P R O C E E D I N G S

TWENTY FIRST HARD WINTER WHEAT WORKERS WORKSHOP

January 28-30, 1998

U. S. Department of Agriculture
Agricultural Research Service
Lincoln, Nebraska

and

Department of Agronomy
Colorado State University
Fort Collins, Colorado

Sponsored by
The Hard Winter Wheat Improvement Committee

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Institute of Agriculture and Natural Resources

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Note of Appreciation

Those of us who work in the Great Plains Winter Wheat Region owe a great deal to Dr. C. James (Jim) Peterson who ably served as the USDA-ARS regional coordinator and as Secretary of the National Wheat Improvement Committee for many years. This proceedings will be the last that he served as editor. It is with heartfelt appreciation that we acknowledge his many years of tireless service to this region and to wheat workers in general, and wish him every success in Oregon.

P. Stephen Baenziger
Chair, Great Plains Winter Wheat Region

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FOREWORD

Over 140 wheat workers representing public and private wheat research programs from throughout the U.S., Canada, Mexico, and Turkey participated in the 21st Hard Winter Wheat Workers Workshop held in Denver, Colorado on January 28-30, 1998. This was the 21st Workshop since the initiation of the cooperative state-federal hard red winter wheat investigations in 1929. The Workshop has been held on three-year intervals and is sponsored by the Hard Winter Wheat Improvement Committee (HWWIC).

The format of this workshop follows the traditions established by the HWWIC for past Workshops. Priority research areas and topics were identified through open discussions among the HWWIC members, then session chairs were identified to organize the respective sessions of the Workshop. Our appreciation goes out to all those who participated in organizing this Workshop, the session chairs, and the many speakers who deserve credit for the overall success of this Workshop.

Submission of written material for this Proceedings was optional and the format and length of submission was left up to the authors. As such, the Proceedings do not reflect the scope of the presentations, nor the scope of intensity of discussions. A business meeting of the HWWIC was held during the Workshop and minutes of that meeting are included.

The HWWIC and Workshop organizers wish to express their sincere appreciation to Dr. Jim Quick and Colorado State University for hosting the meeting, and for financial support provided by Agripro Seeds, Hybritech Seed, Cargill-Goertzen Seed Research, and Trio Research.

Special thanks to Jan Preston for her help with organization and mailings for the Workshop, and for publication of these Proceedings.

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USDA-ARS, Lincoln, NE
Secretary, HWWIC

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WHEAT WORKER'S CODE OF ETHICS

Adopted by the
National Wheat Improvement Committee
November 5, 1994

This seed is being distributed in accordance with the "Wheat Workers' Code of Ethics for Distribution of Germplasm", developed and adopted by the National Wheat Improvement Committee on Nov. 5, 1994. Acceptance of this seed constitutes agreement.

1. The originating breeder, institution, or company has certain rights to the unreleased material. These rights are not waived with the distribution of seeds or plant material but remain with the originator.

2. The recipient of unreleased seeds or plant material shall make no secondary distributions of the germplasm without the permission of the owner/breeder.

3. The owner/breeder in distributing unreleased seeds or other propagating material grants permission for its use in tests under the recipient's control or as a parent for making crosses from which selections will be made. Uses for which written approval of the owner/breeder is required include:

(a) Testing in regional or international nurseries;
(b) Increase and release as a cultivar;
(c) Re-selection from within the stock;
(d) Use as a parent of a commercial F1 hybrid, synthetic, or multiline cultivar;
(e) Use as a recurrent parent in backcrossing;
(f) Mutation breeding;
(g) Selection of somaclonal variants; or
(h) Use as a recipient parent for asexual gene transfer, including gene transfer using molecular genetic techniques.

4. Plant materials of this nature entered in crop cultivar trials shall not be used for seed increase. Reasonable precautions to ensure retention or recovery of plant materials at harvest shall be taken.

HARD WINTER WHEAT IMPROVEMENT COMMITTEE
Membership, December 5, 1997

Colorado

Rob Bruns
Cathy Butti
Gordon Cisar
Blaine Johnson
J. P. Hill
John Moffatt
Jim Quick
Jim Reeder
John Shanahan

Kansas

Wally Bates
Bill Bockus
Bob Bowden
G. Brown-Guedira
O. K. Chung
Merle Eversmeyer
Dale Fjell
Bikram Gill
Jim Hatchett
Bill Heer
Ray Lamond
George Lookhart
Joe Martin
Pat McCluskey
Maureen Olewnick
Gary Paulsen
Sid Perry
John Raupp
Craig Roozeboom
Rollie Sears
Dallas Seifers
Jim Shroyer
Virgil Smail
Gerald Wilde
Jerry Wilson
Jim Wilson
Merle Witt

Nebraska

Stephen Baenziger
Roy French
Bob Graybosch
Jim Peterson
David Shelton
John Watkins

Oklahoma

Cheryl Baker
Brett Carver
Arron Guenzi
Robert Hunger
David Porter
Larry Singleton
Ed Smith
James Webster

South Dakota

Scott Haley
Yue Jin
Marie Langham

Texas

Allan Fritz
Mark Lazar
David S. Marshall
Jerry Michels
B. McDonald
Henry Nguyen
Charlie Rush
David Worrall

Montana

Phil Bruckner
Don Mathre

Washington

Ed Donaldson

Wyoming

Jim Krall

Idaho

Ed Souza

REGIONAL BUSINESS MEETING

Hard Winter Wheat Improvement Committee
January 29, 1998
Denver, CO

MINUTES

The meeting was called to order by Chairman Joe Martin at 3:30 p.m. Jim Peterson read current list of Committee members and established proper voting procedures for approving Committee actions. A list of Committee members is included in the minutes.

Members voted to approve minutes of the last meeting held at Stillwater, OK on January 26, 1995, and dispense with reading of the minutes. The minutes are printed in the Proceedings of the 20th Hard Red Winter Wheat Workers Conference, January 25-27, 1995, Oklahoma City, OK.

Status of Hard Winter Wheat Regional Nursery Program

Peterson reviewed changes in the HWW Regional Nursery program adopted by the HWWIC in 1995. The 'Wheat Worker's Code of Ethics' was adopted as formal policy for entry, distribution, and evaluation of germplasm through the Regional Nursery program. Private companies were approved to receive seed, grow performance nurseries, and contribute data to the regional report. Three condition must be met for a company to fully participate in the program: 1) the company must be active in germplasm development and breeding in the HWW region; 2) the company must show evidence that is conducts crossing and manages all segregating generations for evaluation and selection through commercial cultivar or hybrid release; and 3) the company must sign and document their acceptance of the Wheat Workers Code of Ethics in regard to handling of any seed through the Regional Nursery Program. The Regional Germplasm Observation Nursery was initiated in 1995 as a replacement for the Uniform Winterhardiness Nurseries.

Peterson indicated that no major changes were being proposed at this time in format or general operations of the Regional Nursery program. He then reviewed current checks in the Performance Nurseries and asked for input. Check varieties for the SRPN are to remain Kharkof, Scout 66, and TAM-107 with a maximum of 45 entries in the nursery. Haley proposed replacing Abilene with Nekota in the NRPN. Concern was expressed over loss of Abilene as a quality check in the nursery. Quick suggested that Abilene remain as a check and add Nekota as an additional check for a period of 2-3 years. Replacement of Abilene with Nekota would then be re-evaluated at a later date. Motion to do so was approved and NRPN checks will be Kharkof, Roughrider, Abilene and

Nekota, with review of the NRPN check cultivars scheduled for the next Workshop. Quick made a motion to replace Lamar with the new variety Prowers in the WPRPN. Prowers was developed from backcrossing Russian wheat aphid resistance into Lamar. Motion was seconded by Baenziger and approved. Baenziger suggested adding a hard white wheat check variety to the WPRPN in consideration of increasing interest and testing of hard whites in the western plains. It was proposed that KS95HW62-6, which is on track for release in 1999, be included as a check in the WPRPN and to replace the current check variety Siouxland. Motion was approved. Peterson noted that the new Regional Germplasm Observation Nursery (RGON) had been very well received and expressed his appreciation for the commitment and testing efforts of the many collaborators. Peterson did express concern, however, with the rapidly increasing number of entries in the RGON; from 340 entries in 1996 to 450 entries in 1998 . Baenziger motioned that the RGON be limited to 500 entries in total with a limit for any one breeder/geneticist of 40 entries. Motion was seconded by Quick and approved, with provision for the Regional Coordinator to truncate entries beyond 30/program as needed to meet the entry limit.

Seed requirements for the regional nurseries are currently 17 lb/entry in the SRPN; 11 lb/entry in NRPN; 2,000 gms in WPRPN; and 140 gms in the RGON. Seed is to be untreated. Seed of check varieties are increased and distributed with new entries each year from Lincoln, NE.

Moffatt proposed that all new entries in the Regional Performance Nursery entries be simultaneously entered into the National Small Grains Collection. There was much discussion and concern regarding impact on future PVP eligibility if experimental lines were made freely available through the Collection prior to official release. Moffatt indicated he would discuss the matter with Alan Atchley and Harold Bockelman and update the Committee at a later date. No other action was taken on the proposal.

Peterson reported on the web site developed for the Regional Nursery program. Items currently available for viewing/downloading include nursery lists and preliminary reports, the final nursery report for 1996, summary lists on release varieties and 1RS screening efforts, Nursery policies and cooperators, and information regarding HWWIC activities. The site address is 'HTTP://ianrwww.unl.edu/ianr/agronomy/region/' and can also be accessed through graingenes. In addition, Scott Haley has set up a list server to facilitate email communications among wheat researchers in the region. Contact Scott Haley directly to be added to the server email address list.

Quality Analyses for Regional Nursery Samples

Okky Chung reported on current status of quality testing for SRPN, NRPN, and WPRPN nurseries at the U.S. Grain Marketing Research Laboratory. The SRPN

and NRPN are being composited and evaluated for baking quality on an 'intraregional production zone' basis. As such, there are four composites evaluated for the SRPN and three composites for the NRPN. In addition, mixograph, SDS sedimentation, and single kernel characterization are being evaluated for individual sites within each production zone. The goal is to provide more comprehensive quality analyses and a measure of genotypic stability for end-use quality over environments. A complete review of the new testing approach and resulting data is planned for the next regional Workshop to be held in 2001.

Scott Haley has developed a new database system for management and reporting of data from regional nursery quality evaluations. The program effectively identifies lines with unique or defective quality attributes, allows flexibility in weighting variables used to measure end-use quality, and provides data summaries over years and nurseries. The database program is freely available and will be updated each year with new quality data from the USDA-GMPRL regional nursery evaluations. A more complete description of the database is included in the abstracts section of this Proceedings.

U.S. Wheat Associates

Ron Maas reported on concerns of the U.S. Wheat Associates regarding quality and competitiveness of our wheat in the export market. U.S. Wheat has appointed a Wheat Quality Committee to establish and recommend minimum quality standards for all classes of wheat grown in the U.S. The Committee also will be collecting samples of major varieties for evaluation by overseas customers. The Committee will suggest new standards and provide feedback to U.S. breeders with the goal of improving end-use quality and uniformity among new wheat varieties.

National Wheat Improvement Committee

Sears provided an update on activities and efforts of the National Wheat Improvement Committee. Sears reported that new funds have been obtained to support USDA-ARS pathology research efforts at Manhattan, KS; St. Paul, MN; Pullman, WA; and Raleigh, NC. The funds were obtained both through ARS's 'Emerging diseases' initiative, which was targeted to enhance research on Scab and Karnal bunt, and Congressional efforts. Sears indicated that the NWIC will join in support of the National Scab Initiative. The Initiative proposes funding of a multi-state effort on breeding, pathology, and toxin research related to fusarium head scab. A steering committee, chaired by Rick Ward, Michigan State University, is organizing efforts and support for the Initiative. The NWIC also will be working to support funding of the Wheat and Barley Genome Initiative, with the goal to develop molecular markers for public use.

Sears reported that the National Association of Wheat Growers has proposed establishment of a National Wheat Research Council. The Council is intended to bring together all components of the wheat industry, from grower through exporter, to speak with one voice on high priority issues and research needs of the industry. The NWIC will meet in conjunction with NAWG in January, 1999, to facilitate organization of the Council and provide input on key research needs and issues.

Election of Regional Officers

Stephen Baenziger was elected as Chair of the Hard Winter Wheat Improvement Committee. Scott Haley and John Moffatt were elected as regional representatives to the National Wheat Improvement Committee. A resolution of appreciation to Joe Martin, past chair, and past NWIC representatives Brett Carver, Stephen Baenziger, and David Worrall will be drafted by Peterson.

Site of Next Wheat Breeders Field Day

The 1998 Regional Breeders Field Day was set for June 30th at Sidney Nebraska. Based on history of past field days, the 1999 field day will be scheduled for Oklahoma.

Site of Next Regional Workshop

The next Workshop is to be hosted by Kansas State University in 2001; the date and exact location to be determined.

Martin and Peterson expressed the Committee's appreciation to the Local Organizing Committee for a very successful 21st Wheat Workers Workshop and a formal resolution of appreciation will be drafted by Peterson.

Respectfully submitted,

C. J. Peterson
Secretary, HWWIC

Resolutions

The following resolutions were unanimously adopted:

No. 1. Whereas, Joe Martin has provided superior and active leadership to the Hard Red Winter Wheat Improvement Committee; and

Whereas, Dr. Brett Carver, Dr. Stephen Baenziger and Dr. David Worrall, along with Joe Martin, have served as excellent and conscientious representatives of the Hard Red Winter Wheat Improvement Committee to the National Wheat Improvement Committee;

Be it therefore resolved, that the Hard Red Winter Wheat Improvement Committee expresses its sincere appreciation to past-Chairman Martin, Brett Carver, Stephen Baenziger and David Worrall for their efforts and superior contributions on behalf of the committee.

No. 2. Whereas, the 21st Hard Red Winter Wheat Workers Workshop has been an excellent and informative meeting and our hosts have expended much time and effort to ensure the success of the workshop;

Be it therefore resolved, the Hard Red Winter Wheat Workers express their sincere appreciation to Colorado State University researchers for serving as hosts in this workshop; to Jim Quick for organization and leadership in the local arrangements; to Sally Clayschulte, John Stromberger, and Bruce Clifford for local arrangements; to Cheryl Baker, Bob Bowden, Scott Haley, David Worrall, Brett Carver, John Moffatt, Jim Quick, Joe Martin, Tom Peeper, and Allan Fritz for serving as session chairs; and to regional officers Joe Martin, Stephen Baenziger, Brett Carver, David Worrall, and Jim Peterson for contributions to workshop planning.

Be it further resolved, the Hard Winter Wheat Workers express their sincere appreciation for financial support of the workshop from Agripro Seeds, Hybritech Seed, Cargill-Goertzen Seed Research, and Trio Research.

SESSION I

BIOTIC STRESSES - ENTOMOLOGY

Development of Control Strategies for Wheat Stem Sawfly

Phil L. Bruckner
Department of Plant Science, Montana State University

Wheat stem sawfly (*Cephus cinctus* Norton), a wasp endemic to North America, remains a major threat to wheat production in Montana. Originally, a stem-boring insect of the large-stemmed wild grasses, wheat stem sawfly adapted readily to spring wheat, and more recently to winter wheat. In today's presentation I will discuss the life cycle of wheat stem sawfly, how it damages wheat, take a brief look at sawfly from a historical perspective, then concentrate on control strategies, particularly host plant resistance and the development of resistant cultivars.

Life cycle and damage to wheat

The adult wheat stem sawfly is a nonfeeding wasp that emerges in Montana over a 4 to 6 week period beginning in late May or early June. The wasp is a weak flier with limited dispersal potential and infestation generally occurs in close proximity to the emergence site. Female wasps deposit a single egg within the lumen of the stem after penetrating the stem with a "saw-like" ovipositor. An elongating internode of the proper diameter is the preferred oviposition site. A single female wasp may lay 30 to 40 eggs. The eggs hatch in approximately 7 days and larvae begin feeding within the wheat stem on parenchyma and vascular tissue, eventually completing 4 to 5 instars. The completion of larval development corresponds closely to the beginning of plant senescence. At this time larvae migrate to an overwintering site at the base of the stem near the soil surface. Larvae girdle the stem with a V-shaped notch and plug the stem with frass below the notch, creating an overwintering chamber where the diapausing larvae remains until the next spring. The wheat stem usually breaks at the notch leaving a short stub. In May the larvae pupate, chew emergence exits out of the stub, and emerge as adult wasps to reinitiate the cycle.

Damage to wheat by wheat stem sawfly occurs in two ways. Direct damage due to larval feeding on vascular tissue results in reduced vascular flow for kernel growth and a 11 to 22% decrease in yield as a result of reduced kernel number and size. Grain protein decreases in the range of 0.6 to 1.2 percentage points in response to sawfly infestation are

also documented. A second type of damage, the damage the producer notices, results from stem lodging and associated harvest losses. Lodging and harvest losses are extremely variable and environ-mentally dependent. In 1992, Morrill documented sawfly-induced harvest losses in four fields ranging from 1.7 to 33.2 bu/acre. Lodging also increases harvest costs by reducing harvest speed and forcing some producers to swath their grain. Although wheat stem sawfly is widely distributed in western U.S. it is not an economic problem except in the northern Great Plains where wasp emergence is closely synchronized with the susceptible wheat growth stages from jointing to heading. In Montana, losses to wheat stem sawfly have exceeded $25 million annually in 1995, 1996, and 1997.

Historical perspective

Wheat stem sawfly was originally found in many of the large-stemmed grasses endemic to the North American Great Plains. As wheat culture increased on the prairies during the early 1900's, sawfly adapted easily to wheat and spring wheat gradually became a preferred host plant. Losses to wheat stem sawfly gradually increased with deployment of rust-resistant cultivars, adoption of shallow tillage techniques, strip cropping, and wheat monoculture. By the 1920's and 1930's the wheat industry in the northern Great Plains was threatened by the wheat stem sawfly. The first sawfly-resistant wheat cultivar, Rescue, was released in 1946. Rescue was readily accepted by producers and grown on wide-spread acreage. In 1954, wheat stem sawfly populations were substantially reduced by the 15B stem rust epidemic that killed off much of the sawfly population along with its wheat host plants.

During the 1960's and 1970's in Montana, damage to wheat stem sawfly occurred primarily in spring wheat. Damage was held in check by use of solid-stemmed spring wheat cultivars such as Fortuna, Lew, and Tioga. From 1960 to 1980 these and other resistant cultivars accounted for 30 to 70% of the spring wheat acres in Montana. Since the 1980's, widespread and heavy damage to winter wheat has occurred in Montana. In Montana, wheat stem sawfly has adapted from grasses to spring wheat and more recently to winter wheat. Although increases in conservation tillage (enhanced overwinter sawfly survival) and CRP (host reservoir) have been associated with increased prevalence of wheat stem sawfly in the state, the adaptation to winter wheat as a preferred host is likely a result of earlier seasonal activity of the sawfly wasp which enhanced the synchrony to winter wheat

susceptible growth stages.

The reproductive mode of wheat stem sawfly is arrhenotokous, diploid females (2n=18) arise from fertilized eggs and haploid males (n=9) from unfertilized eggs. Limited dispersal potential, short adult life span, low fecundity, and host plant distribution and phenology influence wheat stem sawfly gene flow. There is evidence for high levels of genetic diversity in wheat stem sawfly and multiple lines of evidence for population structuring. Evidence of population structuring within wheat stem sawfly includes parthenogenic reproductive behavior, virulence differences among populations, developmental (emergence date) differences among populations, and RAPD variation among and within geographically-dispersed populations.

Control strategies

Wheat stem sawfly can increase by a factor of ten each year; a 7-9% infestation can result in a 70 to 90% infestation the next season. Because of this potential increase, management strategies or methods that affect <90% of the sawfly population may not impact infestation levels the following year. To achieve 90% control, a combination of control strategies is likely necessary. Wheat stem sawfly control strategies include insecticides, crop rotation, biological control, residue management, trap crops, escape strategies, swathing, pheromone manipul-ation, and resistant varieties.

With exception of insecticides which are not effective, and pheromones whose effects are currently unknown, all other control strategies provides some increments of wheat stem sawfly control. Crop rotation to nonhost crops is effective, however rotation options in Montana are limited, rotation is most effective on a farm scale rather than a field scale, and native grasses which could be in ditches, along roads, and along field borders are also included in the host range. Biological control is effective in some areas of Montana. Two species, *Bracon cephi* and *Bracon lissogaster* parasitize sawfly within the wheat stem by laying eggs within the larvae. The second generation of the parasitoids which occurs in August is most effective, however, wheat in Montana often matures before the second parasitoid generation is completed. Residue management can have detrimental or positive effects on wheat stem sawfly populations. Reduced tillage programs that trap snow and reduce erosion also enhance sawfly overwintering populations by leaving overwintering sites intact. Deep plowing is effective in burying larvae below depths from

which they can emerge but is not an option due to erosion potential. Burning is not effective since a large proportion of the sawfly population is protected below the soil surface. Fall tillage can effectively reduce sawfly populations providing the stubs containing sawfly larvae are brought to the soil surface and allowed to desiccate and be exposed to subzero temperatures during the winter.

Trap crops are sometimes used and can be effective. The technique attempts to concentrate the infestation in a small area at the interface of stubble and the new crop. Many types of trap crops can be used provided they are in the host range. After infestation the trap crop can be destroyed, treated with insecticide, or harvested by various methods. Escape strategies attempt to modify crop maturity and phenology so that the susceptible wheat growth stages do not coincide with the emergence time of the sawfly. Escape strategies with winter wheat attempt earlier maturity with early heading cultivars, while escape strategies in spring wheat delay maturity either with delayed planting date or late heading cultivars. In Montana late maturity is a risky strategy since water and high temperature stress often occur late in the growing season. Swathing or early windrowing is a commonly used management options to reduce harvest losses to wheat stem sawfly. Grain can be swathed with no yield loss anytime after physiological maturity which occurs 10 to 14 days before harvest maturity. By manipulation of the timing and height of the swathing process, larvae can be isolated from their overwintering sites. Host plant resistance and resistant cultivars is the best current control option.

Host plant resistance and resistant cultivars

Although there have been reports in the literature on germplasm with resistance to wheat stem sawfly these reports are quite limited. In 1954 it was reported that PI170924 wheat was a source of a single dominant gene conditioning an antibiosis response to wheat stem sawfly. Other reports indicate solid- and hollow-stem durums, *Agropyron elongatum*, and *Triticum tauchii* as possible sources of resistance to wheat stem sawfly. However for all practical purposes, stem solidness is the only known source of resistance to wheat stem sawfly. As Noble summarized at the 1963 International Wheat Stem Sawfly Conference in Great Falls, MT, "In our search for resistant germplasm we have worked all the way through the world collection of wheats and part way back again without finding any resistance different or better than that of the Canadian variety Rescue". Holmes stated it more clearly, "Resistance boils down to stem solidness".

Indeed stem solidness seems to be the only known source of effective resistance. From 1949 to 1965, 12-15,000 lines were screened for resistance to wheat stem sawfly and no other resistance types were found. Diverse sources of stem solidness have been found quite readily. For example, in 1969 Wallace reported the results of screening trials of 1339 Portuguese introductions, reporting 31 were solid stemmed and resistant, 100 intermediate, and 1208 susceptible to sawfly. In recent years in Montana we have found that stem solidness is fairly common in "modern" foreign germplasm, although it is unknown whether new sources of solidness are genetically distinct from stem solidness already deployed through S615 and Rescue.

Solidness is the result of undifferentiated parenchyma cells within the lumen of the wheat stem. Stem solidness sometimes, but not always, reduces infestation rate. Stem solidness disrupts the normal life cycle of the sawfly resulting in increased mortality in the egg through larval stages. The solid stem trait is highly heritable, with most reports indicating genetic control by 2 to 4 genes. Expression of stem solidness is environmentally sensitive, expressed to the greatest degree in drier, lower-yielding environments. Expression of stem solidness in F_1 hybrids is intermediate to hollow and solid-stemmed parents.

All solid-stemmed, sawfly-resistant cultivars trace back to the same source of stem solidness even though multiple sources of stem solidness in diverse genetic backgrounds have been identified. Approximately 20 to 25 solid-stemmed cultivars have been released from breeding programs in Montana, North Dakota, and Canada. Significant spring wheat sawfly-resistant cultivars include Rescue, Fortuna, Tioga, and Lew. The current trend is toward higher-yielding cultivars with intermediate stem solidness. In 1995 and 1996, respectively, Vanguard and Rampart were released as sawfly-resistant winter wheats for Montana. In the 50 years since the release of Rescue, only an additional 3 to 4 breeding cycles have been completed with solid-stemmed germplasm, suggesting further genetic progress may be possible.

Solid stem cultivars have effectively reduced losses to wheat stem sawfly over a 50 year period. However, stem solidness as a resistance mechanism is not without limitations. Major limitations of solid-stem cultivars include lower yield potential and the fact that stem solidness is differentially expressed and not effective in all environments. In 1978, N.D.

Holmes reported that over a 26 year period Rescue spring wheat expressed adequate resistance (<20% cut) in 9 years, moderate resistance (20-39% cut) in 5 years, and inadequate resistance (>40% cut) in 12 years. As another example, Vanguard and Rampart winter wheats are superior to most hollow-stemmed cultivars in sawfly infested environments but are not yield-competitive where sawfly is not a consistent problem. Improvement in yield potential of solid-stem germplasm has not occurred at the same rate as in hollow-stemmed germplasm. Weiss and Morrill in 1992 reported that based on standard yield trial results and a hypothetical major infestation (15.5% yield loss) by wheat stem sawfly, resistant varieties provided a yield advantage over hollow-stemmed varieties 42%, 26%, 82%, and 90% of the time at Williston ND, Minot ND, Conrad MT, and Havre MT, respectively. However, at the same sites, resistant varieties provided a yield advantage to a minor infestation (2.7% yield loss) by wheat stem sawfly only 5%, 0%, 55%, and 30% of the time, respectively. Thus solid-stem cultivars are a useful option only when sawfly infestations are consistent and moderate to heavy.

Are we more prepared for sawfly than we were 50 years ago? N.D. Holmes, who spent a lifetime working with the insect said in 1978, "During the past 70 years we have learned many things about the wheat stem sawfly - some of them pretty intimate - and yet some mysteries remain." "Zero tillage and other developments could create an environment in which the sawfly could again flare up. We should be better prepared than we were 40 years ago."

Greenbug Biotypes: Victims of selection or little green bandits?

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Abstract

Future advances in wheat, *Triticum aestivum* (L.), resistance to greenbug, *Schizaphis graminum* (Rondani), will likely come from introduction of resistance transgenes into high-performance cultivars. First-generation resistance transgenes will be single genes that impart antibiosis traits (similar to *Bacillus thuringiensis* endotoxins in transgenic corn, *Zea mays* L.). This approach to pest management is incompatible with interpretations of simulation models that predict that deployment of antibiosis resistance controlled by single genes drives the development of new, virulent pest biotypes. This dichotomy must be addressed if full advantage is to be taken of the new, powerful tools of molecular biology for plant protection against insects. In this paper, the specific insect-plant interactions of greenbugs on wheat were examined to understand the relationship between the deployment of plant resistance and the development of new greenbug biotypes. From this analysis, there was no relationship between the use of resistant wheat and the development of new greenbug biotypes. Similar analysis of sorghum, *Sorghum bicolor* (L.) Moench, revealed that with only three of the 11 biotypes could there be any correlation between the use of resistant hybrids and the development of new biotypes. Even with these three biotypes, no clear cause-and-effect relationship was established. Based on analysis of these specific insect-plant interactions, we propose that future plant resistance efforts focus on the use of the most effective resistance genes, despite past predictions of what effect these genes may have on aphid population genetics.

Introduction

Strategies for deploying crop varieties with durable genetic resistance to insects have been for years the focus of considerable debate and conjecture. Elaborate simulation models to predict endurance of resistance take into account a myriad of considerations in the deployment of insect-resistant. Interpretations of information from these simulation models have become commonly accepted by plant breeders and entomologists who interact to develop improved plant varieties. By far the most

common inference drawn from these simulation models is that widespread use of an insect-resistant cultivar with a single, major gene for antibiosis resistance will be selective for new, virulent biotypes.

The information derived from simulation models has been used to develop principles and practices to enhance the durability of resistance. In general, tolerance and antixenosis resistance are believed to be less selective than antibiosis resistance for virulent biotypes of insects, including greenbug. Also, using several genes that confer minor resistance effects is believed to provide more stable resistance than using a single gene conferring a major effect. While these approaches are thought to provide durability of resistance, they are rarely practical in a typical plant breeding program or even, in some cases, possible given the current state of plant transformation technology.

Genetic transformation will be the vanguard of breeding cereal crops for resistance to insect pests in the future. Current transformation technology in cereal crops is limited to the transfer of relatively short strands of DNA. As such, first-generation transgenes are single genes that impart antibiosis resistance to the plant (e.g., *Bacillus thuringiensis* endotoxins in transgenic corn (*Zea mays* L.), and GNA, the mannose-specific lectin from snowdrop, *Galanthus nivalis* L., in wheat). These single genes have major plant resistance effects expressed through the production of highly antibiotic products. These attributes (single major gene and antibiosis) are inconsistent with the central tenet of currently recommended resistance deployment strategies. While now in its infancy, genetic transformation technology will improve, and the use of transgenes for plant improvement will become more common. Eventually, an arsenal of plant resistance genes will be available for moving into high-performance cultivars for rapid deployment in agriculture. Consequently, resistance deployment strategies should continue to be examined. An examination of the relationship between the release and use of greenbug-resistant wheat and sorghum varieties and the development of new greenbug biotypes is relevant to this subject.

In this paper we will show through a compilation of information covering plant resistance development, greenbug biotype history, biotype genetics, and greenbug host range adaptation that greenbug biotypes occurred independently of selective pressure from resistant cultivars. Based on this analysis, we suggest that in the past, proper strategies were used for deployment of greenbug-resistant cultivars. The fact that biotypes appeared need not be an obstacle to deploying greenbug-resistant wheat and sorghum. We question the tenet that places emphasis on releasing tolerant, multigenic cultivars and de-emphasizes antibiotic, simply inherited greenbug resistance.

History of Greenbug Biotypes

Greenbug has been a serious, perennial aphid pest of small grains in North America since the 1880s and of sorghum since 1968. The aphid has been particularly damaging to sorghum in the Southern Plains. It was not until the 1950s, when resistant wheat began to be developed, that populations of the insect were identified that differed in their ability to damage resistant plants. This was the first evidence that greenbug populations differed genetically in ability to damage resistant plants. These genetically distinct populations are called "biotypes," and each biotype is a phenotypic expression of an indefinite number of genotypes.

A system of identification that now differentiates 11 biotypes (A through K) of greenbug has been developed over the years. We reviewed the chronology of biotype reports to determine, when possible, dates biotypes first were collected from the field or publication dates of the report of a new biotype. Population size and distribution of the biotype at the time of detection generally were not quantified and will not be the subject of speculation here. We chronicled the detection of new greenbug biotypes to document the extent of genetic variability for virulence within the greenbug populations and also to highlight the periodicity of detection as related to plant resistance deployment.

The chronology of greenbug biotype reports is summarized in Table 1 and shows a total of 11 biotypes detected and described within a span of 35 yr (1961 through 1996). Biotypes D and J are not virulent on wheat or sorghum and therefore will not be included in the discussion of insect-plant interactions. Also, biotype A can no longer be recovered from the field and is, therefore, presumed extinct. This leaves eight biotypes (B, C, E, F, G, H, I, and K) detected within greenbug populations that are able to damage one or more wheat or sorghum sources of resistance. Of these eight biotypes, three (F, G, and H) are rarely found on wheat and sorghum. These biotypes have low intrinsic rates of increase and, therefore, probably would not reach damaging infestation levels.

History of Breeding Wheat for Resistance

The chronology of efforts to develop wheat with greenbug resistance is presented in Table 2. Porter et al. (1997) describes the history of breeding wheat for resistance in detail. There appears to be a long period of inactivity in development of greenbug resistance in wheat from the mid-1950s until the late 1970s (Table 2). Beginning with the report of the greenbug resistance of Amigo in 1978, a series of 5 wheat resistance sources was reported every 2-6 yr. With the availability of these resistance genes, wheat cultivars now can be developed by incorporating specific resistance genes against any or all known greenbug biotypes. The relationships between the six known wheat resistance genes and the eight important greenbug biotypes are presented in Table 3.

Wheat Resistance/Greenbug Biotype Relationship

Dates of greenbug biotype reports were compared with the dates greenbug resistance was identified in wheat (Tables 1 and 2). The objective of this comparison was to determine whether use of wheat cultivars with resistance to greenbugs affected the development of greenbug biotypes.

Data in Tables 1 and 2 would appear to indicate that the regular identification of greenbug biotypes since 1961 was induced by use of greenbug-resistant wheat. This relationship is better shown by data in Table 4. For example, biotype B was reported 6 yr after the report of biotype A resistance of DS 28A. There was no biotype B-resistant wheat reported before biotype C was detected in 1968. As mentioned previously, biotype C was referred to as the greenbug originating on sorghum. Biotype E was detected 1 yr after the report of biotype C-resistant Amigo wheat. Biotypes F, G, H, and I were detected or reported 6 or more years after the report of biotype E-resistant Largo wheat. Finally, biotype K was detected in 1992, 1 yr after the report of multibiotype-resistant GRS-1201 (Table 4). These data seem to confirm the hypothesis that deployment of plant resistance influenced, or even directed, development of new biotypes. However, despite this appearance, none of the biotypes reported could have been affected by greenbug resistance in wheat because there was never a wheat cultivar in field production that was resistant to the greenbug biotype prevalent at the time. DS 28A was not used to develop commercial wheat cultivars. The biotype C resistance gene (*Gb2*) in Amigo wheat was first made available to growers in August 1984 in the form of TAM 107. However, the new virulent biotype E was detected in 1979, 5 yr before biotype C-resistant TAM 107 was released to growers. Currently, the greenbug resistance in CI 17959 (*Gb4*), CI 17882 (*Gb5*), and GRS-1201 (*Gb6*) has not been made available to growers in the form of greenbug-resistant wheat cultivars. Biotype E resistance provided by Largo (*Gb3*) is just now being made available to growers in the form of TAM 110. Therefore, the regular development of greenbug biotypes, listed by report date in Table 1, could not have been affected by use of greenbug-resistant wheat.

Summary

Significant genetic variability for virulence to resistant wheat and sorghum exists naturally within greenbug populations. This variability probably existed long before the introduction of greenbug-resistant wheat and sorghum. However, virulence alone apparently is not enough of an adaptive advantage to enable a given genotype of aphid to become established as the predominant and lasting biotype. Reproductive fitness and environmental adaptation capacity of the aphid are arguably the keys to if, and how, a particular greenbug genotype eventually infests crop production areas. It also is clear from the literature that noncultivated hosts play an important role in maintenance of greenbug genetic diversity. Greenbug genotypes, with potentially different virulence genes and fitness characteristics, are exposed to various selection

pressures on perennial, noncultivated grasses for much longer periods than on greenbug-resistant wheat and sorghum. Yet, the dogma persists that deployment of greenbug-resistant wheat and sorghum placed selective pressure on greenbug populations and resulted in establishment of new, virulent biotypes.

The use of greenbug-resistant wheat cultivars could not have contributed to the development of new biotypes. However, if one wishes to disregard the information presented on greenbug biotype genetics and the impact of noncultivated hosts on greenbug genetic diversity, then the sequence of events in which new greenbug biotypes were detected following deployment of greenbug-resistant sorghum hybrids indicates a classic case of cause-and-effect. It appears that this conclusion has been made by many and is now the dogma of greenbug resistance in sorghum.

Without careful experimentation and supporting data, we cannot say conclusively that greenbug-resistant sorghums had no impact on greenbug biotype formation any more than we can say the opposite. However, in defense of sorghum greenbug-resistance efforts, it can be said that deployment of biotypes C, E, and I-resistant sorghums was, by most measures, in conformance with idealized resistance deployment strategies. That is, the resistance was intermediate, manifested by tolerance or a mix of resistance components, and controlled by one or more genes. Also, at the height of their popularity, these resistant sorghums occupied only about 50% of the total acreage planted to sorghum. This approach, based on interpretations of simulation models, should have exerted a minimum of selection pressure on the aphid population, thus ensuring maximum durability of resistance. It should be emphasized here that while greenbug-resistant hybrids were effective for only a few years, each year a resistant cultivar is used prevents millions of dollars in crop losses and insecticide use.

In the final analysis, the goal should be a team approach to quickly develop and deploy wheat and sorghum varieties resistant to greenbug. This likely will involve resistance sources that are highly antibiotic and simply inherited. This kind of resistance is much easier and faster to incorporate into improved varieties than are multiple genes for tolerance. More greenbug-resistant germplasm will be coming on-line as resistance genes are moved across species barriers into wheat and sorghum. As this is done, greenbug population genetic shifts should be monitored and efforts taken to search for new resistance sources if changes are detected. In the future, we should be as concerned with greenbug fitness as we are now concerned with greenbug virulence when developing and deploying greenbug-resistant varieties.

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*Due to space limitation all references have been omitted except for Porter et al., 1997. Complete references are listed in this publication.

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Table 1. Chronology of greenbug biotype status reports

Biotype	Host collected from	Year collected or reported
A	wheat	1961
B	wheat	1961
C	sorghum	1968
D	sorghum	1975
E	wheat	1979
F	Canada bluegrass	1986
G	wheat	1987
H	wheat	1987
I	sorghum	1990
J	wheat	1995
K	sorghum	1992

Table 2. Chronology of greenbug resistance reports in wheat

Resistance source	Resistance gene	Year reported
DS 28A	*gb1*	1955
Amigo	*Gb2*	1978
Largo	*Gb3*	1980
CI 17959	*Gb4*	1982
CI 17882	*Gb5*	1985
GRS 1201	*Gb6*	1991

Table 3. Wheat germplasm sources of resistance to greenbug biotypes

Germplasm	Gene designations	Reaction to greenbug biotype							
		B	C	E	F	G	H	I	K
DS 28A	*gb1*	S	S	S	R	S	S	S	S
Amigo	*Gb2*	R	R	S	S	S	S	S	S
Largo	*Gb3*	S	R	R	S	S	R	R	R
CI 17959	*Gb4*	S	R	R	S	S	S	R	R
CI 17882	*Gb5*	S	R	R	S	S	S	R	R
GRS1201	*Gb6*	R	R	R	S	R	S	R	R

R and S indicate resistant and susceptible reactions, respectively.

Table 4. Comparison of chronologies of wheat resistance gene identifications and greenbug biotype status reports

Plant resistance between resistance source and new biotype report	Year reported	New biotype reported	Biotype report date	Years report
DS 28A (A-resistant)	1955	B	1961	6
		C	1968	
Amigo (B-, C-resistant)	1978	E	1979	1
Largo (C-, E-resistant)	1980	F	1986	6
		G	1987	7
		H	1987	7
		I	1991	11
GRS-1201 (multiresistant)	1991	K	1992	1

NEW DEVELOPMENTS IN BREEDING FOR RWA RESISTANCE

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Breeding for host plant resistance has been one of the most important objectives in the effort to reduce losses by the Russian wheat aphid (RWA). The development of resistant cultivars involves consideration of genes in the wheat plant, genes in the pest, and their interaction with the environment. The purpose of this paper is to describe (i) the economic justification, (ii) sources and uses of resistance genes in the region, and (iii) breeding progress for the development of Russian wheat aphid resistant cultivars.

Since the initial detection of the Russian wheat aphid (Diuraphis noxia, Mordvilko) in the Texas Panhandle of the USA in 1986, it has been found in 17 western states of the USA and three provinces in western Canada. The economic impact during 1986-1996 in the US has been estimated at more than $850 million. Losses caused by the RWA during 1990-93 were small and variable compared to 1986-1989, but when favorable conditions for the aphid occurred, losses increased dramatically in 1994 and 1997 (Table 1). In the United States, the first significant level of resistance found in wheat was in PI 372129 (Turcikum 57 = T-57) in Colorado. Subsequently, several other wheats from various countries expressed significant resistance levels in regional uniform seedling screening programs and in many other screening programs. All introductions from the regions of RWA origin possess several undesirable traits for hard winter or spring wheat breeding programs.

Research on breeding for resistance to the RWA was summarized by Quick in 1995 (6). Cultivar development is proceeding well using the T-57 (PI 372129) and other sources. 'Halt' was the first RWA-resistant cultivar released in the USA in August 1994 (7). Halt is an awned, semidwarf height, white-glumed cultivar which has been most similar to 'Yuma' in appearance at maturity. Halt has averaged a grain yield about equal to Yuma and TAM 107 over all eastern Colorado dryland trials. Milling and baking quality have been superior to TAM 107 and equal to 'Lamar'(Table 2).

Screening procedures developed by entomologists for screening breeding materials are very efficient. At least seven different major genes have been associated with RWA resistance (2,3,4,8). However, the allelism and gene number associated with the genes in PI 294994 (Dn5+), and similar problems with Dn1 and Dn2, have been observed. Baker, et al. (1) and Zhang, et al. (9) have reported solutions to these problems. Dn1 and Dn2 have associated modifier genes, and PI 294994 is a mixture of resistant genotypes having variable numbers of genes.

Significant breeding advances have been made and host plant resistance has become the key to integrated management of the RWA. An understanding of the mechanisms of resistance associated with the major resistance genes, and/or molecular markers associated with them will be very valuable in developing durable resistance through gene pyramiding and deployment. Three molecular markers have been reported (5), and other studies are underway(Table 3).

Information on the regional breeding effort for RWA resistance was obtained through a survey conducted by the author in December 1997. The sources of resistance being used to develop resistant wheats for the southern Great Plains and the western regions are shown in Table 4. The regional effort on size and type of program, anticipated germplasm and variety release, and genetic sources and studies are shown in Tables 5 and 6.

During the past three years, germplasms have been released by programs in Colorado, Montana, Oklahoma (USDA-ARS), Kansas, and Idaho (Table 7). Three cultivars have been released by Colorado and their grain yield performance is shown in Table 8.

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Table 1. Economic impact of Russian wheat aphid in Colorado, 1986 - 1997.

YR	PROD LOST	ACRES SPRAYED	IMPACT $$	% SPRAYED	% CO GROWN
1986	5.50	90	13.2	NA	NA
1987	7.10	1150	27.1	48.0	5.03
1988	3.00	350	14.0	15.0	4.35
1989	4.00	850	22.8	38.0	4.63
1990	3.00	475	10.8	25.0	6.77
1991	0.73	115	3.5	42.0	5.98
1992	1.70	100	5.8	15.5	4.93
1993	0.03	6	0.1	NA	NA
1994	2.02	430	12.1	NA	NA
1995	0	220	2.3	NA	NA
1996	0	35	0.5	NA	NA
1997	(est.)	750			
TOTALS	27.08	3586	112.2	33.7	6.28

Production lost in millions of bushels, acres sprayed x 1000,impact in $millions, % of total Colorado acres sprayed, and % of U.S. acres grown. Source: Frank Peairs, Dep. of Entomology, CSU.

Table 2. New hard red winter wheat cultivar performance in Colorado; relative data.

	HALT	AKRON	TAM 107	YUMA	LAMAR
Yield(D),%	100	102	100	100	95
TW, lb/bu	60	60	60	60	61
HT, in	30	31	30	30	36
DH	142	144	142	144	146
W.Surv.,%	60	80	90	50	80
L Rust, 0-9	5	2	7	1	1
RWA	R	S	S	S	S
Qual, Bk	EX	EX	AC	VG	EX

Halt planted on 4% of 1998 crop acreage in Colorado.

Table 3. Molecular markers reported for RWA resistance genes.

Gene	Marker	PI Source	Reference
Published			
Dn2	KsuA1	262660	Ma, et al.. 1998
Dn4	abc156	372129	Ma, et al., 1998
In progress			
??	KsuD2	220127	Gill, pers.comm.
Dn1 ?	Ch 7D etc.	225245,	Linscott, 97 ASA abstr.
??	Ch 4R T'cale	386156	Fritz 97 ASA abstr.

Table 4. Resistance sources used by regional wheat programs.

PROGRAM	SOURCES
CALIFORNIA	IRANIAN, PI94460, PI94375, PI137739, PI294994
COLORADO	Halt, PI243781, PI294994, PI262660
IDAHO	PI372129, PI137739, PI294994, PI94365, PI140207, PI151918
KANSAS	YILMAZ-10, PI220127
MONTANA	PI372129, PI294994
NEBRASKA	CORWA1, PI137739, PI262660
OKLAHOMA	PI149898, PI140207, PI366616, PI245462, PI225217, PI366520, PI366525,PI366515 plus 16
OREGON	PI294994
CARGILL	PI372129, PI149898, STARS 9302W, KS92WGRC24, PI294994, PI262660
HYBRITECH	T-57, PI137739, PI294994
AGRIPRO	CORWA1, PI294994, PI262660, PI372129

Table 5. Genetics of resistance to the Russian wheat aphid.

PI/SEL	GENE SYMBOL	CLASS	GENETICS REF.
137739	Dn1	HWS	SA, CO
262660	Dn2 ?	HWW	SA, CO
SQ 24	dn3	T. TAUS.	CO
262605	Dn1	HRW	CO
372129	Dn4	SWW	CO
294994	Dn1,4,5,6 ?	HRW	SA, CO, OR
243781	Dn6	HWW	CO
CORWA1	Dn4	HRW	CO
KS92WGRC24	Dn6	HWW	CO
STARS9302W	Dn5	HRW	CO
CI 2401	Dn4, *	HRW	CO
CI 6501	Dn6	HRW	CO
151918	Dn4	winter	CO
94355	*	winter	CO
94365	*	winter	CO
222666	*	HRW	CO
222668	*	HRW	CO
225245	*	HWW	CO
225262	*, *	HWW	CO
225271	*	HRW	CO
220127	Dn_	winter	KS
149898	Dn_, Dn_	winter	OK
225217	Dn_	winter	OK
245462	Dn_, Dn_	winter	OK
386148	Dn_	triticale	CO
AUS-VAV1	Dn5	spring	CO
140207	Dn_	spring	OK
366515	Dn_, Dn_	spring	OK
366616	Dn_, Dn_	spring	OK

*: allelism unknown, but not Dn4, Dn5, or Dn6
Dn_: allelism unknown
CO, OK, OR, SA: Colorado, USDA/Oklahoma, Oregon, South Africa

Table 6. Regional breeding effort for RWA resistance-1.

PROGRAM	% OF PROGRAM	TYPE OF SCREEN	WHEAT CLASSES
CA	5	GH, F	HRS,HWS
CO	100	GH, F	HRW, HWW, HRS
ID	5	GH	FIVE
KS	15	GH	HRW, HWW
MT	10	GH, F	HRW, HWW, HRS
NE	5	GH	HRW, HWW
OK/OSU	10	GH, F	HRW
OK/ARS	70	GH, F	HRW, HWW, HRS, HWS, SWS
OR	10	GH, F	CLUB
CARG	15	GH, F	HRW, HWW
HYBR	5	GH, F	HRW, HWW, HRS
AGRIPRO	10	GH	HRW, HWW

Table 6. Regional breeding effort for RWA resistance-2.

PROGRAM	GP RELEASE	CV RELEASE	RES SOURCES	STUDIES ALLEL.	STUDIES MECH.
CO	1991	1994,97	FOUR	Y	Y
ID	1996	1998	SEVEN	Y	Y
KS	1993	1999	THREE	Y	N
MT	1992	2001	FOUR	N	N
NE	2004	2004	FOUR	N	N
OK/OSU	?	2001	FOUR	N	N
OK/ARS	1993	--	24	Y	Y
OR	?	?	FOUR	Y	N
TX	?	?	CORWA1	N	N
CARG	?	2001	SIX	N	N
HYBR	?	?	TEN	N	N
AGRIPRO	?	?	FOUR	N	N

Table 7. Regional germplasm and cultivars released.

YEAR	LOCATION	NAME	CLASS	GENE
1991	Colorado	CORWA1	HRW	DN4
1992	Montana	14 HRSW	HRS	DN4
1993	OKLA-ARS	STARS-9302W	HRW	DN5
		STARS-9303W	HRW	DN5
1993	Kansas	KS92WGRC24	HRW	DN6
		KS92WGRC25	HRW	DN6
1994	Colorado	HALT	HRW	DN4
1995	Kansas	KS94WGRC29	HWW	?
		KS94WGRC30	HRW	?
		KS94WGRC31	HRW	?
1996	Idaho	ID471a, b	HRS	PI294994
		ID472	HRS	DN1
1997	Colorado	YUMAR	HRW	DN4
		PROWERS	HRW	DN4
1998	Idaho	ID498	?	DN4
		ID10085-5	SWW	PI294994

Table 8. Grain yields (bu/a) of RWA-resistant wheats in Colorado, 1996 - 1997.

	LMVT*	HMVT*	HMVT**	HMVT***
Locations:	(5)	(10)	(4)	(1)
YUMA	52.2	44.5	44.2	10.6
YUMAR	51.7	47	47.2	22.4
LAMAR	49.7	46	45	7.1
PROWERS	50	-	44.3	12.3
TAM 107	50.7	48.4	48	23.8
HALT	52.7	45.3	44.3	30.8
AKRON	52.9	47.9	47.2	13.5

* LMVT = Lower Moisture Variety Trial; HMVT = Higher Moisture Variety Trial

** 1997 ave. without Burlington

*** Burlington 1997 with serious RWA, WSMV, and drought

A New Technique for Screening for Bird Cherry-Oat Aphid Resistance in Wheat and Barley

by

C.A. Baker, K.A. Mirkes, J.A. Webster, and D.R. Porter

ABSTRACT

The bird cherry-oat aphid, *Rhopalosiphum padi* (L.), has been shown to reduce the yield of wheat, *Triticum aestivum* (L.), and barley, *Hordeum vulgare* (L.), yet it causes no obvious visual symptoms. This lack of obvious symptom development makes it impossible to use the standard screening test, which is effective in screening for resistance to several other aphids. Therefore, a new technique was developed to identify resistance to the bird cherry-oat aphid. This technique uses transparent seed growth pouches that allow a clear view of both shoot and root development. A rapid visual comparison of infested vs. noninfested plants makes it possible to identify genotypes that are less impacted by the aphid at the seedling stage.

INTRODUCTION

The bird cherry-oat aphid (BCO), *Rhopalosiphum padi* (L.), is recognized as a significant problem of cereal crops in many areas of the world (Blackman and Easthop, 1984). In the United States it is primarily recognized as an efficient vector for barley yellow dwarf virus (BYDV), which causes the most economically important viral disease of cereals worldwide (Lister and Ranieri, 1995; Gourmet et al., 1994). Recognition of the damage-causing potential of BCO, even when aviruliferous, is less widespread. Economic thresholds for BCO are not well established and recommendations vary from state to state, ranging from "control is rarely warranted" (Oklahoma and Nebraska), to recommendations that are at least as rigorous as those for greenbug (GB), *Schizaphis graminum* (Rondani), and Russian wheat aphid (RWA), *Diuraphis noxia* (Mordvilko) (Colorado, Montana, North Dakota).

In contrast to the common perception that BCO causes few problems on its own, research on plant damage and economic losses due to BCO have shown that its effect can be significant. BCO infestation has been shown to reduce the winter hardiness of winter wheat (Wellso et al., 1985). Seedling infestations have caused significant yield losses in spring wheat (Riedell and

Kieckhefer, 1995; Kieckhefer and Kantack, 1980), spring barley (Kieckhefer and Kantack, 1986), and winter wheat (Kieckhefer and Gellner, 1992; Pike and Schaffner, 1985). Maximum yield losses in these studies ranged from 26-60 per cent, depending on aphid numbers and number of days infested. In some cases, BCO was shown to have a more deleterious effect on yield than GB (Kieckhefer and Gellner, 1992; Kieckhefer and Kantack, 1980; Pike and Schaffner, 1985).

In an effort to identify the manner in which BCO affected plant growth and caused yield reductions, Riedell and Kieckhefer (1995) compared damage caused by BCO, RWA and GB at the seedling stage. All three aphids caused similar reductions in shoot growth, root length and dry weight. However, once aphids were removed, it took the BCO infested plants nearly three times longer to recover (that is, to reach the same size as control plants), than the GB and RWA infested plants.

Many different attempts have been made to identify cereal genotypes that are resistant to BCO. Standard seedling screening tests that have been highly effective in screening for resistance to other aphids (Starks and Burton, 1977; Webster et al., 1987) are impractical to use with BCO due to the lack of obvious symptom development. Therefore, most attempts to identify plant genotypes resistant to BCO have concentrated on the effect of the plant on the aphid. Methods to determine different levels of antibiosis have included measurements of:

- number of nymphs produced per female (Hsu and Robinson, 1962, 1963; Tremblay et al., 1989; Lamb and MacKay, 1995),
- effects on alate formation (Weibull, 1987; Haley et al., 1996),
- aphid biomass after specified feeding times (Tremblay et al., 1989, Weibull, 1994; Lamb and MacKay, 1995),
- duration of the prereproductive period (Tremblay et al., 1989),
- intrinsic rate of increase and population growth over time (Weibull, 1987; Tremblay et al., 1989; Thrackay et al., 1990; Lamb and MacKay, 1995; Haley et al., 1996).

Antixenosis/preference has been measured with a binary choice test (Tremblay et al., 1989) and by determining the number of aphids per host genotype in natural field infestations (Papp and Mesterházy, 1993, 1996; Weibull, 1994). Only two reports have specifically tried to identify host plant tolerance to the aphid: Papp and Mesterházy (1993,1996) measured per cent loss in grain yield and thousand kernel mass, and Lamb and MacKay (1995) used a biomass conversion ratio to compare the dry biomass gained by the aphids to the simultaneous reduction in the biomass of the plant.

Use of host plant resistance would be a potentially effective way of controlling losses due to BCO. However, all of the screening methods described

above are extremely laborious, time consuming and would be unwieldy in large scale attempts to locate new and different sources of resistance. They would also be very difficult or impossible to use in the long term breeding programs needed to incorporate BCO resistance into new varieties. This paper describes the development of a rapid new technique that can visually assess the effect of BCO on many different cereal genotypes at the seedling stage.

Materials and Methods

Aphids - Aviruliferous BCO aphids were obtained from R. Kieckhefer, Brookings, South Dakota in 1994. Colonies are maintained on Clintland oat; this oat variety turns red when infected by BYDV and so serves as an indicator if the aphids ever become viruliferous.

Plants- Initial attempts to identify BCO resistance have focused on wheat and barley lines that have been classified as resistant to BYDV. Since BCO is a primary vector of this serious viral disease, it would be advantageous to combine BYDV resistance and aphid resistance in a single line. There is also the possibility that resistance to BYDV could be due to resistance to aphid feeding.

Seed used in the screening test was obtained through the GRIN (Germplasm Resources Information Network) System, maintained by USDA-ARS in Aberdeen, Idaho. To date, only germplasm classified as resistant to BYDV has been screened. It is our intent to continue screening the entire wheat and barley germplasm collection, as was done for both RWA and greenbug.

Technique- In order to observe both shoot and root growth, test plants are grown in clear plastic seed growth pouches held by racks (Mega International, Minneapolis, MN). A hole is punched in the bottom of each pouch with a standard paper hole punch to allow water to penetrate the pouch. Infested and noninfested control racks each contain 55 pouches, five seeds per entry, one entry per pouch. Each rack is placed in a clear plastic storage box (18 cm wide , 30 cm long, 9.5 cm deep) containing 3 gm Peter's 20-20-20 fertilizer mixed with 3.5 liters of tap water. Twenty-four hours later, both infested and noninfested racks are treated with 1 ml of fungicide solution (1.5 gm Arasan, (Du Pont Agricultural Products, Wilmington, DE) per 500 ml water) per pouch. The noninfested rack is also treated with 1 ml insecticide solution (0.05 ml Gaucho 480 (Gustafson, Inc., Plano, TX) per 500 ml. water) per pouch. Gaucho 480 is an effective insecticide when applied as seed treatment (Mullins, 1993). It has both repellent and insecticidal activity. Preliminary tests have shown Gaucho 480 to have no significant effect on plant growth.

Approximately 7 days after planting, when seedlings are approximately 6 cm tall, infestation is accomplished by laying leaves from a BCO colony across the tops of the pouches in the treatment (infested) rack. This results in approximately 5 aphids per seedling; infestation levels have been found to be uniform with this technique.

Fourteen days after planting each entry is rated by comparing the infested pouch and noninfested control pouches. Entries are visually rated as being equal to, better than, or worse than the noninfested control for both shoot and root growth. With this simple technique it is possible to rapidly evaluate many entries.

RESULTS AND DISCUSSION

To date, 2146 wheat and barley entries have been screened for resistance to BCO. Of these, 86% have shown visually apparent stunting in both shoot growth and root growth, approximately 8% have shown slight reductions in shoot and/or root growth, 5% are apparently unaffected by BCO infestation, and surprisingly, 1% may even show an increase in shoot and/or root growth with aphid infestation. Further tests on the apparently resistant entries are needed to determine if there are any genotypic interactions with the Gaucho treatment.

In order to determine if these growth differences at the seedling stage are reflected in actual yield differences, the next step will be to transplant these lines from the screening test to the greenhouse, and to follow plant growth through maturity to determine if seedling response is correlated with yield. These tests are planned for the 1997-1998 growing season.

So far, results are very promising. Over 100 lines have been selected that show no stunting or that even show increased growth with BCO infestation. Since these lines are also resistant to BYDV, they may prove a valuable resource in future breeding programs. This technique has made it possible to easily and rapidly categorize plant response to BCO infestation.

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SESSION 2

BIOTIC STRESSES - PATHOLOGY

Changes in Leaf Rust Virulence Frequencies

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Table 1 - Wheat leaf rust code for the North American differential host.
The coding method used to identify North American leaf rust races. This is a tool for coding the leaf rust genes in a package that can be used easily and quickly by anybody who has a interest in the leaf rust gene virulences in the North American population.

Table 2 - Races of wheat leaf rust identified from collections made in 1997.
The races of wheat leaf rust identified from collections made in 1997 in the U.S. Fifty-six different races were identified from 989 isolates differentiated on 14 leaf rust isogenic lines. The two races that were the most widely identified were MDRL and MBRL. Race MBRL has been the predominant race identified the past 4 years. The third most widely identified race MCDL (virulent to *Lr*1,3,10,17 and 26) is the race that is generally identified from rust collections made from Jagger which is grown on significant acreage from Texas to Kansas. Jagger has *Lr*17 as part of its leaf rust resistance package. Other races that also have *Lr*17 virulence are MBJL, MCTL, MGDL, SBDB, SCDG, TDDL and TDSL.

Table 3 - Percentage of wheat leaf rust isolates virulent to the single gene differential lines used in 1978-1997.
A few points of significance would be the increase in *Lr*1, 3ka, 11, 24, 26 and 30 virulences and decreases in *Lr*9 and 16 virulences in the last 20 years. Again the increase in *Lr*17 virulence the past 2 years is evident.

Table 4 - Postulated seedling leaf rust resistance in cultivars grown in the hard red winter wheat region.
The "+" symbol in the postulated *Lr* gene column indicates other genes are present in this cultivar but they have not been identified. *Lr*3, 10, and 24 are the most common genes identified in these cultivars. Postulated leaf rust genes in other cultivars have been identified and this information will eventually be available on the Cereal Disease Lab's web page: http://www.crl.umn.edu.

Fig. 1 - Wheat leaf rust virulence frequencies in 1997 in the Great Plains.
There is not much difference in the virulence frequencies as you progress northward in the U.S. A higher virulence frequency is noted on *Lr*2a,2c,26 and 18 in the southern Great Plains. More different races would be expected in the southern Great Plains because that is where leaf rust generally increases and mutates throughout most of the year.

Fig. 2-4 - Wheat leaf rust virulence frequency in the Great Plains, 1988-97.
In Fig. 2 the *Lr*2a and 2c percent virulence has been decreasing since 1988. *Lr*1 and 2a

virulences have been close to 90% over the past ten years. Wheat leaf rust virulence frequencies for the genes used most commonly for leaf rust resistance in wheat breeding programs are presented in Fig3. *Lr*9 and 16 virulence frequencies have almost been nonexistent while *Lr*24 and 26 frequencies have fluctuated between 20 and 50%. The *Lr*9 gene is used in the soft red winter wheat breeding programs for leaf rust resistance while the *Lr*16 rust resistant gene is used in many of the wheat breeding programs from Texas through to Canada. Every year virulence to *Lr*9 and 16 is identified but generally is less than 1% of the frequency total. *Lr*24 and 26 resistances are found in the parentage of many cultivars in the Great Plains. Six *Lr* genes are presented in Fig. 4. There has been an increase in virulence frequency in *Lr*3ka,11 and 30 since 1988. In the past year, there has been an significant increase in *Lr*17 which was due to the increase in acreage of Jagger which has *Lr*17 resistance. *Lr*10 virulence frequency has been consistently above 90% the past 10 years while virulence to *Lr*18 has been less than 10 percent.

What are the methods for obtaining longer lasting wheat leaf rust resistance? 1) Pyramid seedling and adult plant genes; 2) Find new genes from wide crosses; 3) Gene deployment and 4) Genetic engineering. Many of these methods were discussed at the Tuesday night meeting.

Table 1. Wheat leaf rust code for the North American differential hosts

		Infection type[b] produced on near isogenic *Lr* lines:			
	Host set 1:	1	2a	2c	3
	Host set 2:	9	16	24	26
	Host set 3:	3ka	11	17	30
Code[a]	Host set 4:	10	18	-[c]	-[c]
B		L	L	L	L
C		L	L	L	H
D		L	L	H	L
F		L	L	H	H
G		L	H	L	L
H		L	H	L	H
J		L	H	H	L
K		L	H	H	H
L		H	L	L	L
M		H	L	L	H
N		H	L	H	L
P		H	L	H	H
Q		H	H	L	L
R		H	H	L	H
S		H	H	H	L
T		H	H	H	H

[a] Code consists of the designation for set 1 followed by that for set 2, etc. For example, race MGB; set 1 (M) - virulent to Lr 1, 3; set 2 (G) - virulent to Lr 16; set 3 (B) - avirulent.

[b] L=low infection type (avirulent pathogen); H=high infection type (virulent pathogen).

[c] Isogenic Lr lines in these two positions change each year.

Table 2. Races of wheat leaf rust identified from collections made in 1997

	Percent of isolates per state[a]																							
Code[b]	AL	AR	FL	GA	LA	MS	NC	SC	TN	VA	IL	IN	MO	OK	TX	KS	NE	MN	SD	ND	MT	CA	WA	USA
CBGB				5																				0.2
CBRG											20													0.2
CCMQ												9												0.2
MBBL	2											5						2		1		19		0.9
MBBQ																						31		0.1
MBDL												**9**		**1**	**2**	**2**	**1**		**7**	**20**				**3.8**
MBGL	**8**		**5**	**5**												**1**		**2**		**2**		**23**		**1.7**
MBJL																		**4**		**2**			**50**	**0.7**
MBRB																		4		1				0.3
MBRL	**26**	**47**	**10**	**30**	**16**		**25**				**10**	**9**		**4**	**10**	**14**	**1**	**34**	**24**	**24**	**33**	**4**		**16.6**
MBRQ	17	2	14	10	35	50				29	10			2	5	4								6.4
MCBL				**5**											**1**					**2**		**15**	**25**	**1.1**
MCDL							**25**					**14**	**17**	**10**	**13**	**20**	**7**	**13**	**7**	**9**	**17**			**8.3**
MCGL				5											1									0.3
MCRL	4		5	3					50						1		1			2		4		1.3
MCRQ	11	5	29		18			25		14		9			3									3.5
MCTL															**4**	**1**								**0.7**
MDBL					4	17									4	5	1			1				1.7
MDGL	6														1									0.4
MDRB															1									0.1
MDRL	**15**	**11**		**5**							**10**		**33**	**47**	**10**	**35**	**66**	**23**	**48**	**19**	**50**		**25**	**22.6**
MDRQ														4		8								1.1
MFBL		9													5		1	5						1.6
MFRL	**2**													**2**						**1**				**0.4**
MGDL														**1**										**0.1**
MGRL														**1**										**0.1**
PBGQ		5																						0.3
PBRG											20													0.2
PBRQ									50			27		1										0.9
PCGL																						4		0.1
PCMG												9												0.2
PCRQ										14														0.2
PLMQ		5											33											0.5
PLRQ																		7						0.4
PMRQ										14														0.2
PNMQ				**7**	**2**										**1**									**0.5**
SBDB														**1**										**0.1**
SCDG																		2						0.1
TBBL	**4**		**5**	**3**											**1**				**5**	**2**				**1.1**
TBGL		4				17						5												0.5
TBRQ		4									10													0.3
TCBL				5														2		4				0.8
TCGL					**4**								**17**		**1**	**1**		**2**	**3**	**4**				**1.4**
TCGQ										14														0.2
TCRL																		2		2				0.3
TDBL	**2**	**4**	**14**											**2**	**12**	**3**	**3**		**3**	**2**				**3.5**
TDDL																**1**		**2**		**1**				**0.3**
TDRL					**5**									**11**	**14**	**5**	**15**			**1**				**5.1**
TDSL															**3**					**1**				**0.5**
TFBL												**5**		**5**	**5**		**2**							**1.7**
TFCL				3																				0.1
TFGL															1	1			2					0.4
TFRL				5										6										0.7
TGBL															**1**									**0.1**
THBL															**1**									**0.2**
TLGG	**4**	**2**	**10**	**10**	**18**	**17**	**50**	**75**		**14**	**20**									**2**				**3.5**
No. isol	53	55	21	40	57	12	4	8	4	14	10	22	6	81	151	103	73	56	58	125	6	26	4	989

[a] States grouped according to agroecological area (Plant Dis. 76:495-499).

[b] In fourth host set, L is virulent on *Lr*10, G is virulent on *Lr*18, and Q is virulent on *Lr*10 and *Lr*18.

Table 3. Percentage of wheat leaf rust isolates virulent to the single gene differential lines used in 1978-1997 surveys in the U.S.

	Percentage of isolates virulent to *Lr* gene														No. of isolates
	1	2a	2c	3	3ka	9	10	11	16	17	18	24	26	30	
1997	99	22	25	99	64	5	95	72	*	**16**	20	42	24	64	989
1996	100	16	19	99	53	4	93	70	1	9	14	19	17	53	276
1995	98	22	25	97	42	8	88	82	2	2	13	22	17	39	700
1994	98	23	28	98	43	3	96	67	*	2	6	30	16	44	683
1993	92	34	43	97	**19**	**13**	84	51	2	5	20	37	24	16	674
1992	88	47	54	96	4	4	93	57	*	3	4	30	24	5	723
1991	86	39	47	98	8	5	83	51	*	1	8	12	19	8	647
1990	85	39	46	95	8	5	88	62	2	3	16	17	14	6	906
1989	85	43	55	98	11	9	91	34	0	3	14	17	13	12	983
1988	87	40	55	96	9	1	92	16	1	2	13	18	15	9	705
1987	83	46	58	95	7	4	91	17	7	6	10	16	6	8	947
1986	77	37	51	91	6	7	81	16	**17**	6	12	10	1	5	972
1985	54	52	68	98	11	9	83	9	**11**	9	19	2	*	-	1148
1984	62	32	51	94	10	6	80	21	-	9	18	2	-	-	836
1978-83	34	25	53	95	26	**25**	73	-	-	11	10	4	-	-	1928

* = Less than 0.6%.

Table 4. Postulated seedling leaf rust resistance in cultivars grown in the hard red winter wheat region

Cultivar	Lr gene(s)	Cultivar	Lr gene(s)
2137	3,+	Longhorn	24,+
2163	3,10+	Mankato	3
2174	3,+	Nekota	+
2180	10,+	Newton	1,+
Abilene	24,+	Niobrara	10,+
Agseco 7853	0	Ogallala	24
Agseco 9001	24,+	Pecos	+
Akron	10	Pronghorn	+
Alliance	0	Redland	3,16
Arapahoe	10,16,24	Rowdy	+
Arlin	0	Siouxland	24,26
Big Dawg	+	Scout 66	10,+
Champ	3,+	TAM 107	0
Chisholm	0	TAM 110	0
Colby 94	11,+	TAM 200	24,+
Coronado	+	TAM 202	24,26,+
Custer	24,26	TAM 300	1,2a,10,16
Dominator	3,11,+	TAM 301	1,2a,26,+
Hickok	+	Tomahawk	10,+
Ike	3Ka,+	Tonkawa	+
Jagger	17,+	Victory	10,+
Karl 92	3Ka, 10, 11+	Vista	16,+
Laredo	10,+	Voyager	2a,+
Larned	10	Windstar	+

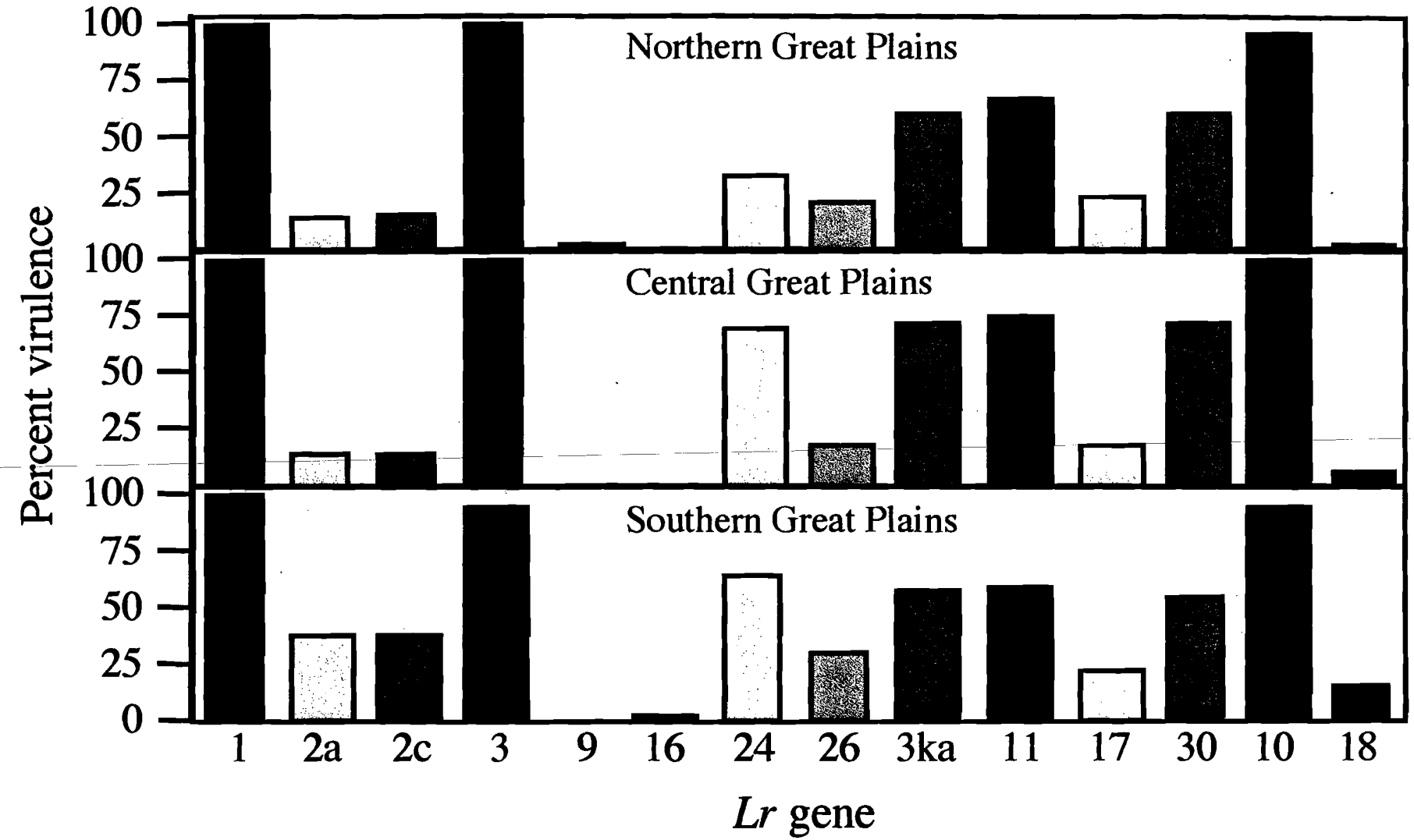

Fig 1. Wheat leaf rust virulence frequency in 1997

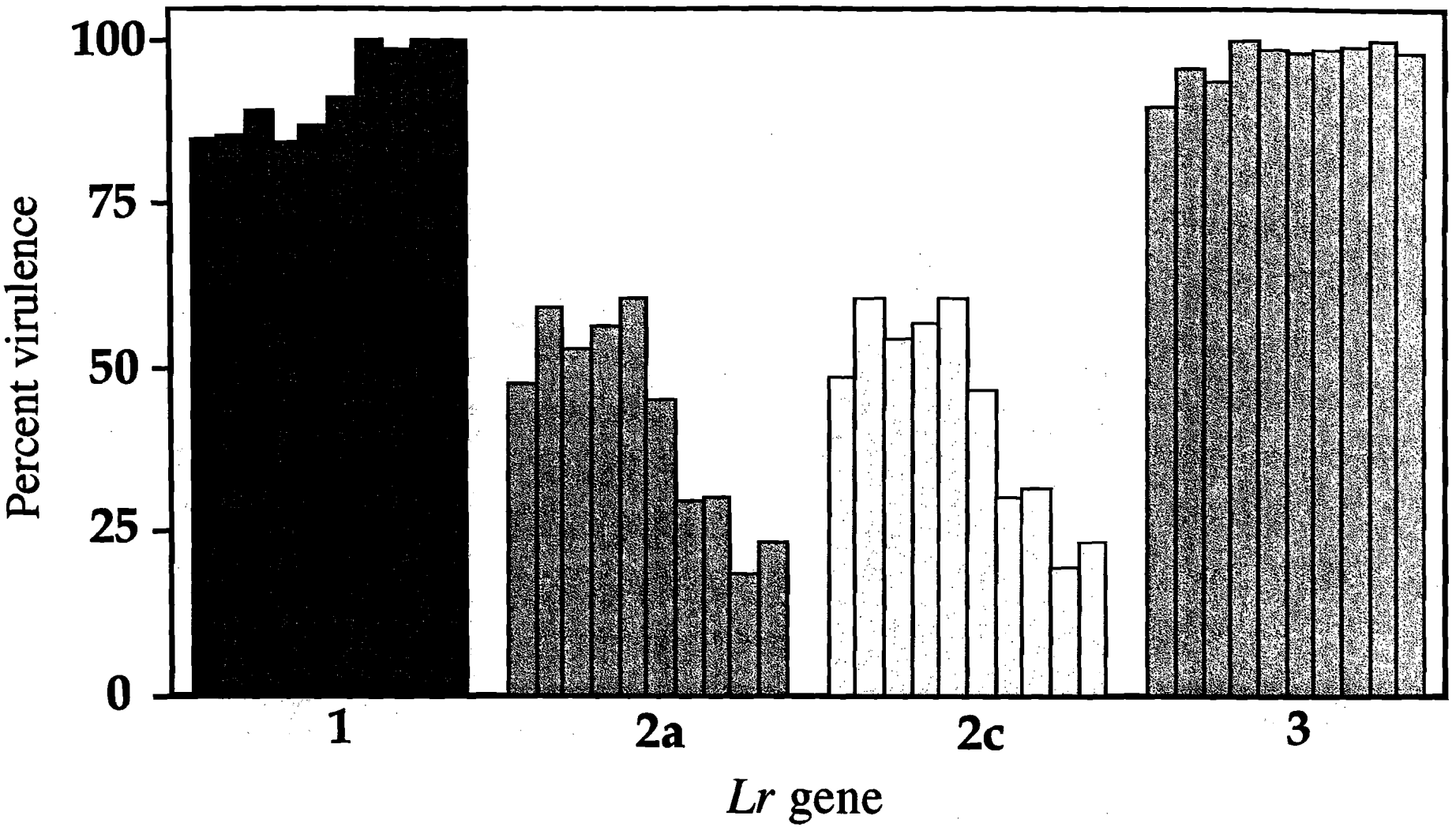

Fig 2. Wheat leaf rust virulence frequency in the Great Plains, 1988-97

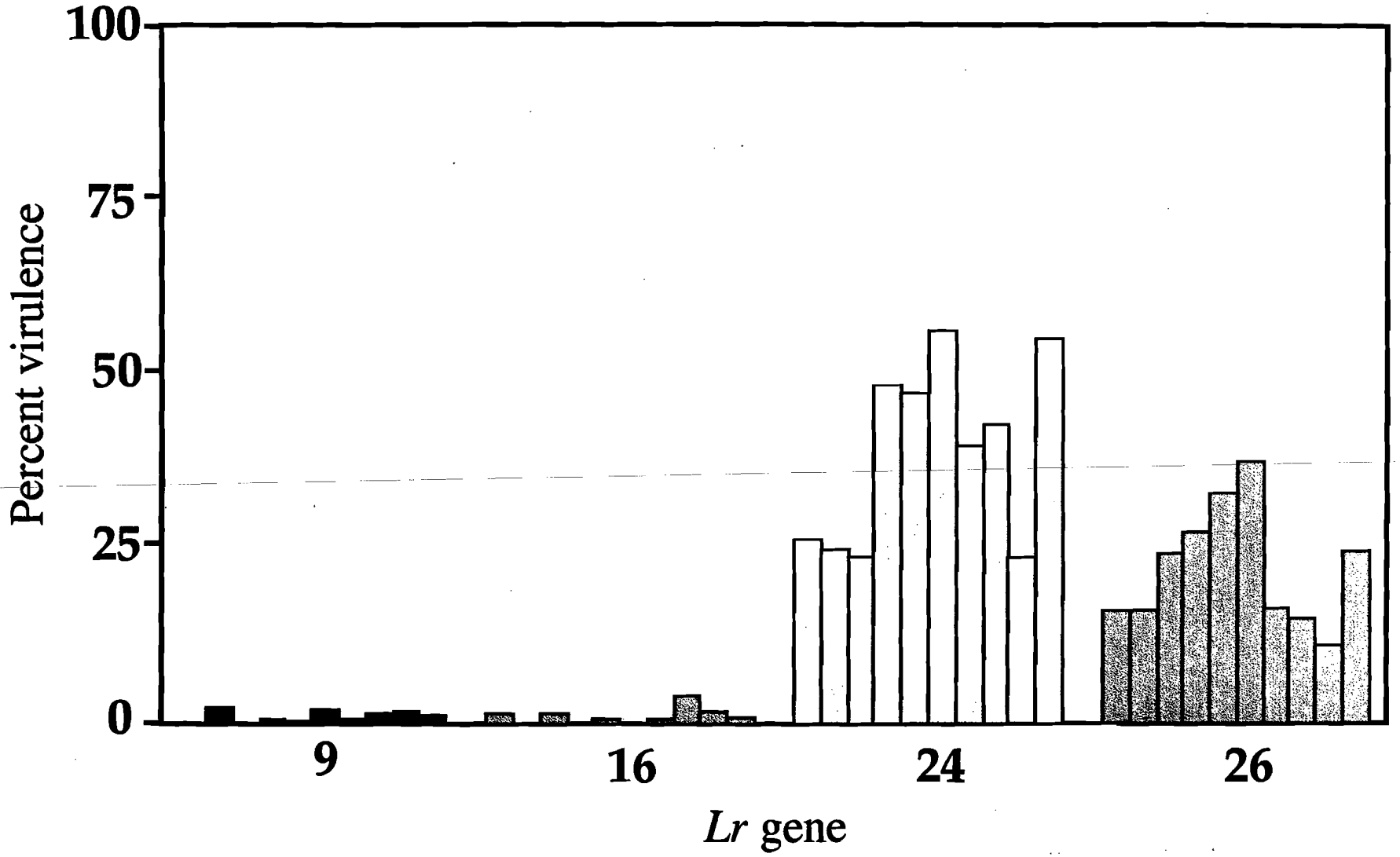

Fig 3. Wheat leaf rust virulence frequency in the Great Plains, 1988-97

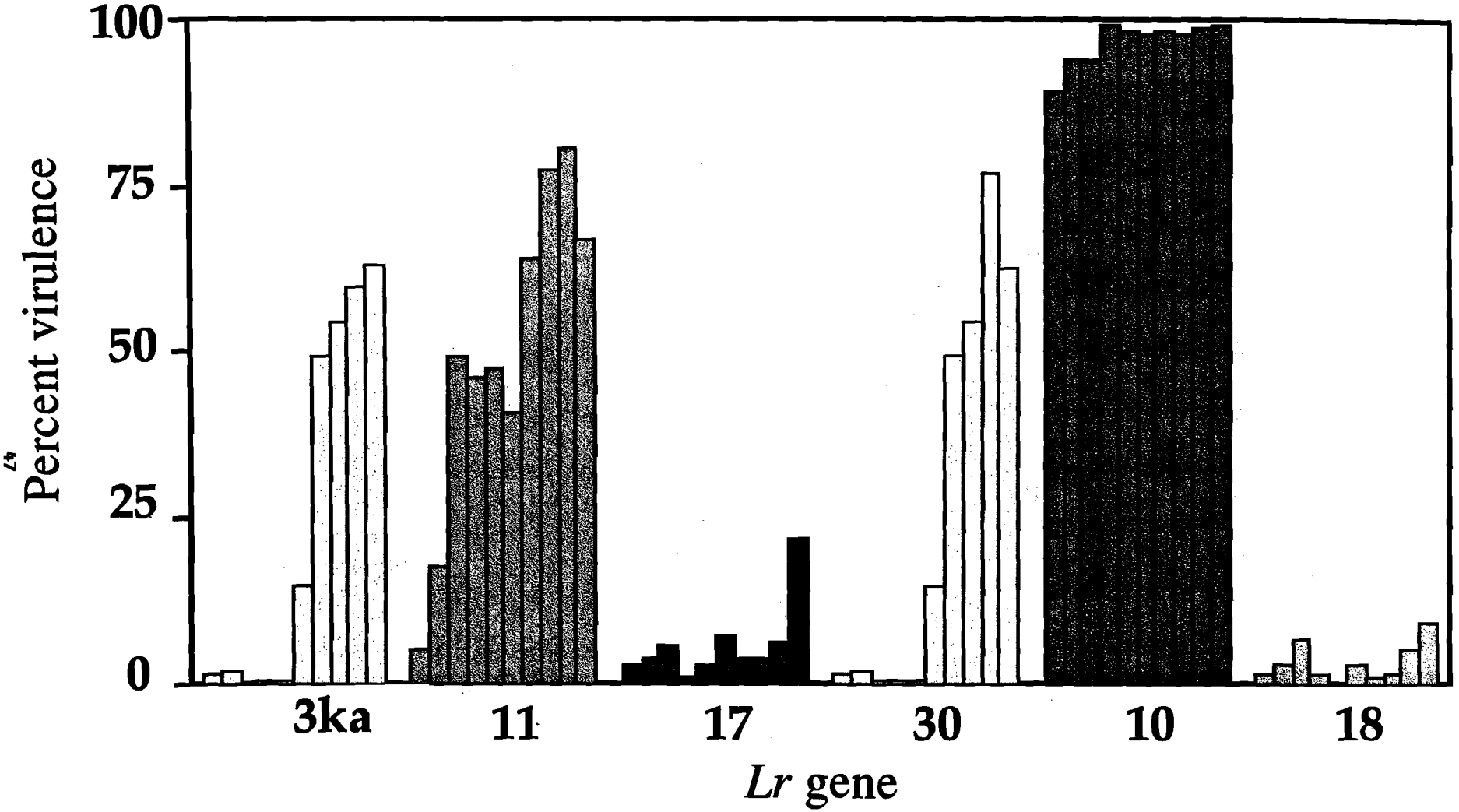

Fig 4. Wheat leaf rust virulence frequency in the Great Plains, 1988-97

Observations Concerning Wheat Streak Mosaic Virus, the High Plains Virus, and a Pathogen Isolated From Wheat with Mosaic Leaf Symptoms and Yellow Heads

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Wheat with a high level of resistance to wheat streak mosaic virus (WSMV) derived from Agropyron intermidium was inoculated with large numbers of WSMV isolates from different geographic locations and also with those capable of infecting sorghum and pearl millet. The wheat remained resistant to all WSMV isolates within the temperature range in which the resistance is effective, indicating that the resistance should be stable against WSMV isolates throughout the Great Plains.

Pure cultures of the High Plains Virus (HPV) from 5 state were established using vascular puncture inoculation (VPI). Experiment with viruliferous and aviruliferous wheat curl mites (WCM) from 5 different states showed that not all WCM could vector a given HPV isolate while other WCM could vector all HPV isolates. VPI tests using corn considered resistant to HPV were conducted. Results indicated that some, but not all of the HPV isolates could infect such corn. Thus, variability exists for WCM to vector HPV and in HPV isolates to infect a given germplasm..

Wheat having mosaic symptoms on the flag leaf and yellow heads was observed in Kansas in 1997 and 1998. Such plants tested negative in enzyme-linked immunosorbent assay against WSMV, HPV and many other antisera, and extracts from such tissue were not infective using traditional mechanical inoculation procedures. Using VPI, a pathogen was isolated from symptomatic wheat and has been successfully maintained in corn through successive serial transfer. Analysis of such such symptomatic corn by minipurification and sodium dodecyl sulfate polyacrylamide electrophoresis (SDS-PAGE) demonstrated a protein band unique to only symptomatic plants. Cesium chloride purification of symptomatic corn resulted in a single light scattering band, which when analyzed by SDS-PAGE resulted in a band migrating to the same position as that observed for minipurified tissue extracts.

SOILBORNE DISEASES OF CEREALS

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Soilborne diseases of cereals are generally associated with inhibition and/or dysfunction of a plant's root system. In Oklahoma, soilborne pathogens are widely distributed, and cause economic losses in wheat. Most wheat soilborne pathogens exert there influence on the wheat plant throughout the crop season from planting to harvest. Typically pre- and post- emergence damping-off during the first and second week after planting do not commonly occur in our environment, thus soilborne pathogen affects are more subtle on plant growth, and disease symptoms may not appear until the later stages of crop maturity. These effects are manifested in root-rot-prone areas in the state after heading when white-headed patches begin to appear. These whiteheads are prematurely ripened heads filled with immature kernels as a result of the damage in the crown and root tissues of the plant caused by root rot pathogens. The white-headed areas in a field can cover up to 50% or greater, and result in a significant yield loss. The severity of the damage is greater in years when drought stress occurs concurrently with heading.

With soilborne pathogens in wheat, there are major technical problems associated with accurately determining disease incidence and severity, and the amount of yield loss. Root pathogens diagnosis is more complicated because: a) symptoms are not directly observable because the roots and crown tissues have to be extracted from the soil; b) their above ground symptomology such as yellowing, stunting etc. is indistinct, and can be mistaken with nutrient deficiencies, and poor soil drainage; and 3) more than one pathogen can be present.

From this viewpoint, root health then becomes the major issue as stressed by Bolley in 1913 with the following quote **"When a valuable fertilizer is present and the roots are dead by disease, the wheat plant cannot make use of it. If the roots are healthy, they can make use of it."** . Thus, Bolley had recognized the importance of a healthy root system. As a result, in root-rot-prone areas of the state, we stress the following components of wheat production: 1) use of balanced fertility program; 2) good soil preparation, weed and insect control practices. However, the effectiveness of these practices will be diminished depending on the incidence and severity of the root rot pathogens that are present.

In Oklahoma, hard red winter wheat (*Triticum aestivum* L.), a versatile and profitable crop, is grown as a cash grain crop (averaging 150 million bushels), and with proper management can also serve a dual purpose in providing winter grazing for livestock (50-60% of 6-7 million acres). Oklahoma's production systems can be categorized as: 1) cash grain production, 2) forage production only and 3) combination forage/grain production. With the latter, the producer has the option for utilizing his forage production for grazing, and also taking a cash grain crop. These production options for wheat production place our producers in a position of greater economic competitiveness.

Wheat production is not without risks because of environmental constraints imposed by the variable rainfall patterns and temperature extremes. In addition to the environmental risks, there are hazards associated with soilborne pathogens, insects and weed pests. In Oklahoma, wheat root rot nematode disease research has shown that forage and grain yields are being conservatively reduced by an average of 77% and 16% in root rot prone areas; respectively (two years and five locations; Russell and Singleton). Thus, grain and forage production is not just simply environmentally limited, but is being confounded by the effects of soilborne fungi and nematode pathogens. Thus, effective measures for soilborne disease control are of critical importance.

In Oklahoma, soilborne pathogens encompass a complex of soilborne fungi and nematodes as pathogens. These soilborne pathogens attack plant tissues associated with the roots and crown of the wheat plant. The ultimate result is the destruction of root and crown tissues that interferes with soil water and nutrient utilization. The most severe damage by these pathogens occurs in association with forage and grain production systems where early September planting is necessary. The following fungal pathogens are important components in our root rot disease complex.

Common root rot [causal agent *Bipolaris sorokiniana*] and, **dryland foot rot** (causal agent *Fusarium* spp.)-- both pathogens cause damage to subcrown internode and crown tissues of wheat plants, and are widely distributed. Common root rot damage is evident as brown to black colored lesions on subcrown internodes. Dryland foot rot results in a uniform light to dark tan discoloration of the basal internodes. The most conspicuous symptom associated with these pathogens is the occurrence of "whitehead" some weeks prior to normal senescence as described above.

Pythium root rot (causal agent[s] several *Pythium* spp.)-- causes damage to crown and root tissues. *Pythium* infected root tissues are stunted, brown and water soaked in appearance. Above ground symptoms are yellowing and stunting and can be confused with nitrogen deficiency symptoms. This pathogen is as widely distributed as common and dryland foot rot pathogens.

Take-all (causal agent *Gaeumannomyces graminis*) is not as widely distributed in our wheat soils, and is most frequently a problem in on irrigated soils in the Panhandle counties where wheat is grown in rotation with other crops. This pathogen causes more damage with wet soil conditions in contrast to common and dryland foot rot pathogens that are favored by a dry soil environment. Diseased root and crown tissues will have a black carbonaceous like appearance. This material can actually be scraped from tissue because the fungous is growing epiphytically on the surface of the root and crown tissues. Damage will be evident as patchy areas of whiteheads as previously described.

Sharp eyespot (causal agent *Rhizoctonia cerealis*) is widely distributed and occurs in conjunction with dryland foot rot. In the past years, we associated occurrence of this pathogen with acid soil situations. In recent years, a sharp eyespot is more commonly found, and we do not have an explanation as to why this is occurring. Sharp eyespot produces an elliptical eye-spot like lesion on the outer leaf sheaths that later penetrate directly through to the basal culms tissue proper. This type of damage weakens the stem and also results in white head development as previously described.

Cephalosporium stripe (causal agent *Cephalosporium gramineum)* rare in occurrence in our northern tier of counties, but is more common in Kansas because of the need for alternate freezing and thawing of wheat soils. Soil heaving is necessary to create the root wounding that is critical for root infection by this pathogen. Severe areas of white-heading occur with this disease. The lower culm and crown tissues will be clean and healthy in appearance on white-headed plants. Since this is a vascular wilt pathogen, vascular occlusion and discoloration occurs several internodes above the basal internodes.

As shown in Fig.1, planting dates are a critical component of the expression of root rot disease damage. With early planting (late Aug.- earl Sept.), soil temperatures at planting depth are high (90F) and soil pathogens are more aggressive in attacking seedlings. As shown, maximum and minium soil temperatures decline as planting dates are delayed by ~two week intervals. Also, disease incidence values decline as the planting dates were delayed. Thus, there was apparently a lessened amount of infection of wheat seedlings as soil temperatures were declining. By contrast, grain yields were increasing as planting dates were delayed. Thus, the later plantings were escaping the effects of damage by soilborne pathogens as a result of the lower soil temperatures at planting. These results are representative of the fact that greater disease and yield loss from soilborne pathogens occurs with early planting for forage and grain production. Thus in root rot prone areas, the following grower recommendations are suggested:

- In areas of chronic root rot disease pressure, cultural control by delayed planting (October 15th) is suggested as an effective alternative to early planting.
-In our environment, grows will have to be educated as to the risks associated with early planting practices.

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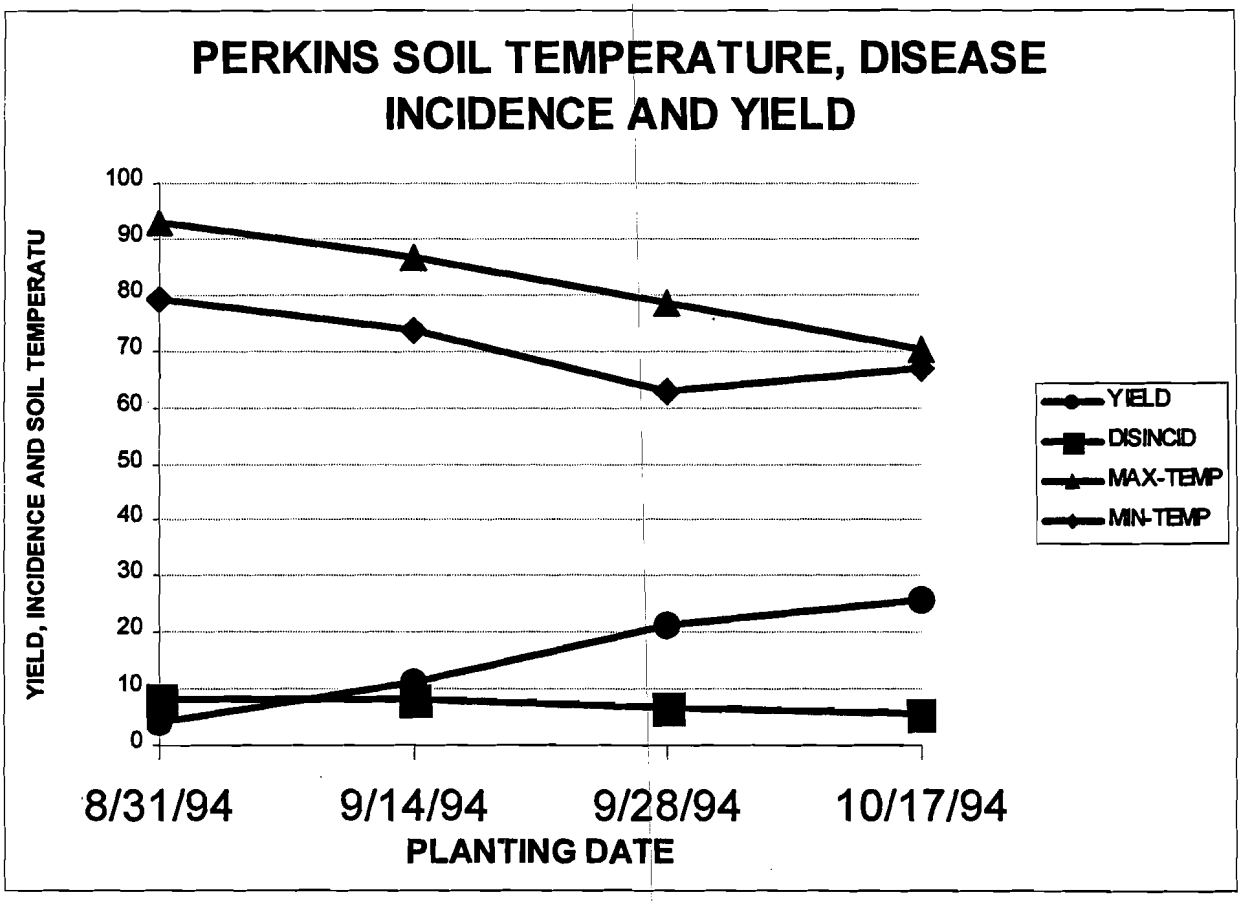

Figure 1. Perkins, OK maximum and minium soil temperatures, disease incidence, and grain yield data for four planting dates.

SESSION 3

ABIOTIC STRESSES

Perspectives on Physiology and Genetic Improvement for Freeze Resistance

David Livingston, USDA-ARS/North Carolina State University

Introduction:

Severe winters can be devastating to winter cereals, particularly to those species most susceptible to freezing, such as winter oat. Because oats are the most susceptible winter cereal to freezing we have used it as a model crop in which to improve winter hardiness. I am convinced that most, if not all, the discussion presented here can be directly applied to wheat.

We have taken a two pronged approach in our effort to improve the winter hardiness of oat. First, we have taken an applied approach with crossing decisions based on historical winter survival data and screening progeny that are grown and frozen under controlled conditions. Second, we are studying biochemical changes which occur in the extracellular region (apoplast) of the crown at below freezing temperatures.

Applied:

The Uniform Winter Hardiness Nurseries (wheat, barley and oats) can provide a large amount of winter survival data with a minimum of effort. In 1992, data from the Oat Winter Hardiness Nursery was discovered in the attic of a laboratory in State College, PA. After compiling 65 years of these data we found that the average survival of 2 of the most hardy checks (Wintok and Norline) was not significantly ($p = 0.05$) different from each other. However, in several years at certain locations they were significantly ($p = 0.05$) different.

Gullord, et al (1975) found that the freezing tolerance of wheat genotypes ranked significantly differently depending on the type of freeze test used. He hypothesized that different sets of genes were conferring resistance under the different freezing regimes. We likewise hypothesized that the two oat cultivars differed from each other because different sets of winter hardiness genes were being expressed under the particular conditions of certain location/years. To see if transgressive segregants for freezing tolerance could be produced from the two parents, Dr. Paul Murphy at North Carolina State University made a cross between the two cultivars in 1993. In 1997 F4 progeny from the cross were grown and hardened in growth chambers and test frozen at -13 to -15°C in custom designed freezers. We have so far performed 12 separate freeze tests on the progeny and have found that in each test about 10% of the individual plants survive while both parents are completely killed. Field testing on the survivors will begin in the fall of 1998.

Biochemical:

Trunova (1965) reported that a below freezing treatment (-3°C) conferred additional hardiness to wheat plants beyond that achieved by the traditional cold hardening at temperatures just above freezing. We have concentrated on this "second phase of hardening" since it seemed likely that changes occurring in the plant while it is freezing would more directly relate to its survival than those occurring prior to freezing. We have focused on changes that occur in the apoplast because this is where ice is formed initially in the plant. Using a non-destructive apoplast sampling technique (crowns can be replanted after sampling and will eventually set seed) we analyzed apoplastic fluid for carbohydrates, carbohydrate metabolic enzyme activity and apoplastic proteins.

Table 1. Fructan, sugars, and carbohydrate metabolic enzyme activity in apoplast fluid from Wintok oat.

	Fructan activity						Sugars			Enzyme	
	DP>7	DP7	DP6	DP5	DP4	DP3	S	G	F	Hydrolase	Invertase
				mg/g	fresh	wt.				Nmoles fructose/min/g fr	
unhardened	0.06	0.02	0.02	0.03	0.03	0.03	0.05	0.08	0.03	3	14
1PH	0.10	0.04	0.05	0.07	0.09	0.08	0.14	0.12	0.04	5	15
2PH	0.43	0.21	0.36	0.46	0.48	0.27	0.94	1.27	0.82	20	60

DP = Degree of polymerization. DP3 = fructose-glucose-fructose.
S = sucrose, G = glucose, F = fructose.
Enzyme activity was measured by the amount of fructose released in the reaction per min.
1,2PH = first, second phase hardened.

The level of carbohydrates and enzyme activity in unhardened and first phase hardened (above freezing) apoplastic fluid was less than 3% of the total carbohydrate concentration and enzyme activity in the whole crown tissue. This amount was about the same as was expected from cellular rupture during the extraction procedure.

During second phase hardening, levels of all carbohydrates and their enzymes in the apoplast increased many-fold (Livingston and Henson, 1998). Levels of glucose and fructose increased more than any other carbohydrate during second phase hardening and were 18 and 15% respectively, of the total glucose and fructose in the whole tissue. These levels were considerably higher than expected from cellular rupture. We also found levels of the fructose polymer, fructan to increase significantly ($p=0.01$) in the apoplast during second phase hardening, in addition to fructan exohydrolase activity which is the enzyme that cleaves fructose molecules from the polymer. Invertase (cleaves sucrose, which releases glucose and fructose) activity also

increased significantly in the apoplast; this may explain the high glucose and fructose levels observed in the apoplast fluid of second phase hardened plants.

While these sugar and fructan increases in the apoplast would only lower the freezing point of the apoplast fluid by a fraction of a degree, if the sugars were concentrated in critical regions of the apoplast, as suggested by Canny (1995), those areas could be highly resistant to damage from freezing and thus protect the whole plant.

We are currently analyzing apoplast fluid for proteins used SDS gels and have discovered at least 6 protein bands which appear in apoplast fluid during second phase hardening. We are currently trying to identify these proteins and see if they are present in less hardy cultivars.

If any of these biochemical changes are related to freezing tolerance then apoplast sampling could be a simple means of screening exotic germplasm for individual components of freezing tolerance as well as identifying those components which may be missing in agronomically adapted cultivars which would benefit from additional freezing tolerance.

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High Temperature Effects on Physiology and Productivity of Wheat

Gary M. Paulsen*

What Limits Wheat Yields in the Great Plains?

Average grain yields are much lower in the hard winter wheat region of the U.S. than in many other parts of the world (Paulsen, 1994). Yields in the Great Plains, for instance, generally range from 1.5 to 3.2 tons/ha compared with 8 to 10 tons/ha or more in western Europe. Reasons for the low yields in the region, which has excellent varieties, skilled producers, and deep fertile soils, are disputed. Identifying the causes of the differences in yields might suggest some traits for improving productivity of wheat.

Low yields in the Great Plains often are attributed to inadequate moisture. However, yields are frequently lowest in parts of the region that have the most precipitation. In Kansas, for instance, yields are usually lowest in the southeastern crop reporting district, which receives the most moisture in the state, and highest in the northcentral district. Irrigation does benefit yields, but average yields of irrigated wheat are still low and the response relative to dryland conditions is poor compared with other crops.

Diseases and insects are important problems in production of wheat in the region. They destroy, on average, about 14% and occasionally as much as 22% of the wheat crop in Kansas each year (R.L. Bowden, personal communication). Although important, this loss is still much less than the difference in average yields between Kansas and more favorable areas.

The technology for growing wheat is certainly much different in high-yield areas of the world than in the Great Plains. In western Europe, tram lines, high seeding rates, narrow rows, multiple applications of high rates of N fertilizer, and routine use of pesticides and growth regulators are essential components of intensive management systems for high yields. Wheat in the Great Plains rarely responds economically to these intensive practices even under irrigation, suggesting that some factor other than management is limiting.

Importance of High Temperature

Increasing evidence suggests that high temperatures during the grain-filling period are

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critical for wheat yields in the Great Plains (Paulsen, 1994). Maximum temperatures during most of the growth cycle of winter wheat -- from planting in autumn to heading in spring -- are low. Winterkill and spring freeze injury are much more common than damage from high temperature during this period. Unfortunately, this period of low, generally benign temperatures is followed by high temperatures during maturation. As noted by one observer, the yield potential of wheat in the region appears highest around heading, and the actual yield is determined by how rapidly the crop deteriorates before it reaches physiological maturity (R.G. Sears, personal communication).

Monthly average maximum temperatures exceed 25°C everywhere in the region during grain-filling and, on some days, the high temperature reaches 35 to 40°C or greater (Mary Knapp, Kansas State University Weather Library). Wind and low humidity often accompany high temperatures, causing even well-watered plants to desiccate. The soil temperature follows the same pattern as the air temperature, with the temperature at the 5-cm soil depth sometimes exceeding the air temperature and the temperature at 10 cm only slightly lower.

Wheat is poorly adapted to high temperatures during maturation. It is a typical temperate species with the C_3 photosynthetic pathway and evolved in a climate that is greatly different than that of the Great Plains. Although the light reactions are more sensitive than the dark reactions of photosynthesis, both are diminished by the rapid senescence that follows the onset of high temperatures (Al-Khatib and Paulsen, 1984). This rapid senescence is initiated by a marked increase in the activity of protease enzymes in leaves, which is considered the first biochemical sign of senescence. It causes wheat plants to quickly "turn" as the leaf area for photosynthesis is lost. Rapid senescence is very detrimental because most of the grain weight comes from current assimilation, i.e., photosynthesis during grain filling, and yield is correlated highly with leaf area duration after anthesis (Evans et al., 1975).

In the grain, activities of enzymes involved in synthesis of starch are affected as greatly as photosynthesis in leaves by high temperature (Bhuller and Jenner, 1986). Considerable evidence suggests that responses to high temperature that originate in the grain might "trigger" the rapid senescence and other effects in the shoots (Jenner, 1994). Early cessation of enzymatic activities in the grain shortens the duration of grain filling, the period from anthesis to physiological maturity. This period is only about four weeks in the Great Plains compared with eight to 10 weeks or longer in much of western Europe. Grain yield is correlated highly with the duration of the grain filling period as it is with the duration of the leaf area (Evans et al., 1975).

Roots in the upper soil layers are exposed to nearly the same temperature regime as shoots during maturation. However, little is known about responses of roots to high temperature or the consequences of high root temperatures on other plant parts. Nielsen (1974) noted that, compared with shoots, roots have a lower temperature optimum, are less adapted to temperature extremes, and are more sensitive to sudden temperature fluctuations. He also concluded that root temperatures are the critical temperatures for plant survival. Studies in which the temperatures of shoots and roots of wheat were varied independently during maturation ascribed even greater

importance to the root environment (Kuroyanagi and Paulsen, 1988). In these studies, low root temperature delayed senescence of the shoot regardless of its temperature, whereas high root temperature always accelerated senescence of the shoot.

Effects on Yield

The magnitude of yield losses from high temperature is difficult to estimate. The most firm estimates come from controlled environment studies, but it is difficult to extrapolate them to field conditions. Controlled environment studies usually impose continuous high temperatures on plants, whereas the stress in the field is episodic. Some controlled studies also consider only the primary tillers, whereas secondary tillers might be affected more severely in the field. Finally, by their design, controlled studies lack the other factors such as wind that often coincide with high temperature. Much of the difficulty in estimating losses in the field comes from not knowing the probable yield under favorable conditions. Calculating the effect of something is difficult when it is never absent to provide a standard.

One of the best estimates of yield losses from high temperatures in controlled environments was made by Wardlaw et al. (1989). They estimated a reduction in yield of 3 to 4% per degree Celsius above an optimum of 15°C during maturation for 28 cultivars. Most of the loss was from reduced kernel weight. Shpiler and Blum (1986) found nearly a four-fold difference in yield between summer and winter field crops, mostly from a change in kernel number. It is intriguing to speculate on the potential yield of winter wheat in the Great Plains under more favorable temperatures during maturation, particularly if the calculation included the benefits in plant water relations and production technology that would result.

Interactions with High Temperature

The problem of estimating yield losses is compounded by the coincidence of other environmental stresses with high temperature. Dry winds that occur with high temperature are so common that they have their own names: *Gan Zhe Feng* in China, *Sukhovei* in the CSIR, *Sharav* in Israel, *Siroccu* in North Africa, *Khamsin* in the Middle East, and *Larrech* in Spain (Paulsen, 1994). As an example of their effect, hot dry wind reduced the final yield of well-watered wheat in the field up to 57% when applied at the foot stage and 65% when applied at the milk stage (Smika and Shawcroft, 1980).

Drought frequently occurs with high temperature and greatly exacerbates injury. Nicolas et al. (1984) found that wheat yields were reduced up to 18% by moderately high temperature, 44% by severe drought, and 65% by the combined stresses applied for 10 days during maturation. Most of the loss, as expected, was from reduced kernel weight. High temperature briefly accelerated dry matter accumulation but shortened the duration of grain growth. High temperature alone had no effect on starch granules, whereas drought and the combined stresses decreased cell numbers and the number and size of starch granules. Ample sucrose was present

in the endosperm under all treatments, suggesting that the responses occurred in the grain instead of the leaves.

Another interaction that might indirectly involve high temperature is an association between spring freeze injury and wheat yields. Record grain crops were harvested in Kansas, Nebraska, and Oklahoma during years dating to 1931 when spring freeze injury was reported (Paulsen and Heyne, 1983). The association recurred in Kansas this past year, when a severe freeze in April was followed by a record harvest. Excellent yields after spring freeze injury were attributed in part to the precipitation and generally lower temperatures from the cold fronts that caused the injury.

Grain Quality and High Temperature

Hard winter wheat in the Great Plains is used predominantly for bread and must meet strict quality criteria. High temperature affects the quality of wheat for bread as severely as it affects yield. Finney and Fryer (1958), for instance, studied grain from 17 crop years and concluded that temperatures of 32°C or higher during the last 15 days of maturation adversely affected most flour properties. They concluded that low loaf volume because of weak dough was determined more by high temperature than by flour mixing time or protein content.

Adverse effects of high temperature on flour quality were confirmed by many investigators. Randall and Moss (1990) found that high temperatures after anthesis decreased kernel weight (a change that might reduce flour yield), dough strength, loaf score, and loaf volume independently of effects on protein. They recommended an index to identify grain from areas where high temperature might cause weak dough.

Heat shock proteins were investigated extensively, but their role in high-temperature responses of wheat remains uncertain. They are induced by many conditions and are not specific to high temperatures and, with few exceptions, no functions have been identified for them (Harrington et al., 1994). Some evidence supports a relationship with exposure of seedlings to high temperature and differences in thermotolerance among genotypes (Nguyen et al., 1994). All 20 varieties tested in the field, some of which presumably differed in tolerance to high temperature, expressed heat shock proteins.

Blumenthal et al. (1991) proposed that high temperature weakened dough strength by activating heat shock elements of gliadin genes. An increase in gliadin proteins was associated with notably weak dough when field temperatures exceeded 35°C. Gliadin synthesis also was enhanced greatly in excised spikes incubated at 40°C.

Breeding for Thermotolerance

Little direct attention has been given to breeding for high-temperature tolerance in wheat

in the Great Plains until recently. However, wheat breeders obviously have selected for the trait in improved cultivars for many decades. Experimental lines that are not tolerant of high temperatures are not adapted to Great Plains conditions and would never be considered for release. Although genetic differences in thermotolerance are readily apparent (Rawson, 1986; Al-Khatib and Paulsen, 1990), the challenge is to identify genotypes that are superior to cultivars that are already grown in the region.

The few genetic studies on the problem demonstrated that thermotolerance is a complex quantitative trait in wheat. This suggests that many features must be combined in a tolerant genotype. Some of the important features that must be present in the spikes, leaves and stems, and roots can be surmised from the literature.

In the spikes, soluble starch synthase, the most sensitive enzyme in the pathway of starch biosynthesis, was inactivated rapidly (Jenner, 1994). Grain contained several forms of the enzyme, some of which were more thermotolerant than others. Selecting for the tolerant isozymes might increase resistance of wheat to high temperatures; however, no information is available on genetic variability of the trait or its relationship to grain yield under stress conditions. Breeding for stable grain quality under high temperatures might be more successful. A group of genotypes among 45 tested maintained stable quality at 40°C, a trait associated with the Glu-D1d allele, glutenin-to-gliadin ratio, and large glutenin polymers (Blumenthal et al., 1995).

Active photosynthesis and persistent, viable leaves during maturation seem essential for satisfactory performance at high temperatures, given the direct relationship between leaf area duration and grain yield in wheat. Steady photosynthesis might involve both resistance of Photosystem II, the most sensitive component, to inactivation and resistance of all photosynthetic enzymes to proteolysis. Maintenance of viable leaf area also might require low activity of protease enzymes. These changes, if possible, might necessitate some revision of N management practices, because most of the grain protein in wheat comes from remobilization of N in photosynthetic enzymes in leaves (Evans et al., 1975).

A low leaf temperature, which is a function of stomatal conductance and indicates leaf cooling, might help plants avoid injury from heat (Reynolds et al., 1994). Canopy temperature depression is measured easily and correlates highly with grain yield.

Mobilization of stem reserves to support grain filling is a constitutive trait that enables plants to maintain yield under high-temperature stress (Blum et al., 1994). Genotypes differed in the content of stem reserves, the degree of depletion of reserves, and the duration of grain filling.

Extreme sensitivity of roots to temperature and the importance of roots to other plant parts all suggest, that underground organs must be considered. It would seem to be of little benefit to improve resistance of grain and leaf activities to high temperature if the plant parts that support these activities are ignored.

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Cropping System Intensification and Impacts on Wheat Production

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Traditional Wheat-Fallow Systems:

Alternating wheat with fallow has been the predominate dryland farming system in the Great Plains since the 1930's for both spring and winter wheats. In the Central Great Plains alone, there are about 9,000,000 acres of wheat per year, and an equal acreage of fallow. Most of this fallow is managed with tillage to control weeds, and it is common for a producer to till the fallow 5 to 6 times between wheat crops. Summer fallow has many negative attributes such as:

1) Inefficient precipitation use efficiency
2) High soil erosion potential by both wind and water;
3) Accelerated loss of soil organic matter;
4) Increasing problems with winter annual weeds;
5) Increasingly smaller profit margins

Intensive Cropping Systems:

Beginning in the 1980's Great Plains scientists began to investigate increasingly intensive cropping systems to minimize summer fallow and improve overall productivity. Earlier work had shown that 3-year rotations like wheat-sorghum-fallow and wheat-corn-fallow could be successful if some or all of the tillage was eliminated by adoption of no-till methodology (Smika and Wicks, 1968). Peterson, et al. (1993) report increases in annualized grain production of >70% with intensified systems compared to wheat-fallow. The more intensive cropping systems increased water use efficiencies by 28% compared to wheat-fallow (Peterson, et al., 1996).

Furthermore, more intensive cropping are increasing soil organic matter relative to wheat-fallow (Figure 1). All systems with less summer fallow have increased soil organic C . Opportunity cropping, a continuous cropping system, has markedly improved soil organic C

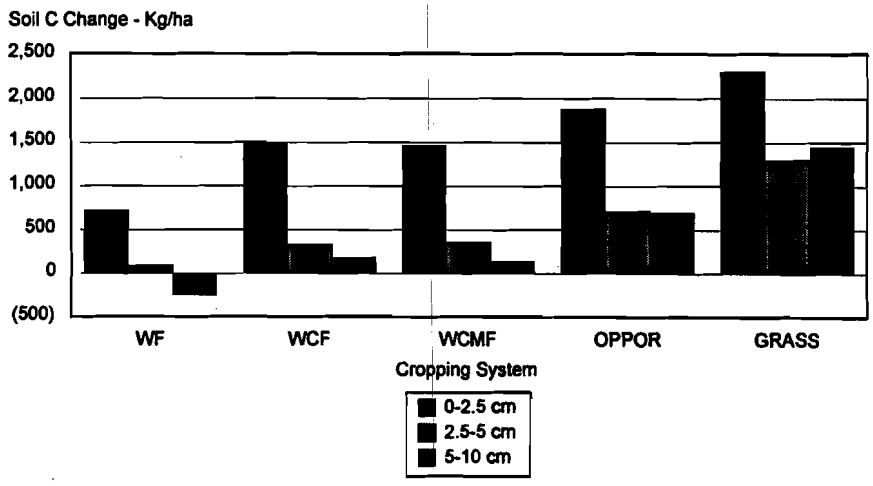

Figure 1. Soil carbon change after 10 years of intensive cropping as related to cropping system and soil depth in eastern Colorado. [WF = wheat-fallow; WCF = wheat-corn-fallow; WCMF = wheat-corn-millet- fallow; OPPOR = Opportunity cropping]

supplies. Increased organic C will eventually translate into improved water infiltration and water use efficiency as surface soil aggregates are stabilized by the organic compounds formed during residue decomposition.

Economics are making the intensive systems increasingly attractive. Data from Dhuyvetter, et al. (1996), provided in Table 1, shows that intensive cropping increased the net economic return relative to wheat-fallow in all instances, except in Southeastern CO. They also report that without government subsidies wheat-fallow is not economically sustainable. With the *"Freedom to Farm"* legislation it is only a matter of a few years before producers may be forced out of alternate wheat-fallow.

Table 1. Net economic return to wheat-fallow in contrast to net return for intensive rotations for several Great Plains States (Dhuyvetter, et al., 1996)

State	Wheat-Fallow Net	Intensive Rotation Net	Change in Net
	----$----	----$----	----%----
TX	9	47	406
CO (Southeast)	22	15	- 30
CO (Northeast)	30	43	43
KS (Southwest)	35	47	36
KS (North Central)	- 4	11	+ ∞
KS (North Central)	- 22	11	+ ∞
ND	32	44	38

Wheat in Intensive Cropping Systems:

How does increasing cropping intensification affect wheat production and the plant attributes necessary to maximize the overall yield potential of the new systems? First of all we must recognize that intensive cropping requires substitution of herbicidal weed control of tillage so precipitation capture and water retention in the soil can be maximized. Conversion to no-till, or at least very minimum tillage, means that there will be moderate to heavy surface residue cover on the soil surface most of the time. The more intense the rotation, the greater the surface residue cover. For example in eastern CO wheat-fallow has averaged 2.8 t/ha of surface residue at wheat planting time, while the wheat-corn-fallow rotation has averaged 5t/ha at wheat planting.

Residue cover causes soil temperatures to be lower, which is beneficial in terms of reducing soil water evaporation loss, but a cooler soil in the spring may delay wheat growth and result in later maturity. The later maturity can force the reproductive period of wheat to occur in a potentially hotter and more arid part of the summer season, which in turn can result in poor grain fill and reduced yields relative to wheat that matures earlier in low residue systems.

Data in Figure 2 (Bouzerzour, 1983) show that soil temperatures from mid February to mid April, months when the wheat usually begins to grow, are 3 to 5 degrees cooler under 100% cover than with no cover. Wilhelm, et al. (1989) reported that temperature depressions of this magnitude retarded wheat plant development to some degree. By anthesis the 100% cover treatment still had a smaller leaf area index (LAI) than did the no cover situation. This is but one example of how no-till, intensively cropped systems will affect wheat plant growthh. Obviously there could be multiple interactions with root diseases and other pathogens in these systems that would not be present in conventionally tilled systems.

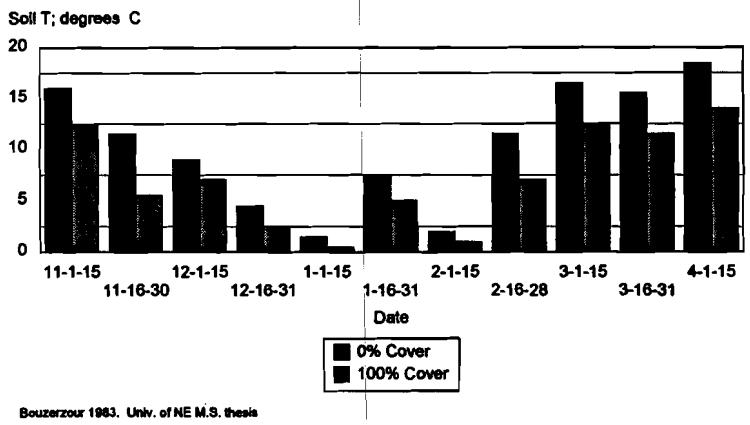

Figure 2. Soil temperature at the 50 mm depth as affected by residue cover at Sidney, NE (Bouzerzour, 1983).

Conclusions:

As no-till intensive cropping systems replace conventional wheat-fallow it is important that wheat breeders consider the potential effects of these moister, cooler, high residue systems when making selections for the wheats of the future. Table 2 is a list of possible characteristics that might be of interest for intensive cropping systems in the Central Great Plains. Breaking dormancy at lower soil temperatures in high residue situations could help avoid some heat stress during grain fill. Wheats must be able to thrive at lower soil temperatures; particularly if planted late in the growing season. For example wheat seeded after corn harvest requires the ability to tolerate less than an optimal temperatures. These are only a few considerations in a set of many unknowns. It is definitely important that wheat breeders become members of research teams that are investigating cropping systems for the Great Plains.

Table 2. Characteristics to consider for wheat in intensively managed dryland cropping systems.

✓	Ability to break dormancy at a lower soil temperature
✓	Ability to thrive under cooler soil temperatures
✓	Ability to over winter when planted late
✓	Spring wheats for southern latitudes

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SESSION 4

HARD WHITE WHEAT QUALITY

Waxy Genes in Wheat

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Starch from wild-type bread wheat (*Triticum aestivum* L.) contains 25-30% amylose. Amylose is synthesized in amyloplasts through the activity of an enzyme known as the granule-bound starch synthase (GBSS, E.C.2.4.1.21), also known as the "waxy" protein. Three structural genes, *wx-A1*, *wx-B1*, and *wx-D1*, encode wheat GBSS. The *wx-A1* and *wx-D1* loci are located on chromosomes 7A and 7D, respectively. The *wx-B1* locus is found on chromosome 4A. Null alleles, which produce no detectable isoforms of GBSS, are known from all three loci. Null alleles at the *wx-A1* locus occur at a high frequency in wheats from Asia, especially Japan and Korea. The *wx-B1* null commonly occurs in wheats of western Australia, especially those derived from 'Gabo'. Only two lines, 'BaiHuo' and 'BaiHuoMai', both from China, are known to carry *wx-D1* nulls. Null alleles at the *wx-A1* and *wx-B1* loci have been found within the Great Plains wheat gene pool (Table 1).

Japanese scientists produced the world's first waxy wheats by selecting waxy (triple null) progeny from the mating 'Kanto107' (*wx-A1* null + *wx-B1* null) / BaiHuo (*wx-D1* null). At Lincoln, waxy wheats also have now been produced after mating waxy progeny of Kanto107/BaiHuo to Ike, and from the cross BaiHuoMai/Ike. These initial waxy lines are poorly adapted, and significant breeding work is necessary before adapted types will be available. Waxy wheats have no starch amylose, and may be easily detected by staining starch or cut seed with an iodine solution (1 gm iodine + 1 gm potassium iodide in 100 mls H_2O). Waxy starch will stain red-brown, as opposed to the purple-black color of wild-type starch.

Wheats with one or two waxy null alleles often have reduced amylose content relative to wild-type; such wheats are often referred to as "partial waxy". The reduction in amylose content appears to be influenced by genetic backgrounds. In hard winter wheats, some, but not all, lines with single nulls have significantly lower amylose contents than wild-types; the double null line 'Ike' typically has only 15-20% amylose (Figure 1). Biotypes of the cultivar 'Norin1' having two null alleles have significantly lower amylose content than biotypes with only one null allele (Figure 2). This difference was observed whether amylose content was measured by enzymatic digestion following amylopectin precipitation by concanavalin A or by iodine binding after dissolution of starch in DMSO (conA vs I2/DMSO, Figure 2).

Reduced amylose content may alter starch cooking properties. A study was designed to determine the effects of GBSS null alleles on the starch pasting properties of hard winter wheats, and to verify the effects of double nulls through their examinations in additional genetic backgrounds. In 1996, head selections were made from F_3 bulk populations grown at Mead, Nebraska. Pedigrees of the three populations were:

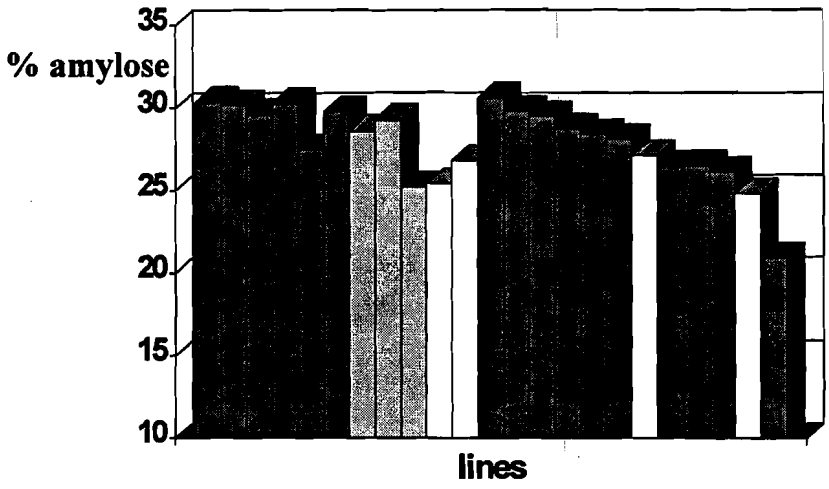
population 1 = MT8713/NE87612//Ike, population 2 = NE90476/Ike, population 3 = SD88137/Ike. Starch was purified from a bulk sample of 8 seed per head; starch-granule proteins were purified and separated by gel electrophoresis. Remnant seed of 150 lines was seeded in unreplicated head-rows at Berthoud, Colorado, in February, 1997. Ike and 'Vista' (wild-type) were seeded as checks in replicated plots randomized throughout the study. Sixty-one entries, plus checks, provided sufficient seed for both re-planting and analysis of starch pasting properties. Starch pasting properties were determined with a Rapid Viscoanalyzer. The following variables were recorded: gelatinization temperature, peak viscosity, breakdown, set-back, and final viscosity. A typical starch pasting curve from the RVA is shown in Figure 3. Based on GBSS status, lines were assigned to one of four genotypic classes: wild-type, *wx-A1* null, *wx-B1* null, and *wx-A1* + *wx-B1* null (double null). Analysis of variance and Duncan's multiple range test were used to compare genotypic means to each other, and to Vista and Ike, across all populations. Within- population comparisons were made in populations 2 and 3. In population 2, *wx-A1* null and *wx-A1* + *wx-B1* null (double null) lines were present, while in population 3, all four genotypic classes were represented.

With the exception of Vista, starches from all genotypes had nearly identical pasting temperatures (Table 2). Differences in peak viscosity were detected; in general, the presence of null alleles increased RVA peak viscosity, though distinct differences between genotypic classes were not observed. Double null lines and Ike did display significantly higher breakdown, lower setback and lower final viscosity than wild-type lines and Vista; single null lines displayed intermediate values for these variables.

Populations 2 and 3 afforded direct comparisons of the effects of *wx* null alleles within common genetic backgrounds. Together, the results (Table 3) suggest: a) pasting temperature is not altered by one or two null alleles; b) the presence of one or two null alleles increases peak viscosity relative to wild-type, but the difference between single and double null lines is dependent upon genetic background; c) null alleles increase breakdown and decrease setback in an additive manner; and, d) final viscosity decreases with the presence of null alleles.

In summary, null alleles at the *wx* loci can be used to effect significant changes in starch amylose content, and in starch pasting properties as measured by the RVA. Additional experimentation is required to determine the extent to which end-product quality might be altered by these changes in starch properties. GBSS null alleles will be used to develop both partial waxy and waxy wheats; both types will assist in the development of flours with altered cooking properties. Waxy types may provide new uses for U.S. wheats.

Figure 1. Amylose contents of select hard winter wheats



l.s.d. = 4.0

wt wx-A1 wx-B1 wx-A1 + wx-B1, lke

Figure 2. Amylose contents of Norin 1 biotypes.

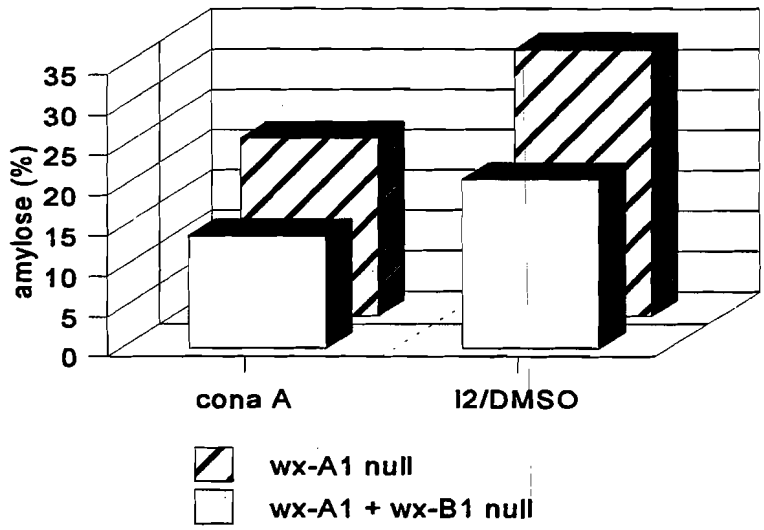


Figure 3. Typical RVA curve showing measured variables.

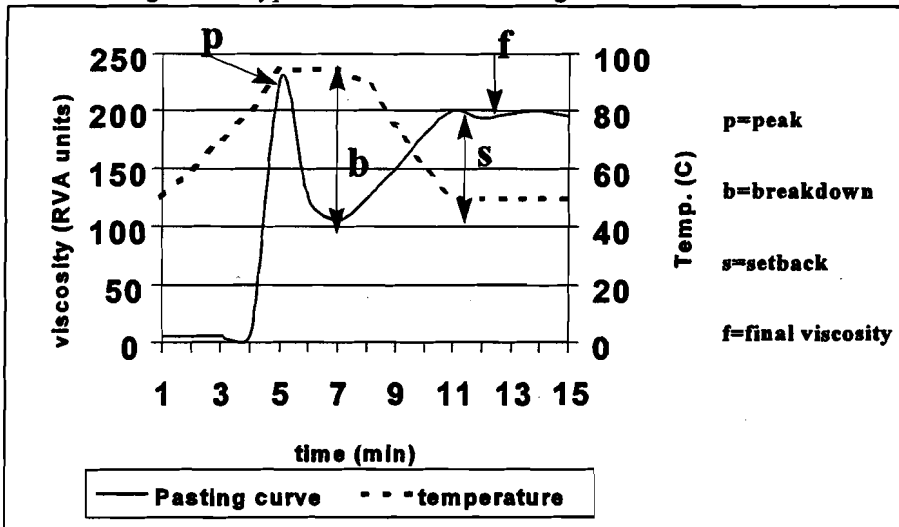


Table 1. U.S. hard winter wheats found to carry *wx* null alleles.

Genotype	Lines
wx-A1 null	Chisholm, Cimarron, KS801072 Colt, NE86501, Laredo, Custer, Sturdy, Payne (heterogeneous)
wx-B1 null	TAM200, TAM202, TX92V3108, TX93V5919, TX93V5922, TX93V4927, RioBlanco, WI93335, WI93339, K94H115, K94H400, K94H402, CO910748
wx-A1 null + *wx-B1* null	Ike

Table 2. Starch pasting properties of hard winter wheats from three breeding populations compared to Ike (double null) and Vista (wild-type). Means followed by the same letter were not significantly different.

Class or entry	n	Pasting temp. (C)	Peak Viscosity (RVA units)	Breakdown (RVA units)	Setback (RVA units)	Final Viscosity (RVA units)
Vista	1	71.2a	209.7c	60.5c	113.6a	262.8bc
Ike	1	64.1b	265.0a	111.3a	83.8d	242.0cd
wild-type	8	65.2b	239.2b	66.5c	111.4ab	287.0a
wx-A1 null	16	63.7b	250.7ab	86.3b	107.4ab	271.8ab
wx-B1 null	15	63.9b	262.2a	92.4b	99.7bc	269.5ab
double null	17	64.0b	256.5ab	109.5a	83.8d	231.0d

Table 3. Starch pasting properties of sister lines from two populations. Means followed by the same letter were not significantly different.

Genotype	n	Pasting temp. (C)	Peak Viscosity (RVA units)	Breakdown (RVA units)	Setback (RVA units)	Final Viscosity (RVA units)
Pop. 2						
wx-A1 null	9	64.2a	255.5a	93.0b	111.7a	274.2a
double null	8	63.9a	253.3a	115.7a	86.9b	224.5b
Pop. 3						
wild-type	6	65.5a	227.7c	58.2c	112.8a	286.1a
wx-A1 null	6	63.4a	244.4bc	76.4b	101.0b	269.0b
wx-B1 null	8	63.5a	263.1a	87.0ab	99.5b	275.7ab
double null	7	63.5a	259.3ab	99.8a	79.78c	239.3c

ASSESSING HARD WHITE WHEAT SAMPLES FOR END-USE QUALITY

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White wheats may be classified as soft white club, soft white common or hard white. There is currently no distinction between winter or spring types. However, with the exception of hardness, this method of classifying wheats is purely artificial and arbitrary. From an end-use quality standpoint, white wheats should be evaluated in terms of the following criteria:

- Grain hardness, *i.e.* whether the grain is "soft" or "hard." Hardness is the single most important trait governing end-usage. Harder grain typically has increased starch damage during milling, increased availability of oligosaccharides for fermentation and higher optimum water absorption. Another dimension of grain hardness is the relationship with level of soluble pentosans.
- Protein quality and quantity, *i.e.* gluten strength and mixing properties. Gluten properties affect most end-products and are critically important for most yeast-leavened products. Club wheats, as a class, have typically been among the weakest gluten wheats.
- Starch pasting properties. This trait is primarily governed by the presence or absence of the "waxy" gene(s) and is a direct manifestation of the amylose/amylopectin ratio of starch. Starch quality is the primary determinant of suitability in Japanese Udon noodles. "Second-generation" wheat genetics will undoubtedly characterize minor genes involved in amylopectin branching, amylose chain length, etc.
- Color attributes, especially PPO (polyphenol oxidase enzymes) and other sources of color that confer consumer acceptability of end-products; also, the preference for white seed coat color. A bright, stable color of alkaline noodles (high L*-value of raw, sheeted dough at 24 hr) is currently one of the primary criteria for developing hard white wheats.
- Water relations. Actually a summation of all physical-chemical traits (some known and some unknown); primarily a function of grain hardness (starch damage and pentosans) and protein content. Generally, hydration properties are assessed using mixing instruments (such as Mixograph and Farinograph) or solvent retention capacities.
- End-product tests. Laboratory-scale end-product tests such as cookie, bread, sponge cake and noodle attempt to emulate commercial practice, predict potential quality, and discern (or amplify) differences between flours.

Following is a list of routine tests conducted at the WWQL on breeding samples:

Test weight	Grain protein (NIR & Dumas)
SKCS 4100 single-kernel hardness, weight	NIR grain hardness
Grain moisture (NIR & oven)	Quadrumat flour milling
Buhler flour milling	Flour ash
Flour protein	Flour moisture
Mixograph	Rapid Visco-Analyzer
Alkaline noodle color (Minolta)	Flour swelling volume
Sugar-snap cookie	Pup loaf pan bread
Japanese sponge cake	Japanese Udon noodle

For most tests, more than one parameter is measured and reported to the breeder. The following table describes the main selection criteria for all of the major market classes of wheat in the PNW.

Table 1. Current quality criteria for each major wheat type and their primary end-use.

Market Class	Grain Hardness	Protein	Starch Pasting	Color Factors	Water Relations	Main End-Uses
Soft White Common	Soft	Weak, Low	N/A	N/A	Low Water Absorption	Cookies, Cakes, Flat Breads
Soft White Club	Soft	Very Weak, Low	N/A	N/A	Very Low Absorption	Cookies, Cakes
Hard White†	Hard	Strong, High for Bread(?)	High or Normal?	Low PPO, Alk. Noodle	High for Bread	Noodles, Bread(?)
Hard Red	Hard	Strong, High for Bread	N/A	N/A	High Water Absorption	Bread

† Criteria for hard white wheat have not been fully established by the industry.

A dilemma has arisen with this class structure regarding the targeted end-use of hard white wheat. My current opinion is that hard white wheat varieties should essentially be equivalent to current hard red varieties, but with white seed coat (obviously) and low PPO/bright alkaline noodle color. These can be either high pasting, "one-gene waxies" or normal pasting, normal amylose types. But, if both types are developed in the hard white market class, they will need to be segregated for noodle use, essentially creating two "sub-classes." If the two types cannot be accommodated, then the preference will be for normal amylose types. Consequently, there is an inherent problem with the current U.S. market class system: there exists no easy way to accommodate these two "starch types" of hard white, nor does there exist a way to easily accommodate the market demand for high pasting, soft, moderate-strength types for Japanese Udon, and to some extent, Korean white salted noodles.

A further consideration in any discussion of hard white wheats is the large flat-bread market, which I believe may be targeted as a "sink" for low protein or "out of spec." hard white production. Again, segregation will be the key to customer satisfaction.

Questions and comments are welcome:
WWQL home page: *http://www.wsu.edu:8080/~wwql/*
e-mail: WWQL@wsuinx.it.wsu.edu

Recent publications of interest:

Morris, C. F. and S.P. Rose. 1996. Chapter 1. Wheat. (In) Cereal Grain Quality. R.J. Henry and P.S. Kettlewell (eds.) Chapman Hall, London. pp. 3-54.

Wrigley, C. W. and C. F. Morris. 1996. Chapter 11. Breeding cereals for quality improvement. (In) Cereal Grain Quality. R.J. Henry and P.S. Kettlewell (eds.) Chapman Hall, London. pp. 321-369.

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EVALUATION OF ASIAN NOODLE TEXTURE AND COLOR

by
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Asian noodles are evaluated for their texture, appearance, processing quality, and cooking characteristics. This talk focuses on four noodle types: Chinese raw (white salted), instant fried, hokkien, and bamee (Cantonese style noodles). The relative importance of texture, appearance, etc., varies according to the noodle type. For example, processing quality and texture are more important for instant noodles, while appearance (color and brightness) are weighted more heavily for hokkien and bamee noodles. Noodle color is measured objectively using a colorimeter, and results are compared to subjective evaluations by noodle makers. Correlations are quite good between these two measures. Noodle brightness is a characteristic more difficult to test using a colorimeter. Key Asian noodle texture attributes are bite (hardness), springiness (visco-elasticity), and smoothness (slipperiness). Noodle texture is tested using a TA.XT2 texture analyzer (other texture instruments are available). One of the techniques is Texture Profile Analysis (TPA), which quantifies attributes such as firmness, springiness, cohesiveness, etc. Results are compared to noodle makers' subjective ratings of these attributes. Good correlations have been obtained relating noodle bite to TPA firmness, and noodle springiness to TPA chewiness. Future efforts will focus on objective measurement of noodle brightness and standardizing instrumental texture techniques.

Marketing Hard White Wheat

Mr. Rob Bruns
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Agripro Seeds, Inc.

Summary:
Hard White Wheat ***Value*** will drive the acceptance and commercialization of this exiting new crop. In my opinion, the perceived value of hard whites among the wheat research community and grower groups is greater than real value. As the wheat community moves forward with hard white wheat development, it is time to "GET REAL" about the marketing value of Hard White Wheat.

What is the real value of Hard Whites?

- Is there extra **milling** value? As long as the ash standard is used, the only extra value is really related to a 1% - 2% increase in flour yield. This would equate to a $.03 to $.07 per bushel value.
- Is there value in export **preference**? There are a number of key markets that genuinely prefer white wheat, but those same customers are very price sensitive. The export industry cannot participate in supplying these markets unless the preference is great enough to command a higher price than currently paid to the Australians.
- Is there value in improved **taste**? Improved flavor has been demonstrated in controlled studies, but to date, no one to date has been able to successfully market taste to the baking industry or to the consumers.
- Is there value in **special utilization**? There are currently a number of smaller markets that utilize Hard White Wheat and generate enough extra value to cover the costs of production, segregation, storage and distribution. These would include AWWPA, ConAgra Flour Milling, Cargill Flour Milling, and Pro Mar in Idaho.

To be successful, hard white wheat has to create enough extra value somewhere in this chain of industries to overcome the inherent added cost of project development. Some examples of inherent added cost could be: technology costs, grain production costs, transportation & storage costs, special handling costs, market development costs, and non-grade disposition costs.

Based upon my experience, I would propose the following formula for successful hard white wheat market development:

Objective: ***"create enough value to overcome development costs"***
Strategies:

1. Develop multiple Special Utilization projects to create industry awareness and minimum scales.
2. Blend in mini-commodity programs on the coat tails of the Special Utilization projects.
3. Once the industry is familiar with Hard White Wheat, the true commodity value will level out naturally.

As the wheat community moves forward with hard white wheat development, we need to be realistic and seek out the true value it offers to the entire industry.

SESSION 5

HYBRID WHEAT

Heterosis in Hard and Soft Red Winter Wheat.

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Berthoud, CO 80537

Presented to:
21st Hard Winter Wheat Workers Workshop
Denver, CO
January 28 - 30, 1998

Abstract:

Best parent heterosis for grain yield in Soft Red Winter (SRW) wheat has been calculated for some 2787 hybrid combinations grown over multiple locations in the Corn Belt region of the USA in different years from 1984 - 1994. Average heterosis for this sample of hybrids was 106.5%, with a standard deviation of 9.1%. There appears to be an interesting year effect associated with the expression of heterosis, which ranged from a low of 96% (averaged over 84 hybrids) in 1984 to a high of 111% (averaged over 173 hybrids) in 1986. The most heterosis for a given hybrid within each of these years ranged from a low of 111% (12 location average) in 1995, to a high of 149% in 1991 (8 location average).

Best parent heterosis for grain yield in Hard Red Winter (HRW) wheat averaged 103.4% for 85 hybrids grown in two years (1993 and 1997) in the Great Plains. The standard deviation of this distribution was 6.9%.

Heterosis for other traits was evaluated as well. Average best parent heterosis for these traits is as follows:

Soft Red Winter Wheat Trait:	Best Parent Heterosis:
Test Weight:	101 %
Flour yield:	100 %
Break flour yield:	98 %

Hard Red Winter Wheat Trait:	Best Parent Heterosis:
Norris Hardness :	98 %
Flour protein :	98 %
Flour Yield :	99 %
Absorption :	100 %
Tolerance :	91%
Loaf Volume :	98 %

With the notable exception of tolerance for Hard Red Winter Wheat quality, all traits approach or occasionally exceed the best parent value.

Gene pools were established in SRW germplasm in 1984, with the objective of improving the average expression of heterosis. Based on a sample of hybrids grown during the first five years (1984-1988), and comparing their average heterosis to a different sample of hybrids during the last five years (1991-1995), it

appears that the average improvement in heterosis between the three gene pools was 2.7 % for the 12 year time frame.

Inbreeding depression in SRW wheat averaged 5.3 bu./A. (6.4%) over several different hybrids grown in six different years from 1981 to 1995. However, in several cases, the F-2 yielded more than its respective F-1 hybrid. The most inbreeding depression was 12.5 bu./A. (13.3%) for a hybrid grown in 1987-88. The least inbreeding depression was for a hybrid grown in 1991, where the F-2 yielded 4.8 bu./A. more than its respective F-1 hybrid. Average inbreeding depression in HRW wheat was 9.9 bu./A. (11%) for nine hybrids grown in three reps and four locations in 1993.

The ability to produce hybrid seed is at least as important as the need to exploit the phenomenon of heterosis in the development of hybrid wheat. Average hybrid seed yield from the Crossing Block nursery in Lafayette, IN, as measured on a number of different hybrids over 10 years was 29.2 bu./A. This compares to an average yield of an elite line trial on the same farm of 60. 7 bu./A. Heritability of hybrid seed yield was estimated for two hybridizing agents using data from the Crossing Block nursery in Lafayette, IN. Heritability for the CHA (Chemical Hybridizing Agent) RH-0007 was estimated to be 0.29, but was not significantly different from zero. Heritability for "GENESIS" hybridizing agent was estimated to be 0.83. Thus it appears very likely that progress in developing good seed yielding parents is possible.

Lessons for Hybrid Wheat: As Learned From Corn Breeding.

Blaine Johnson
HybriTech USA
A Unit of Monsanto

Presented to:
21st Hard Winter Wheat Workers Workshop
28-30 January, 1998
Denver, Colorado

Abstract:

Single Stage Evaluation: Selection of superior of individuals with superior genetic value is obviously critical to the success of any plant breeding project. When selection is made on basis of the phenotypic value of a metric trait, the probability of correctly identifying genetic superior individuals is a function of the true genetic value of each individual, the environmental effect, and any genotypic by environmental interaction. In a study of 285 S_2 maize lines, evaluated as testcross progeny for the trait grain yield, a 20% selection intensity resulted in a probability of over 0.999 of selecting at least one individual which had a genetic value in the upper 10% of all individuals. When the goal was retaining at least one individual with a genetic value in the upper 2.5%, and phenotypic selection intensity increased to 5%, the probability dropped to 0.609. When the goal was identification of at least five individuals from the upper 5% phenotypic tail that had genetic values in the upper 2.5% genetic value, the probability dropped to less than 0.003. The lesson: During a single stage of selection, high selection intensities result in low probabilities of correctly identifying individuals with superior genetic value for traits such as grain yield in corn which have relatively low heritabilities.

Multiple Generation Evaluations: During parental inbred line development in corn, limited resources force breeders to make decisions as to the generation in which testcrossing is initiated, and number of testers to be used within each stage of development and evaluation. A simulation program was developed to investigate various scenarios in which testcross evaluation was initiated in successively later generations of inbreeding, and the effect of using multiple versus a single tester during the inbreeding and selection process. The stochastic model simulated phenotypic, genetic, and environmental variances, as well as expected means. Results of evaluations of S_4 testcross hybrid progeny showed that greatest genetic value of the hybrid progeny resulted from initiating testcrossing in the S_0 generation. Genetic value of S_4 hybrid progeny was consistently decreased with each successive generation in which testing was delayed. Likewise, use of multiple testers over generations resulted in greater genetic value of S_4 hybrid progeny than did using a single tester over the multiple generations of evaluations. In contrast, genetic value of the parental lines *per se* increased with each successive generation in which testcross evaluations were delayed, with this increase being greater when multiple testers were used than when single testers were used. Averaged over all multiple generation evaluations, over 55% of the selected parental lines were found to trace back to only three S_0 plants. The lessons: Genetic value of hybrid progeny is maximized by beginning testing of experimental lines as early in the selfing and development of new inbreds as is possible; Because few original

S_0 plants contribute to selected parental lines, sufficient numbers must be retained during initial selection among S_0 to ensure that selfed progeny of genetically superior S_0 plants will be identified in future generations of selfing and testing.

Value of Parental Lines as Determined by Value of Hybrid Progeny: In hybrid programs the ultimate value of a parental line is determined by the value of the hybrid progeny of the parental line. During inbred line development in maize programs virtually all evaluations, other than selection among plants within a selfing nursery, is based upon hybrid progeny performance. In wheat where production and testing of hybrid progeny is more difficult than in maize, substitution of line *per se* testing for hybrid progeny testing is often suggested. In an evaluation of seven quality traits in wheat, the performance of parental line R1287 exceeded the performance of W94-042 for five of the seven traits, when both were evaluated as lines *per se*. Yet when averaged over all hybrid progeny, the average hybrid progeny values of W94-042 exceeded the average hybrid progeny values of R1287 for all of the seven traits. The highest ranking hybrid of all progeny of both R1287 and W92-042 for total aggregate quality score was the hybrid W94-391 x W94-042. Average hybrid progeny values (HPV) were estimated for total aggregate quality score for all 46 parental lines in the study. Based upon rankings of HPV, W92-042 was ranked 6^{th} while R1287 ranked 26^{th} even though line *per se* performance of R1287 was significantly better than W94-042 . The lesson: In hybrid wheat, as in corn, the value of a parental line is determined by the performance of hybrid progeny of the parental line, and that value cannot necessarily be predicted on basis of line *per se* performance of the parental line.

Marketing Hybrid Wheat in the Central Plains.

Scott Dyer
HybriTech Seed
Plains Business Team Leader

Presented to:
21st Hard Winter Wheat Workers Workshop
Denver, CO
January 28 - 30, 1998

Abstract:

Issues: Grower feedback provides HybriTech with two key points:

1. Hybrids produce more wheat than varieties
2. They cost too much.

Other market feedback indicates the feeling that hybrids are for the irrigated producer only. At HybriTech our objective is to differentiate hybrid from varieties by selling by the seed count, recommending by the seed count and pricing by the acre using average rainfall as our predictor of seeding rate. By selling by seed count we place a value on the seed that is in direct relationship to the seeding rate. This allows pricing by the acre and setting of target price per acre. When a target price is set, we are then able to sell the other benefits hybrids deliver, such as improved stand, straw strength, consistency of yield and improved disease package. Recommended seed counts remove seed size as the determining factor to population. Rainfall determines a component of the yield equation and thus fertility and yield expectation. Differentiating hybrids by population and selling by the acre will allow HybriTech to be successful. Seed for 1998 will be available in bulk from regional bulk stations. Market research tells us that 95% of the market is bulk seed and that bulk in the preferred package size of choice.

SESSION 6

MANAGEMENT OF INTELLECTUAL PROPERTY

OVERVIEW OF INTELLECTUAL PROPERTY RIGHTS ISSUES

Hard Winter Wheat Workers Workshop
January 28-30, 1998
Fred A. Cholick

First and foremost, I am not an "expert" with regard to Intellectual Property Rights (IPR) issues but rather a plant breeder and, by position, an Agricultural Experiment Station Director. I am now required to deal with this critical issue from a different perspective in our ever changing world. The following, from the IPR III Workshop, summarizes where we are today: "Intellectual Property Rights have become a standard element in the conduct of agricultural research. The rise of biotechnology has shifted the focus from breeders rights to patent rights." Also from IPR III: "Genetic diversity is essential for the future production of an abundant, safe, and reliable supply of food and fiber. Genetic diversity - a genetically diverse supply of breeding material - is the most critical element in the process of developing improved plant varieties." In order to have and conduct a breeding program with this required genetic diversity today, plant breeders and the institutions with which they are associated must understand the shift from "breeders rights" to "patent rights".

The Convention on Biological Diversity (CBD) redefined genetic resources from being considered as a public good and as part of our common global heritage, to a commercial good with market potential. This has resulted in numerous individuals, institutions, and governments expecting monetary return from genetic resources. There appears to be the idea that genetic resources will be a great source of revenue.

Plant breeders rights are not new and are based on UPOV (International Union for the Protection of New Varieties of Plants) 1961, 1991, and PVPA (Plant Variety Protection Act) 1970. (Note - as of July 1997 UPOV had 32 members and 4 more under examination). In some circles, Plant Breeders Right and Plant Variety Protection have become synonymous and provide a minimum form of protection in today's world. This approach does not impact the use of the protected variety (genetic resources) and thus respects the breeders' traditional reliance of building on one another's advances without any additional agreements. The 1991 UPOV convention introduced the concept of "essentially derived" to clarify which genetic resources are eligible for protection. The concept of "essentially derived" is not difficult to define from a technical perspective but can be difficult with regard to determining ownership. Variety protection also does not address the issue of access of germplasm that is not marketed, but deposited in germplasm collections.

Patent rights are different from breeders rights in many ways but two are of particular importance - first, no research exemption, and second, ability to make further modification under the same protection (patent). Both of these impact the use of germplasm. The first one directly because agreements must be developed to make crosses or even to conduct research. The second can result in continued

modification and thereby continued restriction of availability and use of the genetic resource.

The use of patent rights has developed a new order (paradigm) within the plant breeding community, and this is an agreement on "use" of germplasm - typically called licensing. Licenses are needed because patents provide monopoly rights to the patent holder. Material Transfer Agreements (MTA) are becoming the licensing format of choice. The MTA's can, and do, take many farms from open-end free use to extremely restricted, i.e. the breeding methodology to be used. In addition to MTA's, Freedom To Operate (FTO) agreements are being developed that allow two identities to conduct research in a given area with the understanding that financial consideration will be determined through future negotiations. MTA's and FTO's can, and are, being put together into one agreement.

The private sector of plant breeding has addressed IPR issues head on and as a way of life. The following quote from Byron illustrates this: "...assessment should include: preparation of a product-specific technology profile, identification of relevant patents and patent applications and determination if licenses can be obtained from intellectual property owners...failure to acquire "freedom to operate" with key intellectual property components can result in a barrier to conducting research and commercialization of products."

In contrast, public institutions generally are not organized in such a way that IPR issues are addressed consistently. The accountability of public institutions to the taxpayers coupled with its role to provide unbiased information and knowledge are factors creating conflicts within public institutions. The lack of public investment into agricultural research in general, and specifically plant breeding, will likely increase public institutions' effort to obtain funding from licensing IPR including genetic resources. I recently heard a high level administrator in a public institution indicate they are patenting everything that moves.

The question of impact of utility patents on access and exchange of genetic resources was discussed at IPR III with no consensus on if this type of protection had a positive or negative influence on the flow of genetic resources.

Related issues

1. Farmers Rights - rights arising from the past, present, and future contributions of farmers in conserving, improving, and making available plant genetic resources.

2. NGO - Non Governmental Organizations.

3. Conservation, classification and preservation of genetic resources - deposits - access.

Everyone appears to agree that diverse genetic resources are the life blood of a plant breeding program, but ownership issues, and therefore IPR issues, have created questions on how this life's blood will and should flow.

There is little question that IPR issues have added complexity to the plant breeding profession and a new team member to the plant breeding team - Lawyers.

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Evolution of Intellectual Property Rights Issues in Soybean[1]

Bill Schapaugh[2]

I appreciate the opportunity to share with you events that have occurred over the past several years to the exchange of soybean germplasm and describe briefly how these changes have impacted the Kansas State University Soybean Breeding program. I thank Rollie Sears and Jim Quick for asking me to address this topic. I'll begin by describing the primary method of germplasm exchange that dominated the industry when I began my career as a plant breeder. I'll review some of the events that began to impact the germplasm exchange in soybean and then discuss the impact those changes had on our program and on the testing and use of germplasm entered in the Uniform Tests. Using input received from private soybean breeders, I also will summarize the current policies regarding germplasm use in the industry, share some general concerns and conclude with comments about future expectations.

Breeders' Exemption and Free Exchange

I began my breeding career when the Plant Variety Protection Act (PVPA) of 1970 was in place. In 1994, the amended PVPA made significant changes regarding farmer use of protected varieties but left intact the breeders' exemption, which automatically allows the use of protected varieties for further breeding and research. The PVPA stimulated significant private investment in soybean development. As the number of programs grew and competition increased, it was probably inevitable that alternatives would be explored to enhance the intellectual property protection available through the PVPA.

Enhanced Breeders' Rights

In the mid to late 1980's, several major events occurred that began to shape the current state of germplasm exchange. In 1985, the Patent Office Board of Appeals and Interference ruled that utility patents could be granted for plant material in the case Ex Parte Hibberd (227 USPQ 443). Although this ruling did not immediately impact germplasm exchange in soybean, it certainly raised questions about the extent to which utility patents would be used in the future to protect soybean varieties. For several years, I believed the general feeling in both the public and private sectors was that utility patents would be used infrequently to protect a variety, but that assumption is turning out to be incorrect.

A few years after the Hibberd case, a public breeding program released two varieties developed by backcrossing Phytophthora root rot resistance into a popular private variety. Both public releases were protected under the PVPA, as was the private variety used as the recurrent parent. It was evident that with only the PVPA, breeding programs could easily duplicate substantial gains made by anyone in the industry. These releases certainly contributed to the discussion of essentially derived varieties and minimum distance. In 1994, the amended PVPA addressed the concepts of essentially derived varieties and inappropriate use of germplasm. But utility patents, or the PVPA didn't have the first impact on germplasm utilization in the Kansa

State breeding program. It was technology use agreements that made us closely examine how we were gaining access to germplasm used in our program.

During the spring of 1988, our experiment station director received a letter from the associate director of an experiment station in a neighboring state. That station had been informed by a private soybean breeding company that it expected to receive compensation for soybean varieties developed from crosses that contained varieties that it developed. Several varieties developed by the company had been used as parents in single crosses by the public program. We were asked to provide a complete list of crosses in our program that traced back to some 31 restricted private varieties. The form of intellectual property protection used to protect these varieties was not the PVPA or utility patents, but restrictions specified on seed use agreements, in this case, between a public and a private breeding program.

We were working with several populations in which the parentage traced to the restricted varieties. Most of the populations in question were created using lines entered in the Uniform Tests as parents, but some populations had been created using the restricted varieties directly as parents. The Kansas Agricultural Experiment Station at that time chose not to attempt entering into a royalty arrangement with a private company, and all of the populations and lines tracing to the restricted varieties were discarded from our program. Attempts are now made to obtain written permission for the use of privately developed varieties for use as parents in our breeding program. If permission is denied, the germplasm is not used. With technology use agreements and utility patents, we had entered a new era in soybean breeding.

Germplasm Exchange Agreements

Most publicly developed soybean germplasm is available on an unrestricted basis to establish single or more complex cross populations. So, a majority of the dynamic activities in germplasm exchange is taking place in the private sector and impacting the exchange among private companies and between the private and public sectors. To be as up-to-date as possible on the current polices, I contacted representatives from most of the private soybean breeding programs and requested information on their germplasm use agreements and/or purchase agreements that address using their germplasm for breeding and variety improvement purposes. I received input from about half of the over 30 organizations I contacted. I appreciate their cooperation.

I received three basic responses to the request for information on germplasm use agreements. A few companies indicated that they will provide seed of a variety for unrestricted use in biparental crossing. This represented the standard policy for a small percentage of respondents and was one of several options offered by a few others. Included in this group are varieties protected under the PVPA, and the companies were recognizing the breeders' exemption provided under that form of protection. A few companies indicated that they were not exchanging germplasm for use as parents. A majority of the companies have taken the position that they will exchange germplasm under a controlled agreement.

These "controlled" exchanges may specify how the material can be utilized in a breeding program, how the new variety can be released, geographical area of release of the new variety, a royalty schedule, and possibly other factors. Some organizations prefer agreements that involve the exchange of varieties between participants. Royalty payments may be linked to the contribution the exchanged germplasm makes to a new variety.

Technology use agreements and utility patents are providing enhanced protection to a growing sector of the soybean industry. Some companies have taken the position that all their soybean varieties should be patented. Germplasm is definitely becoming more tightly controlled. An increasing percentage of the germplasm that is shared will be available only through transfer agreements, which likely will involve royalties. Apparently, soybean breeders have accepted the requirement to pay a royalty to another germplasm originator to enhance diversity in their breeding programs. Although these agreements are certainly more restrictive than was common a few years ago, germplasm is being exchanged.

Impact on Uniform Tests

The Uniform Tests represent an extremely important component of our evaluation system. Through this cooperative effort, we have access to as many as 25 testing locations per season for evaluation of elite material. To enter experimental lines in the Uniform Tests, including the Preliminary Tests, the lines must be free of restrictions on their use as parents in biparental crosses or in recurrent selection programs. If an entry is not free of these restrictions, then the germplasm is excluded from the testing program. Conflict with this policy first appeared in 1988, in the situation I previously described. Since that time, test participants have avoided submitting entries with restrictions.

State release mechanisms also are impacting the utility of the Uniform Tests. Current policy requires that any state or province participating in the Uniform Test must be offered the opportunity to participate in the release of any entry proposed for release. Entries can be released on a restricted basis or a contractual basis only after participants have been offered the opportunity to participate in their release. Several releases over the past few years have not been offered to all or any of the test participants or have been offered only with some type of restriction or qualification. The Kansas Agricultural Experiment Station has placed restrictions on recent cooperative releases. In most cases, cooperating states were required to establish a marketing group if they wanted access to the variety. The strength and success of the Uniform Testing program can be attributed to tremendous cooperation, which has allowed each state access to the best public germplasm both for crossing and release, regardless of where the germplasm was developed. These recent release restrictions have limited producer access to some our the public varieties.

A growing percentage of the germplasm base is now available through restricted access to breeders. Many of these restrictions prohibit public breeders from using the germplasm to develop populations and still gain access to the Uniform Tests to evaluate the progeny. If the current polices for entries in the Uniform Tests are maintained, new alliances will develop to accomplish the needed testing of restricted germplasm. If the polices are amended to permit the

evaluation of restricted germplasm, some participants will not realize the benefit of using specific entries for crossing or participating in the release of specific varieties. Regardless of the path the breeders choose to take with Uniform Testing policy, some of the benefits and strengths of the cooperative program likely will diminish.

Concerns

National and international attention is focused on the protection and impact of germplasm use and exchange. The International Association of Plant Breeders is considering a discussion paper that specifically addresses breeders' and research exemptions and compulsory licensing for plant varieties protected using utility patents. The development of patenting plant varieties in the USA appears to be the impetus to address these issues. Discussions will progress, and intellectual property rights for plants will continue to evolve, but protection offered by the PVPA probably will not sustain the level of investment currently experienced in soybean breeding. The industry recognizes the need for germplasm exchange, but in breeding as in most endeavors, it is easier to catch up than to break new ground. Restrictions on exchange will continue.

Operating under a system of restricted germplasm exchange raises concerns about the impact on genetic gain. These concerns have merit, but hopefully the present variety protection alternatives will increase incentives to pursue other germplasm sources and develop germplasm that would be developed less quickly or not at all. This outcome could offset the potentially negative impact of limited exchange. Also, public soybean programs can play a key role in complementing activities in the private sector and help diversify the germplasm base.

Expectations

We will continue to experience diversity in germplasm use agreements. Organizational positions will change and evolve as decisions are made on the appropriate types of protection to utilize with each variety developed. Many of these decisions will result in tighter controls on an increasing portion of the germplasm. Some organizations will flourish, and others will struggle in response to these changes. But look at the opportunities that are becoming a reality with the genetic manipulation of plants and the evolution of intellectual property protection for plant varieties that we are experiencing today: advancements that dramatically impact the production and utilization of soybean and soybean products. Advancements that are needed to effectively serve the producers and, more importantly, the consumers.

SESSION 7

GERMPLASM DEVELOPMENT AND BIOTECHNOLOGY

USDA-ARS Regional Germplasm Development Efforts

Gina Brown-Guedira
USDA-ARS Plant Science and Entomology Research Unit
Manhattan, Kansas

USDA-ARS Units involved in the development of germplasm for the hard winter wheat growing area of the Great Plains include the Plant Science Research Lab at Stillwater, OK, the Plant Science and Entomology Research Unit at Manhattan, KS, and the Wheat, Sorghum, and Forage Research Unit at Lincoln, NE. Each ARS program has specific research objectives, but all work to identify diverse sources of traits of economic value, study the genetics and expression of the traits of interest, incorporate useful genes into adapted germplasm for use in breeding programs, and test and evaluate breeding lines and varieties.

Development of wheat germplasm with enhanced end-use quality:
C. J. Peterson, Bob Graybosch, USDA-ARS, Lincoln, NE

Modified starch characteristics Germplasm lines with altered amylose/amylopectin ratios are being developed from crosses of Nebraska wheats with the variety Ike, which has null alleles at two of the three waxy protein genes of wheat. Partial waxy lines have been recovered and will be made available in the fall of 1999. Small quantities of triple null, waxy grain lines derived from crosses involving the Japanese varieties Bai Huo and Kanto 107 are currently available. This material will not be formally released. More agronomically desirable full waxy-grain types developed from crosses with Ike are being developed and should be available in 2-3 years. (See paper by Bob Graybosch, this volume, for information on the genetics and quality aspects of waxy wheat).

Advanced lines have been developed that had reduced levels of polyphenol oxidase activity in preliminary tests. This will be confirmed with grain from 1998 trails and lines would be made available in the fall of 1999.

Enhanced protein quality
Germplasms with increased glutenin content are being developed through the introduction of additional genes encoding high-molecular-weight (HMW) glutenin subunits into hexaploid bread wheats, which normally do not have more than five active HMW-glutenin genes. Populations that have a sixth active glutenin gene from *T. dicoccoides* backcrossed into an adapted wheat background should be available in the fall of 1999 or 2000. Lines developed from crosses with a hexaploid landrace from Israel having a duplication of one of the HMW-glutenin genes will also be available at that time. Transgenic spring wheats developed at the USDA-ARS Western Regional Research Center at Albany, California, with genetically engineered HMW-glutenin genes are also

being used as a source of genes to alter protein composition. Introgression of the transgenes into Great Plains wheats is in early stages.

The translocated chromosome t1BL/1RS is being backcrossed into N86L177, a very strong gluten line, to overcome some of the quality deficiencies associated with the rye chromosome arm. These lines are currently available and are scheduled for formal release in 1999. A new source of the T1AL/1RS translocation is available in the germplasm GSR1201 developed by the ARS unit at Stillwater, Oklahoma. Lines identified from the cross GSR1201/TAM 200 with improved agronomic performance and the new T1RS/1AL chromosome with be released this year.

Development of wheat germplasm with resistance to cereal aphid pests:
James A. Webster, David R. Porter, Cheryl A. Baker, USDA-ARS, Stillwater, Oklahoma

A description of most of the work done on aphid resistance was presented during the entomology session of these meetings. Readers are referred to papers by Jim Quick, Colorado State University, and David Porter and Cheryl Baker, ARS-OK, in these proceedings. The status of the development of germplasm resistant to the Russian wheat aphid (RWA) is briefly described below.

Russian wheat aphid
RWA resistance derived from 14 different plant introductions has been incorporated into wheat lines of five different market classes (hard red winter, hard white winter, hard white spring, soft white spring, and hard red spring). Most of this material was at the fifth backcross this past crossing season (1997) and are being advanced for production and identification of homozygous lines. Additional introductions with exceptional levels of resistance have been entered into the crossing program, but are not as far advanced. The availability of different RWA resistance genes in high performance wheats of all market classes gives breeders a greater selection and will broaden genetic diversity in newly developed RWA-resistant wheat cultivars.

Development of wheat germplasm resistant to biotic stress:
Merle Eversmeyer, Jim Hatchett, and Gina Brown-Guedira, USDA-ARS, Manhattan, KS

Before discussing the germplasm development efforts of the unit, I would like to update the group on personnel changes. In addition to my appointment as the Wheat Research Geneticist this past July, the unit has received funds to fill a newly created Molecular Plant Pathologist position. Also, Dr. Jim H. Hatchett, who led the ARS research program on the Hessian fly since 1976, retired earlier this month. As was the case with my position, a new scientist coming into the position left vacant by Dr. Hatchett will assume an active and productive research program. Current and future priorities of the Hessian fly research program are noted below.

Hessian Fly

The unit plans to continue research on Hessian fly resistance strategies in wheat and plant-insect interactions. Identification of new sources of resistance and development of resistant germplasm via interspecific crosses will continue to receive emphasis. The goal of the research is to introgress resistance genes from progenitor species into elite genotypes and develop diverse resistant germplasms for use in breeding programs. With the aid of molecular markers, greater effort will be made to develop multiple pest resistant germplasms.

Evaluation of wheat breeding lines for Hessian fly resistance, and the development of resistant varieties in collaboration with breeders will continue to be an important part of the program. Presently, the project cooperates with public or private breeders in Kansas, Nebraska, Texas, South Dakota, Washington and Idaho in the development of resistant varieties. As a result, the acreage of resistant varieties in the Great Plains has more than doubled in the last ten years. However, the Hessian Fly continues to invade areas where it was not previously known or is not considered a serious pest. In some of these areas, infestations have caused serious losses that continue to go unchecked because resistant varieties are not available. Recent tests of wheats in the Regional Germplasm Observation Nursery showed that all breeding programs contained unselected lines that carried some resistance to Hessian fly. Thus, early generation selection for resistance would be valuable to breeders that are developing varieties for areas where Hessian fly is or may become a serious problem.

New Hessian fly-resistant germplasm is being developed. Backcross lines having a single, dominant gene for resistance to Hessian fly derived from *T. turgidum* spp. *dicoccon* have been recovered with both winter and spring growth habits. Material is currently being evaluated to isolate cytologically stable, homozygous resistant lines. Work is underway to determine the relationship of this resistance with other genes that have been transferred to common wheat from *T. turgidum* spp. *durum.*

Leaf rust

Leaf rust resistance genes from six accessions of *Ae. tauschii*, one accession of *T. monococcum* ssp. *monococcum*, and one accession of *T. monococcum* ssp. *aegilopoides* are being transferred into wheat. Tests of alellism in the progenitor species suggest that the two A-genome sources represent new genes for resistance, and that three of the *Ae. tauschii* accessions have a leaf rust resistance gene different from *Lr21*. Homozygous resistant lines were recovered from crosses between wheat and eight different accessions of *T. timopheevii* spp. *armeniacum*. Studies in the wild species indicated the presence of at least three unique leaf rust resistance genes in these eight accessions. Currently the genetic relationship of the resistance genes in a hexaploid background is being tested. Two germplasms with leaf rust resistance derived from *T. armeniacum* have been released, KS96WGRC35 and KS96WGRC36. The release of additional germplasm with resistance from this species will depend upon the results of genetic studies and the agronomic performance of resistant lines.

The USDA world wheat was screened for seedling resistance to virulence in the leaf rust pathogen populations to *Lr* genes 1, 2a, 2b, 2c, 2d, 3, 9, 10, 11, 15, 17, 18, and 24. Low infection types (0-3 on a scale of 0-9) were observed for 1973 landraces in the collection. Accessions with low infection types to the mixture of viruleneces tested were identified in each of the cultivated diploid, tetraploid, and hexaploid species tested. These landraces are being tested further with individual isolates to *P. recondita* to characterize the resistance.

Greenbug Evaluation of the 1996 and 1997 Regional Germplasm Observation Nursery at Stillwater, OK, for reaction to greenbug, identified germplasms with resistance to greenbug derived from three different accessions of *Ae. tauschii*. One resistant line, KS95U306, was crossed with TAM 110, which has the *Ae. tauschii*-derived resistance gene *Gb3*. No segregation was observed a F2 population of 300 plants infested with greenbug biotype K. Resistance in KS95U306 is determined by a single dominant gene. Inheritance and uniqueness of resistance derived from the two other *Ae. tauschii* accessions is being determined.

Other disease and insect resistances Material in early stages of development include transfer of resistance to wheat curl mite from *T. monococcum* spp. *aegilopoides* and *T. armeniacum*, and resistance to wheat curl mite and powdery mildew from a spring wheat line having the short arm of chromosome 6V (T6VS/6AL) from *Haynaldia villosa*. Attempts are also being made to transfer resistance to leaf rust, powdery mildew, and greenbug from *Ae. speltoides*.

IMI-WHEAT AS A SOLUTION FOR BROAD SPECTRUM WEED CONTROL

by
Bob Morrison
American Cyanamid
Princeton, NJ

IMI™ wheat describes wheat varieties that are tolerant to imidazoline herbicides. American Cyanamid has realized considerable commercial success with this class of herbicides, most notably for broad spectrum weed control in soybeans. Since soybeans and other legume crops are naturally tolerant to imidazolinone herbicides, application of this weed control tool in other crops required the development of tolerance through genetic-based selection. Tolerance to imidazolinone herbicides in wheat was reported by Newhouse et al. (1991). Seed mutagenesis with subsequent selection for tolerant plants using imazethapyr was employed. The resulting lines were shown to be tolerant to several imidazolinone herbicides. Generic analysis confirmed the tolerance to be due to a mutation to the gene coding for the AHAS enzyme. Subsequent tolerance testing with imidazolinone herbicides and other herbicides that inhibit AHAS activity confirmed the imidazolinone tolerance conferred by the trait designated as FS2 and FS4. These traits did not confer tolerance to other herbicides such as chlorsulfuron. Cyanamid is now working with wheat breeding programs around the world to transfer the imidazolinone tolerance to adapted wheat varieties. The breeding collaboration comprises provision of germplasm with the imidazolinone tolerance trait as well as assistance in greenhouse and field selection and seed increase. The principle herbicide being developed for weed control in IMI wheat crops is imazamox. This is a new imidazolinone herbicide characterized by weed control efficacy across a broad spectrum of broadleaf and grass weeds. While it exhibits sufficient residual soil activity to provide season-long control of most key weeds, the residual activity is shorter than most other imidazolinone herbicides resulting in a broad crop rotational profile. Imazamox is particularly efficacious on key grass weeds including brome species, foxtails, jointed goat grass and wild oats. Imazamox also controls many key broad leaf weeds.

Potential for Genetically Engineered Wheat

Mark J. Messmer Ph.D.

Hard Winter Wheat Workers Conference
Denver, Colorado
January 28-30, 1998

Introduction

Although genetically engineered wheat has not yet arrived on the commercial scene, much research work is ongoing to try to make genetically engineered wheat a reality. For this paper a general review of the current status of genetically engineered crop plantings will be presented. Then the possible commercial potential of genetically engineered wheat will be established by reviewing the uptake and acceptance of the genetically engineered Roundup Ready (RR) trait in another high acreage food crop (soybeans). The paper then will examine some possible applications of genetic engineering to wheat from the HybriTech/Monsanto standpoint. Finally, a brief discussion of the status of RR wheat will be presented.

Current Status of Genetically Engineered Crop Plantings

The United States broke ground on significant commercial planting of transgenic crops in 1996 with upwards of five million acres planted. In 1997, commercial transgenic plantings rose to more than 23 million acres in the US, and approximately 30 million acres worldwide. It is projected that in 1998 transgenic crops will cover more than 50 million acres worldwide, almost doubling the 1997 planted acreage.

The general level of transgenic crop distribution and uptake is amazing given the commercial risk imposed by tight development timelines and technical challenges associated with inventory bulk-up in addition to the rigorous regulatory standards these crops must meet before commercial release. The level of uptake and acceptance generated also speaks well for the potential of genetically engineered wheat when all the technical and commercial development hurdles have been cleared.

It is expected that over the next few years, the current emphasis on genetically engineered agronomic traits will shift to grain quality and added value food traits. By the 2005-2010 timeframe the emphasis will shift to even higher value products such as genetically engineered plants producing pharmaceuticals or enhanced nutritional factors. Wheat will lag 5-10 years behind the introduction of

these traits in leading crops like corn and soybeans, but will nevertheless enter the scene and be a force in the future transgenic expansion.

By the year 2005 it is projected that the plant biotechnology industry will generate more than six billion dollars in revenue. The initial driver in this value expansion will be herbicide resistant crops, but herbicide resistance will quickly be replaced as the expansion leader by other agronomic traits and quality traits which together will account for more than 80 percent of the value of transgenic crops by the year 2005.

Of this six billion dollar value in the year 2005, over 50 percent will be accounted for by soybean and corn traits. The contribution of wheat will be less than five percent of the total, but will be a quickly growing component of the overall value of transgenic crops.

Roundup Ready Soybeans as a Proxy for Genetically Engineered Wheat

At present, RR soybean is the largest contributor to transgenic crop plantings worldwide. RR soybean was introduced commercially on a large scale in 1996 in the US with around one million planted acres supplied by three seed companies. In 1997, the expansion reached 9 million acres in the US, 3.5 million acres in Argentina, and 5000 acres in Canada. In 1998, it is expected that over 20 million acres of RR soybeans supplied by over 85 companies will be planted worldwide.

The non-US potential of RR soybeans is extremely large as potential in South America tops 22 million acres. China and India account for an additional 10 million potential acres.

Based on 1997 market research in the US, a customer satisfaction rate of 97 percent indicates that farmers were extremely pleased with the results they achieved with RR soybeans. Overall, 90 percent of growers were more satisfied with the results they achieved with RR soybeans than with the results they achieved with non-RR soybeans. The only problem with RR soybeans to date has been the limited number of varieties containing the RR trait. However, this is being quickly rectified.

So given the extremely favorable acceptance of RR soybeans, what sort of potential exists for genetically engineered wheat?

Possible Applications of Genetic Engineering in Wheat

HybriTech/Monsanto believes that a number of genetically engineered wheat traits will impact the 10-20 million acres of hybrid wheat target market in both the

US and Europe over the next decade or so. In addition, potential exists to see genetically engineered wheat traits impact a substantial number of acres in other appropriate markets as well.

Relatively short term opportunities exist in both agronomic and quality traits. Viral and fungal disease control genes will be important initial contributors to the genetic enhancement of wheat through transgenics. In particular, Fusarium Head Blight (Scab) is a very important target.

Monsanto owns a number of proprietary anti-fungal protein (AFP) genes which hold potential to help control Scab and other important fungal diseases if the genes are effectively expressed in wheat. The Monsanto Scab program has achieved transformation of some of these genes into wheat. There were initial greenhouse tests of this material in 1996. The first field tests of this material were carried out in 1997 with promising results. This program will continue on the development track with the eventual objective being to insert multiple AFP genes into a single genotype in order to achieve a very high and stable level of disease control.

In addition to the AFP approach, Monsanto is also looking at a number of other novel approaches to disease resistance.

Another key area of research in wheat genetic engineering is that of quality enhancement for key end user traits. Bakers are interested in better bread making quality and specifically longer shelf life, higher nutritional quality, better taste and texture as well as better water absorption characteristics for flour. In addition, in-store baking is an increasing trend with more and more customers desiring higher quality fresh specialty breads. The in-store baking trend leads to the need for enhancement in frozen dough characteristics which promise to add significantly to the baker's bottom line through the reduction of stale bread or fresh dough losses.

Solutions to these quality challenges potentially come in many forms. The production of "designer proportions" of various protein and gluten components contributing to desired quality characteristics in either fresh or frozen dough products is possible. Enzymes which provide for unique and valuable starch characteristics providing expanded end user value in terms of reduced staling or other characteristics could be engineered. Looking further into the future it is possible to imagine the production of key nutritional factors in wheat to help address nutritional needs in the developing world.

Beyond the potential to enhance wheat for human consumption through genetic engineering, the possibility also exists to enhance wheat as an animal feed as well. Optimization of amino acid ratios in wheat for enhanced poultry production is one possible target. Another possible target might be to engineer wheat which

could provide an enhanced environmental waste profile after being fed through livestock. Higher energy wheat may be possible though the enhancement of oil levels in the grain, or alteration of metabolic pathways impacting production of some of the less digestible components of the wheat kernel.

The potential list of traits is nearly endless. The challenge is to focus on development of the most useful and valuable traits.

Roundup Ready Wheat

Roundup Ready wheat has the potential to be a major transgenic crop worldwide and in fact the first transgenic wheat trait introduced by Monsanto. In North America the potential of RR wheat could be very large although it is still somewhat undefined. Because of various issues surrounding cultural practices and the maintenance of Roundup herbicide as a viable component of some of the important crop rotation systems employed especially in the western US, a decision to market RR wheat in the US remains under consideration.

Although commercialization decisions have not been made, the first RR wheat field trials are underway. Roundup Ready wheat could potentially be launched in North America sometime after the year 2002.

Initial specifications for development of RR wheat include hybrid tolerance at a minimum of two times the maximum field application rate. This equates to a minimum tolerance of 64 ounces per acre with no measurable yield loss. Initial experimental transgenic events supplying this level of tolerance are currently in hand. In addition to sufficient tolerance levels in the presence of the herbicide, yield potential of the selected transgenic events in the absence of the herbicide must be at least equal to similar non-transgenic parents. Finally, because Jointed Goatgrass is a wild relative and shares the D genome with hexaploid wheat, the transgene must have been inserted in either the A or the B wheat genomes in order to be considered for commercial development.

As mentioned earlier, although RR wheat has excellent potential as a broadly utilized transgenic product, there are some significant issues from an environmental and cultural practice standpoint which should be considered.

The first of these is the wild outcrossing issue just mentioned. Targeted placement of the transgene in addition to responsible application of chemical rotations to minimize the development or maintenance of resistant weed species should allow management of the wild outcrossing issue.

The second issue is that of possible development of natural resistance. This is probably not an issue since development of natural resistance to Roundup herbicide has never been reported.

Another possible issue is that of the presence of RR wheat in fallow rotations treated with Roundup herbicide. It is interesting to note that this issue was considered when IMI tolerant corn was developed due to concern about resistant volunteer corn and the prevalent use of IMI herbicides in soybeans. This has been managed with prudent use of alternative herbicide strategies when volunteer herbicide resistant crops are an issue in rotation systems.

The final key issue is that wheat is a major food crop for direct human consumption. Never before has a crop with the extensive direct human food profile of wheat been commercialized. Because of this, food safety of RR wheat must be considered. Although the regulatory track has not yet been developed for RR wheat, there is no reason to suspect that the food safety profile of RR wheat will be different from that of RR soybeans or corn which are also directly consumed by humans. In any case, safety assessments will be appropriately rigorous, and only absolutely safe products will ever be released.

At the end of 1997 a research agreement was announced between Agriculture and Agri-Food Canada (AAFC) and Monsanto for the development of RR spring wheat for Canada. This agreement indicates that Monsanto is in fact serious about development of genetically engineered wheat. It also indicates that Monsanto is interested in establishing key strategic partnerships to develop transgenics in wheat.

The decision to pursue development of a RR wheat product for Canada was driven in part be the desire Canadian wheat growers had to have available the same weed control flexibility that was provided by RR canola in western Canada.

This agreement specifically provides for insertion of the RR trait into AAFC elite spring wheat germplasm. The agreement also signals the commercial potential of the RR wheat trait in other parts of the world although as mentioned earlier, the decision to develop the trait elsewhere has not been made. Evaluation of these opportunities is a continuing process.

Summary

Based on the benefits provided and the uptake on transgenics demonstrated in other food crops, the potential for genetic engineering in wheat seems to be great. Given this observation, technical challenges in wheat transgenics still remain, but will undoubtedly be resolved as knowledge of the genome progresses. Many of these challenges have already been resolved.

Work in genomics will in the relatively short term provide more and more transgenic traits which can be manipulated as oligogenic traits in breeding programs. Quality, insect and disease resistance, and possibly even additional

herbicide tolerance traits will result. Genes for nutritional value and even plant produced pharmaceuticals could be possible.

As genomics knowledge accumulates, interactions among known genes will become better understood and polygenic or quantitative traits may become much more amenable to application of high tech breeding strategies. This knowledge will allow better application of advanced genetic techniques in the extremely complex wheat genome as well.

Finally, it is very probable that the seed industry will go the way of the chemical industry by trading technology in mutually beneficial ways which will generate more sources of revenue for industry specialists.

All things considered, the potential of genetic engineering is great, and wheat is positioned to take full advantage of pioneering research accomplished in other crops.

WHEAT TRANSFORMATION: A MOLECULAR BREEDING APPROACH

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Introduction

Cereals are the most important crops in the world and were among the first plants to be domesticated. Cereal grains have contributed to the diet of man and livestock for nearly 10,000 years. The great success of cereals is largely due to a number of factors including high seed return ratio, protein content, and the ability to be grown in wide range of climatic conditions. Some examples of important cereal grain crops include wheat, rice, corn, barley, oats, rye, sorghum, and millets. These cereals have been an important source of carbohydrates, proteins, and minerals in both human and animal diets. Of the cereals, wheat production is the largest in the world because of its relatively high protein content. The importance of wheat and other cereals, mostly due to diet consumption and economic value, have led to the employment of biotechnological approaches for crop improvement.

The goal of plant breeding is to develop new cultivars through the selection of progeny derived from sexual crosses (intraspecific and interspecific) with enhanced or altered characteristics. Traditional breeding is a numbers game involving the number of crosses made, number of individuals and generations produced, recording of yield parameters and analysis of agronomic traits. Traditional breeding methods (gene pool, computers, data technology) have been and will continue to be main source of genetic improvement, but face increasing amount of biological and environmental problems. Continued problems include resistant cultivars becoming more susceptible to insects and diseases as well as being cultivated in unfavorable conditions. Recently, genetic engineering is being used for the production of transgenetic plants that may overcome these problems and potentially open up new methods to modify plants to meet specific needs.

Genetic engineering technology will complement plant breeding efforts by increasing the diversity of genes and germplasm available for incorporation into crops. This technology will help with the cloning of new genes and gene families having agronomic importance, understanding the physiological, biochemical and genetic basis of agronomic traits, creating new cultivars by introducing genes from unrelated plant species and tailoring promoters and

genes for a specific tissue or purpose. In addition, genetic engineering can improve crop productivity and enhance environmental conservation by decreasing the dependence on harmful chemicals and moving towards sustainable agriculture. Finally, genetic engineering of plants allows for the development of new products and manufacturing processes from agrichemical, food processing, and pharmaceutical industries.

Transformation Systems

Development of an efficient and reliable transformation system is a prerequisite for the genetic engineering of cereals. Although the first reports of transformation for dicots occurred over ten years ago, progress towards cereal transformation has been relatively slow up to now. But recent progress and developments in the field of biotechnology have overcome difficulties previously encountered in cereal transformation. These developments include the use of highly regenerable tissue or explant, efficient methods of DNA introduction (DeBlock, 1993; Songstad *et al.*, 1993) and reliable selection agents (Wilmink and Dons, 1993). These improved procedures have attributed to the transformation of all major cereals including rice, maize, wheat, oats, sorghum, barley and rye. In addition, it is now possible to analyze monocot gene expression and traits using transgenic monocots instead of dicot plant systems (Shimamoto, 1994). To be successful, transformation must obtain the delivery, integration, expression and inheritance of foreign genes into regenerated plants. It should also satisfy a number of general requirements including being reliable, efficient, and reproducible. In addition, it should be technically easy to implement.

For most dicotyledonous species, *Agrobacterium tumefaciens* vector system is the most commonly used and efficient transformation method used to transform plants such as soybeans (Hinchee *et al.*, 1988) and cotton (Umbeck *et al.*, 1987) for herbicide resistance, disease resistance, and viral protection (Grant *et al.*, 1991). *Agrobacterium* transformation offers several advantages over other transformation systems in that it is simple to use, comparatively efficient, and inexpensive (DeBlock, 1993). In addition, transgenic plants usually contain a limited number of transgene sequences and rearrangements when compared with transgene integration observed from direct DNA delivery systems. A number of monocot crops such as rice, corn, barley and wheat have been reported transformed using *Agrobacterium*.

The invention of particle bombardment which was developed by Sanford *et al.* (1987) and the delivery of DNA into living cells by Klein *et al.* (1987) has become the most widely used procedure for the transformation of monocot species. Particle bombardment transformation is based upon the principle of gene transfer by the use of high-velocity microprojectiles which are coated with DNA (Sanford, 1990). The projectiles penetrate cells and tissues introducing

DNA accelerated by a biolistic device. Birch and Bower (1994) suggested several uses of particle bombardment which include: 1) efficient inoculation with infectious nucleic acids; 2) studies of gene regulation based on transient expression of introduced DNA in target cells; 3) cell lineage analysis using chimeric transformants expressing visual marker genes; 4) reproducible transformation of cellular organelles; and 5) regeneration of transgenic organisms expressing useful new genes, following selection for stable transformed target cells. Three additional methods of transformation (protoplasts, intact tissue electroporation, silicon carbide fiber) have been reported and all three show promise for cereal transformation.

Technical Aspects and Questions

Although wheat transformation technology has advanced rapidly over recent years, there are many of unknowns and questions yet to be answered. In addition, there are only a limited number of published reports on the stability and heredity of the transgenes. In order to evaluate the effects of introduced transgenes such as copy number and site of gene integration, a large number of independently produced transgenic plants are needed. The efficiencies obtained in wheat transformation of laboratories having an established particle bombardment method is in the range of one to two percent. Even though wheat transformation efficiencies are lower when compared to those obtained in some dicot systems, the efficiency will still allow wheat transformation to be used as a basic tool for the study of wheat biochemistry, development, and engineering of new cultivars.

A matter of some concern has been the genotype specificity of regeneration in wheat. It has been documented (Sears and Deckard, 1982) that there is a wide variation of tissue culture response in wheat genotypes. This presents a small problem in the fact that most of the genotypes that respond well in culture are not in commercial production, requiring additional time to backcross the trait of interest into an elite line.

In addition, public acceptance of the engineered wheat plants and their products must be taken in account. The licenses, patents and proprietary rights of this new technology will also have to be considered.

Conclusion and Prospects

Before transformation technology can be fully utilized for the development of new cultivars, there are several issues that will have to be addressed (expanded from Lindsey, 1990): 1) problems associated with the routine transformation and tissue culture of plants; 2) identification and isolation of genes which are involved in cell regulation, developmental and metabolic

processes; 3) unpredictability of integration site and level of foreign gene expression; 4) gene stability; 5) integration of transgenic wheat into breeding programs; and 6) consumer acceptance of genetically engineered products.

Now that the technical aspects of wheat transformation have been established, scientific research efforts may be redirected towards identification and cloning of new genes and gene families having agronomic importance. The immediate focus of current efforts in wheat transformation will be the bioengineering of plants with a one or few genes that have been isolated, characterized and are available. Examples of these genes are for herbicide resistance, pathogen resistance, and insect resistance. Other genes of interest include quality characteristics for breads, pasta, crackers and cakes, and nutritional quality (amino acids) for both livestock and human consumption (Anderson *et al.*, 1994; Shewry *et al.*, 1995).

Future targets of wheat transformation may include the engineering for pharmaceuticals (human serum albumin), industrial enzymes (alpha-amylase), oils (lubricates), plastics (polyhydroxybutyrate) and abiotic (drought) tolerance stress. A better understanding of the process of gene function and regulation, as well as factors involved in position effect, co-suppression and co-transformation will be required for the multi-gene traits mentioned above.

With the tremendous accomplishments in recombinant DNA technology, molecular cell biology and transformation technology over the last decade, one can only imagine what will be accomplished in the future with plant genetic engineering. It is projected that the supply and demand balance for all major food, feed grains and protein crops will become critical within ten years. The potential for new agricultural technologies may ensure healthier, better quality, affordable, and increased availability of food is met. It is hoped that from these technologies we will benefit and provide both industrialized and developing countries with means to sustain increasing populations and provide new resources for the producer and consumer.

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Molecular mapping of durable leaf rust resistance

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Introduction

Leaf rust is an important disease of wheat worldwide and most wheat breeding programs have and continue to incorporate resistance into cultivars in order to reduce the losses caused by the disease. More than 40 leaf rust resistance genes (*Lr*) have been identified in either wheat or related species. Most of these confer a hypersensitive reaction at the seedling stage and have been overcome by new races of *Puccinia recondita*. In addition to those well characterized major genes, a number of minor genes have been found to confer a more durable resistance; usually, these are non-race specific and act in the adult plant. Cultivars containing such minor resistance alleles show a slow increase of rusting during the growing season rather than immunity. An example of such an adult plant resistance (APR) allele is *Lr34*, shown to increase the latency period, and decrease both the size and number of uredia (Singh, personal communication). Although *Lr34* alone does confer slow rusting resistance, it may not be adequate under high disease pressure. The combination of *Lr34* with two to three additional APR alleles have resulted in high levels of resistance. This *Lr34*-complex has been introduced into CIMMYT wheat from the cultivars Frontana and TZPP. The *Lr34* allele is known to be either pleiotropic or closely linked with leaf tip necrosis (LTN) of adult plants (Dyck, 1991; Singh, 1992) and has been located on the short arm of chromosome 7D (Dyck 1987). Our efforts in breeding for disease resistance in general, and leaf rust resistance in particular, have focused on the use of durable resistance (Van Ginkel and Rajaram, 1993).

Our objectives have been to determine the number and location of genes conferring APR to leaf rust, and to identify molecular markers tightly linked to the resistant alleles. Such markers would allow us to (1) determine which wheat accessions contain particular resistance gene(s), (2) transfer these genes to various backgrounds using marker-assisted selection (MAS), (3) combine different sources of APR genes, and (4) perform selection at earlier stages of the breeding program.

Molecular markers closely linked to eight major genes for leaf rust resistance have been reported (Table 1). In addition, Nelson et al. (1995 and 1997) reported a region on 7DS which significantly reduced leaf rust severity in field experiments, possibly representing *Lr34*.

Table 1. Reported molecular markers for leaf rust resistant genes

Resistance gene	Marker type(s)	Chrom. location	Reference
Lr1	RFLP	5DL	Feuillet et al. 1995
Lr9	RAPD, RFLP	6BL	Schachermayr et al. 1994
Lr10	Candidate gene	1AS	Feuillet et al. 1997
Lr19	isozyme, RFLP	7DL	Autrique et al. 1995, Winzeler et al. 1995
Lr24	RFLP, RAPD	3DL	Schachermayr et al. 1995, Dedryver et al. 1996
Lr25	RAPD	4A	Procurnier et al. 1995
Lr29	RAPD	7DS	Procurnier et al. 1995
Lr32	RFLP	3DS	Autrique et al. 1995

Our work has involved a search for markers for APR genes in the resistant cultivars Parula and Frontana, as well as for *Lr34* in Jupateco73 near-isogenic lines (NILs) for *Lr34*.

Parula x Siete Cerros population

Initially we examined durable leaf rust resistance genes in a population of recombinant inbred lines (RILs) from a cross between a resistant line, Parula (*Lr13*, *Lr34*, and 2-3 other APR genes) and a partially susceptible line, Siete Cerros (1-2 minor gene(s)). The population of 77 RILs along with the parental lines was evaluated for reaction to leaf rust pathotypes TCB/TD, TBD/TM and MCD/SM in replicated trials in the Yaqui valley near Ciudad Obregón, Sonora, Mexico, during 1992-93 and 1993-94. Leaf rust severity was recorded three times following the modified Cobb Scale and LTN was scored after flowering. The area under disease progress curve (AUDPC) was then computed for each line from the three severity ratings (Knott and Padidam, 1988). We started our mapping using RFLPs. However, too few RFLP loci were available then to allow the construction of a good map. Of 242 probes screened on the parental lines, only 52 loci were scored in the population (Acevedo, 1993).

We then decided to use RAPDs and bulk segregant analysis (BSA) where each bulk was formed of 10 RILs (William et al. 1997). The resistant bulk contained the lines with the lowest disease severity scores and which showed leaf tip necrosis (*Ltn+*), while the susceptible bulk had the lines with the highest severity scores and did not show leaf tip necrosis (*Ltn-*), based on the

two year evaluations. Four hundred Operon decamer primers were screened on DNA enriched for low copy sequences of the parental lines, and 30-35% of those revealed a polymorphism between the parents as compared to 8-10% of primers when total genomic (non-enriched) DNA was used. Primers showing a polymorphism were then used on enriched DNA of the two bulks and three of those revealed the same polymorphism as between the parents. The polymorphic bands were isolated from the gels and cloned for use as RFLP probes on all RILs and on nullitetrasomic wheat stocks in order to determine the linkage to APR and the chromosomal location of these loci. Genetic analysis of the segregation of these three loci and of *Ltn* showed that none were linked to *Ltn* and two (detected by OPG-05 and OPI-16) were tightly linked to each other (2% recombination). Cytogenetic analysis showed these loci to be located on 7BL while the third probe (OPR-03) detected loci on 1BS and 1DS. One-way ANOVA using the segregation data from the RFLPs, LTN, and leaf rust scores from the two years, showed that these three loci were significantly associated with durable leaf rust resistance (Table 2).

Table 2. QTL for durable leaf rust resistance in the population of Parula x Siete Cerros

Marker	Chrom. location	Phenotypic variance (R^2)*
Ltn	7DS	20 - 31%
OPG-05	7BL	18 - 29%
OPI-16	7BL	22 - 34%
OPR-03	1BS or 1DS	7 - 10%
	Total	**45 - 55%**

* Proportion of phenotypic variance for leaf rust explained by each locus, range indicates variation in estimates based on the two ratings (LR rating and AUDPC) in the two trials (92/3, 93/4)

Results of this study have been discussed in more detail in a recent publication (William et al. 1997). The marker on group 1 could be indicating the presence of a slow rusting resistance gene, *Lr46*, detected in Pavón76, another cultivar showing adult plant resistance (Singh et al., unpublished data).

Frontana x INIA66 population

We then decided to identify slow rusting genes of Frontana by mapping. Frontana is a Brazilian cultivar with proven durable leaf rust resistance that has been holding up for over 40 years in many areas of the world. Frontana contains *Lr13* and *Lr34* and is estimated to have an additional 2-3 minor genes (Singh and Rajaram, 1992). The population being used for mapping is composed of 248 RILs from a cross between Frontana and the susceptible cultivar INIA66 which has *Lr13* and *Lr17*. In addition to its larger size, this population has the advantage of segregating for other characters including resistance to yellow rust, *Fusarium* head scab, *Septoria tritici*, BYDV, and sprouting, allowing us to map these traits as well. We opted for constructing a full linkage map in search of the quantitative trait loci (QTL) controlling the expression of durable leaf rust resistance.

The RILs and parental lines were grown in replicated trials at Ciudad Obregón, during 1991-92 and 1994-95 and were inoculated with pathotype TBD/TM. In the first trial, leaf rust severity was recorded three times and the AUDPC computed. In the second trial, unusually high temperatures during the early stages of growth caused the premature drying of leaves and therefore, the leaf rust severity was confidently scored only once. LTN was recorded in trials where fungicide applications were used to control diseases.

The genotyping of the population was done using RFLPs, simple sequence repeats (SSRs, microsatellites), and amplified fragment length polymorphisms (AFLPs). Of 822 RFLP probes used on DNA from the parental lines and digested with five restriction endonucleases, 543 were of good enough quality to be usable in mapping. Of those, 158 detected a polymorphism between the parents (29%). From these, a total of 125 RFLP loci were scored in the segregating population. Of 68 SSR primer pairs screened on the parental lines, 56 resulted in clear amplification products and of those, 27 detected a polymorphism (48%) (Guillén-Andrade, 1998). Recently, we have used AFLPs as additional markers. For each primer combination used, between 70 and 130 fragments are resolved, of which 7 to 16 are polymorphic between Frontana and INIA66. Todate, 66 AFLPs have been scored in part of the population.

A linkage map was constructed based on the genotyping of a maximum of 117 of the RILs using the 218 molecular markers and the morphological marker, LTN and resulted in 26 linkage groups containing 189 loci (some markers did not link to any other). Composite interval mapping (CIM) was used for QTL detection using the constructed map and the phenotypic evaluations in year 1991-92 (AUDPC) and 1994-95 (severity score) as well as the joint analysis of both years. A QTL of major effect and with a very high likelihood ratio (LR=72 equivalent to LOD=15.7) was found in the vicinity of *Ltn* and explained about 50% of the phenotypic variance for leaf rust resistance. This was the only area where the likelihhod ratio exceeded the set threshold

equivalent to a LOD score of 2.5. However, there was a peak very close to the threshold value near an AFLP marker mapped to chromosome 5B. The percentage variance explained by that putative QTL was 5%. When the data was analyzed by one-way ANOVA, another three genomic regions were found to be associated with leaf rust resistance at the probability level of 0.01. These were on groups 2, 6, and 7 and each explained between 5 and 9% of the phenotypic variance for the disease. These are not necessarily *Lr* alleles and could be genes indirectly affecting the resistance reaction especially considering the low R^2 values on some.

We believe that the linkage map obtained does not contain enough markers for a good coverage of the genome and are working on placing more markers on it. With a more complete map, we should be able to identify by CIM the QTL involved in the expression of durable leaf rust resistance.

Jupateco NILs

We are also using a RIL population from a cross between NILs for *Lr34*: Jupateco73S x Jupateco73R to find a marker for *Lr34*. Here we are doing a BSA using AFLPs. So far, of 48 primer combinations tested on the Jupateco bulks (almost 5,000 fragments resolved), none showed a clear polymorphism between the bulks.

Perspectives

As mentioned, we are continuing to add new markers on the FxI map in order to identify all the genomic regions responsible for durable leaf rust resistance in Frontana and determine their genetic effects. More primers can be assayed on the Jupateco bulks in search for a close marker to *Lr34*. We are also in the process of developing segregating populations between the susceptible cultivar Lalbahadur and single chromosome intervarietal substitution lines in Lalbahadur in order to tag specific APR genes, for example, 1B from Pavón76 to tag *Lr46* and 7B from Parula to tag the allele determined to contribute to adult rust resistance.

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Molecular Tagging of Russian Wheat Aphid Resistance Genes In Wheat

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The Russian wheat aphid (RWA) is the most serious insect pest of wheat in the United States at present. The most effective and economically sound approach for controlling the RWA is through the use of resistant cultivars. The objectives of this study were: 1) to identify DNA markers for two RWA resistance genes, *Dn2* and *Dn4*, in wheat and 2) to evaluate the use of these markers for pyramiding *Dn2* and *Dn4* in a single wheat cultivar. F2 populations were made from a cross between PI372129 (contains *Dn4*) and 'Yuma' (susceptible) and between PI262660 (contains *Dn2*) and 'Carson' (susceptible). A total of 167 and 200 RFLP markers were screened for polymorphisms between PI372129 and Yuma and between PI262660 and Carson, respectively. ksua1 was linked to *Dn2* at a distance of 9.8 cM. Three RFLP markers (abc156, ksue18, and ksud14) were linked to *Dn4*. abc156 was the closest marker at a distance of 11.7 cM from *Dn4*. The use of DNA markers for pyramiding would cut by half the number of years to obtain an advanced line containing two genes compared to conventional selection methods. Crosses were made to incorporate *Dn2* and *Dn4* in the susceptible cultivar 'Lamar'. To be useful for pyramiding, a DNA marker linked to a gene must distinguish the parent containing that gene from the other two parents not containing the gene. The RFLP patterns of abc156 and ksua1 did not provide polymorphisms that could distinguish each resistant parent from the other two parents. PCR products from the three parents were identical and digestion with several 4-bp and 6-bp cutting enzymes did not reveal useful polymorphisms. Finally, the PCR products from the three parents were sequenced. The 1126 bp fragment containing the ksua1 sequence and the 686 bp fragment containing the abc156 sequence were identical among the three parents. These results indicate that the three wheat lines used are very similar and that markers more tightly linked to the genes are required for tagging and pyramiding. The use of other DNA marker systems which can uncover greater levels of polymorphisms in wheat , such as AFLP and microsatellites, may be useful for finding markers tightly linked to the genes.

ABSTRACTS OF POSTER PRESENTATIONS

YIELD TESTING: YEARS OR LOCATIONS?

Kraig Roozeboom, Kansas State University

21st Hard Winter Wheat Workers Workshop Poster Session
January 28-30, 1998

Each year Kansas State University Research and Extension distributes over 10,000 reports summarizing Kansas Wheat Performance Test results. This poster evaluates several variety selection strategies based on multi-year yield averages or multi-location yield averages for their effectiveness in selecting a high-yielding wheat variety using information from the Kansas Wheat Performance Tests.

Strategies:

1. 1L1Y The top variety at a location in 1 year
   1. 2L1Y The top variety averaged over 2 locations in one year
   2. 3L1Y The top variety averaged over 3 locations in one year
   3. 4L1Y The top variety averaged over 4 locations in one year
   4. 1L2Y The top variety averaged over 2 years at one location
   5. 1L3Y The top variety averaged over 3 years at one location
   6. 1L4Y The top variety averaged over 4 years at one location
   7. 2L2Y The top variety averaged over 2 locations and 2 years

Success was measured by the relative performance (% of test mean) of the selected variety at the location of interest in the following year and by the frequency distribution of that performance over time and over locations. Variety selections were made using yield information from 16 testing locations for 1982 - 1996. Performance of the selected varieties was evaluated for 1986 - 1997. For the statistical analysis, years were replications, locations were whole plots and selection strategies were subplots. The 16 locations were grouped into 4 regions of 4 locations each: East, Central, West, and Irrigated.

The 16-location ANOVA revealed a significant effect for selection strategy, but also a significant interaction effect for strategy x location. Analysis by region and by individual location showed that the selection strategies behaved slightly differently at different testing locations. However, in general, multi-location averages tended to do a better job than multi-year/single location averages in predicting variety yield performance. The strategies utilizing 3 or 4-location averages selected varieties which yielded an average of 109% of the test mean in the following year. The strategies utilizing 3 or 4-year averages from a single location selected varieties which yielded an average of 105% of the test mean in the following year. The remaining strategies provided intermediate performance, averaging 107% of the test mean in the following year.

The frequency of selected varieties yielding 110% or more than the test mean in the following year followed a similar pattern. Strategies utilizing 3 or 4-location averages selected varieties which yielded 110% or more than the test mean in the following year at a frequency of 43% compared to 31% for strategies utilizing 3 or 4-year averages. The frequency of the selected variety yielding 110% or more than the test mean in the following year was 38% for the remaining strategies.

Regardless of which strategy was used, selecting a variety based on yield results from the Kansas Crop Performance Tests resulted in a variety that yielded 107% of the test average in the following year. The selected varieties yielded above average in the following year more than 7 out of 10 times.

Relationship of heterosis on components of partial resistance of wheat to *Stagonospora nodorum.*

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Breeding wheat for reduced disease level of *S. nodorum* blotch (SNB) often involves measuring components of partial resistance (CPR). Most commonly used CPR are incubation period, latent period, lesion size, and necrosis. The effect of heterosis on SNB and it's interaction with wheat has not been studied. The objectives of this study were to determine 1) the effect of SNB on CPR, and 2) the effect of the interaction between heterosis and SNB on CPR, and on grain yield.

A greenhouse study was conducted in 1992-93. Five F1 hybrids and their 10 parents were obtained from HybriTech Seed International, Inc. Plants (heading stage or between 51 to 60 of Zodoks scale) were inoculated with a *S. nodorum* spore suspension (3.2 X 10^6 spores/ml). A replication consisted of all parents and F1's which had both inoculated and non-inoculated plants (8 replications). All pots were placed in inoculation chambers for 72 hrs at near 100% humidity. Data were collected on incubation period, latent period, and % necrosis at 10 and 15 days after inoculation. Upon maturity kernel wt/plant (KWP), kernel wt/spike (KWS), and 100 kernel weight were recorded. For incubation period (IP) in the inoculated treatment, the mean IP was 4.5 days. The hybrid, PS 8424/LB 291 had a significantly longer IP than its high parent (LB 291). Three of the other four hybrids had an IP equal to or longer than their parents. Y 89-7A/PS 8424 had a mean IP midway between its parents. These data indicate that for most of these hybrids, that there may have been some heterosis for IP. Differences between entries for latent period (LP) were quite small, but significant. Three hybrids (U 88-9/LB 291, Y 89-7A/PS 8424, and PS 8424/LB 291) had LP shorter than both of their parents. The other hybrids were between their parents in length of LP. No heterosis was apparent for LP. For percent necrosis (PN), no significant heterosis could be measured on either the leaves or spikes at either 10 or 15 days after inoculation. Four of the five hybrids had leaf PN values either below both parents, or a mid-parent value. The longer IP of the hybrids did not result in a longer LP of the hybrids compared to their parents. We have no explanation why this should occur. This does indicate that the heterosis measured as IP would not be useful in lengthening the disease cycle or slowing down the disease pyramid, at least with these parents.

Yield loss: Both KWP and KWS were reduced by the SNB treatment due to premature death of the plant. There appears to be heterosis for KWP for four of the five F1's in the noninoculated treatment. In the inoculated treatment, heterosis was not measured in an additional cross (U88-9/LB291). Never the less, it appears that heterosis for KWP was present in most hybrids, but that KWP for all genotypes was reduced no matter whether they are parents or hybrids. KWS and hundred kernel weight were reduced for all genotypes by the inoculation treatment. In the inoculated or noninoculated treatment, heterosis for HKW was not significantly greater than the high parent in any cross. Results of this study indicated that heterosis, if present in hybrids, increased grain yield of hybrid plants of healthy or diseased (SNB) wheat genotypes. Further, grain yields in this study were reduced about equally in both parents and hybrids by the presence of SNB. Therefore, heterosis will not overcome the SNB pathogen and maintain grain yields of diseased plants or provide the hybrids with tolerance to SNB. In regard to heterosis for components of partial resistance to SNB, we could only measure slight heterosis (high parent) for incubation period. The longer IP of some of the hybrids, was not translated into a longer latent period for those hybrids, and therefore is of no practical use in a breeding program.

Coleoptile Length Characterization of Semidwarf and Standard Height Winter Wheat Germplasm **Frederic Hakizimana*, Scott Haley, and Steve Kalsbeck Plant Science Department. South Dakota State University Brookings, SD 57000**

In the northern Great Plains, optimum fall stand establishment is critical for winter survival of winter wheat (Triticum aestivum L.). The length of the coleoptile (protective sheath that covers the shoot during emergence) has been associated with fall stand establishment, most notably with semidwarf wheat cultivars that possess the Rht1 and/or Rht2 dwarfing genes. While coleoptile length evaluation and improvement is an objective of many breeding programs, little information is known on the coleoptile length of the winter wheat germplasm grown in the Great Plains region. The objective of this study was to identify sources of adapted semidwarf winter wheat germplasm that are compatible with long coleoptile development. Conversely, information about coleoptile length of the standard height winter wheat genotypes would be also obtained. Greenhouse- and field-grown seed samples of 143 winter wheat genotypes from the 1995 Uniform Winterhardiness Nursery (UWHN)-Southern Great Plains section and 131 winter wheat genotypes from the Northern Great Plains section were used for this study. Forty seeds of each genotype were germinated for coleoptile length evaluation. The 3Z values 2,also called 3 standard normal deviate 2 showed that eighteen genotypes with Rht1 and/or Rht2 semidwarfing genes (based on gibberellic acid reaction) from the southern Great Plains section and eight from the northern Great Plains section had long coleoptiles. Four semidwarf gibberellic acid-sensitive genotypes from the northern Great Plains section were found to have long coleoptiles, suggesting the presence or Rht8 or Rht9 semidwarfing genes. The results from our study indicate that semidwarf winter wheat genotypes with long coleoptiles are present among the southern and northern Great Plains winter wheat breeding programs. These genotypes may be useful to the wheat breeders especially when they are trying to incorporate Rht gene (s) into their breeding materials.

Yield and Agronomic Traits Linked to RFLP's in a Winter Wheat Population

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Selection for yield agronomic traits in wheat is a primary function in most wheat breeding programs. Identifying genetic markers linked to quantitative variation for these traits would improve the understanding of and possibly the selection of yield. For this purpose, 78 recombinant inbred lines derived from the winter wheat cross Clarks Cream/NY6432-18 (CC/NY18) were grown at Moscow and Aberdeen, Idaho, in 1996 and 1997. Genotypes were planted in a two replication design with experimental plots of 3.9 m^2, with the Aberdeen location irrigated to replace 80% evapotranspiration loss while the Moscow location was rain-fed only. Plots were evaluated for yield and test weight in all four environments and height, heading date and lodging in all environments except Moscow 1996. Average line performance was compared to a previously developed molecular map of the CC/NY18 population consisting of 181 markers across all 21 chromosomes. Using individual marker regression, 56 markers were significantly associated with one of more of the agronomic traits ($p<0.01$). All of the markers associated with yield (11 of 11) and almost all of the markers associated with test weight (26 of 29) and lodging (5 of 7) were also associated with height or heading date. The association between short stature and yield was greatest in the irrigated trials and least in the Moscow 1997 environments. In Moscow 1997, the RFLP xbcd18a was the most important predictor of yield. Xbcd18a also accounted for 25% of the variation in the 3 location-average heading date. The largest factor associated with line performance was a group of linked loci on a group 2 chromosome. Multiple regression models identified the best markers explaining 18% of the variation in Moscow yield to 70% of the variation in plant height.

A New Technique For Screening For Bird Cherry-Oat Aphid Resistance in Wheat and Barley. C.A. BAKER*, K.A. MIRKES, J.A. WEBSTER and D.R. PORTER, USDA-ARS, Stillwater, Oklahoma

The bird cherry-oat aphid, *Rhopalosiphum padi* (L.), has been shown to reduce the yield of both wheat, *Triticum aestivum* (L.), and barley, *Hordeum vulgare* (L.), yet it causes no obvious visual symptoms. This lack of obvious symptom development makes it impossible to use the standard screening test which is effective in screening for resistance to several other aphids. Therefore, a new technique was developed to identify resistance to the bird cherry-oat aphid. This technique uses transparent seed growth pouches that allow a clear view of both shoot and root development. A rapid visual comparison of infested vs. noninfested plants makes it possible to identify those genotypes that are less impacted by the aphid at the seedling stage.

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SEEDLING LEAF RUST REACTION OF WHEAT ENTRIES IN THE 1998 REGIONAL GERMPLASM OBSERVATION NURSERY

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Seedling reaction to wheat leaf rust was determined for entries in the 1998 Regional Germplasm Observation Nursery (RGON). Leaf rust reaction was determined using a mixture of *Puccinia recondita* f. sp. *tritici* (PRT) urediospores collected in May, 1997, from the wheat cultivars Chisholm, Danne, and Karl growing at three locations in Oklahoma [Apache (southwestern OK), Stillwater (northcentral OK), and Lahoma (northcentral OK)]. The avirulence/virulence formula of this urediospore mixture as determined by three replicate inoculations of a set of single-gene differentials was 9 17 19 26 'Siouxland' (*Lr24* + *Lr26*) / 1 2a 2c 3 3ka 11 16 24 'Century' (*Lr24*) 30. First leaves of 10-15 seedlings of each wheat entry were inoculated by brushing with infected Danne seedlings. Inoculated seedlings were kept in a mist chamber at 68-72 F for 24 hr and then moved to greenhouse benches. Leaf rust reaction was rated 10-12 days later (Stakeman. USDA Bull. #E617, 1962, 53 pp), and values from Stakeman's system were translated into one of six categories:

1. R=resistant=Stakeman's 0 / ; /1 / X;3=n / X;1.
2. MR=moderately resistant=Stakeman's X;3= / X;3- / X;3=c / X;3-n / X;3-c / X;3 / 3=cn / 3=n.
3. MS=moderately susceptible=Stakeman's X3; / 3= / 3=c / 3-c / 3-n / 3c / 3n.
4. S=susceptible=Stakeman's 3- / 3 / 3+ / 3+c / 4.
5. Seg-R=segregating, with most seedlings resistant (minimum of four susceptible seedlings in a clump of 10-15).
6. Seg-S=segregating, with most seedlings susceptible (minimum of four resistant seedlings in a clump of 10-15).

The 1998 RGON consists of 450 entries, of which 396 are breeder lines and 54 are checks. All replications of check entries (nine/entry) 'Tam-107', Karl, and Danne scored 'S' except for two 'MS' reactions. All replications of the check entry 'Arapahoe' scored 'MS' except for one 'S' reaction. All replications of check entry 'Siouxland' scored 'MR' and all Thatcher (*Lr19*) replications scored 'R'.

Forty-six percent (184) of the 396 breeder entries scored 'S', 13% (53) scored 'MS', 18% (71) scored 'MR', 20% (78) scored 'R', 2% (6) scored 'Seg-R', 1% (3) scored 'Seg-S', and there was one entry with no seed. These results indicate the reaction of these entries to PRT in the seedling stage. Some of the entries that scored in the 'S' or 'MS' category may have adult plant resistance, which may not be detected in the seedling tests. Another consideration is that PRT spores collected only from Oklahoma were used in this test. Inoculation with PRT races or spores collected from other locations may detect the presence of other resistance genes.

Hybrid Hard Red Spring Wheat..... An Economic Analysis

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Introduction: Hybrid wheat consistently out yields varieties, but in order for hybrid wheat to become a fixture on farms it must also be capable generating an increased net return per acre. In the Hard Red Spring Wheat (HRSW) region income from wheat is a function of yield, test weight and grain protein, in addition to the basis price. A set of 16 HRSW hybrids and 8 leading check varieties were tested head-to-head in replicated trials over a two year period (1995-96) and ranked for economic return using the ten year average Minneapolis Grain Exchange prices for various protein levels and typical local elevator test weight discount schedules. Total input costs per acre were estimated at $150/acre for certified varieties and $171/acre for hybrids, assuming a typical ~1.2 MM kernels/acre seeding rate. **Results and Discussion**: The mean yields of hybrid wheat were 4.70 Bu./acre greater than the mean of the variety checks over the two years of testing. The top yielding hybrid exceeded the top variety by 8.2 Bu./acre. Test weight averaged 58.8 for hybrids and 59.4 Lb./Bu. for the varieties. Protein averaged 14.2% for hybrids & 14.1% for varieties. The net value per bushel and net return per acre are shown in Table 1. This table assumes no additional cost for hybrid seed over certified variety seed. This type of ranking is analogous to an economic selection index for "breeding value" and reflects the relative importance of yield, test weight and protein. A second ranking in Table 2. Shows the net return per acre assuming an additional $21/acre input cost for hybrid seed compared to certified variety seed. The mean hybrid net return per acre was $111.27/acre compared to $110.67/acre for the mean of the variety checks. The top hybrid produced an additional $6.81/acre over the top variety ($136.80 vs. $129.99). In this particular data set varieties had slightly better Return On Investment (ROI) even though they had lower net returns per acre. This data set is also strongly influenced by Fusariurn head blight infections at several of the location x years of testing. The top net returning variety was Gunner which has shown significantly less damage than most varieties to FHB. **Conclusion**: This data set established that HRSW hybrids can be competitive on an economic basis compared to the leading varieties in the Red River valley. It remains to be seen whether or not the magnitude of the increased net return per acre is sufficient to justify a farmer switching to hybrid wheat should it become commercially available. However, based on this data it would appear that farmers should feel comfortable in planting at least a portion of their acreage to hybrids with out any greater risk of losing money. It is anticipated that many new biotechnology advances will best be delivered to the market place in the form of hybrids which allow a greater measure of investment recovery and acreage control.

Stable Basta-Resistant Transgenic Hard Red Winter Wheat Obtained Via Particle Bombardment

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Transgenic wheat plants have been produced by several laboratories in spring or soft white classes of wheat. Hard red winter (HRW) wheat is the largest class of wheat produced in the U.S. and has been one of the crops most recalcitrant to the application of available transformation methods. The presence of escapes, low frequencies of stable DNA integration and plant regeneration, and various vernalizaton requirements after transformation have made the transformation system for HRW wheat very inefficient. We conducted experiments designed to optimize transformation efficiency for HRW wheat and produce fertile transgenic plants with high levels of marker-gene expression. Immature embryos of commercial HRW wheat cultivars were bombarded with pAHC25 for comparison of GUS expression 2 and 30 days after bombardment, and an *in vitro* regeneration and selection system was developed that permits transgenic plants to be regenerated directly from bombarded immature embryos without going through selection for transgenic embryogenic callus. We have recovered the first transgenic HRW wheat, which expresses the Bar gene for resistance to phosphinothricin (Basta). The regeneration process used minimized time *in vitro* and thus minimized production of sterile plants. Stringent *in vitro* selection produced only fertile transgenic plants and eliminated escapes and low transgene expression. Recovered plants have expressed resistance to 0.5% phosphinothricin over three generations.

EFFECT OF HIGH TEMPERATURE STRESS ON SINGLE KERNEL HARDNESS OF HYBRID AND PURELINE VARIETIES IN HARD RED WINTER WHEAT.

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High temperature stress of winter wheat in the Great Plains is a frequent occurrence, especially during the grain filling period. It is the most important environmental variable affecting yield and end use quality. The purpose of this research was to examine the direct effect of high temperatures at specific stages of grain filling on kernel weight, kernel diameter, and single kernel hardness, and to compare the effects of high temperature stress on F_1 hybrids, their parents, and pureline varieties grown in greenhouse environments. Seven hybrids, their parents, and four varieties were grown in the greenhouse in a randomized complete block design. Four days after anthesis (Feekes 10.52), plants were transferred to greenhouses set at control (25/20 ^{0}C), or high temperature stress (35/25 ^{0}C) 16-h daylength. Data were collected on kernel hardness, diameter, and weight using the single kernel characterization system (SKCS) 4100. Overall, hardness increased from 71.5 to 80.5 under high temperatures. Diameter of the kernel decreased from 2.35mm to 1.92mm under high temperatures. Kernel weight decreased from 29.4mg to 22.6 mg under high temperature. There were no significant differences among hybrids, parents or check varieties for kernel hardness. Hybrids had a significant advantage in kernel diameter over both parents and check varieties. Also hybrids and their parents had a significant advantage over check varieties in kernel weight. Although high temperature stress increased kernel harness the magnitude was small. Kernel hardness was increased 12% compared to a 30% reduction in kernel weight in these experiments

Performance of Hard White Winter Wheat Lines in Colorado

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Abstract

Low, uneven distribution of rainfall and high temperature are major limiting factors for winter wheat production. We studied the response of hard white winter wheat (HWWW) lines to abiotic factors that affect yield and yield components in diverse environments; assessed white lines for post-anthesis drought tolerance, desiccant tolerance, and heat tolerance of seedlings. Strong associations were observed between grain yield and biomass in all environments. Grain yield and spike number had a strong correlation at the high stress environment. Shortage of rainfall during grain filling was the cause of variation among entry means across environments (R^2=0.89, P≤ 0.01). Traits were reduced more by low available water than by desiccation. Grain yield was strongly correlated with biomass in both treatments. The range in relative heat injury (RI) was 60 to 87%. Among HWWW cultivars, Arlin with 82% RI was the highest and Rio Blanco with 60% RI was the least injured.

A Relational Database System for Summarization and Interpretation of Hard Winter Wheat Regional Quality Data

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ABSTRACT

Achieving acceptable end-use (milling and baking) quality is a fundamental objective of wheat (Triticum aestivum L.) breeding programs throughout the U.S. hard winter wheat region. Numerous analytical methods have been developed to measure quality. Few tools are available, however, to assist in the decision-making process when faced with a large number of parameters from comprehensive milling and baking tests. Our objective was to develop a relational database system for summarization and interpretation of wheat end-use quality data from the USDA-ARS hard winter wheat quality laboratory. The database system uses a graphical interface with a series of 31ayouts2 that require input from the user, guide the user to a successive layout, or provide a data report. The database system provides simultaneous assessment of multiple quality traits on a standardized scale, user-specified prioritization of end-use quality traits for numerical and qualitative ratings of genotypes, tabulation of major quality deficiencies of genotypes, and summarization of quality ratings for a genotype across multiple nurseries. The database system has specific application to the hard winter wheat regional testing program. The basic principle and design, however, could be readily extended to nursery-based end-use quality testing programs in other wheat regions and market classes.

Desiccation Tolerance and its Association with Assimilate Partitioning in Spring Wheats for Eastern Colorado. A.A. SALMAN* and J.S. QUICK, Colorado State University.

There are major needs for adapting spring wheats to the eastern Colorado winter wheat area. Post-anthesis drought stress is a major problem in spring wheat production. The objectives of this study were: 1) to evaluate the feasibility of chemical desiccation for identifying post-anthesis drought resistance genotypes in spring wheats, and 2) to determine the relationship between tolerance to chemical desiccation, grain yield and dry matter partitioning in spring wheats. The study was conducted at Akron and Fort Collins, Colorado in 1997. The experimental design was a split-plots. Nine genotypes (3 tall, 3 medium, and 3 short) were assigned to the main plots and two desiccation treatments were assigned to the sub-plots. There were significant differences among genotypes in grain yield, biological yield, kernel weight and head weight. Correlation coefficients for head weight with grain yield and biological yield were 0.86** and 0.90** at Akron and 0.97** and 0.87** at Fort Collins, respectively, while stem weight and sheath weight (10 PAA) showed a similar trend at both locations. The results of this study supported the effectiveness of chemical desiccation as a tool for identifying differences among genotypes under drought stress.

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HMW and LMW Glutenin Subunit Transcripts Levels in Wheat Grains Subjected to High Temperature Stress, Susan B. Altenbach and Sitsari Kitisakkul, USDA-ARS, Western Regional Research Center, 800 Buchanan Street, Albany, CA 94710.

End-use quality of wheat is often compromised by the environmental conditions under which the grain has developed and matured. Numerous reports suggest that episodes of heat stress during grain fill result in wheat flour that produces doughs that are weaker than expected. The molecular basis for the effect of heat stress on wheat flour quality is unresolved at the present time, although it has been suggested that high temperature growth may result in a lower ratio of polymerized glutenin to monomeric gliadin in the mature grain as well as comparatively shorter glutenin polymers. Such alterations could be caused by changes in expression of individual seed storage protein genes. To investigate this possibility, we have done a careful analysis of steady-state RNA levels for some of the major gluten proteins in wheat plants (T. aestivum cv. Arapahoe) subjected to periods of high temperature stress at defined stages of seed development. We have used both hybridization analysis and competitive reverse-transcriptase polymerase chain reaction (RT-PCR) to compare the levels of transcripts for HMW-GS and LMW-GS in grains from individual heads of plants grown under different temperature regimes. Hybridization analysis measures the response of many closely-related genes within the complex gene families that encode the wheat storage proteins, whereas RT-PCR provides a way to quantitate the levels of transcripts corresponding to individual genes within those families. Our results indicate that transcript levels for five HMW-GS and 7 LMW-GS are stable during episodes of high temperature stress.

Chemical desiccation tolerance of winter wheat in the field and greenhouse

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Abstract

Chemical desiccation has been proposed as a promising technique to screen for post-anthesis drought tolerance. This study was conducted to determine the potential of chemical desiccation in the greenhouse (GH) as a selection tool for post-anthesis drought tolerance in the field. Nine cultivars of winter wheat varying in yield potential, water stress tolerance, height and maturity were evaluated under field and GH conditions. The desiccant, sodium chlorate, was applied 10 days after anthesis. Grain yield and kernel weight were the most sensitive traits to both chemical desiccation and drought stress; however chemical desiccation caused more reduction than drought. Cultivars with larger seed size generally suffered less chemical desiccation injury for grain yield and kernel weight, both in the GH and the field. A strong positive association between grain yield injury and both kernel weight injury and test weight injury were found. There was no association between kernel weight and kernel weight injury both in the field and the GH. Above-ground biomass of desiccated field plants was positively associated with desiccated grain yield and untreated grain yield of field grown plants. Harvest index of untreated field grown plants was positively correlated with grain yield injury in field, but negatively associated with grain yield injury of GH plants.

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Genet ic Transformation Can Be Used to Either Increase or Decrease the Levels of Wheat HMW-Glutenin Subunits. Ann E. Blechl. Susan B. Altenbach, Hung Q. Le, Peter W. Gras', Frank Bekes~ and Olin D. Anderson. Agricultural Research Service - USDA, Western Regional Research Center, 800 Buchanan St., Albany, CA 947 1 0- 1105 and ~CSIRO Division of Plant Industry, Grain Quality Research Lab, PO Box 7, North Ryde NSW 2113 Australia

The strength and elasticity of bread doughs made from wheat flours are correlated to their highmolecular-weight (HMW-) glutenin subunit compositions. In an effort to increase the levels of these storage proteins above their natural range of 5-10% of total seed protein, genes encoding hybrid and native HMW-glutenins have been added to wheat by genetic transformation. Most of the lines from these experiments exhibited increased overall levels of HMW-glutenin accumulation due to the additive contributions of the transgene(s). In some lines, however, decreases in the expression of native homologous genes, a phenomenon known as transgene-mediated suppression, were apparent. Various degrees of suppression were observed, ranging from partial to complete inhibition of endogenous subunit accumulation, usually accompanied by high levels of the transgene product. One case of complete co-suppression was observed: neither endogenous nor transgene-encoded HMW-glutenins were evident in protein gels even though intact genes for all were present in the genome. RT-PCR using gene-specific primers demonstrated that suppression also occurred at the level of steady state mRNA accumulation. Decreases in specific transcripts were quantified by competitive RT-PCR. In two transgenic lines characterized in more detail, the transgene-mediated suppression was heritable, behaved as a trans-dominant trait in outcrosses, and was completely reversed upon segregation of the transgene. In 2 g mixograph tests, flours from these lines exhibited decreased mixing times and tolerances in proportion to the decreases in their HMW-glutenin subunit levels. These results show that addition of transgenes to the wheat genome can both increase and decrease the levels of homologous gene products and that sense suppression can be used to mimic dominant loss-of-function mutants in wheat gene expression.

APPENDIX I
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