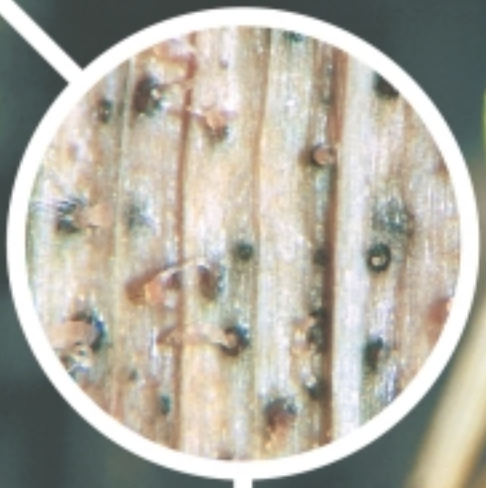
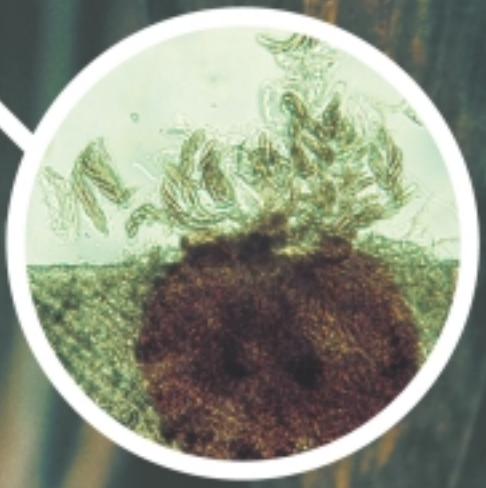
# Septoria and Stagonospora Diseases of Cereals:

## A Compilation of Global Research





M. van Ginkel,
A. McNab,
and J. Krupinsky,
editors

CIMMYT
International Maize and
Wheat Improvement Center

# Septoria and Stagonospora Diseases of Cereals:

## A Compilation of Global Research

**Proceedings of the Fifth International Septoria Workshop**

**September 20-24, 1999**

**CIMMYT, Mexico**

M. van Ginkel, A. McNab, and J. Krupinsky, editors

Dedicated to the memory of
Dr. Zahir Eyal

**The Organizing Committee expresses it sincere thanks to the Workshop Sponsors: Bayer de México, S.A., and Zeneca Mexicana, S.A.**

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Printed in Mexico.

**Correct citation:** van Ginkel, M., A. McNab, and J. Krupinsky, eds. 1999. *Septoria and Stagonospora Diseases of Cereals: A Compilation of Global Research*. Mexico, D.F.: CIMMYT.

**ISBN**: 970-648-035-8
**AGROVOC descriptors**: Wheats; *Triticum*; *Triticum aestivum*; Soft wheat; *Triticum durum*; Hard wheat; Winter crops; Plant diseases; Fungal diseases; *Septoria*; *Stagonospora*; Blotches; *Mycosphaerella*; Epidemiology; Plant breeding; Selection; Disease resistance; Genetic control; Gene location; Cultural control; Plant response; Research projects
**Additional Keywords**: *Triticum tauschii*
**AGRIS category codes**: H20 Plant Diseases
F30 Plant Genetics and Breeding
**Dewey decimal classification**: 632.4

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# In Memoriam, Dr. Zahir Eyal

Our friend and colleague, Professor Zahir Eyal, died Friday, July 30, 1999. Zahir was intimately involved in all of the International Septoria Workshops, from the first in 1976 held in Griffin, Georgia, USA, until the fifth, and present, one held in CIMMYT, Mexico. He put forward his many ideas for program and participants in a forceful, but thoughtful way, and was able to settle disputes with good humor and a smile. Until the last few days of his life, Dr. Eyal continued to work on plans for this *Septoria/Stagonospora* workshop.

After finishing agricultural high school in Israel, Zahir went to the USA, where he earned his B.Sc. degree from Oklahoma State University and his Ph.D. from Rutgers. This was followed by a post-doctoral term at Purdue, where he studied with Jack Schafer and the late Ralph Caldwell. His work on septoria of cereals began when he joined the Department of Botany at Tel Aviv University in 1967. He developed an integrated program of fundamental and applied research aimed at minimizing the economic impact of cereal pathogens, particularly *Septoria tritici*, on production. He reached out to colleagues in many countries and to international organizations, most especially CIMMYT, for cooperation. Over the years, Zahir contributed greatly to those programs. During his tenure at Tel Aviv University, Professor Eyal served two terms as Head of the Department of Botany (1984-87 and 1992-94).

Professor Eyal was an enthusiastic teacher, well-loved by students, both undergraduate and graduate. He taught in English or in Hebrew with equal facility, sharing his knowledge and insights with students and faculty during two sabbaticals at Montana State University and at several other institutions. The numerous publications he authored with his students and the important posts those students occupy today attest to the excellence of his teaching abilities.

Zahir's research and outreach programs incorporated ideas that were new to his country; they were solidly anchored in basic science and innovative to the end. These programs not only improved wheat production in Israel but had positive effects on cereal improvement programs throughout the world. At the time of his death, Professor Eyal was Director of the Institute for Cereal Crop Improvement at Tel Aviv University, where germplasm of wild ancestors of cultivated small grains are being preserved, characterized, and utilized in breeding improved cultivars.

Dr. Eyal's contributions to research, teaching, university administration, and international agriculture are many and far reaching. He received the Hazera Seed Co. Melamed Award in 1968, the A.C. Cohen Award in 1978, and in 1995 was made a Fellow of the American Phytopathological Society. Professor Eyal served as President of the Israeli Phytopathological Society from 1979 to 1982. He will be fondly remembered and sadly missed by his multitude of friends, colleagues, and students throughout the world.

# Foreword

In the mid-1970s the idea of holding a septoria workshop began to take hold among a small group of scientists in the USA. They were interested in exchanging ideas and finding ways to manage the septoria diseases that affect wheat and other cereals all over the world. The first workshop was organized in a matter of a few months and held in Griffin, Georgia, in 1976. Among the 50 scientists who attended were a few researchers from outside the US. The enthusiasm of that first workshop led to the development of the second, which was a truly international meeting attended by more than 100 scientists from many countries, held in Bozeman, Montana, in 1983.

Since then, international septoria workshops have been held about every five years: in Zurich, Switzerland, in 1989; in Radzikow, Poland, in 1994; and this year at CIMMYT in El Batan, Mexico. Each workshop has expanded the network of scientists who share their knowledge and pose the many questions that remain to be solved about these diseases and their management.

The Zurich workshop had increased participation by workers from Europe and Africa. The Radzikow workshop brought increased participation from scientists in eastern Europe. The early workshops focused on the biology of the pathogens and breeding strategies, subjects in which there remain many unanswered questions. The 1994 workshop and the current one emphasize molecular approaches to the genetics of the pathogens.

The Fifth International Workshop provides another opportunity to focus on the *Septoria/ Stagonospora* diseases, but also to see them in the context of the worldwide programs of CIMMYT, which emphasize collaboration with developing countries with the aim of developing stable high yielding wheat varieties that possess durable resistance to the diseases.

This workshop also gives us the opportunity to remember our friend and colleague, Zahir Eyal, who passed away not long ago. An integral part of the program development process and the discussions at each workshop, he organized the scientific program for this workshop as well. Zahir Eyal was an enthusiastic supporter of the septoria workshops and the international exchange of ideas. He will be missed.

We would like to express our appreciation for the efforts of Ravi Singh, Maarten van Ginkel, and Linda Ainsworth, who organized the workshop. We wish to thank Diana Godínez, María Luisa Varela, and Laura Rodríguez for managing the logistical support. We also recognize the efforts of Arnoldo Amaya, María Garay, Lucy Gilchrist, Monique Henry, Gilberto Hernández, Reynaldo Villareal, Juan José Joven, Marcelo Ortíz, Eliot Sánchez, Kelly Cassaday, Miguel Mellado, Wenceslao Almazán, and Antonio Luna, as well as many other members of CIMMYT staff who contributed to the success of this event.

The International Organizing Committee
September 21, 1999

## *Opening Remarks*

# Historical Aspects and Future Challenges of an International Wheat Program

S. Rajaram
Wheat Program, CIMMYT, El Batan, Mexico

*I am immensely honored and grateful to the organizing committee of the 5th International Septoria Workshop for asking me to deliver this lecture in the opening session. Even though my presentation is very broad and covers many issues, I assure you that I have been involved in breeding for resistance to septoria tritici blotch for at least 25 years, with some remarkable success. In this attempt, I would like to recognize the contribution of Prof. Zahir Eyal, who served as a consultant on septoria issues to CIMMYT in the 1970s and 1980s. Indeed, he and I have some common intellectual roots through Prof. Ralph Caldwell of Purdue University. Prof. Eyal's untimely death and departure from the scientific community is a loss to us all. I dedicate this opening lecture to him.*

Wheat is the most widely grown and consumed food crop in the world. It is the staple food of nearly 35% of the world population, and demand for wheat will grow faster than for any other major crop. The forecasted global demand for wheat in the year 2020 varies between 840 (Rosegrant et al., 1995) to 1050 million tons (Kronstad, 1998). To reach this target, global production will need to increase 1.6 to 2.6% annually from the present production level of 560 million tons. Increases in realized grain yield have provided about 90% of the growth in world cereal production since 1950 (Mitchell et al., 1997) and by the first decade of the next century most of the increase needed in world food production must come from higher absolute yields (Ruttan, 1993). For wheat, the global average grain yield must increase from the current 2.5 t/ha to 3.8 t/ha. In 1995, only 18 countries world worldwide had average wheat grain yields of more than 3.8 t/ha, the majority located in Northern Europe (CIMMYT, 1996).

The formidable challenge to meet this demand is not new to agricultural scientists who have been involved in the development of improved wheat production technologies for the past half century. For all developing countries, wheat yields have grown at an average annual rate of over 2% between 1961 and 1994 (CIMMYT, 1996). In Western Europe and North America the annual rate of growth for wheat yield was 2.7% from 1977 to 1985, falling to 1.5% from 1986 to 1995. Recent data have indicated a decrease in the productivity gains being achieved by major wheat producing countries (Brown, 1997). In Western Europe, where the highest average wheat grain yield is obtained in the Netherlands (8.6 t/ha), yield increased from 5 to 6 t/ha in five years, but it took more than a decade to raise yields from 6 to 7 t/ha. Worldwide, annual wheat grain yield growth decreased from 3.0% between 1977-1985, to 1.6% from 1986-1995, excluding the USSR (CIMMYT, 1996). Degradation of the land resource base, together with a slackening of research investment and infrastructure, have contributed to this decrease (Pingali and Heisey, 1997). Whether production constraints are affected by physiological or genetic limits is hotly debated, but future increases in food productivity will require substantial research and development investment to improve the profitability of wheat production systems through enhancing input efficiencies. Due to a continuing necessity for multi-disciplinary team efforts in plant breeding, and the rapidly changing development of technologies, three overlapping avenues can be considered for raising the yield frontier in wheat: continued investments in "conventional breeding" methods; use of current and expanded genetic diversity; and investigation and implementation of biotechnology assisted plant breeding.

## Conventional Wheat Breeding

It is likely that gains to be achieved from conventional breeding will continue to be significant for the next two decades or more (Duvick, 1996), but these

are likely to come at a higher research cost than in the past. In recent surveys of wheat breeders (Braun et al., 1998; Rejesus et al., 1996), more than 80% of respondents expressed concern that plant variety protection (PVP) and plant or gene patents will restrict access to germplasm. This may have deleterious consequences for future breeding success. Rasmusson (1996) has stated that nearly half of the progress made by breeders in the past can be attributed to germplasm exchange. Regional and international nurseries have been an efficient means of gathering data from varied environments and exposing germplasm to diverse pathogen selection pressures, while providing access and exchange of germplasm. Breeders utilize these cooperative nurseries extensively in their crossing programs (Braun et al., 1998). However, the number of cooperatively distributed wheat yield and screening nurseries has been greatly reduced during the past decade.

Investments needed for breeding efforts will increase with increasing yield levels. Further, progress to develop higher yielding cultivars is reduced with every objective added to a breeding program. Though the list of important traits may get longer and longer, little if any assistance has been provided by economists to prioritize breeding objectives. Considering that a wheat breeding program like CIMMYT allocates around 60% of its resources to durable resistance breeding, the need for research in this field is obvious. Due to high costs, we see durable resistance breeding as one of the first fields where transformation should be applied by breeders through introgression of one or more genes controlling disease resistance.

## Adoption of CIMMYT-Based Germplasm

CIMMYT's breeding methodology is tailored to develop widely adapted, disease resistant germplasm with high and stable yield across a wide range of environments. The impact of this approach has been significant. The total spring bread wheat (*Triticum aestivum* L.) area in developing countries, excluding China, is around 63 million ha, of which 36 million ha or 58% are planted to varieties derived from CIMMYT germplasm (Table 1) (Byerlee and Moya, 1993; Rajaram, 1995). During the 1966-90 period, 1317 bread wheat cultivars were released by developing countries, of which 70% were either direct releases from CIMMYT advanced lines or had at least one CIMMYT parent (Byerlee and Moya, 1993). For the 1986-90 period, 84% of all bread wheat cultivars released in developing countries had CIMMYT germplasm in their pedigrees. Simultaneously the use of dwarfing genes has continued to increase over time. Today, regardless of the type of wheat, more than 90% of all wheat varieties released in developing countries are semidwarfs, which covered 70% of the total wheat area in developing countries by the end of 1990 (Byerlee and Moya, 1993). The continuous adoption of semidwarf spring wheat cultivars in the post-Green Revolution period (1977-90) resulted in about 15.5 million tons of additional wheat production in 1990, valued at about US$ 3 billion, of which 50%, or US$ 1.5 billion, is attributed to the adoption of new Mexican semidwarf wheat cultivars (Byerlee and Moya, 1993). In 1990, an estimated 93% of the total spring bread wheat production in developing countries, excluding China, came from semidwarf spring wheats, which covered about 83% of the total spring bread wheat area in developing countries (Byerlee and Moya, 1993).

**Table 1. Origin of spring bread wheat varieties in developing countries.**

| | | NARS cross | | |
|---|---|---|---|---|
| | CIMMYT cross | CIMMYT parents | CIMMYT ancestor | No CIMMYT |
| 1966-90 | 45% | 28% | 3% | 24% * |
| 1991-97 | 58% | 30% | 3% | 9% |

* Estimated.
Note: Excluding China. NARS=national agricultural research system.

The cornerstone of CIMMYT's breeding methodology is targeted breeding for the mega-environments, the use of a diverse gene pool for crossing, shuttle breeding, selection for yield under optimum conditions, and multi-locational testing to identify superior germplasm with good disease resistance. In this paper we would like to present some recent developments in CIMMYT's Wheat Program.

### Targeted breeding: The mega-environment concept

To address the needs of diverse wheat growing areas, CIMMYT introduced in 1988 the concept of mega-environments (ME) (Rajaram et al., 1994). Mega-environments are defined as a broad, not necessarily contiguous areas, occurring in more than one country and frequently transcontinental, defined by similar biotic and abiotic stresses, cropping system requirements, consumer preferences, and, for convenience,

by volume of production. Germplasm generated for a given ME is useful throughout that environment, accommodating major stresses but perhaps not all the significant secondary stresses. Within an ME, millions of hectares are addressed with a certain degree of homogeneity as relates to wheat. By 1993, 12 ME had been defined, 6 for spring wheats (ME1-ME6), 3 for facultative wheats (ME7-ME9), and 3 for winter wheats (ME9-ME12). Details of each ME are given in Table 2.

## Use of diverse genepools to maintain genetic diversity

Recent surveys conducted by the CIMMYT Economics Program have found that 58% of all wheat varieties in developing countries derive from CIMMYT germplasm; this percentage rises to more than 80%, if varieties with parents of CIMMYT origin are also included (Table 1). This spectacular success puts an enormous burden on CIMMYT to continually diversify its germplasm base for resistance and stability parameters.

Broad-based plant germplasm resources are imperative for a sound and successful breeding program. Utmost attention is given to the genetic diversity within CIMMYT germplasm to minimize the risk of genetic vulnerability, since it is grown on large areas and is widely used by national programs. I believe that the use of genetically diverse material is mandatory to increase yield potential and yield stability in the future. In any year 500-800 parental lines are considered for crossing.

**Table 2. Classification of megaenvironments (MEs) used by the CIMMYT Wheat Program.**

| ME | Latitude[a] | Area (m ha)[b] | Moisture regime[c] | Temperature regime[d] | Growth habit | Sown[e] | Major breeding objectives[f, g] | Representative locations/ regions | Year breeding began at CIMMYT |
|---|---|---|---|---|---|---|---|---|---|
| **SPRING WHEAT** | | | | | | | | | |
| 1 | Low | 32.0 | Low rainfall, irrigated | Temperate | Spring | A | Resistance to lodging, SR, LR, YR | Yaqui Valley, Mexico; Indus Valley, Pakistan; Gangetic Valley, India; Nile Valley, Egypt | 1945 |
| 2 | Low | 10.0 | High rainfall | Temperate | Spring | A | As for ME1 + resistance to YR, *Septoria* spp., sprouting | North African Coast, Highlands of East Africa, Andes, and Mexico | 1972 |
| 3 | Low | 1.7 | High rainfall | Temperate | Spring | A | As for ME2 + acid soil tolerance | Passo Fundo, Brazil | 1974 |
| 4A | Low | 10.0 | Low rainfall, winter dominant | Temperate | Spring | A | Resistance to drought, *Septoria* spp., YR | Aleppo, Syria; Settat, Morocco | 1974 |
| 4B | Low | 5.8 | Low rainfall, summer dominant | Temperate | Spring | A | Resistance to drought, *Septoria* spp., *Fusarium* spp., LR, SR | Marcos Juárez, Argentina | 1974 |
| 4C | Low | 5.8 | Mostly residual moisture | Hot | Spring | A | Resistance to drought, and heat in seedling stage | Indore, India | 1974 |
| 5A | Low | 3.9 | High rainfall / irrigated, humid | Hot | Spring | A | Resistance to heat, *Helminthosporium* spp., *Fusarium* spp., sprouting | Joydepur, Bangladesh; Londrina, Brazil | 1981 |
| 5B | Low | 3.2 | Irrigated , low humidity | Hot | Spring | A | Resistance to heat and SR | Gezira, Sudan; Kano, Nigeria | 1975 |
| 6 | High | 5.4 | Moderate rainfall/ summer dominant | Temperate | Spring | S | Resistance to SR, LR, *Helminthosporium* spp., *Fusarium* spp., sprouting, photoperiod sensitivity | Harbin, China | 1989 |

(cont'd.)

**Table 2. Continued.**

| ME | Latitude[a] | Area (m ha)[b] | Moisture regime[c] | Temperature regime[d] | Growth habit | Sown[e] | Major breeding objectives[f, g] | Representative locations/ regions | Year breeding began at CIMMYT |
|---|---|---|---|---|---|---|---|---|---|
| **WINTER/FACULTATIVE WHEAT** | | | | | | | | | |
| 7 | High | – | Irrigated | Moderate cold | Facultative | A | Rapid grain fill, resistance to cold, YR, PM, BYD | Zhenzhou, China | 1986 |
| 8A | High | – | High rainfall / irrigated, long season | Moderate cold | Facultative | A | Resistance to cold, YR, *Septoria* spp. | Chillan, Chile | 1986 |
| 8B | High | – | High rainfall / irrigated, short season | Moderate cold | Facultative | A | Resistance to *Septoria* spp., YR, PM, *Fusarium* spp., sprouting | Edirne, Turkey | 1986 |
| 9 | High | – | Low rainfall | Moderate cold | Facultative | A | Resistance to cold, drought | Diyarbakir, Turkey | 1986 |
| 10 | High | – | Irrigated | Severe cold | Winter | A | Resistance to winterkill, YR, LR, PM, BYD | Beijing, China | 1986 |
| 11A | High | – | High rainfall /irrigated, long season | Moderate cold | Winter | A | Resistance to *Septoria* spp., *Fusarium* spp., YR, LR, PM | Temuco, Chile | 1986 |
| 11B | High | – | High rainfall / irrigated, short season | Severe cold | Winter | A | Resistance to LR, SR, PM, winterkill, sprouting | Lovrin, Romania | 1986 |
| 12 | High | – | Low rainfall | Severe cold | Winter | A | Resistance to winterkill, drought, YR, bunts | Ankara, Turkey | 1986 |

Source: Adapted from Rajaram et. al. (1995).
a Low = less than about 35-40 degrees.
b Exact area distribution for winter/facultative wheat is not available.
c Refers to rainfall just before and during the crop cycle. High = >500mm; low = <500mm.
d Hot = mean temperature of the coolest month > 17.5°C; cold = <5.0°C.
e A = autumn, S = spring.
f Factors additional to yield and industrial quality. SR=stem rust, LR=leaf rust, YR=yellow (stripe) rust, PM=powdery mildew, and BYD=barley yellow dwarf.
g Further subdivided into (1) optimum growing conditions, (2) presence of Karnal bunt, (3) late planted, and (4) problems of salinity.

Twice a year around 30% of the parental stocks are replaced with outstanding introductions. About 2000 out of 8000 crosses per year are made to these introductions. In addition, commercial varieties from NARS, and non-conventional sources such as durum wheat and alien species, are used to incorporate desired traits by recombination or translocation. The introductions are mostly used as female parents to preserve cytoplasmatic diversity.

The most recent example of the potential impact of generating new diversity is the reconstitution of bread wheat by the CIMMYT wide crossing program by crossing durum wheat (*Triticum durum*) with the D-genome donor *T. tauschii*. Lines derived from backcrosses to bread wheat showed substantial morpho-agronomic variation, resistance to Karnal bunt *(Tilletia indica)* and scab *(Fusarium graminearum)*, plus a 1000-grain weight of up to 53 g (Villareal, 1995). Yield potential is close to that of bread wheat, with grain yield of the best synthetic wheat reaching 7.7 t/ha (Table 3).

Another source being exploited for new variability is *T. dicoccoides* (emmer wheat), a source of resistance to stripe rust, leaf rust, powdery mildew, *Septoria* spp., and wheat streak mosaic virus, plus drought tolerance, high protein content, and higher yield potential. Bread wheat is crossed with durum

**Table 3. Grain yield and thousand kernel weight (TKW) of two crosses of bread wheat with synthetic wheats in yield trials at Cd. Obregon, Mexico in 1993.**

| Entry | Grain yield (kg/ha) | TKW (g) |
|---|---|---|
| Chen/*T. tauschii*//BCN | 7740a[1] | 53a |
| Cndo/R143//Ente/Mexi/ 3/*T.tauschii*/4/Weaver | 6830b | 52a |
| Bacanora 88 (BW check) | 6770b | 40b |

[1] Means within columns followed by different letters are significantly different at the 0.05 level of probability.
Source: Villareal (1995).

wheat to increase grain size. The six highest yielding lines derived from this program outyielded their breadwheat parent by 5-20% in yield trials in Cd. Obregon, Mexico.

### Shuttle breeding within Mexico

Young and Frey (1994) provide two factors that influence the success of a shuttle program: a) the use of a germplasm pool encompassing genotypes with broad adaptation, and b) the use of selection environments eliciting different responses from plant types. They also state that the wheat breeding program of N.E. Borlaug met these conditions. When Borlaug started the shuttle breeding approach in 1945, his only objective was to speed up breeding for stem rust resistance. Since then, segregating populations have been shuttled 100 times between the two environmentally contrasting sites in Mexico, Cd. Obregon and Toluca (Braun et al., 1992).

Some of the salient points of this shuttle breeding program are:

- Cd. Obregon is situated at 28º N at 40 masl, in the sunny, fertile, and irrigated Yaqui Valley of Sonora. Wheats are planted in November when temperatures are low and harvested in April/May when temperatures are high. The yield potential of location is high (±10 t/ha); wheat diseases are limited to only leaf rust, Karnal bunt, and black point.
- The Toluca location is characterized by high humidity (precipitation: ±1000 mm). The nursery is planted in May/June when temperatures are high and harvested in October when they are low. High humidity causes incidence of many diseases including rust, septorias, BYD, and fusarium.

An important result of shuttle breeding was the selection of photo-insensitive wheat genotypes. Initially, selection for photoperiod insensitivity was unconscious, but only this trait permitted the wide spread of the Mexican semidwarfs (Borlaug, 1995). Today, this trait has been incorporated into basically all spring wheat cultivars grown below 48º latitude and is now also spreading to wheat areas above 48 º N (Worland et al., 1994).

### Multi-locational testing and wide adaptation

About 1500 sets of yield trials and screening nurseries consisting of around 4000 advanced bread wheat lines are annually sent to more than 200 locations. Multi-locational testing plays a key role in identifying the best performing entries for crossing. Since the shuttle program permits two full breeding cycles a year, it takes around five to six years from crossing to international distribution of advanced lines to cooperators. This "recurrent selection program" ensures continuous and rapid pyramiding of desirable genes.

Ceccarelli (1989) pointed out that the widespread cultivation of some wheat cultivars should not be taken as a demonstration of wide adaptation, since a large fraction of these areas are similar or made similar by use of irrigation and/or fertilizer. Therefore, the term wide adaptation has been used mainly to describe geographical rather than environmental differences. If this is true, the genotypic variation should be considerably higher than GxE interaction in ANOVAs of CIMMYT trials. Braun et al. (1992) showed that this is not the case. When subsets of locations were grouped by geographical and/or environmental similarities, GxE interaction was mostly greater than the genotypic variance. The environmental diversity of sites where CIMMYT's 21st International Bread Wheat Screening Nursery was grown and the diversity among genotypes in this nursery were demonstrated by Bull et al. (1994). They classified similarities among environments by forming subsets of genotypes from the total dataset and comparing them with the classification based on the remaining genotypes. Using this procedure they concluded that it was not possible to form a stable grouping of environments, because little or no relationship existed among them.

Conclusions drawn from trials carried out on research stations are always open to critics who argue that these results do not necessarily reflect conditions in farmers' fields. However, the wide acceptance of CIMMYT germplasm by farmers in MEs 1-5 does not support the view that the wide adaptation of CIMMYT germplasm is based on geographical rather than environmental differences.

## Breeding for High Yield Potential and Enhanced Stability

Selection of segregating populations and consequent yield testing of advanced lines are paramount for identifying high yielding and input responsive wheat genotypes. The increase in yield potential of CIMMYT

cultivars developed since the 1960s is shown in Figure 1 (Rees et al., 1993). The average increase per year was 0.9%, and there is no evidence that a yield plateau has been reached. This progress in increasing genetic yield potential is closely associated with an increase in photosynthetic activity (Rees et al., 1993.). Both photosynthetic activity and yield potential increased over the 30-year period by some 25%. These findings may have major implications for CIMMYT's future selection strategy since there is evidence that wheat genotypes with a higher photosynthetic rate have lower canopy temperature, which can be easily, quickly, and cheaply measured using a hand-held thermometer. If this is verified in future trials, this trait may be used by breeders to increase selection efficiency for yield potential. This technique may be particularly useful in selecting wheat genotypes adapted to environments where heat is a production constraint.

Yield *per se* is closely associated with input responsiveness. Increasing the input efficiency at low production levels can shift crossover points, provided they exist, and enhance residual effects of high genetic yield potential. Furthermore, combining input efficiency with high yield potential will allow farmers to benefit from such cultivars over a wide range of input levels. The increase in nitrogen use efficiency is shown in Figure 2 (Ortiz-Monasterio et al., 1995).

CIMMYT's breeding strategy has resulted in the development of widely grown varieties, such as Siete Cerros, Anza, Sonalika, and Seri 82, which at their peak were grown on several million hectares. Seri 82 was released for irrigated as well as rainfed environments. Reynolds et al. (1994) reported that Seri 82 was the highest yielding entry in the 1st and 2nd International Heat Stress Genotype Experiment. Seri 82 can be considered as the first wheat genotype truly adapted to several MEs, particularly to ME1, ME2, ME4, and ME5. A comparison between Seri 82 and Pastor, a recently developed CIMMYT cultivar, demonstrates the progress made in widening adaptation during the last ten years. Figure 3 shows the performance of Pastor (Pfau/Seri//Bow) in CIMMYT's 13th Elite Spring Wheat Yield Nursery. In 50 trials grown in all 6 MEs, Pastor yielded significantly (P=0.01) lower than the highest yielding entry only in eight trials. This figure also demonstrates that Pastor has no tendency to crossover at any yield level. While we do not reject that crossover may exist for some cultivars, Pastor and Seri 82 are clear examples that it is possible to combine abiotic stress tolerance with high yield potential. Figure 4 shows the yield difference between Seri 82 and Pastor. Only in 16 out of 50 trials did Seri outyield Pastor. The latter cultivar proves that breeding for wide adaptation has not yet reached its limit.

Apart from the physiological basis of yield potential, the yield gains in CIMMYT wheats are due to the utilization of certain genetic resources. The germplasm has been paramount to increase yield in CIMMYT's Wheat Program and in

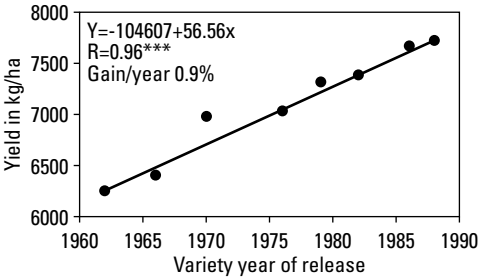


**Figure 1. Mean grain yields for the historical series of bread wheat varieties for the year 1990-93 at Cd. Obregon, Mexico (data from Rees et al., 1993).**

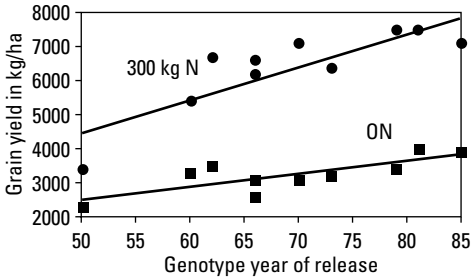


**Figure 2. Grain yield of the historical series of bread wheats at Cd. Obregon, Mexico, at 0 and 300 kg/ha N application (data from J.I. Ortiz-Monasterio et al., 1995).**

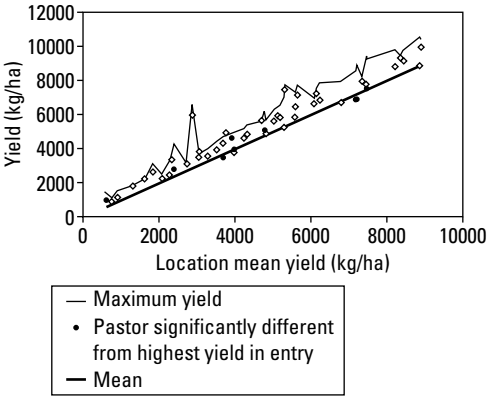


**Figure 3. Yield of Pastor at 50 locations of the 13th ESWYT.**

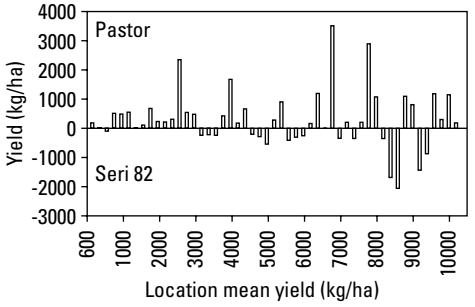


**Figure 4. Yield difference between Pastor and Seri 82 at 50 locations of the 13th ESWYT.**

Minnesota's barley program (Rasmusson, 1996). Some examples are listed next.

- The incorporation of Norin 10 x Brevor germplasm not only produced dwarf wheats, but also simultaneously gave high yield.
- Spring and winter crosses involving the variety Kavkaz resulted in Veerys, representing high yield potential and enhanced yield stability (Figure 5).
- The incorporation of the *Lr*19 gene and *Aegilops squarrosa*-derived synthetic wheats has further increased yield potential. The variety Super Seri has the *Lr*19 gene (Figure 6) and a derivative of *Ae. squarrosa* is given in Table 3.

## Breeding for durable disease resistance

From the beginning, incorporating durable, non-specific disease resistance into CIMMYT germplasm was a high priority, since breeding widely adapted germplasm with stable yields without adequate resistance against the major diseases would be impossible. The concept goes back to Niederhauser et al. (1954), Borlaug (1966), and Caldwell (1968), who advocated developing general resistance in the CIMMYT program versus the specific or hypersensitive type. Very diverse sources of resistance for rusts and other diseases are intentionally used in the crossing program. The major sources are germplasm from national programs, advanced CIMMYT lines, germplasm received from the CIMMYT or other genebanks, and CIMMYT's wide crossing program.

CIMMYT's strategy in the case of cereal rusts is to breed for general resistance (slow rusting) based on historically proven stable genes. This non-specific resistance can be further diversified by accumulating several minor genes and combining them with different specific genes to provide a certain degree of additional genetic diversity. This concept is also applied to other diseases like septoria leaf blotch, helminthosporium spot blotch, and fusarium head scab. The present situation of CIMMYT germplasm regarding resistance to major diseases may be summarized as follows:

- Stem rust *(Puccinia graminis* f.sp. *tritici)* resistance has been stable after 40 years of utilization of the genes derived from the variety Hope. Losses due to stem rust have been negligible since the late 1960s. The resistance is based on the gene complex *Sr2*, which actually consists of *Sr2* plus 4-5 minor genes pyramided into three to four gene combinations (Rajaram et al., 1988). *Sr 2* alone behaves as a slow rusting gene. Since there has been no major stem rust epidemic in areas where CIMMYT germplasm is grown, the resistance seems to be durable.

- Leaf rust *(Puccinia recondita* f.sp. *tritici)* resistance has been stabilized by using genes derived from many sources, in particular the Brazilian cultivar Frontana (Singh and Rajaram, 1992). No major epidemic has been observed in almost 20 years. Four partial resistance genes, including *Lr 34,* give a slow rusting response and have been the reason for the containment of leaf rust epidemics in the developing world during the last 15 years. About 60% of CIMMYT germplasm carries one to four of these partial resistance genes. *Lr34* is linked to *Yr18* as well as to a morphological marker (leaf tip necrosis) that makes the gene particularly attractive for

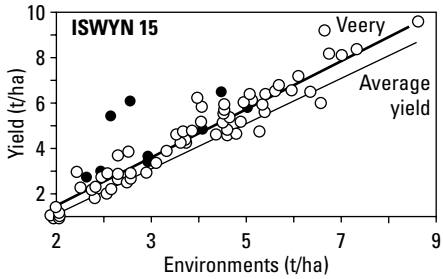


**Figure 5. Performance of Veery in 73 global environments (ISWYN 15).**

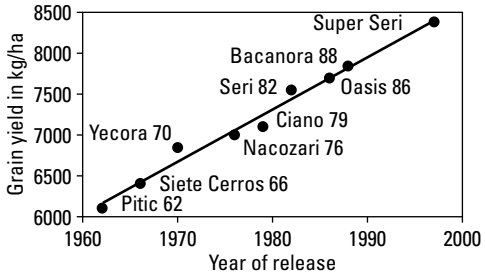


**Figure 6. Increase in grain yield potential of CIMMYT-derived wheats as a function of year of release.**

breeders (Singh, 1992a, b). CIMMYT continues to look for new sources of partial resistance.

- Stripe rust *(Puccinia striiformis)*: Slow rusting genes like *Yr18* have been identified (Singh, 1992b); however, their interaction is less additive than for leaf and stem rust. More basic research is needed to understand the status of durable resistance in high yielding germplasm. The breakdown of *Yr9* in West Asia and North Africa and the present yellow rust epidemics underline the need for the release of cultivars with accumulated durable resistance.

- *Septoria tritici*: Initially all semidwarf cultivars developed for irrigated conditions were susceptible. Today more than eight genes have been identified in CIMMYT germplasm and two to three genes in combination provide acceptable resistance. Future activities will concentrate on pyramiding these genes and spreading them more widely within CIMMYT germplasm (Jlibene, 1992; Matus-Tejos, 1993).

- Karnal bunt (*Tilletia indica*): More than five genes have been identified and most of them are partially dominant. Genes providing resistance to Karnal bunt have been incorporated into high yielding lines (Singh et al., 1995).

- Powdery mildew (*Erysiphe graminis* f.sp. *tritici*): CIMMYT germplasm is considered vulnerable to this disease. The disease is absent in Mexico and the responsibility to transfer resistance genes has been delegated to CIMMYT's regional breeder in South America.

## Breeding for Drought Tolerance

There has been a large transformation in the productivity of wheat due to the application of Green Revolution technology. This has resulted in a doubling and tripling of wheat production in many environments, but especially in irrigated areas. High yielding semidwarf wheats have continuously replaced the older tall types at a rate of 2 million ha per year since 1977 (Byerlee and Moya, 1993).

There is a growing recognition that the dissemination, application, and adoption of this technology has, however, been slower in marginal environments, especially in the semiarid environments affected by poor distribution of water and drought. The annual gain in genetic yield potential in drought environments is only about half that obtained in irrigated, optimum conditions. Many investigators have attempted to produce wheat varieties adapted to these semiarid environments with limited success. Others have criticized the Green Revolution technology (Ceccarelli et al., 1987) for failing to adequately address productivity constraints in semiarid environments, although their own recommended technology has had limited impact, in particular in farmers' fields. This criticism is in clear contrast to the actual acceptance of semidwarf wheat cultivars in rainfed areas, since most of the 16 million ha increase in the area sown to Mexican semidwarf wheats in the mid-1980s occurred in rainfed areas; in 1990, more than 60% of the dryland area in developing countries were planted with semidwarfs (Byerlee and Moya, 1993).

### Definition of semiarid environments and description of drought patterns

In Table 2, the major global drought patterns observed in wheat production are presented (Rajaram et al., 1994, Edmeades et al., 1989). Through respectively dealing with spring (ME4A), facultative (ME9), and winter wheat (ME12), these three MEs are characterized by sufficient rainfall prior to anthesis, followed by drought during the grain-filling period. In South America, the Southern Cone type of drought (ME4B) is characterized by moisture stress early in the crop season, with rainfall occurring during the post-anthesis phase. In the Indian Subcontinent type of drought stress (ME4C), the wheat crop utilizes water reserves left from the monsoon rains during the previous summer season. In the Subcontinent the irrigated wheat crop (ME1) may also suffer drought due to a reduced or less than optimum number of irrigations.

The traditional methodology for breeding for drought stress is typified by handling all segregating populations under target conditions of drought, and the use of local landraces is recommended in the breeding process (Ceccarelli et al., 1987). The methodology rests

on the assumption that the agro-ecological situation facing the farmer does not vary in its expression over time and that responsiveness of varieties to improved growing conditions will not be needed. It also assumes that crossover will always occur below a certain yield level under dry conditions, where modern high yielding varieties of a responsive nature would always yield less than traditional landrace-based genotypes. Such crossovers may occur for selected genotypes, and one should always be open to the possibility that there are real "drought tolerance" traits operating at the 1 t/ha and below yield level that adversely affect high yield potential at the 4 t/ha and higher yield levels. So far such traits have not been identified at CIMMYT. In any case, crossover would be restricted to such harsh conditions, where in fact farmers choose—rightfully so—not to grow wheat at all, but to produce other, more drought tolerant crops such as barley or sorghum, or resort to grazing (van Ginkel et al., 1998).

At CIMMYT we advocate an "open-ended system" of breeding in which yield responsiveness is combined with adaptation to drought conditions. Most semiarid environments differ significantly across years in their water availability and distribution pattern. Hence it is prudent to construct a genetic system in which plant responsiveness provides a bonus whenever conditions improve due to higher rainfall. With such a system, improved moisture conditions immediately translate into greater gains for the farmer.

## The Veerys

In the early 1980s, when advanced lines derived from the spring x winter cross Kavkaz/Buho//KAL/BB (CM33027) were tested in 73 global environments of the 15th International Wheat Yield Nursery (15th ISWYN) (Figure 5), their performance was quite untypical compared to any previously known high yielding varieties. In later tests, we found that these lines, called Veerys, carry the 1B.1R translocation from rye and that the general performance of such germplasm was superior not only in high yielding environments but particularly under drought conditions (Villareal et al., 1995; Table 4). From the Veery cross 43 varieties were released, excluding those released in Europe.

The Veerys represent a genetic system in which high yield performance in favorable environments and adaptation to drought could be combined in one genotype. The two genetic systems are apparently not always incompatible, although others have claimed that their combination would not be possible. However, it is possible to hypothesize a plant system in which efficient input use and responsiveness to improved levels of external inputs (in this case, available water) can be combined to produce germplasm for marginal (in this case, semiarid) environments that at least maintains minimum traditional yields and expresses dramatic increases whenever conditions improve. The impacts described below support the utilization of this methodology.

- By the mid-1980s CIMMYT germplasm occupied 45% of the semiarid wheat areas with 300-500 mm of rainfall, and 21% of the area less than 300 mm (Morris et al., 1991), including large tracts in West Asia/North Africa (WANA). By 1990 63% of the dryland areas, especially in ME4A and ME4B, was planted with semidwarf wheats (Byerlee and Moya, 1993), many carrying the 1B/1R translocation.

- To support the above assumptions, an experiment was conducted (Calhoun et al., 1994; Tables 5 and 6) to determine how the most modern and widely (spatially) adapted germplasm compared to commercial germplasm from countries representing the Mediterranean region (ME4A), the Southern Cone of South America (ME4B), and the Indian

**Table 4. Effect of the 1BL.1RS translocation on yield characteristics of 28 random F2-derived F6 lines from the cross Nacozari 76/Seri 82 under reduced irrigated conditions.**

| Plant characteristics | 1BL.1RS | 1B | Mean diff. |
|---|---|---|---|
| Grain yield | 4945 | 4743 | 202* |
| Above-ground biomass at maturity (t/ha) | 12600 | 12100 | 500* |
| Grains/$m^2$ | 14074 | 13922 | 152NS |
| Grains/spike | 43.5 | 40.6 | 2.9* |
| 1000-grain weight (g) | 37.1 | 36.5 | 0.5* |

Source: Villareal et al. (1995).
Note: NS: not significatnt, * : significant at the 0.05 level.

**Table 5. Wheat genotypes representing adaptation to different moisture environments.**

| | | |
|---|---|---|
| $ME_1$ | Irrigation | Super Kauz, Pavon 76, Genaro 81, Opata 85 |
| $ME_4A$ | (Mediterranean) | Almansor, Nesser, Sitta, Siete Cerros |
| $ME_4B$ | (Southern Cone) | Cruz Alta, Prointa Don Alberto, LAP1376, PSN/BOW CM69560 |
| $ME_4$ | (Subcontinent) | C306, Sonalika, Punjab 81, Barani |

Source: Calhoun et al. (1994).

**Table 6. Grain yields of selected wheat genotypes grouped by adaptation and tested under moisture regimes in the Yaqui Valley, Mexico, 1989-90 and 1990-91**

| | | Adaptation group | | | |
|---|---|---|---|---|---|
| | | Full irrigation[1] | Late drought[2] | Early drought[3] | Residual moisture[4] |
| $ME_1$ | Irrigation | 6636 a* | 4198 a | 4576 a | 3032 a |
| $ME_4C$ | Mediterranean | 6342 b | 3990 ab | 4390 b | 2883 b |
| $ME_4B$ | Southern Cone | 5028 c | 3148 bc | 4224 b | 2359 c |
| $ME_4C$ | Subcontinent | 4778 c | 3245 bc | 3657 c | 2704 b |

Source: Calhoun, et al. 1994

[1] Received 5 irrigations; [2] received 2 irrigations early before heading; [3] received one irrigation for germination and two post heading; [4] received one irrigation for germination only.

* Means in the same column followed by the same letter are not significantly different at P=0.05.

Subcontinent (ME4C), under conditions artificially simulating those three MEs. The most widely adapted CIMMYT lines outyielded the commercial varieties in all artificially simulated environments.

- Nesser is an advanced line with superior performance in drought conditions bred at CIMMYT/Mexico and identified at ICARDA/Syria. The cross combines a high yielding CIMMYT variety Jupateco and a drought tolerant Australian variety W3918A. The performance of Nesser in WANA's ME4A environments has been widely publicized (ICARDA, 1993), and the line is considered by ICARDA to represent a uniquely drought tolerant genotype. However, it was selected at CIMMYT/Mexico under favorable environments, and carries a combination of input efficiency and high yield responsiveness. It performs similarly to the Veery lines in the absence of rust.

## The breeding scheme

The breeding scheme described below is used to combine the two genetic systems. Two contrasting selection environments are alternated, allowing alternate selection for input efficiency and input responsiveness.

F1 Crosses involving spatially widely adapted germplasm representing yield stability and yield potential, with lines with proven drought tolerance in the specific setting of either ME4A, ME4B or ME4C. Winter wheats and synthetic germplasm are emphasized.

F2 Individual plants are raised under irrigated and optimally fertilized conditions and inoculated with a wide spectrum of rust virulence. Only robust and (horizontally) resistant plants are selected. These may represent adaptation to favorable environments.

F3, F4 The selected F2 plants are evaluated using a modified pedigree/bulk breeding system (Rajaram and van Ginkel, 1995) under rainfed conditions or very low water availability. The selection is based on individual lines rather than individual plants. The progenies are selected based on such criteria as spike density, biomass/vigor, grains/$m^2$, and others (van Ginkel et al., 1998) (Table 7). This index helps identify lines which may adapt to low water situations.

F5, F6 The selected lines from F4 are further evaluated under optimum conditions.

**Table 7. Genotypic correlation (rg) between agronomic traits and final grain yield, for optimum environment (full irrigations) and reduced water regime (late drought, Mediterranean type) in wheat.**

| | Moisture regime | |
|---|---|---|
| Trait | Full irrigation | Late drought |
| Days to heading | 0.40 | 0.19 |
| Days to maturity | 0.29 | 0.27 |
| Grain fill period | -0.32 | 0.36 |
| Height | -0.39 | 0.05 |
| Peduncle length | -0.46 | 0.22 |
| Relative peduncle extrusion | -0.51* | 0.25 |
| Spike length | -0.28 | -0.50* |
| Spike/$m^2$ | -0.12 | 0.64** |
| Grains/spike | 0.62* | -0.42 |
| Grains/$m^2$ | 0.74** | 0.68** |
| Yield/spike | 0.55* | -0.64** |
| 1000 grain weight | 0.08 | -0.45 |
| Test weight | 0.13 | 0.05 |
| Harvest index | 0.83** | -0.39 |
| Biomass | 0.90** | 0.94** |
| Straw yield | 0.52* | 0.86** |
| Yield / day (planting) | 0.99** | 0.57* |
| Yield / day (heading) | 0.94** | 0.44 |
| Biomass / day (planting) | 0.86** | 0.69** |
| Biomass / day (heading) | 0.74** | 0.63** |
| Vegetative growth rate | 0.32 | 0.63** |
| Spike growth rate | 0.62** | -0.58* |
| Grain growth rate | 0.17 | -0.44 |

*, ** indicate significance at the 0.05 and 0.01probability level, respectively.

Source: van Ginkel et al. (1998).

F7, F8 Simultaneous evaluations under optimum and low water environments. Selection of lines showing outstanding performance under both conditions. Further evaluation in international environments is carried out for purposes of verification.

The proposed breeding methodology is supported by research published in recent years by others, not only on wheat (Bramel-Cox et al., 1991; Cooper et al., 1994; Duvick, 1990, 1992; Ehdaie et al., 1988; Uddin et al., 1992; Zavala-Garcia et al., 1992), where the importance of testing and selecting in a range of environments, including well-irrigated ones, has shown to identify superior genotypes for stressed conditions. The methodology aims at combining input efficiency with input responsiveness by alternating selection environments during the breeding process.

## Future Research Directions

### Yield stability and yield potential

Traxler et al. (1995) analyzed grain yield increases and yield stability of bread wheat cultivars released during the last 45 years. In the early period of the Green Revolution, when rapid yield increases occurred, variance for yield concomitantly increased. Since the early 1970s, yield stability has increased at the cost of increases in yield. However, steady progress was made in developing varieties with improved stability, grain yield or both. For the developing world, yield stability increased since the beginning of the Green Revolution (Smale and McBride, 1996). While price policy, input supplies, and environmental variation contribute more to yield stability than the genotype, the increasing yield stability reflects the emphasis given by breeders to develop germplasm with tolerance to a wider range of diseases and abiotic stresses. Sayre et al. (1997) concluded that from 1964 to 1990, yield potential in CIMMYT-derived cultivars increased at a rate of 67 kg/ha/yr or 0.88% per year. The data did not suggest that a yield plateau had been reached and the performance of recently released lines, such as Attilla (pb343) and Babax (Baviacora M92) indicates that yield potential has been further enhanced. Improvements made by breeding for yield stability and adaptation may be illustrated by data for the advanced line Pastor, which outyielded the hallmark check cultivar Seri 82 in 34 out of 50 locations where the 13$^{th}$ Elite Spring Wheat Yield Nursery was grown (Figure 4). Discussion on how to increase the yield potential of wheat often still centers around traits that contributed to the success of the Green Revolution varieties more than 30 years ago, e.g., photoperiod and dwarfing genes (Worland et al., 1998; Sears, 1998).

CIMMYT has made a modest investment in restructuring and creation of a new plant type characterized by robust stem, broad leaf, long spike (30 cm), and large numbers of grain per spike. The new plant type still suffers due to diseases and is deficient in quality and certain agronomic characteristics. In 1994, we launched a dynamic breeding program to correct these deficiencies.

### Plant nutrition

Selecting for yield potential and yield stability under medium to high levels of nitrogen has indirectly increased efficiency for nutrient uptake. Recently released CIMMYT bread wheat cultivars require less nitrogen to produce a unit amount of grain than cultivars released in the previous decades (Ortiz Monasterio et al., 1997). Under low N levels in the soil, N use efficiency increased mainly due to a higher N uptake efficiency—the ability of plants to absorb N from the soil—whereas under high N levels, the N utilization efficiency—the capacity of plants to convert the absorbed N into grain yield—increased. In spite of the increased N use efficiency of recently released wheat cultivars, the response to nitrogen of wheat production systems has been observed to be declining in many areas of Southeast Asia. In Turkey, where zinc deficient soils are common, recently released winter bread wheat cultivars have improved Zn uptake and consequently higher grain yield than local landraces (M. Kalayci, pers. comm.).

### Physiology

A recent survey of wheat breeders suggested that research in plant physiology has had a limited impact on wheat improvement (Jackson et al., 1996). A strong body of evidence now indicates that physiological traits may have real potential for complementing early generation phenotypic selection in wheat. One of the more promising traits identified is canopy

temperature depression (CTD). CTD refers to the cooling effect exhibited by a leaf as transpiration occurs. While soil water status has a major influence on CTD, there are strong genotypic effects under well-watered, heat-stressed, or drought-stressed conditions. CTD gives an indirect estimate of stomatal conductance and is a highly integrative trait affected by several major physiological processes, including photosynthetic metabolism, evapotranspiration, and plant nutrition. CTD and stomatal conductance, measured on sunny days during grain filling, showed a strong association with the yield of semidwarf wheat lines grown under irrigation, in both temperate (Fischer et al., 1998) and subtropical environments (Reynolds et al., 1994). In addition, CTD, as measured on large numbers of advanced breeding lines in irrigated yield trials, was a powerful predictor of performance not only at the selection site but also for yield averaged across 15 international sites. CTD has been shown to be associated with yield differences between homozygous lines, indicating a potential for genetic gains in yield, in response to selection for CTD (Reynolds et al., 1998).

## Germplasm is paramount

Three-quarters of recently surveyed wheat breeders felt that a lack of genetic diversity would limit future breeding advances (Rejesus et al., 1996), though genetic diversity was not considered an immediately limiting factor in most programs. This concern was greater from breeders in developing and former USSR countries (>80%) than from higher income countries (59%). Furthermore, in countries where privatization of wheat breeding programs has occurred, investments in strategic germplasm development that may be risky or important only in the long term have declined (McGuire, 1997).

A wide range of opinion has been expressed concerning the abundance or availability of usefully exploitable genetic variability. Allard (1996) emphasized that the most readily useful genetic resources were modern elite cultivars, since these lines possessed relatively high frequencies of favorable alleles. Rasmusson and Phillips (1997) have shown that the assumption that all genetic variability is a result of the inherent exclusive contribution of two parents, *per se*, is not necessarily true, considering results from molecular analysis. They discuss mechanisms by which induction of genetic variability may involve altering the expression of genes, the possible mechanisms of single allele change, intragenic recombination, unequal crossover, element transpositions, DNA methylation, paramutation, or gene amplification. They also stressed the possible importance of epistasis effects which may have been underestimated in the past.

Introduction of genetic variability from distantly related wheat cultivars, or related or alien species, has often been specifically aimed at the introduction of simply inherited traits (e.g., genes for disease resistance), but it has appeared to be of limited value in quantitative trait improvement (Cox et al., 1997). incorporated genes for leaf rust resistance from *T. tauschii* into bread wheat. With two backcrosses to the recurrent wheat parent, leaf rust resistant winter wheat advanced lines with acceptable quality and equal in yield to the highest yielding commercially grown cultivars were identified. In addition, it has been postulated that since recombination between the D genomes of *T. aestivum* and *T. tauschii* occurred at a level similar to that in an intraspecific cross (Fritz et al., 1995), *T. tauschii* could be considered another primary source of genes for wheat improvement.

The number of wheat/rye translocations that have had a significant impact on wheat improvement are actually few in number. The majority of the 1BL.1RS translocations occurring in more than 300 cultivars worldwide can be traced to one German source and all 1AL.1RS translocations, widely present in bread wheat cultivars grown in the Great Plains of the US, trace to one source, "Amigo" (Schlegel, 1997a,b; Rabinovich, 1998). Other translocations carry genes for copper efficiency (4BL.5R) and Hessian fly resistance (2RL.2BS, 6RL.6B, 6RL.4B, 6RL.4A; McIntosh, 1993). Chromosomes 2R and 7R enhance zinc efficiency in wheat-rye addition lines (Cakmak and Braun, unpublished). Considering the impacts which have come from the use of wheat/rye translocations, further exploitation of these translocations may be warranted.

While there have been reports indicating a positive effect of 1BL.1RS translocations on yield performance and adaptation (Rajaram et al., 1990), Singh et al. (1998) determined that with Seri 82, replacing the translocation with 1BL from cv. Oasis resulted in a yield increase of 3.4 and 5.0% in

irrigated and moisture stress conditions, respectively. A further increase in grain yield in disease free conditions of about 5% was observed in the irrigated trials through the introgression of 7DL.7Ag translocation carrying the *Lr19* gene (from *Agropyron elongatum*). This yield increase was attributed to higher rate of biomass production in the 7DL.7Ag lines. However, under moisture stress conditions 7DL.7Ag lines were associated with a 16% yield reduction, possibly due to excessive biomass production in early growth stages. This would suggest that the effect of the 1BL.1RS translocation is genotype specific and that 7DL.7Ag could be a useful translocation for enhancing yield potential at least in irrigated conditions.

Recent efforts to generate newly accessible genetic diversity have involved the reconstitution of hexaploid wheat by producing "synthetic wheat" by crossing durum wheat (*T. turgidum),* the donor of the A and B genomes, with *T. tauschii,* the donor of the D genome (Mujeeb-Kazi et al., 1996). Villareal (1995) and Villareal et al. (1997) showed that lines derived after two backcrosses to *T. aestivum* showed increased morpho-agronomic variation, and resistance to Karnal bunt and head scab. Under full irrigation in northwestern Mexico, the yield of this material was nearly 8 t/ha. When tested under drought conditions for two years, nearly all of the synthetic derivatives had significantly higher 1000-kernel weight, with grain yield varying between 84 to 114%, when compared with the bread wheat checks.

The more focused the breeding objective, the more restricted a breeder is in the choice of suitable parents. The use of genetically diverse material will continue to be a prime genetic source for increasing yield potential, a complex trait still not well understood genetically or physiologically. As long as breeders have no other readily accessible tools, genetic diversity and the opportunity for its recombination through crossing will be important to break undesired linkages and increase the frequency of desirable alleles. Future breakthroughs in yield potential will likely come from such genetically diverse crosses.

## Hybrid wheat

Pickett (1993) and Picket and Galwey (1997) evaluated 40 years of wheat hybrid development and concluded that hybrid wheat production is not economically feasible because of a) limited heterotic advantage; b) lack of advantage in terms of agronomic, quality, or disease resistance traits; c) higher seed costs; and d) probably most importantly, heterosis could be "fixed" in polyploid plants and consequently hybrids would have no advantage over inbred lines.

The use of hybrid crops is usually targeted to higher yield potential environments. Results from South Africa (Jordaan, 1996), however, show that hybrids outyield inbred lines by 15% at a 2 t/ha mean production potential when narrow row spacing and low seeding rates (<25 kg/ha) are used. Mean grain yield of hybrids tested in the Southern Regional Performance Nursery (SRPN), across locations in the southern Great Plains, were significantly higher than for inbred lines (Peterson et al., 1997). Bruns and Peterson (1998) calculated a yield advantage of hybrid wheat at 10-13% and attributed this advantage, in part, to better temporal and spatial stability and improved heat tolerance.

In contrast, recent reports of hybrid performance in Europe indicate lower levels of heterosis (5-12%) (Eavis et al., 1996). Gallais (1989) stated that provided overdominance is of little importance in wheat, in the long term, inbred line development will be more effective than $F_1$ hybrids. If biotechnological methods can identify increased expression of heterosis by more effective selection of favorable alleles, this impact will likely have equal advantage to inbred and hybrid development. Whether hybrids have a higher absolute yield potential than inbred lines has to be seen in light of inbred bread wheat cultivars with an observed grain yield of 17 t/ha (Hewstone, 1997).

## Biotechnology

Techniques such as doubled haploids were considered "biotechnology" ten years ago but have became routine in many programs. Lack of genetic polymorphism in crops like wheat and soybeans and the consequent problems to identify molecular markers have been a major limitation to the impact of marker assisted selection (MAS) in wheat breeding. The identification of a high number of polymorphisms in single sequence repeats (SSR) should therefore greatly enhance the potential to find molecular markers in wheat.

Conventional plant breeders adopt breeding methods that increase their breeding efficiency but are conservative when making methodological changes. A small survey of wheat programs having unrestricted access to new biotechnological methods found that few research programs, and no main-line wheat breeding programs, routinely used MAS or quantitatively inherited trait loci (QTL). Limitation in use is caused by lack of markers for traits of interest, population specificity of a given marker, or markers'relatively high costs when compared with conventional selection techniques. These limitations may lessen in the next decade.

Modern cultivars are the product of recombinations among the high number of landraces in their pedigrees (Smale and McBride, 1996). In contemporary breeding programs, however, landraces are used directly only as a source of qualitatively inherited traits. Tanksley and McCouch (1997) argued that the lack of success from crosses involving landraces for the improvement of grain yield was mainly due to evaluation on a phenotypic basis, an imprecise indicator of genetic potential. Analyses of QTLs have revealed that loci controlling a quantitatively inherited trait do not contribute equally to the observed variation for the trait, and often a few QTLs explain most of the observed variation. In rice, QTLs for yield were identified in a wild, low yielding relative. After introgression into modern hybrid rice cultivars, yield increases of 17% compared to the original hybrid were observed. Based on the observed gains, Tanksley and McCouch (1997) identified the need to more thoroughly evaluate exotic germplasm. Those accessions most distinct from modern cultivars may contain the highest number of unexploited, potentially useful alleles.

The comparative genetic mapping of cereal genomes has identified a vast amount of conserved linearity of gene order (Devos and Gale, 1997). This observation will likely accelerate the application of QTLs in wheat, as well as aid in the identification of genes required for introgression from alien species. Considering the low number of loci tagged today in wheat, the problems related to developing a high density map for wheat (Snape, 1998), and consequently the limited progress to identify QTLs for yield in wheat, we believe that the impact from this linearity on wheat improvement will be significant.

Wheat has been successfully transformed for herbicide resistance and high molecular weight (HMW) glutenins, using both the ballistic and *Agrobacterium tumefaciens* systems (Cheng et al., 1997). Barro et al. (1997) inserted two additional HMW glutenin subunits, 1Ax1 and 1Dx5, and observed a stepwise improvement of dough strength. Altpeter et al. (1996) introduced 1Ax1 into Bobwhite and increased total HMW glutenin subunit protein by 71% over Bobwhite. However, the effects of transformation are not necessary additive as was shown by Blechl et al. (1998), who identified trangenics for HMW glutenins that also exhibited decreased accumulation due to transgene-mediated suppression.

## Conclusions

The challenge to annually produce one billion tons of wheat within the next 25 years is formidable and can be met only by a concerted action of scientists involved in diverse disciplines (agronomy, pathology, physiology, biotechnology, breeding), as well as economics and politics. I am optimistic that this target will be met. Today, funds are directed from breeding towards biotechnology, often due simply to the novelty required for publication. Eventually, transformation may be a valuable technique to alter the performance of a genotype; however, at least during the next decade, the simple decision of a breeder in the field to "keep or discard" will contribute more to yield increase than any other approach. In conclusion, I agree with Ruttan (1993) who stated that "at least for the next two decades to come, progress through conventional breeding will remain the primary source of growth in crop and animal production."

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## *Session 1: Pathogen Biology*

# Biology of the *Septoria/Stagonospora* Pathogens: An Overview

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## Abstract

*More than 2000 form-species of fungi, mostly plant parasites, comprise the genera* Septoria *and* Stagonospora. *The two most important pathogens in wheat production are* Mycosphaerella graminicola (Septoria tritici) *and* Phaeosphaeria nodorum (Stagonospora nodorum). *Primary inoculum for the wheat diseases caused by these pathogens is most often air-borne ascospores, but may also be wind- and rain-borne conidia. Field diagnoses may be augmented and made more exact by use of rapid immunological tests and molecular genetic methods. Infection processes of* S. tritici *and* S. nodorum *are similar, but penetration by* S. tritici *is known only via stomata. Patterns of occurrence of the two pathogens have changed dramatically in recent years.* Septoria tritici *has become more important in northern Europe, and* S. nodorum *incidence has increased in parts of North Africa. Changes in cultivars and cultural practices are thought to be responsible for the shifts in pathogens and diseases. Genetics of resistance in the wheat host and virulence in the pathogen populations continues to be unclear. Some gene-for-gene interactions have been shown, but in field situations resistance is generally observed as non-specific and pathogen populations vary most in aggressiveness.*

"Scientists build on foundations laid by their predecessors…, but they show great reluctance to inspect those foundations" (Ainsworth, 1965). Such reluctance was evident for many years in the case of the *Septoria/Stagonospora* diseases of cereal grains. Nineteenth and early twentieth century workers in Europe and North America described the pathogens and the diseases they cause in considerable detail. But, only in the years since the advent of cultivars bred for dwarf stature and high yield under conditions of intensive culture have the *Septoria/Stagonospora* diseases been recognized as having major impacts upon yield and quality. The causal organisms are still called by several names, as are the diseases. A concise summary of the latest information on taxonomy and nomenclature was published in 1997 (Cunfer, 1997) and 1999 (Cunfer and Ueng, 1999). I believe that we should urge from this venue, as did those attending the Fourth International Workshop on Septoria of Cereals (July 4-7, 1994, IHAR, Radzikow, Poland), that all workers accept and use the latest nomenclature.

More than 2000 form-species of fungi, most of them plant parasites, have been assigned to the genera *Septoria* and *Stagonospora.* Two of these that attack wheat are of most concern to us. Losses of potential yield world-wide from just these two are estimated in the millions of metric tons of grain and billions of U.S. dollars each year. The common names of the diseases are septoria tritici blotch and stagonospora nodorum blotch. Classification and nomenclature of these and other pathogens of cereals are given in Table 1 (Eyal, 1999). The *Septoria* anamorphic states have a teleomorphic state assigned to the genus *Mycosphaerella,* while the *Stagonospora* states have teleomorphic forms in the genus *Phaeosphaeria*. The teleomorphs are very important because they furnish the primary inoculum for disease development under specific environmental conditions.

Several periods of ascospore deposition (both *Mycosphaerella* and *Phaeosphaeria*) from the atmosphere during the months of August to October in the northern hemisphere and February to April in the southern hemisphere have recently been shown to have critical importance in epidemic establishment (Arseniuk et al.,

**Table 1. Classification and nomenclature of the *Septoria* spp. and *Stagonospora* spp. fungi on small grain cereals.[a]**

| Genus | Teleomorph | Anamorph | Common name | Host |
|---|---|---|---|---|
| *Septoria* spp. | *Mycosphaerella graminicola* | *Septoria tritici* | Septoria tritici blotch of wheat | Wheat |
| | -[b] | *Septoria tritici f. avenae* | | Oats |
| | - | *Septoria tritici* f. *holci* | | Holcus |
| | - | *Septoria tritici* f. *lolicola* | | Lolium |
| | - | *Septoria passerinii* | Speckled leaf blotch of barley | Barley |
| | - | *Septoria secalis* | Leaf spot of rye | Rye |
| *Stagonospora* spp. | *Phaeosphaeria nodorum* | *Stagonospora nodorum* | Stagonospora nodorum blotch of wheat | Wheat |
| | *Phaeosphaeria avenaria* | *Stagonospora avenae* f. sp. *avenae* | | Oats |
| | *Phaeosphaeria avenaria* f. sp. *triticea* | *Stagonospora avenae* f. sp. *triticea* | | Oats, wheat and triticale |

[a] (7)
[b] Teleomorphic stages not found.

1998; Shaw and Royle, 1989). The anamorphic conidia, also called pycnidiospores, are most important as secondary inoculum locally as the crop is growing and are disseminated mainly by rain splash.

Sources of primary inoculum in areas where the teleomorph is not known remain a matter of controversy and speculation, particularly in the case of septoria tritici blotch. Both *S. nodorum* and *S. tritici* are found parasitizing a wide range of graminaceous hosts. (Krupinsky, 1994; Sprague, 1950). Several species of grasses have been suspected as alternative hosts and inoculum sources, but the question is yet unresolved. Conidia from plant debris may act as primary inoculum for disease development in some cases. The fact remains to puzzle us that no case has been reported in which septoria tritici blotch and/or stagonospora nodorum blotch failed to appear because of a lack of primary inoculum when a susceptible crop and favorable environmental conditions prevailed.

As late as the 1960s, diagnosis of *Septoria* diseases on wheat was not well developed even among plant pathologists and plant breeders. Leaf chlorosis and necrosis was often viewed as part of the natural process of maturation. Any of several leaf spotting pathogens could have been present and contributing to leaf death. When *Septoria* was recognized, it was often called "head septoria" which we know now as stagonospora nodorum blotch and "leaf septoria" which we know as septoria tritici blotch. Both often occur together and with other pathogens, and both can infect and cause symptoms on all parts of the wheat plant.

Field diagnosis on the basis of symptomology is regularly done, but often laboratory backup, with microscopic examination of spores, is necessary for accurate diagnosis. As can be seen in Table 2 (Eyal, 1997), spore size and appearance may overlap, so some doubts may remain even after microscopic examination. Rapid tests have been developed that use immunological techniques mainly for early diagnosis in intensive production areas where chemical control is commonly used. Molecular genetic methods have been used recently to show similarities and differences between species and *forma speciales* (Arseniuk et al., 1997; Ueng et al., 1998.). The karyotype of *S. nodorum* suggests 14-19 chromosomes having approximately 0.5-3.5 megabase pairs (Cooley and Caten, 1991). McDonald and Martinez (1991) determined 14-16 bands believed to correspond to chromosomes of 0.33-3.5 megabase pairs in *S. tritici.*

Regardless of the fact that morphologic and genetic differences of considerable magnitude exist between *S. nodorum* and *S. tritici*, histological studies have shown that the

**Table 2. The *Septoria tritici/Stagonospora nodorum* pathogens of wheat.**

| Asexual state | Pycnidium (μm) | Pycnidiospore (μm) | Number of septa | Chromosome number | Lesion |
|---|---|---|---|---|---|
| *Septoria tritici* | 60-200 | 35-98 x 1-3 | 3-5 | 14-19[a] | Irregular to rectangular, elongated between veins |
| *Stagonospora nodorum* | 160-210 | 15-32 x 2-4 | 0-3 | 14-16[b] | Lens shaped, with chlorotic border |
| **Sexual state** | **Pseudothecium (μm)** | **Ascospore (μm)** | **Number of cells** | | |
| *Mycosphaerella graminicola* | 70-100 | 10-15 x 2-3 | 2 | | |
| *Phaeosphaeria nodorum* | 120-200 | 23-32 x 4-6 | 4 | | |

[a] McDonald and Martinez (1991).
[b] Cooley and Caten (1991).

infection process and the production of pycnidia in the host leaf bear some remarkable similarities (Karjalainen and Lounatmaa, 1986; Kema et al., 1996b; Straley, 1979). Usually all observed conidia of *S. tritici* on leaf surfaces of either resistant or susceptible cultivars germinated and produced germ tubes. The same can be said in the case of *S. nodorum,* but in one case (*T. aestivum* Manitou, CI 13775 resistant spring wheat), significant differences were found in germination of conidia on leaves of susceptible and resistant cultivars (Straley, 1979). Hyphae of both species grow extensively over leaf surfaces, branching and often crossing stomata and guard cells without entering them.

According to Kema et al. (1996b), penetrations of *S. tritici* were strictly stomatal and no direct penetrations of the cuticle were observed. Appressorial structures were seen in both pathogens, not associated with particular anatomical features, but most often in epidermal cracks. In the case of *S. nodorum*, direct epidermal cell penetrations under appressorial structures could not be observed with the light microscope, but were confirmed with the scanning electron microscope (SEM). Stomatal penetration by *S. nodorum* was readily observed 72 h after inoculation. Stomata were penetrated in either the open or closed condition, with or without appressoria (Harrower, 1978; Straley, 1979).

Reported patterns of occurrence of *S. nodorum* and *S. tritici* have changed dramatically during the past 15 years. In the early 1980s, *S. nodorum* was considered most important in northern Europe, eastern USA and Western Australia, while *S. tritici* was most prevalent in Mediterranean climates and the Great Plains of North America. Shifts to a greater importance of *S. tritici* have occurred in the UK and are continuing to progress in central Europe. Some of the contributing factors in the UK may have been host susceptibility, earlier sowing, increased N fertilization and high summer rainfall as well as resistance to certain fungicides (Bayles, 1991).

An interesting situation that could benefit from a systematic region-wide study has been reported from the Maghreb in North Africa. Populations of *S. tritici* particularly adapted to *durum* wheats have evolved, along with populations of *S. nodorum* more adapted to *aestivum* wheats. This phenomonon has become particularly obvious in Morocco, where a decided increase in plantings of bread wheats has occurred in recent years (Jlibene et al., 1995; Saadoui, 1987).

Where sexual reproduction plays a role in epidemics, new virulence combinations can be selected by host virulence genes, and recombination of virulence genes will overcome "pyramids" of resistance genes (McDonald, 1997). Ahmed et al. (1996) concluded that *S. tritici* populations adapt to host cultivars and that susceptible cultivars tend to select higher levels of pathogen aggressiveness in the field. Although evidence has been accumulated over a long period for specific gene-for-gene relationships in the *S. tritici* – wheat system (Eyal et al., 1985; Kema et al., 1996a), the question remains whether distinctive races can be identified with specific virulence for particular cultivars and whether the interaction indicates a gene-for-gene system in the *S. tritici* — and *S. nodorum* – wheat pathosystem (Johnson, 1992).

The bulk of evidence continues to indicate that in *S. nodorum* — and *S. tritici* – wheat systems, gene-for-gene interactions probably occur when selected cultures are inoculated on specific host plants under controlled conditions, but when population confronts population in the field, resistance to infection in wheat is generally non-specific and aggressiveness is the principal attribute of pathogen populations.

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# Molecular Analysis of a DNA Fingerprint Probe from *Mycosphaerella graminicola*

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## Abstract

*Clones hybridizing to the* Mycosphaerella graminicola *DNA fingerprint probe pSTL70 were identified from subgenomic libraries and sequenced. Analyses of the DNA sequences of these clones plus the original pSTL70 clone revealed that pSTL70 contains part of the open reading frame for a probable homologue of an osmosensing histidine kinase gene from yeast. The remaining portion of the clone contained a partial reverse transcriptase gene sequence and a 29 base pair direct repeat, which could mean that the clone is a transposable element. Methods for converting transposable elements into improved DNA fingerprinting techniques are discussed.*

DNA fingerprinting is a powerful tool for analyzing the genetic structure of fungal populations. Several fingerprinting strategies have been employed, including those based on the polymerase chain reaction (PCR) such as random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP) and DNA amplification fingerprinting (DAF). These techniques can provide information on hundreds of potential genetic loci in a very short time. However, each method has problems that can limit its utility. The RAPD technique relies on annealing of short (only 10 base) primers at low temperatures. This leads to high variability and low transportability to other labs. AFLPs require a reasonably high degree of sophistication in expertise and facilities, and also can suffer from problems with repeatability. DAF is operationally simple but the large number of bands produced can be difficult to separate and interpret.

The most widely used DNA fingerprint technique is restriction fragment length polymorphism analysis using small pieces of repetitive genomic DNA as probes. This technique has been used extensively to analyze the population biology of the ascomycetes *Magnaporthe grisea* (Hamer et al., 1989) and *Mycosphaerella graminicola* (McDonald and Martinez, 1991), and the oomycete *Phytophthora infestans* (Goodwin et al., 1992). Thousands of isolates of each species have been analyzed. The *M. grisea* repeat (MGR) 586 probe contains part of an inverted repeat transposon (Farman et al., 1996). However, the nature of the repeating elements in the *M. graminicola* pSTL70 and *P. infestans* RG57 probes has not been determined.

This study was initiated to test whether the *M. graminicola* pSTL70 probe was part of a transposable element. The long-term goal is to clone individual DNA fingerprint loci and convert them to a PCR-based system by designing specific primers to unique regions at each genetic locus.

## Materials and Methods

Subgenomic libraries were constructed from isolates IPO 323 and 94269. These are the parent isolates of the *M. graminicola* mapping population (Kema et al., 1996). Approximately 2 µg of genomic DNA from each isolate was digested to completion with the restriction enzyme *Pst* I. DNA fragments from 0.5-3 kb and from 3-9 kb for each isolate were excised from gels and purified using Wizard PCR Prep (Promega, Madison, WI). The DNA fragments were then ligated into pBluescript vector and transformed into competent cells of *E. coli* strain INValphaF′. White colonies were transferred into 200 µL LB+amp medium in 96-well Microtest tissue culture plates and grown at 37°C overnight. The 96 cultures from each plate were transferred onto large (150 x 15 mm) LB+amp agar

plates using a replica plater, and incubated upside down at 37°C overnight.

Colony lifts were made onto 8 x 12 cm pieces of Zeta Probe (BioRad) membranes by briefly laying the membrane pieces over the 96 colonies and lifting to pick up the bacteria. Membranes were placed colony side up onto blotting paper soaked with 10% SDS for 3 min, then 0.5 M NaOH/1.5 M NaCl for 5 min, 0.5 M Tris, pH 8.0/1.5 M NaCl for 5 min, and 6x SSC for 5 min. Finally, a UV Stratalinker (Stratagene) was used to crosslink the plasmid DNA to the membrane.

For Southern analysis, pSTL70 DNA was labeled using the Random Primer Fluorescein labeling kit with antifluorescein-HRP (DuPont NEN) and hybridized according to the manufacturer's instructions. Approximately 4,000 clones (2,000 from each isolate) were screened. Positive hybridizations were verified by digesting each positive clone with *Pst* I to release the insert, separating the fragments on agarose gels, blotting and probing as described above. Clones that hybridized after two rounds of screening were sequenced using a Pharmacia ALF automated DNA sequencer.

## Results

Among 4,000 clones screened, five hybridized strongly to pSTL70. Complete sequences have been obtained for the original pSTL70 clone and three others. Clones 2E11, 9A5 and 11E8 each had large regions of near identity with pSTL70 (Table 1). However, the three clones shared no similarity with each other. BLASTX searches of the putative translation products identified a number of GenBank accessions with similarity to clones pSTL70, 2E11 and 11E8 (Table 1). The original pSTL70 clone had high similarity to a two-component regulator gene from yeast, *Sln1*, and a similar gene from *Candida albicans*.

Clone 2E11 had even higher similarity to the same genes. This clone corresponded to and extended the 5′ end of pSTL70 (Figure 1). Clone 11E8 corresponded to and extended the 3′ end of pSTL70 (Figure 1). This sequence may code for a reverse transcriptase. The final 239 bases of pSTL70 contained part of the putative reverse transcriptase coding sequence, but not enough of the gene to obtain positive BLAST hits in GenBank.

The first 917 bases of 11E8 are not related to the sequence of pSTL70. Clone 9A5 had no similarity to any sequences in GenBank. The first 1103 bases of this clone were identical to pSTL70, but the final 1537 bases were unrelated (Figure 1). Clones pSTL70 and 9A5 both contained a 29 bp sequence that was tandemly repeated approximately 3.5 times (Figure 1). Southern analysis of genomic DNA from the parents and several progeny isolates of the *M. graminicola* mapping population revealed that 9A5 also gave a DNA fingerprint pattern, while 2E11 and 16D7 did not (data not shown).

## Discussion

The *M. graminicola* fingerprint probe pSTL70 (McDonald and Martinez, 1991) contains part of the open reading frame for the yeast two-component regulator gene *Sln1*. This gene has been shown to function as an osmosensing

**Table 1. Analysis of the *Mycosphaerella graminicola* DNA fingerprint clone pSTL70 and three additional clones that hybridized to pSTL70 in Southern analysis.**

| Clone | Size (bp) | Overlap with pSTL70 | Blastx results | E value | Comments |
|---|---|---|---|---|---|
| pSTL70 | 2860 | N/A | *Sln1* | 8e-14 | 29 bp repeat |
| 2E11 | 3073 | 1278 | *Sln1* | 2e-15 | Gene sequence |
| 9A5 | 2640 | 1103 | No hits | N/A | 29 bp repeat |
| 11E8 | 1636 | 417 bp | Reverse transcriptase | 0.006 | Transposable element (?) |

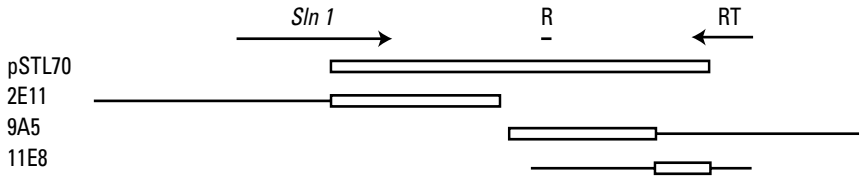


**Figure 1. Relationships among the *Mycosphaerella graminicola* DNA fingerprint probe pSTL70 and related clones. Regions of overlap are indicated by open boxes, unique regions by single lines. Clones are labeled on the left. Approximate locations of the *Sln1* and reverse transcriptase (RT) open reading frames are indicated by arrows. The tandem repeats are indicated by R.**

histidine kinase in yeast, although its function in *M. graminicola* remains unknown. Clone 2E11 includes an even larger portion of this gene containing the first 500 amino acids of the putative yeast homologue.

Other parts of the pSTL70 clone contain a 29 bp tandem repeat and a partial coding sequence for a reverse transcriptase. These indicate that pSTL70 may contain part of a transposable element. Hybridization analysis confirmed that this region of the clone was responsible for the DNA fingerprint pattern, which strengthens the transposable element hypothesis. The MGR586 probe of *M. grisea* also has been shown to contain a transposable element flanked by 16 bp tandem repeats and 9 bp inverted repeat sequences (Farman et al., 1996). If pSTL70 is a transposable element, it is truncated partway through the reverse-transcriptase sequence. Cloning and analysis of the remaining portion of the reverse-transcriptase gene will be necessary to test whether it is flanked by direct and/or inverted repeats.

It is surprising that the three additional clones identified using pSTL70 as a probe did not overlap with each other. Two of the clones, 9A5 and 11E8, contained unique regions that did not overlap with pSTL70. There are two likely explanations for this result. One is that these clones were from different loci in the *M. graminicola* genome that shared segments due to independent movements of the putative transposable element. The other possibility is that one or more of these clones is a chimera with multiple inserts. The chimera hypothesis is being tested by additional analyses of the cloned sequences.

If the DNA fingerprint pattern of pSTL70 is due to a transposable element, it may be possible to make specific PCR primers to amplify single DNA fingerprint loci. This could be accomplished using two different strategies. If the element is relatively small, primers could be made in the single-copy regions flanking the element. These should give a large amplification product when the element is present and a small one when it is not. Such differences could be resolved easily on agarose gels without the need for autoradiography, and should be easily transportable to other laboratories.

The other approach would be to design one primer within the transposable element and the other in the flanking region. This would give a plus/minus polymorphism at each locus: plus when the transposable element is present and minus when it is absent. The advantage of this approach is that the primers could be designed so that the sizes of the PCR products would not overlap. It then may be possible to use multiplex PCR to perform DNA fingerprinting of individual genetic loci from small quantities of starting genomic DNA in single PCR reactions. This would be much faster and simpler than Southern analysis and would avoid the repeatability, transportability, and interpretation problems of other PCR-based methods for fungal population genetic analyses.

## Acknowledgments

We thank Bruce McDonald for providing the original pSTL70 DNA fingerprint clone and for encouragement during the course of this project.

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# Characterization of *Septoria tritici* Variants and PCR Assay for Detecting *Stagonospora nodorum* and *Septoria tritici* in Wheat

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## Abstract

*Pathogenic specialization of* Septoria tritici *was studied by inoculating 14 isolates of the fungus, of which seven were obtained from durum wheat and seven were isolated from bread wheat. Isolates obtained from durum wheat were more virulent on durum wheat, while those isolated from bread wheat were more severe on bread wheat, which revealed physiologic specialization of* S. tritici *to either bread or durum wheat. The sequence coding for the nuclear 5.8S rDNA and the internal transcribed spacer (ITS1 and ITS2) were amplified by polymerase chain reaction and sequenced for five isolates adapted to bread wheat and five isolates adapted to durum wheat. These sequences were identical between both variants, resulting in the absence of divergence inside tritici species.* Septoria tritici *and* Stagonospora nodorum *isolates collected from Tunisia were tested for amplification with specific primers to these pathogens. This revealed the primer's efficiency to distinguish* Septoria *species and detect* Stagonospora nodorum *DNA with as little as 2 pg of DNA. Time course quantification of* S. tritici *mycelia after inoculation of resistant and susceptible cultivars distinguished those cultivars by the earliest date of apparition of a PCR product.*

Extensive genetic variation for virulence in *Septoria tritici* or its telomorph *Mycosphaerella graminicola,* characterized by differential interaction between host and pathogen genotypes, suggested the involvement of specific factors for virulence and resistance in the pathosystem (Kema et al., 1996a). Specific interaction between *M. graminicola* and wheat was well demonstrated by statistical evidence (Kema et al., 1996b), which suggested gene-for-gene interaction between resistance and virulence in host and pathogen, respectively. *Mycosphaerella graminicola* specialization is much more pronounced on bread wheat and durum wheat than differential specificity on a particular cultivar. In particular when considering the presence of pycnidia as a disease parameter, bread wheat and durum wheat isolates were particularly virulent on bread and durum wheat, respectively. Therefore bread wheat and durum wheat variants in *M. graminicola* were considered. The identical sequence of the internal transcribed spacer ribosomal DNA (ITS rDNA) observed by Kema et al. (1996a) showed that both variants could not be distinguished using their ribosomal DNA and that both were therefore from a similar taxonomic rank.

PCR has been shown to be a powerful technique to detect small amounts of fungal plant pathogens. The technique is now commonly used by field pathologists and growers for diagnosing several plant pathogens. The early detection of plant disease allows the judicious use of agricultural fungicides; early treatment is often more effective because the pathogen is less well established in its host. PCR amplification using specific primers for *S. tritici* and *Stagonospora nodorum* or *Septoria nodorum* was able to distinguish these pathogens and detect a few fungal cells in infested wheat tissues well before disease symptoms were apparent (Beck and Ligon, 1995). Since both pathogens may infect the same plant and visually distinguishing them is difficult, the specific detection of *S. tritici* and *S. nodorum* is well circumvented using the PCR technique and facilitates the decision of whether or not to treat with fungicides and which fungicides to use.

According to the previous observations, this paper describes physiologic specialization of bread and durum wheat isolates collected from Morocco and Tunisia, respectively, and the sequence of their ITS rDNA. Specific primers determined by Beck and Ligon (1995) for PCR detection of *S. tritici* and *S. nodorum* were used to differentiate those pathogens from other fungal pathogens of wheat and to detect their presence in asymptomatic wheat plants. Time course PCR quantification of *S. tritici* in infected wheat was assessed to analyze the evolution of mycelia in resistant and susceptible cultivars.

## Materials and Methods

### Plant material and experimental design

Thirty-six durum wheat and eight bread wheat cultivars (Table 1) were employed to study genetic variation for virulence. Seeds of the cultivars were sown in trays with 228 alveolus with 3 cm x 3 cm surface and 5 cm height. Each alveolus contained four seeds. The experiment was conducted in an arbitrary complete block design with three replicates for each isolate. Replicates were blocked in the same tray, separated by two rows of alveolus.

### Experimental procedure

Seven isolates of *S. tritici* taken from durum wheat and seven isolates from bread wheat collected in different regions of Tunisia and Morocco, respectively, were used to inoculate durum and bread wheat cultivars (Table 2). Eleven-day-old seedlings with emerging second leaves were inoculated with a monosporal suspension till run-off. The inoculum was prepared from monosporal culture cultivated on potato dextrose agar (PDA) medium. The spores were scraped from the agar, re-suspended in distilled water, filtered, and adjusted to $10^6$-$10^7$ spores/ml. After inoculation the trays were incubated in a humid chamber for 72 hours and returned to a growth chamber at temperature 20-25°C during the day, 17°C during the night, and 12 hours photoperiod.

**Table 1. List of durum and bread wheat cultivars used to study genetic variation for virulence in *Mycosphaerella graminicola*.**

| Code | Cultivars | Code | Cultivars |
|---|---|---|---|
| 01 | Florence aurore* | 23 | Medea AC3 |
| 02 | Florence Aurore x PUSA* | 24 | Medea AC4 |
| 03 | Derbessi x Biskri | 25 | Medea AP1 |
| 04 | Guelma* | 26 | Medea AP2 |
| 05 | EAPC6326127* | 27 | Allorea* |
| 06 | Tunis9* | 28 | Richelle* |
| 07 | Tunis23* | 29 | Sebei glabre |
| 08 | Abdelkader | 30 | Sebei pubescent |
| 09 | Agili pubescent AC1 | 31 | Souri AC8 |
| 10 | Bidi AP4 | 32 | Souri AC9 |
| 11 | Bidi AP1 | 33 | Medea AC1 |
| 12 | Bidi AP3 | 34 | Medea AC2 |
| 13 | Bidi AP | 35 | Medea AP6 |
| 14 | Biskri glabre | 36 | Medea AP10 |
| 15 | Biskri glabre AP3 | 37 | Medea RP1 |
| 16 | Derbessi AC1 | 38 | Biancullida ICM27 |
| 17 | Derbessi AP1 | 39 | Mahmoudi ICM28 |
| 18 | Derbessi AP2 | 40 | Mahmoudi ICM67 |
| 19 | Hamira AC2 | 41 | Khotifa x ICM75 |
| 20 | Hamira AC3 | 42 | ICM313 |
| 21 | Hamira AC4 | 43 | ICM314 |
| 22 | Hamira AC5 | 44 | Hamira |

* Bread wheat cultivars.

### Disease evaluation and statistical analysis

Disease severity was evaluated at 21 days after inoculation on the second leaf of the plant and using the presence of pycnidia as the disease parameter (Kema et al., 1996a). The collected data were treated with SAS software (1993 version). The presence of pycnidia was subjected to analysis of variance. The tables of means of the 14 isolates were subjected to hierarchical clustering using the statistical analysis software CSTAT.

### DNA extraction from fungal spores and infected plants

Spores of *S. tritici* and *S. nodorum* cultivated on PDA medium were subcultured in 100 ml flasks of yeast extract and glucose liquid medium. Five-day spore cultures were centrifuged and DNA extraction of fungal spores was performed using CTAB extraction buffer according to the protocol described by Morjane et al. (1995). DNA extraction from three infected leaves was performed according to the protocol described by Möller et al. (1992). DNA pellet after extraction was re-suspended in 50 µl Tris-HCl; 10 mM EDTA; 1 mM (TE) buffer.

### PCR amplification and sequencing

The sequence of ITS rDNA concerned 5 isolates (MAR 1, 2, 3, 4, 5) collected from Morocco (Table 2) and 5 isolates (TUN 1, 2, 3, 4, 5) collected from Tunisia (Table 2). ITS amplification was performed with ITS1 (5′-TCCGTAGGTGAACCTGCGG-3′) and ITS 4 (5′-TCCTCCGCTTATTGATATGC-3′) universal primers. PCR reactions

**Table 2. Experimental code and origin of *Mycosphaerella graminicola* collected from Morocco and Tunisia to study genetic variation for virulence.**

| Isolates collected from Tunisia | | | Isolates collected from Morocco | | |
|---|---|---|---|---|---|
| | Origin | | | Origin | |
| Code | Location | Wheat species | Code | Location | Wheat species |
| TUN1 | Beja | DW | MAR1 | Jemaa Sham | BT |
| TUN2 | Mateur | DW | MAR2 | Douyet | BT |
| TUN3 | Mornag | DW | MAR3 | Agadir | BT |
| TUN4 | El Agba | DW | MAR4 | Ain Orma | BT |
| TUN5 | Zaghouane | DW | MAR5 | Azrou | BT |
| TUN6 | Siliana | DW | MAR6 | Essaouira | BT |
| TUN7 | Mjez El Bab | DW | MAR7 | Tetouan | BT |

were performed in a volume of 50 µl containing 50 mM KCl, 10 mM Tris-HCl, pH8.3, 1.5 mM MgCl2, 200 µM of dNTP, 50 pmole of primer, 2.5 units of *Taq* polymerase (Boerhringer-Manheim) and 25 ng of genomic DNA. Reactions were run in a Thermolyne, Temptronic model thermal cycler for 30 cycles, each consisting of 15s at 94°C, 15 s at 50°C, and 45 s at 72°C. An additional 5 min polymerization step at 72°C ended the amplification. The products were analyzed by electrophoresis of 20 µl aliquot of each PCR sample on 0.8% agarose gel.

Specific DNA amplifications were performed as determined by Beck and Ligon (1995). ITS1 and JB446 (5′-CGAGGCTGGAGTGGTGT-3′) primers were used for specific amplification of *S. tritici* DNA and JB433 (5′-ACACTCAGTAGTTTACTACT-3′) and JB434 (5′-TGTGCTGCGTTCAATA-3′) were used to amplify *S. nodorum* DNA. PCR was performed as described above, except the annealing temperature was 57°C.

ITS sequencing was performed using 90 ng of 500 bp amplified product with ITS1 and ITS4. The sequence was determined by the dideoxynucleotide chain termination method using an automated sequencer (Perkin Elmer) at the Darwin Building of University College London (London, United Kingdom).

### Fungal DNA amplification in resistant and susceptible cultivars

To evaluate the competition of plant DNA with fungal DNA using ITS1 and ITS4 primers, amplification was performed using 1 µl and 5 µl of plant DNA extract mixed with several amounts ( 0.001, 0.01; 0.1; 1; 10; 100 and 1000 ng) of fungal DNA. The PCR conditions were the same as described before with a 57°C annealing temperature.

The time course amplification using ITS1 and ITS4 primers was realized using 2 µl of DNA extract from one infected leaf at 3, 6, 9, 14, 18, and 22 days after inoculation. The PCR conditions were the same as described before, with a 57°C annealing temperature.

## Results and Discussion

Bread wheat derived isolates almost exclusively produced pycnidia in the bread cultivars, whereas pycnidial production by durum wheat derived isolates was almost entirely restricted to durum wheat isolates. The analysis of variance (Table 3) shows highly significant differences ($p<0.001$) between varieties and isolates, which suggest the existence of different factors of resistance and virulence. The interaction between isolates and cultivars was highly significant ($p<0.001$) suggesting specificity of interaction between *M. graminicola* and its host. The specificity of the interaction is not limited to the observation that bread wheat and durum wheat isolates almost exclusively produce pycnidia in their respective host. Specificity is observed within durum wheat cultivars for durum wheat derived isolates. For example, TUN5 caused pycnidial coverage of 15% and 60% of the leaf area in cultivar Bidi AP4 and Bidi AP3, respectively (data not shown).

The aggressivity of bread wheat derived isolates on bread wheat cultivars was weaker than the aggressivity of durum wheat derived isolates on durum wheat

**Table 3. Analysis of variance of the pycnidia (P) disease parameter caused by 14 isolates of *Septoria tritici* on 44 wheat cultivars.**

| Source of variation | DDL | MS | F | Probability |
|---|---|---|---|---|
| Block | 2 | 241.33 | 2.39 | 0.0925 |
| Isolates | 13 | 42440.68 | 419.50 | 0.0001*** |
| Cultivars | 43 | 2757.42 | 27.26 | 0.0001*** |
| Isolates x cultivars | 556 | 570.82 | 5.64 | 0.0001*** |
| Error | 1230 | 101.17 | | |

*** Difference highly significant at p=0.001, DDL: degrees of freedom, F: F test.

cultivars. The presence of pycnidia of bread wheat derived isolates does not exceed 15% of the leaf surface (data not shown). An explanation of the observed weak virulence would be long time conservation of these isolates in glycerol. Aggressivity of these isolates would be recovered after their direct isolation from infected leaves and inoculation from fresh spore culture.

Cluster analysis of the 14 isolates according to the presence of pycnidia resulted in nine different clusters. Bread wheat derived isolates are clearly separated from durum wheat derived isolates (Figure 1). Durum wheat isolates are clustered into six groups. Two isolates (TUN7 and TUN6) originated from distant regions (Mjez El Bab and Siliana, respectively) belong to the same group, whereas isolates from the same regions belong to different groups, indicating genetic variation for virulence within local populations.

## ITS sequence analysis

Physiological specialization of *S. tritici* to bread wheat and durum wheat led to the hypothesis of the existence of two subspecies inside tritici. For that purpose sequencing ITS rDNA would provide evidence about the taxonomy of those variants. The comparison of the consensus sequence of the 550 pb ITS region fragments obtained from five bread wheat derived isolates and five durum wheat derived isolates shows 100% homology (Figure 2). Therefore *S. tritici* variants belong to the same taxonomic rank and cannot be considered as two different subspecies of tritici. Since both variants coexist in the same field (McDonald, personal communication), permanent

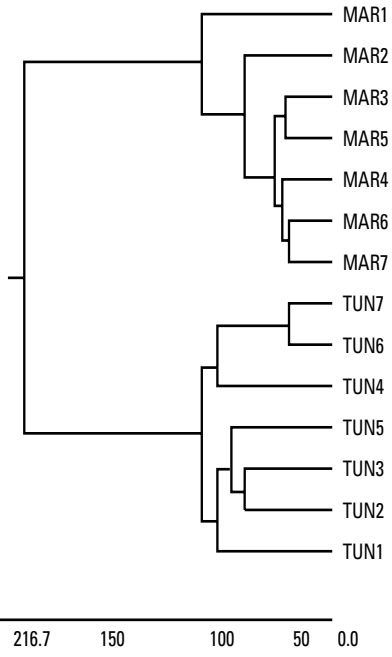


**Figure 1. Hierarchical classification of 14 *Septoria tritici* isolates as determined by CSTAT software.**

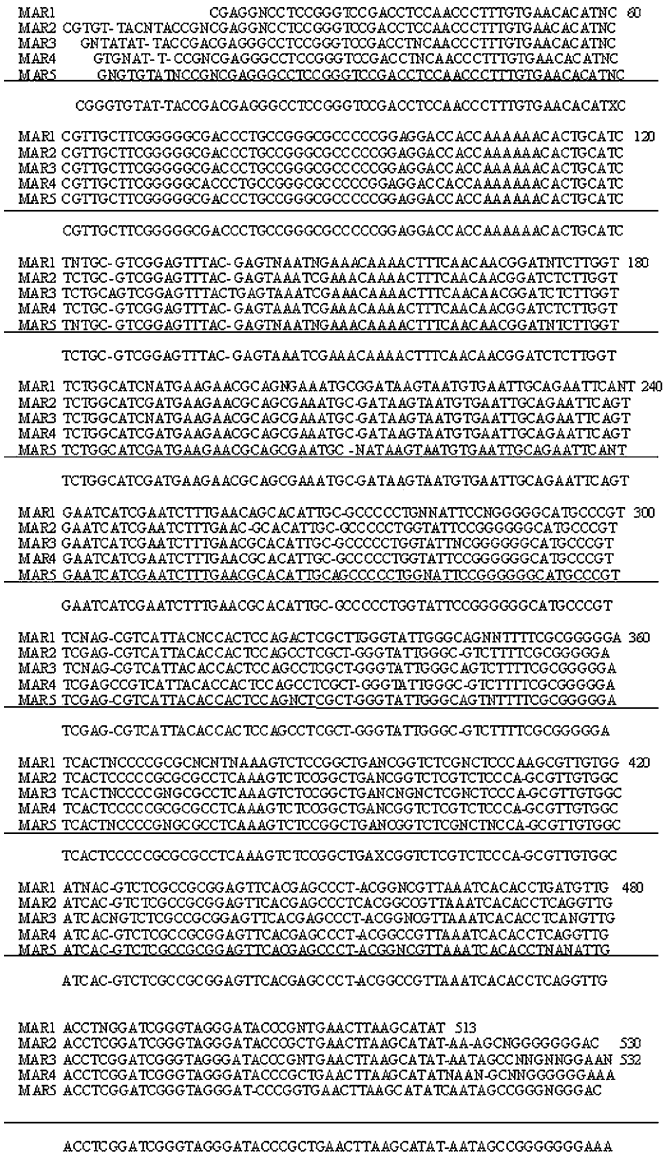


**Figure 2. Nucleotide sequence of the internal transcribed spacer region derived from bread wheat (MAR) and durum wheat (TUN) derived isolates of *Septoria tritici*. The sequence includes the 5.8S rDNA gene and the internal transcribed spacer (ITS1 and ITS2). The consensus sequences were deduced from bread wheat and durum wheat derived isolates each.**

**Figure 2. cont.**

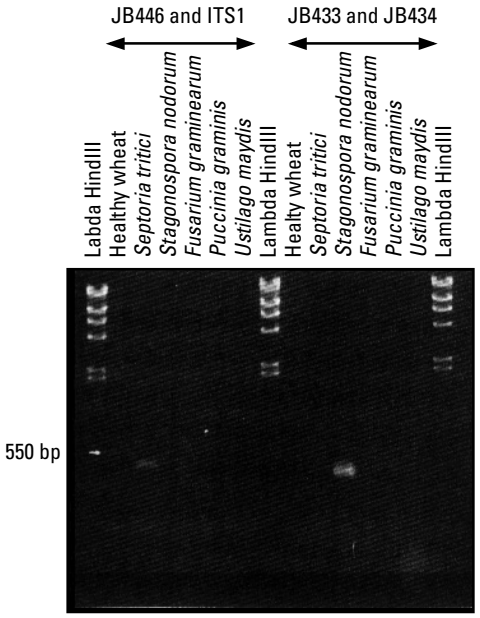


**Figure 3. Ethidium bromide stained agarose gel of polymerase chain reaction amplification product using *Septoria tritici* specific primers (JB446 and ITS1) and *Stagonospora nodorum* specific primers (JB433 and JB434) with healthy wheat DNA and fungal DNA of wheat pathogen.**

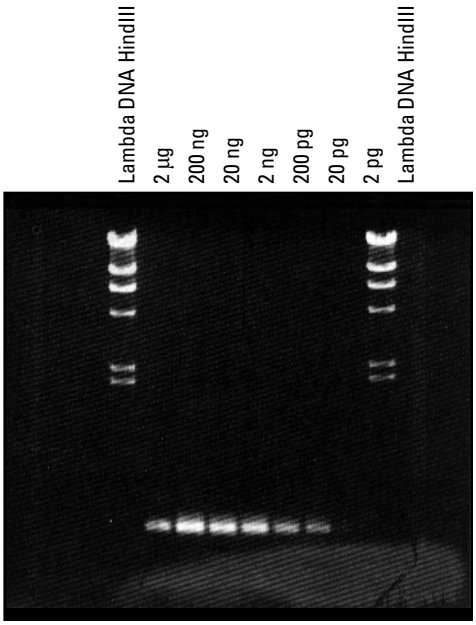


**Figure 4. Ethidium bromide stained agarose gel of polymerase chain reaction products from amplification of 0.2 pg to 2 µg of genomic DNA of *Stagonospora nodorum* DNA using *S. nodorum* specific primers JB433 and JB434.**

conversion of one variant to the other by genetic exchange of virulence genes may occur through sexual reproduction.

## Diagnosis of *S. tritici* and *S. nodorum*

In order to investigate the specificity of *S. tritici* and *S. nodorum* specific primers determined by Beck and Ligon (1995), we examined whether they produce PCR products from other fungal pathogens of wheat, such as *Fusarium graminearum*, *Ustilago maydis,* and *Puccinia graminis*. The *S. tritici* specific primers JB446 and ITS1, and *S. nodorum* specific primers JB433 and JB434 did produce PCR products only from those pathogens (Figure 3) revealing that these primers can be used to diagnose *S. tritici* and *S. nodorum* isolates collected from Tunisia.

Amounts ranging from 2 µg to 2 pg of *S. nodorum* were tested by PCR amplification with their respective specific primers (JB433, JB434) to determine the DNA threshold that can be detected. PCR amplification with JB433 and JB434 was able to detect 2 pg of *S. nodorum* DNA (Figure 4), which is almost the same amount detected by Beck and Ligon (1995).

Provided that *S. nodorum* fungal genomes do not exceed 100 Mbp in those conditions, PCR is able to detect as little as 20 fungal cells.

### Fungal DNA amplification in resistant and susceptible cultivars

Different fragment sizes 600 bp and 550 bp amplification products were obtained from plant and fungal DNA, respectively, using ITS1 and ITS4 primers (Figure 5). PCR amplification with ITS primers and using a mixture of both fungal and plant DNA templates resulted in the amplification of both fragments. However, the intensity of the fungal PCR product is relative to the amount of fungal DNA (Figure 5). These result suggest that plant DNA could be used as competitor of fungal DNA amplification with ITS primers. Time course PCR amplification using ITS1 and ITS4 primers at 0, 3, 6, 9, 14, 18, and 22 days after inoculation of susceptible (Karim) and resistant (Jennah Khotifa) cultivars with *S. tritici* is shown in Figure 6. ITS rDNA amplification products from fungal DNA (550 bp) appeared at 3 and 18 days from infected susceptible and resistant cultivars, respectively.

This result showed that the multiplication of *S. tritici* inside the plant is delayed in the resistant cultivar. Therefore, the time interval corresponding to the beginning of PCR product apparition may be an indicator of resistance. Furthermore, the intensity of the PCR product increased with the days post-inoculation (Figure 6), indicating a quantitative response of the PCR

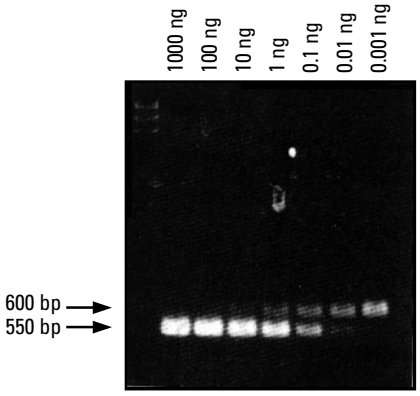


**Figure 5. Ethidium bromide stained agarose gel of polymerase chain reaction products using ITS1 and ITS4 primers with 0.0001 to 1000 ng fungal DNA of *Septoria tritici* and 1 µl plant DNA extract from healthy wheat leaves. 550 bp and 600 bp are ITS rDNA amplification products from *Septoria tritici* and wheat, respectively.**

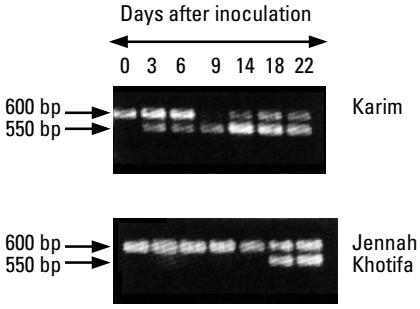


**Figure 6. Ethidium bromide stained agarose gel of time course PCR amplification products using ITS1 and ITS4 primers with *Septoria tritici* infected wheat (susceptible; Karim and resistant; Jennah Khotifa) DNA. 550 bp and 600 bp are ITS rDNA amplification products from *S. tritici* and wheat, respectively.**

assay. These observations are preliminary results towards a quantification of fungal proliferation within tissue to rapidly characterize varying levels of host resistance. Characterization of host resistance by measuring the fungal biomass inside wheat leaves was tested using ELISA (Kema et al., 1996c). This technique did not detect the fungal antigen within the 48 h-8 dpi interval, although a slight increase of the fungal tissue was observed microscopically during that interval. Thus the ability of PCR to detect fungal mycelia in a susceptible cultivar at 3 dpi better reflects the colonization of wheat cells by the fungus. These preliminary results will also help establish an efficient protocol for fungicide utilization. Effective utilization of fungicides would decrease or maintain the intensity of the PCR product a long time after its application.

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# Populations of *Septoria* spp. Affecting Winter Wheat in the Forest-Steppe Zone of the Ukraine

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## Abstract

*Data in the literature and results of our investigations indicate that septoria leaf blotch of winter wheat has been reported annually in the forest-steppe zone of the Ukraine.* Septoria tritici *is the predominant species among the causal agents of septoria leaf blotch of winter wheat. However, the portion caused by* Stagonospora nodorum *increased to 23-44% in recent years.*

*Septoria tritici,* the pathogen that causes leaf blotch, and *Stagonospora nodorum*, the pathogen that induces spike and leaf blotch, are the most widespread pathogens of winter wheat in the forest-steppe zone of the Ukraine.

*Septoria* spp. induce a decrease in assimilation surface, developmental retardation, premature leaf desiccation, and 1000-grain weight. In epidemic years, yield losses may reach 30–50%. Total yield losses caused by these pathogens all over the world are estimated at 9 million tons.

Developing cultivars resistant to the pathogens and establishing their cultivation is impossible without investigating the composition of pathogenic species in a given area and systematically recording its changes. Climatic conditions, the composition of biocenoses, and the substrate where pathogens develop significantly affect the ratio between species. In the literature data on the areas occupied by the pathogens are quite limited, but can be found in articles published by Kovalenko (1975), Vasetskaja et al. (1983), Dyak (1990), and Sanina and Antsiferova (1991).

In 1995–97, we investigated the composition of *Septoria* pathogens in the forest-steppe zone of the Ukraine (Kyiv, Cherkasy, Vinnytsya, Khmelnytskyy, Ternopil, Zhytomyr, and Poltava regions). Studies were conducted on promising and cultivated cultivars of winter wheat using routine methodologies. The species were identified via evaluation of stable traits: form, length, and width of conidia and ends.

Our research demonstrated that the forest-steppe zone of the Ukraine is occupied by both *Septoria* pathogens, but *S. tritici* predominated in the 1970s, while *S. nodorum* was found only in certain years (Kovalenko, 1975). In the 1980s *S. nodorum* was reported in Kyiv (30-36%) and Ternopil (13-23%) regions (Dyak, 1990). In Cherkasy region (6.6%) the pathogen was reported only in certain years and was not detected at all in Vinnytsya region.

In the mid 1990s, the proportion of *S. nodorum* increased among the species. *Septoria tritici* dominated in all investigated regions: the highest percentage was reported in Vinnytsya region (76%), while the lowest was reported in Ternopil region (53%). In Kyiv, Cherkasy, Poltava, Khmelnytskyy, and Zhytomyr regions, it reached 68, 65, 69, 72, and 72%, respectively (Figure 1). Data from the literature and our own research results indicate an increase in the area occupied by *S. nodorum,* as well as in its ratio of *Septoria* pathogens present.

Changes in the ratio of the pathogens may be explained by changes in climatic conditions, the range of cultivars used and, possibly, by the spread of *S. nodorum* infection through seeds, especially in recent years, when seeds were not properly treated. Thus the spread of *S. nodorum*–the most aggressive and damaging *Septoria* pathogen–increased under the above conditions.

* Author prevented from attending workshop by unforeseen travel problems.

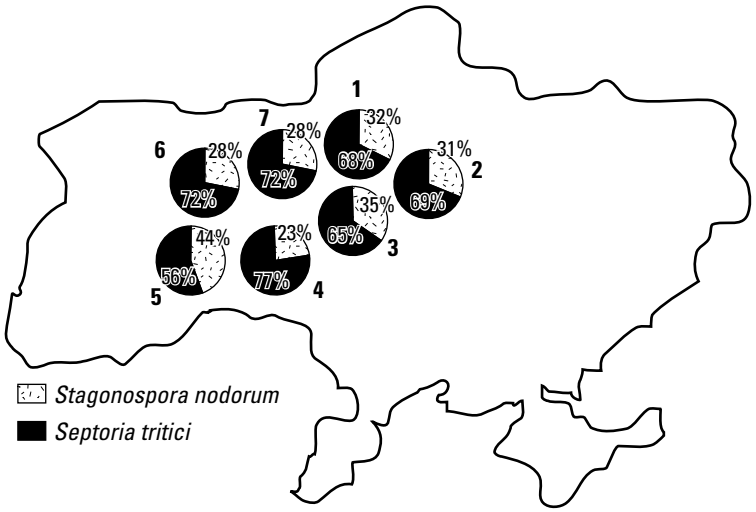


**Figure 1. The proportion between leaf septoriose pathogens of winter wheats in the forest-steppe zone of the Ukraine.**
**1-Kyiv, 2-Poltava, 3-Cherkasy, 4-Vinnytsya, 5-Ternopil, 6-Khmelnytskyy, 7-Zhytomyr regions.**

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# *Septoria passerinii* Closely Related to the Wheat Pathogen *Mycosphaerella graminicola*

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## Abstract

Septoria passerinii *is known only from its anamorphic* Septoria *state; no teleomorph has been identified. In culture,* S. passerinii *looks very similar to* Mycosphaerella graminicola *from wheat. Comparisons of the nucleotide sequences of the internal transcribed spacer (ITS) regions of the ribosomal DNA of both species and of many other fungi in the Dothideales revealed that the ITS regions of* S. passerinii *and* M. graminicola *differed by only 10 bases out of 571 total. Therefore, these species are very closely related. Phylogenetic analysis showed that* S. passerinii *and* M. graminicola *grouped together within a large cluster of* Mycosphaerella *species. Thus,* S. passerinii *almost certainly has a* Mycosphaerella *teleomorph.*

*Septoria passerinii* causes speckled leaf blotch on barley. The colony and conidial morphologies of *S. passerinii* and the wheat pathogen *Mycosphaerella graminicola* are very similar. Both are pathogens of cereals, and they may be closely related. However, nothing is known about the evolutionary relationships of *S. passerinii* (Cunfer and Ueng, 1999); no fruiting structures have been found and its teleomorph remains unknown.

Analyses of the internal transcribed spacer (ITS) region of ribosomal DNA have been very useful for elucidating the phylogenetic relationships of fungi. This region contains the highly variable ITS1 and ITS2, separated by the conserved 5.8S ribosomal RNA gene, and is bounded by the highly conserved 18 and 28S ribosomal RNA genes. This greatly facilitates analysis, because polymerase chain reaction (PCR) primers within the conserved regions of the 18 and 28S genes amplify the intervening ITS region of approximately 600 bp. Furthermore, many fungal ITS sequences are available in GenBank, which expands the number of species available for comparison.

The objective of this research was to assemble a database of ITS sequences to test the hypothesis that *S. passerinii* is a close relative of *M. graminicola*. The alternative hypothesis is that *S. passerinii* could be more closely related to other cereal pathogens, such as the septoria nodorum pathogen of wheat and barley, *Phaeosphaeria nodorum*.

## Materials and Methods

Isolates of species of *Mycosphaerella*, *Leptosphaeria,* and *Phaeosphaeria* were obtained from various sources (Table 1). DNA was extracted from lyophilized tissue and the ITS regions were amplified using primers ITS4 and ITS5. Amplification was with the following cycling parameters: 94 C for 2 min, 30 cycles of 93 C for 30 s, 53 C for 2 min, 72 C for 2 min, and a final extension of 10 min at 72 C. Amplification products were purified and cloned. Each clone was sequenced in both directions on an automated DNA sequencer and several clones were sequenced per isolate.

DNA sequence alignment, genetic distance calculation, bootstrap analysis (1000 replications), and a neighbor-joining tree was prepared using Clustal X (http://www-igbmc.u-strasbg.fr/BioInfo/ClustalX/Top.html). The final tree was printed using NJplot.

## Results

The ITS regions of all *S. passerinii* isolates were 571 bases long, including the primer regions, and were essentially identical. A BLAST search on GenBank identified *M. graminicola* as the closest match to *S. passerinii*. Sequences of other species showing good matches with *S. passerinii* in the BLAST search were downloaded from GenBank, along with those of species of *Leptosphaeria*, *Phaeosphaeria,* and other fungi in the Dothideales (Table 1). A multiple alignment revealed that the ITS sequences of *S. passerinii* and *M. graminicola* differed by only 10 bases (data not

**Table 1. Sources of isolates or DNA sequences for the internal transcribed spacer database of *Septoria passerinii, Mycosphaerella graminicola,* and other fungi in the Dothideales.**

| Species | Isolate | Growth medium | Source |
|---|---|---|---|
| *Cladosporium caryigenum* | LCF | — | GenBank |
| *Cladosporium herbarum* | MZKI B-1002 | — | GenBank |
| *Cladosporium sphaerospermum* | MZKI B-1005 | — | GenBank |
| *Dothidea hippophaeos* | CBS 186.58 | — | GenBank |
| *Dothidea insculpta* | CBS 189.58 | — | GenBank |
| *Hormonema dematioides* | — | — | GenBank |
| *Leptosphaeria bicolor* | — | — | GenBank |
| *Leptosphaeria conteca* | — | Malt broth | ATCC |
| *Leptosphaeria doliolum* | — | — | Genbank |
| *Leptosphaeria maculans* | — | — | Genbank |
| *Leptosphaeria microscopica* | — | — | Genbank |
| *Mycosphaerella citri* | — | Yeast malt broth | Tobin Peever |
| *Mycosphaerella fijiensis* | — | PDB | ATCC |
| *Mycosphaerella graminicola* | — | — | GenBank |
| *Mycosphaerella graminicola* | IPO323 | Yeast malt broth | Gert Kema |
| *Mycosphaerella graminicola* | T1 | Yeast malt broth | Minnesota |
| *Mycosphaerella graminicola* | T48 | Yeast malt broth | Indiana |
| *Mycosphaerella musicola* | — | PDB | ATCC |
| *Mycosphaerella pini* | — | — | GenBank |
| *Mycosphaerella tassiana* | CBS 111.82 | — | GenBank |
| *Ophiosphaerella herpotricha* | — | — | GenBank |
| *Ophiosphaerella korrae* | — | — | GenBank |
| *Phaeosphaeria avenaria* | — | — | GenBank |
| *Phaeosphaeria halima* | — | Emerson YpSs | ATCC |
| *Phaeosphaeria nodorum* | — | — | GenBank |
| *Phaeosphaeria spartinae* | — | Emerson YpSs | ATCC |
| *Phaeosphaeria typharum* | — | Emerson YpSs | ATCC |
| *Phaeotheca triangularis* | MZKI B-950 | — | GenBank |
| *Rhizopycnis vagum* | — | — | GenBank |
| *Septoria passerinii* | 22585 | Yeast malt broth | ATCC |
| *Septoria passerinii* | 26516 | Yeast malt broth | ATCC |
| *Septoria passerinii* | P70 | Yeast malt broth | North Dakota |
| *Septoria passerinii* | P71 | Yeast malt broth | North Dakota |
| *Septoria passerinii* | P78 | Yeast malt broth | Minnesota |
| *Septoria passerinii* | P83 | Yeast malt broth | North Dakota |
| *Trimmatostroma salinum* | MZKI B-962 | — | GenBank |

shown). The neighbor-joining tree showed that *S. passerinii* and *M. graminicola* clustered together along with most of the other *Mycosphaerella* species (Figure 1). *Phaeosphaeria nodorum* was in a very different cluster that mostly contained species of *Phaeosphaeria*, *Leptosphaeria,* and *Ophiosphaerella* (Figure 1). Bootstrap analysis indicated strong support for all of the major clusters (Figure 1).

## Discussion

These data clearly indicate that *S. passerinii* and *M. graminicola* are very closely related. Because these two taxa were deeply embedded within a large cluster of *Mycosphaerella* species, it seems certain that *S. passerinii* has, or was derived from a progenitor that had, a *Mycosphaerella* teleomorph. This must be confirmed by searching for the *Mycosphaerella* stage on infected barley tissue.

In addition to elucidating the evolutionary relationships of *S. passerinii*, the cluster analysis raises a number of interesting questions. For example, *Phaeosphaeria*, *Leptosphaeria,* and *Ophiosphaerella* form a large cluster. However, it is not clear whether *Phaeosphaeria* warrants a separate generic designation. Clarifying the relationships among the mitosporic Dothideales (those with no known sexual stage) will require a larger number of species and a more comprehensive analysis. Aligning the 5.8S and ITS2 regions was fairly straightforward. However, ITS1 varied greatly in length, which made alignment difficult. The alignment used for the cluster analysis presented here has not been optimized and, thus, should be considered preliminary. However, the close relationship between *S. passerinii* and *M. graminicola* is certain.

## Reference

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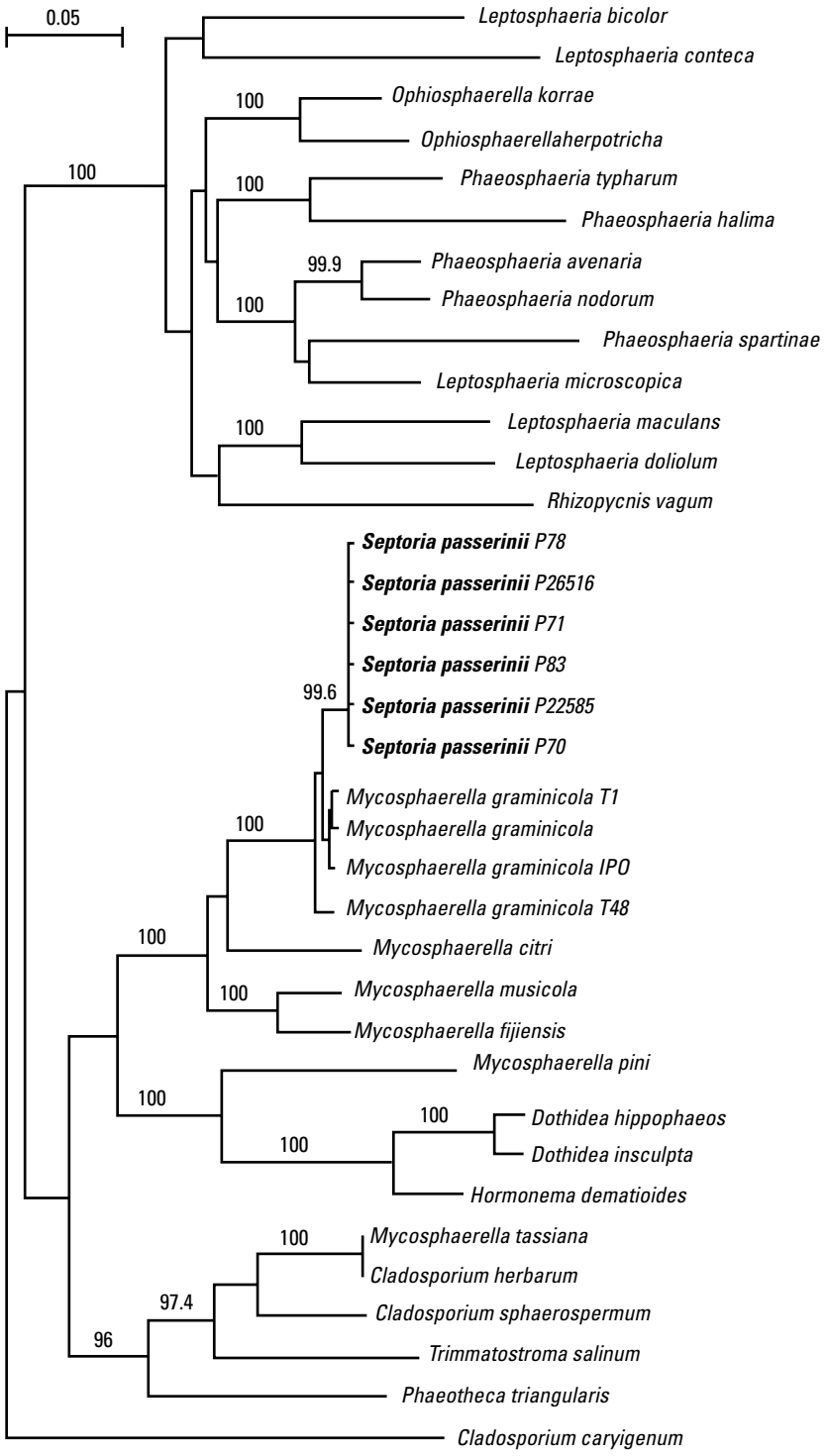


**Figure 1. Phylogenetic analysis of sequences of the internal transcribed spacer region of the ribosomal DNA of *Septoria passerinii, Mycosphaerella graminicola* and other fungi in the Dothideales. All bootstrap values above 90% are indicated.**

# *Septoria/Stagonospora* Leaf Spot Diseases on Barley in North Dakota, USA

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## Abstract

*Diseased barley leaves were collected from fields in North Dakota in 1998. The most common* Septoria/Stagonospora *diseases were septoria speckled leaf blotch (*Septoria passerinii*) and stagonospora avenae leaf blotch (*Stagonospora avenae *f. sp.* triticea*). Net blotch (*Drechslera teres*) and spot blotch (*Bipolaris sorokiniana*) were also commonly present. Stagonospora nodorum blotch (*Stagonospora nodorum*) and tan spot (*Drechslera tritici-repentis*), which are major diseases on wheat in the area, were detected on barley but at rather low levels. When isolates of* S. nodorum *from barley were tested on wheat and barley in greenhouse inoculations, higher symptom severity ratings were obtained on wheat compared to barley, indicating that the isolates obtained from barley were probably wheat-type isolates. Because of the importance of both* Septoria passerinii *and* Stagonospora avenae *f. sp.* triticea *on barley, selecting barley for* Septoria/Stagonospora *resistance will require screening with both organisms.*

Barley (*Hordeum vulgare* L.) can be affected by a number of plant diseases that can cause economic losses in yield and quality. The *Septoria/Stagonospora* diseases common on barley are septoria speckled leaf blotch (*Septoria passerinii* Sacc.), stagonospora avenae leaf blotch (*Stagonospora avenae* Bissett f. sp. *triticea* T. Johnson [syn. *Septoria avenae* A.B. Frank f. sp. *triticea* T. Johnson]), and stagonospora nodorum blotch (*Stagonospora nodorum* [Berk.] Castellani & E.G. Germano [syn. *Septoria nodorum* {Berk.} Berk. in Berk & Broome]) (Kiesling, 1985; Mathre, 1997). Barley-type isolates of *S. nodorum* have also been identified (Cunfer and Youmans, 1983; Smedegard-Peterson, 1974). A cooperative survey was undertaken to determine the importance of *Septoria/Stagonospora* leaf spot diseases common on barley in North Dakota.

## Materials and Methods

Diseased barley leaves (green leaves with leaf spots) were gathered from naturally-infected barley plants in the field in 1998. Leaves were collected from 70 fields located in the southwestern, central, northeastern, and eastern areas of North Dakota. Collected leaves were dried and stored dry at 4C in a refrigerator until processed. Leaf sections, 2 cm long, from approximately 8 leaves per collection, were processed. Leaf sections were surface-sterilized for 3 min in a 1% sodium hypochlorite solution containing a surfactant, rinsed in sterile distilled water, plated on 2% water agar in plastic Petri dishes, and incubated under a 12-h photoperiod (cool-white fluorescent tubes) at 21C. After 7 days, leaf sections were examined for fungi. Pycnidiospores from pycnidia on the leaf sections were identified microscopically. The presence of *Drechslera teres* (Sacc.) Shoemaker, *Bipolaris sorokiniana* (Sacc.) Shoemaker, and *D. tritici-repentis* (Died.) Shoemaker was also noted. Two to four fungal species were present on some leaf sections. Isolates were obtained, grown, stored, and inoculum was prepared as described by Krupinsky (1997).

Nine isolates of *S. nodorum* obtained from barley were compared in glasshouse inoculations of wheat and barley seedling plants. Two wheat cultivars, Eureka and Fortuna, and two barley cultivars, Bowman and Hector, were used. In a glasshouse, seedlings were planted, grown, fertilized, inoculated, incubated, and assessed for percentage necrosis of the first leaf as previously reported (Krupinsky 1997).

## Results and Discussion

Of the 531 leaf sections processed, 45% were infected with *S. passerinii*, 37% with *S. avenae* f. sp. *triticea*, and 14% with *S. nodorum. Drechslera teres, B. sorokiniana,* and *D. tritici-repentis*

were identified on 55, 51, and 10% of the leaf sections, respectively. Using the number of leaf sections infected with a particular fungus as an indicator of the relative importance of a fungus, the most common diseases making up the leaf-spot disease complex on barley in North Dakota were in descending order of importance net blotch, spot blotch, septoria speckled leaf blotch, and stagonospora avenae leaf blotch. This ranking was similar to those in Saskatchewan, Canada, north of western North Dakota, where *D. teres* was considered the most common leaf spotting pathogen, followed by *B. sorokiniana,* which was more common than *Septoria* spp. (Fernandez et al., 1999). In Manitoba, Canada, north of eastern North Dakota, leaf spot diseases were considered to cause minimal damage in 1998. *Drechslera teres* and *B. sorokiniana* were found in 90-93% of the barley fields and *S. passerinii* was recovered in 22% of the fields (Tekauz et al., 1999).

Two of these diseases, spot blotch and stagonospora avenae leaf blotch, are usually minor diseases on wheat in central North Dakota. Stagonospora nodorum blotch and tan spot, although major diseases on wheat in North Dakota, were only isolated at low levels from barley and are not considered to be serious problems on barley at the present time. Because of the importance of both *S. passerinii* and *S. avenae* f. sp. *triticea* on barley in North Dakota, selecting barley for *Septoria/Stagonospora* resistance will require screening with both pathogens.

The nine isolates of *S. nodorum* obtained from barley consistently produced higher symptom severity, as measured by percentage necrosis, on wheat than on barley. Overall, Fortuna averaged 28% necrosis and Eureka averaged 29% necrosis, compared to less than 1% for Bowman and Hector.

## Acknowledgments

The authors thank D. Wetch for technical assistance, as well as D.E. Mathre and M. Babadoost for their reviews and constructive comments.

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# Interrelations among *Septoria tritici* Isolates of Varying Virulence

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Reduction in pycnidial coverage on Seri 82 was found by Halperin et al. (1996) after inoculation with two *Septoria tritici* isolates: ISR398 (avirulent) and ISR8036 (virulent). Cross protection was suggested as a mechanism for the suppression phenomenon, triggered by the avirulent isolate. The aim of the current study was to investigate possible interactions between new isolates of *S. tritici* on Seri 82 and other wheat cultivars.

Co-inoculations with mixtures of isolates were conducted on seedlings and maturing plants of various cultivars. The following *S. tritici* isolates and wheat cultivars were used:

- ISR398 - avirulent on Seri 82, KK, Bobwhite "S" and IAS20; virulent on Shafir.
- ISR8036 - avirulent on KK, Bobwhite "S" and IAS20; virulent on Shafir and Seri 82.
- ISR9812 - avirulent on IAS20; virulent on Shafir, Seri 82, KK and Bobwhite "S".
- ISR9840 - avirulent on KK and Bobwhite "S"; virulent on Shafir, Seri 82 and IAS20.

Pairs of isolates were used for inoculation with 1:1 mixtures on each of the five cultivars. Pycnidial coverage by the virulent isolate of each pair was used as a reference. The results are shown in Table 1. Significant suppression of pycnidial coverage was observed when seedlings were inoculated with mixtures composed of a virulent and an avirulent isolate. However, no suppression was found when isolate ISR9840 was used as the virulent component. Since all isolates were virulent on Shafir, no suppression was found on this cultivar.

Suppression of pycnidial coverage was observed on maturing plants of Seri 82 in the field following co-inoculation at GS 37 with a 1:1 isolate mixture of ISR398+ISR8036. The other five isolate mixtures on all wheat cultivar combinations are currently being tested in field trials.

The identity of pycnidia developed on leaves of Seri 82 and Shafir, co-inoculated with ISR398+ISR8036, was verified using specific PCR primers. Isolate ISR8036 dominated (>70%) the pycnidial population on seedlings and from the onset of the epidemics in field plots of both Shafir and Seri 82. The low fraction of ISR398 on the susceptible cultivar Shafir is explained by competition. In the mixture ISR398+ISR8036, isolate ISR8036 has a competitive advantage over ISR398, attributed to its higher aggressiveness. The same experimental approach will be adapted to the other isolate mixtures under investigation, once specific PCR primers are obtained for ISR9812 and ISR9840.

The structure of natural populations of this pathogen is determined by both cultivar-isolate interactions and isolate-isolate interactions.

**Table 1. Reduction in pycnidial coverage on seedlings of the wheat cultivars Shafir (SHF), Seri 82 (SER), IAS 20, Bobwhite "S" (BOW) and Kavkaz/K4500 (KK), inoculated with 1:1 mixture of *Septoria tritici*. Data are percent reduction as compared to pycnidial coverage by the more virulent isolate in each case.**

| | Wheat cultivars | | | | |
|---|---|---|---|---|---|
| **Isolate mixture** | **SHF** | **SER** | **IAS20** | **BOW** | **KK** |
| ISR398 + ISR8036 | 52.2 | 98.7 | -[a] | - | - |
| ISR398 + ISR9812 | 38.3 | 78.0 | - | 99.5 | 99.7 |
| ISR398 + ISR9840 | 11.0 | 7.0 | 21.6 | 21.9 | - |
| ISR8036 + ISR9812 | 16.3 | 28.9 | - | - | 71.8 |
| ISR8036 + ISR9840 | 26.7 | 24.3 | 33.8 | 27.4 | - |
| ISR9812 + ISR9840 | 26.1 | 24.3 | 32.8 | 63.4 | 100.0 |

[a] Cultivar resistant to both isolates.

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## *Session 2: The Infection Process*

# *Stagonospora* and *Septoria* Pathogens of Cereals: The Infection Process

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## Abstract

*Definitive information on the infection process has been reported for* Stagonospora nodorum, Septoria tritici, *and* Septoria passerinii. *Like other necrotrophic pathogens, they do not elicit the hypersensitive reaction. A significant difference in the infection process between* Septoria *and* Stagonospora *pathogens is that spore germination and penetration proceed much faster for* S. nodorum *than for* S. tritici *and* S. passerinii. *The* Septoria *pathogens penetrate the leaf primarily through stomata, whereas* S. nodorum *penetrates both directly and through stomata.* Stagonospora nodorum *kills the epidermal cells quickly, but* S. tritici *and* S. passerinii *do not kill epidermal cells until hyphae have ramified through the leaf mesophyll and rapid necrosis begins. Resistance slows host colonization but has no appreciable effect on the process of lesion development. The mechanisms controlling host response are still unclear. The infection process for ascospores is probably very similar to that for pycnidiospores. Ascospores of* Phaeosphaeria nodorum *germinate over a wide range of temperatures and their germ tubes penetrate the leaf directly. However, unlike pycnidiospores, the ascospores do not germinate in free water.*

The infection process has been studied most intensely for *Stagonospora nodorum* and *Septoria tritici*. One in-depth study on *Septoria passerinii* is available. Nearly all of the information reported is for infection by pycnidiospores. However, the infection process for other spore forms is quite similar. The information presented is mostly for infection of leaves under optimum conditions. Some studies were done with intact seedling plants, whereas others were conducted with detached leaves. Infection of the wheat coleoptile and seedling by *S. nodorum* was described in detail by Baker (1971) and reviewed by Cunfer (1983). Although no precise comparisons have been made, it appears that the infection process has many similarities in each host-parasite system and is typical of many necrotrophic pathogens. Information on factors influencing symptom development and disease expression are excluded but have been reviewed by other authors (Eyal et al., 1987; King et al., 1983; Shipton et al., 1971). A summary of factors affecting spore longevity on the leaf surface is included.

## Role of the Cirrus and Spore Survival on the Leaf Surface

The most detailed information on the function of the cirrus encasing the pycnidiospores exuded from the pycnidium is for *S. nodorum*. The cirrus is a gel composed of proteinatous and saccharide compounds. Its composition and function are similar to that of other fungi in the Sphaeropsidales (Fournet, 1969; Fournet et al., 1970; Griffiths and Peverett, 1980).

The primary roles of cirrus components are protection of pycnidiospores from dessication and prevention of premature germination. The cirrus protects the pycnidiospores so that some remain viable at least 28 days (Fournet, 1969). When the cirrus was diluted with water, if the concentration of cirrus solution was >20%, less that 10% of pycnidiospores germinated. At a lower concentration, the components provide nutrients that stimulate spore germination and elongation of germ tubes. Germ tube length increased up to 15% cirrus concentration, then declined moderately at higher concentrations (Harrower, 1976). Brennan et al. (1986) reported greater germination in dilute cirrus fluid. Cirrus components reduced germination at 10-60% relative humidity. Once spores are

dispersed, the stimulatory effects of the cirrus fluid are probably negligible (Griffiths and Peverett, 1980). At 35-45% relative humidity, spores of *S. tritici* in cirri remained viable at least 60 days (Gough and Lee, 1985). The cirrus components may act as an inhibitor of spore germination, or the high osmotic potential of the cirrus may prevent germination.

Pycnidiospores of *S. nodorum* did not survive for 24 hours at relative humidity above 80% at 20 C. Spores survived two weeks or more at <10% relative humidity (Griffiths and Peverett, 1980). When the cirrus fluid of *S. nodorum* was diluted with water, about two-thirds of the pycnidiospores lost viability within 8 hours, and after 30 hours in daylight, only 5% germinated. When spores were stored in the dark, 40% remained viable after 30 hours (Brennan et al., 1986). Dry conidia of *S. nodorum*, shaded and in direct sunlight, survived outdoors at least 56 hours (Fernandes and Hendrix, 1986a). Germination of *S. nodorum* pycnidiospores was inhibited by continuous UV-B (280-320 nm), whereas germination of *S. tritici* was not. Germ tube extension under continuous UV-B was inhibited for both fungi, compared with darkness (Rasanayagam et al., 1995).

## Infection by *Stagonospora nodorum*

The process of host penetration and development of *S. nodorum* within the leaf was examined in detail by several investigators (Baker and Smith, 1978, Bird and Ride 1981, Karjalainen and Lounatmaa, 1986; Keon and Hargreaves, 1984; Straley, 1979; Weber, 1922). Pycnidiospores tend to lodge in the depressions between two epidermal cells, and many attempted leaf penetrations begin there. Spores germinate on the leaf surface in response to free moisture (Fernandes and Hendrix, 1986b). They begin to germinate 2-3 hours after deposition, and after 8 hours germination can reach 90%. Leaf penetration begins about 10 hours after spore deposition (Bird and Ride, 1981; Brönnimann et al., 1972; Holmes and Colhoun, 1974).

At the onset of germination, the germ tube is surrounded by an amorphous material that attaches to the leaf. Germ tubes growing from either end of a spore and from intercalary cells tend to grow along the depressions between cells and are often oriented along the long axis of the leaf (O'Reilly and Downes, 1986). Hyphae from spores not in depressions grow randomly with occasional branching (Straley, 1979). An appressorium forms with an infection peg that penetrates the cuticle and periclinal walls of epidermal cells directly into the cell lumen, resulting in rapid cell death.

Many penetrations first are subcuticular or lateral growth of a hypha occurs within the cell wall before growth into the cytoplasm (Bird and Ride, 1981; O'Reilly and Downes, 1986). Penetration through both open and closed stomata also occurs and may be faster than direct penetration (Harrower, 1976; Jenkins, 1978; O'Reilly and Downes, 1986; Straley, 1979). Germ tubes branch at stomata and junctions of epidermal cells. Penetration of a germ tube into a stomate may occur without formation of an appressorium. Penetration sometimes occurs through trichomes (Straley, 1979). Apparently, most penetration attempts fail, with dense papillae formed in the cells at the site of attempted penetration (Karjalainen and Lounatmaa, 1986; Bird and Ride, 1981).

After penetration, epidermal cells die quickly and become lignified, and the hyphae grow into the mesophyll. Mesophyll cells become misshapen, and lignified material is deposited outside of some cells, which then collapse. Lignification occurs before hyphae reach the cell. The process is the same in all cultivars but develops more slowly in resistant cultivars. The hyphae grow intercellularly among epidermal cells, then into the mesophyll. When the mesophyll is penetrated, chloroplast deterioration begins in 6-9 days (Karjalainen and Lounatmaa, 1986). However, the photosynthetic rate begins to decline within a day after infection and before symptoms are visible (Krupinsky et al, 1973).

Sclerenchyma tissue around vascular bundles prevents infection of vascular tissue. The vascular bundles block the spread of hyphae through the mesophyll except when sclerenchyma tissue is young and not fully formed (Baker and Smith, 1978).

*Stagonospora nodorum* releases a wide range of cell wall degrading enzymes including amylase, pectin methyl esterase, polygalacturonases, xylanases, and cellulase *in vitro* and during infection of wheat leaves (Baker, 1969; Lehtinen, 1993; Magro, 1984). The information related to cell wall degradation by enzymes agrees with histological observations. These enzymes may act in conjunction with toxins. Enzyme sensitivity may be related to resistance and rate of fungal colonization (Magro, 1984).

Like many necrotrophs, *Septoria* and *Stagonospora* pathogens produce phytotoxic compounds *in vitro*. Cell deterioration and death in advance of hyphal growth into mesophyll tissue (Bird and Ride, 1981) is consistent with toxin production. However, a definitive role for toxins in the infection process and their relation to host resistance has not been established (Bethenod et al, 1982; Bousquet et al, 1980; Essad and Bousquet, 1981; King et al, 1983). Differences in host range between wheat and barley-adapted strains of *S. nodorum* may be related to toxin production (Bousquet and Kollmann, 1998).

Initiation of spore germination and percentage of spores germinated are not influenced by host susceptibility (Bird and Ride, 1981; Morgan 1974; Straley, 1979; Straley and Scharen, 1979; Baker and Smith, 1978). Bird and Ride (1981) reported that extension of germ tubes on the leaf surface was slower on resistant than on susceptible cultivars. This mechanism, expressed at least 48 hours after spore deposition, indicates pre-penetration resistance to elongation of germ tubes. There were fewer successful penetrations in resistant cultivars, and penetration proceeded more slowly on resistant cultivars (Baker and Smith, 1978; Bird and Ride, 1981).

Lignification was proposed to limit infection in both resistant and susceptible cultivars, but other factors slowed fungal development in resistant lines. In susceptible lines, faster growing hyphae may escape lignification of host cells. Four days after inoculation of barley with a wheat biotype isolate of *S. nodorum*, hyphae grew through the cuticle and sometimes in outer cellulose layers of epidermal cell walls. Thick papillae were deposited beneath the penetration hyphae and the cells were not penetrated (Keon and Hargreaves, 1984).

## Infection by *Septoria tritici*

Pycnidiospores of *S. tritici* germinate in free water from both ends of the spore or from intercalary cells (Weber, 1922). Spore germination does not begin until about 12 hours after contact with the leaf. Germ tubes grow randomly over the leaf surface. Weber (1922) observed only direct penetration between epidermal cells, but others concluded that penetration through both open and closed stomata is the primary means of host penetration (Benedict, 1971; Cohen and Eyal, 1993; Hilu and Bever, 1957). Kema et al. (1996) observed only stomatal penetration. Hyphae growing through stomata become constricted to about 1 µm diameter, then become wider after reaching the substomatal cavity.

Hyphae grow parallel to the leaf surface under epidermal cells, then through the mesophyll to cells of lower the epidermis, but not into the epidermis. No haustoria are formed and hyphal growth is limited by sclerenchyma cells around the vascular bundles, except when hyphae are very dense. Vascular bundles are not invaded. Hyphae grow intercellularly along cell walls through the mesophyll, branching at a septum or middle of a cell. No macroscopic symptoms appear for about 9 days except for an occasional dead cell, but mesophyll cells die rapidly after 11 days. Pycnidia develop in substomatal chambers. Hyphae seldom grow into host cells (Hilu and Bever, 1957; Kema et al, 1996; Weber, 1922).

Successful infection only occurs after at least 20 hours of high humidity. Only a few brown flecks developed if leaves remained wet for 5-10 hours after spore deposition (Holmes and Colhoun, 1974) or up to 24 hours (Kema et al., 1996). Host-parasite relations are the same on resistant or susceptible wheats. Spore germination on the leaf surface is the same regardless of susceptibility. The number of successful penetrations is about the same, but hyphal growth is faster in susceptible cultivars, resulting in more lesions. Hyphae extend

beyond the necrotic area in all cultivars. A toxin may play a role in pathogenesis (Cohen and Eyal, 1993; Hilu and Bever, 1957). In contrast, colonization was greatly reduced on a resistant line (Kema et al., 1996).

## Infection by *Septoria passerinii*

Green and Dickson (1957) present a detailed description of the infection process of *S. passerinii* on barley. The infection process is similar to *S. tritici*. Like *S. tritici*, the length of time required for leaf penetration is considerably longer than for *S. nodorum*.

Germ tubes branch and grow over the leaf surface at random, but sometimes along depressions between epidermal cells. Leaf penetration is almost exclusively through stomata. Germination hyphae become swollen, and if penetration is unsuccessful, hyphae continue to elongate. No penetration occurs 48 hours after spore deposition. After 72 hours, germ tubes thicken over stomata, grow between guard cells and on surfaces of accessary cells and into the substomatal cavities. Direct penetration between epidermal cells is seen only rarely.

Spore germination and host penetration are the same on resistant and susceptible cultivars. There is much less extension of hyphae within leaves on resistant cultivars and papillae are observed on many but not all cell walls. Hyphae grow beneath the epidermis from one stoma to another, but do not penetrate between epidermal cells. The mesophyll is colonized, but no haustoria form. After the mesophyll cells become necrotic, epidermal cells collapse. Mycelial development in the leaf is sparse and usually blocked by vascular bundles. In younger leaves, if the vascular sheath is less developed, hyphae pass between the bundle and the epidermis. Pycnidia form in substomatal cavities, mostly on the upper leaf surface (Green and Dickson, 1957).

## Factors Affecting Spore Longevity on the Leaf Surface

Among the *Stagonospora* and *Septoria* pathogens of cereals, definitive information on the infection process has been reported only for *S. nodorum*, *S. tritici*, and *S. passerinii*. Like many other necrotrophic pathogens, neither group of pathogens elicit the hypersensitive reaction. A significant difference in the infection process between *Septoria* and *Stagonospora* pathogens is that spore germination and penetration proceeds much faster for *S. nodorum* than for *S. tritici* and *S. passerinii*. This has a significant influence on disease epidemiology. The *Septoria* pathogens penetrate the plant primarily through stomata, whereas *S. nodorum* penetrates both directly and through stomata. *S. nodorum* penetrates and kills the epidermal cells quickly, but *S. tritici* and *S. passerinii* do not kill epidermal cells until hyphae have ramified through the leaf mesophyll and rapid necrosis begins.

Histological studies of fungal growth following host penetration match the data generated from epidemiological studies of host resistance. Resistance slows the rate of host colonization but has no appreciable effect on the process of lesion development. The mechanisms controlling host response, whether related to enzymes and toxins or other metabolites released by the pathogens during infection, are still unclear.

There is little information about infection by ascospores. The infection process is probably very similar to that for pycnidiospores. Ascospores of *Phaeosphaeria nodorum* germinate over a wide range of temperatures, and their germ tubes penetrate the leaf directly. However, according to Rapilly et al. (1973), ascospores, unlike pycnidiospores, do not germinate in free water.

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# Aggressiveness of *Phaeosphaeria nodorum* Isolates and Their *In Vitro* Secretion of Cell-Wall-Degrading Enzymes

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## Abstract

*The relationship between the* in vitro *production of cell-wall-degrading enzymes and the aggressiveness of three* Phaeosphaeria nodorum *isolates was studied. When grown in liquid medium containing 1% cell wall from wheat leaves as the only carbon source, the isolates secreted xylanase, α-arabinosidase, β-xylosidase, polygalacturonase, β-galactosidase, cellulase, β-1.3-glucanase, β-glucosidase, acetyl-esterase, and butyrate-esterase. Time course experiments showed different levels of enzyme production and different kinetics between isolates. Xylanase, cellulase, polygalacturonase, and butyrate-esterase were positively correlated with isolate aggressiveness. The most aggressive isolate produced a higher proportion of xylanase than the other two isolates, suggesting the role of this enzyme in the pathogenesis of* P. nodorum.

Studies have revealed that *Phaeosphaeria nodorum* populations have significant variability in pathogenicity that has been measured by the ability of isolates to cause symptoms on wheat (Griffith and Ao, 1980; Allinghan and Jackson, 1981;Yang and Hughes, 1986; Krupinsky, 1997).

As other plant-pathogenic fungi, *P. nodorum* produces a range of cell-wall-degrading enzymes (CWDE) that enables it to penetrate and infect host tissues (Lehtinen, 1993; Magro, 1984). Very little is known about the variability of enzyme production in *P. nodorum* populations; there is, however, a need to assess the diversity of these wall-degrading enzymes and, in particular, the extent to which this diversity could lead to differences in pathogenicity.

The objective of this study was to compare enzymatic variation between two wild isolates and one mutant isolate of *P. nodorum* by examining CWDEs, and to establish the possible relationship between enzymes associated to pathogenesis and the aggressiveness of fungal strains.

## Materials and Methods

### Fungal strains

Two wild strains (A/5 and 6/T) that differed significantly in their pathogenic behavior were isolated as described by Rapilly et al. (1992). The other isolate (300.2) is a carbendazim (MBC) resistant strain that was obtained using the protocol described in a previous study (Halama et al., 1999).

### Growth conditions and preparation of fungal extracts

The pycniospores used for inoculation were produced on synthetic (S) medium (Halama and Lacoste, 1992). For enzyme production, isolates of *S. nodorum* were grown in liquid medium (Lehtinen, 1993) in which sucrose was replaced by 1% wheat cell walls. Wheat cell wall material was taken from 1-week-old seedlings of cv. 'Soissons' and prepared according to Lehtinen's modified procedure (Lehtinen, 1993). After incubating cultures at 24°C under constant shaking (72 rpm), liquid filtrates were centrifuged at 8000 g for 15 min at 4°C. Supernatants were used for enzyme activity assays, and each culture experiment was replicated three times.

### Enzyme assays

Cell-wall-degrading enzymes of *P. nodorum* were assayed from the crude culture filtrates using different methods. The dinitrosalicylic acid (DNS) modified method, described by Miller (1959) for determining the reducing group, was used for the endo-enzyme assay. Xylanase, endo-polygalacturonase, cellulase, and β-1.3-glucanase activities were measured respectively on oat spelts xylan, galacturonic acid, carboxymethyl cellulose, and laminarin used as substrates. Absorbances were measured at 540 nm. The reference sugar was glucose.

Glycosidase activities were measured using p-nitrophenyl glycoside as a substrate (Poutanen, 1988). β-glucosidase, β-

galactosidase, β-xylosidase, and α-arabinosidase activities were assayed using p-nitrophenyl-β-D-glucopyranoside, p-nitrophenyl-β-D-galactopyranoside, p-nitrophenyl-β-D-xylopyranoside, and p-nitrophenyl-α-L-arabinopyranoside, respectively, as substrates.

Esterase acetate activity was tested as described by Biely et al. (1988) using p-nitrophenylacetate. Degradation of p-nitrophenylbutyrate was measured using p-nitrophenylbutyrate according to the procedure of Kolattukudy et al. (1981). In each case, p-nitrophenol was used for the standard microtitre plate. For all enzyme assays, one unit of enzyme was defined as the amount of enzyme that hydrolyzed 1 mmol of appropriate substrate, and each activity was expressed in $mU.ml^{-1}$.

### Pathogenicity test

Isolates were tested for their relative pathogenicity on detached seedling leaves of the cultivar 'Soissons' as previously described by Halama et al. (1999).

### Statistical analysis

The analysis of variance was performed with the aid of the STAT-ITCF statistical software. Differences between the isolates were assessed using the Newman-Keuls test. Correlations between data for aggressiveness rates and for enzyme secretion were examined.

## Results

The maximum secretions of CWDEs are presented in Table 1, which shows that *P. nodorum* isolates were able to produce a wide range of enzymes on synthetic medium supplemented with isolated cell wall. The secreted enzymes were determined every second day over the growth period (14 days).

### Time course for production of CWDEs

The A/5 isolate produced, in order of decreasing activity, xylanase, butyrate-esterase, β-glucosidase, β-1.3-glucanase, acetyl-esterase, cellulase, polygalacturonase, β-galactosidase, and β-xylosidase (Table 1). Culture filtrates of the A/5 isolate had significantly higher levels of xylanase, butyrate-esterase, cellulase, and polygalacturonase activities than those of 6/T and 300.2 isolates. Levels of enzyme activity between 6/T and 300.2 isolates were significantly different mainly for two peaks of β-1.3-glucanase and acetyl-esterase activity on days 10 and 14 produced respectively by the 6/T and the 300.2 isolates. Xylanase activity by the A/5 isolate on day 10 was approximately seven times higher (1574 $mU.m1^{-1}$) than that of the two other isolates.

No α-arabinosidase activity could be detected in the filtrate of A/5 isolate, and very low levels were detected for 6/T and 300.2 isolates. Very low β-xylosidase activity was detected both by A/5 and 6/T isolates.

The polygalacturonase activities were highest on day 6 for A/5 isolate (56 $mU.ml^{-1}$), after which the activity decreased. For 6/T and 300.2 isolates, the highest activities were 27 and 12 $mU.ml^{-1}$ respectively, showing differences in their kinetics. As early as the second day, isolate 6/T produced a large amount of polygalacturonase, although isolate 300.2 secreted maximum polygalacturonase only after 12 days.

β-1.3-glucanase assay was detected mainly for A/5 and 6/T isolates. β-glucosidase activity was produced by the three isolates with maximum activity on day 14 for the A/5 and 300.2 isolates and on day 10 for the 6/T isolate. However, A/5 and 300.2 isolates may not have reached their peaks of β-glucosidase activity at the end of the time course.

Where detected, the cellulase activity secreted by *P. nodorum* occurred transiently, and the highest activity took place during the early stages of fungal culture for A/5 isolate. The butyrate-esterase activity was observed for the three isolates with a maximum

**Table 1. Maximum enzyme activity expressed by three isolates of *Phaeosphaeria nodorum* (A/5, 6/T, and 300.2) on medium supplemented with wheat cell walls.**

| | Isolate | | |
|---|---|---|---|
| **Enzyme** | **A/5** | **6/T** | **300.2** |
| Xylanase | 1574[1] (10)[2] | 213 (6) | 200 (14) |
| α-arabinosidase | 0 | 5 (8) | 7 (14) |
| β-xylosidase | 6 (10) | 16 (14) | 8 (14) |
| Polygalacturonase | 56 (6) | 27(2) | 12 (12) |
| β-galactosidase | 17 (12) | 21 (10) | 20 (14) |
| Cellulase | 106 (6) | 0 | 3.5 (10) |
| β-1,3 – glucanase | 145 (12) | 165 (10) | 12 (12) |
| β-glucosidase | 153 (14) | 158 (10) | 118 (14) |
| Acetyl esterase | 126 (10) | 44 (6) | 124 (14) |
| Butyrate esterase | 527 (10) | 409 (6) | 379 (14) |

[1] Data are expressed as $mU.ml^{-1}$ culture medium filtrate.
[2] Enzyme activity was measured on the day given in parentheses.

at day 10, 6 and 14 for A/5, 6/T and 300.2 isolate respectively. Acetyl-esterase and β-galactosidase activities were also detectable for all three isolates.

### Aggressiveness of *P. nodorum* isolates

The pathogenicity test allowed us to distinguish two groups of isolates according to their aggressiveness (Table 2). Significant differences in the average of necrosis symptoms were conclusive for differentiating the A/5 isolate from isolates 6/T and 300.2. The A/5 isolate of *P. nodorum* produced longer lesions on detached leaves than did the other two isolates 6/T and 300.2. Furthermore, there was partial correlation between the incubation period and length of necrotic lesions; the longer lesion of A/5 was related to a short latent period, but the similar lengths of necrotic lesions caused by 6/T and 300.2 were unrelated to their incubation periods.

## Discussion

Results reported here constitute the first report on the relationship between aggressiveness and *in vitro* production of CWDEs by *P. nodorum.* Our study indicated that *P. nodorum* isolates are able to secrete enzymes of which xylanases were the most abundant (Table 1). Xylans are constituents of secondary walls of dicots but in contrast form the main component of some monocot primary walls (Keon et al., 1987). During the enzymatic degradation of wheat cell walls by the pathogen's attack mechanisms, xylan degradation prevails. Xylanase synthesis occurs early in the infection process of graminaceous plants, which suggests its key role in the infection of cereals. Xylanases were found to be involved in the pathogenicity of cereal pathogens (Cooper et al., 1988), especially in the case of *P. nodorum*. In the cell walls of gramineae, α-arabinosidase, β-xylosidase, and acetyl-esterase, which cleave the side chain of xylans, may contribute synergistically to xylan degradation. In our study, the absence or low levels of arabinosidase and β-xylosidase activity are surprising, given that these enzymes, together with xylanase, are required for efficient degradation of xylan.

**Table 2. Lesion length and incubation period on detached leaves of wheat (cv. 'Soissons') caused by isolates of *Phaeosphaeria nodorum* 10 days after inoculation.**

| Isolate | Lesion length (mm) | Incubation period[1] (hours) |
|---|---|---|
| A/5 | 9.78 a[2] | 43 |
| 6/T | 3.60 b | 63 |
| 300.2 | 3.53 b | 41 |

[1] The incubation period was determined by measuring the period between inoculation and the moment when 50% of the detached leaves presented visible lesions.

[2] Within a column, means followed by different letters are significantly different according to *t* test (P<0.001).

Our results showed clear differences among the production kinetics of the three isolates: in contrast to the 300.2 isolate, the polygalacturonase activity for A/5 and 6/T isolates occurred transiently and peaked during the early stages of fungal culture. Polygalacturonases are the first CWDEs produced *in vitro* by *Verticillium albo-atrum* and *Colletotrichum lindemuthianum* (Cooper, 1977). Transient high level expression of polygalacturonases has also been reported in *F. oxysporum* f.sp. *melonis* (Martinez et al., 1991).

Isolates of *P. nodorum* were significantly different in their infection efficiencies of detached wheat leaves. Differences in aggressiveness may be explained partially by differences in enzymatic activity, as shown from *in vitro* studies. Within the range of activities recorded, there is a strong positive correlation between mean activity levels of the four enzymes and their aggressiveness. Indeed, the size of necrotic lesions was highly correlated with the levels of xylanase and cellulase (correlation coefficient of 0.99), and with the levels of polygalacturonase and butyrate-esterase (correlation coefficient of 0.94 and 0.93, respectively). However, these correlations are not absolute. Isolates A/5 and 6/T, for example, produce large quantities of β-1.3-glucanase and β-glucosidase, although they are in two distinct aggressiveness groups. Correlation between pathogenicity of fungi and their ability to secrete wall-degrading enzymes has also been observed by other authors (Chan and Sackston, 1972; Howel, 1975; Carder et al., 1987; Senna and Goodwin, 1996).

Although strong correlation between polysaccharidase activities and isolate aggressiveness was observed in this study (suggesting an active role for these enzymes in *P. nodorum* pathogenesis), the relationship between enzyme production and various parasitic abilities is complex. Many factors may act upon enzymatic production, such as *in vitro* conditions compared to enzymatic activities *in situ,* or upon pathogenesis, such as the ability to also produce toxic compounds. Despite this limitation, xylanase activity is the enzyme activity most intensely expressed by *P. nodorum* and the enzyme typically secreted by some cereal pathogens.

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# Growth of *Stagonospora nodorum* Lesions

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Disease severity is often expressed as a percentage or proportion of leaf area showing symptoms. Increase in disease severity of stagonospora nodorum glume blotch is caused by either an increase in the number of lesions as a result of new infection or by expansion of existing lesions. While new infections are still in the incubation period, existing lesions have time to expand considerably (Lannou et al., 1994). Berger et al. (1997) suggested that lesion expansion should become the sixth component in describing polycyclic epidemics, and should be added to the already existing "epidemic quintet." Lesion expansion has, for a long time, been considered as one of the most important factors for stagonospora nodorum disease increase (Rapilly et al., 1977; Rapilly et al., 1982).

## Materials and Methods

In a field experiment with winter wheat inoculated with *Stagonospora nodorum,* length and width of glume blotch lesions were measured repeatedly on the three uppermost leaves of tagged plants. The area of lesions was calculated assuming that they had an ellipsoidal shape. Lesion growth rates were analyzed in relation to current weather, lesion size, when a lesion was formed (first observed), and lesion age.

## Results and Discussion

The results show that lesions followed a pattern of exponential growth and thus the rates were not constant over time. Among weather factors, mean temperature was the only factor that had a small effect on lesion growth rate, aside from the actual size of the lesions. Lesions that were formed (first observed) before or at heading (DC<=55) grew more slowly than lesions that were first observed after heading. There was no difference in lesion growth rate between the three leaf levels. A slight decrease in growth rate with increasing age of lesions was noticed. The time period when the lesions were formed and the age effects are partially confounded. Lesion growth (length, width, and area) is a process that continues almost undisturbed by environmental factors, under normal field conditions, once it has been initiated. The results will have future applications in simulation modeling of stagonopora nodorum glume blotch.

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## *Session 3A: Host-Parasite Interactions*

# Genetic Control of Avirulence in *Mycosphaerella graminicola* (Anamorph *Septoria tritici*)

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*Mycosphaerella graminicola* is a plant pathogenic bipolar heterothallic ascomycete (Kema et al., 1996c) that causes septoria tritici leaf blotch of wheat. The disease is currently considered the major threat to European wheat crops. Our main interest is to understand the genetic control of specificity in mating and pathogenicity in this pathosystem. Therefore, we previously studied the histopathology of specific interactions and the genetic variation for virulence and resistance in pathogen isolates and host species and cultivars, respectively. We concluded that resistance to *M. graminicola* in the tested wheat cultivars is most probably not based on hypersensitivity (Kema et al., 1996d).

Like others we observed numerous host-pathogen interactions, which were confirmed in repeated field experiments (Eyal and Levy, 1987; Saadaoui, 1987; van Ginkel and Scharen, 1988; Ahmed et al., 1995; Kema et al., 1996a, b; Kema and van Silfhout, 1997). The next step was to understand the genetic control of these phenomena. Therefore, we had to determine the mating system of *M. graminicola* and find a way to cross particular isolates of the fungus, which eventually succeeded (Kema et al., 1996c).

Since then, our group continues to study genome plasticity in *M. graminicola* and is exploring the possibility of dissecting the genetic factors that control the interaction between host and pathogen, as well as mating between pathogen strains (Kema et al., 1999). The current status and future prospects of this work will be discussed.

## Materials and Methods

*Wheat cultivars*. Cultivars Shafir, Veranopolis, and Kavkaz were used as differentials and cvs. Taichung 29 and Kavkaz/K4500 were used as susceptible and resistant checks, respectively, in seedling experiments. In field experiments cvs. Vivant and Hereward and the breeding lines NSL92-5719, RU51A, and CH76337 were used.

*Fungal isolates*. Isolate IPO323 [MAT1-1] and IPO94269 [MAT1-2] were crossed to generate an F1 population. Individual F1 progeny isolates were 1) backcrossed to both parents for mating type identification and to generate BC1 populations, and 2) intercrossed to generate F2 populations. The avirulence/virulence of these parental isolates to the aforementioned cultivars is shown in Table 1.

*Experiments.* F1, BC1, and F2 populations were tested at the seedling stage in growth rooms and repeated twice. Individual progeny isolates have been tested several times on the differential cultivars in the course of additional experiments. The F1 mapping population was also evaluated on differential wheat cultivars in the field. Each plot, with randomized

**Table 1. Responses of wheat cultivars to the parental *Mycosphaerella graminicola* strains IPO323 and IPO94269 in seedling and adult plant experiments conducted in growth rooms and in the field, respectively.[a]**

| | Seedling | | Adult plant | |
|---|---|---|---|---|
| **Cultivars** | **IPO323** | **IPO94269** | **IPO323** | **IPO94269** |
| Taichung 29 | + | + | | |
| Kavkaz/K4500 | - | - | | |
| Kavkaz | - | + | | |
| Veranopolis | - | + | | |
| Shafir | - | + | - | + |
| Hereward | | | - | + |
| Vivant | | | - | + |
| NSL92-5719 | | | - | + |
| RU51A | | | +/- | - |
| CH76337 | | | +/- | - |

[a] Pycnidial coverage: +=susceptible, -=resistant.

cultivars, was replicated twice and was inoculated with an individual F1 isolate.

## Results and Discussion

In the seedling experiments all progeny isolates were virulent on cv. Taichung. None of these isolates carried virulence for cv. Kavkaz/K4500, indicating that the parental isolates carry the same avirulence factor(s) for this cultivar. Avirulence for each of the differentiating cultivars is inherited as a single gene. However, these avirulences co-segregated. Thus the entire F1, BC1, and F2 progenies showed the parental types. This suggests that the avirulences for these cultivars are tightly linked.

There are two reasons for this hypothesis: 1) additional data indicate that cvs. Shafir, Veranopolis, and Kavkaz carry different resistance factors (Kema et al., 1996a, b) and 2) identification of recombinant F1 progeny isolates in another cross (USDA50*IPO323.69.1) with avirulence for either Kavkaz or Veranopolis (unpublished data). Still, two alternative hypotheses cannot be excluded: 1) IPO323 carries an avirulence factor for a common resistance factor in the tested differentials or 2) an avirulence gene product from IPO323 is recognized by different resistance factors in these cultivars.

In order to investigate whether the avirulence in IPO323 for other wheat cultivars (Table 1) would segregate independently from the identified locus, a field experiment was conducted. Again, avirulence for the individual cultivars was controlled by a single locus. The avirulences for cvs. Vivant, Hereward, NSL92-5719, and Shafir co-segregated, indicating that they map to the same position as the locus identified in the seedling experiments. However, in addition to that, the avirulences for the breeding lines RU51A and CH76337 inherited independently from that locus. Hence, recombinant progeny isolates were identified that were entirely virulent or avirulent on all cultivars.

Hence, we hypothesize the presence of a complex major effect locus of tightly linked avirulence genes in *M. graminicola* IPO323 and two additional independent loci carrying minor effect avirulence factors in IPO94269. The former locus was mapped on the *M. graminicola* genome and is currently being isolated following map-based cloning strategies.

## Acknowledgments

We thank Jos Koeken, Suzanne Verhaegh, and the IPO-DLO technical and experimental farm staff for contributing to the presented data. Stimulating discussions with the "Wageningen" *Mycosphaerella* group, the "John Innes" *Mycosphaerella* group, and the EU-SIS consortium are kindly acknowledged. Part of this project is funded by EU-BIOTECH PL096-352.

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# Cytogenetics of Resistance of Wheat to Septoria Tritici Leaf Blotch

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The John Innes Centre holds the world's most comprehensive collection of precise genetic stocks of wheat. Elements of this collection, including intervarietal substitution and alien addition lines, are ideal for detecting chromosomes carrying agronomically important genes. These lines are being used in research on the genetics of resistance to septoria tritici leaf blotch caused by *Mycosphaerella graminicola* (anamorph *Septoria tritici*).

A synthetic hexaploid wheat (Synthetic) was developed from a cross between *Triticum dicoccoides* (AABB) and *Aegilops squarrosa* (DD) (Sears, 1976). Synthetic carries genes for resistance to *Stagonospora nodorum* (Nicholson et al., 1993), and a complete set of substitution lines of Synthetic chromosomes into a susceptible wheat variety, Chinese Spring (CS), has been developed (Worland et al., 1996).

To study resistance to *M. graminicola*, a detached seedling leaf technique (Arraiano et al., 1999) was used to test both Chinese Spring and Synthetic. Individual Dutch and Portuguese *M. graminicola* isolates supplied by IPO-DLO (Netherlands) were tested. Synthetic was completely resistant to all isolates, except for IPO92006, whereas Chinese Spring was susceptible to all isolates except IPO323, to which it was moderately resistant. The results for Chinese Spring are consistent with field trial data (Brown et al., 1999). Baldus and Longbow, the susceptible controls, were very susceptible to all isolates.

Based on these results, CS (Synthetic) substitution lines were tested using the detached leaf technique. Leaves were inoculated with the Dutch isolates IPO323 and IPO94269, to identify the Synthetic chromosomes that carry genes for resistance to *M. graminicola*. The line carrying chromosome 7D (i.e., CS background with the 7D chromosome substituted by that of Synthetic) showed complete resistance to both isolates. One-hundred 7D single-chromosome recombinant lines are now being tested to locate Synthetic's resistance gene more precisely in relation to microsatellite and RFLP markers.

Bezostaya 1 has been identified as a source of resistance to septoria tritici leaf blotch (Danon et al., 1982), and field trials showed it to be specifically resistant to IPO323 (Brown et al., 1999). Substitution lines of Bezostaya 1 into Dwarf A, a susceptible line, were tested as adult plants, and chromosome 3A was identified as carrying genes for resistance to IPO323.

## Acknowledgment

This research was supported by PRAXIS XXI – Fundação para a Ciência e a Tecnologia, Portugal, and the Ministry of Agriculture, Fisheries and Food, UK.

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# A Possible Gene-for-Gene Relationship for Septoria Tritici Leaf Blotch Resistance in Wheat

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## Abstract

*In an F2 population of a cross between the resistant variety Flame and the susceptible variety Longbow it was shown that Flame carried one partially recessive gene for resistance to* Septoria tritici *isolate IPO323, as measured in a detached leaf assay. Work is underway to determine whether this resistance gene has a gene-for-gene relationship with the single avirulence locus identified in the IPO323 isolate.*

Septoria tritici leaf blotch, caused by the fungus *Mycosphaerella graminicola* (anamorph *Septoria tritici*), is one of the most serious foliar pathogens of wheat in Europe. In recent field trials using individual *M. graminicola* isolates, specific variety x isolate interactions have been observed (Kema and van Silfhout, 1997). A number of wheat varieties show specific resistance to the Dutch isolate IPO323, including the British varieties Flame and Hereward (Brown et al., 1999). In Holland, crosses have been made between IPO323 and another isolate, IPO94269. Avirulence of IPO323 on three specifically resistant cultivars, Shafir, Kavkaz, and Veranopolis, appears to be controlled at a single locus with simple inheritance (Kema et al., 1999). To investigate whether the specific resistances of Flame and Hereward to IPO323 conform to a gene-for-gene relationship and also whether Flame and Hereward share the same resistance gene, crosses between Flame, Hereward, and a susceptible variety, Longbow, have been made.

Eighty $F_2$ seedlings from a cross between Flame and Longbow were tested in a detached leaf assay (Arraiano et al., 1999) in which primary and secondary leaves were inoculated with IPO323. Leaf sections were scored according to the percentage leaf area covered with lesions bearing pycnidia 15-28 days after inoculation. Each $F_2$ plant was represented by four leaf sections tested in separate boxes. The parental varieties, Flame and Longbow, were also included in each box. Comparison of infection levels on all four replicate leaf sections allowed individual plants to be rated as resistant, susceptible or intermediate. Flame was always resistant and Longbow always susceptible. Of the 80 progeny tested 23 were scored as resistant (less than 10% infection on all leaves). The other 57 progeny were scored as either susceptible (all leaf sections heavily infected) or intermediate (variable levels of infection). The 1:3 ratio of resistant:susceptible/intermediate is consistent with a single, partially recessive resistance gene in Flame.

$F_3$ families generated from the 80 $F_2$ individuals are being tested as seedlings (GS 25) in a polytunnel to test the prediction of a single gene for resistance to IPO323 in Flame. If this prediction is correct, $F_3$ families from resistant or susceptible $F_2$ individuals are expected to be uniformly resistant or susceptible, whereas intermediate $F_2$ individuals should produce $F_3$ families segregating 1:3 for IPO 323 resistance:susceptibility.

To investigate whether Hereward carries the same IPO323 resistance gene as Flame, $F_2$ progeny of crosses between Hereward and Longbow and between Hereward and Flame will be tested as detached leaves. The Hereward x Longbow cross will determine whether Hereward's resistance is controlled by a single gene. The Hereward x Flame cross will test for allelism. The results from both tests will be presented.

As IPO323 avirulence is controlled at a single locus (Kema et al., 1999) and Flame's resistance to this isolate may be due to a single gene, we hypothesize that a gene-for-gene relationship exists between Flame and IPO323. To confirm this hypothesis, we are testing 61 progeny isolates from the IPO323 x IPO94269 cross on Flame and Longbow as detached leaves.

## Acknowledgments

This work was funded by the EU Framework 4 Biotechnology program and the Ministry of Agriculture Fisheries and Foods (MAFF).

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# Diallel Analysis of Septoria Tritici Blotch Resistance in Winter Wheat

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## Abstract

*In the winter wheat area of the northern Great Plains, leaf spot complex has been problematic in the past decade. In years with high precipitation from late April to July, septoria tritici blotch (STB), caused by* Septoria tritici, *is most prevalent. As part of our effort to improve STB resistance, inheritance of STB resistance was investigated by an eight-parent full diallel scheme. Parents, $F_1$, and reciprocal $F_1$ were planted on three different dates. Within each planting date, three to five seeds of each experimental unit were planted in the greenhouse. Materials were arranged in a randomized complete block design (RCBD) with three replicates. Plants at the second-leaf stage were inoculated with a bulk of six* S. tritici *isolates. Significant general combining ability (GCA), specific combining ability (SCA), and reciprocal effects were observed in the analysis of variance. The ratio of GCA sum of squares relative to SCA sum of squares suggested that GCA was more important than SCA. Additive effects played the major role in host response to STB, while non-additive effects were also detected. General combining ability effects of individual genotypes were in close agreement with parental performance. KS94U338, a genotype with resistance derived from* Triticum tauschii, *had the lowest STB score and the highest general combining ability. This result indicates that this genotype, possessing resistance distinct from other known sources, should prove useful in breeding efforts to improve STB resistance in wheat.*

Winter injury is the most adverse factor for winter wheat production in the northern Great Plains of the USA. Adoption of conservation tillage practices, with winter wheat planting into spring wheat stubble, has increased steadily over the past decade. While this practice improves winter survival, accumulation of wheat residue on the soil surface has promoted the development of leaf spot diseases in this region. Severe epidemics of leaf spot complex on winter wheat have occurred during the last decade. Septoria tritici blotch has been predominant in years with above average precipitation from late April to July. Improvement of STB resistance was intensified in our project beginning in 1995. As part of this effort, we initiated studies to investigate combining ability of STB resistance in known resistant genotypes. A better understanding of resistance could lead to more efficient deployment of germplasm resources.

## Materials and Methods

Eight winter wheat genotypes (Table 1) were selected based on the level of resistance (field and greenhouse) and their diverse origins of resistance. A total of 64 genotypes (parents, $F_1$, and reciprocal $F_1$) were included in the test. The study consisted of three planting dates at three-day intervals. In each planting, three to five seeds of each experimental unit were planted in plastic conetainers filled with a peat-moss and perlite mixture. Each planting

**Table 1. Parental means, general combining ability (GCA), specific combining ability (SCA), and reciprocal effects of septoria tritici blotch scores on the second leaf in an eight-parent diallel.**

| | Resistant | | | Moderately resistant | | Susceptible | | |
|---|---|---|---|---|---|---|---|---|
| **Parent** | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** |
| 1 KS94U338 | —† | 0.5 | 0.1 | 0.3 | -1.9** | 0.9** | 0.6* | -0.6* |
| 2 Jagger | 0.8* | — | -0.5 | -1.0** | -2.0** | 1.0** | 1.1** | 2.0** |
| 3 KS91W005-1-4 | -0.9* | -0.9* | — | -2.0* | 1.6** | 0.8** | 1.0** | 0.7* |
| 4 KS91W0935-29-1 | 1.3** | -0.6 | 0.0 | — | 1.3** | 0.4 | 0.5 | -0.6* |
| 5 KS87822-2-1 | 0.3 | 0.1 | -0.6 | -0.5 | — | 0.1 | 0.3 | 0.5 |
| 6 SD93493 | -0.1 | -0.4 | -0.2 | 0.1 | 0.2 | — | -0.8* | -1.3** |
| 7 Tandem | -1.1* | 0.3 | 0.2 | -0.2 | 0.1 | -0.2 | — | -0.9* |
| 8 SD93500 | -1.2** | -0.3 | -0.1 | -1.1** | -0.2 | -0.2 | -0.1 | — |
| Parental means | 1.4 | 1.4 | 1.9 | 5.8 | 5.8 | 8.0 | 8.3 | 8.7 |
| Parental GCA | -2.2** | -1.6** | -1.1** | -0.5** | 0.0 | 1.8** | 1.6** | 1.8** |

*, ** Significantly different from zero at 0.05 and 0.01 probability levels, respectively.
† SCA effects are above the diagonal line, and reciprocal effects are below.

* First author prevented from attending workshop by unforeseen travel problems.

consisted of three replicates arranged in a randomized complete block design (RCBD).

Six isolates were used throughout this study. Fresh inoculum was prepared each time before inoculation. Conidia of each isolate were produced on acidic potato dextrose agar petri plates. The plates were incubated on a laboratory bench for 12 h under cool white fluorescent lights at room temperature. When the edge of pink colonies tended to darken, conidia were harvested by flooding the plates with doubled-distilled water and gently scraping the colonies with a rubber spatula attached to a glass rod. The conidial suspensions from the six isolates were combined, filtered through two layers of cheesecloth, and adjusted to approximately 5-10 x $10^6$ conidia $ml^{-1}$.

Plants were inoculated when the second leaf was fully expanded. A volume of 100 ml of the conidial suspension was sprayed evenly onto 300 plants using an atomizer. Plants were maintained in a mist chamber for 96 h at 100% relative humidity and 21±1°C under a 12-h photoperiod. To avoid leaf senescence, the mist chamber was opened 30 min every 24 h while the plants were kept wet. Then the plants were incubated in a growth chamber with a 12-h photoperiod at 21±1°C.

Twenty-one days after inoculation, disease ratings of the second leaf were taken based on a 1 to 9 scale (Table 2). The average disease scores of resistant genotypes (R) were 1.0 to 4.9; of moderate resistant genotypes (MR) from 5.0 to 6.9; of moderate susceptible genotypes (MS) from 7.0 to 7.9; and of susceptible genotypes from 8.0 to 9.0. The data were analyzed with the "Diallel analysis and simulation" program designed by Burow and Coors (1994) according to Griffing's Model 1 (fixed model), Method 1 (Griffing, 1956). Because these experiments were conducted in one controlled environment, the genotype by environment interaction was theoretically negligible. Thus, sets (planting dates) were treated as replicates, and the mean value of the three observations within each set represented the estimated STB score of the replicate.

A *t*-test was used to test whether the GCA, SCA, and reciprocal effects were significantly different from zero. The degrees of freedom for estimated GCA effects ($g_i$) were (p-1), where p = number of parents. The degrees of freedom for estimated SCA effects ($s_{ij}$) and estimated reciprocal effects ($r_{ij}$) were p(p-1) (Kang, 1994).

## Results and Discussion

No significant differences between replicates (different planting dates) were detected (Table 3). However, highly significant effects ($P<0.01$) due to general combining ability (GCA), specific combining ability (SCA), and reciprocal effects were observed. The GCA effects were larger than SCA. Since GCA effects accounted for the largest portion of the genotype variation, additive gene effects were most important in the inheritance of resistance to STB in this group of genotypes.

Parents varied significantly in their reaction to STB (Table 1). Three reaction groups (resistant, moderate resistant, and susceptible) were observed in the parents. GCA effects were generally in agreement with parental performance (Table 1). A negative GCA value indicated that the corresponding parent reduced the disease score, while a positive value was associated with susceptibility. 'KS94U338' had the lowest GCA value (-2.2) and the lowest STB score (1.4), indicating that resistance to STB was consistently inherited in crosses with this parent. This genotype,

**Table 2. Septoria tritici blotch reading scales used in this study.**

| Rating | Symptom description |
|---|---|
| 1. | No visible symptoms are observed, and the leaf remains green and healthy. |
| 2. | A few chlorotic lesions are present, and the infection site is a tan-colored spot. |
| 3. | Extensive chlorotic lesions are present. Lesions occasionally have necrosis at the infection sites. |
| 6. | Extensive chlorotic lesions merge with each other, and individual lesions are identified by initial infection sites. Necrosis and chlorosis both exist on the leaf. |
| 7. | Lesions fully merge, and more than half of the leaf is desiccated by necrosis. |
| 8. | The entire leaf is desiccated, and water-soaked lesions occupy the entire leaf. |
| 9. | A few pycnidia are visible on the infected sites, and less than 30% of the leaf is occupied by pycnidia covered lesions that remain dry and green. |
| 10. | Pycnidia occupy 50 to 70% of the leaf, and infected sites are dry yet still green. |
| 11. | The entire leaf is covered by pycnidial lesions, and the leaf is dry yet still green. |

**Table 3. Analysis of variance for STB resistance of an eight-parent diallel cross.**

| Source | df | Mean squares |
|---|---|---|
| Replication | 2 | 0.2 |
| Entries | | |
| GCA | 7 | 128.5** |
| SCA | 28 | 7.5** |
| Reciprocal | 28 | 2.0** |
| Error | 126 | 0.6 |

**Significant at the 0.01 probability level.

with resistance derived from *T. tauschii*, should prove useful in breeding efforts to improve STB resistance in wheat. The other two resistant genotypes, 'Jagger' and 'KS91W005-1-4', had large, negative, highly significant GCA effects. Of the moderate resistant parents, 'KS91W0935-29-1' and 'KS87822-2-1', only KS91W0935-29-1 had significant negative GCA effects. Each of the susceptible parents ('SD93493', 'SD93500', and 'Tandem') had high GCA values.

Specific combining ability effects were observed in 20 of the 28 possible combinations, indicating that non-additive effects may exist for STB resistance. Reciprocal effects were present in five of the eight combinations involving KS94U338, crosses of KS91W005-1-4 with Jagger, and SD93500 with KS91W0935-29-1. When crossed with KS94U338 as female, the hybrids of KS91W005-1-4 and the two susceptible parents (SD93500 and Tandem) had lower disease score than those of the reciprocal crosses (with reciprocal effects of -0.9, -1.1, and -1.2). Apparently KS91W005-1-4, Tandem, and SD93500 could contribute additional resistance when used as female. The maternal effects of SD93500 were also observed when it was crossed with KS91W0935-29-1. The maternal effects in combinations of KS94U338 with Jagger and KS94U338 with KS91W0935-29-1 are ascribed to KS94U338, due to the positive reciprocal effects between crosses of KS94U338 used as male and female. The existence of reciprocal effects in resistance to STB was also observed by Jlibene et al. (1994).

With the observation of predominant GCA effects and reciprocal effects for enhanced resistance, improvement of STB resistance can be achieved by crossing two parents having good resistance, while selecting resistant progeny from particular crosses based on the direction of the crosses is also predictable.

## Acknowledgments

The authors gratefully acknowledge Drs. Rollin Sears, Joe Martin, and Stan Cox (Kansas State University, USDA-ARS) for providing some of the germplasm used in our studies.

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# Analysis of the Septoria Monitoring Nursery

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## Abstract

*The Septoria Monitoring Nursery (SMN) was divided into two sections: 1) the best sources of resistance identified by CIMMYT and national agricultural research breeding programs (renewed every three years) and 2) a tentative group of differentials. The nursery included two susceptible durum varieties and a resistant one, as well as two susceptible bread wheat varieties and a resistant one. Readings of septoria leaf blotch were taken using the double digit modified scale, and analyses were done separately by digits. The variance component estimate and the F test for the first and the second digits were highly significant for site, as well as for the site x genotype interaction component for the 1st and 2nd SMN. An analysis of the group of tentative differentials across the seven years and all sites also gave significant differences between sites and between sites and genotypes. The contrast between durum and bread wheat for the 1st SMN was not significant, which indicates that at least at those locations there were no host-specific pathogen populations. The contrast between durum and bread wheat was significant in the 2nd SMN, indicating the existence of specific pathogen populations for each of the two crops in some sites.*

*The most resistant lines across sites for the 1st SMN were Trap#1/Bow and #1959, of Chinese origin, and for the 2 nd SMN, Eg-AH567.71// 4*Eg-A/3/2*CMH79.243, Cal/NH/H567.71/3/2*Ning 7840/4/CMH83.2277/5/Bow/2*Ning 7840// CMH83.2277, and Sha5/Bow. A more detailed analysis of genotype x site interaction will be carried out to identify the differential response of some lines to the pathogen populations in different sites.*

The Septoria Monitoring Nursery was initiated in 1992 (Gilchrist, 1994). Based on virulence studies on seedlings involving *Septoria tritici* isolates obtained from hot spot locations in many countries around the world, Kema et al. (1996; 1997) found significant interaction between pathogen isolates and host cultivars. The same authors also confirmed the specificity of the relationship between bread wheat genotypes and isolates taken from bread wheat, and between durum wheat genotypes and isolates collected from durum wheat, as demonstrated by Eyal et al. (1973), Saadaoui (1987), and van Ginkel and Scharen (1988).

A better understanding of specificity in the *Septoria*-wheat pathosystem may facilitate quantifying its magnitude and understanding its role in providing protection at the crop level rather than under controlled conditions (Eyal, 1999). According to Eyal (1999), "the International Monitoring Nursery philosophy executed by CIMMYT can provide information on pathogen x cultivar interaction in the regional, national and international domain. Futhermore, it may serve as a common basis for broad-base evaluation of germplasm to diverse pathogen populations under variable environmental conditions."

The information generated by this nursery could be extremely useful to CIMMYT's wheat breeding program and those of national agricultural research systems (NARS) in their attempts to increase stable resistance over time.

## Material and Methods

The Septoria Monitoring Nursery was divided into two sections: 1) one (renewed every three years) with the best sources of resistance identified by CIMMYT and NARS breeding programs and 2) a tentative group of differentials proposed by Dr. Z. Eyal, Tel Aviv University, as a fixed group. They were Ald/Pvn, Enkoy (K=4500), Colotana, IAS 20, Bow CM33203-K-9M-2Y-1M-1Y-2M-0Y-1Ptz, Don Ernesto (Bow) CM33203-K-9M-33Y-1M-500Y-0M-OJ-1J, Seri 82, Kvz-K500.L.6.A.4, Beth Lehem, Lakhish, Kauz, Penjamo 62, Etit 38 (D), Inbar (D), Glennson 81, and Barrigon Yaqui (D).

The same nursery with two replications was sent to each site during three years to avoid environmental and year effects, and to obtain more reliable data. The first Septoria Monitoring Nursery was sent to 23 locations. The following group of resistant lines selected under Patzcuaro (Michoacan state) and Toluca (Mexico state) conditions were included:

1. Trap #1/Bow
2. Thb//Ias 20/H567.71
3. PF70354/Bow
4. Lfn/1158.57//Prl/3/Hahn
5. Ias 58/4/Kal/Bb//Cj/3/Ald/5/Bow
6. Br 14*2/Sumai 3
7. Sushoe #6//Ald/Pav
8. Br 14*2//Nobre*2/Tp
9. M2A/Cml//Nyubay/3/CMH 72A .576/Mrg
10. M2A/Cml//2* Nyubay
11. CMH80A.253/Sx
12. CMH80.278/3/Ssfm/H567.71//2*Ssmf
13. #1959
14. Sumai#3
15. CS/Th. curv.//Glenn81/3/Ald/Pvn

The second Septoria Monitoring Nursery was sent to 22 locations and included the following resistant lines:

1. Bobwhite (CM 33203-K-10M-7Y-3M-2Y-1M-0M)
2. Cno79/4/CS//Th. curv.//Glenn81/3/Ald/Pvn
3. TIA.2/4/CS/Th. curv.//Glenn81/3/Ald/Pvn
4. CS/Th. curv.//Glenn81/3/ Ald/Pvn/4/ CS/Le. Rac//*…
5. Chirya 3
6. Chirya 1
7. Chirya 4
8. CS/Th. curv.//Glenn81/3/Ald/Pvn/4/ Suz8
9. CS/Th.curv.//Glenn81/3/Ald/Pvn/4/Nanjing 8401
10. Eg-A/H567.71//4*Eg-A/3/2*CMH79.243
11. Cal/NH//H567.71/3/2*Ning 7840/4/…
12. CMH72A.576/Mrng//CMH78.443/3/CMH79.243/…
13. CMH77.308/CMH82.205
14. Ald/Pvn//Ymi #6
15. Sha 5/Bow

The nursery included two susceptible durum varieties and a resistant one, as well as two susceptible bread wheat varieties and a resistant one. Readings of septoria tritici leaf blotch were taken using the double digit modified scale (Eyal et al., 1987), and the analysis was done separately by digits. Height and spike emergence measurements were used as covariants in the analysis.

## Results and Discussion

Seven of the 23 locations were included in the analysis of first Septoria Monitoring Nursery. Practically no infection was detected at the other locations due to low moisture conditions. The sites where weather conditions produced medium to good epidemics were Argentina (Balcarce and Pergamino), Chile (Hidango and Chillan), Guatemala (Quetzaltenango), Mexico (Toluca, four years), and Uruguay (La Estanzuela). Some sites were eliminated because there was strong interference from other diseases. Toluca was the only site that had no such interference, and it allowed four diseases evaluations.

The variance component estimate and the F test for the first and the second digits were highly significant for the factor site, as was the site x genotype interaction component, although the latter was much smaller than the former (Table 1).

Infection averages per country and site are presented in Table 2. Results indicate that La Estanzuela (Uruguay) and Toluca (Mexico) had the heaviest epidemic compared with the other sites.

**Table 1. Variance and F test for digits 1 and 2 of the response to infection by *Septoria tritici* for site and genotype x site interaction (1st SMN).**

| | Variance component | F test |
|---|---|---|
| **Digit 1** | | |
| Site | 2.68 | 82.98*** |
| Site x genotype | 1.12 | 1.95*** |
| **Digit 2** | | |
| Site | 1.34 | 40.53*** |
| Site x genotype | 1.64 | 2.44*** |

The responses of durum and bread wheat lines in the first Septoria Monitoring Nursery were not significantly different, which indicates that at least at those locations there were no host-specific pathogen populations. There were no significant differences between the susceptible bread wheat checks (Seri 82 and Lakish) and the resistant check (Beth Lehem). This suggests that the resistant entries were not always resistant, and that our universal resistant check was not always resistant. In the case of durum wheat, this difference was significant. The resistant variety Etit 38 was always resistant, while susceptible varieties Inbar and Barrigon Yaqui were always susceptible.

The mean infection (first and second digit) of the varieties across sites and years are shown in Table 3. The most resistant varieties across sites were Trap#1/Bow and #1959, of Chinese origin.

Fourteen of a total of twenty-two sites sampled were included in the second Septoria Monitoring Nursery data analysis. The sites not included had problems with interference by rust infection or experienced low moisture conditions. The countries and

**Table 2. *Septoria tritici* infection average per country and site, and disease evaluation for digits 1 and 2 (1st SMN).**

| | Infection average | |
|---|---|---|
| **Country and site** | **Digit 1** | **Digit 2** |
| Toluca (Mexico) | 7.25 | 6.63 |
| La Estanzuela (Uruguay) | 6.06 | 6.96 |
| Chillan (Chile) | 6.91 | 5.32 |
| SNA-Hidango (Chile) | 3.08 | 5.67 |
| Balcarce (Argentina) | 5.64 | 4.32 |
| Pergamino (Argentina) | 6.07 | 4.92 |
| Quetzaltenango (Guatemala) | 2.45 | 4.07 |

**Table. 3. Infection averages (digits 1 and 2) across sites and years (1st SMN).**

| Variety | Digit 1 | Digit 2 |
|---|---|---|
| Trap #1/Bow | 3.56 | 1.49 |
| Thb//Ias 20/H567.71 | 4.78 | 2.70 |
| PF70354/Bow | 4.62 | 2.73 |
| Lfn/1158.57//Prl/3/Hahn | 4.97 | 2.61 |
| Ias 58/4/Kal/Bb//Cj/3/Ald/5/Bow | 5.81 | 3.25 |
| Br 14*2/Sumai 3 | 4.25 | 2.06 |
| Sushoe #6//Ald/Pvn | 4.42 | 2.92 |
| Br 14*2//Nobre*2/Tp | 4.72 | 2.70 |
| M2A/Cml//Nyubay/3/ CMH 72A.576/Mrg | 5.36 | 3.75 |
| M2A/Cml//2* Nyubay | 5.39 | 3.34 |
| CMH80A.253/Sx | 6.24 | 4.79 |
| CMH80.278/3/Ssfm/ H567.71//2*Ssmf | 5.51 | 3.93 |
| #1959 | 4.03 | 1.94 |
| Sumai#3 | 5.25 | 4.20 |
| CS/Th. curv. //Glenn81/3/ Ald/Pvn | 5.37 | 3.55 |
| **Tentative differentials** | | |
| Ald/Pvn | 5.03 | 3.72 |
| Enkoy (K=4500) | 5.18 | 3.09 |
| Colotana | 3.06 | 1.56 |
| IAS 20 | 3.67 | 2.31 |
| Bow CM33203-K-9M-2Y-1M-1Y-2M-0Y-1Ptz | 6.29 | 4.52 |
| Don Ernesto(Bow)CM33203-K-9M-33Y-1M-500Y-0M-0J-1J | 6.62 | 5.50 |
| Seri 82 | 7.07 | 6.33 |
| Kvz-K500.L6.A.4 | 6.16 | 4.48 |
| Beth Lehem | 7.17 | 6.77 |
| Lakhish | 7.03 | 6.78 |
| Kauz | 6.92 | 6.50 |
| Penjamo 62 | 7.28 | 6.75 |
| Etit 38 (D) | 4.04 | 3.06 |
| Inbar (D) | 4.37 | 3.15 |
| Glennson 81 | 6.44 | 5.53 |
| Barrigon Yaqui (D) | 7.00 | 5.74 |

locations selected were Argentina (Tres Arroyos, La Plata), Chile (Temuco), Ethiopia (Holetta), Mexico (Toluca, Patzcuaro), Portugal (Elvas), Russia (Krasnoda), Switzerland (Zurich), and Uruguay (La Estanzuela, Tarariras). Some of them were included for more than one year.

The variance component estimate and the F test for digits 1 and 2 *S. tritici* evaluation were highly significant for the factor site, as was the genotype x site interaction component (Table 4). Again, the interaction component was much smaller than the main factor component.

The *S. tritici* infection averages per country and site for digits 1 and 2 are presented in Table 5. Mexico (Toluca and Patzcuaro) and Uruguay (La Estanzuela) had the lowest infection averages. This was to be expected, given that data for these three locations were considered in selecting resistant lines.

The contrast between durum and bread wheats was significant, which indicated that most sites included in the second nursery had specific pathogen populations for every crop (Table 6). As in the first nursery, there were no differences between the susceptible bread wheat checks (Seri 82 and Lakish) and the resistant check (Beth Lehem). This confirms that the resistant check was not always resistant, and that our resistant check is not universally resistant and should be changed. Results confirmed that the resistant durum variety Etit 38 was always resistant, and varieties Inbar and Barrigon Yaqui were always susceptible(Table 6).

Infection averages (digits 1 and 2) of the varieties in the second Septoria Monitoring Nursery across sites and years are shown in Table 7. The most resistant lines across sites were: 1) Eg-AH567.71//4*Eg-A/3/2*CMH79.243, 2) Cal/NH/H567.71/3/2*Ning 7840/4/CMH83.2277/5/Bow/2*Ning 7840//CMH83.2277, and 3) Sha 5/Bow.

**Table 4. Variance and F test for digits 1 and 2 of the response to infection by *Septoria tritici* for site and genotype x site interaction (2nd SMN).**

| | Variance component | F test |
|---|---|---|
| **Digit 1** | | |
| Site | 1.12 | 45.07*** |
| Site x genotype | 1.20 | 2.64*** |
| **Digit 2** | | |
| Site | 0.78 | 22.55*** |
| Site x genotype | 1.18 | 2.11*** |

**Table 5. *Septoria tritici* infection averages per country and site for digits 1 and 2 (2nd SMN).**

| | | Infection average | |
|---|---|---|---|
| **Country and site** | | **Digit 1** | **Digit 2** |
| Argentina | Tres Arroyos | 7.81 | 4.05 |
| | La Plata | 6.45 | 3.46 |
| Chile | Temuco | 6.43 | 5.11 |
| Ethiopia | Holetta | 7.64 | 5.04 |
| Mexico | Toluca | 4.87 | 3.52 |
| | Patzcuaro | 6.75 | 4.90 |
| Portugal | Elvas | 7.40 | 3.94 |
| Russia | Krasnovar | 7.36 | 5.10 |
| Switzerland | Zurich | 7.45 | 3.23 |
| Uruguay | La Estanzuela | 6.45 | 4.47 |
| | Tarariras | 7.37 | 4.98 |

**Table 6. Differences between average durum and bread wheat and resistant (R) and susceptible (S) checks for each crop for the 1st SMN, 2nd SMN, and the combination of all sites for the 1st and the 2nd SMN.**

| | Significance levels | |
|---|---|---|
| **Source** | **Digit 1** | **Digit 2** |
| **1st SMN** | | |
| Bread and durum wheat | 1.93 NS | 2.64 NS |
| Bread wheat (S) and bread wheat (R) | 0.04 NS | -0.22 NS |
| Durum wheat (S) and durum wheat (R) | 1.64 ** | 1.38 * |
| **2nd SMN** | | |
| Bread and durum wheat | 1.77 *** | 1.89 NS |
| Bread wheat (S) and bread wheat (R) | -0.07 NS | 0.25 NS |
| Durum wheat (S) and durum wheat (R) | 2.02 *** | 0.99 ** |
| **1st and 2nd SMN combined** | | |
| Bread and durum wheat | 1.83 *** | 2.16 ** |
| Bread wheat (S) and bread wheat (R) | -0.08 NS | 0.23 NS |
| Durum wheat (S) and durum wheat (R) | 1.91 *** | 1.11 *** |

NS: Not significant.
* Significant at the 5% level.
** Significant at the 1% level.
*** Significant at the 0.1% level.

An analysis of the differential group across the seven years and all sites also gave significant differences between sites and a significant sites x varieties value. The values of the *S. tritici* infection averages for digits 1 and 2 for each variety are in Table 8.

**Table 7. Infection averages (digits 1 and 2) across sites and years (2nd SMN).**

| Variety | Digit 1 | Digit 2 |
|---|---|---|
| Bow CM 33203-K-10M-7Y-3M-2Y-1M-0M | 6.10 | 3.26 |
| Cno79/4/CS/Th. curv.//Glenn81/3/Ald/Pvn | 6.61 | 3.62 |
| TIA.2/4/CS/Th. curv.//Glenn81/3/Ald/Pvn | 6.81 | 3.92 |
| CS/Th. curv//Glenn81/3/Ald/Pvn/4/CS/Le.Rac//*… | 6.64 | 3.56 |
| Chirya 3 | 6.87 | 3.75 |
| Chirya 1 | 6.82 | 4.27 |
| Chirya 4 | 6.94 | 3.86 |
| CS/Th. curv. //Glenn81/3/Ald/Pvn/4/ Suz8 | 6.26 | 3.37 |
| CS/Th.curv. //Glenn81/3/Ald/Pvn/4/Nanjing 8401 | 6.74 | 4.08 |
| Eg-A/H567.71//4*EG-A/3/2*CMH79.243 | 5.25 | 2.60 |
| Cal/NH//H567.71/3/2*Ning 7840/4/… | 5.47 | 3.38 |
| CMH72A.576/Mrng//CMH78.443/3/CMH79.243/… | 7.04 | 5.53 |
| CMH77.308/CMH82.205 | 6.67 | 3.78 |
| Ald/Pvn//Ymi #6 | 6.27 | 3.12 |
| Sha 5/Bow | 5.96 | 3.17 |
| **Tentative differentials** | | |
| Ald/Pvn | 6.10 | 3.34 |
| Enkoy (K=4500) | 5.37 | 2.52 |
| Colotana | 3.74 | 2.16 |
| IAS 20 | 4.51 | 2.34 |
| Bow CM33203-K-9M-2Y-1M-1Y-2M-0Y-1Ptz | 6.35 | 3.87 |
| Don Ernesto (Bow) CM33203-K-9M-33Y-1M-500Y-0M-OJ-1J | 6.85 | 4.45 |
| Seri 82 | 7.27 | 5.39 |
| Kvz-K500.L.6.A.4 | 6.60 | 3.67 |
| Beth Lehem | 7.61 | 5.62 |
| Lakhish | 7.82 | 5.35 |
| Kauz | 7.27 | 4.74 |
| Penjamo 62 | 7.78 | 5.60 |
| Etit 38 (D) | 4.44 | 2.90 |
| Inbar (D) | 5.89 | 3.35 |
| Glennson 81 | 6.74 | 4.75 |
| Barrigon Yaqui (D) | 7.42 | 4.44 |

A more detailed analysis of variety x site interactions will be carried out in the near future to identify the differential response of some lines to the pathogen populations in different sites.

## Acknowledgments

The authors fondly remember Dr. Zahir Eyal and recognize his positive influence, his wise advice to follow this line of research, and his keen visualization of its potential impact. They also thank the NARS cooperators who sent in the data that made it possible to obtain the reported results. Last, but not least, the authors wish to thank Dr. Jesse Dubin for contributing his well-considered ideas on the data analyses.

**Table 8. *Septoria tritici* infection averages for differential varieties across the seven years of evaluation covering 33 locations.**

| Variety number | Digit 1 | Digit 2 |
|---|---|---|
| Ald/Pvn | 5.71 | 3.43 |
| Enkoy (K=4500) | 5.29 | 2.69 |
| Colotana | 3.51 | 1.96 |
| IAS 20 | 4.23 | 2.33 |
| Bow CM33203-K-9M-2Y-1M-1Y-2M-0Y-1Ptz | 6.30 | 4.08 |
| Don Ernesto (Bow) CM33203-K-9M-33Y-1M-500Y-0M-OJ-1J | 6.76 | 4.78 |
| Seri 82 | 7.19 | 5.70 |
| Kvz-K500.L.6.A.4 | 6.44 | 3.90 |
| Beth Lehem | 7.45 | 5.98 |
| Lakhish | 7.55 | 5.81 |
| Kauz | 7.15 | 5.30 |
| Penjamo 62 | 7.61 | 5.96 |
| Etit 38 (D) | 4.28 | 2.93 |
| Inbar (D) | 5.37 | 3.26 |
| Glennson 81 | 6.59 | 4.96 |
| Barrigon Yaqui (D) | 7.01 | 4.83 |

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## *Session 3B: Host-Parasite Interactions*

# Host – Parasite Interactions: *Stagonospora nodorum*

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## Abstract

*Relative virulence of 15* Stagonospora *(*Septoria*)* nodorum *(SNB) monopycnidiospore isolates and their mixture was studied under field conditions on a differential set of triticale and bread wheat cultivars. The isolates originated from diseased triticale and wheat plants sampled in diverse geographical regions of Poland. Significant effects of isolates, cultivars, and cultivar by isolate interactions were detected. The interaction between* S. nodorum *isolates and wheat and triticale genotypes appeared to be to a great extent a statistical interaction, rather than a well defined physiological specialization. The detection of the interaction was influenced by the amount of disease inflicted by the pathogen isolates on selected host genotypes and the effect of environmental conditions on disease development, as well as by the tools and precision with which the host was examined. Nonetheless, since the isolate by cultivar interaction was significant in most cases, the term virulence is used. The mixture of isolates was classified among isolates showing intermediate virulence on the cultivars tested. In comparison to single isolates, the differential capacity of the isolate mixture was also reduced. Relationships between mean isolate virulences and cultivar variances of SNB reactions were not significant for leaves (moderate SNB level) and significant for heads (low SNB level). A single pathogenic isolate used for screening breeding materials may provide reliable information on their SNB resistance.*

*Stagonospora nodorum* (Berk.) Castellani et E.G. Germano (=*Septoria nodorum* (Berk. Berk. in Berk. & Broome) [teleomorph *Phaeosphaeria nodorum* (E. Müller) Hedjaroude (=*Leptosphaeria nodorum* E. Müller) is a necrotrophic pathogen of graminaceous plant species. The pathogen, together with *S. avenae* and *Septoria tritici*, belongs to a group of cereal pathogens sometimes called *Septoria* complex. Among these pathogens special forms have been recognized only in *S. avenae*: f.sp. *avenae* and f.sp. *triticea*. In the other two pathogens, i.e. *S. nodorum* and *S. tritici*, host specificity is generally low, but more pronounced in *S. tritici* (Eyal et al., 1987; Skajennikoff and Rapilly, 1983).

The presence of specificity in these pathogen populations is based on the analyses of the statistical interaction terms between cultivars and isolates. The isolate by cultivar interaction terms in *S. nodorum* are usually of a smaller magnitude than for *S. tritici* (Yechilevich-Auster et al., 1983; Scharen and Eyal, 1983; Scharen et al., 1985). Therefore, distinguishing specificity (virulence) from aggressiveness is more difficult for *S. nodorum*. Since specificity in the *S. nodorum*/cereal systems at the cultivar level is not so clear, it might be called genome specificity.

Isolate by genotype interactions between *S. nodorum* isolates and cereal species have been reported by a number of authors under controlled environment as well as field conditions (Arseniuk et. al., 1994; Krupinsky, 1986, 1994, 1997a,b,c). Information on the magnitude of such interaction is important for inoculum composition and resistance management in breeding programs. Although genetic protection is considered the main pillar of host defense, the majority of commercial wheat cultivars do not have suitable protection.

The lack of resistant germplasm, high pathogen variability, and low specificity have all contributed to the slow progress in breeding for resistance and the deficient genetic protection in wheat and triticale. Therefore, the objective of this study was to compare the screening efficiency of triticale and wheat cultivars for stagonospora

nodorum blotch (SNB) resistance with single isolates and their mixture under field conditions. Concurrently, the relative virulence of *S. nodorum* isolates, the magnitude of cultivar by isolate interaction, and the relative resistance of the cereal genotypes used in the study were examined.

## Materials and Methods

The relative virulence of 15 single pycnidiospore isolates of *Stagonospora* (= *Septoria*) *nodorum* and their mixture was studied in 1993-1995 under field conditions on both winter and spring triticale and bread wheat. For the purpose 11 winter triticale and 4 winter wheat cultivars and germplasm lines (Tables 1 and 2) and 5 spring triticale and 3 spring wheat cultivars (Tables 3 and 4) were used. The genotypes had been preselected earlier based on their capacity to differentiate *S. nodorum* isolates in regard to their virulence/aggressiveness (pathogenicity). (The term virulence will be used from this point on in this paper, since the interaction of isolates by cultivars was significant.)

Fourteen isolates used for the study originated from different geographical regions of Poland and one from Switzerland (87TS-1-1). As for host association, of those 15 isolates, 14 were derived from diseased hexaploid triticale plants and one (Wen-1-1) from a hexaploid bread wheat plant cv. 'Weneda' (Table 5). No record was made of the specific triticale genotypes from which the isolates were derived. All isolates used in the study were derived from single pycnidiospores

**Table 1. Analysis of variance for the amount of SNB on leaves and heads of 11 winter triticale and 4 winter wheat cultivars scored on a 0-9 digit scale (0 = resistant, 9 = susceptible) under field conditions.**

| | Leaves | | | | Heads | | | |
|---|---|---|---|---|---|---|---|---|
| Source of variation | D. f. | Mean Square | F value | Proba-bility | D. f. | Mean Square | F value | Proba-bility |
| Years (Y) | 2 | 427.5 | 21.8 | 0.01 | 2 | 1957.8 | 2696.1 | 0.00 |
| Blocks (B) | | | | | | | | |
| Error 1 (Y × B) | 3 | 19.6 | | | 3 | 0.7 | | |
| Scoring dates (S.d.) | 3 | 4217.7 | 866.7 | 0.00 | 2 | 650.3 | 4.1 | 0.07 |
| Y × S.d. | 6 | 77.7 | 16.0 | 0.02 | 4 | 55.0 | 0.3 | 0.83 |
| Error 2 (Y×B×S.d.) | 9 | 4.9 | | | 6 | 159.7 | | |
| Cultivars (C) | 14 | 113.1 | 321.3 | 0.00 | 14 | 139.3 | 346.4 | 0.00 |
| Isolates (I) | 15 | 157.3 | 446.7 | 0.00 | 15 | 131.4 | 326.8 | 0.00 |
| C × I | 210 | 1.4 | 3.9 | 0.00 | 210 | 1.1 | 2.8 | 0.00 |
| Y × C | 28 | 17.1 | 48.6 | 0.00 | 28 | 20.2 | 50.2 | 0.00 |
| Y × I | 30 | 26.5 | 75.4 | 0.00 | 28 | 3.4 | 8.5 | 0.00 |
| S.d. × C | 42 | 4.5 | 12.7 | 0.00 | 30 | 46.3 | 115.1 | 0.00 |
| S.d. × I | 45 | 2.2 | 6.3 | 0.00 | 30 | 3.7 | 9.1 | 0.00 |
| Y × C × I | 420 | 0.7 | 1.9 | 0.00 | 56 | 1.2 | 3.1 | 0.00 |
| Y × S.d. × I | 90 | 1.4 | 3.9 | 0.00 | 60 | 1.8 | 4.6 | 0.00 |
| Y × S.d. × C | 84 | 1.8 | 5.2 | 0.00 | 420 | 0.3 | 0.7 | 1.00 |
| S.d. × C × I | 630 | 0.3 | 0.8 | 1.00 | 420 | 0.7 | 1.8 | 0.00 |
| Y × S.d.× C × I) | 1260 | 0.3 | 0.7 | 1.00 | 840 | 0.3 | 0.7 | 1.00 |
| Error(Y×B×S.d.×C×I) | 2868 | 0.4 | | | 2151 | 0.4 | | |

**Table 2. The expression of virulence by 15 *Stagonospora nodorum* isolates and their mixture (a composite isolate) on leaves and heads of 11 winter triticale (t) and 4 winter wheat (w) cultivars under field conditions.**

| No. | Isolate | Almo (t) | Ugo (t) | Malno (t) | Lasko (t) | Grado (t) | Dagro (t) | Bolero (t) | Presto (t) | Largo (t) | LAD-285 (t) | DAD-187 (t) | Alba (w) | Jawa (w) | Begra (w) | Liwilla (w) | Mean |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Lb-2-1 | 3.9* | 4.1 | 3.7 | 2.9 | 4.3 | 4.5 | 3.0 | 3.1 | 3.5 | 4.4 | 3.8 | 3.5 | 4.2 | 4.9 | 3.4 | 3.8 |
| 2 | Lb-3-1 | 4.8 | 4.7 | 4.1 | 3.6 | 4.8 | 5.0 | 4.0 | 3.8 | 4.2 | 5.1 | 4.7 | 3.9 | 4.7 | 5.3 | 3.5 | 4.4 |
| 3 | Lb-4-1 | 4.8 | 4.6 | 4.3 | 3.5 | 4.9 | 4.9 | 3.8 | 3.8 | 4.3 | 5.0 | 4.5 | 3.9 | 4.4 | 5.6 | 3.6 | 4.4 |
| 4 | Z-20-1 | 3.4 | 3.5 | 3.4 | 2.6 | 3.8 | 3.9 | 2.9 | 2.8 | 3.3 | 3.6 | 3.5 | 3.3 | 4.1 | 4.9 | 3.1 | 3.5 |
| 5 | Z-22-1 | 4.1 | 4.2 | 4.2 | 3.6 | 4.9 | 5.0 | 3.3 | 4.1 | 4.6 | 5.4 | 4.8 | 3.8 | 4.7 | 5.6 | 3.5 | 4.4 |
| 6 | Gw-3-1 | 5.9 | 5.8 | 5.5 | 4.9 | 6.2 | 5.9 | 4.6 | 5.3 | 5.3 | 5.9 | 5.6 | 4.5 | 5.4 | 6.1 | 4.3 | 5.4 |
| 7 | TS87-1-1 | 5.4 | 5.4 | 5.3 | 4.8 | 5.8 | 5.9 | 4.3 | 5.2 | 5.0 | 5.7 | 5.1 | 4.7 | 5.6 | 6.1 | 4.7 | 5.3 |
| 8 | Wen-1-1 | 5.1 | 5.3 | 5.3 | 4.3 | 6.0 | 5.9 | 4.5 | 5.3 | 5.1 | 5.2 | 5.4 | 4.3 | 5.2 | 5.6 | 4.1 | 5.1 |
| 9 | Ml-2-1 | 3.5 | 3.3 | 3.4 | 2.9 | 3.9 | 4.0 | 2.8 | 2.8 | 3.7 | 3.7 | 3.5 | 3.2 | 4.3 | 5.0 | 3.1 | 3.5 |
| 10 | Ch-9-1 | 3.2 | 3.5 | 3.3 | 2.8 | 3.7 | 3.7 | 2.6 | 2.8 | 3.2 | 3.3 | 3.3 | 2.8 | 4.0 | 4.8 | 3.3 | 3.3 |
| 11 | Wr-6-1 | 4.4 | 4.2 | 4.0 | 3.5 | 4.5 | 4.5 | 3.5 | 3.6 | 4.2 | 4.5 | 4.4 | 3.8 | 4.6 | 4.9 | 3.8 | 4.2 |
| 12 | Ch90-3-1 | 5.1 | 4.9 | 4.8 | 4.1 | 5.8 | 5.9 | 4.1 | 4.8 | 4.8 | 5.6 | 8.6 | 3.8 | 5.1 | 5.4 | 3.8 | 5.1 |
| 13 | Rd93-1-1 | 4.0 | 3.8 | 3.7 | 3.0 | 4.3 | 4.3 | 3.1 | 3.2 | 3.5 | 4.0 | 4.0 | 3.4 | 4.3 | 5.4 | 3.7 | 3.8 |
| 14 | T91-1-4 | 3.4 | 3.9 | 3.3 | 2.8 | 3.9 | 4.1 | 2.8 | 2.7 | 3.5 | 4.1 | 4.0 | 3.3 | 4.3 | 4.9 | 3.0 | 3.6 |
| 15 | Lu90-1-1 | 4.3 | 4.5 | 4.3 | 3.4 | 5.3 | 5.3 | 3.4 | 4.4 | 4.7 | 4.8 | 4.3 | 3.8 | 4.8 | 5.2 | 3.6 | 4.4 |
| 16 | Mixture | 5.0 | 4.6 | 4.5 | 3.9 | 5.4 | 5.3 | 3.7 | 4.3 | 5.0 | 5.4 | 4.8 | 4.0 | 4.9 | 5.4 | 3.5 | 4.6 |
| | Mean | 4.4 | 4.4 | 4.2 | 3.5 | 4.8 | 4.9 | 3.5 | 3.9 | 4.2 | 4.7 | 4.6 | 3.7 | 4.7 | 5.3 | 3.6 | 4.3 |

$LSD_{0.05}$ = 0.114 for virulence of isolates on leaves; $LSD_{0.01}$ = 0.110 for reaction of cultivars on leaves.

* Mean score on a 0-9 scale of 4 SNB scorings per season × 6 blocks over 3 growing seasons.

and, as determined after the research was completed, all were of the wheat biotype.

The isolates used for inoculation were increased on bread wheat grain. The composite isolate (a volumetric mixture of isolates) was prepared by mixing equal volumes of spore suspensions of all isolates adjusted to equal concentrations of spores/ml. Plants were inoculated at the late boot stage ($5 \times 10^6$ spores/ml) and after heading ($2 \times 10^6$ spores/ml). SNB was rated visually on a 0 (resistant) - 9 (susceptible) scale at about weekly intervals. Separate notes were taken for leaves and heads four and three times, respectively, on winter types, and four and two times, respectively, on spring types. All the tabulated data (Tables 2, 6, 3, 4) are averages of the numbers of replications and disease scoring dates.

**Table 3.The expression of virulence by 15 *Stagonospora nodorum* isolates and their mixture (a composite isolate) on leaves and heads of 5 spring triticale and 3 spring wheat cultivars un-der field conditions.**

| No. | Isolate | Jago (t) | Maja (t) | Gabo (t) | Grego (t) | Migo (t) | Henika (w) | Eta (w) | Sigma (w) | Mean |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Lb-2-1 | 2.8* | 2.6 | 2.2 | 2.9 | 2.8 | 3.6 | 2.4 | 2.8 | 2.8 |
| 2 | Lb-3-1 | 2.7 | 2.8 | 2.4 | 3.2 | 2.7 | 3.5 | 3.0 | 2.8 | 2.9 |
| 3 | Lb-4-1 | 2.8 | 2.1 | 2.3 | 2.8 | 2.8 | 3.4 | 2.6 | 2.6 | 2.7 |
| 4 | Z-20-1 | 1.9 | 2.1 | 2.2 | 2.5 | 2.7 | 3.2 | 2.2 | 2.3 | 2.4 |
| 5 | Z-22-1 | 2.2 | 2.2 | 2.3 | 2.3 | 2.5 | 2.8 | 2.2 | 2.1 | 2.3 |
| 6 | Gw-3-1 | 3.3 | 3.3 | 3.6 | 3.4 | 3.7 | 4.2 | 3.2 | 3.0 | 3.4 |
| 7 | TS87-1-1 | 4.4 | 4.3 | 4.4 | 4.3 | 4.9 | 4.9 | 4.1 | 4.1 | 4.4 |
| 8 | Wen-1-1 | 3.8 | 3.8 | 3.9 | 4.2 | 4.0 | 4.8 | 3.9 | 3.8 | 4.0 |
| 9 | Ml-2-1 | 2.7 | 2.8 | 2.5 | 2.5 | 3.2 | 2.9 | 2.7 | 2.9 | 2.8 |
| 10 | Ch-9-1 | 2.8 | 2.9 | 2.7 | 2.5 | 3.2 | 2.7 | 3.1 | 2.9 | 2.8 |
| 11 | Wr-6-1 | 2.7 | 3.1 | 2.6 | 2.3 | 2.9 | 2.9 | 2.6 | 2.7 | 2.7 |
| 12 | Ch-90-3 | 2.2 | 2.4 | 2.2 | 2.0 | 2.4 | 2.6 | 2.3 | 2.1 | 2.3 |
| 13 | Rd91-1-3 | 2.1 | 2.5 | 2.1 | 2.0 | 2.3 | 2.2 | 2.0 | 2.2 | 2.2 |
| 14 | T91-1-4 | 2.9 | 3.6 | 3.4 | 2.9 | 3.9 | 3.8 | 3.3 | 3.6 | 3.4 |
| 15 | Lu90-1-1 | 3.8 | 4.5 | 4.1 | 4.3 | 4.9 | 4.8 | 4.2 | 4.4 | 4.4 |
| 16 | Mixture | 3.6 | 4.1 | 3.7 | 3.6 | 4.3 | 4.6 | 3.7 | 3.9 | 3.9 |
| | Mean | 2.9 | 3.1 | 2.9 | 3.0 | 3.3 | 3.6 | 3.0 | 3.0 | 3.1 |

LSD0.01 = 0.243 - for virulence of isolates on leaves; LSD0.01 = 0.172 - for reactions of cultivars on leaves.
* Mean score on a 0-9 scale of 4 SNB scorings per season x 5 blocks over 3 growing seasons.

**Table 4. The expression of virulence by 15 *Stagonospora nodorum* isolates and their mixture (a composite isolate) on heads of 5 spring triticale and 3 spring wheat cultivars under field conditions.**

| No. | Isolate | Jago (t) | Maja (t) | Gabo (t) | Grego (t) | Migo (t) | Henika (w) | Eta (w) | Sigma (w) | Mean |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Lb-2-1 | 0.2 | 0.0 | 0.1 | 0.3 | 0.1 | 1.3 | 2.0 | 2.0 | 0.7 |
| 2 | Lb-3-1 | 0.1 | 0.1 | 0.3 | 0.2 | 0.0 | 2.2 | 2.0 | 2.0 | 0.9 |
| 3 | Lb-4-1 | 0.1 | 0.0 | 0.3 | 0.0 | 0.0 | 2.6 | 3.1 | 2.0 | 1.0 |
| 4 | Z-20-1 | 0.0 | 0.5 | 0.1 | 0.4 | 0.0 | 2.6 | 2.8 | 2.0 | 1.0 |
| 5 | Z-22-1 | 0.3 | 0.2 | 0.1 | 0.3 | 0.1 | 1.7 | 2.9 | 2.0 | 0.9 |
| 6 | Gw-3-1 | 0.7 | 0.2 | 0.0 | 1.0 | 0.8 | 2.9 | 3.5 | 2.0 | 1.4 |
| 7 | TS87-1-1 | 0.3 | 0.2 | 0.3 | 0.3 | 0.1 | 2.2 | 3.3 | 3.5 | 1.3 |
| 8 | Wen-1-1 | 0.2 | 0.2 | 0.6 | 0.4 | 0.3 | 2.9 | 3.2 | 3.5 | 1.4 |
| 9 | Ml-2-1 | 0.3 | 0.0 | 0.1 | 0.1 | 0.1 | 1.8 | 2.2 | 2.5 | 0.9 |
| 10 | Ch-9-1 | 0.0 | 0.1 | 0.1 | 0.0 | 0.0 | 1.6 | 1.6 | 2.0 | 0.7 |
| 11 | Wr-6-1 | 0.1 | 0.2 | 0.1 | 0.1 | 0.0 | 1.6 | 1.8 | 1.5 | 0.7 |
| 12 | Ch-90-3 | 0.4 | 0.4 | 0.2 | 0.3 | 0.3 | 1.9 | 1.9 | 2.5 | 1.0 |
| 13 | Rd91-1-3 | 0.0 | 0.0 | 0.0 | 0.6 | 0.0 | 1.8 | 2.0 | 2.5 | 0.9 |
| 14 | T91-1-4 | 0.1 | 0.2 | 0.2 | 0.0 | 0.0 | 2.2 | 1.9 | 2.0 | 0.8 |
| 15 | Lu-90-1 | 0.1 | 0.1 | 0.1 | 0.0 | 0.2 | 2.1 | 1.8 | 2.0 | 0.8 |
| 16 | Mixture | 0.0 | 0.3 | 0.0 | 0.3 | 0.2 | 2.0 | 2.4 | 3.0 | 1.0 |
| | Mean | 0.2 | 0.2 | 0.2 | 0.3 | 0.1 | 2.1 | 2.4 | 2.3 | 1.0 |

LSD 0.01 = 0.268 - for virulence of isolates on heads; LSD 0.01 = 0.189 - for reactions of cultivars on heads.
* Mean score on 0 - 9 scale of 2 SNB scorings per season ¥ 5 blocks over 3 growing seasons.

A split-plot experimental design was used. One experimental block comprised a number of main plots equal to the number of individual *S. nodorum* isolates studied. The main plots (rows of parcels) contained 1-$m^2$ parcels (subplots) planted to assigned cereal cultivars and germplasm lines. Individual isolates of the pathogen, considered as the main plots, were randomly assigned to the sets of subplots. The number of subplots within the main plot was equal to the number of cereal genotypes studied (15: 11 triticales + 4 wheat genotypes) and 8 (5 triticales + 3 wheat genotypes) for both winter and spring types, respectively. Each isolate (main plot) had its own control, treated with a fungicide (Tilt 250 EC, 0.1% a.i., 500 l/ha). In fact, the disease scores were actually differences between disease levels on cereal subpolts inoculated with individual isolates and its mirror images protected chemically. Isolate main plots containing the cereal subplots (cultivars/lines) were replicated twice for both winter and spring types (in 1994 only once).

Data were subjected to an analysis of variance using an MSUSTAT computer program, Version 5.25 (developed by R. Lund, Montana State University, MT 59715-002, USA) for a split-plot experiment. The variance among cereal cultivars/germplasm lines for disease reaction to individual *S. nodorum* isolates was regressed

**Table 5. Origin of *Stagonospora nodorum* isolates.**

| No. | Isolate name | Host | Geographic origin (location) |
|---|---|---|---|
| 1 | Lb-2-1 | triticale | Lubinicko, woj. zielonogórskie |
| 2 | Lb-3-1 | triticale | Lubinicko, woj. zielonogórskie |
| 3 | Lb-4-1 | triticale | Lubinicko, woj. zielonogórskie |
| 4 | Z-20-1 | triticale | Zadabrowie. woj. przemyskie |
| 5 | Z-22-1 | triticale | Zadabrowie. woj. przemyskie |
| 6 | Gw-3-1 | triticale | Grodkowice, woj. krakowskie |
| 7 | TS87-1-1 | triticale | Z¸rich, Switzerland |
| 8 | Wen-1-1 | winter wheat Weneda | Elblag, woj. torunskie |
| 9 | MI-2-1 | triticale | Malyszyn, woj. gorzowskie |
| 10 | Ch-9-1 | triticale | Choryn, woj. poznanskie |
| 11 | Wr-6-1 | triticale | Wrocikowo, woj. olsztynskie |
| 12 | Ch90-3-1 | triticale | Chelm, woj. chelmskie |
| 13 | Rd93-1-1 | triticale | Radzików, woj. warszawskie |
| 14 | T91-1-4 | triticale | Torun, woj. torunskie |
| 15 | Lu90-1-1 | triticale | Lucmierz, woj. lódzkie |

against mean virulence of the isolates. Similarly, the variance among isolates for virulence to individual cultivars/lines was regressed against mean resistance of the cultivars/lines expressed on the 0-9 scale.

# Results

All isolates of *S. nodorum* used in the study were pathogenic to leaves and to heads of all triticale and bread wheat cultivars (Tables 2, 6, 3, 4). On average, the disease on leaves was 2-3 times more severe than on heads. Some of the isolates did not cause disease on heads of spring triticale genotypes (Table 4). The lack of disease was due to low rainfall and drought during the summers. In spite of the adverse weather, the same isolates were able to cause the disease on heads of more SNB susceptible wheat cultivars.

**Table 6. The expression of virulence by 15 *Stagonospora nodorum* isolates and their mixture (a composite isolate) on heads of 11 winter triticale (t) and 4 winter wheat (w) cultivars under field conditions.**

| No. | Isolate | Almo (t) | Ugo (t) | Malno (t) | Lasko (t) | Grado (t) | Dagro (t) | Bolero (t) | Presto (t) | Largo (t) | LAD-285 (t) | DAD-187 (t) | Alba (w) | Jawa (w) | Begra (w) | Liwilla (w) | Mean |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Lb-2-1 | 0.7* | 0.8 | 1.5 | 1.4 | 1.3 | 1.4 | 0.7 | 0.8 | 0.9 | 1.6 | 1.1 | 1.8 | 2.9 | 2.7 | 1.8 | 1.4 |
| 2 | Lb-3-1 | 1.2 | 1.0 | 1.7 | 1.7 | 1.7 | 2.2 | 1.2 | 1.5 | 1.5 | 2.5 | 1.6 | 2.7 | 3.6 | 3.6 | 1.9 | 2.0 |
| 3 | Lb-4-1 | 1.3 | 1.4 | 2.2 | 2.1 | 2.5 | 2.8 | 1.8 | 1.8 | 1.8 | 3.1 | 1.8 | 2.3 | 3.6 | 3.2 | 2.3 | 2.3 |
| 4 | Z-20-1 | 0.5 | 0.6 | 0.7 | 0.6 | 1.9 | 1.1 | 0.7 | 0.8 | 0.9 | 1.2 | 0.9 | 1.6 | 2.3 | 2.0 | 1.6 | 1.2 |
| 5 | Z-22-1 | 0.9 | 0.8 | 1.1 | 1.2 | 1.2 | 1.8 | 0.8 | 1.3 | 1.0 | 2.1 | 1.6 | 2.3 | 3.2 | 2.9 | 1.9 | 1.6 |
| 6 | Gw-3-1 | 2.1 | 2.1 | 3.2 | 2.7 | 2.5 | 3.6 | 2.1 | 2.8 | 2.0 | 3.5 | 2.6 | 4.2 | 5.2 | 4.4 | 3.8 | 3.1 |
| 7 | TS87-1-1 | 1.8 | 2.1 | 3.2 | 2.8 | 3.3 | 3.7 | 2.2 | 2.8 | 2.6 | 4.0 | 2.5 | 3.3 | 4.9 | 4.1 | 3.7 | 3.1 |
| 8 | Wen-1-1 | 1.6 | 2.2 | 2.7 | 2.6 | 3.3 | 3.4 | 1.8 | 2.3 | 2.2 | 3.6 | 2.6 | 3.4 | 4.5 | 3.9 | 2.7 | 2.9 |
| 9 | MI-2-1 | 0.7 | 0.6 | 0.9 | 0.9 | 2.3 | 1.3 | 0.6 | 0.6 | 0.8 | 1.8 | 1.0 | 1.7 | 2.7 | 2.2 | 1.7 | 1.3 |
| 10 | Ch-9-1 | 0.7 | 0.5 | 0.6 | 0.7 | 0.9 | 0.9 | 0.7 | 0.7 | 0.6 | 1.3 | 1.0 | 1.2 | 2.1 | 1.9 | 2.9 | 1.1 |
| 11 | Wr-6-1 | 1.4 | 1.3 | 1.8 | 1.6 | 1.5 | 2.4 | 1.3 | 1.7 | 1.4 | 2.4 | 1.7 | 2.4 | 3.5 | 3.2 | 2.8 | 2.0 |
| 12 | Ch90-3-1 | 1.4 | 1.3 | 2.2 | 2.3 | 2.8 | 3.2 | 1.5 | 1.8 | 1.6 | 3.1 | 2.1 | 2.7 | 4.3 | 3.3 | 2.6 | 2.4 |
| 13 | Rd93-1-1 | 0.8 | 0.9 | 0.8 | 1.1 | 2.3 | 1.8 | 0.8 | 1.2 | 1.1 | 1.9 | 1.4 | 2.1 | 2.6 | 2.4 | 3.6 | 1.7 |
| 14 | T91-1-4 | 0.6 | 0.5 | 1.0 | 1.4 | 1.6 | 1.7 | 0.8 | 1.0 | 1.1 | 1.8 | 1.2 | 2.1 | 2.8 | 2.6 | 1.8 | 1.5 |
| 15 | Lu90-1-1 | 1.0 | 0.9 | 1.6 | 1.5 | 1.7 | 2.1 | 1.0 | 1.4 | 1.6 | 2.6 | 1.4 | 2.4 | 3.5 | 2.7 | 2.4 | 1.9 |
| 16 | Mixture | 1.1 | 1.3 | 2.4 | 2.3 | 2.4 | 3.0 | 1.6 | 1.7 | 2.1 | 2.6 | 1.7 | 2.7 | 4.0 | 3.3 | 2.8 | 2.3 |
| | Mean | 1.1 | 1.1 | 1.7 | 1.7 | 2.1 | 2.3 | 1.2 | 1.5 | 1.4 | 2.4 | 1.6 | 2.4 | 3.5 | 3.0 | 2.5 | 2.0 |

LSD0.01 = 0.141 - for virulence of isolates on heads; LSD0.01 = 0.136 for reaction of cultivars on heads.

* Mean score on a 0-9 scale of 3 SNB scorings per season ¥ 6 blocks over 3 growing seasons.

Due to a large number degrees of freedom and low error values, most of the sources of variation included in the analysis of variance (Tables 1 and 7) proved to be significant. The main effects of cultivars and isolates were highly significant. It is noticeable that the effect of isolates was smaller than that of cultivars. This would indicate that the reaction of cultivars to inoculation with different isolates of *S. nodorum* was determined mainly by the

**Table 7. Analysis of variance for the amount of SNB on leaves and heads of 5 spring triticale and 3 spring wheat cultivars scored on a 0-9 digit scale (0 = resistant, 9 = susceptible) under field conditions.**

| | Leaves | | | | Heads | | | |
|---|---|---|---|---|---|---|---|---|
| Source of variation | D. f. | Mean Square | F value | Proba-bility | D. f. | Mean Square | F value | Proba-bility |
| Blocks (B) | 4 | 844.03 | 18.13 | 0.01 | 4 | 13.17 | 1.84 | 0.28 |
| Scoring dates (R.d.) | 3 | 1553.60 | 33.37 | 0.00 | 1 | 39.55 | 5.52 | 0.08 |
| Error (B × S.d.) | 12 | 46.56 | | | 4 | 7.17 | | |
| Cultivars (C) | 7 | 193.32 | 272.50 | 0.00 | 7 | 238.49 | 552.86 | 0.00 |
| Isolates (I) | 15 | 11.3 | 15.65 | 0.00 | 15 | 4.58 | 10.62 | 0.00 |
| C × I | 105 | 0.636 | 0.90 | 0.76 | 105 | 0.71 | 1.65 | 0.01 |
| S.d. × C | 21 | 4.95 | 6.97 | 0.00 | 7 | 2.55 | 5.91 | 0.00 |
| S.d. × I | 45 | 0.693 | 0.98 | 0.51 | 15 | 0.167 | 0.39 | 0.98 |
| S.d. × I × C | 315 | 0.239 | 0.34 | 0.00 | 105 | 0.129 | 0.30 | 1.00 |
| Error (B×S.d.×C×I) | 2032 | 0.709 | | | 1016 | 0.431 | | |

cultivar genotype. Nonetheless, the differences in virulence among isolates were large enough to make cultivar response range from resistant to susceptible, depending on the isolate used (Tables 2, 6, 3, 4).

The effect of cultivar by isolate interaction was not significant only for SNB assessment on leaves of spring genotypes (Table 7). In all other cases, the latter effect, though highly significant, contributed only slightly to the total variance (Tables 1 and 7).

The significance of cultivar by isolate interaction indicated that there is some degree of specificity in the host-pathogen relationship. The low specificity in the pathosystem was confirmed through Spearman rank correlations. They revealed that rankings of the genotypes on their disease reaction to inoculation with individual *S. nodorum* isolates, and conversely, the rankings of isolates on their virulence expressed on the genotypes were correlated.

The analyses of variance of the disease scores revealed significant differences between disease scoring dates, primarily on leaves. This means that the disease progressed with the passing of time. For SNB on heads, scoring dates were bordering on significance for both winter and spring genotypes. This indicates that, due to adverse environmental conditions (primarily summer droughts), disease progress over time was slower on heads than on leaves (Tables 1 and 7). The highly significant interactions between disease scoring dates and other components of the analysis of variance indicate that the disease response progressed at different rates (Tables 1 and 7) on individual genotypes (S.d. by C) between consecutive scoring dates, and that the isolates (S.d. by I) caused more disease during the intervals between consecutive scoring dates.

This may have something to do with the susceptibility of plant tissue at different growth stages. Older tissue may be more susceptible. The significance of second and third degree interactions among years, scoring dates, isolates, and cultivars indicated that disease progress was influenced by all factors involved, i.e. host and pathogen genotypes, as well as the environment.

It should be pointed out that the interactions of years with isolates and cultivars (Table 1 and 7), although significant, were low and contributed only slightly to total variation. Thus rankings of cultivars based on their SNB reaction and isolates on their virulence were not substantially changed. Over consecutive years of the study they were consistently classified into the same resistance or pathogenicity groups (patterns). Only intragroup changes were observed over years during the study.

It is noticeable that the association between virulence patterns and the isolate's geographic origin in regard to location was stronger on leaves than on heads, e.g. isolates Lb-2-1, Lb-3-1, and Lb-4-1 from the same location showed more similarity on leaves (Tables 2 and 3; Figures 1 and 2) than on heads in regard to virulence patterns (Tables 6 and 4; Figures 3 and 4).

Of importance is the finding that the composite isolate (a volumetric mixture of isolates) was never the most virulent. It was usually classified into a group of isolates showing sometimes little more than an intermediate level of virulence (Tables 2, 6, 3, 4; Figures 1, 3, 2, 4).

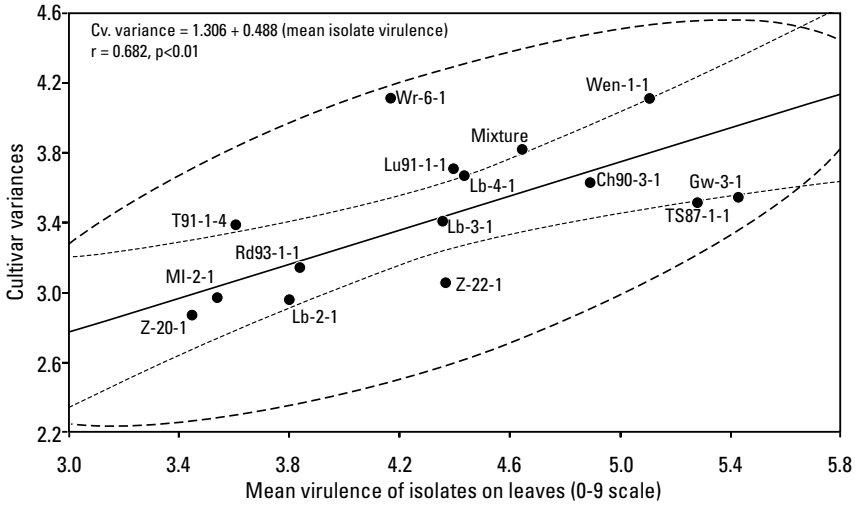


**Figure 1. Relationship between mean virulences of 15 *Stagonospora nodorum* isolates and their mixture (composite isolate) on leaves and variances of SNB reactions of 11 winter triticale and 4 winter wheat cultivars (3 years' data).**

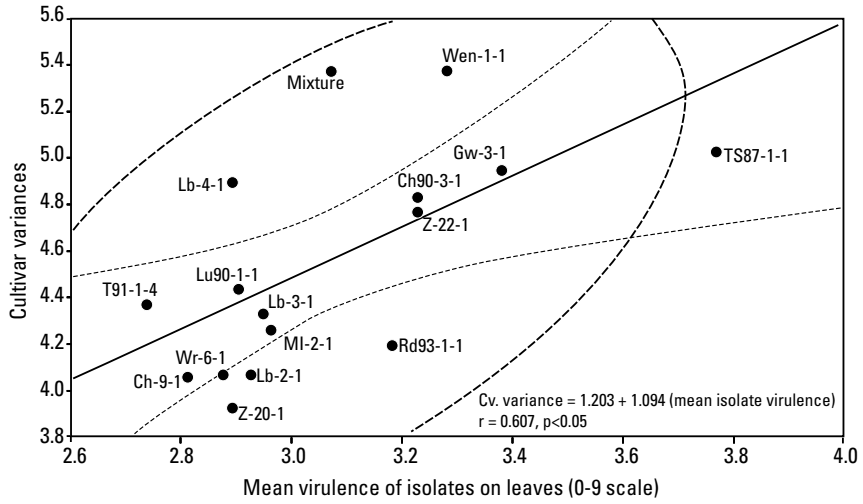


**Figure 2. Relationship between mean virulences of 15 *Stagonospora nodorum* isolates and their mixture (composite isolate) on leaves and variances of SNB reactions of 5 spring triticale and 3 spring wheat cultivars (3 years' data).**

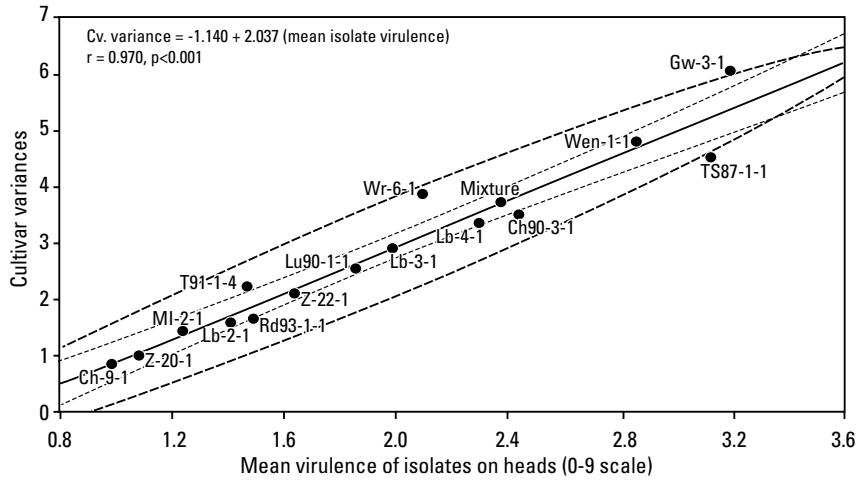


**Figure 3. Relationship between mean virulences of 15 *Stagonospora nodorum* isolates and their mixture (composite isolate) on heads and variances of SNB reactions of 11 winter triticale and 4 winter wheat cultivars (3 years' data).**

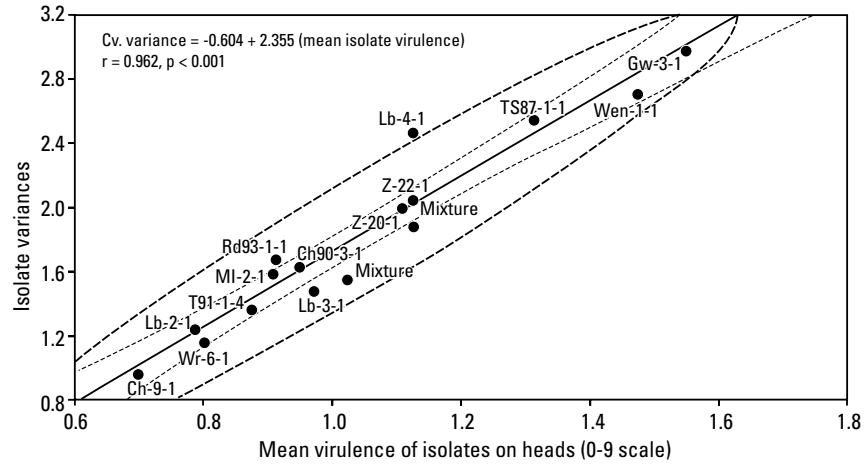


**Figure 4. Relationship between mean virulences of 15 *Stagonospora nodorum* isolates and their mixture (composite isolate) on heads and variances of SNB reactions of 5 spring triticale and 3 spring wheat cultivars (3 years' data).**

A positive correlation was found between the mean virulence of *S. nodorum* isolates on leaves and heads and the variances in SNB reaction among cultivars to the respective isolates (Figures 1, 3, 2, 4). Three years' data, especially on heads, show that highly virulent isolates were also efficient in differentiating the genotypes based on their resistance, e.g. isolates Wen-1-1, Gw-3-1, TS87-1-1 (Figures 1, 3, 2, 4). The differentiating capacity of the composite isolate appeared to be stronger on leaves (Figures 1 and 2) than on heads (Figures 3 and 4).

The data obtained do not support the conclusion that using a mixture of *S. nodorum* isolates is the most efficient way to differentiate triticale and wheat breeding materials for their SNB resistance. Instead, the data suggest that screening triticale and wheat genotypes using one or just a mixture of a few virulent isolates of *S. nodorum* seems to be most appropriate for practical breeding purposes. However, the statistically significant correlations of the cereal genotype ranks based on reactions to any pathogen isolate, as observed in the present study, suggest that a single isolate used for a prescreening breeding materials may provide reliable information on their SNB resistance.

The leaf and head reactions to SNB of the winter genotypes were spread throughout the scale (Figures 5 and 6) showing the whole range of reactions. In contrast, the spring cultivars

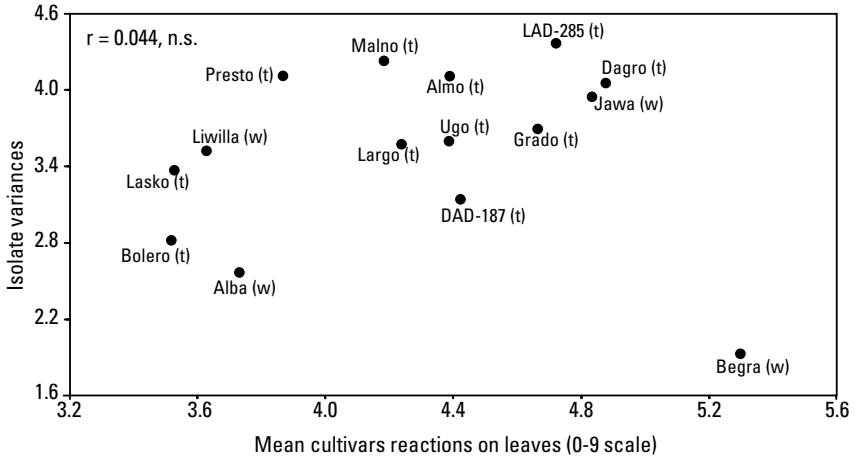


**Figure 5. Relationship between the mean reactions of 11 winter triticale (t) and 4 winter wheat (w) cultivars and variances of SNB induction on leaves of the cultivars by 15 *Stagonospora nodorum* isolates and their mixture (3 years' data).**

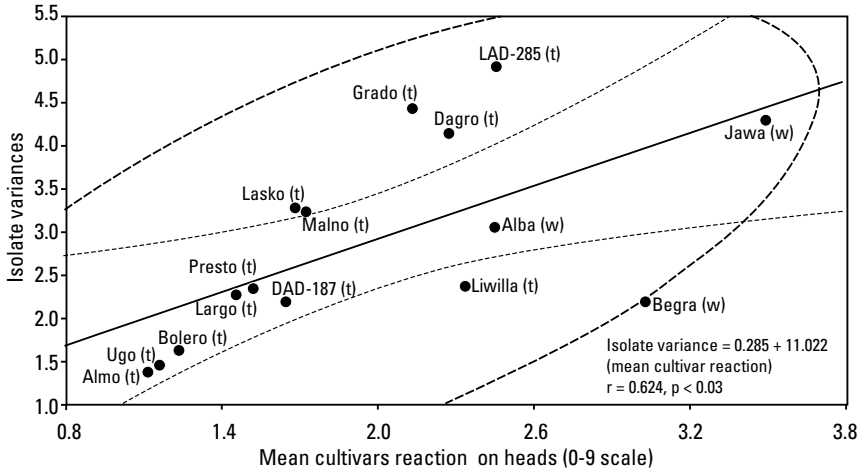


**Figure 6. Relationship between the mean reactions of 11 winter triticale (t) and 4 winter wheat (w) cultivars and variances of SNB induction on heads of the cultivars by 15 *Stagonospora nodorum* isolates and their mixture (3 years' data).**

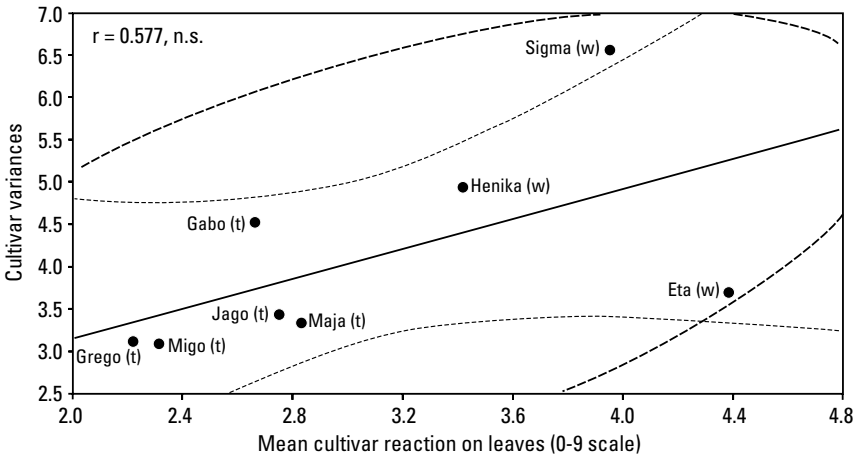


**Figure 7. Relationship between mean reactions of 5 spring triticale (t) and 3 spring wheat (w) cultivars and variances of SNB induction on leaves of the cultivars by 15 *Stagonospora nodorum* isolates and their mixture (3 years' data).**

showed two distinct resistance groups (Figures 7 and 8). Correlations between mean host reactions on leaves and heads and the variances in SNB induction among isolates were significant only for heads (Figures 5 and 7) and not for leaves (Figures 6 and 8). It was found that a highly susceptible genotype is not necessarily efficient in differentiating virulence among isolates (Figures 5, 4, 7, 8).

## Conclusions

The results produced in the study do not seem to support that using a mixture of *S. nodorum* isolates is the most efficient way to differentiate cereal breeding materials on their SNB resistance. The finding for *S. nodorum* is in accordance with results obtained by Zelikovitch and Eyal (1991) that inoculation of wheat seedlings and adult plants with a mixture of *S. tritici* isolates resulted in significant suppression of symptoms (necrosis and pycnidial coverage) compared to the most virulent single isolate in the mixture.

1. Screening for SNB resistance with a few virulent isolates that also show good differential capacity seems most appropriate. Screening breeding materials with a single highly virulent isolate may provide reliable information on their SNB resistance.
2. Sets of selected isolates and cultivars differentiated each other sufficiently well, but according to cluster analyses similar results could have been obtained with a lower number of both isolates and germplasm lines.

3. The reported data indicate that the interaction between *S. nodorum* isolates of the wheat biotype and wheat and triticale genotypes to a great extent is more a matter of statistics than a well defined physiological specialization. Whether the interaction is detected or not depends upon the amount of disease inflicted by the pathogen isolates on selected host genotypes, the effect of environmental conditions on disease development, and the tools and precision with which the host has been examined.

## Acknowledgments

This study was financially supported by the ICD-RSE-FAS-USDA (Project no. PL-AES-241, Grant no. MR-USDA-93-139) and by the Plant Breeding and Acclimatization Institute. The technical assistance of T. Bajor, E. Matyska, and E. Pawióska is gratefully acknowledged.

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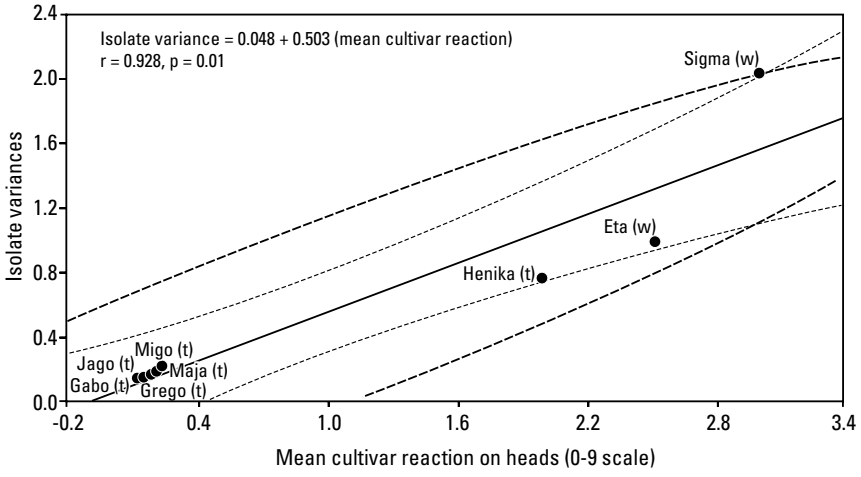


**Figure 8. Relationship between mean reactions of 5 spring triticale (t) and 3 spring wheat (w) cultivars and variances of SNB induction on heads of the cultivars by 15 *Stagonospora nodorum* isolates and their mixture (3 years' data).**

# Identification of a Molecular Marker Linked to Septoria Nodorum Blotch Resistance in *Triticum tauschii* Using F2 Bulked Segregant

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## Abstract

*A search was conducted for a molecular marker linked to a gene for resistance to septoria nodorum blotch in the* Triticum tauschii *accession RL5271. DNA was extracted from leaves of F2 plants which were progeny tested to identify homozygous resistant and homozygous susceptible F2 plants. The DNA from the homozygous resistant plants was pooled together and the low copy sequences were enriched using renaturation kinetics and hydroxyapatite to remove the repetitive DNA sequences. The pooled DNA from the homozygous susceptible plants was treated in the same manner. The pooled DNA and the parental DNA were screened using RAPD primers. Two markers present in the pooled resistant DNA and in the parental DNA were identified and cloned. These markers were verified using RFLPs with the cloned marker as a probe. One of the probes did not produce any polymorphism as a RFLP, even when a number of different restriction enzymes were used. The second marker was polymorphic between the two parents when the DNA was restricted with* Hin*dIII and then* Mse*I. In the 14 homozygous F2 plants tested, the marker was completely linked to the resistance gene. This marker may be valuable in introgressing the resistance gene from* T. tauschii *into a commercial bread wheat cultivar.*

Septoria nodorum blotch is the major disease of wheat (*Triticum aestivum* L.) in the Western Australian wheat belt. It is caused by *Stagonospora nodorum* (Berk.) Castellani & E.G. Germano (teleomorph *Phaeosphaeria nodorum* (E. Müller) Hedjaroude). The preferred method of control is the use of resistant cultivars, but this has been limited in the past by the lack of major genes for resistance. In bread wheat inheritance of resistance to septoria nodorum blotch is frequently complex (Mullaney et al., 1982; Scharen and Krupinsky, 1978).

The complex and additive nature of septoria nodorum resistance in bread wheats has made the selection for resistance difficult. Relatively small improvements in the overall resistance can be difficult to identify, as they can be masked by strong environmental effects. Selection of resistant plants is complicated by the association of resistance and both plant height and maturity (Rosielle and Brown, 1980; Scott et al., 1982). The use of a molecular marker would help overcome these problems by avoiding interpretation of phenotype under environmental influences (Allen, 1994). Furthermore, if the molecular marker is co-dominant, it will make it possible to identify an individual plant as homozygous for resistance without further progeny testing. This ability to identify homozygous plants would be particularly useful in any program where extensive backcrossing would be required, such as the introgression of a resistance gene from wild wheats.

The aim of this work was to identify a molecular marker that is linked to a single gene conferring resistance to septoria nodorum blotch identified in the accession RL5271 of the wild wheat *Triticum tauschii*.

## Materials and Methods

A cross between the resistant accession RL5271 and the susceptible accession CPI110889 was progeny tested. The F2 plants were progeny tested using F3

families to identify homozygous F2 plants. The DNA of eight homozygous resistant F2 plants and 14 homozygous susceptible F2 plants was selected for the analysis.

Equal amounts of DNA from the homozygous resistant F2 plants were bulked together as was the DNA from the homozygous susceptible F2 plants. Each bulked sample and the DNA from each parent were divided into two aliquots. One aliquot was left untreated. The second aliquot was treated to enrich the proportion of low copy sequence DNA by removing the repetitive DNA sequences using hydroxyapatite after selective renaturation. The technique followed Eastwood et al. (1994). The double-stranded DNA was removed from the samples after a Cot value of 120 had been achieved (Smith and Flavell, 1975). The DNA was re-suspended in 0.1xTE buffer and diluted to a final concentration of 5ng/ml of DNA.

RAPD reactions were carried out on a Perkin-Elmer PE9600. The random primers were produced by Operon Technologies (Alameda, Ca.). The RAPD products were electrophoresed on polyacrylamide gels, and the gels were stained using ethidium bromide with gel images recorded digitally using a Bio-Rad Gel Doc 1000 system. For each primer nine reactions were carried out: a negative control, sterile distilled water, was used instead of template DNA, the next four reactions used the total genomic DNA as template, and the final four reactions used the non-repetitive DNA as template.

If a polymorphism was identified, the polymorphic band was stabbed with a sterile syringe needle. The needle was washed in sterile distilled water which was used as template for a further RAPD reaction using the same primer and reaction conditions that had generated the polymorphism. The amplified fragment was ligated into the plasmid pGEM-T. The plasmids were transformed into *E. coli* cells by heat shock. The following day colonies were screened using PCR primers, and permanent cultures were established from insert-containing clones.

The cloned polymorphic band was used as an RFLP probe to verify and determine the linkage of the potential marker to the resistance gene. Total genomic DNA of the parental accessions was digested with a number of restriction enzymes, singly and in combination, to find a restriction enzyme that would produce a polymorphism with the marker between the resistant and the susceptible parent. The marker band was prepared using an alkali-SDS minprep, double digested with *Nco*I and *Sac*I, separated on an agarose gel and purified using agarase. The purified band was labeled using random nonamer primers with $^{32}$P as dCTP. The membranes were hybridized overnight, washed the following morning, and exposed to autoradiograph film for up to one week.

When a suitable restriction enzyme had been selected, the DNA from the selected seven homozygous resistant F2 plants and seven homozygous susceptible F2 plants was digested, electrophoresed and blotted using an alkali capillary blot. The membranes were autoradiographed for up to two weeks.

## Results

Sixty random primers were used to screen the DNA samples. Two polymorphic bands were identified by comparing the banding pattern of the resistant parent and the resistant bulk with the susceptible parent and the susceptible bulk. The first was amplified using primer OPJ6 and was approximately 770bp long ($OPJ6_{770}$). The second polymorphic band was amplified using primer OPJ18 and the fragment was approximately 350bp ($OPJ18_{350}$). Both bands were ligated into pGEM-T.

For the first marker, $OPJ6_{770}$, no restriction enzyme nor combination of enzymes tested was able to produce a polymorphism between the resistant and the susceptible parent. Consequently the marker could not be tested for its linkage to resistance. The second marker, $OPJ18_{350}$, produced a polymorphism between the resistant and susceptible parent when the DNA was double restricted with *Hin*dIII and followed by *Mse*I. The polymorphism was present as an extra band in the resistant parent. All of the F2 plants tested were correctly classified as resistant or susceptible when the marker was used.

## Discussion

A marker linked to a resistance gene to septoria nodorum blotch in the *Triticum tauschii* accession RL5271 has been found. The marker and the resistance gene appear to be completely linked; however, a greater number of plants need to be tested to develop a more accurate estimate of the linkage. The multiple bands produced when the marker was used as an RFLP probe indicate that regions of repetitive DNA exist within the marker, which is not uncommon (Eastwood et al., 1994).

The RFLP probe is currently being sequenced and primers designed that will allow the development of a sequence characterized amplified region (SCAR). This will allow a simpler and faster test to be developed. The simpler assay will be required if it is to be used extensively in a marker-assisted selection program. If the designed primers do amplify a polymorphism between the resistant and the susceptible plants, it may be one of several types. The primers may amplify a region that is present in the resistant plants but not in the susceptible plants. This marker will be dominant and will not allow for the identification of heterozygotes; however, this will enable simple alternatives to running the PCR product on a gel to determine if the band is present or not to be used (Dedryver et al., 1996). This will remove one of the major time constraints and limiting factors in the screening of large numbers of plants. Alternatively, the SCAR primers could amplify different size fragments between the resistant and the susceptible plants. This type of polymorphism will allow heterozygotes to be identified (co-dominant), as both alleles will be amplified. This in turn will allow the selection of homozygotes for use in a backcrossing population, where the direct and accurate selection of homozygotes will be of greatest benefit.

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# Inheritance of Septoria Nodorum Blotch Resistance in a *Triticum tauschii* Accession Controlled by a Single Gene

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## Abstract

*A potentially useful source of resistance has been identified in an accession of* Triticum tauschii. *A cross was made between the resistant* T. tauschii *accession, RL5271, and a susceptible accession, CPI110889, to study the genetics of resistance from this source. The parental accessions and the F1 and F3 progeny were screened in the glasshouse as seedlings. The resistant parent took significantly longer to develop symptoms, developed significantly fewer lesions, and expressed significantly lower levels of disease than the susceptible parent. The F1 mean response for disease severity indicated there was no complete dominance. The genotypic ratios generated by screening the F3 families were not significantly different from the genotypic ratio expected for a single gene. The effectiveness and simple genetic control of the resistance in the* T. tauschii *accession RL5271 may be useful as a resistance source in a bread wheat breeding program.*

Septoria nodorum blotch, caused by *Phaeosphaeria nodorum* (E. Müller) Hedjaroude (anamorph *Stagonospora nodorum* (Berk.) Castellani, and E.G. Germano), is the principal foliar disease of wheat (*Triticum aestivum* L.) in the cereal belt of Western Australia (Murray and Brown, 1987). The preferred method of control is the use of resistant cultivars. In bread wheat inheritance of resistance to septoria nodorum blotch is reported to be complex, with an additive action of the genes (Mullaney et al., 1982; Scharen and Krupinsky, 1978). Resistance is often linked to a number of traits such as height and maturity (Rosielle and Brown, 1980; Scott et al., 1982), which makes breeding for resistance difficult.

Promising levels of resistance to septoria nodorum blotch were found when a collection of *T. tauschii* accessions was investigated for resistance to septoria nodorum blotch. The ability to use these potential resistance sources in a wheat breeding program will be influenced by the number of genes controlling the resistance and its expression in a bread wheat background. The aim of this work was to investigate the differences between the parents in the components of resistance and to determine the number of genes controlling resistance to septoria nodorum blotch in the *Triticum tauschii* accession RL5271.

## Materials and Methods

The resistant *T. tauschii* accession RL5271 was crossed to a susceptible accession CPI110889. The 17 F1 progenies were selfed, producing 300 F2s which were selfed to produce the F3 families. The F1 plants were screened for resistance to assess dominance, and the F3 plants were screened to determine the genotypic ratio, which was used to estimate the number of genes controlling resistance.

The *T. tauschii* seed was germinated on sterile filter papers in petri dishes and vernalized before being sown into pots in a glasshouse. In the F3 generation, a random selection of approximately 200 F3 families was screened sequentially in two sub-populations. Each sub-population was grown on a single bench in the glasshouse, and each F3 family contained 10 plants.

The plants were inoculated when the third leaf on the main stem had emerged. A single isolate of *Stagonospora nodorum* was used. The inoculum was produced using V8 Czapek-Dox agar (V8CDA) as a

modified method of Cooke and Jones (1970). The plants were inoculated with $10^6$ spores/mL, with a surfactant, until the point of run-off using an airless spray-painting gun while the pots were revolving on a turntable at 33 rpm. After inoculation the plants were placed in a humidity chamber for 48 hours and then returned to the bench. The plants were rated using the youngest fully emerged leaf at the time of inoculation on the main tiller eight days after inoculation. The leaves were rated using disease score (DS), a 0 to 5 severity scale where 0 was an immune response and a score of 5 was given if the leaves were fully necrosed. The DS is based upon the number, size, and type of lesions, as well as the area of the leaf covered by the lesions.

The two parental accessions were assessed twice for two components of resistance, and the F1 plants were assessed once. The components were the incubation period (IP, the number of days from the time of inoculation till symptoms first appear) and the infection frequency (IF, the number of lesions per square centimeter of leaf tissue at IP). In the first screening the IF had to be transformed using a natural logarithmic function, and during the second screening it was transformed using a square root function to remove the trends in the residuals.

The F3 families were classified using the response of individual plants within an F3 family. During the rating it was evident from the parental accession's response that a score of less than 2.5 was resistant and a score of equal to or greater than 2.5 was susceptible. A F3 family was classified as resistant if all its plants had a DS of less than 2.5 and as susceptible if all the plants had a disease score greater than or equal to 2.5. Any family with plants with a DS of less than 2.5 and greater than 2.5 were classified as segregating.

## Results

In both tests the components of resistance showed significant differences between the resistant and susceptible parents in their response to infection. The IP for the parents was not significantly different in the first screening but the resistant parent (RL5271) had a significantly longer IP than the susceptible parent (CPI110889) in the second screening. The mean response of the F1 for the IP was significantly longer than both parental accessions (Table 1). The IF was significantly higher in the susceptible parent than the resistant parent in both screenings. The F1 mean response for the IF was not significantly different from the resistant parent, but it was significantly lower than the susceptible parent (Table 1). In both screenings the resistant parent had a significantly lower DS than the susceptible parent. The mean DS of the F1 plants was intermediate between the two parents and was significantly different from both parents (Table 1).

**Table 1. The incubation period (IP), infection frequency (IF), and disease score (DS) of the F1 mean response during screening 1, and the parental accessions, RL5271 and CPI110889, during screenings 1 and 2.**

| | IP (days) | IF (lesions/ $cm^2$)[i] | DS |
|---|---|---|---|
| Screening 1 | | | |
| F1 | 8.5b[ii] | 0.8a | 1.3b |
| RL5271 (Resistant parent) | 6.8a | 0.8a | 0.4a |
| CPI110889 (Susceptible parent) | 5.9a | 2.0b | 2.2c |
| Screening 2 | | | |
| RL5271 (Resistant parent) | 5.5a | 2.4a | 0.9a |
| CPI110889 (Susceptible parent) | 3.5b | 5.8b | 3.3b |

i Back transformed values are presented for the IF in both screenings.

ii Numbers from the same screening and for the same trait followed by different letters are significantly different from each other at $p<0.05$.

During the screening of the F3 families, the coefficient of variation was low and the broad sense heritability high when both sub-populations were screened (Table 2). In the first sub-population, 25 F3 families were classified as resistant, 49 as segregating, and 26 as susceptible. The observed genotypic ratio was not significantly different from the genotypic ratio expected for a single gene ($X^2=0.060$, $p=0.97$) (Table 2). In the second sub-population, there were 20 resistant F3 families, 48 segregating, and 26 susceptible families. Again the observed ratio was not significantly

**Table 2. Genotypic ratios of the two F3 family sub-populations and the combined population, and their comparison with the genotypic ratio expected for a single gene.**

| Population | Resistant | Segregating | Susceptible | Total | 1:2:1 ($X^2$, p-value) |
|---|---|---|---|---|---|
| Sub-population 1 | 25 | 49 | 26 | 100 | 0.06, 0.97 |
| Sub-population 2 | 20 | 48 | 26 | 94 | 0.81, 0.67 |
| Combined | 45 | 97 | 52 | 194 | 0.51, 0.78 |

different from the expected genotypic ratio for a single gene ($X^2$=0.81, p=0.67) (Table 2). When the genotypic ratios of the two sub-populations were compared using a Pearson's chi-squared test for homogeneity, they were not significantly different ($X^2$=0.84, p=0.66). The combined ratio (45 resistant F3 families, 97 segregating, and 52 susceptible families) was not significantly different from the genotypic ratio expected for a single gene ($X^2$=0.50, p=0.78) (Table 2).

## Discussion

Resistance to septoria nodorum blotch in the *Triticum tauschii* accession RL5271 is controlled by a single gene. From the F1 mean response there was no evidence of complete dominance or recessiveness for resistance. The resistant parent took significantly longer to develop symptoms, developed significantly fewer infections and expressed significantly lower levels of disease than the susceptible parent. The variation in the resistance components between the two parents was consistent with previous studies reporting a resistance response. The IP has been shown to vary significantly among wheats with longer IP being associated with resistance (Wilkinson et al., 1990; Bruno and Nelson, 1990). The IF was significantly lower for the resistant parent, which is consistent with previous work where a low IF has been associated with resistance (Wilkinson et al., 1990; Ma and Hughes, 1993; Loughman et al., 1996). The inheritance of the IF has been found to be clearly dominant in the F1 (Ma and Hughes, 1993) which is consistent with this study.

Resistance in *Triticum tauschii* accession RL5271 is the first single-gene resistance to septoria nodorum blotch identified in the D genome. Resistance to septoria nodorum blotch has been previously identified in another *Triticum tauschii* accession (=*Aegilops squarrosa* Tausch). It was found to be controlled by three genes, located on chromosomes 3D, 5D, and 7D, with 3D being the most important of the three (Nicholson et al., 1993). The simplicity of the inheritance and strong expression of the resistance gene in *T. tauschii* warrants making an attempt at introgressing the resistance into a bread wheat background.

## Acknowledgment

This work was funded by the Grains Research and Development Corporation of Australia.

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## *Session 4: Population Dynamics*

# Population Genetics of *Mycosphaerella graminicola* and *Phaeosphaeria nodorum*

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## Abstract

*Restriction fragment length polymorphisms (RFLPs) in the nuclear (nu) and mitochondrial (mt) genomes were used to determine the genetic structure of populations of* Mycosphaerella graminicola *and* Phaeosphaeria nodorum *from around the world. Both fungi have genetic structures consistent with a regular sexual cycle and a high degree of gene flow occurring on a global scale. Gene as well as genotype diversity in the nuDNA are high for both fungi. There was no evidence for widespread clones within field populations of either fungus. While both fungi had less diversity in the mtDNA,* M. graminicola *exhibited significantly less diversity for the mtDNA compared to* P. nodorum. Mycosphaerella graminicola *populations from Patzcuaro, Mexico, and Australia exhibited significantly lower gene diversity, suggesting that these populations originated from a limited number of founders. Collections of* M. graminicola *taken from the same field between 1990 and 1995 showed that genetic drift is negligible, suggesting that effective population sizes are very large. A replicated field experiment showed that selection can cause significant changes in genotype frequencies during the course of a growing season, and that the contributions of immigration and recombination to genetic diversity in field populations can change over the growing season.*

Ten years ago, we began developing DNA-based markers as tools to learn about the population genetics of the wheat leaf blotch pathogen *Mycosphaerella graminicola*. One year later, we began parallel studies using the same genetic tools for the wheat glume blotch pathogen *Phaeosphaeria nodorum*. We began with two elementary questions regarding the population genetics of both fungi. How much genetic diversity is present within populations? How is genetic diversity distributed within and among populations? As our knowledge of the genetic structure of both pathogens deepened, we addressed more complex questions. What are the relative contributions of sexual and asexual reproduction to the genetic structure of populations? How stable are populations over time? Does selection for specific pathogen genotypes occur on particular host genotypes? Is there evidence for host specialization in these pathosystems?

To address the latter questions, we utilized increasingly sophisticated field experiments to differentiate among the various evolutionary forces acting on populations of these fungi. In this manuscript, I will briefly review our understanding of the population genetics of both fungi at this point in time. The majority of this manuscript was distilled from a review chapter written for the Long Ashton Symposium on Septoria in Cereals held in 1997. Detailed data to support our interpretations are presented in that chapter (McDonald et al., 1999).

## Materials and Methods

### DNA markers

The RFLP markers utilized for these studies were developed in the same way for both fungi utilizing the methods described in McDonald and Martinez (1990b). Single-locus probes were used to measure gene diversity for individual RFLP loci and to measure population subdivision and genetic similarity among populations (McDonald and Martinez, 1990a; Boeger et al., 1993; McDonald et al., 1994; Keller et al., 1997a,b). Probes that hybridized to repetitive elements were used for

DNA fingerprinting and to make measures of genotype diversity (McDonald and Martinez, 1991; Keller et al., 1997a,b). To measure variation in the mitochondrial (mt) genome, we purified mtDNA and used it as a probe to hybridize to the entire digested mitochondrial genome for each individual.

### Sampling methods

Hierarchical sampling methods now are commonly used in plant pathology (Kohli et al., 1995; McDonald et al., 1995; McDonald, 1997). For many of the collections described in this manuscript, a standardized six-site hierarchy was used to sample field populations (McDonald et al., 1999). For other field collections, long transects were arranged in a field and leaves were sampled at 1-2-meter intervals along each transect. For some collections, leaves were collected at random in a field. All isolates in a collection were taken from a single field at a single time point for the majority of collections. I refer to these collections as field populations. Four collections of isolates came from the same country or geographical region, but did not originate from a single field. I refer to these as regional populations to distinguish them from field populations.

For *P. nodorum*, the Arkansas population was made up of isolates collected from infected seed from two different seed lots distributed in Arkansas. The crested wheatgrass population consisted of isolations made from the weed crested wheatgrass over a two-year period in the state of North Dakota. The goal for each collection was to have a large enough field sample to be confident that allele frequencies for individual RFLP loci were representative of the entire field population. Only populations having at least 25 isolates were included in the analysis. A summary of the *M. graminicola* and *P. nodorum* isolates in our collection was presented previously (McDonald et al., 1999).

### Data analysis

Methods used to measure gene and genotype diversity, genetic similarity, population subdivision, and gametic disequilibrium have been published elsewhere (McDonald and Martinez, 1990a; Boeger et al., 1993; Chen et al., 1994; Chen and McDonald, 1996). The DNA fingerprint probes were validated for both fungi in previous experiments (McDonald and Martinez, 1991; Keller et al., 1997b). Here we will pay special attention to the collection of *M. graminicola* isolates from Mexico.

## Results and Discussion

### Genetic diversity and mating/reproduction systems

Both gene and genotype diversity are high for both fungi. On average, 18 alleles were present for 11 RFLP loci across ~2000 isolates of *M. graminicola,* and five alleles were present for seven loci across ~950 isolates of *P. nodorum*. In each population, between 3-4 alleles were present in ~90% of the isolates (McDonald et al., 1999). Nei's measure of gene diversity across all populations averaged 0.44 across eight RFLP loci for *M. graminicola* and 0.51 across seven RFLP loci for *P. nodorum*. Though both fungi exhibited high gene diversities, *M. graminicola* had a significantly greater number of alleles per locus then *P. nodorum* and *P. nodorum* had a more even distribution of allele frequencies.

Comparisons of gene diversities across populations revealed some interesting patterns. The Israeli population had significantly higher gene diversity than other populations around the world (McDonald et al., 1999). We interpret this as evidence that the Middle East is a center of diversity for *M. graminicola*, and the likely center of origin for this fungus. The Mexican population exhibited lower gene diversity than other populations. It was made from leaves collected by Dr. Lucy Gilchrist at a CIMMYT disease nursery in Patzcuaro, a location remote from other wheat fields (L. Gilchrist, personal communication).

The Mexican population also exhibited a lower genotype diversity than expected (McDonald et al., 1999), having a higher incidence of a few widespread clones than any other population surveyed. These findings are consistent with a small founding population that has not undergone regular sexual reproduction. We hypothesized that these isolates represent an historic inoculation of the Patzcuaro site with a few isolates that were not sexually compatible. We hope to collect another *M. graminicola* field population from wheat-growing areas of Mexico to obtain a more representative sample of this fungus in that region.

The gene diversity in the *P. nodorum* population was lowest in the collections from crested wheatgrass and from Mexico (McDonald et al., 1999). These were the only two collections that were fixed for one allele at an RFLP locus. These populations also exhibited substantial differences in allele frequencies for some RFLP loci when compared to other populations (McDonald et al., 1999). It is possible that the lower gene diversity and different allele frequencies in the crested wheatgrass population resulted from selection due to host specialization. However, the finding that common alleles are shared between isolates taken from crested wheatgrass and wheat suggests that crested wheatgrass may serve as a reservoir of inoculum for *P. nodorum* that infects wheat. The differences in the Patzcuaro population may reflect founder events and the geographic isolation of this location as described previously for *M. graminicola*.

*Genotype diversity.* Genotype diversity in the nuclear genome was high for both fungi (McDonald et al., 1999). In the majority of populations surveyed, each leaf was colonized by a unique fungal genotype. When isolates shared the same DNA fingerprint, they usually were sampled from the same leaf. The only evidence for a widespread clone in *M. graminicola* was in the Patzcuaro population. The only evidence for a widespread clone in *P. nodorum* was from our first collection in a Texas field (McDonald et al., 1994).

It appears that the typical population of both fungi exhibits a low degree of clonality at the field level. Clones were usually distributed across an area of approximately 1 square meter and did not become widespread. This finding is consistent with limited spread of the splash-dispersed conidia for both fungi.

Our interpretation of the high degree of genotype diversity is that populations of both fungi undergo regular sexual cycles and that the primary inoculum for both fungi is likely to be ascospores (Chen and McDonald, 1996; Keller et al., 1997b). We have used tests for the randomness of associations among RFLP loci to indicate the frequency of sexual recombination for both fungi (Chen and McDonald, 1996). Associations were random for both fungi in the cases where sample sizes were adequate to make robust tests for disequilibrium, supporting the hypothesis of random mating. Though the sample size was smaller (N=160), the Israel population also showed the gametic equilibrium and high genotypic diversity typical of a random mating fungus (McDonald et al., 1999), suggesting that the teleomorph is present in the Middle East.

After a field experiment provided evidence that the teleomorph was contributing significantly to infection during the growing season (Zhan et al., 1998), we sampled lower leaves in the crop canopy in Oregon and found the teleomorph existing as a significant fraction of all fruiting bodies on wheat leaves (C. Cowger, C.C. Mundt, and B.A. McDonald, unpublished).

*mtDNA diversity.* For both fungi, the mtDNA exhibited less diversity then the nuDNA. The mtDNA genome ranges from 48-62 kb in size for both fungi. Among 385 isolates of *P. nodorum* from Switzerland, Texas, and Oregon, we found only 46 different mtDNA haplotypes compared to over 300 nuDNA haplotypes. Fifteen of the mtDNA haplotypes were shared among these field populations (S. Keller and B. A. McDonald, unpublished data).

The mtDNA of *M. graminicola* exhibited even lower diversity. We routinely found only 2-3 mtDNA haplotypes in field populations of *M. graminicola* (McDonald et al., 1999). We sometimes found only one *M. graminicola* mtDNA haplotype in a field (McDonald et al., 1999). We picked the three most common mtDNA haplotypes from around the world and digested the mtDNA with 10 different restriction enzymes that sampled ~5% of the entire 48 kb of mtDNA sequence in an attempt to detect cryptic variation. Types 1, 2, and 3 mtDNA haplotypes produced identical digestion patterns with all enzymes in all populations tested, suggesting that these mtDNA haplotypes are identical globally (B.A. McDonald and K. Hogan, unpublished). We have proposed that the limited mtDNA diversity is due to a selective sweep that occurred as *M. graminicola* populations became specialized to infect bread wheat (McDonald et al., 1999).

## Genetic drift

The high nuclear gene diversity found in most populations for both fungi suggests that population sizes are very large and the effects of genetic drift are small. In a previous experiment, we showed that the population genetic structure of an *M. graminicola* population did not change over a three-year period of 1990-1992 (Chen et al., 1994). We have extended data from this population to 1995 and find the same result. The frequencies of neutral RFLP alleles did not change significantly over time (Table 1), and the degree of population subdivision across years is less than 1% (Table 2), demonstrating that population sizes are large enough to make genetic drift insignificant as an evolutionary force in populations of this fungus.

## Population subdivision and gene flow

The distribution of genetic diversity across populations was consistent with a significant level of gene flow for both fungi. Common alleles at individual RFLP loci were shared among nearly all populations (McDonald et al., 1999), and allele frequencies often were quite similar though populations were separated by thousands of kilometers (McDonald et al., 1999). We have interpreted this similarity as indicating a significant degree of gene flow among populations of both fungi on an international scale (McDonald et al., 1995; Keller et al., 1997). Global estimates of $G_{ST}$ were 0.06 and 0.07 for *M. graminicola* and *P. nodorum*, respectively. These values of $G_{ST}$ are consistent with movement of 11 and 7 individuals among populations globally every generation.

The present lack of subdivision in the nuclear genome probably reflects historic movement of both fungi around the world. It is possible that gene flow is not a

**Table 1. Allele frequencies for seven RFLP loci in *Mycosphaerella graminicola* collections from Corvallis, Oregon, in 1990, 1991, 1992, and 1995. Alleles present at a frequency of less than 0.05 in all four populations were pooled into one category (allele "p"). Sample sizes (N) used to calculate allele frequencies are shown for each locus. The chi-square values and their degrees of freedom (in parentheses) for measurement of population differentiation are also included.**

| | | Populations | | | | |
|---|---|---|---|---|---|---|
| RFLP Locus | Allele | 1990 | 1991 | 1992 | 1995 | $c^2$ |
| *pSTS192-PstIA* | 1 | 0.943 | 0.877 | 0.911 | 0.953 | 6.36(6) |
| | 2 | 0.030 | 0.088 | 0.054 | 0.047 | |
| | p | 0.027 | 0.035 | 0.036 | 0.000 | |
| | N | 401 | 57 | 56 | 43 | |
| *pSTS192-PstIB* | 1 | 0.980 | 1.000 | 0.981 | 1.000 | 1.99(3) |
| | P | 0.020 | 0.000 | 0.019 | 0.000 | |
| | N | 406 | 56 | 56 | 44 | |
| *pSTS14-PstI* | 1 | 0.808 | 0.825 | 0.821 | 0.818 | 0.85(6) |
| | 2 | 0.187 | 0.175 | 0.179 | 0.182 | |
| | p | 0.005 | 0.000 | 0.000 | 0.000 | |
| | N | 407 | 57 | 56 | 44 | |
| *pSTS2-PstI* | 1 | 0.648 | 0.667 | 0.571 | 0.818 | 8.87(9) |
| | 2 | 0.085 | 0.056 | 0.125 | 0.023 | |
| | 3 | 0.203 | 0.204 | 0.214 | 0.136 | |
| | p | 0.065 | 0.074 | 0.089 | 0.023 | |
| | N | 400 | 54 | 56 | 44 | |
| *pSTL10-PstI* | 1 | 0.680 | 0.649 | 0.518 | 0.643 | 14.51(9) |
| | 2 | 0.051 | 0.053 | 0.107 | 0.000 | |
| | 3 | 0.220 | 0.281 | 0.357 | 0.286 | |
| | p | 0.049 | 0.018 | 0.018 | 0.071 | |
| | N | 409 | 57 | 56 | 43 | |
| *pSTL53-PstI* | 1 | 0.475 | 0.561 | 0.536 | 0.465 | 26.86(18) |
| | 2 | 0.119 | 0.070 | 0.107 | 0.023 | |
| | 3 | 0.161 | 0.158 | 0.232 | 0.070 | |
| | 5 | 0.042 | 0.053 | 0.000 | 0.047 | |
| | 6 | 0.119 | 0.140 | 0.107 | 0.209 | |
| | 7 | 0.027 | 0.000 | 0.000 | 0.047 | |
| | p | 0.057 | 0.018 | 0.018 | 0.140 | |
| | N | 404 | 57 | 56 | 43 | |
| *pSTS197-PstI* | 1 | 0.632 | 0.649 | 0.589 | 0.591 | |
| | 2 | 0.227 | 0.175 | 0.286 | 0.364 | 9.65(9) |
| | 3 | 0.108 | 0.158 | 0.107 | 0.045 | |
| | p | 0.033 | 0.018 | 0.018 | 0.000 | |
| | N | 259 | 57 | 56 | 44 | |

**Table 2. Nei's measures of gene diversity and population subdivision for 7 RFLP loci in *Mycosphaerella graminicola* populations collected from Corvallis, Oregon, in 1990, 1991, 1992, and 1995.**

| | Hi [a] | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| RFLP Loci | 1990 | 1991 | 1992 | 1995 | HT[b] | HS[c] | Dst[d] | Gst[e] |
| *pSTS192A-PstI* | 0.110 | 0.222 | 0.166 | 0.091 | 0.126 | 0.125 | 0.001 | 0.008 |
| *pSTS192B-PstI* | 0.039 | 0.000 | 0.104 | 0.000 | 0.039 | 0.039 | 0.000 | 0.007 |
| *pSTS14-PstI* | 0.312 | 0.289 | 0.294 | 0.298 | 0.307 | 0.307 | 0.000 | 0.000 |
| *pSTS2-PstI* | 0.531 | 0.508 | 0.610 | 0.323 | 0.524 | 0.520 | 0.004 | 0.007 |
| *pSTL10-PstI* | 0.486 | 0.497 | 0.592 | 0.526 | 0.506 | 0.501 | 0.005 | 0.010 |
| *pSTL53-PstI* | 0.716 | 0.633 | 0.636 | 0.722 | 0.704 | 0.700 | 0.004 | 0.006 |
| *pSTS197-PstI* | 0.537 | 0.523 | 0.560 | 0.517 | 0.540 | 0.536 | 0.004 | 0.007 |
| Pooled | 0.390 | 0.382 | 0.423 | 0.354 | 0.392 | 0.390 | 0.003 | 0.006 |

Notes: a= gene diversity for each population; b= combined gene diversity across all collections; c= gene diversity within populations; d= gene diversity among populations; e= population differentiation

significant evolutionary force at present. However, we consider it more likely that some gene flow continues as a result of the global commerce in grain. The obvious mechanism for gene flow on a regional basis is air-dispersed ascospores. In the case of *P. nodorum*, the most likely mechanism for intercontinental dispersal is infected seed (King et al., 1983). Since it has been shown that *M. graminicola* can infect seed (Brokenshire, 1975) we consider it likely that this also is the mechanism for long distance gene flow in *M. graminicola*. Whatever the mechanism, the high degree of similarity in populations around the world for both fungi suggests that they have been transported around the world by humans.

### Evidence for selection

We recently completed an experiment to measure competition among 10 genotypes of *M. graminicola* in a field setting. The 10 isolates were inoculated onto three host treatments consisting of a moderately resistant wheat variety (Madsen), a susceptible wheat variety (Stephens) and a 1:1 mixture of these cultivars. Our most important finding in this experiment was that intense competition appeared to occur among the different genotypes. Significant changes in the frequencies of specific pathogen genotypes occurred over the season (McDonald et al., 1999). Some isolates showed evidence for adaptation to particular hosts. The results from this experiment provided our first direct evidence that selection operates on specific *M. graminicola* pathogen genotypes in a field setting.

We have not yet conducted similar replicated field experiments to measure selection in *P. nodorum*. But we have indirect evidence that selection does not result in widespread clones that are adapted to specific host genotypes. In an experiment conducted in Switzerland in collaboration with Martin Wolfe's group, we sampled 50 isolates of *P. nodorum* from each of nine wheat fields near Zurich. Three different wheat varieties were represented three times each among the nine wheat fields. Though fields planted to the same variety used the same source of seed, no genotypes were shared among field populations. Only six pairs of clones were found among the 432 isolates that were assayed. Isolates with the same DNA fingerprints always came from the same site within a field (Keller et al., 1997b).

Taken together, all of our experiments suggest that nuclear genotypes do not persist through time for either fungus. Instead, the genes are the units of selection that are carried forward across generations. Selection operates on the population instead of the individual. In order to gain a representative spectrum of the diversity for virulence in natural populations, plant breeders should include the widest possible diversity of strains when screening germplasm for resistance to these fungi. Similarly, chemical companies should include at least several hundred strains in their screens for resistance to fungicides.

## Conclusions

Given our present data, we have drawn the following conclusions regarding the evolutionary forces that affect the population genetics of *M. graminicola* and *P. nodorum*:

- For both fungi, the mating system includes both sexual and asexual reproduction. Asexual reproduction may have an important impact over an area of a few square meters, but the sexual reproduction has much greater consequences for the evolutionary biology of both fungi. Genotypes are ephemeral but genes persist in populations through time.
- Population sizes are large enough to make genetic drift negligible for both fungi. Large population sizes also ensure that ample mutations are present in every population to allow for a rapid response to selection, e.g. mutations from avirulence to virulence for major resistance genes. The population from Patzcuaro, Mexico, exhibits a genetic structure consistent with a founder effect.
- Gene flow is sufficient to unite large geographical areas into a single genetic population. If gene flow is ongoing, then breeders should continue to test their resistant lines over the widest possible geographical area. If gene flow is episodic, continued vigilance is needed to limit the spread of new virulence genes and fungicide resistance genes. Quarantines in areas with low gene diversity, such as Australia, should be enforced to limit the evolutionary potential of these populations. If CIMMYT continues to use Patzcuaro as a field site to screen for resistance

to *M. graminicola* and *P. nodorum*, it may want to consider introducing more diverse fungal populations from other parts of Mexico into this disease nursery.

- Selection appears to operate on both nuclear and mitochondrial genomes in *M. graminicola*. Though selection may increase the frequency of particular genotypes over the course of a growing season, it appears that particular genotypes are unlikely to reach high frequencies within field populations because of the limited dispersal potential for conidia. But the genes in the most fit individuals will persist and be recombined to create novel genotypes in the next growing season. Over the course of many growing seasons, selection will change the frequency of genes that affect adaptation to the wheat host, but new genotypes will appear each season. It is too early to say if selection operates the same way in *P. nodorum*.

In summary, the population genetics of *P. nodorum* and *M. graminicola* are very similar. This probably reflects the similarity in their life histories. Both fungi produce airborne sexual ascospores and splash-dispersed asexual spores. They both infect above-ground plant parts on the same host and they both infect seeds that can be transported globally as part of the world commerce in wheat. Use of multi-allelic, neutral genetic markers combined with hierarchical sampling has allowed us to achieve a much greater understanding of the population biology of both fungi.

## Acknowledgments

BAM gratefully acknowledges the many collectors and collaborators around the world who responded to his request for infected leaf material. Funding for this project came from the USDA National Research Initiative Competitive Grants Program (Grant # 93-37303-9039), the National Science Foundation (Grant # DEB-9306377), the Texas Agricultural Experiment Station (Hatch project #6928), and the Swiss National Fund (Grant # 5002-38966).

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# Characterization of Less Aggressive *Stagonospora nodorum* Isolates from Wheat

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## Abstract

*Two less aggressive* Stagonospora nodorum *isolates, 9074 and 9076, were characterized by inoculation tests, mating ability, and molecular tools. As expected, they caused mild symptom severity on wheat, triticale, and rye. They crossed with other* S. nodorum *isolates and had "+" mating type determinant(s). These two isolates also had the same restriction patterns and sequences in the internal transcribed spacer (ITS) region of rDNA similar to other* S. nodorum *isolates. With AFLP analysis, it was concluded that these less aggressive* S. nodorum *isolates were closely related and genetically different from highly aggressive ones.*

*Stagonospora nodorum* (Berk.) Castellani & E.G. Germano (teleomorph *Phaeosphaeria nodorum* (E. Müller) Hedjaroude) causes septoria nodorum blotch on wheat, barley, and other small grains (Sprague, 1950). Genetic variation of *S. nodorum* isolates has been reported (Allingham and Jackson, 1981; Krupinsky, 1997a,b; Scharen et al., 1985). Understanding this genetic variation would help in the selection and/or development of resistant germplasm. This study was undertaken to determine whether less aggressive isolates could be characterized and how they relate to highly aggressive isolates.

## Materials and Methods

### Inoculation tests

*Stagonospora nodorum* isolates were obtained from lesions on infected wheat (Sn26-1, 9074, and 9076) and rye (Sn48-1) in Poland and USA. Isolates 9074 and 9076 were collected from Richland and Gallatin counties in Montana, respectively. These two isolates were associated with mild symptom severity on detached wheat leaves and seedlings, and considered to be less aggressive isolates (Krupinsky, 1997a,b). Plants were inoculated by spraying a 3-ml pycnidiospore suspension (3 x $10^6$ spores/ml) per pot onto 10-day-old seedlings. Three wheat cultivars (Alba, Begra, and Liwilla), two triticales (Bogo and Pinokio) and one rye (Zduno) were used as host plants. The plants were maintained in a high humidity environment for 72 h after spraying. Fourteen days after inoculation, 10 primary leaves were assessed for percentage necrosis and scored using a 1 (resistant) to 9 (susceptible) scale. The experiments were repeated three times.

### Determination of mating type

Isolates 9074 and 9076 were mated *in vitro* with reference "+" and "–" strains of the pathogen with a procedure developed in the lab (Arseniuk et al., 1997).

### DNA isolation and characterization of ITS of nuclear rDNA

Growth of fungal culture in a liquid medium, isolation and purification of genomic DNA mainly followed the procedures described earlier (Ueng et al., 1992). The internal transcribed spacer (ITS) region of the nuclear rDNA repeat units was amplified by PCR following the protocol reported earlier (Ueng et al., 1998; White et al., 1990). Two highly aggressive *S. nodorum* isolates, 8408 and 9506-2 (Krupinsky, 1997a; Krupinsky, unpublished data) were compared with the two less aggressive ones, 9074 and 9076. The PCR products of the ITS region were restricted with endonuclease enzymes, *Dra*I, *Hae*III, *Hpa*II, and *Pvu*II. The sequences of PCR-amplified ITS regions were determined using a DNA sequencer (Applied Biosystem 373A, Perkin Elmer, Foster City, CA).

### AFLP analysis

The amplified restriction fragment polymorphism (AFLP) technique was used to explore DNA polymorphisms in four *S. nodorum* isolates with different degrees of aggressiveness on wheat. The AFLP™ Analysis System I (Life Technologies, Gaithersburg, MD) kit was used following the protocol provided by

the manufacturer. Cluster analysis using the unweighted pair group method with an arithmetic average (UPGMA) algorithm was performed to produce a phenogram based on the banding patterns produced by AFLP (Rohlf, 1990).

## Results and Discussion

In the greenhouse seedling inoculations, isolates 9074 and 9076 generally showed lower symptom severity on wheats, triticales, and rye (Table 1). The results agreed with the previous work on detached wheat leaves and seedlings (Krupinsky, 1997a,b). It was also shown that Polish isolate Sn48-1, originally isolated from rye, caused severe symptom severity on rye (Table 1) and less symptom severity on triticale (a cross between wheat and rye).

After the isolates mated, pseudothecia with mature ascospores were formed after 50-80 days of incubation. Isolates 9074 and 9076 were both shown to be "+" mating types. The ITS regions of rDNA in these two isolates had the same endonuclease restriction patterns and DNA sequences as the two highly aggressive isolates 8408 and 9506-2, and other wheat-biotype *S. nodorum* isolates (Beck and Ligon, 1995; Ueng et al., 1998). This would confirm that these two less aggressive isolates are wheat-biotype *S. nodorum* isolates. Of a total 390 bands generated by 48 AFLP reaction combinations, only 78 bands were shared by all four isolates in this study. The genetic diversity was high in highly aggressive isolates, and between highly and less aggressive ones (Figure 1). Cluster analysis showed great similarity between the two less aggressive isolates. The presence of differential AFLP fragments in the highly aggressive isolates may be related to aggressiveness and may be useful to identify virulent elements in the future.

## Acknowledgments

The authors thank Yan Zhao of USDA-ARS, MPPL, Beltsville, MD, for his technical support and Weidong Chen of the University of Illinois for his comments and suggestions.

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**Table 1. Comparison of *Stagonospora nodorum* isolates on cereal seedlings.**

| | Wheat | | | Triticale | | Rye |
|---|---|---|---|---|---|---|
| **Isolates** | **Alba** | **Begra** | **Liwilla** | **Bogo** | **Pinokio** | **Zduno** |
| Sn26-1 | 4.1±1.63 | 6.2±1.70 | 1.8±0.70b | 4.3±1.24 | 3.1±1.55 | 3.0±1.97f |
| Sn48-1 | 2.2±1.00 | 4.3±1.46 | 2.1±0.96b | 6.6±0.94 | 6.4±1.72 | 8.3±1.03 |
| 9074 | 1.5±0.57 | 3.1±1.92a | 1.0±0.56c | 1.7±0.99d | 1.8±0.73e | 2.2±2.02f, g |
| 9076 | 2.1±0.52 | 2.9±0.92a | 1.0±0.53c | 1.4±0.57d | 1.8±0.71e | 2.0±1.80g |

The rating scale for fungal infection is from 1 (resistant) to 9 (susceptible).With T-test at the confidence level of 0.05, the pairs with the same letters are not significantly different.

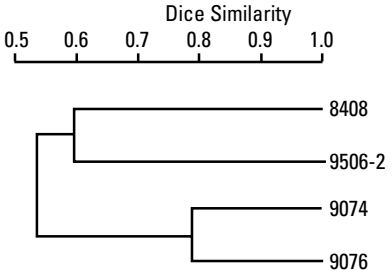


**Figure 1. UPGMA phenogram of four *Stagonospora nodorum* isolates based on the Dice Similarity Coefficients ($S_d$) of 390 individual AFLP DNA fragments.**

# A Vertically Resistant Wheat Selects for Specifically Adapted *Mycosphaerella graminicola* Strains

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## Abstract

*At its commercial release in Oregon, USA, in 1992, the winter bread wheat cultivar Gene was highly resistant to* Mycosphaerella graminicola. *Within just four years, that resistance had substantially disintegrated. In 1997, we collected* M. graminicola *isolates from experimental field plots of Gene and two other cultivars in Corvallis, Oregon, and in a greenhouse experiment inoculated seedlings of the same three cultivars with those isolates. Gene seedlings were resistant to all isolates derived from the other two cultivars, but were susceptible to six of seven isolates derived from Gene. A similar experiment using a small number of 1992 isolates from the same three cultivars had previously shown no isolates virulent on Gene, in keeping with other published data on Gene's early resistance. Commercial cultivation of Gene, which increased to about 15% of the local wheat area during the period 1992-1995, appears to have selected for strains of* M. graminicola *adapted to specific virulence on Gene. Cultivar specificity is a feature of the* M. graminicola*-wheat pathosystem.*

The issue of whether *M. graminicola* is specific in its interactions with cultivars of a single wheat species (wheat or durum) has been widely debated. Recent studies (Ahmed et al., 1995; Ahmed, et al., 1996; Kema, et al., 1996; Kema, et al., 1997) consistently indicate the existence of interactions between cultivars of a single species and isolates derived from that species. However, most experiments where interaction has been measured have utilized tester cultivars and isolates not originating on them. Such experiments can reveal only the potential for cultivar-specific adaptation. Here, we report the appearance of cultivar specificity when tester cultivars were challenged with isolates selected on those same cultivars in the field.

## Materials and Methods

We conducted two experiments involving the soft white winter wheat cultivars Gene (P.I. 560129), Madsen (P.I. 511673), and Stephens (C.I. 017596). Monopycnidial isolates of *M. graminicola* were obtained from Corvallis, Oregon, field plots of these cultivars in 1992 and again in 1997. Greenhouse experiments were performed with these isolates in 1994 and 1998, respectively. We used two isolates derived from each cultivar in 1992, and seven or eight isolates from each cultivar in 1997. Seedlings of each cultivar were grown in pots in the greenhouse, and each tester pot was inoculated at 21 days with an individual isolate in a factorial design. Both experiments were replicated four times. The percent of leaf area covered by lesions was assessed at 21 days after inoculation. Data were $\log_e$-transformed and subjected to analysis of variance.

## Results

Of the six 1992 isolates tested, including two from Gene, none was virulent to Gene (data not shown). By contrast, the 1997 isolates derived from Gene were generally virulent to Gene, while the 1997 isolates derived from the other two cultivars were avirulent to Gene (Table 1). Analysis of variance showed no significant cultivar-of-origin x tester interaction for the 1992 isolates, and a significant cultivar-of-origin x tester interaction for the 1997 isolates (not shown). Linear contrasts (not shown) were used to dissect the cultivar-of-origin x tester interaction, and they revealed a highly significant interactive effect of the tester Gene vs. testers Madsen and Stephens ($P = 0.0068$).

## Discussion

From its release in 1992, Gene expanded to occupy 15% of the Willamette Valley wheat area by 1995, but then declined (Figure 1) as its resistance to *M. graminicola* broke down (and incidence of septoria nodorum blotch, to which Gene was always susceptible, increased).

Our first greenhouse experiment used only six isolates, but it supports published field data on Gene's resistance during the years leading up to its release in 1992 (Ahmed et al., 1995; Mundt et al., 1999). Our data suggest that cultivar specificity can develop in the *M. graminicola*-wheat pathosystem, and that this pathogen is capable of rapidly adapting to qualitative host resistance. Past observations suggested that resistance to *M. graminicola* does not break down rapidly or completely (Johnson, 1992; Kema et al., 1996). However, the resistance of Gene succumbed within less than five years. We believe that Gene's resistance is probably under monogenic or oligogenic control, and is perhaps due to the *Stb4* gene (Kronstad et al., 1994). Further, *M. graminicola* strains able to defeat Gene's vertical resistance appear to persist in the local population despite the limited continuing commercial production of Gene.

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**Table 1. Percent diseased leaf area of greenhouse-grown wheat seedlings inoculated with *Mycosphaerella graminicola* isolates collected in 1997.[a]**

| Cultivar of origin & isolate nbr. | | Tester cultivar Gene | Madsen | Stephens | Mean |
|---|---|---|---|---|---|
| Gene | | | | | |
| | 1 | 19.9 | 26.4 | 9.4 | 18.6 |
| | 2 | 3.4 | 18.5 | 16.7 | 12.8 |
| | 3 | 13.0 | 29.5 | 23.4 | 21.9 |
| | 4 | 11.2 | 17.9 | 29.6 | 19.5 |
| | 5 | 43.6 | 63.4 | 63.4 | 56.8 |
| | 6 | 21.1 | 32.9 | 33.0 | 29.0 |
| | 7 | 37.2 | 61.3 | 56.6 | 51.7 |
| Mean[b] | | 21.3a[c] | 35.7a | 33.1a | 30.1a |
| Madsen | | | | | |
| | 8 | 0.9 | 77.1 | 56.0 | 44.7 |
| | 9 | 3.0 | 7.4 | 11.5 | 7.3 |
| | 10 | 4.2 | 30.6 | 28.4 | 21.0 |
| | 11 | 10.7 | 38.0 | 31.5 | 26.8 |
| | 12 | 1.0 | 30.1 | 28.7 | 19.9 |
| | 13 | 1.3 | 30.1 | 22.6 | 18.0 |
| | 14 | 0.2 | 19.6 | 7.8 | 9.2 |
| | 15 | 0.7 | 15.1 | 13.4 | 9.8 |
| Mean | | 2.8b | 31.0a | 25.0a | 19.6b |
| Stephens | | | | | |
| | 16 | 3.3 | 22.7 | 24.7 | 16.9 |
| | 17 | 1.0 | 13.2 | 7.9 | 7.4 |
| | 18 | 0.4 | 31.0 | 21.7 | 17.7 |
| | 19 | 2.5 | 31.4 | 24.9 | 19.6 |
| | 20 | 2.7 | 22.0 | 16.1 | 13.6 |
| | 21 | 0.7 | 15.5 | 14.3 | 10.2 |
| | 22 | 2.2 | 36.1 | 28.3 | 22.2 |
| Mean | | 1.8b | 24.6a | 19.7a | 15.4b |
| Grand mean | | 8.4 | 30.5 | 25.9 | 21.6 |

[a] Experiment conducted in 1998.
[b] Means are the untransformed percent diseased leaf area over four replications. Statistical analyses were conducted on $\log_e$-transformed data.
[c] Within a column, means followed by the same letter are not significantly different at the 95% confidence level based on Fisher's protected least significant difference.

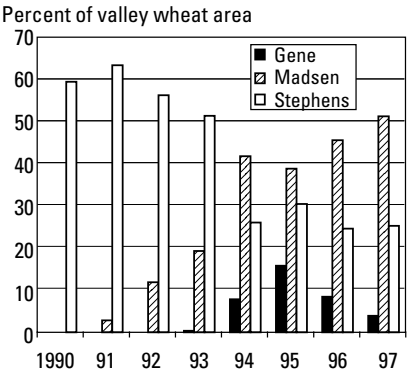


**Figure 1. Percent of soft white winter wheat area occupied by three cultivars in the Willamette Valley of Oregon during the period 1990-97.**

# Genetic Variability in a Collection of *Stagonospora nodorum* Isolates from Western Australia

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## Abstract

Stagonospora nodorum *isolates were collected from the Western Australian cereal-belt during 1993. These isolates and a subset of isolates taken from a single location were used to assay the level of variation within the pathogen population. The isolates were compared using anonymous nuclear DNA markers. Three low copy number and a single high copy number probe were used to generate restriction fragment length polymorphisms (RFLPs). The collection exhibited a high genotypic diversity for the high copy number probe. This is consistent with the high level of sexual reproduction previously found in the fungal population. Only minor differences between the total collection of isolates and the subset of isolates taken from the single location were found.*

Septoria nodorum blotch is one of the most important leaf diseases of wheat (*Triticum aestivum* L.) in the Western Australian cereal-belt (Murray and Brown, 1987). It is caused by *Stagonospora nodorum* (Berk.) Castellani & E. Germano (teleomorph *Phaeosphaeria nodorum* (E.Muller) Hedjaroude). Previous work on the variability of *S. nodorum* has been conducted principally on phenotypic traits such as aggressiveness (Allingham and Jackson, 1981) and culture color (Scharen and Krupinsky, 1970). These studies have shown that there is considerable variation between isolates collected within relatively small distances (Allingham et al., 1981). More recently studies have assayed the genotypic variability using molecular techniques.

RFLPs were used to look at two populations of *S. nodorum* in the USA using a hierarchical system of sampling (McDonald et al., 1994). Considerable genetic variation was found to occur on a relatively small scale. The same set of eight probes were used to study a population of *S. nodorum* isolates gathered in Switzerland and two populations from the USA using a similar hierarchical sampling system. The Swiss population was genetically similar to the populations from the USA, providing evidence for gene flow between the populations (Keller et al., 1997a) and the gene diversity for four of the seven RFLP loci tested was not significantly different between the populations (Keller et al., 1997a).

The aim of this investigation was to examine the level of genetic diversity among a collection of Western Australian *S. nodorum* isolates using RFLPs and to compare the results obtained with similar work done in other regions of the world.

## Materials and Methods

A total of 118 isolates from 38 locations were used in the study. Thirty-nine of the isolates were obtained from an existing collection. Of these 39 historical isolates, 26 were from a single site at Badgingarra Research Station, recognized as an area where high levels of disease severity could be expected. These isolates were captured as single ascospores in spore traps during 1991. The remaining 79 isolates were collected from 26 locations throughout the cereal belt in 1993. They were isolated as ascospores from wheat stubble remaining from the 1992 crop.

The cultures of *S. nodorum* were revived on wheat meal agar plates to confirm their identity through pycnidiospore production. Mycelia was cultured in liquid broth and the DNA extracted (Lagudah et al., 1991).

The genomic DNA was digested with *Hin*dIII and run on an agarose gel. A capillary alkali method was used to transfer the DNA onto the membrane. Three low copy number probes (pASN15, pASN125 and pJSN73) were used to produce a multilocus haplotype, and a single high copy number probe (pSNS4) was used to generate a fingerprint for

each isolate. Two of the probes used were from the genomic library of a Western Australian isolate of *S. nodorum* (pASN15 and pASN125) and the remaining two probes were a gift from Bruce McDonald of Texas A&M University (pJSN73, pSNS4). Both genomic libraries were set up by the restriction of genomic DNA from a *S. nodorum* isolate using *Pst*I. Each probe was labelled with alpha $^{32}$P. Hybridization was carried out for a minimum of 12 hours. The membranes were washed and placed on x-ray film for up to 14 days.

Each probe was assumed to represent a single RFLP locus and each restriction fragment length variant were treated as an allele (McDonald et al., 1994). Each allele for each RFLP locus was designated an arbitrary number. The high copy number probe (pSNS4) was used to identify clones within the same multilocus haplotype (McDonald et al., 1994). The subset of the isolates from Badgingarra was treated as a separate collection to determine if the same level of genetic diversity present in the total collection was also present at a single location. The allelic frequencies, Nei's measure of gene diversity (Nei, 1973), the genotypic diversity, and the gametic disequilibrium were all calculated as described in McDonald et al. (1994).

## Results

There were two alleles for probes pASN125 and pJSN73 and four for probe pASN15. Only four *S. nodorum* isolates had the same DNA fingerprint using the high copy number probe pSNS4. Nei's measure of gene diversity was 0.23 for pJSN73-*Hin*dIII, 0.50 for pASN15-*Hin*dIII and 0.56 for pASN15-*Hin*dIII. The average over all three loci was 0.43 in the clone-corrected data. For the Badgingarra subset no clones of *S. nodorum* were identified. The allelic frequencies were very similar for the three loci compared with the total population (being 0.27, 0.49, and 0.60, respectively) and Nei's measure of gene diversity averaged 0.45 over the three loci.

For the clone-corrected data of the total population, 25% of the allele pairs were in significant gametic disequilibrium (Table 1). When all of the alleles were added together for each locus pair in the clone-corrected data, one of the locus pairs was in significant gametic disequilibrium (Table 1). In the Badgingarra subset, 20% of all the allelic combinations had a significant disequilibrium coefficient, and for each locus pair, one pair were in significant gametic disequilibrium (Table 1).

There were 100 different fingerprint haplotypes produced from 103 isolates, giving a genotypic diversity of 92.2, 90% of its maximum value. In the subset collection from Badgingarra, the genotypic diversity was 15, 100% of its maximum value.

**Table 1. Gametic disequilibrium for the clone-corrected data of the total population and the Badgingarra subset for each of pair of alleles between each pair of loci and for each pair of loci.**

| Probe | pASN15 | pJSN73 | pASN125 |
|---|---|---|---|
| pASN15 | | 4/8<br>15.7** (3) | 0/8<br>7.15 (3) |
| pJSN73 | 0/8[1,2]<br>2.05 (3) | | 1/4<br>3.70 (1) |
| pASN125 | 3/8 | 0/4<br>9.62 (3) | |

[1] Clone-corrected data only; values above the diagonal are for the total population and values below the diagonal are for the Badgingarra subset.

[2] For each set of comparisons, the first line shows the number of allele pairs that are in significant gametic disequilibrium ($p<0.05$) out of the total number of allele pairs; the second line of data is the total chi-squared value for the loci pair. The number in brackets is the degrees of freedom.

** Significant at $p<0.01$.

## Discussion

Considerable genetic variation was observed within isolates of *Stagonospora nodorum* collected throughout the cereal belt of Western Australia. This genetic variation was evident from the high genotypic diversity of the DNA fingerprints and the high values obtained using Nei's measure of gene diversity. The Badgingarra subset and the total collection were similar for all of the parameters measured. As there was no sign of population differentiation, it would appear that there is gene flow between the Badgingarra collection and the rest of the collection of *S. nodorum* within the cereal belt.

The level of genetic variation within the Western Australian collection of *S. nodorum* isolates is consistent with the levels previously found internationally. Nei's measure of gene diversity from this collection is consistent with previously published data on *S. nodorum* (McDonald et al., 1994; Keller et al., 1997a). The genotypic diversity was 90% of its maximum for the Western Australian collection, which was consistent with previous studies, which found the genotypic diversity ranged from 42% to 100% of its maximum for the DNA fingerprints (Keller et al., 1997a).

This study has provided some insight into the genetic variation within *S. nodorum* in Western Australia. The prevalence of the sexual stage means that the ascospores are a potential and probably significant source of inoculum in Western Australia (Bathgate and Loughman, 1995), and the considerable genotypic variability of the DNA fingerprints is a reflection of the role sexual

recombination plays in the population dynamics. This level of variation may have implications for the cereal plant breeding and selection program if it is a reflection of the potential variability for aggressiveness in the pathogen. If the level of genotypic variation does reflect the level of variation in aggressiveness, selecting resistance using the widest possible range of *S. nodorum* isolates would be desirable.

The most practical method to screen breeding lines for a wide selection of *S. nodorum* isolates is to use straw from previously infected wheat as a source of inoculum (Holmes and Colhoun, 1975). Previous work investigating the effect of the host genotype upon the selection of *S. nodorum* provided no evidence of specialization of the pathogen with the host cultivar (Keller et al., 1997b) according to neutral RFLP markers. It would also be unnecessary to use straw from numerous sites, as the genotypic variation encountered at a single site can account for as much as 95% of the total genotypic variation within a population (Keller et al., 1997b). Using straw from a collection of cultivars and from a number of sites may be unnecessary, as it would provide only a modest increase in the overall genotypic diversity.

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# Mating Type-Specific PCR Primers for *Stagonospora nodorum* Field Studies

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## Abstract

*Conserved regions of the mating type genes (*MAT*) in* Cochliobolus heterostrophus, Mycosphaerella zeae-maydis, *and* Alternaria alternata *were used to design primers to identify the mating type genes and thus assign mating types to* Stagonospora nodorum. *These primers successfully distinguished between mating types of* S. nodorum *isolates that had been identified through traditional crosses. The primers were used to screen a small sample of* S. nodorum *isolates from New York, thus revealing the presence of both mating types. This efficient method of identifying mating types may help determine the role of sexual recombination in the epidemiology of* S. nodorum, *as well as elucidate phylogenetic relationships among related species.*

*Stagonospora nodorum* (Berk.) Castellani & E.G. Germano (teleomorph = *Phaeosphaeria nodorum* (E. Müller) Hedjaroude) is the causal agent of stagonospora nodorum blotch, a major disease of wheat around the world. *Phaeosphaeria nodorum* is heterothallic and thus requires strains of the two different mating types to produce ascospores (Müller, 1989). The potential significance of sexual reproduction in the epidemiology of stagonospora nodorum blotch has been heightened by the high RFLP variability found among field isolates (McDonald et al., 1994). Fungi that regularly reproduce sexually have more opportunities to develop fungicide resistance and to overcome cultivar resistance. Furthermore, airborne ascospores would permit dissemination over longer distances than splash-dispersed conidia.

*MAT* genes have been identified for several species of Loculoascomycetes including *Setosphaeria turcica, Mycosphaerella zeae-maydis, Alternaria alternata,* and several *Cochliobolus* spp. (Arie et al., 1997). Both genes, *MAT-1* and *MAT-2*, contain conserved regions that allow the relatively rapid identification of these genes from an increasing group of ascomycetes. A high mobility group (HMG) and a-box motif are the conserved regions in MAT-2 and MAT-1, respectively (Turgeon, 1998). These sequences, besides elucidating sexual mechanisms and phylogeny, may also help address epidemiological questions.

We have developed primers to identify the mating types of *P. nodorum* from the conserved *MAT* regions of related fungi. Preliminary data on the mating type prevalence in a small sample of New York field isolates is also presented.

## Materials and Methods

### Fungal isolates and media

Three *S. nodorum* isolates of different mating types (two (-) (SN435PL-98, SN437GA-98) and one (+) (SN436GA-98)) (Arseniuk et al., 1997a,b) were grown on V-8 juice agar (200 ml V8 juice, 3 g $CaCO_3$, 15 g agar per 800 ml of distilled water). After approximately seven days, mycelial plugs were taken and placed into yeast-malt-sucrose broth (5 g yeast extract, 5 g malt extract, 20 g sucrose per 1 liter of distilled water), and placed on a shaker (150 rpm) at room temperature. Mycelia were harvested after 5-7 days by filtering through 3-ply sterile cheesecloth lining a Buchner funnel and were rinsed with distilled water. The samples were then frozen and lyophilized overnight. *MAT-1* and *MAT-2 C. heterostrophus* laboratory strains (C5 and C4, respectively) were used as controls.

Thirty-eight arbitrarily chosen field isolates of *S. nodorum* collected from New York in different places and years were grown on cellophane discs overlaid on V-8 juice agar. After approximately seven days, mycelia were scraped off the cellophane and placed in microcentrifuge tubes to be lyophilized.

### DNA extraction

Lyophilized mycelia were ground in liquid nitrogen. The field isolates were ground directly in microcentrifuge tubes with a small amount of white quartz sand (-50 + 70 mesh, Sigma Chemical Company, St. Louis, MO). DNA was extracted by a miniprep procedure (Wirsel et al., 1996).

### Amplification of the HMG and a-boxes by PCR

PCR primers (Sn-HMG1 and Sn-HMG2) for the HMG box (Table 1) were designed from conserved regions in the HMG box of the *MAT* gene of *Cochliobolus heterostrophus*, *M. zeae-maydis,* and *A. alternata*, whereas the primers (Sn-ab1 and Sn-ab2) for the a-box were designed from the a-boxes of *M. zeae-maydis* and *A. alternata.* The primers were synthesized by the Cornell University DNA Services Facility and were dissolved (100 µM) in sterile distilled water and stored at –20C. Each PCR reaction mixture had approximately 20 ng of genomic DNA in 50 µl reaction buffer [1X PCR Buffer (Perkin Elmer, Norwalk, CT), 0.2 mM dNTPs, 2.5 mM $MgCl_2$, 2 µM each primer, and 0.025 U Taq polymerase]. The samples were denatured at 95C for 2 min, and then subjected to 30 cycles of 95C for 1 min, 50C for 30 sec, and 72C for 1.5 min. After extension at 72C for 10 min, the samples were kept at 4C. 5 µl aliquots of the PCR products were analyzed on a 2% agarose gel in TAE buffer.

**Table 1. PCR primers used to amplify fragments of *MAT* genes in *Stagonospora nodorum.***

| | |
|---|---|
| MAT-1 | |
| Sn-ab1 | 5′ AA(A/G)GCN(C/T)TNAA(C/T)GCNTT (C/T)GTNGG 3′ |
| Sn-ab2 | 5′ TC(C/T)TTNCC(A/G/T)AT(C/T)TG(A/G)TCNCG(A/G/T)AT 3′ |
| MAT-2 | |
| Sn-HMG1 | 5′ AA(A/G)GCNCCN(AC)GNCCNATGAA 3′ |
| Sn-HMG2 | 5′ TT(C/T)TT(C/T)TT(C/T)T(CG)NCCNGG(C/T)TT 3′ |

### Cloning, sequencing, and analysis of PCR products

The *MAT* sequences from related fungi predicted that a PCR product of ~270 bp for the HMG box and a product of ~230 bp for the a-boxes would result if the amplifications were successful. Products of these sizes were cloned from the PCR reaction into the vector pCR2.1, using the guidelines of the manufacturer (Invitrogen Co., San Diego, CA). DNA sequences were determined at the Cornell University DNA Services Facility. Sequences were aligned with the LaserGene program MegAlign (DNASTAR Inc., Madison, WI), using the clustal method. A BLAST search was done using the NCBI/Genbank internet databases.

### Gel blot hybridization

A standard DNA gel blot hybridization procedure was followed (Sambrook et al., 1989).

## Results

The primers for the HMG box were used with genomic DNA of (+) and (-) *S. nodorum* strains as templates. A PCR product approximately 0.3 kb, matching the ~270 bp predicted, was present in the (+) (*MAT-2*) strain, but was not present in the (-) (*MAT-1*) strains (Figure 1). The primers for the a-box, however, amplified the

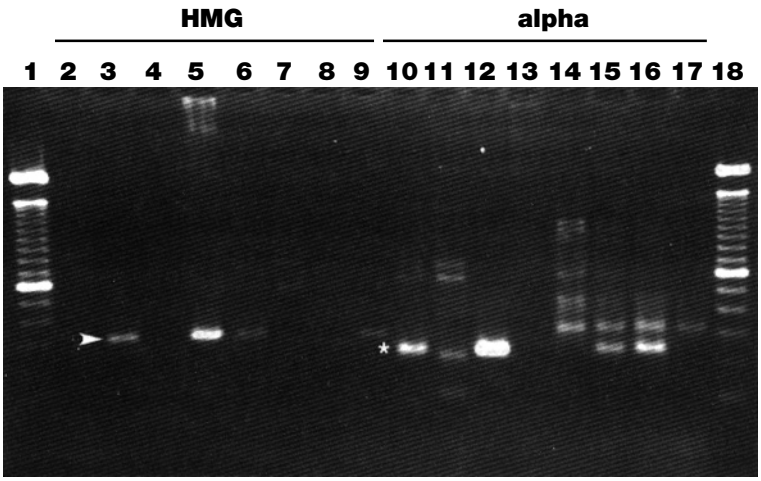


**Figure 1. PCR products amplified with the HMG and a-box primers. Lanes 2-9 were amplified using HMG (*MAT-2*) primers, and lanes 10-17 were amplified with a-box (*MAT-1*) primers. Lanes 2,10 and 3,11: *C. heterostrophus* C5 (*MAT-1*) and *C. heterostrophus* C4 (*MAT-2*), respectively; lanes 4,12 and 5,13: plasmid with cloned a-box from SN435PL-98 and plasmid with cloned HMG box from SN436GA-98, respectively; lanes 6 and 14: SN436GA-98; lanes 7 and 15: SN437GA-98; lanes 8 and 16: SN005NY-85; lanes 9 and 17: SN197NY-88. A ~0.3-kb band (arrowhead) was amplified in the *MAT-2* isolates only, and a band above 0.2-kb (asterisk) was amplified only in the *MAT-1* isolates.**

expected product (~230 bp) from only the (-) (*MAT-1*) strains. The primers were also able to distinguish between the *MAT-1* and *MAT-2 C. heterostrophus* controls. The BLAST search of the *S. nodorum* cloned products showed highest homology to the *MAT* genes of *Cochliobolus* spp.

In gel blot hybridization, a single band was obtained from DNA of the (+) SN436GA-98 isolate only when probed with the HMG box; no hybridization occurred on the (-) SN435PL-98 isolate. Likewise, a single band was obtained when SN435PL-98 was probed with the a-box, and no hybridization occurred to the DNA of SN436GA-98.

When these primers were used to test the 38 field isolates, 20 were shown to be of the *MAT-1* mating type and 18 were of the *MAT-2* mating type.

## Discussion

A PCR technique that readily identifies the mating type can be a useful tool for studying *S. nodorum*. Surveying for the distribution of mating types in the field may result in a better understanding of the role of sexual reproduction in the disease cycle. The preliminary data given here tested *S. nodorum* isolates that were collected in New York wheat fields between 1985 and 1995. These isolates are not only temporally random, but spatially unrelated. More informative surveys of spatially distributed isolates from single fields are being conducted. However, the preliminary data presented here do indicate that both mating types exist in New York and that sexual recombination is possible.

Mating types may also serve as an additional marker for experiments requiring genetically characterized isolates. As previously established (Arie et al,.1997), we refer to (-) isolates containing the a-box as *MAT-1,* and (+) isolates containing the HMG box as *MAT-2.* Finally, as outlined in a review by Turgeon (1998), *MAT* gene sequences may provide valuable information for phylogenetic analyses, particularly of related species, the study of pathogenesis, and the underlying mechanisms of reproductive life styles in fungi.

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## *Session 5: Epidemiology*

# Epidemiology of *Mycosphaerella graminicola* and *Phaeosphaeria nodorum*: An Overview

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## Abstract

*The within-season and between-crop methods of multiplication and survival, and their environmental relations are reviewed.* Mycosphaerella graminicola *multiplies within a season by conidia which are primarily but not exclusively dispersed by rain. Arguments are given that the influence of ascospores within a crop will be minor, but they are the major source of movement of the pathogen into new crops.* Phaeosphaeria nodorum *also multiplies within a season by conidia, but has clearer associations with wet weather. The role of ascospores in movement between crops may vary geographically; seed transmission seems to be very important in some areas.*

In this contribution, I have tried to summarize what is understood of the epidemiology of these two diseases. My aim has been to produce a concise summary to introduce the detailed and novel contributions that follow. I have tried to give slightly more extended discussion of those areas where new ideas have arisen or our understanding has changed substantially in the last few years.

The relevant questions for both diseases fall into two classes. First, qualitative: what conditions allow inoculum transfer, permit infection, and encourage sporulation? Second: quantitative: in a given agro-ecosystem, what factors in practice regulate pathogen population size? The two questions are related, but both need to be answered if the diseases are to be managed most effectively. The answers also depend greatly on scale: within a region and over several years, very different processes and factors may need to be considered from those operating within a crop and within a season. The paper is restricted to wheat.

## *Mycosphaerella graminicola*

### Within a crop

As discussed later, infection of a crop is usually initiated by airborne ascospores (Shaw and Royle, 1989). The density of initial infections is such that once a few sporulating lesions per square meter exist, a polycyclic epidemic on successive leaf layers follows (Shaw and Royle, 1993). Pycnidia are produced within roughly 14 to 40 days, depending on both temperature and host cultivar. Conidia may be dispersed by single rain splashes within a circle of about 1 m radius, the number dispersed decreasing exponentially with distance, with half distances of the order of 10 cm (Bannon and Cooke, 1998; Brennan et al., 1985a; Brennan et al., 1985b). Initial dispersal is to the ground or to surface water on a leaf, whence further dispersal is possible. However, spores contacting leaf surfaces are bound to the surface within a short time. The average number of splashes moving a spore during rain of given intensity and duration is hard to estimate, but is unlikely to be large, so half distances for effective horizontal dispersal will be of the order of 20-50 cm at most. Each initial infection may plausibly produce 50,000 to 500,000 conidia (10-100 pycnidia of ca. 5000 spores) (Eyal, 1971). Although most of these are not dispersed far, considering them as evenly dispersed over a circle of 0.5 m radius gives 5-50 spores per square centimeter from initial infections spaced at about $1/m^2$. If they had 2-20% infection efficiency, the crop would be saturated with latent lesions. Fortunately, infection efficiency is usually lower than this, but if few spores are present, 1% of those applied may cause infection under good infection conditions. The actual environmental conditions permitting infection are lax because the pathogen tolerates extended breaks in humidity during the infection process (Shaw, 1991a; Shaw and Royle, 1993).

Certainly, within two infection cycles the pathogen population in crops with moderate initial amounts of disease will be limited by the rate of growth of leaf area

and the favorability of the environment rather than by shortage of inoculum. However, spread by splash is very inefficient over other than short distances, and if initial infections are widely spaced, then the disease should remain patchy and therefore cause limited damage.

The preceding paragraph ignored vertical spread, which has been argued to be the key factor determining severe disease on the uppermost leaf layers of a wheat crop. Vertical movement of splash droplets is limited, declining exponentially with typical half-distances of 5 cm, but varies between storms (Bannon and Cooke, 1998; Shaw, 1987; Shaw, 1991b). Probably more critically, the distance between leaf layers with and without infection varies greatly according to both the architecture of the wheat cultivar and the latent period of the pathogen on the cultivar. Lovell et al. (1997) have shown how the interaction of these causes great variation in the potential for spread of pathogen to the upper part of the crop, where it becomes damaging.

Once a few sporulating lesions are present on a reasonable proportion of the uppermost leaves of a wheat crop, there is potential for multiplication leading to widespread infection and premature leaf death, except in very dry weather.

The major advance since the last Workshop is the recognition that pseudothecia are produced regularly throughout the year under at least some conditions on at least some cultivars (Hunter et al., 1999; Kema et al., 1996). This means that sparse initial infections could spread much more effectively by windblown ascospores, and that multiplication of the disease could take place efficiently in the absence of rain, dew alone providing wetness for infection by dry dispersed spores. This is a potentially dramatic change to our view of the ecology of the organism. However, the effect on epidemics within a season is probably limited, because the pseudothecia are always produced long after pycnidia. Two extreme situations can be imagined: one in which pycnidia can infect often during the season, one in which they can never infect.

In the first situation, even in the fastest quoted latent periods for perithecia, the effect on the epidemic rate of ascospore production is equivalent to perhaps 1000-10,000 extra spores from a single infection, produced at roughly the time when the next generation of lesions from the pycnidia produced earlier are themselves producing pycnidia. If the multiplication ratio of lesions from one generation to the next is *R*, then the pycnidial route is producing of the order of R * 10,000 conidia in the time during which the sexual route is producing 10,000 spores: a ratio of 1:*R*. Order of magnitude estimates of *R* can be obtained in at least two ways. First, the ratio between the number of lesions a latent period after disease first entered a crop and the initial number of lesions. For the crops observed by Shaw and Royle (1989) this ratio was about 100. Second, the ratio between the initial number of lesions on the uppermost leaves of a crop, and the number one latent period later (Shaw and Royle 1993): this too suggests a large number. The effect on the epidemic rate must be very small. This is borne out by detailed modelling (Eriksen et al., in prep.).

In the second situation, pseudothecia are the only route by which infection can progress. In this case, the latent period is at least double the latent period for an epidemic otherwise similar but driven by pycnidia. Since the latent period is at least doubled, and any gains in infection or dispersal efficiency are offset by the reduced number of ascospores produced, the rate of the epidemic is at least halved. A rather more qualitative prediction would be that where conditions prevent conidial dispersal, epidemics will develop very slowly, and be damaging only where initial disease is rather widespread. It will clearly be very interesting to study epidemic progress in areas where pycnidial progression is not possible, and see whether this prediction is true.

In fact, even this may overstate the case, since the fungus is heterothallic (Kema et al., 1996) and presumably therefore requires two infections of opposite mating type to be adjacent in order to produce ascospores. It is not known how close together infections must be to mate effectively. Metcalfe (1999) found internal hyphae spanning an average maximum of 11 mm in a susceptible wheat cultivar just before pycnidia were produced, so infections more than 20 mm apart may be isolated. In a sparse, slow epidemic this may be rare. Mating will be commonest in situations where inoculum is not limiting the pathogen population in any way, but in these cases, the extra inoculum provided by ascospores will not affect epidemic rate.

When inoculum is common low in the canopy, but transport is potentially limiting infection of upper leaves, ascospores could profoundly alter the risk of severe infection on the uppermost leaves, because rainfall is not required for their movement to these leaves. However, recent work confirms (under UK conditions at least) the apparent association of severe *M. graminicola* with rain (Lovell et al., 1997); and windblown transport of spores from close to the ground to the upper leaves of a dense canopy is not efficient because windspeeds are not high within the canopy. Nonetheless, the simple picture of rain moving spores from the base of the canopy onto the upper leaves as the only risk factor requires modification and may well be misleading under some circumstances.

Interactions between genetics and ecology are of interest, and could complicate the population dynamics of the pathogen considerably, at worst rendering forecasts almost impossible. The fittest isolate on a given cultivar can vary with temperature (B. al-Hamar, University of Reading, personal communication). This means that equivalent sized populations could have intrinsically different growth-rates according to the combination of weather conditions which gave rise to them, so that identical environmental conditions and initial population sizes would give rise to different population responses.

## Between crops

Windblown ascospores appear to be the main mechanism by which *M. graminicola* moves between crops, both from one season to the next and within a season. However, there is little documentation of the relative importance of local debris and widely dispersed, generalized sources of inoculum for new crops. In northern Europe, with most wheat grown in rotation and with full tillage, the generalized air spora appear to dominate sources. However, this situation must differ in different farming systems. Where wheat is a scarcer crop, the generalized concentration of ascospores in the air must be lower, and where no-till and continuous cropping is common but the wheat fields are scattered, one would expect local sources to dominate. There is relatively little evidence published about this outside Europe and northern America. A curious feature of the disease in, for example, the UK seems to be that the extremely efficient control of the pathogen achieved on the upper part of the plant by fungicide does not appear to have reduced the ability of the fungus to produce ascospores and infect subsequent crops. This could have two explanations: 1) in stubbles, ascospores are produced as much on the lower parts of plants, never managed by fungicide, as on the upper parts; or 2) the dominant triazole fungicides act only as fungistats and after the death of leaves, fungal development and the production of pseudothecia can continue as if the treatment had never taken place. Genetic and physiological evidence suggests that alternate hosts are unimportant in perpetuating the disease on wheat.

As noted in a previous review (Shaw, 1999), the proportion of land devoted to wheat in a region could affect the importance of *M. graminicola* as a pathogen. As the proportion increases, so the distance between last year's fields and this year's will decline. Even by wind, the number of spores moving a given distance declines rapidly with distance, so moderate changes in average distance between fields could cause substantial changes in the density of initial infections. In turn, at low densities, this could affect the importance of the disease at the end of the cropping season. The detail depends on how cropland is organized and how new wheatlands tend to be disposed relative to old, as well as on the strength of linkages between initial and end-of-season disease.

At the Long Ashton conference in 1997, a crucial question in understanding the uniformity in genetic composition of *M. graminicola* worldwide was whether seed-borne transport could ever occur. This apparently simple question remains open, and is far from simple, since very, very rare events could provide enough migration to leave populations genetically linked. Suppose a million seeds each from a diseased crop and a fungicide treated crop are grown, and a lesion appears on one plant from the diseased crop. Can we confidently say that lesion arose from seed transmission or could it have been attributable to a failure of isolation and a stray ascospore? Some form of gene exchange between widely dispersed populations apparently occurs, so the geneticists tell us, but it may be almost impossible to distinguish the various categories of very rare events giving rise to this.

The pathogen appears to have been becoming more widespread worldwide. A most interesting series of data is provided by Gilbert (1998) showing a more or less linear increase in *M. graminicola* incidence between 1990 and 1995 in southern Manitoba, from a very scarce disease to the most prevalent. Coupled with the genetic evidence that populations worldwide are genetically very similar (McDonald et al., 1995) it does seem possible that a novel form of the pathogen has been spreading worldwide. If true, this would raise the interesting question as to what epidemiological characteristic confers the new form's invasiveness.

## *Phaeosphaeria nodorum*

### Within crops

Multiplication is more rain dependent than *M. graminicola*, since pycnidia are produced in response to rain as well as dispersed by it. Conidia require wet periods to infect and do not tolerate interruptions well. The pathogen also appears to multiply ineffectively in cold conditions. Correlations with weather conditions are much better (Djurle et al., 1996; Gilbert et al., 1998) and more closely linked in time to serious outbreaks than for *M. graminicola*. In wet, warm weather, typical of summer in some regions, latent periods can be shorter than in *M. graminicola*. Progress up the growing plant is correspondingly faster and more regular (Mittermeier and Hoffmann, 1984).

Like *M. graminicola* spores, spores of some isolates of *P. nodorum* infect with a startlingly high probability when rare on a leaf surface, the infection efficiency per spore falling rapidly with total numbers attacking in a droplet (Jeger et al., 1985)(MWS, unpublished). The ecological advantage is presumably that reinfection of a new crop is very effective, after which survival of the isolate is more or less certain. The epidemiological consequence is surprisingly rapid early spread of the pathogen with subsequent rapid decline in apparent epidemic rate.

### Between crops

*Phaeosphaeria nodorum* can be seedborne, and before routine fungicide treatment of seed and the upper parts of the crop, substantial numbers of seeds were infected. In some regions, such as northern Europe, the disease appears to have become much less important during the 1980s and 1990s. It is tempting to ascribe this decline to the increased use of fungicides reducing seed infection both in the ear and at planting. Recent experiments in other parts of the world suggest that seedborne inoculum is crucial in many places (Milus and Chalkley, 1997).

Alternate hosts seem to harbor isolates of the pathogen capable of attacking wheat, but there is sufficient partial specialization that these do not act as the source of most infections on wheat as well as isolates clearly partially specialized to other hosts (Krupinsky, 1997a; 1997b). Crop residues have been shown to be important (Cunfer, 1998) but international survey evidence does not suggest they are the dominant source of inoculum (Leath et al., 1994).

Ascospores have been found, but the evidence for their ubiquity and epidemiological importance seems to vary geographically. Trapping experiments like those used to show how airborne inoculum of *P. nodorum* varies through the year have been published by Cunfer (1998). These showed that in Georgia, USA, inoculum persisted for long periods in stubble, detectable sporadically for at least 18 months after the crop was harvested. As little as 17 m from a field edge, inoculum was trapped only once, in March, even though both mating types of the fungus were present and could potentially form perithecia. This suggests that sexual reproduction is unusual, though possibly not absent.

These data are consistent with the relatively old published data on ascospore release in France (Rapilly et al., 1973). Anecdotally, near Reading in 1994 we observed a sudden patch of the disease in May in a first wheat. Although all isolations were from a single plot, and the disease had not been previously observed in that crop, 13 genotypes tested using RFLPs were of different genotypes (C.N.F.M.R.J Pijls and M.W. Shaw, unpublished).

However, the most extensive study made, in Poland, demonstrated a quite different picture, with ascospores of *P. nodorum* present throughout the year and at densities that appeared to be independent of distance from the nearest infected plot (Arseniuk et al., 1998). Ascospores were also found throughout the year in northern Germany, but the trap was operated over an unharvested field, so it is not possible to say whether

the spores were from local or distant sources (Mittelstadt and Fehrmann, 1987).

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# Spore Dispersal of Leaf Blotch Pathogens of Wheat (*Mycosphaerella graminicola* and *Septoria tritici*)

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## Abstract

*The spatial and temporal patterns of discharge and dissemination of air-borne* Mycosphaerella graminicola *spores and of* Septoria tritici *spores through rain splash were studied. Spore traps were used to monitor both ascospores and pycnidiospores when the wheat crop was in the vegetative and debris states. Relationships between distance from a point source and weather variables such as rainfall, relative humidity, and air temperature were analyzed. The release of pycnidiospores was favored by rainfall, as was explained through the multiple regression model (18% of the variation). Air dispersal of ascospores and splash dispersal of pycnidiospores were significantly influenced by each of the weather variables. The number of air-borne ascospores increased in association with rainfall. The multiple regression model explained 59% of the variation. The correlation analysis showed significant association with temperature, humidity, and rainfall; the regression coefficients of the climatic variables were significant. The effect of different distances from the inoculum source on the density of rain-splashed pycnidiospores and wind-borne ascospores was not significant. Pycnidiospores were the omnipresent inoculum in the cereal-producing area during the observed period. Thus this inoculum poses a risk to crop production and may be important to the epidemiology of septoria diseases under the climatic conditions in the wheat-producing areas of Argentina.*

Leaf blotch, caused by *Septoria tritici* Rob.ex Desm. (teleomorph *Mycosphaerella graminicola* (Fuckel) Schroeter, in Cohn) is an important wheat (*Triticum aestivum)* disease that causes yield losses in different regions of the world every year (Shipton et al., 1971; Eyal, 1981; Eyal et al., 1987). Its incidence depends on cultivar susceptibility, inoculum availability, crop management practices, and favorable environmental conditions (cool temperature, high humidity, and frequent rain). Climatic factors, especially precipitation, affect fungal growth and the amount and timing of spore production, as well as the release, dispersal, and deposition of spores. Unusually intense rain may cause the onset of a *S. tritici* epidemic in a wheat crop. The greatest risk to a crop is related to the occurrence of conditions that favor spore dispersal during and shortly after flag leaf emergence. Spore dispersal and infection at this time favors a second generation of pathogens. Spore dissemination patterns of *Phaeosphaeria* spp. and *Stagonospora* spp. have been described (Arseniuk and Góral, 1998; Góral and Arseniuk, 1998; 1991). The release of *M. graminicola* ascospores occurred at two peak times of the year (at crop emergence and emergence of the upper two leaves) (T. Hunter unpublished).

This work aimed at producing a mathematical model of pycnidiospore and ascospore dispersal by:

1. determining the pattern of spore dispersal during a 6-month period;
2. investigating the effect of climatic variables (rainfall, temperature, humidity) on density of air-borne spores; and
3. doing a preliminary analysis of the dispersal distance for both types of spores.

## Materials and Methods

In the experiment station situated in Los Hornos, near La Plata, Buenos Aires Province, air-borne spores were collected on 13 spore traps made of PVC capsules containing slides covered with petroleum jelly. The capsules were fixed to wooden stakes 0.7 m above the soil surface. Glass tubes 0.03 m in diameter and 0.16 m long were

placed near the capsules to catch rain-splashed spores. The capsules moved with the wind to ensure that the slides caught spores in different weather conditions. The spore traps were located at different distances (11 of them at 1.5 m and 2 at 12 m) from a wheat plot 30 m long and 10 m wide. Spore samplers were arranged parallel to the length of the test plot.

The plot was sown on 24 June 1998 and inoculated twice (17 July and 12 August) to obtain high disease incidence. Plants were inoculated when the second leaf unfolded (GS12). The main shoot and second side shoot (GS 22) (Zadoks et al., 1974) were inoculated by spraying a pycnidiospore suspension (5 x $10^6$ spores/ml) of one *S. tritici* isolate. Slides were collected and examined weekly from October 1998 to March 1999. The rain-splashed spores were identified by observing one drop of rain water stained with aniline blue (1/100) solution under the microscope. Four spore counts (1 $cm^2$ each) were done on every sample. Furthermore, ascospores and pycnidiospores on the entire slide area covered with petroleum jelly were counted.

Different populations of spores were observed under a light microscope at 100x magnification. Identification was based on the morphology of *Mycosphaerella* spp. and *Phaeosphaeria* spp. ascospores and *Septoria* spp. pycnidiospores. Viability of air-borne spores was determined by recording their germination directly on the petroleum jelly on the slides.

Climatic variables (rainfall, temperature, and relative humidity) were recorded at the meteorological observatory in La Plata.

Multiple regression analyses were used to explore the relationship between the number of pycnidiospores in rain water and petroleum jelly, and ascospores in petroleum jelly, and the three weather variables. An analysis of variance was performed to determine significant differences between spore traps placed at different distances. Pycnidiospore and ascospore dispersion was plotted in relation to climatic variables.

## Results and Discussion

During the observed period, the weekly mean temperature ranged between 14ºC and 25ºC, relative humidity between 64 and 84%, and rainfall between 0 and 135 mm.

Figure 1a and b, and Figure 2a, b, and c show the distribution of pycnidospores in rain water and petroleum jelly and of ascospores in rain water with weather variables throughout the 24 weeks of sampling.

During the 6-month period, pycnidiospores were always present in both types of sampling. Figure 1a shows an increase in rain-splashed pycnidiospores around the week of 21 December. This increase was associated with a high rainfall regimen in which intense rains lasting as long as five hours without interruption occurred. However, correlation coefficients and partial correlation coefficients with weather variables were not significant (Table 1). Application of a multiple regression model did not yield significant numbers either (Table 2). The increased amount of pycnidiospores was associated with the increase in rainfall between 22 January and 9 February 1999. The pycnidiospore pattern showed a marked reduction from 30 December to 9 February. This was

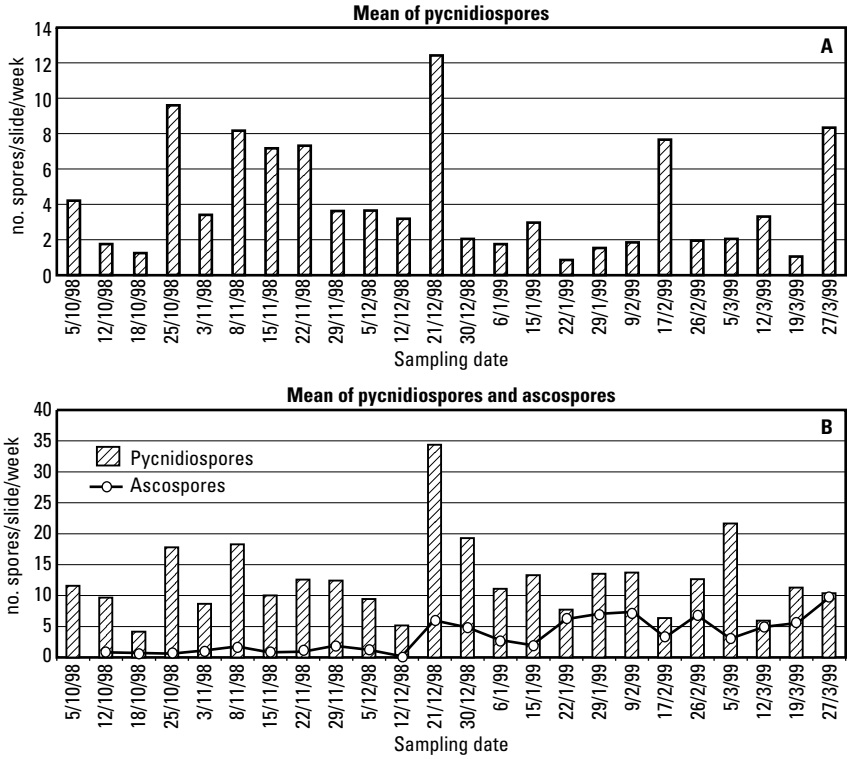


**Figure 1. Mean counts of pycnidiospores in rainwater (a), and pycnidiospores and ascospores in petroleum jelly (b) settled on microscope slides.**

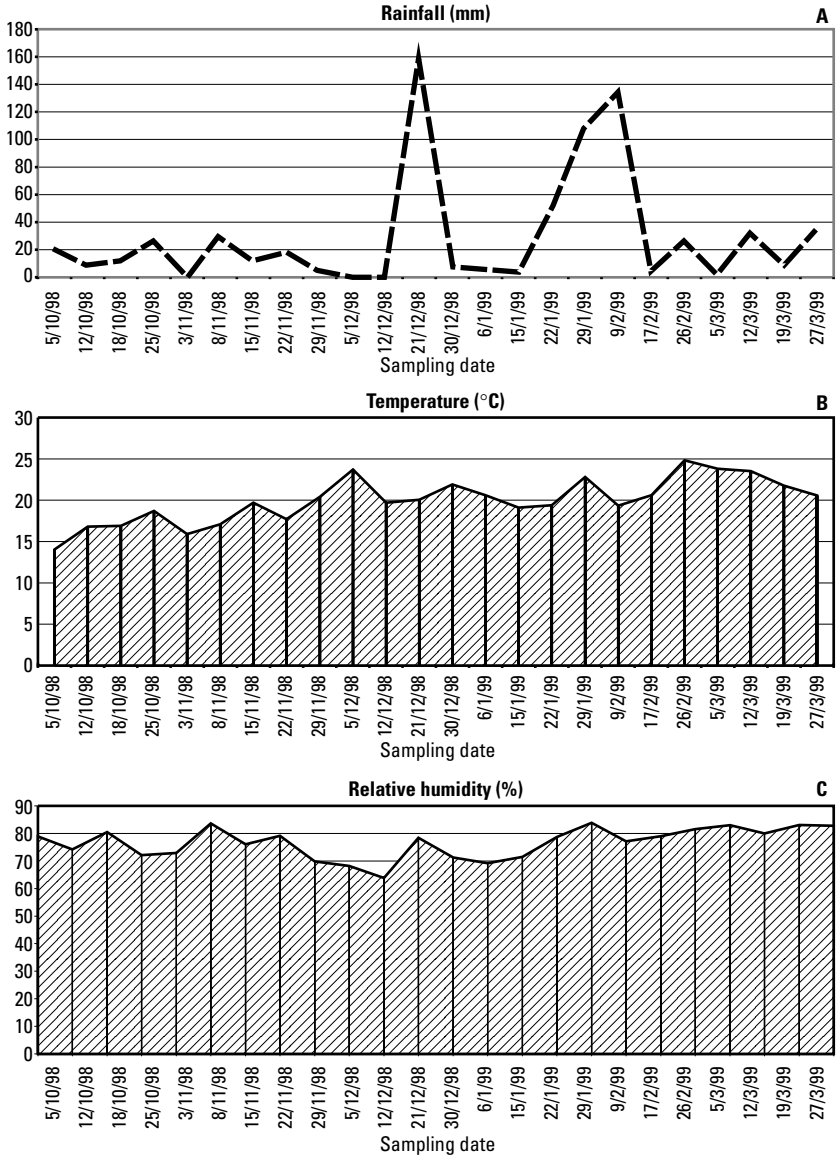


**Figure 2. Weekly mean rainfall (a), weekly mean temperature (b), and weekly mean relative humidity (c).**

**Table 1. Correlation coefficients and partial correlation coefficients between number of pycnidiospores in rain water and petroleum jelly and number of ascospores of *Septoria tritici* and *Mycosphaerella graminicola* with weather variables.**

| | Temperature | Relative humidity | Rainfall |
|---|---|---|---|
| Pycnidiospores/ rain water | -0.20 ns [a] | 0.06 ns | 0.26 ns |
| | -0.19 ns [b] | 0.04 ns | 0.04 ns |
| Pycnidiospores/ p. jelly | 0.12 ns | 0.11 ns | 0.53** |
| | 0.27 ns | 0.01 ns | 0.46* |
| Ascospores | 0.53** | 0.46* | 0.57** |
| | 0.62** | 0.42* | 0.60** |

a= correlation coefficient.
b= partial correlation coefficient.

**Table 2. Multiple regression model of weather variables and number of pycnidiospores in rain water, petroleum jelly, and ascospores of *Septoria tritici* and *Mycosphaerella graminicola.***

| | Coefficient | Significance level |
|---|---|---|
| **No. of pycnidiospores in water** | | |
| Constant | 9.33 | |
| Temperature | -0.26 | ns |
| Relative humidity | -0.01 | ns |
| Rainfall | 0.02 | ns |
| **No. of pycnidiospores in p. jelly** | | |
| Constant | 11.31 | ns |
| Temperature | 0.20 | ns |
| Relative humidity | 0.07 | ns |
| Rainfall | 0.08 | P<0.01 |
| **No. of ascospores** | | |
| Constant | -18.02 | P<0.00 |
| Temperature | 0.49 | P<0.00 |
| Relative humidity | 0.14 | P<0.05 |
| Rainfall | 0.03 | P<0.00 |

related to the end of the crop vegetative period and the advent of saprophytic pathogens on the wheat debris. A new increase in pycnidiospores on 5 March was related to the first natural infection of seedlings by septoria leaf blotch.

The pycnidiospore pattern in petroleum jelly was constant from the first week of observation (Figure 1b). The increased density was associated with the amount of rain water. The correlation coefficient and the partial regression coefficient between pycnidiospores and rainfall were significant (Table 1), as was the regression coefficient for rainfall in the multiple regression model. This model explained 18% of the variance (Table 2). Temperature and humidity during this period were probably not so high as to affect the number of pycnidiospores released. Similar to observations by Arseniuk and Góral (1998), we recorded high densities of pycnidiospores in water microdroplets dispersed by wind during short periods of time, mainly in the summer.

The first ascospores on the spore samplers were detected on 12 October. The number increased gradually until the twelfth week of observation (13-21 of December), as rainfall increased to its maximum intensity. After that, the number of spores was more or less constant, with increments recorded when

rainfall increased. Periods of low rainfall were associated with low ascospore density (30 December to 15 January, 17 February, and 5 March). Temperatures after week 12 were in general above 20ºC, whereas in the previous period they were generally lower. It is thus likely that both the disease cycle and the increase in temperature affected ascospore release. Analysis of the partial correlation, which considers variables as being independent, showed significant association with temperature, relative humidity, and rainfall (Table 1). The multiple regression model (Table 2) explained 59% of the variance, and the regression coefficients for climatic variables were significant. As Arseniuk and Góral established in 1998, the abundance of ascospores reached its peak at harvest time. After harvest, plant debris decomposed and the amount of air-borne spores was reduced. Despite this reduction, ascospores were also released when there was low rainfall and adequate relative humidity. Arseniuk and Góral (1998) observed that less than 1 mm of rainfall and high relative humidity were enough to increase the number of air-borne ascospores.

Finally, the effect of different distance from the inoculum source (1 m and 12 m) on the density of ascospores and pycnidiospores was not significant, in agreement with Góral and Arseniuk (in press 1998; Arseniuk and Góral, 1998) (Table 3). The number of spores near the inoculum source and at 12 m was similar.

It is concluded that air dispersal of ascospores and pycnidiospores plays a role in the epidemiology of *Mycosphaerella* (*Septoria*) blotch under the climatic conditions east of Buenos Aires, Argentina. Pycnidiospores were produced in greater abundance than ascospores and they were the predominant source of inoculum. The abundance of pycnidiospores probably reflects their greater importance in the epidemiology of leaf blotch of wheat.

**Table 3. Analysis of variance for mean values of pycnidiospores and ascospores sampled at different distances from an inoculum source.**

| Source of variation | Mean square |
|---|---|
| Distance | 53.7 ns |
| Error | 35.0 |

ns= not significant according to the F test. P=0.05.

## Acknowledgments

We are grateful to the Comisión de Investigaciones Científicas, Buenos Aires Province, and the Consejo Nacional de Investigaciones Científicas y Tecnológicas for their financial support. We also thank Ing. Agr. D. Bayo, Mrs. N. Kripelz, Mr. B. Cordo López, and J. Balonga for their technical assistance.

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# Epidemiology of Seedborne *Stagonospora nodorum:* A Case Study on New York Winter Wheat

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## Abstract

*Stagonospora nodorum blotch is the most important component of the foliar disease complex that attacks New York winter wheat. Ascospores of the teleomorph* Phaeosphaeria nodorum *are not commonly observed in New York, where wheat is generally preceded in sequence by several years of nonhost, rotational crops. Wheat seed infection by* Stagonospora nodorum *is common, the extent and range depending mainly on rainfall during the production season. Transmission of the pathogen from infected seeds to coleoptiles can approach 100% over a wide soil temperature range; transmission to the first leaves is less than 50% and is most efficient at soil temperatures below 17 °C. Nevertheless, under the high densities at which wheat is sown, a significant number of infected seedlings per unit area can originate from relatively low initial seed infection levels and transmission efficiencies. Stagonospora nodorum blotch epidemics arising from infected seed potentially can be managed by reducing initial seedborne inoculum and its transmission. Wheat cultivars exhibit differential responses to infection of seed by* S. nodorum, *and this seed resistance appears to be under genetic control separate from foliar resistance. Breeding for reduced frequencies of seed infection and transmission, along with improved disease management in seed production fields and the application of seed fungicides, may comprise an effective integrated strategy for managing stagonospora nodorum blotch in wheat production systems similar to that in New York.*

## The Pathosystem in New York

Approximately 53,000 hectares of soft winter wheat (mainly white cultivars for pastry flour) are cultivated annually as a rotational crop on vegetable, dairy, and cash grain farms in western and central New York. Stagonospora nodorum blotch is the most prevalent and severe foliar disease affecting the crop (Schilder and Bergstrom, 1989). The disease is currently undermanaged. Adapted wheat cultivars are susceptible, and because of low grain prices and inconsistent returns on fungicide expenditure, few producers apply foliar fungicide.

Several observations led to the hypothesis that infected seed is the primary inoculum source for stagonospora nodorum blotch epidemics in New York. Firstly, infected wheat debris, regarded as the primary inoculum source in continuously or frequently cropped wheat systems, is eliminated from wheat fields by three- to six-year rotations. New York wheat fields are also relatively small and scattered, reducing the chances of infected debris blowing from one wheat field to another. *Stagonospora nodorum* infects several grasses, but their role in epidemic initiation on wheat is likely minimal (Krupinsky, 1997), and there is some evidence of host specificity (Ueng et al., 1994; Krupinsky, 1997). The teleomorphic stage, *P. nodorum*, formed typically on wheat straw, has not been recovered in New York, despite concerted searches of debris and aerial spore trapping from fields affected heavily by stagonospora nodorum blotch (Bergstrom, unpublished). Moreover, most straw is baled and removed from fields shortly after harvest, and the remaining stubble is sometimes plowed under in late summer before planting alfalfa or grass, or is plowed the following spring prior to planting of corn, vegetables, or soybean.

About 40% of New York winter wheat is underseeded with red clover, which serves as a winter cover crop. Clover attains a height of 30 to 40 cm, enough to completely cover unplowed stubble 20 to 25 cm high, thus impeding spore dispersal. Assuming that the *P. nodorum* stage does occur undetected, current cultural practices would diminish substantially its role in disease epidemiology in New York wheat. Shah et al. (1995), using DNA fingerprinted isolates of the pathogen, demonstrated that seedborne *S. nodorum* could initiate foliar epidemics under New York field conditions and foliar disease severity during grain development may be correlated with seed infection incidence (Shah et al., 1995).

## Seed Surveys

Most New York wheat producers sow certified seed that has passed minimal standards for germination, cultivar purity, contamination by weed seed and foreign materials, and infection by smuts and bunts. Infection by *S. nodorum* is not part of the certification standard. Winter wheat seedlots were surveyed from 1990 to 1996 for *S. nodorum* by assaying seed samples on SNAW medium (Manandhar and Cunfer, 1991). Parts of the survey have been published (Shah and Bergstrom, 1993). In some years, all sampled lots contained seed infected by *S. nodorum*, and no cultivar was completely resistant to seed infection. Seasons with higher rainfall during wheat elongation through grain formation stages resulted in elevated seed infection incidences compared to low rainfall seasons (Figure 1).

## Transmission from Infected Seed

Four lots (representing the cvs. Cayuga, Geneva, and Harus), ranging in infection incidence from 53 to 96%, were sown in soil at five temperatures (9, 13, 17, 21, 25 ºC), at 90 % relative humidity and a 16-h photoperiod. All seedlings were harvested at the second leaf emerging stage, and coleoptiles were inspected for lesions induced by *S. nodorum*. Segments of the first leaf were plated onto Bannon's medium (Bannon, 1978). Plates were incubated for 14 d under near ultraviolet light at room temperature. Leaf pieces were then inspected for *S. nodorum* pycnidia, which confirmed infection of the leaf. Transmission frequencies to the coleoptiles and first leaves were analyzed by SAS Proc Mixed (version 6.12; SAS Institute Inc., Cary, NC).

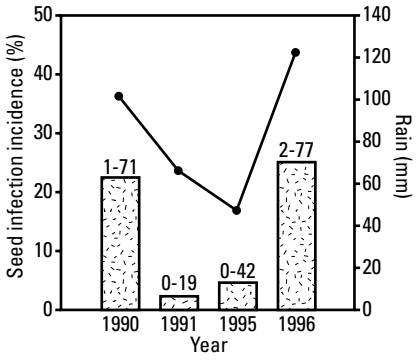


**Figure 1. Mean seed infection incidence by *Stagonospora nodorum* in New York winter wheat lots in four production seasons. Incidence of infection range is shown over each bar. Rainfall averaged over the months of April-June and the 15 major wheat growing counties in western and central New York are shown by the line graph.**

Germination was slightly, but not significantly, lower at 9 ºC. Transmission to the coleoptiles was high at each temperature, but the effect of temperature was significant ($P = 0.0001$), reflecting differences in transmission at the extremes of 9 and 25 ºC. Pycnidia were found sometimes on the coleoptiles. Transmission to the first leaf was much less frequent and was temperature dependent. The average transmission frequency at 9 ºC was 0.39, but dropped to 0.03 at 25 ºC. The pathogen was not distributed evenly in leaf tissue. It was recovered at higher frequency from sections of the first leaf proximal to the stem than from sections taken from the leaf apex region. Approximately 50% of infected first leaves were asymptomatic.

At a transmission frequency of 0.1, based conservatively on our results, a seedlot with 5% infected seed would give rise to an average of one infected seedling per $m^2$ in a stand of 200 seedlings per $m^2$.

## Simulations

We simulated stagonospora nodorum blotch epidemics from infected seedlings at main shoot and 3 tillers to early dough, a period of about 60 to 70 d for New York wheat, using apparent infection rates (*sensu* van der Plank, 1963) derived from or reported in the literature (Jeger et al., 1983; Rambow, 1989) and the logistic model (van der Plank, 1963). Near 100% incidence of infection by the early dough stage is possible from as low as one infected plant per $m^2$ (Figure 2). Relatively low seed infection incidences and transmission rates, without any increase in disease incidence over the winter, could conceiveably result in 50% of all plants infected by flowering (Figure 3). Decreasing the initial seedling disease incidence can delay the epidemic significantly (Figure 3).

## Differential Seed Infection Among Cultivars

To examine the possibility of cultivar differential response to seed infection, we assayed seed samples

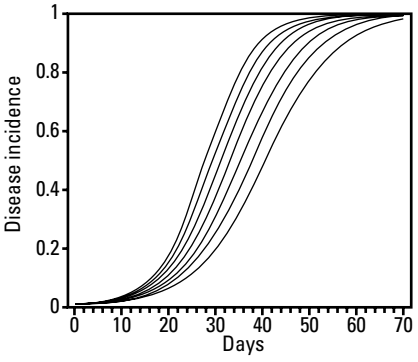


**Figure 2. Increase in stagonospora nodorum blotch incidence with apparent infection rates of 0.13, 0.14, 0.15, 0.16, 0.17, 0.18, or 0.19, starting from an initial seedling disease incidence of 0.005, which corresponds to 1 infected plant per $m^2$ at a density of 200 per $m^2$. A period of 70 days corresponds to the interval from main shoot and 3-tiller stage to early dough stage under New York winter wheat production conditions.**

from each of four New York winter wheat trial locations in 1995 and 1996. Data for two 1995 sites are shown in Figure 4. Results for 1996 were similar. The effect of local environment is significant; seed infection at site B is greater for all cultivars than at site A. The initial inoculum sources were the same at both sites since seed used for sowing came from the same lot for any given cultivar. Some cultivars (Delaware and Houser, for instance) had consistently less seed infection than others, even across different production environments (Figure 4).

Screening of the cultivars for differential responses to seed infection by *S. nodorum* was continued under glasshouse conditions. Flag leaves and ears were inoculated with spore suspensions of a single isolate at $10^6$ spores/ml. Flag leaves were assessed for percent leaf area diseased 15 d post-inoculation, and seeds were assayed on SNAW medium upon maturity. Seed infection was high, but again, some cultivars had less seed infection than others. There was a strong correlation between the field based observations and the results of the quantitative glasshouse inoculations when cultivars were ranked according to seed infection level. There appears to be genetic mechanisms of resistance to seed infection by *S. nodorum*. The poor correlation between flag leaf disease severity and seed infection incidence suggests that seed and foliar resistance are under separate genetic control.

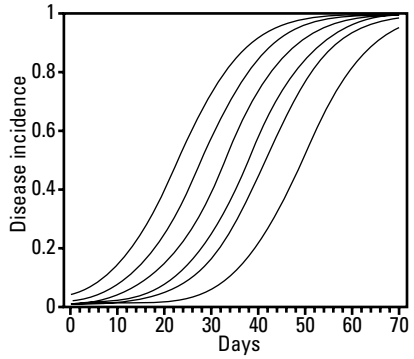


**Figure 3. Increase in stagonospora nodorum blotch incidence from initial seedling disease incidences of 0.001, 0.003, 0.005, 0.01, 0.02, or 0.04, given an apparent infection rate of 0.14. The 70 day period corresponds to the interval from main shoot and 3-tiller stage to early dough stage under New York winter wheat production conditions.**

## Potential for Integrated Management

No single control tactic is likely to reduce seedborne inoculum below levels that could initiate epidemics under favorable environments. The most effective triazole seed fungicides can reduce pathogen transmission in grossly infected seedlots so that only 1 to 2% of seedlings are infected, still enough for epidemics to develop (Bergstrom, unpublished). A strategy integrating breeding for reduced seed infection and transmission frequencies, improved disease management in seed production fields, and the application of seed fungicides may be employed to manage stagonospora nodorum blotch effectively in wheat production systems similar to that in New York.

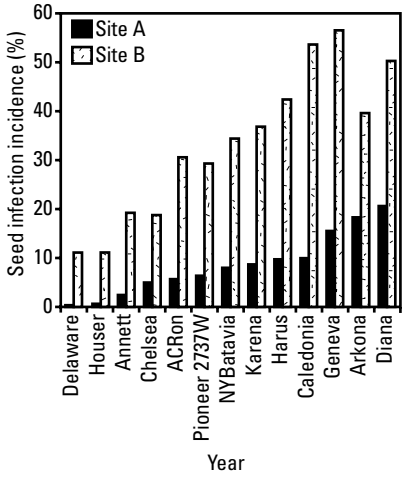


**Figure 4. Differential seed infection of winter wheat cultivars by *Stagonospora nodorum* at two New York sites (A and B), located 5 km apart but differing in microclimate, in 1995.**

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## *Sessions 6A and 6B: Cultural Practices and Disease Management*

# Influence of Cultural Practices on *Septoria/Stagonospora* Diseases

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## Abstract

*The use of cultural management practices is probably one of the oldest approaches to controlling plant diseases. How cultural management practices such as crop rotation, tillage (residue level), fertilizer application, seeding operations, and disease-free seed can influence the incidence and severity of* Septoria/Stagonospora *diseases are reviewed. Considering that early reports on cultural practices have been reviewed previously, recent research will be emphasized. Management practices such as the use of resistant cultivars, cultivar blends, and the use of fungicides will be covered by others at the workshop.*

From ancient times various cultural practices have been used to reduce plant diseases and have paralleled the development of agriculture (Howard, 1996). Disease severity can be modified by various cultural practices, but the magnitude and direction of these effects are not always consistent (King et al., 1983; Shipton et al., 1971; Tompkins et al., 1993). In some studies, disease severity can be affected more by trial location than by the agronomic practices being tested (Tompkins et al., 1993).

Considering that early reports on cultural practices have been reviewed (Eyal, 1981; King et al., 1983; Shipton et al., 1971), more recent research will be emphasized in this paper. A number of cultural practices such as crop rotation, tillage, fertilization, seeding, and disease-free seed will be reviewed. Other practices, including the use of resistant cultivars, cultivar blends, and fungicides are management practices that are covered by other authors in these proceedings.

## Crop Rotation: A Key Factor

The survival of *Septoria/ Stagonospora* spp. on residue from a previous wheat crop is the most important means of carryover from one crop to the next. Researchers have recommended proper crop rotations in combination with other practices to reduce the severity of *Septoria/Stagonospora* diseases. Plowing and burning have also been used to reduce the amount of residue-borne inoculum, but these practices alone may still leave sufficient inoculum for the next wheat crop (Eyal, 1981; King et al., 1983; Shipton et al., 1971). Although resistant cultivars are covered by other authors in these proceedings, the impact of resistant cultivars on the carryover of pathogens should also be considered. Murray et al. (1990) pointed out with septoria tritici blotch (STB) that resistant cultivars not only reduce crop losses but the residue can also influence the next crop. Residue from resistant crops reduces the potential disease severity the following year because less inoculum is provided to initiate the epidemic.

Early researchers, such as Rosen (1921), included proper crop rotation in their recommendations for control of stagonospora nodorum blotch (SNB). Crop rotations take advantage of the fact that plant pathogens important on one crop may not cause disease problems on another crop. Appropriate crop rotations lengthen the time between crop types so that pathogen populations have time to decline. Although pathogens may not be completely eliminated, there is a reduction in the pathogen population. By rotating among crop types, the pathogens on residue from the previous crop will not infect the crop being grown (Krupinsky et al., 1997). Crop rotation allows time for the decomposition of residue on which pathogens carry over, and natural competitive organisms reduce the pathogens on the remaining residue while unrelated crops are being grown. Crop rotation is a key factor affecting the health and productivity of future wheat crops (Cook and Veseth, 1991). Crop rotation and residue management are most effective with pathogens that are disseminated only short distances but not as effective for pathogens

disseminated over long distances. When inoculum is produced within a field and is disseminated only short distances, as with *Stagonospora nodorum* in the southeastern USA, crop rotation is an important management practice (Cunfer, 1998).

When evaluating methods of reducing the risk of disease severity with reduced tillage, Bailey and Duczek (1996) indicated that the most effective means of reducing disease is crop rotation. In Saskatchewan, Canada, Pedersen and Hughes (1992) reported that crop rotation was effective in reducing the severity of epidemics caused by a leaf spot disease complex of *S. nodorum* and *Septoria tritici*. In general, *Septoria/ Stagonospora* disease severity is greater when wheat is grown in monoculture, compared to wheat following an alternative crop. In Germany, Käesbohrer and Hoffmann (1989) reported that winter wheat was infected earlier and more frequently by *S. nodorum* in wheat after wheat, compared with wheat in a crop rotation. Sutton and Vyn (1990) found that the severity of SNB on spikes of winter wheat was higher after wheat compared to wheat after soybeans.

Disease severity was significantly higher under monoculture than under rotation, particularly with no tillage, with a leaf-spot disease complex composed of *Pyrenophora tritici-repentis*, *Bipolaris sorokiniana*, *and S. nodorum* (Reis et al., 1992, 1997). With *P. tritici-repentis* and *S. tritici* as the dominant pathogens, STB was significantly higher on winter wheat when grown in monoculture (Odorfer et al., 1994). With *P. tritici-repentis* and *S. nodorum* as the dominant pathogens, Fernandez et al. (1998) observed that wheat after wheat had a higher disease severity than wheat grown after flax or lentil. This was particularly evident in years with high disease pressure but not in years with low disease pressure.

In general, most carry-over inoculum of *Septoria/Stagonospora* diseases is minimized by crop rotations that include wheat or other cereals every third year (Wiese, 1987). Considering that plant residue decays more rapidly in warm humid conditions than in drier and cooler conditions, the amount of time necessary between wheat crops may vary depending on the environment and location. In Israel, Eyal (1981) reported that a 3-5 year rotation is needed to decrease the incidence of STB. Under favorable disease conditions in Saskatchewan, Canada, a rotation of two years between spring wheat crops reduced disease severity and provided adequate control of STB and SNB (Pedersen and Hughes, 1992). Also in Saskatchewan, Fernandez et al. (1998) recommended two years of spring wheat followed by two years of a non-cereal crop, or by a non-cereal crop and summer fallow to reduce disease severity (*P. tritici-repentis* and *S. nodorum*).

Since Fernandez et al. (1993) indicated that *S. nodorum*, along with *Fusarium graminearum* and *B. sorokiniana*, survived longer on wheat residue after summer fallow compared to wheat residue after corn or soybean crops, summer fallow may not be as effective as an alternative crop in reducing inoculum levels. In the southeastern USA, *S. nodorum* inoculum may be present on wheat stubble on the soil surface 22 months after a wheat crop is harvested, leading to recommendations for crop rotation and tillage (Cunfer, 1998). In addition to crop rotation, intercropping can be used to reduce inoculum movement in the field. In Ireland, Bannon and Cooke (1998) reported that the dispersal of pycnidiospores of *S. tritici* was reduced 33% horizontally and 63% vertically in a wheat-clover intercrop.

## Tillage

Infested residue provides an important mechanism for the carryover of *Septoria/Stagonospora* spp. from one crop to the next. Tillage promotes residue decomposition by fracturing the residue and exposing it to residue-decomposing microorganisms. Thus, tillage operations such as plowing have been recommended as a means to reduce residue (King et al., 1983; Shipton et al., 1971).

In recent years, conservation tillage practices have been promoted to reduce soil erosion and conserve soil moisture. These minimum or no tillage operations increase the quantity of crop residue on the soil surface. Increased levels of crop residues may influence the incidence and severity of plant diseases, depending upon the disease and region. Conservation tillage or reduced tillage practices increase, decrease, or have no effect on plant diseases (Bailey and Duczek, 1996; Sumner et al., 1981). Conservation tillage may have different effects on plant diseases depending on the soils, region, or prevailing environment, and the biology of the disease organism.

Differences in weather cycles in wheat growing areas are often dramatic, which may account for some of the differences in the impact of diseases described in the literature. When comparing tillage effects in northeastern North Dakota, USA, Stover et al. (1996) observed that different diseases dominated each year with different weather conditions. They found that *P. tritici-repentis* dominated in the first two years of the study, and septoria foliar diseases and fusarium head blight dominated in the third year.

Tillage or lack of tillage can also affect the severity of individual diseases. Sutton and Vyn (1990) reported that *P. tritici-repentis* and *S. nodorum* were promoted when wheat was grown after wheat and minimum or no tillage was used, whereas *S. tritici* was suppressed. The reverse occurred when wheat followed alternative crops in all tillage treatments or following wheat under conventional tillage. With airborne ascospores of *S. tritici* implicated as the source of primary inoculum in Oklahoma, USA, Schuh (1990) concluded that the amount of residue after different tillage operations did not have a strong effect on disease severity.

In western Canada, Bailey and Duczek (1996) indicated that foliar diseases increase under reduced tillage but not always to damaging levels. With conditions for low disease development, Bailey et al. (1992) did not observe consistent tillage effects on foliar diseases. In Kentucky, USA, Ditsch and Grove (1991) reported that SNB was not affected by tillage but powdery mildew infection (*Erysiphe graminis*) was higher under no tillage. In North Dakota, USA, with a leaf-spot disease complex composed mainly of *P. tritici-repentis* and *S. nodorum*, Krupinsky et al. (1998) found generally higher levels of necrosis and chlorosis associated with no tillage compared to minimum or conventional tillage. However, in some trials the tillage effect varied depending on the nitrogen treatment. With a leaf-spot disease complex composed of *P. tritici-repentis*, *B. sorokiniana*, *and S. nodorum,* Reis et al. (1992, 1997) reported that disease incidence and severity were higher with minimum and no tillage treatments in Brazil. In the eastern USA, both tillage and crop rotation are recommended to avoid losses to SNB (Cunfer, 1998; Milus and Chalkley, 1997).

## Burning of Residues

Although burning crop residue was recommended in the past (King et al., 1983; Shipton et al., 1971), it is no longer recommended because of environmental concerns. In addition, burning may not be hot enough to eliminate all residue, leaving sufficient infested residue to provide carryover of inoculum for another wheat crop (Eyal, 1981). In northeastern North Dakota, USA, Stover et al. (1996) compared chisel plowing (high residue) to moldboard plowing and burning followed by moldboard plowing. The effect of chisel plowing on early season foliar disease did not consistently carry over to late season severity ratings. In the first year, chisel plowing resulted in the highest late season disease severity, in the second year, there were no differences, and in the third year, the chisel plow treatment had the lowest late season disease severity. Overall, yields were not affected by a tillage (burning)-related foliar disease effect.

## Plant Nutrition: Nitrogen Fertilizer

As with other management practices discussed above, environmental conditions influence disease development and treatment responses, leading to variation in research results among different geographical regions. The severity of *Septoria/Stagonospora* diseases may increase or decrease with increasing nitrogen rates depending on the region. Tiedemann (1996) suggested that inconsistencies of *Septoria/Stagonospora* disease response to nitrogen fertilization may be due to in part to environmental factors such as ozone.

High rates of nitrogen fertilizer have been reported to increase the severity of *Septoria/Stagonospora* diseases. In addition, increased nitrogen can increase the danger of lodging and delayed maturity (Shipton et al., 1971). In Pennsylvania, USA, Broscious et al. (1985) reported that the severity of SNB and powdery mildew increased significantly on winter wheat as the rate of spring applied nitrogen fertilizer increased. Similarly, Ditsch and Grove (1991) in Kentucky, USA, reported that SNB and powdery mildew were lowest on winter wheat at the zero nitrogen treatment but increased as nitrogen rates increased. In New York, USA, under conditions of low disease severity, Cox et al. (1989) suggested that high rates of nitrogen have the potential to increase yield and foliar disease severity on winter wheat in the northeastern USA. In Tennessee, USA, Howard et al. (1994) reported that the severity of three foliar

diseases (*S. tritici*, *S. nodorum*, and leaf rust [*Puccinia recondita*]) increased on winter wheat with higher nitrogen rates, especially when fungicides were not applied. In the United Kingdom, Leitch and Jenkins (1995) reported that *Septoria/Stagonospora* disease (principally STB) development on winter wheat was enhanced with the application of nitrogen throughout the season. A wide range of timing and splits of nitrogen application did not significantly influence the level of disease severity after anthesis. Also in the United Kingdom, Jenkyn and King (1988) found an increase in *Septoria/Stagonospora* diseases (mostly STB) on winter wheat after fallow compared to winter wheat after ryegrass. They attributed the increase in disease severity to an increased accumulation of available nitrogen during the fallow period.

There have also been reports of no effect or a decrease in the severity of *Septoria/Stagonospora* diseases on wheat with increased nitrogen rates. In Germany, assessing disease damage by the number of pycnidia and number of latent infections of *S. nodorum* on winter wheat, Büschbell and Hoffmann (1992) reported that the influence of nitrogen rates was not significant. Also in Germany, Tiedemann (1996) reported that increased nitrogen reduced the severity of SNB on spring wheat, while increasing the severity of powdery mildew and leaf rust. This nitrogen effect on the disease severity of SNB was reversed at elevated ozone concentrations. In the United Kingdom, late season applications of urea solution reduced the severity of STB on the flag leaf of winter wheat (Gooding et al., 1988). Naylor and Su (1988) reported that the severity of SNB on winter wheat was not affected by increased nitrogen levels early in the season and even decreased with increasing nitrogen later in the season.

In Maryland, USA, SNB was reduced on winter wheat with a higher nitrogen fertility rate (Orth and Grybauskas, 1994). They suggested that the reduction of SNB was apparently due to interference of splash dispersal of spores in a denser canopy and the suppressive effect of high nitrogen fertility. In glasshouse trials, they also reported that increased nitrogen fertility decreased the severity of SNB on the same winter wheat cultivars tested in the field. In North Dakota, USA, with a leaf-spot disease complex composed mainly of *P. tritici-repentis* and *S. nodorum*, Krupinsky et al. (1998) reported that with low nitrogen levels disease severity was higher in no tillage compared to conventional tillage. At higher nitrogen levels, the difference in disease severity for tillage treatments was greatly reduced.

In Saskatchewan, Canada, the development of *Septoria/ Stagonospora* diseases on winter wheat was influenced by nitrogen fertility in one trial out of nine (Tompkins et al., 1993). Greater disease severity was associated with low nitrogen fertility. They suggested that lesion development may be promoted by nitrogen deficiency or a nutrient imbalance. Also in Saskatchewan, Fernandez et al. (1998) reported an increase in disease severity with an increase in nitrogen deficiency in dry years with a leaf-spot disease complex composed mainly of *P. tritici-repentis* and *S. nodorum*. Disease severity declined with treatments receiving no phosphorus.

## Seeding Operations

A higher disease severity of STB was associated with earlier sowings in New South Wales, Australia (Murray et al., 1990). The longer time between sowing and heading probably leads to a higher disease severity. When studying seeding rates, row spacing, and depth of seeding in Pennsylvania, USA, Broscious et al. (1985) reported that increased seeding rates increased SNB in four out of 13 trials and decreased SNB in one trial. They suggested that growers could reduce row spacing from 18 cm to 13 cm to increase yields without increasing disease severity. In Saskatchewan, Tompkins et al. (1993) reported that the severity of *Septoria/ Stagonospora* diseases increased with a higher seeding rate. Disease severity was not influenced by row spacing. Narrow row spacing (10 cm) with increased nitrogen rates reduced SNB on winter wheat and increased yields in Maryland, USA (Orth and Grybauskas, 1994).

## Use of Disease-Free Seed

Seed infected with *S. nodorum* can be a source of primary inoculum and a probable source of transmission from one wheat crop to the next. Inoculum can survive in seed for extended periods of time. *Septoria tritici* can be seed-borne but is not a significant source of inoculum (Eyal, 1981; King et al., 1983; Shipton et al., 1971). Although the use of disease-free seed may not be considered a cultural practice, it should be evaluated by the producer as a management practice for reducing disease severity. Cultural practices such as crop rotation may not be effective if seed infected with *S. nodorum* is used for planting (Luke et al., 1983). The beneficial effect of disease-free seed can be negated by sowing into residue from a previous wheat crop (Luke et al., 1983, 1985; Milus and Chalkley, 1997).

In Germany, Rambow (1990) indicated that infected seed was the main source of primary inoculum. In Poland, Arseniuk et al. (1998) reported that higher seed infestation by *S. nodorum* resulted in higher disease levels in the field. *Stagonospora nodorum* was found in 98% of the wheat seed samples tested in North Carolina, USA, and the fungus survived for more than two years on stored seed (Babadoost and Hebert, 1984). Using seed lots infected 1% to 40% with *S. nodorum*, Luke et al. (1986) reported that 10% seed infection was adequate to cause a severe epidemic in the southeastern USA. In the northeastern USA, Shah et al. (1995) found that in 1990-91, a season mildly conductive to disease development, plots sown to seed with less than 1% infection by *S. nodorum* developed epidemics. In 1991-92, epidemics were initiated with seed infection levels as low as 0.5%. They demonstrated that the same isolates in the seed used for planting were found in the seed harvested. This indicated that seed populations of *S. nodorum* could initiate epidemics of SNB in new locations and could provide year-to-year perpetuation of these populations. In Manitoba, Canada, plants from shriveled seed caused by *S. nodorum* had lower seedling vigor but with increased number of tillers on plants from the shriveled seed, the yield from plump or shriveled seed plots could not be differentiated (Gilbert et al., 1995).

When disease-free seed is not available, chemical treatments and fungicides can be used to reduce or eliminate *Septoria/Stagonospora* pathogens from seed used for planting (Eyal, 1981; King et al., 1983; Shipton et al., 1971). The use of fungicides for seed treatment are covered by other authors in these proceedings.

## Conclusion

With relatively low value crops such as small cereal grains, there is a need for cultural management practices that can reduce the impact of diseases (Howard, 1996). With the adoption of conservation tillage practices, additional long-term research is needed to minimize disease severity with integrated diverse cropping systems. Hopefully new experiments that study the interaction of cultural practices, including the development of new crop rotation sequences, will provide additional information in the future. Adapted resistant cultivars, which are an important factor in reducing disease epidemics, should be used when available. The best strategy to minimize the impact of *Septoria/Stagonospora* diseases is to integrate several cultural practices into one system. The integration of several cultural practices should minimize disease impacts on the economics for the grower without increasing the cost of production.

## Acknowledgments

I thank B. M. Cunfer and D. E. Mathre for their reviews and constructive comments. Mention of a trademark, proprietary product, or company by USDA personnel is intended for explicit description only and does not constitute a guarantee or warranty of the product by the USDA and does not imply its approval to the exclusion of other products that may also be suitable. USDA-ARS, Northern Plains Area, is an equal opportunity/affirmative action employer and all agency services are available without discrimination.

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# Disease Management Using Varietal Mixtures

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## Abstract

*Few data are available that evaluate the impact of variety mixtures on the* Septoria/Stagonospora *diseases of cereals. The splash-dispersed nature of the pathogens and the predominance of quantitative variation for host resistance and pathogenicity in the* Septoria/Stagonospora *diseases may make interactions more complex than for the rusts and mildews, where major gene interactions have been the main object of study. We have found that epidemic progression of septoria tritici blotch can be substantially suppressed in mixtures of a susceptible and a moderately resistant variety, sometimes to below the level of the more resistant variety grown in pure stand.* Mycosphaerella graminicola *populations sampled from variety mixtures have been found to be reduced in pathogenicity in all mixtures that have been investigated thus far. Disruptive selection may be an important mechanism affecting disease progression and evolution of* M. graminicola *in variety mixtures. Major genes for resistance have the potential to contribute substantially to the use of variety mixtures for control of* Septoria/Stagonospora *diseases, both through their epidemiological impacts on disease spread, and through effects of induced resistance between avirulent and virulent genotypes of the pathogen that may occur in variety mixtures.*

Variety mixtures have been investigated to the greatest extent for obligate parasites of small grains (Garrett and Mundt, 1999; Wolfe, 1985), and are being utilized commercially for control of these diseases (Garrett and Mundt, 1999; Mundt, 1994). Much less information is available regarding the impact of variety mixtures on diseases caused by non-obligate pathogens.

The effects of variety mixtures may differ for the *Septoria/ Stagonospora* diseases compared to rusts and mildews for at least two reasons. First, steep dispersal gradients caused by splash dispersal of conidia may decrease inoculum exchange among host genotypes in mixture and, hence, reduce the efficacy of mixtures for disease control (Fitt and McCartney, 1986; Mundt and Leonard, 1986). Second, qualitative incompatibility reactions are much less common for septoria diseases in current agricultural systems compared to the rusts and mildews.

Though there clearly is much more host/pathogen specificity than has been suggested in the past for *Mycosphaerella graminicola* (Ahmed et al., 1995; Ahmed et al., 1996; Cowger et al., this volume; Kema et al., 1986; 1987), the general lack of use of major genes for resistance to the *Septoria/ Stagonospora* diseases in agriculture places much greater emphasis on quantitative interactions. For *M. graminicola*, there clearly can be substantial quantitative variation for pathogenicity that is under the influence of host selection (Ahmed et al., 1995; 1996; Mundt et al., 1999) and may be influenced by host diversity. In addition, Jeger et al. (1981a) have derived models which indicate, based on epidemiological considerations, that variety mixtures can impact disease severity even in the absence of host/pathogen specificity.

There are a limited number of studies that address the impact of variety mixtures on *Septoria/ Stagonospora* diseases in the field. Jeger et al. (1981b) found that mixing wheat varieties with apparent non-specific resistance reduced the severity of stagonospora nodorum blotch by more than 50% relative to the mixture components grown separately in pure stands, though overall severity levels were very low. Mundt et al. (1995) studied all possible equiproportional mixtures among two susceptible, one moderately resistant, and one highly resistant wheat variety. Mean disease reductions late in the

epidemics were 27, 9, and 15% for the three seasons, with substantial differences among specific mixtures.

The purpose of the work reported herein was to investigate in greater detail the effects of wheat variety mixtures on epidemic progression of septoria tritici blotch and to determine the influence of host diversity on pathogenicity of *M. graminicola* populations derived from field plots of pure and mixed stands of wheat varieties in the Willamette Valley of Oregon, USA. This location is highly conducive for development of the *Septoria/ Stagonospora* blotches of wheat, with frequent rains from November through June. Naturally occurring epidemics have varied from moderate to severe over the past decade, and commercial fields of susceptible varieties are sprayed every year.

In Oregon, septoria tritici blotch is currently more important than stagonospora nodorum blotch, perhaps due to competitive exclusion caused by earlier ascospore showers of *M. graminicola* as compared to *Phaeosphaeria nodorum* (DiLeone et al., 1997). Septoria tritici blotch epidemics in Oregon begin from a very dominant sexual stage, with primary infections resulting from heavy ascospore showers (Mundt et al., 1999). Ascospore showers often peak in November, after fall rains have begun, but continue at some level throughout the crop season (DiLeone et al., 1997), including secondary cycles of sexual recombination (Zhan et al., 1998).

## Materials and Methods

### Field plots

Plots were grown at the Botany and Plant Pathology Field Laboratory near Corvallis, OR, during the 1997-98 winter wheat season. Plots were sown at a rate of 322 seeds/$m^2$ on 20 October 1997. Each plot was 3.0 m (12 rows) x 5.2 m, and planted within a "checkerboard" of barley (*Hordeum vulgare*) plots of equal size. Standard fertilization and weed control practices were used, supplemented by hand-weeding. Epidemics were initiated from naturally occurring ascospore showers.

Treatments included two susceptible (Stephens and W-301) and two moderately resistant (Cashup and Madsen) varieties, and the four possible equiproportional mixtures of a susceptible and a moderately resistant variety. Treatments were replicated four times in a randomized complete block design. Percent of leaf area covered by lesions, on a whole-canopy basis, was recorded from early February through mid-June. Each plot received a rating that was the average of two observers.

At the end of the season, leaves were sampled from each plot to obtain isolates for greenhouse tests of pathogenicity, as described below. The pathogen was sampled along a diagonal across the inner 10 rows of each plot, with one sampling point per row. Two or three flag leaves were collected from each sampling point. Leaf samples were collected on 12 June 1998, a time when the pathogen had been exposed to repeated generations of selection on the host varieties.

### Greenhouse experiment

A greenhouse experiment was conducted in spring 1999 to determine pathogenicity of the field-collected isolates. Plants of Stephens, W-301, Cashup, and Madsen were raised in 10-cm plastic pots filled with a greenhouse mix that included a slow-release fertilizer. Approximately 15 seeds of a cultivar were sown in each pot. Before inoculation, plants were thinned to 10 per pot. Daytime greenhouse temperature was maintained from 20-25°C, and sodium-halide lights were used to extend daylength to 16 h.

Cultures were obtained from field-collected leaves, stored, and increased by standard methods that have previously been described (Ahmed et al., 1996). Equal numbers of spores of the 10 isolates (one from each of the sampling points of each plot) were combined and the total suspension adjusted to $10^5$ spores/ml.

At 21 days after seeding, groups of four pots (one of each of the four varieties) were inoculated with a suspension derived from each field plot by placing them on a turntable at 16 rpm and applying 33.3 ml of the suspension with a hand sprayer. Surfactant (Tween) was added at the rate of one drop per 50 ml of spore suspension. After inoculation, the plants were kept in

a moist chamber (wooden frame covered with a polyethylene sheet) for 72 hours. High humidity (95% or above) was maintained using a humidifier (ultrasonic, cool mist type) inside the moist chamber. The pots were subsequently returned to a greenhouse bench in a randomized complete block design.

Percent lesion area on the second leaf from the base of each of the 10 plants in each pot was estimated visually at three weeks after inoculation. Each of two observers half of the plants in each pot.

### Effects of pathogen diversity on epidemic progression

A field experiment that allowed us to examine the influence of pathogen diversity on epidemic increase in variety mixtures was conducted in the 1994-95 season. These plots were treated in a manner highly similar to those described above, and have been discussed in more detail elsewhere (Zhan et al., 1998). Treatments were a complete factorial of three host treatments (pure stand of Madsen, pure stand of Stephens, and a 1:1 mixture of Madsen:Stephens) and two inoculation treatments (natural and artificial) in a completely random design with three replications. One set of plots was naturally inoculated, while the other was sprayed in November 1994 with a mixture of 10 isolates to competitively exclude the highly diverse inoculum provided by outside ascospore showers.

## Results

The Madsen/Stephens and Cashup/Stephens mixtures suppressed epidemic development below that of the more resistant component in each mixture (Figure 1). In contrast, the mixtures containing the variety W-301 as the susceptible component performed less well, with the Cashup/W-301 mixture showing no epidemic suppression relative to the mean of the component pure stands (Figure 1).

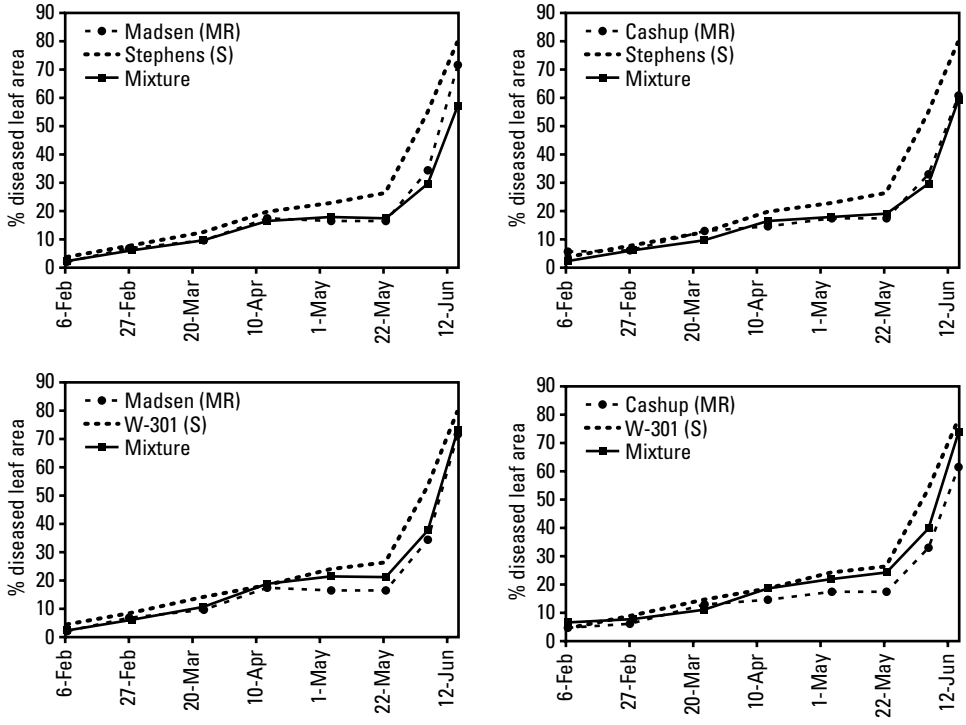


**Figure 1. Disease progress of septoria tritici blotch in naturally-occurring epidemics of pure stand varieties and 1:1 variety mixtures. MR = moderately resistant and S = susceptible.**

Greenhouse tests indicated that host diversity had a substantial impact on pathogenicity of *M. graminicola* (Table 1). All populations derived from the mixtures produced less disease on its component varieties in the greenhouse than the mean of the populations derived from the pure stand components in the field.

Molecular analyses (Zhan et al., 1998) have previously shown that the artificial inoculation used in the 1994/95 season competitively excluded 97% of potential ascospore infections early in the season, though immigration and sexual recombination among inoculated and/or naturally occurring genotypes within plots resulted in non-inoculated genotypes comprising 35-40% of the population later in the season. In contrast, natural inoculum has been estimated to result in hundreds of unique genotypes per $m^2$ (McDonald et al., 1996). For the artificially-inoculated plots, epidemic progression for the Madsen/Stephens mixture was approximately midway between the two pure stands throughout the epidemic (Figure 2). By contrast, in the naturally-inoculated plots, epidemic progression in the mixture began midway between the pure stands, but became suppressed to near that of the moderately resistant variety as the season progressed (Figure 2).

## Discussion

Our results indicate that some variety mixtures suppress epidemic progression of septoria tritici blotch even in the absence of major genes for resistance. There are at least two plausible explanations for this result. First, the models of Jeger et al. (1981a) have shown that, in absence of host/pathogen specificity, mixtures can decrease, increase, or have no effect on epidemic progression, depending on the relative levels of sporulation and infection frequency in the component varieties. Thus, differences between mixtures and pure stands in our experiment might be explained by differences in resistance components between cultivars, though we have not measured these components.

**Table 1. Disease severity (percent of second leaf area covered by septoria tritici blotch lesions) caused by *Mycosphaerella graminicola* populations derived from single wheat varieties and variety mixtures in replicated field plots.**

| | Source of population | |
|---|---|---|
| Variety mixture | Mixture[a] | Pure stands[b] |
| Madsen/Stephens | 9.5 | 15.9 |
| Madsen/W-301 | 9.3 | 11.6 |
| Cashup/Stephens | 10.8 | 13.4 |
| Cashup/W-301 | 6.6 | 9.0 |

[a] Mean disease severity for populations derived from a given mixture when tested separately on each of the component varieties. For example, populations collected from the Madsen/Stephens mixture in the field were tested separately on Madsen and Stephens in the greenhouse.

[b] Mean disease severity for populations derived from the component pure stands and tested on the same variety. For example, to compare with populations derived from the Madsen/Stephens mixture, populations collected from pure stands of Madsen in the field were tested on Madsen in the greenhouse and populations derived from pure stands of Stephens in the field were tested on Stephens in the greenhouse.

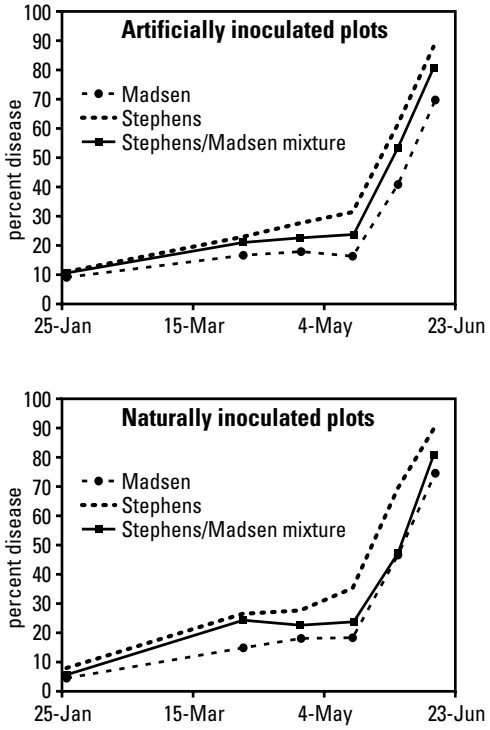


**Figure 2. Disease progress of septoria tritici blotch on a moderately resistant variety (Madsen), a susceptible variety (Stephens) and a 1:1 mixture of the two varieties in epidemics initiated from artificial inoculation with 10 isolates or from naturally occurring inoculum.**

A second explanation is that disruptive selection may reduce the fitness of pathogen populations that cycle between host genotypes in mixtures. The disruptive selection hypothesis is supported by the reduced fitness of *M. graminicola* populations derived from the variety mixtures as compared to the pure stands. It should be noted that the mean 27% fitness reduction of populations from mixtures that was found in our study is likely an underestimation, as disease severity in a monocyclic test accounts for only infection efficiency and lesion expansion, but not other fitness components such as sporulation rate or latent period.

Additional support for the disruptive selection hypothesis is that variety mixtures suppressed epidemic development under conditions of natural inoculation, but not when artificially inoculated with a limited number of isolates. This result would be expected under the disruptive selection hypothesis, as there would be insufficient pathogen variation within the artificially inoculated plots for disruptive selection to occur. Such disruptive selection was earlier suggested to increase the efficacy of barley variety mixtures for control of powdery mildew (caused by *Erysiphe graminis*) (Wolfe et al., 1981) and to reduce the fitness of mildew genotypes that were able to overcome more than one major resistance gene in the mixtures (Chin and Wolfe, 1984).

The levels of disease suppression contributed by our variety mixtures may not be sufficient as the sole control practice, but would certainly be useful in an integrated management program. In addition, we are currently more interested in the potential for varietal diversification to slow pathogen evolution towards increased virulence and/or aggressiveness (sensu Vanderplank, 1968) than we are for the immediate effects on epidemic progression. This is crucial in the Willamette Valley of Oregon, as there is evidence that even quantitative resistance to *M. graminicola* may erode in our environment (Mundt et al., 1999).

Though not addressed in the research reported here, major genes for resistance may contribute substantially to control of septoria diseases in wheat variety mixtures. Our earlier studies showed that mixtures containing both a susceptible and a highly resistant variety provided greater disease control than other types of mixtures (Mundt et al. 1995). The subsequent "breakdown" of the high-level resistance in the variety Gene (Cowger et al., this volume) might be seen to increase the overall level of disease in such mixtures. On the other hand, mixtures of a virulent and avirulent pathogen isolate have been shown to reduce pycnidial production of *M. graminicola* very substantially (Halperin et al., 1996), which could greatly increase the value of "defeated" major genes in variety mixtures.

In summary, there appears to be some potential for variety mixtures to suppress septoria blotch epidemics and to slow pathogen evolution. Currently available data are very scant, however, and substantially greater field evaluation is required to determine the short- and long-term value of the diversity approach for control of *Septoria/Stagonospora* diseases.

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## *Session 6C: Breeding for Disease Resistance*

# Breeding for Resistance to the Septoria/Stagonospora Blights of Wheat

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## Abstract

*Genetic resistance remains the first line of defense against the septoria foliar blights, especially in developing countries. Resistance genes with major or minor effects may be either recessive or dominant. A few genes may be enough to confer resistance, as in the case of partial resistance. Additive gene effects contribute more to resistance than dominance effects. Among normally maturing semidwarf wheats possessing resistance, those carrying* Rht2 *may be more resistant to* Septoria tritici *than those with* Rht1. *In the case of* Stagonospora nodorum, *resistance of the flag leaf and of the spike may be at least partly under separate genetic control.*

*Five confounding factors complicate selection for resistance: 1) maturity and plant height affect the expression of resistance; 2) the relationship between seedling and adult plant responses is highly inconsistent; 3) the correlation between disease response and yield loss is very variable; 4) there is interaction among fungal isolates on the leaf surface; and 5) it is essential to determine the implications of the existence of races for resistance breeding.*

*Durability of resistance across years and locations is a particular concern, and examples of both erosion of resistance and stable resistance have been put forward. There is clear proof of differential variety by isolate interactions at the seedling stage and on adult plants in the field. However, almost without exception, the size of the interaction component is about a magnitude smaller than that of the main effects due to varieties and isolates. Recent molecular experiments on the genetic structure of* S. tritici *populations have found a lack of adaptation to the host genotype.*

*The methodology applied at CIMMYT for pyramiding resistance genes in wheat includes utilizing proven resistance sources as parents, selecting for resistance using a shuttle breeding program between contrasting locations, and confirming resistance through global multilocation testing. The best resulting lines are fed back into the crossing program as parents. The first genepool exploited for resistance to* S. tritici *by CIMMYT breeders originated in Brazil and Argentina, followed by winter wheats and, subsequently, Chinese materials. New sources of resistance, such as highly promising synthetic wheats, are currently being exploited in combination with the previous sources.*

Globally the septoria diseases have increased in importance over the past decades, and as Walter Spurgeon Beach wrote in 1919: "The genus is the more worthy of study on account of its high economic importance." The two main septoria blights addressed in this paper are caused by *Septoria tritici* and *Stagonospora nodorum*. However, inferences can be drawn for the septoria blights in other cereals, such as *Septoria passerinii* on barley. Several excellent overviews have been written on the genetics of resistance to the septoria diseases of cereals and on how to breed for such resistance (Shipton et al., 1971; King et al., 1983; Nelson and Marshall, 1990; Lucas et al., 1999; Eyal, 1999). Most of those reviews were published 10 years or more ago, but a great deal of the information they contain is still correct and relevant today.

This paper emphasizes some of the more recent literature (post 1990) on the genetics and breeding of resistance, and how these new insights may be applied in developing varieties that express durable resistance to the septoria blights.

## The Need for Resistance

In most wheat production environments, although not in all, genetic resistance is the most economical approach to control fungal diseases. There are, however, two other control methods—cultural and chemical—that may be utilized.

In the case of the septoria foliar blights, crop residues play a critical role in the over-seasoning of the pathogen and in supplying the primary inoculum for the subsequent cropping cycle. In recent years zero and reduced tillage practices have been gaining popularity for reasons ranging from the economics of production to protection of the environment, and the trend is likely to become stronger in the near term. Hence cultural control methods involving specific residue removal seem less of an option than a few decades ago. However, in the more distant future, we may once again see a contraction, particularly in the use of the zero tillage option, as the problems of excessive fungicide use and disease buildup both above and below the soil become more intractable.

Chemical control will always need to be available as an option. In particular when conditions are unexpectedly conducive to disease—for example, due to excessive rainfall—a well-researched chemical option must be close at hand. Under such circumstances chemical control may often be justified because it can avoid production losses of great economic value.

Considering the above mentioned control options, breeding for resistance must remain the first line of defense, especially in developing countries. In such countries other options, while attractive in theory, often cannot be implemented in a timely fashion and/or the resources needed for their development and large-scale application are lacking, even in an emergency.

Where resistance is not effective, tolerance can be sought (McKendry and Henke, 1994a). Despite more than 25 years of interest in tolerance to the septoria blights, its mechanism(s) in general remains vague (Zuckerman et al., 1997). There is, however, little doubt that tolerance is widely available and operational in modern-day germplasm.

## Sources of Resistance

Many sources of resistance to the septoria foliar blights, including some wild relatives of wheat, have been studied over the years. The interest in "alien sources" is not new; in the early part of this century, wild relatives resistant to the *Septoria* spp. were already being tested and identified (Beach, 1919; Mackie, 1929). Some of those sources were reported in the official literature, while others were used in breeding programs and reported less in written form.

Although grouping the sources of resistance based on origin or wheat class is to a certain extent arbitrary, commonly used classifications are: derived from South American (in particular Brazilian) sources, winter wheats, Chinese origin, and wild relatives of wheat. Some of the major sources confirmed by several authors are listed in Table 1.

**Table 1. Sources of resistance to *Septoria tritici* and *Stagonospora nodorum* confirmed by several authors. Generally only one key reference is given.**

| ***Septoria tritici*** | **Reference** |
|---|---|
| Anza | Wilson (1994) |
| Bezostaya 1 | Danon et al. (1982) |
| Bobwhite | Gilchrist et al. (1995) |
| Bulgaria 88 | Rillo and Caldwell (1966) |
| Carifen | Lee & Gough (1984) |
| Colotana | Danon et al. (1982) |
| Fortaleza 1 | Danon et al. (1982) |
| Israel 493 | Wilson (1979) |
| Milan | Gilchrist et al. (1995) |
| Nabob | Narvaez & Caldwell (1957) |
| Oasis | Danon et al. (1982) |
| Seabreeze | Rosielle & Brown (1979) |
| Sheridan | Danon et al. (1982) |
| Synthetic wheats (some) | May & Lagudah (1992) |
| Tadinia | Somasco et al. (1996) |
| Tinamou | Gilchrist et al. (1995) |
| *T. dicoccon* | Gilchrist & Skovmand (1995) |
| *T. speltoides* | McKendry & Henke (1994) |
| *T. tauschii* | Appels & Lagudah (1990); May & Lagudah (1992); McKendry & Henke (1994b) |
| Veranopolis | Rosielle & Brown (1979) |
| Vilmorin | Gough & Smith (1985) |
| ***Stagonospora nodorum*** | |
| *Aegilops longissima* | Ecker et al. (1990a) |
| Atlas 66 | Kleijer et al. (1977) |
| Blueboy II | Nelson (1980) |
| Cotipora | Bostwick et al. (1993) |
| Frondoso | Mullaney et al. (1982) |
| Fronthatch | Mullaney et al. (1982) |
| Oasis | Nelson (1980) |
| *T. monococcum* | Ma & Hughes (1993) |
| *T. speltoides* | Ecker et al. (1990b) |
| *T. tauschii* | Ma & Hughes (1993) |
| *T. timopheevii* | Ma & Hughes (1993) |

## Inheritance of Resistance

Resistance genes having major effects have been identified in wheat (Nelson and Marshall, 1990). They have been found to transmit

their resistance in either a recessive or dominant fashion (Eyal, 1999). However, in most cases, resistance appears dominant, with the F1 from a cross between a resistant and a susceptible parent expressing an intermediate level of resistance that is more similar to that of the resistant parent. A few genes may be enough to confer resistance that will hold up in farmers' fields (Dubin and Rajaram, 1996). While heritabilities tend to be only of moderate magnitude, progress in breeding for resistance is evident.

Although the number of genes available may be quite high, accumulating a few key ones may be sufficient to achieve resistance. As has been shown for durable resistance to leaf rust (Singh et al., 1991), several of the alleged components of partial resistance to *S. tritici* may also be controlled by only one or a just a few genes (Jlibene and El Bouami, 1995). It would seem that those components that are genetically different could be combined into the same genetic background by crossing. Once available, molecular markers will be of tremendous use for accumulating resistance to such environmentally sensitive diseases.

The expression of *S. tritici* resistance was studied in *T. tauschii* accessions, synthetic wheats derived from them, plus derivatives of synthetic wheats crossed to common wheats. The results obtained all pointed to inheritance based on one or a few dominant genes (Appels and Lagudah, 1990; May and Lagudah, 1992).

Chromosome 5D of *T. tauschii* contributed a high level of resistance to *S. nodorum* in a synthetic cross with a *T. dicoccum* line and in derived substitution lines (Nicholson et al., 1993). Chromosomes 5D, 3D, and 7D contributed resistance in decreasing order of importance. *Aegilops longissima* and *Ae. speltoides* were shown to contribute *S. nodorum* resistance based on two to four partially dominant to over-dominant genes (Ecker et al., 1990a and b). *Triticum timopheevii* transmitted one *S. nodorum* resistance gene located on chromosome 3A to its progeny from a cross with a susceptible durum parent (Ma and Hughes, 1995).

In most published accounts of quantitative analyses, additive gene effects or general combining ability (GCA) effects contributed more to resistance than dominance or special combining ability (SCA) effects (van Ginkel and Scharen, 1987; Bruno and Nelson, 1990; Danon and Eyal, 1990; Wilkinson et al., 1990; Jonsson, 1991; Jlibene et al., 1994). However, significant non-additive effects were often identified in the same studies. SCA effects tend to be an order of magnitude weaker than GCA effects. Indirectly Nelson and Fang (1994) confirmed these conclusions when they observed an absence of heterosis for components of *S. nodorum* resistance, indicating a general lack of over-dominance effects that enhance resistance. Jlibene et al. (1994) found a small cytoplasmic effect in a few of their crosses.

Triticales were found not to be any more resistant than most wheats, and a range of reactions to both *S. tritici* and *S. nodorum* was observed (Scharen et al., 1990). Tritordeum amphiploids (doubled derivatives from crosses between *Hordeum chilense* and *Triticum* spp.) expressed the *S. tritici* resistance of the barley parent both as seedlings in greenhouse tests and adult plants in field trials (Rubiales et al., 1992).

Tolerance to *S. nodorum* was shown to be a mechanism that is genetically different from (partial) resistance and involves several chromosomes (Rapilly et al., 1988). Distinct characters were identified within each of these two defense mechanisms.

Although the introduction of alien cytoplasm from wild wheat relatives reduced partial resistance somewhat, it did increase tolerance as measured by reduced yield losses (Keane and Jones, 1990).

## Agronomic Traits and Resistance

After a period in the 1960s and 1970s of seemingly increased levels of disease susceptibility in the semidwarf wheats (Santiago, 1970), modern semidwarf germplasm carrying high levels of resistance was subsequently identified. Many are now widely grown as commercial varieties.

As in previous studies, Camacho-Casas et al. (1995) showed that it is possible to breed normally maturing semidwarf wheats if proper disease conditions

are created during the selection process and attention is paid to achieving the desired maturity. Nevertheless, tall and/or late versions of similar isogenic lines tend to be less affected by the septoria/stagonospora blights than their shorter, earlier sister lines.

The roles in conferring *S. tritici* resistance of the two most common dwarfing genes may not be the same (Baltazar et al., 1990). In the four crosses studied, the *Rht2* dwarfing gene tended to be associated more often with increased levels of resistance. Plants carrying *Rht1* were more susceptible.

In the case of *S. nodorum*, resistance of the flag leaf and of the spike may be at least partly under separate genetic control (Rosielle and Brown, 1980; Bostwick et al., 1993; Hu et al., 1996). Resistance of the flag leaf was found to be coded for by genes on chromosomes 3A, 4A, and 3B, while spike resistance was located on the same chromosomes and on 7A (Hu et al., 1996).

## Determination of Resistance

One of the most confounding factors in determining resistance to the septoria blights has been and continues to be the interaction between resistance and maturity and plant height, as mentioned above. Parlevliet (1990) describes how the ranking of resistance may even change dramatically, once these factors have been corrected for.

Deviation from regression was proposed by van Beuningen and Kohli (1990) to correct for the confounding factors of heading and plant height. Loughman et al. (1994a) proposed a numerical classification approach that creates clusters of similar entries based on response patterns including disease reaction, maturity, and height. If proper checks are included, cluster analysis helps to identify resistant material.

A second confounding factor in quantifying resistance is the relationship between seedling and adult plant responses. Somasco et al. (1996) showed that long-term resistance to *S. tritici*, such as that found in Tadinia (derived from the winter wheat Tadorna), was strongly expressed in both seedlings and adult plants. Kema and van Silfhout (1997) showed that not all isolates respond similarly to seedling and adult plant infection.

Several studies have found that resistance to *S. nodorum* is expressed differently in seedlings than in adult plants (Koric, 1988; Arseniuk et al., 1991). These studies concluded that while only preliminary seedling data should be considered, subsequent adult plant screening is imperative.

In a set of bread wheats and durum wheats, seedling response to a crude toxic extract of *S. tritici* produced a varietal ranking different from the adult plant reaction to field inoculation with a spore mixture of the same fungus (Harrabi et al., 1995).

However, media containing crude extracts from grain inoculated with *S. nodorum* elicited a differential response that correlated well with field resistance or susceptibility of the spike (Keller et al., 1994). Resistant lines showed a higher percentage of embryo-forming callus in the presence of the extract.

A third major confounding factor is the correlation between disease response and yield loss. Under field conditions visually observed symptoms of *S. nodorum* on the flag leaf or spike were not always well correlated to yield losses (Tvaruzek and Klem, 1994). Lack of consistently high correlations between *S. nodorum* disease scores and final yield loss motivated Walther (1990) to study a more extensive scoring method. When flag leaves of protected and non-protected plots were scored on three dates around the middle of the epidemic, correlations rose to 0.73.

*Stagonospora nodorum* severity scores on the flag leaf had the highest correlations with yield loss, followed by those on the flag leaf -1 (Walther and Bohmer, 1992). Spike infection was poorly correlated with yield. Heritability of disease variables could be further increased if plant height and maturity were corrected for, with heritability values for weighted assessments reaching 0.89.

The practice of using detached leaves to determine resistance appears to have diminished in the past decade. Most work is now carried out on seedlings and adult plants in the greenhouse or the field.

## Durability of Resistance

Durability of *S. nodorum* resistance within a crop cycle decreased with leaf age, and the older lower leaves proved more susceptible than the younger upper leaves (Jonsson, 1991). Due to this effect, late maturing germplasm is often wrongly considered more resistant than early maturing lines. Thus it is crucial to measure resistance at the same adult growth stage.

More debated than durability within a crop cycle is the issue of durability of resistance across years and locations. Johnson in his 1992 general review of breeding for disease resistance highlights the difficulty of establishing the presence of differential host-pathogen interaction in the septoria foliar blights. He compares data gathered by the Eyal group (Eyal et al., 1973) with those of van Ginkel (van Ginkel and Scharen, 1985) and concludes that even the interpretation of quite similar data may differ. In practice, he concludes, no unequivocal demise of varieties due to truly differential races of the septoria foliar blights has been reported.

The increase of disease severity over time on the same variety has been noted in the Netherlands, Australia, Israel (Jorgensen and Smedegaard-Petersen, 1999), and the USA (Mundt et al., 1999). However, it was not unequivocally shown whether this was due to an evolution of aggressiveness in the pathogen population or due to differential host-pathogen interaction based on the evolution of new virulence patterns. Mundt et al. (1999) conclude that the cultivation of susceptible hosts will result in selection for aggressiveness. In Germany several varieties were shown to retain their resistance to isolates of *S. nodorum* collected annually from all over the former East Germany for more than 10 years, except for small variations over years due to specific weather conditions (Walther, 1993).

Clear proof of differential variety by isolate interaction at the seedling stage was presented by Kema et al. (1996). However, as in previous studies by this group and others, the size of the interaction component was about a magnitude smaller than that of the main effects due to varieties and isolates. In a later monocyclic field experiment, Kema and van Silfhout (1997) applied isolates specifically selected for their virulence differences at the seedling level, to adult plant plots. Again, small but significant interactions between isolates and varieties were apparent in this monocyclic situation. The significant interaction effect was largely due to two of the three isolates interacting differentially with two of the 22 varieties tested.

Recent work on Kenyan isolates indicated that variety by isolate interaction in the field, while present, was small, with the interaction component accounting for only about 5% of the main effects (Arama, 1996).

Recent experiments on the genetic structure of *S. tritici* populations in response to host differences found a total lack of evidence for adaptation to the host genotype (McDonald et al., 1996). Different hosts had no differential effects on the dynamics of the pathogen population. On the other hand, Ahmed et al. (1996) found that susceptible varieties selected for more aggressive pathogen populations. However, the virulence levels of the pathogen isolates were associated with the variety of origin.

Varietal mixtures of resistant and susceptible varieties have met with mixed success. Recent comparisons by Loughman et al. (1994b) of pure lines and mixtures did not find that mixtures have any advantage when it comes to durability of resistance.

Interaction among isolates on the leaf, which can enhance or reduce expected disease severities (Eyal, 1992; Gilchrist and Velazquez, 1994), adds another component to the mix of virulence and aggressiveness.

The implications for resistance breeding of the above findings on variety by isolate interactions remain to be tested further in real agricultural settings. However, it does appear that the response of the septoria/stagonospora foliar blights to their hosts cannot compare to the highly volatile population dynamics we see in the wheat rusts.

It is essential to determine the practical relevance of the presence of races of the septoria foliar blights when applying a resistance breeding strategy. Indeed, the importance of this topic cannot be overstated. New insights into the genetic structure of the pathogen population and its dynamics, especially as supported by biotechnological tools, may increase our understanding significantly in the near term.

## Breeding for Resistance at CIMMYT

The methodology applied at CIMMYT for breeding for disease resistance in wheat has been outlined in previous publications, including the proceedings of several international septoria workshops (Mann et al. 1985; van Ginkel and Rajaram, 1989; van Ginkel and Rajaram, 1993; Gilchrist et al., 1995; van Ginkel and Rajaram, 1995). The methodology consists of three key components: utilizing proven resistance sources as parents, selecting for increased resistance in a shuttle breeding program, and confirming resistance by multilocation testing. The best identified lines are fed back into the crossing program as parents to further accumulate resistance, bringing the process full circle.

The materials that provide the best resistance are made available by CIMMYT to all collaborators around the world who request them. Lists of such materials are published periodically (Gilchrist, 1994). Gilchrist et al. (this volume) have provided a new overview of lines found to be consistently resistant by CIMMYT researchers.

Every year CIMMYT distributes to collaborators the newest wheat lines that combine *S. tritici* resistance with a full "agronomic package," including adaptation to high rainfall environments, superior yield, a certain level of resistance to other prevalent diseases such as the rusts and scab, and acceptable grain quality.

The parental stocks emphasized in developing these lines have a relatively long history of proven resistance in many locations around the world. The first genepool exploited for resistance to *S. tritici* by CIMMYT breeders was that represented by such lines as IAS20 from Brazil and Klein Atlas from Argentina. The Tinamou line is an example of a widely adapted, resistant line that was developed from those resistance sources. Then followed the winter wheats, in particular those from the former USSR, such as Aurora. Examples of elite lines emanating from that work are the group of Bobwhite lines, such as the cultivar PROINTA Federal and the Milan lines, which have shown strong disease resistance throughout the world, including in the hotspots of eastern Africa and the Southern Cone of South America (Kohli, 1995; Dubin and Rajaram, 1996). Subsequently, Chinese materials, such as the Ning, Shanghai, and Suzhoe series, plus other varieties from China, were heavily crossed by CIMMYT breeders. A key resistant line that resulted from that effort was Catbird. This history is discussed in more detail by Gilchrist et al. (this volume).

New sources of resistance, such as highly promising synthetic wheats, are currently being exploited. CIMMYT's Wide Crosses Program produces synthetic wheats by crossing CIMMYT elite durum wheats to accessions of *Aegilops squarrosa* (syn. *Triticum tauschii* and *Ae. tauschii*). The resulting triploid seedlings are treated with colchicine to double the number of chromosomes, resulting in man-made (hence "synthetic") hexaploid bread wheats. Certain of those wheats have shown remarkable levels of *S. tritici* resistance, and low severities bordering on immunity have also been observed. Since synthetic wheats are relatively easy to cross with common wheats, their resistance can be readily introgressed into agronomically acceptable plant types, and combined with other resistances.

CIMMYT's breeding methodology emphasizes regularly exposing segregating materials to disease epidemics. Since the breeding program also targets resistance to several other diseases besides *S. tritici*, straw is used as the source of primary inoculum. Straw residues may carry inoculum for tan spot, fusarium head scab, fusarium foliar blight, and different species of bacteria in addition to the septorias. In special cases and in specific studies, pure liquid *S. tritici* spores are applied.

CIMMYT applies a shuttle breeding methodology in which materials are alternately grown in a high rainfall site in the Mexican highlands (Toluca) and an irrigated site in northwestern Mexico (Cd. Obregon). As a result, the material

targeted for *S. tritici*-prone regions is exposed to the disease 3-4 times during the segregating phase when grown in Toluca. During the selection process, selection intensity is increased as the segregating populations are advanced. At the end of the shuttle breeding process, all homozygous lines are exposed for two additional cycles at three sites in the high rainfall Mexican highlands: Toluca, Patzcuaro (Gomez and Gonzalez, 1987), and El Tigre.

Shuttle breeding is followed by multilocation testing at key locations around the world through a network of cooperators. The global data allow truly outstanding material (see Gilchrist et al., these proceedings) to be identified and used as new parents in the ongoing process of recombining genetically different sources of resistance, and they also offer the prospect of combining different resistance mechanisms.

Future work will concentrate on combining accumulated resistances that are based on different genes and/or different resistance mechanisms, with superior yield, scab resistance, shattering tolerance, and industrial quality.

## Conclusions

Several conclusions in regard to the impact of our increased understanding of resistance on related breeding aspects can be drawn from the combined research of the past decade:

1. There are many sources of genetic resistance available that have either been shown to be different or seem to behave differently. Many of those resistances have proven to be quite stable.
2. Seedling data at best are an indication of adult plant resistance, and testing adult plants in a field situation appears crucial.
3. A variety is rarely replaced solely or primarily because it succumbs to one of the septoria/stagonospora blights.
4. The relative role of virulence and aggressiveness in the field may soon be elucidated using molecular tools in combination with the ability to cross among isolates. The outcome should have direct impact on breeding strategy.
5. The diversity of isolates at the field level makes gaining a deeper understanding of interactions among these isolates of paramount importance. The breeder needs to take this into consideration when planning to artificially inoculate, or rely on multisite testing.
6. Multilocation confirmation of advanced lines remains a necessity, while the debate on possible specificity continues.

While several of these exciting issues remain in debate, advances in breeding for resistance to the septoria/stagonospora pathogens have not ceased. Breeders have continued to produce varieties with higher yields, better industrial quality, and improved resistance to multiple diseases.

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# Breeding for Resistance to Septoria and Stagonospora Blotches in Winter Wheat in the United States

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This report will concentrate on resistance breeding efforts in the winter wheat region of the United States, where leaf blotch, caused by either *Septoria tritici* or *Stagonospora nodorum*, has been a chronic disease. Leaf blotch has been a recognized problem in the eastern soft winter wheat region of the US for more than 50 years. Historically, *Stagonospora nodorum* was the major pathogen in the southeastern US and *Septoria tritici* was the major pathogen in the north-central regions. Since the mid 1980s, however, *S. nodorum* has been at least as damaging in the north-central region as *S. tritici*.

The Purdue University-USDA small grain improvement program provides an example of efforts to manage leaf blotch by use of genetic resistance. During the mid 1950s, wheat breeders began to incorporate resistance to *S. tritici* into adapted soft red winter wheat cultivars. At that time, *S. tritici* was clearly the dominant leaf blotch pathogen. The primary sources of resistance were wheat cultivars from South America, e.g. Bulgaria 88, Sao Sepe, and Sudeste. Eventually, most effort concentrated on Bulgaria 88 as the source of resistance. Cultivars Oasis and Sullivan, released in 1973 and 1977, respectively, carried this resistance (Patterson et al., 1975; 1979). When Oasis or Sullivan are inoculated at the adult plant stage in the greenhouse with spores of *S. tritici*, they are highly resistant. Infection results in small chlorotic flecks, with little or no chlorosis and no formation of pycnidia. Resistance in these cultivars segregates as a single, dominant Mendelian factor when they are crossed to a susceptible cultivar.

Although Oasis derived its resistance to *S. tritici* mainly from Bulgaria 88, it evidently carried additional factors for resistance to S. *nodorum* because it exhibited a degree of resistance and performed well in areas of the southeastern US where this species was the dominant leaf spotting pathogen. This additional resistance may have resulted from the fact that selection for resistance in Indiana was entirely in the field under conditions of natural infection, and there may have been more *S. nodorum* present than was suspected.

Since the latter half of the 1980s, *S. nodorum* has emerged as the dominant leaf spotting pathogen in Indiana (Shaner and Buechley, 1995). This shift in pathogen populations has evidently occurred throughout the north central region of the US, and leaf blotch must now be regarded as a complex involving both *S. tritici* and *S. nodorum*. In more southern regions, *S. nodorum* continues to be the major leaf spotting pathogen.

Management of this disease complex by genetic resistance requires resistance to both pathogens. Despite at least 50 years of effort in breeding for resistance to leaf blotch, progress has been modest. Evidence for this conclusion comes from descriptions of cultivars developed in this region and from direct observation of many cultivars in field trials.

An estimation of the degree of resistance available in currently available cultivars of soft red winter wheat can be obtained from data collected in statewide wheat performance trials conducted each year in Indiana. For many years, entries in this trial have been evaluated for disease. Leaf blotch is encountered every year to some degree in Indiana wheat fields, but at varying severity. For the period 1973–91, leaf blotch symptoms reached the flag leaf of susceptible cultivars within 26 days of heading in 10 of the 19 years (Shaner and Buechley, 1995).

An examination of leaf blotch severity data from 1986 through 1996, collected from two locations in Indiana (Tippecanoe and Daviess Counties), can be used to estimate progress in development of cultivars with resistance to this disease complex. The number and identity of entries in these trials varied from year to year. Typically, a cultivar would be included for several consecutive years, but not for the full period covered by these trials. Each year, the trial included most cultivars of soft red winter wheat offered for sale in Indiana and adjacent states. The cultivars Arthur and Caldwell were included in every trial as long-term checks. Severity of leaf blotch in these trials was estimated using a 0 to 9.5 scale, modified from one developed at CIMMYT (Shaner and Buechley, 1995). This scale is reproduced here as Table 1 for convenient reference. The ratings of leaf blotch severity discussed here were made when wheat was in the early to mid dough stage of development.

There are several ways that the question of improvement in resistance to leaf blotch can be investigated using data from these trials. If the general level of resistance among cultivars increased over the 14-year period, then the trial mean leaf blotch severity, based on all cultivars, should decline relative to the severities for the check cultivars. Among the 16 trials, trial means ranged from 5.4 to 9.2, reflecting variation among years in the suitability of weather for leaf blotch development. This variation in trial means was closely followed by the values for the two long-term check cultivars, Arthur and Caldwell. The trial mean was always less than the severity for Caldwell and usually less than the severity for Arthur, but there was no evidence for a greater disparity between the trial mean and the checks over time (Figure 1). The conclusion from this is that the newer cultivars are not more resistant collectively than those included in trials during earlier years, as represented by the two check cultivars.

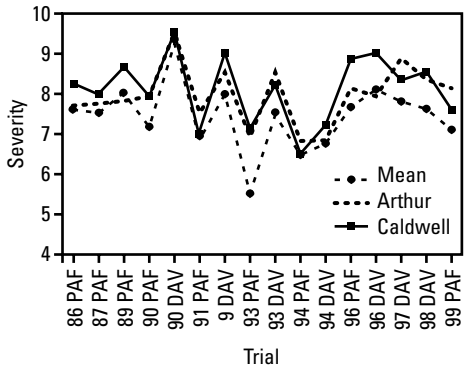


**Figure 1. Trial means and means for check cultivars Arthur and Caldwell for leaf blotch ratings, 1986 through 1999, at two locations in Indiana. PAF = Purdue Agronomy Farm (Tippecanoe County); DAV = Daviess County.**

If only a few cultivars in a trial had substantially improved resistance, then the mean of all entries in that trial might not reflect this improvement, but the standard deviation of cultivar means should be greater than in trials lacking exceptionally resistant cultivars. If there has been progress in

**Table 1. Leaf blotch severity scale for wheat.**

| | Range in percent severity on indicated leaf | | | | |
|---|---|---|---|---|---|
| **Scale value** | **Flag** | **Flag-1** | **Flag-2** | **Flag-3** | **Mean severity[x]** |
| 1 | | | | 0-5 | 0.1 |
| 2 | | | | 5-20 | 2.9 |
| 3 | | | | 20-40 | 8.1 |
| 4 | | | 1-10 | 40-70 | 15.6 |
| 5 | | 0-1 | 10-25 | 70-90 | 25.5 |
| 6 | | 1-10 | 25-75 | 90-100 | 37.8 |
| 7 | | 10-50 | 75-100 | 100 | 52.3 |
| 8 | 1-20 | 50-90 | 100 | 100 | 69.3 |
| 9 | 20-90 | 90-100 | 100 | 100 | 88.5 |
| 9.5 | 90-100 | 100 | 100 | 100 | 99.1 |

[x] Mean severity is the average for the four leaves, based on midpoint values for each range. Mean severity ($P$) can be calculated from the scale value ($S$) according to: $P = -0.38253 - 0.69435\ S + 1.17499\ S^2$ ($R^2 = 0.999$).

development of cultivars resistant to leaf blotch, then the variance of cultivar means should be seen to increase over the years. Plotting trial means and standard deviations over time revealed no tendency for the standard deviation to increase with time or to vary with the trial mean (Figure 2).

Examination of data from individual trials may reveal to what extent resistance is expressed under conditions of severe, moderate, or mild disease pressure. Results from three trials are presented here for illustration. The trial with the greatest severity of leaf blotch was in Daviess County during 1990. The trial mean severity was 9.2. The cultivars with the least disease in this trial had a rating of 8. Thus, under conditions of great disease pressure, symptoms progressed to the flag leaves of all cultivars (Figure 3). The entire range in resistance was confined to the degree of flag leaf area that was blighted. All lower leaves were completed destroyed by leaf blotch.

Mean leaf blotch severity was very low in the trial conducted in Tippecanoe County during 1993 (Figure 4). The range among cultivar severity means was somewhat greater than in the previous example, because severity values were not truncated by the maximum possible value. The most severely affected cultivars showed only a few lesions on the flag leaf. On most of the cultivars, leaf blotch was at most only 10% on leaf F-1.

The trial conducted during 1997 in Daviess County represents moderately severe disease pressure. The trial mean was 7.8 (Figure 5). Blotch symptoms reached the flag leaf on fewer than half of the cultivars in this trial, but the cultivars with the least amount of disease had symptoms on leaf F-1. Regardless of overall disease pressure, the range in leaf blotch severity among cultivars within a trial was not great.

Analysis of these field trial data provides no evidence for substantial increases in resistance in a region of the United States where leaf blotch is a chronic and often severe disease of wheat. At best, a resistant cultivar will hold symptom development back by a few days on the flag and flag-1 leaves.

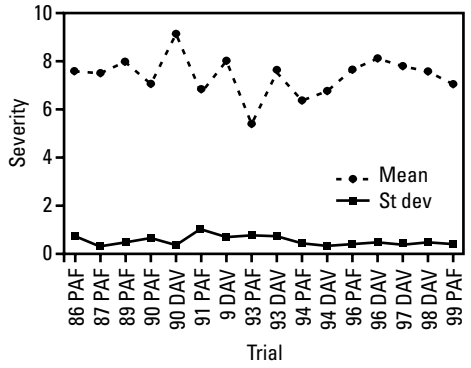


**Figure 2. Mean and standard deviation of leaf blotch severity on wheat in cultivar trials from 1986 through 1999 at two locations in Indiana.**

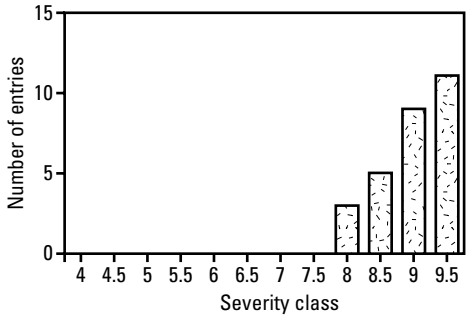


**Figure 3. Frequency distribution of mean leaf blotch severities on wheat cultivars evaluated during 1990 in Daviess County, Indiana.**

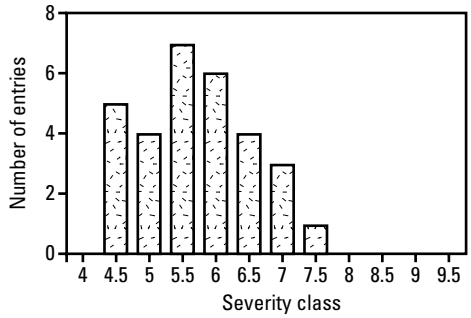


**Figure 4. Frequency distribution of mean leaf blotch severities on wheat cultivars evaluated during 1993 in Tippecanoe County, Indiana.**

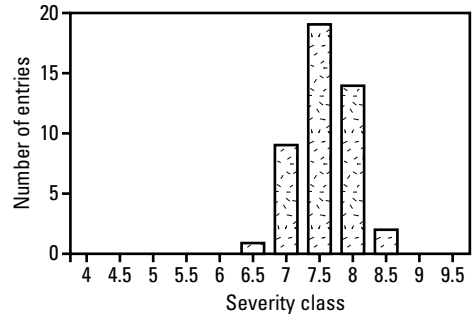


**Figure 5. Frequency distribution of mean leaf blotch severities on wheat cultivars evaluated during 1997 in Daviess County, Indiana.**

Another approach to determining progress in breeding winter wheat for resistance to leaf blotch is to examine cultivar registrations in the journal Crop Science over a period of years. Registrations for 131 wheat cultivars were published in volumes 28-39, of which 96 were for winter wheat. Of these winter wheat cultivars, 25 were reported to have some degree of resistance to *S. tritici* or *S. nodorum* and 2 were reported to be susceptible. For the other 69 cultivars there was no mention of reaction to either of these pathogens. The summary for winter wheat cultivars includes both hard and soft, and red and white classes, covering the eastern portion of the US, the Great Plains, and the Pacific Coast states. Because leaf blotch has historically been a greater problem in the eastern region of the US, data were summarized for this region. Of 39 wheat cultivars developed in the eastern soft wheat region of the US, 15 were described as having some resistance, none were described as susceptible, and there was no mention of reaction for 24 cultivars. The absence of any comment about reaction to leaf blotch is interesting, because for many other wheat pathogens and pests (e.g. rusts, powdery mildew, viruses, Hessian fly), the registration articles commonly documented susceptibility as well as resistance. Of the 15 cultivars described as being resistant, 12 were described as having resistance to *S. nodorum*, 7 of which were described as having resistance to *S. tritici* as well. The other three cultivars were described as being resistant only to *S. tritici*.

An association has long been noted between tall stature, late maturity, and resistance to leaf blotch caused by either *S. tritici* or *S. nodorum* (Camacho-Casas et al., 1995; Scott et al., 1982). Late maturity and tall stature per se may confer a significant degree of leaf blotch in such cultivars. Where leaf blotch is the greatest threat in North America, the eastern soft wheat region, breeders have emphasized development of short (90 to 100 cm), early maturing cultivars, as a way to reduce risk from rust infection, to permit grain filling before the excessive hot and humid conditions of summer, and to permit double-cropping with soybeans. The desired standards of early maturity and short stature may be an important reason for the lack of progress in achieving adequate levels of resistance to the leaf blotch pathogens.

Hectares of soft wheat production in the eastern US have been declining for many years. Poor yields and poor grain quality because of disease have been a major reason for the abandonment of wheat production by many farmers. Unless greater degrees of resistance can be bred into high-yielding, early-maturing, short-statured wheat cultivars, this downward trend in production will likely continue.

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# *Septoria tritici* Resistance of Wheat Cultivars at Different Growth Stages

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## Abstract

*Nine wheat cultivars planted in the greenhouse were inoculated with a mixture of three isolates of* Septoria tritici *at four different growth stages. The first evaluation, done six weeks after inoculation, showed that the facultative wheats at growth stage (GS) 39 (flag leaf ligule just visible) and spring wheats at GS 65 (flowering halfway complete) reached the highest levels of infection, with two exceptions, cvs E. Pelón 90 and P. Oasis. In the second evaluation a week later, the highest level of infection was reached when facultative wheats were inoculated at GS 32-33 (stem elongation-first node detectable) and spring wheats at GS 39 (flag leaf ligule just visible). In both evaluations, the effects of cultivars, seeding date/GS at inoculation, and the interaction between cultivars and GS at inoculation were significant.* Septoria tritici *susceptibility increased with the age of the plants. The increase in the level of infection was significantly lower in cv P. Superior (Bobwhite), indicating a certain level of adult plant resistance.*

Wheat is an important crop in the western region of Uruguay, where it is grown for grain and forage (double purpose) in the mixed farming system, or solely for grain production. Among diseases present in the country, *Septoria tritici* leaf blotch is one of the most important, and it can be particularly severe during the wheat-growing season in this temperate high rainfall environment. The wide range of planting dates from May (or even earlier for double purpose crops) to August determines the growth stage (GS) at the time of primary infection, later in the fall or in early spring. Facultative wheats, sown in the fall, are infected very early (seedling stage) or at the beginning of spring (boot stage), depending on the year. Spring wheats, sown later than the facultative types, are exposed mainly to spring infections.

Reduced disease severity associated with tall plant stature and late maturity has been observed (Danon et al., 1982; Eyal and Talpaz, 1990; van Beuningen and Kohli, 1990; Tavella, 1978). However, the relationship between the resistance expressed at the seedling stage and the adult plant stage, or vice versa, is not fully understood. Kema and van Silfhout (1995) found significant correlations for one of three isolates inoculated on 22 wheat cultivars at the seedling and adult plant stages. On the other hand, Arama, Parlevliet, and van Silfhout (1994) described three types of resistance: resistance only in seedling (seedling resistance), resistance only in adult plant (adult plant resistance), and resistance in both seedling and adult plant (overall resistance).

The objective of this study was to determine the level of resistance in nine wheat cultivars, representing different reactions to *S. tritici* in the field, at different growth stages and under controlled conditions.

## Materials and Methods

Nine wheat cultivars with different growth habits and field reactions to *Septoria tritici* were planted in three pots each, five plants per pot, on four seeding dates: 28 April, 16 May, 31 May, and 27 June 1995. The pots measured 29 and 20 cm in their upper and lower diameters, respectively, and 20 cm in height. The cultivars' growth habit, progenitors, and field reaction to *S. tritici* are presented in Table 1. The concentration of *S. tritici* spore suspension was adjusted to $10^6$ conidia per ml, with the aid of a hemacytometer. The cultivars were inoculated with a mixture of three isolates (26S, 4407, and E. Federal) on 17 July, at the growth stages (Zadoks et al., 1974) indicated in Table 2. The selected isolates were characterized by Diaz de Ackermann et al. (1994). The plants were kept in a humid chamber until 20 July. Due to the delayed

appearance of symptoms, two evaluations were done, the first on 28 August and the second on 5 September.

The infection was scored as percent of leaf area affected by the disease, on two tillers per plant and five leaves per tiller. Transformed data ($Log_e$+0.5) for the flag leaf, F-1, and F-2, as well as the average of the three leaves, were analyzed using SAS GLM procedure (Version 6.12; SAS Institute, Cary, NC). Infection on the lower two leaves was not included in this analysis due to many missing values.

**Table 1. Cross, growth habit and field reaction to *Septoria tritici* of nine wheat cultivars.**

| Cultivar | Cross | Growth habit | Reaction |
|---|---|---|---|
| E. Cardenal | Veery#3 | Spring | S[1] |
| INIA Mirlo | Car 853/Coc//Vee#5/3/Ures | Spring | R |
| E Pelón 90 | Kavkaz/Torim73 | Spring | R-MR |
| ProINTA Superior | Bobwhite | Spring | MR |
| ProINTA Oasis | Oasis/Torim | Spring | S |
| E. Federal | E.Hornero/CNT8 | Facultative | MR |
| E Halcón | Buck6/MR74507 | Facultative | MR |
| Buck Charrúa | RAP/RE//IRAP/3/LOV/4/RAP/RE//IRAP | Facultative | R |
| LE 2196 | E. Jilguero/ND526 | Facultative | R |

[1] S: susceptible, R: resistant, MR: moderately resistant.

**Table 2. Growth stages of spring and facultative wheat varieties at time of inoculation.**

| Seeding date | 28/04 | 16/05 | 31/05 | 27/06 |
|---|---|---|---|---|
| Spring wheats | 65 | 39 | 30-31 | 22-23 |
| Facultative wheats | 39 | 32-33 | 30 | 24-25 |

**Table 3. Average level of infection of top three leaves, comparison among date of seeding (small letter) and cultivars (capital letter). Evaluation August $28_{th}$ and September $5_{th}$.**

| Sowing date | 28/04 | 16/05 | | 31/05 | | 27/06 | |
|---|---|---|---|---|---|---|---|
| GS Facultative | 39 | 32/33 | | 30 | | 24/25 | |
| GS Spring | 65 | 39 | | 30/31 | | 22/23 | |
| Cultivar | 1st. eval | 1st. eval | 2nd. eval | 1st. eval | 2nd. eval | 1st. eval | 2nd. eval |
| E.Cardenal | 11.65 aA | 3.81 bA | 46.24 aA | 0.16 c | 3.57 bA | 0.00 c | 0.45 c |
| I. Mirlo | 1.98 aCD | 2.17 aAB | 23.93 aB | 0.00 b | 1.96 bB | 0.00 b | 0.66 c |
| E. Pelón 90 | 1.32 aDE | 2.20 aAB | 16.86 aC | 0.02 b | 1.76 bB | 0.00 b | 0.19 c |
| P. Superior | 2.92 aC | 1.50 bB | 8.14 aD | 0.00 c | 1.86 bB | 0.00 c | 0.11 c |
| P. Oasis | 1.79 bCD | 4.25 aA | 27.22 aB | 0.26 c | 2.18 bB | 0.02 c | 0.44 c |
| E. Federal | 7.64 aB | 3.17 bA | 19.15 aC | 0.00 c | 0.50 bD | 0.00 c | 0.43 b |
| E. Halcón | 1.25 aE | 0.23 bC | 3.61 aE | 0.03 b | 1.13 bC | 0.00 b | 0.17 c |
| B. Charrúa | 0.14 aE | 0.03 aC | 2.91 aE | 0.00 a | 0.56 bCD | 0.00 a | 0.44 b |
| LE 2196 | 1.66 aDE | 0.22 bC | 6.65 aD | 0.03 b | 1.23 bB | 0.00 b | 0.33 c |
| **Average** | 3.37 | 1.95 | 17.19 | 0.06 | 1.64 | 0.00 | 0.36 |

## Results and Discussion

The analysis of variance (ANOVA) for each leaf and the average of the three leaves showed significant differences between cultivars and seeding dates, as well as significant interaction between seeding dates and cultivars in both evaluations. As the results were similar for individual leaves and the average of the three top leaves, only data for the latter are presented (Table 3).

Differences among cultivars were found on the first two seeding dates (28/04 and 16/05) during the first evaluation, and on the intermediate dates (16/05 and 31/05) during the second evaluation.

All the cultivars except B. Charrúa showed different infection levels on different seeding dates at first evaluation. This interaction is explained by the cultivars' different behavior on the different seeding dates/GS at inoculation. Early seedling infection demonstrated higher sensitivity for detecting differences among cultivars. During this evaluation, the facultative wheat varieties were found to have higher *Septoria* susceptibility at GS 39 (flag leaf ligule just visible) and spring varieties at GS 65 (flowering halfway complete). These were followed by GS 32/33 (stem elongation-first node detectable) and GS 39 for facultative and spring varieties, respectively. Inoculations at GS 22 to GS 31 did not produce adequate infection levels.

At the second evaluation on 5 September, the group seeded on the first date was already dry. For the remaining three sowing dates, the highest infection level was shown by facultative wheat varieties inoculated at GS 32-33 and by spring wheat varieties inoculated at GS 39.

Comparison of the cultivars' field reactions to *S. tritici* with infection under controlled conditions showed differences in I. Mirlo, P. Superior, E. Halcón, and P. Oasis. These cultivars are considered resistant (R), moderately resistant (MR), moderately resistant (MR), and susceptible (S), respectively, under field conditions; under controlled conditions they were

MS, R, R, and MS, respectively. The interaction between cultivar and growth stage at inoculation indicated the lack of precision that may occur while evaluating resistance of a variety.

In the first evaluation, with relatively low levels of infection, cultivars such as P. Oasis showed significantly lower infection levels at GS 65 than at GS 39. E. Pelón 90 showed similar behavior without being significantly different at these two growth stages. However, in contrast to P. Oasis and E. Pelón 90, P. Superior presented a significantly higher infection level at GS 65 than at GS 39 (Table 3; Figure 1). Although the infection level of P. Superior at the initial stages was almost equal to that of susceptible varieties P. Oasis and E. Cardenal at the second evaluation, P. Superior showed the slowest increase over time to become the most resistant genotype at the adult plant stage. This suggests that P. Superior (Bobwhite) may have a certain degree of adult plant resistance.

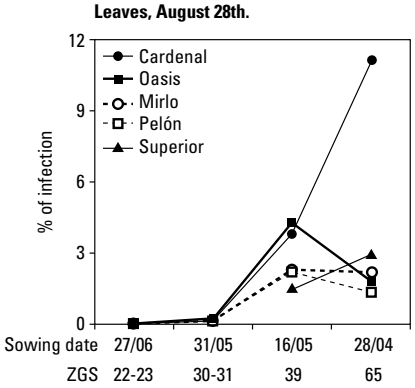


**Figure 1. Level of infection, average of three**

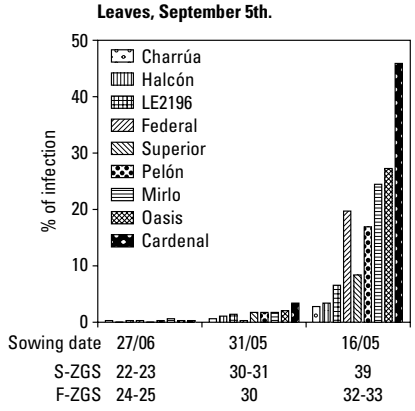


**Figure 2. Level of infection, average of three**

In general, spring habit, high yielding, early maturing semidwarf wheats showed higher levels of *Septoria* infection than the facultative wheats (tall and late maturing). The highest infection level observed in a facultative wheat (E. Federal) is equivalent to the level demonstrated by a moderately resistant spring variety such as E. Pelón 90 (Figure 2). As per the definition put forward by Arama, Parleviet, and van Silfhout (1994), Buck Charrúa seems to possess overall resistance. A high degree of resistance was observed at the juvenile stage in all varieties. Only P. Superior demonstrated adult-plant resistance. Further studies are required to evaluate the level of resistance in stages earlier than GS 22-25.

## Conclusions

The *S. tritici* susceptibility of different wheat varieties seems to increase with the age of the plants. However, the rate of this increase (the slope of the curve) is different for each cultivar. As a result interactions were observed between the cultivars' susceptibility (infection level) and growth stage at the time of infection (inoculation). This suggests that several evaluations may be required to determine a cultivar's potential as a parent in a crop improvement program.

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# *Septoria tritici* Resistance Sources and Breeding Progress at CIMMYT, 1970-99

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## Abstract

*An overview is provided of the sources of* Septoria tritici *resistance used in the CIMMYT bread wheat program, and of progress in breeding for resistance for wheat mega-environment 2 (high rainfall) in 1970-99. Several phases can be distinguished: use of Russian winter wheat, lines from the Southern Cone of South America (Brazil, Chile and Argentina) and USA as resistance sources; introduction of disease resistance from Brazilian sources into semi-dwarf and early backgrounds; introduction of genes from triticale and* Triticum diccocon *into common wheat; introgression of resistance from synthetic hexaploids and derivatives into susceptible bread wheat stocks; pyramiding of Chinese resistance sources with the sources mentioned above, and combining them into adapted agronomic types. Some preliminary work on resistant durum wheat sources, including some of the best ones from Tunisia, is presented.*

## Bread Wheat Program

The semidwarf wheats developed in Mexico in the 1960s were widely distributed. The modern wheat varieties that replaced local wheats were successful because of their wide adaptation, high yield potential, and disease resistance (mainly to stem and leaf rust) for favorable, irrigated production conditions (Rajaram et al., 1994).

The emphasis of CIMMYT's bread wheat breeding program on disease resistance was expanded in the 1970s to include resistance to *Puccinia striiformis* and *Septoria tritici* for the high rainfall wheat growing areas of the world (mega-environment 2). These are temperate environments with an average precipitation of >500 mm. The search for *S. tritici* resistance, the accumulation of resistance genes, and the incorporation of resistance into high yielding advanced lines have been very successful in the CIMMYT bread wheat program.

Three main groups of sources were used to introduce resistance: a) Russian winter wheat, b) lines from the wheat growing areas of Brazil, Chile, and Argentina (Southern Cone of South America), and c) to a lesser extent, lines from the USA (Mann et al., 1985) (Table 1). The resistance of these lines was identified and/or confirmed in different countries through the International Septoria Nursery (ISEPTON), beginning in 1971.

The breeding program focused on combining the semidwarf plant type with high yield potential and resistance to leaf and stripe rust plus septoria leaf blotch. Germplasm was screened and selected for septoria leaf blotch at two locations in Mexico: Patzcuaro and Toluca. Patzcuaro is located at 2,200 m altitude in the western Mexican highlands (Michoacan state). It proved suitable for testing advanced lines, since epidemics of *S. tritici* occur naturally every year. In some years we can also select for resistance to *Pyrenophora tritici-repentis* and *Stagonospora nodorum*. The Atizapan experiment station in Toluca (Mexico state) at 2,600 m altitude has an intense rainy season during the summer months (800-900 mm). Artificial epidemics are created by inoculating with infected straw or a spore suspension involving a mixture of isolates.

Numerous lines with acceptable resistance levels were derived from crosses using the above mentioned sources and subsequent testing in Toluca and Patzcuaro (Table 2).

Another set of resistant lines was created by the wheat germplasm enhancement section. The *S. tritici* resistance of lines from Brazil (IAS 20, Pelotas Arthur, Colotana, IAS 58, and Maringa), which are tall and late-maturing with weak straw, was incorporated into early-maturing, high yielding semidwarf lines. Thus early-maturing, short, high yielding lines were developed that had a level of resistance similar to that of the original resistant parent (Table 3).

Bread wheat lines were also crossed with triticale, and the resulting lines showed high levels of *S. tritici* resistance (Table 4). Subsequently, some of the lines listed in Table 3 were combined with triticale-derived lines (Table 5) in an attempt to pyramid several resistance genes.

CIMMYT's wheat wide crosses program has generated a wide range of resistant germplasm from D genome synthetics and their synthetic derivatives (Mujeeb-Kazi et al., 1996; 1998; 1999). These materials express high levels of resistance to several leaf pathogens, including *Bipolaris sorokiniana*, *Pyrenophora tritici-repentis*, and *S. tritici***.** Their resistance may be to one or a combination of these pathogens. Table 6 shows a group of representative synthetic wheats with high levels of resistance to *S. tritici.*

**Table 1. Main sources of *Septoria tritici* resistance used by the CIMMYT wheat breeding program in the 1970s.**

| Ex-USSR winter wheat | Southern Cone of SA spring wheat | USA spring wheat | Other sources |
|---|---|---|---|
| Aurora | IAS 55 | Chris | Enkoy |
| Kavkaz | IAS 58 | Era | Salomoni Seafoam |
| Bezostaja 1 | PF 70254 | Frontana-Kenya x Newthatch | Kvz/K4500 L.6A.6 |
| | Maringa | | |
| | Carazinho | | |
| | IAS 63 | | |
| | Lagoa Vermelha | | |
| | Tizano Pinto Precoz | | |
| | IAS 62 | | |
| | CTN 7 | | |
| | CTN 8 | | |
| | Gaboto | | |
| | IAS 20 | | |

**Table 2. CIMMYT-derived lines with acceptable *Septoria tritici* resistance from the ex-USSR, USA, France, Brazil, Chile, and Romania.**

| Advanced line | Pedigree cross | Number | *S. tritici* score[a] | Origin |
|---|---|---|---|---|
| Veery | Kvz/Buho//Kal/Bb | CM 33027 | 76-86 | Ex-USSR |
| Bobwhite | Au//Kal/Bb/3/Wop | CM 33203 | 32-76 | Ex-USSR |
| Musala | Lee/Kvz/3/Cc//Ron/Cha | CM16780 | | Ex-USSR |
| Sunbird | Gll/Cuc//Kvz/Sx | CM34630 | 42-77 | Ex-USSR |
| Milan | VS73.600/Mirlo/3/ Bow//Ye/Trf | CM75113 | 21-74 | France/Ex USSR |
| Attila | ND/VG9144//Kal/Bb/ 3/Yaco/4/Veery | CM85836 | 73-76 | USA/Ex-USSR |
| Bagula | Ore F1 158/Fdl// Mfn/2*Tiba63/3/Coc /4/Bb/GLl//Carp/3/Pvn | CM59123 | 53-75 | USA/Romania |
| Prinia | Prl/Vee#6/Myna/Vul | CM90722 | 74-76 | Ex-USSR |
| Chilero | 4777*2//Fkn/Gb54/3/ Vee#5/4/Buc/Pvn | CM66684 | 74-76 | Ex-USSR |
| Corydon | Car 853/Coc/Vee/3/ E7408/Pam//Hork/PF73226 | CM81074 | 41-75 | Chile/ Brazil/ Ex - USSR |
| Tinamu | IAS58/4/Kal/Bb// Cjm71/Ald/5/Au// Kal/Bb/3/Wop | CM81812 | 32-75 | Brazil/Ex-USSR |

[a] Double digit scale (Saari and Prescott, 1975; Eyal et al., 1987) indicating the range of damage during two screening cycles in Toluca.

**Table 3. Short, early-maturing, *Septoria tritici* resistant lines derived from tall, late-maturing, resistant Brazilian varieties, with a resistance similar to that of the original resistant parents.**

| Lines | Cross number | *S. tritici* score[a] |
|---|---|---|
| IAS 20/H567.71//4*IAS 20 | CMH 79.243 | 35-52 |
| IAS 20/H567.71//IAS 20/3/IAS 58 | CMH 78.390 | 31-42 |
| IAS 20/H567.71//IAS20/3/3*MRNG | CMH 78.443 | 32-63 |
| CLT/H471.71A//4*CLT | CMH 83.2277 | 32-53 |
| H.567.71/3*P.Ar | CMH 77.308 | 32-74 |
| IAS 20 | – | 11-62 |
| IAS 58 | – | 31-54 |
| Maringa | – | 31-54 |
| Colotona | – | 11-51 |
| Pelotas Arthur | – | 21-71 |

[a] Double digit scale (Saari and Prescott, 1975; Eyal et al., 1987) indicating the range of damage during two screening cycles in Toluca.

**Table 4. *Septoria tritici* resistant lines derived from bread wheat x triticale crosses.**

| Lines | Cross number | *S. tritici* score[a] |
|---|---|---|
| INIA 66/RYE*2//ARM/3/H277.69 | CMH 72.A576 | 21-42** |
| M2A/CML//2*NYUBAY | CMH 80.A1267 | 22-52 |
| M2A/CML//Nyubay/3/ CMH72A576/MRG | CMH 82.961 | 32-43 |

[a] Double digit scale (Saari and Prescott, 1975; Eyal et al., 1987) indicating the range of damage during two screening cycles in Toluca.

**Table 5. *Septoria tritici* resistant lines combining resistance from Brazilian sources and bread wheat x triticale derived lines.**

| Lines | Cross number | *S. tritici* score[a] |
|---|---|---|
| H.567.71/3*P. Ar/MRNG//IAS20/H567.71/ IAS20/3/3*MRNG/79.243/4/M2A/CML// NYUBAY/3/INIA66/Rye*2//ARM/3/H277.69 | CMH 85.3215 | 31-42 |
| EAGLE/H567.71//4*EAGLE/3/2*IAS20 /H567.71//4*IAS20 | CMH 79.243 | 11-43 |

[a] Double digit scale (Saari and Prescott, 1975; Eyal et al., 1987) indicating the range of damage during two screening cycles in Toluca.

**Table 6. Synthetic hexaploid wheats (*Triticum turgidum* x *Aegilops tauschii*; 2n=6x=42) with high levels of resistance to *Septoria tritici*.**

| Synthetic hexaploids | Cross number | *S. tritici* score [a] |
|---|---|---|
| Aco 89/*Ae. tauschii* (309)[b] | CIGM90.595 | 11-11 |
| Croc1/*Ae. tauschii* (879) | CIGM89.479 | 11-21 |
| Doy1/*Ae. tauschii* (372) | CIGM93.229 | 11-21 |
| Sty-US/Celta//Pals/3/SRN-5/4/*Ae. tauschii* (502) | CIGM93.261 | 11-11 |
| Altar 84/*Ae. tauschii* (502) | CIGM93.395 | 11-11 |

[a] Double digit scale (Saari and Prescott, 1975; Eyal et al., 1987) indicating the range of damage during two screening cycles in Toluca.
[b] Accession number in the wide cross working collection in CIMMYT's wheat genebank.

**Table 7. Synthetic hexaploids crossed with susceptible and moderately susceptible bread wheats to develop advanced derivatives resistant to *Septoria tritici*.**

| Advanced derivative | Cross number | *S. tritici* score[a] |
|---|---|---|
| Altar 84// *Aegilops tauschii* (191)[b] //Yaco/3/2*Bau | CIGM92.337 | 21-52 |
| Croc 1/ *Aegilops tauschii* (205)//Kauz | CIGM90.248 | 11-31 |
| Croc 1/ *Aegilops tauschii* (205)/5/Br12*3/4/ Ias 55*4….. | CIGM90.252 | 11-32 |
| Altar 84/*Aegilops tauschii* (219)//Opata | CIGM90.429 | 11-32 |
| Altar 84/*Aegilops tauschii* (219)//2*Seri | CMSS92Y01855M | 21-32 |
| Altar 84/*Aegilops tauschii* (224)//2*Yaco | CIGM91.191 | 11-32 |
| Bcn/3/Fgo/USA2111//*Aegilops tauschii* (658) | CASS94Y00146S | 11-22 |
| Opata/6/68111/Rgb-V//Ward/3/Fgo/4/ Rabi/5/*Aegilops tauschii* (878) | CASS94Y00247S | 11-22 |
| Filin/4/Snip/Yan79//Dack/Teal/3/ *Aegilops tauschii* (633) | CASS94Y00072S | 32-32 |
| Altar 84/*Aegilops tauschii* (191)// Opata/3/ Altar 84/*Aegilops tauschii* (224)//Yaco(1)… | CIGM93.566 | 11-22 |
| Mayoor//TkSN1081/*Aegilops tauschii* (222) | CASS94Y00009S | 11-22 |

[a] Double digit scale (Saari and Prescott, 1975; Eyal et al., 1987) indicating the range of damage during two screening cycles in Toluca.
[b] Accession number in the wide cross working collection in CIMMYT's wheat genebank.

The high levels of resistance present in the synthetic hexaploids were incorporated into susceptible bread wheat advanced lines and in some cases were used to reinforce the intermediate susceptibility present in some lines (Table 7). To pyramid the resistance genes, resistant alien germplasm possessing tertiary gene pool diversity was crossed with resistant Chinese germplasm (Table 8).

Another effort to diversify the sources of resistance focused on the *Triticum dicoccon* collection in the wheat genebank (Gilchrist and Skovmand, 1995). Early tall accessions with resistance to *P. striiformis* and *S. tritici*, and weak straw were identified from genebank collections. One of these accessions was selected and crossed to a very susceptible selection of the line Kauz. The resulting lines combine high levels of resistance to *P. striiformis* and *S. tritici* with good agronomic type (Table 9).

In a second effort of the bread wheat breeding program (1986-87) to increase variability of the resistance base, a group of advanced bread wheat lines consisting of resistance sources in an adapted background (Table 10) was crossed with a group of Chinese lines having acceptable levels of resistance (Sumai #3, Suzhoe #8, Suzhoe #6 Ningmai #4, Yangmai #4, Ning 8401, YMI #6, Shangai #5, Shangai #, Nanjing 7840, Wuhan 2, and Wuhan 3).

Once the resistance of the Chinese materials had been introduced, a new effort was

initiated to combine high yield potential and pyramid resistance genes (Table 11).

Shuttle breeding between different sites is very important for selection and helps to increase effective resistance across different locations where *S. tritici* is a problem. A large number of advanced lines showing very high levels of resistance have been selected through shuttle breeding. New sources have also been selected with this methodology. A representative example of new sources coming from the Southern Cone of South America that were identified in the region through the LACOS (Advanced Lines for the Southern Cone) nursery is given in Table 12.

## Durum Wheat Program

In the countries of West Asia and North Africa (WANA), severe epidemics of septoria leaf blotch have occurred, especially in Morocco, Tunisia, and Turkey (Saari, 1974). Isolates of the pathogen have been identified in different regions, along with their specificity for durum and bread wheat. Breeding efforts have increased resistance levels, but selection for resistance is complicated by different disease reactions at different locations and in different years. This may be due to different environmental conditions or to the variability of *S. tritici* populations (Arama et al., 1988); van Silfhout et al. (1989) have reported specificity in some durum and bread wheat isolates. Complementary information was

**Table 8. Advanced wheat lines resistant to *Septoria tritici* and derived from crosses of perennial Triticeae species and Chinese germplasm.**

| Lines | Crosses | *S. tritici* score[a] |
|---|---|---|
| CS/*Thinopyrum curvifolium*//Glenn.81/3/Ald/Pvn | CIGM84295 | 31-43 |
| Cno79/4/CS/*Thinopyrum curvifolium*//Glenn.81/3/Ald/Pvn | CIGM88829 | 32-62 |
| Cno 79/4/CS/*Thinopyrum curvifolium*//Gen/3/Ald/Pvn/4/CS/*Leymus racemosus*//2*CS/3/Cno 79 | CIGM8876 | 32-42 |
| Inia66/*Thinopyrum distichum*//Inia66/3//4/Gen/CS/*Thinopyrum curvifolium*//Glenn.81/3/Ald/Pvn | CIGM88734 | 31-62 |
| CS/*Thinopyrum curvifolium*//Glenn 81/3/Ald/Pvn/4/Ningmai#4/Oleson//Ald/Yangmai#4 | CIGM87.116 | 51-72 |
| CS/*Thinopyrum curvifolium*/Glenn 81/3/Ald/Pvn/4/Suzhoe#8 | CIGM87.123 | 31-42 |
| CS/*Thinopyrum curvifolium*//Glenn 81/3/Ald/Pvn/4/Ningmai 8401 | CIGM87.1109 | 41-53 |
| Chir3/5/CS/*Thinopyrum curvifolium*//Glenn 81/3/Ald/Pvn/4/Cs/*Leymus racemosus*//2*CS/3/Cno79 | CIGM93.612 | 21-22 |

[a] Double digit scale (Saari and Prescott, 1975; Eyal et al., 1987) indicating the range of damage during two screening cycles in Toluca.

**Table 9. *Triticum dicoccon*/Kauz advanced lines with resistance to *Septoria tritici*.**

| Advanced lines and pedigree | Cross number | *S. tritici* score[a] |
|---|---|---|
| *T. dicoccon* PI 1254156/2*Kauz | GRSS93SH18-0M-12SH-2M-1Y | 11-51 |
| *T. dicoccon* PI 1254156/2*Kauz | GRSS93SH18-0M-13SH-3M-1Y | 11-21 |
| *T. dicoccon* PI 1254156/2*Kauz | GRSS93SH18-0M-13SH-3M-1Y | 11-21 |
| *T. dicoccon* PI 1254156/2*Kauz | GRSS93SH27-0M-10SH-3M-1Y | 11-72 |
| *T. dicoccon* PI 1254156/2*Kauz | GRSS93SH29-0M-5SH-3M-1Y | 32-73 |
| Kauz (susceptible parent) | | 89-99 |
| Bobwhite (moderately resistance check) | | 41-46 |
| *T. dicoccon* PI 1254156 (resistant parent) | | 11-71 |

[a] Double digit scale (Saari and Prescott, 1975; Eyal et al., 1987) indicating the range of damage during two screening cycles in Toluca.

**Table 10. Advanced lines consisting of sources of *Septoria tritici* resistance in an adapted background, crossed with a group of resistant Chinese lines.**

| Advanced line | Cross | *S. tritici* score[a] |
|---|---|---|
| Shangai#5/Bow | CM91100 | 32-72 |
| Ald/Pvn//YM#6 | CM91065 | 21-72 |
| Suzhoe#6//Ald/Pvn | CM91128 | 32-71 |
| YM#6/Thb*2 | CMH87.2698 | 42-72 |
| Ald/Pvn//Ning#7840 | CMH91325 | 11-47 |
| Sha#5/Weaver | CM95103 | 11-32 |
| Sha#8/Gen | CM95124 | 11-32 |
| Milan/Sha#7 | CM97550 | 52-63 |
| Sabuf | CM95073 | 53-32 |
| Catbird | CM91045 | 42-76 |

[a] Double digit scale (Saari and Prescott, 1975; Eyal et al., 1987) indicating the range of damage during two screening cycles in Toluca.

**Table 11. Bread Wheat Program advanced lines with high yield potential containing different combinations of *Septoria tritici* resistance sources in their pedigrees.**

| Advanced lines | Cross | *S. tritici* score[a] |
|---|---|---|
| Trap#1/Bow | CM 84548 | 21-35 |
| PF70354/Bow | CM 67910 | 21-54 |
| Gov/Az//Mus/3/Dodo/4/Bow | CM 79515 | 31-64 |
| Ald/Pvn//YMI#6/3/Ald/Pvn | CM 96723 | 11-41 |
| Ias20*3/H567.71//Sara | CM 81021 | 11-31 |
| Ning8675/Catbird | CMSS92Y00639S | 32-52 |
| Thb/CEP7780//Suz#9/Weaver/3/Ng8675 | CMSS92Y02302T | 52-63 |
| Cbrd/5/Cs/*Thinopyrum curvifolium*//Glen/3/Gen/4/L2266/1406.101//Buc/3/Vpm/Mos83.11.4.8//Nac | CMBW91MO3723 | 11-21 |
| Sha3/Seri//Kauz/3/Sha4/Chil | CMBW91M03723 | 11-21 |
| Bau/Milan | CM103873 | 11-21 |
| Lfn/II58.57//PRL/3/Hahn | CM77224 | 31-42 |
| Sha #3/Seri//Psn/Bow | CMBW90M2470 | 31-42 |
| Suz #6/Weaver//Tui | CMBW90M2474 | 11-21 |

[a] Double digit scale (Saari and Prescott, 1975; Eyal et al., 1987) indicating the range of damage during two screening cycles in Toluca.

**Table 12. Bread wheat germplasm resistant to *Septoria tritici* that was selected through shuttle breeding and collaborative nurseries (CIMMYT/national agricultural research programs).**

| Lines and crosses | *S. tritici* score[a] | Origin |
|---|---|---|
| T00011/T00007 A8972-1T-2N-1B-2T-2T-0T | 53 | Argentina |
| L.A.CIAT(Santa Cruz) | 63 | Bolivia |
| Talhuen INIA | 31 | Chile |
| Br14/CEP847 B31615-0A-0Z-1A-15A-0A | 21 | Brazil |
| Iapi/Fink CP3409-1E-0Y-0E-18Y-0E | 31 | Paraguay |
| E Fed/F.5.83.7792 (Bajas) | 42 | Uruguay |

[a] Double digit scale (Saari and Prescott, 1975; Eyal et al., 1987) indicating the range of damage during two screening cycles in Toluca.

found by Eyal et al. (1985), whose studies revealed that isolates from Syria and Tunisia were more virulent on tetraploid than on hexaploid varieties.

Based on this information, elite durum wheat lines were selected in Toluca and Patzcuaro, as was described earlier for bread wheat. A representative group of these selections is listed in Table 13. This germplasm is under observation in the WANA region, but the dry conditions that have prevailed in recent years have not permitted accurate information to be obtained on the resistance of germplasm pre-selected in Mexico. However, information from Tunisia (Table 14) has contributed greatly to enhancing the effective resistance in durum wheat germplasm, and the disease data from that location have been used to design crosses.

## Conclusion

In conclusion, there are numerous sources of resistance to *S. tritici*. For the most part, the genetic constitution of these sources remains unclear, but the information contained in this paper suggests that they are genetically different. Resistance sources in CIMMYT's genebank collections are available on request.

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**Table 13. Elite durum wheat lines with resistance to *Septoria tritici* selected at Toluca and Patzcuaro.**

| Lines | Cross | *S. tritici* score[a] |
|---|---|---|
| Aaz77 3/Olus | CD 94270 | 21-22 |
| Ajaia 12/F3Local (Sel.Ethio.135.85)//Plata 13 | CD 98331 | 21-32 |
| Eco/CMH76A.722//Yav/3/ Altar 84/4/Ajaia 2/5/Kjove 1 | CD 91B2636 | 32-42 |
| Gs/Cra//Sba81/3/Ho/4/Mex 1/ 5/ Memo/6/2*Altar 84 | CDSS 92B1193 | 32-33 |
| Himan9/ Bejah 6 | CD91B2096 | 21-21 |
| Kucuk | CD 91B2620 | T-11 |
| Lhnke//Gs/Str/3/Altar 84/4/Focha 1 | CDSS92B1076 | 21-22 |
| Liro 2 | CD 93352 | 31-31 |
| Lotus 1/Espe | CD97015 | 11-21 |
| Lymno 8 | CD92314 | 21-42 |
| Nus/Silver//CMH82A.1062/ Rissa/3/Chen/Altar84/4/Don | CD91B2664 | 21-32 |
| Patka 3 | CD 78995 | 11-21 |
| Plata1 Smn//Plata 9 | CD97899 | T- 11 |
| Plata10/6/Mque/4/Usda573//Qfn/ Aa 7/3/Alba-D/5/Avohui/7/ Plata 1 | CD98581 | 32-43 |
| Plata 6/ Green17 | CD96789 | 11-21 |
| Plata7/Fillo 9//Sbak | CD99545 | 31-32 |
| Plata 8/4/Garza/Afn//Cra/ 3/Gta/5/Rascon | CD91B2061 | 32-42 |
| Rascon 21/Long | CDWS91M377 | 32-42 |
| Rascon 37/Green 2 | CD91B1975 | 31-32 |
| Rascon 37/Tarro 2//Rascon 37 | CDSS92B1022 | 31-32 |
| Rascon 39/Tilo 1 | CDSS92B61 | 21-22 |
| Sn Turrk Mi 83-84 503/ Lotus 4//Lotus 5 | CD97775 | T - 11 |
| Sooty 9/Rascon 37 | CD91B1938 | 31-32 |
| Sora/2*Plata 12 | CD92011 | T - 11 |
| Topdy 18/Focha 1//Altar 84 | CDSS92B1034 | 22-32 |

[a] Double digit scale (Saari and Prescott, 1975; Eyal et al., 1987) indicating the range of damage during two screening cycles in Toluca.

**Table 14. Durum wheat germplasm with resistance to *Septoria tritici* from Tunisia.**

| Lines | Cross | *S. tritici* score[a] |
|---|---|---|
| Karim (Check S ) | CM9799 | 98-97 |
| Khiar (check S) | CD57.005 | 97-97 |
| BD 2337 | – | 53-42 |
| BD 2338 | – | 64 |
| BD 2339 | – | 64 |
| Src2/Src1 | ICD88.66 | 75-74 |
| Gdfl/ T. dicoccoides-SY20013/Bcr | – | 64-53 |
| Bcr/Guerou 1 | ICD87.0572 | 75-76 |
| Zeina 2 | ICD88.1233 | 44-32 |
| Zeina 4 | ICD88.1233 | 64-32 |
| Aus 1/5/Cndo/4/Bry*2/ Tace//II27655/3/Tme/Zb/2*W | ICD88.1120 | 53-76 |
| Srn 3/Ajaia 15//Don 87 | CD96855 | 73-42 |
| Porron 1 | CD85328 | 54 |
| Poho/Ajaia | CD99818 | 64 |
| Cali/ship 2// Fillo 7 | CD98278 | 42 |
| Plata 6/Green 17 | CD96789 | 52 |
| CMH82A.1062/3/Ggovz394//Sba81/ Plc/4/Aaz 1/Crex/5/Hui//Cit71/CII | CD97395 | 43 |

[a] Double digit scale (Saari and Prescott, 1975; Eyal et al., 1987) indicating the range of damage during two screening cycles in Toluca.

# Selecting Wheat for Resistance to *Septoria/Stagonospora* in Patzcuaro, Michoacan, Mexico

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## Abstract

*The research reported in this paper was conducted in the humid, temperate area of Patzcuaro in the state of Michoacan, in Mexico, where there is a high natural incidence of* Septoria/Stagonospora *spp. Nine wheat genotypes that had previously been selected for resistance to* Septoria/Stagonospora *spp. were included in the study. They were compared to two check varieties, one tolerant and one susceptible. The materials were tested with and without chemical protection. Three years' data were analyzed and relative yield losses of 10-32% were found. Two different responses were observed among the outstanding genotypes. On the one hand, several lines expressed little reduction in yield when challenged by the septoria pathogens. Depending on their yield potential, final yield could be moderate to quite good. These could be classified as resistant or tolerant to* Septoria/Stagonospora *spp. Some of these same materials did show a considerable level of severity; their response would thus be better classified as tolerance rather than resistance. On the other hand, certain materials with high yield potential did lose yield following the attack by the septoria foliar blights, but retained sufficient expression of yield potential to compete well with the local check variety. These should not be classified as resistant nor tolerant, but may well be desirable from a production standpoint. The line that best combined these traits is IAS20/H567.71/5*IAS 20. It is being considered for release under the name Patzcuaro. Severity of foliar disease on the flag leaf proved to be well correlated with yield loss.*

Over the past 15 years, Mexico's National Livestock and Agricultural Research Institute (INIFAP) and CIMMYT have worked together on breeding bread wheats for resistance to *Septoria tritici* and *Stagonospora nodorum* in the Patzcuaro, Michoacan, region of Mexico. Natural conditions in this area favor the yearly development of these two *Septoria* species (Gomez and Gonzalez, 1987). The area enjoys a temperate climate, with mean temperatures of 8-21ºC, > 800 mm annual rainfall, and > 85% relative humidity. The isolates of *Septoria/Stagonospora* spp. that thrive under these conditions are very aggressive, which makes this a stress environment for wheat. According to Eyal et al. (1985), the *Septoria* isolates present in the Patzcuaro area are among the most virulent in the world.

## Materials and Methods

In this study, 11 advanced wheat lines from CIMMYT nurseries (Table 1) targeted for high rainfall production environments (e.g., HRWSN and ASWSN) were evaluated with and without chemical protection during 1994-96.

The variety Curinda M-87, which has functioned as reference in our work for the past 10 years, was used as the resistant/tolerant check variety in the trials (Table 1). The variety Batan was the susceptible check. Although both *S. tritici* and *S. nodorum* were present, the former was found in a higher proportion in most years.

Fungicides Terbuconazole and Tecto were used for chemical control. Terbuconazole was applied every 10 days starting from tillering to grain milk stage at a dosage of 0.5 l/ha. Tecto was applied in the same dosage every eight days, starting at the end of the booting stage.

Days to flowering, percentage flag leaf area affected by *Septoria/Stagonospora* spp. (% flag leaf severity, FLS) at grain milk stage, plant height, grain yield, and test weight were measured. Yield losses and correlations were calculated for individual years.

## Results and Discussion

Several lines expressed higher levels of resistance than the resistant check variety Curinda (29% FLS) (Table 1). Severities as low as 1-3% were noted in individual years. Across the three

**Table 1. Response of 11 wheat lines to natural infection by *Septoria/Stagonospora*, in regard to flag leaf severity (FLS in %), yield, percentage loss, in field plots either protected or unprotected with fungicide, in Pátzcuaro, Michoacan. México, during 1994, 1995, and 1996.**

| | | 1994 | | | | | |
|---|---|---|---|---|---|---|---|
| Entry | Cross/Selection history | Yield (kg/ha) CC * | % ** | UP | % | % Yield loss | % Flag leaf |
| 1 | CURINDA<br>Tolerant check | 2875 e *** | 100 | 2042 d | 100 | 29 | 28 |
| 2 | BATAN<br>Susceptible check | 3521 b | 122 | 2271 c | 111 | 36 | 23 |
| 3 | IAS20/H567.71/5* IAS20<br>CM78A-544-7B-1Y-1B-1Y-2B-1Y-0B | 3826 b | 133 | 3694 a | 181 | 4 | 1 |
| 4 | CHIL//ALD/PVN<br>CM92801-65Y-0M-0Y-4M-0RES | 2820 e | 98 | 1590 e | 78 | 44 | 36 |
| 5 | THB/CNT 7<br>CM 830057-0Z-0A-1A-1A-1A-0Y | 2632 f | 92 | 2243 c | 110 | 15 | 10 |
| 6 | LIRA/TAN//SPB<br>CM96824-U-0Y-0H-0SY-4M-0RES | 2792 f | 97 | 1847 d | 90 | 34 | 18 |
| 7 | ALD/PVN//YMI 6<br>CM 91065-2M-0M-0Y-0M-2Y-0B-5PZ | 3681 b | 128 | 2681 b | 131 | 27 | 3 |
| 8 | BAU/MILAN<br>CM103873-2M-030Y-020Y-010M-4Y-0M | 3312 c | 115 | 1986 d | 97 | 40 | 15 |
| 9 | ISEPTON89-82E/BR6//PF83144<br>F32938-2M-0AL-0AL-0AL-4Y-0M | 3049 d | 106 | 3014 b | 148 | 1 | 1 |
| 10 | BOW/MII//RES/NAC/3/PFAU<br>CM1033644-2FC-0M-1FC-0C | 4432 a | 154 | 2688 d | 132 | 39 | 8 |
| 11 | CAR853/COC//VEE/3/E7408/PAM//HORK/PF73226<br>CM80174-32Y-04M-0Y-3M-1M-0M | 3854 b | 134 | 2292 d | 112 | 41 | 24 |
| | LSD | 498 | | 545 | | | |
| | Mean | 3344 | | 2396 | | | |
| | C.V. | 10 | | 16 | | | |

| | | 1995 | | | | | |
|---|---|---|---|---|---|---|---|
| Entry | Cross/Selection history | Yield (kg/ha) CC * | % ** | UP | % | % Yield loss | % Flag leaf |
| 1 | CURINDA<br>Tolerant check | 4153 c | 100 | 3125 a | 100 | 25 | 18 |
| 2 | BATAN<br>Susceptible check | 3492 e | 84 | 2285 c | 73 | 35 | 53 |
| 3 | IAS20/H567.71/5* IAS20<br>CM78A-544-7B-1Y-1B-1Y-2B-1Y-0B | 4521 b | 108 | 3375 a | 108 | 35 | 26 |
| 4 | CHIL//ALD/PVN<br>CM92801-65Y-0M-0Y-4M-0RES | 4007 d | 96 | 2722 b | 87 | 32 | 45 |
| 5 | THB/CNT 7<br>CM 830057-0Z-0A-1A-1A-1A-0Y | 4035 d | 97 | 3153 a | 101 | 22 | 21 |
| 6 | LIRA/TAN//SPB<br>CM96824-U-0Y-0H-0SY-4M-0RES | 3854 d | 92 | 2986 a | 96 | 22 | 30 |
| 7 | ALD/PVN//YMI 6<br>CM 91065-2M-0M-0Y-0M-2Y-0B-5PZ | 4722 b | 114 | 2875 a | 92 | 39 | 16 |
| 8 | BAU/MILAN<br>CM103873-2M-030Y-020Y-010M-4Y-0M | 3952 d | 95 | 3333 a | 102 | 20 | 13 |
| 9 | ISEPTON89-82E/BR6//PF83144<br>F32938-2M-0AL-0AL-0AL-4Y-0M | 4007 d | 97 | 3368 a | 108 | 17 | 5 |
| 10 | BOW/MII//RES/NAC/3/PFAU<br>CM1033644-2FC-0M-1FC-0C | 5292 a | 127 | 3236 a | 104 | 39 | 13 |
| 11 | CAR853/COC//VEE/3/E7408/PAM//HORK/PF73226<br>CM80174-32Y-04M-0Y-3M-1M-0M | 4549 b | 110 | 3222 a | 103 | 29 | 21 |
| | LSD | 484 | | 592 | | | |
| | Mean | 4235 | | 3045 | | | |
| | C.V. | 8 | | 13 | | | |

* (CC) Chemical control and (UP) Unprotected.

** Percentage relative to the tolerant check, Curinda M87.

*** Values followed by the same letter are not significantly different at the 0.05 probability level.

**Table 1. Continued...**

| | | 1996 | | | | | |
|---|---|---|---|---|---|---|---|
| | | Yield (kg/ha) | | | | % | |
| Entry | Cross/Selection history | CC * | % ** | UP | % | Yield loss | % Flag leaf |
| 1 | CURINDA<br>Tolerant check | 2896 c | 100 | 2826 c | 100 | 3 | 40 |
| 2 | BATAN<br>Suscepible check | 2038 e | 70 | 1637 f | 57 | 19 | 61 |
| 3 | IAS20/H567.71/5* IAS20<br>CM78A-544-7B-1Y-1B-1Y-2B-1Y-0B | 3359 b | 116 | 3173 a | 112 | 6 | 27 |
| 4 | CHIL//ALD/PVN<br>CM92801-65Y-0M-0Y-4M-0RES | 2644 d | 91 | 2289 e | 81 | 13 | 75 |
| 5 | THB/CNT 7<br>CM 830057-0Z-0A-1A-1A-1A-0Y | 3206 b | 111 | 2766 c | 98 | 14 | 31 |
| 6 | LIRA/TAN//SPB<br>CM96824-U-0Y-0H-0SY-4M-0RES | 2710 d | 94 | 2655 c | 94 | 2 | 62 |
| 7 | ALD/PVN//YMI 6<br>CM 91065-2M-0M-0Y-0M-2Y-0B-5PZ | 3961 a | 137 | 3291 a | 116 | 17 | 28 |
| 8 | BAU/MILAN<br>CM103873-2M-030Y-020Y-010M-4Y-0M | 3028 c | 105 | 2326 d | 82 | 23 | 10 |
| 9 | ISEPTON89-82E/BR6//PF83144<br>F32938-2M-0AL-0AL-0AL-4Y-0M | 3146 b | 109 | 2847 b | 101 | 10 | 22 |
| 10 | BOW/MII//RES/NAC/3/PFAU<br>CM1033644-2FC-0M-1FC-0C | 3456 b | 119 | 3185 a | 113 | 26 | 38 |
| 11 | CAR853/COC//VEE/3/E7408/PAM//HORK/PF73226<br>CM80174-32Y-04M-0Y-3M-1M-0M | 3156 b | 110 | 2852 b | 101 | 28 | 33 |
| | LSD | 412 | | 345 | | | |
| | Mean | 3094 | | 2713 | | | |
| | C.V. | 9 | | 11 | | | |

* (CC) Chemical control and (UP) Unprotected.
** Percentage relative to the tolerant check, Curinda M87.
*** Values followed by the same letter are not significantly different at the 0.05 probability level.

years Curinda, when unprotected, yielded on average 2688 kg/ha, expressing a 19% loss (Table 2). Several lines were also superior to the check in yield, and suffered less yield loss.

In this study percent flag leaf area affected correlated negatively with yield loss (-0.71 to -0.83; Table 3). This negative correlation appears strong and reliable over years.

Although grain yield of most lines tested varied over years depending on climatic and production conditions (Figure 1), in general their performance relative to the resistant check Curinda showed impressive consistency over time. In contrast, the susceptible check Batan yielded less every year as a percentage of the check (Tables 1 and 2; Figure 1), and seemed to be losing its resistance progressively.

The plots protected with fungicide provide a partial expression of each line's yield potential in the absence of disease stress. However, other abiotic stresses, such as soil acidity and nutrient imbalances related to leaching following excessive rain, also reduce yield in this high rainfall environment. The difference between the protected and unprotected plots provides a measure of yield loss due to disease. Mean yield losses were in the 10-32% range. The line ISEPTON89-82E/BR6//PF83144 experienced the lowest loss (10%), while the susceptible check Batan suffered the highest (32%).

Comparing protected and unprotected plots provides a measure of the contribution of resistance or tolerance to yield. If the yield difference is small (i.e. the loss is small), the genotype tested apparently has a certain level of resistance/tolerance that enables it to express most, if not all, of its yield potential. However, useful information can also be gained from direct comparison of lines in a disease-prone setting, in companion plots without the use of fungicides, in particular when screening germplasm to identify potential varieties. This is less expensive than also growing protected plots, and hence allows larger numbers of entries to be tested.

To be acceptable to farmers, a variety must yield the same or better than the commercial check variety. Candidate varieties can be acceptable

and appear well adapted to the point of being releasable to farmers for two reasons, or a combination of both. On the one hand, a line may express true resistance or tolerance. In that case challenge by the pathogen does not result in great reductions in yield (Tables 1 and 2). Yield potential is roughly maintained due to resistance, with only minimum disease severity, or due to tolerance when considerable disease severity may be noted but losses are little. The line ISEPTON89-82E/BR6//PF83144 is a good example of a resistant genotype (9% FLS) with minimum yield loss (10%) and a high mean yield of 3034 kg/ha, 13% more than the tolerant check, Curinda, in the presence of disease. Most lines suffered yield losses which percentage-wise were roughly equivalent to about two-thirds of their percentage-wise flag leaf severity scores. Good examples of very sensitive lines are ALD/PVN//YMI 6 and BAU/MILAN, whose mean % yield losses (27%-28%) were almost twice that of their FLS scores (13%-16%).

On the other hand, a line may have a very high intrinsic yield potential in the absence of biotic stresses, such as attacks by *Septoria/Stagonospora* spp. Even if susceptible to the fungus, such lines may express a residual yield that competes well with that of the check variety (and may even outyield it in certain cases). These lines are not resistant—on the contrary, they may be quite susceptible. Their virtue lies in their high initial yield potential, which allows a considerable loss due to disease, and still produces

**Table 2. Mean response of 11 wheat lines to natural infection by *Septoria/Stagonospora*, in regard to flag leaf severity, yield, percentage loss, in field plots either protected or unprotected with fungicide, in Patzcuaro, Michoacan, Mexico, over 1994, 1995, and 1996.**

| | | Mean values | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Entry | Cross/Selection history | CC * | % ** | UP | % | % Yield loss | % Flag leaf | Overall mean yield kg/ha | % |
| 1 | CURINDA<br>Tolerant check | 3376 c *** | 100 | 2688 d | 100 | 19 | 29 | 3005 c | 100 |
| 2 | BATAN<br>Susceptible check | 3016 e | 89 | 2003 h | 75 | 32 | 46 | 2470 f | 82 |
| 3 | IAS20/H567.71/5* IAS20<br>CM78A-544-7B-1Y-1B-1Y-2B-1Y-0B | 4094 b | 121 | 3380 a | 126 | 13 | 18 | 3709 a | 123 |
| 4 | CHIL//ALD/PVN<br>CM92801-65Y-0M-0Y-4M-0RES | 3062 d | 91 | 2216 g | 82 | 30 | 52 | 2606 d | 87 |
| 5 | THB/CNT 7<br>CM 830057-0Z-0A-1A-1A-1A-0Y | 3298 c | 98 | 2727 c | 101 | 17 | 21 | 2991 c | 99 |
| 6 | LIRA/TAN//SPB<br>CM96824-U-0Y-0H-0SY-4M-0RES | 3098 d | 91 | 2519 e | 94 | 20 | 37 | 2786 d | 93 |
| 7 | ALD/PVN//YMI 6<br>CM 91065-2M-0M-0Y-0M-2Y-0B-5PZ | 4160 a | 123 | 2998 b | 112 | 28 | 16 | 3534 a | 118 |
| 8 | BAU/MILAN<br>CM103873-2M-030Y-020Y-010M-4Y-0M | 3449 c | 102 | 2473 f | 92 | 27 | 13 | 2924 d | 97 |
| 9 | ISEPTON89-82E/BR6//PF83144<br>F32938-2M-0AL-0AL-0AL-4Y-0M | 3486 c | 103 | 3034 b | 113 | 10 | 9 | 3242 b | 108 |
| 10 | BOW/MII//RES/NAC/3/PFAU<br>CM1033644-2FC-0M-1FC-0C | 4441 a | 132 | 3058 b | 114 | 31 | 20 | 3696 a | 123 |
| 11 | CAR853/COC//VEE/3/E7408/PAM//HORK/PF73226<br>CM80174-32Y-04M-0Y-3M-1M-0M | 3870 b | 115 | 2798 b | 104 | 28 | 26 | 3292 b | 109 |
| | LSD | 346 | | 312 | | | | 314 | |
| | Mean | 3577 | | 2717 | | | | 3114 | |
| | C.V. | 12 | | 15 | | | | 18 | |
| | Chemical control (CC) | | | | | | | 3577 a | |
| | Unprotected (UP) | | | | | | 2717 | b | |
| | 1994 | 3344 b | | ns | | | | | |
| | 1995 | 4234 a | | ns | | | | | |
| | 1996 | 3153 c | | ns | | | | | |
| | LSD | 181 | | | | | | | |

* (CC) Chemical control and (UP) Unprotected.
** Percentage relative to the tolerant check, Curinda M87.
*** Values followed by the same letter are not significantly different at the 0.05 probability level.

an impressive final yield when challenged by the fungus. In this study BOW/MII//RES/NAC/3/PFAU is an example of a line with high yield potential, which despite having 20% of its flag leaf area damaged by *Septoria/Stagonospora* spp., resulting in a 31% mean yield loss, still yielded 14% more than the commercial check.

The most desirable genotype is one that combines high yield potential with resistance/tolerance to the fungus; this results in relatively low losses in the presence of disease, and outstanding yields in its absence. The line IAS20/H567.71/5*IAS 20 combines these traits; only 18% of its foliage was affected, which translated into a minimal yield loss of 13% and resulted in the trial's top mean yield of 3380 kg/ha, 26% over the commercial check, Curinda, in the presence of disease (Table 2 and Figure 1). It produced a very respectable yield of 4094 kg/ha, the third highest of the group, in the absence of disease. This line is now being considered for release under the name Patzcuaro.

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**Table 3. Correlation coefficients of flag leaf severity and yield with various other characters, Patzcuaro, Michoacan, Mexico, during 1994, 1995, and 1996.**

| Variables | Variables | 1994 | 1995 | 1996 |
|---|---|---|---|---|
| Flag leaf severity due to infection by *Septoria/Stagonospora* | Yield | -0.81 | -0.83 | -0.71 |
| | Test weight | -0.72 | -0.34 | -0.44 |
| | Plant height | -0.48 | -0.55 | -0.62 |
| | Yield loss (%) | 0.71 | 0.33 | 0.71 |
| | Days to flowering | -0.42 | -0.71 | -0.84 |
| Yield | Test weight | 0.72 | 0.85 | 0.76 |
| | Plant height | 0.62 | 0.44 | 0.55 |
| | Yield loss (%) | -0.75 | -0.49 | -0.86 |
| | Days to flowering | 0.46 | 0.86 | 0.79 |

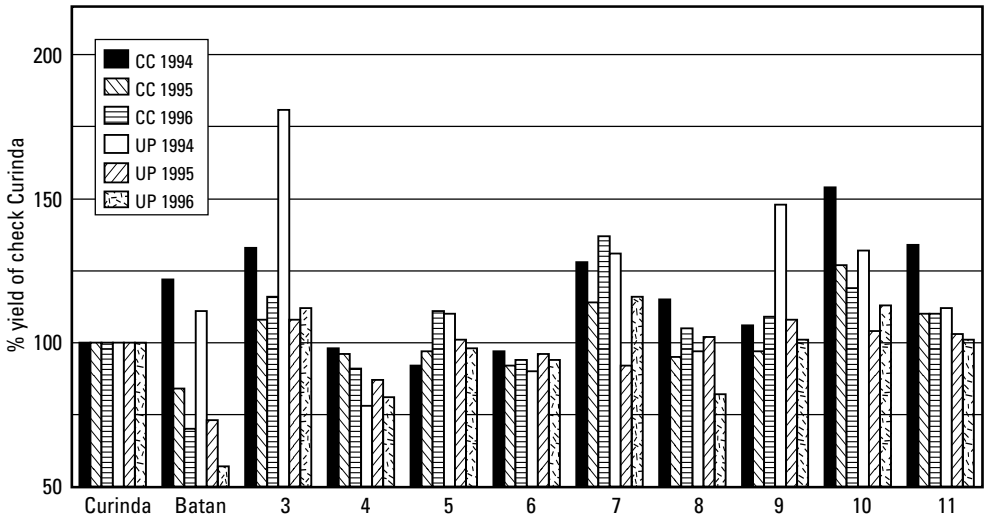


**Figure 1. Yield as percentage of the tolerant check Curinda, in chemically controlled plots (CC) and unprotected plots (UP) of nine wheat lines, during 1994, 1995 and 1996. Patzcuaro, Michoacan, Mexico.**

# Varieties and Advanced Lines Resistant to Septoria Diseases of Wheat in Western Australia

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## Abstract

*The development of bread wheats in Western Australia has resulted in the release of several varieties with improved response to the commonly occurring leaf spot diseases caused by* Phaeosphaeria nodorum, Mycosphaerella graminicola, *and* Pyrenophora tritici-repentis. *In a yield trial evaluating resistance to* M. graminicola, *susceptible varieties suffered 20-50% yield loss, while yield loss of resistant crossbreds and varieties was 0-15%. Similar responses were observed in most lines for grain weight when assessed in* M. graminicola*-infected row plots. In* P. nodorum*-infected row plots, susceptible varieties suffered 30-50% grain weight loss, while resistant lines suffered 0-30% loss in grain weight depending on year of testing.*

In Western Australia resistance to *Phaeosphaeria nodorum* is sought in combination with *Mycosphaerella graminicola* and *Pyrenophora tritici-repentis* resistance, as these pathogens frequently occur in combination (Loughman et al., 1994). Improved resistance to these diseases is sought by continually crossing the most resistant, best adapted lines with resistance donors. A selection of varieties, advanced lines, and potential resistance sources were assessed for resistance in replicated field experiments using susceptible adapted wheats with comparable maturities as benchmarks.

## Materials and Methods

Lines were evaluated for response to infection with *M. graminicola* or *P. nodorum* in separate experiments in 1995-96 using split plot designs of three replicates. At Mt. Barker lines were sown as 5-row, 5-m subplots. Main plots were established in early spring as either fungicide protected (three applications of 125 g tebuconazole/ha) or inoculated twice with aqueous pycnidiospore suspensions of the pathogen. Growth stage (Zadoks et al., 1974) and % infection of the flag-3 leaves were assessed in late spring. Yields were assessed. At South Perth lines were sown as 40-cm, 2-row subplots with 18-cm row spacings, each subplot separated from its neighbor by a single row of barley. Main plots were established with fungicide or inoculation as above. Separate experiments were established for *M. graminicola* and *P. nodorum*. Heading date (as day of the year) and disease severity as percent infection of the flag or flag and second top leaves were recorded in spring. Grain weights were assessed from a random subsample of harvested grain. Relative grain weights of infected plots were calculated as a percentage of fungicide protected plots.

## Results and Discussion

### Resistance to *M. graminicola*

Cascades (Tadorna.Inia / 3*Aroona), Tammin (Bod/Eradu sib.xbvt//Atlas66.2Madden), and Nyabing (Ar-3Ag3(WT329)/ (IW753)Jab.Ag4C) developed low to moderate infection and yielded 5.4, 5.2, and 4.1 t/ha, respectively in the presence of disease. These yields were not significantly different from fungicide sprayed plots (yield diseased was 89-99% relative to the fungicide protected plots). In contrast, two susceptible varieties of similar maturity, Gamenya and Datatine, developed high infection scores and yielded 1.5 and 3.2 t/ha, respectively when diseased. These yields were significantly different from fungicide sprayed plots (yield diseased was 48-72% relative to fungicide protected).

**Table 1. Maturity (growth stage), percent leaf disease (Dis), yield, hundred-grain weight (HGW), and relative yield (RY) and relative grain weight (RGW) of wheat varieties infected with *Mycosphaerella graminicola* in a yield trial at Mt. Barker and small row plots at South Perth, 1995. Comparisons for percent flag leaf disease should be made between varieties of similar maturity.**

| | Mt. Barker | | | | | South Perth | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Growth stage | Dis Flag-3 | Yield(t/ha) Fung | Inoc | RY % | Dis Flag | HGW (g) Fung | Inoc | RGW % |
| **Resistant and moderately resistant lines** | | | | | | | | | |
| 77Z893 | 44 | 2 | 5.4 | 5.3 | 98 | 86 | 3.6 | 2.5 | 70 |
| Nyabing | 42 | 30 | 4.1 | 4.1 | 99 | 38 | 4.2 | 3.4 | 81 |
| Cascades | 41 | 23 | 6.0 | 5.4 | 89 | 86 | 4.2 | 2.5 | 57 |
| Tammin | 41 | 3 | 5.8 | 5.2 | 90 | 23 | 4.2 | 3.1 | 76 |
| 483W3 | 40 | 0 | 5.1 | 5.2 | 101 | 21 | 3.2 | 3.4 | 105 |
| 486W189 | 39 | 0 | 4.6 | 4.3 | 92 | 32 | 3.9 | 4.2 | 106 |
| 78Z976 | 39 | 5 | 5.7 | 5.0 | 89 | 40 | 3.1 | 2.7 | 87 |
| Calingiri | 38 | 1 | 5.9 | 5.0 | 85 | 43 | 4.0 | 3.7 | 94 |
| **Resistant and moderately resistant control varieties** | | | | | | | | | |
| Millewa | 43 | 9 | 4.5 | 3.9 | 86 | 78 | 3.3 | 2.2 | 69 |
| Aroona | 40 | 13 | 3.9 | 4.4 | 114 | 99 | 3.6 | 2.5 | 72 |
| Corrigin | 40 | 2 | 5.7 | 5.2 | 91 | 70 | 3.1 | 2.5 | 80 |
| **Susceptible control varieties** | | | | | | | | | |
| Kulin | 55 | 85 | 4.0 | 2.2 | 55 | 99 | 2.7 | 1.6 | 63 |
| Tincurrin | 42 | 90 | 4.5 | 3.6 | 80 | 99 | 3.6 | 2.2 | 68 |
| Datatine | 41 | 50 | 4.5 | 3.2 | 72 | 73 | 3.4 | 2.5 | 72 |
| Gamenya | 41 | 88 | 3.2 | 1.5 | 48 | 99 | 3.6 | 1.9 | 59 |
| Stiletto | 38 | 23 | 4.5 | 3.2 | 70 | 98 | 4.1 | 2.8 | 69 |
| lsd 5% | 2.5 | 23 | 1.0 | | 32 | 0.7 | 23 | | |
| cv % | 4 | 41 | 13 | | 44 | 13 | 19 | | |

**Table 2. Maturity (heading day of year, Hd), percent leaf disease (Dis), hundred-grain weight (HGW), and relative grain weight (RGW) of wheat varieties infected with *Phaeosphaeria nodorum* in short row plot experiments in South Perth, 1995-96 (- denotes testing discontinued). Comparisons for percent flag leaf disease should be made between varieties of similar maturity.**

| | 1995 | | | | | 1996 | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Hd d.o.y. | Dis Flag | HGW (g) Fung | Inoc | RGW % | Dis F,F-1 | HGW(g) Fung | Inoc | RGW % |
| **Moderately resistant lines** | | | | | | | | | |
| Brookton | 225 | 64 | 3.6 | 3.7 | 101 | 52 | 3.5 | 2.8 | 80 |
| W48795 | 228 | 46 | 3.6 | 3.6 | 101 | 4 | 3.8 | 2.9 | 76 |
| 81W1137 | 230 | 43 | 3.8 | 3.6 | 97 | - | - | - | - |
| Tammin | 231 | 32 | 3.9 | 3.9 | 103 | 8 | 4.3 | 3.0 | 71 |
| W48433 | 232 | 33 | 3.2 | 3.1 | 98 | 30 | 3.8 | 3.0 | 81 |
| **Resistant and moderately resistant controls** | | | | | | | | | |
| ALMRES-83-18 | 225 | 72 | 4.3 | 3.6 | 83 | 56 | 3.9 | 2.8 | 74 |
| CNT2 | 236 | 21 | 4.6 | 4.2 | 93 | 45 | 4.1 | 3.9 | 103 |
| Aus20917 | 236 | 40 | 3.1 | 2.5 | 81 | 27 | 2.8 | 2.4 | 87 |
| Qualset601-68 | 238 | 18 | 3.6 | 3.0 | 85 | 20 | 4.0 | 2.9 | 73 |
| **Intermediate lines** | | | | | | | | | |
| W486110 | 220 | 50 | 4.9 | 4.2 | 87 | 39 | 4.0 | 3.3 | 83 |
| Westonia | 220 | 83 | 4.1 | 3.3 | 80 | 76 | 3.2 | 2.2 | 69 |
| Cascades | 226 | 83 | 4.1 | 3.2 | 80 | 83 | 3.7 | 2.0 | 54 |
| Carnamah | 226 | 68 | 3.4 | 2.9 | 86 | 32 | 4.1 | 2.1 | 51 |
| Cunderdin | 228 | 50 | 3.7 | 3.0 | 82 | 41 | 2.9 | 2.4 | 81 |
| **Susceptible control varieties** | | | | | | | | | |
| Kulin | 221 | 99 | 3.4 | 2.2 | 66 | 91 | 3.2 | 1.5 | 46 |
| Aroona | 225 | 87 | 4.6 | 2.2 | 49 | 92 | 3.2 | 1.6 | 50 |
| Millewa | 225 | 96 | 2.6 | 2.0 | 77 | 97 | 3.3 | 1.3 | 44 |
| Gamenya | 228 | 77 | 3.4 | 2.1 | 64 | 51 | 3.2 | 2.0 | 63 |
| Cranbrook | 236 | 71 | 3.1 | 2.5 | 80 | 48 | 3.4 | 2.0 | 61 |
| Spear | 242 | 59 | 4.2 | 2.6 | 63 | 1 | 4.1 | 3.0 | 75 |
| lsd 5% | | 23 | 0.6 | | 24 | 18 | 0.8 | | 28 |
| cv % | | 26 | 10 | | 19 | 23 | 16 | | 26 |

Calingiri (Chino/Kulin// Reeves) also exhibited resistance and while some of this low disease expression may have been due to later maturity, the performance was measurably superior to the similar maturing but susceptible variety Stiletto. Crossbreds 483W3, 78Z976 and 77Z893 (Corrigin sib) also expressed high levels of resistance, as indicated by low disease scores, the non-significant yield losses, and the resulting high relative yields (yield diseased was 89-101% relative to fungicide protected) (Table 1).

### Resistance to *P. nodorum*

Five moderately resistant lines achieved high grain weights in diseased plots relative to fungicide protected plots. Variety Brookton (Torres/Cranbrook//76W596/ Cranbrook) developed significantly less disease and no significant grain weight reduction when compared with susceptible varieties of equivalent maturity such as Aroona or Millewa. W48795, Tammin, and sister line 81W1137 had similar maturity to susceptible variety Gamenya. These lines developed significantly less disease than Gamenya and had smaller grain weight reductions in the presence of disease. Line W48433, derived from CNT2, was similar to Tammin and developed low disease in 1995 and high relative grain weights in 1995 and 1996. This line was comparable with the resistant parent CNT2 for disease severity and relative grain weight in 1995, though relative grain weight appeared less robust than that of CNT2 in 1996 (Table 2).

Westonia (SpicaTimg.Tosc/ Cr:J2.Bob), Cunderdin (Cranbrook sib/Sunfield sib), Carnamah (Bolsena-1ch(RAC529)/77W660), and Cascades gave responses that were intermediate between the resistant and susceptible lines. Although hundred grain weight reductions in the presence of disease were generally significant, the grain weight reductions were less than for susceptible lines.

Although excellent levels of *M. graminicola* resistance from diverse sources have been observed in lines such as 483W3 and 78Z976, moderate resistance derived from WW15 via Aroona and Condor (Wilson, 1994) has had the greatest impact in the development of commercial varieties. While this moderate resistance can be more difficult to identify in intensive disease nurseries, its effect has been observed often in practice and previously reported (Loughman and Thomas, 1992).

Significant progress has been made in developing moderate resistance to *P. nodorum*. Commercial varieties currently exhibit improved responses compared with their susceptible predecessors, and some varieties and advanced lines perform at similar levels to resistant control varieties. In some cases moderate resistance to different leaf spot diseases has been combined. Cascades combines moderate resistance to both *M. graminicola* and *P. tritici-repentis* (Loughman et al., 1998). Brookton, Cunderdin, and Westonia have moderate resistance to *P. tritici-repentis* (Loughman et al., 1998), in combination with partial resistance to *P. nodorum*. Because *P. nodorum*, *M. graminicola,* and *P. tritici-repentis* frequently occur as disease complexes in Western Australia, combinations of resistance are important for effective disease management.

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# Field Resistance of Wheat to Septoria Tritici Leaf Blotch, and Interactions with *Mycosphaerella graminicola* Isolates

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## Abstract

*The resistance of 71 varieties of bread wheat to six isolates of* Mycosphaerella graminicola *was studied in field trials in the Netherlands, Switzerland, and the UK, carried out over three years. There was a wide range of* Septoria tritici *infection. Some varieties had especially good resistance, including lines from Europe (especially Switzerland) and Latin America. Many interactions between varieties and isolates were detected. In particular, 27 varieties of diverse origins were specifically resistant to the isolate IPO323. Variety-by-isolate interactions were stable over years and locations. The existence of these interactions and the fact that they are stable over environments implies that certain widely used resistances to* Septoria tritici *might break down through the evolution of specific virulence in the fungus. Breeders should take these interactions into account in their efforts to develop varieties with durable resistance.*

Septoria tritici leaf blotch is now the most economically important foliar disease of wheat in Europe, and controlling it with fungicides costs several hundred million dollars a year. Resistance to septoria tritici leaf blotch is therefore a major target for most European wheat breeders. However, if resistant varieties are to be economically worthwhile, resistance must be both effective and durable.

Strong, specific interactions between wheat varieties and isolates of the pathogen, *Mycosphaerella graminicola*, have been found in both seedlings and adult plants (Kema and van Silfhout, 1997), while the resistance of at least one variety, Gene, has "broken down" through the evolution of a virulent pathogen population (Mundt et al., 1999). This means that breeders need to know not only which varieties may be good sources of resistance in breeding programs, but also whether or not resistance genes in these varieties are at risk from virulent isolates of *M. graminicola*.

This paper reports a series of field trials carried out in England, The Netherlands, and Switzerland between 1994 and 1997. The aims were to investigate resistance to septoria tritici leaf blotch in a representative set of European varieties, to evaluate potential new sources of resistance, to study the responses of varieties to different isolates of *M. graminicola* and to test the stability of variety-by-isolate interactions over a range of environments.

## Materials and Methods

A total of 71 wheat varieties of diverse origins were included in the field trials. The majority were cultivars or breeding lines developed by European breeders. Most of these were winter wheats. Several varieties that are potential sources of septoria tritici leaf blotch resistance were also included, as were a number of varieties that are parents of precise genetic stocks held by the John Innes Centre.

Trials were inoculated with six monospore isolates of *M. graminicola* from the IPO-DLO collection. Six trials were conducted, three in the Netherlands, one in Switzerland, and two in England. Each trial was sown in a split-plot design with two replicate blocks. Within each block, there were five or six main

plots, each inoculated with one isolate, such that isolates were randomized within blocks, while varieties were randomized within main plots. The percentage of the leaf area that was necrotic or covered by lesions bearing pycnidia was estimated visually.

## Results

Levels of necrosis and pycnidia were highly correlated, so further analysis was based on pycnidia scores. There was highly significant variation between varieties in the level of disease and highly significant interactions between varieties and isolates. However, there was no significant variation in variety-by-isolate interactions among the six trials. This implies that variety-by-isolate interactions were stable over the range of environments used in this series of trials.

There was a wide range of resistance among the varieties grown in the trials. The existence of strong variety-by-isolate interactions and the small number of isolates used means that one cannot be certain which varieties have good, overall resistance to the European population of *M. graminicola*. However, some lines were identified that should be tested further, preferably with more isolates. The most resistant was the Brazilian cultivar Veranopolis, a well-known source of resistance. Four of the top ten varieties were from the FAP breeding program in Switzerland, including the popular cultivar Arina. Other varieties which had very good resistance to the isolates used in these trials included cultivars and breeding lines from several European countries, as well as another well-known source of resistance, Kavkaz-K4500 l.6.a.4, and Frontana, one of the parents of Veranopolis.

Variety-by-isolate interactions were most pronounced in tests with the isolate IPO323. A total of 27 cultivars and breeding lines, from several European countries, China, Israel, and the USA, showed specific resistance to this isolate. One or more varieties showed specific resistance to four of the other five isolates, while specific susceptibility to four isolates was also detected.

## Discussion

There is good resistance to septoria tritici in a wide range of European wheat germplasm from several countries. Plant breeders may be able to exploit this resistance by recombining quantitative resistance from different sources. There may be some additional value in introducing resistance from sources outside Europe. The striking success of the Swiss breeding program may be attributed to a long tradition of selection for foliar disease resistance, using both artificial and natural inoculation. Furthermore, trials are conducted without fungicides in several locations with diverse climatic conditions.

The existence of strong variety-by-isolate interactions, as well as the fact that they are stable over environments, means that breeders must take account of the possibility that certain resistances may not be durable. This is particularly the case with resistance to IPO323, although it is not known if this resistance is controlled by the same genes in different varieties. One strategy for breeding might be to use several specific resistance genes in a breeding program and aim to select varieties with combinations of resistances. An alternative would be to avoid selecting for specific resistances altogether, aiming instead for a high level of quantitative resistance.

## Acknowledgments

This research was supported in part by the EU Framework 4 Biotechnology program, the UK Ministry of Agriculture, Fisheries and Food, and Praxis XXI (Portugal).

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# Using Precise Genetic Stocks to Investigate the Control of *Stagonospora nodorum* Resistance in Wheat

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## Abstract

*A substitution series of 'Synthetic 6x' into 'Chinese Spring' had previously been studied to determine which of the 21 chromosomes of 'Synthetic 6x' conferred resistance to* Stagonospora nodorum*; from this single chromosome recombinant lines (SCRs) have been developed for substitution lines that improve the resistance of 'Chinese Spring' to the pathogen. The SCRs were screened for their response to infection. Marker assisted screening was also employed on the SCR populations; micro-satellite, RFLP and iso-electric focusing, coupled with conventional QTL analysis and morphological character scoring (vernalization response). As these markers had been previously mapped, it would be possible to pinpoint the resistance gene(s) and find a linked marker. Chromosome 5D had been shown to be the most effective against* Stagonospora nodorum *and is also known to carry the isozyme genes,* Ibf-1 *and* Mdh-3*, a range of micro-satellite markers, a sparse selection of RFLP markers, and a vernalization response gene (*Vrn 3*). Screening revealed that the resistance gene (*Srb3*) was located on the long arm between* Ibf-1 *and the RFLP probe* Xpsr 912*, but being 15.6 and 12.0 cM, respectively, away from* Srb3*, they could not be used as markers for resistance. SCRs have been developed for the remaining chromosomes, and screening continues to precisely map the resistance gene(s).*

*Stagonospora nodorum* (*Septoria nodorum* Berk.) is the causal agent of leaf and glume blotch in wheat with infection reducing yield by up to 40%. Conventional fungicide control has significant environmental and economic consequence, thus alternative methods of control are being sought. Several *Aegilops* species including *Ae. squarrosa* exhibit partial resistance to the pathogen. This resistance is expressed in a synthetic amphiploid 'Synthetic 6x' derived from a cross between *Ae. squarrosa* and *Triticum dicoccum* (McFadden and Sears, 1946; Sears, 1976). A substitution series of 'Synthetic 6x' chromosomes into 'Chinese Spring' was developed and is maintained at the John Innes Centre. This series was utilized in disease tests to determine the chromosomal location of the resistance gene(s) (Nicholson et al., 1993) as 'Chinese Spring' was shown to be susceptible to *Stagonospora nodorum* (Scott and Benedikz, 1977).

From the D genome *Ae. squarrosa* parent, chromosome 5D conferred a high level of resistance, as did 3D and 7D. Resistance was most pronounced in 5D where the percentage leaf infection was near to that of the amphiploid. Three chromosomes from the *T. dicoccum* parent (AB genome) 2A, 3B, and 5A conferred resistance but to a lesser extent than that promoted by the D genome chromosomes. SCRs were then developed for these chromosomes. This paper reports the results of experiments to localize the resistance genes and assign markers linked to the genes.

## Materials and Methods

Single chromosome recombinant lines were developed by crossing the individual chromosome substitution lines, previously identified as conferring resistance, to 'Chinese Spring' euploid (or ditelocentric), and then backcrossing the $F_1$ with the corresponding monosomic from the 'Chinese Spring' monosomic series (Sears, 1954). Monosomic plants were then selected in the backcrossed $F_1$ progeny and selfed for disomic extraction (Figure 1).

Infection response was scored on juvenile plants. Seeds had been placed on moist filter paper in petri dishes and incubated at 25ºC for 2 days (with 2 days cold shock at 4ºC to promote germination if necessary) and then planted individually in pots of John Innes no. 2 compost. In all experiments the plantlets were grown fully randomized over 10 replications with an additional uninfected replicate as a control.

The seedlings were inoculated when the second leaves were fully expanded with a 1 x $10^6$ spore/ml suspension of an aggressive isolate (S353/88). The plantlets were then placed in propagators and transferred to growth chambers for 72 h at 15ºC in complete darkness to establish infection. Once this had been completed, the plants were moved to a containment glasshouse and removed from their propagators. Disease was scored at 10 and 17 days post-inoculation and expressed as the percentage of leaf area lesioned (at the intervals 1, 5, 10, 25, 40, 60, 75, 90, and 100%). The scores were transformed using the angular transformation and an analysis of variance carried out using the GENSTAT 5 statistical package. The significance of differences in the mean scores relative to 'Chinese Spring' and 'Synthetic 6x' was estimated using a two-tailed t-test.

Morphological characters were scored on field plots, with three replicates of 11 plants in randomized blocks to enable QTL analysis and screening for *Vrn3*.

Biochemical analysis involved the isoelectric focusing technique, using seed embryo or endosperm on flat bed electrophoresis apparatus, showing separation of the proteins at their relevant isoelectric points (pI) (Liu and Gale, 1989).

Molecular marker techniques required extraction of DNA (phenol chloroform method) and in the case of RFLPs digesting with enzymes that have a 4 base-pair recognition sequence (Bam HI, Eco RI) and then hybridizing with $P^{32}$ labeled CTP (Southern, 1975). The micro-satellite technique was PCR-based and used micro-satellite probes developed at IPK-Gatersleben. Both techniques clearly revealed the SCR progeny as either 'Chinese Spring' or 'Synthetic 6x' type.

Marker results were input into Mapmaker (Lincoln et al., 1992) to compare the segregation of markers in the progeny, and a map of resistance gene(s) location

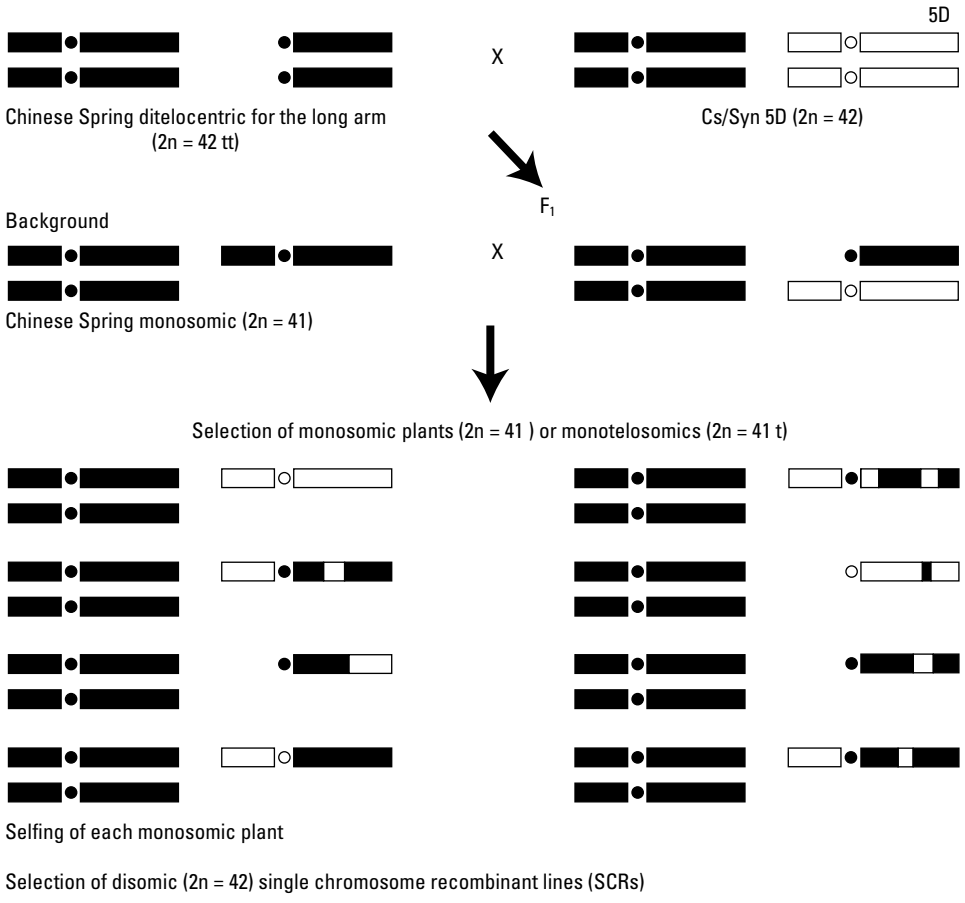


**Figure 1. Development of single chromosome recombinant lines (SCRs) for the long arm of chromosome 5D (18).**

related to the markers analyzed was compiled. QTLs were also assigned to markers on the chromosome using the Students t-test.

To date only the 5D SCR population has been analyzed for disease resistance and associated markers.

## Results and Discussion

Following analysis of the 5D population (85 lines), it was found that *S. nodorum* resistance was under the control of a single gene *Srb3*, which was located between *Ibf-1* and *Xpsr 912*, with a QTL for plant height (Qph) being linked to *Ibf-1* (Figure 2). However, these genes are too remote from *Srb3* to be useful as flanking markers. Work is continuing to locate more markers on chromosome 5D, as the region in which *Srb3* is located currently has a low marker concentration. RFLP screening is also underway with recently developed markers and with the collaboration at IPK-Germany; any new 5D micro-satellites produced will also be screened. It is hoped

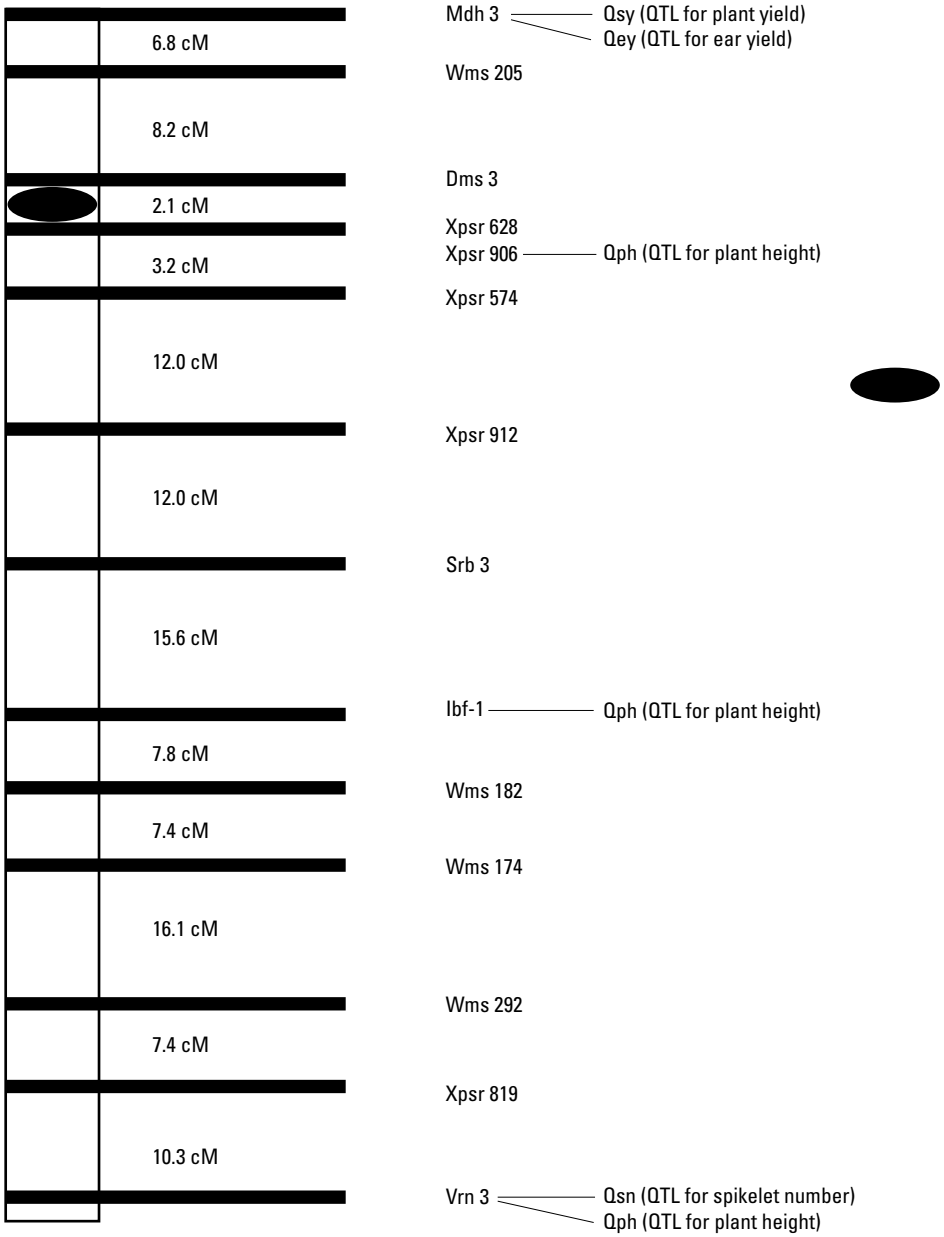


**Figure 2. Relative position of the *Stagnospora nodorum* resistance gene *Srb3* and previously mapped markers.**

that this continuing work will help to complete the map more successfully.

Mapping, field trials, and disease screening are now in progress on the SCR populations for chromosomes 2A, 3D, and 7D.

Our results have shown that *S. nodorum* resistance in wheat is inherited in a complex manner involving several genes. By studying them in single chromosome recombinant lines, we should be more able to fully understand their heritability as well as highlighting linked markers.

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# Evaluating *Triticum durum* x *Triticum tauschii* Germplasm for Resistance to *Stagonospora nodorum*

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## Abstract

*This study was conducted to determine resistance of 50 CIMMYT* Triticum durum *x* Triticum tauschii *populations to septoria glume blotch. Seedlings were inoculated at the 2-leaf stage, and data were recorded on disease severity and latent period. Several lines from this population demonstrated good resistance compared to check entries and are being used as sources of resistance to septoria glume blotch in the wheat breeding program.*

Septoria glume blotch caused by *Stagonospora nodorum* is a significant disease of wheat in the southern and eastern USA. Immunity of wheat to *S. nodorum* has not been found (Dantuma, 1955), but variation in resistance is apparent. New sources of genetic resistance are needed in order to reduce yield losses cause by this fungal disease. In our research we have studied components of partial resistance (Nelson and Marshall, 1990). A relatively simple test to evaluate resistance is based on measuring incubation period and disease severity at a different length(s) of time after inoculation. In this study, we utilized these two components to evaluate resistance of wheat germplasm to septoria glume blotch. The objective of this study was to screen a collection of 50 exotic CIMMYT germplasm accessions for resistance to septoria glume blotch, and compare any resistance found with that of moderately resistant checks to determine the potential of exotic germplasm.

## Materials and Methods

Seed of 50 CIMMYT populations and a susceptible (TAM 107) and a resistant (TX76-40-2) check (Nelson et al., 1994) were planted in rows in soil in flats. The CIMMYT germplasm consisted of 50 amphiploids from crosses between *Triticum durum* and *Triticum tauschii* obtained from Dr. A. Mujeeb Kazi, CIMMYT, via Dr. Mark Sorrells at Cornell University. The wheat was germinated and grown in the greenhouse until the 2-leaf stage. At that time spores of *S. nodorum* were sprayed on the wheat, which was then placed in a humidity chamber. The plants were removed from the humidity chamber after 50 hours. During this 50 hour period, a cool mist blower operated for 15 minutes every 2 hours during the day, and for 15 minutes every 4 hours during the night, which kept the leaves moist, but did not cause run-off. After the 50-hour period, the flats were removed from the chamber and allowed to continue growing in the greenhouse. Five days after being removed from the humidity chamber, some plants began to show leaf necrosis. Disease ratings were recorded for each row, or entry, on a 0 to 9 disease rating (0 = no symptoms; 9 = severe necrosis) on day 5, 8, and 11, to obtain an estimate of incubation period and disease severity. A mean rating over the above three days was also calculated.

## Results

Data are presented in Table 1 for each day's rating and the mean disease rating. The susceptible and resistant checks provided a benchmark or standard upon which to compare these lines. Any lines that have disease ratings similar or less than the resistant check (TX76-40-2) may be useful in a wheat-breeding program and could provide new resistance. Any lines similar to the susceptible check TAM 107 will not be useful for increasing resistance to septoria glume blotch. TAM 107 was already highly diseased by day 5, indicating a very short incubation period. Some lines had low disease ratings on day 5, but by day 11, had high disease ratings, similar to TAM 107. This indicates a longer incubation period, and this resistance may be useful in a breeding program to delay the buildup of the disease.

**Table 1. Disease rating of CIMMYT durum x *T. tauschii* wheat lines for septoria glume blotch on a 0 to 9 scale where 0 = no disease and 9 = very high disease rating.**

| Entry number | Rating day 5 | Rating day 8 | Rating day 11 | Mean rating |
|---|---|---|---|---|
| TAM 107 Susc. Ck | 7 | 7 | 7 | 7 |
| Syn-1 Altar 21 | 3 | 7 | 7 | 6 |
| Syn-2 Altar 219 | 1 | 4 | 6 | 4 |
| Syn-3 Altar 219 | 4 | 6 | 6 | 5 |
| Syn-4 Altar 219 | 7 | 6 | 6 | 6 |
| Syn-5 Altar 224 | 7 | 7 | 7 | 7 |
| Tx76-40-2 Res. Ck | 0 | 1 | 1 | 1 |
| Syn-6 Altar 224 | 4 | 3 | 4 | 4 |
| Syn-7 Altar 224 | 3 | 3 | 6 | 4 |
| Syn-8 Altar 221 | 3 | 4 | 6 | 4 |
| Syn-9 Altar 223 | 1 | 2 | 3 | 2 |
| Syn-10 Altar 223 | 2 | 3 | 4 | 3 |
| Syn-11 Altar 192 | 1 | 3 | 4 | 3 |
| Syn-12 Altar 198 | 1 | 3 | 2 | 2 |
| Syn-13 Altar 198 | 0 | 0 | 1 | 1 |
| Syn-14 Altar 198 | 1 | 2 | 2 | 2 |
| Syn-15 Altar 211 | 1 | 3 | 2 | 2 |
| Syn-16 Chen 205 | 7 | 6 | 7 | 7 |
| Syn-17 Chen 205 | 8 | 8 | 8 | 8 |
| Syn-18 Chen 205 | 6 | 7 | 7 | 7 |
| Syn-19 Chen 205 | 5 | 5 | 5 | 5 |
| Syn-20 Chen 205 | 3 | 3 | 5 | 4 |
| Syn-21 Chen 205 | 2 | 3 | 5 | 3 |
| Syn-22 Chen 205 | 5 | 5 | 7 | 6 |
| Syn-23 Chen 205 | 4 | 4 | 6 | 5 |
| Syn-24 Chen 205 | 3 | 3 | 3 | 3 |
| Syn-25 Chen 205 | 5 | 5 | 5 | 5 |
| Syn-26 Chen 215 | 3 | 3 | 3 | 3 |
| Syn-27 Chen 215 | 0 | 2 | 2 | 1 |
| Syn-28 Chen 224 | 7 | 6 | 6 | 6 |
| Syn-29 Chen 224 | 7 | 7 | 4 | 6 |
| Syn-30 Chen 224 | 7 | 7 | 4 | 6 |
| Syn-31 Chen 224 | 4 | 5 | 5 | 5 |
| Syn-32 Chen 224 | 3 | 3 | 4 | 3 |
| Syn-33 Chen 224 | 5 | 5 | 5 | 5 |
| Syn-34 Chen 224 | 3 | 5 | 5 | 4 |
| Syn-35 Chen 224 | 6 | 6 | 6 | 6 |
| Syn-36 Chen 224 | 6 | 6 | 4 | 5 |
| Syn-37 Chen 224 | 7 | 7 | 7 | 7 |
| Syn-38 Chen 224 | 5 | 6 | 6 | 6 |
| Syn-39 Cndo/R143// Ent "S"/Mex 214 | 7 | 6 | 6 | 6 |
| Syn-40 " " | 5 | 6 | 5 | 5 |
| Syn 41 " " | 3 | 5 | 5 | 4 |
| Syn-43 Cndo/R143// Ent"S"/Mex 221 | 5 | 5 | 5 | 5 |
| Syn-44 Cndo/R143// Ent"S"/Mex 221 | 5 | 4 | 4 | 4 |
| Syn-45 " " | 4 | 7 | 7 | 6 |
| Syn-45 " " | 3 | 4 | 5 | 4 |
| Syn-46 " " | 6 | 7 | 6 | 6 |
| Syn-45 Laru's 309 | 3 | 5 | 5 | 4 |
| Syn-48 | 5 | 6 | 6 | 6 |
| Syn-49 " " | 8 | 7 | 8 | 8 |
| Syn-50 " " | 2 | 3 | 3 | 3 |

Highly resistant lines were Syn 9, Syn 12, Syn 13, Syn 14, Syn 15, and Syn 27. Their resistance was similar to TX76-40-2. Moderately resistant lines were Syn 10, Syn 11, Syn 32, and Syn 50. Several other lines also demonstrated a moderate degree of partial resistance. Lines Syn 1 through 15 may have a similar genetic background for the A and B genomes because they all have Altar as the durum parent. Of the resistant lines, the *T. tauschii* parents were 223 for Syn 9 and 10, 192 for Syn 11, 198 for Syn 12-14 and 211 for Syn 15. 'Chen' and 'Laru' "S" were the durum parents for Syn 27 and 50 and the *T. tauschii* lines were 215 and 309, respectively. It appears that at least some of the resistance in this germplasm originated in the *T. tauschii* lines used to make the amphiploids.

## Discussion

It appears that we have several promising new sources of resistance in a wheat background that can be crossed with our adapted winter wheat. We are presently increasing seed of these 50 lines in our greenhouse. We have crossed several of the best lines for resistance to septoria glume blotch with both hard and soft red winter wheat in our crossing program during 1998/99. Since this germplasm contains sources of resistance to septoria glume blotch which are likely not present in our soft red winter wheat, there may be an opportunity to pyramid genes and make significant improvement in resistance to this disease.

## Acknowledgment

Appreciation is expressed to CIMMYT for developing and sharing the germplasm.

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# Sources of Resistance to *Septoria passerinii* in *Hordeum vulgare* and *H. vulgare* subsp. *spontaneum*

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## Abstract

*Septoria speckled leaf blotch (SSLB), incited by* Septoria passerinii *and* Stagonospora avenae *f. sp.* triticea, *has become one of the most serious diseases of barley in the Upper Midwest region of the USA. In barley SSLB can cause significant losses in both the yield and quality. The major malting and feed barley cultivars in the Upper Midwest are very susceptible to SSLB. Resistance breeding is the most effective strategy for controlling this disease. A diverse group of barley germplasm including* Hordeum vulgare *subsp.* spontaneum *accessions, advanced midwestern breeding lines, and commercial cultivars was evaluated at the seedling stage in the greenhouse for reaction to* S. passerinii. *Of 200 accessions tested, 79 were found resistant. Most of* H. vulgare *subsp.* spontaneum *accessions (17 of 24) were resistant to* S. passerinii. *These accessions all originated from the Middle East, except one that was from Tibet. Most of the advanced midwestern breeding lines found resistant to this pathogen have Gloria"S"/Copal"S" (an ICARDA/CIMMYT barley line) in their pedigree, which is believed to be a source of resistance to* S. passerinii. *From this study, it is evident that many barley accessions possess resistance to* S. passerinii. *Additional evaluations will be made on this germplasm to* S. avenae *f. sp.* triticea *to identify accessions that possess effective resistance to both SSLB pathogens.*

Septoria speckled leaf blotch (SSLB), caused by *Septoria passerinii* and *Stagonospora avenae* f. sp. *triticea*, has become an increasingly important disease of barley (*Hordeum vulgare* L.) over the past decade in the Upper Midwest region of the USA. In surveys of North Dakota barley fields in 1998, *S. passerinii* and *S. avenae* f. sp. *triticea* were recovered from 45% and 37% of leaves exhibiting leaf spot symptoms, respectively (Krupinsky and Steffenson, this volume). Yield losses of 20% have been recorded in barley due to *S. passerinii* infection (Green and Bendelow, 1961). In recent trials conducted in North Dakota, yield losses of 23% to 38% were observed in Robust barley infected with SSLB (J. Lukach and B. Steffenson, unpublished data). In addition to reducing yield, SSLB also reduces kernel plumpness and malt extract, which are important malt quality characters (Green and Bendelow, 1961).

Host plant resistance provides the most practical and environmentally safe method of disease control. Unfortunately, all major malting and feed barley cultivars in the Upper Midwest region are susceptible to SSLB. The objectives of this study were to 1) identify sources of resistance to *S. passerinii* in commercial barley cultivars, agronomically advanced midwestern breeding lines, and *Hordeum vulgare* subsp. *spontaneum* accessions; and 2) evaluate previously reported sources of *S. passerinii* resistance to a midwestern isolate of this pathogen.

## Materials and Methods

In total, 200 barley entries including 24 *H. vulgare* subsp. *spontaneum* accessions were evaluated at the seedling stage in the greenhouse. Five seeds of each entry were planted in pots (10 x 10 cm) filled with a potting mix consisting of peat moss (75%) and perlite (25%). Slow-release (14-14-14, N-P-K, 2 g/pot) and water-soluble (15-0-15, N-P-K, 2 g/pot) fertilizers were added at the time of planting. All seedlings were grown in the greenhouse at 20±3ºC with a 14-h photoperiod. The fungal isolate (ND97-15) used in this study was obtained from naturally infected barley leaves collected from a commercial field in Bottineau county, North Dakota, in 1997. For inoculum production, the isolate was grown on yeast malt agar (YMA) (Eyal et al., 1987) in plastic Petri dishes at 21ºC with a 12-h photoperiod (cool-white fluorescent tubes). When pycnidia developed and sporulated, mass spore transfers were made by removing with a sterile needle cirrhi from pycnidia and transferring them to other YMA plates. After 4-5 days of incubation under the same conditions, pycnidia were harvested by flooding the surface of the plates

with double-distilled water and scraping the agar surface with a rubber spatula. This suspension of pycnidia was blended for 30 s in a blender to release pycnidiospores and then filtered through four layers of cheesecloth. Tween-20 (polyoxyethylene-20-sorbitan monolaurate) was added to the pycnidiospore suspension at a rate of 100 µl/l to facilitate the uniform distribution and adsorption of inoculum onto the leaf surfaces.

Barley seedlings were inoculated with the pycnidiospore suspension ($5 \times 10^5$ pycnidiospores/ml) at the two-leaf stage (10-12 days old). Inoculated seedlings were incubated at 21ºC/dark and 25ºC/light for 72 h in mist chambers, where the relative humidity was maintained near 100%. The first 40 h of incubation was in darkness, followed by a photoperiod of 5 h for next two days. Plants were allowed to dry off slowly before being transferred to the greenhouse under the same conditions previously described. The reaction of the entries to *S. passerinii* was assessed on the second leaves of seedlings 17 days after inoculation using a 0-5 rating scale where 0, 1, and 2 are indicative of resistance and 3, 4, and 5 of susceptibility. Resistant (cv. Atlas) and susceptible (cv. Betzes) checks were included in the experiment.

## Results and Discussion

*Hordeum vulgare* and *H. vulgare* subsp. *spontaneum* accessions were classified as susceptible or resistant based on their reaction to *S. passerinii* at the seedling stage. Marked differences were observed in the reaction of barley accessions to *S. passerinii* infection. In total, 79 lines were found resistant to *S. passerinii*. Of the 24 *H. vulgare* subsp. *spontaneum* accessions tested, 17 were resistant. These accessions all originated from the Middle East, except one, which was from Tibet. Similar results were obtained by Metcalfe et al. (1977) who found that all *H. vulgare* subsp. *spontaneum* accessions collected in the Middle East were resistant to *S. passerinii*.

All of the major 6-rowed malting (Foster, Stander, and Robust), and 2-rowed feed (Bowman, Conlon, and Logan) cultivars grown in the Upper Midwest region were susceptible. Of 120 advanced midwestern 6- and 2-rowed breeding lines, 41 were resistant. Most of these breeding lines have Gloria"S"/Copal"S" (an ICARDA/CIMMYT barley line) in their pedigree. This line exhibited *S. passerinii* resistance under field conditions in North Dakota and is presumed to be the source of resistance to *S. passerinii* in these breeding lines (J. Franckowiak, personal communication).

Nine accessions (AC Hamilton, Atlas, Bolron, CIho 4439, CIho 4780, CIho 10644, Feebar, Nomini, and Starling) previously reported to have resistance to *S. passerinii* were also resistant to the North Dakota isolate (ND97-15) used in this study. Other barley accessions resistant to this isolate were: Atlas 54, Baronesse, Belford, CIho 4428, CIho 4940, Flynn 1, Glacier, and Vaughn. Only a few studies have been advanced on the genetics of resistance in barley to *S. passerinii*. Peterson (1956) indicated that the variety Atlas possesses dominant resistance genes to *S. passerinii*. Buchannon (1961) found two recessive genes conferring resistance to *S. passerinii* in the cultivar Feebar, and Rasmusson and Rogers (1963) reported two different dominant resistant genes, *Sep2* and *Sep3,* in the accessions CIho 4780 and CIho 10644, respectively. Metcalfe et al. (1970) found that a single dominant gene governs resistance to *S. passerinii* in CIho 4439. It is evident that many barley accessions possess resistance to this pathogen; however, none has been exploited in midwest barley breeding programs, as all of the major malting and feed cultivars are susceptible to *S. passerinii*.

Segregating populations derived from some of the described sources of resistance and susceptible commercial cultivars are under evaluation in our laboratory to study the genetics of resistance in barley to *S. passerinii* and to identify molecular markers linked to *S. passerinii* resistance gene(s). All accessions found resistant to *S. passerinii* in this study will also be evaluated to *S. avenae* f. sp. *triticea*. This test will enable us to identify sources of resistance to both *S. passerinii* and *S. avenae* f. sp. *triticea* and to determine whether resistance to both pathogens is governed by the same or by different gene(s). The development of barley cultivars with resistance to both *S. passerinii* and *S. avenae* f. sp. *triticea* is necessary, as both pathogens are common in the Upper Midwest production area.

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# Soft Red Winter Wheat with Resistance to *Stagonospora nodorum* and Other Foliar Pathogens

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Soft red winter wheat germplasm adapted to the southeastern USA that has a high level of resistance to *Stagonospora nodorum* has been difficult to identify. Some partially resistant cultivars with long-lasting resistance have been developed, but these are replaced frequently due to loss of resistance to powdery mildew (*Blumeria graminis*), leaf rust (*Puccinia recondita*), and Hessian fly (*Mayetiola destructor*). Four lines, GA 84202, GA 85240, GA 85410AB, and GA 861460, with good agronomic traits have been selected and included among elite lines in the Georgia wheat breeding program in the past eight years. These lines have resistance to *S. nodorum* which is equal or better than that of older cultivars such as Oasis. They are also resistant to current populations of leaf rust present in the southeastern USA, most races of powdery mildew, and Hessian fly.

The lines selected were evaluated in the greenhouse and field over a six-year period against 100 or more advanced and elite lines and standard check cultivars each year. Seedling plants were inoculated in the greenhouse each year. Adult plants were inoculated in the field with *S. nodorum* and exposed to natural infection. Data were collected in field trials at Griffin and Plains, GA, under moderate to severe disease pressure from powdery mildew, leaf rust, and leaf and glume blotch. Results from the greenhouse and field were generally in good agreement.

Seeds of each line have been deposited in the USDA Small Grains Collection, Aberdeen, ID. These lines may be useful in other regions where they are adapted. For example, in addition to being agronomically adapted, 85410AB and 84202 were found to be resistant to stripe rust (*P. striiformis*) and leaf rust in a field trial at Colonia, Uruguay, in 1995. These lines are facultative wheats adapted to winter wheat culture in regions with a mild to moderate winter climate. They have been used as parents in several advanced and elite breeding lines currently being evaluated in the Georgia breeding program.

# Partial Resistance to *Stagonospora nodorum* in Wheat

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## Abstract

*Seedling plants of parents, $F_1$ crosses, and $F_2$ populations were inoculated at the two-leaf stage with spores of* Stagonospora nodorum *in a humidity chamber to determine incubation period (IP), latent period (LP) and necrosis percentage (NP). Incubation period, LP, and NP exhibited polygenic inheritance and were controlled by 2-3, 3, and 1-4 genes, respectively. Each of the components of partial resistance showed moderate to high heritabilities.*

Breeding wheat for resistance to *Stagonospora nodorum*, common name septoria glume blotch (SGB), has been difficult because no genes for immunity exist. There are numerous sources of genetic resistance; however, most have been labeled as partial resistance and in some manner delay the growth of the pathogen. In this study our objectives were to conduct a quantitative genetic analysis of three components of resistance to SGB in wheat. Second, to compare 10 wheat parents, 21 $F_1$, and 21 $F_2$ crosses for foliar disease reaction induced by inoculation, and third, to investigate the heritability estimates and gene numbers governing SGB resistance.

## Materials and Methods

Six soft winter wheat and four hard wheat genotypes that varied greatly in resistance to SGB were used in two incomplete diallel crosses. Soft wheat lines were TX92D7374, TX82-11, L890682, 18NT, 'Coker 9803', and 'Coker 9543'. Hard wheat lines were TX91V3308, TX84V344, 'TAM 300', and SWM14240. SWM14240 is a wheat line selected in the northwestern US from CIMMYT germplasm and therefore is likely to have different genes for SGB resistance. Seeds were germinated on moist filter paper for two days and then transplanted into pots containing a soil/peat moss mixture. Two plants in each pot were treated as two samples of the particular hybrid or parent. Plants were inoculated and placed in a humidity chamber for 50 h, as previously described (Nelson, 1980). Hayman's approach (1954) was used to calculate components of genetic variations.

## Results

### Estimation of components of variation

*Hard winter wheat: Incubation period.* Parents with the highest frequency of dominant alleles have the smallest Vr (variance) and Wr (parent-offspring covariance); thus their relative position along the Vr/Wr regression line reflects the frequency of dominant alleles. Sorting the parents used in these crosses in order of decreasing dominance gave the follow sequence: TX91V3308, TAM 300, TX84V344, and SWM14240. SWM14240 had least dominant effect (Figure 1). The fixable variation D and the dominance component H were calculated (Table 1). Narrow sense heritability was 66% and 61% in $F_1$ in $F_2$ generations, respectively. Gene number governing the incubation period was 2-3 (Table 1).

*Latent period*. The analysis of Wr, Vr, and Wr/Vr graphical statistics in both $F_1$ and $F_2$ provided detail information on the interrelation between the parents. The order of decreasing dominance was TX84V344, TAM 300, TX91V3308, and SWM14240 for $F_1$ (Figure 1); TAM 300, TX91V3308, TX84V344, and SWM14240 for $F_2$ . Average degree of dominance was partial dominance because of the positive intercepts. SWM14240 had the least dominant effect. TAM 300 had the

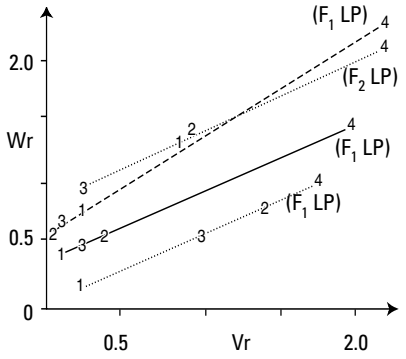


**Figure 1. Parent-offspring covariance (Wr)/ variance (Vr) for SNB incubation period and latent period of $F_1$ and $F_2$ hard winter wheat crosses.**
**1 = TX91V3308 2 = TX84V344**
**3 = TAM 300 4 = SWM14240**

largest dominant effect. Narrow sense heritability was 64% and 68% in $F_1$ and $F_2$ generations, respectively (Table 1). There were about three genes governing the resistance of latent period. The additive effect D and dominance component H1 and H2 were also calculated. Additive D was 3.74 for the $F_1$ and 3.75 for the $F_2$ generation.

*Soft winter wheat: Incubation period.* The order of decreasing dominance was TX82-11, TX92D7374, 18NT, Coker 9803, Coker 9543, and L890682 for $F_1$, and TX82-11, 18NT, Coker 9803, Coker 9543, L890682, and TX92D7374 for $F_2$ generations. Mean square of Wr-Vr was not significant. Dominance seems to account for the major proportion of the non-additive variation. The additive variation D and the dominant component H were estimated in Table 2. The dominance ratio H1/D, an estimate of the average level of dominance, was 0.7 and 0.6 for $F_1$ and $F_2$ generations, respectively. This indicates that resistance for incubation period was partially dominant. There were 2-3 genes controlling IP in the soft wheat.

*Percent necrosis.* The order of decreasing dominance was TX82-11, 18NT, Coker 9803, TX92D7374, Coker 9543, and L890682. Dominance and non-allelic interaction was important in necrosis. H/D>1 indicates that necrosis had over-dominance effects (Table 2). Heritability estimated for necrosis was similar by different methods of calculation. Narrow sense heritability for necrosis was relatively low. Only one gene controlled % necrosis in soft winter wheat. It was different from hard winter wheat and may have resulted from the genotype *X* environment interaction-over dominance effects.

## Discussion

Nelson and Marshall (1990) stated that resistance that reduced the infection rate had polygenic inheritance. Jeger (1980) indicated that resistance to SGB may involve four independent polygenes. The results of this study agree with these previous studies. IP, LP and PN had polygenic inheritance. Incubation period, LP, and NP were controlled by 2 to 3, 3, and 1 to 4 genes in the hard and soft wheat studies, respectively. Previous studies (Nelson 1980; Mullaney et al., 1982; Scott et al., 1982) also provided evidence for polygenic control of host reaction to *S. nodorum* both at the seedling plant stage and the mature plant stage. The most accepted hypothesis is that these polygenes are independently inherited. Our research may indicate some of the polygenes may have multi-effects. For example, one of the latent period resistant genes may also be a resistant gene in incubation period polygenes.

*Analysis of components of variation.* The H1/D value of $F_1$ hard wheat, and $F_1$ and $F_2$ soft wheat were less than one with the exception of $F_2$ hard winter wheat (1.1). This means that additive gene action was of greater importance in the components in this study. Therefore, incubation period could be a very useful selection criterion for SGB resistant breeding. Both $F_1$ and $F_2$ hard winter wheat crosses had larger additive value D than dominant value H1. It is likely that the non-additive genetic effects were due predominantly to contribution from additive x additive epistatic gene action rather than dominance.

Additive gene action and additive x additive epistatic gene action are most easily utilized and exploited in homozygous genotypes. Latent period would also be a good selection criterion for SGB resistant breeding. H1/D values for hard and soft wheat crosses were greater than 1. Over dominance may have been present for necrosis. Nelson (1980),

**Table 1. Genetic variation of components of partial resistance for hard winter wheat crosses.**

| | Incubation period | | Latent period | | Necrosis | |
|---|---|---|---|---|---|---|
| **Component** | **F1** | **F2** | **F1** | **F2** | **F1** | **F2** |
| D | 2.51 | 2.38 | 3.74 | 3.75 | 365.76 | 360.37 |
| H1 | 2.03 | 2.51 | 2.16 | 2.34 | 459.48 | 441.15 |
| H2 | 0.88 | 1.34 | 1.33 | 0.52 | 265.07 | 59.52 |
| F | 2.40 | 1.87 | 3.10 | 3.24 | 475.17 | 350.18 |
| E | 0.32 | 0.51 | 0.76 | 0.70 | 37.12 | 42.52 |
| $H_{narrow}$ | 66% | 61% | 64% | 68% | 30% | 77% |
| Number of genes | 3 | 2 | 3 | 3 | 4 | 3 |

**Table 2. Genetic variation of components of partial resistance for soft winter wheat crosses.**

| Component | Incubation | period | Necrosis |
|---|---|---|---|
| | F1 | F2 | F1 |
| D | 1.19 | 1.60 | 3.66 |
| $H_1$ | 0.83 | 0.92 | 148.27 |
| $H_2$ | 0.50 | 1.44 | 123.54 |
| F | 0.55 | 1.24 | -4.00 |
| E | 0.91 | 0.72 | 26.99 |
| $H_{narrow}$ | 32% | 39% | 22% |
| Number of genes | 2 | 3 | 1 |

Wilkinson et al. (1990), and Bostwick et al. (1993) had similar results. It is reasonable for the components of resistance to show dominant gene action in the $F_1$ generation because of gene interaction. From the breeder's viewpoint, necrosis should not be an early generation selection criterion for SGB resistant breeding.

*Heritability*. Estimates of heritability in all crosses studied were moderate to high. Relatively high heritability for spike and flag leaf reaction to SGB have been estimated in previous studies (Rosielle and Brown, 1980; Fried and Meister, 1987; Bostwick et al. 1993). Therefore, early generation selection for SGB should be effective.

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# Comparison of Methods of Screening for *Stagonospora nodorum* Resistance in Winter Wheat

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## Abstract

*Isolates of* Stagonospora nodorum *that varied in levels of aggressiveness were used in both controlled environment and field tests to determine whether isolate aggressiveness enhanced selection of resistant wheat genotypes. Two segregating wheat populations that differed in mean levels of resistance to* S. nodorum *were developed. Components of resistance measured in controlled environments indicated significant differences among isolate treatments. Measurement of disease severity in field studies also indicated significant differences among the isolate treatments. For the breeding population (random selections of genotypes in $F_3$, $F_4$, $F_5$, and $F_6$ generations), incubation period measured in both juvenile and adult plant tests were most highly correlated with resistance measured in the field. For the biparental population (progeny of a resistant by susceptible cross), incubation period and percent leaf area diseased on the flag leaf for adult plant tests were most highly correlated with resistance measured in the field. Rankings of genotypes based on their resistance in both field and controlled environment tests indicated that five of the ten most resistant genotypes measured under controlled conditions were also identified as resistant in field tests. Similarly, four of the ten most susceptible genotypes measured under controlled conditions were also identified as susceptible in field tests. Combinations of adult and juvenile screening tests may allow for the identification of up to eight of ten genotypes resistant in controlled environments that are also resistant in field tests.*

Glume blotch, caused by the ascomycete fungus *Stagonospora nodorum* (teleomorph *Leptosphaeria nodorum*) is an economically important disease of wheat. Resistance to *S. nodorum* is controlled by multiple genes (Jeger et al., 1983; Mullaney et al., 1982; Nelson and Gates, 1982; Wilkinson et al., 1990) and expression of resistance is strongly affected by the environment.

Previous studies attempting to correlate components of host resistance measured in controlled environments with host resistance under field conditions have reported variable results (Wilkinson et al., 1990; Rufty et al., 1981). A better understanding of the relationship between isolate aggressiveness and host response could provide more effective selection methods for resistance to *S. nodorum*. The objective of this study was 1) to use isolates with different levels of aggressiveness to measure differences in components of resistance among genotypes in two segregating wheat populations, under both field and controlled environments and 2) to determine which components are the most effective in selection of resistant genotypes.

## Materials and Methods

Two wheat populations were used in all studies. Population 1 (biparental) consisted of the F3:5 progeny of NKC 8427 (resistant) by Caldwell (susceptible) cross. Population 2 (breeding) was composed of random F3, F4, F5, and F6 lines from a southern US soft red winter wheat nursery in North Carolina. A total of 50 lines from each population were used in all studies. A collection of 60 NC field isolates was screened in a seedling assay and the most aggressive isolate and least aggressive isolate in the collection were selected for further study. The same isolate treatments were used in all subsequent studies and consisted of the most and least aggressive isolates (A and D, respectively) applied singly, in combination (AD), and a non-inoculated control.

### Field tests

Hill-plots were planted on a 0.3-m square grid in three replications at Laurel Springs, NC, in 1997 and at both Laurel Springs and Kinston, NC, in 1998. Each population of 50 genotypes was inoculated with a spore suspension of a different isolate treatment at either Feekes growth stage (GS) 10.1 in 1997 and Feekes GS 9 in 1998. Percent leaf area diseased (% LAD) was recorded at four bi-weekly intervals after inoculation using the Saari-Prescott scale.

### Greenhouse tests

Seeds of each genotype were over-planted and thinned to two plants per pot and allowed to grow in alternating 12-hour light and dark periods at 16 to 26ºC. Experimental design was a randomized complete block with four replications in time.

At GS 10 the penultimate leaf of one plant/pot was marked and a 2 ml droplet of a single isolate spore suspension standardized to 1 x $10^6$ spores $ml^{-1}$ applied to the leaf. Presence/absence of a lesion at 5 and 10 days was recorded. Following the last rating on the penultimate leaf of one plant, the flag leaf of the other plant/pot was marked. Spore suspensions of each isolate treatment were applied to 50 genotypes per population using hand atomizers until run-off occurred. Glass slides placed in the plant canopy during inoculation indicated that 50-75 spores were deposited per $mm^{-2}$. Incubation period and % LAD on each plant were recorded.

### Growth chamber

Seeds of each genotype were planted and grown for 21 days in 12 h light and darkness periods in day/night temperatures of 22/18ºC. At the 2-leaf stage, spore suspensions of the same four isolate treatments as described previously were applied to 50 genotypes per population using hand atomizers until run-off occurred, with approximately 65 spores deposited per $mm^{-2}$ of leaf tissue. Incubation period and % LAD on each plant were recorded. Experimental design was a randomized complete block with four replications.

### Detached leaf test–adult and juvenile

Seeds of each genotype were planted and grown in the greenhouse for three weeks (juvenile) and ten weeks (adult), respectively. For the juvenile test, fully expanded second leaves were cut from each genotype in both populations. For the adult test, plants were grown to Feeke's GS 10.1, and the flag leaf was excised from each plant. Each single leaf was cut into four equal sized pieces and placed on 150 ppm benzimidazole agar in a box divided in 4-cm grid squares. Each grid square represented each of the isolate treatments on a single genotype. A 2 ml droplet of a single isolate spore suspension was placed in the center of each leaf piece. Incubation period, lesion expansion rate, and final lesion size was recorded. Both the adult and juvenile tests were replicated four times.

## Results and Discussion

The level of resistance in the breeding population was generally higher than the biparental population in all tests. The AUDPC values indicated significant differences among isolate treatments in the field at Laurel Springs in 1997 and at both locations in 1998 (Table 1). Genotype by environment interaction was significant.

Isolate treatments in greenhouse evaluations were significantly different for most of the components of resistance measured (Tables 2 and 3). Results of inoculations with the most and least aggressive isolates in combination (AD) were similar to inoculations with the most aggressive isolate alone and both of these treatments resulted in higher numbers of lesions, more rapid rates of lesion expansion and higher % LAD than inoculation the least aggressive isolate. Shorter incubation periods were not consistently associated with the most aggressive isolate or the most and least aggressive isolate together. Components of resistance measured in controlled environments were highly correlated with each other (Table 4).

Few components of resistance measured in controlled environments correlated with field measured disease severity. A comparison of the controlled environment studies with field data from Kinston in 1998 indicated that adult plant incubation period and % LAD on the flag leaf for adult plant spray inoculation were highly correlated (P>0.01) with field

**Table 1. Area under the disease progress curve values for disease severities measured on hill plots inoculated with isolates of *Stagonospora nodorum*.**

| | Laurel Springs 1997 | Laurel Springs 1998 | | Kinston 1998 | |
|---|---|---|---|---|---|
| Isolate treatments | Breeding | Biparental | Breeding | Biparental | Breeding |
| A (most)[a] | 2724b | 1669b | 2196a | 1678a | 1587a |
| D (least) | 2783a | 1667b | 1972c | 1557c | 1561c |
| AD (both) | 2770a | 1806a | 1733d | 1608b | 1628a |
| NI (control) | 2694c | 1829a | 2087b | 1367d | 1452d |

[a] 'Most' and 'least' aggressive refer to isolate reaction measured in the isolate screening assay done on seedlings in controlled conditions.

disease severity in the biparental population. Adult and juvenile plant incubation period and lesion expansion rate measured in the detached leaf test were highly correlated with field disease severity measured at Kinston in 1998 in the breeding population.

Similar results were obtained for disease measured at Laurel Springs in 1997. Components of resistance were not highly correlated with field disease measured at Laurel Springs in 1998.

## Resistant genotypes

A comparison of genotype rankings measured in the field with those measured in controlled environments indicate that for the biparental population at Kinston in 1998, five of the ten most resistant genotypes in the field were also identified by the measurements of the incubation period and % LAD on the flag leaf in the adult plant tests. Similarly, rankings based on % LAD measured in the adult plant spray test ranked five of the same ten most resistant genotypes identified by the field disease severity values at Laurel Springs in 1998. The numbers were slightly lower for the breeding population. At Kinston in 1998, four of the ten most resistant genotypes in the field were also identified as resistant by measurements of % LAD for adult plant spray inoculation. Combining adult plant greenhouse tests and juvenile detached leaf tests could increase this number to seven or eight of the top ten resistant genotypes identified in controlled

**Table 2. Components of resistance to *Stagonospora nodorum* measured on $F_{3:5}$ lines of NKC 8433 x Caldwell cross in controlled environments.**

| | Dlt[a] Juvenile | | Dlt Adult | | Phytotron(Juvenile) | | Greenhouse | |
|---|---|---|---|---|---|---|---|---|
| Isolate treatments[b] | Incubation (days) | # of lesions | Incubation (days) | Lesion expansion | Incubation ( days) | # of lesions | Droplet incubation | Spray %LAD |
| A (most) | 7.9 a | 2.4 b | 12.7 c | 0.14 b | 12.9 a | 2.4 bc | 3.8 a | 5.5 ab |
| D (least) | 8.1 b | 2.3 b | 10.4 b | 0.13 a | 13.1 a | 1.7 a | 4.3 a | 3.9 a |
| AD (both) | 7.6 a | 2.7 c | 9.4 a | 0.20 c | 12.9 a | 1.8 b | 5.4 b | 4.8 a |
| NI(control) | 20.0 c | 0.0 a | - | - | 17.1 b | 1.2 a | 20.0 c | - |

[a] Detached leaf test.
[b] 'Most' and 'least' aggressive refer to isolate reaction measured in the isolate screening assay done on seedlings in controlled conditions.

**Table 3. Components of resistance to *Stagonospora nodorum* measured on random $F_3$, $F_4$, $F_5$ and $F_6$ lines from a southern US winter wheat nursery in controlled environments.**

| | Dlt[a] Juvenile | | Dlt Adult | | Phytotron(Juvenile) | | Greenhouse | |
|---|---|---|---|---|---|---|---|---|
| Isolate treatments[b] | Incubation (days) | # of lesions | Incubation (days) | Lesion expansion | Incubation ( days) | # of lesions | Droplet incubation | Spray %LAD |
| A (most) | 8.4 a | 2.5 b | 12.7 b | 0.11 a | 13.5 a | 2.1 c | 5.1 b | 3.7 a |
| D (least) | 9.0 b | 2.4 b | 10.8 a | 0.12 b | 12.8 a | 1.6 b | 3.4 a | 2.8 a |
| AD (both) | 8.3 a | 2.4 b | 11.0 a | 0.19 c | 13.7 a | 2.2 c | 4.9 b | 4.1 ab |
| NI (control) | 20.0 c | 0.0 a | - | - | 17.5 b | 0.9 a | 20.0 c | - |

[a] Detached leaf test.
[b] ' Most' and 'least' aggressive refer to isolate reaction measured in the isolate screening assay done on seedlings in controlled conditions.

**Table 4. Correlations among resistance components measured in controlled environments and in field tests.**

| | Treatment A (most)$_a$ | | Treatment AD(most/least) | | Treatment D(least) | |
|---|---|---|---|---|---|---|
| Resistance component | Biparental | Breeding | Biparental | Breeding | Biparental | Breeding |
| Adult dlt – incubation | 0.23 * | 0.30 * | NS | NS | NS | 0.25 * |
| Adult dlt- lesion expansion | NS | -0.34 ** | NS | NS | 0.2 0* | 0.20 * |
| Juvenile dlt – incubation | NS | -0.40 ** | NS | NS | NS | NS |
| Juvenile dlt – lesion size | NS | NS | 0.20 * | 0.20* | 0.27 * | NS |
| Adult spray test - %LAD | NS | NS | 0.23 * | NS | 0.33** | 0.14 |

[a] 'Most' and 'least' aggressive refer to isolate reaction measured in the isolate screening assay done on seedlings in controlled conditions.

environment tests also being identified as resistant under field conditions.

### Susceptible genotypes

Of the ten most susceptible genotypes in each population, four were also identified as susceptible by measurement of juvenile plant incubation period. Combining results from both the adult plant and juvenile plant detached leaf tests resulted in as high as seven of the ten most resistant genotypes in the field also being identified as resistant under controlled conditions.

In general, evaluation of components of resistance may provide a more efficient selection method by allowing for the removal of extremely susceptible types and identification of resistant types before large-scale field selection. Based on our results and practicability to the plant breeder, a combination of juvenile detached leaf tests and adult plant inoculations (droplet or spray) in the greenhouse could aid in the selection of parental materials with resistance to *S. nodorum*. These methods could also be used for screening the progeny of segregating populations. Highly aggressive isolates or combinations of isolates with different levels of aggressiveness help to maximize differences among genotypes in both field and controlled environments

### Epidemiology

In addition to measuring components of resistance to *S. nodorum* in the field and in controlled conditions, molecular analyses of the development of the epidemics in the field were also studied using these same isolate treatments and populations. The information provided by the molecular analyses may explain differences among treatments in the field and help elucidate the role of the natural inoculum in epidemic development.

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# Response of Winter Wheat Genotypes to Artificial Inoculation with Several *Septoria tritici* Populations

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## Abstract

*A study was conducted to identify genotypic differences in the response of winter wheat to artificial inoculation with several* Septoria tritici *populations. Twenty-two genotypes were grown in two locations and artificially inoculated with four* Septoria tritici *populations. Significant effects of host genotype, pathogen population, and host-pathogen interaction were found. Most released cultivars were susceptible. Several entries, including some* Aegilops *spp. derivatives, were resistant to all* Septoria *populations in both locations.* Septoria tritici *populations differed both in aggressiveness and virulence. A strong host-pathogen interaction was found in several cultivars with medium average resistance.*

*Septoria tritici* is a major foliar disease of wheat in Romania. Yield losses caused by septoria leaf blotch can reach 25% in years that favor the disease. Most currently grown wheat cultivars are more or less susceptible to *Septoria tritici*. Therefore, resistance to this pathogen is a high-priority breeding goal.

## Material and Methods

Twenty-two winter wheat genotypes were grown on 2-m+ plots using three replications in each of two locations (Fundulea in the south and Brasov in the central part, near the mountains). Artificial inoculation was conducted by spraying the plants at heading with a spore suspension diluted to 10 spores/ml, from four *Septoria tritici* populations, collected from several regions of Romania. After spraying, the plants were covered with plastic sacks to maintain the humidity necessary for infection. The intensity of the attack was read 30 days after inoculation, using the 0 to 9 scale developed by Saari and Prescott at CIMMYT (1995). For statistical analysis, the arc sin transformation was used. Significance of differences among genotypes was determined using the Duncan test.

## Results and Discussion

The analysis of variance (ANOVA) shows there are significant effects of wheat genotypes, pathogen populations, and host x pathogen interaction (Table 1). Genotype classifications for each pathogen population in the two locations are presented in Tables 2 and 3. Most released cultivars were susceptible to all populations and in both locations. On the other hand, several entries showed low intensities of attack with all populations and in both locations (e.g. F91552G8-01, Admis, ZE16547, KS93U134, G1662-51, G557-5, G557-6). The last four are derivatives from crosses with *Aegilops* spp. Finally, some entries (such as Turda 81) showed a very variable response to different *Septoria* populations.

The differences between host genotypes are more pronounced than between pathogen populations. The pathogen populations produced different attack intensities in the two locations. In Fundulea, all *Septoria* populations had a higher pathogenicity than in Brasov. In Brasov, the pathogenicity of all populations was approximately equal, but in Fundulea Population 4 was less pathogenic, compared with the other populations. The host-pathogen interactions in the two locations are exemplified in Figures 1-4. A strong host-pathogen interaction was noticed for several cultivars in both locations.

## Reference

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**Table 1. Analysis of variance (MS) for the intensity of *Septoria tritici* attack on the leaf in Fundulea and Brasov.**

| Source of variation | Fundulea | Brasov |
|---|---|---|
| Genotypes (G) | 1479.276*** | 696.308*** |
| Error (a) | 81.600 | 5.582 |
| Populations (P) | 3041.026*** | 688.315*** |
| G x P | 339.740*** | 69.820*** |
| Error (b) | 30.020 | 3.300 |

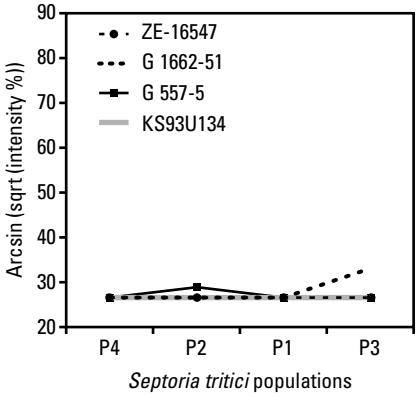


**Figure 1. Interaction between genotypes and population (intensity of the attack on the leaf), Fundulea - sources of resistance.**

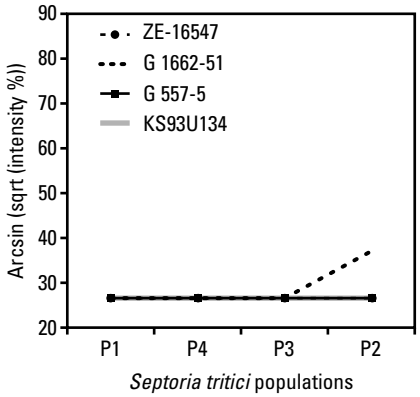


**Figure 2. Interaction between genotypes and population (intensity of the attack on the leaf), Brasov - sources of resistance.**

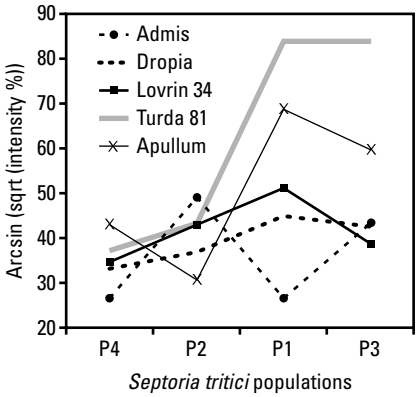


**Figure 3. Interaction between genotypes and population (intensity of the attack on the leaf), Fundulea - cultivars.**

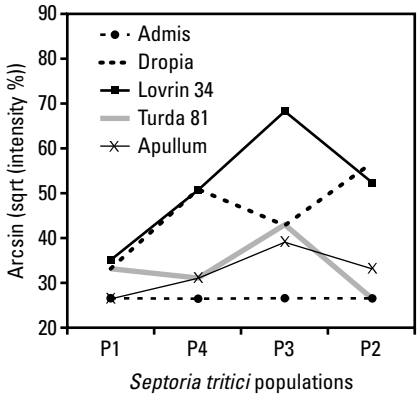


**Figure 4. Interaction between genotypes and population (intensity of the attack on the leaf), Brasov - cultivars.**

**Table 2. The reaction of genotypes to *Septoria tritici* infection–intensity of attack on the leaf in Fundulea.**

| Genotypes | Population 1 | Population 2 | Population 3 | Population 4 | Mean |
|---|---|---|---|---|---|
| ZE16547 | 26.6 a † | 26.6 a | 26.6 a | 26.6 a | 26.6 |
| G557-5 | 26.6 a | 26.6 a | 26.6 a | 26.6 a | 26.6 |
| G557-6 | 26.6 a | 26.6 a | 26.6 a | 26.6 a | 26.6 |
| KS93U134 | 26.6 a | 26.6 a | 26.6 a | 26.6 a | 26.6 |
| 91552G8-01 | 28.8 a | 28.8 a | 30.8 a | 26.6 a | 28.7 |
| G1662-51 | 26.6 a | 26.6 a | 37.2 b | 26.6 a | 29.2 |
| 649T2-1 | 28.8 a | 33.0 ab | 39.2 bc | 26.6 a | 31.9 |
| Lovrin 34 | 51.2 bc | 43.1 cde | 38.9 bc | 34.9 ab | 32.3 |
| Rapid | 45.0 b | 28.8 a | 31.0 ab | 26.6 a | 32.8 |
| Admis | 26.6 a | 49.2 ef | 43.3 cd | 26.6 a | 36.4 |
| 577U1-106 | 46.9 b | 48.9 ef | 50.8 d | 26.6 a | 36.6 |
| Dropia | 44.9 b | 36.9 bc | 42.7 c | 33.0 a | 39.4 |
| 93396G1-1 | 26.6 a | 52.8 ef | 59.0 ef | 26.6 a | 41.2 |
| 92033G2-1 | 55.0 bcd | 28.8 a | 51.2 de | 33.2 ab | 42.0 |
| 91375GA-13 | 57.0 cd | 31.0 b | 63.9 fg | 26.6 a | 44.6 |
| 93009G11-12 | 59.0 de | 35.2 abc | 61.2 ef | 26.6 a | 45.5 |
| Fundulea – 4 | 61.2 def | 46.9 de | 55.0 e | 35.2 ab | 49.6 |
| 93122G6-2 | 26.6 a | 61.2 gh | 63.5 f | 48.9 d | 50.0 |
| Apullum | 68.9 f | 30.8 ab | 59.7 ef | 43.0 bc | 50.6 |
| 508U3-201 | 26.6 a | 56.6 fg | 72.8 g | 46.9 cd | 50.7 |
| Turda 81 | 83.9 g | 43.0 cd | 83.9 h | 37.2 b | 62.0 |
| Cadril 249 | 66.2 de | 63.9 h | 72.3 g | 48.9 d | 62.8 |

† Means in columns followed by the same letter are not significantly different by Duncan's test at the 0.05% level.

**Table 3. The reaction of genotypes to *Septoria tritici* infection–intensity of attack on the leaf in Brasov.**

| Genotypes | Population 1 | Population 2 | Population 3 | Population 4 | Mean |
|---|---|---|---|---|---|
| ZE16547 | 26.6 a † | 26.6 a | 26.6 a | 26.6 a | 26.6 |
| G557-6 | 26.6 a | 26.6 a | 26.6 a | 26.6 a | 26.6 |
| KS93U134 | 26.6 a | 26.6 a | 26.6 a | 26.6 a | 26.6 |
| 91552G8-01 | 26.6 a | 26.6 a | 26.6 a | 26.6 a | 26.6 |
| Admis | 26.6 a | 26.6 a | 26.6 a | 26.6 a | 26.6 |
| 649T2-1 | 26.6 a | 28.8 a | 26.6 a | 26.6 a | 27.1 |
| G557-5 | 26.6 a | 26.6 a | 26.6 a | 28.8 a | 27.1 |
| Cadril 249 | 26.6 a | 31.0 b | 26.6 a | 26.6 a | 27.7 |
| G1662-51 | 26.6 a | 33.2 b | 26.6 a | 26.6 a | 28.2 |
| Rapid | 26.6 a | 28.8 a | 37.2 c | 28.8 a | 30.3 |
| 93396G1-1 | 26.6 a | 37.2 c | 33.2 b | 26.6 a | 30.9 |
| 93009G11-12 | 26.6 a | 39.2 c | 33.2 b | 26.6 a | 31.4 |
| 508U3-201 | 26.6 a | 37.2 c | 35.2 b | 26.6 a | 31.4 |
| Apullum | 26.6 a | 33.2 b | 37.2 c | 31.0 b | 32.0 |
| Turda 81 | 33.2 b | 26.6 a | 43.1 d | 31.0 b | 33.5 |
| 91375GA-13 | 31.0 b | 45.0 d | 35.2 b | 26.6 a | 34.5 |
| 93122G6-2 | 26.6 a | 45.0 d | 37.2 c | 31.0 b | 35.0 |
| 577U1-106 | 33.2 b | 39.2 c | 37.2 c | 33.2 c | 35.7 |
| 92033G2-1 | 37.2 d | 48.9 e | 45.0 d | 37.2 d | 42.1 |
| Dropia | 33.2 b | 56.8 g | 43.1 d | 50.8 e | 46.0 |
| Fundulea – 4 | 50.8 e | 52.8 f | 52.8 e | 39.2 d | 48.90 |
| Lovrin – 34 | 35.2 c | 52.8 f | 68.9 f | 50.8 e | 51.92 |

† Means in columns followed by the same letter are not significantly different by Duncan's test at the 0.05% level.

# Comparison of Greenhouse and Field Levels of Resistance to *Stagonospora nodorum*

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## Abstract

*A biparental population of 150 recombinant wheat inbred (RI) lines segregating for reaction to* Stagonospora nodorum *was developed and tested in the greenhouse and field for levels of resistance. The $F_{3:4}$ lines were tested in the greenhouse for incubation period and percent leaf infection 5 and 10 days after initial symptoms of disease. Plants were tested at both the juvenile and adult stages. Adult plants were also tested for percent disease on the spike. The $F_{3:5}$ lines were tested in the field over four locations for level of adult leaf and head resistance. Heritability of leaf resistance to* S. nodorum *was calculated to be 0.56 on an entry means basis and 0.17 on a per plot basis. Heritability of resistance of the spike was calculated to be 0.57 on an entry means basis and 0.20 on a per plot basis. Both heritability estimates are based on field data. Correlations between field data and greenhouse data were generally weak, notably between field data and greenhouse performance of juvenile plants. However, resistance of the spike in the field and the greenhouse were highly correlated (r = 0.74).*

Resistance to *Stagonospora nodorum* is an economical way to control glume blotch as well as a component of integrated management of the disease. Resistance to *S. nodorum* is complex and polygenic, particularly in adult plants. There is some evidence that resistance in the spike and at the juvenile growth stage are under different genetic control than adult leaf resistance (Eyal et al., 1987). By understanding the relationships between juvenile, adult leaf, and resistance of the spike as well as field and greenhouse resistance, one could improve progress in selecting the most resistant lines. Also, an understanding of the various components of resistance and the epidemiology of glume blotch in a given area would allow deploying the most useful component of resistance in that environment.

## Materials and Methods

A cross of the soft red winter wheat cultivars Caldwell= (CItr 17897) ( mod. susceptible) x Coker 8427 (9766) (PI601429) (mod. resistant) was made, and progeny were advanced in bulk to the $F_3$ generation. Individual spikes were taken from the $F_3$ generation and used to form 150 $F_{3:4}$ derived lines.

The $F_{3:4}$ lines were tested in the greenhouse for incubation period and percent leaf area diseased at both the juvenile and adult plant growth stages. Percent diseased tissue on the spike was measured at the adult growth stage.

A test consisted of a single plant from each $F_{3:4}$ line using a completely randomized design. A total of four tests were replicated in time during the winter of 1997-98.

Plants were inoculated with a mixture of three *S. nodorum* isolates at a concentration of $1.0 \times 10^6$ conidia/ml plus 0.01% Tween 80. Inoculations on juvenile plants were performed at the three-leaf stage, while adult inoculations occurred at anthesis. After inoculation, plants were kept in high humidity for 72 hours. Plants were rated each day for incubation period, and then at five and ten days after the incubation period was determined for each individual line. Leaf and spike ratings in the greenhouse were recorded as a percentage of the total leaf or head tissue displaying symptoms. Spike ratings underwent square root transformation prior to statistical analysis.

Field testing was performed during the 1998-99 growing season at three locations: Johnston Co., Lenoir Co., and Ashe Co., North Carolina, USA. $F_{3:5}$ lines were planted as hillplots, consisting of 25 seeds planted together as a group. Hillplots were planted on a grid, with 0.3 m separating each plot. Three repetitions of each line were planted in a randomized complete

block design within each location. Dried wheat straw cut the previous season from a field heavily infected with *S. nodorum* was distributed at each location to induce disease. Overhead irrigation was used at the Lenoir and Ashe County locations, but was unavailable at the Johnston County location. Heritability estimates were calculated as described by Fehr (1991).

Each hillplot was rated for level of disease on the leaf and on the spike. Leaf disease ratings were done using the double digit scale (Eyal et al., 1987), indicating both the highest point of disease in a hillplot and the percentage of disease at that point. Disease on the spike was recorded as the percentage of diseased tissue and underwent square root transformation prior to statistical analysis. Statistical analysis was performed using SAS release 6.12. Correlation analysis used the Spearman ranked test.

## Results and Discussion

A range of values for each measured trait was produced among lines in both the greenhouse and in the field (Table 1). The analysis of variance demonstrated significant differences among lines ($p < 0.001$) for each trait in both the greenhouse and the field (data not shown). Using field data, heritability of adult leaf resistance was calculated and found to have a value of 0.56 on an entry means basis and a value of 0.17 on a per hillplot basis. Heritability of resistance of the spike was 0.57 on an entry means basis and 0.20 on a per plot basis. These data indicate the genetic component of resistance in this population has a large effect on disease expression. Ranking of mean resistance scores among lines was consistent over three locations.

Correlation analysis demonstrated a negative correlation between adult incubation period and leaf resistance in the greenhouse and field, as well as a small positive correlation between adult greenhouse leaf resistance and field leaf resistance (Table 2). The largest and most significant correlation was between greenhouse and field resistance of the spike (0.74). This indicates screening for resistance of the spike in the greenhouse may provide an alternative to field testing. In the greenhouse, juvenile plant reactions demonstrated little correlation with adult plant resistance traits either in the greenhouse or in the field, indicating a possible separate genetic mechanism for juvenile resistance. Resistance of the spike and leaf resistance in the field produced a correlation value of 0.55, indicating some relationship between the traits, but a large degree of variation between the traits remained unexplained.

We are currently using AFLP and RAPD markers in an attempt to match polymorphisms at the molecular level with traits measured in the field and greenhouse to gain some insight into the genetics of resistance to this disease.

## References

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**Table 1. Means, standard deviations, and ranges of *Stagonospora nodorum* traits measured in the greenhouse and field.**

| Trait | Growth Stage and Environment | Mean | Std. Dev. | Min. | Max. |
|---|---|---|---|---|---|
| Incubation period | Juvenile, greenhouse | 5.8 days | 0.94 days | 4.0 days | 10.0 days |
| Leaf area disease after 5 days | Juvenile, greenhouse | 5.5% | 4.7% | 0.5% | 30.0% |
| Leaf area disease after 10 days | Juvenile, greenhouse | 10.2% | 7.8% | 0.4% | 42.5% |
| Incubation period | Adult, greenhouse | 5.4 days | 0.86 days | 3.5 days | 9.5 days |
| Leaf area disease after 5 days | Adult, greenhouse | 8.4% | 7.2% | 1.0% | 48.8% |
| Leaf area disease after 10 days | Adult, greenhouse | 27.8% | 19.0% | 3.0% | 90.0% |
| Head area disease | Adult, greenhouse | 3.2% | 0.8% | 0% | 23.0% |
| Leaf rating | Adult, field | 72.1% | 7.7% | 48.0% | 88.8% |
| Head rating | Adult, field | 25.0% | 1.0% | 0% | 100% |

**Table 2. Spearman correlation coefficients between *Stagonospora nodorum* traits measured in the greenhouse and field.**

| | Adult[a] incub. | Adult[b] day 5 | Adult[c] day 10 | Adult[d] head | Juv.[e] incub. | Juv.[f] day 5 | Juv.[g] day 10 | Field[h] leaf | Field[i] head |
|---|---|---|---|---|---|---|---|---|---|
| Adult incub. | 1.0 | -0.40*** | -0.36*** | -0.11 | 0.04 | -0.12 | -0.16* | -0.26** | -0.13 |
| Adult day5 | -0.40*** | 1.0 | 0.75*** | 0.12 | 0.00 | 0.16* | 0.20** | 0.18* | 0.21** |
| Adult day10 | -0.36*** | 0.75*** | 1.0 | 0.13 | -0.03 | 0.14 | 0.18* | 0.23** | 0.19* |
| Adult head | -0.11 | 0.12 | 0.13 | 1.0 | 0.11 | 0.03 | 0.06 | 0.48*** | 0.74*** |
| Juv. incub. | 0.04 | 0.00 | -0.03 | 0.11 | 1.0 | -0.05 | -0.15 | -0.08 | 0.00 |
| Juv. day5 | -0.12 | 0.16* | 0.14 | 0.03 | -0.05 | 1.0 | 0.69*** | 0.10 | 0.08 |
| Juv. day10 | -0.16* | 0.20* | 0.18* | 0.06 | -0.15 | 0.69*** | 1.0 | 0.09 | 0.08 |
| Field leaf | -0.26** | 0.18* | 0.23** | 0.48*** | -0.08 | 0.10 | 0.09 | 1.0 | 0.55*** |
| Field head | -0.13 | 0.21** | 0.19* | 0.74*** | 0.00 | 0.08 | 0.08 | 0.55*** | 1.0 |

*, **, *** $p < 0.05, 0.01, 0.0001$, respectively.

a. Incubation period of adult plants in greenhouse test.

b. Percent leaf area disease of adult plants five days after first sign of disease in greenhouse test.

c. Percent leaf area disease of adult plants ten days after first sign of disease in greenhouse test.

d. Percent area head disease of adult plants in greenhouse test.

e. Incubation period of juvenile plants in greenhouse test.

f. Percent leaf area disease of juvenile plants five days after first sign of disease in greenhouse test.

g. Percent leaf area disease of juvenile plants five days after first sign of disease in greenhouse test.

h. Leaf disease rating in field tests.

i. Head disease rating in field tests.

## *Session 6D: Chemical Control*

# Adjusting Thresholds for Septoria Control in Winter Wheat Using Strobilurins

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## Abstract

*In semi-field trials in spring wheat, azoxystrobin has shown a longer residual and preventive effect on* Stagonospora nodorum *than the triazole propiconazole. Three weeks after application, more than 90% control was still obtained with one quarter of the recommended rate of azoxystrobin. Propiconazole gave in comparison only 50% control. In another semi-field trial using* Septoria tritici, *azoxystrobin and propiconazole had a similar curative effect, both being significantly better than chlorothalonil. Different dosages (12-100% of normal rate of azoxystrobin) were tested in field trials. When taking into account the different timing, the most profitable dose has varied between 50 and 100% of normal rate, depending on growth stage and disease pressure. If optimal timing is used, 25-50% of normal rate has generally been sufficient. In 1998 in field trials azoxystrobin and the co-formulation propiconazole + fenpropimorph or tebuconazole provided similar control of* S. tritici *when applied after 4 or 8 days of precipitation, respectively. The test model for azoxystrobin recommended between 36 and 45% of the normal rates. Azoxystrobin gave an increase in yield of 100-600 kg ha$^{-1}$ above the co-formulation or tebuconazole. So far azoxystrobin has shown profitable yields in all trials carried out in Denmark since 1994. However, it is still not known how many days of precipitation are required to make spraying with azoxystrobin profitable.*

Based on historical trial data, it has been shown that in Denmark an economically important attack of *Septoria tritici* or *Stagonospora nodorum* requires more than 7-8 days of precipitation (>1 mm rain) between GSZ 32 and 30 days thereafter (Hansen et al., 1994). This model has been incorporated into the decision support system PC-Plant Protection and is widely used by Danish farmers as a guide for septoria control (Secher et al., 1995). Under validation the model has given correct answers in 75% of the events studied (Jørgensen et al., 1999). The experience using triazoles for control of septoria diseases in Denmark has been that their application is justified in approximately half of the years.

Adjusting the model for strobilurins requires knowledge of their preventive and curative effect, the residual effect, the dose response at different timing, and the yield responses measured in relation to fungicides. Several of these aspects are under investigation using azoxystrobin as representative of the strobilurins.

## Materials and Methods

In outdoor semi-field trials, spring wheat (cv. Dragon) was grown in 8-liter pots using 20 seeds per pot and 4 replicates. The plants were exposed to normal weather conditions (May, June, and July). Two types of trials were carried out using either artificial inoculation of *S. nodorum* or *S. tritici* at growth stage 39-45. The plants were inoculated by spraying each pot with 25 ml of a suspension containing 2.5 x $10^6$ spores ml$^{-1}$of either *S. nodorum* or *S. tritici*. After inoculation the pots were covered with polyethylene for two days to provide high humidity conditions for infection. Plants were treated with fungicide either after inoculation to investigate the curative effect, or before inoculation to examine the preventive and residual effect. Azoxystrobin, propiconazole, and chlorothalonil were tested at 100-25% of the normal rate using a laboratory pot sprayer with flat-fan nozzles (Hardi 4110-14) and a water volume of 167 l ha$^{-1}$. Percent total coverage of the plants by disease as well as percent severity on individual leaves were assessed.

Dose response field trials were carried out by the Danish Institute of Agricultural Sciences (DIAS), and the trials testing the septoria model using either 4 or 8 days of precipitation were conducted by DIAS and the Danish Agricultural Advisory Centre (DAAC). The design of the trials was in the first case split plot and in the second

systematic complete block design with 5 replicates and a plot size of 20-32 $m^2$. The fungicides were applied with knapsack sprayers at low pressure (2-3 bar) using flat fan nozzles in a volume of 200-300 l $ha^{-1}$. The following products were tested: Tilt top (125 g propiconazole + 375 g fenpropimorph per liter), Folicur (250 g/l tebuconazole per liter), and Amistar (250 g azoxystrobin per liter). Septoria attacks were observed in all trials, with *S. tritici* being dominant, but mixed infections with *S. nodorum* were also seen. Plots were harvested with a plot combine harvester, and grain yield was corrected to 15% moisture content. When calculating net yields (profit), the prices used were: Tilt top 375 DKkr per liter and Folicur 400 DKkr per liter, Amistar 583 dkr per liter, and 60 dkr per application; grain price, 750 dkr per ton. Danish fungicide prices include a 33% tax.

## Results and Discussion

Semi-field trials have shown that azoxystrobin has a curative effect on *S. tritici* similar to propiconazole and much better than chlorothalonil (Figure 1). Compared to very effective triazoles like epoxiconazole, the effect of azoxystrobin is, however, lower (data not shown). Azoxystrobin showed a very effective, long preventive effect against *S. nodorum* (Figure 2), lasting for three weeks or more. In comparison propiconazole + fenpropimorph had only a residual preventive effect for 10 days. In field trials the early season effect (GSZ 31) of azoxystrobin has been found to be inferior to the effect of the co-formulation propiconazole + fenpropimorph, something which has not been observed following applications carried out around heading (Jørgensen and Nielsen, 1998).

Field trials using four different dosages of azoxystrobin, along with several other trial series (Jørgensen and Nielsen, 1998), have shown that azoxystrobin and other strobilurins may be used at a reduced dose (Figures 3 and 4). Different dosages, 12-100% of the normal azoxystrobin rate, have been tested in trials. When taking

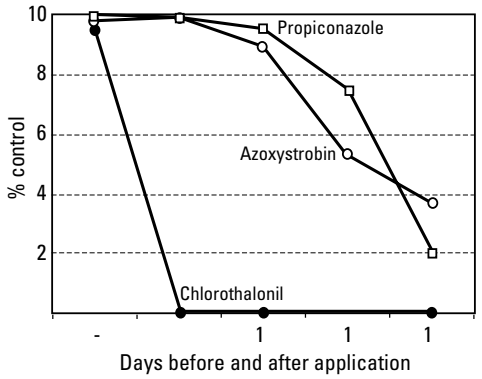


**Figure 1. Curative control of *Septoria tritici* using ½ rate of three different fungicides at different timing. 12% attack in untreated.**

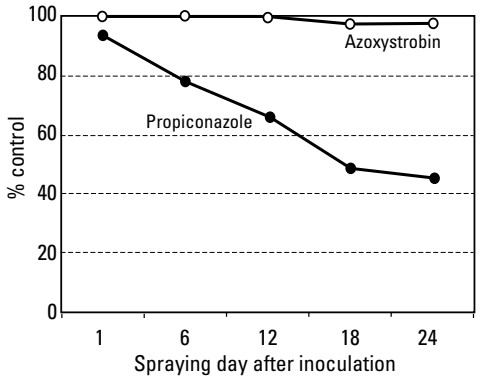


**Figure 2. Control of *Stagonospora nodorum* preventatively using ½ rate of two fungicides. 35% attack in untreated.**

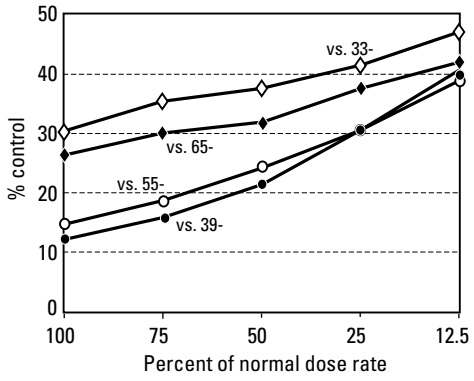


**Figure 3. Percent attack of *Septoria tritici* at GS 75 after application of different dosages of azoxystroin at different growth stages. 2 trials, 1998.**

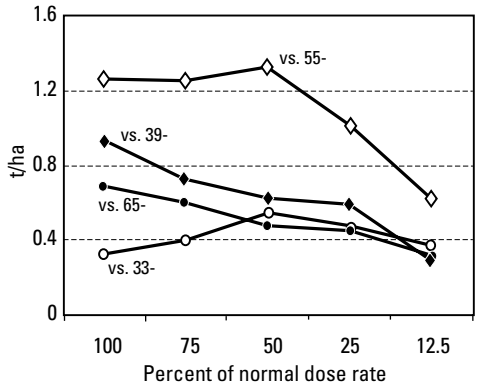


**Figure 4. Net yield in winter wheat after one application af azoxystrobin for control of *Septoria tritici* using different dosages and timing. 2 trials, 1998.**

into account different timing, the most profitable dose under severe disease pressure in 1998 varied between 50 and 100% of the normal rate, depending on growth stage and disease pressure. Using optimal timing, 50% was sufficient. When summarizing results from all trials with azoxystrobin, the most profitable treatment is most likely applied between GSZ 39 and 55, using 25 to 50% of the normal dose.

In three seasons, different thresholds have been investigated as background for recommending spraying azoxystrobin for septoria control. In 1998 the difference between using a threshold of 4 or 8 days of precipitation above 1 mm was minor (Table 1). Similar results were found in two trials in 1997 (data not shown). In two trials in 1996, four days was the optimal threshold (Jørgensen et al., 1999). Mixing azoxystrobin with tebuconazole has given results similar to azoxystrobin applied alone at both 4 and 8 days. These results indicate that the curative effect of azoxystrobin against *S. tritici* under Danish conditions are similar or better than the triazoles propiconazole and tebuconazole used on the Danish market.

Based on collected information, so far the test model for strobilurins recommends spraying after four days of precipitation starting at GSZ 32. If spraying is carried out before GSZ 51, 10 days' protection can be expected before days of precipitation have to be counted again. If applying after GSZ 51, 20 days' effect can be expected from azoxystrobin, which in practice means that no further application is needed. The recommended rates of azoxystrobin in the model vary between 35 and 45% of the normal rate.

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**Table 1. Results from trials using 4 and 8 days of precipitation as a threshold model for control of septoria diseases in winter wheat.**

| Treatment 9 trials 1999 | No. of appl. | TFI[a] | % septoria GS 69-71 | Yield and yield inc. (t/ha) | Net yield (t/ha) |
|---|---|---|---|---|---|
| Untreated | 0 | 0 | 21 | 6.67 | |
| Tilt top 1.0 GSZ 45 | 1 | 1 | 8 | 1.05 | 0.47 |
| PC-P Tilt top 8 days | 0.9 | 0.36 | 12 | 0.80 | 0.55 |
| PC-P Tilt top 4 days | 1.4 | 0.65 | 7 | 0.91 | 0.47 |
| Amistar 1.0 GSZ 45 | 1 | 1 | 7 | 1.67 | 0.81 |
| PC-P Amistar 8 days | 0.9 | 0.36 | 12 | 1.20 | 0.85 |
| PC-P Amistar 4 days | 1.3 | 0.43 | 7 | 1.19 | 0.75 |
| PC-P Amistar+Folicur 8 days | 0.9 | 0.44 | 12 | 1.26 | 0.90 |
| PC-P Amistar+Folicur 4 days | 1.3 | 0.52 | 8 | 1.18 | 0.74 |
| $LSD_{95}$ | | | | 0.35 | |

| | | | % septoria | | | |
|---|---|---|---|---|---|---|
| Treatment 3 trials 1998 | No. of appl. | TFI[a] | Flag leaf | 2nd leaf | Yield and yield inc. (t/ha) | Net yield (t/ha) |
| Untreated | 0 | 0 | 33.2 | 80.6 | 7.40 | - |
| Folicur 1.0 GSZ 45 | 1 | 1 | 2.0 | 32.5 | 1.70 | 1.09 |
| PC-P Folicur 8 days | 1 | 0.41 | 4.1 | 43.6 | 1.16 | 0.86 |
| PC-P Folicur 4 days | 2 | 0.84 | 5.6 | 28.7 | 1.76 | 1.15 |
| Amistar 1.0 GSZ 45 | 1 | 1 | 2.8 | 42.3 | 2.00 | 1.14 |
| PC-P Amistar 8 days | 1 | 0.41 | 5.5 | 55.7 | 1.79 | 1.39 |
| PC-P Amistar 4 days | 1.3 | 0.42 | 9.2 | 44.7 | 1.89 | 1.46 |
| PC-P Amistar+Folicur 8 days | 1 | 0.5 | 3.6 | 43.8 | 1.88 | 1.47 |
| PC-P Amistar+Folicur 4 days | 1.3 | 0.5 | 10.9 | 35.8 | 1.91 | 1.45 |
| $LSD_{95}$ | | | 6.8 | 10.9 | 5.3 | |

[a] TFI = Treatment frequency index.

## *Concluding Remarks*

# The Septoria/Stagonospora Blotch Diseases of Wheat: Past, Present, and Future

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## Abstract

*Septoria tritici and stagonospora nodorum blotch of wheat are regarded as major diseases because of their impact on crop management and wheat production. Both pathogens mainly affect the crop's grain filling processes. Early research dealt mostly with genetic, cultural, and chemical control measures. Chemical control remains one of the major means of protecting wheat production, mostly through the use of new families of systemic fungicides. Emphasis has been given to epidemiological studies, in many cases associated with chemical control; however, in recent years epidemiological studies have expanded to include the effect of primary inoculum initiated from dispersal of ascospores of both pathogens. That shift in focus has introduced issues such as disease cycle, mating types, and their effect—together with pathogenicity—on each pathogen's population structure. Recent findings attributed population structure to the pathogens' sexual rather than asexual stage. The association between virulence spectrum and pathogenicity and the contribution of the sexual stage remains to be investigated. The impact of such studies on population structure may dictate adapting the proper breeding strategies. A population that is ever changing due to recombination and gene flow may influence the use of sources for specific resistance. As for the introduction of DNA technology into* Septoria/Stagonospora*: wheat so far has been able to identify population genetic trends in both pathogens, but they have yet to be linked to virulence patterns. The use of genetic transformation in both pathogens makes it possible to follow post-penetration processes by following reporter genes. This may reveal that synchronous events associated with the disease cycle, such as the building of fungal biomes and the initiation of picnidia formation, are all under the control of host-parasite interaction. Mutations in virulence genes facilitate studying their function and genome mapping. Concurrently resistance genes can be identified, studied, and mapped. This is an exciting era for* Septoria tritici/Stagonospora nodorum *x wheat interaction.*

The septoria/stagonospora blotch diseases of wheat are incited by *Septoria tritici* Roberge in Desmaz. (teleomorph: *Mycosphaerella graminicola* (Fückel) J. Schrot. in Cohn) and by *Stagonospora nodorum* (Berk) E. Castellani and E. G. Germano (teleomorph: *Phaeosphaeria nodorum* (E. Müller) Hedjaroude), respectively. The two pathogens cause major foliar diseases of wheat, inflicting considerable yield losses in many countries worldwide (King et al., 1983; Pnini-Cohen et al., 1998). It is of interest to note that these two pathogens are rare in many rice-wheat management systems in southeast Asia or others in Africa.

The increased economic importance of these pathogens, especially of *S. tritici*, can be attributed to the cultivation of susceptible cultivars with a concomitant enhancement of resistance to other foliar pathogens (e.g., rusts, powdery mildew), predominance of wheat in crop management systems usually characterized by poor management of crop residues, increased nitrogen fertilization, high summer rainfall, earlier sowing, and resistance to MBC fungicides (Eyal, 1999).

A surge in scientific research on these two pathogens occurred in the early 1980s, when a wide range of scientific data were published in control-related disciplines (mostly chemical control and epidemiology) and less on biological and genetic aspects associated with the pathogens (Eyal, 1999). Breeding for disease resistance lagged due to scarcity of resistant germplasm and poor understanding of host-pathogen relationships and of how to manipulate resistance sources in breeding schemes.

The shift in importance from stagonospora nodorum blotch to septoria tritici blotch (Pnini-Cohen et al., 1998) that occurred in certain European countries was explained by the biological differences in their disease cycles (shorter for *S. tritici*)

and possibly due to differential sensitivity of the two pathogens to commercially used fungicides (Shaw, 1999). These explanations—which may reflect the situation in certain Western European countries—may not hold true in other wheat management systems. Still, the verification of the mechanism(s) affecting such shifts is extremely important, since it may dictate adoption of different control strategies.

The sources of primary inoculum are rather variable: airborne ascospores, pycnidiospores from plant refuse, wild grasses, and, possibly, infested (infected?) seeds (Brokenshire, 1975). Several wild grass species can serve as sources of inoculum to cultivated wheat, and some of them can contribute to speciation in pathogen populations. Artificial inoculation of wheat seedlings by isolates secured from grasses may not provide information on their contribution under natural infection. The possible contribution of infected seeds to the onset of disease was confirmed for *S. nodorum*, but is not well established for *S. tritici*. The current world trade of grain and, for that matter, of infested/infected grain provides ample opportunities for the dissemination of inoculum worldwide if such a distribution system is operative.

Global virulence surveys conducted by Eyal et al. (1985) and Kema et al. (1996) have shown considerable diversity at the species level (*Triticum* spp.) and differential response on the selected "differential" set of wheat cultivars. McDonald et al. (1999) stated that "since it has been shown that *M. graminicola* can infect seed (Brokenshire,1975), we consider it likely that this is also the mechanism for long distance gene flow in *M. graminicola*." The authors quoted King et al. (1983) that in the case of *S. nodorum*, the most likely mechanism for intercontinental dispersal is infected seed. It should be noted that Brokenshire (1975) stated that "infection of seedlings from infected untreated seed samples has proved unsuccessful with *Triticum dicoccum*." The report on seed infection of *S. tritici* by Brokenshire (1975) was not further verified and thus introduced ambiguity in the understanding of the possible dissemination of this pathogen by seeds. The epidemiological implications of naturally-infected grasses for short-scale dissemination and seeds (especially for *S. tritici*) for long-distance transport, warrant detailed investigations.

The supposition that population genetics of *M. graminicola* using single-locus probes to measure gene diversity for individual RFLP loci , population subdivision, and genetic similarity among populations (McDonald et al., 1999) can be superimposed on virulence patterns was not supported in studies on populations from California and Oregon (Mundt et al., 1999). Isolates of *S. tritici* from these states differed significantly in their pathogenicity (Ahmed et al., 1996), yet they manifested similarity in allele frequencies as measured by RFLPs (Chen and McDonald, 1996). The finding that the genetic diversity measured among 122 isolates collected in an *S. tritici* nursery at Patzcuaro, Mexico, was among the lowest, does not imply that the pathogen has a narrow virulence pattern. The Patzcuaro *S. tritici* population was reported to possess virulence to some of the most resistant wheat cultivars (e.g., Bobwhite"S" and Kavkaz/K4500 L.6.A.4) (McDonald et al., 1999).

Based on genetic population studies, McDonald et al. (1999) suggest that "if CIMMYT continues to use Patzcuaro as a field site to screen for resistance to *M. graminicola* and *S. nodorum*, it may want to consider introducing more diverse fungal populations from other parts of Mexico into this disease nursery." It should be mentioned that some of the testing sites in the Patzcuaro area were artificially inoculated in the early 1980s with a mixture of Mexican *S. tritici* isolates to ensure selection pressure on breeding materials (Santiago Fuentes, personal communication). The suggestion made by McDonald et al. (1999) implies that genetic diversity measured by random RFLP alleles can be used as an estimator of virulence pattern. It is therefore proposed that unless genetic diversity measured by random alleles is not highly correlated with diversity in virulence patterns, the former should not be used as an estimator of virulence in disease resistance breeding schemes. The issue of selecting relevant fungal isolates in screening for disease resistance will be further discussed.

The quantitative estimation of host response percent (pycinidia and/or necrosis) for both *S. tritici* and *S. nodorum* and thereafter analyses of the interaction term of cultivar x isolates by statistical means (ANOVA) have been used to estimate genetic variation in these pathosystems (Eyal and Levy, 1987). Such a statistical measure

requires a predetermined set of "differentiating cultivars," which was not agreed to by *Septoria tritici/ Stagonospora nodorum* workers, suitable methodology (sampling, culturing of pathogen, inoculum age and dosage, incubation categories, disease assessment, etc.), and controlled environmental conditions to ensure reliability of results. The lack of a uniform methological approach and, in particular, of a common and agreeable set of differentials introduces difficulties in comparing and drawing conclusions on a wider basis.

The suggested speciation of *M. graminicola* on durum (*Triticum durum*) and common wheat (*T. aestivum*) may require the inclusion of differentiating cultivars of both species when they are grown together or in studies where both are tested (Kema et al., 1996; Saadaoui, 1987). Isolates from one species may not be pathogenic to the other *Triticum* species; still, they may show a significant interaction term when a proper set of cultivars of a species is being inoculated with isolates from the same species. Cross infection of the two species was reported for isolates secured from either *Triticum* species (Eyal, 1999; Kema et al., 1996). The specific pathogenicity towards *T. durum* may have bearing on the structure of *S. tritici* populations (Saadaoui, 1987). Alternate cropping of bread wheat cultivars in a durum wheat management system may express a lower than expected infection level on the former than under uninterrupted bread wheat management.

Today it is generally accepted that the specificity in virulence is low in *S. nodorum*, but can be detected in *S. tritici* populations provided proper differentiating germplasm is used (Eyal, 1995). The magnitude of specificity in the *S. tritici* - wheat pathosystem and its implications for breeding for disease resistance requires elaboration. Few dominant single genes conferring resistance were identified (e.g. $Stb_1$ - Bulgaria 88, $Stb_2$ - Veranopolis; $Stb_4$ - Tadinia) (Somasco et al., 1996). There are several reports that these resistant sources are not providing the claimed protection when moved across *S. tritici* populations with wide virulence patterns (Ballantyne and Thomson, 1995; Eyal, 1999).

It is proposed that specificity and inheritance studies should be conducted with germplasm that is agronomically relevant to breeding, preferably exhibiting resistance to a wide virulence pattern (such as "Bobwhite"S", IAS20-IASSUL, or other Frontana derivatives, Kavkaz/K4500 L.6.A.4, and other sources identified through multilocation testing) (Eyal et al., 1987). Special attention should be given to resistant wheat accessions developed in wide-cross programs. Some of these accessions may be susceptible to other foliar diseases that may need attention when incorporating *Septoria/Stagonospora* resistance. Germplasm used in virulence studies is usually derived from disease nurseries naturally or artificially infected with the pathogen of interest. Only in a few cases has the virulence spectrum of these populations been categorized.

It is therefore likely that identified resistant germplasm may have only limited use either as a breeding source or as "differentials." Resistant germplasm that has withstood prolonged testing to variable pathogen populations can be considered a potential source for breeding for resistance. Special attention should be given to avoid selecting tall stature and late maturing germplasm that may introduce such non-genetic factors into germplasm evaluation, or germplasm with poor agronomic characteristics.

Isolates possessing virulence to important resistance sources can become the "core virulence spectrum" for screening germplasm. There is no criterion as yet for selecting "relevant" isolates in artificially inoculated breeding trials. The criteria dictating the choice of isolates for genetic studies of virulence (Kema et al., 1999) may use considerations other than breeding. The choice by Kema et al. (1999) of *S. tritici* isolates such as IPO323 (avirulent on Veranopolis, Kavkaz, and Shafir) and IP094269, which is virulent on these cultivars, in studying the genetics of avirulence in this pathogen merits adoption by other *Septoria tritici / Stagonospora nodorum* investigators.

The correlation between seedling and adult host response has been substantiated by several investigators (Eyal, 1999; Kema and van Silfhout, 1997). Screening for resistance at the seedling stage does not provide an integral view of the tested germplasm. Seedling tests can serve as a supplementary measure and are an excellent tool for detailed, controlled studies on a multitude of biological issues associated with host-pathogen interactions. The seedling test as a screening measure is hampered by not knowing whether the used isolates are relevant to the virulence spectrum of the

populations to which the test germplasm will be subjected under field conditions.

Cross testing of germplasm to both seedling and field conditions is usually performed with predetermined isolate(s) that can serve as a "basic virulence set" (Eyal, 1999). Its composition may change by introducing isolates found to be virulent on specific resistant germplasm, or omitting others. The set should include isolate(s) whose virulence on specific accessions has been repeatedly confirmed. The multilocation Septoria Monitoring Nursery initiated by CIMMYT can provide information as to the magnitude of protection of some of the identified sources and contribute information on worldwide virulence patterns.

The contribution of the sexual stage to the virulence spectrum needs special attention. Evidence from studies on population genetics emphasizes the major influence of the sexual stage on the genetic diversity of the population (Chen and McDonald, 1986; Eyal and Levy, 1987; Eyal et al., 1985; Jlibene et al., 1994; McDonald et al., 1999; Zhan et al., 1998). McDonald et al. (1999) stated that asexual reproduction may have an important impact over a limited area (e.g. few square meters), whereas sexual reproduction has much greater consequences for population genetics.

The limited scope of clonality in *M. graminicola* and *P. nodorum* populations strengthens the need for comparative studies on virulence. The limitation of such studies resides with selected "wheat differentials." This difficulty can be partly overcome by tracing specific virulences within the pathogen population on certain resistance accessions (e.g. Kavkaz/K4500 L.6.A.4). Low frequency of virulence to Kavkaz/K4500 L.6.A.4 was reported for the first time in Israel (Ezrati et al., 1998), interestingly, in Nahal Oz, where McDonald et al. (1999) detected the greatest genetic diversity using random RFLP alleles. Low frequency of virulence on Kavkaz/K4500 L.6.A.4 was reported in the global virulence surveys conducted by Eyal et al. (1995) and Kema et al. (1996). This germplasm was not used in international and Israeli breeding programs in the past and therefore no selective advantage can explain its detection. It is expected that the presence of virulence on this accession at Nahal Oz can be accounted for by the fact that the sexual stage is operative there, as implied by McDonald et al. (1999).

Chen and McDonald (1996) hypothesized that *M. graminicola* isolates can be produced via genetic recombination with the same combination of virulence genes that may not have the same recent ancestors, thus contributing to multiple clonal lineages in the population. The distribution of this virulence in space (locations) and time (years) is under study.

The expression of virulence on certain wheat germplasm was reported to be altered upon inoculation with mixtures of *S. tritici* isolates (Ezrati et al., 1998; Zelikovitch and Eyal, 1991). When a resistant cultivar (e.g., Seri 82) is inoculated with a mixture of avirulent (ISR398) and virulent (ISR8036) isolates, the level of pycnidia produced on the seedlings and adult plants in the field resembles that produced by the avirulent isolate inoculated singly (Ezrati et al., 1998). The virulent isolate ISR8036 predominated in the population of pycnidia on both seedlings and adult plants of the susceptible cultivar Shafir inoculated with a 1:1 mixture of the two isolates. It was suggested that isolate ISR8036, which induced the same level of pycnidia on Shafir as ISR398, was more aggressive than the latter, giving it a competitive advantage in the population.

The suppression phenomenon was also operative when an array of resistant cultivars (e.g. Bobwhite"S", IAS 20-IASSUL, and Kavkaz/K4500 L.6.A.4) were inoculated with appropriate mixtures of avirulent and virulent isolates (Ezrati et al., 1998). These findings are indicative of the commonality of the phenomenon where resistance induced by an avirulent *S. tritici* isolate can provide tissue protection against the virulent isolate. The level of protection, the conditions under which it is expressed, and the mechanism(s) associated with the induction needs further investigation.

It is possible that under field conditions, induced resistance may be operative under low to moderate *S. tritici* epidemics, provided a certain level of genetic resistance is present in the infected wheat cultivar. The reported differences in aggressiveness among isolates (Ahmed et al., 1996; Ezrati et al., 1998; Mundt et al., 1999) suggest that the structure of an asexual population progressively developed on wheat plants can be strongly affected by both virulence and aggressiveness. If the sexual stage is the sole

provider of primary inoculum from distant sources (Shaw and Royle, 1989) or from within the crop (Gilchrist and Velazquez, 1994), the structure of the population will probably change annually.

The vertical progression within the crop of pseudothecia from lower to upper leaves (Gilchrist and Velazquez, 1994) may give an advantage to "local" populations, though it does not exclude the possibility that the primary inoculum may come from a distance and then perpetuate itself within the crop. Hunter et al. (1999) stressed that the continuous development of a functional sexual stage suggests that there is genetic exchange throughout the growing season that can respond over time to selection pressure exerted by resistant germplasm. The role of the asexual stage in epidemics and in formulating the virulence structure of the population under such conditions is not clear.

The genetic variation for virulence and resistance in the wheat-*S. tritici* pathosystem, in addition to having implications for breeding for disease resistance, provides an opportunity to expand our knowledge on the biology and genetics of the interaction. The recognized specificity in the relationship linked with the implementation of new DNA technology allows for a more thorough understanding of the pathogen, the host, and the interaction. This makes it possible to adopt methodologies from other host-pathogen systems and apply them to the economically important wheat-*S. tritici*/*S. nodorum* pathosystems.

Issues associated with the ability to infect wheat and processes related to the infection-cycle (biochemical, involvement of toxins, tissue necrosis, induction of pycnidia formation, and suppression of production), virulence (genotypic diversity and population structure), pathogen migration, the potential of genetic recombination on pathogen structure and then on disease management, and genetic host resistance (specific, non-specific) are currently being investigated with the aid of molecular tools (Caten, 1999; Eyal, 1999; Kema et al., 1999; Baker et al., 1997; Eyal et al., 1985). Genetic diversity and population structure are being revealed with the aid of RFLP, RAPD probes, and DNA fingerprinting (McDonald et al., 1999).

The issue of mating types and possible sequencing of genes associated with these types, and mapping of the *M. graminicola* and *P. nodorum* genomes are being investigated by Caten (1999) and Kema et al. (1999). Genetically transformed *S. tritici* isolates and tagged mutants with altered virulence are being investigated by Pnini-Cohen et al. (1998). The use of reporter gene(s) (e.g. GUS, GFP) in genetically transformed *S. tritici* and *S. nodorum* isolates and immunological assays can greatly contribute to the understanding of *in planta* qualitative and quantitative post-inoculation events. The interrelations between isolates in a population and the effect of induced resistance and competition on a pathogen population are elucidated with the aid of isolate-specific PCR primers (Ezrati et al., 1998). The elucidation of loci associated with host resistance employ QTL analyses, and genomic analysis may some day contribute to the identification of sequences linked to resistance.

It is likely that genes associated with resistance to *S. tritici* and *S. nodorum* share products with structural similarities to other plant defense systems (Baker et al., 1997). The structural features shared by several R (resistance) gene products are a leucine-rich repeat (LRR) motif or a serine-threonine kinase domain. Some of these genes encode cytoplasmic receptor-like proteins that contain an LRR domain and a nucleotide binding site (NBS). It is therefore possible that these gene products are operative in wheat and can be tagged. The identification of specific interactions between avirulence genes in *S. tritici* (AVR*mg*) and genes for resistance in the wheat plant may pave the way for the "genetic dissection of R gene-mediated induction of hypersensitive (?)" (or suppression of symptoms in isolate mixtures ?) host defense. This insight can be used to genetically engineer wheat cultivars resistant to a broad spectrum of pathogens. It will require a better understanding of avirulence, specific resistance, and host-pathogen interactions and their products in those pathosystems, prior to the formulation of a protection strategy. As a consequence, the economic and ecological (chemical protection) impact on wheat production will be reduced.

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**ISBN: 970-648-035-8**

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