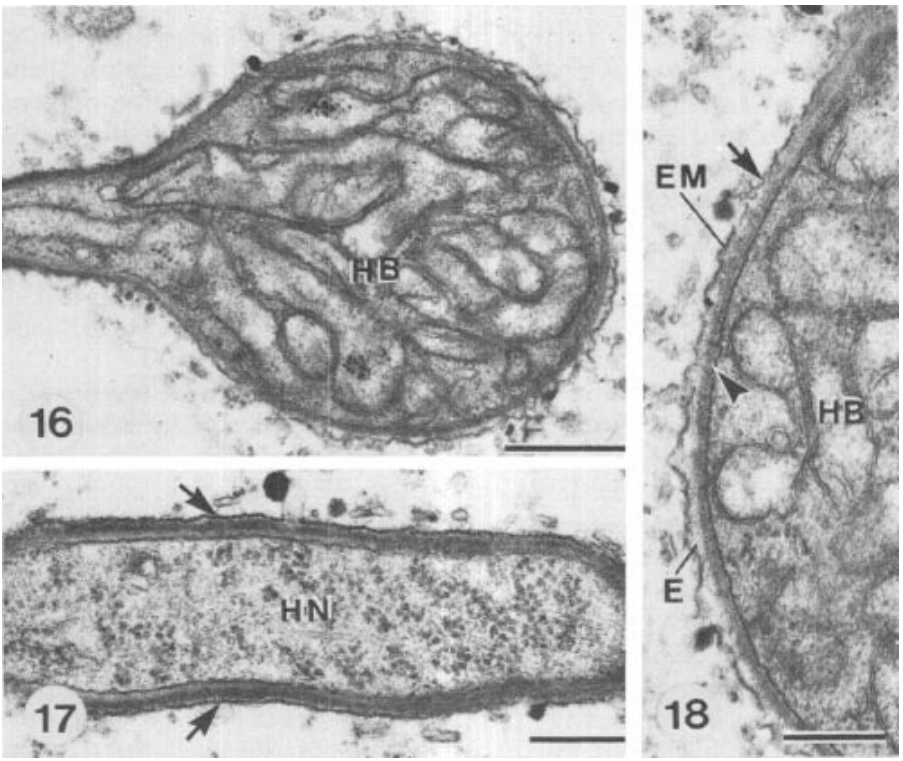


Fig. 16. A young haustorium of *Puccinia coronata* in the early expansion phase. The young haustorial body (HB) is packed with mitochondria (Glt/OsO_4; UA/PbC) (×30,400; bar, 0.5 μm). (From Chong, 1981.) **Fig. 17.** Part of the haustorial neck (HN) from the same haustorium shown in Fig. 16. Two moderately stained layers separated by a middle electron-dense layer can be seen in the entire neck wall. The extrahaustorial membrane (arrows) adheres tightly to the entire length of the neck. A neck ring is not present at this stage (Glt/OsO_4; UA/PbC) (×47,100, bar, 0.25 μm). (From Chong, 1981.) **Fig. 18.** Part of the haustorial body (HB) of the same haustorium shown in Fig. 16. The body wall (arrowhead) is composed of only one layer. The extrahaustorial membrane (EM) is separated from the wall near the base (arrow) of the body to form the extrahaustorial matrix (E) (Glt/OsO_4; UA/PbC) (×51,400; bar, 0.25 μm). (From Chong, 1981.)

When the haustoria of *P. coronata* or *P. graminis* f. sp. *tritici* attain a size of about 5 μm in diameter, an electron-dense band appears in the neck wall and forms a ring around the neck. The neck ring forms approximately midway along the neck of *P. graminis* f. sp. *tritici* and about one-third of the way from the base of the body in *P. coronata* (Chong and Harder, 1980). The neck ring

has been found in the D-haustoria of all rusts so far examined. The neck ring in the rusts has been consistently interpreted as a single, intensely osmiophilic band. However, a major variation in neck ring structure has been observed for *P. coronata*(Chong and Harder, 1980). When the neck ring in this fungus is first formed, it appears as a single broad band (Fig. 19), but in mature haustoria a second narrower band is clearly evident (Fig. 20). These two bands have been respectively designated as the α and β bands (Chong and Harder, 1980). The significance of the PA–TCH–SP "staining" of the α band in Fig. 19 is discussed later in Section IV,E. In contrast, only a single band has been resolved in the haustorial necks of *P. graminis* f. sp. *tritici* (Chong, 1981).

A possible explanation for the function of the neck ring was provided by Heath (1976) when it was shown that uranyl acetate crystals occurred between the host and fungal cell membranes up to but not beyond (proximal end of the neck) the neck ring of *P. sorghi.* This indicates that there is an apoplastic flow of materials along the neck wall that is stopped by the neck ring. This observation strengthened an earlier suggestion that the tight association of host and fungal membranes with the neck ring is reminiscent of the Casparian strip of endodermal cells in roots of higher plants (Littlefield and Bracker, 1972). The tight association of the extrahaustorial membrane and the neck ring of *P. coronata* is clearly demonstrated after protease treatment where the neck wall is extracted, but the neck ring remains intact, and the extrahaustorial membrane remains bound to the neck ring (see Fig. 24). The neck ring is arguably a structure that has been evolved by the rust fungi to force a symplastic route (through the haustorium) for the movement of solutes from host to parasite.

Despite the importance of the view just advanced, little is known about the chemical composition of the neck ring. In *P. coronata* the α band is resistant to periodic acid digestion (Fig. 19), but the β band is extracted. Similarly, the entire single bands of mature haustoria of *P. graminis* f. sp. *tritici* (Fig. 21) or *Melampsora lini* (Littlefield and Bracker, 1972) are extracted by periodic acid. It was shown (Chong andHarder, 1980) that both bands of *P. coronata* are inherently electron-dense, and using energy-dispersive X-ray (EDX) analysis, the α band was found to have a high silicon content, while the β band had iron and phosphorus, probably in the form of ferric pyrophosphate (Chong and Harder, 1980). Although the single bands of *P. graminis* f. sp. *tritici* or *M. lini* have not been subjected to EDX analysis, the fact that they are periodic acid-extractable, similar to the β band of *P. coronata,* suggests that they may be similar in composition.

A haustorium is considered to be mature when nearly all of the cytoplasm of the HMC has migrated into the haustorium, leaving the HMC largely vacuolate. In these haustoria the mitochondria tend to retain the peripheral distribution as in the HMC (Chong, 1981), but they again assume the more irregular, less compact form similar to that in the intercellular hyphae (see Harder, Chapter 11, this volume). The peripheral distribution of the haustorial mitochondria

appears to offer an advantage in the active transport of materials into the haustorium.

The nuclei in the mature haustoria become more irregular in shape as compared to their more compact ovoid form in the HMCs. The nucleoli in haustoria of either *P. coronata* or *P. graminis* f. sp. *tritici* (Harder *et al.*, 1978, and unpublished) are smaller and without intranucleolar lacunae, and are less granular than those in active intercellular hyphal cells. Nucleolar granules are considered to be precursors of cytoplasmic ribosomes (Smetana and Busch, 1974) and thus are associated with synthetic activity. The reduced granular component of nucleoli in haustoria suggests that the haustorium is not actively involved in the synthesis of new materials.

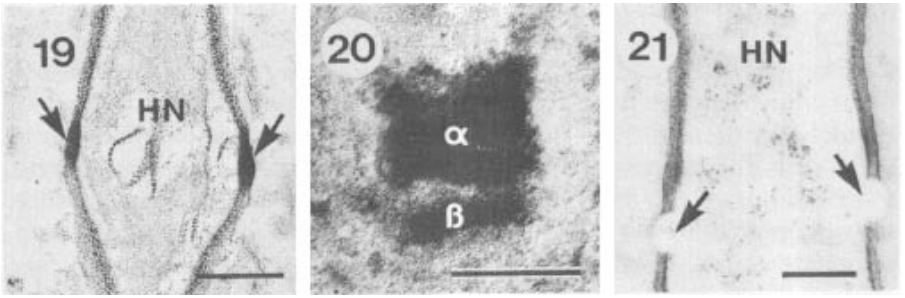


Fig. 19. A near-longitudinal section of the neck (HN) of a young haustorium of *Puccinia coronata* with only a single band present (Glt/OsO_4; PA–TCH–SP) (×44,600; bar, 0.25 µm). (From Chong and Harder, 1980. Reproduced by permission of the National Research Council of Canada.) **Fig. 20**. An oblique tangential section of a mature haustorial neck of *Puccinia coronata*. Note the presence of two bands. The larger band closer to the haustorial mother cell is designated as the α band, the smaller one closer to the haustorial body, the β band (Glt/OsO_4; UA/PbC) (×66,400; bar, 0.25 µm). (From Chong and Harder, 1980. Reproduced by permission of the National Research Council of Canada.) **Fig. 21**. A near-longitudinal section of a haustorial neck (HN) of a mature haustorium of *Puccinia graminis* f. sp. *tritici* after PACP staining. The entire neck ring (arrows) is electron-lucent (Glt; PACP) (×36,400; bar, 0.25 µm). (From R. Rohringer and J. Chong, unpublished.)

By light microscopy, the mature haustoria of *P. coronata* (Ruttle and Fraser, 1927) and *P. recondita* (Allen, 1926) were found to contain only one nucleus. We have examined numerous haustoria of the former fungus by electron microscopy and have never seen more than one nucleus, although two nuclei are always found in the young HMCs. A similar observation was made for *P. poarum* by Al-Khesraji and Lösel (1981). It has not been particularly difficult to find two nuclei in haustoria of other rusts by electron microscopy. The significance of the observation of a single nucleus in haustoria of *P. coronata* or *P. poarum* awaits further investigation.

E. ORGANIZATION AND CYTOCHEMISTRY OF THE HAUSTORIAL WALLS

The organization and cytochemistry of the haustorial walls is of considerable interest because these form part of the host-pathogen interface (Bracker and Littlefield, 1973), and they may be involved in plant and fungus recognition (Rohringer *et al.*, 1982). A number of histochemical tests have been performed to identify components of walls of both immature and mature haustoria of *P. coronata* (Chong, 1981; Chong *et al.*,1981) and *P. graminis* f. sp. *tritici* (Chong, 1981; Rohringer *et al.*, 1984). The results of these tests for *P. graminis* f. sp. *tritici* are summarized in Fig. 22. For a description of the tests used, see Section II. The diagram in Fig. 22 is included to facilitate identification of different portions of the neck or body walls and to indicate that the inner layer (IL) of the neck wall becomes thicker to comprise the bulk of the body wall, while the thick outer layer (OL) remains as a narrow band around the haustorium. The haustorial walls of *P. coronata* responded similarly to the tests in Fig. 22 except for the response to protease. In *P. coronata* the entire outer layer of the neck wall between the penetration peg and haustorial body is digested by protease (Fig. 24), whereas in *P. graminis* f. sp. *tritici* the portion of the neck wall between the neck ring and penetration peg is resistant to this enzyme (Figs. 22 and 23). These results indicate two types of neck wall organization: One in which the neck ring marks an abrupt transition in the properties of the neck wall (P. *graminis* f. sp. *tritici*), and the other in which the entire neck wall appears to be uniform in composition (P. *coronata*). Similar conclusions regarding two different types of neck wall organization in the rust fungi were made by Littlefield and Heath (1979).

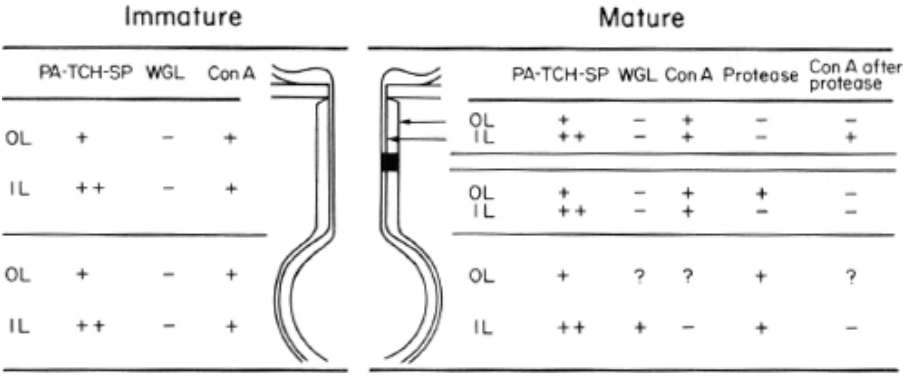


Fig. 22. Positive (+) or negative (–) reactions of the outer (OL) or inner (IL) wall layers of immature or mature haustoria of *Puccinia graminis* f. sp. *tritici* after treatment with PA–TCH–SP, WGL, Con A, or protease. Note that the OL and IL of the neck wall become reversed in relative thickness around the haustorial body. The treatments (also see Section II) are for detection of substances as follows: PA–TCH–SP, Polysaccharides with vicinal

hydroxyl groups; WGL, chitin; Con A, α-linked glucose or mannose; protease, protein; Con A after protease, α-linked carbohydrates that are not bound to proteins.

The conclusions drawn from the application of the cytochemical tests in various combinations were that the major components of the walls (i.e., protein, carbohydrate, and lipid) exist in complex forms, probably as glycoproteins, lipoproteins, or glycolipoproteins (Chong, 1981; Chong *et al.*, 1981). Also, the properties of the walls change as the haustoria mature; the haustorial body walls become more resistant to protease and acquire a chitin component as indicated by the increased wheat germ lectin binding. Probably the most significant finding is that the neck wall is unique in composition relative to the walls of any other part of the rust fungal thallus, particularly in its apparent lack of chitin. It has been suggested (Rohringer *et al.*, 1982) that the neck wall may carry host–rust recognition factors in the determination of compatibility or incompatibility in the interaction between wheat and P. *graminis* and f. sp. *tritici* containing the *P6* gene for avirulence.

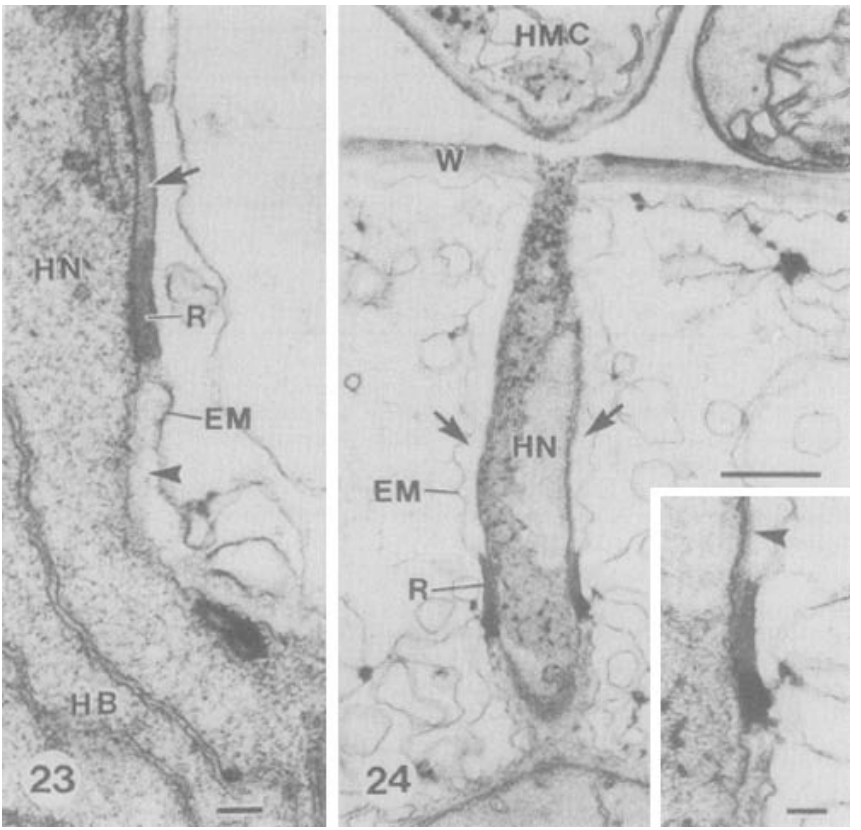


Fig. 23. Differential extraction of the haustorial walls of a mature haustorium of *Puccinia graminis* f. sp. *tritici* after protease treatment. The part of the neck (HN) wall (arrows) between the neck ring (R) and the penetration peg is resistant to protease, but the portion (arrowhead) above the neck ring and the body (HB) wall have been largely digested. The extrahaustorial membrane (EM) adheres tightly to the neck ring and to the part of the neck wall that is not affected by protease, but is convoluted where the wall has been digested (Glt-protease-OsO_4; UA/PbC) (×62,500; bar, 0.1 μm). (From R. Rohringer and J. Chong, unpublished.) **Fig. 24.** The haustorial neck (HN) wall (arrows) of a mature haustorium of *Puccinia coronata* is largely electron-lucent after protease treatment. The extrahaustorial membrane (EM) has been freed from the neck except at the neck ring (R), where it remains tightly bound. Note separation of the haustorium mother cell (HMC) from its haustorium. W, Host cell wall. Inset shows the remaining thin layer (arrowhead) of fungal wall material along the neck (Glt-protease-OsO_4; UA/PbC) (Fig. 24: ×26,300; bar, 0.5 μm. Inset: × 53,000; bar, 0.1 μm). (Fig. 24: From Chong *et al.*, 1981. Inset: From Chong and Harder, 1980. Reproduced by permission of the National Research Council of Canada.)

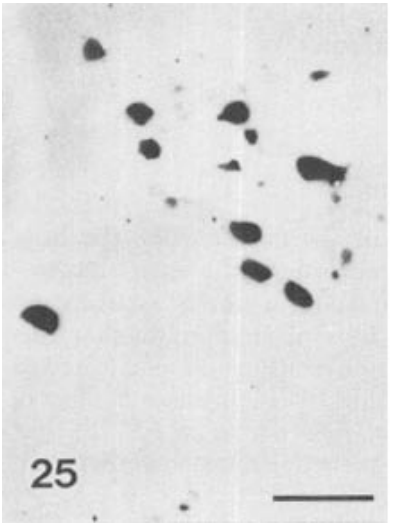
F. POLYPHOSPHATES

In older haustoria and in young HMCs, small vacuoles that contain electron-dense granules are frequently observed. In glutaraldehyde-fixed, unstained sections of haustoria of *P. coronata*, these granules are electron-dense (Fig. 25), suggesting that they have a mineral composition. With EDX analysis these granules were found to be rich in phosphorus and to contain some iron and sulfur (Chong and Harder, 1982a), indicating that they may contain polyphosphate. Polyphosphates commonly occur in the fungi, and in *P. graminis* f. sp. *tritici* much of the polyphosphate occurs in the urediospores (Bennett and Scott, 1971).

G. HAUSTORIA IN THE CENTERS OF AGED COLONIES

Ruttle and Fraser (1927) noted by light microscopy that the haustoria and HMCs near the center of older, well-developed colonies of *P. coronata* appeared to be aberrant. In particular, the HMCs were distorted and the walls were glassy in appearance, and in extreme cases the lumen of these cells was almost completely obliterated by the swollen wall. Electron microscopy showed that similarly located haustoria were distorted and densely staining (Chong, 1981), similar to necrotic haustoria in incompatible interactions. When the tissue was fixed only in glutaraldehyde and the sections left unstained, electron-dense deposits were found in the walls of the HMCs (Fig. 26) as well as the HMC septa. These modified walls were subsequently shown by EDX analysis to be heavily silicified (Chong, 1981). This finding explains the “glassy” appearance noted by Ruttle and Fraser (1927). This also provides a clue to a possible unique mechanism evolved by this fungus to protect itself from toxic by-products. During the late stages of rust infection many of the host cells become disorganized, presumably releasing products that

are detrimental to the fungus. This results in a physiologically incompatible situation, in which the haustoria die (Chong, 1981). However, to prevent deleterious products from reaching the remainder of the fungal thallus, the HMC walls and the HMC septum become heavily silicified, likely making them resistant to the passage of these products. Similar electron-dense deposits were also found in the walls and septa of many of the HMCs of *P. graminis* f. sp. *tritici* located at or near the center of the colonies.



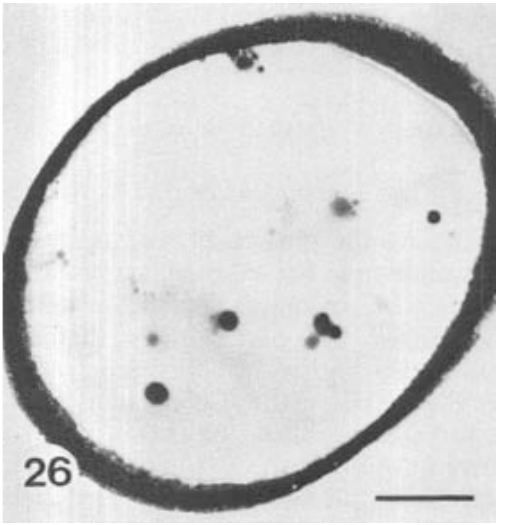


Fig. 25. A semithin unstained section of part of an old haustorium of *Puccinia coronata* showing the presence of large electron-dense granules in the protoplast. This section had been subjected to EDX analysis (Glt; unstained; (×12,600; bar, 1 µm). (From Chong, 1981.) Fig. 26. A semithin unstained section of an old haustorium of *Puccinia coronata* showing the heavy accumulation of electron-dense deposits in the wall. This section had been subjected to EDX analysis (Glt; unstained) (×25,700; bar, 0.5 µm). (From Chong, 1981.)

H. THE HOST–HAUSTORIAL INTERFACE

1. The Extrahaustorial (EH) Membrane

During the growth of rust haustoria in their host cells, the host plasmalemma becomes invaginated and surrounds the entire haustorium. The invaginated plasmalemma consists of newly synthesized membrane. The part of the invaginated host plasmalemma that surrounds the haustorium, beginning at the penetration site, is referred to as the extrahaustorial (EH) membrane. The EH membrane is closely associated with the haustorial neck wall (Harder *et*

al., 1978), but around the haustorial body a matrix of material intervenes between the body wall and EH membrane.

In most conventionally processed tissue the EH membrane is undulated (Fig. 27), but preliminary results from freeze-substitution indicate that this membrane is in fact smooth (D. E. Harder and K. Mendgen, unpublished). The fixation with glutaraldehyde, as performed for conventional electron microscopy, may result in alteration of membrane conformation (Willison and Brown, 1979). The conformation of the membrane may also be affected by the age of the haustorium. In freeze-etched preparations, EH membranes range from smooth to very rough under similar conditions of preparation, apparently varying with age (D. E. Harder and K. Mendgen, unpublished). The EH membrane in Fig. 28 represents a view of a moderately rough EH membrane.

In most profiles seen by electron microscopy the EH membrane is thicker than the other host membranes, and continues to thicken and may attain a more diffuse outline as the haustorium ages (Harder *et al.,* 1978). The EH membrane is continuous with the host plasmalemma and presumably would share some of its properties. The PACP stain, which is specific for plant plasma membrane, also intensely stains the EH membrane in*P. coronata* (Fig. 27) as well as in *P. graminis* f. sp. *tritici* infections (Harder *et al.,* 1978). In this respect the EH membrane is similar to the plasmalemma. However, Harder and Mendgen (1982) showed by freeze-etch electron microscopy after filipin treatment that the EH membrane contains considerably less sterol than does the host plasmalemma. Also, Spencer-Phillips and Gay (1981) demonstrated a lack of ATPase activity at the extrahaustorial membrane as compared to the noninvaginated host plasmalemma in *U. appendiculatus* infections. It was suggested by the latter workers that an enzyme-deficient host plasma membrane is developed around the haustoria.

A specific role, if any, of the EH membrane in rust-host interactions is still speculative. One possible role is the control of the flow of metabolites into or out of the haustorium through alterations in permeability. Membrane sterol is known to play a role in membrane permeability, thus the change in sterol content may reflect such a role. A more intriguing possibility involves the association of the EH membrane with other host membranes. As will be seen (Section IV,H,3) there is an extensive association of the EH membrane with host endoplasmic reticulum. Such direct associations between the plasmalemma and endoplasmic reticulum are rare, and the change in sterol content of the EH membrane may result in greater compatibility between these two types of membranes. An alternate view to the control of metabolite flow directly through the EH membrane will be presented in Section IV,H,4.

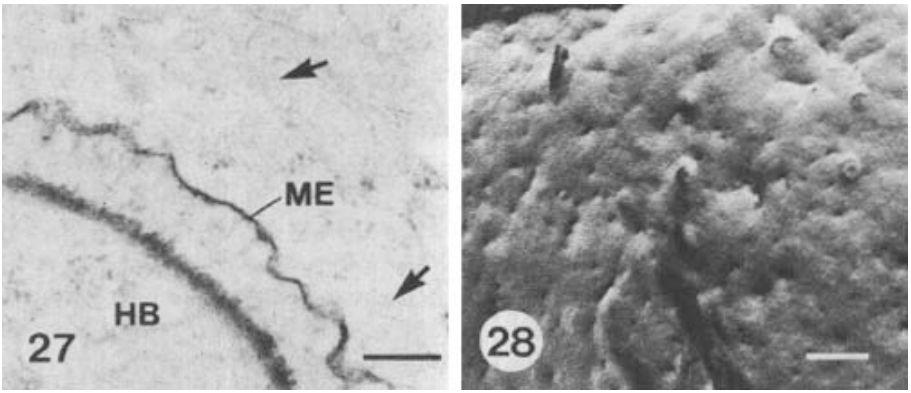


Fig. 27. The extrahaustorial membrane (ME) around a haustorial body (HB) of *Puccinia coronata* is undulated and stains more densely than tubule membranes (arrows) and other membranes of the host (Glt/OsO_4; PACP) (×40,000; bar, 0.5 µm). (From Chong *et al.*, 1981.) **Fig. 28**. A freeze-etch replica of the extrahaustorial membrane around a haustorium of *Puccinia coronata*. The extrahaustorial membrane has a moderately rough profile (Glt; freeze-etch) (×30,900; bar, 0.5 µ.m). (D. E. Harder and K. Mendgen, unpublished.)

2. The Extrahaustorial (EH) Matrix

No part of the haustorial apparatus has led to more speculation than the EH matrix. In the rust fungi, the EH matrix is of universal occurrence around the body of the haustorium but is highly variable in appearance. The matrix ranges from a narrow, nearly electron-lucent band to an apparently broader zone containing various amounts of fibrillar or granular electron-dense substances. The variability in appearance has frequently been related to the age of the haustorium or degree of compatibility with the host. The matrix has variously been considered to be derived from the fungal wall, to be of host origin, or to be an artifact resulting from histological preparation procedures. Despite the attention paid to the EH matrix, there is little definitive information in the literature on its composition.

Recent work on *P. coronata* (Chong, 1981; Chong *et al.*, 1981) and *P. graminis* f. sp. *tritici* (Rohringer *et al.*, 1984) has provided some information on the composition of the matrix. The EH matrices of *P. coronata* and *P. graminis* f. sp. *tritici* were shown to contain mixtures of lipid, larger amounts of polysaccharide, and protein. At least two types of polysaccharides were apparent: cellulose, which may be a response of the host to build a wall at this interface, and protein-bound polysaccharide (glycoprotein). The variability in electron density that is normally encountered in the matrix is probably due to the level of solubilization, or accumulation and polymerization of its components. The latter appears to increase with increasing age of the

haustorium. In any case, the contents of the matrix are clearly not an artifact of preparation procedures. Some preliminary work on freeze-substitution of haustoria of *P. coronata* or *U. appendiculatus* var. *appendiculatus* (D. E. Harder and K. Mendgen, unpublished) has indicated that the matrix is structurally a uniform and easily recognizable entity.

The differentiation of the matrix from the haustorial wall is frequently unclear. Many micrographs show a diffuse, somewhat frayed zone at the juncture of the matrix and the body wall. This led Littlefield and Heath (1979) to suggest that although the wall and matrix appeared distinct, perhaps matrix material impinged into the outer surface of the haustorial wall (or vice versa). Histochemical tests (Chong, 1981; Chong *et al.,* 1981) showed that the wall and matrix are clearly distinguished in mature haustoria of the two rusts studied: There were no WGL receptor sites (i.e., chitin) in the matrix, but they were common in the wall. In mature haustoria of *P. graminis* f. sp. *tritici*, there were no Con A receptor sites in the body wall, but they were common in the matrix. However, the outer surface of the wall is probably less smooth than the inner surface; whether this is an introduced artifact is not certain, but it may represent a larger wall surface area for solute transfer.

3. Association of Host Endoplasmic Reticulum (*ER*)

Invasion of the host cell results in marked alteration of the distribution and configuration of host ER. This appears to be a generalized phenomenon throughout the rusts (see Littlefield and Heath, 1979). Endoplasmic reticulum occurs around the bodies of young haustoria of *P. graminis* f. sp. *tritici*, but the greatest association of ER is in the neck region of young developing haustoria (Ehrlich and Ehrlich, 1971; Harder *et al.,* 1978). The extent of ER association in *P. graminis* f. sp. *tritici* is seen in Fig. 29, where much of the ER radiates from the haustorial neck region into the surrounding host cytoplasm. The association of ER with young haustoria is the most striking in *P. graminis* f. sp. *tritici* infections, and has not been observed to such an extent in other host–rust interactions. In *P. coronata* infections the ER cisternae tend to be parallel to the EH membrane (Harder, 1978) (Fig. 30). In *P. graminis* f. sp. *tritici* the extensive association of ER with the haustoria in the neck region tends to diminish as the haustoria mature. Although the host ER has been shown to contact the EH membrane extensively in the neck region, direct-line continuity between these membranes has not been established. However, there appears to be direct-line continuity between ER and the EH membrane around the haustorial body where the EH matrix is apparent (Harder *et al.,* 1978). Convincing evidence for such continuity is difficult to find, and only in a few cases is it readily apparent.

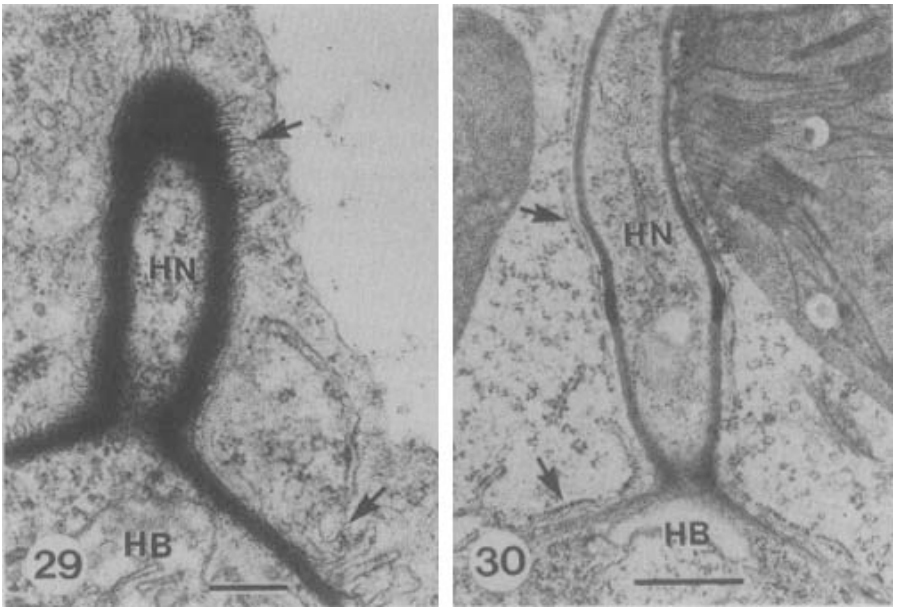


Fig. 29. A nonmedian section of a young haustorium of *Puccinia graminis* f. sp. *tritici* showing the extensive association of host endoplasmic reticulum (arrows) with the neck (HN) and body (HB) (Glt/OsO_4; UA/PbC) (× 19,300; bar, 0.5 µm). (From D. E. Harder and R. Rohringer, unpublished.) **Fig. 30**. The host endoplasmic reticulum cisternae (arrows) tend to lie parallel to the extrahaustorial membrane in *Puccinia coronata*. HN, Haustorial neck; HB, haustorial body (Glt/OsO_4; UA/PbC) (×27,900; bar, 0.5 µm). (From Chong and Harder, 1980. Reproduced by permission of the National Research Council of Canada.)

4. Association of Host Membrane Complexes

A number of reports describe vesicles or tubular membranous structures in the host cytoplasm near the haustoria (Chong *et al.,* 1981; Ehrlich and Ehrlich, 1963, 1971; Harder, 1978; Harder *et al.,* 1978; Rijkenberg, 1975; Van Dyke and Hooker, 1969; Yudkin and Reiter, 1979). The vesicular configurations as noted in several of those articles are probably parts of tubules, as indicated in work on *P. graminis* f. sp.*tritici* (Harder *et al.,* 1978) and *P. coronata* (Harder, 1978; Chong *et al.,* 1981). (See 2 in Note Added in Proof.) In *P. coronata* the tubules develop as an irregular network (Fig. 31), whereas in *P. graminis* f. sp.*tritici* there is a more highly organized complex of small and large tubules (Figs. 33 and 34). The latter complexes were shown to be derived from the host ER (Harder *et ah.,* 1978), and the same appears to be true for*P. coronata* (J. Chong and D. E. Harder, unpublished). The small and large tubules in *P. graminis* f. sp. *tritici* infections are interconnected, and the entire complex may surround part of the haustorium. A three-dimensional

interpretation of this complex is shown in Fig. 36. The membranes of these complexes, both in *P. coronata* and *P. graminis* f. sp. *tritici* infections, have frequently been observed to be continuous with the EH membrane (Fig. 32, inset). In *P. coronata* infections the tubular complexes were most commonly found in the host cytoplasm between the haustorium and adjacent host nucleus (Fig. 32) (Chong, 1981). This has not been observed for *P. graminis* f. sp. *tritici* infections. For the latter, individual complexes were observed to be interconnected by ER, ramifying extensively around the haustorium (Harder *et al.*, 1978).

Membrane configurations similar to the complexes in the *P. graminis* f. sp. *tritici* or *P. coronata* infections have never been seen in the absence of infection, thus the complexes are probably specifically induced by the invading fungus. The type of complex induced in oats by *P. graminis* f. sp. *avenae* (Fig. 35) is similar to those induced in wheat by *P. graminis* f. sp. *tritici*, as distinct from the type induced in oats by *P. coronata.*This demonstrates alteration of host processes that are specific to the species of the invading fungus; that is, the fungus is able to pass a message(s) into the cell to alter specifically the metabolic processes in that cell.

The structure of the components of the membranous–tubular complexes are reminiscent of the transfer apparatus associated with the HMC septum (Section IV,B). The main feature involves an electron-dense core bound by a membrane. This was consistent for the two rusts studied regardless of the organization of the complexes. As mentioned earlier, membrane structures of somewhat similar shape have been regarded as functional sites where intensive secretion or absorption may take place (Berridge and Oschman, 1972; Gunning, 1977). We interpret the haustorium-associated membranous complexes to be synthetic or secretory bodies related directly to the requirements of the fungus. In regard to the host–pathogen interface, the concept that the EH membrane forms the most immediate interface between the host and pathogen protoplasts requires revision in view of the large ramification of the complexes around the haustorium. These complexes are open directly to the EH matrix, and are themselves interconnected via the host ER system. This network most likely includes the nucleus, as the ER is also continuous with the outer membrane of the nuclear envelope (Morré and Mollenhauer, 1974). The “functional” interface in effect extends throughout the host cell. Gunning (1977) suggested that the undulated nature of the EH membrane around fungal haustoria was to increase the surface area to facilitate transfer of substances. However, this may be relatively less important, for as noted earlier, the undulation of the EH membrane may be largely artifactual (D. E. Harder and K. Mendgen, unpublished). In view of the extensive access to the host cell's metabolic machinery through the EH membrane-associated tubular complexes, emphasis should also be placed on the complexes to provide and facilitate the flow of metabolites.

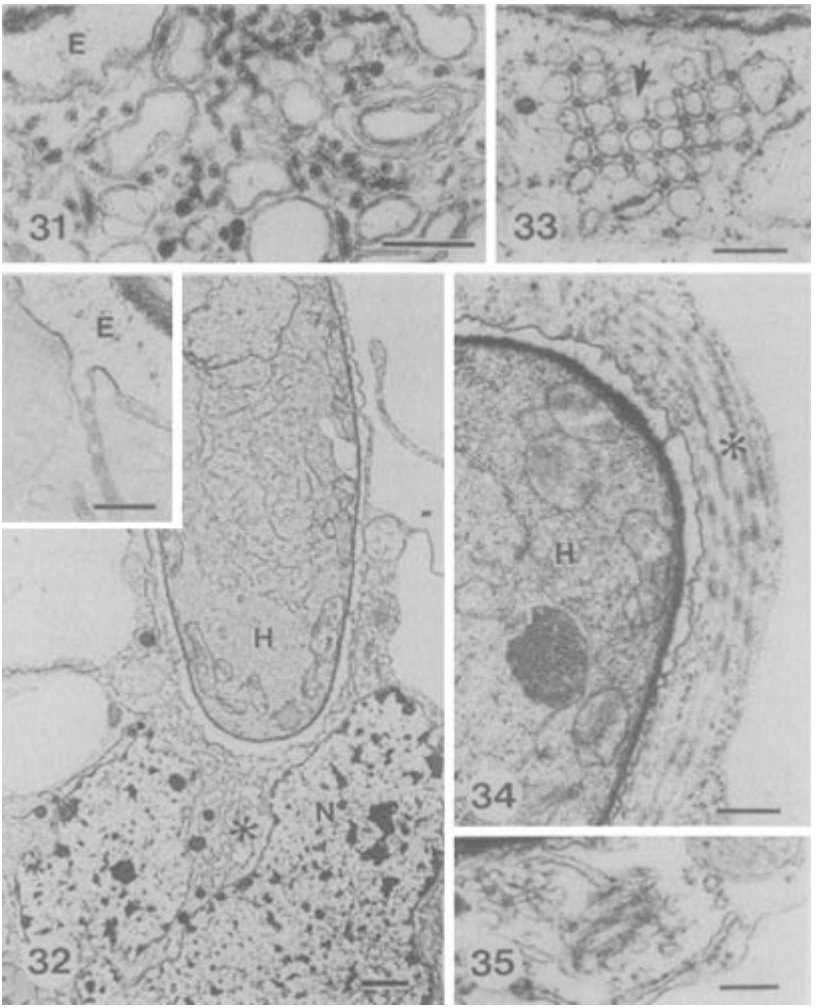


Fig. 31. Development of an irregular network of tubules in the host cytoplasm near the haustorium of *Puccinia coronata*. The tubules contained an electron-dense core. E, Extrahaustorial matrix (Glt/OsO_4; UA/PbC) (×25,700; bar, 0.5 µm). (From Chong, 1981.) **Fig. 32**. Close association between a haustorium (H) of *Puccinia coronata* and the host nucleus (N). Cytoplasmic tubules are found in the region (asterisk) between the haustorium and the host nucleus. The portion of the host nucleus surrounding the tubule complex is lobed (Glt/OsO_4–$K_3Fe(CN)_6$) (×6900; bar, 1.0 µm). Inset shows the continuity of a tubule with the extrahaustorial matrix (E) in *Puccinia graminis* f. sp.*tritici* (Glt/OsO_4; PA–TCH–SP) (×34,300; bar, 0.25 µm). (From Chong, 1981.) Fig. 33. Cross section of a membrane complex near a haustorium of *Puccinia graminis* f. sp. *tritici.* Note the orderly arrangement of two types of tubules: smaller ones containing an electron-dense core and larger ones (arrow) with electron-lucent contents

(Glt/OsO_4; UA/PbC) (×41,400; bar, 0.25 µm). (From Harder *et al.*, 1978. Reproduced by permission from the National Research Council of Canada.) **Fig. 34.** A near-longitudinal section of a large membrane complex (asterisk) similar to that shown in Fig. 33, in the host cytoplasm near a mature haustorium (H) of *Puccinia graminis* f. sp. *tritici*(Glt/OsO_4; UA/PbC) (×18,000; bar, 0.5 µm). (From D. E. Harder and R. Rohringer, unpublished.) **Fig. 35**. An oblique section of a membrane complex induced in an oat mesophyll cell by *Puccinia graminis* f. sp. *avenae.* This complex is characteristic of those induced in wheat cells by *Puccinia graminis* f. sp. *tritici* (Glt/OsO_4; UA/PbC) (×33,200; bar, 0.25 µm). (From D. E. Harder, unpublished.)

V. Monokaryotic Haustoria

Observations by light microscopy on the basidiospore-derived monokaryotic infections of several cereal rusts (Allen, 1930, 1932a,b) showed that their intracellular structures were more filamentous than the haustoria in the dikaryotic infections. Electron microscopy has supplemented these findings and has clearly shown that the monokaryotic (M) intracellular structures of a number of rusts have little of the structural specialization of the D-haustorial apparatus. The type of intracellular structure formed is considered to be dependent on the karyotic state of the thallus and not on the host species infected (Gold *et al.*, 1979). Within the cereal rusts, the M-intracellular structure has variously been designated as haustorium (Allen, 1930, 1932a,b), P-haustorium (Harder, 1978), or intracellular hypha (Rijkenberg and Truter, 1973; Al-Khesraji *et al.*, 1980; Al-Khesraji and Lösel, 1980, 1981; Gold *et al.*, 1979). Littlefield and Heath (1979) introduced the term *M-haustorium* to cover the M-intracellular structures of all the rusts, and this terminology will be retained here. (See 3 in Note Added in Proof.)

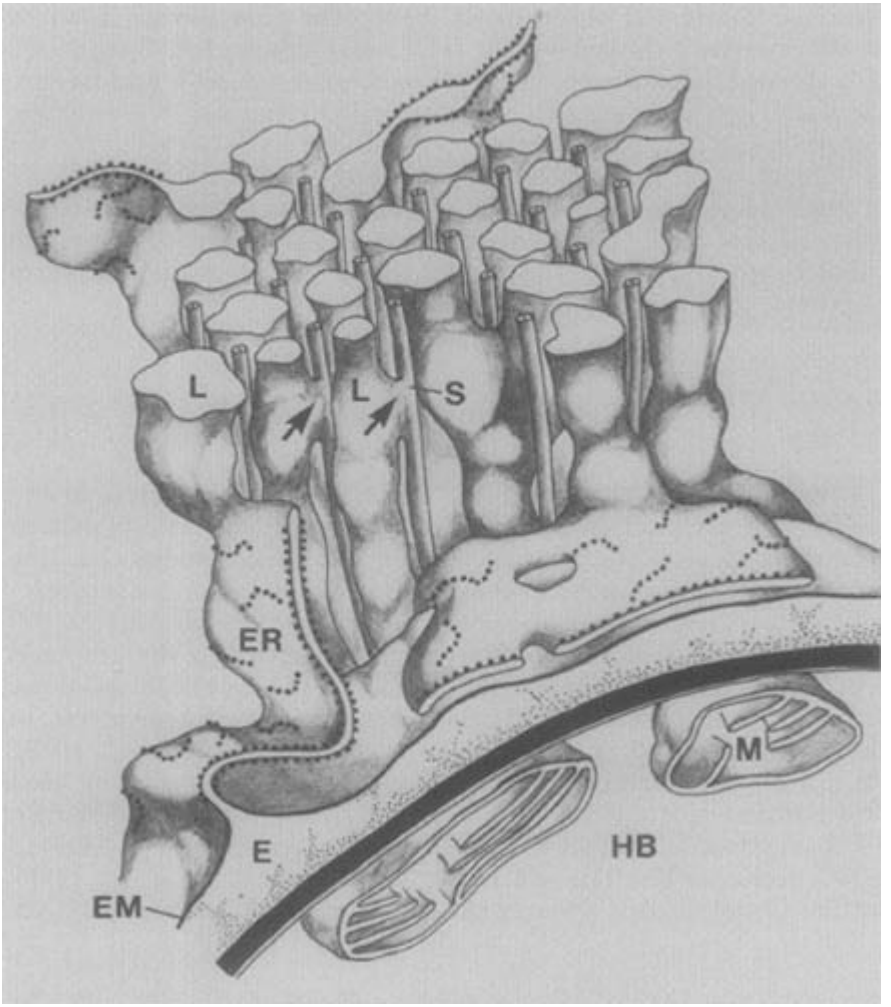


Fig. 36. Diagram of a haustorium (*Puccinia graminis* f. sp. *tritici*)-associated organized membrane complex (reconstructed from a series of serial sections) cut open to show the principal components and their interrelationships. Note the connections (arrows) between the large (L) and small (S) tubules. The large tubules are also connected to the surrounding host endoplasmic reticulum (ER), which in turn is continuous with the extrahaustorial matrix (E). EM, Extrahaustorial membrane; HB, haustorial body; M, mitochondria.

The features that typify the development and structure of a M-haustorium of *P. coronata* are illustrated in the drawing in Fig. 37. Although this drawing is summarized from observations involving *P. coronata*, the structural features, except as noted later, are generally representative of the M-haustoria of the

cereal rusts known so far from ultrastructural studies (Chong, 1981; Chong *et al.*, 1981; Al-Khesraji and Lösel, 1980, 1981; Al-Khesraji *et al.*, 1980; Gold *et al.*, 1979; Harder, 1978; Rijkenberg and Truter, 1973). The following description of M-haustoria is based on the morphological features of D-haustoria to illustrate how these two intracellular structures compare and where they differ.

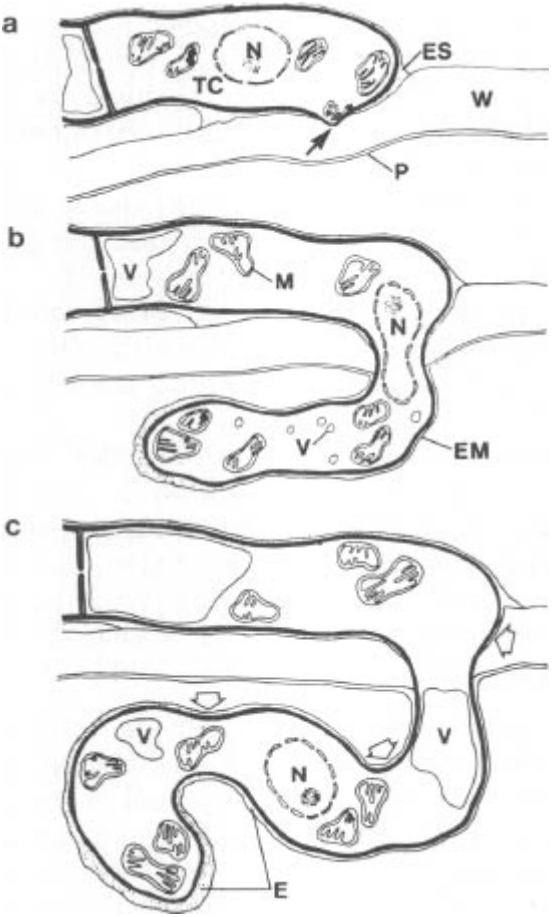


Fig. 37. A diagrammatic, chronological representation of M-haustorium development in *Puccinia coronata.* (a) M-haustorium formation is initiated by a protuberance (arrow) from a terminal cell (TC). (b) After penetration, subsequent growth of the protuberance forms the haustorium. (c) Old M-haustoria are often septate, and possible location of septation is indicated (open arrows). E, Extrahaustorial matrix, EM, extrahaustorial membrane; ES, extracellular coating substance; M, mitochondrion, N, nucleus, P, plasmalemma; V, vesicle; W, host cell wall.

1. The M-haustorium arises from a terminal intercellular hyphal cell.
2. There is no differentiation of a specialized HMC nor development of a specialized HMC septum with its transfer apparatus.
3. Penetration is likely accomplished by enzymatic digestion of the host wall.
4. There is only a slight constriction of the penetration peg.
5. The wall of the terminal intercellular hyphal cell is continuous with that of the M-haustorium and remains unmodified.
6. The growth of the M-haustorium is filamentous, and no neck ring is formed.
7. Centripetal septum formation may occur at any point intracellularly or at various points outside the penetration region.
8. There is no redistribution of mitochondria.
9. The host plasmalemma becomes invaginated to form an EH membrane, and an EH matrix is evident. In *P. coronata* the EH matrix is most pronounced around the distal end of the M-haustorium.
10. A collar may or may not form around the "neck."
11. There is less extensive association of host ER with the M-haustorium, and the development of membranous transfer-type complexes in the host cytoplasm has not been observed.
12. Growth is usually terminal in the invaded host cell, although it has been reported (Gold *et al.*, 1979) that the M-haustoria of *P. reconduct* may exit from their host cells.

In cytochemical tests the EH matrix of the M-haustoria of *P. coronata* exhibits some unique characteristics (Chong *et al.*, 1981). It is more pronounced around the distal end, and in this respect it resembles the EH matrix around the D-haustoria of the same fungus. However, in the M-haustoria the EH matrix stains more intensely and more uniformly with UA/PbC or PA–TCH–SP, indicating a higher concentration of substances, particularly polysaccharides. Interestingly, the EH matrix stained differentially and oppositely with UA/PbC and PACP at the proximal or distal ends, indicating regional specialization of composition of the matrix (Chong, 1981; Chong *et al.*, 1981).

The designation of the M-haustoria as haustoria or intracellular hyphae has been a problem since Allen's light microscopic descriptions (1932a,b, 1933). Electron microscopy has shown the M-haustoria to be essentially unaltered hyphae that invade and grow inside a host cell. One of the criteria outlined by Bushnell (1972) to define haustoria was that they are terminal in their host cells. In this sense the M-haustoria of *P. coronata* (Allen, 1932b; Harder, 1978) or *P. poarum* (Al-Khesraji and Lösel, 1980) fit the definition of a haustorium. However, the morphologically similar M-haustoria of *P. recondita* (Gold *et al.*, 1979) or others (see Littlefield and Heath, 1979) have been observed to exit from invaded host cells. The latter are more suitably defined as intracellular

hyphae. A functional definition is also not without difficulties, as there is little direct information concerning the functions of either D- or M-haustoria. Thus far, association with the host cytoplasm and apparent nutrient uptake are the most obvious features shared by D- and M-haustoria, and this provides the basis for the designation of both as haustoria (Littlefield and Heath, 1979). However, the latter authors also pointed out that either a haustorial or intracellular hyphal designation could be used if the respective structures are known.

VI. Collars

A common response of the host cell to the invasion of the rust fungi is the deposition of a collar of material around the fungus in the region of host cell penetration. Collars are not an integral part of the haustorial apparatus; thus they are considered separately here.

A. DIKARYOTIC INFECTIONS

Collars are not formed in every invaded cell, and their formation is frequently linked to the degree of host-rust fungal compatibility (Heath, 1974; Heath and Heath, 1971). Collar formation is seen as a nonspecific response by the host to wall off the fungus, and in some cases of incompatibility, the entire invading haustorium may be encased by the collar (Heath, 1971; Heath and Heath, 1971).

In genotypically compatible interactions a collar may form at the point of penetration, but it rarely extends beyond the haustorial neck. Collars are more frequently observed in older infections in a variety of host–rust interactions (Chong, 1981; Coffey *et al.*, 1972; Ehrlich *et al.*, 1968; Heath and Heath, 1971), which is probably related to a general decrease in the degree of host–rust fungal compatibility in older infections (Chong, 1981; Littlefield and Heath, 1979). In our observations, collars have been more frequently observed in compatible interactions involving *P. coronata* than those of *P. graminis* f. sp. *tritici* (D. E. Harder and J. Chong, unpublished).

The following description of collars and their formation in infections of *P. coronata* or *P. graminis* f. sp. *tritici* is summarized from Chong and Harder (1982b) and Harder (1978). The mode of collar formation most frequently observed is interpreted in the drawing in Fig. 38. These collars are formed after fungal penetration and correspond to the type I collar designated by Littlefield and Heath (1979). Although these collars surround the haustorial neck, a zone of host material normally intervenes between the collar and the neck.

Where collars occur in *P. coronata* or *P. graminis* f. sp. *tritici* infections, they are initiated by the deposition of material against the host wall in the region where the fungus enters the host cell. In one version of collar formation, small membrane-bound vesicles, some containing electron-opaque material, aggregate near the base of the neck. These vesicles appear to be derived from Golgi bodies that have aggregated at this site (see also Littlefield and Bracker, 1972). The vesicles then apparently coalesce to form the bulk of the collar. Mature collars frequently contain trapped membranes, which probably reflects this mode of formation. However, some collars do not contain the trapped membranes, and there may be variations in their mode of formation (see Littlefield and Heath, 1979). The more homogeneous type of collar may be the result of fusion of vesicular contents (a reverse pinocytotic process) rather than direct fusion of the entire vesicles.

In *P. coronata* infections in particular, the collars are often variable in shape, which may reflect stages in their formation. Collars frequently have long projections radiating into the host cytoplasm. Host ER and Golgi bodies are associated with these projections (Fig. 41). The involvement of ER and vesicles in collar formation is not unexpected, because the collars are essentially a wall apposition, and secretory vesicles have been implicated in wall thickening induced either artificially (Wheeler, 1974; Wheeler *et al.*, 1972) or pathogenically (Tu and Hiruki, 1971). For further discussion of wall appositions in plant pathogenesis see Aist (1976) and Bracker and Littlefield (1973).

The collars in most rust infections are of variable electron density after conventional processing. Callose-like compounds have been suggested to be a major component of collars, although other carbohydrate substances may also be present (Littlefield and Heath, 1979). Collars in *P. coronata* (Fig. 39) or *P. graminis* f. sp. *tritici* (Fig. 40) show intense staining with PA–TCH–SP, particularly for *P. coronata*. The PA–TCH–SP procedure does not stain callose [which is a β(1→3) -glucan], thus the heavy PA–TCH–SP staining is due to polysaccharides other than callose. Similarly, Heath and Heath (1971) indicated that collars in an immune bean rust interaction were rich in polysaccharides as shown by periodic acid–silver hexamine staining. In other cytochemical tests (Chong, 1981), treatments with protease, cellulase, lipid solvents, or for glycogen were negative. From Fig. 39 it is clear that the collar is integral with the inner layer of the host wall. In *P. graminis* f. sp. *tritici* infections the collar is more distinct from the inner wall layer, although this may represent a difference in concentration of PA–TCH–SP stainable polysaccharides. The conclusions from these tests are that the collars are mainly composed of carbohydrate, and particularly in *P. coronata* infections, much of this carbohydrate is in a form other than callose. The amount of callose in the latter infection remains to be determined.

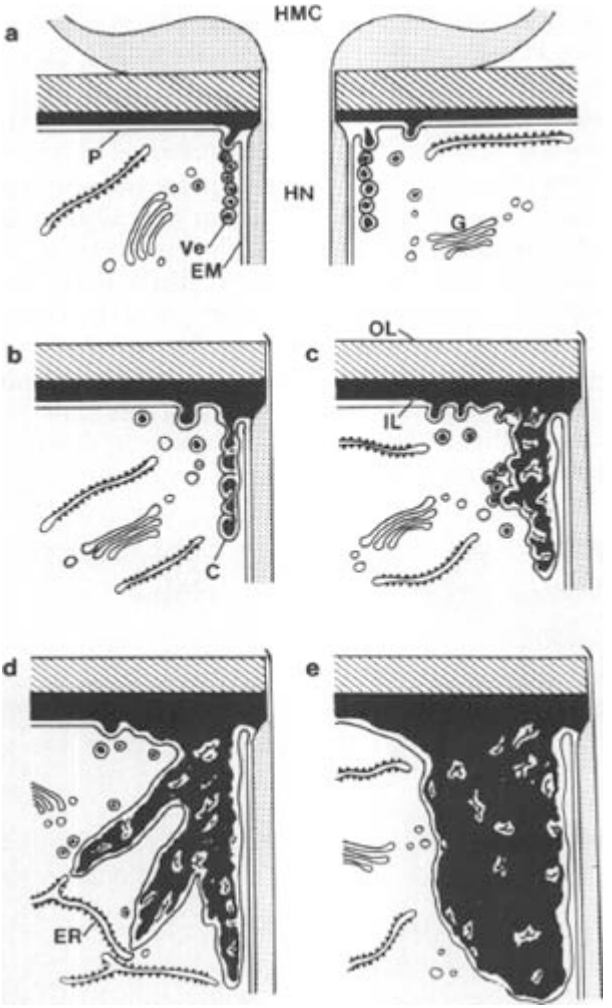


Fig. 38. A diagrammatic chronological representation of one possible mode of collar formation in the D-infection of *Puccinia coronata*. (a) Vesicles (Ve) containing collar material are found adjacent to the extrahaustorial membrane (EM). (b) Vesicles coalesce to form a small collar (C). (c) The collar grows as more material is being deposited into the collar and to the inner layers (IL) of the host wall. (d) The growth of the collar is enhanced by the presence of large projections that are interconnected by host endoplasmic reticulum (ER). (e) Subsequently, a large collar is formed around the haustorial neck, and is integral with the inner layer of the host wall. G, Golgi body; HMC, haustorial mother cell; HN, haustorial neck; OL, outer layer of host cell wall; P, plasmalemma.

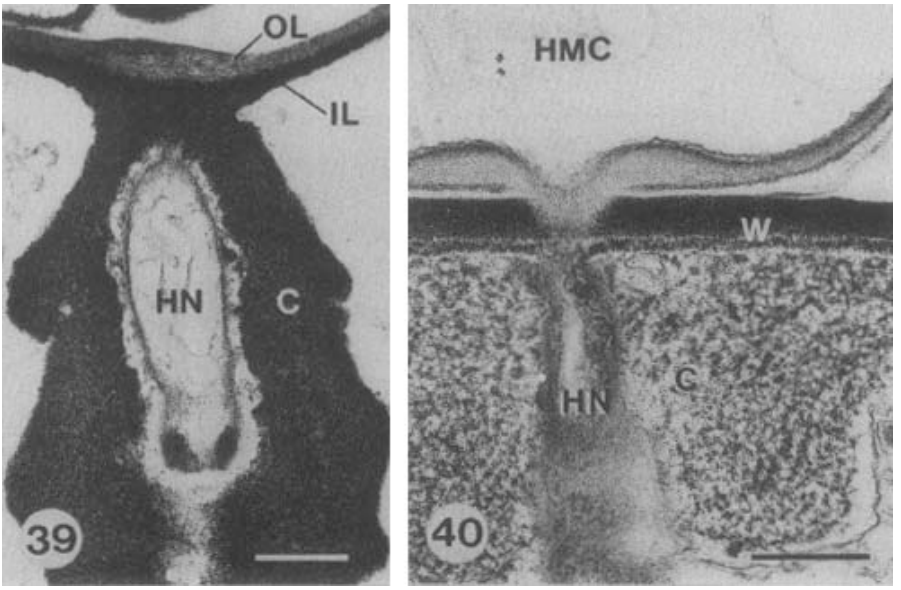


Fig. 39. A large collar (C) around a haustorial (HN) neck of *Puccinia coronata.* Material making up most of the collar is intensely stained except for the small area immediately adjacent to the neck. The collar material is integral with the inner layer (IL) of the host cell wall. OL, Outer layer of host cell wall (Glt/OsO_4; PA–TCH–SPI (×24,300; bar, 0.5 µm). (From Chong, 1981.) **Fig. 40.** A well-developed collar (C) around a haustorial (HN) neck of*Puccinia graminis* f. sp. *tritici.* Collar material is stained, but it has a diffuse and granular appearance and is more lightly staining than the host wall (W). HMC, Haustorium mother cell (Glt/OsO_4; PA–TCH–SP) (×30,000; bar, 0.5 µm). (From Chong, 1981.)

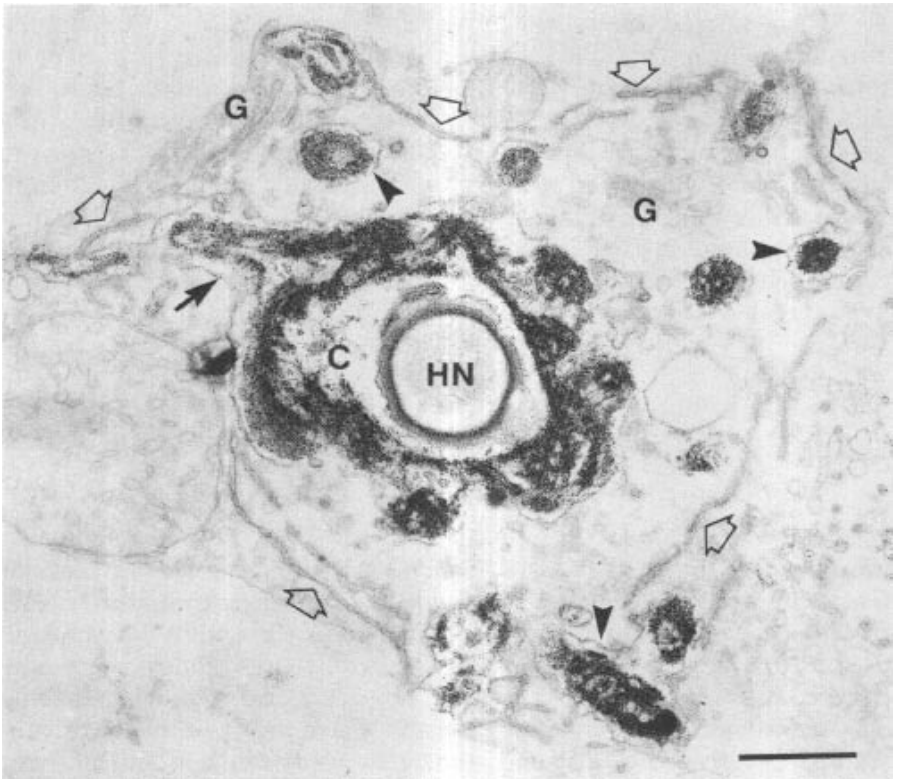


Fig. 41. A developing collar (C) around a haustorial neck (HN) of *Puccinia coronata* with a projection (arrow). Serial sections showed that the nearby large vesicles (arrowheads) containing densely staining material were cross sections of projections radiating out from the collar. Host endoplasmic reticulum (open arrows) and Golgi bodies (G) are associated with these projections (Glt/OsO_4; PA–TCH–SP) (×31,400; bar, 0.5 μm). (From Chong, 1981.)

B. MONOKARYOTIC INFECTIONS

Similar to D-infections, collars have been observed to occur to a greater or lesser extent around the M-haustoria of *P. poarum* (Al-Khesraji and Lösel, 1981), *P. coronata* (Harder, 1978), and *P. sorghi*(Rijkenberg and Truter, 1973). In all these cases the collar appears continuous with and is morphologically poorly distinguished from the host wall. The collar also fuses with the wall of the M-haustoria to a greater or lesser distance from the penetration site. Thus these collars are quite distinct from those of D-infections, and correspond to the type II collars of Littlefield and Heath (1979). Rijkenberg and Truter (1973) considered the collars in M-infections of *P. sorghi* to be indistinguishable from

the EH matrix, although it is evident from *P. coronata* infections (Harder, 1978) that the collars are discrete and of limited extent. The morphology of the collars may be a reflection of the growth habit of the M-thallus. Al-Khesraji and Lösel (1981) show the intercellular hyphae of *P. poarum* to be embedded in the host walls and to grow through the middle lamellar layer between host cells. A similar growth habit is exhibited by M-hyphae of *P. coronata* (D. E. Harder, unpublished). The collars then may result from the fungus growing between or within the walls, and where the hyphae turn into the host cell, the host wall extends around the penetration site.

VII. Haustorial Function

In this section we will discuss the possible function(s) of rust haustoria on the basis of what is currently known of their structural features and their development from early formation to maturity. The fact that rust haustoria are intracellular organs generally leads to the assumption that their primary function is nutrient absorption from the invaded host cells. However, there is as yet no direct evidence for this role. Although autoradiographic studies have shown transfer of substances between the host and some rust fungi (Ehrlich and Ehrlich, 1970; Favali and Marte, 1973; Manocha, 1975; Mendgen and Heitefuss, 1975; Mendgen, 1977, 1979; Onoe *et al.*, 1973; see Littlefield and Heath, 1979), it has not been verified that the route of transfer has occurred directly through the D-haustoria. There are claims that intercellular mycelial growth can occur to some extent in the absence of haustoria (Onoe *et al.*, 1973; Pady, 1935). This implies that the intercellular rust mycelium is able to obtain at least some nutrients directly from the host without passage through the haustorium. Also, the axenic culturability of some of the rusts (see Williams, Chapter 13, this volume) indicates that nutrient uptake via the hyphae is sufficient for a certain amount of growth. The D-haustorium may have a more specific role than that of extracting basic nutrients from the invaded host cell. As shown by Onoe *et al.* (1973), the D-haustoria of *P. coronata* can take up more complicated substances than can the intercellular mycelium. The specific types of nutrients and perhaps the efficiencies of their uptake are the more important factors to consider when dealing with the nutritional role of D-haustoria.

Evidence from ultrastructural studies supports an absorptive role for haustoria at least in D-infections. As described earlier, haustorium-associated host tubular complexes are found in *P. graminis* f. sp. *tritici*, *P. coronata*, and perhaps in other uredial infections (Rijkenberg, 1975; Van Dyke and Hooker, 1969; Yudkin and Reiter, 1979). In P. *graminis* f. sp. *tritici* in particular, the buildup of the organized tubular complexes is extensive. These

complexes are interconnected via the host ER system, and the tubules in turn are open to the extrahaustorial matrix. In effect, the host–pathogen interface extends throughout the entire host cytoplasm. The net result is a large amplification of the "functional" interfacial area, thereby effecting a more efficient transport of materials, presumably from the host cell to the haustorium.

Further evidence, though indirect, that supports a nutritional role for haustoria is the unique structure of the D-haustorium. Although the D-haustoria are intracellular, they do not in fact penetrate the host plasmalemma. After host wall penetration, the D-haustorium invaginates the host plasmalemma as it grows into the host cell. Thus except for blockage by the neck ring, the region between the extrahaustorial membrane and the haustorial wall is open to the host cell wall. This would allow materials that are transported from the host cytoplasm to the extrahaustorial matrix to flow along the haustorial wall into the host wall region (apoplastic flow), thus to be lost to the fungus. The neck ring appears to be a unique structure evolved by the rust fungi to prohibit this apoplastic "escape" of host solutes (Heath, 1976). The mineral composition of the neck ring suggests its ability to act as a barrier. This, combined with the tight adherence of the extrahaustorial membrane to the ring, argues for a forced route of metabolites from the extrahaustorial matrix through the haustorium (the symplast route), thus increasing the efficiency of metabolite transfer. Further, the peripheral distribution of the mitochondria in the haustoria would appear to offer an advantage in the active transport of materials.

Comparison of growth and reproduction between the M- and D-life cycle phases is instructive relative to haustorial physiology. Intercellular growth in the M-phase of *P. poarum* (Al-Khesraji and Lösel, 1980) and*P. sorghi* (Rijkenberg and Truter, 1973) was much more profuse, but with a relative paucity of intracellular structures as compared to the D-phase of either fungus. Rijkenberg and Truter (1973) concluded that the M-phase could subsist largely on substances diffusing from host cells. Further, the M-haustoria are able to invade vascular tissue (Al-Khesraji and Lösel, 1980; Harder, 1978) and thereby have direct access to the host's nutritional resources. In the macrocyclic rusts the uredial stage is the main reproductive phase, whereas the pycnial–aecial stage is more short-lived. Thus the M-haustoria may be less important for nutrition of the fungus. This is reflected in the low level of specialization of the M-haustoria. The D-haustoria by contrast are highly differentiated structurally, and their differentiation appears to be adapted for efficiency of metabolite uptake and transfer. The relatively greater amount of intracellular growth and sporulation in the D-phase indicates that the thallus is more dependent on the haustoria, implicating a nutritional role for them.

The D-haustorium may also have a role in altering the metabolism of the host to suit its own requirements. During the early stages of haustorium formation there is extensive association of host ER with the young haustorium,

and formation of the haustorium-associated host membranous complexes is initiated. The configurations of the host membrane alterations appear to be related to the rust fungal species rather than the host species (see Section IV,H,4). This demonstrates that during the initial stages of haustorium development, information is passed into the host cell that results in alterations of the host endomembrane system. These alterations could have two effects: One may be to alter metabolism and to synthesize metabolites peculiar to the requirements of the fungus; the other is to provide for an efficient and controlled means of transport of metabolites into the haustorium. The latter possibility would provide a means for the fungus to draw on the resources of the host with a minimum of physical disruption to the host cell. In effect, the host alterations are accomplished in a subtle way so as to favor the fungus but to keep the host cell functioning. These observations are consistent with the suggestion by Spencer-Phillips and Gay (1981) that the host cooperates in passing solutes to the fungus (which could be via the membranous complexes), but the pathogen actually controls the efflux from the host. The latter authors indicated that the control activity may occur at the level of the haustorial plasma membrane.

The D-haustorial apparatus is thus a remarkably specialized adaptation of the rusts. Not only does it appear to be structurally specialized to conduct functions required for a compatible host–fungus interaction, but when the infection of a cell has run its course and moribundity sets in, the haustorial apparatus becomes encased in silicon, presumably to limit now deleterious metabolites from spreading through the thallus.

Note Added in Proof

1. Haustoria are the only known intracellular structures in the dikaryotic life cycle stage of most rusts. However, *Physopella zeae*, an example of a direct-penetrating (uredial stage) tropical rust, has been shown to grow extensively as intracellular hyphae and to form typical D-haustoria from the same thallus (Heath and Bonde, 1983).
2. Heath and Bonde (1983) demonstrated that vesicles with electron-dense contents, along with tubules, formed near haustoria of the maize rust fungus *Physopella zeae*.
3. Recently Gold (1983) introduced the term *haploid* (*H*)-*haustorium* as a possibly more appropriate term than monokaryotic-haustorium. This was to emphasize more strongly the haploid stage of the life cycle during which they occur rather than their nuclear complement.

References

Aist, J. R. (1976). Papillae and related wound plugs of plant cells. *Annu. Rev. Phytopathol.* **14**, 145–163.

Al-Khesraji, T. O., and Lösel, D. M. (1980). Intracellular structures of *Puccinia poarum* on its alternate hosts. *Trans. Br. Mycol. Soc.* **75**, 397–411.

Al-Kesraji, T. O., and Lösel, D. M. (1981). The fine structures of haustoria, intracellular hyphae and intercellular hyphae of *Puccinia poarum. Physiol. Plant Pathol.* **19**, 301–311.

Al-Khesraji, T. O., Lösel, D. M., and Gay, J. L. (1980). The infection of vascular tissue in leaves of *Tussilago farfara* L. by pycnial-aecial stages of *Puccinia poarum* Niels. *Physiol. Plant Pathol.* **17**, 193–197.

Allen, R. F. (1926). A cytological study of *Puccinia triticina* physiologic form II on Little Club wheat. *J. Agric. Res.* (*Washington, D.C.*) **33**, 201–222.

Allen, R. F. (1930). A cytological study of heterothallism in *Puccinia graminis. J. Agric. Res.* (*Washington, D.C.*) **40**, 585–614.

Allen, R. F. (1932a). A cytological study of heterothallism in *Puccinia triticina. J. Agric. Res.* (*Washington, D.C.*) **44**, 733–754.

Allen, R. F. (1932b). A cytological study of heterothallism in *Puccinia coronata. J. Agric. Res.* (*Washington, D.C.*) **45**, 513–541.

Allen, R. F. (1933). Further cytological studies of heterothallism in *Puccinia graminis. J. Agric. Res.* (*Washington, D.C.*) **47**, 1–16.

Bennett, J., and Scott, K. J. (1971). Inorganic polyphosphates in the wheat stem rust fungus and in rust-infected wheat leaves. *Physiol. Plant Pathol.* **1,** 185–198.

Berridge, M. J., and Oschman, J. L. (1972). "Transporting Epithelia." Academic Press, New York.

Bracker, C. E., and Littlefield, L. J. (1973). Structural concepts of host-pathogen interfaces. *In* "Fungal Pathogenicity and the Plant's Response" (R. J. W. Byrde and C. V. Cutting, eds.), pp. 159–318. Academic Press, New York.

Bushnell, W. R. (1972). Physiology of fungal haustoria. *Annu. Rev. Phytopathol.* **10**, 151–176.

Chong, J. (1981). Ontogeny of mono- and dikaryotic haustoria of *Puccinia coronata avenae:* Ultrastructure, cytochemistry and electron-probe X-ray analysis. Doctoral Thesis, University of Manitoba.

Chong, J., and Harder, D. E. (1980). Ultrastructure of haustorium development in *Puccinia coronata avenae.* I. Cytochemistry and electron probe X-ray analysis of the haustorial neck ring. *Can. J. Bot.* **58**, 2496–2505.

Chong, J., and Harder, D. E. (1982a). Ultrastructure of haustorium development in *Puccinia coronata* f. sp. *avenae:* cytochemistry and energy dispersive X-ray analysis of the haustorial mother cells. *Phytopathology* **72**, 1518–1526.

Chong, J., and Harder, D. E. (1982b). Ultrastructure of haustorium development in *Puccinia coronata avenae:* some host responses. *Phytopathology* 72, 1527–1533.

Chong, J., Harder, D. E., and Rohringer, R. (1981). Ontogeny of mono- and dikaryotic rust haustoria: Cytochemical and ultrastructural studies. *Phytopathology* **71**, 975–983.

Coffey, M. D., Palevitz, B. A., and Allen, P. J. (1972). The fine structure of two rust fungi, *Puccinia helianthi* and *Melampsora lini. Can. J. Bot.* **50**, 231–240.

De Bary, A. (1863). Recherches sur le développement de quelques champignons parasites. *Ann. Sci. Nat, Bot. Biol. Rev.* (4) **20**, 5–148.

Ehrlich, H. G., and Ehrlich, M. A. (1963). Electron microscopy of the host-parasite relationships in stem rust of wheat. *Am. J. Bot.* **50**, 123–130.

Ehrlich, M. A., and Ehrlich, H. G. (1970). Electron microscope radioautography of ^{14}C transfer from rust uredospores to wheat host cells. *Phytopathology* **60**, 1850–1851.

Ehrlich, M. A., and Ehrlich, H. G. (1971). Fine structures of the host-parasite interfaces in mycoparasitism. *Annu. Rev. Phytopathol.* **9,** 155–184.

Ehrlich, M. A., Schafer, J. F., and Ehrlich, H. G. (1968). Lomasomes in wheat leaves infected by *Puccinia graminis* and *P. recondita. Can. J. Bot.* **46**, 1020–1027.

Favoli, M. A., and Marte, M. (1973). Electron microscope-autoradiography of rust-affected bean leaves labelled with tritiated glycine. *Phytopathol. Z.* **76**, 343–347.

Gold, R. E. (1983). Fungal cytology and host-pathogen relations: activation and pattern of teliospore germination in *Uromyces appendiculatus* and basidiospore infection of *Phaseolus vulgaris.* Doctoral Thesis, Universität Konstanz, Federal Republic of Germany.

Gold, R. E., Littlefield, L. J., and Statler, G. D. (1979). Ultrastructure of the pycnial and aecial stages of *Puccinia recondita. Can. J. Bot.* **57**, 74–86.

Gunning, B. E. S. (1977). Transfer cells and their roles in transport of solutes in plants. *Sci. Prog.* (*Oxford*) **64**, 539–568.

Harder, D. E. (1978). Comparative ultrastructure of the haustoria in uredial and pycnial infections of *Puccinia coronata avenae. Can. J. Bot.* **56**, 214–224.

Harder, D. E., and Mendgen, K. (1982). Filipin-sterol complexes in bean rust- and oat crown rust-fungal/plant interactions: Freeze-etch electron microscopy. *Protoplasma* **112**, 46–54.

Harder, D. E., Rohringer, R., Samborski, D. J., Kim, W. K., and Chong, J. (1978). Electron microscopy of susceptible and resistant near isogenic (*sr*6/*Sr*6) lines of wheat infected by *Puccinia graminis tritici.* I. The host pathogen interface in the compatible (*sr6/P6*) interaction. *Can. J. Bot.* **56**, 2955–2966.

Heath, M. C. (1971). Haustorial sheath formation in cowpea leaves immune to rust infection. *Phytopathology* **61**, 383–388.

Heath, M. C. (1974). Light and electron microscope studies of the interactions of host and nonhost plants with cowpea rust-*Uromyces phaseoli* var. *vignae. Physiol. Plant Pathol.* **4,** 403–414.

Heath, M. C. (1976). Ultrastructural and functional similarity of the haustorial neckband of rust fungi and the Casparian strip of vasclar plants. *Can. J. Bot.* **54**, 2484–2489.

Heath, M. C, and Bonde, M. R. (1983). Ultrastructural observations of the rust fungus *Physopella zeae* in *Zea mays. Can. J. Bot.* **61**, 2231–2242.

Heath, M. C, and Heath, I. B. (1971). Ultrastructure of an immune and a susceptible reaction of cowpea leaves to rust infection. *Physiol. Plant Pathol.* **1,** 277–287.

Heath, M. C, and Heath, I. B. (1975). Ultrastructural changes associated with the haustorial mother cell during haustorium formation in *Uromyces phaseoli* var. *vignae. Protoplasma* **84**, 297–314.

Heath, M. C, and Heath, I. B. (1978). Structural studies of the development of infection structures of cowpea rust, *Uromyces phaseoli* var. *vignae.* I. Nucleoli and nuclei. *Can. J. Bot.* **56,** 648–661.

Horisberger, M., and Rosset, J. (1977). Colloidal gold, a useful marker for transmission and scanning electron microscopy. *J. Histochem. Cytochem.* **25,** 295–305.

Horisberger, M., and Vonlanthan, M. (1977). Location of mannan and chitin on thin sections of budding yeasts with gold markers. *Arch. Microbiol.* **115,** 1–7.

Littlefield, L. J., and Bracker, C. E. (1972). Ultrastructural specialization of the host-pathogen interface in rust-infected flax. *Protoplasma* **74**, 271–305.

Littlefield, L. J., and Heath, M. C. (1979). "Ultrastructure of the Rust Fungi." Academic Press, New York.

Manocha, M. S. (1975). Autoradiography and fine structure of host-parasite interface in temperature-sensitive combinations of wheat stem rust. *Phytopathol. Z.* **82**, 207–215.

Mendgen, K. (1977). Reduced lysine uptake by bean rust haustoria in a resistant reaction. *Naturwissenschaften* **64**, 438.

Mendgen, K. (1979). Microautoradiographic studies on host-parasite interactions. II. The exchange of 3H-lysine between *Uromyces phaseoli* and *Phaseolus vulgaris. Arch. Microbiol.* **123,** 129–135.

Mendgen, K. (1982). Differential recognition of the outer and inner walls of epidermal cells by a rust fungus. *Naturwisen schaften* **69**, 502–503.

Mendgen, K., and Heitefuss, R. (1975). Micro-autoradiographic studies on host-parasite interactions. I. The infection of *Phaseolus vulgaris* with tritium labelled urediospores of *Uromyces phaseoli. Arch. Microbiol.* **105,** 193–199.

Morré, D. J., and Mollenhauer, H. H. (1974). The endomembrane concept: A functional integration of endoplasmic reticulum and Golgi apparatus. *In* "Dynamic Aspects of Plant Ultrastructure" (A. W. Robards, ed.), pp. 84–137. McGraw-Hill, New York.

Onoe, T., Tani, T., and Naito, N. (1973). The uptake of labeled nucleosides by *Puccinia coronata* grown on susceptible leaves. *Rep. Tottori Mycol. Inst.* **10,** 303–312.

Pady, S. M. (1935). The role of intracellular mycelium in systemic infections of *Rubus* with the orange rust. *Mycologia* **27**, 618–637.

Reynolds, B. A. (1975). Cytology and ultrastructure of brown rust in barley cultivars of varied resistance. Doctoral Thesis, University of London.

Rice, M. A. (1927). The haustoria of certain rusts and the relation between host and pathogens. *Bull. Torrey Bot. Club* **54**, 63–153.

Rijkenberg, F. H. J. (1975). The uredial stage of maize rust. *Proc.—Electron Microsc. Soc. South Afr.* **5,** 33–40.

Rijkenberg, F. H. J., and Truter, S. J. (1973). Haustoria and intracellular hyphae in the rusts. *Phytopathology* **63**, 281–286.

Rohringer, R., Chong, J., Kim, W. K., and Harder, D. E. (1982). Cytochemistry of the host/parasite interface and the use of gold markers in cereal/rust interactions. *In* "Active Defence Mechanisms in Plants" (R. K. S. Wood, ed.) Plenum, New York.

Rohringer, R., Chong, J., and Harder, D. E. (1984). In preparation.

Roland, J. C, Lembi, C. A., and Morré, D. J. (1972). Phosphotungstic acid-chromic acid as a selective electron-dense stain for plasma membranes of plant cells. *Stain Technol.* **47**, 195–200.

Ruttle, M. L., and Fraser, W. P. (1927). A cytological study of *Puccinia coronata* Cda. on Banner and Cowra 35 oats. *Univ. Calif., Berkeley, Publ. Bot.* **14**, 21–72.

Savile, D. B. O. (1939). Nuclear structure and behavior in species in the Uredinales. *Am. J. Bot.* **26**, 585–609.

Smetana, K., and Busch, H. (1974). The nucleolus and nucleolar DNA. *In* "The Cell Nucleus" (H. Busch, ed.), Vol. 1, pp. 73–147. Academic Press, New York.

Spencer-Phillips, P. T. N., and Gay, J. L. (1981). Domains of ATPase in plasma membranes and transport through infected plant cells. *New Phytol.* **89**, 393–400.

Thiery, J. P. (1967). Mise en évidence des polysaccharides sur coupes fines en microscopie électronique. *J. Microsc.* (*Paris*) **6,** 987–1018.

Tu, J. C, and Hiruki, C. (1971). Electron microscopy of cell wall thickening in local lesions of potato virus-M infected red kidney bean. *Phytopathology* **61**, 862–868.

Van Dyke, C.'G., and Hooker, A. L. (1969). Ultrastructure of host and parasite in interaction of *Zea mays* with *Puccinia sorghi. Phytopathology* **59**, 1934–1946.

Ward, H. M. (1882). On the morphology of *Hemileia vastatrix* Bark. and Br. Q. *J. Microsc. Sci.* **22**, 1–11.

Wheeler, H. (1974). Cell wall and plasmalemma modification in diseased and injured plant tissues. *Can. J. Bot.* **52**, 1005–1009.

Wheeler, H., Baker, B. L., and Hanchey, P. (1972). Pinocytosis in root cap cells exposed to uranyl salts. *Am. J. Bot.* **59**, 858–868.

Willison, J. H. M., and Brown, R. M., Jr. (1979). Pretreatment artifacts in plant cells. *In* "Freeze Fracture: Methods, Artifacts and Interpretations" (J. E. Rash and C. S. Hudson, eds.), pp. 51–57. Raven Press, New York.

Wynn, W. K., and Staples, R. C. (1981). Tropisms of fungi in host recognition. *In* "Plant Disease Control: Resistance and Susceptibility" (R. C. Staples and G. H. Toenniessen, eds.), pp. 45–69. Wiley, New York.

Yudkin, L. Y., and Reiter, B. G. (1979). Electron microscopy study of the haustorial apparatus of the causal agent of brown rust in wheat. *Mikol. Fitopatol.* **12**, 479–483.

15

Structural and Physiological Alterations in Susceptible Host Tissue

W. R. Bushnell

Cereal Rust Laboratory, Agricultural Research Service, U.S. Department of Agriculture, University of Minnesota, St. Paul, Minnesota

I. Introduction

Rust fungi have a biotrophic relationship with their cereal hosts, in which host cells undergo many changes but continue to live while the fungus grows and sporulates. The changes in the host are assumed to benefit the fungus but usually in ways that are not entirely clear. Each rust uredium is the site of intense metabolic activities of both host and parasite. Changes in the host tend to be obscured by activities or substances in the fungus. Nevertheless, through diverse experimental approaches, many changes in the host have been described, and some are beginning to be understood.

The cereal host seems to go through two distinct responses to rust infection: (1) an initial juvenile, antisenescence response in which host cells are kept physiologically young and (2) an autolytic response in which cytoplasmic organelles slowly disappear and cells become highly senescent. This chapter will describe the principal structural and physiological changes associated with these responses.

II. Structural Changes in Rusted Host Tissues

A. THE JUVENILE HOST RESPONSE

For the first 4–6 days after inoculation, colonized host tissues at each infection site are maintained in a juvenile condition in which normal leaf senescence is retarded. This is directly visible if senescence is accelerated by detaching or shading infected leaves. As non-colonized tissues become yellow, the colonized tissue remains green, producing a "green island" at each infection site (Bushnell, 1967; Dekhuijzen, 1976; Durbin, Chapter 16, this volume; Ruttle and Frazer, 1927). As the fungus starts to sporulate, the central tissues, in contrast, may become chlorotic, leaving a green ring. The green island sometimes associated with infection type 2 in stem rust (Allen, 1926; Stakman *et al.*, 1962) is a special case in which the island becomes visible because peripheral yellowing is promoted by incompatibility.

Host cell cytoplasm in the juvenile response generally resembles cytoplasm of young healthy leaves. This is most evident in the vicinity of haustoria within infected cells. An extensive network of endoplasmic reticulum (ER) develops (Ehrlich and Ehrlich, 1971b), which sometimes touches the extrahaustorial membrane, and unique tubular complexes, thought to be synthetic or secretory structures related to requirements of the fungus, may be present in host cytoplasm (Harder and Chong, Chapter 14, this volume). Golgi bodies increase in number (Ehrlich and Ehrlich, 1971a; Shaw and Manocha, 1965b; Mares,

1979; Reiter *et al.,* 1976; Van Dyke and Hooker, 1969), and the volume of cytoplasm seems to increase (Ehrlich and Ehrlich, 1971a; Mares, 1979; Shaw and Manocha, 1965b). These changes may be related to migration of cytoplasm and organelles to the haustorium instead of synthesis of new structures. J. L. Gay (personal communication) noted that the abundant ER and organelles near haustoria may be normal for cytoplasm near nuclei where haustoria are located.

The rust haustorium is universally found in contact with the host nucleus in cereal or grass hosts (Allen, 1923, 1926; Hilu, 1965; Mares, 1979; Ruttle and Frazer, 1927; Van Dyke and Hooker, 1969). Sometimes the nucleus and haustorium are partially enfolded with one another. Why the nucleus migrates to the haustorium and remains there is not known.

Rust infection of cereal cells generally stimulates a marked increase in volume of the host nucleus (Allen, 1923; Bhattacharya and Shaw, 1967; Hilu, 1965; Ruttle and Frazer, 1927; Whitney *et al.,* 1962). The increase begins in the juvenile host response stage, but extends into the beginnings of the autolytic, chlorotic stage. Thus Allen (1923) showed that host nuclear volume doubled with wheat stem rust by 7 to 10 days after inoculation. The increase in volume is greatest at the colony center, tapering to little change at the margin of the colony (Whitney *et al.,* 1962). The increase in nuclear volume is accompanied by an increase in volume of the host nucleolus with wheat stem rust (Bhattacharya *et al.,* 1968; Whitney *et al.,* 1962) and by a shift from several nucleoli per nucleus to a single nucleolus in a corn rust (Hilu, 1965). For wheat leaf rust, Allen (1926) noted that host nuclei did not increase in volume but that they elongated, producing a narrow, tapering lobe that frequently came in contact with the haustorium.

The period in which the host nucleus and nucleolus usually enlarge coincides with increased synthesis of nucleolar and extranucleolar RNA, and of cytoplasmic protein in host cells (see Section IV,B and C). Apparently, the enlarged nucleoli and nuclei produce increased amounts of ribosomes and messenger RNA (mRNA) that are used for protein synthesis in the cytoplasm. As noted, these changes are not strictly associated with the juvenile host response and instead may provide enzymes active in the autolytic stage to follow.

B. THE AUTOLYTIC HOST RESPONSE

By 10 days after infection, the cytoplasmic organelles of host cells at pustule centers begin to degenerate and eventually disappear in what seems to be a slow autolytic digestion process. The ER becomes less abundant, breaking into vesicles, and mitochondria lose their inner membranes (cristae), swell, and become vesiculate (Shaw and Manocha, 1965b). Mitochondria may gain small electron-dense inclusions (Reiter *et al.,*1976). With wheat stem rust, the volume of host chloroplasts is usually reduced (Allen, 1923; Whitney *et al.,* 1962),

although chloroplasts sometimes appear swollen (Ehrlich and Ehrlich, 1971a). Volume of chloroplast stroma can increase (Reiter *et al.*, 1976). The chloroplast lamellae may become less compacted as grana structure becomes disorganized. The outer chloroplast membrane can become vesiculate (Shaw and Manocha, 1965b) and eventually rupture (Ehrlich and Ehrlich, 1971a). Leaf rusts of wheat and barley cause a less detrimental response in which chloroplasts may not shrink (Allen, 1926) or show signs of structural degeneration other than loss of chlorophyll (Calonge, 1967). Electron-dense materials have been seen in chloroplasts with wheat leaf rust (Reiter *et al.*, 1976) and stripe rust (Mares, 1979).

As part of the autolytic stage, the host nucleus shrinks rapidly. With wheat stem rust, Allen (1923) showed a decrease in nuclear width and volume by 14 days after inoculation. This occurred about 4 days after the chloroplasts began to shrink. With wheat leaf rust, Reiter *et al.* (1976) found a reduction in host cell nuclear volume as chromatin within the nucleus condensed.

As autolysis progresses, most host cells remain alive and turgid for as long as 3 to 4 weeks after inoculation. Vacuoles can form that contain residues of degenerated organelles, membranes, and electrondense bodies (Reiter *et al.*, 1976). In advanced stages of autolysis, virtually the only host cell contents remaining are the host nucleus and the fungal haustorium (Hilu, 1965), as the cytoplasmic layer lining the cell wall becomes highly attenuated (Mares, 1979).

The autolytic host response resembles normal senescence of cereals and other plants (Shaw and Manocha, 1965b; Stoddart, 1981). The sporulating fungus seemingly accelerates the senescence of the host tissues; the result is digestion of host constituents, which are then probably utilized largely by the sporulating fungus.

C. THE PHYSICAL PRESENCE OF THE FUNGUS

Rust mycelium develops so abundantly in the intercellular spaces of the cereal leaf that its physical presence could be a factor in changes induced within host cells. As much as half the volume of spongy mesophyll can be intercellular space. Allen (1923) described a zone perhaps 1 mm in diameter at the center of young wheat stem rust pustules in which "… each chink and cranny of intercellular space becomes filled with the fungus, which forms little masses of pseudoparenchyma conforming closely to the shape of the irregular passages they occupy." In older pustules, "some of the host cells are crowded out of shape and almost obliterated, but in many cases are still living." Similar fungal development has been described for a corn rust (Hilu, 1965) and oat crown rust (Ruttle and Frazer, 1927). Host cells are especially likely to be deformed or crushed in the layer of mesophyll immediately below the epidermis.

The engulfed host cells are thus subject to damage by the physical presence of the growing fungus and also by any metabolites that might leak or be secreted from the fungus. The gaseous environment around host cells is undoubtedly changed. The shift from the juvenile to the autolytic host response occurs at the onset of sporulation when hyphae begin to rupture the epidermis, separating it from the underlying fungal–host complex. Still, experimental evidence is lacking to show how the physical forces exerted by the fungus affect host cells.

D. CHANGES IN HOST MEMBRANES

During the juvenile response, host cells probably retain a full capacity for active uptake of metabolites. Later, as disease progresses into the autolytic stage, host tissues become leaky and readily lose ions, sugars, amino acids, and probably other substances if the tissues are immersed in water (Hoppe and Heitefuss, 1974a). Such leakiness is a characteristic of uninfected, naturally senescing tissues as enzymes located on membranes lose activity (Stoddart, 1981). Thatcher (1942) showed for wheat stem rust that the host cell plasmalemma has increased permeability to nonelectrolytes, which are now thought to enter cells through the phospholipid portions of the membrane. Some evidence for changes in phospholipid components of host membranes in bean rust was obtained by Hoppe and Heitefuss (1974b). Elnaghy and Heitefuss (1976) implicated the germination self-inhibitor of bean rust urediospores (methyl-3,4-dimethoxycinnamate) as a possible cause of the change in host membranes. The importance of membrane alterations to the movement of nutrients from host to parasite is discussed further by Durbin (Chapter 16, this volume).

E. DIFFERENCES IN HOST RESPONSE AMONG CEREAL RUSTS

The sequence of qualitative changes in host tissue is similar among the cereal rusts, but stem rusts induce more pronounced host changes than do leaf rusts. Allen (1923) provided a clear example in wheat. The host cell in which the first haustorium was produced at each infection site was killed with stem rust, not leaf rust. Also, the changes in host nuclei and chloroplasts were greater with stem rust than leaf rust. Furthermore, the stem rust fungus produced a massive concentration of cells at pustule centers with vigorous runner hyphae at colony borders, whereas the leaf rust fungus grew less and lacked runners. Thus physiological changes may be correspondingly greater in stem rusts than in leaf rusts.

III. Hormonal Changes in Rusted Host Tissues

A. OVERVIEW OF GROWTH HORMONES IN RUSTS

Plant growth hormones generally increase greatly in rusted tissues. These hormones could be produced by the host, the fungus, or both. Most types of hormones have been found in rust spores or mycelium, and the quantities of hormones tend to parallel growth of the fungus, often peaking at the time of sporulation. This suggests that the hormones in rusted tissues are produced by the fungus and that they may have a role in fungal growth and sporulation, although the hormones could be produced by the host and transferred to the fungus in some cases. In addition to effects on the fungus, growth hormones may induce some or all of the host responses described in Section II. The somewhat sparse data suggest that rust fungi modify infected tissues either by producing the hormones themselves or by changing the local concentrations of host-produced hormones.

B. CYTOKININS

Cytokinins are implicated as inducers of the juvenile host response to rust infection. If a cytokinin solution is applied as a drop to the surface of a detached leaf, tissues near the site of application remain green as the rest of the leaf rapidly senesces as a result of detachment. The green zones are metabolically active and closely resemble the green islands produced at rust pustules on detached leaves as described in Section II,A (Bushnell, 1967). The cytokinin-induced green zone acts as a sink for substances translocated from the yellow, senescing parts of the detached leaf as do rust-induced green islands (Durbin, Chapter 16, this volume). Furthermore, Shaw and Manocha (1965a,b) concluded from ultrastructural comparisons that cytokinin-treated tissues closely resemble tissues infected with *P. graminis* f. sp. *tritici.* Cytokinins seem to delay leaf senescence by maintaining protein synthesis (Stoddart, 1981) through mechanisms that are not understood but that possibly act through effects on cell membranes (Durbin, Chapter 16, this volume).

Extracts from rusted leaves of bean (Dekhuijzen and Staples, 1968; Király *et al.,* 1967) and wheat (Sziráki *et al.,* 1976) have had more activity in cytokinin bioassays than have extracts from healthy leaves. Increases were principally due to a single chromatographic fraction in each case. In bean, this fraction chromatographically resembled cytokinin from the host and was unlike cytokinin from rust mycelium, implicating the host as the source of the increased cytokinin. The increases in amount of cytokinins, and the similarities in effect of rust and cytokinin, provide considerable evidence that cytokinins contribute to maintenance of the juvenile state in rusted tissues.

Nevertheless, the evidence that cytokinins control the juvenile state is incomplete, especially in cereal rusts. Extracts from spores of P. *graminis* f. sp. *tritici* and *avenae, P. coronata,* and P. *recondita* have all produced green zones in detached cereal leaves (Bushnell, 1967; Johnson *et al.,* 1966), but the green zones were not sinks for translocation of ^{32}P as expected. Several substances other than cytokinins can delay leaf senescence (Bushnell, 1967; Dekhuijzen, 1976; Durbin, Chapter 16, this volume; Stoddart, 1981); one or more of these could have a role in rust-induced senescence delay.

C. Auxins

Auxins generally increase in rusted tissues, particularly in the rusts that produce galls and overgrowths of host tissue (Pegg, 1976a). In rusted cereal tissues, auxin apparently also increases, especially at sporulation, but the pattern of increase is incompletely documented. The frequently cited data of Shaw and Hawkins (1958) include only one value for a compatible host–parasite combination, showing a 20-fold increase in free indoleacetic acid (IAA) 10 days after inoculation. Shaw (1963) cited data of B.I.S. Srivastava showing 10- to 50-fold increases in free IAA with wheat stem rust at sporulation and smaller increases in bound IAA. Artemeko *et al.* (1980) reported a 2.5-fold increase in free IAA with wheat stem rust from 6 to 144 hr after inoculation, whereas esterified IAA did not increase. The surprising initial increase in free IAA was attributed to IAA in the spores used to inoculate the plants.

The increase in IAA in rusted cereal leaves may possibly be a consequence of decreased IAA oxidation by oxidases (decarboxylases) or peroxidases (Daly and Knoche, 1976; Pegg, 1976a). Shaw (1963) listed other possibilities, including synthesis of IAA by the rust fungus. Indoleacetic acid has been found in urediospores of *Puccinia graminis* (Umnov *et al.,* 1978).

What is the role of IAA in rusted tissues? It may cause a small part of the respiratory increase thought to occur in host tissues (Section IV,F). It probably acts in concert with other growth hormones, especially cytokinin, to control the metabolic state of the host cell in the juvenile stage of host response. In addition, auxin may have a direct function in growth and sporulation of the rust fungus.

D. ETHYLENE

Emission of ethylene from wheat leaves can increase 10-fold or more as a consequence of rust (Daly *et al.,* 1971), with the highest rates at the start of sporulation. The large increases in emitted ethylene in rusted tissues are difficult to evaluate because the amounts within leaves can be large compared to amounts released (Pegg, 1976b). Chigrin *et al.* (1978) found increases in

emitted ethylene with wheat stem rust to be small compared to the large, fluctuating amounts within leaves.

Ethylene is commonly emitted from injured or diseased plant tissues in which cells are killed (Williamson, 1950). Ethylene is sometimes emitted in bursts before or during expression of hypersensitive cell death in rusts (Chigrin *et al.*, 1978; Montalbini and Elstner, 1977). In compatible hosts, ethylene emission might relate to cell injury when the epidermis is ruptured at the start of sporulation. However, the ethylene is unaccompanied by ethane, a usual sign of injury (Montalbini and Elstner, 1977).

If wheat leaves are exposed to ethylene, peroxidase activity of the leaves increases. Nevertheless, the peroxidase content of rusted, susceptible leaves is low, despite high rates of ethylene emission (Daly *et al.*,1971).

Could ethylene have a controlling role in the autolytic response of susceptible hosts? Premature senescence and yellowing are among the many physiological effects of ethylene (Archer and Hislop, 1975), and ethylene is emitted rapidly during the initial stages of chlorophyll loss in senescing leaves (Stoddart, 1981). The largest increases in ethylene in rusted leaves occur as the autolytic stage begins at pustule centers. Heath (1974) cautioned that the changes in chloroplasts apparently triggered by ethylene in cowpea rust are more like changes in ripening fruit than in senescent leaves. Stoddart (1981) attributed some ethylene-induced changes to injury instead of senescence. In any case, the role of ethylene deserves further investigation.

E. ABSCISIC ACID

Abscisic acid has been shown to increase in wheat stem rust (Chigrin *et al.*, 1981). Abscisic acid has been postulated to have an indirect role in promoting senescence in healthy leaves and can promote premature yellowing of detached leaves (Stoddart, 1981). Along with ethylene, abscisic acid deserves investigation as a possible cause of the yellowing and autolysis of rusted tissues.

IV. Metabolic Changes in Rusted Host Tissues

A. THE HETEROGENEOUS RUSTED LEAF

The typical rusted leaf sampled for physiological purposes contains many uredia that may coalesce as they enlarge. With time, an increasing proportion of the leaf comes under the influence of the rust. Additional heterogeneity results from changes within each uredium. Tissues at uredial centers are usually at a more advanced stage of response than tissues at the edges. At certain times

after infection, host tissues may be briefly homogeneous as zones of influence coalesce, but this homogeneity is difficult to achieve reproducibly. [Patterns of starch deposition can be useful for this purpose (Bushnell, 1967).] Because of this heterogeneity, results obtained at different infection densities are often inconsistent.

The growing fungus contributes to heterogeneity of the rusted leaf. Unfortunately, no entirely satisfactory way has been found to measure the amount of rust fungus present in infected leaves (Rohringer and Heitefuss, Chapter 7, and Rowell, Chapter 10, this volume). This is one reason estimates are poor for the relative contributions of host and parasite to an activity or substance common to both.

Changes in weight present another obstacle to interpreting results from rusted leaves. Dry weight per unit leaf area increases 20–100% in cereal rusts (Johnson *et al.*, 1968; Owera *et al.*, 1981; Quick and Shaw, 1964; Shaw and Colotelo, 1961; Samborski and Shaw, 1956). Fresh weight can decrease abruptly in advanced stages of infection. Consequently, data reported on a weight basis are difficult to interpret if weight per unit area is not given.

Finally, the natural senescence of cereal leaves complicates interpretation of changes reported for rusts. As soon as the leaves are fully grown they begin to senesce, slowly losing protein and many metabolic components. Because of this, rusted tissues may have more of a substance than do nonrusted tissues at a given sampling time, because the loss was retarded and not because of an actual gain. To distinguish between the two possibilities, samples must be taken near the time of inoculation and periodically thereafter.

B. NUCLEIC ACIDS

How nucleic acids (and proteins) in rusted tissues relate to whether host and parasite will be compatible or incompatible is treated by Rohringer and Heitefuss (Chapter 7, this volume). Here we focus on compatible host-parasite combinations and the series of changes that occur within the host as disease progresses.

1. RNA

As part of the juvenile host response before the fungus sporulates as postulated in Section II,A, host cells should maintain or possibly increase their ability to synthesize protein. They should maintain the machinery needed for DNA-dependent transcription of mRNA and production of ribosomes. Protein synthesis occurs in cytoplasm, synthesis of ribosomes occurs in nucleoli, and synthesis of mRNA occurs in the extranucleolar portion of nuclei.

Enhanced metabolic activity in both the nucleolar and extranucleolar portions of host nuclei has been demonstrated cytologically for wheat stem rust

by M. Shaw and co-workers. As the volumes of host cell nuclei and nucleoli increase (see Section II,A), the amount of both nucleolar and extranucleolar RNA doubles (Bhattacharya *et al.*, 1965; Whitney *et al.*, 1962). Incorporation of radioactively labeled uridine and cytidine (precursors of RNA) into nuclei was doubled (Bhattacharya and Shaw, 1967), as was incorporation of leucine into nuclear protein (Bhattacharya and Shaw, 1967). Heitefuss (1970) showed that actinomycin D inhibited the incorporation of labeled uridine, indicating that incorporation depended on transcription. Furthermore, the diffuse interchromatin network of the nucleus, where transcription occurs, increased in electron density (Manocha and Shaw, 1966). Finally, the amount of histone within the nucleus decreased, and apparently also the incorporation of amino acids into histone (Bhattacharya *et al.*, 1965, 1968). Histones are thought to repress transcription non-specifically (Rohringer and Heitefuss, Chapter 7, this volume). Together, these cytological studies indicate that transcriptional activities increase in host nuclei as a consequence of rust.

The incorporation of ^{32}P into total RNA of rusted leaves increased two- to five-fold 3 to 6 days after inoculation in wheat stem rust (Dmitrieva and Zhukov, 1971; Rohringer and Heitefuss, 1961) and oat crown rust (Tani *et al.*, 1970), probably reflecting synthesis of RNA by both host and parasite. However, the total amount of RNA either increases modestly or not at all, even though the fungus is growing and probably synthesizing RNA. This suggests that the total amount of RNA in the host declines.

Trends in total RNA from the time of inoculation indicate that the loss of RNA that normally occurs in uninfected leaves is retarded by disease, resulting in 20 to 40% higher amounts in rusted than non-rusted tissues (Heitefuss, 1964; Johnson *et al.*, 1967; Quick and Shaw, 1964; Tani *et al.*, 1970). Fractions of RNA such as rRNA also tend to show retarded loss instead of actual gains, whereas chloroplast rRNA clearly declined with oat crown rust (Tani *et al.*, 1973a). Because amounts of RNA decline in the host while rates of RNA synthesis are enhanced (as described earlier), it follows that the rate of RNA degradation is increased.

2. RNase

In line with the probable enhancement of RNA degradation, the activity of RNase increases in rusted tissues (Rohringer and Heitefuss, Chapter 7, this volume). With wheat stem rust, RNase activity doubles at 1 to 4 days after inoculation [which could be an artifact of handling at inoculation (Nielsen and Rohringer, 1963)] and later peaks again at about 6 days at levels two to five times those of uninfected leaves (Chakravorty *et al.*,1974; Sachse *et al.*, 1971). Apparently, the new RNase is the type found in uninfected leaves and not a fungal type (Rohringer and Heitefuss, Chapter 7, this volume). Furthermore, the RNase in rusted flax was of the type that degrades RNA and not of the type

involved in posttranscriptional processing of RNA (Sutton and Shaw, 1982). The large amount of RNase activity in cereal rusts is probably involved in rapid RNA turnover.

3. DNA

Because cereal host cells do not enlarge or divide in rusted tissues, no increase in the amount of host DNA is expected; indeed, the amount of DNA in host nuclei as measured microspectrophotometrically does not change with wheat stem rust until 9 days after inoculation when a slow decline begins (Bhattacharya *et al., 1965,* 1968). Total DNA of host and parasite combined tends to remain constant (Heitefuss and Wolf, 1976; Quick and Shaw, 1964; Tani *et al.,* 1970), because it has usually been expressed on a dry-weight basis, and because the amount of nuclear host DNA eventually declines as new fungal DNA is produced. In wheat stem rust, the rate of ^{32}P incorporation into a DNA fraction increased (Heitefuss, 1965, 1966), probably a result of fungal DNA synthesis. Measurable DNase activity also increased (Heitefuss and Wolf, 1976), in line with DNA degradation in the host, at least late in pustule development.

C. PROTEINS

The total amount of protein in rusted cereal tissues sometimes increases 20–50% on a fresh-weight basis, paralleling increases in dry weight, at least in the first few days after inoculation (Quick and Shaw, 1964; Shaw and Colotelo, 1961). More frequently, the total protein of host and parasite either remains fairly constant (Johnson *et al.,* 1968; Samborski *et al.,* 1961) or declines (Gassner and Franke, 1938). Much of the total protein can be assumed to be in the developing fungus, especially at sporulation and thereafter. Probably little protein is left in the highly autolyzed host cell described in Section II,B.

Although total host protein declines, the evidence tor accelerated RNA metabolism suggests that synthesis of some host proteins might be enhanced by rust infection, especially before sporulation. Surprisingly, there is little evidence showing what preexisting kinds of proteins have increased rates of synthesis or if new kinds are synthesized. With wheat stem rust, Fric and Heitefuss (1970) could not detect new kinds of host protein 5 days after inoculation using immunochemical and electrophoretic methods. New proteins were judged to be of fungal origin. With flax rust, von Broembsen and Hadwiger (1972) could find no change or only slight decreases in incorporation of radioactively labeled leucine into soluble protein in two compatible host-parasite combinations 6–18 hr after inoculation. In contrast, incorporation into several protein fractions was increased in incompatible combinations. Similar results were obtained by Tani and Yamamoto (1979) with oat crown rust 10–24

hr after inoculation. Blasticidin S, an inhibitor of protein synthesis, had no effect on crown rust development in a compatible combination, evidence that protein synthesis in the host was not required for the early stages of fungus growth. In samples taken after sporulation in wheat stem rust, Wrigley and Webster (1966) found reduced amounts of two protein peaks as detected on polyacrylamide gels, one of which was thought to be largely ribulose-l,5-bisphosphate carboxylase, an important enzyme of photosynthesis and photorespiration (Section IV,E). Using similar methods, Staples and Stahmann (1964) found a decrease in an unidentified host protein in bean rust.

As rust develops in host tissues, several new isozymes can be detected on polyacrylamide gels. In most cases, these appear to be of fungal origin (Johnson *et al.,* 1968; Staples, 1965; Staples and Stahmann, 1964). The amount of a host isozyme may change as with acid phosphatase in bean rust (Williams and Staples, 1964; Staples and Stahmann, 1964), but most of the work with isozymes has been qualititative and does not clearly indicate quantitative changes. However, Sadler and Shaw (1979a) showed a change in a host glutamate dehydrogenase in flax rust at 1 and 7 days after inoculation. Although its molecular weight was apparently unchanged, the new form of the enzyme was distinct in degree of inhibition by ATP or pyridoxal phosphate, suggesting to Sadler and Shaw that the protein molecule had changed conformation or that subunits of the enzyme had been rearranged. Whether the enzyme had been modified during or after synthesis was not established.

Protein synthesized in cell-free translation systems using template from mRNA, chromatin, or polysomes from leaves have differed in kind and amount as a result of infection with oat crown or wheat stem rust (Chakravorty, 1982; Pure *et al.,* 1979; Tani *et al.,* 1973b). Such experiments are described by Rohringer and Heitefuss (Chapter 7, this volume). The results suggest that changes preceding translation, either before or after mRNA is produced by transcription, lead to changes in the proteins that are synthesized by the host. Although cell-free translation techniques have great potential, the results with rusts are still of a preliminary nature, and the new proteins are yet to be identified.

It seems that the juvenile host response is not accompanied by large qualitative or quantitative changes in host proteins. We know at least that host proteins are changed less in compatible than incompatible host–parasite combinations the first day after inoculation. How host proteins are changed at the beginning of the autolytic stage is unclear. As indicated in Section II,A, perhaps increased synthesis of host mRNA leads to synthesis of enzymes involved in the autolytic degeneration of cytoplasmic components of the host.

D. AMINO ACIDS AND AMIDES

Changes in rusted tissues seem to assure that generous amounts of amino acids and amides are available for nutrition of the fungus. During the juvenile host responses, these substances are probably synthesizedlocally from photosynthates and ammonia, and also translocated from tissues distant from the infection site (Durbin, Chapter 16, this volume). Later in the autolytic stage, significant amounts of amino acids and amides probably also come from local degradation of protein.

Soluble nitrogen compounds (mostly amino acids and amides) can increase threefold in rusted tissues with wheat stem rust, especially in the first 3–6 days after inoculation (Shaw and Colotelo, 1961; Samborski *et al.,* 1961). Gassner and Franke (1938) found little or no increase, but they showed that the decline in soluble nitrogen as leaves aged was not as rapid in rusted as in nonrusted leaves. Glutamine generally increases in rusted tissues, and at least two investigators have reported increases for each of the following amino acids or amides: asparagine, arginine, phenylalanine, leucine or isoleucine, and valine (Farkas and Király, 1961; Rohringer, 1957; Shaw and Colotelo, 1961; Siebert, 1961). Tryptophan increased four- to fivefold when measured by procedures to conserve it during extraction (Kim and Rohringer, 1969). Ammonia also has accumulated in significant amounts (Farkas and Király, 1961; Siebert, 1961). Several amino acids have been reported to increase as early as 2 days after inoculation.

What amino acids or amides does the rust fungus require? In axenic culture, *Puccinia graminis* requires nitrogen in a reduced form as its principal source of nitrogen. Ammonia, aspartic acid, or glutamine can meet this need (Maclean, 1982; Mendgen, 1981). In addition, sulfur must be supplied in reduced form as cysteine, cystine, glutathione, or—with well-established cultures—methionine (Maclean, 1982). Rust fungi apparently do not have absolute amino acid requirements beyond these. They can synthesize several amino acids from glucose, either on artificial media (Maclean, 1982) or when growing as parasites (Mitchell and Shaw, 1968; Pfeiffer *et al.,* 1969; Reisener *et al.,* 1970). Despite those indications of minimal amino acid requirements, other evidence suggests that rust fungi take up and utilize many diverse amino acids from their hosts. Reisener and co-workers (Jäger and Reisener, 1969; Reisener and Ziegler, 1970) showed that *P. graminis* takes up arginine, glutamic acid, lysine, and tyrosine from wheat leaf tissue. Furthermore, rust fungi grow best on artificial media containing rich mixtures of amino acids, for example, certain peptones, casein hydrolysates, or a mixture resembling the amino acids of wheat leaves (Maclean, 1982; Mendgen, 1981). With the reservation that nutritional requirements may differ between artificial culture and leaf culture, rust fungi probably grow and sporulate at maximum

rates in host tissues when amino acids and amides of many kinds are present in abundant supply.

Glutamine is probably the most important of the amino acids or amides utilized by rust fungi in host tissues. As noted earlier, it accumulates consistently in rusted tissues. It is readily translocated from place to place within plants and, along with ammonia and asparagine, can be a major product of proteolysis (Lea and Miflin, 1980). Furthermore, glutamine can be the favored source of bulk nitrogen for axenic cultures of *P. graminis*(Maclean, 1982). Glutamine is a precursor for the synthesis of fungal chitin (Farkas and Király, 1961; Raggi, 1974).

Synthesis of several amino acids in higher plants is linked to photosynthesis and photorespiration. For example, glycine and serine are produced directly in the pathway for photorespiration (Tolbert, 1980). Raggi (1975) concluded from $^{14}CO_2$ incorporation into amino acids in rusted bean that decreases in amounts of glycine and serine probably relate to a decrease in photorespiration. Likewise, a decline in amount of alanine was linked to a decline in photosynthesis. In flax rust, Sadler and Shaw (1979b) showed that ammonia is assimilated via the glutamate synthase cycle, which requires reduced ferredoxin supplied by photosynthesis. These examples show that the amounts of some amino acids can relate to activities of chloroplasts, which, in turn, generally decline as part of the progressive autolysis of rusted hosts (Section IV,E). This does not seem to be detrimental to the fungus, which apparently is not highly dependent on the amino acids derived from chloroplast activities.

E. PHOTOSYNTHESIS AND PHOTORESPIRATION

The rate of net photosynthesis in heavily rusted leaves by 8 to 12 days after inoculation is generally reduced to rates one-third to two-thirds those of corresponding uninfected leaves (Doodson *et al.,* 1965; Livne, 1964; Mitchell, 1979; Owera *et al.,* 1981). To determine the actual (gross) rates of photosynthesis, the rates of both dark and photorespiration must be added to the net rate. Although rust is known to increase dark respiration (Section IV,F), its effect on photorespiration is less certain. Both photorespiration and photosynthesis use many of the same enzymes, most notably ribulose-l,5-bisphosphate carboxylase, which catalyzes the first step in CO_2 fixation in photosynthesis. Thus photorespiration usually declines concomitantly with photosynthesis (Kosuge, 1978), as it did with bean rust at high infection densities (Raggi, 1978), and with powdery mildews of beet and oak (Gordon and Duniway, 1982b; Hewitt and Ayers, 1975). However, this pattern has not been found with cereal rusts. Instead, photorespiration increased 1.5-fold with barley leaf rust (Owera*et al.,* 1981) and remained virtually unchanged with wheat stem rust (Mitchell, 1979), whereas photosynthesis declined in both cases.

Although we cannot safely generalize about rates of photorespiration in cereal rusts, declines in net photosynthesis are always greater than increases in dark and photorespiration combined, so that the rate of gross photosynthesis declines. For example, despite an apparently large increase in dark and photorespiration with barley leaf rust, gross rates of photosynthesis were calculated to be 81–89% of rates in healthy leaves (Owera *et al.*, 1981). Gross rates are probably reduced more than this in most cases.

Net photosynthesis was temporarily stimulated in wheat by stripe rust in the first few days after inoculation (Doodson *et al.*, 1965) at 0.5% CO_2. Similar stimulation occurred with powdery mildew of barley at 0.5% CO_2, but not at 0.04% (Edwards, 1970). The increased photosynthesis at high CO_2 concentration was attributed to impairment of glycolic acid oxidase. However, chloroplasts in rusted tissues may temporarily have an enhanced ability to synthesize proteins used in photosynthesis. The normal decline in rRNA within chloroplasts was temporarily retarded with oat crown rust (Tani *et al.*, 1973a). In addition, a temporary decrease in photorespiration could contribute temporary increases in net photosynthesis, as indicated for powdery mildews of oak and pea (Ayres, 1976; Hewitt and Ayres, 1975).

As disease develops into the stage of definite decline in gross photosynthesis, cytological evidence indicates that chloroplasts become degenerate, especially with wheat stem rust (Section II,B). However, rates of photosynthesis start to decline before structural changes are conspicuous. To learn what limits the process in the early stages of the decline, several aspects of photosynthesis have been investigated.

1. *Resistance to diffusion of CO_2 into the leaf.* Diffusion of CO_2 into the leaf was not an important limiting factor for photosynthesis in barley leaf rust (Owera *et al.*, 1981). Resistance to diffusion decreased as pustules broke the leaf surface.

2. *Amounts of chlorophyll.* The amount of chlorophyll declines in rusted cereal tissues (Calonge, 1967; Doodson *et al.*, 1965; Mitchell, 1979), but the amounts of chlorophyll do not correlate closely with rates of photosynthesis. However, Owera *et al.* (1981) concluded that loss of chlorophyll was a principal limiting factor for photosynthesis with barley leaf rust.

3. *Photosynthesis per unit chlorophyll.* The rate of photosynthesis per unit of chlorophyll declined with wheat stem rust to 70 to 85% of rates in healthy leaves, roughly comparable to the percentage decline in gross photosynthesis (Mitchell, 1979). The amount of chlorophyll also declined, suggesting that both factors contribute to the loss in photosynthetic capacity. In contrast, Owera *et al.* (1981) calculated the rate of photosynthesis per unit of chlorophyll to increase substantially because rates of photorespiration were estimated to be high. This illustrates the importance of quantitating photorespiration in interpreting photosynthesis in rusted leaves.

4. *Amounts of ribulose-1,5-bisphosphate carboxylase.* A protein in rusted wheat judged by Wrigley and Webster (1966) to be ribulose-1,5-bisphosphate carboxylase decreased (as noted in Section IV,C). Loss of this enzyme has also been implicated in the decrease in photosynthesis in powdery mildew of sugar beet (Gordon and Duniway, 1982a).

5. *Photophosphorylation.* Although Wynn (1963) detected no change in photophosphorylation with oat crown rust, Buchanan and coworkers found rates of noncyclic photophosphorylation reduced in host tissues with both broad bean rust and powdery mildew of sugar beets (Magyarosy *et al.,* 1976; Montalbini and Buchanan, 1974). Activity was reduced to 70% of that in uninfected leaves. For powdery mildew, the reduced activity was attributed to a reduction in amount of the cytochromes used for electron transport in noncyclic photophosphorylation (Magyarosy and Malkin, 1978). More work on photophosphorylation in rusted cereals is needed, including comparisons with uninfected senescing leaves.

In summation, the reduction in photosynthetic activity in rusted leaves seems to be due to loss of chlorophyll and to key proteins such as ribulose-l,5-bisphosphate carboxylase or possibly cytochromes. Ribosomal RNA within chloroplasts was reduced in amount with oat crown rust (Tani *et al.,* 1973a), suggesting that synthesis of chloroplast proteins may be generally depressed. Increased protein degradation may also contribute to the loss of protein, especially in the late autolytic stage of disease when decompartmentation probably allows hydrolytic enzymes to reach the chloroplast.

F. RESPIRATION

Respiration as measured in the dark can increase severalfold in rusted cereal leaf tissues. This phenomenon was described thoroughly in the 1950s and 1960s when manometric methods for measuring gas exchange were popular and when respiratory pathways in healthy higher plants were being intensively investigated. For reviews of this era see Allen (1959, 1966), Daly (1976), and Shaw (1963).

1. Combined Respiration of Host and Parasite

Respiratory rates of heavily rusted leaf tissues are usually two to three times the rates of uninfected tissues with wheat stem rust (Antonelli and Daly, 1966; Heitefuss, 1965; Mitchell, 1979; Shaw and Samborski, 1957), wheat leaf rust (Staples, 1957), barley leaf rust (Owera *et al.,* 1981), or wheat stripe rust (MacDonald and Strobel, 1970). Respiratory increases are first detected about 5 days after inoculation. Tissues excised from pustule centers can have rates 10–15 times those of uninfected tissues (Bushnell, 1970; Samborski and Shaw,

1956), reflecting the intense respiratory activity of the compacted, sporulating fungus. The respiratory quotient (ratio of volume of CO_2 released to volume of O_2 used) of rusted tissues is near 1.0, indicating that lipids are not the principal substrate for respiration (Daly, 1976). The ratio of carbons in respired CO_2contributed by C_6 and C_1 from hexose substrates (the C_6:C_1 ratio) declines from about 0.5 in healthy tissue to 0.3 at sporulation in rusted tissue (Antonelli and Daly, 1966; Shaw and Samborski, 1957), suggesting that a part of the enhanced respiration occurs by the oxidative pentose phosphate (PP) pathway instead of via glycolysis and the tricarboxylic acid (TCA) cycle. Confirming this, the activities of two key enzymes of the PP pathway, glucose-6 phosphate (G6P) dehydrogenase and 6-phosphogluconate (6PG) dehydrogenase, were found to increase with wheat stem rust (Lunderstädt, 1964; Lunderstädt *et al.*, 1962).

2. Respiration of the Rust Fungus

Much, and perhaps most, of the increased respiratory activity in rusted tissues is contributed by the fungus. The PP pathway is known to be important in both rust mycelium (Williams and Shaw, 1968) and urediospores (Staples and Wynn, 1965). The pathway reduces NADP + to NADPH, which is thought to be used in synthesis of fungal lipids as well as in synthesis of mannitol and arabitol, two of the principal carbohydrates found in rust fungi (Section IV,G). Because the fungus cannot be separated from the host in rusted tissues, the actual rates of fungal respiration cannot be estimated accurately, nor can the proportion of total fungal respiration by way of the PP pathway be determined.

3. Respiration of the Host

Before the rust fungus sporulates, host tissues probably undergo respiratory increases of 20 to 30%. Rates are increased 20 to 60% at 5 to 6 days after inoculation (Antonelli and Daly, 1966; Daly *et al.*, 1961), when the amount of fungal mass is small relative to that of the host, and the fungus is therefore not likely to contribute significantly to total respiratory activity. Daly (1976) has emphasized that the C_6:C_1 ratio remains unchanged prior to sporulation, instead of decreasing as would be expected if the fungus with its predominant PP pathway contributed significantly to respiratory activity.

The small, putative respiratory increase in the host prior to sporulation occurs as part of the juvenile host response when senescence is delayed (Section II,A). Auxins and cytokinins, both tentatively implicated in disease-induced senescence delay (Section III,B and C), may induce part of the respiratory increase. Each has increased respiratory rates of uninfected wheat or barley leaves by 20 to 30% (Bushnell, 1967; Daly *et al.*, 1962). Increased

concentrations of carbohydrates (Section IV,G) may also cause small increases in respiration.

As the rust fungus sporulates and total respiratory activity of host and parasite increases two- to threefold, respiratory rates in host tissues are suspected to increase. Much of the respiration in the rusted host is probably by way of the TCA cycle coupled to cytochrome electron transport. Evidence for this has been previously summarized (Shaw, 1963; Daly, 1976). In addition to continued activity of the TCA cycle, activity of the oxidative PP pathway is postulated to increase in the host. This has been shown to be the case in powdery mildew of barley, in which most of the fungus can be removed so that host respiratory activities can be measured without major interference by the fungus. The respiratory rates of such host tissues are two to three times those of uninfected tissue (Bushnell and Allen, 1962; Scott, 1965). Activities of G6P and 6PG dehydrogenases increase in the mildewed host (Scott, 1965), indicating that the enhanced respiration is by way of the PP pathway. Furthermore, the respiratory alterations seem to be coupled to changes in chloroplasts. Respiratory increase coincided with onset of chlorosis and decline in photosynthesis in the host (Scott and Smillie, 1966). No increase in respiration or activities of G6P or 6PG dehydrogenases occurred in tissues lacking chloroplasts, even though powdery mildew developed abundantly if the leaves were supplied White's culture medium with sucrose. Postulating that $NADP^+$ lost from chloroplasts stimulated the PP pathway in cytosol, Ryrie and Scott (1968) obtained evidence that $NADP^+$ moved from chloroplasts to cytosol in mildewed tissues, although the separation of chloroplasts from cytosol was incomplete in their preparations.

Several lines of evidence suggest that respiratory activities in rusted hosts are the same as those in mildewed hosts:

1. Activities of G6P and 6PG dehydrogenases were shown cytologically and by enzyme assay to increase in host tissues at the borders of bean rust pustules (Tschen, 1974; Tschen and Fuchs, 1968), an indication that PP-pathway activity had increased in the host. Indirect evidence for increased activity of the pathway in the host was obtained from patterns of enzyme activity in rusted plants with and without potassium deficiency (Lunderstädt and Fuchs, 1968).
2. Respiratory increase coincided with decrease in photosynthesis with wheat stem rust (Mitchell, 1979), as with powdery mildew of barley. To be meaningful, such correlations must be general over a wide range of infection densities, environmental conditions, and cultivars. Indeed this requirement is yet to be met for powdery mildews.
3. Rusts, like powdery mildews, develop abundantly in the absence of photosynthesis if leaves are supplied sugars and other nutrients (Section IV,G). Whether fungus development occurs under such conditions

without respiratory increase in the host as reported for powdery mildew is unknown.

4. The amount of NAD increases in diseased tissue with rust (Rohringer, 1964) and with powdery mildew (Ryrie and Scott, 1968). Part of the increase was judged to be in host tissue in both diseases. Ryrie and Scott (1968) suggested that NAD has a role in the breakdown of chloroplasts that was thought to lead to release of $NADP^+$ from the chloroplast.

These findings indicate that the PP pathway is enhanced in rusted tissues and that its activity might be linked to degradation processes in chloroplasts as postulated for powdery mildew of barley.

How would the fungus benefit by increased respiration via the PP pathway? There is no evidence that NADPH or other intermediates of the pathway in the host are utilized directly by the fungus. As noted earlier, the fungus respires via the PP pathway and apparently uses it to supply NADPH for synthesis of lipids, mannitol, and arabitol. These products are unlikely to be synthesized in the host and transferred to the fungus; in fact, mannitol and arabitol do not support rapid rust fungus growth when supplied to rusted leaves in the dark (Silverman, 1960; Samborski and Forsyth, 1960).

Alternatively, enhanced operation of the PP pathway may not be of direct benefit to the fungus, but instead may be only an early manifestation of decompartmentation that eventually leads to host cell autolysis. Some enzymes of the PP pathway increase in activity during senescence of uninfected, detached wheat leaves (Farkas *et al.*, 1964). Respiratory increase associated with senescence of uninfected tissues excised from barley leaves can depend on light (Allen, 1966), suggesting that senescence-induced respiratory increase is related to photosynthesis, as Scott and co-workers have found for powdery mildewinduced respiratory increase.

For powdery mildew, Scott (1982) postulated that $NADP^+$ enhances the PP pathway either in the cytosol (as noted earlier) or possibly in the chloroplast itself. In addition, activity of the PP pathway can be controlled by amounts of G6P and 6PG dehydrogenases (Turner and Turner, 1980), which could be synthesized as part of a general activation of protein synthesis in rusted host cells.

G. CARBOHYDRATES

"A plentiful supply of carbohydrates to the host is a *sine qua non* for the development of obligate parasites on a genetically congenial host plant or leaf." So wrote P. J. Allen (1954), a statement that still applies accurately to rusts and powdery mildews.

1. Carbohydrate Requirements of the Rust Fungus

Rust mycelia in artificial culture can grow on any of several carbohydrates including glucose, fructose, mannose, sucrose, raffinose, cellobiose, and soluble starch (Maclean, 1974). Several indirect lines of evidence indicate that the needs of rust fungi growing as leaf parasites are met by one or more of these carbohydrates, most likely glucose, fructose, and sucrose. Glucose fed to rusted wheat leaves was utilized by the fungus without rearrangement of carbons 1 and 6, indicating that the intact glucose molecule was taken up (Pfeiffer *et al.*, 1969). Glucose, fructose, or sucrose have given the most abundant rust development when supplied to rusted corn or wheat leaves in darkness (Dickson *et al.*, 1959; Silverman, 1960), to rusted albino corn leaves (Dickson *et al.*, 1959), or to rusted wheat leaves in which photosynthesis is inhibited (Mashaal *et al.*,1981). Finally, Lewis (1976) implicated sucrose as a principal carbohydrate source for *Puccinia poarum* on leaves of *Poa pratensis*, by showing that sucrose infiltrated into rusted leaves specifically inhibited movement of ^{14}C-labeled sucrose from host to parasite.

Whether sucrose is taken up directly by the rust fungus or first hydrolyzed to glucose and fructose is not clear. The amount of invertase in rusted cereal leaves increases severalfold, concomitantly with fungal growth (Lunderstädt, 1966; Mitchell, 1982; Mitchell *et al.*, 1978). Urediospores apparently do not have invertase (Lunderstädt, 1966), but the wheat stem rust fungus grown in artificial culture is thought to hydrolyze sucrose before uptake (Maclean, 1982). Higher plants produce invertase, especially in young leaves or in response to wounding (Long *et al.*, 1975; Lewis, 1976), so that invertase in rusted leaves could be of both fungal and host origin.

Rust fungi do not accumulate host carbohydrates as such but, instead, convert them mainly into arabitol, mannitol, trehalose, and glycogen, none of which are common host constituents (Daly, 1967; Lewis, 1976). Glucitol and ribitol were found in *P. graminis* grown on artificial media (Maclean, 1982). Conveniently, this means that most of the glucose, fructose, sucrose, and starch found in rusted tissues can be assumed to be from the host.

2. Carbohydrates in the Host

Sucrose, glucose, and fructose often increase severalfold in rusted cereal leaves as part of the juvenile host response prior to fungus sporulation, and then decline rapidly thereafter (Lunderstädt, 1966; Mitchell *et al.*,1978; Syamananda and Staples, 1963). Similar patterns occur with bean rust (Inman, 1962) and rust of *Poa pratensis* (Lewis, 1976). The tissues tend to retain photosynthate and to favor import of sugars from distant tissues. Reasons for these changes in sugar translocation patterns in rusted tissues are discussed by Durbin (Chapter

16, this volume). The sugars are later depleted during the autolytic host response as the sporulating fungus uses increasing amounts of carbohydrate.

Starch tends to accumulate along with sugars, so that host tissues within and near pustules stain with iodine (Bushnell, 1970). The starch is found cytologically to be in chloroplasts. However, the amount of starch can show large, puzzling day-to-day fluctuations with cereal rusts (MacDonald and Strobel, 1970; Mirocha and Zaki, 1966). Host tissues seem to shift rapidly between starch synthesis and degradation.

Inorganic phosphate inhibits starch synthesis and favors starch degradation in higher plants (Preiss and Levi, 1980). Supporting this, MacDonald and Strobel (1970) showed a negative but incomplete correlation between fluctuating levels of starch and inorganic phosphate with stripe rust. Inorganic phosphate can be sequestered as polyphosphate by rust fungi, which could favor starch synthesis in host cells (Lewis, 1976; Scott, 1982). Starch synthesis also could be favored by the accumulation of sugars in host cells, which would favor movement of triose phosphate into chloroplasts, which in turn would promote starch synthesis allosterically (Preiss and Levi, 1980). An unidentified activator of host β-amylase was found in bean rust urediospores, which possibly related to a temporary disappearance of starch soon after bean plants were inoculated with the spores (Schipper and Mirocha, 1969). Similar activators were found in urediospores of *Puccinia graminis, P. coronata,* and *P. recondita.*

Uninfected cereal leaves usually do not contain starch. By inducing the host to store starch early in pustule development when carbohydrate supplies are abundant, the rust fungus presumably adds to the total carbohydrate available when supplies are eventually depleted. More work is needed on mechanisms controlling starch synthesis and degradation within host chloroplasts.

V. Concluding Statement

Rust physiologists working in the 1950s and early 1960s were motivated in part by the mysteries of obligate parasitism. How did the dependence of the rust fungus on living hosts relate to metabolic processes in the host and to changes induced therein by the fungus? Were host and parasite intimately linked with respect to metabolic pathways and intermediates? Since then, the culture of rust fungi on relatively simple culture media (Williams, Chapter 13, this volume) has suggested that such complex metabolic interactions may not exist. Instead, the changes induced in the host seem only to ensure a generous supply of simple substrates, principally sugars and amino acids. The diverse investigations of rusted leaves reviewed here have not revealed intimate metabolic interdependencies between host and parasite. Furthermore, the

changes in the cereal host are not unique to biotrophic disease as was once suspected. Host responses are well within the normal range of plant capabilities. Events in the host may be delayed, hastened, or amplified, but not changed qualitatively.

A reduction in efforts devoted to understanding the physiology of interactions between rust fungi and compatible cereal hosts has occurred in the last 10 years as emphasis has shifted to specificity and recognition phenomena (Rohringer and Heitefuss, Chapter 7, this volume). Because of the many recent advances in understanding the physiology of healthy plants, a return now to responses in susceptible hosts would appear productive. In special need of investigation are (1) identification of host enzymes that are possibly synthesized in response to infection, (2) changes in photosynthesis in relation to changes in photorespiration and dark respiration, and (3) the role of growth hormones and other substances in controlling alteration within rusted hosts. A better understanding of these phenomena in rusted cereals will ultimately help us manipulate host–parasite interaction to minimize parasite development and yield loss.

Acknowledgment

This chapter is dedicated to the memory of Paul J. Allen, who set high intellectual standards for the study of host–parasite interaction and who did so with sensitivity and good cheer.

References

Allen, P. J. (1954). Physiological aspects of fungus diseases of plants. *Annu. Rev. Plant Physiol.* **5**, 225–248.

Allen, P. J. (1959). Metabolic considerations of obligate parasitism. *In* "Plant Pathology, Problems and Progress 1908–1958" (C. S. Holton, G. W. Fischer, R. W. Fulton, H. Hart and S. E. A. McCallan, eds.), pp. 119–129. Univ. of Wisconsin Press, Madison.

Allen, P. J. (1966). The role of the host in the development of disease symptoms—a review. *Phytopathology* **56**, 255–260.

Allen, R. F. (1923). Cytological studies of infection of Baart, Kanred, and Mindum wheats by *Puccinia graminis tritici* forms III and XIX. *J. Agric. Res.* (*Washington. D.C.*) **26**, 571–604.

Allen, R. F. (1926). Cytological studies of forms 9, 21, and 27 of *Puccinia graminis tritici* on Khapli emmer. J. *Agric. Res.* (*Washington, D.C.*) **32**, 701–725.

Antonelli, E., and Daly, J. M. (1966). Decarboxylation of indoleacetic acid by near-isogenic lines of wheat resistant or susceptible to *Puccinia graminis* f. sp. *tritici*. *Phytopathology* **56**, 610–618.

Archer, S. A., and Hislop, E. C. (1975). Ethylene in host–pathogen relationships. *Ann. Appl. Biol.* **81**, 121–126.

Artemenko, E. N., Umnov, A. M., and Chkanikov, D. I. (1980). Changes in the level of indolyl-3-acetic acid and possible paths of its regulation in leaves of wheat infected with stem rust. *Sov. Plant Physiol.* (*Engl. Transl.*) **27**, 447–451.

Ayres, P. G. (1976). Patterns of stomatal behavior, transpiration, and CO_2 exchange in pea following infection by powdery mildew (*Erysiphe pisi*). *J. Exp. Bot.* **27**, 1196–1205.

Bhattacharya, P. K., and Shaw, M. (1967). The physiology of host–parasite relations. XVIII. Distribution of tritium-labeled cytidine, uridine, and leucine in wheat leaves infected with the stem rust fungus. *Can. J. Bot.* **45**, 555–563.

Bhattacharya, P. K., Naylor, J. M., and Shaw, M. (1965). Nucleic acid and protein changes in wheat leaf nuclei during rust infection. *Science* **150**, 1605–1607.

Bhattacharya, P. K., Shaw, M., and Naylor, J. M. (1968). The physiology of host–parasite relations. XIX. Further observations on nucleoprotein changes in wheat leaf nuclei during rust infection. *Can. J. Bot.* **46**, 11–16.

Bushnell, W. R. (1967). Symptom development in mildewed and rusted tissues. *In* "The Dynamic Role of Molecular Constituents in Plant–Parasite Interaction" (C. J. Mirocha and I. Uritani, eds.), pp. 21–39. Am. Phytopathol. Soc. Press, St. Paul, Minnesota.

Bushnell, W. R. (1970). Patterns in the growth, oxygen uptake, and nitrogen content of single colonies of wheat stem rust on wheat leaves. *Phytopathology* **60**, 92–99.

Bushnell, W. R., and Allen, P. J. (1962). Respiratory changes in barley leaves produced by single colonies of powdery mildew. *Plant Physiol.* **37**, 751–758.

Calonge, F. D. (1967). Chlorophyll and total nitrogen in barley rust infection. *Trans. Br. Mycol. Soc.* **50**, 397–401.

Chakravorty, A. K. (1982). Biochemistry of host rust interaction. Part A. Primary metabolism: Changes in the gene expression of host plants during the early stages of rust infection. *In* "The Rust Fungi" (K. J. Scott and A. K. Chakravorty, eds.), pp. 179–205. Academic Press, New York.

Chakravorty, A. K., Shaw, M., and Scrubb, L. A. (1974). Ribonuclease activity of wheat leaves and rust infection. *Nature* (*London*) **247**, 577–580.

Chigrin, V. V., Chumakovskii, N. N., and Zhigalkina, T. E. (1978). Dynamics of ethylene production in the pathogenesis of wheat stem rust. *Mikol. Fitopatol.* 12, 141–145 (in Russian, transl. by D. H. Casper).

Chigrin, V. V., Zhigalkina, T. E., Yukhnov, A. I., and Savtich, M. A. (1981). Abscisic acid in wheat leaves during infection with stem rust. *Sov. Plant Physiol.* (*Engl. Transl.*) **28**, 45–51.

Daly, J. M. (1967). Some metabolic consequences of infection by obligate parasites. *In* "The Dynamic Role of Molecular Constituents in Plant–Parasite Interaction" (C. J. Mirocha and I. Uritani, eds.), pp. 144–164. Am. Phytopathol. Soc. Press, St. Paul, Minnesota.

Daly, J. M. (1976). The carbon balance of diseased plants: Changes in respiration, photosynthesis and translocation. *Encycl. Plant Physiol., New Ser.* **4**, 450–479.

Daly, J. M., and Knoche, H. W. (1976). Hormonal involvement in metabolism of host–parasite interactions. *In* "Biochemical Aspects of Plant–Parasite Relationships" (J. Friend and D. R. Threlfall, eds.), pp. 117–133. Academic Press, New York. Daly, J. M., Bell, A. A., and Krupka, L. R. (1961). Respiratory changes during development of rust diseases. *Phytopathology* **51**, 461–471.

Daly, J. M., Krupka, L. R., and Bell, A. A. (1962). Influence of hormones on respiratory metabolism of healthy and rust-affected tissues. *Plant Physiol.* **37**, 130–135.

Daly, J. M., Ludden, P., and Seevers, P. (1971). Biochemical comparisons of resistance to wheat stem rust disease controlled by the Sr6 or Sr11 alleles. *Physiol. Plant Pathol.* **1**, 397–407.

Dekhuijzen, H. M. (1976). Endogenous cytokinins in healthy and diseased plants. *Encycl. Plant Physiol, New Ser.* **4**, 526–559.

Dekhuijzen, H. M., and Staples, R. C. (1968). Mobilization factors in uredospores and bean leaves infected with bean rust fungus. *Contrib. Boyce Thompson Inst.* **24**, 39–52.

Dickson, J. G., Syamananda, R., and Flangas, A. L. (1959). The genetic approach to the physiology of parasitism of the corn rust pathogens. *Am. J. Bot.* 46, 614–620.
Dmitrieva, K. O., and Zhukov, B. I. (1971). Effect of stem rust on rate of nucleic acid synthesis in tissues of wheat leaves. *Sov. Plant Physiol.* (*Engl. Transl.*) **18**, 356–359.

Doodson, J. K., Manners, J. G., and Myers, A. (1965). Some effects of yellow rust (*Puccinia striiformis*) on ^{14}carbon assimilation and translocation in wheat. J. *Exp. Bot.* **16**, 304–317.

Edwards, H. H. (1970). Biphasic inhibition of photosynthesis in powdery mildewed barley. *Plant Physiol.* **45**, 594–597.

Ehrlich, M. A., and Ehrlich, H. G. (1971a). Fine structure of *Puccinia graminis* and the transfer of C^{14} from uredospores to *Triticum vulgare*. *In* "Morphological and Biochemical Events in Plant–Parasite Interaction" (S. Akai and S. Ouchi, eds.), pp. 279–307. Phytopathol. Soc. Jpn., Tokyo.

Ehrlich, M. A., and Ehrlich, H. G. (1971b). Fine structure of the host–parasite interfaces in mycoparasitism. *Annu. Rev. Phytopathol.* **9**, 155–184.

Elnaghy, M. A., and Heitefuss, R. (1976). Permeability changes and production of antifungal compounds in *Phaseolus vulgaris* infected with *Uromyces phaseoli* 1. Role of the spore germination self-inhibitor. *Physiol. Plant Pathol.***8**, 253–267.

Farkas, G. L., and Király, Z. (1961). Amide metabolism in wheat leaves infected with stem rust. *Physiol. Plant.* **14**, 344–353.

Farkas, G. L., Dézsi, L., Horváth, M., Kisbán, K., and Udvardy, J. (1964). Common pattern of enzymatic changes in detached leaves and tissues attacked by parasites. *Phytopathol. Z.* **49**, 343–354.

Fric, F., and Heitefuss, R. (1970). Immunochemische und elektrophoretische Untersuchungen der Proteine von Weizenblättern nach Infektion mit *Puccinia graminis tritici*. *Phytopathol. Z.* **69**, 236–246.

Gassner, G., and Franke, W. (1938). Untersuchungen über den Stickstoffhaushalt rostinfizierter Getreideblätter. *Phytopathol. Z.* **11**, 517–570.

Gordon, T. R., and Duniway, J. M. (1982a). Effects of powdery mildew infection on the efficiency of CO_2 fixation and light utilization by sugar beet leaves. *Plant Physiol.* **69**, 139–142.

Gordon, T. R., and Duniway, J. M. (1982b). Phytosynthesis in powdery mildewed sugar beet leaves. *Phytopathology* **72**, 718–723.

Heath, M. C. (1974). Chloroplast ultrastructure and ethylene production of senescing and rust-infected cowpea leaves. *Can. J. Bot.* **52**, 2591–2597.

Heitefuss, R. (1964). Veränderungen im Gehalt an Ribonucleinsäure in Weizenpflanzen unterschiedlicher und temperaturgesteuerter Resistenz gegen *Puccinia graminis tritici*. *Z. Pflanzenkr.* (*Pflanzenpathol.*) *Pflanzenschutz* **71**, 154–158.

Heitefuss, R. (1965). Untersuchungen zur Physiologie des temperaturgesteuerten Verträglichkeitsgrades von Weizen und *Puccinia graminis tritici*. I. Veränderungen von Sauerstoffaufnahme und Phosphatstoffwechsel. *Phytopathol. Z.* **54**, 379–400.

Heitefuss, R. (1966). Untersuchungen zur Physiologie des temperaturgesteuerten Verträglichkeitsgrades von Weizen und *Puccinia graminis tritici*. II. Veränderungen des Nukleinsäurestoffwechsels. *Phytopathol. Z.* **55**, 67–85.

Heitefuss, R. (1970). Der Einfluss von Actinomycin auf *Puccinia graminis tritici* auf Weizen und den Einbau von Orotsäure-C^{14} und Uridin-H^3 in Wirtspflanze und Parasit. *Phytopathol. Z.* **69**, 107–114.

Heitefuss, R., and Wolf, G. (1976). Nucleic acids in host–parasite interactions. *Encycl. Plant Physiol., New Ser.* **4**, 480–508.

Hewitt, H. G., and Ayres, P. G. (1975). Changes in CO_2 and water vapor exchange rates in leaves of *Quercus robur* infected by *Microsphaera alphitoides* (powdery mildew). *Physiol. Plant Pathol.* ***7***, 127–137.

Hilu, H. M. (1965). Host–pathogen relationships of *Puccinia sorghi* in nearly isogenic resistant and susceptible seedling corn. *Phytopathology* **55**, 563–569.

Hoppe, H. H., and Heitefuss, R. (1974a). Permeability and membrane lipid metabolism of *Phaseolus vulgaris* infected with *Uromyces phaseoli*. I. Changes in the efflux of cell constituents. *Physiol. Plant Pathol.* **4**, 5–9.

Hoppe, H. H., and Heitefuss, R. (1974b). Permeability and membrane lipid metabolism of *Phaseolus vulgaris* infected with *Uromyces phaseoli*. III. Change in relative concentration of lipid bound fatty acids and phospholipase activity. *Physiol. Plant Pathol.* **4**, 25–35.

Inman, R. E. (1962). Disease development, disease intensity and carbohydrate levels in rusted bean plants. *Phytopathology* **52**, 1207–1211.

Jäger, K., and Reisener, H. J. (1969). Untersuchungen über Stoffwechselbeziehungen zwischen Parasit und Wirt am Beispiel von *Puccinia graminis* var. *tritici* auf Weizen. I. Aufnahme von Aminosauren aus dem Wirtsgewebe.*Planta* **85**, 57–72.

Johnson, L. B., Schafer, J. F., and Leopold, A. C. (1966). Nutrient mobilization in leaves by *Puccinia recondita. Phytopathology* **56**, 799–803.

Johnson, L. B., Zscheile, F. P., Jr., and Brannaman, B. L. (1967). Effect of *Puccinia recondita* infection on the RNA composition of wheat leaves. *Phytopathology* **57**, 632–638.

Johnson, L. B., Brannaman, B. L., and Zscheile, F. P., Jr. (1968). Protein and enzyme changes in wheat leaves following infection with *Puccinia recondita. Phytopathology* **58**, 578–583.

Kim, W. K., and Rohringer, R. (1969). Metabolism of aromatic compounds in healthy and rust-infected primary leaves of wheat. III. Studies on the metabolism of tryptophan. *Can. J. Bot.* **47**, 1425–1433.

Király, Z., El Hammady, M., and Pozar, B. I. (1967). Increased cytokinin activity of rust-infected bean and broad bean leaves. *Phytopathology* **57**, 93–94.

Kosuge, T. (1978). The capture and use of energy by diseased plants. *In* "Plant Disease" (J. G. Horsfall and E. B. Cowling, eds.), Vol. 3, pp. 85–116. Academic Press, New York.

Lea, P. J., and Miflin, B. J. (1980). Transport and metabolism of asparagine and other nitrogen compounds within the plant. *In* "The Biochemistry of Plants" (B. J. Miflin, ed.), Vol. 5, pp. 569–607. Academic Press, New York.

Lewis, D. H. (1976). Interchange of metabolites in biotrophic symbioses between angiosperms and fungi. *In* "Perspectives in Experimental Biology" (N. Sunderland, ed.), Vol. 2, pp. 207–249. Pergamon, Oxford.

Livne, A. (1964). Phytosynthesis in healthy and rust-affected plants. *Plant Physiol.* **39**, 614–621.

Long, D. E., Fung, A. K., McGee, E. E. M., Cooke, R. C., and Lewis, D. H. (1975). The activity of invertase and its relevance to the accumulation of storage polysaccharides in leaves infected by biotrophic fungi. *New Phytol.* **74**, 173–182.

Lunderstädt, J. (1964). Die Aktivität einiger Enzyme des Kohlenhydratstoffwechsels in Weizenkeimpflanzen nach Infektion mit *Puccinia graminis tritici. Phytopathol. Z.* **50**, 197–220.

Lunderstädt, J. (1966). Effect of rust infection on hexokinase activity and carbohydrate dissimilation in primary leaves of wheat. *Can. J. Bot.* **44**, 1345–1364.

Lunderstädt, J., and Fuchs, W. H. (1968). Beitrag zum dissimilatorischen Kohlenhydratstoffwechsel von Weizenprimärblättern unter dem Einfluss von Kali und Befall durch *Puccinia graminis tritici. Phytopathol. Z.* **63**, 247–258.

Lunderstädt, J., Heitefuss, R., and Fuchs, W. H. (1962). Aktivität einiger Enzyme des Kohlenhydratstoffwechsels aus Weizenkeimpflanzen nach Infektion mit *Puccinia graminis tritici. Naturwissenschaften* 49, 403.

MacDonald, P. W., and Strobel, G. A. (1970). Adenosine diphosphate-glucose pyrophosphorylase control of starch accumulation in rust-infected wheat leaves. *Plant Physiol.* **46**, 126–135.

Maclean, D. J. (1974). Cultural and nutritional studies on variant strains of the wheat stem rust fungus. *Trans. Br. Mycol. Soc.* **62**, 333–349.

Maclean, D. J. (1982). Axenic culture and metabolism of rust fungi. *In* "The Rust Fungi" (K. J. Scott and A. K. Chakravorty, eds.), pp. 37–120. Academic Press, New York.

Magyarosy, A. C., and Malkin, R. (1978). Effect of powdery mildew infection of sugar beet on the content of electron carriers in chloroplasts. *Physiol. Plant Pathol.* **13**, 183–188.

Magyarosy, A. C., Schurmann, P., and Buchanan, B. B. (1976). Effect of powdery mildew infection on photosynthesis by leaves and chloroplasts of sugar beets. *Plant Physiol.* **57**, 486–489.

Manocha, M. S., and Shaw, M. (1966). The physiology of host–parasite relations. XVI. Fine structure of the nucleus in the rust-infected mesophyll cells of wheat. *Can. J. Bot.* **44**, 669–674.

Mares, D. J. (1979). A light and electron microscope study of the interaction of yellow rust (*Puccinia striiformis*) with a susceptible wheat cultivar. *Ann. Bot.* (*London*) [N.S.] **43**, 183–189.

Mashaal, S. F., Bama, B., and Király, Z. (1981). Effect of photosynthesis inhibitors on wheat stem rust development. *Acta Phytopathol. Acad. Sci. Hung.* **16**, 45–48.

Mendgen, K. (1981). Nutrient uptake in rust fungi. *Phytopathology* **71**, 983–989.

Mirocha, C. J., and Zaki, A. I. (1966). Fluctuation in amount of starch in host plants invaded by rust and mildew fungi. *Phytopathology* **56**, 1220–1222.

Mitchell, D., and Shaw, M. (1968). Metabolism of glucose-^{14}C, pyruvate-^{14}C, and mannitol-^{14}C by *Melampsora lini.* II. Conversion to soluble products. *Can. J. Bot.* **46**, 453–460.

Mitchell, D. T. (1979). Carbon dioxide exchange by infected first leaf tissues susceptible to wheat stem rust. *Trans. Br. Mycol. Soc.* **72**, 63–68.

Mitchell, D. T. (1982). Invertase activity in leaf tissues susceptible and resistant to wheat stem rust. *Trans. Br. Mycol. Soc.* **78**, 173–176.

Mitchell, D. T., Fung, A. K., and Lewis, D. H. (1978). Changes in the ethanol-soluble carbohydrate composition and acid invertase in infected first leaf tissues susceptible to crown rust of oat and wheat stem rust. *New Phytol.* **80**, 381–392.

Montalbini, P., and Buchanan, B. B. (1974). Effect of a rust infection on photophosphorylation by isolated chloroplasts. *Physiol. Plant Pathol.* **4**, 191–196.

Montalbini, P., and Elstner, E. F. (1977). Ethylene evolution by rust-infected, detached bean (*Phaseolus vulgaris* L.) leaves susceptible and hypersensitive to *Uromyces phaseoli* (Pers.) Wint. *Planta* **135**, 301–306.

Nielsen, J., and Rohringer, R. (1963). Incorporation of cytidine-H^3 into the primary leaf of wheat following infection with *Puccinia recondite* Rob. ex Desm. *Can. J. Bot.* **41**, 1501–1508.

Owera, S. A. P., Farrar, J. F., and Whitbread, R. (1981). Growth and photosynthesis in barley infected with brown rust. *Physiol. Plant Pathol.* **18**, 79–90.

Pegg, G. F. (1976a). Endogenous auxins in healthy and diseased plants. *Encycl. Plant Physiol., New Ser.* **4**, 560–581.

Pegg, G. F. (1976b). The involvement of ethylene in plant pathogenesis. *Encycl. Plant Physiol., New Ser.* **4**, 582–591.

Pfeiffer, E., Jäger, K., and Reisener, H. J. (1969). Untersuchungen über Stoffwechselbeziehungen zwischen Parasit und Wirt am Beispiel von *Puccinia graminis* var. *tritici* auf Weizcn. II. Aufnahme von Hexosen aus dem Wirtsgewebe. *Planta* **85**, 194–201.

Preiss, J., and Levi, C. (1980). Starch biosynthesis and degradation. *In* "The Biochemistry of Plants" (J. Preiss, ed.), Vol. 3, pp. 371–423. Academic Press, New York.

Pure, G. A., Chakravorty, A. K., and Scott, K. J. (1979). Cell-free translation of polysomal messenger RNA isolated from healthy and rust-infected wheat leaves. *Physiol. Plant Pathol.* **15**, 201–209.

Quick, W. A., and Shaw, M. (1964). The physiology of host–parasite relations XIV. The effect of rust infection on the nucleic acid content of wheat leaves. *Can. J. Bot.* **42**, 1531–1540.

Raggi, V. (1974). Free and protein amino acids in the pustules and surrounding tissues of rusted bean. *Phytopathol. Z.* **81**, 289–300.

Raggi, V. (1975). Incorporation of $^{14}CO_2$ in amides and amino acids in rusted bean leaves. *Can. J. Bot.* **53**, 1244–1254.

Raggi, V. (1978). CO_2 release in light, dark and open light-dark transition (CO_2 outburst) in rust-infected bean leaves. *Phytopathol. Mediterr.* **17**, 135–138.

Reisener, H. J., and Ziegler, E. (1970). Über den Stoffwechsel des parasitischen Mycels und dessen Beziehungen zum Wirt bei *Puccinia graminis* auf Weizen. *Angew. Bot.* **44**, 343–346.

Reisener, H. J., Ziegler, E., and Prinzing, A. (1970). Zum Stoffwechsel des Mycels von *Puccinia graminis var. tritici* auf der Weizenpflanze. *Planta* **92**, 355–357.

Reiter, B. G., Yudkin, L. Y., and Yudnika, N. B. (1976). Submicroscopic structure of *Puccinia recondita* f. sp. *tritici* and leaf cells of *Triticum aestivum. Mikol. Fitopatol.* 10, 257–260 (in Russian, transl. by D. H. Casper).

Rohringer, R. (1957). Untersuchungen zur Biochemie von Weizenkeimpflanzen nach Infektion mit *Puccinia graminis tritici,* Erikss. und Henn., ph. R. 126 A. *Phytopathol. Z.* **29**, 45–64.

Rohringer, R. (1964). Drifts in pyridine nucleotide levels during germination of rust spores and in wheat leaves after inoculation with leaf rust. Z. *Planzenkr.* (*Pflanzenpathol.*) *Planzenschutz* **71**, 160–170.

Rohringer, R., and Heitefuss, H. (1961). Incorporation of P^{32} into ribonucleic acid of rusted wheat leaves. *Can. J. Bot.* **39**, 263–267.

Ruttle, M. L., and Frazer, W. P. (1927). A cytological study of *Puccinia coronata* Cda on Banner and Cowra 35 oats. *Univ. Calif., Berkeley, Publ. Bot.* **14**, 21–72.

Ryrie, I. J., and Scott, K. J. (1968). Metabolic regulation in diseased leaves. II. Changes in nicotinamide nucleotide coenzymes in barley leaves infected with powdery mildew. *Plant Physiol.* **43**, 687–692.

Sachse, B., Wolf, G., and Fuchs, W. H. (1971). Nukleinsäure abbauende Enzyme in Blättern von *Triticum aestivum* nach Infektion mit *Puccinia graminis tritici. Acta Phytopathol. Acad. Sci. Hung.* **6**, 39–49.

Sadler, R., and Shaw, M. (1979a). Studies on glutamate dehydrogenase from rust infected flax cotyledons. Z. *Pflanzenphysiol.* **91**, 211–224.

Sadler, R., and Shaw, M. (1979b). Pathways of nitrogen assimilation in rust infected flax cotyledons. Z. *Pflanzenphysiol.* **93**, 105–115.

Samborski, D. J., and Forsyth, F. R. (1960). Inhibition of rust development on detached wheat leaves by metabolites, antimetabolites, and enzyme poisons. *Can. J. Bot.* **38**, 467–476.

Samborski, D. J., and Shaw, M. (1956). The physiology of host–parasite relations. II. The effect of *Puccinia graminis tritici.* Eriks. and Henn. on the respiration of the first leaf of resistant and susceptible species of wheat. *Can. J. Bot.* **34**, 601–619.

Samborski, D. J., Rohringer, R., and Person, C. (1961). Effect of rust-inhibiting compounds on the metabolism of wheat leaves. *Can. J. Bot.* **39**, 1019–1027.

Schipper, A. L., Jr., and Mirocha, C. J. (1969). The mechanism of starch depletion in leaves of *Phaseolus vulgaris* infected with *Uromyces phaseoli. Phytopathology* **59**, 1722–1727.

Scott, K. J. (1965). Respiratory enzymic activities in the host and pathogen of barley leaves infected with *Erysiphe graminis. Phytopathology* **55**, 438–441.

Scott, K. J. (1982). Biochemistry of host rust interactions. B. Intermediary metabolism. *In* "The Plant Rusts" (K. J. Scott and A. K. Chakravorty, eds.), pp. 206–222. Academic Press, New York.

Scott, K. J., and Smillie, R. M. (1966). Metabolic regulation in diseased leaves. I. The respiratory rise in barley leaves infected with powdery mildew. *Plant Physiol.* **41**, 289–297.

Shaw, M. (1963). The physiology and host–parasite relations of the rusts. *Annu. Rev. Phytopathol.* **1**, 259–294.

Shaw, M., and Colotelo, N. (1961). The physiology of host–parasite relations. VII. The effect of stem rust on the nitrogen and amino acids in wheat leaves. *Can. J. Bot.* **39**, 1351–1372.

Shaw, M., and Hawkins, A. R. (1958). The physiology of host–parasite relations. V. A preliminary examination of the level of free endogenous indoleacetic acid in rusted and mildewed cereal leaves and their ability to decarboxylate exogenously supplied radioactive indoleacetic acid. *Can. J. Bot.* **36**, 1–16.

Shaw, M., and Manocha, M. S. (1965a). Fine structure in detached, senescing wheat leaves. *Can. J. Bot.* **43**, 747–755.

Shaw, M., and Manocha, M. S. (1965b). The physiology of host–parasite relations. XV. Fine structure in rust-infected wheat leaves. *Can. J. Bot.* **43**, 1285–1292.

Shaw, M., and Samborski, D. J. (1957). The physiology of host–parasite relations. III. The pattern of respiration in rusted and mildewed cereal leaves. *Can. J. Bot.* **35**, 389–407.

Siebert, R. (1961). Biochemische Untersuchungen zum Wirt-Parasit-Verhältnis am Beispiel von *Puccinia graminis tritici. Phytopathol. Z.* **40**, 221–244.

Silverman, W. (1960). The development of stem rust on wheat leaves treated with some sugars and sugar alcohols. *Phytopathology* **50**, 114–119.

Stakman, E. C., Stewart, D. M., and Loegering, W. Q. (1962). Identification of physiologic races of *Puccinia graminis* var. *tritici. U. S. Agric. Res. Serv.*, **E617.**

Staples, R. C. (1957). Changes in the organic acid composition of wheat leaves infected with the leaf rust fungus. *Contrib. Boyce Thompson Inst.* **19**, 1–18.

Staples, R. C. (1965). Acid phosphatases from healthy and rust infected pinto bean leaves. II. Effect of removing apical meristem. *Contrib. Boyce Thompson Inst.* **23**, 83–91.

Staples, R. C., and Stahmann, M. A. (1964). Changes in proteins and several enzymes in susceptible bean leaves after infection by the bean rust fungus. *Phytopathology* **54**, 760–764.

Staples, R. C., and Wynn, W. K. (1965). The physiology of uredospores of the rust fungi. *Bot. Rev.* **31**, 537–564.

Stoddart, J. L. (1981). Leaf senescence. *In* "Aspects and Prospects of Plant Growth Regulators" (B. Jeffcoat, ed.), pp. 51–63. British Plant Growth Regulatory Group, ARC Letcombe Laboratory, Wantage, Oxfordshire.

Sutton, B. C. S., and Shaw, M. (1982). Changes in two ribonuclease isozymes during rust infection of flax cotyledons. *Plant Physiol.* **69**, 205–209.

Syamananda, R., and Staples, R. C. (1963). The carbohydrate content of rusted corn leaves. *Contrib. Boyce Thompson Inst.* **22**, 1–8.

Sziráki, I., Barna, B., Waziri, E., and Király, Z. (1976). Effect of rust infection on the cytokinin level of wheat cultivars susceptible and resistant to *Puccinia graminis* f. sp. *tritici. Acta Phytopathol. Acad. Sci. Hung.* **11**, 155–160.

Tani, T., and Yamamoto, H. (1979). RNA and protein synthesis and enzyme changes during infection. *In* "Recognition and Specificity in Plant Host–Parasite Interaction" (J. M. Daly and I. Uritani, eds.), pp. 273–287. University Park Press, Baltimore, Maryland.

Tani, T., Onoe, T., and Naito, N. (1970). Drifts in ^{32}P distribution and its incorporation into nucleic acid in oat leaves after inoculation with *Puccinia coronata*. *Tech. Bull. Fac. Agric., Kagawa Univ.* **21**, 40–49.

Tani, T., Yoshikawa, M., and Naito, N. (1973a). Effect of rust infection of oat leaves on cytoplasmic and chloroplast ribosomal ribonucleic acids. *Phytopathology* **63**, 491–494.

Tani, T., Yoshikawa, M., and Naito, N. (1973b). Template activity of ribonucleic acid extracted from oat leaves infected by *Puccinia coronata*. *Ann. Phytopathol. Soc. Jpn.* **39**, 7–13.

Thatcher, F. S. (1942). Further studies of osmotic and permeability relations in parasitism. *Can. J. Res., Sect. C* **20**, 283–311.

Tolbert, N. E. (1980). Photorespiration. *In* "The Biochemistry of Plants" (D. D. Davies, ed.), Vol. 2, pp. 487–523. Academic Press, New York.

Tschen, J. (1974). Changes of several enzyme activities of carbohydrate catabolism in bean leaf tissues infected with *Uromyces phaseoli typica. Bot. Bull. Acad. Sin.* **15**, 1–7.

Tschen, J., and Fuchs, W. H. (1968). Endogene Aktivität der Enzyme in rostinfizierten Bohnenprimärblättern. *Phytopathol. Z.* **63**, 187–192.

Turner, J. F., and Turner, D. H. (1980). The regulation of glycolysis and the pentose phosphate pathway. *In* "The Biochemistry of Plants" (D. D. Davies, ed.), Vol. 2, pp. 279–316. Academic Press, New York.

Umnov, A. M., Artemenko, E. N., and Chkanikov, D. I. (1978). [The presence of indolyl-3-acetic acid in uredospores of wheat stem rust.] *Mikol. Fitopatol.* **12**, 222–227.

Van Dyke, C. G., and Hooker, A. L. (1969). Ultrastructure of host and parasite in interactions of *Zea mays* with *Puccinia sorghi. Phytopathology* **59**, 1934–1946.

von Broembscn, S. L., and Hadwiger, L. A. (1972). Characterizations of disease resistance responses in certain gene-for-gene interactions between flax and *Melampsora lini. Physiol. Plant Pathol.* **2**, 207–215.

Whitney, H. S., Shaw, M., and Naylor, J. M. (1962). The physiology of host–parasite relations. XII. A cytophotometric study of the distribution of DNA and RNA in rust-infected leaves. *Can. J. Bot.* **40**, 1533–1544.

Williams, P. G., and Shaw, M. (1968). Metabolism of glucose-^{14}C, pyruvatc-^{14}C, and mannitol-^{14}C by *Melampsora lini*. I. Uptake. *Can. J. Bot.* **46**, 435–440.

Williams, P. H., and Staples, R. C. (1964). Acid phosphatases from healthy and rust infected pinto bean leaves. *Contrib. Boyce Thompson Inst.* **22**, 269–282.

Williamson, C. E. (1950). Ethylene, a metabolic product of diseased or injured plants. *Phytopathology* **40**, 205–208.

Wrigley, C. W., and Webster, H. L. (1966). The effect of stem rust infection on the soluble proteins of wheat leaves. *Aust. J. Biol. Sci.* **19**, 895–901.

Wynn, W. K., fr. (1963). Photosynthetic phosphorylation by chloroplasts isolated from rust-infected oats. *Phytopathology* **53**, 1376–1377.

16

Effects of Rust on Plant Development in Relation to the Translocation of Inorganic and Organic Solutes

Richard D. Durbin

Agricultural Research Service, U.S. Department of Agriculture, and Department of Plant Pathology, University of Wisconsin, Madison, Wisconsin

I. Introduction

The interaction between a cereal and a rust fungus leads to many diverse changes in the growth and development of the host. One of the most profound of these changes is in the distribution patterns of inorganic and organic solutes. The significance of this change arises from the proposition that (1) the distribution of solutes constitutes "a key factor in productivity" (Loomis *et*

al., 1976), and (2) cereal rusts create major imbalances in these distribution patterns. The evidence emerging for this proposition is largely indirect but compelling. Actually, observations bearing on this view have been accumulating for many years. Cornu (1881), for example, was commenting on this when he spoke of the "activité vitale" of rust infections, and even earlier reports can be found in the writing of medieval herbalists; stunting of cereals, presumably due to rusts, is even mentioned in the Bible.

Because rust fungi are biotrophic, they have had by necessity to evolve ways to superimpose themselves upon the host without killing it—at least in the beginning of the infection cycle. Second, because they cannot adequately develop throughout their life cycle solely at the substrate levels found in parasitized cells, they also have had to evolve mechanisms for obtaining nutrients at some distance away from these cells. How this comes about is the topic of this chapter.

II. Distribution of Solutes during Plant Development

To appreciate how rust diseases affect solute distribution patterns in cereals, it is first necessary to understand the patterns of metabolite movement in healthy plants, as well as the underlying mechanisms responsible for them (Lüttge and Pitman, 1976; Stocking and Heber, 1976; Zimmerman and Milburn, 1975). These patterns vary from one metabolite to another and depend on many factors (e.g., developmental stage of the plant, external stresses). Furthermore, they can change within minutes if the external conditions change (Fondy and Geiger, 1980; Geiger, 1976; Wyse and Saftner, 1982).

Some inorganic solutes are extremely mobile, as exemplified by phosphorus and potassium, taking part in reactions in one cell, then likely as not being translocated to another cell—either nearby or relatively distant—where the process is repeated. Others, calcium for example, are the antithesis of this. Once it enters the cereal leaf, it appears to remain essentially immobilized until the leaf dies. However, a considerable amount of the calcium that remains in the stem may ultimately be transported to the developing grain (Martin, 1982).

Organic compounds also are in a continual state of flux, their rates of turnover (relative rates of synthesis and degradation) depending on the compound in question. However, essentially all compounds that have been carefully studied have been found to turn over, even those secondary products that once were thought to serve only as metabolic end products for disposal of toxic substances. Thus an individual carbon or nitrogen atom may sequentially be a constituent of a large array of compounds within a single cell before passing on to another cell. It is only when senescence of the leaves and

maturation in the grain itself occurs that metabolic turnover slows and essentially stops in these organs.

Translocation patterns within the plant are governed by “sources,” that is, regions that export solutes and water, and “sinks,” regions that import solutes and water for metabolic utilization or storage (Loomis *et al.*,1976; Sutcliffe, 1976; Wareing and Patrick, 1975). Meristems in vegetative and reproductive organs are the principal sinks, but storage pools and regions of high respiration are also important sinks. The activity of these sinks is determined by many factors, as cited previously; some are intrinsic to the sink itself, whereas others are determined by the sources and/or environmental conditions. In any event, sinks play a large role in determining the plant's priorities for the distribution of solutes and water.

During germination, the seed's endosperm initially acts as the major source for inorganic solutes, but soon the root system takes over this function. Although the roots continue to obtain and translocate inorganic solutes throughout the cereal plant's life, it is important to note here that as the plant body develops, an increasing proportion of these solutes entering the shoot meristem, and ultimately the grain, are translocated from older leaves and the stem rather than directly from the roots (Durbin, 1967). In wheat, for instance, Martin (1982) found that 75% of the nitrogen, 86% of the phosphorus, 22% of the potassium, and 37% of the magnesium in the vegetative portion of the plant were translocated into the grain. In oats, more than 90% of the phosphorus and nitrogen of the grain has been accumulated by the plant before it reaches 25% of its maximum dry weight (Williams, 1955). Likewise, in corn 60% of the kernel nitrogen comes directly from the leaves (Hay *et al.*, 1953).

The leaf blade, together with the sheath that covers most of the stem in cereals and, to a greater or lesser extent, the glumes, awns, and stem, produce most of the organic solutes (i.e., photosynthates and from them the primary and secondary metabolites) (Durbin, 1967). These are either metabolized *in situ* or exported elsewhere, particularly to the developing root and shoot apices. The lower leaves tend to translocate most of their organic solutes basipetally, whereas upper leaves translocate them acropetally.

Nitrogen in the form of nitrate, certain amino acids, and asparagine and glutamine is first supplied from the root to mature leaves via the xylem. Some of the nitrogen is then loaded into the phloem and retranslocated, chiefly as amides and amino acids, to the developing shoot and root. The nitrogen-containing compounds entering the developing shoot are thus thought to have come mainly from mature leaves rather than directly from the root because, having a relatively small surface area, the developing shoot's requirements cannot be met by transpirationally derived nitrogen. A large proportion of the nitrogen is continually being cycled through the plant. In wheat, Simpson *et al.* (1982) envision this process as constituting a dynamic nitrogen reserve that can increase or decrease depending on the prevailing source–sink relationships.

Solutes entering the leaf are partitioned in various ways, depending on the kind of solute and the stage of plant development. Some are transferred readily from the xylem to the phloem for retranslocation out of the leaf (Pate, 1975, 1980). Others are retained in the leaf either in storage pools or actively utilized in anabolic processes. The path of solute movement within the leaf is not definitely known. Two pathways appear to be probable candidates: an apoplastic one within the aqueous continuum of the leaf's free space (i.e., cell wall) and a symplastic one (i.e., metabolic space) through the plasmodesmata between the mesophyll cells and the sieve elements (Läuchli, 1976; Spanswick, 1976). The prevailing view currently hypothesizes a mixed pathway in which solutes are first unloaded from the symplast (phloem or veinal tissues associated with the phloem) into the apoplast. They then move in the apoplast to the mesophyll cells, and finally enter the symplast of the mesophyll cell cytosol. The phloem is loaded by solute movement along the same pathway in the opposite direction (Geiger, 1976; Madore and Webb, 1981). As an example of this, Kuo *et al.* (1974) have suggested that in wheat during phloem loading there may be a movement of sucrose into the apoplast from within the mestome sheath that surrounds all the longitudinal veins. Phloem loading from the apoplast has also been demonstrated in corn (Cronshaw, 1981), and there is evidence that such loading is an active process (Heyser, 1980).

As the plant grows, each leaf goes through a developmental cycle in which, when juvenile, it behaves as a sink; then, as growth slows and maturity approaches, it increasingly serves as a source. A period of maturity follows during which the leaf acts as a net exporting organ. Eventually, it declines in metabolic activity (i.e., becomes senescent), and then dies. During senescence, reserves and proteins are hydrolyzed and, along with mineral nutrients, retranslocated to developing tissues so that, when the leaf dies, much of its nonstructural components have already been transported elsewhere.

Changes in inorganic solute levels in cells can also regulate organic solute movement to some extent. A mild potassium deficiency, for instance, will cause an accumulation of photosynthates in leaves and, conversely, in excess it can stimulate translocation. These effects appear to be mediated via an interaction of potassium with a membrane-bound ATPase, which presumably is involved in phloem loading (Giaquinta, 1979). To complicate the matter, carbohydrate movement, in turn, appears largely to control phosphorus movement (Marshall and Wardlaw, 1973).

Beginning early and continuing at an increasing rate throughout the plant's vegetative phase, inorganic and organic metabolites begin to accumulate or be produced at levels above those needed for metabolism. In cereals, most of these metabolites are sequestered in stem tissues in the form of secondary metabolites or in storage pools. Subcellular sites for such temporary storage are found in the chloroplast, cytosol, and vacuole. There they remain until after anthesis, when they are mobilized and retranslocated to the developing grain.

During plant growth, the solute distribution patterns, governed by all these source–sink relationships, become more complex as additional sources and sinks are formed. The patterns and the role of the xylem and phloem in translocation change in a series of timely transitions. They undergo their most radical alterations after anthesis, when the developing grain begins to supersede all other sinks. At this point, further vegetative growth almost ceases, the plant increasingly directs its metabolic resources toward grain development, and the grain ultimately becomes, for all practical purposes, the sole sink.

III. Effects of Rust on Solute Distribution

The impact of a rust on this multivariate, dynamic, and adaptive system is manifold and profoundly affects the plant's subsequent growth and development. Such factors as the stage of plant development at the time of infection, the cultivar, the presence or absence of stresses (e.g., other diseases or insect infestations), infection type, disease intensity, location of the infection(s) on the plant, and environmental conditions can modify the magnitude of the effect. The rust has a skewed effect on the host, such that (1) low disease levels have disproportionately large effects, and (2) imbalances created during early growth can have a particularly large effect, as has been shown with powdery mildew (Carver and Griffiths, 1981).

Because plant processes are intimately related, alterations in solute distribution patterns induced by localized infections of rust fungi will have physiological and metabolic repercussions in other organs of the plant and at later times in its development. As an instance of this, Bushnell and Rowell (1968) found that in severely rusted wheat the major reason for shoot desiccation and death was a drastic decline in the root's capacity to provide water. This decline apparently had originally come about because of a decrease in organic solute transfer to the root from the rusted shoot. This example illustrates one of the general effects that rusts have on their hosts: The developmental cycle of the host or one or more of its organs is foreshortened. For this reason, when infection occurs during especially critical periods such as anthesis or grain filling, productivity can be drastically curtailed because the maturation period is reduced.

Another instance of this linkage among organs in the diseased plant is exemplified by the effect of rust on the photosynthetic rate of healthy leaves. Livne (1964) found that the rate of photosynthesis of a trifoliate leaf on a bean plant with infected primary leaves was higher than that of a corresponding leaf on a healthy plant. A considerable amount of evidence has accumulated indicating that the removal of photosynthates from the healthy leaf under the influence of the rust is responsible for this increase (Durbin, 1967; King *et*

al., 1967; Wyse and Saftner, 1982). Hartt (1963), working with sugarcane, first showed that a variety of conditions that lead to sucrose depletion increase photosynthesis. Conversely, when sucrose accumulates, the rate of photosynthesis decreases. Her results also help to explain why photosynthesis declines in infected tissues (Doodson*et al.*, 1965), for here, there is an accumulation of photosynthates.

It was evident quite early that rust infections must have a significant effect on the translocation patterns of the host. Shaw and Samborski in 1956 stated, "The relative rates of transport into and utilization of metabolites within the infection zone may well be an important factor in determining both the degree of development of the parasite and the reaction of the host." This view came about because of observations on infected tissues that showed that (1) they had elevated respiration rates and depressed photosynthetic rates (Shaw, 1963), (2) they increased in fresh and dry weights (Yarwood and Childs, 1938; Bushnell, Chapter 15, this volume), (3) they remained alive longer than surrounding tissues, (4) the host cells (in certain rusts) around the pustule sometimes began to divide (Yarwood and Cohen, 1951), and (5) the growth rate of the rest of the plant decreased (Durbin, 1967).

Later studies using radionuclides (e.g., ^{3}H, ^{14}C, ^{32}P, and ^{35}S) conclusively showed that both inorganic and organic substances accumulated at the site of infection, an area that Shaw (1963) has called the rust's "field of dominance." At first, this accumulation of metabolites was thought to occur predominantly in the host cells at the site. This view was buttressed by the then prevalent hypothesis of Allen (1953) that host respiration might be accelerated by an uncoupling agent elaborated by the pathogen. However, additional research showed that this was not entirely the case and that much of the observed increases in respiration rate and weight were due to the pathogen (Bushnell, Chapter 15, this volume; Daly, 1967; Shaw, 1963). Closer inspection also has shown that as the time after administration of a radionuclide increases, a larger amount of the label is incorporated into the fungal mycelium and spores (Durbin, 1967; Mendgen, 1977; von Sydow and Durbin, 1962).

By administering the radiolabeled substances to different parts of locally infected plants (Doodson *et al.*, 1965; Durbin, 1967; Holligan *et al.*, 1974), it was shown that accumulation was due to two factors: an increase in the rate of solute movement toward the infection site and a decrease in their movement away from the site. Using bean rust as a model system, Livne and Daly (1966) found that rusted primary leaves imported 40-fold more photosynthate from the trifoliate leaf than did corresponding healthy primary leaves, and Zaki and Durbin (1965) found that photosynthate movement to the stem apex from infected primary leaves is reduced fivefold, and to the root eightfold. [Livne and Daly (1966) found a 40-fold decrease in the latter case.] Studying yellow rust of wheat (*Puccinia striiformis*), Doodson *et al.* (1965) found that ^{14}C translocation over a 3-hr period was reduced as much as *99%* from an infected

leaf. Thus, much of what appears to be taking place can be explained if one simply assumes that the fungus creates an extremely efficient sink.

The host cells immediately around the infection site also play a role in enhancing the accumulation process. As indicated earlier, these cells begin to photosynthesize less but respire more as the infection develops (Owera *et al.*, 1981; Bushnell, Chapter 15, this volume); thus they become more dependent on surrounding cells for their nourishment. Their starch (Schipper and Mirocha, 1969) and other reserves also are depleted (Bushnell, Chapter 15, this volume), processes biochemically controlled from within the cell (Huber, 1981). An important attribute of cells in this region is that they remain in a juvenile state much longer than would normally be the case. This tends to intensify the sink effect, and, especially in older leaves, minimizes these cells' capacity to act as a source (Durbin, 1967). The net result is that the vegetative and/or reproductive meristems are starved (Doodson *et al.*, 1965; Siddiqui and Manners, 1971). Just how great an effect this "starvation" has is undetermined, but certainly it is of major consideration, especially during grain development.

These and other kinds of studies clearly show that accumulation at the infection site is at the expense of the remainder of the plant (Doodson *et al.*, 1965; Holligan *et al.*, 1974; Siddiqui and Manners, 1971). Although not designed to measure flux rates of ingress and egress, they did describe overall changes in the translocatory processes. However, it is not clear if these changes involve an increase in the mobilization of reserves followed by their export, or whether there is simply a quantitative change in the solute distribution patterns to the various sinks already present. Also a factor to be considered is the influence rust has on both the photosynthetic and dark CO_2-fixation rates (Rick and Mirocha, 1968) in the various parts of the plant. Quantitative analyses need to be done on these systems using, for example, such approaches as plant growth modeling (Causton and Venus, 1982). This kind of approach has already proved useful in barley for studies on the relationship between brown rust (Owera *et al.*, 1981) or powdery mildew, and yield.

A significant portion of the accumulated nutrients is probably required to support the growth and reproduction of the pathogen; particularly in susceptible combinations where there is a large mass of the pathogen that, during sporulation, rapidly depletes the soluble nutrient pools in the mycelium (von Sydow, 1966; von Sydow and Durbin, 1962). However, we do not know what proportion of these nutrients are metabolically utilized by the pathogen, or what the exact compounds are that traverse the interface between the fungal haustorium and the host plasmalemma (Bushnell, Chapter 15, this volume). Are all these individual chemical species actually required or do some simply accumulate because of mass flow toward the infection site? These considerations are of some moment because, if compounds are non-specifically directed to the infection site and then transferred to the pathogen, then fungitoxic substances could also accumulate within the fungus to

concentrations significantly higher than would be found in the surrounding tissues. In essence, the fungus' requirement for nutrients could be turned against it! Such a strategy is the basis for the application of Ni-containing compounds on cereals by low-volume spraying (Peturson *et al.*, 1958).

Although it is thought that the majority of solutes pass into the vegetative hyphae of the pathogen across the haustorial–plasmalemmal interface, there are no detailed quantitative studies showing what proportion of the solutes pass through this interface. On the basis of results with powdery mildews (Bracker and Littlefield, 1973; Heath, 1972, 1976), one might presume that this is the paramount, if not only, portal of entry. Possibly, however, in the rust fungi such exchange may not be restricted to the haustorial region, but may also encompass a portion, or all, of the intercellular mycelial complex. In support of this, Ehrlich and Ehrlich (1970), using ^{14}C-labeled urediospores, concluded that there is an outward movement of ^{14}C from the pathogen, and that it probably occurs along the intercellular mycelia. Assuming that this movement of solutes could be bidirectional, the host, via the apoplastic space, might be able to provide a significant amount of nutrients to the pathogen. Certainly the water film lining the intercellular spaces and saturating the cell wall (i.e., the apoplast) is replete with inorganic and organic nutrients. In tobacco, for example, the intercellular fluid of leaf tissue contains, per square centimeter of surface area, about 6 µg of carbohydrate, 20 nmol of assorted amino acids and ammonia, and 50 nmol of inorganic solutes (R. D. Durbin, unpublished data). To put this in some perspective, these values would roughly correspond to a very dilute microbial culture medium.

Another factor that helps a rust pustule to become a significant sink is the movement of water. Initially after infection, there appears to be a transient decrease in water loss because of a decrease in stomatal aperture (as measured by an increase in the diffusive resistance of leaves). However, once the epidermis is ruptured by the sporulating fungus, an abrupt and dramatic increase in water loss may occur (Duniway, 1976; Suksayretrup *et al.*, 1982). In contrast to other changes in moisture flux, the plant has very little control over this loss. Such a rapid and localized loss results in an increase in water movement toward the pustule-containing area, an effect that could lead to the accumulation of even more solutes by mass flow. It has been suggested that one way to minimize damage of this type might be to breed for high stomatal resistance (Suksayretrup *et al.*, 1982).

IV. Factors Responsible for Pathogen-Induced Imbalances

One of the major unanswered questions concerns which mechanism(s) are involved in solute accumulation by rust fungi. When considering this question,

we need to realize that the process is a continuum involving the directed mobilization and translocation of solutes, as well as their movement across the host–pathogen interface, and their subsequent movement and metabolism within the parasite. Taking this view, it seems probable that a number of mechanisms acting in parallel as well as in concert contribute to solute accumulation. Unfortunately, detailed information about how rust fungi are able to cause accumulation is necessarily incomplete, because the mechanisms that operate even in healthy plants are not clearly understood. In addition, these fungi, because of their essentially obligate nature (i.e., they adapt poorly to artificial media and lack normal haustoria *in vitro*), are difficult experimental subjects. Furthermore, it is not clear to what extent analogies can be made among the different pathogens causing biotrophic diseases. For instance, although haustoria of the Uredinales share several features with those of the Erysiphales (e.g., structure of the haustorial complex) (Bushnell and Gay, 1978), there are many differences that may alter or invalidate comparisons (e.g., host tissues in which haustoria are formed, kinds and quantity of fungal polyols, and the presence of intercellular hyphae). The evidence presented by Harder and Chong (Chapter 14, this volume) indicates that the haustoria of cereal rust fungi have associated with them a complex of microtubule-like structures extending into the host's cytoplasm. Such structures have not as yet been seen in powdery mildews. Also, Spencer-Phillips and Gay (1981) found differences between the two groups with respect to ATPase activity of the haustorial plasma membranes. Thus rusts may fundamentally differ from the powdery mildews in how their haustoria obtain nutrients from the host.

One may be able to gain some insight into what factors are important in redirecting translocation patterns by considering work done in related areas. Accordingly, listed here are some potentially important factors that might be involved in rust-induced imbalances in solute distribution in cereal plants. As yet, there are very few direct experimental findings to support any one of them. However, this is mainly because appropriate experimental systems have not yet been developed rather than because of any accumulation of negative scientific results.

The ability of rust fungi to delay the senescence of the tissues around the infection site, which otherwise would act as a source for the developing meristems and grain, appears to be one of the major factors responsible for creating imbalances in solute translocation patterns. It is clear that senescence is regulated by a number of interactive processes operating either to promote or retard the process (Thimann, 1979; Thomas and Stoddart, 1980). Accordingly, when rust is superimposed upon this framework, it seems likely that it could affect senescence at one or several points by diverse mechanisms also acting in either a positive or negative manner. This can be seen in the following listing, in which several of the factors mentioned exert their influence, at least in part, on senescence.

A. HORMONE LEVELS

Rust researchers have long been intrigued by plant growth hormones because their effects on solute accumulation in cereals mimic, at least superficially, some of those exhibited by rust diseases. From these observations it has been postulated that the fungus in some way regulates hormone levels at and around the infection site so that solute accumulation is favored. Illustrative of this are the cytokinins, which by themselves can substantially alter translocation patterns by reducing the mobilization of reserves and their export from treated areas, especially in senescing leaves (Gilbert *et al.*, 1980). The result of this is a green zone surrounded by senescing, bleaching tissue. A phenomenon of similar appearance, called "green islands," is commonly observed in leaves infected by obligate parasites (Bushnell, 1967; Bushnell, Chapter 15, this volume).

Cytokinins apparently exert their delaying influence on senescence in part through a depression of specific enzyme systems involved in membrane function and solute transport (Gilbert *et al.*, 1980). Alterations in the balances among these enzymes, brought about either by changes in cytokinin levels or by some other factor that alters enzyme levels or their activities, might be involved in rust diseases. However, whereas accumulation in green islands on detached leaves can involve a net import, net accumulation in attached leaves is not very great, especially when compared with the amounts of nutrients going into spores at the infection site (Bushnell, 1967). Still unanswered is whether the changes resembling those induced by cytokinins have any role in making infected tissue such an effective sink.

Cytokinins as well as auxins and other growth hormones are known to undergo large increases in rusted tissues of cereals, although the reasons for the increases are not known (Bushnell, Chapter 15, this volume). Presumably these changes in the levels of plant growth hormones, particularly of cytokinins and auxins, may be involved with maintaining the host tissues adjacent to the pustule in a juvenile state such that senescence and its associated shift to an exporting status is both delayed and decreased in intensity. Whether the newly produced hormones' originate in the host or the parasite is uncertain. We know essentially nothing about how the pathogen might initiate host responses that could lead the host to produce elevated hormone levels, or whether the host can affect the pathogen's ability to produce these hormones and/or degrade them. Certainly, the complex changes in growth hormones deserve further study in relation to their role in controlling translocation processes in rust diseases.

B. TOXINS

Sempio (1959) has called attention to various ways in which toxins might be responsible for creating nutrient imbalances. They include (1) impaired

transport, (2) changes in membrane permeability, (3) inhibition of metabolite synthesis, and (4) inhibition of metabolite utilization. If, in rust diseases, toxins produced by the pathogen are indeed important for creating such effects, they probably are acting quite close to and on either side of the host–parasite interface (see next section). There is no experimental evidence to show that toxins are translocated any distance away from the infection site, although by analogy with those from other pathogens, this is conceivable (Durbin, 1981).

C. MEMBRANE STRUCTURE

Currently, major advances are being made in our understanding of membrane structure as well as the driving forces responsible for the transmembrane movement of various types of solutes. From work done in this field, it seems possible that rust fungi may synthesize proteins or other compounds that can increase the membrane permeability of the host cell by inducing its plasmalemma to pass through a sequence of conformational states. These changes in state can be effected by molecules that bind either noncovalently (i.e., allosteric effectors) or by covalent bonding to functional groups present on the membrane surface. Evidence for this type of mechanism has been found with tobacco mosaic virus coat protein (Banerjee *et al.*, 1981), peptide hormones (Poss *et al.*, 1978), and toxins (Sessa *et al.*, 1969), all of which interact with and destabilize membranes. Such alterations are known to modulate the ionic conductance of membranes, as for example in vision (Montal *et al.*, 1977) and egg fertilization (Ridgeway *et al.*, 1977). Similar membrane effects could be produced by toxins or other compounds acting as ionophores (Durbin, 1981). The possibility that obligate parasites may alter membrane composition and/or structure is suggested by recent work on barley powdery mildew. Changes in the pattern of cell plasmolysis and plasmalemma permeability after infection have led to the postulation that infection alters the neutral lipids but not the phospholipids of the plasmalemma (Lee-Stadelmann *et al.*, 1982).

The classical work of Thatcher (1939, 1942, 1943) showed that infection of susceptible, but not resistant, wheat cultivars by *Puccinia graminis* f. sp. *tritici* caused an increase in host cell permeability to several nonelectrolytes as well as to water. In this connection, we now know that various types of compounds enter plant cells via different portals. Some utilize the phospholipid bilayer, whereas others are transported by proteins. Consequently, it is an oversimplification to speak of a general increase in the permeability of host cells. Rather, the different pathways for transmembrane movement should be examined separately to see what role they might play in the effect Thatcher observed. Obviously, further study of this phenomenon could be very informative.

D. ENZYME ACTIVITIES

The pathogen may also be able to initiate other changes in the host membrane that could aid in its acquisition of nutrients. For example, Borochov *et al.* (1982) have suggested that senescence may be controlled by membrane fluidity. They found that the fluidity of the lipid core decreased with age because of a reduced capacity of the plant cells to synthesize membrane phospholipids and their enhanced capacity to degrade them via phospholipase A. When this happens the membrane becomes "leaky." A similar phenomenon in rust infections could be a contributing factor to solute translocation out of plant cells (Gilbert *et al.*, 1980), or for the alterations described by Thatcher.

Enzymes involved in the transmembrane movement of solutes might be another example. For example, changes in membrane-bound transport ATPases appear to be important factors for solute transfer across the haustorial–plasmalemmal interface. According to Spencer-Phillips and Gay (1981) in their studies on bean rust, the host's plasma membrane in the structural domain of the haustoria (i.e., extrahaustorial region bounded by the haustorial neckband) lacks normal ATPase activity. There was also no evidence of ATPase activity in the haustorial plasma membrane. Thus transport across this portion of the host–parasite interface is thought to be passive.

Still another possibility involves changes in the activities of enzymes responsible for the synthesis and degradation of translocatable metabolites. Such changes, if they occur under the influence of the rust fungus, could play a significant role in determining source–sink activities. For instance, reactions that lead to the removal of sucrose at the infection site (i.e., the sink) would increase translocation, because the sucrose gradient between the site and its sources has now been increased. Thus increases in invertase and amylase activities noted in rusted tissues may be particularly pertinent. In the former case, Clancy and Coffey (1980) found up to a 24-fold increase for this enzyme in rusted flax leaves. Although the cellular location of these alterations is not known, their net result would be to enhance the accumulation of soluble substances in the infected regions. Also possibly related to this problem are the findings that *Uromyces phaseoli* produces an activator of β-amylase (Schipper and Mirocha, 1969), and *Puccinia recondita* causes both a localized and systemic activation of peptidases (Huber, 1978).

Some years ago, Atkin and Neilands (1972) showed that various siderophores would induce the formation of green islands. They postulated that in rust diseases these substances might play a role in the formation of this symptom by complexing iron and transporting it into the fungus at the host's expense. The result of this could be a massive alteration in the content of iron-containing enzymes in the host. Unfortunately, this idea has not been further studied.

E. WATER POTENTIAL

Nutrients are withdrawn from water-stressed tissues at a reduced rate (Hocking, 1982). Thus, if a rusted leaf is under water stress, the stress of itself will tend to reduce nutrient export from that leaf. This reduction could, in turn, slow protein degradation, and hence delay senescence of the leaf, if the two processes were linked by some kind of feedback mechanism. Because water potential is a central factor in controlling the plant's biochemical and physiological processes, many of the alterations observed with rust infection may basically result from changes in water potential (Duniway, 1976). A pressure gradient could be the driving force for movement toward the rust fungus. Likewise, osmotic uptake of water by the pathogen could be required for solute translocation within the fungus. Unfortunately, we do not know enough about the influence of rusts on the water relations of the host. Such information would be very pertinent for determining to what extent water potential may be linked to solute imbalances.

V. Applications

Tolerance can be defined as the ability of a plant to yield more than would normally be expected considering the amount of disease present. Under this general heading there appear to be grouped a number of diverse physiological phenomena that contribute to disease tolerance in different ways. Some of these ways involve interactions with the mechanisms governing translocation. In some cases tolerance appears to be due to the host's ability to continue to “fill” the developing grain in spite of a moderate to high number of pustules of an infection type that ordinarily would categorize the cultivar as susceptible. In other cases the pathogen's development is obviously retarded. Such cultivars are referred to as “slow-rusting” types. Here, the host appears to be able to divert nutrients away from the pathogen to the extent that the pathogen's rate of development is markedly reduced. Perhaps in these cultivars the developing grain is such an effective sink vis à vis the rust infection that normal translocation patterns are largely maintained. Alternatively, or additionally, the host may be producing some factor(s) that in some way hinders substrate utilization by the fungus or specifically delays its sporulation.

In some cultivars the flag leaf, glumes, and/or awns contribute substantial amounts of photosynthate to the developing grain (Durbin, 1967). Because of their proximity to the developing grain, they develop very strong source–sink relationships with the grain. Also, such structures, being younger than the remainder of the shoot, tend to be more lightly infected and hence less subject

to the “sink effect” of rust infections. Even more emphasis needs to be placed on exploiting this important type of tolerance.

We need to identify the kinds of physiological mechanisms that operate in these cases and determine how they might be used to minimize disease losses. At present, it is not clear if restricting the pathogen's influence on translocation patterns can be a cause of host resistance, or whether it merely reflects an effect following from the expression of some other resistance mechanism. Still, it is feasible to look for mechanisms and substances that could act on sources and/or sinks, and upset the pathogen's effect on translocation patterns (i.e., reorder the priorities for solute distribution). Another strategy to minimize rust effects might be to develop cultivars with extended or shifted heading periods. If we could somehow manipulate these host processes, we might be on the threshold of developing a very useful control procedure.

VI. Conclusion

Although our understanding is incomplete on major aspects of how solute distribution patterns are altered by rust fungi, there is a growing body of information indicating that this phenomenon is central to controlling their growth potential, and that the effect of solute redistribution in the host constitutes one of the major ways in which rusts affect productivity, and conversely that solute redistribution can be a controlling factor in the growth of the pathogen. Assuming that these views are sustained, we should direct increasing efforts toward the study of solute redistribution mechanisms, employing a coordinated effort by diverse disciplines to develop control strategies.

References

Allen, P. J. (1953). Toxins and tissue respiration. *Phytopathology* **43**, 221–229.

Atkin, C. L., and Neilands, J. B. (1972). Leaf infections: Siderochromes (natural polyhydroxamates) mimic the “green island” effect. *Science* **176**, 300–302.

Banerjee, S., Vandenbranden, M., and Ruysschaert, J. M. (1981). Interaction of tobacco mosaic virus protein with lipid membrane systems. *FEBS Lett.* **133**, 221–224.

Borochov, A., Halevy, A. H., and Shinitzky, M. (1982). Senescence and the fluidity of rose petal membranes: Relationship to phospholipid metabolism. *Plant Physiol.* **69**, 296–299.

Bracker, C. E., and Littlefield, L. J. (1973). Structural concepts of host–pathogen interfaces. *In* “Fungal Pathogenicity and the Plant's Response” (R. J. W. Byrde and C. V. Cutting, eds.), pp. 159–318. Academic Press, New York.

Bushnell, W. R. (1967). Symptom development in mildewed and rusted tissues. *In* "The Dynamic Role of Molecular Constituents in Plant–Parasite Interactions" (C. J. Mirocha and I. Uritani, eds.), pp. 21–39. Am. Phytopathol. Soc. Press, St. Paul, Minnesota.

Bushnell, W. R., and Gay, J. (1978). Accumulation of solutes in relation to the structure and function of haustoria in powdery mildews. *In* "The Powdery Mildews" (D. M. Spencer, ed.), pp. 183–235. Academic Press, New York.

Bushnell, W. R., and Rowell, J. B. (1968). Premature death of adult rusted wheat plants in relation to carbon dioxide evolution by root systems. *Phytopathology* **58**, 651–658.

Carver, T. L. W., and Griffiths, E. (1981). Relationship between powdery mildew infection, green leaf area and grain yield of barley. *Ann. Appl. Biol.* **99**, 255–266.

Causton, D. R., and Venus, J. C. (1982). "The Biometry of Plant Growth." University Park Press, Baltimore, Maryland.

Clancy, F. G., and Coffey, M. D. (1980). Patterns of translocation, changes in invertase activity, and polyol formation in susceptible and resistant flax infected with the rust fungus *Melampsora lini*. *Physiol. Plant Pathol.* **17**, 41–52.

Cornu, M. (1881). Prolongation de 1'activité végétative des cellules chlorophylliennes sous l'influence d'un parasite. *C. R. Hebd. Seances Acad. Sci.* **93**, 1162–1164.

Cronshaw, J. (1981). Phloem structure and function. *Annu. Rev. Plant Physiol.* **32**, 465–484.

Daly, J. M. (1967). Some metabolic consequences of infection by obligate parasites. *In* "The Dynamic Role of Molecular Constituents in Plant–Parasite Interactions" (C. J. Mirocha and I. Uritani, eds.), pp. 144–164. Am. Phytopathol. Soc. Press, St. Paul, Minnesota.

Doodson, J. K., Manners, J. G., and Myers, A. (1965). Some effects of yellow rust (*Puccinia striiformis*) on ^{14}carbon assimilation and translocation in wheat. J. *Exp. Bot.* **16**, 304–317.

Duniway, J. M. (1976). Water status and imbalance. *Encycl. Plant Physiol., New Ser.* **4**, 430–449.

Durbin, R. D. (1967). Obligate parasites: effect on the movement of solutes and water. *In* "The Dynamic Role of Molecular Constituents in Plant–Parasite Interactions" (C. J. Mirocha and I. Uritani, eds.), pp. 80–99. Am. Phytopathol. Soc. Press, St. Paul, Minnesota.

Durbin, R. D., ed. (1981). "Toxins in Plant Disease." Academic Press, New York.

Ehrlich, M. A., and Ehrlich, H. G. (1970). Electron microscope radioautography of ^{14}C transfer from rust uredospores to wheat leaf cells. *Phytopathology* **60**, 1850–1857.

Fondy, B. R., and Geiger, D. R. (1980). Effect of rapid changes in sink-source ratio on export and distribution of products of photosynthesis in leaves of *Beta vulgaris* L. and *Phaseolus vulgaris* L. *Plant Physiol.* **66**, 945–949.

Geiger, D. R. (1976). Phloem loading in source leaves. *In* "Transport and Transfer Processes in Plants" (I. F. Wardlaw and J. B. Passioura, eds.), pp. 166–183. Academic Press, New York.

Giaquinta, R. T. (1979). Phloem loading of sucrose. Involvement of membrane ATPase and proton transport. *Plant Physiol.* **63**, 744–748.

Gilbert, M. L., Thompson, J. E., and Dumbroff, E. B. (1980). Delayed cotyledon senescence following treatment with a cytokinin; an effect at the level of membranes. *Can. J. Bot.* **58**, 1797–1803.

Hartt, C. E. (1963). Translocation as a factor in photosynthesis. *Naturwissenschaften* **21**, 666–667.

Hay, R. E., Earley, E. B., and DeTurk, E. E. (1953). Concentration and translocation of nitrogen compounds in the corn plant (*Zea mays*) during grain development. *Plant Physiol.* **28**, 606–621.

Heath, M. C. (1972). Ultrastructural of host and non-host reactions to cowpca rust. *Phytopathology* **62**, 27–38.
Heath, M. C. (1976). Ultrastructural and functional similarity of the haustorial neckband of rust fungi and the Casparian strip of vascular plants. *Can. J. Bot.* **54**, 2484–2489.
Heyser, W. (1980). Phloem loading in the maize leaf. *Ber. Dtsch. Bot. Ges.* **93**, 221–228.
Hocking, P. J. (1982). Effect of water stress on redistribution of nutrients from leaflets of narrow-leaved lupin (*Lupinus angustifolius* L.). *Ann. Bot.* (*London*) [N.S.] **49**, 541–543.
Holhgan, P. M., Chen, C., McGee, E. E. M., and Lewis, D. H. (1974). Carbohydrate metabolism in healthy and rusted leaves of coltsfoot. *New Phytol.* **73**, 881–888.
Huber, S. C. (1978). Disturbed mineral nutrition. *In* "Plant Disease" (J. G. Horsfall and E. B. Cowling, eds.), Vol. 3, pp. 163–181. Academic Press, New York.
Huber, S. C. (1981). Inter- and intra-specific variation in photosynthetic formation of starch and sucrose. *Z. Pflanzenphysiol.* **101**, 49–54.
King, R. W., Wardlaw, I. F., and Evans, L. T. (1967). Effect of assimilate utilization on photosynthetic rate in wheat. *Planta* **77**, 261–276.
Kuo, I., O'Brien, T. P., and Canny, M. J. (1974). Pit-field distribution, plasmodesmatal frequency, and assimilate flux in the mestome sheath cells of wheat leaves. *Planta* **121**, 97–118.
Läuchli, A. (1976). Apoplastic transport in tissues. *Encycl. Plant Physiol., New Ser.* **2**, 3–34.
Lee-Stadelmann, O. T., Bushnell, W. R., and Stadelmann, E. J. (1982). Plasmolysis form and membrane permeability of *Hordeum vulgaris* coleoptile cells by *Erysiphe graminis*. *Plant Physiol., Suppl.* 69, 795.
Livne, A. (1964). Photosynthesis in healthy and rust-infected plants. *Plant Physiol.* **39**, 614–621.
Livne, A., and Daly, J. M. (1966). Translocation in healthy and rust-infected beans. *Phytopathology* **56**, 170–175.
Loomis, R. S., Ng, E., and Hunt, W. F. (1976). Dynamics of development in crop production systems. *In* "CO_2 Metabolism and Plant Productivity" (R. H. Burris and C. C. Black, eds.), pp. 269–286. University Park Press, Baltimore, Maryland.
Lüttge, V., and Pitman, M. G., eds. (1976). "Transport in Plants II. Part B. Tissues and Organs," Encycl. Plant Physiol., New Ser., Vol. 2. Springer-Verlag, Berlin and New York.
Madore, M., and Webb, J. A. (1981). Leaf free space analysis and vein loading in *Cucurbita pepo*. *Can. J. Bot.* **59**, 2550–2557.
Marshall, C., and Wardlaw, I. F. (1973). A comparative study of the distribution and speed of movement of ^{14}C assimilates and foliar-applied ^{32}P-labelled phosphate in wheat. *Aust. J. Biol. Sci.* **26**, 1–13.
Martin, P. (1982). Stem xylem as a possible pathway for mineral retranslocation from senescing leaves to the ear in wheat. *Aust. J. Plant Physiol.* **9**, 197–207.
Mendgen, K. (1977). Reduced lysine uptake by bean rust haustoria in a resistant reaction. *Naturwissenschaften* **64**, 438–439.
Montal, M., Darszon, A., and Trissl, H. W. (1977). Transmembrane channel formation in rhodospin bilayer membranes. *Nature* (*London*) **267**, 221–225.
Owera, S. A. P., Farrar, J. F., and Whitbread, R. (1981). Growth and photosynthesis in barley. *Physiol. Plant Pathol.* **18**, 79–90.
Pate, J. S. (1975). Exchange of solutes between phloem and xylem and circulation in the whole plant. *Encycl. Plant Physiol., New Ser.* **1**, 453–473.
Pate, J. S. (1980). Transport and partitioning of nitrogenous solutes. *Annu. Rev. Plant Physiol.* **31**, 313–340.

Peturson, B., Forsyth, F. R., and Lyon, C. B. (1958). Chemical control of cereal rusts. II. Control of leaf rust of wheat with experimental chemicals under field conditions. *Phytopathology* **48**, 655–657.

Poss, A., Deleers, M., and Ruysschaert, J. M. (1978). Evidence for a specific interaction between GT! ganglioside incorporated into bilayer membranes and thyrotropin. *FEBS Lett.* **86**, 160–162.

Rick, P. D., and Mirocha, C. J. (1968). Fixation of carbon dioxide in the dark by the malic enzyme of bean and oat stem rust uredospores. *Plant Physiol.* **43**, 201–207.

Ridgeway, E., Gilkey, J. C., and Jaffe, L. (1977). Free calcium increases explosively in activating Medaka eggs. *Proc. Natl. Acad. Sci., U.S.A.* **74**, 623–627.

Schipper, A. L., Jr., and Mirocha, C. J. (1969). The mechanism of starch depletion in leaves of *Phaseolus vulgaris* infected with *Uromyces phaseoli. Phytopathology* **59**, 1722–1727.

Sempio, C. (1959). The host is starved. *In* "Plant Pathology" (J. G. Horsfall and A. E. Dimond, eds.), Vol. 1, pp. 277–312. Academic Press, New York.

Sessa, G., Freer, J. H., Colacicco, G., and Weissmann, G. (1969). Interaction of a lytic polypeptide, melittin, with lipid membrane systems. *J. Biol. Chem.* **244**, 3575–3582.

Shaw, M. (1963). The physiology and host–parasite relations of rusts. *Annu. Rev. Phytopathol.* **1**, 259–294.

Shaw, M., and Samborski, D. I. (1956). The physiology of host–parasite relations. I. Accumulation of radioactive substances at infections of facultative and obligate parasites including tobacco mosaic virus. *Can. J. Bot.* **34**, 389–405.

Siddiqui, M. Q., and Manners, J. G. (1971). Some effects of general yellow rust (*Puccinia striiformis*) infection on carbon assimilation, translocation and growth in a spring wheat. J. *Exp. Bot.* **22**, 792–799.

Simpson, R. J., Lambers, H., and Dalling, M. J. (1982). Translocation of nitrogen in a vegetative wheat plant (*Triticum aestivum*). *Physiol. Plant.* **56**, 11–17.

Spanswick, R. M. (1976). Symplasmic transport in tissues. *Encycl. Plant Physiol., New Ser.* **2**, 35–53.

Spencer-Phillips, P. T. N., and Gay, J. L. (1981). Domains of ATPase in plasma membranes and transport through infected plant cells. *New Phytol.* **89**, 393–400.

Stocking, C. R., and Heber, V., eds. (1976). "Transport in Plants. III. Intercellular Interactions and Transport Processes," Encycl. Plant Physiol., New Ser., Vol. 3. Springer-Verlag, Berlin and New York.

Suksayretrup, K., Kirkham, M. B., and Young, H. C., Jr. (1982). Stomatal resistance of five cultivars of winter wheat infected with leaf rust (*Puccinia recondita* f. sp. *tritici*). *Z. Acker- Pflanzenbau* **151**, 118–127.

Sutcliffe, J. F. (1976). Regulation in the whole plant. *Encycl. Plant Physiol., New Ser.* **2**, 394–417.

Thatcher, F. S. (1939). Osmotic and permeability relations in the nutrition of fungus parasites. *Am. J. Bot.* **26**, 449–458.

Thatcher, F. S. (1942). Further studies of osmotic and permeability relations in parasitism. *Can. J. Res., Sect. C* **20**, 283–311.

Thatcher, F. S. (1943). Cellular changes in relation to rust resistance. *Can. J. Bot.* **C21,** 151–172.

Thimann, K. V., ed. (1979). "Senescence in Plants." CRC Press, Boca Raton, Florida.

Thomas, H., and Stoddart, J. L. (1980). Leaf senescence. *Annu. Rev. Plant Physiol.* **31**, 83–111.

von Sydow, B. (1966). Uber den Einbau von $C^{14}O_2$ in Weizenblätter nach Infektion mit *Puccinia gramanis tritici*. I. Histologische Untersuchungen. *Phytopathol. Z.* **56**, 78–96.

von Sydow, B., and Durbin, R. D. (1962). Distribution of C^{14}-containing metabolites in wheat leaves infected with stem rust. *Phytopathology* **52**, 169–170.

Wareing, P. F., and Patrick, J. W. (1975). Source–sink relations and the partition of assimilates in the plant. *In* "Photosynthesis and Productivity in Different Environments" (J. P. Cooper, ed.), Int. Biol. Programme 3, pp. 481–499. Cambridge Univ. Press, London and New York.

Williams, R. F. (1955). Redistribution of mineral elements during development. *Annu. Rev. Plant Physiol.* **6**, 25–42.

Wyse, R. E., and Saftner, R. A. (1982). Reduction in sink-mobilizing ability following periods of high carbon flux. *Plant Physiol.* **69**, 226–228.

Yarwood, C. E., and Childs, J, F. L. (1938). Some effects of rust infection on the dry weight of host tissues. *Phytopathology* **28**, 723–733.

Yarwood, C. E., and Cohen, M. (1951). Hypertrophy from the uredial stage of bean rust. *Bot. Gaz.* (*Chicago*) **112**, 294–300.

Zaki, A. I., and Durbin, R. D. (1965). The effect of bean rust on the translocation of photosynthate from disease leaves. *Phytopathology* **55**, 528–529.

Zimmerman, M. H., and Milburn, J. A., eds. (1975). "Transport in Plants I. Phloem Transport," Encycl. Plant Physiol., New Ser., Vol. 1. Springer-Verlag, Berlin and New York.

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