

7

Histology and Molecular Biology of Host-Parasite Specificity

R. Rohringer

*Agriculture Canada Research Station, Winnipeg, Manitoba,
Canada*

R. Heitefuss

*Institut für Pflanzenpathologie und Pflanzenschutz,
Göttingen-Weende, Federal Republic of Germany*

I.	Introduction	193
	A. Impact of Genetics	194
	B. Levels of Specificity	194
II.	Histology	196
	A. General Remarks	196
	B. Early Inhibition of Fungal Growth	200
	C. Indeterminant Hypersensitivity	203
	D. Diffusible Fungal Determinant of Hypersensitivity ("Rust Toxin")	208
	E. Delayed Expression of Incompatibility	210
III.	Molecular Biology	212
	A. General Remarks	212
	B. Proteins and Macromolecular Glycosubstances	212
	C. Nucleic Acids	215
IV.	Present Trends, New Technology	218
	A. Histology as an Aid to Biochemical Work on Host-Parasite Specificity	218
	B. Biochemical Approaches	219
	References	223

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 interac-

tions (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 "nonhost 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 impor-

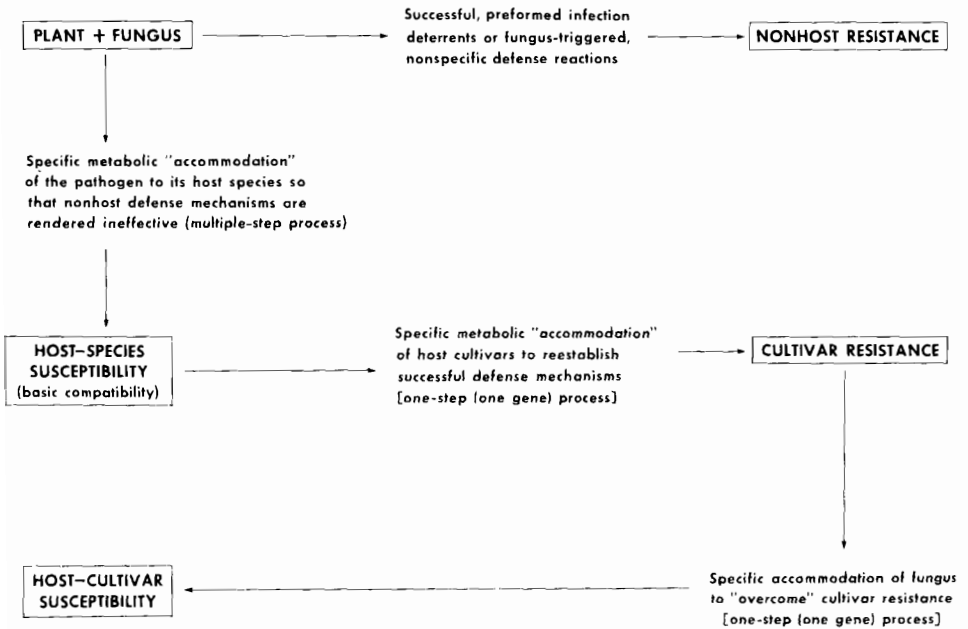


Fig. 1. Postulated events leading to species and cultivar specificity of a fungal pathogen (redrawn from Heath, 1981b).

tant 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.

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 histopathological 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 mor-

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- <i>Puccinia graminis</i> f. sp. <i>tritici</i> ^b			
<i>Sr6/P6</i>	1+	≤24	≤24
<i>Sr5/P5</i>	0	≤24	≤24
<i>Sr8/P8</i>	1+	72	60
<i>Sr22/P22</i>	2	96	72
Wheat- <i>Puccinia recondita</i> ^c <i>Lr20/P20</i>	;	48	36 ^e
Oats cv. Shokan I- <i>Puccinia coronata</i> race 226 ^d	0	20	28

^aFor 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.

^bRohringer *et al.* (1979).

^cJones and Deverall (1977a).

^dTani and Yamamoto (1979).

^eFirst physiological changes detected 20 hr after inoculation.

phologically 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.

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 autofluorescence is not specific to incompatible interactions between host and pathogen was demonstrated by treatment of healthy leaves with diethylaminoethyl-dextran (DEAE-dextran), which induced necrosis in

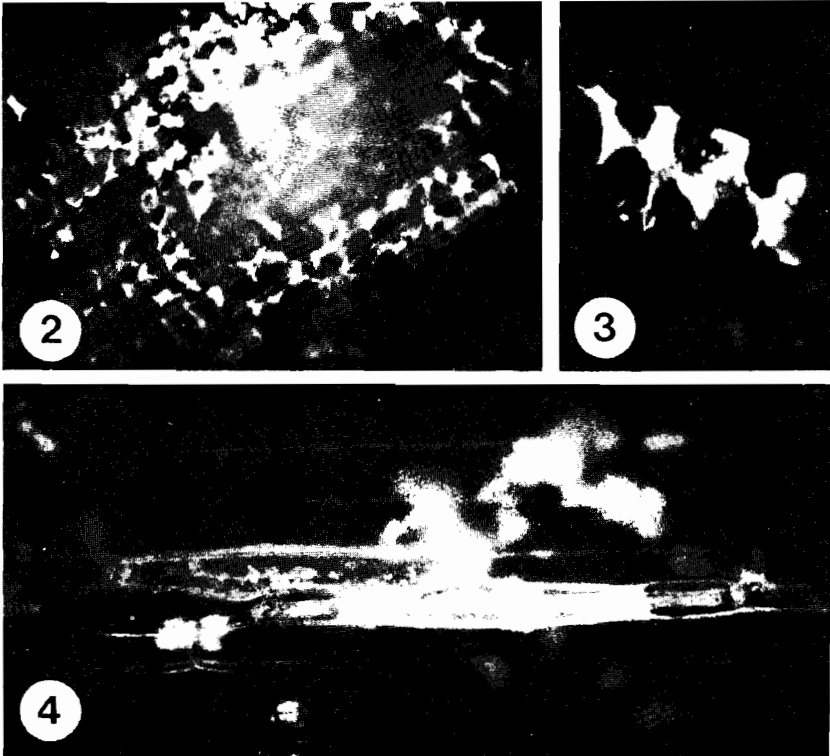


Fig. 2. Fluorescence photomicrograph of autofluorescing wheat leaf cells from incompatible interactions with stem rust of wheat. A fungal colony is shown, isolated by macerating enzymes from an infected leaf and surrounded by necrotic and collapsed mesophyll cells [*Str6/P6* interactions; W. K. Kim, unpublished].

Fig. 3. Fluorescence photomicrograph as in Fig. 2. A single collapsed mesophyll cell is shown, isolated by macerating enzymes from infected tissue [*Str6/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).

cells that then showed the same type of autofluorescence and similar ultrastructural changes as could be observed in hypersensitively reacting cells (Harder *et al.*, 1979b).

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 (Kuck *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 *P5* 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 haustorium 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. coronata* 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

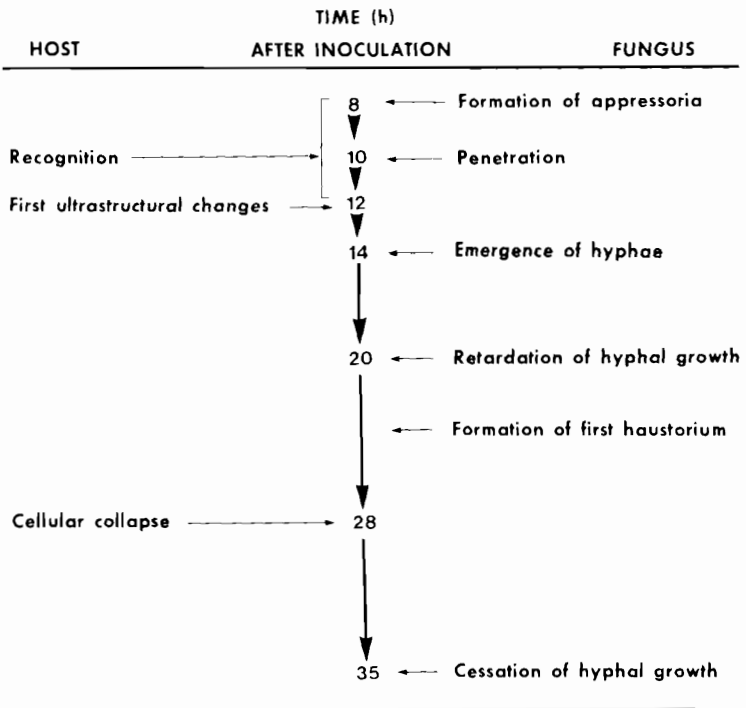


Fig. 5. Schematic representation of events in the incompatible interaction between oat leaves (cv. Shokan I) and crown rust of oats (race 226) (after Tani and Yamamoto, 1979).

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.

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 autofluorescence 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*

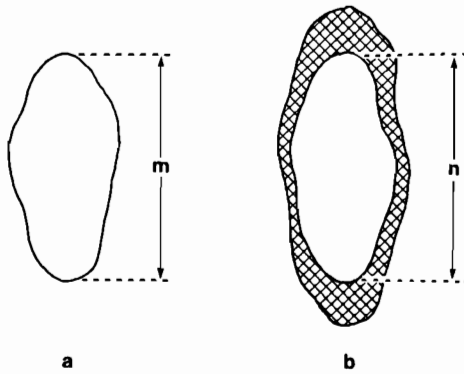


Fig. 6. Schematic representation of the "necrotic ring effect" in the temperature-dependent *Sr6/P6* interaction between wheat and stem rust of wheat. This effect is observed when the genotypically avirulent race is grown initially at 25°C (compatible) and then transferred to 19°C (incompatible). (a) 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).

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" ultrastructurally. 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.

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

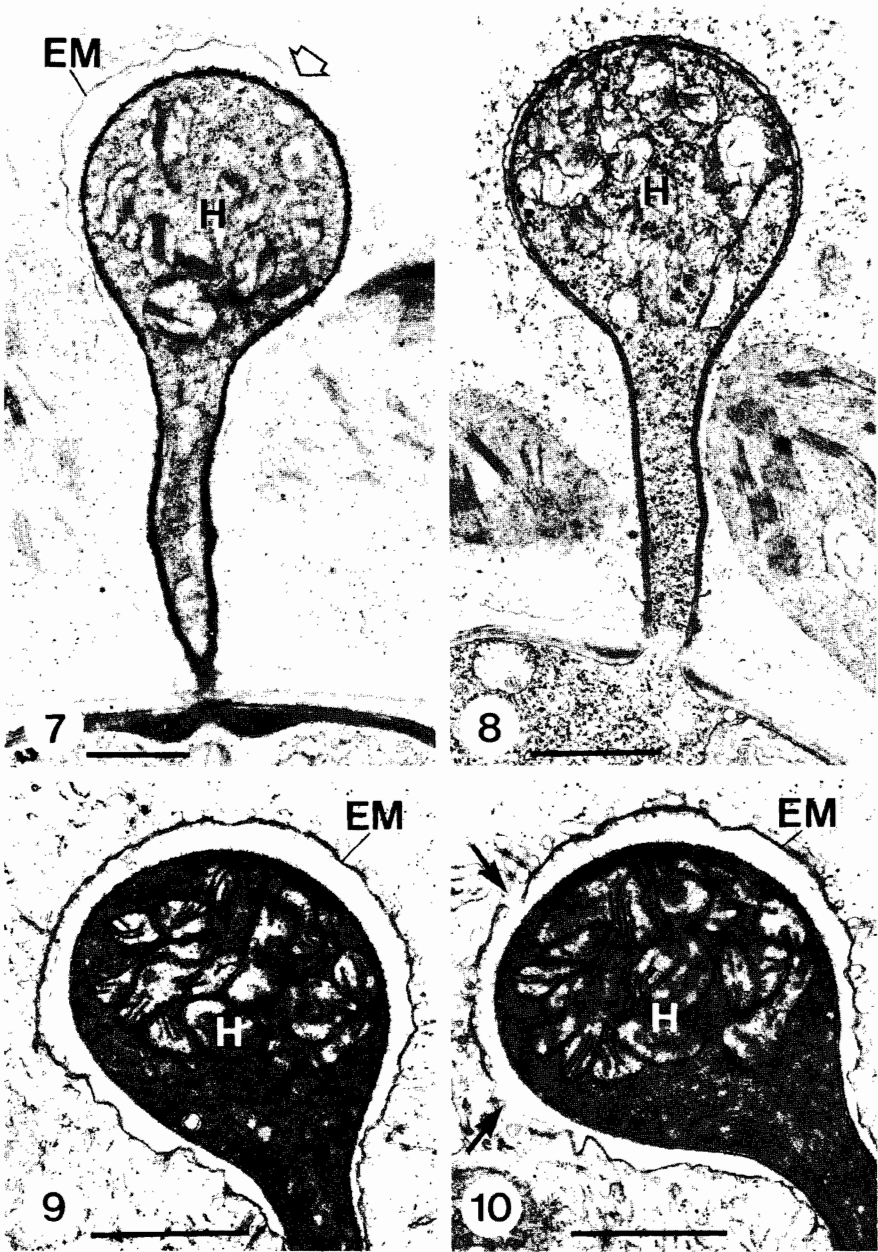


Fig. 7. Electron photomicrograph of a young haustorium [H] of wheat stem rust in the *Sr6/P6* interaction [incompatible]; the extrahaustorial membrane [EM] is discontinuous [open arrow] around a portion of the haustorial body ($\times 13,700$; bar $1 \mu\text{m}$; Harder *et al.*, 1979b).

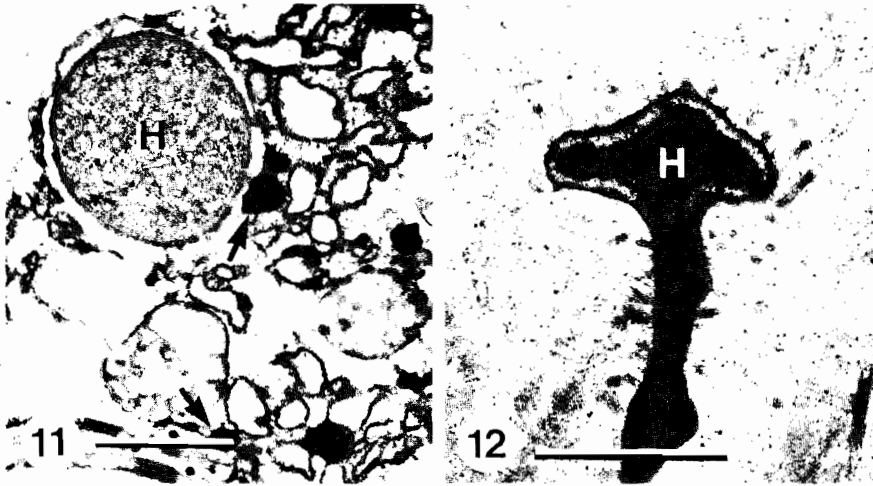


Fig. 11. Electron photomicrograph of an apparently "normal" haustorium (H) in the *Sr6/P6* interaction (incompatible) between wheat and stem rust of wheat. The electron-dense deposits [arrows] indicate the onset of host cell necrosis ($\times 18,200$; bar $1\ \mu\text{m}$; Harder *et al.*, 1979b).

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 ($\times 24,900$; bar $1\ \mu\text{m}$; Harder *et al.*, 1979b).

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.

In the incompatible interaction between stem rust of wheat and

Fig. 8. Electron photomicrograph of a typical young haustorium of wheat stem rust in the compatible (*sr6/P6*) interaction, here illustrated for comparison ($\times 17,100$; bar $1\ \mu\text{m}$; 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 ($\times 20,600$; bar $1\ \mu\text{m}$; 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) ($\times 20,600$; bar $1\ \mu\text{m}$; Harder *et al.*, 1979b).

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 [³H]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 in-

compatible 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 noninfected 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 fur-

ther 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 appressorium 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*

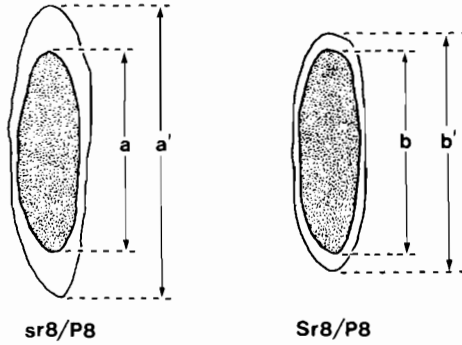


Fig. 13. Schematic representation illustrating delayed expression of incompatibility in stem rust of wheat. Wheat leaves near-isogenic with respect to the *Sr8* gene for resistance were inoculated with a race containing the *P8* gene for avirulence; gross colony structure was observed 72 hr after inoculation. 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).

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.

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-carboxyanilido-6-methyl-1,3-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 con-

tribute to the expression of resistance, for example, in the *Phytophthora megasperma*-soybean system. Albersheim and co-workers reported that low molecular weight β -(1 \rightarrow 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 -1,3-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 phytoalexins 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 avenalumin 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, systemically resistant leaves or following injection of leaves with polyacrylic 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 *Puccinia 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 ribosomal 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 V_{max} . 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 macroscopical 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 glycosubstances 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). Ultrastructural localization of enzymes, particularly that of glycosyltransferases (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 immunoglobulin 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-bean 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 in-

formation 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" (Söding, 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 (Rathmell 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 par-

ticipant 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 non-specific, 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 gene-specific. 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., Balázs, E., and Király, 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 lignification 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 arabinogalactans 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 Kuć, 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-¹⁴C, L-tyrosine-U-¹⁴C, and ferulate-U-¹⁴C. *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 Kuć, 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 nonsusceptibles 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 host-pathogen 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., Hümme, 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. 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. (1979). Hyphal tip cell ultrastructure of the fungus

- Fusarium*: Improved preservation by freeze-substitution. *J. Ultrastruct. Res.* **66**, 224–234.
- Howes, N. K., Kim, W. K., and Rohringer, R. (1982). Detergent-soluble polypeptides extracted from urediospores of 4 physiologic races of *Puccinia 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., Ser. A* **37**, 19–38.
- Jermyn, M. A., and Ycow, 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**, 275–284.
- Jones, D. R., and Deverall, B. J. (1977b). Experimental manipulation of the hypersensitive response 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, N. 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 may function 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). β -1,3-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 carbohydrates 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. Histochem. Cytochem.* **26**, 772–781.
- Kuč, J. (1982). Phytoalexins from the Solanaceae. In "Phytoalexins" (J. A. Bailey and J. 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 alla ruggine. *Phytopathol. Z.* **75**, 59–73.
- Mayama, S., Daly, J. M., and Rehfeld, D. W. (1975a). Hypersensitive response of near-isogenic 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 avenalumin 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 anfälligen und resistenten Bohnensorten. *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 rust 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., Dinoo, 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 tobacco 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., Lunderstädt, I., and Samborski, D. J. (1967). Metabolism of

- aromatic compounds in healthy and rust-infected primary leaves of wheat. I. Studies with $^{14}\text{CO}_2$, quinate- $\text{U-}^{14}\text{C}$, and shikimate- $\text{U-}^{14}\text{C}$ as precursors. *Can. J. Bot.* **45**, 863–889.
- 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.
- 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 Garavito, 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 coronata* 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). Nucleinsäure 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. 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 [*Sr6* 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.
- Söding, H. (1941). Über den Nachweis einer aus dem Interzellularraum von *Escheveria*blättern 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*-soybean 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 coronata 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.