Helminthosporium Blights of Wheat:

Spot Blotch and Tan Spot

E. Duveiller,
H.J. Dubin, J. Reeves,
and A. McNab, editors

CIMMYT/UCL/BADC

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Proceedings of an International Workshop Held at CIMMYT El Batan, Mexico

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UCL
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Foreword

Over the last 30 years in developing countries, the outstanding advances in wheat productivity attained by CIMMYT and its partners have contributed greatly to the well-being of millions of people. As growing numbers of farmers have taken advantage of new, superior wheat varieties, wheat has been introduced into warmer areas where it was not previously planted or has been grown in increasingly complex, intensive farming systems in the developing world's traditional wheat growing areas.

These changing circumstances for wheat production have brought new challenges for farmers, including the increased incidence of helminthosporium blights of wheat: spot blotch, caused by *Bipolaris sorokiniana*, and tan spot, caused by *Pyrenophora tritici-repentis*. In South Asia's intensive rice-wheat cropping systems, particularly in the heavily populated eastern Gangetic Plains, spot blotch has become the major disease constraint. In reduced tillage cropping systems, tan spot is an increasingly important concern, as the pathogen survives on crop residues and alternate hosts. To complicate matters, these diseases may occur together in the field, where they are often very difficult to distinguish from one another. For this reason, it is desirable to study their incidence and control together.

The complexity of these nontraditional wheat diseases and of the conditions in which they occur makes it essential for us to seek a holistic approach for their control. Such an approach includes breeding for durable disease resistance; developing appropriate crop management practices, including nutrient applications; monitoring pathogen diversity; and applying recent advances in biotechnology to overcome disease losses. In addition, researchers need to develop a better understanding of the cropping systems and the many interactions that can influence the spread of disease. Better protocols for ensuring the production of healthy seed are needed as well.

To strengthen research partnerships directed at reducing yield losses to these diseases, and to foster a more holistic view of potential strategies for disease control, CIMMYT organized an international workshop, Helminthosporium Blights of Wheat, at its headquarters in Mexico from 9 to 14 February, 1997. Sponsored by the Belgian Administration for Development Cooperation and CIMMYT, in close collaboration with the University of Louvain, Belgium, this workshop was an important component of a larger, collaborative research project on nonspecific foliar pathogens of wheat.

The meeting assembled key researchers from the national agricultural research systems of developed and developing nations to review recent advances in pathology and breeding for resistance to spot blotch and tan spot of wheat. Fifty-four participants from 21 countries attended the workshop; participants represented most of the wheat-growing areas where tan spot and spot blotch limit yields. The workshop enabled researchers to bring each other up to date on the global incidence of foliar blights caused by *B. sorokiniana* and *P. tritici-repentis*, particularly in South Asia's rice-wheat system and under reduced tillage. Research results were presented and discussed, future research collaborations defined, and networking activities strengthened, all with the goal of obtaining better disease control.

This proceedings documents the results of a unique opportunity for CIMMYT scientists and their colleagues to exchange information on these two important diseases. The papers and workshop discussions presented here should provide a useful record for workshop participants. The proceedings also should prove to be a valuable reference for scientists who could not attend these meetings, researchers who work to reduce grain losses in the warmer production areas of developing countries, and their counterparts in other parts of the world.

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Helminthosporium Diseases of Wheat: Summary of Group Discussions and Recommendations

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Introduction

Four working groups were formed on the last day of the conference to brainstorm on the different topics raised during the presentations. The topics discussed were: pathogen variability, disease scoring, the effect of cropping practices, and breeding methodologies including breeding strategy and multilocation testing. The final goals were to reach an agreement on research needs in relation to spot blotch and tan spot, harmonize research methodologies, and ultimately define a set of recommendations for the coming years. The workshop gave scientists from NARSs in regions where *Helminthosporium* diseases of wheat are a constraint a unique opportunity to exchange research ideas with other scientists, and to update the information on those diseases. This forum, attended by scientists working on foliar blights in 21 countries, will help to refine CIMMYT's future strategy for better control of these diseases in partnership with NARSs and advanced research centers. A summary of group discussions and final recommendations is presented.

Pathogen Variability

It was agreed that CIMMYT's Helminthosporium Monitoring Nursery (HMN) should continue. Particular attention must be given to the inclusion of differential entries and a core of genotypes maintained over years in order to monitor pathogenicity changes. The nursery should contain a maximum of 20 entries with sources of resistance to both *Bipolaris sorokiniana* and *Pyrenophora tritici-repentis*. Scoring time is important for comparing results among locations. Concerns were raised about how to include results in a database for maximum global access.

A working group would be useful to exchange information on inoculation methods since the standardization of experimental conditions appears to limit extensive screening for spot blotch and tan spot resistance under controlled conditions. Information on growing conditions and inoculation techniques needs to be more widely exchanged, and conducting a "ring test" among several scientists using the same methods was proposed to evaluate trial repeatability among laboratories.

Global studies on pathogen populations are needed to anticipate future breeding requirements because pathogenic specialization may change over time and across locations. CIMMYT plays an important role in catalyzing sample collections on a worldwide scale. It is important that this effort continue and a wide range of strains be studied. During the last five years, a global collection of strains has been maintained in Belgium and is easily accessible. However, additional funding is required to continue research on strain diversity. A smaller group of scientists showed specific interest in analyzing the effect of storage conditions (using mineral oil or lyophilization) on strain variation and pathogenicity.

Disease Scoring

It is desirable but not always easy to reach a consensus on scoring approach. Various approaches were discussed. Lesion size may be useful in some situations. The advantage of the double digit scale is that the individual components are used to produce an index. This approach, extensively used by some researchers, is quick and easy. Percentage diseased leaf area can be used as a parameter or to calculate the AUDPC (area under disease progress curve). It is easy to teach, to manipulate mathematically, and to analyze. This scoring method is the most universally applicable and particularly useful for comparing epidemic severity across locations and seasons, provided that similar checks are used. On the other hand, it is time consuming. The 00-99 double digit scale will probably continue to be used for rapid screening.

When adult heads are evaluated, a scale such as "lesion type" can be used quite effectively, particularly under controlled conditions. Simple percentage assessment and the 0-9 scale are probably the most realistic methods but the complication of black chaff in samples may require the grain to be directly assessed.

Since spot blotch and tan spot diseases may occur as a complex within nurseries and across genotypes, it is important to include differential genotypes in the nursery that may help to determine which pathogen prevails in each environment.

The most useful parameters for selection are yield with and without disease control (for tolerance/intolerance evaluation), and grain weight. In some environments, however, tolerance may be difficult to assess. The complication comes from the fact that highly susceptible check lines may not respond well to fungicide control and thus confound tolerance estimates. It is important to have appropriate checks in control plots and to be aware of agronomic influences. It is most important to include in the breeding material check varieties that range in maturity and height.

If any scoring adjustment in terms of maturity is going to be made, disease should be contrasted within different maturity categories. It would be useful to

have a physiologically relevant growth stage scale that has some arithmetic relationship with thermal time.

Tan spot toxins may be used for disease scoring, but the problem with this approach is that the toxin has to be infiltrated and there is no other approach at present. The necrosis toxin is qualitative and so gives only a plus-minus response; thus, it can give a good idea of one gene interaction. In expert hands and with the appropriate equipment, 150 inoculations by infiltration can be performed per hour. At present, the chlorosis toxin appears to be important; however, additional information is needed. Another suggestion is to make some of these assays quantitative and protoplast assays with electrolytes leakage should be considered, although this may be quite intensive in terms of resources.

Resistance genes are expected to be tagged in the next 10 years. To be worthwhile, molecular screening should be done simultaneously for several genes. In the case of tan spot, the necrosis toxin resistance gene should be tagged first, and then others that may be related to infection frequency and other parameters. They can be useful if combined in the same molecular screening.

Glasshouse evaluation and advancing the material in a glasshouse are useful. Australia's infection type approach can be followed, but measuring the percentage of diseased leaf area is also useful. If you rely on codes where leaf necrosis is important, a standardized way of applying inoculum is needed. Also, in the greenhouse, screening using the toxin directly is feasible, or the pathogen can be inoculated on one leaf and toxin infiltration done on another leaf.

Effect of Cropping Practices

Cropping systems, crop rotations, and tillage conditions are so variable that one of the first things to be done to measure the effect of cropping practices on foliar blights is to standardize procedures for assessing the diseases. This includes, first, an evaluation of the seed status, for instance, using the freezing blotter test. Once this is status is known, a fungicide seed treatment can be used. Disease incidence is assessed by observing the top two leaves. Disease severity should be assessed whenever possible, but members of the discussion group indicated that it is not feasible in some locations.

Pathogen identification remains important because clear and unique symptoms are not always caused by a particular fungus. Root rot, caused by *B. sorokiniana*, should be evaluated on pulled plants at the soft dough stage, using Tinline's method published in Canada. At harvest time, evaluation can be standardized by using the freezing blotter test to see if there is any carryover of pathogen at the seed level. An alternative would be to use trap cultivars to help monitor conidia production during the season. Other crops can also be used as traps and taken back to the greenhouse for evaluation.

Long term agronomic studies have been conducted in some countries, but often the pathologists have not been involved in these trials. It is important to quantify the impact of cropping practices on disease incidence. This does not require additional resources or much extra effort. These are ready-made trials for assessing foliar blights and the effects of agronomic practice on disease.

Another approach is to look at fertilizer effects; more research is needed to understand the relationship between soil fertility and foliar blight development using standardized methods. Conservation tillage studies should include assessment of disease development to identify appropriate cropping practices and to define germplasm improvement needs under these practices. Also, the effect of legumes or crucifers in some wheat-based cropping systems needs to be evaluated, as these crops may affect disease pressure and may play a significant role in local economies where foliar blights are increasing. In general, the role of alternative hosts in pathogen survival should be better understood. Biological control should be considered, since there are successful examples with take-all, caused by *Gaumannomyces graminis*.

Finally, the movement of infected seed should be minimized, and nurseries for spot blotch resistance screening should be multiplied in a clean area.

Breeding Methodologies

Breeding strategy

The names of both diseases, spot blotch and tan spot, and their causal pathogens are confusing to breeders, who feel that names of diseases and pathogens should be clarified and standardized.

The breeding strategy for spot blotch needs to rely on the infusion of resistance from alien species such as *Triticum tauschii* (*Ae. squarrosa*) and *Thinopyrum curvifolium*. Selection for spot blotch resistance must be combined with heat tolerance. There is a need for testing at key sites (at least 5-10) in order to identify suitable parental material and to exchange advanced and segregating materials with good agronomic type and high resistance levels.

Tan spot and leaf rust resistance need to be combined; however, a better understanding of the relationship between tan spot development and zero tillage is needed to make breeding for this resistance more efficient. To develop wheat populations with the desirable characters, the use of backcrosses and multiple crosses will be most advantageous, as well as understanding the correlation between greenhouse and field testing.

Multilocation testing

Shuttle breeding has been successful at CIMMYT and at national centers. It should continue to be emphasized, along with the exchange of germplasm and information on a regional and global

scale. Initiating a newsletter has been proposed. The exchange of parental and segregating materials not only from CIMMYT but also from national programs should be further encouraged. Key sites and key checks should be identified based on the last HMN, already available at CIMMYT.

Recommendations of the Working Groups

- A short note on the names and taxonomy of pathogens causing spot blotch and tan spot will be included at the beginning of these proceedings. The name *B. sorokiniana* for the spot blotch pathogen seems appropriate, given that the anamorph plays the main role in nature. Similarly, since the teleomorph is effective during the disease cycle, *P. tritici-repentis* should be used for tan spot.
- There is a need to develop a set of 10-15 CIMMYT lines, plus others, in order to monitor helminthosporium blights. The HMN must continue at key sites and emphasis should be put on accurate scoring.
- Assessment of diseased leaf area is the most accurate way to evaluate disease severity and should be recommended.
- Developing a database on pathogens is becoming necessary, and global studies on pathogen virulence are needed.
- Research on the effect of zero and reduced tillage on disease development is needed.
- Integrated approaches to limit disease spread need to be studied, and more pathology studies of long term trials have to be conducted to understand the effect of soil fertility and cropping practice on foliar blights.
- Testing for foliar blight needs to be standardized.
- Multilocation testing is critical. More effort should be invested in testing segregating material and sharing earlier-generation material.
- Genetic marker studies should be encouraged to identify and understand the genetics of resistance to spot blotch and tan spot.
- Human resource development and training are critical to increase the capacity of NARSs to better control foliar blights of wheat.

It is proposed that a working group on helminthosporium blights of wheat be formed to further exchange information on spot blotch and tan spot.

Evolution of the Nomenclature Used for *Helminthosporium* spp. Causing Leaf Blight of Wheat

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Abstract

This paper reviews the evolution in nomenclature of Helminthosporium sativum *Pammel, King & Bakke to* Cochliobolus sativus *(Ito & Kurib.) Drechsler ex Dastur, anamorph* Bipolaris sorokiniana *(Sacc.) Shoem., and of* H. tritici-repentis *Drechsler to* Pyrenophora tritici-repentis *(Died.) Drechsler, anamorph* Drechslera tritici-repentis *(Died.) Shoem.*

The names *Helminthosporium sativum* and *Helminthosporium tritici-repentis* are still widely used for the fungi that cause spot blotch and tan spot of wheat, respectively. This is despite the fact that Shoemaker (1959) considered *Helminthosporium* an illegitimate orthographic variant of *Helmisporium* Link ex S.F. Gray (meanwhile *nomen rejiciendum*) and in 1959 already proposed the anamorph names actually recommended for graminicolous *Helminthosporium* species, which include the fungi associated with leaf blight of wheat. This is due, on one hand, to a deep-rooted attachment of plant pathologists to the name *Helminthosporium* linked to important textbook diseases and, on the other hand, to the difficulty and reluctance to follow the never ending discussions of systematists and changes in taxonomy and nomenclature. Meanwhile, however, some of these changes have become widely adopted and, in order to favor mutual understanding, it is wise to reach a consensus on using the same name for the same organism. The aim of this paper is to briefly explain the reasons behind the numerous revisions and to unravel the nomenclatural evolution in this group of fungi for plant pathologists. Sivanesan (1987) and Alcorn (1988) provided useful reviews of the taxonomy of the graminicolous hyphomycetes placed in the genus *Helminthosporium* and their teleomorphs.

The graminicolous *Helminthosporium* differ fundamentally from the species type *H. velutinum* Link:S.F.Gray. Conidia are singly formed through a pore at the apex of the conidiophore, which resumes growth by sympodial extension from the sub-apical region. This leads to a geniculate conidiophore at the site of conidiogenesis. A scar surrounds the pore through which the conidium is produced. For *H. velutinum*, the conidiophores are straight or flexuous and produce conidia through small pores in the walls of distal and intercalary cells. The conidia are

formed laterally, often in verticels below septa, while the conidiophore is elongating at the apex. Conidia bear a conspicuously darkened hilum but there are no corresponding scars on the conidiophore at the sites of conidium production (Alcorn 1988). These differences have justified the renaming of the graminicolous *Helminthosporium* species.

The name *Helminthosporium sativum* Pammel, King & Blakke (1910) was given without taking into account the earlier description of *H. sorokinianum* Sacc. in Sorokin, Trans. Soc. Nat. Univ. Kazan 22: 15 (1890) (Sivanesan 1987). When Shoemaker (1959) proposed the generic name *Bipolaris* for the *Helminthosporium* species with fusoid, straight, or curved conidia, germinating by one germ tube from each end (bipolar germination), the spot blotch pathogen was renamed *Bipolaris sorokiniana* (Sacc.) Shoem. This name is presently widely adopted. *H. acrothecioides* Lindfors, *H. californicum* Mackie & Paxton, and *Drechslera sorokiniana* (Sacc.) Subram. & Jain are synonyms.

The ascigerous state (teleomorph) was first observed in the laboratory on natural media in the presence of opposite mating types and described as *Ophiobolus sativus* Ito & Kurib. It was later renamed *Cochliobolus sativus* (Ito & Kurib.) Drechsler ex Dastur (1942). *Cochliobolus* Drechsler is characterized by globose ascomata usually with a long cylindrical neck, obclavate-cylindric asci, and helically coiled filiform ascospores. This genus is associated with *Bipolaris* and *Curvularia* anamorphs. Teleomorphs of *C. sativus* were only recently reported from Zambia (Raemaekers 1991).

The name given to the tan spot fungus has also evolved considerably. Studying the link between *Helminthosporium* anamorphs and *Pleospora* teleomorphs on various grasses, Diedicke first described, in 1902, *H. gramineum* Rab. ex Schlecht. f.sp. *Tritici repentis* on *Triticum repens*, an ancient name for quack grass (*Agropyrum repens* (L.) Beauv.). The anamorph was linked with the teleomorph *Pleospora trichostoma* (Fr.) Wint. f.sp. *Tritici repentis*. Mainly based on pathogenicity studies and association of *Alternaria* spp. with *P. trichostoma* (Fr.) Wint., he changed the names to *Helminthosporium Tritici repentis* Died. and *Pleospora Tritici repentis*, respectively (Diedicke 1903). He also pointed out the pathogenic differences between these fungi and *H. Bromi* Died, and erected *Pleospora Bromi* Died., *P. teres* Died., *P. Avenae* Died., and *P. graminea* Died. for the ascus stages of different *Helminthosporium* species. However, Noak (1905) did not accept this conclusion because of morphological similarities of the teleomorphs, and reduced the parasites on quack grass and *Bromus* to biological forms of *P. trichostoma* (Fr.) Wint. f.sp. *tritici-repentis* Noak. *Pleospora tritici repentis* was renamed *Pyrenophora tritici-repentis* (Died.) Drechsler (1923) because of the presence of setae on the ascocarp.

Wehmeyer questioned the exclusion by Diedicke of all forms having a *Helminthosporium* state from *Pleospora trichostoma*, the erection of the five binomials for the ascus state of the different species of *Helminthosporium*, and the descriptions of Drechsler. Based on morphological similarities of the ascocarp, he reintroduced *Pleospora trichostoma* (Fr.) Ces. & de Not. (Wehmeyer 1953) and later on *Pyrenophora trichostoma* (Fr.) Fck. as the teleomorph of the various species of *Helminthosporium* on *Bromus*, *Hordeum*, *Poa*, *Secale*, and *Triticum* (Wehmeyer 1961). This name has been widely used for the tan spot and yellow leaf spot pathogen of wheat (Wiese 1977; Hosford 1982). Nevertheless, this created confusion from the phytopathological point of view because the different species of *Helminthosporium* on the various hosts are associated with very different diseases with regard to pathogenicity, ecological requirements, and cultivar resistance, and because *H. tritici-repentis* also has, *per se*, a wide host range (Shoemaker 1962).

In his description of *H. tritici-repentis*, Drechsler (1923) described the subhyaline, straight-cylindrical conidia as "most distinctive peculiarity ... the shape of the basal segment ... remotely suggestive of the horizontal aspects of the head of a snake". Nisikado (1928) associated leaf spots on wheat with *H. tritici-vulgaris* Nisikado, characterized by conidia with uninflated, long-conical basal cell; *H. tritici-repens* being considered to have inflated basal cells. Ito (1930) proposed the generic name *Drechslera* Ito for *Helminthosporium* species with conidia germinating from every cell and renamed *H. tritici-vulgaris* as *Drechslera tritici-vulgaris* (Nisikado) Ito. However, the hyphomycete genus *Drechslera* Ito was not taken up until the indepth taxonomic studies of this group by Shoemaker (1959, 1962). The latter considered the difference between *H. tritici-repentis* and *H. tritici-vulgaris* as not consistent and proposed *Drechslera tritici-repentis* (Died.) Shoem. as the anamorph of *Pyrenophora tritici-repentis*. By this simplification, *Pyrenophora tritici-vulgaris* Dickson (1956) became the synonym of *P. tritici-repentis*.

Further studies have been undertaken during the last decades on the taxonomy of this group, taking other criteria into account including the type of hilum, the conidial wall, and the known or unknown teleomorph, but the terminology proposed by Shoemaker for the above graminicolous *Helminthosporium* has not been changed (Sivanesan 1987; Alcorn 1988).

The international code of botanical nomenclature permits the different states of fungi with pleomorphic life-cycles to be given separate names; if a teleomorph is present, the name automatically refers to that morph even if the anamorph is also present (Hawksworth *et al.* 1995). The correct names for helminthosporium leaf blight of wheat are accordingly: *Cochliobolus sativus* (Ito & Kurib.) Drechsler ex Dastur, anamorph *Bipolaris sorokiniana* (Sacc.) Shoem., and

Pyrenophora tritici-repentis (Died.) Drechsler, anamorph *Drechslera tritici-repentis* (Died.) Shoem.

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Crop Management and Breeding for Control of *Pyrenophora tritici-repentis* Causing Yellow Spot of Wheat in Australia

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Abstract

Pyrenophora tritici-repentis *is a major leaf disease in the northern wheat growing areas of Australia and also occurs sporadically in southern wheat growing areas. In Western Australia, the disease occurs in combination with* Phaeosphaeria nodorum, *and losses of 5-20% are frequent in medium to high rainfall areas. Pink grain occurs sporadically in seasons that are very favorable for disease development. The disease has increased in the last 20 years due to intensified cropping and adoption of stubble retention/reduced tillage. Stubble burning and crop rotation are the main means of control. Single applications of triazole fungicides, such as propiconazole and tebuconazole at 62 g ai* ha^{-1} *applied around the time of flag leaf emergence, can provide economic control but are rarely practiced because of uncertainty of seasonal growing conditions. Breeding programs for white grained, high quality wheats are pursuing resistance as a high priority in Western Australia (WA), Queensland (QLD), and northern New South Wales (NSW). Approaches to breeding in Australia include complex crossing and backcrossing strategies combined with glasshouse and field based screening. In WA, resistance to* P. tritici-repentis *is sought in combination with* P. nodorum. *Moderate resistance has been developed from a broad range of genetic material. This has resulted in the release of cvs. Cascades (Tadorna.Inia/3*Aroona), Carnamah (Bolsena-1CH/77W:660), and Cunderdin (Flicker sister/Sunfield sister) with moderate resistance to* P. tritici-repentis. *In QLD and NSW, a backcrossing program using known sources of recessive resistance has resulted in the release of Leichhardt (CNT2/4*Hartog). In some cases, susceptible parents appear to contribute to the resistance. Among pathogen isolates* nec^{+} chl^{+} *types predominate.*

In the northern wheat growing areas of Australia, *Pyrenophora tritici-repentis* causes yellow spot, a major leaf disease of wheat. It also occurs sporadically in southern wheat growing areas. In Western Australia (WA), the pathogen occurs in combination with *Phaeosphaeria nodorum*, and losses of 5-20% are frequent in medium to high rainfall areas. Pink grain occurs sporadically in seasons that are very favorable for pathogen development. Disease levels have

increased in the last 20 years due to intensified cropping and adoption of stubble retention/reduced tillage practices. Fungicides can provide economic control but applications are rare because of uncertainty of seasonal growing conditions. Disease resistance is of high priority in breeding for white grained, high quality wheats in WA, Queensland (QLD), and northern New South Wales (NSW). Moderately resistant varieties have been recently released, thereby providing better opportunities for control. In the absence of varietal resistance, crop rotation and stubble burning are the main means of control.

Disease Management

Stubble management

The effects of stubble management and tillage practices on *P. tritici-repentis* in Australia are well understood (Rees 1987; Summerell and Burgess 1989). In QLD and northern NSW, stubble retention is important for erosion control of high clay-content soils during the wet non-cropping period. In WA, stubble is retained to prevent wind erosion of light textured, sandy soils during the dry non-cropping period. In the WA environment, disease induced by stubble-borne pathogens can cause yield losses of up to 46% under favorable conditions; losses of up to 15% are typical of normal conditions in medium to high rainfall areas in WA (Table 1). To manage these erosion risks and, at the same time, reduce the risk of yellow spot, stubble is frequently burnt immediately preceding the cropping phase, providing most of the benefits of erosion control but reducing inoculum of *P. tritici-repentis* and other diseases. Farmers are seeking systems in which stubble can be retained through cropping phases.

Crop rotation

Another important disease management option in WA is crop rotation. This practice reduces the impact of leaf disease by separating the new wheat crop from existing inoculum sources in time and space. The most common rotations involve leguminous crops (primarily *Lupinus angustifolius*) or self-regenerating pastures. Stubble retention is routinely practised in these situations because of erosion control benefits, but also because surface stubble

Table 1. Yield response to control of leaf disease caused by *Pyrenophora tritici-repentis* and *Phaeosphaeria nodorum* associated with stubble at three locations in Western Australia, 1995/96.

Treatment	Yield kg ha^{-1} (%) East Chapman 1995	Yield kg ha^{-1} (%) Wongan Hills 1996	Yield kg ha^{-1} (%) Mingenew 1996	% Leaf disease[1] W. Hills 1996	% Leaf disease[1] Ming. 1996
Nil stubble + Folicur 250EW[2]	1502 (100)	3019 (100)	2201 (100)	31	36
10-50 g m^{-2} stubble		2751 (91)	1466 (67)	64	
80-100 g m^{-2} stubble	973 (65)	2571 (84)	1196 (54)	71	88
LSD 5%	120	200	140	20	

[1] %F-2 GS74 at Wongan Hills, %F-3 GS32 at Mingenew.
[2] Either 2 or 4 applications of 125 g tebuconazole ha^{-1}, depending on location.

protects lupins from rain-splashed soilborne *Pleiochaeta setosa*, causing brown spot disease (Sweetingham *et al.* 1993). Since stubble-borne inoculum retained on the soil surface can remain viable for over two years (Summerell and Burgess 1989), there can be some risk of yellow spot development in the return wheat cropping phase of year-in year-out rotations. In these situations, new crops are established 18 months after the harvest of the previous wheat crop. A survey of stubble paddocks showed that the infectivity of 18-month-old stubble was similar to that of 6-month-old stubble in some situations (Table 2). While the frequency of such an occurrence in the field is low, disease carryover may occur, despite rotations, if significant quantities of surface wheat straw persist through the rotation phase.

Chemical control

Rotation effectively reduces the amount of early inoculum on crops; however, wheat grown in rotation does not remain disease free, presumably due to the movement of airborne inoculum from adjacent areas. Well managed crops grown in rotations still exhibit some yield loss from leaf disease. Fungicide seed dressings appear ineffective (Rees and Platz, unpublished data). Over the last five years, tebuconazole and flutriafol sprays have been registered for broadacre use in Australia and now compete with propiconazole. In-crop control represents another management option for *P. tritici-repentis*. In WA, single applications of these fungicides at rates of 31-62 g ai ha^{-1} are the most cost effective, and are being commercially evaluated and adopted on a small scale. At costs of around A$ 25 ha^{-1}, treatments are only economic with minimum yield increases of around 150 kg ha^{-1} at present wheat prices. This is most readily achieved in high yielding crops (around 3t ha^{-1}, compared with the WA state average of 1.5 t ha^{-1}) where yield responses over 5% are observed (Table 3). Treatments applied around flag leaf emergence can provide economic control but are generally not practised because of uncertainty with seasonal growing conditions.

Table 2. The effect of 18-month- and 6-month-old wheat stubble on early leaf disease of wheat.

Age of stubble	% Disease (range)		
	1992	1993	1994
Nil stubble	2	5	0
18 months	1-12	5-19	0-80
6 months	40-75	87-95	88-97
LSD 5%	20	8	16

Note: Plots were sown and then field-collected straw applied to give 200 g wheat stubble m^{-2}. Disease was assessed on juvenile plants (Loughman, unpublished). For each year, the data show the range of disease intensities observed from a range of stubble samples in the two age classes. In one year (1994), some 18-month-old stubble induced similar disease as 6-month-old stubble.

Breeding for Resistance

Genetics and sources

Rees and Platz (1990) reported that resistance was available in Brazilian and CIMMYT material, and that some varieties combined yellow spot resistance with resistance to other diseases. Yellow spot resistance sources include BH1146 (selection), Chinese Spring, CNT2, Fink'S', Genaro81, Norin26, Ponta Grossa1, Red Chief, and Vicam71 (Rees

and Platz 1992). Resistance may be common in different sources (Rees and Platz, unpublished).

Resistance utilized in QLD has been found to be recessive and usually conditioned by several genes (Rees and Platz 1992). Between 250 and 300 F2 populations frequently fail to generate homozygous F3 families with resistance levels as high as the resistant donor. These studies are supported by those of Roake (unpublished) on crosses between Janz (susceptible) x Fink 'S' (resistant), Sunco (susceptible) x Fink 'S', Fink 'S' x Banks (susceptible), and Vicam71 (resistant) x Banks. The F3 populations from these crosses show that resistance is determined by at least three to four recessive complementary genes. A cross between two susceptible varieties, Sunelg- (toxin insensitive Sunelg selection) and Suneca (nec+ chl-, same infection type as cv. Glenlea), produced lines more resistant than either parent, though no lines were as resistant as the control Fink 'S'. Inheritance of this resistance was probably determined by three to four recessive complementary genes. Background effects are apparent in backcrossing, with resistance being more easily recovered with some recurrent parents than others. It is possible that such parents carry one or two minor genes for resistance.

Priorities, methods, and progress

The regional significance of yellow spot varies greatly throughout Australia and is reflected in the priorities of the respective breeding programs. Resistance is a priority in the breeding programs of QLD, northern NSW, and WA. Approaches to breeding in Australia include complex crossing and backcrossing strategies combined with glasshouse- and field-based screening. In the southeastern state of Victoria, stripe rust and septoria tritici blotch are the most important leaf diseases. Selection for yellow spot resistance is not practised, resulting in higher susceptibility levels (Table 4).

In QLD and northern NSW, yellow spot resistance is being developed in a backcrossing program using locally adapted Prime Hard quality parents. The backcrossing strategy enables the recovery of recessive resistance at levels similar to the resistant donor, and rust

Table 3. Effect of tebuconazole (Folicur 250EW) on disease caused by *Pyrenophora tritici-repentis* and *Septoria nodorum* measured over two years at Mingenew, Western Australia.

	1995			1996	
Fungicide rate	**% Flag (Z75)**	**Yield**	**Yield %**	**Yield**	**Yield %**
Nil	72	3.61	100	3.20	100
31 g ai ha^{-1} @ Z49	61	3.66	101	3.48	109
63 g ai ha^{-1} @ Z49	38	3.75	104	3.38	106
63 g ai ha^{-1} @ Z49&71	28	3.90	108	3.54	111
125 gai ha^{-1} @Z49	39	3.82	106	3.48	109
LSD 5%	17	0.16		0.185	

resistance and quality similar to the recurrent parent. Resistant x adapted crosses are made during winter and the F1 grown in summer. In the pre-winter period, more than 1500 F2 plants are screened at the two-leaf stage by inoculating with field-collected conidia (2 x 10^4 ml^{-1}). Leaf wetness is maintained for 40 h and seedling response assessed after 8 days at 14/23°C. Plants are then transplanted to the field where the first back cross is made to the six most resistant lines. The cycle is repeated over at least the next three years including progeny testing of the selected BCF2s at each stage and selection for agronomic type on field transplants. Based on seedling tests, the best 50 BC3F2s are selected and BC3F3s are produced in the winter of year 5. Single plant selections of these families are progeny tested through two more generations, and preliminary yield evaluation is begun in year 7, progressing through wide scale testing to potential release in year 12.

In 1993, line QT5360 (Vicam71/3*Hartog) was in wide scale testing but did not meet quality requirements. In 1995, Leichhardt (CNT2/4*Hartog) was released in QLD and has shown moderate resistance. Both lines express less yield loss in the presence of disease than the recurrent parent Hartog (Table 5). Leichhardt shows a high level of seedling resistance (Table 6). In 1997, seedling tests of lines from the Northern Wheat Improvement Program (QLD and northern NSW) will include 30 F2 families for selection and backcrossing, and progeny tests of 3300 BCF2 lines and 1100 BCF3 lines.

In WA, *P. tritici-repentis* resistance is sought in combination with *Phaeosphaeria nodorum* resistance as these diseases frequently occur together (Loughman *et al.* 1994). Moderate resistance has been derived from a broad range of genetic material using different, but often

Table 4. Mean responses to *Pyrenophora tritici-repentis* of current advanced lines from Australian regional breeding programs evaluated in the Australian Septoria Nursery AUSENXXI (1996).

Regional breeding program	Mean response (0-10 scale)	Std error	No. of lines evaluated
Victoria	8.0	0.6	8
Southern NSW	6.9	0.3	18
South Australia	6.4	0.4	17
Queensland[1]	6.1	0.4	7
Western Australia	5.4	0.4	13
Check varieties			
Aus20917(R)	4.3	0.3	
Leichhardt(R)	3.5	0.5	
Millewa(S)	6.5	0.6	

[1] Not including lines from the backcrossing program.
Source: Loughman and Wilson, unpublished.

Table 5. Comparison of moderately resistant lines QT5360 (Vicam 71/3*Hartog) and Leichhardt (CNT2/4*Hartog) with the susceptible recurrent parent Hartog and the very susceptible control Banks, infected with *Pyrenophora tritici-repentis*.

	Yield ($g\ m^{-2}$)		Relative yield Dis/fung (%)	% Flag leaf diseased
	Diseased	Fungicide protected		
1990				
QT5360	607	686	88	6
Hartog	519	672	77	56
1995				
Leichhardt	440	500	88	18
Hartog	317	398	80	81
Banks	176	274	64	33[1]
LSD 5% (1995)	29			

[1] Banks is later maturing.
Source: Rees and Platz, unpublished.

complex, crossing strategies. The program aims to improve resistance to both diseases (as well as to septoria tritici blotch and rusts) by continually crossing the most resistant, best adapted lines with resistance donors. Some resistance may have been obtained through the use of aluminum-tolerant parental material that also carries a range of disease resistance. Lines are selected for agronomic type, yield, and quality in early generations, and screening for *P. tritici-repentis* resistance commences prior to re-selection in the F5. Evaluation is field based. Septoria nodorum blotch development can reduce discrimination between lines varying in yellow spot resistance (Loughman *et al.* 1994), so disease is encouraged with strawborne inoculum in which *P. tritici-repentis* is naturally dominant. In this way, epidemics are generated in which *P. tritici-repentis* is estimated to cause 90% of infections.

Severe ascospore infections provide a simple, reliable non-labor intensive basis for discrimination of resistance in young plants up to stem elongation, while secondary infections are usually adequate to determine resistance expression at adult stages. However, like septoria diseases, adult plant responses are moderated by effects of plant maturity, and assessment of this character may be required to assist interpretation of host response. Around 2000-2500 lines are screened each year.

Line 84Z:1374 (Tables 7 and 8) has a high level of moderate resistance and was used in wide scale testing in 1992 but did not meet quality requirements. Variety Cascades (Tadorna.Inia/3*Aroona; Tables 7 and 8) was released in 1995, while Carnamah (Bolsena-1CH/77W:660; Tables 6 and 8) and Cunderdin (Flicker sister/Sunfield sister; Tables 6 and 8), moderately resistant to *P. tritici-repentis*, were released in WA in 1996. Two additional lines released in 1997 are Westonia (Spica Timgalen.Tosca/ Cranbrook:Jacup*2.Bobwhite) and Brookton (Torres/Cranbrook//76W596/ Cranbrook; Table 8), which have moderate yellow spot resistance. Other

Table 6. Seedling glasshouse responses (0-10) of moderately resistant wheats and susceptible check varieties to *Pyrenophora tritici-repentis*.

Wheat variety	Disease score (0-10)	
	Rep 1	Rep 2
Banks (VS)	10.0	10.0
Hartog (S)	9.0	8.5
Leichhardt (MR)	4.0	3.2
Aroona (S)	8.0	8.0
Cascades (MR)	6.0	5.0
Carnamah (MR)	7.0	5.0
Cunderdin (MR)	4.5	4.8

Source: Rees and Platz, unpublished.

Table 7. Yield (kg ha^{-1} and relative to fungicide protected plots) of wheat varieties affected by *Pyrenophora tritici-repentis* and *Septoria nodorum* at East Chapman, Western Australia, 1995.

Wheat variety	Yield (diseased, kg ha^{-1})	Relative yield (diseased, % of fungicide protected)
84Z:1374	1393	77
Cascades	1317	71
Leichhardt 1014	1014	69
Aroona	892	61
Gutha	835	53
Hartog	704	50
LSD 5%	280	18

Source: Loughman and Wilson, unpublished.

lines undergoing final evaluation with moderate yellow spot resistance include W487118 (a sister to Westonia), 86Z:1494 (77W660/Spear.12IBWSN362), and W486197 (a sister of 86Z:1494). The resistance sources of these varieties or advanced lines are not known but are diverse.

Pathogen variation

Thirty-seven isolates from WA and northern NSW were tested using the differentials of Lamari and Bernier (1989) and selected Australian lines (Roake, unpublished). All isolates except one (R15 from Western Australia) were nec+ chl+ type described by Lamari and Bernier (1989). Isolate R15 was the nec+ chl- pathotype, being avirulent on 6B365 but virulent on Glenlea. Tests conducted on other lines showed differences in the isolates not revealed by the Canadian differentials. R15 was avirulent on the toxin-insensitive lines and selections Columbus-, Cranbrook, Excalibur-, and Sun 234A, but virulent on toxin-insensitive lines Sun 124A and Sunelg-. Isolate R40 was virulent on 6B365, but avirulent on Columbus- and Sun 234A, and less aggressive on resistant lines and susceptible toxin-insensitive lines compared to other isolates. Isolate 8.3 appeared to be avirulent on Columbus-, but virulent on all other susceptible toxin-insensitive lines. Isolate Su6 was as virulent on Columbus- as on Columbus+,

Table 8. Heading day (day of year), % flag leaf diseased, and relative grain weight (grain weight of diseased plots as % of fungicide sprayed plots) of moderately resistant wheat varieties affected predominantly by *Pyrenophora tritici-repentis* in small row plots, South Perth, 1995 and 1996.

	1995			1996		
Wheat variety	HD[1] (d.o.y.)	Flag[2] (%)	RGW[3] (%)	HD (d.o.y.)	Flag (%)	RGW (%)
Gutha (S)	220	68	81	227	89	81
Westonia	219	29	92	236	25	92
Leichhardt	225	28	91	227	84[4]	68[4]
Cunderdin	229	24	84	243	16	96
Hartog (S)	229	85	70	237	70	81
Aroona (S)	231	39	81	238	48	77
Cascades	232	13	80	240	19	91
486WW197	233	20	87	242	10	93
Vicam71 (R)	-	-	-	243	36[4]	74[4]
Carnamah	233	18	78	245	17	91
84Z:1374	235	6	87	245	14	93
Millewa (S)	236	41	70	238	49	79
Brookton	236	12	99	245	16	107
CNT2 (R)	236	6	8	245	10	106
LSD 5%	13	13	17		27	19

[1] Heading date: day of year.
[2] % flag leaf diseased.
[3] Relative grain weight.
[4] High disease and low grain weight for Leichhardt and Vicam71 are due to the presence of *S. nodorum*, to which these varieties are very susceptible.

Note: Control varieties have long term responses to *P. tritici-repentis* shown in brackets. Comparisons for % flag leaf disease should be made between varieties with similar heading dates because maturity influences disease expression. Varieties are listed in 1995 heading date order.

whereas all other isolates appeared less virulent on Columbus- than on Columbus+. Necrosis was apparent on Columbus- with isolate Su6. In conclusion, some specificity is present in these isolates, though none were virulent on the range of resistance sources that were tested. Variation in aggressiveness of isolates was also apparent.

Summary

In Australia, the impact of yellow spot is very regionally and seasonally dependent. Disease can be managed by rotation, stubble reduction, and varietal resistance. Stubble reduction is unsustainable on some soil types. In these situations, moderately resistant varieties are assisting growers in developing economic and sustainable farming systems.

References

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Constraints on the Integrated Management of Spot Blotch of Wheat

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Abstract

Spot blotch of wheat, caused by Bipolaris sorokiniana, *occurs in all Latin American countries and affects all plant parts. The pathogen is transmitted through contaminated or infected seed and can adversely affect germination and development of the root system or kill the seedling within a few days. The disease is capable of causing up to 100% yield loss. Integration of different disease control methods, although partially adopted, is being done in most countries of the Southern Cone region. Methods include varietal resistance, induced resistance, varietal management, soil and residue management, crop rotation, and chemical control. Nonetheless, the disease is still problematic and poses a potential threat to wheat cultivation in the region, mainly because of political, technological, and socioeconomic constraints.*

Spot blotch of wheat, caused by *Bipolaris sorokiniana* (Sacc.) Shoem., occurs throughout the world, but severe losses occur in Bangladesh, Bolivia, Brazil, Paraguay, and Zambia. The disease affects all plant parts and can cause up to 100% yield loss (Mehta 1993). The pathogen is transmitted through contaminated or infected seed, may adversely affect germination and development of the root system, and can kill the seedling within a few days, depending on the severity of infection. Yield losses due to the common root rot phase of the disease have been reported at 5-20% (Diehl *et al.* 1983). No major resistance is available, and disease control depends on an integrated management approach.

Integrated disease management (IDM) is not a discipline but a technique. We do not do research on IDM, as such, but instead focus on individual control measures for a particular disease or group of diseases. Later, we determine whether or not those control measures fit a particular production system and whether they interfere with other existing technologies (Figure 1). Once efficient disease control methods are developed, efforts are made to integrate them to form a disease management system. It then becomes necessary to ensure the appropriate use of the IDM system and to identify the constraints for its adoption. In recent years, IDM has been partially practiced in Brazil and includes the following components.

Varietal Resistance

Generally speaking, highly susceptible cultivars are quickly replaced by less susceptible cultivars. Nonetheless, most wheat cultivars are still quite susceptible or else their degree of

resistance is insufficient (Hetzler *et al.* 1991; Mehta *et al.* 1992). Some sources of resistance are already available (Mehta 1993). Wheat cultivars with some resistance to spot blotch also offer some resistance to tan spot (Mehta *et al.* 1996).

Triller and Mehta (1997) studied the influence of flag leaf age on expression of *B. sorokiniana* resistance in wheat under controlled environmental conditions. The results of their study using 17 genotypes indicated that the older the flag leaves were, the higher their susceptibility to infection by *B. sorokiniana*. The correlation coefficient (r) between flag leaf age and the average percentage of infected leaf area was 0.94 (Figure 2). These authors suggested that in conducting studies on virulence/sources of resistance, plant growth stage DC 56 (3/4 of the inflorescence emerged) should be used because flag leaves show the highest susceptibility to the pathogen at that stage.

The higher predisposition at an advanced growth stage such as DC 56 is very problematic for the wheat crop. Under favorable weather conditions at this growth stage, i.e., continuous rains for 5-6 days followed by relatively higher temperatures (daily average of 20-23°C), a spot blotch epidemic develops very

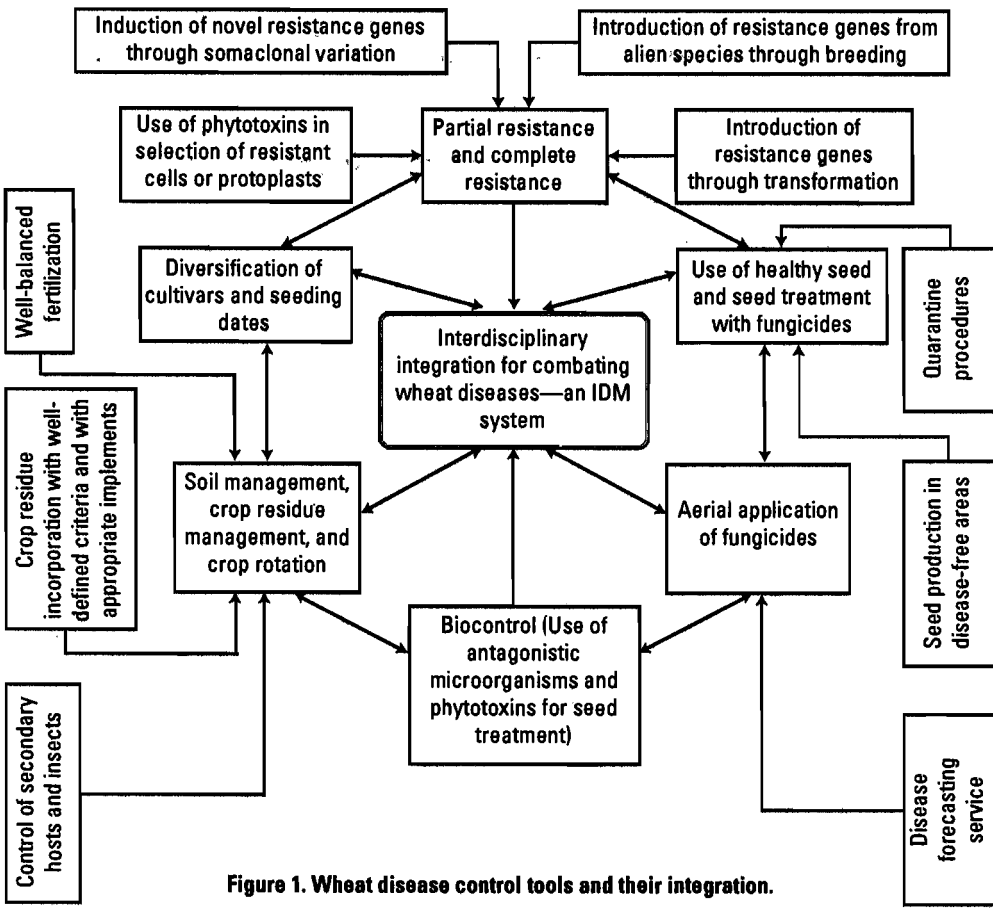

Figure 1. Wheat disease control tools and their integration.

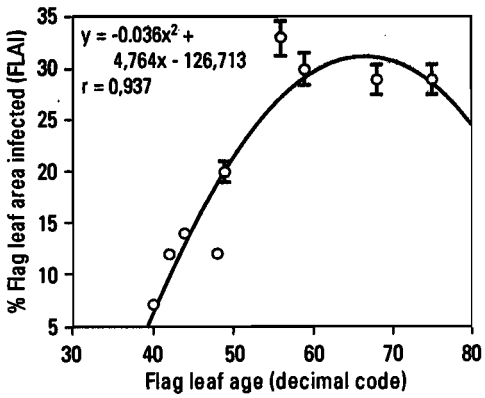

Figure 2. Effect of flag leaf age of 16 wheat genotypes on the average percentage of FLAI by *B. sorokiniana* seven days after inoculation.
Source: Triller and Mehta 1997.

rapidly and is capable of causing almost total yield losses and very poor grain quality, especially in more susceptible cultivars. Such epidemics were recorded several times during the past two decades (Mehta 1993).

The effect of flag leaf age on the severity of *B. sorokiniana* infection is significant in studies aimed at identifying resistance sources and incorporating their resistance into agronomically desirable cultivars. For virulence studies conducted in the laboratory using leaf portions, leaf age should be considered in order to obtain consistent and comparable results.

In 1989-1994 the level of resistance of 31 wheat genotypes was studied using the area under the disease progress curve (AUDPC) developed at CIMMYT, Mexico, (Mehta 1993; Table 1). Attempts were made to transfer resistance from genotypes that showed higher levels of resistance to agronomically desirable cultivars of wider adaptation using two backcrosses. Fixed lines showing higher levels of resistance and desirable agronomic characters were selected in bulk and included in the preliminary yield trial (Mehta *et al.* 1996).

Although new sources of resistance may be available, breeders and pathologists still face the challenge of pinpointing genes that offer better levels of resistance.

Transferring resistance genes from alien species is problematic because of the ploidy problem. Nonetheless, some progress in transferring resistance genes from alien species (*Thinopyrum curvifolium, Elymus curvifolius,* and *T. tauschii*) to wheat germplasm has been achieved (Mujeeb-Kazi *et al.* 1996a, 1996b).

Biotechnological Approach

It has been long suspected that spot blotch resistance in wheat is governed by quantitative traits, and no major resistance genes are known to exist (Mehta 1993). As stated earlier, though sources of higher levels of resistance have been identified, little progress has been achieved using conventional breeding methods, mainly because of the polygenic nature of this resistance. It is therefore necessary to identify the quantitative trait loci (QTLs) where segregation does not follow Mendelian laws. Only then will it be possible to clone such genes. Once the genes are cloned, transformation methods may offer new options for combating the disease in the future.

In recent years several transformation techniques have been developed, e.g., *Agrobacterium*-mediated transformation, protoplast-mediated transformation, microprojectile-mediated transformation, tissue electroporation, and microinjection of DNA. Generally speaking, these techniques are highly sophisticated and depend on the availability of an efficient and reproducible tissue culture system (Jahne *et al.* 1994). Induced variation for higher levels of resistance is also expected

Table 1. Sources of resistance to *Bipolaris sorokiniana* identified in Paraná, Brazil, 1992-1994, at three locations, considering the area under the disease progress curve (AUDPC).

Wheat genotype	Average AUDPC at three locations during 1992-1994[1]									Overall average
	Londrina			Palotina			S.M. do Iguacu			
	1992	1993	1994	1992	1993	1994	1992	1993	1994	
1. CEP 14(PEL 72380/ATR)	148	114	292	614	120	50	504	328	334	278
2. YM#6	135	114	424	890	360	139	604	788	335	421
3. IA 814 (T.AEST/4/TP//CNO/NO/3/ CNO/7C/5/JUP)	179	77	392	1044	248	158	1040	657	387	465
4. CNT 8	213	114	684	600	203	179	554	1480	234	473
5. 8230-54/32*FR/KAD//CB/4PAT(B) /5/CKA "S"	218	121	342	694	360	217	1000	1084	335	485
6. CNT 1(PF11.100-62/BH1146	135	57	392	1420	120	99	850	920	646	515
7. MON "S" MN 72131-CM52721-4Y-2Y-5M-1Y-1M-1Y-0M	192	121	442	960	278	89	1174	1117	387	529
8. SUZHOE F3#8-18B-0Y	161	261	442	1110	248	159	1050	1117	335	542
9. BR 8(IAS/TP//PF70100)	192	191	474	970	240	317	1174	888	840	587
10. (NADXTMP-C112406/BJY"S") TIP	218	702	702	894	278	217	1060	887	335	588
11. CEP 76146	347	77	442	1304	278	129	1710	657	417	595
12. LD 8254	217	147	392	1150	488	217	1200	1117	440	596
13. MON "S" MN 72131-CM52721-4Y-2Y-6M-1Y-1M-1Y-0M	187	202		864	398	217	1124	1183		596
14. VEE"S"	226	191	442	1040	713	80	1124	1314	335	607
15. BON/YR/3/F 3570//KAL/BB	193	121	762	1100	375	80	1024	1183	640	608
16. SUZHOE F3#8-28B-0Y	225	267	618	1240	360	80	1314	1083	307	610
17. PAT 7219(S12/J 9280.67//NB/TP)	347	109	652	1110	278	317	1490	920	335	617
18. IAS 20(COLONIAS/FRONTEIRA/ KENIA 58)	315	235	524	1444	240	229	1430	854	387	628
19. BH 1146(FRONTEIRA/ MENTANA//PG1)	446	114	884		450	279		1215	1109	642
20. KVZ/HD 2009	219	197	652	1300	285	267	1154	1348	387	645
21. IA 7988 (KINGLET "S")	161	197	702	1110	203	217	1414	1513	335	650
22. CEP 11	252	300	606		360	367	1314	1346	692	654
23. SUZHOE F3#8-12B-0Y	225	350	492	1064	450	415	960	1150	840	660
24. LD 7831 (I 73. 4896)	252	223	752	1380	413	456	1620	755	440	699
25. SUZHOE F3#8-22B-0Y	250	375	458	1440	338	465	1224	1413	387	705
26. MON"S"CM 8288-A3-M-6Y-5M-1Y-0M	219	158	1332	1190	413	159	1454	1183	335	716
27. IA 807(KVZ/K4500 L. 6 A. 4)	225	235	492	1680	375	129	1390	1743	640	767
28. ANB "S"/YACO "S"	445	147	1756		585	317		1478	1250	854
29. MITACORE (Susceptible wheat check)	757	790	1390	1380	863	712	3200	2498	1656	1471
30. IT 8037 (Susceptible triticale check)	768	1645	1850	2810	1350	929	3880	3220	1673	2013

[1] Calculated using the AUDPC computer program developed by CIMMYT, Mexico.

through somatic embriogenesis (Vasil and Vasil 1986; Mehta 1996; Bohorova *et al.* 1995).

Phytotoxins as Agents of Specificity

Bipolaris sorokiniana produces toxins made up of different compounds, e.g., helminthosporal, helminthosporol, pre-helminthosporal, and other structurally related compounds. Of these, pre-helminthosporol is the most active and is produced in abundance (Carlson *et al.* 1993; Liljeroth *et al.* 1993; Olbe *et al.* 1995). Although pre-helminthosporol sensitivity is not correlated with known *B. sorokiniana* resistance levels in barley cultivars, it is supposed to play an important role in pathogenesis by killing or weakening plant cells in advance of the growing hyphae (Liljeroth *et al.* 1993; Olbe *et al.* 1995). Similar studies on the wheat-*B. sorokiniana* system are necessary, even though pre-helminthosporol does not show host specificity.

Pyrenophora tritici-repentis, causal agent of tan spot of wheat, is known to produce an HST (Lamari and Bernier 1989). The toxin produced by this fungus is cultivar specific, induces necrosis in the host and is a pathogenic factor (Brown and Hunger 1993; Lamari *et al.* 1995). Thus, if host-selective toxin is the agent of specificity, it should be possible to clone genes for pathogenicity and resistance (Walton and Panaccione 1993). A host- or cultivar-selective toxin is also very useful in tissue culture work involving early selection of resistant cells or protoplasts (Lamari and Bernier 1989).

Cultivation and Crop Rotation

The most common tillage system is still the conventional one where the heavy disc harrow used for the primary tillage causes such serious problems as soil compaction and soil pulverization.

No till is a form of conservation tillage. A conservation tillage system maintains significant crop residues on the soil surface and reduces tillage and field traffic (Elliott *et al.* 1987). Due to its advantages, the no till/conservation tillage system is fast gaining importance on the American continent. The area under conservation tillage in the United States, for example, is projected to be 63-82% of the total planted cropland by the year 2010 (Rothrock and Hargrove 1987; Rothrock 1995).

Changes in cultural practices affect the severity of some diseases (Mehta and Gaudencio 1991; Rees 1987; Reis and Abrão 1983; Reis *et al.* 1983). Mehta and Gaudêncio (1996) studied the effects of crop rotation on root rot severity based on the average of three experiments conducted over a nine-year period (1986-1994). The severity of common root rot was variable and dependent on climatic factors, which vary from year to year (Figure 3). The average degree of infection (DI) increased during the first four years and gradually declined during 1991-1994. In crop rotation treatments where wheat was grown every 2-3 years or in alternate years, average root rot intensity was lower than in rotations

where wheat was grown every year. After the fifth year of testing (1991), there was no difference in the severity of common root rot between rotations where wheat was grown in alternate years and where wheat was grown every 2-3 years (Mehta and Gaudêncio 1996).

In some areas, the recent trend in conservation tillage is to use annual legumes as cover crops to provide mulch, prevent soil erosion, and supply part of the N requirement for the subsequent cash crop. Several alternative legume cover crops are now available (Calegari *et al.* 1993). However, legumes suffer from several diseases caused mainly by fungi that may produce losses of up to 100%. Little information is available on the impact of cover crops on disease damage in a subsequent cash crop such as wheat. Fungal pathogens considered likely to cause serious disease problems are species of *Sclerotium, Rhizoctonia, Phytopthora, Fusarium, Ophiobolus,* and certain other foliage-infecting fungi (Pratt *et al.* 1987).

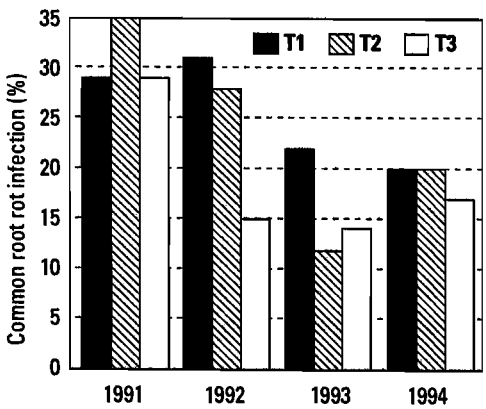

Figure 3. Common root rot of wheat under no-till cultivation during 1991-94, Londrina, PR.
T1 = wheat every year; T2 = wheat every 2 years; T3 = wheat every 3 years.
Source: Mehta and Gaudencio 1996.

Since use of legumes (either as cover or cash crops) influences certain soilborne plant pathogen populations, the importance of such pathogens needs to be examined in terms of damage to specific crops in a particular crop rotation sequence (Rothock and Hargrove 1987). Selection of a particular legume for a crop rotation system should be done cautiously, based on their usefulness in breaking the disease cycle. For example, pigeon pea and lupin (*Lupinus angustifolius*) should not be sown before soybean in problem areas because they increase the probability of soybean stem canker (Galerani 1994). In smaller areas, peas, pigeon pea (*Cajanus cajan*) and chickpea (*Cicer arietinum*) could be grown successfully in a crop rotation system. In northeast Santa Cruz, for example, one of the chickpea varieties introduced from ICRISAT, Hyderabad, India, yielded as much as 3.9 t ha^{-1} with little more than 60 mm rain between germination and harvest.

Very little information is available in the literature on the occurrence, importance, and survival mechanisms of diseases that attack legumes under conservation tillage. Survival of propogules of the spot blotch pathogen on residue of different legume cover/cash crops, for example, needs to be investigated.

Varietal Mixtures

Though so far there is not enough local data on managing spot blotch using varietal mixtures, attempts should be made to generate such information.

Varietal mixtures could be successful since they are already producing promising results in wheat (Browning 1988; Wolfe 1988; Mehta 1993). A field trial on varietal mixtures was carried out in Brazil and Bolivia in 1992 and 1993, respectively. Based on agronomic traits, two wheat cultivars, Genaro and Opata, were selected; they were seeded separately and in a mixture containing equal proportions of seed. The yield of the mixed plots was compared with the average yield of the two cultivars grown separately, with or without fungicide application. During both years, yields of the mixed plots were 15.3-22.7% higher than the average yields of the cultivars grown individually. This was because of lower disease intensity and less water stress during the critical stages of crop development in the mixed plots. The increase in yield was due to the increase in grain number and grain weight per spike. Further testing is necessary to generate local data before official recommendations on varietal mixtures can be made.

Chemical Control

Unless soil inoculum is reduced, seed treatments alone offer no benefit. Though several systemic fungicides for seed treatment are now available, the decision to treat the seed should be carefully considered. In Brazil, for example, seed treatment is recommended based on seed health analyses plus soil condition and crop rotation. The decision to apply seed treatments should be based on the following. Do not apply seed treatment if the seed infection level is less than 20% (as determined by the blotter test), and germination is within standards. Seed lots with less than 20% infection should be treated only if percent germination is low and the standard limit is reached after seed treatment. Seed lots with more than 20% infection should be treated with fungicide only if percent germination is lower than the standard and there is a shortage of seed. Seed lots may be treated with fungicides irrespective of the level of infection, especially for seeding in new areas or in areas where crop rotation is practiced.

However, in recent years only a limited number of farmers have followed such criteria, for two reasons. First, seed health tests are not required for establishing seed standards and, second, seed health tests are expensive and performed by only a few laboratories. Unless seed health tests are a prerequisite for setting seed standards, establishing seed treatment criteria would be pointless.

Foliar fungicides belonging to the dithiocarbamates, such as mancozeb, and triazoles, such as propiconazole, tebuconazole, flutriafol, iprodione, procloraz, and triadimenol, are known to be effective (IAPAR 1996). The first spray should be applied soon after disease symptoms appear, which may be 45-55 days after sowing for the spring wheat cultivars (Mehta and Igarashi 1985).

General Considerations

The increase in the severity of some wheat and soybean diseases has been linked to changes in cultivation practices. A lack of integration among scientists of different disciplines may sometimes be a factor limiting the success of IDM. Undoubtedly, severity of some wheat diseases can also be affected by the inadequate and untimely use of different technologies in the preceding and/or subsequent agro-ecosystems.

The major constraints to IDM are: 1) inadequate resources for research and extension; 2) defective technology transfer mechanism and inadequate extension services; 3) inadequate biological monitoring systems; 4) IDM strategies based on commodity and not usually on production systems; 5) lack of IDM demonstrations; 6) inadequate international cooperation on plant protection/quarantine problems; 7) lack of integration between government organizations and cooperatives, industry representatives and farmers; 8) sophisticated and/or costly IDM programs; and 9) lack of interdisciplinary integration. In fact, all these constraints can be grouped into three major categories: political, technological, and socioeconomic constraints.

Conservation tillage technologies that avoid soil degradation and disease management practices that reduce yield losses and production costs need to be combined and harmonized to achieve successful sustainable IDM programs.

IDM technologies must be compatible with all other technologies used within the cropping systems in a particular agro-ecological zone. IDM systems are regionally based and developed to suit different agro-ecological or crop production systems. Development of IDM systems should include new approaches such as varietal mixtures and crop rotation strategies using non-commercial crops like chickpea, pigeon pea, and common beans wherever suitable. Pesticides are imported in developing countries and crop productivity is generally low. IDM systems should reduce production costs, stabilize productivity, and, at the same time, protect the environment.

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Components of the Tan Spot Disease Cycle

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Abstract

The disease cycle of wheat tan spot is being studied both as a model system to test new information technology and as a means to better understand the epidemiology of this economically important disease. Inoculation, infection, and conidial liberation and dispersal were assessed in the field with a mobile bioassay. Inoculation, conidiation, and liberation were examined in growth chambers and using a crude wind tunnel. Pyrenophora tritici-repentis *normally conidiates diurnally, but conidiogenesis proceeded in culture at 13°C in constant light and on moist wheat leaves at 20°C in constant darkness. Fewer conidia were formed on diseased areas of leaves subjected to interrupted wet periods than on constantly wet leaves, which bore 22 conidia* mm^{-2} *after 96 h. The rate of conidial liberation was lower than expected from studies on detached leaves. While conidia are relatively large, they may be dispersed further than 10 km in turbulent air flow. Conidiophores were about 4% as infective as conidia, and mycelium was largely uninfective when susceptible plants were exposed to a 24-h wet period after experimental inoculation. In North Dakota, environmental conditions conducive to natural inoculation often coincided with a suitable infection period. Dew periods after intermittent rains were conducive to tan spot development, and inoculum potential was much greater after flowering than before. Artificial neural networks, a novel mathematical processing strategy, outperformed statistical regression techniques and discriminated daily tan spot infection periods with as much as 87% accuracy. Neural networks thus appear well suited for disease forecasting.*

The disease cycle of wheat tan spot is being studied at North Dakota State University (NDSU) as a model system to test new disease forecasting technology and to better understand the epidemiology of this economically important disease. The primary disease cycle on wheat is initiated by ascospores and conidia, borne on infested plant residues, that bridge host cropping periods (Adee and Pfender 1989). The secondary disease cycle begins with conidiation on post-latent primary lesions and advances when conidia successfully liberate, disperse (if the same leaf is not re-inoculated), inoculate, and reinfect wheat (Figure 1). The secondary cycle repeats under favorable environmental conditions until host maturity (Wright and Sutton 1990).

Each of the secondary disease cycle components, with the exception of the latent period, has been studied to date in the author's lab; research reports are available elsewhere (De Wolf and Francl 1997; Francl 1995; Francl *et al.* 1995; Francl and Jordahl 1994; Riede *et al.* 1996; Stover

et al. 1996). The secondary cycle of tan spot has received the most emphasis because this phase of the epidemic is usually the most damaging, and a forecasting system may alleviate loss (Rees and Platz 1983). Herein, an overview of tan spot epidemiological research at NDSU and a comparison of recent findings with scientific reports in the literature is given.

Conidiogenesis

Pyrenophora tritici-repentis has unique environmental requirements for conidiation, which makes for an interesting model system. High humidity and moderate temperature promote conidiation in culture under a suitable photoperiod (Platt and Morrall 1980a; Platt *et al.* 1977). *Pyrenophora tritici-repentis* normally forms conidiophores in the light and conidia in the dark (Khan 1971; Platt and Morrall 1980a), but an isolate conidiated *in vitro* under constant light when temperatures were held between 10-15°C (Odvody and Boosalis 1982). This observation was repeated with isolate Pti2 (L. Francl, unpublished); cultures held at 13°C sporulated in constant light but

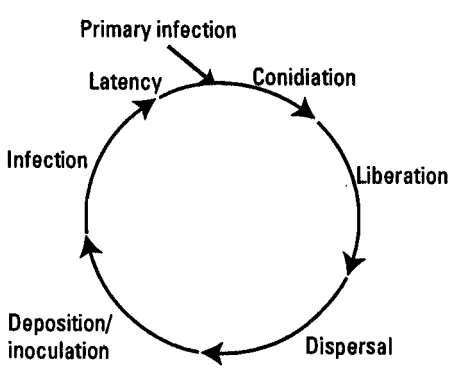

Figure 1. The secondary disease cycle components of tan spot of wheat.

replicate plates at 16°C did not. The physiological mechanism responsible for temperature-mediated diurnal sporulation is unknown. Conidiation *in vitro* in constant darkness has not been reported previously.

Our understanding of conidiogenesis in nature is limited by the few results regarding conidiation on host tissue. Riaz *et al.* (1991) developed a procedure wherein infected wheat leaves were detached and incubated in moist chambers. Conidiation was measured by briefly grinding the leaf in a known quantity of water. Host resistance reduced conidiation per leaf and sporulation was reduced on young leaves on the resistant but not the susceptible genotype (Riaz *et al.* 1991).

A nondestructive leaf measurement (Laser area meter, CID Inc., Vancouver, WA) allowed this technique to be modified at NDSU such that leaves remained attached to the plant until conidia were sampled. The susceptible line ND495 was inoculated after flowering with isolate Pti2 and exposed to moisture for 24 h. After 12 days in a growth chamber, plants were treated to 12-96 h of varying wet and dry conditions and photoperiods. A visual approximation of percent disease area yielded an *in planta* estimate of conidia formed per lesioned area.

Conidiogenesis on continually moist wheat leaves at 20°C was prevented in constant light but proceeded in constant darkness. Constantly wet leaves in alternating 16 h light and 8 h dark

periods bore 12 conidia mm^{-2} lesion after 96 h. Diseased areas of leaves wetted only during the dark period had 1.7 conidia mm^{-2} after 96 h. Conidial levels between constant wetness during alternating photoperiods and dryness during light periods were intermediate when 4-h wet periods interrupted dry periods during the beginning, middle, and end of the light cycle (Figure 2).

These results show that conidiogenesis does not take place on leaves at a temperature above 15°C in constant light and so confirm *in vitro* results. However, conidiation on leaves held in constant darkness differed to *in vitro* results, yet data agreed with previously reported *in vivo* results with another diurnal sporulator (Houston and Oswald 1946). Also, conidiation occurred when leaves were wet only during darkness, which suggests that nighttime dew is sufficient for conidiation in outdoor environments.

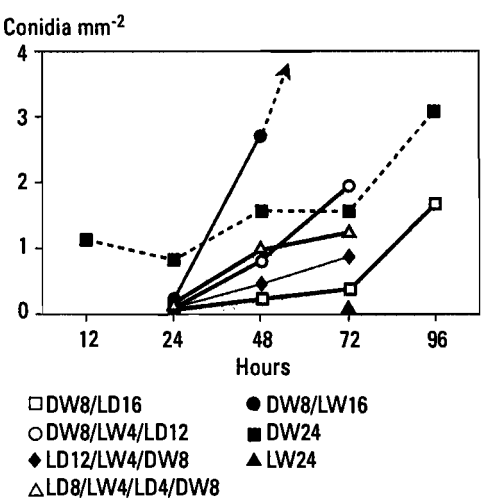

Figure 2. Photoperiod and moisture duration effects on conidiogenesis of *Pyrenophora tritici-repentis* on diseased wheat leaves.
D = Dark, W = Wet/L = Light, D = Dry
i.e., DW8/LD16: Dark Wet 8 h and Light Dry 16 h.

Conidiation levels were increased when a period of wetness occurred at some time during the light period, suggesting that evaporation of dew after sunrise or precipitation during the day may enhance sporulation in nature.

Liberation

Liberation and transport of conidia are necessary for alloinfection in the secondary disease cycle. Platt and Morrall (1980b) observed that most conidia were detached from conidiophores after a 5 min exposure of sporulating lesions to a moderate air flow passing over the leaf surface. Low humidity fostered liberation experimentally and was thought largely responsible for peaks in conidial occurrence commonly observed during dry afternoons (Maraite *et al.* 1992; Morrall and Howard 1975; Platt and Morrall 1980b). Based on their results, Platt and Morrall concluded that in a low humidity environment, essentially all conidia were liberated the day after formation.

The modified procedure of Riaz *et al.* (1991) provided a system to study liberation in a crude wind tunnel. Air speed measurements were taken at the height of flag leaves at fixed distances from a bank of six fans. Potted adult plants of ND495 were infected with isolate Pti2, incubated for 10-12 days, and placed in constant wetness in alternating light and dark cycles for 48 h to promote conidiation. Two flag leaves were initially sampled, pots were placed in a known airflow for 6 or 24 h under ambient RHs <40%, and finally two flag leaves were

sampled after treatment. Although the experiment is incomplete, preliminary results indicate that the rate of conidial liberation was much lower than expected from studies conducted with detached leaves. Further wind tunnel and field experiments will provide an estimate of liberation rates under various conditions.

The putative difference between the preliminary results and those of Platt and Morrall (1980b) may be explained, at least in part, by the different definitions of conidial liberation; i.e., the detachment from conidiophores vs. separation from leaves. Also, leaves still attached to plants twist and flop so most conidia are probably not oriented perpendicularly to the air stream. The conclusion of Platt and Morrall regarding liberation in nature is not supported by these preliminary findings.

Dispersal

Conidia typically follow a steep dispersal gradient because of their large size, but may be transported longer distances if sufficient lift is encountered (Khasonov *et al.* 1990; Schilder and Bergstrom 1992). Gradients of disease spread were also steep when measured from a small source (Sone *et al.* 1994) or from stubble (Rees 1982). Abundance of airborne conidia usually shows both a seasonal and daily periodicity with peak levels generally occurring late in the growing season and during afternoon hours (Maraite *et al.* 1992; Morrall and Howard 1975).

Airborne conidia of *Pyrenophora tritici-repentis* and environmental conditions were monitored at NDSU to infer dispersal distance and temporal pattern. Conidia were measured with Burkard and Hirst samplers in a 1.0 ha wheat field and on a rooftop 15 m above the ground. Conidia became abundant during dry afternoon hours in a wheat field after anthesis, which confirmed earlier reports on periodicity (Figure 3b). A high number of conidia depended on prolonged wet periods from rain and heavy dew (distillation) on preceding days. Conversely, conidia were found infrequently before anthesis and the hourly distribution of conidia lacked a predominant afternoon peak (Figure 3a).

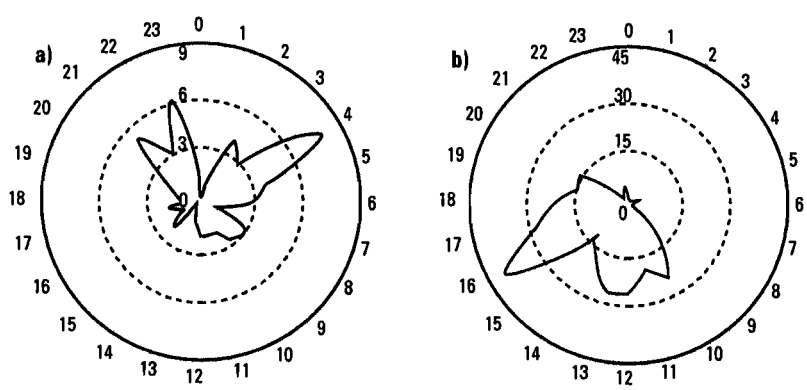

Figure 3. a) Early season and b) late season abundance of *Pyrenophora tritici-repentis* conidia in 1992 by hour of occurrence.

Samples taken from the rooftop showed that conidia could be found remote from any potential source. The rooftop conidial incidence was sparse and independent of field incidence early in the season but was more closely coincident with field incidence after anthesis. Wheat plants became infected by *P. tritici-repentis* after rooftop exposure, indicating viable conidia were being dispersed.

Conidia could have traveled from less than 1 km to more than 10 km after reaching the roof, according to a deterministic forward trajectory model. Also, conidia had to have traveled some distance to reach the roof and more complex models would be needed to reproduce faithfully the complexity of turbulent air flow. Perhaps errors in predicted distances are sufficiently low for demonstrative purposes. While conidia of *P. tritici-repentis* are relatively large, their seasonal pattern, occurrence remote from a source, and the trajectory model indicate that at least some conidia are dispersed moderate to long distances in the northern Great Plains.

Inoculation

Tan spot will develop on a susceptible host if an infective propagule is deposited on a receptive surface in a conducive environment. Although deposition *per se* has not been studied at NDSU, inoculation under controlled conditions has been refined to some degree (Francl and Jordahl 1994). Conidiophore inoculation of wheat seedlings susceptible to tan spot (ND495) produced a lower infection phenotype, fewer infection sites per cm^2, and less severe damage than conidia at equivalent dosages. Hyphal fragments were rarely infectious under a 24-h post-inoculation wet period. The relative reaction of a resistant wheat variety (Erik) to the three types of inoculum was similar to ND495. Inoculum concentration and type particularly influenced categories of the infection phenotype scale that included coalescence as a criterion. The distal leaf half was more severely damaged and usually had more infections per unit area than the proximal half. Infection type data from the middle of the uppermost fully expanded leaf at the time of inoculation should effectively discriminate resistant and susceptible genotypes. Research laboratories that screen germplasm may compare their results with some confidence if inoculum dosage, infection period environment, and collection of information were uniform.

Infection

Controlled climate research

Moisture and temperature are primary determinants of tan spot infection; indoor lighting seems to have little effect. On susceptible genotypes, a minimum wet period of 6 h was necessary for conidial infection of inoculated leaves (Hosford *et al.* 1990). Tan spot severity increased with longer wet periods on susceptible but not resistant genotypes (Lamari *et al.* 1992). Thus, temperatures around 20°C and 24 h of wetness are suitable conditions for reliable infection (Lamari *et al.* 1992). Leaves infected under these conditions

often show water-soaked areas, indicative of infection sites, for 10-20 min after removal from the wet chamber (J. Jordahl and L. Francl, unpublished).

The tan spot screening program at NDSU recently tested wheat germplasm from a variety of sources (Riede *et al.* 1996). Plants were inoculated at the two-leaf stage with a composite of four fungal isolates, and at the adult stage with isolate Pti2. A subset of germplasm was treated at the seedling stage with necrosis toxin, crude culture extract, and conidia; all three of which were produced by *P. tritici-repentis* isolate 86-124 (nec+ chl-). Adult disease reactions most often, but not always, agreed with seedling evaluations, and some of the variation could be explained by sensitivity or a mixed reaction to necrosis toxin (Table 1). Infiltration of leaves by purified necrosis toxin was a reliable way to detect a major factor involved in resistance; however, toxin-insensitive wheats could become diseased, so screening with necrosis toxin alone is inadvisable.

Outdoor studies

Infection was assessed in the field with a mobile bioassay to discretize days conducive or unconducive to infection (Francl 1995). Healthy wheat plants were placed in a wheat field for 24 h and then subjected to a wet period of 24 h or returned directly to a growth chamber. *Pyrenophora tritici-repentis*, *Phaeosphaeria nodorum*, and *Cochliobolus sativus* were the most common pathogens, and *P. tritici-repentis* exhibited the highest infection efficiency of the three (Figure 4). Yearly variation in the predominant disease was apparent, and environmental parameters, especially temperature, rainfall levels, and seasonal rainfall patterns, may have been primary determinants of disease predominance.

In North Dakota, environmental conditions conducive to natural inoculation often coincided with a tan spot infection period. A leaf wetness duration of 7 h was sufficient for tan spot infection on more than one occasion, confirming results obtained at constant

Table 1. Reaction of selected genotypes to purified necrosis toxin, crude filtrate, and conidia produced by isolate 86-124 of *Pyrenophora tritici-repentis*.

	Toxin index of sensitivity[1]		Conidial inoculation	
Genotype	Pure toxin (range)	Crude filtrate (range)	Disease severity (%)	Infection type[2] (mode)
BH1146	3	3-5	24	3
Frontana	0	0-1	17	2
Erik	0	0-2	23	2
ND495	3	3-5	38	5
Ponta Grossa 1	0	1-2	42	5
Columbus	0-3[3]	1-5[1]	25	3

[1] Sensitivity rated as: 0 = no visible reaction; 1 = faint chlorosis; 2 = distinct chlorosis, possibly with some necrosis; 3 = marked necrosis within the infiltrated area, with or without a chlorotic halo; 4 = extensive chlorosis and necrosis within the infiltrated area; and 5 = necrosis and/or chlorosis spreading beyond the infiltrated area.

[2] Rated on a scale of 1-5 (Lamari and Bernier 1989) in which 1 is resistant and 5 is susceptible.

[3] Mixed reaction due to cultivar heterogeneity.

Source: Riede *et al.* 1996.

temperatures. A prolonged period of wetness (21 h) in one case apparently prevented any inoculum from being deposited on the potted plants. Dew periods after intermittent rains were conducive to tan spot development and inoculum potential was much greater after flowering than before.

Models of infection and other disease components derived in controlled environments can be confirmed with the mobile bioassay method, and prediction of tan spot epidemics may become feasible with the discrete daily bioassay. At NDSU, we are working with a novel mathematical processing strategy known as an artificial neural network (ANN) to predict tan spot infection (De Wolf and Francl 1997). ANNs are flexible by design and generally excel at complex pattern detection (Bishop 1995; Ripley 1996). Using environmental variables as input, ANNs outperformed statistical regression techniques and discriminated daily tan spot infection periods with an accuracy as high as 87% (Table 2). ANNs thus appear well suited for disease forecasting and are undergoing further evaluation.

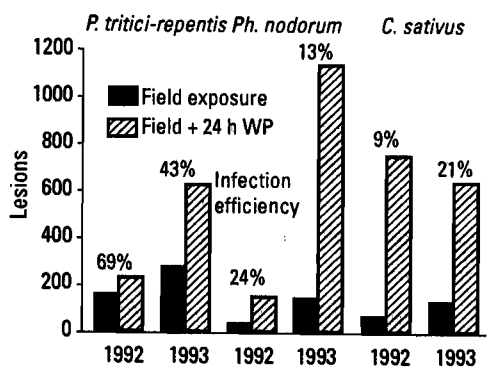

Figure 4. Seasonal predominance of foliar lesions for the three most common wheat pathogens, estimated by mobile bioassays in two growing seasons. Infection efficiency can be estimated by the ratio between the natural environment and a fully conducive environment provided by an additional 24 h wet period (WP) after field exposure.

Lesion Development and Latency

Little has been published on the topic of lesion expansion and latent period, which constitute the final link in the secondary cycle. Lesion expansion on young leaves is slower than in older leaves (Raymond *et al.* 1985; Rees 1982). Riaz *et al.* (1991) found that the latent period on detached leaves lasted about 8 days at 16°C.

Conclusion

Tan spot continues to be an acute problem of wheat production in many areas of the world. The decline of leaf and

Table 2. Accuracy of artificial neural network and statistical models in the prediction of tan spot infection incidence.

	With remote moisture sensor input		Without remote moisture sensor	
	Model development (%)	Model validation (%)	Model development (%)	Model validation (%)
Neural network	99	87	99	81
Stepwise logistic	69	68	74	62
Discriminant	91	50	86	55

Source: De Wolf and Francl 1997.

stem rusts as predominant diseases by deployment of resistant germplasm, the increase in conservation tillage, and the intensification of wheat production through shorter rotations and monoculture contribute to the tan spot dilemma and are unlikely to change. Successful management of tan spot will depend on research efforts stretching from the molecular to the population level.

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Leaf Blight Diseases and Associated Soilborne Fungal Pathogens of Wheat in South and Southeast Asia

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Abstract

There are two helminthosporium leaf blight (HLB) diseases in South Asia: Bipolaris sorokiniana *and* Pyrenophora tritici-repentis, *causing spot blotch and tan spot, respectively. Spot blotch dominates in the warmer, humid areas, whereas tan spot prevails in cooler seasons in north-central India and southern Nepal. In eastern India, Bangladesh, and countries of Southeast Asia,* B. sorokiniana *is often the only HLB species isolated. HLB increased in importance after the "green revolution." Three factors appear to be responsible: the rice-wheat cropping system, an increase in inoculum potential associated with this system, and the incorporation of leaf rust resistance. HLB has been obscured where LR has dominated. Breeding for HLB resistance has been difficult. The spot blotch pathogen is genetically dynamic and overcomes resistance. Tolerance or slow disease development is often associated with late maturity. The economic losses due to spot blotch exceed 20% in South Asia. Losses caused by tan spot are not well documented and often confounded because wheat is either a minor or an experimental crop in Southeast Asia. Spot blotch is a limiting factor to wheat production; it is explosive, and complete crop loss is possible. Without disease resistance or an economical fungicide treatment, wheat production in these environments is hazardous.*

There are numerous foliar leaf blights, and seedborne and soilborne diseases reported on wheat (Mathur and Cunfer 1993; Prescott *et al.* 1986; Wiese 1987; Zillinsky 1983). Many of these are found in South and Southeast Asia (Alam *et al.* 1994; Bhatti and Ilyas 1986; Dubin and van Ginkel 1991; Duy and Dinh 1994; Hafiz 1986; Joshi *et al.* 1978; Karki and Karki 1996; Kulkarni and Naragund 1986; Lapis 1985a; Minh 1991; Nema 1986; Saari 1985, 1986; Saunders 1988; Sharma 1996; Sharma *et al.* 1996; Singh and Srivastava 1997). In these regions, the important leaf blights are caused by fungi. The leaf blight diseases represent a complex, and are collectively referred to as helminthosporium leaf blight (HLB). Two of the most common diseases, spot blotch and tan spot, are caused by the fungi *Bipolaris sorokiniana* (Sacc. in Sorok.) Shoem., and *Pyrenophora tritici-repentis* (Died.) Drechs., respectively.

A common leaf blight fungus reported from India is *Alternaria triticina* Pras. & Prab. It is considered to be an important wheat pathogen that causes significant losses (Joshi *et al.* 1986; Nema 1986; Singh and Srivastava 1997). It is prevalent in the central, eastern, and southern wheat growing areas of India.

In recent surveys, its relative frequency seems to be declining, possibly due to the availability of more resistant varieties (Singh and Srivastava 1997). Symptoms of the three leaf blights are difficult to distinguish in the field. Even microscopic viewing may not clearly resolve cause and effect.

The other reported leaf blight pathogens appear to be more opportunistic or facultative in nature. They are encountered sporadically and are seldom important on a national scale, but can cause losses in individual fields (Joshi *et al.* 1986; Joshi *et al.* 1978; Nema 1986). Results from the questionnaire on the fungal wheat diseases in tropical and subtropical climates, distributed by Dubin and van Ginkel in 1991, has been updated for the fungal pathogens of South and Southeast Asia (Table 1).

The perfect stage (teleomorph) of the HLB and *Alternaria* fungi does not occur in nature in South or Southeast Asia. The teleomorph of spot blotch, *Cochliobolus sativus* (Ito & Kuribay.) Drechs. ex Dastur., has been reported to occur in nature in Zambia (Raemaekers 1988). The teleomorph of the tan spot pathogen is *Pyrenophora tritici-repentis*, which occurs in more temperate climates where zero tillage cultivation is practiced (Hosford 1982; Mehta 1991, 1993; Reis 1991). The tan spot disease has not been reported to occur in Southeast Asia, except for one report from Thailand (Hosford 1982; Schilder and Bergstrom 1993). This means that the three leaf blight fungi survive in the asexual form (anamorph) in their respective areas. Conidia or mycelia are the forms isolated and identified from leaf, soil, and crop debris specimens, and from collateral hosts (Nema 1986; Hafiz 1986; Karki 1982; Karki and Karki 1996; Kishwar *et al.* 1992; Sharma *et al.* 1996; Singh and Srivastava 1997).

Spot blotch is considered to be the most common and widespread disease in the three ecological zones established by Fischer (1985) and used by Dubin and van Ginkel (1991) to distinguish climate and disease in the non-traditional wheat areas. The three zones were separated on the basis of coolest month (January) mean temperature: 1) very hot >22.5 °C, 2) hot >17.5 °C, and 3) warm >12.5°C. In the three zones, where high relative humidity (RH) or dew is common, the spot blotch disease appears to be dominant (Dubin and van Ginkel 1991; Sharma 1996; Sharma *et al.* 1996; Singh and Srivastava 1997). *Bipolaris sorokiniana* has been reported as the most frequently isolated fungus from diseased samples (Alam *et al.* 1994; Sharma 1996; Sharma *et al.* 1996). Tan spot seems to be restricted to the cooler regions of South Asia.

The three leaf blight diseases (spot blotch, tan spot, and alternaria blight) have been recorded in most wheat growing areas of India (Joshi *et al.* 1978, Nema 1986, Sharma *et al.* 1996), Bangladesh (Alam *et al.* 1994), and Nepal (Dahal *et al.* 1992; Karki 1982; Karki and Karki 1996; Sharma 1996). In Pakistan, however, blight diseases of wheat are considered to be of minor importance. Reference to the blight diseases can be found (Bajwa 1985; Bhatti and Ilays 1986; Hafiz 1986), but they are seldom

considered to be factors in wheat production (personal experience). In the southern province of Sindh, where winter temperatures are warmer, HLB has been noted (Bhatti and Ilyas 1986; Hafiz 1986). Observational data on the diseases recorded in wheat breeding nurseries do not include notes on blight diseases (Bajwa 1985; Mustafa *et al.* 1994). Rusts and smuts are, by and far, the diseases of major concern (Bhatti and Ilyas 1986; Hafiz 1986; Roelfs *et al.* 1992; Saari and Prescott 1985; Wilcoxson and Saari 1996).

In central and southern India, where the growing season tends to be warm and somewhat drier (Tandon 1994), the frequency of alternaria blight increases

Table 1. Review of the fungal diseases of wheat reported from South and Southeast Asia, and an estimate of their relative importance in the region (adapted from Dubin and van Ginkel 1991).

Disease	Common name	Scientific name	Importance scale	
			South Asia	Southeast Asia
Foliar	Spot blotch	*Bipolaris sorokiniana*	5	5
	Leaf rust	*Puccinia recondita*	5	3
	Alternaria blotch	*Alternaria triticina*	4	0
	Yellow rust	*P. striiformis*	4	0
	Tan spot	*Drechslera tritici-repentis*	3	0
	Stem rust	*P. graminis*	3	0
	Loose smut	*Ustilago tritici*	3	1
	Flag smut	*Urocystis agropyri*	3	0
	Common bunt	*Tilletia laevis, T. tritici*	3	0
	Karnal bunt	*T. indica*	3	0
	Powdery mildew	*Erysiphe graminis*	3	2
	Septoria leaf blotch	*Septoria tritici*	2	0
	Septoria glume blotch	*S. nodorum*	2	0
	Head blight (scab)	*Fusarium* spp.	2	4
	Fusarium leaf spot	*Fusarium nivale*	2	1
	Downy mildew	*Sclerospora macrospora*	1	0
	Ascochyta leaf spot	*Ascochyta tritici*	1	
	Curvularia spot	*Curvularia* sp.	1	0
	Ephelis spot	*Ephelis* sp.	1	0
	Alternaria leaf spot	*Alternaria* spp.	1	0
	Helminthosporium leaf spot	*Helminthosporium* spp.	1	0
	Cladosporium spot	*Cladosporium herbaurum*	1	0
	Blast	*Pyricularia oryzae*	1	0
	Leptoshaerulina	*Leptoshaerulina trifolli*	1	0
	Chaetomium	*Chaetomium dolichotrichum*	1	0
	Dilophospora leaf spot & twist	*Dilophospora alopecuri*	1	0
Soil	Common root rot	*B. sorokiniana*	4	5
	Fusarium root rot	*Fusarium* spp.	3	3
	Sclerotium	*Sclerotium rolfsii*	2	5
	Rhizoctonia	*Rhizoctonia solani*	2	3
	Pythium	*Pythium* spp.	2	1
	Take all	*Gaeumannomyces graminis*	1	0
	Curvularia	*Curvularia verruciformis*	1	0
	Sclerotinia	*Sclerotinia sclerotiorum*	1	0

Importance scale: 5 most important, 4 Serious, 3 Moderate, 2 Minor, 1 Reported, 0 Absent/not reported.
References: Alam *et al.* 1994, Bhatti and Ilyas 1986, Dubin and van Ginkel 1991, Duy and Dinh 1994, Hafiz 1986, Joshi *et al.* 1978, Joshi *et al.* 1986, Kulkarni and Naragund 1986, Lapis 1985a, Nema 1986, Saari 1985, Saari 1986.

(Nema 1986; Singh and Srivastava 1997). *Bipolaris sorokiniana* is still the principle fungus recovered in laboratory isolations, followed by *A. triticina* (Sharma *et al.* 1996). The tan spot fungus, *P. tritici-repentis*, was recorded much less frequently. The low levels of *P. tritici-repentis* recovered may reflect difficulties encountered in isolating the pathogen from infected leaves (Schilder and Bergstrom 1993), and its slower growth in culture, which can often be overgrown by the prolific spot blotch fungus. It has been reported that *B. sorokiniana* can also be antagonistic to *P. tritici-repentis* (Luz and Bergstrom 1987). Using different observation techniques or isolation procedures, the percentage of isolates reported could be shifted.

Occasionally, the tan spot disease dominates in the cooler areas or seasons in the north central area of India and southern Nepal (Dubin and Bimb 1991; Dubin and van Ginkel 1991; Karki and Hosford 1986; Karki 1982; Ruckstuhl 1994; Saari, unpublished). In warmer seasons, it appears that *B. sorokiniana* establishes early and dominates. Nema (1986), however, reported that McRae in 1922 found *P. tritici-repentis* to be more common at Pusa, Bihar, then *B. sorokiniana*. Nema also reported that, in his studies on spore trapping, higher cumulative spore counts of *P. tritici-repentis* were obtained at Pusa, Bihar. In the warmer areas of eastern India (Bihar, Orissa, West Bengal), Bangladesh, and the countries of Southeast Asia, the dominant leaf blight fungus appears to be *B. sorokiniana*, and, in most cases, few or no other leaf blight causing fungi are reported (Alam *et al.* 1994; Duy and Dinh 1994; Dubin and van Ginkel 1991; Lapis 1985a; Minh 1991; Raymundo 1992; Saari, unpublished data).

The HLB diseases have been present for many years (Joshi *et al.* 1986; Nema 1986; Misra 1973; Saari and Wilcoxson 1974), but were not considered to be of major importance until after the "green revolution" (Alam *et al.* 1994; Devkota 1994; Joshi *et al.* 1978; Karki 1982; Razzaque and Hossain 1991). At least three factors seem to be involved in this observation or perceived change in the disease situation. A primary factor is the adoption and expansion of the rice-wheat cropping system. This system was not widely practiced in South Asia until the introduction of the early maturing semi-dwarf rice and wheat cultivars (Hobbs and Morris 1996).

The strong association of HLB with the rice-wheat system is not fully understood. Two possibilities could be an increase in the inoculum potential, or a predisposition for the disease. The wheat crop sown in November or December becomes infected at an early growth stage and increases rapidly (Alam *et al.* 1994). It is likely that the primary inoculum of the HLB fungi comes from several sources such as weed hosts, soil, crop debris, self-sown plants, and as seedborne infections, as it does in other regions of the world (Duveiller and Gilchrist 1994; Mehta 1996; Nema 1986; Raemaekers 1988; Reis 1991; Schilder and Bergstrom 1993). The role of rice as a passive host, epiphyte, or a supporter of the saprophytic growth of the HLB fungi needs to be determined.

Rice has been reported as a host species for the spot blotch and tan spot fungus in inoculated trials in India (Misra 1973). Rice stubble may also play an important role as a substrate for the fungi after rice harvest is completed.

The role of grass weeds as a collateral host of *B. sorokiniana* in the rice-wheat system must be considered as a possible reason for the perceived increase in HLB. *Phalaris minor* Retz. has become a major weed in the rice-wheat system, and if unchecked can cause major losses (Hobbs and Morris 1996). This weed, and other grass weeds, act as a host for the spot blotch fungus (Nema 1986; Sharma *et al.* 1996), but their role in the epidemiology of the disease has not been determined.

Seed infection is considered to be a major source of primary inoculum for the spot blotch disease (Lapis 1985a; Mehta 1991; Mehta 1993). Sowing infected seeds can result in the early establishment of leaf infection, damping off, and root rot (Duveiller and Gilchrist 1994; Lapis 1985a; Reis 1991; Schilder and Bergstrom 1993).

Leaf rust of wheat was considered to be the most important disease on the subcontinent during the 1970s and early 1980s. Leaf rust establishes itself early on young seedling plants and multiplies rapidly (Joshi 1986; Nagarajan and Joshi 1985). Under such circumstances, leaf rust dominates the leaf surface area, and blight fungi are not apparent. Today there are many cultivars available with good levels of leaf rust resistance; however, if new race(s) of leaf rust evolve that can overcome these resistances, it could again become the dominant disease (Roelfs *et al.* 1992).

The early efforts to breed for resistance or tolerance to *B. sorokiniana* was inadequate, and variation for resistance in the hexaploid wheats has been limited (Dubin and Rajaram 1996; Duveiller and Gilchrist 1994; Gilchrist *et al.* 1991; Hetzler *et al.* 1991). The resistances identified for spot blotch were not robust, and tolerance has been fleeting (personal experience). Many so called resistant or tolerant selections did not persist over time. The pathogenic plasticity of the *B. sorokiniana* fungus seems to be large (Hetzler *et al.* 1991; Singh and Srivastava 1997). The fungus evolves and overcomes resistance, making it difficult to capture. Tolerance, or the slower rate of disease development, appears to be involved, and this has often been associated with later maturity (Duveiller and Gilchrist 1994).

In spite of a lack of good resistance sources in the hexaploid wheats, breeding progress for tolerance or partial resistance has been achieved (Dubin and Rajaram 1996; Dubin and van Ginkel 1991; Duveiller and Gilchrist 1994). By recurrent selection and re-combinations of the best lines, genetic gains for reducing losses to HLB are being experienced. The extensive search for resistance in related and alien species has provided a number of new resistance sources (Dubin and Rajaram 1996; Hetzler 1991; Villareal *et al.* 1995). These sources may require intensive breeding and selection, but in early evaluations, a

number of wide cross derivatives from *Thinopyrum curvifolium* are showing promise, involving several genes for resistance (Dubin and Rajaram 1996). Hopefully, these new sources of resistance will significantly contribute to the development of durable resistance.

Yield losses caused by the blight diseases are considerable in South Asia (Dubin and van Ginkel 1991). Numerous trials estimate the losses to be in excess of 20%, depending on cultivar susceptibility and climate. Older varieties and varieties susceptible to leaf rust tended to suffer greater losses. Late sowing also dramatically increases yield loss. For example, in Bangladesh, the late sowing of the two leaf rust susceptible cultivars Sonalika and Kanchan resulted in a yield loss of 71% and 41%, respectively (Badaruddin *et al.* 1994). Sonalika is the older variety, and Kanchan is now the dominant cultivar. In Nepal, the cultivar RR 21 (=Sonalika) is susceptible to leaf rust but is still grown. In protected yield trials, the yield loss of RR 21 was 34.4%, compared with 16.7% in the more widely grown leaf rust resistant cultivar UP 262 (Bhatta 1997).

Dubin and van Ginkel (1991) summarized the loss data available, and these reports, together with newer studies from the South Asia region, have been combined in Table 2. The average yield loss due to leaf blights for South Asia is calculated at 19.6%. If the two extreme or unusually severe loss figures for Bangladesh are excluded, the calculated average loss in yield is reduced to 16.9%. This figure tends to agree more with the majority of the yield loss estimates, and falls into the general range of loss figures reported.

Soilborne diseases are considered to be important to yield stagnation, and are a major issue in sustainability. A number of known wheat pathogens have been identified from South and Southeast Asia (Joshi *et al.* 1978; Kulkarni and Naragund 1986; Saari 1986). The most commonly reported soil and seedborne pathogens are fungi, although there are occasional bacterial and nematode pathogens encountered (Alam *et al.* 1994; Duveiller *et al.* 1991; Joshi *et al.* 1986; Joshi *et al.* 1978; Kulkarni and Naragund 1986; Mathur and Cunfer 1993; Saari 1986; Sharma *et al.* 1996). In the survey carried out by Dubin and van Ginkel (1991), five pathogenic fungi were mentioned by respondents, with *Sclerotium rolfsii* being considered the most serious soilborne pathogen, followed by *Fusarium* spp. *Bipolaris sorokiniana* has been reported to be the principal fungus involved in the seedling blight and root rot of wheat in Pakistan (Bhatti *et al.* 1986; Hafiz 1986; Kishwar *et al.* 1992). Saksena reported (Wilcoxson and Saari 1974) an increase in the incidence of rhizoctonia seedling blight and root rot in the double crop rice-wheat system. A number of other fungi have been reported (Alam *et al.* 1994; Joshi *et al.* 1986; Lapis 1985a; Nema 1986), and the list of known soil and seed associated fungal pathogens of wheat have been included in Table 1.

Seed and soilborne inoculum are important in the establishment of leaf blight and the initiation of seedling blight

and root rot. Seed and soil treatment trials in Nepal and Bangladesh have shown a significant improvement in plant stand and yields (Alam *et al.* 1994; Badaruddin *et al.* 1994; Meisner *et al.* 1994; Dubin and Bimb 1991). The use of various seed and soil treatments and fungicides suggests that different groups of fungi are involved in causing pre- and post-damping off in wheat plants. Seed treatments with carboxin control *Sclerotium rolfsii* Sacc. and *Rhizoctonia* spp. The metalaxyl soil treatment is effective against Phycomycetes, such as *Pythium* spp. Captan and Thiram are effective against a number of fungi. The losses reported for seed and soil treatment trials are summarized in Table 3.

Table 2. Losses reported for the wheat leaf blight diseases; spot blotch, tan spot, and alternaria blight in South Asia.

Country	Losses (%)	Comments	Reference
India	20.8	V - HD 2329; at Guardaspur; S to YR;	Sharma *et al.* 1996
	11.5	V - UP 2338; at Guardaspur	
	17.3	V - UP 262; at Pantnagar	
	13.2	V - UP 2338; at Pantnagar	
	9.0	V -UP 262; at Faizabad	
	9.8	V-UP 2338; at Faizabad	
	16.3	V - UP 262; at Pantnagar; 2 yr average	Singh *et al.* 1996
	26.5	V - UP 2338; at Pantnagar; 2 yr average	
Average	**15.5**		
Nepal	34.4	V - RR 21 (=Sonalika); Sus. to LR	Bhatta 1997
	20.7	V - Tiveni; R- to LR	
	16.7	V - UP 262; R- to LR	
	10.3	V - Bhrikuti; R- to LR	
	27.0	Foliar spray	Devkota 1994
	26.0	Foliar spray	Dubin and Bimb 1991
	25.0	Foliar spray	Dubin and van Ginkel 1991
	23.8	V - RR 21 (=Sonalika)	Mahto *et al.* 1996
	10.0	V - NL 297	
	8.1	V - Annapurna 1	
	16.2	V - UP 262, foliar spray, farm fields	Mahto and Sedhari 1996
	26.3	Foliar spray	Dangol 1977
	9.1	Farm field trials	Ruckstuhl 1994
Average	**19.5**		
Bangladesh	18.0	V - Sonalika; S- LR; optimum sown	Badaruddin *et al.* 1994
	71.0	V - Sonalika; S- LR; late sown	
	7.4	V - Kanchan; S- LR; optimum sown	
	41.2	V - Kanchan; S- LR; late sown	
	24.0	Spray trial	Dubin and van Ginkel 1991
	14.0	V - Akbar	Razzaque and Hossain 1991
	8.0	V - Kanchan	
	4.0	V - Aghrani	
	21.0	V - Sonalika	
Average	**23.2**		
Grand Mean	**19.6**		

Abbreviations: V= variety, S- = susceptible, R- = resistant, LR= leaf rust, YR= yellow rust.
Comment: By removing the two very high scores in Bangladesh, the overall average loss becomes 16.94 %.

Badaruddin *et al.* (1994) reported results from research station and farm field trials in Bangladesh. Carboxin seed treatment provided an average yield increase of about 14%. Matalaxyl soil treatments also resulted in an average yield gain of about 14%. The two combined produced an average yield gain of approximately 28%. Associated with the yield increase was an increased plant stand of approximately 23%. When all the seed and soil experiments were averaged, the loss approximated 14%.

In Southeast Asia, wheat is, by and large, an experimental crop. The total acreage is less than 200,000 ha, with more than half of this acreage occurring in Myanmar (Dubin and van Ginkel 1991; Winkyi and Kyaw 1994). The acreage in Thailand and Vietnam is nominal, but there is some potential for wheat production (Duy and Dinh 1994; Jongdee 1994; Long *et al.* 1994; Minh 1991; Rajatasereekul *et al.* 1994; Saunders 1988; Somrith 1988). In the Philippines, Indonesia, and Sri Lanka, wheat could be grown in some regions, but there are a number of technical difficulties and the window for cultivation requires good management and reasonable weather (Escano 1994; Liboon 1992; Mann 1992; Saunders 1988; Zaini *et al.* 1991; Saari, personal observation). The production potential with current varieties is approximately 2.5 t ha^{-1} in 90 days or less under rainfed conditions. With some irrigation, yields of up to 5 t ha^{-1}or more are possible (Saunders 1988); however, the available technology and economic incentives are not sufficient to encourage local production (Fischer and Byerlee 1991).

One of the major limiting factors to wheat production in the very hot (< 22.5°C) and humid climates (notably Vietnam, the Philippines, and Indonesia)

Table 3. Wheat yield losses reported or calculated from seed, and soil treatments for control of seed and soilborne pathogens.

Country	Losses (%)	Comments	Reference
Bangladesh	12.0	Seed treat., Carboxin, Res. station	Badaruddin *et al.* 1994
	13.4	Soil treat., Metalaxyl, Res. station	
	29.4	Combined treat., Res. station	
	16.8	Seed treat., Carboxin, Farm fields	
	14.3	Soil treat., Metalaxyl, Farm fields	
	26.4	Combined treat., Farm fields	
	11.0	Seed treat., Farm fields 1989	Meisner *et al.* 1994
	11.5	Seed treat., Farm fields 1990	
	17.0	Seed treat., Farm fields 1991	
	10.0	Carb. + Thiram, Irrigated, V-Kanchan	Razzaque *et al.* 1994
	11.3	Carb. + Thiram, Irrigated, V-Sonalika	
	9.6	Carb. + Thiram, Rainfed, V-Kanchan	
	12.6	Carb. + Thiram, Rainfed, V-Sonalika	
Nepal	12.0	Soil solarization	Dubin & Bimb 1991
	4.0	Soil solarization, Farm fields	Dubin & Bimb 1994
Average	**14.1**		

are the various diseases of wheat. The most important disease is HLB, caused by *B. sorokiniana* (Lapis 1985a; Mann 1992; Saari 1986; Saunders 1988). A number of soilborne pathogens are also present, causing both seedling blights and root rots. The soilborne fungus *S. rolfsii* is a major contributor to the root rot complex, and losses of up to 60% have been noted (Saunders 1988). Other important seedling blights are caused by *Rhizoctonia* spp.; *Fusarium* spp. cause both root rot and head blight where rain showers occur after heading. Leaf rust and powdery mildew are considered to be potential problems in some areas (Danakusuma 1985; Duy and Dinh 1994; Minh 1991).

A number of fungicides have been used to assess losses caused by spot blotch in the Philippines (Saunders 1988). The fungicide propiconazole gave the best control of spot blotch, and resulted in a 65% yield gain (Lapis 1985b). In years when rains occurs late in the crop cycle, especially during grain filling, yield losses can be staggering and complete loss of the crop has been observed (Saunders 1988; Saari, personal observation). In the 1987/88 crop cycle in northern Luzon, the Philippines, unusual rains resulted in severe leaf blight and root rot. The highest yield obtained was 805 kg ha^{-1} compared to the best yield from the year before of 3,419 kg ha^{-1} (Layaoen 1993). This represents a high risk situation which is difficult for the farmers to accept. Even if rains do not occur, high RH seems to be conducive to HLB development. In 1982 there were few late rains, yet severe spot blotch developed, and some wheat trials and farm demonstrations were lost. When mean temperatures in the area average 25-28°C, and the RH is above 70%, spot blotch continues to develop (Lapis 1985a; Raymundo 1992).

Wheat production is limited by disease in the countries of Southeast Asia with very hot and humid climates (Fischer 1985). Spot blotch and associated seed and soilborne disease complexes are major production constraints. Until better resistance and crop management technologies are combined, wheat cultivation remains marginal. In countries where mean temperatures are considered to be very hot (<22.5°C) or hot (<17.5°C), but the RH edges below 70% and dews are less of a factor, some wheat cultivation is possible. Under these conditions, spot blotch development is much reduced. The countries of Myanmar, Thailand, and possibly some areas in Laos and Vietnam fit this situation.

In South Asia, in the hot and the warm (<22.5°C) climates, where dews are a regular feature for extended periods, the blight diseases develop and cause significant disease levels and yield loss. Evidence suggests that older varieties suffer greater loss than newer cultivars. This indicates that some progress has been achieved in breeding for tolerance. The results also suggest that the combination of leaf rust and blight causes more damage, and late sowing magnifies yield losses.

In the central, eastern, and southern regions of India, the terai of Nepal, and

throughout Bangladesh, the most important wheat diseases today are the leaf blight diseases and associated seed and soilborne diseases. This is the result of the control of leaf rust. The importance of leaf blight diseases may also be related to the expansion of the rice-wheat cropping system. It should be pointed out, however, that not all of the area vulnerable to blight diseases is under the rice-wheat system. It is estimated that in South Asia, approximately 11-12 million ha are under the rice-wheat cropping system, while the total wheat area exceeds 30 million ha (Hobbs and Morris 1996).

Yield losses caused by both foliar and soilborne diseases are significant when measured in research and on-farm trials. Losses caused by leaf blights vary greatly, but it seems reasonable to say that they average around 16-17%. Losses caused by seed and soilborne disease complexes can vary greatly from field to field, which has made quantification more difficult. The figures currently available suggest that these losses can average around 14%. If these average losses are extrapolated over the vulnerable area, the total production lost is significant. For South Asia, an attempt has been made to calculate the losses caused by leaf blights, and these results are given in Table 4. The area and production figures were taken from different sources. The state, province, or area production of wheat was multiplied by an average loss value of 8.47%, which represents half of the calculated loss figure of 16.94% (see Table 2). The use of half the measured average loss is a conservative approach, but tends to agree with the differences in the general on-farm yield and demonstration trials where researchers' yields are compared with farmers' yields. For example, the Indian "Front Line" farm wheat demonstrations in Uttar Pradesh, India, averaged 4.5 t ha^{-1} in 1995, whereas the Uttar Pradesh state yield average was 2.2 t ha^{-1} (Nagarajan and Joshi 1995).

The unit area production was multiplied by the average calculated loss (8.47%), which was then multiplied by a vulnerability index. This index is an arbitrary estimate of incidence or overall vulnerability of the crop in a given geographical zone or area. The overall calculated loss comes to between 2-3 million t, and may actually be more. This represents a 3.9% loss for South Asia, based on total production. Little or no wheat is cultivated in Southeast Asia since leaf blight is a limiting disease, hence a calculation of losses was not possible. The calculation of losses caused by the soilborne pathogens is more difficult. The erratic and large variability in losses between farm fields would need additional survey results to transform the measured losses into national or regional averages.

Whether good management and the development of better resistant varieties can eliminate most of these leaf blight losses remains to be seen. However, a realistic and possible goal would be to strive for a reduction of these losses by 50% in the next five to ten years.

Table 4. Wheat areas of South and Southeast Asia, and estimated yield loss caused by leaf blights (calculated using an average loss value of 8.47 %, times the vulnerability index).

Geographic region/country	State/ Province/Area	Area[1] (1000 ha)	Production[1] (1000 mt)	Vulnerability index[2]	Calculated loss (1000 mt)
South Asia					
Pakistan					
	Punjab	5970.0	12430.0	0.1	105.3
	Sindh	1110.0	2340.0	0.2	39.6
	NWFP	860.0	1200.0	0.1	10.2
	Balochistan	340.0	790.0	na [3]	
	Pakistan Total	**8280.0**	**16760.0**		**155.1**
India					
Eastern	Arunachal Pradesh	3.6	6.8		
	Assam	76.4	111.2		
	Bihar	1963.3	3566.0		
	Meghalaya	4.6	5.9		
	Nagaland	0.5	1.6		
	Orissa	30.7	51.3		
	Sikkim	11.4	17.5		
	Tripura	3.7	6.9		
	Uttar Pradesh	8625.8	20155.8		
	West Bengal	269.1	530.2		
	Eastern Total	10989.1	24453.2	1.0	2071.2
Northern	Haryana	1808.0	6502.0		
	Himachal Pradesh	378.1	595.8		
	Jammu & Kashmir	245.0	297.4		
	Punjab	3233.0	12295.0		
	Delhi	32.4	109.4		
	Northern Total	5696.5	19799.6	0.2	335.4
Southern	Andhra Pradesh	8.1	6.7		
	Karnataka	202.6	144.3		
	Tamil Nadu	0.2	0.2		
	Southern Total	210.9	151.2	0.5	6.4
Western	Gujarat	409.0	905.7		
	Maharashtra	627.6	625.7		
	Rajasthan	1779.2	4478.4		
	Dadra & Nagar H.	0.2	0.4		
	Western Total	2816.0	6010.2	0.2	101.8
Central	Madhya Pradesh	3267.4	4673.1	0.5	197.9
	India Total	**22979.9**	**55087.3**		**2712.7**
Bangladesh	**Total**	**634.0**	**1169.0**	**1.0**	**99.0**
Nepal	Mountain	44.6	57.2	0.1	0.5
	Hill	229.6	329.2	0.2	5.6
	Terai	349.9	555.1	1.0	47.0
	Nepal Total	**624.1**	**941.5**	**53.1**	
	South Asia Total	**32519.0**	**73957.8**		
	Total calculated loss				2864.1
	% Loss				3.9
Southeast Asia					
Myanmar		0.1	0.1	na	
Thailand		<1.0	<0.7	na	
Vietnam		<0.1	<0.1	na	
Laos		0?	0?	na	
Indonesia		0.0	0.0	na	
Philippines		0.0	0.0	na	
Sri Lanka		0.0	0.0	na	

[1] Calculated from: CIMMYT. 1996. World Wheat Facts and Trends. CIMMYT, Mexico. 73 pgs. Duy and Dinh 1994. Fertilizer Statistics 1992-93, 1993. Manandhar and Shakya 1996. PARC, Islamabad, Pakistan. Estimates for Wheat Production 1995-96. (Personal communications). Winkyi and Kyaw 1994.

[2] Vulnerability index = 0.1 reported; 0.2 low; 0.5 moderate; 1.0 high.

[3] na = not applicable or available; see text for details.

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Foliar Blights of Wheat in India: Germplasm Improvement and Future Challenges for Sustainable, High Yielding Wheat Production

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Abstract

*Spring wheat is grown over 25 million ha in India, covering six mega environments as a winter crop between November to April. Production is on the order of 65.0 million t. In the northeastern plain zone (NEPZ), a major part of the 9.5 million ha is subject to severe leaf blight development where rice precedes wheat; conditions are warm and humid, nutrient application is poor, and soils are flooded and saline. Since varietal replacement is slow, brown rust (*Puccinia recondita tritici*) severity tends to be high and, when coupled with leaf blight, crop losses are very high. Leaf blight is a disease complex caused by a number of pathogens, with the most serious being Bipolaris sorokiniana (syn.* Helminthosporium sativum*) that causes spot blotch. This pathogen is capable of causing damage from the primary leaf stage, though the plant tends to become more susceptible after flowering. The small spots on the leaf, if numerous, coalesce and the leaf prematurely dries up, reducing the photosynthetic area of the plant. The role of toxin in chlorosis and leaf drying (necrosis) is not well understood. It is further complicated by the slight differences in host response, the need for a scale to separate the more complex levels of host pathogen interaction, and by the wide host range of the pathogen.* Bipolaris sorokiniana *has been found to infect barley, oats, and rice, as well as 12 other grasses. At the Directorate of Wheat Research, a reliable technique has been developed to create artificial epiphytotics under a plastic tunnel and in the field. Also, a new rating procedure has been suggested to score the earhead, flag leaf (F), and F-1 leaf to enable plant breeders to select for more tolerant lines. In the northwest plain zone (NWPZ), tan spot (*Pyrenophora tritici-repentis*) is common on the lower leaves and leaf sheath of wheat plants, and spreads to upper leaves during prolonged wet and warm situations. Symptoms are a narrow halo around the spot and, when severe disease occurs, premature leaf drying and greater levels of grain shriveling. The late sown irrigated wheat of NWPZ that is well fertilized shows severe disease levels by the end of March/early April. Both bread wheat and durum are vulnerable and suffer economic losses either due to tan spot or a combination of tan spot, stripe, and leaf rusts.*

Wheat is cultivated over 25.0 million ha in India, of which nearly 9.0 million ha is exposed to various types of leaf diseases other than rusts and powdery mildew. Of this, it is estimated that more than 6.0 million ha is in the northeastern

plain zone (NEPZ) or the eastern drain of the Indus-Ganges basin where rainfed upland rice, lowland, or medium lowland rice precedes wheat. During the last two decades there has been a substantial increase in the rice-wheat sequence (Figure 1; Huke and Huke 1992). Apart from delayed wheat sowing under the difficult field conditions where the crop stand is invariably poor, the high level of organic matter (rice stubble) and poor nutrient application results in necrotic roots due to *Bipolaris sorokiniana* and other soil dwelling fungi. In the early days, when the cropping intensity was lower, the soil became solarized during the summer, reducing the population of these organisms, as noted in solarization experiments conducted in Nepal (Dubin and Bimb 1994). The solarized soil invariably promoted better rooting of wheat and decreased leaf blight severity. The increased blight severity in eastern India is therefore a second generation problem arising from crop intensification.

In the cooler, well irrigated, fertilized wheat growing area of NW India, leaf blight is a problem on durum and late sown wheat. The leaf blotch/head scab/foot rot syndrome is also observed on durum wheat. It appears that *Fusarium nivale* is involved (J. Kumar, personal communication.).

Disease Epidemiology

The leaf blight complex is caused by *B. sorokiniana* (spot blotch), *Pyrenophora tritici-repentis* (tan spot), *H. bicolor*, and several other less well-known species. All these fungi have been investigated in

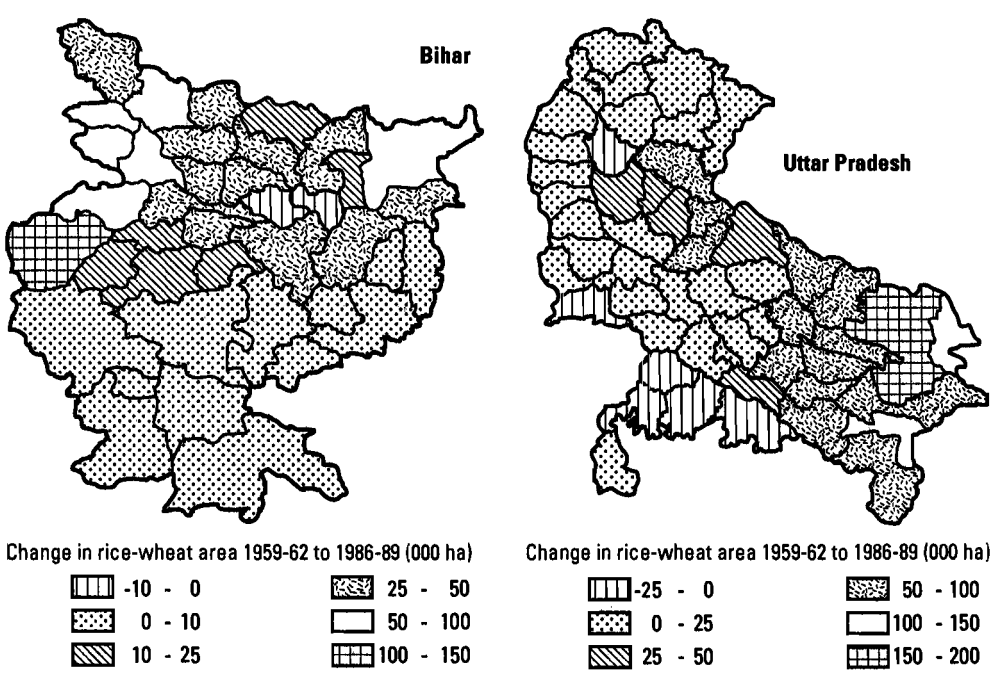

Figure 1. Changes in rice-wheat sequence areas from 1959-62 to 1986-89 in Bihar and Uttar Pradesh. Source: Huke and Huke 1992.

detail by Misra (1973), who failed to note any pathogenic forms, nor any clear variations based on aggregation of isolates. The helminthosporium leaf blights of wheat have a wide host range, often infecting a large number of grasses that coexist in an area. Though the monograph of Misra (1973) indicates that lines Gabo, Khapli, and Thatcher are more susceptible to one species than to another, insufficient evidence does not allow a stronger statement to be made. Unless a reliable seedling/adult plant reaction matrix is developed, it is difficult to quantify pathogenic variation within a species. The different species of *Helminthosporium* produce different types of symptoms, and the toxins produced by the pathogen further complicate the issue. Theoretically, there can be three situations:

1. The genetic basis of resistance is non-specific in *Triticum* and acts to provide resistance to all pathogenic helminthosporia at the late dough stage (LDS) of the crop.
2. Different resistance genes accord resistance to different *Helminthosporium* species and their biotypes. Multiple genes are involved in total resistance to the leaf blight complex.
3. A mechanism such as slow disease development also acts to reduce the disease severity at LDS.

Realizing the difficult nature of addressing the disease, an attempt was made to perfect a protocol to create epiphytotics to screen donor lines and segregating populations.

Disease Scoring Procedures

Until recently, multilocational testing was the only basis for evaluating wheat material for leaf blight resistance. Disease rating was carried out according to Saari and Prescott (1975), where the emphasis is on the plant level (height) that has been affected. The extent to which the leaf is blighted, or leaf area damaged, was added as another dimension, and a double digit system was followed for some years. Both systems are good for rapid evaluation of genetic stocks, but are inadequate for breeding for resistance. The shortcomings are that the diseased leaf area and the type of lesion are not rated. A visual rating scale was developed at the directorate: a 0-9 scale for mean disease expression on leaves, recorded for the entire row of the genotype under evaluation (Figure 2). In cases where the peduncle/earhead are affected, the disease rating data is recorded separately. Invariably, at LDS, only the top two leaves are green and account for grain filling.

Procedure for Creating Epiphytotics

The procedure followed to this time for creating leaf blight epiphytotics to screen germplasm in the multilocational trials was not satisfactory for generating repetitive and reliable results (Figure 3). There were several shortcomings:

- Only local isolates were used which, in some cases, turned out to be saprophytes.

- There was a loss of virulence if the organism was repeatedly cultured on synthetic media.
- Providing the needed level of humidity in the field was a difficult task.
- Early inoculations were invariably not done and so terminal disease severity was always low.

To overcome these, a new methodology was developed. After confirming virulence, the *B. sorokiniana* isolate was multiplied on sorghum seeds soaked in water for a day and autoclaved. The inoculated flasks were incubated at 25°C for 7-10 days, and every day the flask was shaken to mix the grain and to promote good coverage with spores. Mature flask cultures could be stored at 4°C for two weeks or in sterile tinfoil pouches to permit easy handling and mailing to centers even a 1000 km away. Inoculum on sorghum grain was multiplied once or twice following the same procedure in the multilocation sites. These were used for field inoculation by syringe or spray. By suspending the infected sorghum grains in water, and shaking and filtering it on muslin, the inoculum was prepared for syringe inoculation similarly to the way cereal rusts are inoculated.

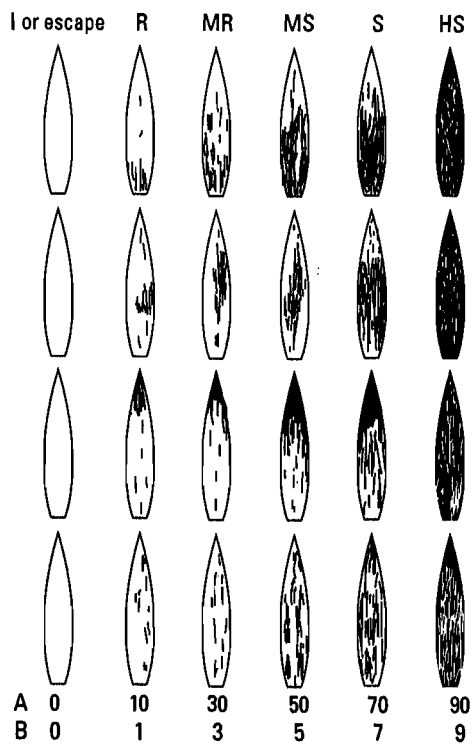

Figure 2. Field key for scoring leaf blight severity.
A = Visual percentage of the leaf surface covered by lesions; B = Numerical score.
I = Immune; R = Resistant; MR = Moderately resistant; MS = Moderately susceptible; S = Susceptible; HS = Highly susceptible.

At inoculated sites, characteristic spots appeared and subsequently spread. A few days after irrigation, the sorghum seeds loaded with inoculum were placed at the base of the tillers of the border rows. This was followed by a few days of spraying with *B. sorokiniana* spores by

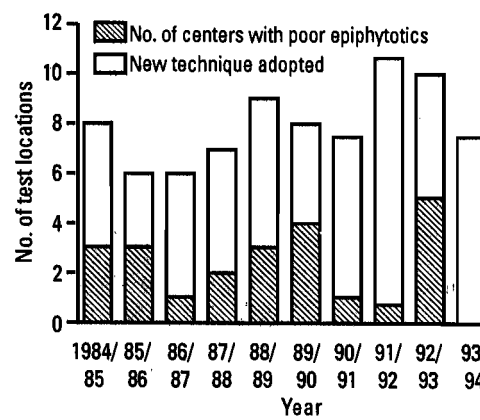

Figure 3. Locational data rejected due to poor artificial epiphytotics under AICWP[1].
[1] All India Coordinated Wheat Improvement Project.
Reasons for poor epiphytotics:
1) non pathogenic inoculum, and
2) irrigation lapses.

power sprayer, ensuring perfect epidemic development. This technique (Figure 4) is now followed at all multilocation test sites and has considerably improved the reliability of testing. Based on the rigorous tests conducted over years, a number of reliable resistant stocks have been identified (Table 1).

Confirming the Donor Line

The identified lines harboring resistance to spot blotch were grown in specially designed plastic houses with misting/sprinkler systems (Figure 5) that have the provision for cooling the temperature by an evapo-cooling device connected to a temperature monitoring device. These plastic houses of 60 m^2 were installed at eight test sites, each costing about US $335. Following the same field procedure, with good moisture levels provided by mist, and using

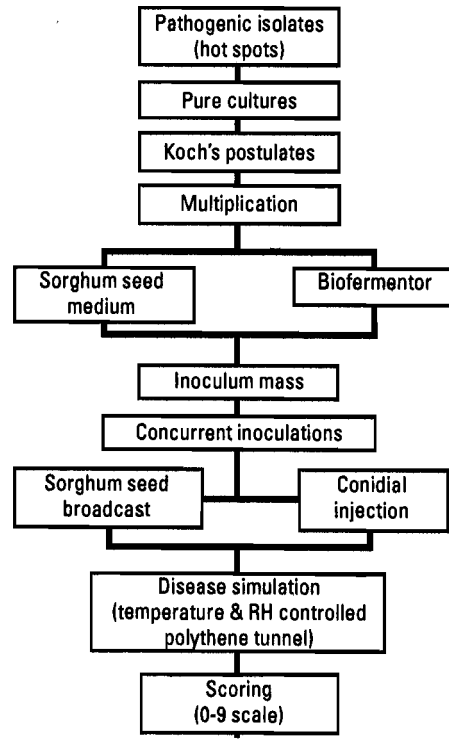

Figure 4. Artificial screening for leaf blight resistance.

Table 1. Genotypes with moderate leaf blight resistance identified in multilocational tests.

Genotype	Pedigree	Origin
Nepal 297	HD2320=HD2137/HD2186//HD2160	Nepal/India
Annapurna 1	Veery 5 =Kvz/Buho//Kal/Bb	Nepal/Mexico
NL644	Au/UP301//Gll/Sx/3/Pew S/4/Mai S/Maya S/Pew S/5/Pea S	Nepal/Mexico
NL 623 (Bhrikuti)	Cmt/Coc75/3/Plo//Fury/Ana75	Nepal/Mexico
HUW206	Veery 7	India/Mexico
Sonalika	II 54-368/An/3/Yt54/N10B//LR64	India/Mexico
A6/Glen (BAW 713)	CM 91220-1ISD-0ISD-1ISD-0ISD	Bangladesh/Mexico
A6/Glen (BAW 714)	CM 91220-1ISD-0ISD-8ISD-0ISD	Bangladesh/Mexico
BAW 599	Crt/Ald S//Seri82	Bangladesh/Mexico
Fang 60	Pi62/Fd/3/Pi62/Mz//Mxp	Pakistan/Thailand/Mexico
K8027	NP875/4/N10B/Y53//Y50/3/Kt54B/5/2*K852	India
VL616	Ska/P46	India
DL153-2	Kundan = Ti71/NP890	India
ACC No. 8528	-	India
Bow "S"	Au//Kal/Bb/3/Wop S	India/Mexico
Vee S/Myna S	-	India/Mexico
BW1052	Zaf/3/Cno67//Lr64*2/Sn64/4/Cno67//Nad/Chr/5/Yr70	India/Mexico
ACC. No. 8450	-	India

[1] Seed source from DWR, Karnal, India, except entries 7-9, which came directly from Bangladesh.
Source: Dubin *et al.*, this proceedings.

Sonalika as the spreader row, a no-escape situation was created to confirm the reaction pattern of the donor lines. Now attempts are underway to evaluate these lines separately against individual pathogens. These donor lines were used by plant breeders in their leaf blight resistance breeding program.

Breeding for Leaf Blight Resistance

Clear differences are evident between susceptible and resistant lines in their ability to reduce leaf blight development. Accordingly a strategy for breeding for leaf blight resistance has been developed (Figure 6). Cross combinations have been attempted and the material subjected to artificial evaluation at DWR, Karnal. Alternate generations are shuttled between DWR and BHU, Varanasi, in eastern India, where leaf blight is a major problem. Material in the fourth cycle shows combined leaf blight resistance and good ideotype. This material will be distributed through the cooperative

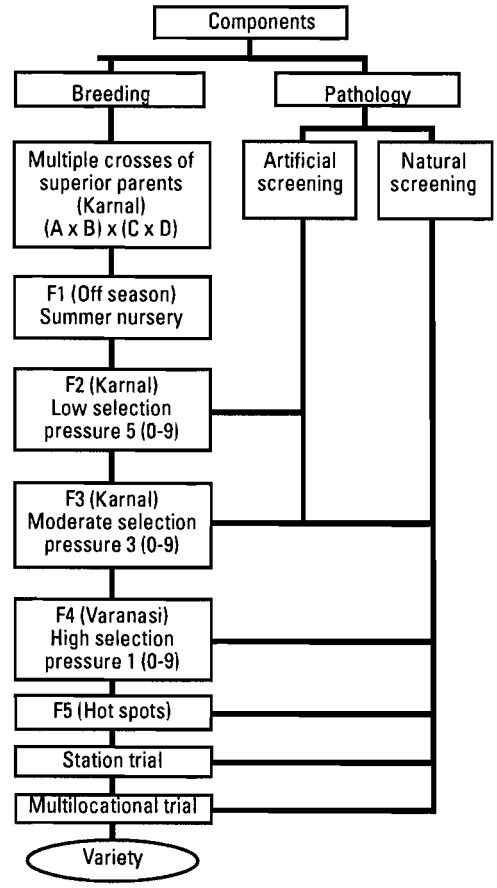

Figure 6. Breeding for leaf blight resistance.

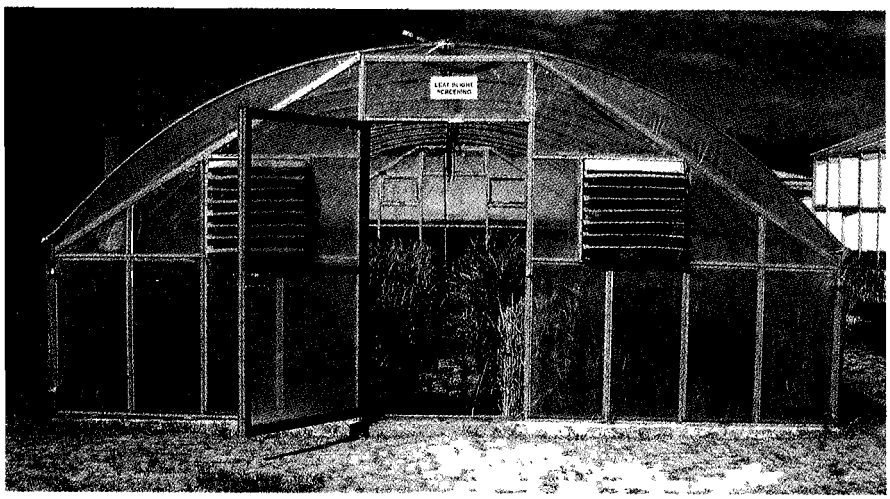

Figure 5. Polythene house designed specially for creating epiphytotics of foliar blight of wheat.

network to Indian wheat breeders for further selection, evaluation, and advancement as varieties (G. Singh, personal communication).

Attempts are also in progress to evaluate the material against each leaf blight separately and the segregating population rising out of crosses between the tolerants that have a leaf blight score below 3.0. In approximately another three crop cycles, there will be adequate material available for the All India Coordinated Wheat Improvement Project centers.

Future Thrust

The disease epidemiology of leaf blight needs to be understood, particularly off-season survival and perpetuation. Role of soil organic matter, zero tillage, proper sowing, and nutrient application all need well planned investigation.

Emphasis will be placed on the standardization of a procedure for screening seedlings for leaf blight pathogens and relating this to mature plant reaction. By developing a seedling resistance based breeding procedure, the incorporation of leaf blight resistant genes can be accelerated. Also, efforts will be made to clarify whether pathogenic forms exist within a species. By measuring disease progress, genotypes that slow disease development will be identified. Developing new genetic stocks will become an institutional activity, and shuttle breeding attempts will be strengthened.

Acknowledgment

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Distribution of Pathogens Causing Foliar Blight of Wheat in India and Neighboring Countries

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Abstract

A large number of leaf blight affected wheat samples were received/collected in every cropping season during 1988-1996, from different coordinating centers in India and neighboring countries. After repeated isolations Bipolaris sorokiniana, Helminthosporium spiciferum, Pyrenophora tritici-repentis, Alternaria alternata, A. triticina, *and* Curvularia lunata *were found associated with foliar blight each year. Of these, all isolates of* B. sorokiniana *and* A. triticina *were pathogenic.* Bipolaris sorokiniana *is the predominant leaf blight pathogen in the Indian states of Bihar, Delhi, Gujarat, Haryana, Karnataka, Maharashtra, Rajasthan, Uttar Pradesh, and West Bengal, and neighboring countries of Bangladesh and Nepal.* Alternaria triticina *predominates in the states of Himachal Pradesh, Punjab, Jammu and Kashmir, and Madhya Pradesh. Maximum disease development is promoted by temperatures around 28°C and relative humidity of 92% at heading and flowering of the crop. Yield loss due to leaf blight is estimated at 18-22%. Application of fungicides Tilt 25 EC (0.5 L* ha^{-1}*) and Indofil M-45 (2.5 kg* ha^{-1}*) achieved satisfactory disease control.*

In India, wheat (*Triticum aestivum* L.), the second most important crop after rice, has received much attention from both policy makers and scientists. Consequently, both productivity and production have increased many times since the mid 1960s; however, yield varies widely from region to region. Yields from eastern Uttar Pradesh are around 2 t ha^{-1} compared with 3-4 t ha^{-1} in western Uttar Pradesh, Haryana, and Punjab. Among other constraints, foliar blight adversely affects wheat yield, particularly under late sown conditions, as reported by Mitra (1931, 1934), Patel *et al.* (1953), Singh and Singh (1967), Joshi *et al.* (1970), Singh and Mukarjee (1981), and Singh *et al.* (1992). Due to the prevalence of rice-wheat rotation and late rice harvest, wheat sowing is often delayed until after mid December. When wheat is sown late, plant development coincides with foliar blight infection during late February and early March, resulting in substantial yield losses. Eastern Uttar Pradesh is therefore considered a hot spot for foliar blight of wheat.

Based on the above situation, a "National Center" for foliar blight disease was established by the Indian Council of Agricultural Research (ICAR) and the

Directorate of Wheat Research (Karnal) at the Department of Plant Pathology, Narendra Deva University of Agriculture and Technology, Kumarganj, Faizabad. Work on foliar blight started during 1987/88 and the findings to date are reported here.

Distribution of Foliar Blight Pathogens

A large number of blight affected wheat samples have been received/ collected from every crop season since 1988 from different coordinating centers in India and neighboring countries. After repeated isolations, *Bipolaris sorokiniana, Helminthosporium spiciferum, Pyrenophora tritici-repentis, Alternaria alternata, A. triticina,* and *Curvularia lunata* were found to be associated with foliar blight each year. Of these, all cultures of *B. sorokiniana* and *A. triticina* were found to be pathogenic, as well as some isolates of *H. spiciferum* and *A. alternata* (Table 1).

Prevalence of Major Foliar Blight Pathogens

Based on observations on the distribution of foliar blight pathogens during 1988-1996 (Table 2) with respect to the two major foliar blight pathogens (*B. sorokiniana* and *A. triticina*), *B. sorokiniana* is the predominant pathogen in the Indian states of Bihar, Delhi, Gujarat, Haryana, Karnataka, Maharashtra, Rajasthan, Uttar Pradesh, and West Bengal, and in the neighboring countries of Bangladesh and Nepal. *Alternaria triticina* predominates in the states of Himachal Pradesh, Punjab, Jammu and Kashmir, and Madhya Pradesh.

Pathogen Behavior

During late December and January, *A. triticina* was isolated from almost all wheat varieties, while only some varieties showed *B. sorokiniana* infection. During this period, the frequency of *A. triticina* was higher than that of *B. sorokiniana.*

Table 1. Distribution and pathogenicity of fungi associated with foliar blight.

Fungi isolated	Pathogenicity[1]
Bipolaris sorokiniana	++
Helminthosporium spiciferum	+
Pyrenophora tritici-repentis	NT
Alternaria alternata	+
A. triticina	++
Curvularia lunata	-
Epicoccum spp.	-

[1] ++ All isolates pathogenic on wheat; + Some isolates pathogenic on wheat; NT Pathogenicity not be tested; - Nonpathogenic.

Table 2. Prevalence of major foliar blight pathogens.

Indian state/Country	Prevalent pathogen
Bihar	*Bipolaris sorokiniana*
Delhi	*B. sorokiniana*
Gujarat	*B. sorokiniana*
Haryana	*B. sorokiniana*
Himachal Pradesh	*Alternaria triticina*
Jammu and Kashmir	*A. triticina*
Karnataka	*B. sorokiniana*
Madhya Pradesh	*A. triticina*
Maharashtra	*B. sorokiniana*
Punjab	*A. triticina*
Rajasthan	*B. sorokiniana*
Uttar Pradesh	*B. sorokiniana*
West Bengal	*B. sorokiniana*
Bangladesh	*B. sorokiniana*
Nepal	*B. sorokiniana*

Incidence of *B. sorokiniana* was found to increase with crop maturity. By late February and March, most of the varieties showed a maximum frequency of *B. sorokiniana*, while that of *A. triticina* was lower.

Symptoms

The disease symptoms of the two major pathogens are as follows:

- *A. triticina*: Lesions on leaves are irregular in shape and dark brown to gray. Initially, the disease appears as small, oval discolored lesions, which coalesce with disease progression, resulting in leaf death.
- *B. sorokiniana*: Lesions on leaves are small, chlorotic, and oval shaped with dark centers. Reddish-brown centers with yellow margins form, and ends of the lesions are tapered.

Foliar Blight Epidemiology

Based on five-year observations, disease data of five wheat varieties (Sonalika, HD 2285, UP 262, HD 2329, and HUW 234) were found to correlate to meteorological parameters for February. During this month, most varieties are at the heading to flowering - the most suitable growth stage for foliar blight infection.

Foliar blight initiation was found to begin as early as the second fortnight in December, but severity increased during heading and flowering stages. Results presented in Table 3 show that a temperature of about 28°C, and relative

Table 3. Foliar blight severity and climatic conditions observed between 1991-96 at Faizabad, India.

Year	Meteorological parameters	Disease severity (%)				
		Sonalika	HD 2285	UP 262	HD 2329	HUW 234
1995/96	Max. temp 24.9°C Min. temp. 10.7°C RH 92.0% Rainfall 9.6 mm	65	60	50	60	50
1994/95	Max. temp 27.7°C Min. temp 9.7°C RH 92.0% Rainfall 20.6 mm	80	75	75	80	60
1993/94	Max. temp 24.1°C Min. temp. 9.7°C RH 93.0% Rainfall 9.6 mm	70	65	60	70	50
1992/93	Max. temp 28.2°C Min. temp. 13.4°C RH 72.0% Rainfall 27.8 mm	75	70	70	75	50
1991/92	Max. temp 23.0°C Min. temp. 11.8°C RH 91.0% Rainfall 2.3 mm	60	50	50	60	40

humidity (RH) of 92% promoted maximum foliar blight development. Rainfall directly increased disease intensity. From the study, 1994/95 was the most favorable year for disease development, with an average temperature of 27.7°C, RH of 92%, and rainfall of 20.6 mm. Other factors which increased foliar blight intensity were minimum tillage, irrigation, late planting, and inappropriate fertilizer regimes.

Yield Loss Assessment

Variety HP 1633 showed a yield loss of 21.72% and a 1000-grain weight loss of 9.80% with a disease reduction of 64.00%. Variety UP 262 showed an 18.34% yield loss and a 9.00% loss in 1000-grain weight with a 68.57% reduction in disease.

Chemical Control

Maximum yield and grain weight, and minimum disease intensity was observed after three sprays of fungicide Tilt 25 EC (0.5 L ha^{-1}) followed by two sprays of Tilt 25 EC (0.5 L ha^{-1}) and three sprays of Indofil M-45 (2.5 kg ha^{-1}).

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Occurrence and Significance of Spot Blotch in Bangladesh

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Abstract

Spot blotch, caused by Bipolaris sorokiniana, *is a destructive disease of wheat in the rice-based cropping system of Bangladesh. The disease has the potential to be epiphytotic on the wheat variety Kanchan, which covers 85% of the total wheat area in the country. The disease appears at the seedling stage and increases in severity with plant age. Incidence of the pathogen in grain is common. At the present time, almost all released varieties in Bangladesh show varying degrees of spot blotch susceptibility but no germplasm has been found to be highly resistant. Yield losses in varieties Akbar, Agrahni, Kanchan, Sawgat, and Sonalika were 16.7%, 9.7%, 19.0%, 23.0%, and 23.6%, respectively. In the 1991/92-1993/94 wheat seasons, the average wheat yield loss due to spot blotch was estimated at 14.9%. Variability among* B. sorokiniana *isolates collected from different locations in Bangladesh has been studied.*

Wheat (*Triticum aestivum* L.) is the second major cereal crop in the rice-based cropping system of Bangladesh. In 1991/92, the area under wheat cultivation was estimated at 0.6 million ha, with an average yield of 2.3 t ha^{-1} (Anonymous 1992); during the early 1970s, the area under wheat cultivation was only 0.1 million ha. The introduction and development of semidwarf, high yielding varieties had a significant impact on wheat area and production: both increased significantly between 1974/75-1984/85. The increase in wheat production during this period was almost 40% per annum; however, both area and production declined by 8% between 1985/86-1988/89 due to a rapid expansion in winter rice cultivation. In recent years wheat area and production have remained almost static (Malaker 1996).

Incidence

Disease is one of the major constraints for wheat production in Bangladesh, where climatic and agro-ecological conditions favor rapid development and growth of various plant pathogens. The most frequently occurring pathogen in Bangladesh is *Bipolaris sorokiniana* (Sacc. in Sorok.) Shoemaker, teleomorph *Cochliobolus sativus* (Ito & Kuribayashi) Drechs. ex Dastur, (syn. *Helminthosporium sativum* Pamm., King & Bakke), which causes spot blotch of wheat, now the major limitation for wheat cultivation in the country (Alam *et al.* 1994). The recommended cultivars are either moderately susceptible or susceptible; Akbar, Ananda, Sawgat, and Prativa are moderately susceptible (MS), and Agrahni, Balaka, Barkat, Kanchan, and

Sonalika are susceptible (S). The pathogen is commonly found in grain. The highest level of seedborne fungal infection was observed for *B. sorokiniana* (46%), followed by *Aspergillus flavus* Link ex Fr. (31%), *Alternaria tenuis* (Syn. *A. alternata*) (Fr.) Keissler (28%), *Rhizopus* spp. (23%), *Curvularia lunata* (Wakker) Boedijn (22%), and *Sclerotium rolfsii* Sacc. (4%) (Kamaluddin 1996; Figure 1).

During the 1980s, several high yielding varieties were released for cultivation under different growing conditions in Bangladesh; however, cv. Kanchan was the most popular in all regions. This variety currently occupies about 80% of the total wheat sown area, whereas it covered only 40% in the 1989/90 growing season (Razzaque *et al.* 1994). Other varieties grown are Akbar, Sonalika, Aghrani, and Prativa. Although almost 100% of the wheat area is under high yielding varieties, the national yield average still remains low compared to many other wheat producing countries.

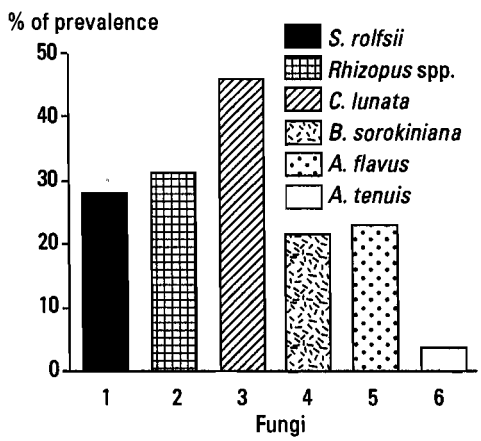

Figure 1. Prevalence of fungi associated with wheat seed.

A 10-year survey conducted in Bangladesh during the 1985/86-1995/96 wheat seasons showed that spot blotch damage occurs late in the season when the crop is approaching maturity. It was also observed that environmental conditions in the south of the country are more favorable for rapid pathogen development and growth than in the north. Sowing time has a marked effect on spot blotch incidence. From our observations, it appeared that disease incidence was higher on late planted (December 15-20) than optimum planted (November 15-30) wheat (Alam and Shah 1991).

Selection for *B. sorokiniana* resistance has been initiated using the best available germplasm at seedling and adult plant stages. To date, high resistance levels have not been found; however, in lines A6/Glen, Prl/Toni, Mayoor, Chirya-3, Chirya-7, Suzhoe-8, Altar84/*T. tauschii*(205)//3*Opata, and Altar84/ *T. tauschii*(224)//4*Yaco, the top leaves were noted to be free from infection, but some isolated lesions were found on lower leaves at later growth stages. Three spot blotch resistant cultivars in Brazil, RH18, BR4, and CEP-11, showed susceptibility when grown in Bangladesh. Available alien species were tested against *B. sorokiniana* strains at all growth stages. Wild species of wheat *T. elongatum*, *T. tauschii*, and *Haynaldia villosa* were found moderately resistant, with *T. elongatum* the most resistant of the three.

Significance

Yield loss due to spot blotch has been reported at 23.6% for old cultivar Sonalika, compared with losses of 16.7, 9.7, 23.0, and 19.0% for recommended cultivars Akbar, Aghrani, Sawgat, and Kanchan, respectively (Razzaque and Hossain 1991; Alam *et al.* 1995). Yield losses in Kanchan were 5.7, 8.2 and 10.5% for the 1985/86, 1986/87, and 1987/88 wheat seasons, respectively (Anonymous 1986, 1987, 1988). The average wheat yield loss due to spot blotch on several farms in Bangladesh over a number of years was estimated at 15%. The experiment was conducted on farms at four different locations during the 1991/92-1993/94 wheat seasons. Kanchan was selected for all trials and two treatments were applied: spraying with 0.5 L ha^{-1} Tilt 250 EC and no spraying (Alam *et al.* 1995).

Pathogen Diversity

The diversity of 27 isolates of *B. sorokiniana* was studied. Seven different morphological and physiological characters were considered in order to determine the divergence level among isolates. Based on multivariate analysis, all 27 isolates clustered in four groups (Figures 2 and 3). Principal component analysis, principal coordinate analysis, cluster analysis, and canonical variate analysis all produced similar results. Three isolates belonged to cluster I, 6 to cluster II, 14 to cluster III, and 4 to cluster IV. The highest inter-cluster distance was observed between cluster I and IV, and the lowest between I and III. Among the seven characters evaluated, cell number per conidium and sporulation at pH 7.0

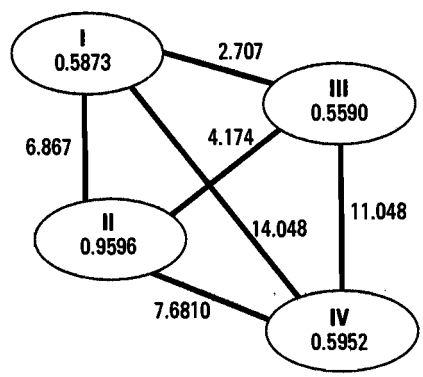

Figure 3. Intra-and inter-cluster distences of 27 isolates of *Bipolaris sorokiniana*.

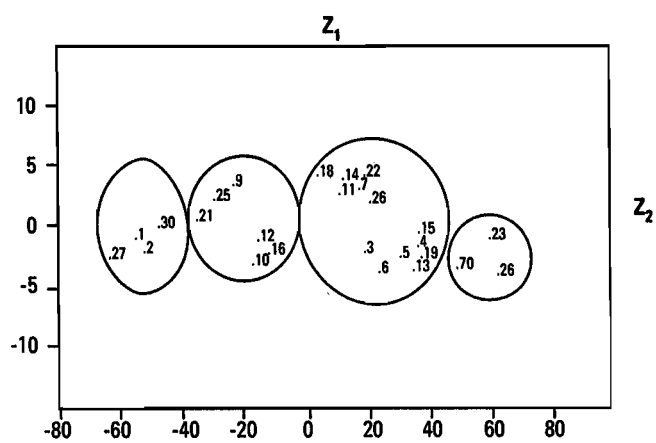

Figure 2. Scatter diagram of 27 isolates of *Bipolaris sorokiniana* based on their principal component scores and cluster analysis.

contributed most to divergence among isolates; however, variability in the degree of isolate pathogenicity needs to be tested in artificial inoculation experiments (Ahmed 1996).

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Disease Incidence and Yield Loss Due to Foliar Blight of Wheat in Nepal

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Abstract

Helminthosporium leaf blight (HLB, tan spot) is a serious disease of wheat in Nepal, caused individually or in combination by Bipolaris sorokiniana *(Sacc.) Shoem. and* Pyrenophora tritici-repentis *(Died.) Shoem. In recent years, the magnitude and severity of HLB have extended from the terai region (100 masl) to the hilly region (2400 masl). Since 1990,* B. sorokiniana *has been the predominant pathogen of the leaf blight syndrome. Incidence of seed infection was higher in samples from eastern terai than western terai and the hills. The relationship between seed infection and germination was significant and negatively correlated. Most of the improved and recommended wheat varieties are severely infected by the disease; however, varieties including NL 644, NL 623, BL 1413, Nepal 297, NL 625, NL 591, and Triveni showed moderate HLB resistance and BL 1420 was observed to be resistant. Eighty-six entries, resistant/tolerant to HLB, were selected from multilocation trials, and could be used as potential sources of resistance. Yield losses as high as 23.8% were recorded. Poor germination was experienced due to high* B. sorokiniana *infection levels in grain harvested during 1996. This problem was solved by treating the seed with Vitavax-200 before distribution to farmers. Disease management has been practiced in Nepal for the last few years. Incidence of HLB was significantly reduced by two irrigation cycles and three applications of propiconazole as a foliar spray at 0.5 L ha^{-1}.*

In Nepal, more than 80% of the total population is engaged directly or indirectly in agriculture or agro-based industries. Wheat occupies the third position among the three major cereal crops with a cultivation area of 653,000 ha and a productivity of 1.48 t ha^{-1}. Over the last 10 years, there has been an increase in wheat area, production, and productivity from 576,000-653,000 ha, 743,000-965,000 t, and 1.29-1.48 t ha^{-1}, respectively (Anonymous 1996; Figure 1).

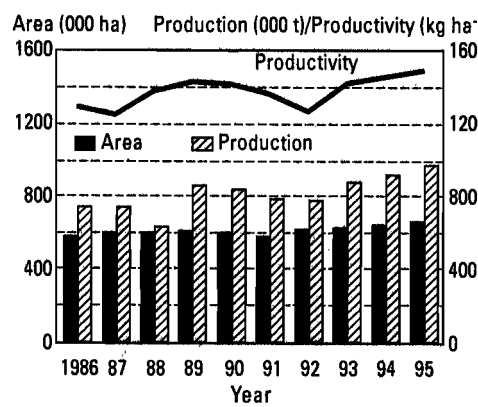

Figure 1. Wheat area, production, and productivity in Nepal.

Helminthosporium leaf blight (HLB, tan spot) is a serious disease of wheat caused individually or in combination by *Bipolaris sorokiniana* (Sacc.) Shoem. (syn. *Helminthosporium sativum* Pamm., King & Bakke) and *Pyrenophora tritici-repentis* (Died.) Drechs. (syn. *Drechslera tritici-repentis* (Died.) Shoem. Helminthosporium leaf blight is considered a disease of major importance because of its potential to cause yield loss. It is widely distributed throughout the wheat growing areas of the country, particularly in the terai (Anonymous 1976). Tan spot, caused by *P. tritici-repentis*, was recorded in 1978 and its incidence has annually increased (Karki 1982). Since this time, disease severity has been high, affecting the leaves of commercial cultivars before the mid dough stage of plant growth in eastern to western regions of the country (Karki and Horsford 1986). Karki and Lohani (1980) reported that a few cultivars were found to be resistant/ tolerant to the disease. Prior to 1990, the pathogen was considered important in causing leaf blight, but later *B. sorokiniana* was found to be predominant and responsible. This change in pathogen dominance may be due to environmental change and the introduction of new exotic wheat varieties. In Nepal, both pathogens were isolated from similar types of lesions on leaves, although different symptoms have been described by Wiese (1977).

Distribution, Severity, and Losses

In recent years, HLB due to *B. sorokiniana* has extended from the lower belt of the terai region (100 masl) to the hilly region (2400 masl); however, incidence and severity are comparatively higher in the terai than in the hills (Anonymous 1991; Karki and Sharma 1990).

Magnitude of yield loss due to HLB may vary among locations and years. Based on a survey conducted in various parts of the country, a disease incidence of up to 90% on cv. RR21 was recorded in the terai belt and 40-50% in the hills (Anonymous 1979). Leaf blight was also reported in the lower belt of the Lumle Agricultural Research Command Areas. A higher disease incidence (74, using the double digit scale) on cv. Annapurna was recorded after a single top dressing of fertilizer than after a double top dressing (53) (Sharma 1995). Disease level on the flag leaf (F) and the F-1 best shows the relationship between disease severity and grain yield loss (Chaurasia 1995). Grain yield loss was reported to vary with genotype (Mahto *et al.* 1995). The highest grain yield loss was reported for RR 21, ranging between 23.2 and 23.8%. Yield losses recorded in the field at Bhairahawa were 7.9%, 3.1%, and 15.2% for Ocepar 7, Ning 8319, and Nepal 297, respectively.

Bipolaris sorokiniana in Wheat Seed

Bipolaris sorokiniana is seed and soilborne, and in nature is seed transmitted (Neergaard 1977; Mehta 1993). In 1977, the pathogen was reported in seed samples of wheat (Shrestha 1977; Shrestha *et al.* 1977). Seed infection by *B. sorokiniana* can adversely affect

germination and root system development and can kill seedlings within a few days (Mehta 1993).

In 1996, the poor germination of wheat seed became a national issue. To investigate the cause, nearly 200 seed samples were collected from different parts of the country (Figure 2) and tested using the standard blotter method (Anonymous 1985).

Heavily infected seeds showed shriveling and black discoloration at the embryonic end; however, normal-looking seeds were also found to be severely infected with *B. sorokiniana*. The data indicate that the average level of seed infection from terai to foot hill regions varies between 5.0-89.1%, irrespective of cultivar, whereas the infection level in the mid hills was quite low. Similarly, the average percentage of seed germination varied between 33.7-94.0% (Table 1). Based on correlation analysis, the relationship between percentage seed infection and seed germination was significant and negatively correlated ($r = -0.789$, $P<0.001$; Figure 3). This confirmed that seed infected by *B. sorokiniana* is responsible for causing poor germination (Table 1). Similarly, seed samples from the eastern hill (Dhankuta) to terai (Jhapa and Dhanusha) region showed an average *P. tritici-repentis* infection level of 4-18.8%, and seed samples from western hill (Pyuthan) to terai (Rupandehi) region showed 3.5-8.1%; however, most samples were free from *P. tritici-repentis*.

Table 1. Distribution of *Bipolaris sorokiniana* in seed samples from different districts of Nepal, and HLB severity in the field, 1995/96.

Districts	No. of samples	Average seed infection[1] (%)	Average germination (%)	HLB severity[2] (00-99)
Jhapa	7	11.0	80.0	88
Morang	1	69.5	75	86
Sunsari	6	44.0	82.0	86
Saptari	1	16.0	63.0	88
Dhanusha	10	33.8	72.3	86
Mohattari	1	56.5	74.0	89
Sarlahi	1	41.0	67.0	88
Rautahat	1	43.0	82.0	88
Bara	10	31.0	80.5	86
Parsa	4	35.9	75.5	88
Makwanpur	2	46.0	62.5	89
Chitwan	2	53.5	67.0	88
Nawalparasi	3	18.6	91.3	88
Tanahun (foot hills)	6	89.1	33.7	87
Rupandehi	12	50.1	70.1	86
Kapilvastu	3	22.7	87.5	83
Bardiya	1	1.5	94.0	85
Dang	1	5.0	90.0	83
Banke	5	4.7	93.7	83
Surkhet	2	8.0	92.0	70
Kailali	5	5.2	88.5	83
Kanchanpur	1	8.5	88.0	79

[1] Source: Shrestha and Timila, unpublished data.
[2] Source: Karki and Karki 1995.

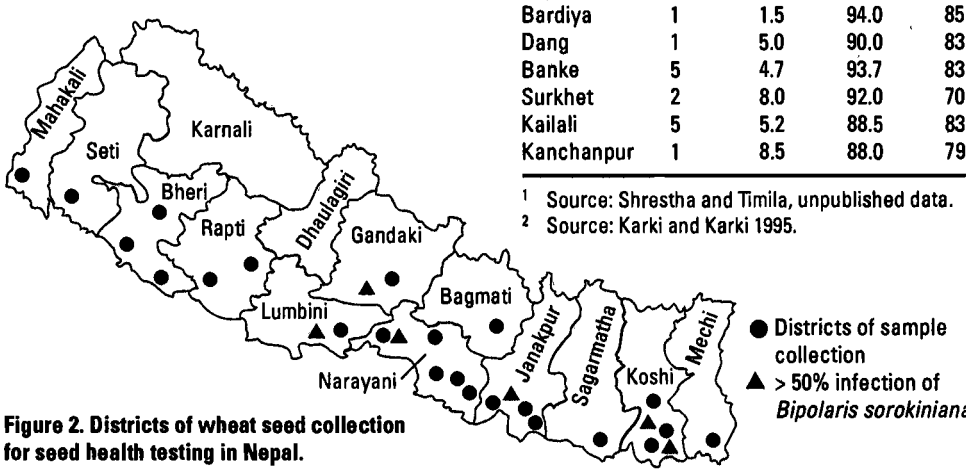

Figure 2. Districts of wheat seed collection for seed health testing in Nepal.

Disease Management

Experiments on integrated disease management for foliar blight control have been conducted over the last few years.

Varietal performance

Most of the improved and recommended wheat varieties in Nepal are severely affected by foliar blight. Generally high HLB infection was observed in check varieties RR 21 and UP 262 during the 1990s. High yielding cultivars had lower AUDPC (area under the disease progress curve). Ning 8319 produced the highest yield (5,108 kg ha^{-1}) in 1994, followed by NL 590, DL 153-2, and Nepal 297 (Mahto *et al.* 1995). Late heading varieties such as WPH 153, WPH 241, WPH 243 had less blight. In the last few years, variety NL 645 has shown good performance in terms of disease resistance and agronomic character (Sharma *et al.* 1995).

Genotypes NL 644, NL 623, BL 1413, Nepal 297, NL 625, NL 591, and Triveni were found to have better levels of HLB resistance than check varieties RR 21 and UP 262, whereas BL 1420 was observed to be resistant but is agronomically unacceptable (Bimb and Mahto 1992a, 1992b, 1992c). Six entries with a score of 93 were found in the helminthosporium monitoring nursery, in which 44 entries with good HLB resistance levels from CIMMYT were included. Of 140 entries from the multilocational helminthosporium leaf blight screening nursery, 86 were selected as resistant/tolerant to HLB and could be used as potential sources of resistance (Mahto 1995). *Thinopyrum curvifolium* derivatives and Chinese lines were also reported to have good HLB resistance.

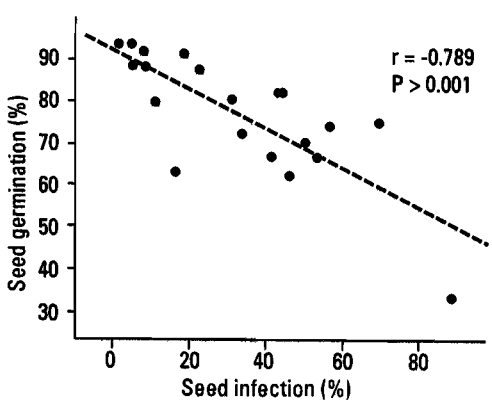

Figure 3. The effect of *Bipolaris sorokiniana* infection level on wheat seed germination.

Soil solarization

The residual effect of soil solarization, occurring in May at Bhairahawa, increased the yield of the wheat crop following rice by 12.44% by significantly reducing HLB. It was also noted that root necrosis and subcrown internode infection were higher in the plot where no solarization occurred (Bimb and Mahto 1992a). Soil solarization in November significantly reduced root necrosis and HLB (Dubin and Bimb 1994).

Irrigation

The application of two irrigation cycles reduced leaf blight levels on the whole plant and on the flag leaf up to the dough growth stage. This practice increased grain yield, spikes m^{-2}, and biomass by significantly reducing foliar blight compared with the non-irrigated plot.

Chemical control

Foliar spray—Several fungicides including Bavistin, Benlate, Blitane,

Dithane M-45, and Dithane Z-78 were tested. Dithane M-45 at 2 kg ha^{-1} in 800 L of water was found to be superior to other chemicals in reducing disease infection (Anonymous 1977; Bimb 1979).

Tilt (propiconazole) was also found to be quite effective in reducing HLB. Foliar blight was effectively controlled by three sprays of Tilt at a rate of 0.5 L ha^{-1} in two-weekly intervals. A 16.19% loss in grain yield was recorded in unsprayed plots (Mahto and Sedhai 1994).

Seed treatment

Rovral (iprodione) at 2.5 g per kg of seed achieved the highest control of *B. sorokiniana*, followed by Vitavax-200 at 2.5 g, and Vitavax-75 at 2.0 g per kg of seed. Since Rovral is not locally available, Vitavax-200 and Vitavax-75 were recommended to farmers. A significant increase in seedling emergence in sterilized soil was also observed after application of these fungicides (Timila and Shrestha, unpublished).

Future Research Strategies

Studies need to focus on whether *B. sorokiniana* and *P. tritici-repentis*, individually or in combination, are responsible for causing economic yield loss. Research into the role of seedborne inoculum and foliar blight incidence in the field should be investigated. The effect of indigenous materials should be tested to determine their efficacy for foliar blight control.

Conclusion

Concerns have been raised over the increased HLB incidence in the terai belt of Nepal due to the planting of carry-over seed by farmers in many districts. Until resistant varieties are developed, it is recommended that Vitavax-200 or Vitavax-75 be provided to farmers and seed producers by the Government at a reasonable cost.

In view of the increasing HLB incidence in the terai region, it is emphasized that seed lots with *B. sorokiniana* infection levels higher than 20% may not be used for planting without seed treatment.

Acknowledgment

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Tan Spot in Western Canada

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Abstract

The main leaf spotting diseases of spring wheat in western Canada are tan spot and the septoria leaf blotch complex. Tan spot is most prevalent in drier areas, whereas septoria leaf blotch is more prevalent under moist conditions. All wheat cultivars currently registered for use in western Canada are susceptible to leaf spots. Some cultivars have been shown to possess adult plant resistance to tan spot. Durum wheat cultivars are more susceptible to tan spot than common wheat, whereas common wheat is mostly affected by septoria leaf blotch. A study conducted in southern Saskatchewan during 1991-1993 showed that the relative prevalence of leaf spotting fungi in durum and common wheat cultivars was affected mainly by weather factors, whereas location affected their relative leaf spotting score. In recent years, there has been a steady increase in the incidence of leaf spotting diseases throughout western Canada, which has been attributed to above-average precipitation and adoption of conservation tillage practices. At present, the most common cropping systems for wheat are a rotation with summer fallow or a broadleaf crop, or continuously cropped wheat. These cropping systems have been increasingly managed using reduced tillage practices. The effect of different crop management practices on leaf spots was examined in two studies conducted at Swift Current, Saskatchewan, during 1993-1996. Leaf spot severity was higher in wheat after fallow than in continuous wheat, under both conventional tillage practices designed to conserve residues and under no-till management. Wheat grown under no-till had similar leaf spot severity as conventional-till wheat. Continuous wheat and wheat grown after a non-cereal crop had similar leaf spot severity, except in years with high disease pressure, when a continuous wheat system had higher disease levels. Examination of crop residues collected at seeding suggested that differences among treatments could be related to the higher density of infective structures of leaf spotting fungi on residues from two seasons before than on those from the immediately previous crop. This is likely due to environmental conditions throughout the winter not being conducive to fungal growth and to the development of infective structures on the new residue. Crop residues in the no-till treatments had a lower density of fungal structures than those in conventional tillage systems. Glyphosate used for weed control in the no-till treatment may have had an inhibitory effect on fungal growth. In addition, there was a higher leaf spot severity in treatments with N deficiency in wet years, and a lower severity of leaf spots in treatments with P deficiency in years that had a cool and wet spring.

Leaf spots have become an increasingly important disease of wheat (*Triticum aestivum* L.) in the western Canadian Prairies (Fernandez *et al.* 1996b, 1997). Above-average precipitation during the growing season, and an

increase in crop residues on the soil surface resulting from a decline in the number of tillage operations used for summer fallow and seedbed preparation, are believed to be the main causes for the increased importance of this disease complex.

The most common leaf spotting diseases in the western Canadian Prairies are tan spot [*Pyrenophora tritici-repentis* (Died.) Drechs., anamorph *Drechslera tritici-repentis* (Died.) Shoemaker) (Ptr)] and Stagnospora blotch [*Phaeosphaeria nodorum* (E. Muller) Hedjaroude, anamorph *Stagnospora nodorum* (Berk.) Castellani & E.G. Germano) (Pn)]. All wheat cultivars currently registered for use in western Canada are susceptible to leaf spots (Saskatchewan Agriculture and Food 1997). Some have shown adult plant resistance to tan spot (Fernandez *et al.* 1994; unreported observations).

Leaf Spot Susceptibility among Wheat Species/ Classes

A three-year study (1991-1993) was conducted at two locations in southern Saskatchewan (Swift Current in the semiarid Brown soil zone, and Outlook in the more moist Dark Brown soil zone) to examine differences in leaf spot severity and in the relative prevalence of leaf spotting fungi among wheat species/ cultivars, locations, and years (Fernandez *et al.* 1996a). Six durum wheat and six common wheat cultivars, currently registered for use in western Canada, were scored for leaf spots at GS 77-84 (Zadoks *et al.* 1974) using a 0-9 scale. Lesioned leaf pieces from each plot were plated on water agar, incubated for a week, and examined under the microscope for fungal structures. The percent leaf area covered by each fungus was calculated relative to its frequency of isolation. Ptr was the most common leaf spotting pathogen at Swift Current, and was more frequently isolated than at Outlook (Table 1). This pathogen was also more prevalent under dry and hot conditions. A similar number of Ptr and Pn isolations were made in the wet years of 1992 and 1993, whereas Ptr was the commonly isolated pathogen in the dry year of 1991.

Ptr was also isolated at a higher frequency from durum wheat than from common wheat cultivars (Table 1). The latter were mostly colonized by Pn when this fungus was favored by environmental conditions. The significant cultivar x year interaction for percent

Table 1. Estimated percent diseased leaf area of durum and common wheat colonized by *Pyrenophora tritici-repentis* and *Phaeosphaeria nodorum* at two locations and three years.

	P. tritici-repentis (%)	*Ph. nodorum* (%)
Location		
Swift Current	67 (1) [1]	28 (1)
Outlook	47 (1)	42 (1)
Year		
1991	74 (2)	8 (1)
1992	53 (2)	45 (1)
1993	44 (2)	49 (2)
Species		
Durum wheat	76 (2)	19 (2)
Common wheat	39 (2)	49 (2)

[1] Percent values (standard errors in brackets) based on six durum and six common wheat cultivars.
Source: Fernandez *et al.* 1996.

isolation of Ptr and Pn (Table 2) indicated that cultivars reacted differently to the different prevalence of the fungi attributed to environmental conditions. In contrast, the significant cultivar x location interaction for leaf spot scores suggested that the different relative severity of leaf spots among cultivars was possibly related to differences in disease pressure between locations.

These observations have implications for establishing protocols for testing germplasm for leaf spot resistance. Selection of the most appropriate locations and useful benchmarks for selecting germplasm for leaf spot resistance in a given type of wheat should involve assessment of the leaf spot reaction of potential checks under different environments, and identification of the most prevalent leaf spotting fungi.

Effect of Management Practice on Leaf Spotting Diseases

The effects of summer fallow, rotation with a noncereal crop, and fertility were examined in a field study, conducted at Swift Current from 1993-1996. Ten crop rotations suitable for southwestern Saskatchewan, involving spring wheat (cv. Lancer) in rotation with other cereal or noncereal crops, or in a 1-5 year rotation with summer fallow, or continuously grown, were examined. All phases of the rotations were represented every year and each rotation was cycled on its assigned plots (10.5 m wide x 40 m long). Treatments were arranged in a randomized complete block design with three replicates. Plots were managed using conventional tillage practices, but designed to conserve surface crop residues. The frequency of soil tillage was kept to a minimum; fallow areas received an average of three tillage operations during the summer season. Fertilizers N and P were applied in accordance with guidelines provided by the Saskatchewan Advisory Council on Soils. The N was broadcast as ammonium nitrate (34-0-0) and soil-incorporated by tillage used to prepare the seedbed. The average rate of N fertilizer applied to wheat was 37 kg ha^{-1}. Phosphorus was applied with the seed as monoammonium phosphate at an average rate of 9.7 kg P ha^{-1}. Some of the treatments were deficient in fertilizer; these were continuously grown wheat with P but no N added, and wheat grown

Table 2. Analysis of deviance of the probability of leaf spot scores not exceeding 6 (on a scale of 0-9) on 12 wheat cultivars at two locations over three years, and of percent leaf area colonized by leaf spotting pathogens (*Pyrenophora tritici-repentis* and *Phaeosphaeria nodorum*).

Source of variation	df[1]	Deviance	Deviance ratio	
		Leaf spot score	*P. tritici-repentis*	*Ph. nodorum*
Cultivar	12	54.49**	34.13**	132.78**
Cultivar x Year	22	8.40	2.59**	3.57**
Cultivar x Location	12	41.24**	1.57	1.40

[1] Degrees of freedom.
** Significant at P<0.01.
Source: Fernandez *et al.* 1996.

in a two-year rotation with fallow with N but no P, or with P but no N. Severity of leaf spots was assessed at GS 74-77 using a 0-11 scale. Lesioned leaf pieces from each plot were plated on water agar and examined under the microscope. Percent isolation of each of the fungi was based on the total area colonized by each of them.

Overall, the average leaf spot severity was lowest in the dry year of 1994 (7.3), and highest in 1995 (9.4). In the latter year, growing season (May to July) precipitation (189 mm) was above average. The second highest mean leaf spot scores were obtained in 1996 (9.1) when dew frequency was high. Average leaf spotting score for 1993 was 8.6. Growing season precipitation in the latter year was above average (185 mm), but temperatures were below average (mean maximum of 20.3°C). Based on analysis of variance, there was a significant difference ($P<0.01$) in leaf spot severity among rotation sequences in all years. Overall, the most prevalent leaf spotting fungus was Ptr, followed by Pn.

Differences between rotations with and without the addition of N, and with and without the addition of P, were highly dependent on environmental conditions. Wheat deficient in N, grown continuously or in the second year after fallow, had higher ($P<0.05$) severity of leaf spots than those that had N and P added in the wetter years of 1993 and 1995, but not in 1994 or 1996 (Table 3). An N deficiency would have been more apparent in wet years. Leaf spot severity in the first year of wheat after fallow in the fallow-wheat-wheat (N, no P) rotation was lower than in fallow-wheat-wheat (N and P) in 1995 and 1996, years that were cool and wet at and after seeding, which is when a response to P would be expected to be highest (Zentner *et al.* 1993). The increase in leaf spot severity with an increase in N deficiency agrees

Table 3. Leaf spot scores of wheat in selected treatments from a crop rotation study at Swift Current, Saskatchewan, 1993-1996.

Rotation sequence[2]	Leaf spots[1]				
	1993	1994	1995	1996	Mean
Fallow-Wheat (N and P)	9.7	8.0	10.0	10.2	9.5
Fallow-Wheat-Wheat (N and P)	8.7	7.8	10.0	10.3	9.2
Fallow-Wheat-Wheat (P, no N)	9.0	7.8	10.0	10.0	9.2
Fallow-Wheat-Wheat (N, no P)	9.0	7.5	8.8	9.2	8.6
Fallow-Wheat-Wheat (N and P)	8.3	7.2	9.7	9.0	8.6
Fallow-Wheat-Wheat (P, no N)	9.0	7.2	10.2	9.3	8.9
Fallow-Wheat-Wheat (N, no P)	8.0	7.0	9.0	8.7	8.2
Continuous wheat (N and P)	8.3	7.2	9.5	8.8	8.5
Continuous wheat (P, no N)	9.0	7.3	10.2	8.8	8.8
Lentils-Wheat (N and P)	8.0	7.2	8.3	8.3	8.0
LSD (0.05)	0.8	0.5	0.5	0.5	

[1] Leaf spot scores based on a scale of 0-11.
[2] Rotation phases underlined refer to those that were sampled.

with Huber *et al.* (1987), Johnston *et al.* (1979), and Orth and Grybauskas (1994), whereas the lower severity of leaf spots in P-deficient plants supports the findings of Cunfer *et al.* (1980) and Leath *et al.* (1993).

Leaf spot severity was higher in wheat grown after fallow than in wheat grown after lentil (*Lens culinaris* Medikus var. Laird) (Table 3). Continuous wheat had a significantly higher leaf spot severity than wheat grown in rotation with lentil only in 1995 and 1996, when disease pressure was high. In all years, wheat grown after fallow (N and P) had higher leaf spot severity than of continuous wheat (N and P). There are no previous reports of a consistent higher severity of leaf spotting in wheat grown after fallow than in continuous wheat.

Differences in leaf spotting severity among treatments could be explained by the density of infective fungal structures on residues. Wheat residues, collected from selected treatments in the spring of 1995 and 1996, were washed, weighed, and observed under the microscope for the presence of infective fungal structures. The number of mature and immature pseudothecia of Ptr, the most prevalent pathogen, was higher in older residues, that is, from two seasons previous, than on residues from the immediately previous crop (Table 4). This was true both on a unit area and a weight basis. The slow development of Ptr on residues from the immediately previous season could be explained by environmental conditions, in particular very low temperatures, in southern Saskatchewan from the harvest of one spring wheat crop to seeding of the next, which would not be adequate for fungal growth and development of fungal structures on crop residues (Odvody *et al.* 1982; Summerell *et al.* 1988). A requirement period of 8 to 9 months from harvest of a winter wheat crop to formation of mature pseudothecia of Ptr on crop residues has been reported under more temperate conditions than in Saskatchewan (Maraite *et al.* 1992; Odvody *et al.* 1982; Wright and Sutton 1990). Our results also indicate that viable

Table 4. Number of mature pseudothecia of *Pyrenophora tritici-repentis* on one- and two-year-old ground wheat residues, collected in May 1995 and 1996 from a rotation study at Swift Current, Saskatchewan.

Year/crop sequence	Year	Residue (g)[1]	*P. tritici-repentis* pseudothecia
1995			
Fallow-Wheat[2]	1993	21	155 [8][3]
Wheat-Wheat	1994	22	51 [2]
	1993	5	109 [19]
1996			
Fallow-Wheat	1994	14	319 [19]
Wheat-Wheat	1995	31	9 [<1]
	1994	5	120 [23]

[1] Average amount of crop residues collected in half square meter samples.
[2] Rotation phases underlined refer to those that were sampled.
[3] Number of fungal structures per half square meter; per gram of residue tissue in brackets.

infective structures are able to survive on residues for at least two years, which agrees with other reports (Krupinsky 1992; Pedersen and Hughes 1992).

The observation that rotating wheat with a noncereal crop for one year did not always result in lower leaf spot levels than in continuous wheat could be explained by infective wheat residues being carried over from previously grown wheat crops, and to the possible survival of wheat pathogens in residues of nonhost species (Fernandez *et al.* 1990).

Based on these observations, we concluded that the best crop rotation to guarantee a low level of leaf spot infection in spring wheat in the Brown soil zone of the western Canadian Prairies would be two consecutive years of wheat followed by at least two years of a noncereal crop, or by a noncereal crop and summer fallow.

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Diseases Caused by *Bipolaris sorokiniana* and *Drechslera tritici-repentis* in Hungary

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Abstract

Of the fungal pathogens of cereal crops in Hungary, Drechslera *and* Bipolaris *species have recently increased in importance. Until 1988,* B. sorokiniana *was considered the most frequently occurring wheat pathogen of the* Helminthosporium sensu lato *genus. The pathogen was found at a frequency of 5-8% in fields, infecting all parts of the wheat plant. During the past few years, the fungus has often been identified on wheat seeds displaying black point disease. In 1988,* D. tritici-repentis *was first reported on winter wheat and rye in Hungary. The pathogen caused a serious epidemic on rye hybrids, whose cultivation was consequently discontinued in some regions. Later the fungus was also isolated from barley and millet. In the early 1990s, the spread of* D. tritici-repentis *decreased to a few percent but has increased again in the past two years. In the autumn seasons of 1991-1994, hundreds of monocotyledonous samples were collected to examine the occurrence and host range of* Bipolaris, Drechslera, *and* Exserohilum *species.* Bipolaris sorokiniana *and* D. tritici-repentis *were collected from 18 and 2 plant species, representing 12 and 2 genera, respectively. The pathogens infected both cultivated and wild plants. Grasses, especially those growing in non-cultivated fields, may act as fungal hosts during overwintering. Susceptibility of 76 wheat genotypes to* B. sorokiniana *and* D. tritici-repentis *at the seedling stage was tested based on sporulation intensity on detached leaves. Types and degree of chlorotic/necrotic symptoms and intensity of mycelium formation were recorded. Plant response to inoculation differed between the two pathogens. In general, an agar plug of* B. sorokiniana *produced higher sporulation levels than that of* D. tritici-repentis. *Inoculation with* D. tritici-repentis, *however, resulted in the formation of more abundant mycelia and necrosis.*

The most important pathogens of cereal crops in Hungary are those that cause powdery mildew, head blight, and rusts. Since their attacks can result in significant yield losses, control measures through chemical applications and/or resistance breeding are usually undertaken. Diseases caused by *Bipolaris* Shoemaker and *Drechslera* Ito species have recently increased in importance. This is partly indicated by a higher incidence of leaf stripe (*D. graminea* (Rabenh. ex Schlecht.) Shoem., teleomorph: *Pyrenophora graminea* Ito & Kurib.), and net blotch (*D. teres* (Sacc.) Shoem., teleomorph: *Pyrenophora teres*

Drechsler) pathogens on barley. A new wheat disease, commonly called tan spot or yellow leaf spot, and its causal agent, *Drechslera tritici-repentis* (Died.) Shoem. (teleomorph: *P. tritici-repentis* (Died.) Drechsler), was first reported in several parts of Hungary in 1988. Until this time, *B. sorokiniana* (Sacc.) Shoem. (teleomorph: *Cochliobolus sativus* (Ito & Kurib.) Drechsler ex Dastur) was considered the most frequent wheat pathogen among those of the *Helminthosporium* Link *sensu lato* genus.

Tan spot control measures include crop rotation, incorporation of crop residues, spraying with fungicides, and planting resistant cultivars (Raymond *et al.* 1985). Incorporation of residues may not be effective enough to prevent pathogens from spreading or from surviving for long periods because they are capable of infecting other gramineous plants that may form a permanent inoculum source. Consequently, host plants growing in non-cultivated fields are a potential infection source and, therefore, there is an increased need for chemical control and tan spot resistant or tolerant cultivars. Unfortunately, fungicides used to control other diseases such as powdery mildew or head blight are not effective enough against spot blotch and tan spot. Furthermore, wheat cultivars currently used by growers are susceptible to both *B. sorokiniana* and *D. tritici-repentis*. For this reason, it is necessary to test the effectiveness of different fungicides against spot blotch and tan spot, and to screen wheat genotypes for resistance/tolerance.

The three main objectives of this paper are to: 1) report on the incidence and host range of *B. sorokiniana* and *D. tritici-repentis* in Hungary, 2) publish results obtained on fungicide effectiveness for controlling these two fungi, and 3) report on the resistance/tolerance of different wheat genotypes to *B. sorokiniana* and *D. tritici-repentis* at the seedling stage.

Materials and Methods

Incidence and host range of *B. sorokiniana* and *D. tritici-repentis*

Plant Health and Soil Conservation Stations annually survey the incidence of spot blotch and tan spot in the field. In 1989 and 1990, after the first report of tan spot in Hungary, a country-wide survey was made to determine the frequency and severity of the blight on winter wheat. The area affected by spot blotch and tan spot was 5700 and 4180 ha, respectively. At the end of May and in the middle of June, plant samples from fields were inspected for tan spot occurrence, and percentage infected leaf area was recorded. Severity of infection was expressed by an index ranging from 0-6 (0 = 0%, 1 = 0.1-5%, 2 = 5.1-10%, 3 = 10.1-25%, 4 = 25.1-50%, 5 = 50.1-75%, 6 = 75.1-100% leaf area infected).

To examine the host range of *B. sorokiniana* and *D. tritici-repentis*, hundreds of monocotyledonous samples were collected in western Hungary during the autumns of 1991-1994. Samples were incubated in moist

chambers for 24-48 h, and microscopic preparations were made from the sporulating lesions. Fungi were identified according to symptoms, host source, and morphology of cultures and conidia formed on the substrate.

Inoculation

Wheat genotypes to be screened for resistance/tolerance were kindly provided by Dr. Gains Falusi (Cereal Research Institute, Táplánszentkereszt, Hungary). Seeds without symptoms were sown into pots containing sterile mold. Pots and seedlings were kept in the laboratory at 22°C under an artificial light/dark cycle for 23 days. Leaves of each genotype were detached from the same height and placed in petri dishes containing wet filter paper. The ends of the leaves were pinched between two glass slides so that the leaves did not touch the filter paper. Leaves were uniformly sprayed with sterile distilled water before inoculation in order to ensure favorable conditions for the fungi.

Bipolaris sorokiniana and *D. tritici-repentis* isolates taken from naturally infected leaves of *Bromus erectus* Huds. and *Agropyron repens* P.B., respectively, were grown on potato-dextrose agar at 25°C in the dark. In a preliminary experiment, several isolates of the two fungi were tested and the most pathogenic isolates were chosen. Agar plugs (3 mm in diameter) were cut from the edge of a seven-day-old colony and placed onto leaf surfaces with the mycelial side in contact with the leaf blade. Each leaf was inoculated at three points (top, middle, and bottom). After two days of incubation at 22°C in the dark, the agar plugs were removed and leaves were further incubated at 22-25°C under artificial light. During evaluation, a 0-3 assessment scale was employed to express sporulation intensity, and symptom types and intensity of mycelium formation on the leaves were recorded (Raymond *et al.* 1985).

Fungicide evaluation

Several fungicides (contact, systemic, and combined), licensed in 1995 in Hungary, have been tested for effectiveness of control on the most important winter wheat fungal diseases including tan spot. Effectiveness was rated according to a 0-10 scale. Duration of effect (weeks) and application costs per ha were recorded. Lastly the technological value was calculated

Results

Incidence and host range of *B. sorokiniana* and *D. tritici-repentis*

Figure 1 shows the *D. tritici-repentis* incidence in 1989 and 1990. In 1989, 70% of the total area was affected by tan spot. Disease severity index ranged from 0-6; however, this value was very low in most fields. Symptoms of primary infection from ascospores were first noticed at the beginning of April when the wheat had two to three nodes (Figure 2a). Symptoms of secondary infection by conidia appeared in the middle of May.

In 1990, tan spot was observed on 51% of the total area (Figure 1). Disease severity index was 0.1-0.9 and 1.0-2.9 on 38% and 13% of the area, respectively. Symptoms of primary infection appeared in the first week of May, but disease frequency and severity did not increase until the third week (Figure 2b). Incidence of *B. sorokiniana* did not change and frequency was similar to that previously observed.

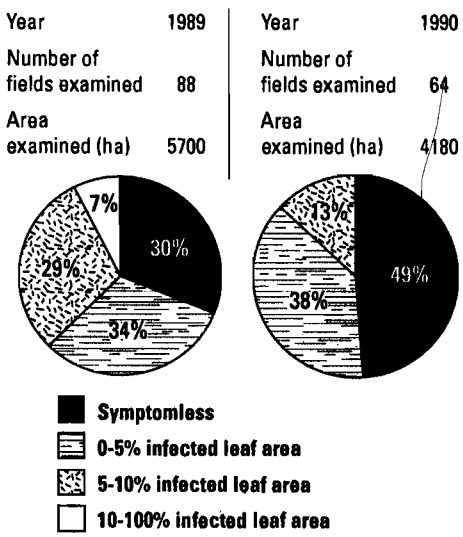

Figure 1. Incidence and severity of tan spot on winter wheat in Hungary.

Drechslera tritici-repentis and *B. sorokiniana* were collected from infected leaves of 2 and 18 monocotyledonous plant species, respectively (Table 1) The tan spot pathogen, however, had been already isolated from rye, triticale, millet, barley, and wild oat in Hungary prior to the survey (Balogh *et al.* 1991).

Table 1. Monocotyledonous hosts of *Bipolaris sorokiniana* and *Drechslara tritici-repentis*.

Hosts	*B. sorokiniana*	*D. tritici-repentis*
Agropyron pectinatum	+	
Agropyron repens	+	+
Alopecurus pratensis	+	
Avena sativa	+	
Beckmannia eruciformis	+	
Bromus erectus	+	
Bromus inermis	+	
Dactylis glomerata	+	
Festuca heterophylla	+	
Festuca ovina	+	
Hordeum murinum	+	
Hordeum vulgare	+	
Lolium perenne	+	
Pennisetum villosum	+	
Poa pratensis	+	
Secale cereale	+	
Seteria viridis	+	
Triticum aestivum	+	+

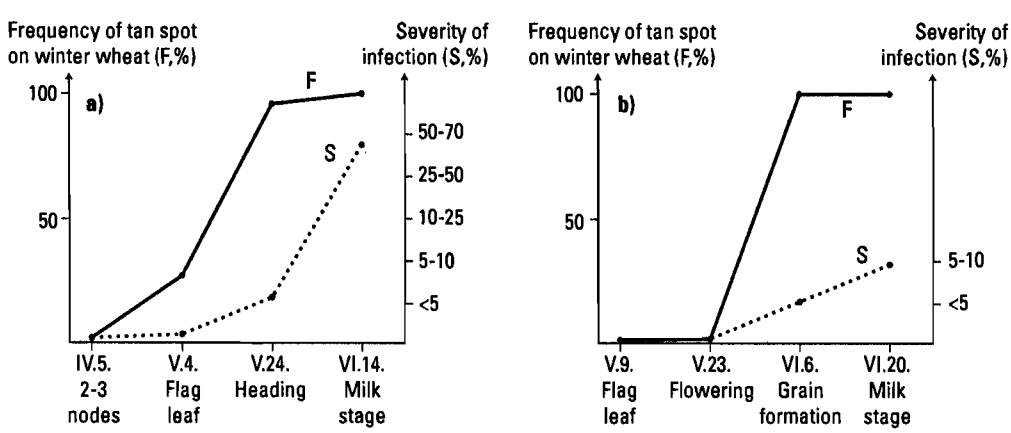

Figure 2. Incidence and severity of tan spot on winter wheat in a) 1989 and b) 1990.

Screening winter wheat seedlings for tan spot and spot blotch resistance

Table 2 shows the average sporulation values calculated after microscopic observation of infection points. *Bipolaris sorokiniana* sporulated abundantly on almost all wheat genotypes. Average sporulation values for the pathogen were lowest on cultivars Alföld, Adriana, and Mv 15. Necrotic symptoms formed around the point of inoculation on the leaves of GK Gereben, Vitka, F 1502, Ilona, and GK Marcal. In contrast, mycelial formation instead of necrosis was noted on FD 80-119/31, GK Zombor, and Viking.

Drechslera tritici-repentis sporulated weakly. The highest sporulation value

Table 2. Seedling resistance of wheat genotypes to *Bipolaris sorokiniana* and *Drechslera tritici-repentis* based on average sporulation values.

Genotypes	*B. sorokiniana*	*D. tritici-repentis*	Genotypes	*B. sorokiniana*	*D. tritici-repentis*
GK Othalom	2.33	0.00	GK Csuros	2.66	0.00
Korona	2.00	0.33	Mv 218-88	3.00	1.00
Mv 19	2.33	0.33	Mv 23	1.66	0.00
GK Kincso	2.66	0.66	GK Orseg	3.00	0.00
Mv 14	3.00	0.00	GK Olt	3.00	0.00
GK Bence	3.00	0.00	F 1502	3.00	0.00
Adriana	1.33	0.00	Mv GK 07-89	3.00	0.00
Alfîld	1.00	0.33	FK 8005	3.00	0.00
Danka	2.66	0.33	GK Ruzsa	2.33	0.00
Kompolti 3	2.66	0.00	Mv 9	3.00	0.66
Mv 18	3.00	0.00	GK Repce	3.00	0.00
GK Barna	2.66	0.00	Mv 10-90	2.00	0.66
GK Bokros	3.00	0.33	Mv 15	1.33	0.00
Ana	2.00	0.00	Bucsanyi 20	2.66	0.00
GK Kata	2.33	0.00	GK Szoke	3.00	0.66
GK Kalaka	2.33	0.00	GK Othalom	3.00	0.00
Mv 23-88	3.00	0.00	FD 5	3.00	0.33
Mv 22	3.00	0.00	Ilona	3.00	0.00
ZG 167-86	3.00	0.00	GK Borzo	3.00	0.00
Mv 36-89	3.00	0.00	Thesee	3.00	0.66
GK Gobe	3.00	0.00	GK 48-90	3.00	0.66
FK 8063	2.66	0.00	GK Perint	3.00	0.66
ZG 1250/86	3.00	0.00	GK Kozar1	2.66	0.00
GK Delibab	2.33	0.00	Florin	2.66	0.66
GK Gereben	3.00	0.66	Soissons	2.66	0.66
Mv 30-90	2.66	1.33	Viking	2.66	0.00
GK Csîrnîc	2.33	0.00	FD 80-119/31	3.00	1.00
GK Zombor	3.00	0.00	P 386/4.88	2.33	0.00
Mv 16	1.66	0.00	P 637.88	2.33	1.00
Mv 17	3.00	0.00	GK Ablanc	2.00	0.00
GK Othalom	2.66	0.00	GK Csencsi	3.00	0.00
Jubilejnaja 50	2.66	0.00	GK Csîrnîc	2.00	0.00
GK Mv Kooperacio	3.00	0.33	GK Kozar2	3.00	1.00
Mv 12	3.00	0.00	GK Marcal	3.00	1.00
GK Istvan	3.00	0.00	GK Orseg	3.00	0.33
Vitka	3.00	0.66	GK Perint	3.00	0.66
GK Orzse	2.33	0.33	GK Pinka	3.00	0.00
Mv 20	2.66	1.00	GK Repce	2.33	0.00

was 1.33 (Mv 30-90). The fungus did not form conidia on 63.1% of the genotypes; however, necrotic lesions formed on the leaves of 40 genotypes and, of these, dense mycelia appeared on 34. Chlorosis was observed on almost all genotypes. Neither conidia, nor necrotic or chlorotic lesions were observed on the leaves of GK Góbé and GK Kozár.

Fungicide evaluation

Data on tan spot control only is discussed here. Figure 3a shows the results obtained for contact fungicides. Four fungicides (Dithane M-45, Vondozeb Plus, Polyram DF and Bravo 500) showed some tan spot control; however, effectiveness was low, ranging from 2-5. Bravo 500 achieved the highest and most prolonged control.

Results of systemic fungicide control are presented in Figures 3b and 3c. Five of the thirteen chemicals were useful for tan spot control. Four of these five (Mirage 45 EC, Sportak 45 EC, Tilt Premium 37.5 WP, and Granit SC) achieved higher control levels than the best contact fungicide. Efficacy of the five effective systemic fungicide lasted from 2-3 to 3-4 weeks. Only Alto 320 SC, Granit SC, and Tilt Premium 37.5 WP remained effective for longer than contact fungicides. Cost of application was US $20-23 ha^{-1} for Alto 320 SC, Tilt Premium 37.5 WP, and Mirage 45 EC; US $23-26 ha^{-1} for Granit SC; and US $16-20 ha^{-1} for Sportak 45 EC.

Data on the five contact and systemic fungicides are shown in Figure 3d. Their effectiveness of tan spot control ranged between 0-10. Milstar had no effect on tan spot levels. Alert and Alto Combi 420 EC achieved control levels of 7 and 5, respectively. The highest score (10) was achieved by Folicur BT and Tango. Effect of control lasted for 3-4 weeks after spraying with Alert or Alto Combi 420 EC and for 4-6 weeks for Folicur BT or Tango. Cost of application was US $23-26 ha^{-1} for Alert and Folicur BT, and US $26-30 ha^{-1} for Alto Combi 420 and Tango. The technological value of the four effective fungicides ranged between 1.6-3.2, with the highest score achieved by both Folicur BT and Tango.

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Raymond, P.J., Bockus, W.W., and B.L. Norman. 1985. Tan spot of winter wheat: procedures to determine host response. Phytopathol 75:686-690.

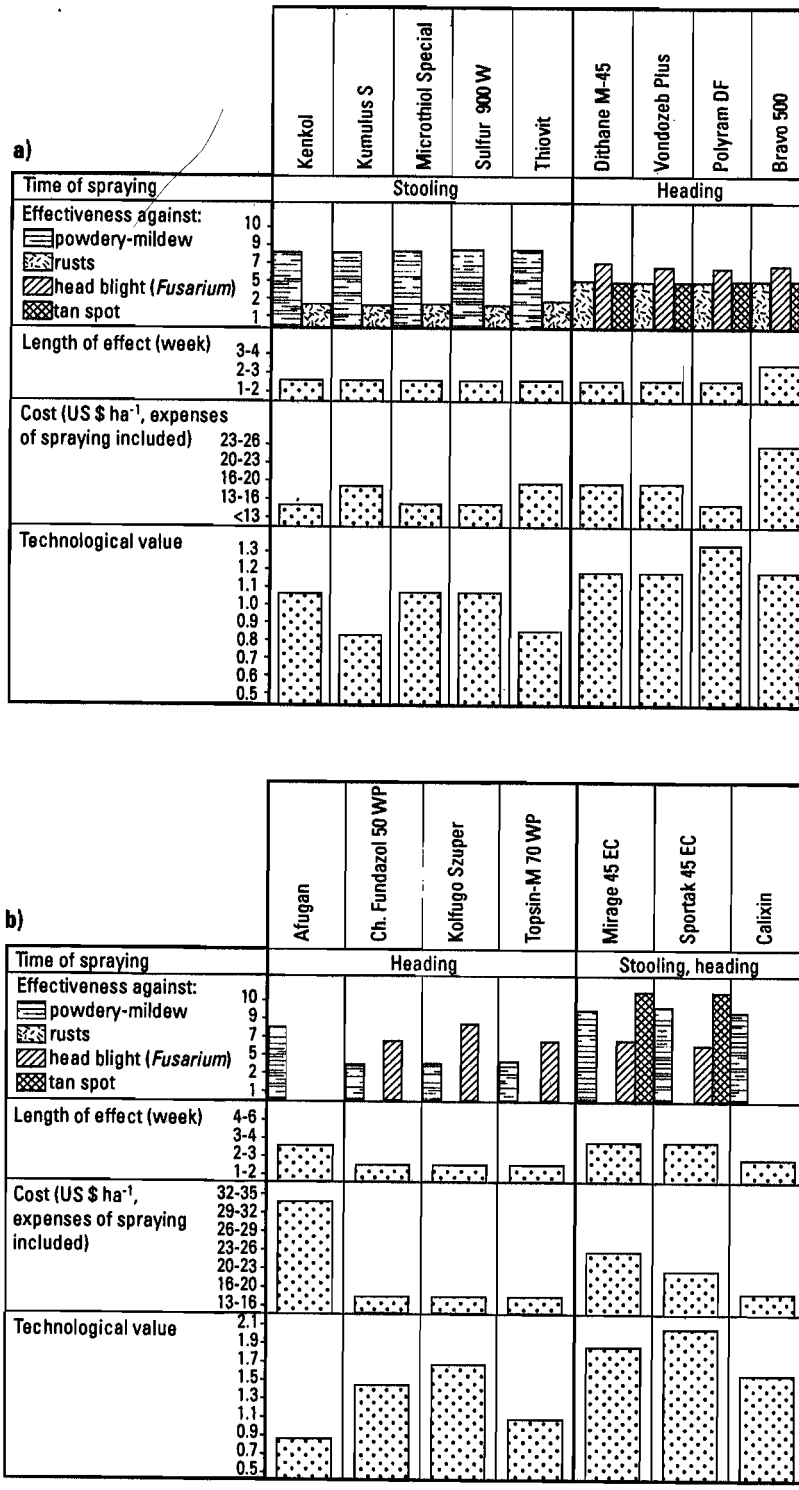
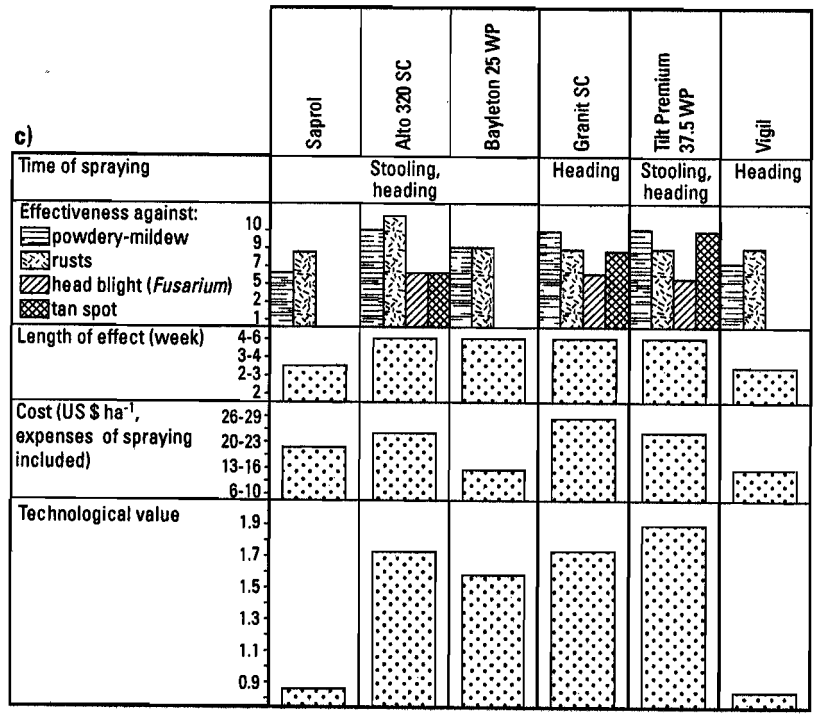
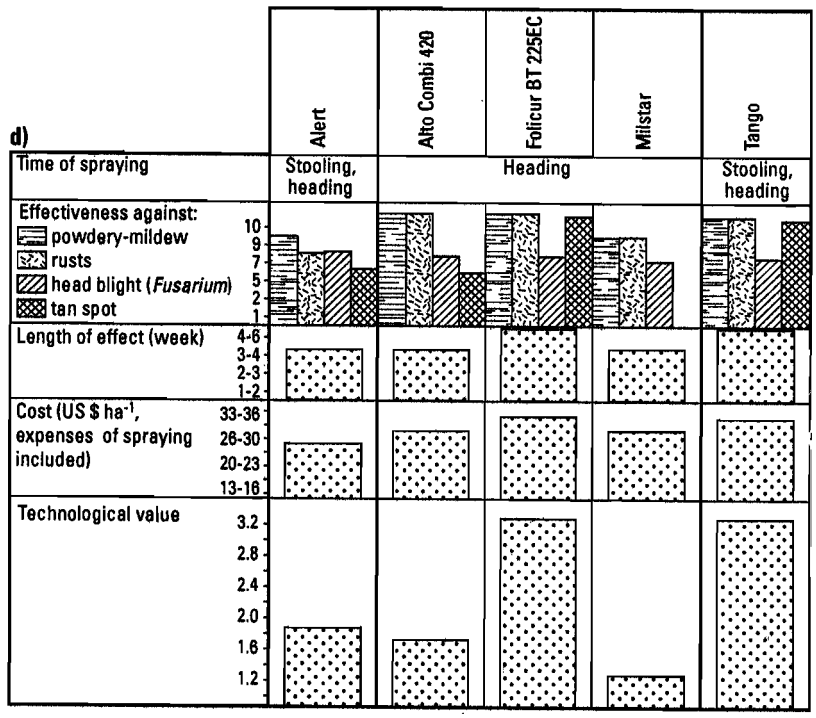


Figure 3. Characterization of a) contact, b) and c) systemic, and d) contact and systemic fungicides licensed in Hungary in 1995 against foliar and head diseases of winter wheat.


c)
Saprol
Alto 320 SC
Bayleton 25 WP
Granit SC
Tilt Premium 37.5 WP
Vigil
Time of spraying
Stooling, heading
Heading
Stooling, heading
Heading
Effectiveness against:
powdery-mildew
rusts
head blight (Fusarium)
tan spot
10 9 7 5 2 1
Length of effect (week)
4-6 3-4 2-3 2
Cost (US $ ha-1, expenses of spraying included)
26-29 20-23 13-16 6-10
Technological value
1.9 1.7 1.5 1.3 1.1 0.9


d)
Alert
Alto Combi 420
Folicur BT 225EC
Milstar
Tango
Time of spraying
Stooling, heading
Heading
Stooling, heading
Effectiveness against:
powdery-mildew
rusts
head blight (Fusarium)
tan spot
10 9 7 5 2 1
Length of effect (week)
4-6 3-4 2-3 1-2
Cost (US $ ha-1, expenses of spraying included)
33-36 26-30 20-23 13-16
Technological value
3.2 2.8 2.4 2.0 1.6 1.2

Population Structure and Epidemiology of *Bipolaris sorokiniana* in the Rice-Wheat Cropping Pattern of Nepal

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Abstract

Fungal species were isolated from foliar blight symptoms on spring wheat cultivars in the rice-wheat cropping pattern in the subtropical terai belt of Nepal. Bipolaris sorokiniana, Drechslera tritici-repentis, *and several* Curvularia *spp. were identified from indistinguishable lesions typically associated with helminthosporium leaf blight (HLB) symptoms. HLB was successfully controlled with three to four foliar applications of propiconazole at a rate of 125 ml ai* ha^{-1}. *Assessment of crop losses in 13 farmers' fields in 1990/91 indicated significant wheat yield losses of 10.7% or 250 kg* ha^{-1} *on the most popular commercial variety UP262 due to HLB. To estimate crop losses of rice and wheat due to soilborne pathogens, solar heating through plastic mulching (soil solarization) was carried out in a series of split-plot experiments, combined with foliar fungicide applications to the wheat crop in farmers' fields in 1992/93. Soil solarization, before rice transplanting in May, increased the mean daily maximum temperature at the soil surface by more than 25 °C and reached 65 °C in the top-soil layer. Subsequent rice yields increased by 32%; yield of the succeeding wheat crop in fungicide protected plots increased by 25%, and in unprotected plots by 15%. Fungicide application enhanced grain yields by 15% in solarized and 6% in nonsolarized plots, mainly through an increase in 1000-grain weight. Information on the population structure of* B. sorokiniana *was improved from a total of 799 fungal isolates divided into 17 populations, collected from five countries (Bolivia, Canada, Nepal, Mexico, and Switzerland). The isolates were characterized by means of randomly amplified polymorphic DNA (RAPD) obtained by polymerase chain reaction (PCR). Cluster analysis, using multidimensional scaling of Nei's genetic distance, clearly segregated populations into five groups according to region of origin. Gene flow, however, may be strong enough to prevent geographic isolation of populations both within and among regions. The percentage of total gene diversity that was allocated to differences among isolates within populations was 83%; of the remaining variance, 3% was attributed to differences among populations within regions, and 14% among regions.*

Rice-wheat cropping sequence

Rice (*Oryza sativa* L.) and wheat (*Triticum aestivum* L.) are sequentially cropped over about 12 million ha on the Indian subcontinent (Singh and Paroda 1994). Typically, the two cereals are grown within a single agronomic year in either a one-rice-one-wheat (RW) or a rice-rice-wheat (RRW) cropping sequence, but also in multi-year rotations

including other crops such as maize, pulses, oilseed, legumes, sugarcane, and jute. In Nepal, the double-cereal cropping sequence is practised on about 500,000 ha in the subtropical terai belt and in the adjacent hill regions, typically with low-input crop management in terms of fertilizer application and crop protection measures. Grain yields are low, averaging around 1.5 t ha^{-1} for wheat and 2.5 t ha^{-1} for rice (Hobbs *et al.* 1996).

Concerns on system sustainability

Longterm observations show declining trends in productivity of the RW cropping sequence in most parts of the Indian Subcontinent, despite the use of improved varieties and a tendency towards higher inputs of inorganic fertilizers. Regmi (1994) analyzed 14 years of yield data from a longterm fertilizer trial with a RRW cropping sequence in Bhairahawa, Nepal, and found declining trends in rice and wheat yields closely related to P and K supply. However, a yield decrease, particularly for both rice crops, was also recorded in treatments with recommended fertilizer dosages (Giri *et al.* 1993). These results supported outcomes from a series of varietal trials over 15 years at six research stations in Nepal: yields of two varieties were observed to decline at annual rates of 6.8% and 2.5%, reflecting different levels of susceptibility to foliar pathogens (Morris *et al.* 1994). Obviously not only nutritional aspects are of concern, crop protection issues also have to be taken into consideration in the discussion on system sustainability.

Low-input crop management

Farmers usually do not consider diseases of rice and wheat to be major production problems, although observed infection levels of both leaf and root diseases can result in crop losses. This may be explained by farmers perceiving plant diseases as a natural phenomenon rather than an avoidable production constraint. However, in most situations this attitude also follows an obvious, economic-based logic: agronomic crop management issues such as irrigation or fertilizer input are ranked as higher priorities; steps that have to be taken before measures of disease prevention will be profitable. However, it can be anticipated that phytopathological issues will attract more attention once monetary inputs reach higher levels, a time when farmers need to minimize the risk of facing even minor crop losses.

Triticum aestivum-Bipolaris sorokiniana pathosystem

This study focused on *Bipolaris sorokiniana* (Sacc. in Sorok.) Shoem. (syn. *Helminthosporium sativum* Pamm., King & Bakke, teleomorph *Cochliobolus sativus* (Ito & Kurib.) Drechsl. ex Dastur), which is considered one of the most important wheat pathogens in the warmer areas of the world (Dubin and van Ginkel 1991).

Fungicide Control of Helminthosporium Leaf Blight

Introduction

Preliminary field observations in Nepal revealed that *B. sorokiniana* was often found on the same leaves, and even

sometimes in mixed lesions, together with *Curvularia* spp. and *Drechslera tritici-repentis* (Died.) Shoem. (syn. *H. tritici-repentis* Died., teleomorph *Pyrenophora tritici-repentis* (Died.) Drechs.), the causal organism of tan spot. For older leaf lesions, it was impossible to unequivocally identify the lesion-causing pathogen. We therefore simplified the disease rating system and hereafter ascribe all leaf blight symptoms to helminthosporium leaf blight (HLB).

Materials and methods

To estimate grain yield losses caused by the HLB disease complex, fungicide control trials were superimposed on 13 farmers' fields in the major RW crop rotation area in the Rupandehi district, Nepal. The HLB susceptible, slow-rusting wheat cultivar UP262 was used. Crop management was carried out by farmers according to their traditional practices. The crop planted in check plots was protected from HLB infection with two to four applications of propiconazole (Tilt 250EC) at a rate of 125 ml ai ha^{-1}. Natural buildup of HLB epidemics was recorded in unprotected plots.

Results

In 1991, initial HLB symptoms on older leaves close to the ground were observed in all fields as early as the end of January, but disease spread was slow throughout February. Affected flag leaf area averaged less than 1% at the end of February. Only two weeks later, HLB lesions covered 6.8-87.8% of the flag leaves; however, in most fields only 15-25% of the flag leaf area was destroyed by the disease complex at early dough stage (GS 83). Periods of rapid disease development occurred immediately after two days of rare spring rainfall. This occurred on two occasions. After March 10, daily maximum temperatures exceeded 30°C, which led to a rapid loss of green leaf area and premature ripening; therefore, the final spread of the blight epidemic was not properly recorded.

Although fungicide application achieved excellent control of HLB infection in the check plots, site-specific analysis showed that treatment effects on grain yield at individual locations were not consistent enough to satisfy significance tests. Overall grain yield losses were 10.7% or about 250 kg ha^{-1}. The 1000-grain weight (TGW) was the yield determining factor primarily affected by the late epidemics. Across all sites, TGW was reduced by 4.1% in untreated plots with a site-specific maximum of 12.6%.

Final disease assessment was made just before heat and moisture stress killed the leaves. Results correlated better with crop parameters than with estimates of the area under the disease progress curve (AUDPC). In seven of thirteen locations, correlations were significant ($P<0.05$) between the final estimates of HLB severity and TGW reduction, and HLB infection accounted for 51-76% of the TGW variation. The other six sites, with weak and non-significant correlations, were from the least diseased fields.

To correlate HLB severity with grain yield or TGW across sites, the relative values of the untreated plots were subjected to simple linear regressions. Both relationships appeared to be significant (grain yield: $P = 0.04$, TGW: $P<0.001$), but only 8% of the yield variation and 39% of the TGW variation were accounted for by the disease severity rate. The number of tillers per m^2 and the number of grains per spike were not affected by the late disease establishment in the fields.

Discussion

In neighboring Bangladesh, 15% wheat yield losses, on average, were attributed to HLB infection for 1991-1994 (Alam, in press). In trials on 13 farmers' fields in 1991, losses in variety UP262 were around 10.7%. However, in an experiment at the Rupandehi research station in the same year using the same variety, 42% HLB infection at late crop stages were recorded, causing a 25% yield loss (Dubin and Bimb 1994). In similar trials in previous years, infection levels were 47% and 88%, resulting in yield losses of 13% and 27%, respectively.

Higher losses at the research station might be explained by the growth of highly susceptible cultivars in breeding plots and by the intensive use of the rice-wheat crop rotation over several decades. This management practice has led to high levels of pathogen inoculum in the soil, and the abundance of wheat straw from previous seasons that serves as an important primary inoculum source. *Bipolaris sorokiniana* can survive in submerged conditions in paddy fields on rotted wheat stubble; however, in farmers' fields, low levels of residue are left over after the monsoon season.

For wheat production, plant stand is the yield component most seriously affected by low input management, as traditionally applied in the terai belt in Nepal. Fertilizer inputs were 69:28:0 $N:P_2O_5:K_2O$ kg ha^{-1}, on average, which are well below recommended doses. Weak plant stand led to an early loss of green leaf material due to increased moisture stress. Plants in these plots experienced premature leaf senescence during periods of heaviest disease pressure. Disease attack was then too late to create treatment differences during the shortened phase in which the remaining green leaf area influenced grain filling.

In an additional experiment, the impact of increased rates of balanced fertilizer dosages on wheat yield and HLB epidemics was tested. Wheat responded with a significant yield boost relative to increases in levels of balanced fertilizer doses up to 150:75:37.5 $N:P_2O_5:K_2O$ kg ha^{-1}. HLB infection was severe in plots without fungicide protection, but remarkably uniform among all but plots with the lowest fertilizer level (50:25:12.5 $N:P_2O_5:K_2O$ kg ha^{-1}). AUDPC indicated that low or imbalanced soil nutrient levels predispose plants to a more severe HLB attack.

Summary

Grain yield losses attributed to HLB infection were less serious than reported from other warm environments (Mehta 1985; Raemaekers 1985). Nevertheless, preventing these losses might compensate for the negative net return of wheat production that farmers face in the RW cropping sequence in the terai belt (Hobbs *et al.* 1996). However, fungicide application is neither environmentally desirable nor economically feasible under low-input crop management, so the use of varieties with higher HLB resistance levels or altered agronomic practices provide more appropriate and promising control strategies.

Soil Solarization

Introduction

Soil solarization denotes the solar heating of moistened soil by mulching with clear polyethylene (PE) sheets. Solarization affects both biotic and abiotic processes and thus determines the chemical and microbial environment for plant growth. It may detect detrimental, but non-lethal, pathogenic root infections of crops, such as common root rot of wheat caused by *B. sorokiniana* or *Pythium* spp. However, increased growth response (IGR) of plants in solarized soils was frequently observed, even in the absence of known pathogens (Katan and DeVay 1991; Stapleton *et al.* 1985).

This mild form of soil disinfestation was tested for its potential impact on the overall performance of both rice and wheat within a long-lasting RW cropping regime. The main goal of soil solarization was the control of harmful soilborne plant pathogens and pests prior to planting. Soil solarization in the hot pre-monsoon period in May was expected to have a strong disinfecting effect, with a positive influence on the performance of the rice crop that might be carried over into the wheat season.

Materials and methods

In four split-plot trials in farmers' fields, soil solarization before rice transplanting was tested for its effect on both the rice and the following wheat crop. Furthermore, propiconazole (Tilt 250EC) was applied as a foliar spray to the wheat crop as the subplot treatment to estimate yield losses due to HLB infection. A high rate of mineral fertilizer was applied to both crops to exclude macronutrients as the major casue for an increased growth response.

Before plots were mulched with PE sheets of 2 mm thickness for 31 days, fields were irrigated up to saturation to improve heat conduction in deeper soil layers. After solarization, plots were aerated for 28-32 days. Fields were then puddled with bullocks, fertilized with a basal dosage of 60:60:40 $N:P_2O_5:K_2O$ kg ha^{-1}, and leveled with a wooden plank. Fertilizer topdressing was 60 kg NH_4-N ha^{-1} amended with 4 kg Zn^{2+} ha^{-1}. The high yielding rice variety Janaki was transplanted after 20-28 days.

It was of special interest to check how long the solarization effect could be carried over into the wheat season. Further, plots were split to study HLB epidemics. The chosen tillage method

allowed only a very shallow plowing, avoiding the mixing of soil from different depths. Fertilizer input was split in a basal application of 100:50:25 $N:P_2O_5:K_2O$ kg ha^{-1} and a topdressing of 50 kg NH_4-N ha^{-1} that was made at GS 21. Variety UP262 was sown at a seed rate of 120 kg ha^{-1}.

Grain yield of rice and wheat and its components was determined based on crop cut samples. The percentage of HLB-infected wheat leaf area was estimated five times during the season, with the final disease rating made at GS 77. Wheat roots of five plants at each subplot were collected at GS 83 and scored for root rot necrosis. Soil samples collected after rice transplanting, at wheat planting, and at wheat harvest were analyzed by the soil department of NWRP, Bhairahawa.

Results

Daily maximum temperatures (DMT) in solarized plots were about 44°C at a depth of 12 cm or, on average, 14°C higher than in unmulched plots, where highest DMT was less than 37°C (the lethal temperature threshold for many fungi) (Pullmann *et al.* 1981). In the upper 6 cm, temperatures under the PE sheets exceeded 50°C at 15 days and at the soil surface exceeded 60°C at 12 days for approximately three hours a day (Tables 1 and 2). Maximum temperature was 65°C, recorded just below the PE sheets on June 12. Up to the puddling plow-pan, soil

Table 1. Temperatures measured during soil solarization in Bhairahawa, Rupandehi district, Nepal.

Temperature	Measurements at	Mean DMT[1] (°C)	No. of days with DMTs ≥60°C	≥50°C	≥40°C	≥37°C
Air	200 cm	36.7	0	0	9	18
Non-solarized plots	-12 cm	30.3	0	0	0	0
Solarized Plots	0 cm	55.7	12	27	30	30
	-6 cm	48.3	0	15	28	30
	-12 cm	44.1	0	2	26	29
	-20 cm	41.5	0	0	21	27

[1] Mean DMT = Mean of the daily maximum temperatures (°C) during solarization periods.
Note: Readings are means of daily maximum temperatures (DMT) at various soil depths during the solarization process from May 16 to June 16, 1992 (31 days).

Table 2. Records of two-hourly temperature measurements during a 24-h period of soil solarization, June 2-3, 1992, Bhairahawa, Rupandehi district, Nepal.

Temperature	Measurements at	DMT[2] (°C)	No. of hours[1] with temperatures ≥60°C	≥50°C	≥40°C	≥37°C
Air	200 cm	37.2	0	0	9	3
Non-solarized plots	-12 cm	29.9	0	0	0	0
Solarized plots	0 cm	61.0	3	7	9	11
	-6 cm	53.9	0	5	10	11
	-12 cm	48.3	0	0	11	14
	-20 cm	44.0	0	0	8	10

[1] Approximate number of hours estimated from the two-hourly measurements.
[2] DMT = Daily maximum temperatures (°C) from June 2 to June 3, 1992.

DMTs of over 40°C were measured at 21 days and over 45°C on 8 consecutive days (Figures 1 and 2).

On average, solarization significantly increased yield of coarse rice from 4.2 to 5.5 t ha^{-1} or 32% across four locations ($P<0.01$), ranging between 14 and 62%. Analysis of yield components revealed that solarization mainly affected number of tillers (+18%) and number of grains per spike (+21%), but to a different extent, depending on location.

In the succeeding wheat crop, the residual effect of soil solarization caused a significant mean increase of 25% in grain yield in disease-free and 15% in HLB-infected subplots (Table 3). Solarization improved plant stand by 11%, whereas number of grains per spike and TGW remained almost unaffected. Solarization significantly reduced root rot infection from 43% to 26%; however, improved root health alone did not explain the observed solarization effects

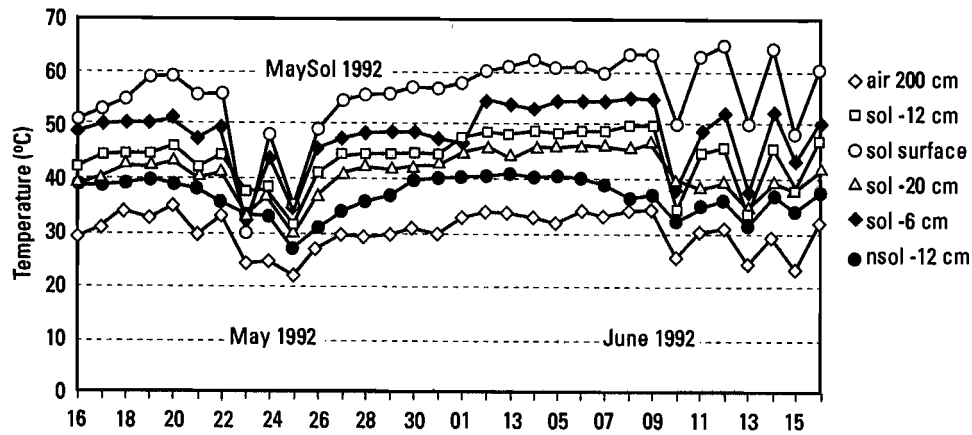

Figure 1. Daily maximum temperatures recorded at soil depths of 6 cm, 12 cm, 20 cm, and at the soil surface during soil solarization in May/June 1992, Bhairahawa, Nepal. In comparison, temperature courses of the air and unmulched soil are shown.
air 200 cm = Air temperature at 2 m; sol = Solarized; nsol = Non solarized.

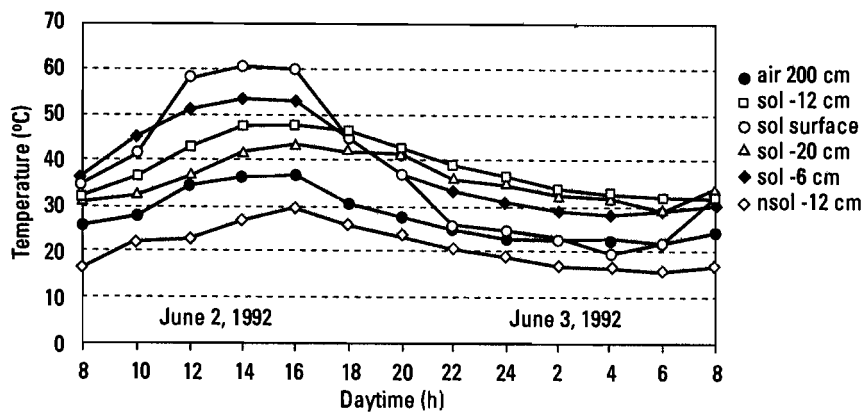

Figure 2. A 24-h temperature profile recorded at two-hourly intervals at soil depths of 6 cm, 12 cm, 20 cm, and at the soil surface during soil solarization, June 1992, Bhairahawa, Rupandehi district, Nepal. In comparison, temperature courses of the air and of unmulched soil are shown.
air 200 cm = Air temperature at 2 m; sol = Solarized; nsol = Non solarized.

because only weak, but highly significant negative correlations (P<0.001) were detected with increases in grain yield (r = -0.43) and number of tillers (r = -0.41).

HLB infection reduced wheat grain yields, on average and over sites, by 13% in solarized and 5.5% in unmulched plots, mainly through a loss in TGW of 7.5% and 8%, respectively (Table 3). HLB infection progress was described using AUDPC (area under disease progress curve) and compared with a final disease severity reading at soft dough stage (GS 77). Both measures revealed very similar, highly significant correlations (P<0.01), with loss of TGW attributed to HLB infection but not to the other two yield components. Final disease severity readings explained 45-89% of the variance in TGW loss. The loss in grain yield across all sites was significantly, but weakly, correlated with HLB infection (r = -0.45; P<0.001).

Discussion

It is not fully understood how the increase in temperature during solarization affects soil microflora. Some effects are direct, such as physical cell death through heat inactivation of cell processes, whereas other mechanisms are indirect, such as a temporary nutrient release. They affect composition and dynamics of pathogen populations by influencing the biological balance

Table 3. Impact of soil solarization (solar vs. non solar) and fungicide application (protected vs. unprotected) on the performance of rice and wheat cropped in sequence.

Rice	Grain yield (dt ha^{-1}; 14% mc)			TGW (g; 14% mc)		
	Solar	Non-solar	% increase[1,2]	Solar	Non-solar	% increase[1,2]
	54.9	41.5	32.3 ***	29.5	28.9	2.1 ***
	No. of tillers m^{-2}			No. of grains/spike		
	Solar	Non-solar	% increase[1,2]	Solar	Non-solar	% increase[1,2]
	239	202	18.3 ***	125	103	21.4***
Wheat	Grain yield (dt ha^{-1}; 12% mc)			TGW (g; 12% mc)		
	Solar	Non-solar	% increase[1,2]	Solar	Non-solar	% increase[1,2]
Protected	31.8	25.4	25.2***	45.4	45.2	0.4 ns
Unprotected	27.7	24.0	15.4***	42.0	41.6	1.0 ns
% increase[2,3]	14.8***		5.8***	8.1***	8.7***	
	No. of tillers m^{-2}			No. of grains/spike		
	Solar	Non-solar	% increase[1,2]	Solar	Non-solar	% increase[1,2]
Protected	303	272	11.4***	34.3	33.4	2.7 ns
Unprotected	312	280	11.4***	34.5	33.8	2.1 ns
% increase[2,3]	-2.9 ns		-2.9 ns	0.6 ns	1.2 ns	
	HLB severity on flag leaves (% infected leaf area at GS 77)			Root necrosis (% infected root area at GS 83)		
	Solar	Non-solar	% reduction[2]	Solar	Non-solar	% reduction[2]
Protected	3.1	3.3	0.2ns	27.9	40.6	12.7***
Unprotected	27.4	23.8	-3.6 ns	24.9	45.9	21.0***
% reduction[c]	24.3***		20.5***	-3.0 ns	5.3 ns	

[1] % change (due to soil solarization) = 100 x (mean of solar plot/mean of non-solar plots) - 100.
[2] Changes were tested for statistical significance with LSD for RCB designs at * P<0.1; ** P<0.05; *** P<0.01.
[3] % change (due to fungicide application) = 100 x (mean of protected plots/mean of unprotected plots) - 100.
Note: The table combines results of RCB split-plot experiments at four locations in Rupandehi district, Nepal, carried out in 1992/93.

between plant pathogens and their antagonists that function as biocontrol agents. Ultimately, this may lead to a reduction or even extinction of pathogenic microorganisms (Katan and DeVay 1991). However, temperatures recorded in the solarization plots were probably beyond the scope of the targeted pathogenic fungi, which are, in general, less heat-tolerant than their antagonists (Cook and Baker 1983).

No differences in total N, available P or K, electrical conductivity, and pH were detected among treatments. The organic matter content of solarized plots was even slightly reduced when measured in the rice season, and indistinguishable in the wheat season. However, chemical analysis of wheat flag leaves sampled at booting stage (GS 41-45) at two experimental sites indicated an increase in Mn content of plants from solarized plots (Graham, unpublished data). Moreover, rice plants with symptoms related to Zn deficiency were commonly observed in all plots, but only plants in solarized plots were able to recover fully after 4 kg Zn^{2+} ha^{-1} was applied as a topdress fertilizer. Thus, if there are nutritional effects associated with the reduction of root rot after solarization, bivalent micronutrients, particularly Mn and Zn, may be the elements to focus on in further studies.

The effect of soil solarization on the rice crop was highly beneficial. The increase in grain yield ranged from 14-62% in the four experimental locations and averaged 32%. Nutritional factors, especially the availability of Zn^{2+}, are likely to contribute to the observed yield increase; however, considering the detrimental temperatures of over 60°C, populations of soilborne pathogens must be greatly reduced. Dubin and Bimb (1994) found a significant correlation between the number of rice root nematodes (*Hirschmanniella oryzae* and *H. mucronata*) and yield after soil solarization before rice transplanting.

The strong residual effect of soil solarization on the wheat crop after the rice season was unexpected. Several field management activities such as puddling, plowing, and irrigation with surface water must have diluted the solarization effect, and the small size of the experimental plots favored reinfestation by pathogens from unmulched areas. Nevertheless, wheat plant stand and plant height were consistently improved, and grain yield increases related to soilborne factors ranged from 10 to 36% in protected and from 5 to 25% in unprotected subplots.

Summary

Although solar heating is a relatively new technique and is restricted to areas with special climatic conditions, Davis (1991) presented a long list of organisms including fungi, bacteria, nematodes, and weeds that were reported to be successfully controlled by soil solarization. In practical application, disease control with solarization has mostly been achieved near the soil surface in regions with high temperatures, especially in Mediterranean countries and California, USA. These two conditions are perfectly

met in the Terai environment with its hot pre-monsoon periods and the very shallow rooting layer above the impervious hardpan.

In the RW cropping sequence, plastic mulching as a management tool could possibly be employed in April or May after plowing proceeding the wheat harvest; however, some aspects are of practical concern. These include: irrigation water required to assure successful heat conduction in lower soil layers is scarce at the end of the dry season, occasional heavy westerly winds may damage the PE sheets, and farmers graze cattle in the fields during the turn-over time between wheat harvest and rice planting.

From an economical viewpoint, the costs of additional irrigation and PE sheets may be lower than the commercial value of the expected yield gains for rice (32%) and wheat (15%); however, these benefits are too variable and unpredictable for farmers who manage their crops with a low-input, low-risk strategy. Consequently, soil solarization will most likely not be adapted as a management tool in the RW cropping sequence in the Terai belt of Nepal, but might be implemented by cereal seed producers or vegetable farmers.

Pathogen Population Structure

Introduction

The most promising measure for *B. sorokiniana* control is the attempt to introduce resistance genes into the host genome. Advanced material from a particular breeding site is either selected under natural infection pressure in disease hot spots or under artificial inoculation. In both cases, information on population structure and biology of the pathogen are important prerequisites to enhance the effectiveness of resistance breeding schemes.

Materials and methods

RAPD marker systems were chosen to detect population substructures of a collection of *B. sorokiniana* isolates. They were sampled in the wheat growing regions of Bolivia, Nepal, Canada, and Switzerland, and at CIMMYT's research site in Poza Rica, on the Mexican Gulf coast where wheat is not grown in farmers' fields. Regional collections consisted of one (Bolivia), two (Canada and Switzerland), or three (Nepal and Mexico) field populations and one district population in each country (Table 4). A total of 799 isolates from 5 regions (MEX, BOL, NEP, CAN, SWI) were grouped in 11 field and 5 district populations. Isolates were analyzed for the presence of 17 polymorphic RAPD marker bands generated by 6 random primers.

Results

Significant differences in allelic frequencies at the same loci were seldom found among populations within 4 of the 5 regions, but quite frequent within the CAN region where, for 7 of 17 loci, the null hypothesis of no differentiation between the populations had to be rejected based on a c^2 homogeneity test ($P<0.05$). However, frequencies of all 17 RAPD markers were significantly

Table 4. Synopsis of the 799 *Bipolaris sorokiniana* isolates used for RAPD-PCR analysis in the study.

Origin of populations	No. of isolates	Host organs	Sampling strategy	Host plants
Mexico	**196**			
District Poza Rica	42	Glumes, leaves	Few isolates from many fields across Poza Rica Research Station	Various wheats
Roots	16	Roots	Every 3 m on a field transect (1994)	Various wheats
Leaves	59	Leaves	Every 3 m on a field transect (1994)	Various wheats.
Glumes	79	Glumes	10 isolates per plot in an RCBD with two varieties (1994)	BH1146, CIANO
Bolivia	**71**			
District Santa Cruz	24	Leaves	Few isolates from many fields within the district of Santa Cruz	Various wheats
Okinawa	47	Leaves	Every 2 m on a field transect (1994)	Chane
Nepal	**268**			
District Rupandehi	124	Leaves	Few isolates from many fields within the Rupandehi district	Various wheats
Parsari	48	Leaves	Every 1 m over a grid (1994)	NL251
Gurwnia	48	Leaves	Every 1 m over a grid (1994)	UP262
Dhakdai	48	Leaves	Every 1 m over a grid (1994)	UP262
Canada	**99**			
District Saskatchewan	50	Subcrown internode	Few isolates from many fields within the province of Saskatchewan	Various cereals
St. Benedict	24	Subcrown internode	Randomly from a field transect (1994)	Various wheats
Saskatoon	25	Subcrown internode	Every 5 m over a grid of a disease nursery (1994)	Various wheats
Switzerland	**165**			
District Zürich	52	Leaves	Few isolates from many fields within the district of Zürich	Various cereals
Herrliberg	65	Leaves	Every 10 m on a field transect (1994)	Arina
Wetzwil	48	Leaves	Every 10 m on a field transect (1994)	Arina

Table 5. The χ^2 homogeneity test applied to detect significant differences in allelic frequencies at individual loci among populations of *Bipolaris sorokiniana*.

Comparison	Region	No. of populations[2]	*fix*	Level of significance for differences in allelic frequencies at RAPD loci[1]			
				ns	P<0.05	P<0.01	P<0.001
Between varieties	Mexico	2 V	0	14	3	0	0
Between fields within regions	Mexico	3 F	0	16	1	0	0
	Nepal	3 F	1	13	3	0	0
	Canada	2 F	0	11	1	3	2
	Switzerland	2 F	3	13	0	1	0
Within regions	Mexico	1 D - 3 F	0	16	1	0	0
	Bolivia	1 D - 1 F	2	14	1	0	0
	Nepal	1 D - 3 F	1	10	4	2	0
	Canada	1 D - 2 F	0	10	2	2	3
	Switzerland	1 D - 2 F	2	11	3	1	0
Between fields among regions	11 fields	11 F	0	0	1	1	15
Between regions	5 regions	5 R	0	0	0	1	16

[1] χ^2 probabilities that allele frequencies among samples within each comparison are different by chance: * P<0.05, ** P<0.01, *** P<0.001, ns Not significant, *fix* Populations with fixed alleles did not permit the use of the χ^2 test.

[2] Column shows the number of populations and hierarchical level used in the χ^2 homogeneity test. Populations are from V = varietal, F = field, D = district, and R = regional samples.

different among the 11 field samples and the 5 pooled regional samples (Table 5).

The mean total gene diversity H_T for all 17 loci across all 11 field populations was relatively high (0.39), partly because only markers known to be polymorphic were analyzed. Hierarchical analysis revealed that the partition of gene diversity varied strongly between loci. Across all loci, 14% of total gene diversity was allocated to variation among regions, 3% to variation among fields within regions, and 83% to variation within fields (Table 6).

Nei's genetic distance (Nei 1972), analyzed over all loci, was smallest between samples within regions and only exceeded 0.04 in the CAN region, emphasizing the special characteristics of the Saskatoon field population. If the genetic distances are subjected to a multiple dimension scaling procedure (Kruskal 1964), populations of the same region are clearly clustered. Distances to the Mexican samples were generally shortest, and distances to the Swiss samples were longest (Figure 3).

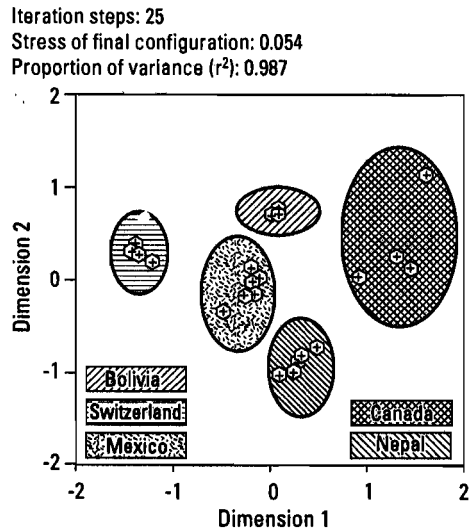

Figure 3. Graphic description of genetic differences among *Bipolaris sorokiniana* populations from five sampling regions. Relative distances between populations are derived from a dissimilarity matrix with Nei's standard genetic distances by a multi-dimensional, monotonic scaling procedure, according to Kruskal (1964). Data points represent 5 pooled regional samples and 5 district samples (one of each per region) and 12 field populations (Bolivia, 1; Switzerland, 2; Mexico, 4; Canada, 2; Nepal, 3).

Table 6. Mean total gene diversity H_T of a worldwide collection of 799 *Bipolaris sorokiniana* isolates and its partition into components assigned to different hierarchical sampling levels.

Source of gene diversity			Percentage of total gene diversity[1]			
Region	Hierarchical levels[2]	Total[3] H_T	within fields	among varieties	among fields within regions	among regions
All regions (range)[4]	12 F-5 R	0.39 (0.20-0.50)	83.2 (66.7-93.6)	0.4 (0.0-1.6)	2.6 (0.7-7.1)	13.8 (1.7-29.3)
Mexico	4 F-2 V	0.36	96.5	1.2	2.4	—
Bolivia	1 F	0.35	—	—	—	—
Nepal	3 F	0.35	97.8	—	2.2	—
Canada	2 F	0.32	91.9	—	8.1	—
Switzerland	2 F	0.25	99.0	—	1.0	—

[1] Partitioning of total gene diversity among hierarchical contributors according to Beckwitt (1980).
[2] Column describes the different hierarchical sampling levels that contributed to total gene diversity. F = field, R = region, V = variety.
[3] Nei's measure of total gene diversity based on 17 RAPD markers.
[4] Range of H_T and its partition, calculated individually for the 17 loci under investigation.

Pairwise population subdivision was determined with Nei's Gst. From this, Nm was calculated, which is an estimation of gene flow and determines the number of migrating individuals per generation among the two populations. The level of gene flow strongly fluctuated between different loci and population pairs, but was always sufficient to prevent geographical separation of populations.

The combination of 17 RAPD markers resulted in the detection of 264 different multilocus haplotypes among the total population of 799 isolates. In field populations, 1.2 to 1.8 isolates with identical haplotype were found in all but the SWI region, where the degree of clonality was higher with 2.3 and 2.8 isolates per haplotype. Among the pooled regional samples, the BOL and CAN populations were of considerably less clonal structure, each with 1.8 isolates per haplotype.

Putative clones of field populations based on the 17-digit multilocus haplotype were identified, and hence all but one isolate of such a clone were eliminated. This resulted in small sample sizes of clone-corrected populations ranging from 13 (MEX-roots) to 47 isolates (MEX-glumes). Statistical tests on dual-loci haplotypes and gametic disequilibrium were performed with a reduced set of markers with intermediate allele frequencies between 0.2-0.8 in the original sample, on both complete and clone-corrected populations, respectively (Table 7). The percentage of marker pairs for which all four possible allele combinations were detected was over 80% in 10 of 13 field populations, while the two Canadian samples were considerably biased due to the small number of isolates. Finally, in the BOL-Okinawa sample, one allele combination was missing in almost 25% of the marker pairs.

Eliminating putative clones in field populations massively increased the percentage of marker pairs in gametic equilibrium. In clone-corrected samples, less than 30% of the pairs of loci showed a significant non-random association among alleles, ranging from 2.9% in MEX-roots to 29.7% in CAN-St. Benedict.

Comparisons of genotypic diversities $G_{obs.}$, from field samples, with mean values $G_{sim.}$, from 1,000 simulated populations, showed significant deviations from the null hypothesis of panmixis at the 1% significance level in all cases where putative clones were not removed (Table 8). However, among clone-corrected populations, panmixis was rejected in only 5 of 13 fields at probability levels of 5% (2 populations), 1% (1 population), and two others at the 0.1% significance level. For the remaining 8 samples, there was not sufficient evidence to reject the null hypothesis, and it was therefore concluded that the observed field populations did not differ in genotypic diversity from the bootstrapped populations simulated under panmictic assumption.

Discussion

For the wheat-spot blotch pathosystem, Hetzler (1992) suggested that a gene-for-gene interaction operates between host resistance response and fungal virulence. Cluster analysis of virulence patterns indicated a differentiation in the pathogen population according to geographical subdivisions.

Table 7. Investigation into the mode of reproduction in 13 field populations of a worldwide collection of *Bipolaris sorokiniana*.

Population	Clone correction[1]	No. of isolates	No. of loci[2]	Percentage of marker pairs in gametic equilibrium[3]	Percentage of marker pairs with all four dual loci haplotypes[4]
Mexico					
Roots	Complete	16	9	91.2	94.4
	Corrected	13	9	97.1	
Leaves	Complete	59	12	68.2	95.5
	Corrected	41	12	83.3	
Glumes	Complete	79	14	62.6	91.2
	Corrected	47	14	78.9	
CIANO	Complete	39	11	54.5	94.5
	Corrected	26	11	78.8	
BH1146	Complete	40	12	75.8	86.4
	Corrected	31	12	81.0	
Bolivia					
Okinawa	Complete	47	10	48.9	75.6
	Corrected	26	10	78.0	
Nepal					
Parsari	Complete	48	11	60.0	94.5
	Corrected	28	11	88.7	
Gurwnia	Complete	48	8	71.4	89.3
	Corrected	29	8	88.5	
Dhakdai	Complete	48	11	48.1	83.6
	Corrected	28	11	78.0	
Canada					
St. Benedict	Complete	24	10	52.5	73.3
	Corrected	19	10	70.3	
Saskatoon	Complete	25	7	50.0	61.9
	Corrected	17	7	85.7	
Switzerland					
Herrliberg	Complete	65	7	42.9	95.2
	Corrected	23	7	90.5	
Wetzwil	Complete	48	8	75.0	89.3
	Corrected	21	8	89.3	

[1] Calculations based on markers with allele frequencies of 0.20-0.80.

[2] Gametic equilibrium was calculated for field populations with both the complete set of isolates (complete) and the clone corrected set (corrected), for which isolates sharing an identical 17-digit multi-locus haplotype were counted only once.

[3] Column shows the percentage of RAPD marker pairs without significant deviation (χ^2-tests; $P>0.05$) of allelic association from that expected in gametic equilibrium.

[4] Column shows the percentage of RAPD marker pairs for which all four allele combinations were detected at least once in the observed population.

Note: Proportions of RAPD loci pairs in gametic equilibrium are listed, together with the percentage of pairs from which all four dual loci haplotypes were detected. Tests were carried out for both complete and clone-corrected populations, and were restricted to loci with marker frequencies of 0.20-0.80.

Do our results support these findings? On first consideration, populations of a common geographic origin were grouping strictly after rescaling Nei's genetic distances in a two-dimensional graph. Therefore, resistance tests in one region of the world would not necessarily be valid for any other region. However, the observed population structure did not allow conclusions on the amount and frequency of genetic exchange among populations. The theory of gene flow,

Table 8. Comparison of genotypic diversities of 13 field populations of *Bipolaris sorokiniana* and means of 1000 simulated populations calculated using RAPD markers of intermediate frequencies.

Population	Clone correction[1]	No. of isolates	No. of loci[2]	$G_{obs.}$[3]	$\hat{G}_{sim.}$[4]	t-test[5]		p[6]
Mexico								
Roots	Complete	16	9	11.6	15.3 (±2.1)	0.001	***	0.000
	Corrected	13	9	13.0	12.6 (±0.6)	0.628	ns	1.000
Leaves	Complete	59	12	25.0	55.1 (±5.2)	0.000	***	0.000
	Corrected	41	12	30.6	39.5 (±2.8)	0.000	***	0.000
Glumes	Complete	79	14	26.1	76.3 (±4.7)	0.000	***	0.000
	Corrected	47	14	41.7	46.1 (±1.6)	0.000	***	0.015
CIANO	Complete	39	11	17.5	37.5 (±3.2)	0.000	***	0.000
	Corrected	26	11	24.1	24.9 (±1.8)	0.600	ns	0.444
BH1146	Complete	40	12	21.6	38.7 (±3.0)	0.000	***	0.000
	Corrected	31	12	27.5	30.5 (±0.9)	0.001	**	0.025
Bolivia								
Okinawa	Complete	47	10	16.1	42.0 (±5.7)	0.000	***	0.000
	Corrected	26	10	21.1	24.5 (±2.5)	0.030	*	0.055
Nepal								
Parsari	Complete	48	11	14.2	45.4 (±4.0)	0.000	***	0.000
	Corrected	28	11	24.5	26.8 (±1.5)	0.122	ns	0.139
Gurwnia	Complete	48	8	16.5	36.0 (±7.0)	0.000	***	0.000
	Corrected	29	8	20.5	24.4 (±6.5)	0.130	ns	0.097
Dhakdai	Complete	48	11	20.2	44.9 (±4.6)	0.000	***	0.000
	Corrected	28	11	26.1	27.0 (±1.8)	0.537	ns	0.441
Canada								
St. Benedict	Complete	24	10	13.7	22.6 (±3.0)	0.000	***	0.000
	Corrected	19	10	15.7	18.2 (±2.2)	0.030	*	0.075
Saskatoon	Complete	25	7	8.8	17.4 (±5.0)	0.001	***	0.000
	Corrected	17	7	10.0	12.6 (±4.4)	0.208	ns	0.145
Switzerland								
Herrliberg	Complete	65	7	11.7	34.9 (±8.0)	0.000	***	0.000
	Corrected	23	7	17.1	16.9 (±5.7)	0.946	ns	0.596
Wetzwil	Complete	48	8	11.2	33.1 (±7.7)	0.000	***	0.000
	Corrected	21	8	16.3	18.6 (±3.6)	0.223	ns	0.189

[1] Figures in the first row of each population are estimates of genotypic diversity with the complete set of isolates (complete), numbers in the second row are estimates of the clone corrected sets (corrected), for which isolates sharing an identical 17-digit multi-locus haplotype were counted only once.

[2] Calculations based on markers with allele frequencies ranging between 0.20-0.80.

[3] Estimate of genotypic diversity for the observed sample.

[4] Expected genotypic diversity (±95% confidence interval) from 1000 simulated populations, generated from the observed allele frequencies of the complete sample under the assumption of random mating.

[5] Probability that $G_{obs.}$ belongs to a population with the same mean as $\hat{G}_{sim.}$, calculated with two-tailed t-tests: * P<0.05, ** P<0.01, *** P<0.001, ns Not significant.

[6] Proportion of the simulated populations with genotypic diversities equal to or smaller than $G_{obs.}$.

Note: Tests were carried out for both complete and clone-corrected populations.

developed by Wright (1969), explains stable subdivisions with a certain amount of migrants among populations, which counteracts the random force of genetic drift. In practice, this means that the observed structure did not exclude the migration of individuals among regions. The data further indicate that between 33 and about 200,000 migrants between regions are necessary to prevent stronger subdivision. If many individuals carry favorable genes to overcome host resistance, they will be readily amplified in the target population.

Do we have indications of host specialization in the pathogen population using our markers? Comparison of the two glume populations isolated from BH1146 and Ciano showed very little difference in gene frequencies at only three loci. The differentiation between the two fields in Canada may be interpreted as differential selection; however, it is unlikely that 6 of 17 markers are directly selected, and therefore differ in frequencies among the two fields. If sexual recombination is rare, or even missing, associations among selected and neutral loci may persist; an effect commonly referred to as hitch-hiking. We can also speculate if migration was less intensive among populations that mainly live on subground parts of the host, and if a specification was therefore more likely to develop.

Do the results give any indication of sexual recombination? The sexual stage of *B. sorokiniana* is named *Cochliobolus sativus* and, to date, has only been found once under field conditions (Raemaekers 1991). However, the results of this study revealed that in all clone-corrected field populations, the vast majority of pairwise allelic association among multi-locus haplotypes did not differ from what would be expected in a randomly mating population. Further, measures of genotypic diversity (Stoddart and Taylor 1988) in 8 out of 13 clone-corrected field populations did not differ from genotypic diversities calculated for bootstrapped populations simulated under the assumption of sexual recombination. And finally, most marker pairs were found to be in gametic equilibrium and therefore indicated that sexual reproduction may be occurring. However, a similar degree of gametic equilibrium could also be expected from genetic drift as a result of the long-term exchange of fungal material through the worldwide seed trade.

Although these results remain somewhat inconclusive, we found evidence for genetic reshuffling through either sexual or asexual recombination processes in the life cycle of *B. sorokiniana*. The amount of sexual recombination is of considerable importance for the incorporation of immigrated virulence factors into the genetic background of locally adapted pathogen populations. It may, in fact, be the crucial factor in determining how long a variety that is susceptible in one region remains resistant in another region.

Summary

Despite the various origins of the isolates, whether sampled from glumes, leaves, or subground parts of the hosts,

from commercial fields or research stations, from traditional (CAN, SWI) or non-traditional (MEX; NEP, BOL) wheat growing areas, genetic substructuring of populations expressed as measures of gene and genotypic diversity was very similar:

- *B. sorokiniana* was found to maintain a large level of variation at a local scale through gene flow, which counteracts population subdivision tendencies and may be part of the very successful survival strategy.
- This study provided conclusive evidence for a substructuring of the *B. sorokiniana* population. However, it remains open if traits of agronomic interest, such as virulence genes, which are under strong selection pressure, would match the clustering found with neutral markers. If so, host genotypes selected for resistance in one region of the world would not necessarily be resistant in other regions.

The presented study probably raised more questions on *B. sorokiniana* population processes than it clarifies. Although geographic substructuring of the pathogen population was observed, regional populations are not strictly separated. Therefore, for spot blotch resistance breeding, shuttle breeding strategies are suggested that may detect regional differences in virulence composition of the pathogen population.

Conclusions

Several observations across the RW cropping belt of Asia raise concern over the sustainability of this multiple cereal cropping system (Paroda *et al.* 1994). Growing two cereal crops such as rice and wheat in an intensive, continuous rotation is not likely to have conflicting nutritional needs, and fertility management is more a question of refining nutrient deposits with respect to uptake patterns. However, from these rotations, no benefits for soil sanitation, structure, fertility, and biodiversity can be expected. Depletion of soil nutrients, especially quantity and quality of the soil organic matter, salinity and waterlogging on irrigated land, falling water tables, and increasing pest and diseases pressure have to be anticipated.

Bipolaris sorokiniana is probably not a major reason for lack of sustainability, but it is definitely an important explanation for the low wheat yields in the RW cropping sequence. Low cost management tools to control the pathogen are not available on the market. Considering the low productivity of the RW cropping sequence and the high losses caused by diseases such common root rot or HLB of wheat, an integrated production approach has to be taken into consideration that includes:

- Crop management practices: non-cereal crop rotation partners; nutrient management, particularly farm yard manure; and crop residue management.
- Disease control strategies: soil disinfestation techniques; promoting fungal antagonists in the soil; and varietal and/or species mixtures

System intensification needed to meet future food demands is unlikely to be successful if the current RW cropping sequence is not considerably diversified. A promising system not only has to ensure food security, but has to increase stability through locally adapted varieties, the use of locally available nutritional inputs, ecologically sound crop protection measures, and well maintained irrigation facilities.

Unfortunately, most agricultural research organizations date back to times where *technology packages*, containing seed, fertilizer, and irrigation, were developed for large areas at centralized research stations and distributed through extension services. These packages were strictly commodity-orientated, and developed for an optimal crop rather than system performance. These packages are not designed to be used in diverse agricultural systems and neglect aspects of system management, further adding to their disappearance.

What is needed are *technology cum knowledge choices* that offer farmers a wide range of management tools for more diverse production "micro-environments". These catalogues might be the result of long-term research activities and ought to be continuously fine-tuned, and not based exclusively on results from research activities, but enriched with experiences gathered from farmers.

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Tan Spot in Central Asia

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Abstract

In Central Asia, surveys are annually conducted to identify fungal pathogens occurring on cereal crops. Tan spot, caused by Pyrenophora tritici-repentis, *was found for the first time in 1986, and has subsequently been recorded throughout Central Asia, mainly in the Djizzakh region of Uzbekistan and Kulyab region of Tadjikistan. To determine possible host plants and sources of* P. tritici-repentis, *Gramineae plants (four cereal and 22 wild grass species) were surveyed under natural and artificial inoculation conditions. Based on observations, it is likely that* Agropyron repens, A. cristatum, Aegilops cylindrica, *and* Bromus inermis *are potential inoculum sources. Field and greenhouse experiments were conducted to identify local resistant cultivars. No immune genotypes were found among 136 cultivars tested. Effect of temperature (2, 5, 10, and 15°C for 2, 4, 6, 8, 10, 15, 20, 25, 30, and 35 h) on* P. tritici-repentis *isolates was investigated. The highest disease intensity was observed at 25 h moisture period at 15°C. In the laboratory, various treatments were tested including light regime, medium formulation, inoculum dose, and three-way inoculum preservation. A technique for producing dry* P. tritici-repentis *inoculum and creating an infection background was developed.*

The government of the Republic of Uzbekistan established a goal to increase cereal productivity and achieve grain self-sufficiency. The area sown to cereals increased to 1.8 million ha in 1996. Cereals are primarily sown on irrigated land where grain yields of 2.5-3.0 t ha^{-1}, compared with 1.2-1.5 t ha^{-1} are possible in rainfed areas.

However, irrigation frequently leads to the creation of conditions more favorable for the development of cereal disease epidemics. Until recently, diseases such as brown rust, yellow rust, smuts, bunts, and powdery mildew were considered the most important cereal diseases in Central Asia. The epidemiology of these diseases has been thoroughly investigated. Of the diseases present in Central Asia, wheat foliar spots and blights have been studied the least. These diseases were usually considered to be a natural physiological withering.

Tan spot of wheat was found for the first time in 1986 in southern Tadjikistan (Khasanov 1988). Subsequently, the disease has been annually reported on winter wheat crops in parts of Uzbekistan, Tadjikistan, and southern and northern Kazakhstan. Based on annual mycological surveys of cereal crops, tan spot, caused by *Pyrenophora tritici-repentis*, was revealed to be one of the main wheat diseases.

The increase in distribution and pathogenicity of tan spot in Central Asia is caused by: 1) continuous wheat cultivation under rainfed conditions over many years, 2) the accumulation of a large

infection source in the form of infected plant residues, and 3) the absence of control measures.

This paper presents the results of long term research initiated in 1986 by Drs. B.A. Khasanov and V.A. Mostovoy, and continued by E. Postnikova from 1988 to the present.

Tan Spot Distribution

Cereal crop surveys were undertaken in Kazakhstan and Tadjikistan during 1986-1992, and in Uzbekistan from 1986 to the present. About 20-50% of cereal crop fields were annually surveyed. Plants sampled included cereal crops, perennial forage grasses, and wild grasses in fields, road borders, meadows, wooded areas, and along rivers. In each field, two or three quadrats of 1 m^2 were checked. Disease severity on 10 plants was determined by the percentage of infected leaf area. Infected plant samples were dried and stored according to the techniques described in CBS Course of Mycology (1971). The samples were stored in paper envelopes in the refrigerator at 5°C. Afterwards, infection parameters (including form, size and color of spots, presence or absence of chlorosis, necrosis, and conidia) were recorded. Pure culture was isolated and conidiophore development was stimulated using fresh plant material. This was carried out usually within a week after sample collection. Species identification was based on microscopic investigation of conidiophores and conidia and the fulfillment of Koch's postulates.

Since 1986, surveys have covered fields in the spring rainfed cereal producing area of northern Kazakhstan and rainfed or irrigated fields in southern Kazakhstan, Uzbekistan, and Tadjikistan. These surveys showed that *P. tritici-repentis* occurs throughout the region but is most widely distributed in Djizzakh region of Uzbekistan, and Kulyab region of Tadjikistan. Disease intensity reached 25-55% in some wheat fields at the flowering to milk wheat growth stage. In some fields under continuous wheat cropping, the proportion of infected plants was 100% and disease intensity was 70-80%. Maximum yield losses were estimated at 20-30% and higher. *Pyrenophora tritici-repentis* was reported in 60-80% of all observed fields in Kazakhstan, with the proportion of infected plants reaching 100%.

Identification of *P. tritici-repentis* Host Plants

Under natural conditions in Central Asia, *P. tritici-repentis* was reported on wheat and the following wild grasses: *Aegilops cylindrica, Agropyron* spp., *Agropyron cristatum, Agropyron interruptum, Elymus junceus, Seratia viridis,* and *Agropyron repens* (Table 1). The highest tan spot intensity was recorded on wheatgrass (*Agropyron repens*), where all above-ground parts of the plant were infected. Oval spots and stripes, 1-2 cm long and grayish-brown in color, occurred on leaves. An increase in the size of the spots and stripes resulted in coalescion and ultimately leaf rot. Conidiophores and conidia, but rarely pseudothecia, were observed on necrotic leaf tissues.

Some pseudothecia contained asci and ascospores.

Various species of Gramineae plants were artificially infected to determine possible host plants of the tan spot fungus. Cereal and wild grass seedlings were inoculated using fungi isolates taken from infected plants. Four cereal species and 22 wild grasses were studied. The inoculum was a mycelial suspension containing 2-3 drops of Tween 80 per 100 ml of water, which was sprayed onto the plants until runoff. Control seedlings were sprayed only with water and Tween 80. Inoculated plants were placed in a moist chamber at 20°C for 30 h. Observations were made on the ninth day after inoculation according to the scale of Rees *et al.* (1988).

The tested fungus isolates showed a high aggressiveness on wheat species (*Triticum aestivum* and *T. durum*), two cultivars of barley (*Hordeum vulgare*), and rye (*Secale cereale*). Among wild grasses, the disease level was severe on brome grass (*Bromus inermis*). High infection levels were recorded on *Aegilops cylindrica, Agropyron repens, Agropyron cristatum, Hordeum spontaneum,* and *Sorghum sudanense.* Leaves of *Agrostis gigantea, Agrostis stolonifera, Anthoxanthum odoratum, Echinochloa crus-galii, Lolium* spp., *Phleum* spp., and *Agropyron* spp. were moderately infected. *Hordeum leporinum, Setaria viridis, Elymus junceus, Elymus giganteus,* and *Festuca rubra* were the least infected. *Avena fatua, Alopecurus pratensis, Lolium perenne,* and *Poa pratensis* showed no infection or only minor infection on their leaves (Table 2).

Based on results from field surveys and artificial inoculation experiments on a wide range of grasses, it can be concluded that in the Central Asian region *Agropyron repens, Agropyron cristatum, Ae. cylindrica,* and *Bromus inermis* can all be considered as tan spot infection sources.

Table 1. Host plants, other than wheat, of *Pyrenophora tritici-repentis*.

Host plant	Regions
Aegilops cylindrica	Uzbekistan, Tadjikistan, South Kazakhstan
Agropyron spp.	Southern Kazakhstan
Agropyron cristatum	Northern Kazakhstan
Agropyron interruptum	" "
Elymus junceus	" "
Setaria viridis	" "
Agropyron repens	" "

Table 2. Artificial inoculation of cereals and wild grasses with *Pyrenophora tritici-repentis*.

Host plants	Disease rate
Triticum aestivum *T. durum* *Hordeum vulgare* (2 cultivars) *Secale cereale* *Bromus inermis*	Very severe
Aegilops cylindrica *Agropyron repens* *Agropyron cristatum* *H. spontaneum* *Sorghum sudanense*	Severe
Agrostis gigantea *Agrostis stolonifera* *Anthoxanthum odoratum* *Echinochloa crus-galii* *Lolium* spp. *Phleum* spp. *Agropyron* spp.	Moderate
H. leporinum *Setaria viridis* *Elymus junceus* *Elymus giganteus* *Festuca rubra*	Weak
Avena fatua *Alopecurus pratensis* *Lolium perenne* *Poa pratensis*	Not infected

Investigation of Tan Spot Resistant Wheat Cultivars

The identification of tan spot resistant wheat cultivars in Central Asia is important because of the increasing incidence of the disease. Experiments using 136 local cultivars were conducted in the field and the greenhouse in 1994. Since attempts to obtain conidium sporulation failed many times, greenhouse plants at the two-leaf stage and plants in the field at booting to tillering were inoculated with a water mycelium suspension. Five isolates of *P. tritici-repentis*, collected from various regions of Central Asia, were used for inoculum production. Inoculated plants were placed in a moist chamber for 24 h. Assessments of the number of infected plants and infected leaf area were made on the ninth day after inoculation.

The results demonstrated that, among those tested, there were no cultivars immune to tan spot. This investigation is considered as preliminary owing to the unavailability of proper inoculum and a convenient inoculation technique.

Improvement of Inoculation Technique

The production of relatively large quantities of inoculum is essential for conducting experiments on the host range of the tan spot pathogen or for evaluating cultivar resistance and effects of disease on yield. Current techniques are time and labor consuming, contain too many stages, and the resulting inoculum is a water conidium suspension that cannot be transported over long distances or stored for long periods. The goal was to develop a simple technique for inoculum production, which involved the testing of several nutrition media, light regimes, methods of preservation, and various rates and formulations of inoculum.

Medium development

Fifteen different media were tested (Table 3). After autoclaving, cooling, and adding an antibiotic (streptomycin sulfate, 1 g L^{-1}), the media were poured into plastic petri dishes. The fungus culture was added either by injection or by placing one drop of the mycelial-conidial suspension onto the media. The drop was evenly spread out over the medium surface using a sterile glass spatula. Dishes were incubated in the growth chamber at 20°C (day) and 18°C (night). Conidium production was

Table 3. Conidia development of *Pyrenophora tritici-repentis* on nutritive media.

No.	Media ingredients (L^{-1})	Sporulation
1	Lactose, 20 g; carbamide, 1.2 g; $MgSO_4$, 0.8 g; KCl, 0.8 g; KH_2PO_4, 0.8 g; agar, 20.0 g	-
2	Medium #1 plus maize extract, 5.2 g	-
3	Medium #2 plus V-8 juice, 50.0 ml	-
4	Medium #1 plus yeast extract, 7.6 g	-
5	Potato, 200 g; dextrose, 20 g; agar, 16.0 g	-
6	Potato, 50 g; dextrose, 20 g; agar, 16.0 g	-
7	Medium 6 plus V-8 juice, 50.0 ml	-
8	Medium #6 plus maize extract, 5.2 g	-
9	Medium #6 plus yeast extract, 7.6 g	-
10	Medium #6 plus peptone, 10.0 g; asparagine, 1.0 g	-
11	Medium #5 plus peptone, 4.9 g; $CaCl_2$, 4.9 g; $CaCO_3$, 1.6 g; dextrose, 20.0 g	-
12	Yeast extract, 7.6 g; sucrose, 20.0 g; agar, 15.0 g	-
13	Peptone, 10.0 g; sucrose, 10.0 g; KH_2PO_4, 2.0 g; agar, 20.0 g	-
14	Lactose, 38.0 g; casein hydrolyzate, 3.2 g; KH_2PO_4, 2.0 g; $MgSO_4$, 0.8 g; microsolution, 2.0 ml; agar, 10.0 g	+
15	Medium #14 plus yeast extract, 8.0 g	+

- No sporulation.
+ Abundant sporulation.

observed on medium #14 and medium #15. The first conidia began to appear on the 5th day of incubation, and abundant sporulation was observed on the 10th day. Up to 40-50 conidia on medium #14 and up to 70 conidia on medium #15 were counted per microscope field (10x10). The research indicated that inoculation should be performed by injection, not with drops of mycelial-conidial suspension because, in the latter, mycelial growth without conidia was observed.

Influence of light regime

The following light regimes were tested during the culture incubation period in the artificial climate chamber:

- 12 h day photoperiod (9,000 lux), illumination with cool white light lamps;
- 12 h day photoperiod (9,000 lux) for 5 days, until mass development of conidiophores. Samples were then exposed to near UV (NUV) light for 6 h;
- 12 h day photoperiod (9,000 lux) for 5 days. Samples were then twice exposed to NUV for 6 h;
- 12 h day photoperiod (6 h NUV light, 6 h 9,000 lux) throughout the incubation period.

On medium #15, which contained yeast extract, lactose, hydrolyzate casein, macronutrients, and microsolution, regardless of light regime, conidiophores appeared on the 3rd day and conidia on the 5th day. Abundant conidia formation was observed on the 10th day. Conidia formation occurred only in plastic petri dishes; there was no sporulation if glass petri dishes were used. No difference was found in the number of conidia per unit area among the various treatments. The mycelium surface in the dishes was a dim velvet color from the produced spores. The light regime of 12 h photoperiod with cool white light lamps (9,000 lux) was chosen because it was the easiest to implement.

Inoculum collection

When abundant conidia production was achieved, lids of the petri dishes were half opened and the temperature in the climate chamber was raised to 22-25°C. After two days, the substrate in the dishes and the fungus culture was air-dried and the inoculum was easily scraped out with a scalpel. The collected inoculum was sifted to remove particles larger than 1 mm, and kept for two days above silica gel. The number of conidia per 1 mg of biomaterial was determined.

Inoculum preservation

Three inoculum preservation techniques were studied: 1) tubes with cotton stoppers at room temperature; 2) tubes with cotton wool above silica gel; and 3) vacuum-sealed ampules at 5°C. Research into conidium viability and inoculum aggressiveness was conducted monthly. It was found that when tubes were stored above silica gel or simply at room temperature, conidium viability was preserved for up to four months. In the ampules, viability was preserved for at least one year and inoculum aggressiveness remained high during storage. This technique allows inoculum to be accumulated in advance for use when needed, thus making phytopathological research on tan spot much easier.

Inoculum formulations and rates

Greenhouse experiments were conducted to determine the optimal concentration and formulation of inoculum for plant infection. Five inoculum rates were tested: 10, 40, 100, 250, and 500 mg m^{-2} in a water-conidium suspension and in dust. The concentration of conidia was 2,300 conidia mg^{-1}. After inoculation, plants were put in a moist chamber for 30 h at 18-20°C. The control plants were sprayed with tap water, dusted with talc, and also put in a moist chamber. All plants (inoculated and control) were then placed on benches in the greenhouse at 15-20°C. The number of infected plants and infected leaf area were assessed on the ninth day after inoculation. Results showed that even though the dried inoculum was easy to use, especially in the field, preference should be given to the water-conidium suspension. To infect wheat plants at the seedling-tillering stage, a rate of 250 mg m^{-2} of biomaterial in the form of water conidium suspension produced an infection severity of 60% on the leaf surface, whereas 500 mg m^{-2} of dust inoculum caused only 40% infection severity.

Environmental Requirements of *P. tritici-repentis*

Effect of moisture period and temperature

Pyrenophora tritici-repentis, as well as other *Deuteromycetes*, can grow and infect plants only within certain temperature and moisture ranges. *Pyrenophora tritici-repentis* has its own pattern of environmental conditions required during the infection period; presence of inoculum and moisture on the leaves of the host plant do not guarantee infection. Tan spot infection is possible within a wide range of temperatures, but the optimal temperature is around 22°C (Lamari *et al.* 1992). Deviation from this range causes a decline in the probability of infection. Dew and raindrops are the moisture sources on wheat crops under natural conditions. Duration of the moist period depends on temperature.

To determine the influence of various combinations of temperature and moisture period duration on the level of tan spot infection on wheat, a series of experiments using one local wheat cultivar (Kyzyl Shark) was carried out in growth chambers with constant temperature and moisture regimes. The effect of low temperature was tested: 2, 5, 10, and 15°C. Plants were sprayed with a water-conidium suspension (250 mg of dried inoculum m^{-2}; 2,300 conidia mg^{-1}) at the two leaf stage and placed in a moisture chamber for 2, 4, 6, 8, 10, 15, 20, 25, 30, and 35 h. A subsequent incubation was conducted in the greenhouse at 15-20°C.

The effect of moisture period duration on tan spot symptoms at certain temperatures was estimated by recording the number of infected plants and infection intensity on the ninth day after inoculation. Tan spot infection was observed at all temperatures except at 2°C. At this temperature, only minor

infection was observed. At 5°C no significant differences were noted among the various dew periods.

The data showed that a 2 h moisture period is not long enough for spore germination and effective plant infection at any of the tested temperatures. Moisture periods of 5, 6, 8, and 10 h caused very weak infection, with the infection level similar at all temperatures (differences were not significant). Where moisture was maintained on the plants for 15 h, the infection level was higher at 10°C and 15°C. At a moisture period of 20 h and temperatures of 10°C and 15°C, a sharp increase in leaf was observed from 14% at 5°C, to 34% and 44% at 10°C and 15°C, respectively. The highest disease intensity (57%) was observed for the 25 h moisture period at 15°C. At moisture periods 30 and 35 h, a further increase in infection rate was not observed at all temperatures.

In late March to early April, wheat is at tillering to booting in Uzbekistan. During this period, the ecological conditions surrounding rainfed and irrigated crops satisfy the requirements of the tan spot pathogen: temperature, 10-15°C; daily average RH, 50-60%; and dew periods, 8-12 h.

Conclusion

The study leads to the conclusion that tan spot epidemics can occur if wheat monoculture is practiced. In this study, the distribution and sources of infection were investigated, and host plants of *P. tritici-repentis* determined. Preliminary investigation of 136 wheat cultivars indicated the absence of tan spot resistance in local cultivars and advanced lines, and highlighted the necessity of screening resistant cultivars during the early stages of the selection process. Also, a simple technique for inoculum production was developed, which created an infection background applicable to regional conditions. This research is essential for the development of new, ecologically adapted cultivars that are resistant to tan spot in Central Asia.

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Breeding for Foliar Blight Resistance in Heilongjiang Province, China

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Abstract

Foliar blight, black point, and root rot of wheat, caused by Bipolaris sorokiniana, B. triticicola, *and* Exserohilum monoceras, *are the second most serious group of diseases in Heilongjiang province, China. Of the three pathogen strains,* B. sorokiniana *is the most important, accounting for more than 80% of fungal strains collected. Annual yield losses due to* B. sorokiniana *are estimated at 10-15%. Greatest damage occurs during the flowering to milk stages of the wheat plant. Disease severity is closely related to temperature and humidity, especially temperature several days before flowering. Breeding for resistance is the best means of reducing damage to wheat production in the province. Some germplasm and varieties with good* B. sorokiniana *resistance have been developed through cross breeding, resistance evaluation after artificial inoculation in the field and laboratory, and screening mutants by phytotoxin selection pressure and cell engineering. Since varieties with different maturation periods are exposed to different ecological conditions, they have different* B. sorokiniana *resistance levels, i.e., late varieties>middle varieties>early varieties.*

Heilongjiang province is the largest area sown to spring wheat in China, with around 2 million ha sown each year. It is located in the northeast of the country at 121° 13′ to 130°E longitude and 43° 32′ to 53° 24′N latitude. Average annual precipitation ranges between 350 and 600 mm. The majority (70%) of total rainfall occurs in the last 10 days of June, July, and August, i.e., during flowering to harvesting stages of the wheat crop, when average temperatures are 22.3-25.8°C. Since the level of *B. sorokiniana* infection is closely related to temperature and humidity, greatest damage occurs during flowering to milking stages.

Stem rust of wheat was a problem in the province until the 1950s, when a breeding solution was found. Since that time, foliar blight, black point, and root rot of wheat, caused by *Bipolaris sorokiniana*, have been an important disease group, second only to scab. In general, annual yield losses due to the disease range between 10 and 15%. Although breeding for disease resistance is considered difficult, it is an effective way to reduce damage to wheat production.

Incidence of *B. sorokiniana*

Of the three fungal species associated with foliar blight, black point, and root rot in Heilongjiang province, *B. sorokiniana* is the most important. The two other species, *B. triticicola* and *Exserohilum monoceras*, can cause symptoms similar to that of *B. sorokiniana* (Zhang Jingchun 1988). Incidence of *B. sorokiniana* was calculated at 86.1-91.2% from 504 plants collected from wheat fields in different areas of Heilongjiang province (Table 1).

Assessment of Disease Severity

Resistance to *B. sorokiniana* is evaluated by assessing the damage on the leaf and kernels of wheat genotypes caused by the pathogen, using the following scale:

Leaf

- Highly resistant (HR): No lesions on the flag leaf, a few spots on the leaf below the flag leaf.
- Resistant (R): Lesion area on the flag leaf between 5 and 10%.
- Moderately resistant (MR): Lesion area on the flag leaf between 25 and 40%.
- Moderately susceptible (MS): Lesion area on the flag leaf ≥65%, and an increase in large lesions.
- Susceptible (S): Lesion area on the flag leaf between 80 and 100% and an increase in large lesions.
- Highly susceptible (HS): Lesions on the leaf sheath, and leaf death.

Table 1. Distribution and incidence of two *Bipolaris* spp. in Heilongjiang.

Region	No. of plants collected	*B. sorokiniana* (no.)	*B. sorokiniana* (%)	*B. triticicola* (no.)	*B. triticicola* (%)
Central	173	149	86.1	24	13.9
Eastern	191	174	91.1	17	8.9
Northern	106	92	86.8	14	13.2
Western	34	31	91.2	3	8.8

Source: Zhang Jingchun 1988.

Kernel

- Highly resistant (HR): No black point evident on the kernel.
- Resistant (R): Average black point <20% on all tested grain.
- Moderately resistant (MR): Average black point between 21 and 35% on all tested grain.
- Moderately susceptible (MS): Average black point between 36 and 50% on all tested grain.
- Susceptible (S): Average black point between 51 and 60% on all tested grain.
- Highly susceptible (HS): Average black point >60% on all tested grain.

Damage and Yield Loss

In Heilongjiang, *B. sorokiniana* can induce three types of symptoms (foliar blight, black point, and root rot) on susceptible wheat varieties, with leaf blotch, at times, causing the greatest damage (Table 2). Black point often affects germination, seedling vigor, root length, and root weight of a wheat variety (Table 3). In addition, we have found that although the leaf and root

system of a wheat variety may have good resistance to *B. sorokiniana*, its kernels often show black point on the embryo, e.g., Bei 87-44 and Bei 88-26, which were developed by the state farm institute in Heilongjiang province.

Breeding for Resistance

Crossing patterns

Breeding for *B. sorokiniana* resistance is currently very important in Heilongjiang to reduce the high yield losses, especially in later maturing wheat varieties. The crossing pattern should be as follows:

- For early and middle maturing varieties: A (MS or MR)/B(MR, R, or HR) or reciprocal.
- For later maturing varieties: A(MR or R)/B(R or HR) or reciprocal.

The *B. sorokiniana* resistance levels of the parental lines mentioned above can be determined after artificial inoculation (spraying) in the field.

Generation selection

In order to achieve disease resistance, the first character selected in the F1 generation must be "good looking plant aspect", meaning maturing with a living stem and flag leaf. Based on our wheat breeding experience in the province, this character reflects good leaf resistance to *B. sorokiniana*, and good root health. Generally speaking, if a plant selected in F1 is not "good looking", it will be difficult to find plants with good *B. sorokiniana* resistance in later generations.

Advanced line selection

An inoculum suspension containing 10-20 spores of *B. sorokiniana* per microscopic field (10x10) is used for leaf inoculation (spray) in the field. The appropriate time for artificial inoculation is 3-4 days prior to flowering. Advanced lines are then selected, according to other breeding objectives and *B. sorokiniana*

Table 2. Effect of foliar blight caused by *Bipolaris sorokiniana* on yield according to disease resistance level, Heilongjiang.

Disease resistance	1000 kernel weight (g)	Weight loss (%)	Yield of 500 spikes (g)	Yield loss (%)
HR	32.9	-	512.5	-
R	32.6	0.9	502.5	2.0
MR	32.4	1.5	441.0	14.0
MS	30.0	8.8	406.8	20.6
S	28.1	14.6	315.0	38.5

Source: Liu Tiruo 1989.

Table 3. Effect of *Bipolaris sorokiniana*-infected seed on seedling growth in Heilongjiang.

Disease resistance	Germination (%)	Diseased seedlings (%)	Seedling height (cm)	Root length (cm)	Fresh root weight (g)
HR	100.0 a[1]	0.0 e	15.82 a	24.51 a	0.31 a
R	97.0 b	9.5 d	14.11 a	24.51 a	0.28 ab
MR	85.7 c	15.4 c	10.20 b	22.00 a	0.15 b
MS	85.0 c	17.7 c	9.20 b	22.00 a	0.14 b
S	72.2 d	48.4 b	6.81 cd	10.40 b	0.06 c
HS	69.7 d	59.4 a	5.72 d	10.21 b	0.05 c

[1] Within a column, readings with the same letter are considered similar ($P<0.05$).
Source: Guo Mei 1996.

resistance levels. These will be further evaluated over the next two years using the same method.

Using the above method of breeding for resistance, several new wheat varieties with good *B. sorokiniana* resistance have been released, such as Kefeng 2, New Kehan 9, Longmai 19, and Longmai 12. Also, some advanced lines were developed from wide crosses between *Agropyron intermedium* and *T. aestivum*.

As indicated, it is necessary for a later maturing variety to have higher *B. sorokiniana* resistance levels than earlier maturing varieties, due to high temperature and humidity during flowering to milking stages in Heilongjiang. Therefore, among varieties, *B. sorokiniana* resistance levels differ with maturation period, with later maturing varieties having the highest resistance levels, and earlier varieties having the lowest levels.

Screening resistant mutants by cell engineering

Since there are only a few wheat genotypes with high *B. sorokiniana* resistance, perhaps it is better to use cell engineering to screen for individual plants that are resistant mutant to *B. sorokiniana*. The screening procedure is as follows:

- Isolate phytotoxin produced by *B. sorokiniana*.
- Place the phytotoxin in a culture medium.
- Cultivate the anther or young spike from wheat varieties with good *B. sorokiniana* resistance on the medium.
- Spray-inoculate the resulting mutants in the field with a suspension containing *B. sorokiniana* spores.
- Select the best mutants with good *B. sorokiniana* resistance, according to two to three years of inoculation results from the field.

Several mutants have been developed by this method in our institute. Of these, RB 500, an advanced line, appears to have higher *B. sorokiniana* resistance than its parent (Table 4). From Table 4, it can be seen that the disease index of RB 500 is 1.75 during the seedling stage, while that of its parent 89K202 is 2.48. During the adult plant stage, the disease index of RB 500 is 1.66 and its parent is 2.47. Yield of the mutant is also superior at 4.2% and 14.5%, compared to its parent and New Kehan 9 (a principal current cultivar in Heilongjiang with good *B. sorokiniana* resistance), respectively. The planting area of improved lines in the province is about 0.6 million ha.

Table 4. Disease response index of mutant and parent line to *Bipolaris sorokiniana* at seedling and adult plant stages.

	Seedling stage			Adult plant stage			
	1990	1991	mean	1989	1990	1991	mean
Parent							
89K202	2.17	2.79	2.48	2.70	2.02	2.70	2.47
Mutant							
RB500	1.64	1.86	1.75	1.29	1.53	2.17	1.66

Source: Wang Guangjin 1994.

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Incidence and Current Management of Spot Blotch of Wheat in China

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Abstract

In recent years, wheat production in China has been severely affected by soilborne disease, including spot blotch, caused by Bipolaris sorokiniana. *The disease was first reported in northern China in the 1950s and is now prevalent throughout the country. Variation in climatic and ecological conditions among wheat growing regions affects disease symptoms, host range of* B. sorokiniana, *resistance levels in wheat cultivars, and, subsequently, wheat yield losses. Disease symptoms include spot blotch; seedling blight, common root rot, head blight, and black point. Of more than 9,400 wheat cultivars evaluated, 8% showed high spot blotch resistance, 21% showed intermediate resistance, and 69% showed low resistance. During growth of the wheat plant, spot blotch resistance levels were noted to change, with flowering and grain filling stages being the most critical. To control spot blotch, it is important to follow an integrated management approach, which combines resistant cultivars, crop rotation, crop management practices, and chemical control. To date, no tolerant or highly resistant cultivars have been found. Wheat rotation with non-gramineae crops for three years were shown to reduce spot blotch occurrence. Disease severity was lower on early sown than late sown wheat. Spot blotch control levels of higher than 60% were achieved by fungicides triadimefon, triadimenol, and diconazole when applied to seed. Triadimefon, applied to foliage, achieved an effective rate of protection of about 60%.*

In China, wheat ranks second only to rice in cultivated area. In recent years, with the change in climate and cropping system, severe damage has been caused by soilborne diseases of wheat including take-all (*Gaeumannomyces graminis*), sharp eye spot and rhizoctonia root rot (*Rhizoctonia* spp.), fusarium root rot (*Fusarium* spp.), common root rot (CRR), and spot blotch (*Bipolaris sorokiniana*). These fungi may infect wheat plants and cause wheat production losses depending on the climatic and ecological conditions in each wheat growing area (Table 1). In this paper, the incidence and management of wheat spot blotch are summarized and discussed.

Spot Blotch Distribution and Damage

Wheat spot blotch is caused by *B. sorokiniana* (syn. *Helminthosporium sativum*, teleomorph *Cochliobolus sativus*). In China in the 1950s, rust resistant wheat cultivars were grown on a large scale and replaced many landraces. Following this, Huang Guichao *et al.* (1957), Wu Yousan *et al.* (1957), and Lu Shiyi *et al.* (1957) reported the occurrence of

helminthosporium root rot and disease damage for the first time in northern and northeastern China. The pathogen was isolated from diseased wheat roots, stems, leaves, and seeds, and identified as *H. sativum* or wheat root rot disease. In addition, Chen Qiben and Pai Chinkai (1957) studied the protection mechanisms of spring wheat seedlings against *B. sorokiniana* infection in northeastern China. From the 1960s to 1990s, the disease gradually spread to other wheat growing regions of China. Disease occurrence and damage were reported in the northwestern area (Ouyang Xiao 1962), Henan province (He Jiabi 1964), Shandong province (Jiang Zehai *et al.* 1978), and Guangdong province (Wu Baichai *et al.* 1983). Today, spot blotch of wheat is prevalent and has gradually expanded from northern to southern China, with the northeastern and northwestern wheat growing areas most severely affected (Shang Hongsheng 1990).

Regional Infection and Symptoms of *B. sorokiniana*

Bipolaris sorokiniana may infect any part of the wheat plant at any development stage. The diseased wheat plants show seedling blight and CRR symptoms at the seedling stage, and spot blotch, foot and root rot, head blight, and black point symptoms during adult plant stages. Changes in climatic and ecological conditions strongly affect disease symptoms. For example, in the wheat growing areas of Ganshu province and the Inner Mongolia Autonomous Region with an arid or semi-arid climate, *B. sorokiniana* causes wheat root and feet rot, causing darkening or brown necrotic spots and reduced tiller number. *Bipolaris sorokiniana* symptoms on wheat show regional variation (Table 2).

Table 1. Distribution of wheat spot blotch in China.

Pathogens	Northeastern China	Northwestern China	Northern China	Southern China	Yellow and Hui River plain	Lower reaches of the Yangtze River
Gaumannomyces graminis		√	√		√	
Fusarium spp.	√	√	√	√	√	√
Rhizoctonia spp.					√	
Bipolaris sorokiniana	√	√	√	√	√	
Drechslera triticicola	√					

Table 2. Climatic conditions and spot blotch distribution in China.

Region	Annual mean temperature (°C)	Mean rainfall (mm)	Wheat type	Spot blotch	CRR	Method of spread
Northeastern China	0-7	350-900	Spring	√		Soil and seed
Northwestern China	<0	120	Winter and spring	√	√	Soil and seed
Southern China	18-24	1200-2400	Spring	√		Seed

Northeastern spring wheat growing region

In this region, the spring wheat growing area includes Liaoning, Jiling, and Heilongjiang provinces. Wheat spot blotch occurs annually, sometimes expressed as seedling blight, CRR, head blight, and black point (Huang Guicao *et al.* 1957; Wu Yousan *et al.* 1957; Chen Hongweng *et al.* 1982; Liu tiruo *et al.* 1987; Zhang Jingchun *et al.* 1988). Wheat yield losses caused by spot blotch are due to a decrease in spikelet number per tiller and in 1000-grain weight. Yield loss has commonly been 20 and 30% or above 75%.

Northwestern spring and winter wheat growing region

This vast area with a dry climate includes Shanxi, Qinghai, and Ganshu provinces, and the Ningxia Hui and Xinjing Uygur Nationality Autonomous Regions.

In the winter wheat growing area, wheat CRR is caused by *B. sorokiniana* combined with *Fusarium* spp., *Gaeumannomyces graminis*, and *Rhizoctonia solani*. Common root rot was a major disease in the area in the 1970s, causing root and foot rot and reduced tiller number. Yield loss due to CRR ranges between 10 and 80% (Ouyang Xiao 1962; Li Qiangkuen *et al.* 1987; Ma Yu *et al.* 1987). With the change in wheat cultivars and crop management practices since the 1980s, helminthosporium spot blotch and fusarium leaf blight have become severe in local wheat growing areas, and CRR severity has declined. In the spring wheat growing areas, two important diseases are spot blotch and CRR.

Yellow and Huai River plain wheat growing region

This region includes most parts of Shandong and Henan provinces. Here, *B. sorokiniana* infects wheat and causes seedling blight, spot blotch, head blight, and black point. Spot blotch occurs mainly on summer sown wheat, and yield loss has been above 30% (He Jiabi 1964; Ma Qixiang *et al.* 1987; Jiang Zhehai *et al.* 1978).

Southern China wheat growing region

In Guangdong Province, the cropping pattern is wheat-rice-rice. *Bipolaris sorokiniana* causes spot blotch or black point late in the growing season. Conidiospores of *B. sorokiniana* are not able to survive for more than 80 days in the soil. The primary infection of *B. sorokiniana* occurs only by seedborne pathogens (Wu Baichai *et al.* 1983).

Helminthosporium Species and Pathogenicity in China

In China, 51 species of *Helminthosporium* have been recorded (Dai Fanglian 1979), and among them 22 can infect Gramineae (Jiang Guangzheng 1957). Reportedly, *B. sorokiniana* can be divided into H_1 and H_2 culture forms (Pai Chinkai *et al.* 1982), according to the appearance of the culture and conidiospore morphology on PDA medium. The H_1 and H_2 culture forms are not stable, however, and do not show differences in pathogenicity and lesion type in the field. In addition, another species, *Dreschslera triticicola*, with weaker pathogenicity, was recorded (Pai Chinkai

et al. 1989). This species was not isolated from samples in Liaoning and Heilongjiang provinces in 1991 and 1992. To date, there has not been a report on physiological specialization in China.

Host Range of *B. sorokiniana*

In northeastern China, *B. sorokiniana* can infect 29 gramineae and other crop species (Pai Chinkai *et al.* 1982). A report from the Plant Protection Institute of Heilonjiang Agriculture Science Academy (1974) suggested that at least 30 plant species could be infected by the pathogen. In the Yellow and Huai River plain region, more than 65 gramineae are hosts of *B. sorokiniana* (Ma Qixiang *et al.* 1987). In Guangdong Province of southern China, 17 plant species can be infected by *B. sorokiniana* (Wu Baichai *et al.* 1987). Of these, *Saccharum officinarum, Zizania caduciflora, Paspalum thumbergii, Ischaemum ciliare,* and *Apluda mutica* were the first hosts reported in China (Table 3). In our laboratory, partially purified HS toxins of *B. sorokiniana* inhibited seed germination and root elongation in 14 species belonging to 6 families (Table 4; Chang Naitao *et al.*, unpublished).

Spot Blotch Resistance Types and Variation

Resistance to spot blotch has differed and changed greatly over years and locations among wheat cultivars. The resistance level of at least 9,408 wheat cultivars from China and abroad has been evaluated in our nursery at Shenyang Agricultural University. The results have shown that 816 (8.67%) cultivars showed high spot blotch resistance, 2004 (21.3%) cultivars showed intermediate resistance, and 6588 (69.4%) cultivars showed low resistance. Tolerant or very highly resistant wheat cultivars have not yet been found. The disease level of resistant cultivars varied from one field to another, and resistant cultivars had lower yield loss than that of susceptible cultivars

Resistance types in wheat cultivars

All parts of the wheat plant can be infected by *B. sorokiniana* in the field. In field evaluation studies, it was found that incidence and severity of spot blotch was not related to head blight or black point. Research in Heilongjiang province reported that wheat yield was negatively

Table 3. Spot blotch of wheat in China.

Early reports of the disease	
Guichao Huang *et al.* 1957	(Northeastern China)
Yousan Wu *et al.* 1957	(Northeastern China)
Shiya Lu *et al.* 1957	(Northern China)
Disease symptoms	
Seedling blight	
Common root rot	
Foliar spot blotch	
Head blight	
Black point	
Method of spread	
Soil and seed	
Hosts	
More than 30 species of plants in northeastern China	
More than 65 species of plants in Yellow and Hui River regions	
At least 17 species of plants in Guandong province, e.g.,	*Saccharum officinarum* *Paspalum thumbergii* *Ischaemum ciliare* *Apluda mutica*

correlated to spot blotch severity after inoculation with conidiospores, and head blight was not correlated to black point (Zhang Jinchun *et al.* 1988).

Resistance variation during wheat development

During development and growth of the wheat plant, level of spot blotch resistance was noted to change from high to low, at the seedling stage and the heading stage, respectively. Flowering and grain filling were the critical times for resistance change in a cultivar. At these growth stages, spot blotch resistance levels were low, and disease severity seriously affected grain formation. At one location in the field it was noted that disease severity on early maturing cultivars was higher than that on late maturing cultivars.

Resistance variation and environment

Bipolaris sorokiniana resistance traits in wheat are polygenic and greatly affected by climatic factors such as rainfall, humidity, and temperature. Cultivar resistance may change under different combinations of year, season, and location. This is important knowledge for our breeding program and germplasm resource development study.

Spot Blotch Management

In recent years, serious outbreaks of wheat spot blotch have occurred and gradually extended in China, even though climatic conditions have not favored the disease. The increased incidence is due to a lack of resistant cultivars, high planting densities, and unbalanced management practices with too much inorganic and too little organic fertilizers. Poor management practices have led to a manure deficiency in the middle or late wheat growth stages, which has lowered *B. sorokiniana* resistance as well as the crop's compensation ability. Integrated management for spot blotch control

Table 4. Inhibition effect of HS toxins on seed germination and root elongation of crop species.

Family	Crop species	Inhibition rate (%)	
		Seed germination	Root elongation
Gramineae	*Triticum aestivum*	86	69
	Zea mays	48	55
	Sorghum vulgare	69	89
	Oryzae sativa	100	100
	Setaria italica	100	100
	Hordeum vulgare	79	87
	Lolium perenne	80	80
Compositae	*Helianthus annuns*	62	47
	Lactuca sativa	100	100
Solanaceae	*Solanum melongena*	100	100
Cucurbitaceae	*Cucumis sativus*	100	100
Fabaceae	*Glycine mas*	64	71
	Vicia faba	75	100
	Pisium sativum	71	100
	Cropolaria juncea	100	100

Note: Partially purified HS toxins were diluted 1:2; treatment conditions 23°C for 48 h.

combines resistant cultivars, crop rotation, crop management practices, and chemical control. Individually, none of these measures provide efficient disease control; hence a combined approach is crucial. Practical spot blotch control measures are listed in Table 5.

Resistant cultivars

Breeding for resistant cultivars is the main disease management measure from a sustainable agriculture perspective. The leading cultivars in each wheat growing region display different levels of spot blotch resistance, i.e., moderate resistance, moderate susceptibility, and susceptibility. Wheat cultivars with stable resistance traits selected in China include Zhongsan No. 6, Dabaimai, Xiumai No. 2, and Xibeizhan No. 5 (Wang Jianxiong 1990).

Crop rotation

In areas of disease epidemics, wheat rotation with non-gramineae crops for three years could reduce spot blotch occurrence. In Guangdong province, for example, the local crop sequence is wheat-rice-rice. Conidiospores of *B. sorokiniana* were observed to survive in the soil for only 80 days and thus could not act as the primary infection source; therefore, disease was caused only by seedborne pathogens.

Table 5. Spot blotch control methods.

1. Resistant and tolerant wheat varieties.
2. Rotation with non-gramineous crops for three years.
3. Crop management
   - Adjust sowing time
   - Manure and water management
   - Sowing
4. Chemical control
   - Seed treatment:
     - Triadimefon
     - Triadimenol
     - Diniconazole
   - Foliar treatment of adult plant
     - Triadimefon
     - Carboxin
     - Mancozeb
     - Thiophanate-methyl
     - Diniconazole

Crop management

In the northeastern and northwestern wheat growing areas, improved crop management, such as changes in sowing time, have contributed to a reduction in disease severity. In the spring growing area of Liaoning, early sown wheat (early to middle March) grows well, with an increased tiller number and lower disease severity. On other hand, late sown wheat (late March-early April) is easily infected by *B. sorokiniana* and showed a higher disease severity and a yield loss of 7-12% (Chang Naitao *et al.*, unpublished). Good crop management practices act to reduce disease severity.

Chemical control

Seed treatment—Triadimefon, triadimenol, and diniconazole were used to treat seed against seedling blight and CRR, as well as wheat smut, seedling rust, and powdery mildew. Triadimefon and triadimenol were used in the northwestern area and the effective rate of control was above 60% (Ma Yu *et al.* 1987). Diniconazole was found to achieve better control than triadimefon in our study (Chang Naitao *et al.*, unpublished).

Foliar treatment—Triadimefon, carboxin, mancozeb, and thiophanate-methyl were used for foliar treatment and sprayed at flowering to reduce disease

rate. Triadimefon was sprayed at elongation or flowering stages. The disease severity percentage at the milk stage was reduced to 30-50% and the effective rate of protection was about 60%, compared to the control (Chang Naitao *et al.*, unpublished).

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Spot Blotch and Tan Spot of Wheat in Paraguay

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Abstract

*Historically wheat has not been a traditional crop in Paraguay. With the significant increase in soybean production over the last two decades, the area of wheat during the winter has also increased. However, high temperatures and humidity are favorable for severe disease epidemics, especially of rusts, powdery mildew, and foliar and head blight of wheat. These diseases are limiting factors for stable wheat production in the country. Foliar blight is caused by spot blotch (*Bipolaris sorokiniana*) and tan spot (*Dreschlera tritici-repentis*), which are two very important wheat diseases. Spot blotch, combined with other diseases, was responsible for a grain yield reduction of almost 70% in the mid 1970s, while tan spot has recently become important. Increase in tan spot prevalence is primarily associated with the increase in cropping area under zero tillage cultivation. An integrated disease management strategy has been implemented to reduce losses caused by the diseases. The national germplasm development program has been successful in releasing commercial varieties including IAN 8, IAN 9, Itapúa 35, and Itapúa 40 that allow only moderate infection levels. Seeding these varieties every third or fourth year on oat or vetch stubble has significantly reduced infection levels; however, in a year of severe disease, chemical control is essential. Chemical control of black point in grain, caused by* B. sorokiniana, *has been successfully achieved using iprodione, guazatine, or triadimenol fungicides at a rate of 200 g/100 kg seed. Foliar applications of systemic fungicides such as tebuconazole, propiconazole, flutriafol, ciproconazole, fluzilazole, epoxiconazole, and metaconazole, applied between heading and grain filling stages, have achieved cost effective disease control. Under severe disease levels, a double application of fungicide can result in a grain yield increase of 38-61%.*

Paraguay, a landlocked country in central South America, between latitudes 19-28°S, is a nontraditional wheat producing country. Although appreciable quantities of wheat were produced by the Jesuit priests during colonial times, until only a decade ago it was widely believed that the country's marginal environment would not permit the production of this cereal grain. However, accompanied by a tremendous increase in soybean acreage during the summer season, wheat acreage has increased during the last 20 years (Pedretti and Kohli 1990; Figure 1). Although both wheat area and production were quite variable until the late 1970s, the availability of newer varieties and production management

technology has allowed the average yield to double, reaching almost 2.1 t ha^{-1}.

Wheat in Paraguay is concentrated in the southeastern part of the country, especially on the banks of the Parana River. Two provinces, Alto Parana and Itapua, with fertile soils and mild temperatures, account for 43 and 40% of the national wheat area, respectively.

Unstable climatic conditions of the tropical and subtropical humid environment during crop growth seriously limit wheat production. Due to irregular precipitation, climatic variation and instability have more impact than temperature variation during the crop cycle (Figures 2a and b). Notably wet years, such as 1972, 1975, and 1983, represent years of severe leaf blight caused by spot blotch and bacterial leaf blight. High precipitation during the wet years can occur during the vegetative period of the crop (June/July), as in 1972 and 1983, or during the reproductive period (August/September), as in 1972 and in 1996. During the years that grain filling and maturity occur under wet conditions, such as 1996, serious pre-harvest sprouting causes further losses in grain quality.

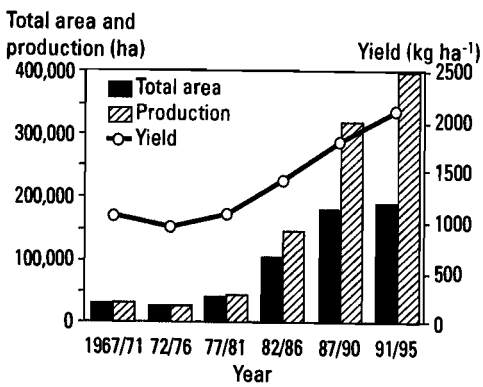

Figure 1. Wheat area and production, and average grain yield in Paraguay, 1967-1996.

Wheat diseases, in general, are one of the most important factors limiting yield in Paraguay. Losses caused by the disease complex in a wet year can reach as high as 70%, as in 1972 (Pedretti and Viedma 1988; Viedma 1989). Apart from the predominance of foliar diseases caused by *Helminthosporium* spp. and *Xanthomonas campestris* pv *undulosa* during wet and hot years, rusts, especially leaf rust, remain very important diseases (Viedma and Bozzano

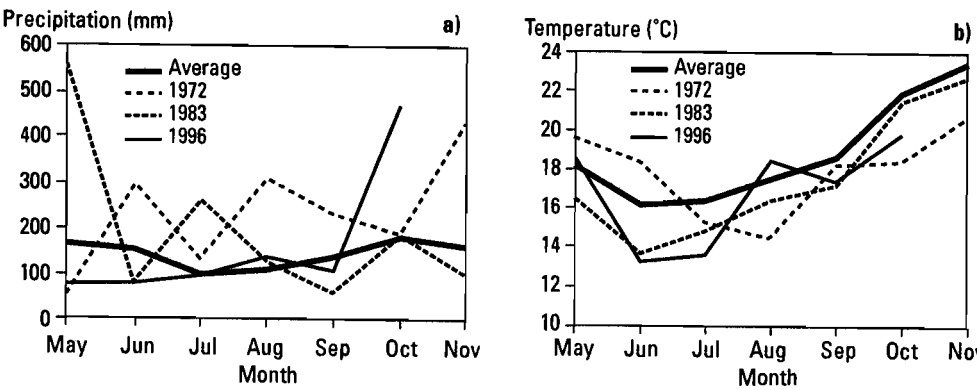

Figure 2. Comparison of a) 20-year average precipitation and b) 20-year average temperature over three critical years.

1986; Viedma and Delgado 1989). The prevalence of powdery mildew and fusarium head blight have also increased during the past few years. The importance of these diseases in Paraguay during 1972-1996 is summarized in Table 1.

It should be noted that tan spot was observed in Paraguay from 1986 when zero tillage cultivation started to increase. Since then, it has become more important (Viedma and Oniki 1986).

Given the importance of the diseases in the country, the wheat breeding program has successfully released new cultivars that are moderately to highly rust resistant and demonstrate lower susceptibility levels to foliar blights. Disease reactions of the most important commercial varieties are presented in Table 2.

Foliar Blights

The most important foliar blights frequently causing damage to wheat are spot blotch, caused by *Bipolaris sorokiniana* (Sacc. in Sorok.) Shoem. (syn. *Helminthosporium sativum* Pamm., King & Bakke) (teleomorph *Cochliobolus sativus* Ito & Kufib. Drechs ex Dastur) and tan spot caused by *Pyrenophora tritici-repentis* (Died.) Drechs and its anamorph *Drechslera tritici-repentis* (Died.) Shoem. (Hosford 1971; Krupinsky 1983).

The spot blotch fungus is not only important in Paraguay, but also in the

Table 1. Wheat diseases prevalent in Paraguay and their importance, 1972-96.

Disease	Year					
	1972-75	1976-79	1980-85	1986-89	1990-95	1996
Leaf rust	X[1]	X	XX	XX	XX	X
Stem rust	X		X			X
Powdery mildew		X	X	X	XX	X
Spot blotch	XX	X	XX	XX	X	
Tan spot				X	XX	XX
Septoria glume blotch	X		X			X
Fusarium head blight	XX		XX		X	XX
Bacterial leaf stripe			XX	XX	X	X

[1] X = Important, XX = Very important.

Table 2. Disease reactions of important commercial wheat varieties in Paraguay.

Disease	Ian-7	Cordillera 3	Cordillera 4	Ian-8	Itapua 35	Itapua 40	Ian 9
Leaf rust	MS[1]	S	R	R	R	MS	MR
Stem rust	MS	R	R	R	MS	R	R
Powdery mildew	MR	MS	S	S	R	MS	MS
Spot blotch	S	S	MS	MS	MR	MS	MR
Fusarium head blight	MR	S	S	MS	MS	S	S
Bacterial leaf stripe	S	S	MS	MS	MS	MS	MS
Tan spot	MS	MR	MS	MS	MR	MS	MR

[1] R = Resistant, MR = Moderately resistant, MS = Moderately susceptible, S = Susceptible.

lowlands of Bolivia and Brazil (Mehta 1987; Mehta and Gaudencio 1991), where it causes serious crop losses during warm and humid winters.

Spot blotch was the most important disease during the initial phases of wheat cultivation in Paraguay during 1967-1972, when it caused serious production losses. During 1972, spot blotch and septoria glume blotch (*Stagonospora nodorum*) were the most important diseases on commercial variety 214, causing severe yield losses of approximately 70% (Pedretti 1990). Another spot blotch epidemic, combined with fusarium head blight, occurred in 1975, and, even though it was as severe as that of 1972, yield losses were less because commercial variety Itapua 1 grown that year was moderately resistant to both diseases. Continuous spot blotch epidemics between 1979-1983 were primarily responsible for the reduction in wheat sown area. In addition, frequent precipitation during crop maturity caused serious black point in harvested grain, thereby reducing industrial quality.

Although the fungus has been known to cause foliar damage in the past (Pedretti and Viedma 1988), incidence of common root rot has significantly increased during recent years.

On the other hand, tan spot has recently predominated in the Southern Cone of South America including Argentina, Brazil, Chile, Paraguay, and Uruguay (Kohli *et al.* 1992). In Brazil, severe losses due to tan spot were reported in the states of Paraná and Rio Grande do Sul since 1982.

In Paraguay, tan spot importance has significantly increased with the increase in area under conservation tillage practices such as zero tillage. Approximately one-third of the 900,000 ha of soybean sown during 1995/96 was under zero tillage. Wheat represents approximately one-quarter of the area sown to soybeans. Therefore, an increase in the area of soybean under zero tillage equates to an increase in the area of wheat sown into soybean stubble using conservation tillage practices.

The alarmingly high tan spot incidence is primarily due to the continual sowing of wheat into the stubble of the previous year's wheat crop. The fungus survives during the summer on wheat stubble; its spores are protected in pseudothecia, even during fallow periods. The pseudothecial density of *D. tritici-repentis* can reach as high as 9,000 pseudothecia m^{-2} under different tillage management systems tested in Paraguay (Table 3). Under local conditions, it takes approximately 17 months for the stubble residue to decompose, which guarantees perpetuation of the fungus, as well as an increase in the inoculum load, year after year (Figure 3).

Table 3. Pseudothecial density of *Drechslera tritici-repentis* under different soil management systems, CRIA, 1994-96.

	Zero tillage	Minimum tillage
Crop residue (g m^{-2})	240	170
Pseudothecia (m^{-2})	9.000	6.120

The teleomorphic phase of *P. tritici-repentis* occurs on crop residue, and the majority of ascospores are released during April-May (seeding time). Their release coincides with lower temperatures and rainy periods, which provide excellent conditions for primary infection very early in the crop cycle.

Disease Management

Genetic resistance

Genetic improvement of germplasm conducted by the Directorate of Agricultural Research (DIA) has achieved significant advances over the past decade. The most important aspect of this was the establishment of a shuttle breeding program in association with CIMMYT, which allowed the possibilities of evaluating two generations a year and multilocation testing. Specific germplasm development activities were also conducted, utilizing the locally adapted and disease resistant parents. The segregating populations were selected under heavy disease pressure at critical locations. As a result, the newer varieties released in the mid 1980s and early 1990s represent a combination of high yield potential and adaptation with moderate to high disease resistance levels.

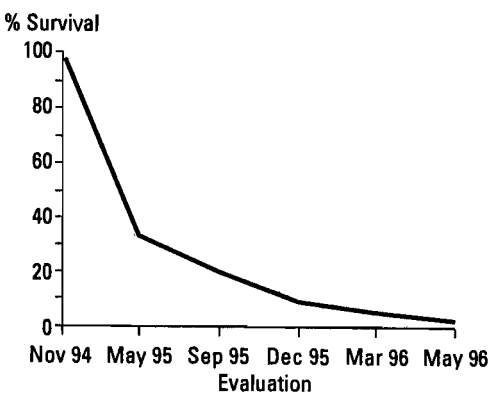

Figure 3. Survival of *Pyrenophora tritici-repentis* on wheat crop residue.

Although the majority of advanced lines in disease resistance breeding are developed for rusts and powdery mildew control, moderate progress has also been achieved in breeding for resistance to foliar blights (Kohli *et al.* 1990). Table 2 shows the disease reactions of important commercial wheat varieties in Paraguay.

For the last several years, tan spot screening of advanced lines and germplasm has been conducted in fields under zero tillage, and, considering the high level of humidity present during the crop cycle, adequate screening levels are being achieved. Regional germplasm demonstrating lower tan spot infection levels over four years are presented in Table 4.

Cultural control

Since 1990, cultural control of foliar blights has been tested at CRIA, comparing crop rotation with wheat monoculture under different tillage practices. The results presented in Table 5 show that lower levels of common root rot and tan spot infection are possible under zero tillage cultivation if wheat is sown every third or fourth year on oat or vetch residue. This practice not only reduces infection levels, but also results in higher wheat yields. As a result, significant advances can be achieved by introducing one or more break crops during the winter cycle under zero tillage.

Table 4. Regional advanced lines demonstrating low tan spot infection levels under natural conditions in a zero tillage field, 1992-96.

ID number	Cross	Pedigree
P91:2470	LAJ2965/ PFED	A11678.4T-0N-2D-1T-1T-0T
P91:2465	LAJ2963/T00026	A11398.T1-0N-1B-2T-1T-0T
CEP8965	CEP14/CEP82113//BR14	B31751-D-0Z-0A-3A-2A-900Y
CEP8966	CEP14/CEP82113//BR14	B31751-D-0Z-0A-3A-2A-900Y
CEP89171	MCR/CEP13//BR14	B31561-G-310Y-0Z-3A-3A-900Y
PF9058	CI1419/2*PF8233	F26562-0R-0F-0F-2F-0F
PF9067	BR35/PF84386//AMIGO/BR14	F30677-0F-1F-0R-3F-0F
PF90120	PF822522222/BR35//IA7998/PF8550	F30872-1F-0R-1F-0R-1F-0R-2F-0R-0F
E. PELON90	QU P-2278	MILAN "S"
88053	BON/YR70/3/F35.70//KAL/BB/4/NAC	CM74553-2E-2E
C-91020	ND/VG9144//KALBB/3/YACO "S"/4/VEE5 "S"	CM8536/4Y/0M/0Y/2M/0Y
CEP8818	BUTUI/BR14//PF79790/CEP75203	B30654-02-0A-3A-0A

Table 5. Severity of common root rot and tan spot of wheat under different tillage and crop rotation systems, CRIA, 1995.

Crop rotation	Tillage system	Infection index		Grain yield (kg ha^{-1})
		Common root rot[1]	Tan spot[2]	
Wheat monoculture	Zero	19.7	29.8	2452 bc
Wheat monoculture	Minimum	13.4	26.9	2467 b
Wheat monoculture	Conventional	7.4	13.3	2663 b
Wheat after oats (one winter without wheat)	Zero	2.5	2.6	2782 ab
Wheat after oats and vetch (two winters without wheat)	Zero	0.9	1.0	2937 a
CV (%)	9.02			
F value	4.28[a]			

[1] Common root rot *(Helminthosporium sativum)* evaluation scale, 0-100.
[2] *Pyrenophora tritici-repentis* evaluation scale, 0-100.

Table 6. Chemical control of tan spot: effect of timing of application on grain yield.[1]

Time of application/ growth stage	Yield increase					
	1992		1993		1995	
	(kg ha^{-1})	(%)	(kg ha^{-1})	(%)	(kg ha^{-1})	(%)
Elongation	2523 bcd[2]	115	1838 cd	155	2238 a	102
Flowering	2903 ab	133	2328 b	146	2267 a	103
Elongation + flowering	3016 a	138	2695 b	161	2337 a	106
Milk stage	2200 bcd	104	1665 cd	105	2205 a	101
Check without application	2180 d	100	1595 d	100	2195 a	100
CV (%)	9.5		11.5		8.7	

[1] Folicur at 500 cc ha^{-1} of commercial product.
[2] Value in the same column followed by different letters are significantly different at the 5% level by Tukey's multiple range test.

Chemical control

The role of complementary chemical control of diseases has been of great importance in stabilizing wheat production in Paraguay; however, given the improved resistance of the present commercial varieties and variable level of disease pressure and climatic conditions over the years, the efficiency of chemical control is not always cost effective. Even so, significant increases in grain yield, varying between 38-61%, can be achieved in years of severe disease, such as 1992 and 1993 (Table 6). On the other hand, in a relatively dry year, such as 1994, the same treatments resulted in only a 6% yield increase. A single fungicide application in a year of severe disease, depending on timing, can result in a 4-6% yield increase and achieve cost effective disease control.

Effective foliar blight control has been possible due to the availability of new systemic fungicides. Experimental results show that most of these fungicides are able to achieve moderate control of spot blotch and tan spot (Table 7). Years of experiments with tebuconazole (Folicur) demonstrate additional moderate control of fusarium head blight.

Table 7. Evaluation of different fungicides for control of foliar blight caused by *Bipolaris sorokiniana* and *Pyrenophora tritici-repentis*, CRIA, 1995/96.

Fungicide	Commercial dosage (cc ha^{-1})	Foliar blight index	Efficiency of control (%)
Tebuconazole	500	12.5	80.1
Propiconazole	1000	15.6	75.2
Flutriafol	500	25.0	58.0
Cyproconazole	1000	17.8	70.2
Fluzilazole	360	17.0	72.7
Epoxiconazole[1]	1000	16.3	73.0
Metaconazole[1]	1200	15.0	75.1
Check		60.5	-

[1] One year of evaluation.

Seed treatment

Bipolaris sorokiniana is the most important pathogen affecting wheat seed, causing black point in virtually all commercial fields (Viedma 1981). Its infection potential varies, depending on year and location, and can reach incidence levels higher than 50%. Several fungicides have been evaluated for pathogen control in grain. Of these, triadimenol, guazatine, and iprodione achieve the highest levels of control (Table 8). New fungicides such as triticonazole are being evaluated with good results.

Table 8. Chemical control of seed infection by *Bipolaris sorokiniana* and subsequent seedling infection.

Commercial name	Technical name	Dosage (g) 100 kg/seed	*B. sorokiniana* infection[1]
Rovrin	Iprodione 20% + thiram 60%	200	2.0
Panoctine	Guazatina 30% + imazalil 20%	200	2.1
Rhodiauram	Thiram 70%	200	20.5
Vitavax	Carboxin 37.5% + thiram 20%	250	24.1
Baytan[2]	Triadimenol 7.8% + imazalil	200	1.0
Check		-	53.6

[1] Incidence of *B. sorokiniana* at the seedlings stage.
[2] Controls rusts and powdery mildew at the seedling stage.

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Research on *Pyrenophora tritici-repentis* Tan Spot of Wheat in Uruguay

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Abstract

Tan spot of wheat, first reported in Uruguay in 1982, did not become a major disease until 1990/91 when high disease incidence was noted in the northwestern coastal region of the country. During those years, identification of germplasm with superior disease resistance was not possible due to severe infection levels, although it was possible to distinguish between symptoms of Drechslera tritici-repentis *and of* Septoria tritici. *Almost all cultivars under evaluation were moderately to highly susceptible to tan spot. The perfect stage of the fungus was found on wheat stubble in 1993. Many of the numerous alternative disease hosts reported in the literature are present in Uruguay; however, none have tested positively for tan spot. The increase in zero-tillage cultivation in the country has made it essential to develop and identify highly resistant cultivars. Local advanced breeding lines and introduced germplasm have been screened for tan spot resistance at key locations since 1990. Of these, Fink and other lines were found to be resistant, but Vicam was found susceptible. Carifen 12 and Red Chief, sources of tan spot resistance elsewhere, were not adapted to local conditions. Newer germplasm from the region and from CIMMYT, such as Milan, Cisne INIA, Coker62/BR14, LE2062/LE2096, LE2091/LE2089, E.Dor/Aepoglom, and Kvz//BB/Cha/3/Trm/4/TEMU36-78/5/Ovacion, has shown stable resistance over years. Recently, a set of advanced lines from CIMMYT selected for high rainfall conditions has been tested at the seedling stage. Given that disease incidence is related to the amount of stubble present, four commercial cultivars (E. Cardenal, ProINTA Queguay, PI. Superior, and INIA Mirlo) were tested for disease development under zero tillage. Disease levels measured (as the area under the disease progress curve, AUDPC) were significantly higher (1363.98) on wheat stubble than on burned wheat stubble (907.34) and oat stubble (366.61). To date, no information on chemical control of tan spot has been locally developed; fungicide for* D. tritici-repentis *control is applied according to recommendations from Brazil.*

Wheat is an important winter crop in Uruguay, planted primarily in the western coastal region. The total wheat sown area during the 1990s has varied from around 110,000 to over 250,000 ha. As a result of the expansion in area seeded in 1996, production was over 600,000 t. Average yield during productive years has reached over 2500 kg ha^{-1} (Anonymous 1995a, 1997; Table 1).

Wheat diseases, in general, seriously affect yield, especially during years with wet spring periods. In the past, leaf and

stem rusts, septoria leaf blotch, and fusarium head blight have been most significant diseases; however, prevalence of tan spot, caused by *Pyrenophora tritici-repentis*, has recently increased in the north of the country under zero tillage conditions. At present, all commercial cultivars are susceptible or moderately susceptible to the disease, and infection level is related more to weather conditions than cultivar behavior (Díaz de Ackermann 1992, 1996).

Tan spot infection was first reported in 1982 (Luzzardi *et al.* 1985). Very high disease incidence and severity levels were recorded in 1990 in Salto region and in 1991 in Young. During these years, it was difficult to identify germplasm with superior tan spot resistance because of the high disease levels, yet it was possible to recognize symptoms caused by the conidial stage of *P. tritici-repentis* from lesions caused by *Septoria tritici* (Díaz de Ackermann 1992, 1996). The perfect stage of the *P. tritici-repentis* fungus was found on wheat stubble in 1993 (Stewart and Díaz de Ackermann 1993).

Table 1. Wheat statistics, Uruguay, 1990-96.

Year	Area (ha)	Production (t)	Yield (kg ha^{-1})
1990	224590	415716	1851
1991	116265	187535	1613
1992	148014	340912	2303
1993	213842	342575	1602
1994	189000	486864	2576
1995	167500	391300	2336
1996	250264	627496	2507

Source: Anonymous 1995a, 1997.

Table 2. Disease impact on wheat yield in Uruguay.

Year	Yield reduction (%)	Leaf blotch[1]	Head blight
1977	44	+	++
1978	23	+	–
1985	26	++	+
1986	24	+	–
1990	4	–	+
1991	19	++	–
1993	26	++	+

[1] *Septoria tritici* and *Pyrenophora tritici-repentis*; + Disease level moderate; ++ Disease level high; – Disease level low.

Economic Impact of Wheat Diseases in Uruguay

In a study conducted over 19 years (1977-1995), a regression analysis demonstrated that wheat production was lower than expected during seven of those years. During this time of reduced wheat production, leaf blotch infection, caused by *Septoria* and *Pyrenophora*, was prevalent in six years, fusarium head blight in four years, and leaf rust in only two years (Table 2). The correlation coefficient (r) between average yield ha^{-1} and severity of different diseases was 0.50, 0.33, and 0.64 for leaf blights, fusarium head blight, and leaf rust, respectively (Díaz de Ackermann 1996).

Research Efforts

Pathogenic variability

As a part of her thesis work at North Dakota State University, the first author studied the pathogenic variability of four isolates of *P. tritici-repentis* in the laboratory (Díaz de Ackermann 1987). Significant differences in growth patterns among isolates were observed on potato dextrose agar medium where strain PYD7 grew fastest, followed by PYR72, 1231CDA, and Pti2. Under culture, PYD7

and Pti2 developed dark green mycelia, while PYR72 developed gray, and 1231CDA developed white mycelia. Differences in sporulation were also observed; PYD7 and Pti2 sporulated abundantly in three days on V-8 medium (14/10 h light/dark period), while PYR72 sporulated poorly, and 1231CDA did not sporulate at all. Growth, color, and sporulation characteristics were not related to virulence; all four isolates were quite virulent.

Although pathogenic variability has not been studied in Uruguay, its occurrence is possible given the presence of the perfect stage of the fungus as well as abundant wild grass hosts. Among alternative hosts cited in the literature, *Hordeum vulgare, Secale cereale, Avena sativa, Dactylis, Poa,* and *Lolium* species are present in the country; however, to date only wheat (*Triticum aestivum*) has tested positively for presence of the disease (Costa Neto 1968; Krupinsky 1982, 1986, 1987). Alternative hosts, together with seedborne inoculum (Schilder and Bergstrom 1992), appear to have been critical sources of inoculum because the disease became important prior to the adoption of zero tillage practices.

During the study in North Dakota, five winter wheats and one spring wheat were tested as differential, and 14 isolates were used to determine host-pathogen interaction (Díaz de Ackermann 1987; Díaz de Ackermann *et al.* 1988). Of these, 11 isolates belonged to an identified racial group and the other three were new, but the host-pathogen interaction was observed to be significant in only one of the five trials. After inoculation with a suspension of 50,000 infective propagules (mycelial fragments, conidiophores, and conidia) per ml, pots were placed in a humid chamber for 30 h at 21± 3°C. Infection evaluation was made by measuring lesion length. Although specific interactions between individual isolates and wheat genotypes have been shown in Canada, other studies have reported conflicting results (Lamari and Bernier 1989b, 1989c; Luz and Hosford 1980).

Sources of resistance

Trials have been sown for many years to evaluate tan spot resistance under natural infection conditions in northern Uruguay. At present, research is underway to identify germplasm with higher tan spot resistance than current varieties using artificial inoculation in the field and on seedlings in the greenhouse. Disease development was visually estimated using the 0-9/0-9 modified Saari-Prescott scale in the field and the Lamari scale (Lamari and Bernier 1989a) in the greenhouse.

Wheat varieties showing tan spot resistance under field conditions are presented in Table 3. Winter wheat

Table 3. Sources, growth habit, and origin of *Pyrenophora tritici-repentis* resistance in Uruguay.

Cultivar	Habit	Origin	Reference
Red Chief	Winter	USA	Raymond 1985
Carifen 12	Winter	Chile	Cox and Hosford 1987
Colotana	Spring	Brazil	Rees and Platz 1990
Veranopolis	Spring	Brazil	Rees and Platz 1990
Fink"S"	Spring	Mexico	Rees and Platz 1990
Genaro"S"	Spring	Mexico	Rees and Platz 1990
Vicam"S"	Spring	Mexico	Rees and Platz 1990

varieties such as Red Chief and Carifen 12 did not head in Young, where disease severity is frequently high, because of their vernalization requirement. Observations on Carifen were also complicated due to the presence of high levels of leaf rust infection. Vicam, another source of tan spot resistance, did not show resistance and was also highly susceptible to fusarium head blight and leaf rust. Under local conditions, Fink was considered the best source of tan spot resistance (Cox and Hosford 1987; Raymond *et al.* 1985; Rees and Platz 1990).

Germplasm of CIMMYT origin reported to be resistant at the Tan Spot Workshop in Fargo, 1992, was tested at Young. Sources that showed resistance in Uruguay are presented in Table 4 (Gilchrist 1992). Based on the results of a regional trial (LACOS) conducted in the Southern Cone region, a wide variation in reaction to *P. tritici-repentis* was observed among cultivars (Table 5). Current commercial cultivars in Uruguay range in tan spot resistance from moderately susceptible to susceptible (Díaz de Ackermann 1996; Table 6).

Table 4. CIMMYT advanced lines showing *Pyrenophora tritici-repentis* resistance.

Cross	Pedigree	Probable source of resistance
CEP7775/CEP8012	B30094-0Z0-0A-1A-5A-0Y	Unknown
MILAN	CM75113-B-5M-1Y-05M-7Y-1B-0Y	BB, BOW, AU, KL.REND., CHR"S"
VEE#7/BOW"S"	CM76736-36Y-06M-013-6B-0Y	KVZ, BB, SR, AU, KL.REND, TZPP, CHR"S", MENTANA
SHANGAI5/BOW"S"	CM91100-3Y-0M-0Y-1M-0Y	AU, BB, KL.REND, TZPP, CHR"S", MENTANA
SUZHOE#10//ALD"S"/PVN	CM91135-9Y-0M-0Y-2M-0Y	CHR"S", BB, VCM

Source: Gilchrist 1992.

Table 5. Advanced lines from the Southern Cone region demonstrating different *Pyrenophora tritici-repentis* resistance levels.

Name	Cross	*P. tritici-repentis* resistance level in Uruguay[1]
Milán	VS73.600/Mrl/3/Bow//Yr/Tr	H
Cisne INIA	Kvz/Cj	H
	Coker62/BR14	H
	LE 2062/LE 2096	H
	LE 2091/LE 2089	H
	Kvz//Bb/Cha/3/Trm/4/TEMU36-78/5/Ovación	H
	E. Dor./Aepoglom	H
Attila	ND/VG9144//Kal/Bb/3/Yaco/4/Vee#5	I
Corydon	Car853/Coc//Vee#5/3/E7408/Pam//Hork/PF73226	I
Tinamou	IAS58/4/Kal/Bb//CJ/3/Ald/5/Bow	I
	Mon"S"/Ald//TEMU3382	I
	KImp/F2-82-344	I
	E. Cal/LI 62	I
—	Trap #1/Bow	L
Burrion	Peg/PF70354/4/Kal/Bb/Ald/3/Mrng	L

[1] L = Low; I = Intermediate; H = High.

During 1996, a large CIMMYT collection (929 lines of PCME2HR) was tested for tan spot resistance at the seedling stage in the greenhouse. Significant differences in infection level were observed in the advanced lines. Since this was the first of this type of greenhouse experiment, the information needs to be confirmed in the coming crop cycle.

The objective of the breeding program in Uruguay is to confirm the effectiveness of sources of tan spot resistance reported in different regions of the world against the local pathogenic population, and to try to introduce the best entries into locally adapted germplasm.

Control measures

In Uruguay, there has been virtually no effort to evaluate losses due to tan spot alone as, in commercial fields, it always appears as part of a leaf blight complex, with *Septoria tritici* the most prevalent disease. As previously mentioned, all Uruguayan cultivars currently recommended are susceptible or moderately susceptible to tan spot. This, together with the increase in wheat area under zero tillage conditions, makes it necessary to generate precise data on the economic importance of the disease.

Cultural practice—Several studies indicated that tan spot disease level is increased by stubble retention (Lamey 1981; Rees and Platz 1992). During 1996, four cultivars (E. Cardenal, ProINTA Queguay, P. Superior, and INIA Mirlo) were planted under zero tillage conditions to test three methods of stubble management. Significant differences ($P<0.01$) in disease development were observed between wheat planted into wheat stubble (AUDPC 1363.98 a), into burnt wheat stubble (AUDPC 907.34 b), and into oat stubble (AUDPC 366.61 c). Disease level was found to be significantly higher on the wheat crop planted into wheat stubble compared with oat stubble; therefore, an oat crop seems to be a viable alternative in the cropping system (Figure 1).

Table 6. Commercial wheat cultivars sown in Uruguay that demonstrate a low to intermediate level of leaf spot infection.

Cultivar	Cross or name	Leaf blight infection	
		ST[1]	PTR[1]
E. Pelón 90	Kvz/Trm	L-I[2]	L-I
E. Benteveo	Bobwhite	L	I
Buck Yapeyú	B.Patacón/T800	L	I-H
INIA Mirlo	Car853/Coc//Vee#5/3/Ures	L	I
LE 2193	E.Federal/Buck6//MR74507	L	I
NEC 909	MAG204/82.64//LAJ942*2/ND501	L	I
INIA Boyero	Aepoglom/MN72II6422//LI7	I	L
Buck Charrúa	RAP/RE//IRAP/3/Lov/4/RAP/RE//IRAP	L-I	I
LE 2196	E.Jilguero/ND 526	L-I	I

[1] ST = *Septoria tritici*; PTR = *Pyrenophora tritici-repentis*.
[2] L = Low; I = Intermediate; H = High.

Chemical control—A wide range of studies has demonstrated the efficacy of chemical application for tan spot control (Buchenau and Cholick 1984; Buchenau and Yahnk 1984a, 1984b; Hosford and Busch 1974; Lamey and Hosford 1982; Luz and Bergstrom 1986; Tekaus *et al.* 1983; Watkins *et al.* 1982, 1985; Williams and Jackson 1985). In general, mancozeb, mancozeb + triadimefon, propiconazole, chlorothalonil, and triadimenol have achieved the best results. Although no local information on the level of control or efficacy of different fungicides against *P. tritici-repentis* is currently available, the use of newer chemicals such as ciproconazole and tebuconazole to control leaf blights has been successful. For general recommendations, data generated and guidelines followed by Brazilian colleagues, where chemical control research is strongly pursued, are being used (Table 7) (Anonymous 1995b).

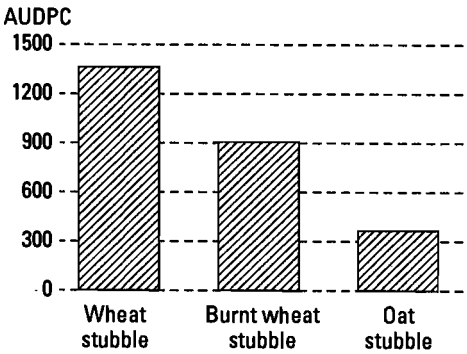

Figure 1. AUDPC (area under disease progress curve) average of four cultivars (Estanzuela Cardenal, P. Superior, INIA Mirlo, and P. Queguay) under zero tillage planted into wheat, burnt wheat, and oat stubble, Mercedes, Uruguay.

Table 7. Fungicides recommended in Brazil for control of *Pyrenophora tritici-repentis*, *Septoria tritici*, *Puccinia recondita*, and *Fusarium* spp., 1995.

Fungicide	Rate (g ai ha^{-1})	PTR[1]	ST	PR	FUS
Benomyl	250	NR[2]	NR	NR	+[3]
Carbendazim	250	NR	NR	NR	+
Ciproconazole	100	+++	+++	+++	NR
Flutriafol	94	NR	NR	+++	NR
Mancozeb	2000	++	++	++	NR
Prochloraz	450	+++	+++	NR	+
Propiconazole	125	+++	+++	+++	+
Tebuconazole	187	+++	+++	+++	+
Triadimenol	125	++	+++	+++	NR

[1] PTR = *Pyrenophora tritici-repentis*; ST = *Septoria tritici*; PR = *Puccinia recondita*; FUS = *Fusarium* spp.
[2] NR = Not recommended; +++ Control over 70%; ++ Control between 50-70%.
[3] Approximately 75% control.
Source: Anonymous 1995.

Because the area under zero tillage practices is significantly increasing in Uruguay, development of tan spot control is essential. In the absence of resistant cultivars, fungicides are a necessary management tool, together with cultural practice, for disease control under zero tillage.

Final Comments

Tan spot has added a further complication to the complex of leaf diseases of wheat present in Uruguay. In spite of its increasing importance, it has been difficult to work with the fungus in the laboratory and the results from seedling resistance tests have shown low repeatability. Furthermore, the pathogen population has not been thoroughly studied. Given the human and research resource limitations in the country, and a multitude of disease complexities, a collaborative research effort through regional and international networks is needed. Uruguay will be prepared to

participate in any international collaborative effort that can be identified or developed at this workshop.

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Improving Control of Tan Spot Caused by *Pyrenophora tritici-repentis* in the Mixteca Alta of Oaxaca, Mexico

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Abstract

Wheat production covers about 14,000 ha in Oaxaca State, Mexico, of which 95% is located in the Mixteca Alta (2,200 masl, 17°30'N and 97°20'W), where soils are characterized by low organic matter and high pH levels. Wheat is mainly grown under rainfed conditions and irrigation is limited. Since erosion is a concern for smallholder producers, reduced tillage cropping practices have increased in recent years. As a result of a lack of adequate rotation, and limited access to fertilizers and technological support, tan spot caused by Pyrenophora tritici-repentis *has become a major concern. Although improved varieties are commonly used (Temporalera M-87, Arandas F-90, and Pavon F-76), they are highly susceptible to foliar diseases, particularly tan spot. In fungicide tests, manzate reduced disease severity by 25%, on average, compared with the control. There were no significant differences between two treatments of cyproconazole or propiconazole and one spray of tebuconazole; however, these five treatments reduced the average disease severity on combined flag leaf (F) and F-1 leaves by more than 75%. Only two sprays of tebuconazole (Folicur) caused a significantly larger reduction in disease severity (86%). Yield obtained after fungicide treatment correlated well with disease severity. At two sites (Yanhuitlán and Nochixtlán), 224 genotypes were screened under zero tillage and natural rainfed conditions, and disease pressure was high as a result of the wheat monoculture. Fifteen entries with higher yield and better disease resistance than the local check were identified.*

Wheat Production in the Mixteca Alta

Wheat production covers about 14,000 ha in Oaxaca State, Mexico, of which 95% is located in the Mixteca Alta (2,200 masl, 17°30′ N and 97°20′ W), where soils are characterized by a low organic matter content and high pH. Wheat is mainly grown under rainfed conditions and irrigation is limited. As erosion is a concern for small farm producers, the use of reduced tillage cropping practices has increased in recent years. Due to a lack of adequate rotation and limited access to fertilizers and technical support, tan spot caused by *Pyrenophora tritici-repentis* has become one of the major concerns for small farmers in the region. Although improved varieties are commonly used (Temporalera M-87, Arandas F-90, and Pavon F-76), they are highly susceptible to foliar diseases, particularly tan spot. In the last two years, chemical control was

required, which dramatically increased production costs. Since more information is needed to evaluate the benefit of fungicide treatments, a trial was conducted to assess different fungicides that are available in the country. However, to make wheat production more sustainable, there is an urgent need to identify genotypes that harbor a higher level of tan spot resistance, which also have good leaf rust resistance. A screening trial was conducted under zero tillage in two locations, Yanhuitlan and Nochixtlan.

On-Farm Fungicide Evaluation

Variety Temporalera M-87A was sown at a rate of 130 kg ha^{-1} in a randomized complete block design with five replicates in a farmer's field at Yanhuitlan on July 9, 1996. Fertilizer regime was 80-60-00 N-P-K. Ten fungicide treatments were applied at booting or booting and flowering stage (Table 1), and were compared to the untreated control plot (check). Each plot was 5 m^2. Five plants per plot were marked at random, and percent diseased leaf area (%DLA) was scored in all plots on the flag leaf (F) and F-1 leaf, eight days after treatment at flowering. Yield (kg ha^{-1}) and 1000-kernel weight were measured at the end of the season.

Significant differences between fungicide treatments were observed ($P<0.01$) for all %DLA measurements (on F, F-1, or the average score on both F and F-l). Thiabendazole did not reduce disease severity. Compared to the control, manzate reduced disease severity by 25%, on average. There was no significant difference between both treatments with

Table 1. Effect of fungicide treatment on tan spot score (%DLA) for flag leaf (F), F-1, and F and F-1 combined; yield; and 1000-grain weight observed in an on-farm trial conducted in Mexico's Mixteca Alta (Yanhuitlan), 1996.

Treatment		Flag (F) leaf (%DLA)[1]	F-1 leaf (%DLA)	Average. F and F-1 (%DLA)	Yield (kg ha^{-1})	1000-grain weight (g)
Tebuconazole	(0.5 L ha^{-1}; B[2], F[3])	0.8	13.0	6.9	3,550	42.4
Cyproconazole	(0.5 L ha^{-1}; B, F)	4.2	17.6	10.8	3,202	39.2
Cyproconazole	(0.5 L ha^{-1}; B)	5.4	28.2	16.8	3,095	38.4
Propiconazole	(0.5 L ha^{-1}; B, F)	2.6	16.0	9.1	3,091	39.2
Tebuconazole	(0.5 L ha^{-1}; B)	1.6	14.4	8.1	2,964	38.4
Propiconazole	(0.5 L ha^{-1}; B, F)	4.0	26.0	14.9	2,766	35.2
Manzate 200	(2 kg ha^{-1}; B)	17.4	62.8	40.1	2,351	31.2
Manzate 200	(2 kg ha^{-1}; B, F)	17.2	58.2	37.8	2,230	32.8
Thiabendazole	(0.4 kg ha^{-1}; B)	21.8	67.0	44.5	2,081	28.0
Control	(No fungicide)	17.6	84.0	50.9	2,016	28.0
Thiabendazole	(0.4 kg ha^{-1}; B, F)	19.4	84.8	52.2	1,996	30.4
LSD ($P<0.01$)		5.4	14.8	8.8	352.8	2.8
F test (df 10 and 40)		15.5	24.8	29.3	16.2	21.9
CV %		46.4	29.9	28.9	11.5	6.9

[1] Diseased leaf area (%).
[2] Booting.
[3] Flowering.

cyproconazole or propiconazole and one spray with tebuconazole based on LSD ($P<0.01$) comparisons; however, the five treatments reduced average severity on combine F and F-1 leaves by more than 75%. Only two sprays of tebuconazole caused a significantly higher reduction in disease severity (86%).

Yield and 1000-grain weight were highly correlated to disease data; however, compared to the unprotected plots, no significant yield increase (LSD = 352.8) was observed when plots were sprayed with manzate and thiabendazole. One spray of propiconazole resulted in a 37% significant yield increase. No significant yield difference was observed between both cyproconazole treatments, two sprays of propiconazole and one tebuconazole spray at booting stage. These four treatments, on average, produced a 53% yield increase ($P<0.01$) compared to the control. Only two sprays of tebuconazole produced a significantly higher yield increase (75% more than control), but this treatment was not significantly different to the two cyproconazole sprays. A farmer's decision to use a particular fungicide may be simplified based on these field results, but the final choice will depend on chemical cost and, most importantly, market grain price.

Screening Bread Wheat Genotypes for Tan Spot Resistance

Bread wheat genotypes (224 entries) previously selected from several CIMMYT nurseries for potential tan spot resistance were screened under natural conditions at two sites (Yanhuitlan and Nochixtlan) under zero tillage and rainfed conditions. Natural disease pressure was high as a result of the wheat monoculture system used for more than two consecutive years. At planting, pseudothecia were clearly evident on over-wintered wheat stubble. No crop had been planted during the winter prior to the trial. Sowing dates were July 10 and 28, 1996, for Yanhuitlan and Nochixtlan, respectively. Plots were 1.4 m^2 and N-P-K fertilization rate was 80-60-00. The cultivar Arandas F-90, commonly sown in the Mixteca, was used as the local check, and Catbird and ND/DG9144//Kal/BB/3/YACO/4/Chil were the resistant and susceptible controls, respectively.

Severe tan spot infection was initially observed at tillering (Zadoks' DC 29), and epidemic development was uniform in both locations. At flowering, tan spot infection was clearly observed up to the flag leaf of most susceptible genotypes, and was confirmed by the consistent detection of *P. tritici-repentis* conidia on samples in the CIMMYT laboratory.

Disease rating, using Saari and Prescott's 00-99 double digit scale, was recorded on October 9, 1996, when epidemic development was highest. Growth stage was measured according to Zadoks' scale at the time of scoring. Yield per plot was converted to kg ha^{-1}. Table 2 presents the disease rating and yield of the 15 top yielding genotypes under these conditions. All genotypes, with the exception of JUP/ZP//COC/3/PVN/4/

TNMU/5/TNMU, showed a lower disease score than the susceptible control. It is expected that tan spot resistance of these genotypes will be confirmed in a coming season. Results are in agreement with observations made at CIMMYT in other growing seasons. Adoption of promising genotypes will depend highly on gluten quality.

Table 2. Crosses and pedigrees of the 15 top yielding genotypes screened under Mixteca conditions.

	Yanhuitlan				Nochixtlan			
	Growth stage	Double Digit		Yield	Growth stage	Double Digit		Yield
Genotype and pedigree	(Zadoks)	D1	D2	(kg ha^{-1})	(Zadoks)	D1	D2	(kg ha^{-1})
SHA3/SERI//G.C.W 1/SERI CMBW91Y01596S-6Y-010M-010Y-015M-1Y-0M	75	4	2	2585	70	4	3	1621
MILAN/SHA7 CM97550-0M-2Y-030H-3Y-3Y-0Y-1M-010Y-0FUS-1FUS	68	3	2	2571	68	3	2	1871
NANJING 82149/KAUZ CM98322-0M-2Y-030M-116M-1Y-0M-1FUS-0Y	69	5	3	2443	70	6	2	2078
NANJING 82149/KAUZ CM98322-0M-2Y-030M-39M-3Y-0M-3FUS-0Y	70	7	2	2407	59	4	2	2064
JUP/ZP//COC/3/PVN/4/TNMU/5/TNMU CMBW89M6328-0TOPY-030M-4Y-010M-010M-010Y-4M-0Y	78	8	3	2378	66	8	2	1071
MILAN/SHA7 CM97550-0M-2Y-030H-3Y-3Y-0Y-2M-010Y-0FUS-3FUS	68	3	2	2371	63	3	2	2214
MILAN/SHA7 CM97550-0M-2Y-030H-3Y-3Y-0Y-1M-010Y-0M	70	3	2	2357	73	3	2	1699
TURACO CM90312-D-2B-15Y-2B-0Y-7M-0Y	60	5	2	2328	58	6	2	714
BOW//BUC/BUL/3/KAUZ CM96492-AA-0Y-0M-0Y-5M-0RES-0SY-0ECU-0Y	70	7	3	2328	70	7	3	850
CBRD/KAUZ CMBW90M2494-14M-010M-010Y-015M-5Y-0M	73	6	2	2321	60	3	2	1928
DUCULA CM80232-28Y-03M-0Y-1M-0Y-0SJ-2PZ-0Y-4SJ-0Y	76	7	2	2314	72	5	2	1335
NANJING8331/KAUZ CMBW89M4524-24M-010Y-010M-010M-010Y-3M-0Y	75	4	2	2257	66	4	2	2400
SHA3/SERI//G.C.W 1/SERI CMBW91Y01596S-6Y-010M-010Y-015M-4Y-0M	76	4	2	2250	68	7	3	1456
THB/CEP7780 CM76635-8Y-0Z-0Y-1M-0Y-2PZ-0Y-0ECU-0Y	74	8	2	2207	66	5	2	1378
NANJING 82149/KAUZ CM98322-0M-2Y-030M-68M-3Y-0M-1FUS-0Y	74	6	2	2200	66	6	2	2228
Checks								
CATBIRD (resistant) CM91045-5Y-0M-0Y-4M-6Y-2M-0M	72	5	2	978	53	4	2	928
ND/DG9144//KAL/BB/3/YACO/4/CHIL (susceptible) CM90461-40Y-0M-0Y-13M-0Y	70	8	4	1600	72	8	5	1128
ARANDAS F-90 (local check) CM74849-2M-2Y-3M-2Y-0B-0MEX	72	8	3	1392	75	8	5	778

Importance of Spot Blotch Caused by *Bipolaris sorokiniana* in Bolivia

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Abstract

In Bolivia, more than 70% of wheat production is grown on the eastern lowlands of Santa Cruz Department. Wheat is an important winter crop grown after the summer soybean crop. In early sown wheat (15 April-10 May), environmental conditions prevailing in the humid and intermediate areas of the region are very favorable for development of spot blotch, caused by Bipolaris sorokiniana. *Yield losses of up to 57% have been observed but are highly dependent on planting date, wheat variety, and climatic conditions. Optimum time for fungicide treatment is 15 days after first observation of symptoms, which corresponds to heading stage for normal sowing times (May).*

In Bolivia, over 70% of wheat production is grown on the eastern plains (Department of Santa Cruz), where spot blotch, caused by *Bipolaris sorokiniana*, is one of the most important wheat diseases.

Importance and Losses

Table 1 shows the estimates of potential losses due to spot blotch for the Department of Santa Cruz. Based on research conducted to determine the damage caused to wheat by this disease, the best time to apply chemical control measures is 15 days after the first symptoms appear. (First symptoms = 50% incidence of plants with spots larger than 2 mm.)

In the wet and moderately humid areas of Santa Cruz, under normal planting conditions (May), the first disease symptoms appear 35-40 days after sowing (tillering-booting). This is the right time to apply fungicides, and it (almost always) coincides with heading. With an early planting date (April-May) and when conditions favor disease development (high humidity and heat), fungicides should be applied twice; the first application when the symptoms appear, and the second application 15-20 days later, especially when growing susceptible varieties. With moderately resistant varieties, one application is enough. Results of trials on losses and economic threshold are shown in Figure 1 (1990-1995).

In 1995, the economic threshold (heading) for the top three leaves of the plant (flag leaf, F; F-1; and F-2) was determined based on disease severity (infected leaf area) (Figure 2). The price of wheat at the mill (US \$180 t^{-1}, the standard price in Bolivia) and the cost of

one fungicide application (195 kg) were used to estimate the following thresholds: 0.27% of infected leaf area (ILA) on the flag leaf, 0.34% on F-1, and 1.77% on F-2, with an average ILA of 0.85% per tiller. The importance in terms of yield of the three top leaves was also determined, showing that the flag leaf is responsible for 78%, second leaf, 18% and third leaf, 4%.

The economic threshold of the three leaves at flowering (Figure 3) was also determined: flag leaf, 2.19%; F-1, 7.31%; and F-2, 23.85% (average ILA/tiller: 11.78%).

Table 1. Estimated wheat yield losses resulting from spot blotch (*Bipolaris sorokiniana*) in Santa Cruz, Bolivia.

Year	Authors	Variety	Spot blotch reaction[1]	Cross	Estimated losses (%)	Locality
1991	Languidey and Barea	Chané	MS-S	VEE#3=KUZ/BUHO"S"/ KAZ/BB	5-57	EEA Saavedra (Humid zone)
1991	Languidey and Barea	Agua Dulce	MS	BJY"S"/JUP	12-41	EEA Saavedra (Humid zone)
1992	Languidey and Barea	Comomoci	S	NACOZARI F 76	13-24	St. Laura-Pailón (Intermediate dry zone)
1992	Toledo	Agua Dulce	MS	BJY"S"/JUP	3-36	Jap. Col. Okinawa 2 (Intermediate humid zone)
1992	Languidey *et al.*	Agua Dulce	MS	BJY"S"/JUP	3-29	St. Laura-Pailón (Intermediate dry zone)
1994	Barea *et al.*	Pailón	S	MOR"S"/VEE"S"	14-18	Jap. Col. Okinawa 1 (Humid zone)
		Guendá	S	NDD/SEL 101(2)/PVN"S"/ SIS"S"	19-22	
		Guapay	MR-MS	TPP/Andes 64/INIA 66/(3)· CNO67 JAR66/KVZ=KEA"S"	24-28	
		Agua Dulce	MS	BJY"S"/JUP	24-25	
		Chané	MS-S	VEE#3=KUZ/BUHO"S"/ KAZ/BB	20-23	
1995	Toledo *et al.*	Pailón	S	MOR"S"/VEE"S"	1-35	EEA Saavedra (Humid zone)

[1] MR = Moderately Resistance, MS = Moderately Susceptible, S = Susceptible.

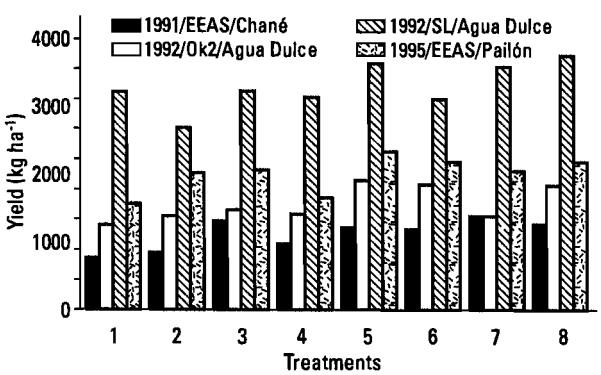

Figure 1. Results of trials on economic thresholds and losses caused by *Bipolaris sorokiniana*. Winter 1990-1995, Santa Cruz, Bolivia.

Treatments:

1. Check (without fungicide application).
2. One fungicide application when first symptoms appear.
3. **One application, 15 days after symptoms appear.**
4. One application, 30 days after symptoms appear.
5. **Two applications, when symptoms appear and 15 days later.**
6. Two applications, when symptoms appear and 30 days later.
7. Two applications, 15 days after symptoms appear and 15 days later.
8. Protected check: Three applications, when symptoms appear, 15 days, and 30 days later.

Breeding for *B. sorokiniana* Resistance

Every year, CIAT's wheat project introduces an average of 2000 wheat lines and varieties, most from CIMMYT, Mexico. From this wide variety of genetic materials, intergeneric lines (from crosses with *Thinopyrum curvifolium*) are being selected more frequently for their

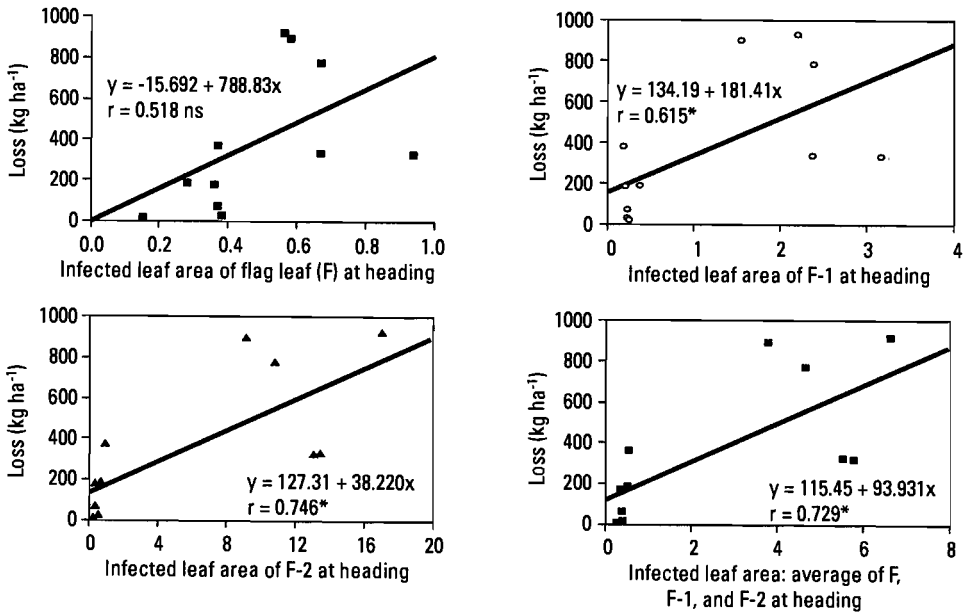

Figure 2. Economic threshold of *Bipolaris sorokiniana* for the three top leaves at heading. EEAS, winter 1995, Santa Cruz, Bolivia.

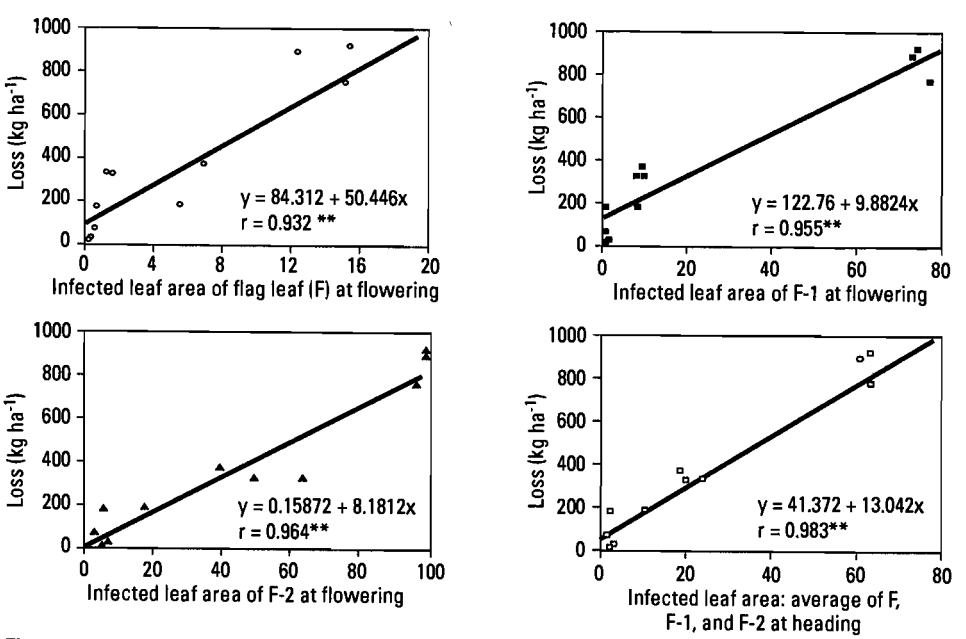

Figure 3. Economic threshold of *Bipolaris sorokiniana* for the three top leaves at flowering. EEAS, winter 1995, Santa Cruz, Bolivia.

resistance to helminthosporium leaf blotch, good agronomic performance, variable cycle, and, more importantly, high yields. Table 2 shows the results of lines selected during the winter of 1994, when incidence of *B. sorokiniana* was relatively high.

Effect of Sowing Date on Spot Blotch

In the Department of Santa Cruz, winter conditions vary from one year to another; however, this does not affect variations in April and May. April is wetter and hotter than May. Spot blotch is greatly influenced by planting date (Figure 4), since early sowing (April 20-May 10) favors the appearance and incidence of the disease, especially under high humidity conditions (>80%), when it can reach epidemic levels. When sowing is carried out after the first two weeks of May, the incidence of *B. sorokiniana* gradually diminishes due to reduced rainfall, which results in lower humidity levels (<70%) and low temperatures.

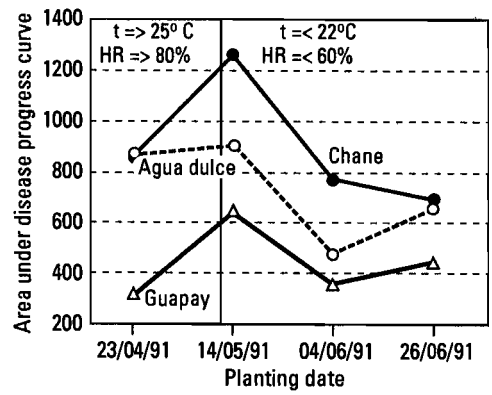

Figure 4. Effect of planting date on the development of spot blotch in three wheat genotypes. Okinawa-2, winter 1991, Santa Cruz, Bolivia.

Table 2. Advanced and promising lines with spot blotch resistance. CETABOL, Japanese Colony, Okinawa-2, winter (May-September), 1994.

Entry number	Line or variety	10th Regional Trial	
		Yield (kg ha^{-1})	*B. sorokiniana* FIC[1]
92054	CS/*Th. Curv.*//GLEN/3/GEN/4/SUZ#8	2290	16
92024	CS/*Th. Curv.*//GLEN/3/ALD/PVN/4/SUZ#8	2480	8
87170	CS/*Th. Curv.*//GLEN 81/3/GEN 81	2102	24
92027[2]	CS/*Th. Curv.*//GLEN/3/ALD/PVN/4/NINGMAI#4/ OLEOSON/YANGMAI#4	2010	16
Local Check[3]	Surutú-CIAT [KEA"S"/BUC"S"]	2120	24
Local Check	Pailón-CIAT [MOR"S"/VEE"S"]	2044	48
Local Check	Guendá-CIAT [NDD/SEL 101(2)/PVN"S"/SIS"S"]	2028	48
Local Check	Guapay-CIAT [TPP/Andes 64/INIA 66/(3) CNO67 JAR66/KVZ=KEA"S"]	1841	48
Local Check	Agua Dulce-CIAT [BJY"S"/JUP]	2036	40
Local Check	Chané-CIAT [VEE#3=KUZ/BUHO"S"/KAZ/BB]	1761	32
Local Check	Pai-Comomoci [NACOZARI F 76]	1789	56
Advanced Yield Trial			
92049	CS/*Th. Curv.*//GLEN/3/ALD"S"/.....YANG#4	2584	32
92052	CS/*Th. Curv.*//GLEN/3/ALD"S"/.....YANG#4	2457	24
92055	CS/*Th. Curv.*//GLEN/3/ALD"S"/PVN"S"	2327	24
92057	CNO/4/CS/*Th. Curv.*//GLEN/3/ALD"S"/PVN"S"	2269	16
92030	CS/E.GIG//2*CS/3/CNO/4/NANJING8401	2059	16

[1] Foliar infection coefficient. [2] Commercial variety. [3] Promising line.

Major Foliar Diseases of Triticale in Morocco

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Abstract

Triticale (x Triticosecale *Wittmack), a new crop in Morocco, is currently attracting more interest from farmers. Though triticale had shown excellent resistance to major diseases and insects under Moroccan conditions in the past, many disease symptoms have recently been observed on several genotypes in breeding nurseries. Therefore, a survey of triticale diseases was conducted during the 1989/90 and 1990/91 crop seasons in different regions of Morocco. The main foliar diseases recorded were tan spot (*Pyrenophora tritici-repentis*) and spot blotch (*Bipolaris sorokiniana*). Speckled leaf blotch (*Septoria tritici*), glume blotch (*S. nodorum*), leaf rust (*P. recondita*), and stem rust (*P. graminis*) were less frequently observed. Another study, including 16 triticale genotypes (three of which had already been released), two bread wheat, and two durum wheat cultivars, was conducted in the greenhouse to determine their reaction to the major cereal pathogens in Morocco. Results showed that genotype reaction ranged from resistant to susceptible for all pathogens except* S. tritici *and* Erysiphe graminis, *isolated from wheat, which were not virulent on triticale genotypes. The virulence of* B. sorokiniana, P. tritici-repentis, *and* S. nodorum *strains isolated from wheat was relatively higher on triticale. This suggests that selection pressure by triticale may induce variation in pathogenicity of some parasitic fungi of wheat.*

Triticale (x *Triticosecale* Wittmack), is a small grain cereal that represents the first successful attempt by man to synthesize a new crop species from intergeneric hybridization. Triticale is the product of a cross between the genera *Triticum* and *Secale* (Zillinski 1974; Varughese *et al.* 1986a, 1986b). It is usually grown for animal feed but also used for human consumption in many parts of the world (Ben Salem 1989; Mergoum *et al.* 1992).

In Morocco, research on triticale started in the early 1970s, mainly on breeding aspects (Mergoum 1988), resulting in the release of many cultivars beginning in 1984. Although triticale is not yet widely grown, the area sown to this crop is increasing significantly every year. Triticale has shown excellent performance in many parts of Morocco, including coastal areas (sandy soils), high elevation zones, and particularly the arid and semi-arid zones (Mergoum *et al.* 1992). One of the main obstacles to cereal productivity in Morocco is disease (Lyamani 1989, 1990); however, few, if no, studies on triticale diseases have been conducted in Morocco. Therefore, this study was conducted to: 1) establish an

inventory of foliar disease of triticale and determine their causal agents; and 2) determine the reaction of 98 triticale genotypes to several diseases during 1989/90 and 1990/91 cropping seasons.

Materials and Methods

Disease inventory

Plant samples—Since triticale is not widely grown by farmers, a survey was conducted in six INRA experimental stations (Sidi El Aydi, Khemis Zmamra, Jemaatt Shaim, Tassaout, Annaceur, and Marchouch) and the National Agricultural School (ENA) at Meknes, where triticale breeding material is grown. Disease incidence (% of genotype infected) and severity were scored at tillering and early maturity stages on genotypes included in two yield trials (EA-I and EA-II) using an appropriate scale (Table 1). Each trial included 24 genotypes (20 advanced lines and 4 checks; Table 2) laid out in a randomized block design with four replications. Each entry was grown in six rows, 5 m long. Plant samples from plots showing disease symptoms were collected and analyzed in the laboratory for disease identification.

Table 1. Keys and scales used to evaluate diseases severity and reaction type.

Disease	Scale/key	Reaction type
Helminthosporiums	0-15 (%)	Resistant
	16-25	Moderate resistant
	26-50	Susceptible
Septorias	0-2 (0-9)	Resistant
	3-5	Moderate resistant
	6-9	Susceptible
	0-19 (%)	Resistant
	20-40	Moderate resistant
	41-100	Susceptible
Rusts	0-14 (0-50)	Resistant
	15-20	Moderate resistant
	21-50	Susceptible

Pathogen isolation and identification—Symptoms on diseased plant samples were used for preliminary identification of the disease (Ellis 1990). Pycnidia of *Septoria* spp. and conidia of *Helminthosporium* spp. from diseased plant parts were placed in petri dishes containing PDA and incubated for seven days at 20°C. *Septoria tritici* was then transferred to YMA, a yeast based medium. *Septoria nodorum* and *Helminthosporium* spp. were transferred to

Table 2. Triticale and wheat lines and cultivars included in EA-I and EA-II experiments during the 1991/92 crop cycle.

EA-I	EA-II
Triticale	**Triticale**
IGUANA"S"	MERINO"S"/JLO"S"//MUS 32
ARDILIA"S"	MUS"S"/LYNX"S"//YOGUI"S"
D7069//PI243741/SPY/3/ ANZA/PI24	GRF"S"//MERINO"S"/MUS"S"
GNU 18-2	YOGUI"S"
MERINO"S"/JLO/3/ BGL"S"/CIN	CARMAN/YOGUI"S"
BANTENG"S"	OCTONV/MA//BTO"S"/3/BGL"S"
IGUANA"S"	RHINO"S"
MERINO"S"/JLO170/// TESMO"S"	ARDILIA"S"
LECHO"S"/TGE"S"	MUS"S"/BTA"S"
MUS"S"/BTA"S"	STIER 4-1
UROM	TA 76/1638//LYNX"S"
ARD"S"	REH"S"/HARE 212
ARD"S"	URON 3
BAN"S"	PFT7717//M2A/BN"S"/3/BOK"S"
BANTENG"S"	GRF"S"/YOGUI"S"
STIER 20	BANTENG"S"
URDN 3	SPD"S" PVN76//YOGUI"S"
MZA/BGL"S"//JLO/6/ FURY/7C/WR	MITHAN"S"
PFT80413/DF"S"//TAPIR"S"	REH"S"/HARE 212
MUS"S"/BTA"S"	HARE 263/CIVET"S"
Delfine 205	Delfine 205
Durum wheat	**Durum wheat**
Marzak	Marzak
Bread wheat	**Bread wheat**
Jouda	Jouda

a V-8 medium. Both preparations were incubated for 12 h at 20°C under near UV light to enhance pathogen sporulation. Leaf rust (*Puccinia recondita*) spores and powdery mildew (*Erysiphe graminis*) mycelium were sampled directly from diseased plant tissue.

Each isolate was tested for pathogenicity by inoculating triticale lines "Iguana "S'", "Banteng 'S", and Uron 3, and bread wheat lines "Nesma" and "Jouda" in order to verify Koch's postulates.

Plant inoculation—A pure spore suspension (*S. nodorum*) or mixed with mycelium (*Helminthosporium* spp.) was prepared by adding 500 ml of distilled sterile water and 0.02% of Tween to the petri dishes containing the cultures. The suspension was passed through a double layer of sterilized cheesecloth. The strained spore suspension solutions were adjusted to 10^6 spores per ml using sterile distilled water and were used to spray-inoculate plants at the two-three-leaf, boot, and heading stages. Inoculated plants were then placed in an incubator at 100% RH for 72 h and transferred to the greenhouse.

Similarly, a 500 ml suspension of *Puccinia recondita* uredospores was used to inoculate plants which were then placed in an incubator at 20°C and 100% relative humidity (RH) in the dark for 17 h and transferred to the greenhouse.

Powdery mildew conidia and conidiophores sampled from infected leaves were used directly for plant inoculation. Inoculated plants were placed under plastic covers in the greenhouse at 18°C and 65 and 100% RH during the day and night, respectively.

Reaction of triticale to major cereal diseases

A second study of 20 genotypes, comparing 16 triticale genotypes (three of which had already been released), 2 bread wheat, and 2 durum wheat cultivars, was conducted in the greenhouse to determine their reaction to the major cereal pathogens in Morocco. The experiment was laid out in a randomized block design with four replications. Each entry was planted in 1-m long row, 30 cm apart. Inoculum preparation and plant inoculation were performed according to the previous experiments.

Results and Discussion

Disease inventory

Evaluation of helminthosporiums (*P. tritici-repentis* and *B. sorokiniana*) and rusts (*Puccinia recondita* and *P. graminis*) was made based on the 0-50 scale. Septorias (*S. tritici* and *S. nodorum*) were evaluated using both 0-9 and 0-100% scales (Table 1).

Both EA-I and EA-II experiments showed that *P. tritici-repentis* and *Puccinia recondita* were the most prevalent diseases on triticale (100 and 95% incidence, respectively), followed by *S. nodorum* (25% incidence) (Table 3). Maximum severity recorded (0-50) was very high for *P. tritici-repentis* (47), and moderate for *Puccinia recondita* (23) and *S. tritici* (25). Except for *B. sorokiniana* at Tassaout, the

highest disease incidence at the other five sites (INRA experiment stations) was due to *P. tritici-repentis* and *B. sorokiniana*, ranging between 43-100% (Table 4). Severity of *P. tritici-repentis* was high (25-47) at Jemaat Shaim and Tassaout. Incidence of other diseases ranged from 0-43% for septorias and 0-24% for *Puccinia recondita*. Severity ranged from 0-27 (0-50), 0-7 (0-50), and 0-5 (0-9 scale) for *B. sorokiniana*, *Puccinia recondita*, and septorias, respectively.

Table 3. Maximum incidence and severity of diseases observed on triticale lines at ENA, Meknes, during the 1989/90 crop cycle.

Disease	Incidence[1] (%)	Average severity (0-50)
Puccinia graminis	2	5
Bipolaris sorokiniana	5	5
Septoria nodorum	25	3
S. tritici	4	25
P. recondita	95	23
Pyrenophora tritici-repentis	100	47

[1] Percent of lines infected.

Results obtained in both experiments were, in general, similar over the five INRA experiment stations (Table 4). Overall, incidence of *P. tritici-repentis* was higher, followed by *B. sorokiniana*, septorias, and leaf rust. Severity, however, varied with disease and location. High disease severity was observed for *P. tritici-repentis*: 20 at Tassaout and 42.5 at Merchouch. *B. sorokiniana* severity ranged from 15 (Tassaout) to 30 (Sidi El Aydi). *Puccinia recondita* was relatively high at Jemaat Shaim (35) and absent at both Merchouch and Tassout. Similarly, septoria severity was high (7.5) at Jemaat Shaim.

We can conclude from this preliminary disease survey that triticale is vulnerable to most wheat diseases prevalent in Morocco. Therefore, sources of genetic resistance to these diseases should be investigated and incorporated into new germplasm to avoid future epidemics.

Table 4. Incidence and severity of diseases observed on triticale lines of the a) EA-I and b) EA-II trials at five locations in Morocco during 1990/91 crop cycle.

Station	PTR[1]		BS		ST and SN		PR	
	Inc.[2]	Sev.[3]	Inc.	Sev.	Inc.	Sev.	Inc.	Sev.
a) EA-I								
Merchouche	100	42.5	86	15.0	0	0.0	0	0.0
Sidi El Aydi	100	42.5	76	25.0	43	3.5	14	17.5
Jemaat Shaim	100	47.5	86	27.5	33	3.0	24	12.0
Khemis Zemamra	100	35.0	86	27.5	5	1.0	5	4.5
Tassaout	43	25.0	0	0.0	9	2.0	0	0.0
b) EA-II								
Merchouche	100	42.5	95	20.0	0	0.0	0	0.0
Sidi El Aydi	100	32.5	71	30.0	52	6.5	24	11.0
Jemaat Shaim	100	40.0	76	22.5	33	7.5	24	35.0
Khemis Zemamra	100	37.5	71	25.0	5	1.0	33	5.0
Tassaout	38	20.0	14	15.0	19	3.5	0	0.0

[1] PTR = *Pyrenophora tritici-repentis*; BS = *Bipolaris sorokiniana*; PR = *Puccinia recondita*; ST = *Septoria tritici*; SN = *S. nodorum*.
[2] % of TCL entries infected.
[3] Scale used: 0-50 for PTR, BS, and PR; 0-9 for ST and SN.

Resistance of triticale to major cereal diseases

The disease resistance levels of 16 triticale genotypes, 2 bread wheat, and 2 durum wheat genotypes to artificial inoculation of the most important cereal pathogens collected from wheat and triticale in Morocco under greenhouse conditions are reported in Table 5. The results suggest that genetic variability for diseases resistance exists among triticales.

Two triticale entries (PFT80413/DF"S"//Tapir"S" and Gnu 18-2) were moderately susceptible and susceptible, respectively, to *P. tritici-repentis* isolated from bread wheat and triticale (Table 5). Iguana"S" was the most resistant triticale to both isolates. All other triticales were moderately resistant to resistant (1-17). Durum and bread wheat checks were moderately resistant (12-23), with durum scoring relatively higher.

Except for triticale line GNU 18-2, which was moderately susceptible to both *B. sorokiniana* isolates, all other triticale genotypes, durum wheat Coccorit and Karim, and bread wheat Nasma were resistant (<12) (Table 5). However, bread wheat Jouda was moderately resistant (16) to both isolates.

Table 5. Reaction of 16 triticale, 2 bread wheat, and 2 durum wheat lines to artificial inoculation by pathogen isolates originating from bread wheat and triticale.

Genotype	PTR[1]		BS		SN		PR
	BW[2]	Trit	BW	Trit	BW	Trit	BW
Triticale							
Juanillo	10.00	10.00	5.00	5.00	2.00	3.33	5.66
Drira out cross	5.67	5.00	1.66	2.66	5.00	3.66	10.00
Delfine 205	3.67	5.00	2.66	3.66	11.66	5.00	41.66
Beagle	7.00	6.67	5.00	3.00	6.66	5.00	56.66
ARDILIA"S"	2.33	5.00	1.00	3.00	16.66	3.66	1.00
IGUANA"S"	0.66	1.33	5.00	3.00	2.00	0.00	0.66
BANTANG"S"	3.33	2.00	2.33	5.00	16.66	10.00	4.00
PFT 80413/DF"S//TAPIR"S"	23.33	26.67	11.66	8.66	6.66	5.00	2.00
MERINO"S"/JLO/3/BGL"S"/CIN	2.00	4.33	5.00	5.00	3.00	3.00	2.00
LECHO"S"/TGE"S"	16.66	15.00	8.00	11.66	10.00	6.66	23.33
D 7069/PI 243741/SPY/3/ANZA/PI 24	5.00	5.00	8.33	9.33	4.33	5.00	4.33
URON 8	6.00	6.67	1.00	2.66	5.00	4.33	2.66
GNU 18-2	31.66	35.00	16.66	20.00	11.66	5.00	11.66
URON-3	6.00	10.00	4.66	5.00	3.00	0.66	0.66
REH"S"/ HARE 212	9.00	13.33	3.66	5.00	2.00	3.00	0.00
SPD"S" PVN76//YOGUI"S"	4.33	6.67	3.66	5.00	13.33	6.66	2.33
Bread wheat							
Nasma	13.33	15.00	6.66	9.00	26.66	13.33	30.00
Jouda	13.33	5.00	16.66	16.66	20.00	10.00	2.33
Durum wheat							
Coccorit	15.00	23.33	5.00	6.00	31.66	30.00	4.33
Karim	18.33	11.67	8.33	9.00	15.00	11.66	2.00
LSD (P = 0.05)	3.10	2.88	3.08	2.22	3.51	2.36	3.33

[1] PTR = *Pyrenophora tritici-repentis*; BS = *Bipolaris sorokiniana*; PR = *Puccinia recondita*; ST = *Septoria tritici*; SN = *S. nodorum*.

[2] Origin of isolates: BW = Bread wheat, Trit = Triticale.

For *S. tritici*, all triticales showed no disease symptoms, and durum wheat cultivars Coccorit and Karim were resistant to both wheat and triticale isolates (Table 6); however, bread wheat Nasma was susceptible to both *S. tritici* isolates and Jouda was susceptible to the wheat isolate only. The different reactions of bread wheat Jouda to both ST isolates showed that the isolates were different. However, all triticales tested showed *S. nodorum* symptoms. Except for triticale lines Ardilia"S" and Bantang"S", which scored 17 for *S. nodorum* bread wheat isolate, all other triticales showed resistant reactions to both isolates (scores of less than 13 and 10 for bread wheat and triticale isolates, respectively). Similarly, both bread wheats were resistant to triticale *S. nodorum* isolate, but were moderately resistant to the bread wheat isolate. Durum wheat Karim appeared resistant and Coccorit was susceptible to both *S. nodorum* isolates. Overall, the bread wheat *S. nodorum* isolate was more aggressive on all three crops, suggesting race specialization.

Immunity was observed on all triticales for powdery mildew (Table 6). Both bread wheat cultivars were also resistant (<5). However, durum wheat cultivars Coccorit and Karim were susceptible, scoring 23 and 45, respectively.

Finally, leaf rust symptoms were recorded on all genotypes (Table 5). Disease severity as high as 57% was recorded on Beagle. Other triticale genotypes such as Delfine 205 and Lecho"S"/TGE"S" showed severities of 42 and 23%, respectively. The rest of the triticale lines were resistant with severity scores of less than 12%. Wheat checks were resistant with the exception of Nasma.

These preliminary results on triticale resistance to several cereal pathogens after inoculation and incubation in greenhouse conditions showed that triticale is suceptible to most prevalent cereal diseases. This confirms other studies on yellow rust (Zillinski 1974; Saari *et al.* 1986), septoria (Zillinski 1983; Saari *et al.* 1986; Skajenikoff and Rapilly 1985; Eyal and Blum 1989), *B. sorokiniana* (Bekele *et al.* 1985; Skovmand *et al.* 1984; Zillinski 1983), and *P. tritici-repentis* (Marteens *et al.* 1988; Saari *et al.* 1986; Felicio *et al* 1988). Virulence of *P. tritici-repentis*, *B. sorokiniana*, and *Septoria nodorum* isolates, from both bread wheat and triticale, suggest that all attack both wheat and triticale. Early work on rusts showed that leaf rust (*P. recondita* f. sp.

Table 6. Reaction of 16 triticale, 2 bread wheat, and 2 durum wheat lines to artificial inoculation by *Septoria tritici* isolates from bread wheat and triticale, and *Erysiphe graminis* isolates from bread wheat (0-50%).

Genotype	*S. tritici* BW[1]	*S. tritici* Trit	*E. graminis* BW
Triticale			
All triticales	0.00	0.00	0.00
Bread wheat			
Nasma	38.33	41.68	4.67
Jouda	28.33	0.00	3.67
Durum wheat			
Coccorit	0.00	4.33	23.33
Karim	5.00	3.68	45.00
LSD (P = 0.05)	1.48	1.58	3.52

[1] Origin of isolates: BW = Bread wheat, Trit = Triticale.

tritici) (Fuentes 1973; McIntosh and Singh 1986) and stem rust (*P. graminis* f. sp. *tritici*) (Lopez *et al.* 1973) have a wide host range including triticale and wheat. However, new recombinants from *tritici* and *secalis* races for certain pathogens can be also considered (Lopez *et al.* 1973; Fuentes 1973).

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Effect of Crop Rotation and Straw Mulch Inoculation on Tan Spot and Root Rot in Bread and Durum Wheat

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Abstract

Tan spot and root rot are two major diseases of wheat in Morocco. They affect durum wheat most severely, especially when planted after another wheat crop. The objectives of this experiment were to evaluate the effects of sowing wheat after a cereal crop and mulching with infested straw on tan spot and root rot development and on wheat yield. Ten durum wheat and five bread wheat cultivars were planted in a split-split-plot trial. Tan spot expression, root rot related symptoms, plant density, and yield were evaluated. Wheat sown after wheat was most susceptible to tan spot and root rot diseases. Mulching with infested straw mostly increased tan spot severity in the wheat-wheat experiment. Yield losses were correlated to severity of tan spot and root rot. Fungicide application reduced tan spot expression but had little to no effect on root rot symptoms.

Tan spot of wheat, caused by *Pyrenophora tritici-repentis* (PTR), has become important in Morocco in the last 10-15 years. Surveys showed this disease to most severely affect durum wheat, while bread wheat was less affected. Yield losses have ranged between 12 and 18%. Several race identification studies showed moderate variability in overall virulence and little physiological specialization of the pathogen. Artificial field and greenhouse inoculations, disease evaluation, and resistance screening methodologies were tested and adapted under Moroccan conditions. Screening for resistance allowed the identification of moderately to highly resistant genotypes. Resistance is linked to late maturing and tall plants. Breeding for resistance to tan spot using the available tall and late maturing resistant parents in crosses with adapted material, coupled with selection under semi-arid conditions, was not effective due to the linkage of resistance with undesired traits.

Root rot of wheat is caused by a complex of pathogens and can annually cause huge losses. In Morocco, root rot is caused by *Cochliobolus sativus* and *Fusarium culmorum*. Studies have shown that durum wheat is most affected in the dry areas of the country. Root rot research in Morocco started in the early 1980s. The responsible pathogens have been determined in numerous surveys, field inoculations techniques have been developed and successfully applied, and small genetic differences have subsequently been observed in durum

wheat. The time is now right to screen for sources of resistance or tolerance to tan spot and root rot, and to implement a genetic improvement program for resistance in durum wheat.

Documentation of environmental conditions that affect the appearance and evolution of tan spot and root rot has been made separately. It is known that root rot is favored by dry conditions that are prevalent in Morocco. Tan spot is favored by humid conditions or by long periods of relatively high humidity that also occur in most of Morocco. The influence of the numerous climatic factors that occur during the growing season on the different diseases is not documented. The objectives of this study were to evaluate the effects of sowing after a cereal crop and mulching with infested straw on tan spot and root rot expression, and yield.

Materials and Methods

A split-split plot experiment with three replicates was planted on a previous rotation experiment in 1992/93 at Sidi El Aydi experiment station. The main plots were wheat after wheat and wheat after fallow. Sub-plot treatments were: 1) protection using Tilt (0.5 L ha^{-1}), a broad spectrum fungicide, at booting and heading; 2) inoculation with infested and weathered straw plus a spray of *P. tritici-repentis* spores; and 3) the natural check. Ten durum and five bread wheat cultivars were sown in each treatment. Straw mulch was applied at the two-three leaf stage and tan spot inoculation was performed at flowering. Elementary plots were 2.5 m long and 1.8 m wide.

Tan spot severity was measured using a scale of 1-100, between 15-20 days after spray inoculation. Root rot symptoms were evaluated throughout the season and combined to give an estimation (1-100%) of severity. Data was collected on plant emergence, tiller number over two linear meters, browning of the lower stem region, and percentage of white heads per plot. Final plant stand and yield were also measured.

Results and Discussion

Although the experiment received supplemental irrigation, the drought that plagued Morocco for the previous and then current growing seasons significantly enhanced root rot development in most wheat plants showing stress symptoms, particularly in the wheat-wheat rotation. Hence the 1992/93 crop season was ideal for root rot development but not for tan spot.

Rotation had a significant effect on the development of both diseases, on plant stand, and on yield (Table 1; Figure 1). Inoculation with infested straw and PTR propagules only had a significant effect on tan spot expression (Table 1; Figure 2). Disease reaction, plant stand, and yield all varied significantly among varieties (Table 1; Figure 3). Data sorted by increasing variety yield showed a positive relationship between grain yield and plant stand, and a negative trend in yield and severity of both diseases.

No significant interaction was observed between rotation and variety or between rotation, variety, and straw

mulch inoculation. The interaction between variety and inoculation with straw and PTR was shown to have a significant effect on disease expression, plant stand, and yield (Table 1).

Straw and PTR inoculation had no effect on yield or on plant stand (Figure 4) in the wheat after fallow experiment; however, it had a significant effect on disease severity and yield in the wheat-

Table 1. Factors affecting tan spot and root rot disease expression, plant stand, and grain yield of wheat, 1992/93.

Source of variation	PTR[1]	Dependent variables*		
		Root rot	Plant stand	Grain yield
Preceding crop (PC)	0.05	0.05	0.01	0.01
Straw inoculation (SI)	0.01	NS	NS	NS
Variety (Var.)	0.01	0.05	0.01	0.01
PC x Var.	NS	NS	NS	NS
SI x Var.	0.01	0.05	0.05	0.01
PC x Var. x SI	NS	NS	NS	NS
Residual	0.85	0.50	0.75	0.83
Mean	52	30	79.16	338.5

* NS, 0.05, 0.01: Not significant, and significant at the 5% and 1% probability levels, respectively.
[1] Tan spot symptoms.

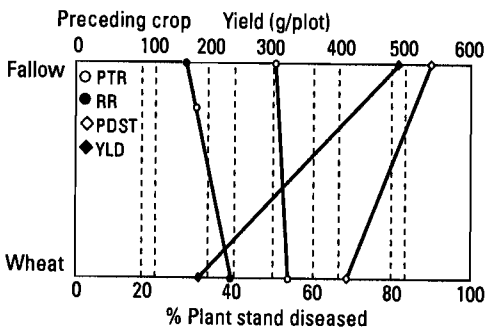

Figure 1. Effect of crop rotation on disease, plant stand, and yield of wheat.

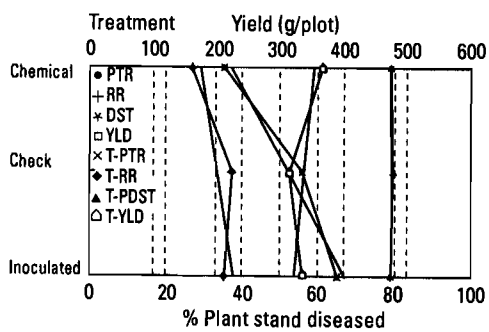

Figure 2. Effect of *Pyrenophora tritici-repentis*/ straw mulch inoculation on disease, plant stand, and yield of wheat.

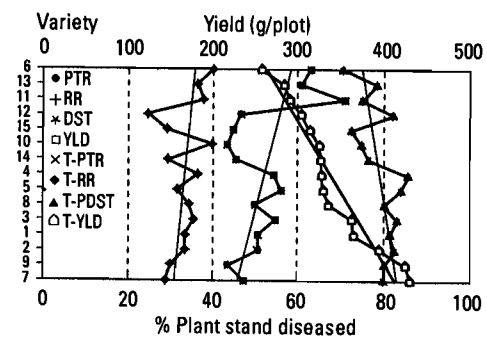

Figure 3. Effect of wheat variety on disease, plant stand, and yield.

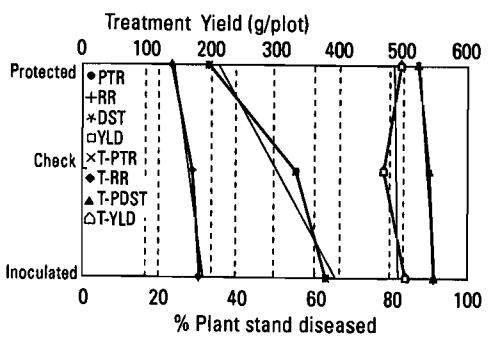

Figure 4. Effect of *Pyrenophora tritici-repentis*/ straw mulch inoculation on disease, plant stand, and yield in a wheat-fallow rotation.

wheat experiment (Figure 5). Plots planted after wheat were apparently more subject to drought than after fallow.

Root rot symptoms were slightly affected by fungicide application and inoculation with PTR and straw (Figures 6, 7, and 8). Tan spot severity greatly increased from the chemically protected plots to the natural check and inoculated plots. Yield and plant stand were not significantly affected by tan spot occurrence (Figures 6, 7, and 8).

Root rot and tan spot levels were higher the wheat-wheat than the wheat after fallow rotation. The increase in root rot was more apparent than that of tan spot. Yield and plant stand both decreased in the wheat-wheat rotation (Figures 9 and 10).

Conclusion

Inoculation with straw mulch and PTR propagules generally increased tan spot occurrence but only affected yield in the wheat-wheat rotation. The wheat-wheat rotation magnified root rot symptoms and the effect of tan spot inoculation, and reduced grain yield and plant stands. Some of these results can be attributed to the effect of the rotation on available soil moisture.

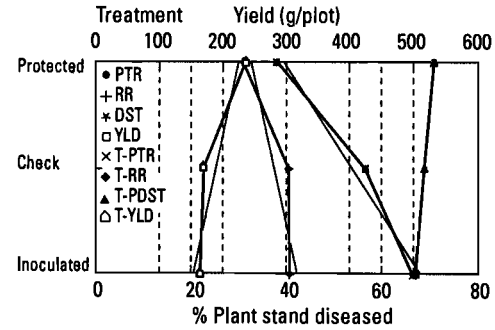

Figure 5. Effect of *Pyrenophora tritici-repentis*/ straw mulch inoculation on disease, plant stand, and yield in a wheat-wheat rotation.

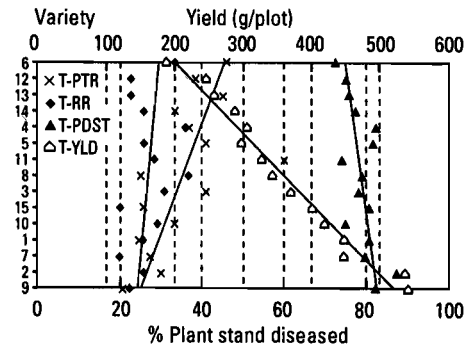

Figure 6. Effect of wheat variety on disease, plant stand, and yield in protected plots.

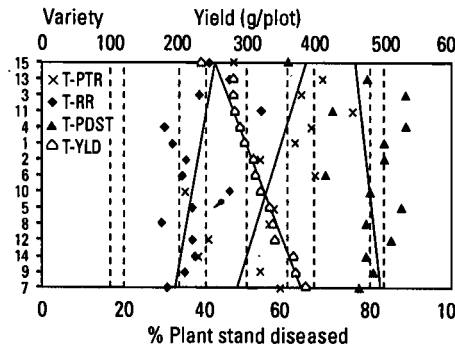

Figure 7. Effect of wheat variety on disease, plant stand, and yield in check plots.

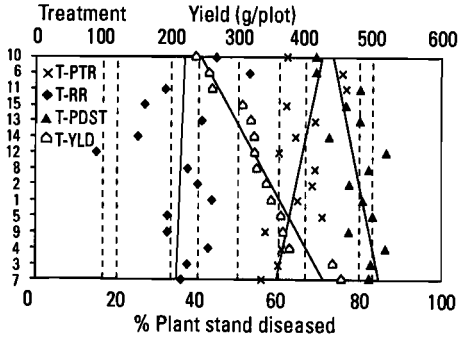

Figure 8. Effect of variety on disease, plant stand, and yield of wheat in inoculated plots.

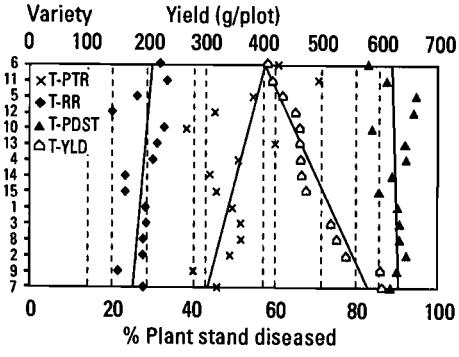

Figure 9. Effect of wheat variety on disease, plant stand, and yield in a wheat-fallow rotation.

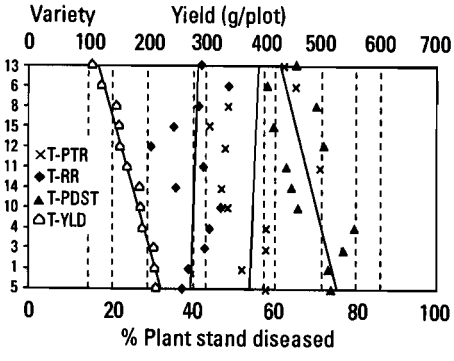

Figure 10. Effact of wheat variety on disease, plant stand, and yield in a wheat-wheat rotation.

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Breeding for Resistance to Spot Blotch in Wheat: Global Perspective

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Abstract

Spot blotch (Bipolaris sorokiniana) *is of increasing concern in developing countries. It mainly occurs in warm, humid wheat growing environments (ME5) in Latin America, Africa, the Indian Subcontinent, Southeast Asia, and China. More recently, spot blotch has also expanded into the cooler, traditional irrigated rice-wheat production areas (ME1), which may be due to pathogen adaptation, changes in varietal spectrum, reduction in tillage practices, a broader host-range, or climate modifications. In the hot and warm wheat production regions (ME5), wheat researchers consider spot blotch the number one economic pathogen. Total affected area is estimated at about 25 million ha. The lack of resistant, adapted cultivars is an urgent problem. Sources of spot blotch resistance are: Latin America (e.g., BH1146, CNT1), China (e.g., Shanghai #4, Suzhoe #8, and Yangmai #6), and wild relatives of wheat or alien species (e.g.,* Aegilops squarrosa, Thinopyrum curvifolium*). One or six genes have been shown to be involved in resistance. No immunity in wheat is known. Low heritabilities, environmental components, and unknown variability in the pathogen may make breeding for resistance difficult. At CIMMYT, directed crosses are made between high yielding germplasm, adapted cultivars from target countries, and distinct sources of resistance. In Mexico, early generations are shuttled between Toluca (central highlands) and Cd. Obregon (coastal plains). Segregating germplasm is selected for resistance to diseases other than spot blotch, such as leaf and stem rusts, foliar blights, head scab, and for agronomic type, potential yield, and relative adaptive traits. CIMMYT's main testing site for spot blotch within Mexico is Poza Rica (21°N, 60 masl). Due to high temperatures and relative humidity, spot blotch is naturally prevalent. In the F4-F7 populations, selected materials are grown in Poza Rica. Lines showing slow disease development, a "good finish", and plump healthy grain are selected. These are grown in yield trials under irrigated conditions at Cd. Obregon under late planting conditions to also allow selection for heat tolerance. Yield is the final deciding factor, plus grain appearance. The top entries are distributed internationally for multilocation testing in the Warm Areas Wheat Screening Nursery to about 50 cooperators around the world. Resistant, widely adapted lines are once again used in crosses at CIMMYT, thus building on what was achieved.*

Target Environments

Spot blotch (*Bipolaris sorokiniana*, previously *Helminthosporium sativum*) is a disease of growing concern in developing countries. Typically, spot blotch occurs in warm and humid environments, such as the nontraditional, subtropical lowland wheat growing areas in the Andean region of Latin America, including

Bolivia and the warmer regions of Brazil, northeast Argentina, and Paraguay. In Africa, similar environments are found in Tanzania and the rainfed wheat growing areas of Zambia and Madagascar. In the Indian Subcontinent, similar conditions occur in the warmer parts of eastern India, most of Bangladesh, and in the terai region of Nepal. In southeast Asia, most small wheat growing areas in Thailand, the Philippines and Indonesia, and the high rainfall and warm wheat growing areas of southern China also have similar growing conditions.

Besides spot blotch pressure, wheat production in these areas is limited by relatively high temperatures. In the areas indicated, mean temperature in the coolest month is generally above 17°C and in some cases is above 22°C (see various contributions in CIMMYT 1985; Klatt 1988; Saunders 1991; Saunders and Hettel 1994).

More recently, spot blotch has begun to dramatically expand into the more moderate and temperate regions of irrigated rice-wheat production, such as the vast central and northwestern regions of the Indian Subcontinent (Diman *et al.* 1994). Whether this is due to pathogen adaptation, changes in the varietal spectrum, the use of reduced tillage practices, or changes in climate remains to be determined in most cases. In Nepal, the increase in spot blotch occurrence was associated with above average warm temperatures in January (Dubin and van Ginkel 1991; Dubin and Bimb 1994). In these rice-wheat growing areas, another factor may also play a role: the spot blotch fungus may be present in small amounts on rice even though it is basically a non-host of the disease. The rice may then serve as a "green bridge" to the subsequent wheat crop (Duveiller and Gilchrist 1994). In particular, rice-wheat rotations in which zero or minimum tillage is adopted are likely to experience increasing spot blotch pressure (Saunders 1989).

Thus, spot blotch is now not only located in the earlier, narrowly defined nontraditional "tropical" wheat growing areas, but has spread into the large, often adjacent areas of traditional wheat production with slightly more moderate climates. In CIMMYT notation, the "nontraditional", tropical environment is referred to as "mega-environment 5 humid" (or ME5A), and the irrigated environment with some heat as "mega-environment 1 with heat" (or ME1HT).

Not only is spot blotch spreading, but a global survey has also established that it is considered by wheat researchers as the number one economic pathogen in the hot and warm wheat production regions (ME5), and in the slightly cooler areas (ME1HT) where mean temperature of the coolest month may drop as low as 12.5°C. Depending on the region, commercial yield losses due to spot blotch are estimated between 1-20% (Dubin and van Ginkel 1991). Total area affected by spot blotch is now estimated at about 25 million ha.

The lack of resistant, adapted cultivars can be critical, such as in Bangladesh, where all commercial varieties (Akbar, Kanchan, and Aghrani) are susceptible (Razzaque *et al.* 1994), Spot blotch is considered the single major disease of wheat in Bangladesh, where disease incidence multiplies annually (Alam *et al.* 1994). In recent years, moderately resistant material has been identified (Hossain Azad 1991; Islam 1991), but it has yet to be found suitable for dissemination to farmers.

Resistance Sources

Sources of spot blotch resistance have been identified over the years, and their origins fall into three categories: Latin America, China, and wild relatives of wheat or alien species.

The Latin American sources are mainly derived from Brazil, and may trace back to Italian ancestry. Older resistant Brazilian commercial varieties are BH1146 and CNT1 (Mehta 1985).

The Chinese sources are mostly materials from the Yangtze River Basin, and in some cases also combine considerable resistance to head scab (caused by various *Fusarium* spp.). Early Chinese sources of resistance used at CIMMYT include Shanghai #4, Suzhoe #8, and Yangmai #6.

Alien resistance sources are many. Alien sources used at CIMMYT have centered around crosses involving *Thinopyrum curvifolium* (Mujeeb-Kazi *et al.* 1996; Villareal *et al.* 1992). Using these alien sources in combination with Chinese resistance sources, outstanding lines such as Mayoor and the Chirya series were developed. It may sometimes be difficult to accurately quantify or determine the exact contribution of alien genes in such multi-parent crosses; however, we feel there is a component of alien-derived resistance present in certain advanced materials developed by Dr. A. Mujeeb-Kazi at CIMMYT. More recently, *Aegilops squarrosa* crosses have shown impressive resistance to spot blotch in Mexico.

Other sources of alien genes should be further exploited (Duveiller and Gilchrist 1994). Researchers at the Punjab Agricultural University in Punjab, India, have studied more than 14,000 entries of wheat and related wild species, representing 13 genera and 136 species, for spot blotch resistance (Dhaliwal *et al.* 1993; Singh and Dhaliwal 1993). They identified several promising entries including *Ae. triuncialis, Ae. speltoides, Ae. squarrosa, Ae. triaristata, Triticum dicoccoides, Ae. cylindrica,* and *T. boeoticum.*

An early Mexican variety, Cocoraque, was found to express field resistance (Mehta 1985). Kea and Opata showed adaptation to spot blotch affected regions.

Little study has been done to determine whether these three sources of spot blotch resistance contribute distinctly different mechanisms of resistance or even different genes. Studies

on the inheritance of spot blotch resistance with Latin American and Indian resistance sources have shown one or two genes to be involved (Srivastava *et al.* 1971; Srivastava 1982; Adlakha *et al.* 1984). Velazquez-Cruz (1994) found six genes to be segregating in four moderately resistant to resistant lines (Gisuz, Cugap, Chirya 1, and Sabuf) developed at CIMMYT, with two to three genes providing good resistance levels. These materials contain various combinations of Chinese and possibly alien sources of resistance.

In preliminary trials, Conner (1990) and Gilchrist *et al.* (1991a, 1991b) have shown that foliar and seed infection by *B. sorokiniana* may be determined by different genetic systems. Although caused by the same fungus, these are very different symptoms that may well be coded by separate genes.

Heritabilities of resistance tend to be low and much influenced by the growing environment, as determined by temperature, humidity, inoculum pressure, planting date, observation date, etc. These environmental components and the unknown variability in the pathogen make breeding for resistance difficult (Duveiller 1993; Duveiller and Gilchrist 1994). No spot blotch immunity in wheat is known.

Germplasm exchange has enabled countries with no indigenous sources of spot blotch resistance to release resistant varieties to their farmers, e.g., in Zambia, where spot blotch is the most devastating disease of rainfed wheat production (Muyanga 1994; Mukwavi 1995). Most resistant cultivars in Zambia contain Brazilian ancestry. Brazilian sources of resistance were first introduced in 1979 from Passo Fundo (Raemaekers 1985); however, initially many were susceptible to leaf rust. In 1984, the introduction PF7748 was released as Whydah, and in 1986 Hornbill (= IAS64/Aldan). PF73339/Hahn, a CIMMYT cross, was released as Coucal in 1988 (Raemaekers 1988). The release of these three varieties allowed yield potential to increase from 1.6-1.7 t ha^{-1} to 2.7 t ha^{-1} (Mukwavi 1995).

The existence of races in the spot blotch causal organism has been reported by Mehta (1981); however, in a study of 206 isolates and 12 test varieties, Hetzler *et al.* (1991) found that only 1-2% of the variance was due to host-isolate interaction. If races do occur, their significance at the field level may be of little practical relevance, and have only minor, occasional effects on varietal resistance in farmers' fields.

Breeding Approach at CIMMYT

Over the past 50 years, the CIMMYT breeding program has evolved from a national focus (in Mexico) to addressing the needs of diverse wheat growing environments on an international scale. Presently the program has a global focus, though emphasizing certain regions and constraints more than others. This mandate centers on the development of

improved germplasm that carries resistance/tolerance to the major stresses, but perhaps not all the significant secondary stresses. Constant vigilance is needed to ascertain whether secondary stresses are becoming major stresses. The latter appears to be increasingly the case for spot blotch, although it was never a truly minor disease in many countries.

Our main testing site within Mexico is located near the city of Poza Rica, situated at 21°N and 60 masl, on the Caribbean coast. During the winter period, temperatures drop and wheat development is possible at this site. From late January onwards, temperature and relative humidity (RH) rise dramatically. In a matter of a few weeks, in most years, endemic spot blotch reaches severe epidemic levels. No artificial inoculation is needed. Yield losses of up to 43% have been noted under these conditions (Villareal *et al.* 1995). Germplasm has been screened at this site since the early 1970s, but only in 1981 did breeding for spot blotch resistance become a stated priority (Kohli *et al.* 1991).

The breeding process starts with the choice of parents to be crossed. Specific crosses are made between high yielding germplasm, adapted varieties from target countries, and sources of spot blotch resistance. Information obtained from international data analysis, based on the international nurseries and trials distributed by CIMMYT, helps to make proper parental choices. The mode of intercrossing may be simple, top, or backcross.

The early generations are shuttled between the Toluca selection site (Mexican Highlands: 19°N, 2640 masl, high rainfall, moderate temperatures) and Cd. Obregon (northwestern Mexican coastal plains: 27°N, 40 masl, irrigated, moderate/warm temperatures) (Rajaram *et al.* 1996). Each crop cycle takes six months. At these two sites, the segregating germplasm is selected for resistance to diseases other than spot blotch, such as leaf and stem rusts, foliar blights, and head scab. In addition, strong selection is applied for agronomic type, potential yield, and adaptation traits in the target environment. Growing the early generations directly under spot blotch pressure in Poza Rica does not appear to allow sufficient rounds of segregation for recombination of desired genes; hence the practice has been de-emphasized. Resistance appears to be based on many minor genes with small, individual but additive effects, requiring the postponement of strong selection pressure to the later generations.

When the necessary traits that can be selected for in Toluca and Cd. Obregon have been increased in frequency in the still segregating populations, they are grown in Poza Rica for the F4-F7 phases. The planting date in Poza Rica is late November to mid December; earlier planting dates result in higher disease levels. Annually in Poza Rica, several hundred segregating populations experience a severe onslaught of spot blotch, starting in late January. During visual progeny selection, emphasis is on

identifying lines that show a slow disease development and a "good finish" (Villareal *et al.* 1985), i.e., a bright, golden peduncle. Following the visual identification of populations and lines with relatively less disease, those materials are harvested. Strict selection against black point (mainly due to the same spot blotch causal organism *B. sorokiniana*) and for plump bold seed is then carried out. The selected materials are planted at El Batan. Here, selection is carried out against leaf rust, following artificial inoculations with highly virulent races. Subsequently the now considerably reduced group of lines is once again planted in Poza Rica.

When the latest advanced lines emerge from this alternating, shuttling process, they are grown as unreplicated yield trials under irrigated conditions at Cd. Obregon. The planting date, normally in late November, is now delayed until January/February. The materials are thus exposed to increasing temperatures (30-39°C) as grainfilling takes place in May/early June. Yield plus grain appearance are the final deciding factors in the selection process. At the same time, a copy of these yield-trial entries are planted in Poza Rica to reconfirm continuing resistance in the field.

The highest yielding materials are shuttled again through El Batan and Toluca for a final round of selection for agronomic type and resistance to disease (other than spot blotch). At this stage, artificial inoculation with *F. graminearum* may be carried out in Toluca to ascertain if that resistance has also been incorporated - an additional benefit.

The selected entries (several hundreds) are then grown in large-scale replicated trials in Cd. Obregon, alongside checks from the target countries, which are again late planted to create heat stress. Final yield and testweight must be high for the best entries to be promoted. At the same time, a set of the same entries is grown in Poza Rica for confirmation of spot blotch resistance.

The top-yielding resistant entries from this final round of selection, numbering 50-100, are distributed internationally for multilocation testing (Rajaram and van Ginkel 1995; Rajaram *et al.* 1996) in the Warm Areas Wheat Screening Nursery (WAWSN). The latter nursery is annually requested by about 50 cooperators around the world. The lines that show wide adaptation, or adaptation specifically in global hotspots, are once again used in crosses at CIMMYT to start a new round of selection, building on what has been achieved. In this way optimal and continuous progress is achieved.

When appropriate, advanced lines and, occasionally, segregating populations, are shuttled with global hotspots, such as Brazil and Zambia, prior to global distribution. Once the spot blotch virulence spectrum is better understood, more early generation

shuttling with hotspots around the world representing different virulences may be needed. Presently, proof for such variability remains to be clearly confirmed.

Elite lines identified in the past few years are listed in Table 1. Upon production, these elite lines are also shared with CIMMYT and other pathologists for more detailed studies, often in collaboration with CIMMYT breeders. These studies may generate an understanding of resistance that will help to identify improved parents that contribute different resistance genes. Thus breeding activities will be facilitated in the future.

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Table 1. Entries most resistant to spot blotch (*Bipolaris sorokiniana*) from recent international Warm Areas Wheat Screening Nurseries (WAWSN).

Nursery	Cross	Pedigree	Score
1st WAWSN	Cno79*2/Prl	CM90312-D-3B-5Y-1B-0Y	4.0
	Ning 7840		4.0
	Wuhan #1	43B-0Y	4.0
2nd WAWSN	Gov/Az//Mus/3/Dodo/4/Bow	CM79515-I-1M-07Y-01M-5Y-2B-0Y	3.5
	Vee#5/3/Gov/Az//Mus	CM79961-18Y-02M-0Y-9M-0Y	3.5
	Thb//IAS20/H567.71	CM81770-1Y-06PZ-1Y-15M-0Y	1.5
3rd WAWSN	Vee#7/Bow	CM76736-36Y-06M-013Y-6B-0Y	3.0
	Sha#4//Myna/Vul	CM91098-24Y-0M-0Y-5M-0Y	3.0
	Milan	CM75113-B-5M-1Y-05M-7Y-1B-0Y	3.3
	Sha#4/Lira	CM91097-1Y-0M-0Y-3M-0Y	3.3
4th WAWSN	Milan	CM75113-B-5M-1Y-05M-7Y-1B-0Y	4.0
	IAS58/4/Kal/Bb//Cj/3/Ald/5/Vee	CM88971-18Y-0M-0Y-5M-0Y	4.8
5th WAWSN	RPB14.68/Pvn//Pho/3/Mon/Ald/4/Parrot	CM90254-B-4M-1PR-0M-2Y-0M	6.0
	Sha#7/Weaver	CM95119-4Y-0M-0Y-2M-0RES	6.0
	Sha#8/Weaver	CM95123-3Y-0M-0Y-3M-0RES	6.0
6th WAWSN	CS/Ag.cu//Glen/3/Ald/Pvn/4/Suz#8	CIGM87.123-3PR-4M-1PR-030M-1M-0Her	4.2
	CS/Ag.cu//Glen/3/Ald/Pvn/4/Suz#8	CIGM87.123-3PR-4M-1PR-030M-2M-0Her	4.2
7th WAWSN	Mayoor	CIGM84.295-24PR-0PR	7.1
	Mayoor	CIGM84.295-4PR-0PR	7.1
	Chirya.1	CIGM87.110-1Y-3M-1PR-1M-3PR-2B-0PR	7.1
New group	Sabuf	CM95073-3Y-0M-0Y-4M-0RES	3.0

Note: Scores used for the first five nurseries are means across locations using the 0-9 scale indicating relative height of the disease. The 6th and 7th WAWSN were scored for spot blotch using the double digit scale (00-99), in which a second digit quantifies disease severity. Epidemic levels varied between nurseries/years, therefore direct comparisons of scores between nurseries cannot be made.

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Evaluating Spot Blotch Resistance of Wheat: Improving Disease Assessment under Controlled Conditions and in the Field

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Abstract

Studies were conducted on spot blotch resistance of wheat at the seedling stage. Results showed that plants inoculated with Bipolaris sorokiniana, *causal agent of spot blotch, should be exposed to conditions of 25°C and 100% relative humidity (RH) for 24 h and then incubated at 24°C and 85% RH for 144 h to allow the detection of statistically significant differences in resistance between genotypes. Among the components of resistance studied, lesion density was difficult to assess. Standardization of experimental conditions was critical for the correct appraisal of genetic resistance at the seedling stage and differences between genotypes tended to be obscured as a result of high data variation. In the field, comparison of AUDPC (area under disease progress curve) was the best approach to separate genotypes based on disease scores repeated over time. Differences in spot blotch resistance observed among genotypes in Poza Rica, Mexico, were generally in agreement with data observed elsewhere; however, the relationship between disease severity, yield loss, plant size, and earliness appeared to be complex. Due to high disease pressure, early genotypes such as NL297, harboring some level of spot blotch resistance, appeared susceptible. Studies on spot blotch resistance under climatic conditions more favorable for the wheat crop are needed in order to detect moderate resistance levels and to better assess the effect of disease on yield.*

Spot blotch, caused by *Bipolaris sorokiniana*, is a serious leaf blight of wheat (*Triticum aestivum*) in Mega-Environment 5A (ME5A), characterized by high temperature (coolest month >17°C) and high relative humidity (RH) (Dubin and van Ginkel 1991; van Ginkel and Rajaram 1993). The disease is one of the major biotic stresses hampering commercial wheat production in some of the warmer tropical areas. Yield losses between 20 and 80% have been reported (Duveiller and Gilchrist 1994). In the humid subtropics of South Asia, where the irrigated rice-wheat rotation covers more than 12 million ha, there is growing evidence that stress conditions are increasing the severity of foliar blights (Dubin and Bimb 1994). Moreover, leaders from the national agricultural research systems (NARSs) have stressed the increasing need for better spot blotch resistance in wheat grown under optimum and irrigated conditions (also

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known as the ME1, i.e., irrigated, low rainfall, and temperate growing conditions) (van Ginkel and Rajaram 1993; CIMMYT 1995).

Several sources of spot blotch resistance have been identified in wheat (Duveiller and Gilchrist 1994; Velasquez 1994; Dubin and Rajaram 1996), but it is generally accepted that resistance of high yielding genotypes is not yet satisfactory. Progress in obtaining germplasm with better disease resistance has been slow due to the quantitative nature of durable spot blotch resistance and the lack of knowledge on screening criteria to achieve a reliable measurement of actual resistance. Few studies have been conducted to detect differences among wheat genotypes based on components of partial resistance (Mehta 1981). Although moisture and high temperature increase disease progress in the field, little is known of the effect of environmental conditions on components of resistance that may allow a better measurement of small, quantitative differences in resistance among wheat genotypes.

Therefore, components of spot blotch resistance were studied in 10 bread wheat genotypes at the seedling stage, incubated under different temperature and moisture regimes. The purpose was to determine experimental conditions that allow better assessment of disease resistance at an early stage. Results were compared with disease observations on adult plants in the field and with yield losses under a natural epidemic.

Materials and Methods

Resistance components at the seedling stage under controlled conditions and adult plant resistance in the field

Genotypes—Ten genotypes grown in geographically separate areas and with distinct selection histories were chosen for their different levels of spot blotch resistance. Ciano and Sonalika are known to be susceptible, and BH1146, Suzhoe#8, K4, and NL297 have some level of resistance. CS/*Th. Curv.*//Glen.81/3/Ald/Pvn Cign84.295-1M-1PR-4M-0PR-0M-0Y-2Y, a genotype resulting from an interspecific cross between wheat and *Thinopyrum curvifolium*, is considered resistant. UP262 is a popular variety largely grown in South Asia. Sawgat has been recently released in Bangladesh. Kvz/K4500 L.6.A.4. SW0176-3M-1Y-10Y-1Y-2M-0Y-0PZ-0Y is known for its tan spot resistance and may also have some resistance to spot blotch.

Experiments under controlled conditions—At the four-leaf stage, all 10 genotypes in four replicates (four pots with five plants per pot) were spray-inoculated with 50 ml of a 7,500 spore ml^{-1} suspension of BS14M1 *B. sorokiniana* monospore strain isolated from wheat collected in Poza Rica, Mexico (60 masl, 21°N). The strain was grown on 30% V8 agar medium under a 12 h photoperiod at room temperature. After seven days, petri dishes were flooded with distilled sterile water and scraped with a spatula before filtration on cheesecloth to obtain a spore suspension without mycelium. A

Fuchs Rosenthal counting chamber was used to adjust spore concentration. Tween-20 (0.01%) was added to the suspension to increase inoculum adherence to the leaf. After inoculation, pots were not moved (about 20 minutes) until all plants were dry to fix the inoculum onto the leaf. Pots were then incubated in a plastic humid room where a gentle mist of distilled water was continuously supplied using a modified ultrasonic humidifier for 4, 24, or 48 h. After exposure to 100% RH and 25 ± 2°C, incubation continued in a controlled Conviron growth chamber at 12, 18, 24, or 30°C and 85% RH for 164, 144, and 120 h. Thus, 12 experiments, hereafter referred to as 'incubation condition', were conducted. In both incubation chambers, pots were arranged according to a randomized complete block design.

Five resistance components were measured 168 h after inoculation on the third leaf of all five plants. Lesion density (lesion cm^{-2}) was calculated after counting the number of lesions per leaf and dividing by leaf area. The latter was estimated as 0.7(L x l) where L = leaf length and l = leaf width, based on earlier observations (Duveiller, unpublished). Lesion size (mm^2) was measured on five randomly spotted lesions per leaf using the assessment key developed by Roumen (1991) for estimating the size of leaf lesions caused by rice blast. Percent diseased leaf area (%DLA) was assessed using the scale proposed by Hetzler (1992). Lesion type was noted according to the following tentative 1-5 key: 1 = distinct black lesions; 2 = distinct brown lesions; 3 = distinct lesions, black or brown, surrounded by a clear yellow chlorotic halo; 4 = diffuse brown inconspicuous lesions with some chlorosis; and 5 = coalescing lesions on more than half of the leaf. Disease severity was also appraised using the 0-5 scale proposed by Adlakha *et al.* (1984): 0 = leaf area free of spots; 1 = necrotic spots without chlorosis, up to 5% of the leaf area involved; 2 = necrosis spots with light chlorosis, 6-20% of the leaf area involved; 3 = necrotic spots with pronounced chlorosis, 21-40% of the leaf area involved; and 5 = spots merging, more than 60% of the leaf area involved. Statistical analysis was conducted with the help of SAS (SAS, Inc.) using the averaged data per pot.

Field trial—The 10 genotypes were planted on November 27, 1994, in Poza Rica, where the humid subtropical climate favors the occurrence of severe spot blotch epidemics every year. Plots (0.75 x 2m) were arranged following a randomized complete block design in three replicates in two adjacent fields with and without fungicide protection. Fungicide protection was done by spraying 0.5 L ha^{-1} of Folicur (ai tebuconazole) every 10 days from early tillering to early dough, in order to avoid any spot blotch development in the controlled plots. Disease scores were taken on January 30, and February 6 and 13, 1995, in the diseased field using the double digit 00-99 assessment scale developed by Saari and Prescott (1975; Eyal *et al.* 1987). Yield (kg ha^{-1}) and 1000-grain weight (g) were measured after

harvesting all plots in both fields. Percent yield and 1000-grain weight losses were calculated.

Assessment of area under disease progress curve (AUDPC) and apparent infection rate

Genotypes—A set of 26 bread wheat genotypes, tentatively divided into four groups based on earliness (late-early) and plant height (tall-short), were sown in Poza Rica on November 17, 1995. Results of the early and short group of entries including Sonalika, Vicam, NL297, Ciano, UP262, Batuira, and Chil/Chumai#18 are presented in this paper.

Disease evaluation—After flowering, %DLA was assessed on five tillers. Three disease scores were taken on the flag (F) and F-1 leaf in order to calculate the AUDPC. The logit of the diseased portion of infected leaf tissue was calculated for each genotype per replicate and regressed over time in order to calculate the apparent infection rate (r) (Zadoks and Schein 1979). The ANOVA was conducted on AUDPC and r, and means were separated based on Tukey's studentized range test.

Results

Resistance components at the seedling stage under controlled conditions and adult plant resistance in the field

Analysis of variance independently conducted for all 12 experiments showed highly significant differences among genotypes ($P<0.01$ and $P<0.05$) for almost all five resistance components when plants were incubated for 24 h at 25°C and 100% RH just after inoculation, independent of the temperature in the second incubation chamber (Table 1). However, as shown in Figure 1, the effect of temperature in the second incubation chamber is notable; incubation at 30°C allowed the detection of highly significant differences between genotypes for all components of resistance except severity when the incubation period in the plastic chamber was 4 or 48 h (Table 1). The lowest P values for differences between genotypes were found for all components of resistance when plants were first incubated at 100% RH for 24 h and then at 24°C and 85% RH. $P = 0.001$ for lesion density and $P = 0.0001$ for %DLA, lesion type, and severity suggested that differences among genotypes at the seedling stage are best detected under these incubation conditions. The lowest P value ($P = 0.005$) for lesion size was observed when temperature was 30°C in the Conviron chamber after the first 24 h incubation.

Figure 1 shows that a minimum of 24 h at 100% RH just after inoculation is advisable for successful infection. Lesion size, type, and severity similarly increased according to temperature in the second incubation chamber (Figures 1a, 1b, and 1c). Temperature increase was associated with higher lesion density and %DLA area after 48 h incubation at 100% RH (Figures 1d and 1e), but not after 24 h at 100% RH, suggesting that these two components of resistance are highly dependent on incubation conditions.

Based on the results obtained in all experiments, lesion type was the resistance component that mainly allowed the detection of differences in spot blotch resistance among genotypes; P<0.01 in 6/12 experiments. Differences among genotypes based on lesion density, lesion size, and %DLA were highly significant (P<0.01) in 5/12 experiments. Severity, estimated using the Adlakha scale (Adlakha *et al.* 1984), was less precise since highly significant

Table 1. Probability (P) of detecting a significant difference in spot blotch resistance between wheat genotypes at seedling stage using five disease resistance components under 12 different incubation conditions (F test, 9 and 27 degrees of freedom).

Incubation conditions RH		Lesion density (cm^{-2})		Lesion size		Diseased leaf area (mm^2)		Lesion type (%)		Severity (1-5)	(0-5)
100%	85%	F test	P	F test	P	F test	P	F test	P	F test	P
4 h	12°C 164 h	1.46	NS	1.75	NS	1.49	NS	1.02	NS	1.30	NS
4 h	18°C	1.00	NS	0.86	NS	0.86	NS	0.86	NS	0.86	NS
4 h	24°C	1.00	NS	1.00	NS	1.00	NS	1.00	NS	1.00	NS
4 h	30°C	3.76	<0.01	4.56	<0.01	4.90	<0.01	4.01	<0.01	4.01	<0.01
24 h	12°C 144 h	3.68	<0.01	4.03	<0.01	6.45	<0.01	5.51	<0.01	2.04	<0.01
24 h	18°C	2.82	<0.05	3.05	<0.05	5.04	<0.01	2.14	<0.01	3.96	<0.01
24 h	24°C	4.48	<0.01	3.96	<0.01	7.23	<0.01	6.86	<0.01	8.40	<0.01
24 h	30°C	2.38	<0.05	5.08	<0.01	2.07	NS	3.59	<0.01	2.19	NS
48 h	12°C 120 h	2.78	<0.05	1.16	NS	1.34	NS	4.09	<0.01	2.80	<0.05
48 h	18°C	2.13	NS	1.53	NS	1.92	NS	2.05	NS	1.60	NS
48 h	24°C	3.67	<0.01	1.16	NS	1.29	NS	1.89	NS	1.56	NS
48 h	30°C	3.24	<0.01	3.86	<0.01	4.07	<0.01	4.21	<0.01	2.09	NS

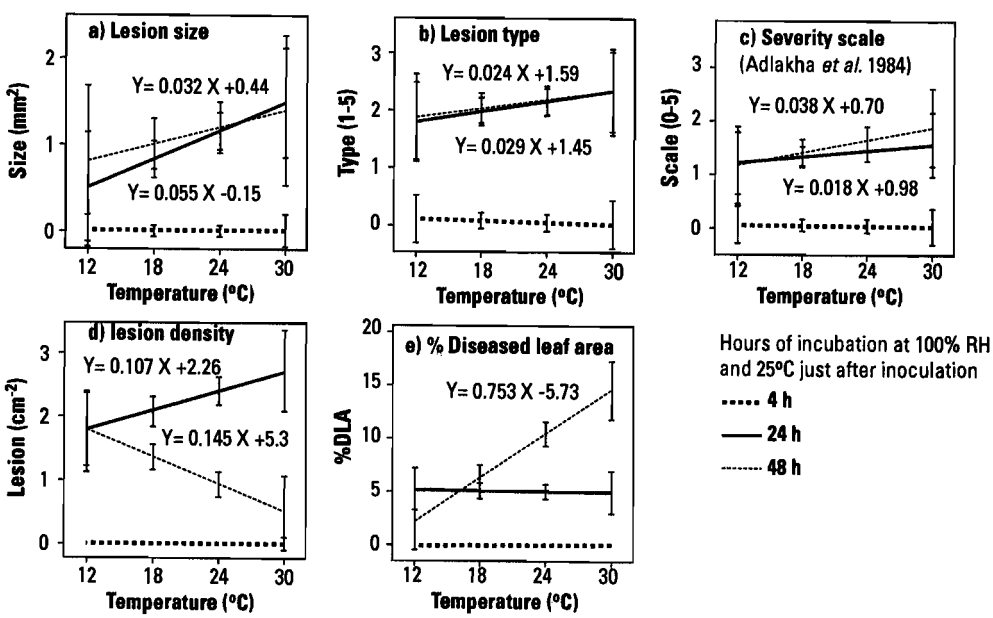

Figure 1. Evolution of (a) average lesion size, (b) lesion type, (c) severity - using the scale of Adlakha *et al.* (1984), (d) lesion density, and (e) percent diseased leaf area according to incubation temperature after plants have been exposed for 4, 24, or 48 h to 100% RH and 25°C.

differences (P<0.01) were only found in 4/12 experiments (Table 1). The ranking of varieties obtained under 24°C for 144 h after an incubation at 100% RH is given in Table 2 according to decreasing average lesion type. Results of Tukey's studentized range test indicated that significant differences in resistance to *B. sorokiniana* are not easily detected between genotypes; LSD values are high compared to the means for all five components. Lower disease scores were obtained for BH1146 (semi-resistant), and Sonalika and UP262 appeared susceptible. However, results for Ciano, NL297, K4 and Cs/*Th. Curv.*//... did not show clear differences between these genotypes.

In the field, Ciano, Sonalika, NL297, and Sawgat appeared more susceptible, whereas Kvz/K4500..., Suzhoe#8, and CS/*Th. Curv.*//... were more resistant. BH1146 and K4 showed intermediate resistance, characterized by a lower disease value than Ciano or Sonalika in the first scoring date which tended to increase dramatically at the end of the season. Based on terminal disease score for both digits, Kvz/K4500..., Suzhoe#8, and CS/*Th. Curv.*//... appeared more resistant. Susceptible genotypes Ciano, Sonalika, and NL297 were short, and Suzhoe#8 and CS/*Th. Curv.*//... were tall; however, Kvz/K4500... had a similar plant height to susceptible genotypes. This suggests that the possible escape effect due to plant height was probably limited and that results show the actual genetic resistance (Table 3). Yield was extremely low for all 10 genotypes but significantly higher for Suzhoe#8 and CS/*Th. Curv.*//... Differences in 1000-grain weight were highly significant but less correlated to disease scores than yield. The highest 1000-grain weight was observed for NL297, Sawgat, Suzhoe#8, CS/*Th. Curv.*//..., and BH1146 (Table 3). Yield losses were much higher than 1000-grain weight losses, suggesting that spot blotch has a higher effect on other yield components (i.e., grains per spike and spikes m^{-2}) under these conditions.

Table 2. Comparison of spot blotch resistance in bread wheat genotypes at seedling stage, seven days after inoculation. Plants were incubated for 24 h at 25°C and 100% RH followed by 144 h at 24°C: minimum significant difference (LSD) using Tukey's studentized range test (P<0.05); means with the same letter are not significantly different.

Genotype	Lesion type	Lesion density	Lesion size	%DLA	Severity
Sonalika	2.45 a	1.15 abc	1.75 a	6.20 a	1.85 a
UP262	2.28 ab	1.05 abc	1.08 ab	2.80 ab	1.50 ab
NL297	2.00 abc	1.72 a	1.35 ab	6.38 a	1.50 ab
K4	1.73 abc	0.95 abc	0.75 ab	1.33 b	1.08 bc
CS/*Th. Curv*//...	1.70 abcd	0.70 bc	0.80 b	1.23 b	1.08 bc
Ciano	1.65 abcd	0.85 bc	1.03 ab	1.18 b	1.08 bc
Kvz/K4500 L.6.A.4.	1.55 bdc	1.02 abc	0.95 ab	1.20 b	1.25 bc
Suzhoe#8	1.38 dc	1.18 abc	0.73 b	2.03 b	1.08 bc
Sawgat	1.35 dc	1.47 ab	0.67 b	1.75 b	1.00 bc
BH1146	0.85 d	0.40 c	0.60 b	0.35 b	0.78 c
LSD (P = 0.05)	0.87	0.86	0.87	0.87	0.53

Results obtained for all resistance components at the seedling stage under the different controlled incubation conditions were studied in relation to each digit of the double digit scale at the different scoring dates in the field (Table 4). Overall, the correlation between field results and studies conducted at the seedling stage was weak. Digit 1 (disease progress from the lower to the top of the plant) did not significantly correlate with any of the results obtained under controlled conditions. Digit 2 (severity) was generally more significantly correlated to lesion size, severity, and %DLA. Interestingly, severity observed in the field on February 6, 1995, was correlated ($P<0.05$) to more resistance components under controlled conditions than field observations on February 13.

Table 3. Average disease scores observed in the field at Poza Rica, Mexico, on three scoring dates using the double digit scale for evaluation of spot blotch severity. Plant height, yield, 1000-grain weight, and percent losses estimated by comparison with a fungicide treated plot. Minimum significant difference (LSD) after Tukey's studentized range test ($P<0.05$); means with the same letter are not significantly different.

Genotype	01/30/95		02/06/95		02/13/95		Height	Yield	1000-grain	Yield	1000-grain
	Digit 1	Digit 2	Digit 1	Digit 2	Digit 1	Digit 2	(cm)	(kg ha^{-1})	(g)	loss (%)	loss (%)
Ciano	8.0 a	3.7 a	9.0 a	9.0 a	9.0 a	9.0 a	67 d	118 d	18.0 d	94 a	49 a
Sonalika	8.0 a	2.7 ab	9.0 a	9.0 a	9.0 a	9.0 a	72 cd	215 cd	26.0 bc	87 abc	43 ab
NL297	8.0 a	3.0 ab	8.7 ab	7.3 ab	9.0 a	9.0 a	67 d	432 bcd	31.3 a	65 bcd	32 abc
Sawgat	7.7 ab	1.7 ab	8.0 ab	3.3 dc	9.0 a	9.0 a	85 bc	595 b	30.0 ab	78 abcd	29 bc
UP262	8.0 a	3.0 ab	8.0 ab	5.0 bc	8.7 ab	6.7 ab	85 bc	288 bcd	24.7 c	91 ab	43 ab
BH1146	7.0 abc	2.0 ab	8.0 ab	3.3 dc	8.0 bc	5.3 bc	105 a	542 bc	28.7 abc	77 abcd	27 bc
K4	4.7 cd	2.3 ab	7.0 bc	2.0 dc	8.0 bc	5.3 bc	98 ab	505 bc	24.7 c	83 abc	39 ab
Kvz/K4500 L.6..	5.3 bcd	1.3 b	6.0 c	1.0 d	8.0 bc	2.0 d	67 d	592 b	24.7 c	61 cd	34 abc
Suzhoe#8	5.7 abcd	2.0 ab	7.0 bc	2.0 dc	7.6 c	3.7 cd	92 ab	1098 a	30.0 ab	61 cd	26 bc
CS/*Th. Curv*//...	3.7 d	1.0 b	5.7 c	1.3 d	7.3 c	2.7 cd	92 ab	1218 a	28.7 abc	49 d	19 c
F test (9-18 df)	12.5	3.3	12.0	19.7	14.3	27.4	21.4	25.7	19.1	6.7	7.2
P	0.01	0.05	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
CV %	12.1	34.9	7.3	27.8	3.48	14.9	6.2	21.7	5.8	13.3	17.7
LSD (P = 0.05)	2.3	2.3	1.7	3.5	0.9	2.7	15	355	4.6	29	17

Table 4. Spot blotch resistance components[1] and controlled conditions for which significant differences in resistance between genotypes were found at the seedling stage, and for which the coefficient of correlation with disease assessment in the field was found significant using one or the other digit of the double digit scale (00-99).

Double digit scale	Observation date	$P<0.05$	$P\geq0.05$, $P<0.10$
Digit 1	01/30/95	DLA_5	
Digit 1	02/06/95	DLA_5	
Digit 1	02/13/95		DLA_5, DLA_7, TLX_5, DEN_7
Digit 2	01/30/95	TLX_{12}	TLX_5
Digit 2	02/06/95	DLA_5, DLA_7, TLX_7, TLX_{12}, TYP_8, TYP_{12}, DEN_5	SEV_7
Digit 2	02/13/95	DLA_5, TLX_{12}	DLA_7, SEV_5

[1] Resistance components: DLA = Diseased leaf area; TLX = Lesion size, SEV = Severity (Adlakha *et al.* 1984); DEN = Density; TYP = Lesion type.

Incubation conditions: 5: 24 h at 100% RH and 25°C, followed by 144 h at 12°C and 85% RH;
7: 24 h at 100% RH and 25°C, followed by 144 h at 24°C and 85% RH;
8: 24 h at 100% RH and 25°C, followed by 144 h at 30°C and 85% RH;
12: 48 h at 100% RH and 25°C, followed by 120 h at 30°C and 85% RH.

This may indicate an optimum date for field observation.

Assessment of AUDPC and apparent infection rate

Table 5 shows the average plant height and number of days to heading of four groups of genotypes. Early and short genotypes, on average, had a significantly higher %DLA than other groups of entries. Late and short genotypes were less diseased than other groups; as a result of slower plant development, leaves were exposed to the pathogen for less time. Although differences between all four groups were not clear, earliness seems to have more effect on disease severity than plant height. Also, it was clearly evident that the disease progressed from the lower part of the canopy toward the upper leaves, because AUDPC on the flag leaf was always lower than on the F-1 leaf.

Within the early and short group of genotypes, significant differences ($P<0.05$) were found among genotypes based on AUDPC and apparent infection rate *r*; however, the ranking of genotypes was different according to the method used (Table 6). Using both approaches, Sonalika appeared in the most susceptible category, which is consistent with observations made in South Asia. Nonetheless, Ciano, which appeared slightly less susceptible than Sonalika based on AUDPC, was not significantly different to resistant genotype Chil/Chumai#18 when *r* was used. Similarly, based on comparison *r*, UP262 appeared to be as resistant as Batuira: a genotype that appeared clearly resistant when AUDPC was analyzed. NL297, which shows a moderate level of resistance under South Asian conditions, showed severe disease levels in Poza Rica, and

Table 6. Comparison of field resistance to spot blotch in a group of early and short spring bread wheat genotypes using AUDPC[1] and apparent infection rate (%) on the flag leaf (three observation dates on five tillers).

Genotype	AUDPC	Apparent infection rate (%)
Vicam	516 a	0.73 a
Sonalika	510 a	0.95 a
NL297	469 a	0.56 b
Ciano	364 b	0.41 c
UP262	285 b	0.54 b
Batuira	172 c	0.44 b
Chil/Chumai#18	68 d	0.37 c

[1] Area under disease progress curve.
Note: Means with a different letter within columns are significantly different using Tukey's studentized range test ($P<0.05$).

Table 5. AUDPC[1] calculated by percent diseased leaf area for flag leaf (F), F-1 leaf, and the combination of F and F-1 leaves in four spring bread wheat genotype groups differing in plant height and earliness.

Genotype group		Height (cm)	Days to heading	No. of entries	AUDPC		
					F leaf	F-1 leaf	F*F-1 leaf
Early	Tall	101	71	6	45 b	194 b	120 b
Early	Short	82	59	7	340 a	610 a	480 a
Late	Tall	121	69	6	47 b	203 b	125 b
Late	Short	96	77	7	36 b	107 b	72 c

[1] Area under disease progress curve.
Note: Means with a same letter in a column are similar based on Tukey's studentized range test ($P<0.01$).

the AUDPC for this entry was not significantly different to that of Sonalika or Vicam.

Discussion

The study conducted under different controlled incubation conditions showed the important effect of moisture and temperature on spot blotch infection. It appeared that 24 h exposure to 100% RH just after inoculation with *B. sorokiniana* was necessary to induce successful infection. Significant differences in spot blot resistance between genotypes were not so clear when plants were exposed to 100% RH for 48 h. Temperature had a strong effect on the different components of resistance, particularly lesion type, lesion size, and severity. This effect was only seen on lesion density and %DLA when the plant had been exposed to 100% RH for 48 h. This may result from the lack of homogeneous distribution of the inoculum during inoculation, incorrect estimated leaf area, or the lack of precise appraisal of coalescing and joint lesions. It illustrates the difficulty in correctly standardizing experimental conditions for identification of spot blotch genetic resistance on seedlings. A strict inoculum quantification that always uses cultures of the same age and perfect control of incubation conditions is required. We recommend incubating plants for 24 h at 100% RH and 25°C just after inoculation with *B. sorokiniana*, followed by incubation at 24°C at 85% RH for 144 h before scoring the plants. Among the criteria used in this study, %DLA, lesion size, and lesion type were resistance components that appeared to be more related to field results. However, results obtained at the seedling stage were not consistently correlated to field observations, and differences between genotypes under controlled conditions were not easily detected. This is due to the high data variability resulting from the difficult standardization of experimental conditions.

In the field, the interpretation of disease scores over time using the double digit scale was not easy, and the comparison of AUDPC calculated on %DLA should be preferred in order to integrate disease data. In Poza Rica, the relationship between disease rating and yield or 1000-grain weight loss appeared unclear. This is due to confounding effects resulting from differing levels of plant adaptation to the environment. Poza Rica is not a wheat growing area and, in addition to spot blotch, other factors such as temperature and light have a strong effect on plant physiology. Vernalization genes present in some genotypes significantly increase the length of vegetative growth under these subtropical conditions and negatively affect yield components.

Although short and early genotypes appeared distinctly more diseased in the field than other groups of entries, further studies are needed to understand the effect of earliness and plant height on disease development or possible escape. Based on heading dates observed in Poza Rica, some entries in our study have been classified in the wrong group; this is

shown by the small difference in averaged days to heading between early and late tall genotypes (Table 5). This may partly result from the lack of adaptation of some entries to Poza Rica conditions indicated above and thus discrepancies with agronomic data obtained in other locations. AUDPC appeared more appropriate than *r* to assess disease progress. When *r* is used, differences in ranking of genotypes are principally due to the weight of the first scoring date in this analysis. If a genotype has a high disease infection score for the first evaluation, the apparent infection rate is expected to be low. As a result of the high value of the first scoring date, the slope of the logit line over time will be low and this genotype will be considered more resistant than it actually is. This explains that, in our study, based on *r*, a susceptible genotype such as Ciano does not appear different than resistant entries.

In general, field results obtained from Poza Rica were in agreement with data obtained from other locations of the world when the double digit scale or AUDPC were used. However, small levels of resistance may be overlooked in Poza Rica, as clearly shown in this study with early genotype NL297. The latter was already severely diseased when disease scoring started for all genotypes because flag leaf exertion time in all genotypes had to be taking into account to begin scoring. Therefore, accurately scoring spot blotch resistance in plants that significantly differ in maturity will remain difficult. Poza Rica is an excellent site to conduct pre-breeding activities for spot blotch and advance material under a severe disease pressure obtained under natural conditions. There is no need to artificially inoculate the field. However, studies should be further developed in commercial wheat growing areas where spot blotch is becoming more severe, such as in South Asia, in order to better detect small differences in resistance and to better understand the impact of spot blotch on yield loss.

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Results of the South Asia Regional Helminthosporium Leaf Blight and Yield Experiment, 1993-94

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Abstract

Helminthosporium leaf blights, caused by Bipolaris sorokiniana *and secondarily by* Drechslera tritici-repentis, *are important in the eastern part of the Indian subcontinent. This paper presents resistance and yield data for 18 wheat genotypes tested at seven locations in Bangladesh, India, and Nepal under natural infections of* B. sorokiniana *and* D. tritici-repentis. *The objective of the experiments was to determine whether the tested genotypes possessed adequate resistance to natural infection by these pathogens and good adaptation to the eastern part of the subcontinent. Superior genotypes had significantly lower AUDPCs (area under disease progress curve), were generally higher yielding than the local check (Sonalika), and headed early to moderately early. The best entries were to be used directly in breeding programs. At least 10 of the genotypes [Bhrikuti (NL623), Kundan, NL644, A6/Glen (BAW 714), K8027, HUW206, Annapurna 1, Fang 60, and NL297] were moderately resistant to resistant to the blights and possessed other required characteristics. It was confirmed that heading was negatively correlated with AUDPC. Covariance analysis or grouping genotypes by heading date, with an internal check in each group, may help reduce this confounding effect.*

Helminthosporium leaf blights (HLB), caused principally by *Bipolaris sorokiniana* (Sacc. in Sorok.) Shoem. (syn. *Helminthosporium sativum* Pamm., King & Bakke.) and secondarily by *Drechslera tritici-repentis* (Died.) Shoem. (syn. *H. tritici-repentis* Died.), are very important in the eastern Indian subcontinent. *Bipolaris sorokiniana* is generally considered to be the most important

foliar blight in the warmer wheat areas of the world (Dubin and van Ginkel 1991). Studies have shown HLB to cause up to 20% yield loss in farmers' fields and close to 30% in experiments in South Asia (Dubin and Bimb 1994; Duveiller and Gilchrist 1994). Villareal *et al.* (1995) noted losses around 40% in experiments in Mexico and higher in other areas of the world.

At present, only moderate levels of HLB resistance are present in some South Asian wheats, and higher levels need to be incorporated. The purpose of the study was to identify germplasm that would be useful progenitors for direct use in breeding programs. Such germplasm should have good yield potential, earliness under South Asian conditions, and measurable HLB resistance. To identify good HLB resistance with acceptable agronomic characters, a group of breeders and pathologists from South Asia agreed to set up a replicated yield experiment especially to test genotypes for HLB resistance in Bangladesh, India, and Nepal.

Materials and Methods

Eighteen bread wheat genotypes were selected for foliar blight analysis by breeding programs in Bangladesh, India, and Nepal in 1993-94 (Table 1). Selections were based on response to foliar blights in experiments conducted the previous year (Table 2). The experiments were planted at two sites in Bangladesh, four sites in India, and two sites in Nepal. One site in India produced incomplete data and could not be included in the analysis. The treatments were replicated four times in a randomized complete block design and were re-randomized at each site. Plot sizes varied among locations according to land area available. All experiments were managed optimally for yield experiments in the area. Foliar blight severity was measured as percent lesions on the flag (F) and F-1 leaves on individual plants in each treatment. Readings were averaged for F and F-1. Three readings were taken and used to calculate area under the disease progress curve (AUDPC):

$$AUDPC = \sum_{i=1}^{n} [(Y_{i+1} + Y_i) \times 0.5] [T_{i+1} - T_i]$$

Table 1. Test sites and cooperators for the South Asia Helminthosporium Leaf Blight Regional Yield Experiment, 1994.

Country/site	Institutions	Latitude	Longitude	Altitude (masl)
Bangladesh				
Dinajpur	Wheat Res. Cent., BARC	25° 38'N	88° 39'E	30
Jessore	Agri. Expt. Stat., BARC	23° 13'N	89° 13'E	8
India				
Faizabad	NDUAT, UP	26° 47'N	82° 12'E	113
Kanpur	CSAU, UP	26° 28'N	80° 24'E	123
Varanasi	BHU, UP	26° 27'N	80° 25'E	150
Nepal				
Bhairahawa	NWRP, NARC	27° 06'N	82° 04'E	105
Rampur	IAAS, Tribhuvan Univ.	27° 37'N	84° 24'E	228

where Y_i = HLB severity (%) at the *i*th observation, T_i = time (days) of the *i*th observation, and n = total number of observations.

The experiment was subjected to natural *B. sorokiniana* infection, and at Bhairahawa and Rampur there was also some *D. tritici-repentis* infection. Combined ANOVA was performed and appropriate LSD (P = 0.05) calculated for key variables. Simple linear correlations were calculated to determine logical relationships.

Results and Discussion

Table 3 gives the average site information for key variables of interest. The Bhairahawa location had the highest average HLB and was one of the earlier sites with respect to heading. Dinajpur and Jessore were very early sites, as expected, with moderate HLB levels. Faizabad and Varanasi were the latest heading and the highest yielding sites. Faizabad had low levels of disease and, on average, the tallest plants. Overall, HLB and yield levels were sufficient to select for resistant and adapted genotypes - the objectives of the regional experiment.

Table 3. Average site data for key variables observed in the South Asia Helminthosporium Leaf Blight Regional Yield Experiment, 1994.[1]

Site	AUDPC	Heading (d)	Yield (t ha^{-1})	Height (cm)
Jessore	246	60	2.1	83
Dinajpur	268	61	2.0	84
Faizabad	97	79	4.7	99
Kanpur	57	76	3.0	89
Varanasi	222	85	4.5	-
Bhairahawa	403	71	2.3	88
Rampur	281	76	2.5	80

[1] Average site data based on 18 genotypes.

Table 2. Genotypes in the South Asia Helminthosporium Leaf Blight Regional Yield Experiment, 1994.[1]

Genotype	Pedigree	Origin
Nepal 297	HD2320=HD2137/HD2186//HD2160	Nepal/India
Annapurna 1	Veery 5 =Kvz/Buho//Kal/Bb	Nepal/Mexico
NL644	Au/UP301//Gll/Sx/3/Pew S/4/Mai S/Maya S/Pew S/5/Pea S	Nepal/Mexico
NL 623 (Bhrikuti)	Cmt/Coc75/3/Plo//Fury/Ana75	Nepal/Mexico
HUW206	Veery 7	India/Mexico
Sonalika	II 54-368/An/3/Yt54/N10B//LR64	India/Mexico
A6/Glen (BAW 713)	CM 91220-1ISD-0ISD-1ISD-0ISD	Bangladesh/Mexico
A6/Glen (BAW 714)	CM 91220-1ISD-0ISD-8ISD-0ISD	Bangladesh/Mexico
BAW 599	Crt/Ald S//Seri82	Bangladesh/Mexico
Fang 60	Pi62/Fd/3/Pi62/Mz//Mxp	Pakistan/Thailand/Mexico
K8027	NP875/4/N10B/Y53//Y50/3/Kt54B/5/2*K852	India
VL616	Ska/P46	India
DL153-2	Kundan = Ti71/NP890	India
ACC No. 8528	-	India
Bow "S"	Au//Kal/Bb/3/Wop S	India/Mexico
Vee S/Myna S	-	India/Mexico
BW1052	Zaf/3/Cno67//Lr64*2/Sn64/4/Cno67//Nad/Chr/5/Yr70	India/Mexico
ACC. No. 8450	-	India

[1] Seed source from DWR, Karnal, India, except entries 7-9, which came directly from Bangladesh.

Previous studies in Nepal indicated that low HLB was generally correlated with late heading or maturing germplasm, and thus there was a tendency to select later heading materials (H.J. Dubin, unpublished; Duveiller *et al.*, this proceedings). Height had a similar but lower correlation. Negative correlations between foliar blight diseases and heading dates are common (van Beueningen and Kohli 1990) and it is not known whether this is due to linkages, pleiotropy, or, in some cases, escape. Table 4 agrees with previous observations in South Asia, and only in Varanasi was there no significant correlation with AUDPC. All others were highly significant. Maturity behaved similarly, as previously observed. Height appeared to be poorly correlated with disease in these experiments although some significant negative correlations were observed. Combined correlations of yield loss vs. HLB severity were very highly significant ($r = -0.42$, $P = 0.001$, $n = 486$).

Under South Asian conditions, HLB-AUDPC and the final HLB reading on the F and F-1 were highly correlated, indicating that one reading at the appropriate time would be sufficient to estimate resistance levels (Table 4). This is consistent with previous observations.

Combined analysis of variance indicated highly significant differences among genotypes and among sites. Although there was a significant difference between genotypes by sites, the mean square was very small compared to that among genotypes. Although other factors may be involved, the analysis tends to indicate that genotypical differences for resistance among the hosts were more important than possible differences in aggressiveness of the pathogen strains present at the different sites. Comparisons of behavior of resistant lines between South Asia and Mexico indicate that genotypes behave similarly, supporting the observations that strong race differences do not exist among infecting strains.

Good resistance coupled with acceptable yield levels and early to moderate early heading (maturity) are expressed by about 10 genotypes, depending on the required lateness or

Table 4. Correlations of helminthosporium leaf blight (HLB) AUDPC[1] and indicated variables at different sites, South Asia HLB Regional Yield Experiment, 1994.

Site	Heading (d)	Maturity (d)	Height (cm)	Last HLB reading (%)	No. of observations
Jessore	-0.70**	-0.74**	-0.25*	0.94**	72
Dinajpur	-0.61**	-0.72**	-0.22	0.96**	72
Faizabad	-0.56**	-0.67**	-0.22	0.95**	72
Kanpur	-0.80**	-0.81**	-0.01	0.86**	72
Varanasi	-0.22	-0.35*	-	0.85**	54
Bhairahwa	-0.64**	-0.75**	-0.32**	0.92**	72
Rampur	-0.78**	-0.75**	-0.25*	0.92**	72

[1] Area under disease progress curve.
* $P = 0.05$, ** $P = 0.01$

resistance of the germplasm. It is important to note that several very early lines such as NL644 and NL297 have significantly better HLB resistance than the check Sonalika and also higher yields (Table 5). Furthermore, lines such as A6/Glen (BAW 714) are relatively early and have excellent HLB resistance. All lines listed in Table 6 have been observed as resistant regionally in South Asia for at least two years. Several lines such as Bhrikuti, Kundan, HUW206, and NL297 have all been released commercially in HLB endemic areas and continue to be more resistant than the older cultivars such Sonalika or UP262. Leaf rust is also very important in South Asia and many of the lines noted in Table 6 combine good characters and leaf rust resistance.

The fact that early lines can be resistant indicates that the correlation between lateness and resistance can be broken. To increase the efficiency of selection for earliness and resistance, genotypes may be grouped by heading time with the inclusion of an internal,

Table 6. Best genotypes for immediate use in breeding programs based on helminthosporium leaf blight resistance, earliness, and good adaptation to areas tested.

Genotype	AUDPC[1]	Yield (t ha^{-1})	Heading (d)	Leaf rust[2]
K8027	157	3.0	76	MR
A6/Glen (BAW 714)	161	2.7	69	R
HUW206	196	3.2	76	R
Annapurna 1	197	3.1	75	R
DL153-2 (Kundan)	199	3.2	72	R
BW1052	208	3.5	70	R
NL623 (Bhrikuti)	235	3.5	70	R
Fang 60	239	3.0	71	S
NL 644	285	3.3	64	S
NL297	339	3.2	63	S
Sonalika (Check)	505	2.7	62	S

[1] Area under disease progress curve.
[2] R = resistant, MR = moderately resistant, S = susceptible.

Table 5. Analysis of variables over sites in the South Asia Helminthosporium Leaf Blight Regional Yield Experiment, 1994.

Genotype	AUDPC[1]	% HLB-flag	Heading (d)	Yield (t ha^{-1})	Height (cm)
Bow "S"	58	6	82	3.3	82
ACC No. 8528	96	8	80	2.5	72
Vee "S"/Myna"S"	112	15	81	2.8	75
A6/Glen (BAW 713)	142	14	75	2.8	84
ACC. No. 8450	152	21	76	2.5	66
K8027	157	17	76	3.0	86
A6/Glen (BAW 714)	161	13	69	2.7	92
HUW206	196	20	76	3.2	79
Annapurna 1	197	26	75	3.1	75
DL153-2	199	14	72	3.2	75
BW1052	208	23	70	3.5	75
NL 623 (Bhrikuti)	235	23	70	3.5	71
Fang 60	239	27	71	3.0	81
NL644	285	29	64	3.3	83
Nepal 297	339	38	63	3.2	69
BAW 599	365	41	69	2.6	84
VL616	398	43	67	2.7	69
Sonalika (check)	505	56	62	2.7	73
LSD (P = 0.05)	26	3	1	0.2	2

[1] Area under disease progress curve.

susceptible check of similar phenology. Covariance analysis can be useful as well (H.J. Dubin and M. Romero, unpublished). In more precise work, flag leaves may be inoculated under controlled conditions to determine if the amount of exposure time to the pathogen affects resistance.

It should be remembered that the goal of these experiments was to select suitable parents for crossing, thus it was considered important to have replication and yield information. The experiments achieved this due to good regional cooperation; however, in segregating populations such precision is not needed. The earliest and most resistant progeny may be selected regardless of the breeding system used. At CIMMYT, selection for resistance and earliness has produced good resistance levels from various sources including alien germplasm (Dubin and Rajaram 1996). Use of three-way crosses seem to work well. In Nepal, we are using several sources of resistance for the first two parents, and crossing with high yielding, well adapted, moderately HLB resistant parents in the third cross.

The results presented indicate that excellent information on HLB resistance can be obtained with a minimum of sophisticated equipment through good regional cooperation among scientists with common problems.

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Breeding for Resistance to Helminthosporium Blights in Nepal: Strategies and Genetic Gains

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Abstract

Helminthosporium leaf blight (HLB) of wheat, caused by Bipolaris sorokiniana *and* Pyrenophora tritici-repentis, *is a disease of economic importance in the terai of Nepal. Most commercial varieties show inadequate HLB resistance. Yield loss assessments conducted by the National Wheat Research Program, Bhairahawa, have shown losses of 23.8-27% in highly susceptible varieties. On-farm studies indicated yield losses of up to 16%. Considering the sizable losses, top priority is being given to breeding for HLB resistance. This paper summarizes the breeding strategy adopted by the National Wheat Research Program to develop and identify HLB resistant wheats and to assess the genetic gains achieved. Breeding begins with the identification of resistant donor lines from different sources. To date, the best materials identified have come from China, CIMMYT alien gene lines from* Thinopyrum curvifolium, *Brazil, and Zambia. Crossing strategies of the hybridization program are designed to involve one parent from our adapted varieties to incorporate earliness, acceptable grains, and other desirable traits into the progeny. Plants in the segregating populations are selected based on resistance governed by quantitative traits such as small lesions on uppermost leaves, low disease severity, 'stay green' at high temperatures during grain filling, freedom from black point, and grain plumpness. A limited backcross program has been initiated, assuming inheritance to be qualitative in some cases; however, progress has remained slow. The best progeny have resulted from three-way and single crosses. Besides hybridization, selection and identification of resistant genotypes from different international collaborative nurseries is continuing. The major outcome of this work is the identification of resistant lines that are now at different stages of multilocation testing. Variety Bhrikuti, released in 1994, is well accepted by farmers, possesses a significant level of HLB resistance, and has 13-39% higher yield than existing varieties. Many other HLB resistant genotypes are in advanced stages of yield testing.*

In Nepal, wheat is the third major cereal crop after rice and maize. Until 1960, it was a minor cereal whose cultivation was limited to a small area in the western hills. With the introduction of Mexican semi-dwarf, high yielding varieties during the mid 1960s, there has been a highly significant increase in both wheat area and production. At present, the wheat sown area is greater than

650,000 ha, with a total production of nearly 1 million t (CBS 1996). Wheat is rotated with rice in Nepal. Rice-wheat (R-W) represents more than 84% of the total wheat area in the country.

In Nepal, *Bipolaris sorokiniana* (Sacc. in Sorok., syn. *Helminthosporium sativum* Pamm., King & Bakke.) and *Pyrenophora tritici-repentis* (Died.) Drechs often occur in combination and, based on symptom observations in the field, it is often impossible to determine if a lesion is caused by one pathogen or the other. Accordingly, both fungi should be considered responsible for the disease complex referred to as helminthosporium leaf blight (HLB) in this paper.

Helminthosporium leaf blight has been a disease of major economic importance, mainly in the terai area where climatic conditions are highly favorable for HLB development. In recent years, it has also extended to hill wheat growing environments. January and February mean temperatures are 16 and 18°C, respectively, with a relative humidity (RH) of more than 90%. Most currently grown commercial wheat varieties have inadequate resistance to the HLB pathogens.

Natural HLB infection generally begins at the tillering stage. Symptoms gradually increase up to and rapidly develop after flowering, covering all above-ground plant parts, depending on genotype. Combined with high temperatures during grain filling, the disease causes premature leaf firing of the wheat plants, resulting in reduced kernel weight and grain yield. These pathogens seem to be very opportunistic in nature and the disease tends to be more severe under stressed plant growth caused by poor nutrition (NPK), low soil organic matter, and heat stress during later growth plant stages (personal observations on long term fertility experiments in R-R-W and R-W systems, Bhairahawa, Nepal).

Both pathogens are commonly observed together on blight affected leaves (Dubin and van Ginkel 1991); however, species predominance has been observed to change with environmental conditions (Dubin and Bimb 1991). For example, *P. tritici-repentis* was the principal pathogen causing foliar blight during 1987/88, whereas *B. sorokiniana* has been dominant since 1989 (Dubin and Bimb 1991; personal observations, 1992-1996).

These pathogens are reported to be highly variable (Mehta 1985; Hetzler *et al.* 1991; Duveiller and Gilchrist 1994). The main inoculum sources are free dormant conidia in the soil, infected seeds, infected crop residues, secondary hosts, and volunteer plants (Reis 1991). Inoculum that persists in the soil has been reported to cause pre-and post-emergence damping off, root rot, sub-crown internode infection, and seedling blight during early crop stages (Saari 1986; personal observations, Bhairahawa).

Yield Losses

Grain yield loss studies carried out at the National Wheat Research Program, Bhairahawa, showed losses as high as 24-27% in susceptible varieties Sonalika and UP 262 (Mahto 1996; Dubin and Bimb 1991). Similarly, studies in farmers' fields in the Rupandehi district showed losses of 9% (Ruckstuhl 1994) and 16% (Mahto *et al.* 1996). Yield loss largely depends on variety and weather conditions during the wheat growing season. Loss estimations calculated under highly favorable conditions in other areas have been as high as 85% (Raemaekers 1988) and 30-80% (Mehta 1985). In Bangladesh, yield losses of up to 29% have been reported (Alam *et al.* 1993).

Breeding Strategies

In the terai of Nepal, foliar blight is not a new problem; however, in recent years, disease severity has significantly increased. Considering the sizable yield loss due to HLB, the National Wheat Research Program has given top priority to the development and identification of wheat varieties that carry HLB resistance. Accordingly, breeding for HLB resistance was initiated in 1989, and good progress has been made in recent years. Breeding methodologies for HLB resistance are described below.

Search for genetic resistance

Breeding work started with the identification of resistant donor lines from different sources. To date, the best materials come from China, CIMMYT wide-cross derivatives (*Thinopyrum curvifolium*), Brazil, Zambia, India, and a few from our own breeding materials (Devkota 1993; Bhatta 1996). A list of resistant wheat varieties/lines from crossing blocks and their disease scores for the last three to four years are presented in Table 1. None are completely

Table 1. Best available bread wheat advanced lines resistant to helminthosporium leaf blight (HLB), Bhairahawa, Nepal, 1990/91-1995/96.

Cross and pedigree	HLB terminal score				
	1990[1]	1992[2]	1993[2]	1994[2]	1995[1]
LONGMAI#10	85	10	5	4	83
YMI#6	82	2	1	4	83
SANGHAI 7	85	1	1	4	82
JINMAI 4058	85	3	1	5	82
NING 8201	84	3	7	4	83
NING 8319	84	3	7	3	83
G162	83	5	5	5	83
SUZHOE F3#4	84	3	10	30	83
LR 25	84	4	10	5	82
DL 153-2	84	10	25	20	85
OCEPAR 7	-	7	20	5	84
DANIAL 88	-	5	15	4	83
K7 (PEL73280/Ttr71/4/...	-	3	5	5	83
MAYOOR(HLB 5)	-	15	15	5	85
MAYOOR (HLB15)	-	5	15	15	84
MAYOOR (HLB19)	-	5	7	30	83
MAYOOR (HLB20)	-	5	7	15	84
MAYOOR (HLB25)	-	3	7	4	82
CS/E.GIG//2*CS/3/CINO 79	-	10	60	20	84
FUFAN 17/VEE#5 (ZSH1)	-	5	15	8	83
TRAP#1/YMI#6 (ZSH13)	-	5	20	7	84
NSIBU (ZSH20)	-	4	15	15	84
SW89-5422	-	1	5	4	82
SW89-3060	-	1	15	10	82
BH1146*3/ALD	-	2	15	35	83
BH1146	-	-	-	15	84
PRL/TONI	-	-	-	20	84
VEE#7	-	5	40	20	84
FRONTANA	-	5	15	4	83
CHIRYA.1	-	-	-	7	82
CHIRYA.3	-	-	-	4	82
CHIRYA.7	-	-	-	4	83
CHIRYA.7	-	-	-	-	81
BHRIKUTI	83	7	35	4	84
NEPAL 297	84	15	35	25	85
UP 262	86	20	60	40	86
SONALIKA	88	30	90	40	88

[1] HLB score using the 00-99 double digit scale.
[2] % HLB on flag leaf.

free of HLB infection. Furthermore, disease severity largely depends on weather conditions, genotypes, time of planting, and inoculum density. For example, some lines which in the past were identified as resistant, such as Mayoor sisters, have shown increased susceptibility levels in recent years and have thus been deleted from the resistant group. Also, wheats with low HLB levels on foliar tissues have shown grain infection. Grain infection is more severe when rain occurs during grain filling (Alam *et al.* 1994; personal observations, Nepal).

As part of varietal screening, resistant and susceptible varieties/lines were studied to observe the degree of *B. sorokiniana* infection on grain and on the sub-crown internode in the laboratory and field. In the laboratory, 40 untreated grains of each variety/line were randomly selected and placed on moistened filter paper in sterilized petri dishes. After 10 days, grains were examined under a light microscope for the presence of *B. sorokiniana* and other fungi. In the field, 200 seeds of each variety/line were selected and half were treated with carboxin at 2.5 g kg^{-1}. The treated and untreated seeds were planted in four 1-m long rows under an unknown inoculum density of *B. sorokiniana*. Fifteen-day-old seedlings from each lot (treated and untreated) were uprooted, washed with clean water, and seedlings with dark brown sub-crown internode counted.

Interestingly, some varieties/lines which showed no *B. sorokiniana* infection of grains showed sub-crown internode infection in the field, indicating the presence of the fungus in the soil. Seed treatment with carboxin was not effective in eliminating sub-crown internode infection by *B. sorokiniana* already present in the soil. Soil samples from the field showed *B. sorokiniana* conidia on microscopic examination. Absence of the fungus in the grains but susceptibility of foliar tissue and sub-crown internode may suggest that resistance to *B. sorokiniana* is governed by two to three independent genes.

Breeding for HLB resistance

Hybridization work in Nepal started in 1989, and multilocational yield testing of HLB resistant advanced lines from different sources has been a regular activity of the wheat breeding program. With the limited knowledge of the genetics of HLB, crosses (40% of total crosses since 1989) involving resistant vs. susceptible and resistant vs. resistant lines are made every year. Some F1s are triple crossed with the best adapted varieties/lines to incorporate earliness, acceptable grains, and other desirable traits. A few back-crosses and multiple crosses were also attempted in the beginning; however, progress was slow. The best progenies have resulted from three-way and single crosses. F2s are space-planted with approximately 2000 plants per cross and are selected based on small lesions on upper-most leaves, low disease severity, 'stay green' at high

temperature regimes during grain filling, absence of black point, and grain plumpness. Modified bulk selection in F3 and F4 generations further helps to obtain clean families. Clean progeny of HLB crosses from F4 progeny are presented in Table 2. In F5, individual plant selection is performed again, and in F6 clean and uniform head row progeny are bulked. Selected F6 lines are evaluated in multilocation experiments for stable resistance over sites, and in F7 the best lines are included in multilocation yield trials.

Besides regular breeding work, studies on selection criteria based on high and low AUDPC (area under disease progress curve), heritability estimates, and inheritance patterns in the segregating populations were initiated in collaboration with CIMMYT and the Institute of Agriculture and Animal Science.

Table 2. F4 progenies selected for helminthosporium leaf blight resistance under field conditions at Bhairahawa, 1995/96.

Cross/pedigree
NEPAL 297*2/DANIAL 88//MAYOOR
G162/NL489//NEPAL 297
NEPAL 251/HLB 11//BHRIKUTI
SW89-5422/NL 251//NL 713
HD 2329//PRL/VEE#6/3/PFAU/VEE#5/4/BL 1135
SW89-5124/3/HE1/3*CNO 79//2*SERI/4/SERI/5/NEPAL 297
K7/MAYOOR//NEPAL 297
SESA/MAYOOR//NEPAL 297
CHIRYA.7/3/LIRA/FFN//VEE#5/4/CBRD
CHIRYA.6/3/SHA3//BUC/FLK/5/CS/E.GIG//CS/3/2*PVN/4/ NINJ 8201
SNB/4/NAC/A.ACUT//3*PVN/3/MIRLO/BUC/5/VEE#7/6/ NEPAL 297
DOVE"S"/BUC"S"//MAYOOR/3/NEPAL 297
K4/HLB 26//NEPAL 297
UP 262/MAYOOR
BHRIKUTI/TRAP#1
BJY/HLB 30
NL 588/BL 1143//DL 153-2
NL 588/HD 2307//BHRIKUTI

Genetic Gains

The major outcomes of breeding for HLB resistance have been the identification of several resistant advanced lines which are now at different stages of multilocation yield testing (Tables 3, 4, and 5), and the release of the

Table 3. The best helminthosporium leaf blight (HLB) resistant bread wheat advanced lines identified and developed at Bhairahawa since 1989.

Variety/cross	HLB terminal score (00-99)	Grain yield (kg ha^{-1})
NEPAL 297/SUZHOE F3#4	81	3000
NEPAL 297/NING 8201	81	4100
NEPAL 251/NING 8319	81	3000
NEPAL 297/OCEPAR 7	82	2800
SONALIKA/ DL 153-2	82	2900
NEPAL 297//MAYA"S"/MON"S"	82	2800
NEPAL 297/3/PIMA 77/SARA//QTZ	82	3000
SANGHAI 4/CHIL/3/MON/IMU/ /ALD/PVN	82	4600
NEPAL 297/NING 8319	82	3600
LAJ 2852/AMSEL	82	3500
SPB/HD 2402	82	3000
ROCK//MAYA/NAC/3/MIL 08P2.9 /4/PGO	82	3500
PARA 2//JUP/BJY/3/VEE#5/JUN /4/NAC	81	3000
DESC//URES*2/PRL	82	3800
DESC/MILAN	81	3000
CHIRYA-3	81	3500
CHIRYA-7	81	800
FRTL	82	3600
SIBIA	82	2600
ROLLER	82	3000
BHRIKUTI (resistant check)	83	3400
SONALIKA (susceptible check)	87	2200
NEPAL 297 (resistant check)	83	2600
UP 262 (susceptible check)	85	800

Note: HLB scores based on field screening, Bhairahawa, 1994/95-1995/96.

moderately resistant wheat variety Bhrikuti in 1994. This variety is well adapted and has a yield gain of more than 13-39% over check varieties UP 262, Nepal 297, and Sonalika (Table 6; unpublished data).

Future Strategies to Minimize Yield Loss

- Release and diversification of additional resistant wheat varieties.

Table 4. Performance of helminthosporium leaf blight (HLB) resistant bread wheat genotypes under multilocation yield testing, 1995/96.

Variety/cross	Grain yield (kg ha^{-1})[1]	HLB (00-99)	Leaf rust severity[2]	Yield gain over best check[3] (%)
NL 751 (CHIRYA.3)	3027	82	0	22.0
NL 785 (CHIRYA.7)	2915	81	0	17.6
NL 784 (CHIRYA.7)	2817	83	0	13.5
NL 791 (MYNA/VUL//PRL)	2827	83	0	14.0
NL 792 (FRTL)	2795	82	TMR	12.6
UP 262 (check)	2482	86	20S MS	-
SONALIKA (check)	2293	88	20S	-

[1] Average of six locations.
[2] MR = Moderately resistant, MS = Moderately susceptible, S = Susceptible, T = Trace.
[3] UP 262.

Table 5. Performance of wheat varieties/lines tolerant to helminthosporium leaf blight (HLB). Advanced varietal trial, 1995/96.

Variety/cross	AUDPC[1]	HLB (%)	Leaf rust[2]	Grain yield (kg ha^{-1})	Yield gain over UP 262	Yield gain over Nepal 297
NL 750 (CHIRYA.3)	178	8.5	0	3534	23	29
NL 748 (FRTL)	245	12.4	5MS, MR	3378	18	24
NL 713	293	14.5	TMR	3130	9	14
NL 731	358	18.8	0	3460	21	26
BL 1473	377	19.0	10MS	3129	9	14
BL 1496	383	19.5	5MS	3340	17	22
Bhrikuti	353	16.9	TMR	3109	8	13
Nepal 297	467	24.0	40S, MS	2735	-	-
UP 262	533	26.7	10MS, S	2867	-	-
SONALIKA	900	46.7	20S	2694	-	-

[1] Area under disease progress curve.
[2] MR = Moderately resistant, MS = Moderately susceptible, S = Susceptible, T = Trace.

Table 6. Results of genetic yield gain studies, Bhairahawa, 1993/94-1995/96.

Variety	Non-protected grain yield (kg ha^{-1})	Protected[1] grain yield (kg ha^{-1})	Grain yield loss (%)	Yield gain over popular checks		
				UP 262	Nepal 297	Sonalika
Bhrikuti	3720	4103	9.3	20.0	13.5	39.0
Nepal 297	3276	3531	7.2	5.5	-	23.0
UP 262	3095	3613	14.3	-	-	16.0
Sonalika	2672	3592	25.6	-	-	-

[1] Propiconazole (125 ml ai ha^{-1}), three foliar spray applications.

- Continuation of the search for better resistance in synthetic hexaploids and other bread wheats, and its transfer to better adapted wheats.
- Inheritance studies on HLB resistance.
- Legume crop establishment between wheat harvest and rice planting to minimize soilborne inoculum.

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Resistance to Spot Blotch in Spring Wheat: Breeding and Genetic Studies

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*Yield of wheat (*Triticum aestivum L.*) is frequently reduced by spot blotch, caused by* Bipolaris sorokiniana, *in warm regions of South Asia. Commercial cultivars possess low levels of resistance to spot blotch. Some exotic wheat genotypes, showing high levels of resistance to spot blotch under field conditions in the lowlands of Nepal, are being used in breeding programs. Studies were conducted to determine genetic control of resistance to spot blotch and response to selection for resistance in selected wheat crosses. Inheritance of spot blotch resistance was conditioned by a single dominant gene in the crosses involving 'Ning 8201' and 'ZSH 22'. Resistance in 'K 7' is controlled by three epistatic loci, whereas in 'Yangmai 6', 'Longmai 10', and 'Ocepar 7' resistance was polygenic. Where resistance was quantitative, heritability estimates of spot blotch were medium to high. Selection for spot blotch resistance in segregating generations was effective in identifying resistant lines in four populations. It was significantly correlated with improved grain yield, biomass, harvest index, thousand kernel weight, and lengthened heading time.*

Effect of Single D-Genome Chromosome Substitutions from Bread Wheat on Spot Blotch Resistance of Hexaploid Triticale

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Abstract

Spot blotch, caused by Bipolaris sorokiniana, *is considered one of the major diseases of triticale (x* Triticosecale *Wittmack), particularly in more humid agroecological zones with high rainfall. Field experiments were conducted at Poza Rica, Mexico, under natural disease epidemics over three years (1994, 1995, and 1996) to determine the effect of single D-genome chromosome substitutions from several cultivars of hexaploid wheat (*Triticum aestivum *L.) on spot blotch resistance of the hexaploid triticale Rhino. Coefficient of infection data across scoring dates were adjusted for growth stage and used to estimate disease severity. Substitutions 1D(1A) and 1D(1B), carrying 1D from cv. Grana, significantly contributed to higher susceptibility relative to Rhino; however, at this point it cannot be entirely excluded that 1A and 1B of Rhino both carry loci for resistance with the same level of expression. Chromosome 1D in substitution for 1R from a hybrid of cultivars Anza and Wheaton, and concomitant absence of 1R, did not affect resistance level; however, when the long arm of 1D from Wheaton was translocated to 1RS of Rhino, replacing 1RL, the resistance level increased. Disease reaction of recombinant chromosomes 1R.1D with long proximal and short interstitial segments of 1D for Wheaton suggests that the factor responsible for increased resistance levels is located in the distal segment of 1DL. Significantly increased susceptibility levels in substitutions 2D(2R) and 4D(4B) indicate that chromosomes 2R and particularly 4B of Rhino carry factors promoting resistance. Substitution line 6D(6B), on the other hand, was more resistant, suggesting that chromosome 6D from Chinese Spring may carry resistance.*

Chromosomal configurations are important factors affecting yield potential and adaptation of triticales, resulting in profound differences in adaptive pattern for 2D(2R) substituted and complete R-genome karyotypes (Varughese *et al.* 1996a, 1996b). International yield nursery results and recent variety release statistics suggest adaptive advantages of complete triticales that carry the 6D(6A) substitution (Figure 1). 6D(6A) is now

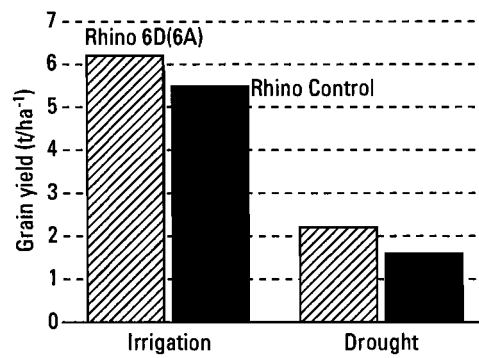

Figure 1. Effect of 6D(6A) chromosome substitutions on grain yield of Rhino triticale.

present in approximately 40% of the spring triticale gene pool at CIMMYT (Pfeiffer 1996). Further, incorporation of *Glu-D1* storage proteins in 1D(1A) and 1D(1B) substitutions and 1A.1D and 1R.1D translocations has dramatically improved the industrial quality of triticale and durum. These associations suggest the exploitation of D(A), D(B), and D(R) substitutions and/or translocations as a useful strategy to increase yield potential, adaptation, and end-use quality of triticale and wheat. Triticale has been used as a "model" crop to detect associations between chromosomes and value-added traits, and as a vehicle for transfer of such traits to wheat. The objective of this study was to determine the effect of D-genome chromosome substitutions from several cultivars of wheat (*Triticum aestivum* L.) on spot blotch (*Bipolaris sorokiniana)* resistance of the spring triticale Rhino.

Materials and Methods

Single D-genome chromosomes from several different cultivars of hexaploid wheat were substituted into hexaploid triticale Rhino. The spot blotch resistance levels of these lines were tested under artificial inoculation over three consecutive years (1994, 1995, and 1996) in Poza Rica, Mexico.

Genetic material

The genetic material consisted of reselections from single D-chromosome substitution and translocation lines in the hexaploid spring triticale Rhino. The Rhino substitution series was developed by Dr. A.J. Lukaszewski at the University of California, Riverside. Several sister lines for each substitution were evaluated:

♦ 1D(1A)	2D(2A)	3D(3A)	4D(4A)	5D(5A)	6D(6D)	7D(7A)
♦ 1D(1B)	2D(2B)	3D(3B)	4D(4B)		6D(6B)	
♦ 1D(1R)	2D(2R)				6D(6R)	

The experiment also involved three translocation lines or segments of chromosome 1DL from cv. Wheaton to the long arm of 1R from Rhino. Translocation 1RS.1DL (1R.1D) involves the entire long arm of 1DL, translocation $1R.1D_{5+10}^{-1}$ (1R.1D #1) involves the proximal 80% of 1DL, while translocation $1R.1D_{5+10}^{-2}$ (1R.1D #2) carries a small interstitial segment of 1DL, with the *Glu-D1* locus in 1RL. The segment length was estimated at 14.5% of 1RL (Lukaszewski and Curtis 1992; Figure 2).

Field experiments were carried out in Poza Rica, Mexico, during 1994, 1995, and 1996, under natural spot blotch epidemics. Each year the experiment was planted with two replications. Disease severity was high in 1994, moderate in 1996, and low in 1995 (Figure 3).

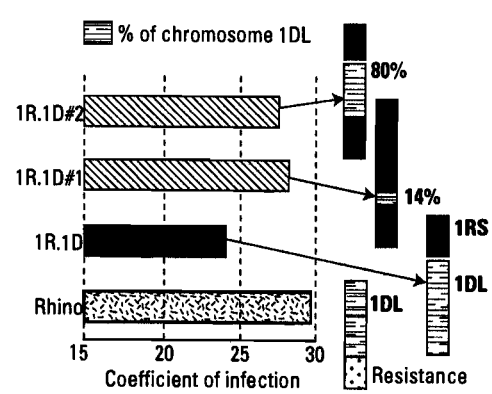

Figure 2. Effect of 1R.1D chromosome translocations on spot blotch resistance of triticale.

Disease was scored at three dates in 1994 and 1996, and at two dates in 1995, using a 00-99 double-digit scale. The first digit represents the vertical disease progress and the second digit, a disease severity estimate. Grain yield and agronomic data from Cd. Obregon, Sonora, and Toluca, Edo. de Mexico, were also used for covariance analysis. Growth stage (Zadoks' decimal code) was recorded for each scoring date (Zadoks *et al.* 1974).

Data analysis

Detailed data analysis was performed, taking into account all variables. Results included a pooled analysis across sister lines, scoring dates, and years using coefficient of infection data (digit 1: disease progress x digit 2: severity) adjusted by growth stage.

Results

Substitutions 1D(1A) and 1D(1B) carried the same chromosome 1D from cv. Grana, a winter wheat from Poland. Both substitution lines show clearly higher susceptibility levels (Figure 4). This suggests that chromosome 1D of Grana contributed to susceptibility; however, at this point it cannot be entirely excluded that both chromosomes 1A and 1B of Rhino carry loci for resistance with the same level of expression. Chromosome 1D in substitution for 1R was from a hybrid between cultivars Anza and Wheaton, and the presence of the chromosome and concomitant absence of 1R did not affect the resistance level; however, when the long arm of 1D from Wheaton was translocated to 1RS of Rhino (i.e., it replaced 1RL of Rhino), the resistance level increased (Figure 2). Two recombinant chromosomes of 1R.1D with a long proximal (80%) and a short interstitial segment (14%) of 1DL from Wheaton did not differ in their spot blotch disease reaction relative to Rhino, indicating that the factor responsible for increased resistance levels in the 1RS.1DL translocation must be located in the distal

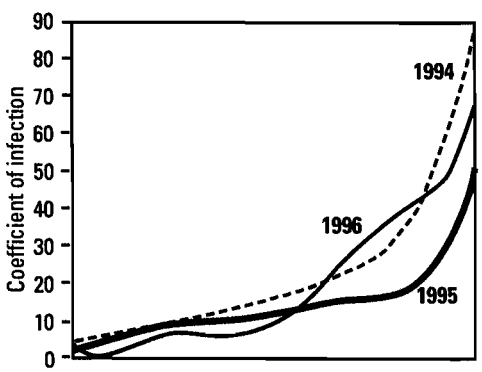

Figure 3. Dynamics of disease epidemics in different years.

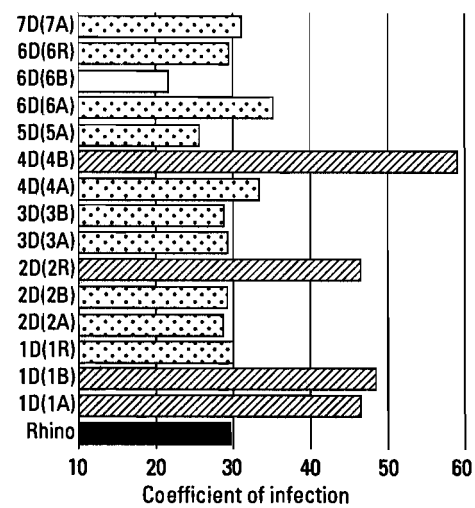

Figure 4. Effect of D-genome chromosome substitutions on spot blotch resistance of triticale.

segment of 1DL (the area which was eliminated when chromosome 1R.1D$_{5+10}^{-1}$ was produced) (Figure 2).

Increased susceptibility levels were observed in substitutions 2D(2R) and 4D(4B), indicating that chromosomes 2R and 4B of Rhino carry factors promoting resistance (Figure 5). The factor on 4B must be particularly stronger. Substitution line 6D(6B), on the other hand, was more resistant, suggesting that chromosome 6D from the hexaploid wheat Chinese Spring may carry resistance.

Conclusions

- 1D of Grana contributes to susceptibility. Substitutions 1D(1A) and 1D(1B) carry 1D from cv. Grana and show clearly higher levels of susceptibility; however, it cannot entirely be excluded that 1A and 1B of Rhino carry both loci for resistance with same level of expression.
- Presence of 1D of Anza/Wheaton in 1D(1R) and concomitant absence of 1R had no effect on the level of resistance.
- 1DL from cv. Wheaton ,translocated to 1RS (replacing 1RL), increased resistance.
- A factor for increased resistance is located in the distal segment of 1DL. Two recombinant chromosomes 1R.1D with long proximal and short interstitial segments did not differ in spot blotch resistance.
- Chromosomes 2R and 4B carry factors promoting resistance. High susceptibility levels were observed in 2D(2R) and 4D(4B).
- 6D of Chinese Spring may carry resistance. Substitution line 6D(6B) showed significantly higher resistance levels.

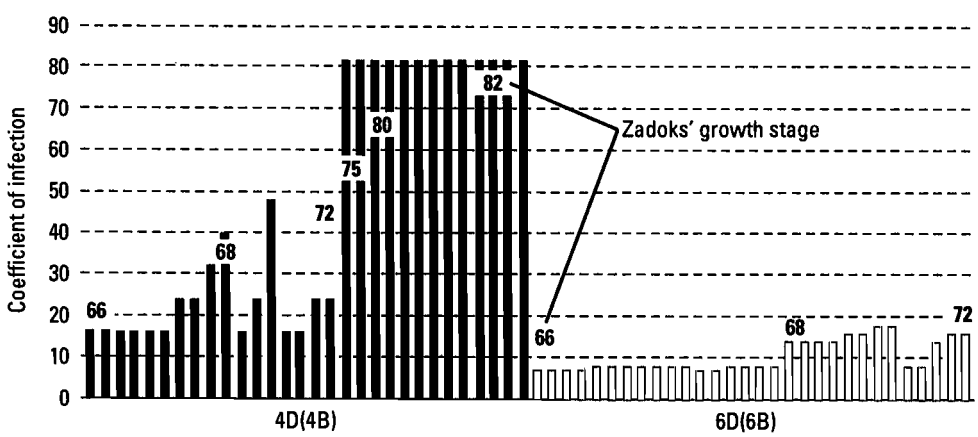

Figure 5. Growth stage and disease development in susceptible 4D(4B) and resistant 6D(6B) substitution lines across scoring dates and years.

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Repeatability of Tan Spot Resistance Evaluation in Wheat

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Abstract

Tan spot is an increasingly important problem for winter wheat production in the northern Great Plains of the USA. Reduced-tillage (or no-tillage) planting into spring wheat stubble, widely credited with contributing to increased winter survival, is largely responsible for the disease increase. The objective of our study was to assess the inherent repeatability of our greenhouse seedling screening procedures as a complement to field screening for improvement of tan spot resistance. Thirty-seven wheat genotypes (released cultivars and experimental lines, both spring and winter growth habit), representing a range of resistance reactions, were tested with a tan spot isolate at four different time periods ("sets"). For each set, materials were planted in a randomized completed block design with two replications. Set inoculations, repeated at three-day intervals, were performed at the two-leaf stage (when the second leaf was fully expanded) with a conidial suspension of about 7,000 spores ml^{-1}. The repeatability of tan spot resistance evaluation obtained by the partitioning of variance components was very high (90.5%), suggesting a high degree of consistency between different evaluation periods. It was concluded that our current greenhouse screening procedure is very reliable and consistently identifies germplasm with a high degree of resistance at the seedling stage. More research is needed, however, to verify the association between tan spot resistance reactions in juvenile and adult plants.

Tan spot, caused by *Pyrenophora tritici-repentis* (Died.) Drechs., is an increasingly important problem for winter wheat (*Triticum aestivum* L.) production in the northern Great Plains of the USA (Hosford 1982). Reduced-tillage (or no-tillage) planting into spring wheat stubble, widely credited with contributing to increased winter survival, is largely responsible for the disease increase. The use of resistant cultivars is the most economic means to reduce yield losses caused by tan spot. Although sources of *P. tritici-repentis* resistance are available from diverse germplasms (Evans *et al.* 1992; Gilchrist 1992; Rees and Platz 1990; Riede *et al.* 1996), the reliability of tan spot evaluation at the seedling stage is unknown.

The objective of this study was to assess the repeatability of our current greenhouse screening procedure for tan spot resistance at the seedling stage. Knowledge of repeatability of the evaluation procedure would be important for determining proper allocation of project resources for screening activities.

Materials and Methods

Plant material and seedling production

Thirty-seven wheat genotypes (released cultivars and experimental lines, both spring and winter growth habit; Table 1), representing a range of resistance reactions, were tested at four different time periods ("sets") at three day intervals. For each set, materials were planted in a randomized completed block design with two replications. Plants were grown in plastic cell cone-tainers filled with Sunshine No. 1 mix. Three seeds from each genotype were planted in each cone-tainer.

Table 1. Wheat genotypes used in the study.

Genotype	Growth habit	Origin/source
BR 34	Spring	Brazil
CEP 11	Spring	Brazil
Halt	Winter	Colorado
2137	Winter	Kansas
Jagger	Winter	Kansas
KS84063-9-39-3-2W	Winter	Kansas
KS84063-9-39-3-4W	Winter	Kansas
KS85W663-11-6-33	Winter	Kansas
KS91044-9-39-3-2W	Winter	Kansas
KS91044-B-1-1	Winter	Kansas
KS950771-24-1	Winter	Kansas
Alliance	Winter	Nebraska
Arapahoe	Winter	Nebraska
Redland	Winter	Nebraska
Siouxland	Winter	Nebraska
Vista	Winter	Nebraska
Elkhorn	Winter	North Dakota
Dawn	Winter	South Dakota
Rose	Winter	South Dakota
SD89119	Winter	South Dakota
SD92174	Winter	South Dakota
SD93104	Winter	South Dakota
SD93113	Winter	South Dakota
SD93128	Winter	South Dakota
SD93267	Winter	South Dakota
SD93336	Winter	South Dakota
SD93340	Winter	South Dakota
SD93352	Winter	South Dakota
SD93407	Winter	South Dakota
SD94110	Winter	South Dakota
SD94112	Winter	South Dakota
SD94118	Winter	South Dakota
SD94167	Winter	South Dakota
SD94188	Winter	South Dakota
SD94189	Winter	South Dakota
SD94208	Winter	South Dakota
TAM 107	Winter	Texas

Inoculum production

A culture of *P. tritici-repentis* (Ptr002, isolated in South Dakota) was used in the study. Conidia were produced following the procedure described by Lamari and Bernier (1989) with slight modification. Three V8-PDA agar pieces of the culture were transferred onto one V8-PDA agar plate and incubated at 23 ± 2°C in the dark. After three days of incubation, the mycelium was flattened with a glass rod, and the culture was incubated at room temperature with constant fluorescent light for 24 h. Plates were then incubated at 15°C in the dark for 12-24 h. Conidia were harvested by flooding the plates with 10 ml water and dislodging the spores by gently scraping colonies with a rubber policeman (a piece of rubber attached to a glass rod). To discard mycelia fragments, the suspension was filtered through two layers of cheesecloth. One drop of Tween 80 was added to every 200 ml of conidia suspension. The spore suspension was adjusted to about 7,000 conidia ml^{-1}.

Inoculation

Plants were inoculated when the second leaf was fully expanded. A volume of 80 ml of the conidia suspension was sprayed evenly onto 300 plants using an atomizer. Plants were placed into a mist chamber where a cool mist ultrasonic humidifier was used to supply constant mist. After 24 h of incubation at 20°C in the dark, plants

were incubated in a growth chamber with a 12 h photoperiod at 20°C.

Disease rating and data analysis

Seven days after inoculation, the lesion type was recorded based on a 0-9 visual rating scale (Table 2). The data were analyzed by split-plot design, with different sets as the main plots and genotypes as subplots. A one-way analysis of variance was used to estimate the between and within mean squares and variance components. Repeatability was estimated according to Falconer (1989).

Results and Discussion

Analysis of variance revealed that differences between replications within evaluation times (sets) were not significant (P = 0.402), while differences among genotypes were highly significant (P<0.001) at each evaluation time. The genotype by set interaction was not significant (P = 0.434). Results suggest that, under our conditions, a single replication provides reliable data for tan spot seedling screening evaluations.

One-way analysis of variance is given in Table 3. The estimate of repeatability is 0.905 (90.5%). The gain in precision from repeating measurements (shown in Figure 1), derived directly from the variance components, indicates that a moderate increase in precision (5%) may be obtained by repeating the screening evaluation a second time.

Repeatability is the ratio of the between individual components of variance to total phenotypic variance. From a breeder's viewpoint, the estimate of repeatability provides an evaluation of consistency of screening procedures

Table 2. Tan spot rating scale used in the study.

Rating	Symptom description
0	Small brown or black spots without any surrounding chlorosis.
1	Small dark spots dominate the leaf but a few spots are surrounded by a chlorosis ring.
2	Small black spots, but more than 50% are surrounded by a yellow expanding area.
3	Yellow spots with tiny brown points at their centers.
4	Yellow brown spots, few of which become necrotic.
5	Majority of the infection spots are small necrotic or chlorotic lesions marked with some tiny black lesions.
6	All lesions are necrotic with a clear border.
7	Some of the necrotic lesions begin to coalesce and become small necrotic coalescing blotches.
8	Most necrotic lesions coalesce into necrotic zones, and only a small portion of spots have distinguishable borders.
9	All lesions coalesce and the leaf is dead.

Table 3. One-way analysis of variance.

Source	df	Mean squares	EMS
Between individuals	36	21.67	$\sigma_w^2+m\sigma_b^2$
Within individuals	108	0.55	σ_w^2

Repeatability = $\sigma_b^2/(\sigma_b^2 + \sigma_w^2)$ = 5.28/(5.28 + 0.55)= 0.905

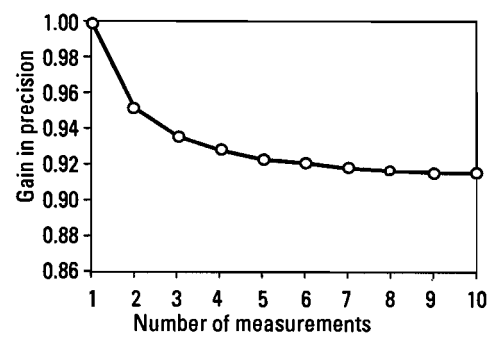

Figure 1. Gain in precision from multiple measurements.

repeated over time, or the reliability of the screening technique (Campbell and Carter 1990). For our greenhouse tan spot seedling screening method, repeatability of tan spot evaluation was high, indicating that sufficient precision exists for tan spot resistance selection in the greenhouse.

Repeatability also establishes the upper ceiling for heritability. As with heritability, repeatability is not a property of the trait *per se* but is a function of the genotype and environment in which the plants are grown and evaluated. When data from different sources are compared, evaluation technique should be taken into consideration. We found that a consistent and high spore concentration (~7000 spores ml^{-1}) could reduce variation. As the tan spot reaction is highly influenced by leaf age (Cox 1987; Hosford *et al.* 1990), evaluation at a specific leaf stage is critical.

The genotype x environment interaction is another reason for data variation. Further studies of plant evaluation in different environments should provide more information on the improvement of tan spot screening procedures. While seedling tan spot resistance evaluation appears to be consistent and reliable, more information on correlation between field adult screening and greenhouse seedling screening is necessary to assess the predictive value of the latter method. Future research in our laboratory will focus on the development of reliable field screening procedures of adult plants.

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New Approach for Clustering Breeding Genotypes Using Production Variables, Yield Losses, and a Double-Digit Disease Scale

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Abstract

Spot blotch resistance was scored in an non-replicated set of 198 bread wheat entries in Poza Rica, Mexico. A 00-99 double-digit scale (DD) was used which can be considered as two separate discrete variables with different disease level categories (0, 1, ..., 9). Continuous production variables were also measured: yield (YLD), 1000-grain weight (TGW), growth stage (GS; Zadoks' decimal code), and percent YLD and TGW loss (after comparison with a fungicide-protected plot in the same field). Genotypes were grouped according to three approaches: 1) using only DD data, 2) using only continuous variables GS, YLD, TGW, and percent loss, and 3) using both types of variables. In the first approach, a cluster analysis was conducted using Ward's minimum variance within groups. In the second method, genotypes were clustered based on the normal mixture method sequential to Ward. The third and new approach used the homogeneous conditional Gaussian (modified) method sequential to Ward. Classifications produced with the three methods were compared in order to choose the best strategy for selecting genotypes based on a simultaneous analysis of discrete disease data, yield, yield loss, and growth stage. In contrast to the two other methods, the third approach presented a balanced distribution of genotypes for both types of variables (discrete and continuous), allowing the identification of three groups of entries: 1) a group (G2) with low values for both disease digits, the highest average value for YLD and TGW under disease pressure, and the lowest average YLD and TGW losses; 2) a group (G4) with intermediate disease scores and the highest average YLD and TWG with fungicide protection; and 3) a group (G6) with the highest disease level, the lowest average YLD and TGW under disease pressure, and the highest average YLD and TGW losses. Breeders should carefully consider genotypes classified under G2, as they can be more useful than entries clustering in a group with a lower disease level.

An unreplicated experiment yields one observation per experimental unit per treatment and does not allow experimental error to be estimated; therefore, mean separation and comparison based on least significant difference (LSD) or Tukey are not possible. In agricultural sciences, it is common to measure several variables in the same experimental unit, i.e., presence

or absence of disease, grain yield response, plant height, etc. Thus, it is possible to use cluster analysis techniques to group treatments (genotypes) into homogeneous groups and be able to quantify and compare "within group" variability and "between group" variability.

In general, hierarchical cluster analyses are descriptive and geometric techniques; however, Wolfe (1970) introduced a more statistical approach to the problem of classification. He proposed the Gaussian mixture model, which is a combination of several multivariate variables with normal probability distributions, to estimate probabilities of membership for each individual into each group.

Materials and Methods

Statistical methods

The Gaussian mixture model (Wolfe 1970; McLachlan and Basford 1988) has been used to classify a random sample of n observations $x_1, x_2, ..., x_n$ obtained from a heterogeneous population into g homogeneous subpopulations, based on the measure of p continuous variables.

Lawrence and Krzanowski (1996) extended the Gaussian mixture model for clustering n observations into g underlying subpopulations using a mixture of continuous and categorical data. The model, called Homogeneous Conditional Gaussian Model (HGCM), combines the q possible levels of the categorical variables in one multinomial variable W, with $m = \prod_{k=1}^{q} C_k$ total levels.

The vector representing the j^{th} observation (individual) in the s^{th} multinomial cell X_{js} [s = 1, 2, ..., m (multinomial cells); j = 1, 2, ..., n_s (observations in the cell s)] has a size of (p+1) x 1, containing the p continuous variables and the multinomial variable. It is assumed that the distribution of the p continuous variables is multivariate, normal and depends upon the multinomial cell in which the observation is placed, the probability of observing an individual in cell s is p_s, and that each observation is drawn from a mixture of g subpopulations π_i, i = 1, 2, ..., g, with a proportion θ_i ($\sum_{i=1}^{g} \theta_i = 1$).

The model defines m x g cells and, assuming homogeneous variance-covariance, the probability density function in the p_i subpopulation is:

$$X_{js} \sim p_{is}\, Np(\mu_{is}, \Sigma) \qquad (1)$$

(i=1, 2, ..., g; s=1, 2, ..., m; j=1, 2, ..., n_s),

where $Np(\mu_{is}, \Sigma)$ is a random variable with vector of means μ_{is} and variance-covariance matrix Σ.

The solution is based on the maximum likelihood estimation of the cell parameters, and is an iterative process that starts with some estimators and continues until the successive values for $\hat{t}_{isj}$ are equal within the defined tolerance limits. The authors propose starting with a random grouping of the data into g groups and m subgroups within groups, and the usual maximum likelihood estimators for each parameter in each subgroup.

Franco *et al.* (1997c) proposed a modification to the HCGM model, called MHCGM, which assumes that the probability distribution function in the π_i subpopulation (Equation 1) is:

$$X_{js} \sim p_{is}\, N_p(\mu_i, \Sigma_i) \text{ or } X_{js} - p_{is}\, N_p(\mu_i, \Sigma) \quad (1a)$$

where the mean and variance-covariance of the continuous variables depend only on the i^{th} subpopulation instead of on the specific $(is)^{th}$ cell. The model assumes that the dispersion matrices and mean vectors are equal for all multinomial cells within each subpopulation, i.e., the distribution of the continuous variables is independent of the categorical variables.

The likelihood estimators of the parameters are:

$$\hat{\theta}_i = \sum_{s=1}^{m} \sum_{s=1}^{n_s} \frac{\hat{t}_{isj}}{n}\,, \quad \hat{p}_{is} = \sum_{j=1}^{n_s} \frac{\hat{t}_{isj}}{n\hat{\theta}_i}$$

$$\hat{\mu}_i = \sum_{s=1}^{m} \sum_{j=1}^{n_s} \frac{\hat{t}_{is} x_{sj}}{n\hat{\theta}_i}\,, \text{ and}$$

$$\hat{\Sigma} = \sum_{j=1}^{g} \sum_{s=1}^{m} \sum_{j=1}^{n_s} \frac{\hat{t}_{isj}(x_{sj} - \hat{\mu}_i)'(x_{sj} - \hat{\mu}_i)}{n}$$

where

$$\hat{t}_{isj} = \frac{\hat{\theta}_i p_{is} \exp\{(x_{sj} - \hat{\mu}_i)' \hat{\Sigma}^{-1} (x_{sj} - \hat{\mu}_i)\}}{\sum_{j=1}^{g} \hat{\theta}_i p_{ts} \exp\{(x_{sj} - \hat{\mu}_t)' \hat{\Sigma}^{-1} (x_{sj} - \hat{\mu}_t)\}}$$

is the estimated probability of membership of the js^{th} observation into the i^{th} subpopulation. Each observation is assigned into the subpopulation with the largest probability of membership.

The modification allows using sequential clustering strategy (Franco *et al.* 1997a, 1997b) where *priori* groups are first defined by a hierarchical method such as the Ward method (1963) with the Gower distance method (1971) implemented by Wishart (1986) using all (continuous and categorical) variables. Secondly, the MHCGM model is applied to the Ward groups in an attempt to improve their structure. This sequential strategy can be used with either the HCGM model (Ward-HCGM) or the MHCGM model (Ward-MHCGM) and allows the optimal number of subpopulations to be estimated using simple rules.

The approximate number of groups is first determined from the results of the Ward method using the upper tail rule (Wishart 1987). Different numbers of groups are then compared using the maximum likelihood criterion (Mardia *et al.* 1979), but always close to the number of groups found by the upper tail rule. The final number of groups is chosen as the g′ with the highest value of $X^2 = -2 \log L$, the largest increment in the likelihood, when comparing $\acute{g}$ vs. g′ using the likelihood criterion $L = L_g / L_{g'}$, $g<g'$.

We implemented two algorithms using the IML procedure in SAS (1990), which will fit either the HCGM model or the MHCGM model, and used the CLUSTAN (Wishart 1987) software for the Ward method with the Gower distance and upper tail rule.

For comparing the different strategies, the measures of affinity and distance between groups were used for the HCGM model proposed by Krzanowski (1983), adapted to the MHCGM model. The affinity coefficient between two groups i and j (ρ_{ij}) has two components: 1) the affinity due to the continuous variables

I_{ij}, associated with the Mahalanobis (1930) distance D^2, $I_{ij} = EXP\{-D^2_{ij}/8\}$, and 2) the square root of the product of the relative frequencies, due to the categorical variables. The coefficient for the MCHGM is:

$$\rho_{ij} = I_{ij} \Sigma^{m}_{s=1} (P_{is} = P_{js})^{1/2},$$

when p_{is} and p_{js} are the proportion of cases with the s^{th} value in the i and j groups, respectively. The affinity is then the product of the affinity due to the continuous variables by the affinity due to the categorical variables. The measure of distance adopted by Krzanowski (1983) is that proposed by Matusita (1956):

$$\Delta_{ij} = \{2(1-\rho_{ij})\}^{1/2}.$$

Experimental data

Resistance to spot blotch caused by *Bipolaris sorokiniana* was evaluated in Poza Rica, Mexico. During the 1994-95 growing season, 198 spring wheat genotypes were sown in an unreplicated experiment with one plot (1 x 0.75 m) per entry. Disease scores were recorded when most entries were at flowering stage (mid February) using the double digit scale for rating foliar disease intensity (Saari and Prescott 1975; Eyal *et al.* 1987). This 00-99 scale can be considered as two discrete variables, where digit 1 (D1) measures the height of the disease progress in the canopy, and digit 2 (D2) measures severity on ordinal 0-9 scale. Seven continuous variables, referred to as production variables in this paper, were also measured. Zadoks' scale (0-100 decimal code) was used to appraise growth stage at disease scoring time (Zadoks *et al.* 1979). Yield (g/plot) and 1000-grain weight were measured in the diseased plot and in a continuous plot sown with the same entries protected by six sprays (0.5 L ha^{-1}) of Folicur (ai tebuconazole). This allowed percent yield loss and 1000-grain weight loss to be evaluated. Spot blotch was assessed in a naturally infected field under the severe epidemic conditions annually prevailing in Poza Rica.

Results and Discussion

Six groups were formed when only D1 and D2 categories were used with the Ward method. Groups G1, G2, and G3 had low D1 and D2 values ($D1 \leq 8$, $D2 \leq 3$; Table 1); groups G4 and G5 had intermediate infection levels ($7 \leq D1 \leq 8$, $4 \leq D2 \leq 6$); and group G6 showed high infection levels ($D1 = 9$, $4 \leq D2 \leq 9$). The groups did not show important differences between means for the continuous variables; the range of group means of the continuous variables were smaller than in any of the other classification strategies. The Gaussian mixture model applied to the groups formed by using the Ward method, and using only continuous variables, produced five groups which showed a clear separation of means of continuous variables. The group means of the continuous variables showed the widest range; however, no clear pattern could be found when D1 and D2 variables were related to the five groups (Table 1).

The six groups formed using both categorical and continuous variables and applying the Ward-MHCGM sequential approach showed differences in the range

of the continuous variables similar to those obtained when only continuous variables were used for grouping. In addition, the groups had an infection pattern similar to that obtained when only categorical variables were used for clustering. It is interesting to note that G2 comprises nine genotypes with high yields under non-protected conditions and low values of grain yield losses; these characteristics are associated with low infection levels for both D1 and D2 (Table 2). Genotypes of group G2 should be the most promising material to be used in future breeding work.

It is interesting to observe that sister lines cluster in two very different groups (G2 and G6): CHIRYA 1, CS/TH.CU//GLEN/3/GEN/4/SUZ8, and MAYOOR (Tables 2 and 3). Results show smaller values for D1, D2, and for losses and higher values for production variables under non-treated conditions for entries belonging to group G2.

Table 1. Percentage of individuals and means of the groups formed using only categorical variables (Ward method), only continuous variables (Gaussian mixture), and both categorical and continuous variables (Ward-MHCGM).

	Categorical[1]							Continuous[2]							
D1	1-3	4-6	7-8		9										
D2	1-3	1-3	1-3	4-6	1-3	4-6	7-9	YU	YP	YL	GWU	GWP	GWL	GS	n
	%							means							
Ward groups using only categorical variables															
G1	10	39	51					58	127	54	22	30	28	69	39
G2		3	94		3			57	124	54	22	32	29	72	33
G3			100					55	127	56	22	31	29	72	47
G4				100				56	131	57	21	32	34	72	17
G5				100				57	153	63	22	33	31	74	22
G6						43	57	31	128	76	18	30	40	74	40
Range								27	30	12	5	3	12	5	
Gaussian mixture groups using only continuous variables															
G1	12	36	32	12		4	4	45	126	65	21	33	36	67	25
G2		62	15			15	8	65	126	48	24	27	11	74	13
G3	1	4	50	25	1	9	11	55	139	60	22	31	31	73	132
G4		20	80					71	87	19	25	31	21	72	10
G5			44	6		11	39	15	93	83	13	28	52	71	18
Range								57	52	64	12	4	41	7	
Ward-MHCM groups using categorical and continuous variables															
G1	15	38	46					51	125	60	21	31	32	66	26
G2		22	78					71	86	18	24	32	23	71	9
G3		3	60	25		10	3	46	110	60	19	30	35	71	40
G4		4	57	37	1			65	148	55	23	32	28	74	68
G5			60			33	7	60	118	49	23	27	14	75	15
G6			18	10		20	53	25	135	81	17	31	44	75	40
Range								46	62	63	7	6	31	8	

[1] D1, D2 measured on a 1-9 scale.

[2] Y = Yield (g/plot); GW = 1000-grain weight (g); GS = Growth stage (scale); U = Non-protected conditions; P = Protected conditions; L = Losses (%); n = Number of individuals in each group.

The mean Mahalanobis distances between groups for the continuous variables were 2.26, 39.40, and 27.30 for the groups formed based only on categorical variables (Ward), continuous variables (Ward-Gaussian mixture), and both categorical and continuous variables (Ward-MHCGM), respectively. The mean distances between groups for the categorical variables were 1.0 (maximum), 0.32, and 0.48, for the groups formed based on Ward, Ward-Gaussian mixture, and Ward-MHCGM, respectively.

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Table 2. Description of genotypes forming group G2.

Genotype	D1[1]	D2	YU	YP	YL	GWU	GWP	GWL	GS	PHT
CHIRYA.1 CIGM87.110-3PR-3M-2PR-1M-3PR-1B-0PR	8	3	79.0	103.0	23.3	28.0	37.0	24.3	74.0	75.0
CHIRYA.7 CIGM87.1017-6Y-2M-1PR-4M-3PR-3B-0PR	8	2	48.0	74.0	35.1	24.0	36.0	33.3	75.0	75.0
CS/TH.CU//GLEN/3/ALD/PVN/4/SUZ8 CIGM87.117-2Y-3M-3PR-1M-3PR-4B-0PR	8	2	40.0	53.0	24.5	20.0	26.0	23.1	72.0	85.0
CS/TH.CU//GLEN/3/GEN/4/SUZ8 CIGM87.524-2Y-3M-3PR-3M-3PR-4B-0PR	7	2	83.0	98.0	15.3	20.0	31.0	35.5	68.0	105.0
CS/TH.CU//GLEN/3/GEN/4/SUZ8 CIGM87.524-1PR-3M-2PR-2M-1PR-3B-0PR	8	2	55.0	69.0	20.3	24.0	32.0	25.0	67.0	110.0
CS/TH.CU//GLEN/3/GEN/4/SUZ8 CIGM87.524-2PR-4M-1PR-3M-1PR-4B-0PR	6	2	84.0	93.0	9.7	28.0	32.0	12.5	72.0	95.0
CS/TH.CU//GLEN/3/GEN/4/SUZ8 CIGM87.527-2Y-1M-1PR-2M-1PR-3B-0PR	8	3	90.0	105.0	14.3	28.0	32.0	12.5	72.0	100.0
MAYOOR CIGM84.295-1M-1PR-4B-0PR-0M-0Y-1PR-0M-2PR-4B-0PR	6	2	78.0	91.0	14.3	24.0	29.0	17.2	70.0	90.0
MAYOOR CIGM84.295-1PR-2B-0PR	8	3	85.0	89.0	4.5	24.0	30.0	20.0	72.0	90.0

[1] D1, D2 measured on a 1-9 scale; Y = Yield (g/plot); GW = 1000-grain weight (g); GS = Growth stage (scale); U = Non-protected conditions; P = Protected conditions; L = Losses (%).

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Table 3. Description of genotypes in group G6 that also appear in group G2.

Genotype	D1[1]	D2	YU	YP	YL	GWU	GWP	GWL	GS	PHT
CHIRYA.1 CIGM87.110-1PR-2M-2PR-4M-2PR-2B-0PR	9	9	16.0	96.0	83.3	16.0	29.0	44.8	74.0	90.0
CHIRYA.1 CIGM87.110-1PR-2M-2PR-4M-2PR-4B-0PR	9	9	22.0	137.0	83.9	12.0	30.0	60.0	74.0	100.0
CHIRYA.1 CIGM87.110-1PR-2M-2PR-4M-3PR-1B-0PR	9	9	13.0	138.0	90.6	12.0	29.0	58.6	74.0	95.0
CHIRYA.1 CIGM87.110-1PR-2M-2PR-4M-3PR-2B-0PR	9	9	18.0	94.0	80.9	12.0	28.0	57.1	74.0	100.0
CHIRYA.1 CIGM87.110-1PR-2M-2PR-4M-3PR-3B-0PR	9	9	18.0	152.0	88.2	16.0	31.0	48.4	72.0	95.0
CHIRYA.1 CIGM87.110-1PR-2M-2PR-4M-3PR-4B-0PR	8	6	19.0	140.0	86.4	16.0	33.0	51.5	72.0	105.0
CHIRYA.1 CIGM87.110-3PR-2M-4PR-4M-2PR-4B-0PR	9	9	30.0	89.0	66.3	16.0	31.0	48.4	75.0	85.0
CHIRYA.1 CIGM87.110-3PR-4M-1PR-2M-1PR-1B-0PR	7	2	39.0	164.0	76.2	16.0	35.0	54.3	76.0	85.0
CHIRYA.1 CIGM87.110-3PR-4M-1PR-2M-1PR-2B-0PR	8	6	38.0	159.0	76.1	20.0	36.0	44.4	75.0	85.0
CHIRYA.1 CIGM87.110-3PR-4M-1PR-4M-1PR-3B-0PR	8	6	40.0	137.0	70.8	20.0	34.0	41.2	75.0	85.0
CHIRYA.1 CIGM87.110-3PR-4M-2PR-4M-3PR-2B-0PR	8	6	26.0	102.0	74.5	16.0	32.0	50.0	76.0	80.0
CHIRYA.1 CIGM87.110-3PR-4M-2PR-4M-3PR-3B-0PR	9	9	36.0	119.0	69.7	16.0	33.0	51.5	76.0	105.0
CHIRYA.1 CIGM87.110-3PR-4M-2PR-4M-3PR-4B-0PR	9	9	21.0	139.0	84.9	20.0	33.0	39.4	76.0	80.0
CS/TH.CU//GLEN/3/GEN/4/SUZ8 CIGM87.524-2Y-3M-1PR-3M-2PR-1B-0PR	8	2	10.0	139.0	92.8	16.0	33.0	51.5	72.0	115.0
MAYOOR CIGM84.295...	8	2	31.0	139.0	77.7	20.0	36.0	44.4	74.0	75.0
MAYOOR CIGM84.295...	8	2	35.0	137.0	74.5	20.0	27.0	25.9	75.0	75.0

[1] D1, D2 measured on a 1-9 scale; Y = Yield (g/plot); GW = 1000-grain weight (g); GS = Growth stage (scale); U = Non-protected conditions; P = Protected conditions; L = Losses (%).

Screening Wheat for *Bipolaris sorokiniana* Resistance in Vietnam

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Abstract

Spot blotch, caused by Bipolaris sorokiniana (Helminthosporium sativum *syn.* Cochliobolus sativus), *is one of the most important diseases of wheat in northern Vietnam, particularly in the Red River Plains (lowlands). In 1987 and 1988, early maturing and spot blotch resistant wheat lines (Long 83-3393, Yiu 83N-6148, Yiu83-5069, and Vee/Buc) were selected from CIMMYT, Thailand, sources. New sources were received from CIMMYT, Mexico. Of 1751 lines introduced between 1991-1995, 198 were selected for spot blotch resistance. Lines showing the best resistance and good agronomic type were: Chyria-1, Chyria-3, SHA3//BUC/FLK, Yang Mai 6, Mayoor, and Chyria-1. They produced yields of up to 5 t ha^{-1}. Most are mid-maturing lines with moderate susceptibility to powdery mildew.*

Vietnam is a tropical country in Southeast Asia, located between 9 and 23°N latitude, with a total area of around 32,955,600 ha. Population has increased from 64 to 74 million people during 1989-1996. Rice is the staple food, but wheat products are becoming more common in the Vietnamese diet. Although rice exports are 1-3 million t per year, wheat flour imports increased from 300,000 to 800,000 t in 1996.

In the northern part of Vietnam (Red River Plains, Hanoi), winter temperatures vary between 24.6°C in October and 18-20°C in November to March. Winter rainfall is low at around 100 mm and relative humidity (RH) levels are 80-88% (Table 1).

An attempt to develop wheat as a commercial crop in Vietnam failed due to a lack of disease resistant material. The climate is favorable for wheat growing but also for disease development. In the Red River Plains (lowlands), wheat production is limited by disease, in particular by spot blotch, caused by *Bipolaris sorokiniana*.

Breeding Objectives

The first wheat breeding objective is selection for resistance to *B. sorokiniana*. Other traits selected for are: early maturity, powdery mildew resistance, fusarium head scab resistance, leaf rust resistance, and yield stability.

Materials and Methods

A total of 1872 bread wheat entries have been introduced, most of them originating from CIMMYT. During 1987-

1992, 765 entries were introduced from CIMMYT, Bangkok. During 1990-1995, 1107 entries were introduced from CIMMYT, Mexico, including the warm areas wheat screening nurseries (941 entries) and the helminthosporium monitoring nurseries (165 entries).

Planting was carried out from October to December to examine disease development and wheat response to climatic conditions. A 0-9 scale was used to evaluate foliar infection level. Four evaluations were made for early maturing materials and six for moderate and late maturing materials. Selection for resistance was made at the soft dough stage (when spikes and peduncle were yellow) based on following traits: a clean and bright head, 'stay green' leaf, and good agronomic type.

Screening Results

Early maturity

Variety Sonalika was used as the early check. Of 1872 bread wheats, the percentage of early maturing varieties was low (12%), with most entries being intermediate to late maturing (40.8% and 47.1%, respectively). Number of days to maturity of early varieties was reduced by sowing in early October (90-95 days; Table 2). Sowing under cooler temperatures (November and December) increased the number of days to maturity to 100-105, some early lines showed spot blotch resistance, and yields higher than that of Sonalika were obtained.

Number of days to maturity of the intermediate materials was also reduced by early October planting (100-110 days) compared with planting in November and December (115-125 days). Intermediate material (100-110 days) was

Table 1. Climatic conditions in northern Vietnam.

Average temperature (°C)							
	Month						
Variety	10	11	12	1	2	3	4
Hanoi	24.6	21.2	17.9	16.6	17.1	19.3	–
Cao Bang	22.4	18.7	15.0	14.0	15.1	18.7	22.6
Son La	21.3	18.1	15.3	14.5	16.5	19.9	22.8
Relative humidity (%)							
Hanoi	85.0	81	81	80	84	88	–
Cao Bang	81	82	80	78	79	81	80
Don La	84	83	82	78	77	73	74
Rainfall (mm)							
Hanoi	123	47	20	18	26	48	111
Cao Bang	84	27	20	16	34	48	118
Son La	6.3	3.9	2.1	2.25	42	103	75
Hours sunshine							
Hanoi	186	148	121	85	54	47	93
Cao Bang	-	38	47.5	57.1	83.4	-	-
Son La	185	150	157	150	125	163	190

accepted for the cropping system: summer rice-wheat-spring rice (the latter transplanted from 25 February).

Bipolaris sorokiniana Resistance

Spot blotch seriously affects wheat in the Red River Plains. The disease affects all plant parts; seedling blight, white heads, and node infection have been observed. In the region, spot blotch affects mainly the leaves and spikes of wheat, usually from heading to grain filling. Climatic conditions vary between years and seasons. Conditions most favorable for spot blotch development are high temperatures (18-24°C) and RH (80-88%). Consequently, natural disease epidemics do not occur frequently in the area from October to January; however, spot blotch has caused serious damage in years of favorable climatic conditions from 20 December to 10 January, 1989/90, 1990/91, and 1992/93.

Spot blotch symptoms vary among wheat genotypes. On resistant material, small dark brown spots initially appear. Approximately 15-20 days after infection, lesions become surrounded by chlorotic margins and spot blotch development is limited. The flag leaf remains green until the peduncle turns yellow. On susceptible material, yellow or large dark brown spots appear on the leaves and quickly develop, causing leaf death (leaf is totally yellow within two weeks).

Diseased leaf samples were observed under the microscope to identify fungi by shape of conidia. Results showed that conidia were *B. sorokiniana*.

A study was carried out on kernel weight per spike at milk stage under different levels of spot blotch infection (Feekes' scale: 11.1, 20 days after heading in Hanoi). Infection level was found to significantly influence kernel weight per spike; the correlation coefficient was negative and high (r = 0.96; Table 3).

Lines CS/A.CUR//GLEN/3/ALD/4/NING MAI No. 4/OLESON//ALD/YANG MAI 4 (F8 MESTE, 387) and (F8 MESTE, 389), which scored 3 on the scale, had high spike weights of 1.4g and 1.26g, respectively. Lerma Rojo 64, which is susceptible, scored 9 and had a low spike

Table 2. Advanced bread wheats lines equal in earliness to Sonalika, but with better *Bipolaris sorokiniana* resistance and higher yield.

Name/pedigree	Spot blotch at milk stage (0-9)	Yield		Days to maturity
		(kg ha^{-1})	% of Sonalika	
Long 83-3393	3	3046	163	92
Vee/Buc	3	2632	141	90
IA 58/4/Kal/BB//CJ/3/ALD"S"	4	2540	136	90
KEA"S"	3	2356	126	90
Kharchea	4	3050	163	95
FCH3/TRT//Vee #9	3	2645	141	95
Sonalika (check)	8	1863	-	95

Note: Average of three years data (1991-1993), Van Dien, Hanoi. Sowing date: 10 October.

weight (0.48g). Among the 1872 bread wheats, 142 lines and varieties showed spot blotch resistance. Some of the best lines are listed in Table 4.

Table 5 shows new sources with the highest yields and *B. sorokiniana* resistance levels in Hanoi. The best line is CS/TH.CU//GLEN/3/GEN/4/SUZ8, yielding 4412 kg ha^{-1} compared with 2100 kg ha^{-1} of check variety Cao Bang. Most new sources have a higher kernel weight per spike (1.2-1.7 g) and good test weight (77-79 kg hl^{-1}).

Selection for only spot blotch resistance was not practical. Resistance to a complex of diseases is required, as well as adaptation traits for humid conditions such as sprouting resistance. Sprouting resistance was tested for in the spot blotch resistant materials. Of 100 entries, three lines and varieties showed the best results after 10 days at 18-24°C and free moisture for emergence. Cao Bang, Long 82-2124-1, and CB (BW) 362 had sprouting levels of 3 %, 5%, and 5 %, respectively.

Conclusions

Traits of early maturity combined with spot blotch resistance were obtained in lines Long 83-3393, Vee/Buc, and FH3/TRT//Vee #9, which are suitable for early sowing in October. New sources of spot blotch resistance from CIMMYT showed the best resistance; however, these are still susceptible to powdery mildew. The supply by CIMMYT of germplasm with improved spot blotch

Table 3. Correlation coefficient between kernel weight per spike and spot blotch infection level of some bread wheats.

Name/pedigree	Weight/ spike (g)	Infection level (0-9)
Cumpas	0.44	9
Nacozari 76	0.59	8
Tobari 66	0.66	6
Cleoparra 74	0.77	7
Lermarojo 64	0.48	9
Sonora 64	0.68	7
YECORA 70	0.59	7
CS/A.CURV ... 170 (F8 MESTE 387)	1.40	3
CS/A. CURV ... 171 (F8 MESTE 389)	1.26	3
Long 83 - 3393	1.30	3
Sonalika (check)	0.70	7
r = -0.96		

Note: Measurements taken 20-25 days after heading, Van Dien, Hanoi.

Table 4. Lines with highest levels of spot blotch resistance.

Name/pedigree	Spot blotch level at milk stage (0-9)
Cao Bang	2
Thanh Uyen	3
Mayoor	3
Chirya - 1	3
Chirya - 3	3
Chirya - 7	3
CN079/4/cs/...195	3
CN079/4/cs/...196	3
Long 83 - 3393	3
Long 82 - 2124 - 1	3
Yiu 83N - 6148	5
Yiu 83 - 5069	3
Yiu 83N - 5262	3
995	4
889	3
KEA/SUzoE - 6	3
SHANG HAI - 8	3
YANG MAI - 6	3
CB (BW) 362	3
SHA3//Buc/FLK	3
FCH3/TRT//vee9	3
CS/A.CUR.../170(Fg MESTE 387)	3

Note: Data averaged over three to eight seasons, Ven Dien, Hanoi.

resistance accelerates our work. However, we hope CIMMYT will be successful in combining this with resistance to other biotic constraints that are part of the disease complex present in Vietnam.

Acknowledgment

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Table 5. Characteristics of lines with the highest *Bipolaris sorokiniana* resistance levels and yields in Hanoi.

Name/pedigree	Yield (kg ha^{-1})	Days to maturity	Height (cm)	Spikes m^{-2}	Weight (g) 1000-grain	Weight (g) Spike	Disease[1] H	Disease[1] E.g	Test weight (kg hl^{-1})
CS/TH.CU//GLEN/3/GEN/4/SUZ8 IBS-15	4412	125	106	277	39.2	1.59	1	0	76.6
CH4227/TRM//MAD/3/VEE IBS-29	3842	115	105	223	35	1.72	1	0	77.6
CHIRYA-3 IBS-85	3628	125	86	237	38	130	3	0	79.2
CHIRYA-3 IBS-84	3619	125	84	219	39	1.65	3	0	77.8
CHIRYA-3 IBS-62	3514	125	80	283	32	1.24	1	0	75.2
SANTA ANA 88 IBS-35	3408	125	87	223	40	1.53	1	0	78.8
MAYOOR IBS-56	3394	125	105	255	32	1.33	1	0	79
A6/GLEN IBS-20	3312	125	100	276	40	1.2	1	0	76.8
CS/TH.CU//GLEN/3/GEN/4/SUZ8 IBS-100	3076	115	95	183	38	1.68	3	5	79.2
CS/TH.CU//GLEN/3/GEN/4/SUZ8 IBS-99	3054	115	95	196	37	1.56	3	5	76.8
CHIRYA-3 IBS-88	3043	125	83	227	34	1.34	3	0	79.0
CS/TH.CU//GLEN/3/GEN/4/SUZ8 IBS-97	2942	115	93	203	34	1.45	3	5	78.6
CS/TH.CU//GLEN/3/GEN/4/SUZ8 IBS-98	2874	115	93	215	36	1.34	3	5	76.8
CHIRYA-3 IBS-87	2861	125	87	210	34	1.36	3	0	77.8
CHIRYA-7 IBS-102	2828	125	90	210	33	1.35	3	0	77.2
CNO79/4/CS/TH.CU//GLEN/3/ALD/PVN N IBS-105	2591	120	96	186	34	1.39	3	0	78.8
CAO BANG (resistant check)	2100	145	106	250	17	0.84	1	0	77.2
CHIRYA-7 IBS-101	2057	130	90	150	36	1.37	3	0	73.8

Note: Sowing date: 5 October 1995.
[1] H = *Bipolaris sorokiniana*; E.g = *Erysiphe graminis.*

Tan Spot Resistance in Tetraploid and Hexaploid Wheat

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Abstract

Tan spot is a member of the leaf-spot complex that attacks wheat in Saskatchewan each year and can cause significant yield losses. Tan spot is particularly important on durum wheat (pink smudge phase) and winter wheat. As part of a program to breed for tan spot resistance in wheat, a range of wheat genotypes was evaluated at the two-to-three-leaf stage for reaction to Pyrenophora tritici-repentis *in growth room tests. Sources of resistance, some believed to be previously unreported, were identified in both durum and common wheat. Resistance to tan spot has also been transferred from* Triticum timopheevii *(PI 290518) into durum wheat cv. Wakooma to produce resistant sib-lines S3-6, S9-10, and S12-1. Preliminary genetic studies of resistant tetraploid and hexaploid wheats indicate that resistance is simply controlled, involving one or two genes. Evidence for linkage of tan spot resistance and resistance to septoria nodorum blotch to red kernel color was found in line S9-10. More detailed inheritance studies are planned. Attempts to identify RAPD markers to use in marker-assisted selection for tan spot resistance have commenced. Preliminary results indicate that marker UBC521-650 is loosely linked (15% recombination) to tan spot resistance in the cross S9-10/Sceptre.*

In the central and northern cropping areas of Saskatchewan, tan spot, caused by *Pyrenophora tritici-repentis*, occurs as part of the wheat leaf spot complex that also includes septoria nodorum blotch and septoria tritici blotch. This leaf spot complex is estimated to cause an average 15% reduction in grain yield and to affect grain bread-making quality (G.R. Hughes, unpublished data). Tan spot is found in most wheat fields each year, but severity varies in this cropping region. However, in recent years, tan spot has become the predominant leaf-spotting disease on durum wheat. Spot blotch, caused by *Cochliobolus sativus*, is generally only a minor disease in this region.

Because none of the current commercially grown bread and durum wheat cultivars possess good resistance to tan spot, some years ago we initiated a research program to identify useful sources of resistance, to determine the genetic control of resistance in these sources, and, more recently, to identify RAPD markers to permit marker-assisted selection for tan spot resistance. Our immediate breeding objectives are to incorporate resistance into adapted durum and winter wheat cultivars. This paper will report briefly on our progress, with particular emphasis on durum wheat.

Screening Methodology

A combination of growth room and field tests is used. In the growth room tests, inoculum is prepared according to the procedure described by Lamari and Bernier (1989). Seedlings are inoculated at the two-leaf stage with a single isolate of *P. tritici-repentis* (Ptr 200, collected at Aylsham, SK) at a concentration of 3-4 $x10^3$ spores per ml. After inoculation, plants are placed in a moist chamber for 24 h and then moved back to growth room benches. The moist chamber is located in the growth room which runs at 21/16°C day/night temperature and a 16 h photoperiod. Plants are rated using the scale proposed by Lamari and Bernier (1989), seven days after inoculation. Plants rating 1 or 2 on this scale are considered resistant.

In field tests, both infected straw and spore suspension are used as inoculum. The infected straw is spread in the plots soon after seedling emergence. Plots are sprayed in the evening with the spore suspension at the three-leaf and flag-leaf stages and irrigated by a low-volume irrigation system for 10 minutes each hour for the next 12 h. To enhance disease spread, this same irrigation schedule is followed twice each week during the growing season. Plots are rated at the flag-leaf and mid-milk stages using a 1-10 scale, which considers both disease severity and spread on the three highest leaves. On this scale, plants rating 6 (5-10% severity on the flag leaf) at the flag-leaf stage are classed as resistant, and rating 7 (10-25%) are classed as moderately resistant.

Screening for Resistance

Both tetraploid wild and adapted cultivars and hexaploid wheats have been screened for resistance. Of 390 tetraploid accessions from the USDA wheat collection tested in growth room seedling tests, only one, *T. dicoccon* PI 254190, gave a resistant reaction (rating 1) in duplicate tests. Fourteen accessions were moderately resistant in both tests: *T. carthlicum* CI 7692; *T. dicoccon* accessions CI 3686, CI 4572, CI 9311, CI 12213, PI 254178, PI 254190, PI 254193; *T. turanicum* accessions CI 11390, PI 254201, PI 254212; and *T. turgidum* accessions CI 3270, CI 7877, CI 8077, CI 13713. Other resistance sources identified are listed in Table 1.

Genetic Control of Tan Spot Resistance

Hexaploid wheat

Preliminary studies based on seedling tests of the F1 generation and F3 or BCF2 families of the crosses 81IWWMN2095, Hadden, and ZG7581-83 with susceptible cv. Kenyon suggested that resistance is partially recessive and controlled by a single gene (Table 2).

Tetraploid wheat

Three resistant, red-seeded sib-lines, S3-6, S9-10, and S12-1, were extracted from the cross *T. timopheevii* (PI 290518)/2*durum cv. Wakooma. Since Wakooma is susceptible, these lines represent a successful attempt to introgress tan spot resistance from *T. timopheevii* into a *T. durum* background.

F2-derived F5 families of crosses of S3-6, S9-10, and S12-1 with susceptible durum cv. Sceptre were tested at the two-leaf stage for reaction to tan spot. In all crosses, family segregation ratios fitted those expected for two-gene control of resistance (Table 3). Red kernel color was also found to be controlled by a single gene in each cross (data not shown).

Table 1. Seedling and field reactions of durum and common wheat cultivars to tan spot in seedling and field tests.

Cultivar	Source	Rating GR[1]	Rating Field[2]
Durum wheat cultivars			
Joda	CIMMYT	2	
Oscar	CIMMYT	2-3	
Pabellon	CIMMYT	1-3	
Sham 3	CIMMYT	1-2	
WL5023	CIMMYT	1-2	
S3-6, S9-10, S12-1 (sib lines) *T. timopheevii*/Wakooma	Saskatoon	1-2	
Spring wheat lines from CIMMYT			
Vee#7/Bow"S"	#3-92MREHTRBW	1	5-7
Car853/Coc//Vee#5"S"/3/Ures	#14-92MREHTRBW	1	7
Car853/Coc//Vee"S"/3/E7408/Pam"S"//Hork"S"/PF73226	#27-92MREHTRBW	1-2	7
Car853/Coc//Vee"S"/3/E7408/Pam"S"//Hork"S"/PF73226	#28-92MREHTRBW	1	5-7
Car853/Coc//Vee"S"/3/E7408/Pam"S"//Hork"S"/PF73226	#29-92MREHTRBW	1	5-7
Car853/Coc//Vee"S"/3/E7408/Pam"S"//Hork"S"/PF73226	#31-92MREHTRBW	1	7
ND/VG9144//Kal/BB/3/Yaco"S"/4/Vee#5"S"	#35-92MREHTRBW	1	7
Other spring wheat cultivars			
86IWMN 2137		1-2	6
Erik		1	5
Winter wheat cultivars			
81IWWMN 2095 (Maris Huntsman/2/VPM/Moisson)		1-2	
Hadden		2	
Red Chief		1	
ZG7581-83 (from Zagreb; pedigree not known)		2	
Kenyon (susceptible)		4-5	9-10

[1] Rating of plants tested at the three-leaf stage (1 = resistant).
[2] Rating of plants after heading using a 1-10 scale.

Table 2. Tests of segregation data for tan spot resistance from seedling tests of three crosses of hexaploid wheat.

Cross	Number tested	Ratio tested[1]	P value
81IWMN2095 x Kenyon BC1F2 families	88	1:1	0.50-0.75
Hadden x Kenyon F3 families	46	1:2:1	0.75-0.90
ZG7581-83 x Kenyon F3 families	58	1:2:1	0.10-0.25

[1] Ratios represent R:Seg:S for F3 families and Seg:S for BC1F2 families.

Linkage of Tan Spot Resistance in Tetraploid Wheat

Since previous work showed that these lines were also resistant to septoria nodorum blotch (snb) and that resistance was controlled by a single gene, *SnbTM* (Ma and Hughes 1995), the F5 families were tested for septoria nodorum blotch reaction to determine if linkage existed between the genes controlling resistance to both diseases. The results suggested that:

- septoria nodorum resistance was linked to red kernel color in lines S3-6, S9-10 and S12-1;
- tan spot resistance was linked to red kernel color in lines S9-10 and S12-1; and
- tan spot resistance was linked to septoria nodorum resistance in line S9-10 and possibly S12-1.

As the gene for snb resistance has been located on chromosome 3A, it is hypothesized that the gene for red kernel color is *R2* and that the snb resistance gene is on chromosome 3AL (the location of *R2*). This also suggests that at least one gene controlling tan resistance is also on chromosome 3AL. The reason for the absence of linkage between tan spot resistance and red kernel color or snb resistance in S3-6 is not known. One possible explanation is a translocation involving the chromosome segment carrying the tan spot gene, but not the other gene loci of interest; this translocation could have occurred during the extraction of the sib-lines.

Table 3. Tan spot reaction tests in F2-derived F5 families of crosses of tetraploid resistant lines S3-6, S9-10, and S12-1 with susceptible cv. Sceptre.

Cross	Families tested	P value[1]
S3-6 x Sceptre	58	0.75-0.90
S9-10 x Sceptre	75	0.10-0.25
S12-1 x Sceptre	71	0.10-0.25

[1] Family segregation ratios were tested for goodness-of-fit to the ratio 0.191 homozygous resistant:0.618 intermediate/segregating:0.191 homozygous susceptible, expected in the F2-derived F4 generation.

Search for RAPD Markers for Tan Spot Resistance

Bulked segregant analysis was used to identify primers producing RAPD markers potentially linked to tan spot resistance in tetraploid lines S3-6, S9-10, and S12-1. After screening over 200 primers on 5-line resistant and susceptible bulks created with F2-derived F5 lines of cross S9-10, primer UBC521 was the only primer found to give reproducible results and produced a polymorphism for a 650 bp fragment associated with tan spot resistance.

Preliminary data suggest marker UBC521-650 is linked to tan spot resistance in cross S9-10 with 15% recombination and to septoria nodorum resistance in all three crosses with an average recombination of 11%. To date, no linkage between UBC521-650 and tan spot resistance in crosses S3-6/Sceptre and S12-1/Sceptre has been found. While these results agree with the genetic linkage study, they need repeating to resolve certain inconsistencies and to provide a better database.

Conclusion and Future Studies

Useful levels of resistance to tan spot were found in both tetraploid and hexaploid wheats. Resistance in tetraploid wheats was found in both durum cultivars and in related species, but in both cases the frequency of occurrence of high resistance levels was less than found in hexaploid wheat.

Based on studies of three crosses of tetraploid wheat, genetic control of resistance derived from the related species *T. timopheevii* was due to two genes. This suggests that the two-gene model proposed by Lamari and Bernier (1991) to explain the genetic control of the necrosis and chlorosis symptoms caused by the tan spot fungus may apply here. However, in tests with the same *P. tritici-repentis* isolate (Ptr 200), resistance in three hexaploid cultivars appeared due to only one gene. Studies on resistance to the necrosis toxin in these crosses may help to elucidate this and additional generations of these crosses will be studied. As well, additional populations have been generated to investigate the control of resistance in other tetraploid and hexaploid resistance sources.

Evidence for linkage between tan spot resistance, red kernel color, and septoria nodorum resistance was found in tetraploid wheat crosses of those studied, and agreed with preliminary data from studies to identify RAPD markers. However, insufficient data was available to permit a reliable estimate of the degree of linkage. The search for other RAPD markers linked to tan spot resistance in both the tetraploid and hexaploid resistance sources will continue. The RAPD marker UBC521-650, which is loosely linked to the S9-10 resistance with about 15% recombination, will be of only limited value for marker-assisted selection.

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Novel Genetic Diversity for Stress Tolerance in the Triticeae: Strategic Avenues and Applied Potentials

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Abstract

Plant breeders exploit conventional crop improvement methods to meet the ever-increasing demands for food production. However, among cultivated crops they are finding less and less germplasm having desired traits with which to make the needed improvements. Fortunately, new and useful genetic resources are being found in wild uncultivated plant species that have a close or distant genetic relationship to food crops. The challenge is to expediently incorporate this alien germplasm into existing food crops and simultaneously sustain genetic diversity. In the Triticeae tribe, these goals are being addressed through intergeneric and interspecific hybridization methodologies. Aided by critical embryo/tissue culture techniques, researchers have ingeniously produced an ample array of wide hybrids among Triticeae species. The current status suggests that hybridization barriers can be readily circumvented and novel germplasm developed. Simultaneously, conventional and molecular diagnostics have evolved to the level where alien introgression detection is no longer a complex process. To speed up alien introgression and production/maintenance of genetic stock programs, use of polyhaploidy through sexual hybridization of bread wheat with maize, pearl millet, sorghum, and Tripsacum *has emerged as a stable technique. The above areas form a package that facilitates exploitation of alien genetic diversity for wheat improvement. These views shall be elucidated while demonstrating our main emphasis on the D genome synthetic hexaploid germplasm developed in CIMMYT.*

Spot blotch (leaf blotch) of wheat (*Triticum aestivum* L.), caused by *Cochliobolus sativus* (Ito & Kuribayashi) Drechs. ex Dastur (syn., *Bipolaris sorokiniana* (Sacc.) Shoemaker, *Helminthosporium sativum* Pammel, King & Bakke), is an important pathogen that limits production in many non-traditional hot and humid wheat producing areas of Asia, Africa, and South America. *Cochliobolus sativus* can attack seedlings, roots, leaves, nodes, spikes, and grains during various stages of plant development.

Estimates of wheat yield loss due to spot blotch vary widely. Losses of 85% were reported from Zambia, and 40% from field trials in the Philippines. In addition, in an experiment conducted at Londrina, Brazil, yield losses in the highly susceptible cultivar Mitacore ranged between 79 and 87%, and grain quality was severely affected. De Milliano and Zadoks (1985) found a 38% yield loss using African wheat cultivars in growth-chamber studies in the Netherlands. Because of the importance of spot blotch,

chemical control is applied in many parts of the world in order to obtain crop production stability. Emphasis is also being given to an integrated pest management approach, utilizing resistant cultivars, healthy seed, cultural practices, and chemical sprays. Though breeding for resistance is a high priority, it is hampered by scarcity of adequate resistance within *T. aestivum*.

Sources of *C. sativus* resistance in species other than *T. aestivum* (i.e., alien gene pools) are of special interest in breeding programs and we, at the International Wheat and Maize Improvement Center (CIMMYT), have been making some effort to incorporate and exploit alien resistance genes in a wheat background.

Our program that has produced significant results so far encompasses two distinct methodologies aimed at introgressing alien genetic diversity:

1. Intergeneric hybridization route for incorporating distant gene pools concentrated around the E genome, and
2. Interspecific hybridization aimed at utilizing closely related genetic diversity focused on the D genome present in *T. tauschii* accessions.

The objectives of this paper are to elucidate the current status of *C. sativus*-resistant germplasm that has emanated from use of the above alien genetic resources, and document the practical potential of some of the incorporated genetic variation.

Materials and Methods

Study 1: Categorization of germplasm resistant to *C. sativus* from intergeneric sources

a) *Thinopyrum curvifolium* (2n=4x=28, $E_1E_1E_2E_2$). The hybridization and advanced procedures of *Th. curvifolium* with wheat have been described by Mujeeb-Kazi *et al.* (1987). Field screening of the derived germplasm under a natural epidemic location of the advanced derivatives was conducted in Poza Rica, Mexico (21°N, 60 masl), during winter cycles (November-March) over three years. Resistance of the selected resistant germplasms to *C. sativus* was based on damage recorded on leaves, nodes, spikes, and grains. Comparison of this resistant germplasm was made with BH1146, a resistant check, and Ciano 79, a susceptible check.

b) Several other basic genetic stocks from wheat/alien Triticeae were also screened in Poza Rica over two years (1994 and 1995) using similar criteria as mentioned above. These genetic stocks have been developed and maintained in CIMMYT since 1980.

Study 2: Evaluation of the D-genome germplasm for resistance to *C. sativus*

Germplasm—*Triticum tauschii* (2n=2x=14, DD) accessions were obtained from the CIMMYT germplasm bank in Mexico (El Batán) and from researchers in

Pakistan (N. Hashmi, National Agriculture Research Council, Islamabad), UK (C. Law, AFRC-IPSR), and USA (B. Gill, Kansas State; G. Kimber, University of Missouri; R. Metzger, then at Oregon State; G. Waines, University of California, Riverside). A total of 490 accessions were acquired and increased for seed quantity prior to their utilization by a vernalization procedure (8°C, 8 h of light for 8 weeks). After the accession seed increase, additional *T. tauschii* seedlings similarly vernalized were also transplanted to the field cycles in Ciudad Obregon, Mexico, during November to May for hybridization to *T. turgidum*.

Hybridization, embryo rescue and plantlet regeneration—Forty-eight elite durum wheat (*T. turgidum*) cultivars were planted over four dates at 10-day intervals in order to correspond with *T. tauschii* pollen availability. Emasculation, pollination, embryo rescue, and regeneration procedures were similar to those reported earlier (Mujeeb-Kazi *et al.* 1987).

Cytology of hybrids, colchicine doubling, and cytology of amphiploids—From each potted hybrid plantlet, root-tips were collected and somatically analyzed (Mujeeb-Kazi and Miranda 1985) to validate hybridity. The hybrid plants possessing 2n=3x=21 chromosomes were treated with 0.1% colchicine + 2.0% dimethyl-sulfoxide for 6 h via aerated root-treatment to double the chromosome number in order to obtain fertile amphiploids (2n=6x=42). All amphiploid seed produced was germinated and somatically analyzed to validate the amphiploid status and to obtain a seed increase.

Cochliobolus sativus *disease screening*—The 570 amphiploid synthetic hexaploid (SH) combinations, their durum parents, and the susceptible Ciano 79 and resistant BH 1146 bread wheat cultivars were each planted in replicated hill plots in Poza Rica, Mexico, November 1994 and 1995, for *C. sativus* screening. Disease evaluations were based on foliar infection and grain blemish at maturity. A double digit scale was used to measure foliar infection, where the first digit equated to the height of infection and the second digit to the infection severity. Scale gradations were 1-9. For height of infection, a score of 5 was for plants with infection up to the plant center, and for a score of 9 the infection had spread to the flag leaf. A disease severity score of 1 was for infected leaves exhibiting low disease symptoms, whereas a score of 9 reflected total leaf destruction. Grain infection at maturity was scored on a 1-5 scale, with 1 being low and 5 representing a high seed blemish at embryo points.

Results and Discussion

Study 1: Intergeneric source of resistance

Five lines were identified and characterized as resistant to *C. sativus* and their agronomic attributes established. Data are presented in Tables 1 and 2. The intergeneric cross pedigree was Chinese Spring/*Th. curvifolium*//Glen/3/Alondra/Pavon. These lines have been

registered (Mujeeb-Kazi *et al.* 1996), two are called Chirya and Mayoor, and all germplasms have been widely distributed. These lines are being utilized for wheat improvement in the breeding programs of CIMMYT, several national programs, and other collaborators. Critical molecular work is needed to possibly diagnose the alien introgression in the germplasm from *Th. curvifolium.*

Table 1. Disease reactions of five spring wheat germplasm lines to *Cochliobolus sativus* at Poza Rica, Mexico.

Germplasm	Leaves[1] a	Leaves[1] b	Spike[2] (1-9)	Grain[3] (1-5)
WX-CIGM-295-1	93	94	2	2
WX-CIGM-295-2	92	93	2	2
WX-CIGM-295-3	93	93	2	2
WX-CIGM-295-4	92	92	2	2
WX-CIGM-295-5	92	94	3	2
BH 1146 (resistant check)	93	95	6	3
Ciano 79 (susceptible check)	99	99	9	5

[1] Two-digit scoring system: first digit = height of infection; 5 = up to mid-plant, 9 = up to flag leaf. Second digit = disease severity on infected leaves: 1 = low, 9 = total leaf destroyed; a = score at early milk stage, b = score at soft dough stage.
[2] 1 = Low infection, 9 = High infection.
[3] 1 = Low grain infection, 5 = Severely infected.

Study 2: Interspecific source of resistance

From screening of several synthetic wheats, diversity of resistance in the various SH wheats was observed and 45 SH *C. sativus* resistant wheats were initially selected. The durum cultivars involved in these SH combinations were generally susceptible with leaf scores of 9-7 to 9-9, grain infection between 3-5, and spike damage score between 7-9. Since these scores are poorer than those of the selected SH wheats, resistance has been interpreted as being a consequence of the respective *T. tauschii* accessions involvement in the synthetics.

Based on the agronomic evaluations (Villareal *et al.* 1995) and *C. sativus* disease screening data, elite resistant SH types have been identified for wheat improvement (Table 3). SH wheats with leaf scores of 9-2 to 9-3 or less, seed damage of 2 or 3 or less, and a spike finish score of 1 or 2 are the preferred resistant donors for wheat improvement. The SH bridge is advantageous for wheat improvement since it allows not only the *T. tauschii* resistance to be exploited, but

Table 2. Agronomic characteristics of *Cochliobolus sativus*-resistant spring bread wheat germplasm grown at Poza Rica, Mexico.

Germplasm	Grain yield (kg ha^{-1})	Days to anthesis[1]	Days to physiol. maturity	Plant height (cm)	1000-grain weight (g)	Test weight (kg hl^{-1})
WX-CIGM-295-1	1997	65	102	87	29.4	73.4
WX-CIGM-295-2	1564	66	106	88	27.4	73.9
WX-CIGM-295-3	1431	67	108	87	25.4	74.2
WX-CIGM-295-4	1580	69	105	88	26.4	69.5
WX-CIGM-295-5	1461	69	110	91	25.3	72.4
BH 1146 (resistant check)	982	59	100	85	27.1	71.7
Ciano 79 (susceptible check)	166	55	103	63	16.7	38.8
LSD (0.05)	526	3	6	6	3.8	8.8

[1] Days from emergence.

also incorporates the genetic diversity of the A and B genomes of the respective durum wheat cultivars. Using this approach, we have developed bread wheat/diverse SH germplasms from which 50 lines resistant to *C. sativus* have been identified. These lines have been further selected for desirable plant type, maturity, and resistance to leaf, stem, and stripe rust. The *C. sativus* resistance attributes of these lines are similar to the SH entries in Table 3. These lines have been made homozygous using the sexual double haploid (DH) methodology (Mujeeb-Kazi and Riera-Lizarazu 1996). The haploid procedure allows for frequencies of about 20% embryo excision, 80% plantlet regeneration, and 70% colchicine induced doubling.

The *T. tauschii* accessions contributing to resistance in the SH wheats have become the parental sources in another approach for their exploitation for wheat improvement through direct susceptible *T. aestivum*/resistant *T. tauschii* accession crosses. This methodology has been advocated as a rapid and efficient technique for exploiting *T. tauschii* variability for wheat improvement.

Table 3. Synthetic hexaploids (SH) for *Triticum turgidum* x *(Ae. squarrosa) T. tauschii;* 2n=6x=42, evaluated for *Cochliobolus sativus* in Poza Rica, Mexico.

T. turgidum cultivar/*Ae. squarrosa* accession*(SH)* combination and CIGM cross number	Disease score		
	Leaves[1] a	Seed[2]	Spike[3] (1-9)
Doy 1/*Ae. squarrosa* (188) CIGM88.1175	9-3	1	1
Rabi//GS/CRA/3/*Ae. squarrosa* (190) CIGM88.1178	9-3	1	1
Gan/*Ae. squarrosa* (236) CIGM88.1228	9-3	2	2
Scoop_1/*Ae. squarrosa* (434) CIGM88.1335	9-3	2	2
Doy 1/*Ae. squarrosa* (447) CIGM88.1344	9-3	1	1
Doy 1/*Ae. squarrosa* (511) CIGM88.1363	9-2	2	1
Doy 1/*Ae. squarrosa* (515) CIGM90.566	9-3	2	1
68.111//RGB-U/Ward/3/FGO/4/Rabi/5/*Ae. squarrosa* (629) CIGM90.590	9-2	1	1
Ceta/*Ae. squarrosa* (895) CIGM89.567-1B	9-3	1	1
Ceta/*Ae. squarrosa* (895) CIGM89.567	9-3	1	1
Gan/*Ae. squarrosa* (890) CIGM90.909	9-3	2	2
Croc_1/*Ae. squarrosa* (224) CIGM86.950	9-3	2	2
68.112/Ward//*Ae. squarrosa* (369) CIGM88.1313	9-3	2	2
Yav3/Sco//Jo69/Cra/3/Yav79/4/*Ae. squarrosa* (498) CIGM88.1356	9-3	2	1
Fgo/USA2111//*Ae. squarrosa* (658) CIGM89.506	9-2	1	1
68.111/RGB-U//Ward Resel/3/Stil/4/*Ae. squarrosa* (783) CIGM89.538	9-3	1	2
68.111/RGB-U//Ward/3/Fgo/4/Rabi/5/*Ae. squarrosa* (878) CIGM89.559	9-3	1	2
Scoop_1/*Ae. squarrosa* (358) CIGM90.820	9-2	1	1
Gan/*Ae. squarrosa* (408) CIGM90.824	9-2	1	2
Sca/*Ae. squarrosa* (518) CIGM90.845	9-3	2	1
Yar/*Ae. squarrosa* (418) CIGM90.846	9-3	2	2
Ciano 79 (susceptible bread wheat)	9-9	5	9
BH 1146 (resistant bread wheat)	9-5	3	6

[1] Two-digit scoring system: first digit = height of infection; 5 = up to center of plant, 9 = up to the flag leaf. Second digit = disease severity on infected leaves: 1 = low, 9 = total leaf destroyed; a = Score at dough stage of grain development.

[2] Grain infection scored as: 1= Low, 5= High seed blemish at embryo points.

[3] Spike infection scored as: 1= Low infection, 9= High infection.

Direct *T. tauschii* hybridization with *T. aestivum* cultivars is accepted as a priority since backcrosses onto the *T. aestivum/T. tauschii* ABDD F1 hybrids (2n=4x=28) by the same *T. aestivum* cultivar readily gives 11/12 (92 %) of the genotype of the recurrent parent in a single growing season. This inference was drawn by Alonso and Kimber (1984) based on stem rust transfers from *T. tauschii* into the *T. aestivum* cultivar Chinese Spring. Using this approach, we have now targeted some resistant *T. tauschii* accessions for direct hybridization with susceptible but elite *T. aestivum* cultivars such as Ciano 79 and Bacanora (see Mujeeb-Kazi and Hettel 1995).

Conclusions

1. The intergeneric hybrid combinations provide a unique genetic pool for wheat improvement, and through the advanced *Th. curvifolium* germplasm coupled with the basic resistant stocks of *Aegilops variabilis, Th. elongatum,* and *Th. scirpeum,* adequate alien diversity has become available.
2. The D genome resistance in SH wheats is a rapid and potent source for wheat improvement. Synthetics covering more *T. tauschii* accessions than the presently screened 250 are being produced, and we anticipate incorporating our entire 490 winter habit *T. tauschii* entry working collection in this SH wheat form. The SH wheats embody the wide array of genetic diversity of *T. tauschii* accessions but are all spring types, hence offering an easier route for their practical utilization, conservation, and global distribution to areas that harbor the *C. sativus* stress constraint. The international distribution of synthetic hexaploids has additional merit in that following screening by national agricultural programs for different objectives, the variation can be readily incorporated into their local adapted germplasm.
3. Results of the with and without fungicide yield tests not reported here unequivocally demonstrate the superiority of the *Th. curvifolium* derivatives over the advanced breeding lines and parental cultivars. We conclude that the *Th. curvifolium* derivatives express superior resistance to *C. sativus* under the Poza Rica conditions in Mexico. Resistance to *C. sativus* obtained from *Th. curvifolium* also exhibits an increased grain yield performance and test weight than our present conventional breeding materials. This germplasm provides a valuable "genetic source" for enhancing spot blotch resistance in *T. aestivum,* and leads to better plant performance and crop adaptation of wheat in warmer humid production areas of the world.

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Evaluating Southern Cone Wheat Germplasm for Spot Blotch and Tan Spot

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Abstract

*Since 1981, advanced lines from wheat breeding programs in the Southern Cone region of South America (Argentina, Brazil, Bolivia, Chile, Paraguay, and Uruguay) have been evaluated in LACOS a regional nursery. The nursery is annually seeded in over 25 key locations. Of these, 1-5 report data on spot blotch (*Bipolaris sorokiniana*) and another 2-4 on tan spot (*Drechslera tritici-repentis*). Based on 10 years of observations, Santa Cruz (Bolivia) and Londrina (Brazil) represent two critical sites for spot blotch evaluation. Correlation coefficients among infection indices from various locations during a severe disease year (1994) show not only low, statistically significant relationships between Bolivia and Brazil (r = 0.142* - 0.214**), but also indicate that pathogen populations may differ within a country such as Brazil. Tan spot infection, on the other hand, has been increasingly evaluated at Pergamino (Argentina), Bella Vista (Paraguay), and Young (Uruguay). While the relationship among these locations is low but statistically significant (r = 0.219** - 0.267**), there is a possibility of a differential pathogen population between Paraguay and Uruguay. Of the commercial varieties tested in 1994/95, most are moderately to highly susceptible; however, some varieties such as Don Ernesto INTA, ProINTA Federal, and ProINTA Guazu from Argentina; Domo INIA, Saeta INIA, and Chagual INIA (durum) from Chile; IAN 7 and Itapua 35 from Paraguay; and ProINTA Superior and E. Pelon 90 from Uruguay demonstrated moderate spot blotch resistance levels. During the same year, IAC 5 - Maringa from Brazil; Domo INIA, Nobo INIA, and Saeta INIA from Chile; E. Cardenal, E. Pelon, and ProINTA Querandi from Uruguay; and IAN 7, IAN 9, Itapua 35, and Itapua 40 from Paraguay showed moderate tan spot resistance. A large number of breeding lines with moderate to high resistance levels to both diseases have been selected. Among these, CIMMYT lines derived from* Thinopyrum curvifolium *combined with Chinese sources of spot blotch resistance and Milan as a source of tan spot resistance have been most outstanding.*

The Southern Cone of South America is one of the most critical regions in the world for disease epidemics of wheat. The region comprising Argentina, Bolivia, Brazil, Chile, Paraguay, and Uruguay sows approximately 10 million ha of wheat every year and, depending on climatic conditions, produces between 16-20 million t of grain. The crop sown area, located between 10° and 40° S latitude, spans vast geographic and climatic conditions that range from tropical/subtropical to temperate regimes

and from flat grasslands to the high Andes mountains. Although spring growth habit is predominant in most of the region, facultative and winter wheats are also grown in the southern latitudes. Except for the Central Valley of Chile and a small area in central Brazil that is under irrigation, the majority of the crop is seeded under rainfed conditions. As a result, unstable climatic conditions and severe drought stress annually affect yield in a large part of the region. However, in the high rainfall areas of the South and Central subtropical region, wheat diseases are probably the most important factor limiting yield. Serious diseases that cause epidemics and production losses include rusts, powdery mildew, leaf blights, bacterial leaf stripe, fusarium head blight, and several viral diseases including barley yellow dwarf and soilborne mosaic virus.

Given the regular frequency of disease epidemics that severely affect various parts of the region, tremendous progress was achieved by the early wheat breeders in selecting and developing new varieties with very high levels of general resistance to a complex of diseases, and other characteristics. Famous examples of such varieties utilized internationally include: Frontana, Surpresa, IAS 20, 38 MA, Barletta, Buck Manantial, Klein Atlas, Tezanos Pintos Precoz, Americano 26, and Litoral. Some of these not only possess a high degree of rust resistance (especially adult plant resistance to leaf rust), but also a significant level of tolerance to foliar blights caused by *Septoria* and *Helminthosporium* species.

Modern wheat breeding programs in the Southern Cone, being aware of their geographic and agroclimatic limitations, in particular the lack of physical barriers to check movement of disease-causing pathogens, have aggressively utilized all opportunities to expand the wheat germplasm base at both the formal and informal levels. With the objective to evaluate adaptation and a wide spectrum of disease resistance, exchange of advanced germplasm within and outside the region has continued since 1950. Some of the formal vehicles of germplasm exchange at the regional level have been: the Latin American Wheat Yield Trial, organized by Dr. Norman Borlaug of the Office of the Special Studies (predecessor institute of CIMMYT); the International Rust Screening Nursery, organized by the US Department of Agriculture; the Southern Cone Wheat Yield Trial (ERCOS), primarily organized by EMBRAPA, Brazil and later by INTA; and the Argentina and Latin American Disease Observation Nursery (VEOLA), organized by the CIMMYT Andean Program.

Under the auspices of the Southern Cone Cooperative Program (PROCISUR), the CIMMYT regional network has organized the Southern Cone Wheat Advanced Lines (LACOS) nursery to increase the annual exchange of germplasm among participating countries. Since 1981, the nursery, consisting of approximately 300 advanced wheat lines from over 20 national breeding programs, has been distributed to approximately 30 locations in the region and another 20 outside the region.

This nursery has become an important vehicle to generate a large set of data on all advanced breeding materials from the participating countries in order to make decisions on commercial releases as well as their utilization as parental stocks.

Based on the information provided on LACOS since its inception, it has been possible to determine that spot blotch, caused by *Bipolaris sorokiniana* (syn. *Helminthosporium sativum*), has been a major disease in central-southern Brazil, northeastern Argentina, Bolivia, and Paraguay. On the other hand, tan spot, caused by *Pyrenophora tritici-repentis*, only appeared as an important disease in 1991. The increase in tan spot incidence can be traced to an increased acreage under conservation tillage practices, especially zero tillage, without consideration of adequate crop rotation.

Spot Blotch

Analysis of 10 years of LACOS data identified Londrina, Brazil, and Santa Cruz, Bolivia, to be two critical sites for spot blotch evaluation in the region. While other locations such as Roque Saenz Peña, Argentina; Okinawa, Bolivia; Cruz Alta, Campinas, and Palotina, Brazil; and Capitan Miranda, Paraguay, have reported spot blotch infection in certain years, Londrina and Santa Cruz have reported severe infection levels during nine and seven years, respectively.

Based on the spot blotch infection index calculated for the 14th LACOS (1994-1995), an effort was made to explore similarities among different locations where the disease was reported. Correlation coefficients used to determine the relationship among Santa Cruz, Bolivia, and four locations in Brazil (Londrina, Palotina, Campinas and Cruz Alta) were found to be positive and statistically significant but of low magnitude (Table 1). A more important aspect of this analysis is probably the lack of a significant relationship among Brazilian locations from the states of Parana, Rio Grande do Sul, and Sao Paulo. While several factors may influence such a relationship, it is possible that these locations represent significant differences in the pathogen populations. A more detailed study will most likely elaborate on these preliminary observations.

In general, very high infection levels were observed on susceptible material every year; however, based on the collection of about 300 lines per year, the

Table 1. Correlation coefficient (r) among spot blotch infection indices at different locations, 14th LACOS, 1994/95.

	Santa Cruz	Londrina	Palotina	Campinas	Cruz Alta
Santa Cruz					
Londrina	0.199**				
Palotina	0.214**	0.054 ns			
Campinas	0.142*	0.173*	0.054 ns		
Cruz Alta	0.173*	0.097 ns	0.147*	0.152*	

** P<0.01 * P<0.05 ns: Not significant

average infection index varied between 20-80% during 1985-1994 (Figure 1). Infection levels in crop years 1985, 1987, 1990, and 1993 were especially severe when the average coefficient of infection over the total number of lines tested was greater than 50%. The maximum coefficient of infection averaged over all locations that reported the disease remained above 80% in all years except in 1988. On the other hand, the minimum infection index varied between 2-21%, except in 1985 when the lowest infection level observed was approximately 50%.

In an effort to classify germplasm from different countries of the region according to disease resistance, no clear distinction in resistance level could be asserted, although minor differences were observed from year to year. In general, germplasm of Brazilian, Bolivian, and Paraguayan origin, as a group, demonstrated a slight tendency to lower infection levels compared with germplasm originating from Argentina, Chile, and Uruguay (Figure 2). Nevertheless, commercial Argentine varieties, such as Don Ernesto INTA and Pro INTA Superior, both Bobwhite lines, and Chilean varieties such as Domo INIA and Chagual INIA (durum), are among the best spot blotch resistant germplasm tested between 1990-1994 (Table 2).

The superiority of these varieties, as well as their stable low reaction to spot blotch infection, was further confirmed at five locations during 1994-1995 (Table 3). Other varieties demonstrating a lower reaction to spot blotch included Pro INTA Guazu, IAN 7, and Estanzuela Pelon 90. During this year, high infection levels were observed at all locations and were most severe at Cruz Alta, Brazil.

Considering that one of the objectives of the LACOS nursery is to identify parental stock for different characteristics, advanced lines selected between 1991-1995 for spot blotch resistance are presented in Table 4. A large set of germplasm from Brazilian crosses dominates the spot blotch resistant

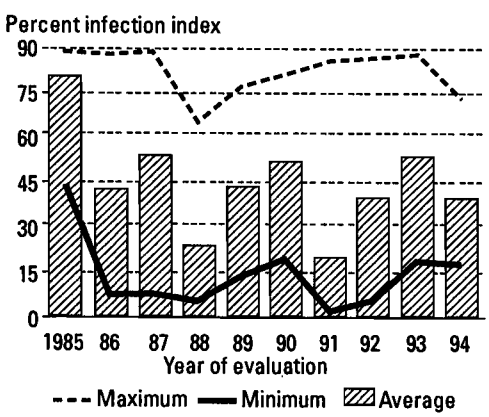

Figure 1. Evolution of spot blotch infection in the Southern Cone region.

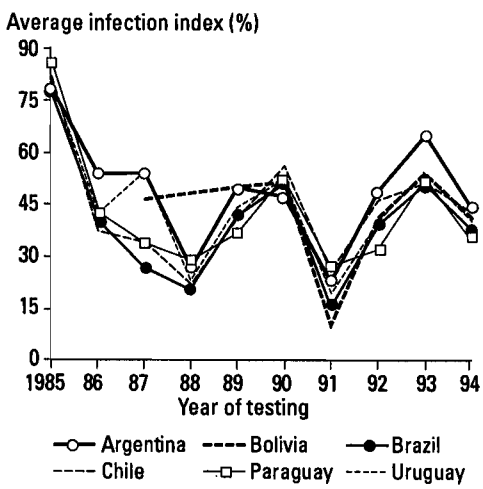

Figure 2. Average spot blotch infection in germplasm from different origins.

materials; however, some late lines from Argentina and Chile have also performed very well. Some CIMMYT derived germplasm, especially Opata lines, were among the first set of materials to combine high yield potential and lower spot blotch infection. As a result, they were commercially released in Bolivia (Agua Dulce CIAT) and in Brazil (IAPAR29-Cacatu).

Although low infection lines have been identified during these years, performance of new germplasm originating from the CIMMYT wide cross program, involving spot blotch resistance from alien *Thinopyrum curvifolium* and Chinese sources, has been most outstanding. Under severe disease epidemics in lowland Bolivia, these lines have shown remarkably higher resistance levels than observed in the past (Table 5).

From studies on approximately 3,000 advanced wheat lines over the past 10 years, it can be concluded that the

Table 2. Spot blotch infection indices of major commercial varieties during 1990-94.

Variety	Country	1990	1991	1992	1993	1994	Average RCI[1]
Nobo INIA	Chile	52	27.0	56.0	-	36	17.6
Domo INIA	Chile	-	-	-	-	27	5.4
IAC 5 Maringa	Brazil	44	3.6	-	20	54	23.6
PI Oasis	Argentina	-	4.5	56	39	36	26.2
Don Ernesto INTA	Argentina	46	3.6	42	59	29	35.2
Agua Dulce CIAT	Bolivia	-	1	41	59	44	29.0
Itapua 35	Paraguay	24	45	25	59	32	37.0
PI Imperial	Argentina	-	-	-	-	37	7.4
OC 18	Brazil	-	-	-	20	56	15.2
Chonta INIA	Chile	-	-	-	39	39	15.6
Trigo BR 23	Brazil	59	2.7	56	39	42	39.2
IAN 7	Paraguay	-	45	42	-	31	23.6
Chagual INIA	Chile	44	-	31	59	25	31.8
M.Juarez INTA	Argentina	22	-	-	59	40	24.2
Itapua 40	Paraguay	-	-	42	-	40	16.4
IA 29 Cacatu	Brazil	44	4.5	-	78	46	33.6
P. Superior	Uruguay	-	-	-	59	26	17.0
Tahuen INIA	Chile	-	-	51	39	38	25.6
E. Cardenal	Uruguay	38	23.0	56	78	42	42.8
IAN 9	Paraguay	-	-	42	-	45	17.4
PI Guazu	Argentina	-	-	31	78	25	26.8
Saeta INIA	Chile	-	-	44	59	31	26.8
E. Pelon	Uruguay	-	-	-	59	31	18.0
PI Isla Verde	Argentina	-	3.6	70	59	52	36.2
P. Querandi	Uruguay	-	-	-	59	35	18.8
Lilen INIA	Chile	-	-	-	59	36	19.0
PI Queguay	Argentina	-	23.0	74	78	44	39.2
Saguayo	Bolivia	54	23.0	56	78	63	50.2
PI Federal	Argentina	-	-	42	78	33	30.6
Rehiue INIA	Chile	-	-	51	59	44	30.8
Guapay CIAT	Bolivia	-	-	-	59	46	21.0
Lican INIA	Chile	-	-	-	78	44	24.4
OC 20	Brazil	-	-	-	78	47	25.0
Granero INTA	Argentina	-	-	-	78	64	28.4

[1] Relative coefficient of infection.

Note: Infection index and relative coefficient of infection are the same. The notes were taken using a double-digit scale, and the two numbers were multiplied. The product was divided by the maximum value at a particular location minus one, and then multiplied by 100, i.e., ((D1 * D2)/Max (D1 * D2)-1)100.

majority of South American germplasm demonstrates only moderate spot blotch resistance levels. However, based on the continuous pyramiding of various sources, as well as newer germplasm identified from the wide cross program, significant advances are expected in the future.

Tan Spot

The increase in tan spot incidence and its establishment as a serious disease in the region has been observed since 1991. As mentioned earlier, the increased incidence in most of the region has been primarily related to the increase in zero

Table 3. Spot blotch infection on commercial wheat varieties from the Southern Cone at five locations, 1994/95.

Variety	Country	Infection score[1]					Average RCI[2]
		Santa Cruz	Londrina	Campinas	Cruz Alta	Palotina	
PI Guazu	Argentina	-	3	10	-	31	24.8
Chagual INIA	Chile	T	2	20	93	82	25.4
P. Superior	Uruguay	3T	2	20	95	31	26.4
Domo INIA	Chile	-	2	10	83	-	27.3
Don Ernesto INTA	Argentina	31	3	20	93	81	28.5
IAN 7	Paraguay	31	2	30	95	51	30.3
E. Pelon	Uruguay	31	2	60	93	31	30.7
Saeta INIA	Chile	3T	3	10	93	83	31.1
Itapua 35	Paraguay	-	2	20	93	-	32.0
PI Federal	Argentina	32	3	30	93	81	32.9
P. Querandi	Uruguay	41	2	40	96	31	34.9
Nobo INIA	Chile	3T	3	10	96	82	35.6
PI Oasis	Argentina	42	3	-	94	3T	35.8
Lilen INIA	Chile	31	3	50	94	32	36.2
PI Imperial	Argentina	32	1	40	97	81	37.0
Tahuen INIA	Chile	31	4	40	94	32	38.0
Chonta INIA	Chile	62	2	20	94	-	39.1
M.Juarez INTA	Argentina	32	4	20	95	81	39.6
Itapua 40	Paraguay	31	3	40	95	82	40.3
E. Cardenal	Uruguay	41	3	30	98	31	41.8
Trigo BR 23	Brazil	31	3	60	96	31	42.1
Agua Dulce CIAT	Bolivia	3T	3	40	98	81	43.6
Lican INIA	Chile	51	3	60	93	83	43.9
Rehiue INIA	Chile	31	4	50	96	31	43.9
PI Queguay	Argentina	32	4	40	96	T	44.2
IAN 9	Paraguay	-	3	40	95	52	44.7
Guapay CIAT	Bolivia	42	3	30	98	51	45.7
IA 29 Cacatu	Brazil	32	2	50	97	82	45.7
OC 20	Brazil	42	2	50	97	82	47.3
PI Isla Verde	Argentina	72	2	20	97	84	51.9
IAC 5 Maringa	Brazil	52	2	60	97	83	53.8
OC 18	Brazil	63	2	40	98	82	55.7
Saguayo	Bolivia	52	3	60	98	84	63.2
Granero INTA	Argentina	73	4	30	97	83	64.4
Maximum		**83**	**5**	**90**	**98**	**96**	

[1] Infection score according to double digit scale (00-99) at Santa Cruz, Cruz Alta, and Palotina; a 0-9 scale at Londrina, and percentage at Campinas.

[2] Relative coefficient of infection.

Note: Infection index and relative coefficient of infection are the same. The notes were taken using a double-digit scale, and the two numbers were multiplied. The product was divided by the maximum value at a particular location minus one, and then multiplied by 100, i.e., ((D1 * D2)/Max (D1 * D2)-1)100.

Table 4. Southern Cone advanced lines selected for low spot blotch infection, 1991-95.

Cross	Pedigree	Country	Origin	RCI[1]
BCIM/T700	BW672X0-0-10-8-4/82	Argentina	10LACOS/ 32	8
COKER 762/BR14	F27304-450F-755F-1Y-900F	Brazil	10LACOS/ 61	8
T800/C33		Uruguay	10LACOS/ 228	13
BW72/TEMU29-80	T21083-T-5P-2T	Chile	10LACOS/ 296	13
MAYA/NAC//TEMU54.82	T21134-T-2P-1T	Chile	10LACOS/ 297	13
BOW"S"/4/COW"S"/3/NAD//BB/INIA	CM70050-11J-1J-1J-0J	Argentina	10LACOS/ 9	14
ANA/4/RRV/WW15/3/BJ/ON*2//BON	CM55679-1B-6B-1B-0P	Argentina	10LACOS/ 40	14
MYNA"S"/VUL"S"	CM 64546-2M-1Y-1M-5Y-0M-10Y-0M	Paraguay	11LACOS/ 279	14
BUC"S"/BJY"S"	CM49641-9Y-1M-2Y-3Y-0M-1P-0P	Chile	11LACOS/ 201	14
URES/BOBWHITE	CM78108-3M-2Y-2M-7Y-1B-0Y	Paraguay	11LACOS/ 254	18
DESCONOCIDO		Brazil	11LACOS/ 113	3
BR14*3//LD*6/FB6628	F28317-D-901F-654F-901Y-905F-Ö	Brazil	11LACOS/ 105	6
IAS58/4/KAL/BB//CJ"S"/3/ALD//YAV	CD58620	Bolivia	12LACOS/ 46	16
CNT10/TAM105//MCR/BR14	B31543-E-900Y-0Z-4A-0A	Brazil	12LACOS/ 126	14
PF7815/LAP689//PF7815/PF80278	F25089-1F-1F-0R-1F-0R-1F-0R-0F	Brazil	12LACOS/ 129	16
TIF SEL/PF79763/3/N.BOZU/3*LD//B7902	F23062-8F-22F-0R-1F-0R-4F-0R-..	Brazil	12LACOS/ 135	16
TEMU49-82/QU-10-096	QU-1864-2C-2C-1C-6C	Chile	12LACOS/ 201	16
B.CHARRUA		Argentina	13LACOS/ 155	20
PEL75135/ALD'S'/5/TIF//KZM.M12/TI/3/ALD..	IP6929-3G-1G-0B-0YL	Brazil	13LACOS/ 74	20
LE2132/LE2134		Uruguay	13LACOS/ 134	20
MON"S"/IMU//ALD"S"/PVN"S"	CM85835-4Y-0H-0KCM-0B-0Y	Paraguay	13LACOS/ 264	20
KLAT/MJI//VI/4/L2266/1406-101/BUC'S'/3Ö	AMX14891.501P-3N-1B-0P	Argentina	13LACOS/ 20	20
PF8237//LAP689/3*CNT10	F25561-199F-0F-0F-0F-12F-0F	Brazil	13LACOS/ 100	20
SPN/NAC//CEP 8386/3/CEP 19	B 32321-(309Y(-0Z-0A-5A-0A	Brazil	14LACOS/ 242	14
MILAN'S'	CM 75113-B-5M-1Y-05M-7Y-1B-0Y	Uruguay	14LACOS/ 212	15
BR 14/CEP 847	B 31615-0A-0Z-1A-15A-0A	Brazil	14LACOS/ 235	18
MILAN'S'	CM 75113-B-5M-1Y-5M-2Y-3B-0Y-..	Chile	14LACOS/ 179	20
BOW/YR 71/3/F35.75//KAL/BB/4/NAC 76	CM 74553-2E-2E-2E	Paraguay	14LACOS/ 70	20
CATBIRD'S'	CM 91045-9Y-0M-0Y-5M-4Y-0B	Uruguay	14LACOS/ 200	22
DOMO INIA		Chile	14LACOS/ 190	15

[1] Relative coefficient of infection.

Table 5. Stability of spot blotch resistance in selected wheat advanced lines of wide cross origin, Santa Cruz, Bolivia, 1992-94.

Code no.	Cross	Infection score		
		Saavedra		Samaipata
		1992	1993	1993-94
92027	CS/AC//Glen/3/Adl../YMI4	62	81	R[1]
92029	CS/AC//Glen/3/Ald/Pvn/4/..	52	81	MR
92050	CS/AC//Glen/3/Ald.. YMI4	73	83	MR
92053	CS/AC//Glen/3/Ald.. YMI4	73	83	MR
92059	CS/A.Curv//Glen/3/Gen/4..	84	81	R
	PAI Comomoci (check)	87	87	S
	Chane CIAT (check)	86	85	S

[1] R = Resistant; MR = Moderately resistant; S = Susceptible.

tillage cultivation with no attention to adequate crop rotation practices. Based on the study of LACOS between 1991-1994, the most critical locations for tan spot infection were Pergamino, Argentina; Bella Vista, Paraguay; and Young, Uruguay. Although other locations, such as Marcos Juarez, Argentina; Cruz Alta, Brazil; and Capitan Miranda, Paraguay, have recorded tan spot infection during certain years, infection levels observed at the former sites are regular and severe every year.

Attempts were made to determine relationships between three locations based on tan spot infection indices observed in the 14th LACOS 1994/95. In the case of spot blotch, the relationship between Pergamino, Argentina (a critical location), and Young, Uruguay, or Bella Vista, Paraguay, was statistically significant, but of very low magnitude (Table 6). On the other hand, the relationship between Bella Vista, Paraguay, and Young, Uruguay, was statistically not significant, thereby indicating a probability that different pathogen populations exist at these two locations. In the future it will be important to elaborate on these preliminary findings with detailed studies on pathogen populations.

Table 6. Correlation coefficient (r) among tan spot infection indices at different locations, 14 LACOS, 1994-95.

	Pergamino	Young	Bella Vista
Pergamino	1		
Young	0.267**	1	
Bella Vista	0.219*	0.109 ns	1

** P<0.01 * P<0.05 ns: Not significant

Tan spot infection levels on LACOS between 1991-1994 were high at all locations. While the maximum infection index on susceptible material reached 80%-100%, the average infection level over all lines in LACOS was 45%-55% (Figure 3). Infection levels of the 1993/94 crop cycle were particularly severe with an average coefficient of infection of 54% observed over approximately 300 advanced lines. The minimum infection index on the most resistant germplasm varied between 2-20% during this period, showing that a significant number of germplasm carried high levels of tan spot resistance even without previous selection for this character.

As in the case of spot blotch, the classification of all advanced lines included in the LACOS for tan spot resistance showed only minor differences among germplasm originating from different countries of the region. Over these years, germplasm originating from Brazil and Uruguay, as a group, showed slightly better performance (Figure 4); however, the commercial varieties such as IAN 7 and Itapua 35 from Paraguay have

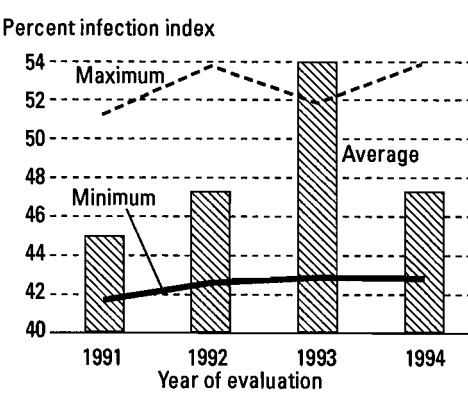

Figure 3. Evolution of tan spot infection in the Southern Cone region.

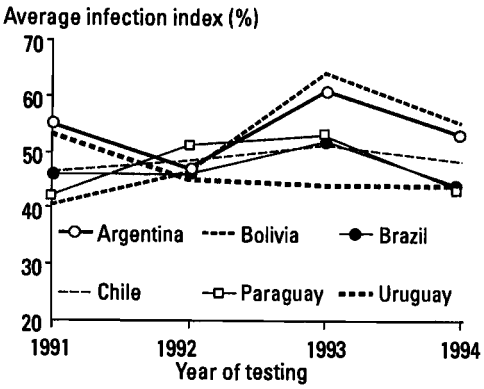

Figure 4. Average tan spot infection in germplasm from different origins.

consistently demonstrated excellent tan spot resistance over the years. Among the commercial varieties derived from CIMMYT germplasm, all Veery selections, as a group, demonstrated a high level of tan spot resistance (Table 7).

Thirty-four commercial varieties used as checks in the 14th LACOS, 1994/95, were studied for tan spot infection at three locations (Pergamino, Bella Vista, and Young). Based on their average

Table 7. Tan spot infection indices of major commercial varieties during 1990-94.

Variety	Country	1991	1992	1993	1994	Average RCI[1]
E. Pelon	Uruguay			26	21	23.5
E. Cardenal	Uruguay	24	24	28	33	27.2
P. Querandi	Uruguay			32	30	31.0
Itapua 35	Paraguay	41	29	38	23	32.8
IAN 7	Paraguay		24	46	31	33.7
Domo INIA	Chile				37	37.0
Tahuen INIA	Chile		28	39	46	37.7
Itapua 40	Paraguay			46	30	38.0
Nobo INIA	Chile	52	34	43	25	38.5
IAN 9	Paraguay			46	36	41.0
Rehiue INIA	Chile		35	46	43	41.3
OC 18	Brazil				43	43.0
Chagual INIA	Chile	41	46	35	51	43.2
IAC 5 Maringa	Brazil		36	70	25	43.7
Saguayo	Bolivia	24	46	60	53	45.8
IA 29 Cacatu	Brazil		55	39	49	47.7
OC 20	Brazil				48	48.0
Chonta INIA	Chile			47	51	49.0
Don Ernesto INTA	Argentina	42	46	65	44	49.2
Saeta INIA	Chile	52	35	79	31	49.2
P. Superior	Uruguay			60	41	50.5
PI Guazu	Argentina		38	57	58	51.0
Trigo BR23	Brazil	64	54	34	53	51.2
Lican INIA	Chile			51	52	51.5
Lilen INIA	Chile			43	70	56.5
PI Federal	Argentina		64	58	52	58.0
PI Imperial	Argentina				62	62.0
PI Oasis	Argentina		55	79	53	62.3
PI Queguay	Argentina	92	63	57	53	66.2
Agua Dulce CIAT	Bolivia		50	64	99	71.0
M. Juarez INTA	Argentina			73	73	73.0
Granero INTA	Argentina			74	73	73.5
Guapay CIAT	Bolivia			64	84	74.0
PI Isla Verde	Argentina	86		80	83	83.0

[1] Relative coefficient of infection.

Note: Infection index and relative coefficient of infection are the same. The notes were taken using a double-digit scale, and the two numbers were multiplied. The product was divided by the maximum value at a particular location minus one, and then multiplied by 100, i.e., ((D1 * D2)/Max (D1 * D2)-1)100.

infection index, apart from Itapua 35 mentioned above, Itapua 40 (Paraguay), Estanzuela Pelon 90 and Pro INTA Querandi (Uruguay), IAC 5 Maringa (Brazil), and Nobo INIA (Chile) demonstrated the highest resistance levels (Table 8). While the maximum infection level was high at all locations, the highest infection on commercial varieties was observed at Bella Vista, Paraguay.

A number of advanced lines were selected from the 12th, 13th, and 14th LACOS (1992-1994) based on their low tan spot infection levels (Table 9). While several Brazilian and Uruguayan lines were included in this group, many of the CIMMYT-derived lines, particularly Veery, Kauz ,and Milan sisters, also demonstrated a very high tan spot resistance levels.

Table 8. Tan spot infection on commercial wheat varieties from Southern Cone at three locations, 1994/95.

Variety	Country	Infection score			Average RCI[1]
		Pergamino	Young	Bella Vista	
E. Pelon	Uruguay	81	32	83	21.2
Itapua 35	Paraguay	82	32	82	22.6
Nobo INIA	Chile			82	24.6
IAC 5 Maringa	Brazil			82	24.6
P. Querandi	Uruguay		32	84	29.9
Itapua 40	Paraguay		32	84	29.9
Saeta INIA	Chile	82	54	82	30.8
IAN 7	Paraguay		T	85	31.2
E. Cardenal	Uruguay			82	32.7
IAN 9	Paraguay		32	85	36.0
Domo INIA	Chile	82	64	83	37.2
P. Superior	Uruguay	82	64	84	41.3
Rehiue INIA	Chile	82	85	82	42.5
Don Ernesto INTA	Argentina	82	75	83	43.7
Tahuen INIA	Chile		64	84	45.7
IA 29 Cacatu	Brazil			84	49.2
Chonta INIA	Chile	83	84	84	51.4
Chagual INIA	Chile	83	84	84	51.4
Lican INIA	Chile	83	85	85	52.0
PI Federal	Argentina	84	64	84	52.2
PI Oasis	Argentina	82	53	88	52.5
Saguayo	Bolivia		84	84	52.7
Trigo BR 23	Brazil		84	84	52.7
PI Queguay	Argentina	84	63	85	52.8
PI Guazu	Argentina	82	64	88	57.8
PI Imperial	Argentina	84	86	83	62.1
Lilen INIA	Chile		64	88	70.3
M.Juarez INTA	Argentina	83	85	88	72.6
Granero INTA	Argentina	83	85	88	72.6
PI Isla Verde	Argentina	84	86	88	82.7
Guapay CIAT	Bolivia		85	88	84.4
Agua Dulce CIAT	Bolivia			88	98.5
Maximum		**86**	**87**	**88**	

[1] Relative coefficient of infection.

Note: Infection index and relative coefficient of infection are the same. The notes were taken using a double-digit scale, and the two numbers were multiplied. The product was divided by the maximum value at a particular location minus one, and then multiplied by 100, i.e., ((D1 * D2)/Max (D1 * D2)-1)100.

Considering the interest and significant increase in conservation tillage practices, particularly zero tillage, in the region, it is quite likely that tan spot will become an established major disease on wheat in the Southern Cone region. Although some commercial varieties have demonstrated lower levels of tan spot infection, it is not certain if they will maintain resistance when the inoculum pressure increases.

In order to reduce yield losses caused by tan spot and spot blotch on commercial crops, farmers are using chemical control measures on a regular basis. However, given the ample genetic variability of resistance to these diseases present in the region and in CIMMYT germplasm, it will be important to use a set of key sites in order to select newly developed germplasm. A nursery such as LACOS will therefore maintain its value as a means to achieve a wide screening range and to identify superior sources of disease resistance.

Table 9. Southern Cone advanced lines selected for low tan spot infection, 1993-95.

Cross	Pedigree	Country	Origin	RCI[1]
E.FEDERAL		Uruguay	13LACOS/156	11
B.CHARRUA		Argentina	13LACOS/155	17
VEE"S"/3/FLN/ACC//ANA	CM67391.2J-2J-1J	Argentina	12LACOS/26	18
IAC5/ALDAN'S'//CEP7780	CO3686-3P-1P-2P-1P-0P	Brazil	13LACOS/120	20
SPN/NAC//CEP 8386/3/CEP 19	B 32321-(309Y)-0Z-0A-5A-0A	Brazil	14LACOS/ 242	21
KAUZ"S"	CM-67458-4Y-2M-1Y-1M-3Y-0B-Ö	Chile	12LACOS/152	22
RFN*2//908/FN/3/MD/4/KKZ/5/BR 23/6/CEP..	B 32300-(900Y)-0Z-0A-4A-0A	Brazil	14LACOS/ 241	24
QUIANG FENG 2		Uruguay	13LACOS/148	26
E.PELON90		Uruguay	13LACOS/158	26
HLN/CNT 7//AMIGO/CNT 7	P 17920-111F-1F-2F-3F-2F-0F	Brazil	14LACOS/ 116	26
BOW'S'/BUC'S'	CM74005-8M-1Y-03M-5Y-2B-0Y	Uruguay	14LACOS/ 202	26
BUTUI/BR14//PF79790/CEP75203	B30654-0Z-0A-3A-0A	Brazil	12LACOS/120	27
CEP11/OASIS//BR14	B31743-0A-0Z-2A-0A	Brazil	12LACOS/124	27
BG/HORK"S"//ALDAN//FOREY	A5151-1B-1B-2B-0B	Argentina	12LACOS/2	28
LILEN INIA		Chile	12LACOS/144	28
PEL75135/ALD'S'/5/TIF//KZM.M12/TI/3/ALD..	IP6929-3G-1G-0B-0YL	Brazil	13LACOS/74	28
CEP14/CEP82113//BR14	B31751-D-0Z-0A-3A-2A-900Y	Brazil	13LACOS/90	28
LE2132/LE2134		Uruguay	13LACOS/134	28
ITAPUA35		Paraguay	12LACOS/295	29
TJB 788-1089/ALDAN//CEP 8385/3/CEP..	B 32335-(301Y)-0Z-0A-12A-0A	Brazil	14LACOS/ 243	29
BR 14/CEP 847	B 31615-0A-0Z-1A-15A-0A	Brazil	14LACOS/ 235	29
ECAL/BOMBU		Uruguay	13LACOS/140	30
MCR/CEP13//BR14	B31561-G-310Y-0Z-3A-3A-900Y	Brazil	13LACOS/96	30
IAS58/4/KAL/BB//CJ'S'/3/ALD'S'/5/BOW'S'	CM81812-12Y-06PZ-6Y-4M-2Y-0MÖ	Uruguay	14LACOS/ 228	30
ITAPUA 40		Paraguay	14LACOS/ 299	30
VS73.600/MRL'S'/3/BOW'S'//YR/TRF'S'=Ö	CM 75113-B-5M-1Y-05M-7Y-1B-0Y	Uruguay	14LACOS/ 212	30
BOW//BUC/BUL	CM90526-1Y-0M-0Y-2M-0Y	Paraguay	14LACOS/ 257	31
PF 74354		Chile	14LACOS/ 172	31
IAC24/BUC	CM 95736-8Y-0M-0AL-1Y-1Y	Chile	14LACOS/ 185	31
SAETA INIA		Chile	14LACOS/ 188	31

[1] Relative coefficient of infection. The notes were taken using a double-digit scale, and the two numbers were multiplied. The product was divided by the maximum value at a particular location minus one, and then multiplied by 100, i.e., ((D1 * D2)/Max (D1 * D2)-1)100.

Variation in Resistance to *Bipolaris sorokiniana* and *Magnaporthe grisea* in Wheat Plants Regenerated through Embryogenesis

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Abstract

Three wheat cultivars (Batuira, Ibiara, Cacatu) with some degree of resistance to Bipolaris sorokiniana, Magnaporthe grisea, *and* Xanthomonas campestris *pv.* undulosa, *and one cultivar (Mirim) susceptible to all three pathogens were used to induce higher resistance levels through somaclonal variation. Floral primordia of 15-20 plants per cultivar at initial development stages were used for callus induction and plant regeneration in petri plates containing 10 ml of MS culture medium and 2.0 mg of 2, 4-D. After acclimatization, the regenerated plants were transferred to pots containing soil in a growth chamber and grown to maturity. Callus initiation was observed within 12-15 days and frequency of callus induction was 100% in all cultivars. Calli were continuously transferred every 30 days to the MS regeneration medium without 2, 4-D. Frequency of regeneration of R1 plants was 10% for Batuira and 15.6% for Mirim. Calli of other cultivars failed to regenerate plants. No albino or sterile plants were regenerated. On average, regenerated plants of Batuira yielded 12 seeds and those of Mirim 15 seeds per spike. Variation in resistance of R2 plants to* B. sorokiniana *and* M. grisea *was observed, indicating the possibility of novel resistance genes through embryogenesis.*

The majority of wheat cultivars in Brazil have high yield potentials but are susceptible to several diseases such as spot blotch, caused by *Bipolaris sorokiniana;* blast, caused by *Magnaporthe grisea;* and bacterial leaf stripe, caused by *Xanthomonas campestris* pv. *undulosa.* Resistance to spot blotch and bacterial stripe is governed by polygenes. Although resistance sources are constantly being identified, their resistance levels are inadequate. Breeding efforts aimed at transferring resistance have received top priority during the past two decades; however, the results are not very encouraging, partly because of the nature of resistance and partly due to the lack of ample genetic diversity among resistance sources. Using somaclonal variation, attempts were made to induce novel resistance genes against *B. sorokiniana, M. grisea,* and *X. c.* pv *undulosa* without drastically altering the beneficial agronomic characteristics of the original genotypes.

Materials and Methods

Major success in callus induction and regeneration of disease resistant somaclonal plants has been obtained through the utilization of explants of genotypes that already possess some degree of resistance (Maddock *et al.* 1983). Cultivars Batuira, Ibiara, and Cacatu were reported to have some degree of resistance to *B. sorokiniana* and *X. c.* pv. *undulosa* (Mehta 1996; Mehta *et al.* 1996), and hence were selected for somaclonal variation, with Mirim the susceptible cultivar.

Fifteen to twenty plants of each cultivar were grown in the glasshouse at three sowing dates. Floral primordia were selected at the initial stage of plant development (inflorescences of 0.5-2.0 cm in length) for callus induction and regeneration of somaclonal plants in petri plates containing 10 ml of MS culture medium with 2.0 mg of 2, 4-D (Murashige and Skoog 1962). The immature inflorescences were surface sterilized in sodium hypocloride solution, excised into small pieces of up to 2-3 mm, placed in petri plates containing the callus induction medium, and incubated in the dark for 30 days at 26°C. Calli were transferred onto fresh medium for a further period of 15 days under a similar set of incubation conditions. After 45 days, calli were transferred to the regeneration MS medium without 2, 4-D, but supplemented with 27.8 mg L^{-1} Fe_2SO_4 $4H_2O$, 37.3 mg L^{-1} Na_2-EDTA, 0.5 mg L^{-1} ANA, and 3.0 mg L^{-1} kinetin, and incubated at 26°C and 8/16 h light/dark (Araujo *et al.* 1995). After reaching the three-leaf stage, regenerated seedlings were acclimatized by flooding the plates with water and incubating them at room temperature for 24 h. Later, the plates were transferred to the growth chamber in earthen pots of 20 cm diameter containing soil and organic manure. Pots were periodically manured with N, P_2O_5, and K at a rate of 4:30:10 (2.5g/pot) and grown to maturity.

R2 plants were inoculated using a mixture of seven monosporic aggressive isolates of *B. sorokiniana* obtained from the 1989 culture collection of IAPAR (strain numbers 9130, 9131, 9132, 9133, 9134, 9140, and 9141). Plants were inoculated with one isolate of *M. grisea*, 15 days after inoculation with *B. sorokiniana*. A separate leaf was inoculated for each pathogen. For *B. sorokiniana*, cultures were grown on potato-dextrose agar (PDA), and for *M. grisea* cultures were grown on oatmeal agar (Araujo *et al.* 1995). Isolates were stored on PDA under room temperature and were used for inoculations in 1989 and 1990. Cultures were initiated one week before inoculation in petri dishes containing PDA. Plates were flooded with distilled water, the spores gently scraped, and the resulting suspension filtered through cheesecloth. Conidial concentration was diluted to 2.0-3.0 x 10^4 conidia ml^{-1}. One drop of Tween 20 per 200 ml of suspension was added and the suspension was sprayed using a small atomizer and a pressure pump. Leaves were first washed with distilled water, gently rubbed between the fingers, and then each one was held upright, given a

quick single round of spray from top to bottom and to the top again. Inoculated pots were incubated for 16 h at about 20°C in a completely dark moist chamber with a water-saturated atmosphere. Later, plants were transferred to another growth chamber and were incubated at about 80% RH and 20°C with an alternating cycle of 12/12 h light/dark. The percentage of flag leaf area infected (FLAI) was visually estimated seven days after inoculation using the disease appraisal scale proposed earlier (Mehta 1981, 1996).

Results and Discussion

Callus initiation was observed at 12-15 days and callus induction frequency was 100% in all cultivars. Floral primordia in their advanced stages (>3 cm) did not produce calli. Embryogenic calli were compact and yellowish-green. Although no albino or sterile plants were regenerated, abnormal pseudofoliar structures and excessive root development without shoots were observed in calli of some cultivars (Vasil and Vasil 1986).

Calli were continuously transferred every 30 days to the MS regeneration medium without 2, 4-D. The frequency of regeneration of R1 plants was 10% for Batuira and 15.6% for Mirim. Calli of other cultivars failed to regenerate plants. On average, regenerated plants of Batuira yielded 12 seeds and those of Mirim 15 seeds per spike. Variation in resistance of the R2 plants to *B. sorokiniana* and *M. grisea* was observed (Table 1), showing the possibility of introducing novel resistance genes through embryogenesis otherwise not available through conventional breeding (De Buyser *et al.* 1987).

Cytological analysis of the resistant somaclones showed a constant chromosome number, as observed in their original hexaploid wheat genotypes (2n=6X=42). The resistant R2 plants are currently being fixed through the "embryo rescue technique" to obtain haplodiploidization (Zhang *et al.* 1996; Matzk and Mahn 1994). The procedure allows complete homozygocity to be obtained in a very short period of time, i.e., within one generation, and facilitates the selection process of somaclonal variants. Resistant doubled haploid (DH) somaclones will be used further for genetic and cytological analysis.

Somaclonal variation exists and has been proved to be genetically inherited. Recently, major problems, such as loss of embryogenic capacity and reduced regenerative potential due to prolonged

Table 1. Somaclonal plants regenerated through embryogenesis.

Cultivar	% Callus induction frequency	% Plant regeneration frequency	No. of R2 plants	No. of plants resistant to *Bipolaris sorokiniana*	No. of plants resistant to *Magnaporthe grisea*
Batuira	100	10	93	3	33
Mirim	100	15.6	188	4	8

time in suspension culture, are presumed to be due to somaclonal variation (Stirn *et al.* 1995). Funatsuki *et al.* (1996) demonstrated that somaclonal problems in suspension cultures of barley, for example, can be overcome by directly regenerating plants from callus-derived protoplasts.

Discrepancies in *in vitro* variation in tissue cultures are encountered in the literature (Larkin *et al.* 1989; Chaudhury *et al.* 1994; Bohorova *et al.* 1995). Some laboratories have shifted their efforts from somaclonal variation to other transformation techniques, mainly because somaclonal variation is unpredictable and hence cannot be pre planned or guaranteed. The desired variation for a particular trait may be achieved within three to four months or may take five to six years. Besides, success in somaclonal variation is genotype dependent. In recent years, several transformation techniques have been developed including: 1) *Agrobacterium*-mediated transformation; 2) protoplast-mediated transformation; 3) microprojectile-mediated transformation; 4) tissue electroporation; and 5) microinjection of DNA. Generally speaking, these techniques are highly sophisticated and depend on the availability of an efficient and reproducible tissue culture system (Jahne *et al.* 1994). Somaclonal variation, on the other hand, is a relatively simple technique and can be used where other methods are not feasible or where resistance genes are not available.

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Evaluating Spot Blotch Resistance Traits in Wheat and Related Species

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Abstract

Spot blotch of wheat, caused by Bipolaris sorokiniana *(syn.* Helminthosporium sativum*), is regarded as an important wheat disease in China due to its high disease frequency in the northwestern, northeastern, and southern regions of the country. Breeding for resistant wheat varieties is considered the most effective method of control. A critical step in breeding wheat for spot blotch resistance is to evaluate resistance traits and their characteristics. Five local spring wheat varieties and 56 species of wheat relatives were selected for resistance using percent diseased area and lesion type methods. After three years of field evaluation, the five local spring wheat varieties were found to be susceptible to spot blotch, whereas the 56 related species showed varying resistance levels, suggesting there is plenty of resistance for spot blotch in each genus of wheat relatives. Drop inoculation for scoring lesion type was, among other things, easy to perform, repeatable, and produced stable disease scores over years and locations. The lesion type method was also more sensitive for reflecting resistance change with growth stage. This method revealed stable resistance traits and high levels of spot blotch resistance in wheat relatives and would be useful in future breeding programs.*

Spot blotch of wheat, caused by *Bipolaris sorokiniana* (*Helminthosporium sativum* syn. *Cochliobolus sativus*), occurs worldwide. In the countries of Southeast Asia, where climates are warm and moist, such as India, Bangladesh, and Thailand, spot blotch is the most severe constraint to wheat production. Several epidemics of the disease have been reported in northeastern, northwestern, and southern China (Wu Yousan *et al.* 1957; Hand Guichao *et al.* 1957; Ouyang Xiao 1962; Ma Qixang, *et al.* 1987; Wu Baichai *et al.* 1983). Due to this high disease frequency, spot blotch is regarded as an important wheat disease in China. However, most leading commercial varieties are not spot blotch resistant. Therefore, the most effective method for future disease control is breeding for resistant wheat varieties.

A critical step in breeding wheat for spot blotch resistance is to evaluate resistance traits and their characteristics. The conventional method is to select for resistance in the field. This method is likely to fluctuate due to climate change and requires further refinement. Another factor that limits breeding efforts is the lack of resistance sources in wheat. Wild sources are rich in resistance genes for various stresses and have been exploited for improving wheat. Disease resistance

traits are thus being studied and compared using different evaluation methods for the special features of wheat and its relatives.

Materials and Methods

Field selection

Spot blotch resistance of wheat and its relatives was evaluated in the nursery at Shenyang Agricultural University during 1992-1994. Five local spring wheat varieties studied were Tiechun No. 1, Tiechun No. 2, Liaochun No. 9, Liaochun No. 10, and Shenmian 85. Fifty-six species of wheat relatives grouped into seven genera were screened, including *Aegilops* L., *Agropyron* Gaerth., *Elytrigia* Desu., *Haynaldia* Schur., *Leymus* Hochst, *Roegneria* C. Koch, and *Secale* L.

Disease severity in the field was recorded on a 0-4 scale according to Zhang Jinchun and Zhu Xinting (1991) and Wu Wei *et al.* (1989) at milk and soft dough stages. The scoring standards were as follows:

0 Immunity (IM) = Free from spotting.
1 Resistance (R) = Necrotic spots on 2-5% of the flag leaf and 5-10% of the penultimate leaf area.
2 Moderate resistance (MR) = Necrotic spots on 25% of the flag leaf and 40% of the penultimate leaf area.
3 Moderate susceptibility (MS) = Necrotic spots on up to 50% of the flag leaf and 80% of the penultimate leaf area.
4 Susceptibility (S) = Necrotic spots on up to 80% of the flag leaf area, the penultimate leaf dead, and leaf sheath infected.

Reaction type on excised leaves *in vitro*

Six excised leaves were placed in a petri dish containing a layer of filter paper moistened with 40 ppm 6 BA water solution. Each leaf was inoculated with 4-6 drops of a suspension solution of conidiospores (*B. sorokiniana*), then transferred and cultured under for 12 h at 25±1°C and 2000 lux. Necrotic spot reaction types were scored at intervals. Control leaves were drop-inoculated with water. Number of *B. sorokiniana* conidiospores in suspension was determined under a microscope at a low magnification (15x4).

Disease severity was recorded on a 0-4 scale, according to the type of necrotic spot on the excised leaf. Resistance level was scored according to disease severity as follows:

0 Immunity (IM) = Free from spotting.
1 Resistance (R) = Necrotic spots without chlorosis.
2 Moderate resistance (MR)= Necrotic spots with light chlorosis but of limited area.
3 Moderate susceptibility (MS) = Necrotic spots with obvious chlorosis along the vein.
4 Susceptibility (S) = Large necrotic spots with a larger area of chlorosis.

Disease severity and lesion type at different growth stages

The flag and penultimate leaves, detached from the wheat plant at elongation, boot, early heading, and heading stages, were used to evaluate resistance reaction by drop inoculation.

Five spring wheat varieties (Tiechun No. 1, Tiechun No. 2, Liaochun No. 9, Liaochun No. 10, and Shenmian 85) grown in the greenhouse were chosen for the study. Lesions were scored at 48, 72, and 120 h after drop inoculation. Trial procedures were repeated three times, and mean disease severity data were compared.

Lesion type in wheat relatives

Six species of wheat relatives were grown in the greenhouse, and the flag and penultimate leaves were detached from the plants at early heading. The species studied after drop inoculation were: *Triticum tauschii* (D genome), *Agropyron desertorum, Elytrigia elongata, Et. intermedia, Haynaldia villosa,* and *Leymus racemosus.*

Resistance reaction was continuously monitored until lesion development on the excised leaves had finished. Lesion type was recorded at 144, 216, 240,and 288 hours. The experiment was repeated three times.

Results

Field selection

During three years of field resistance evaluation, the five local spring wheat varieties were found to be susceptible to spot blotch, whereas the 56 species of wheat relatives grouped into seven genera had varying resistance levels.

Of the 56 wild wheat species, all were infected by *B. sorokiniana* but to different degrees. In terms of disease severity scoring, the number of resistant wild species was 26 (46.4%); moderately resistant, 7 (12.5%); moderately susceptible, 1 (1.7%); and susceptible, 22 (39.2%). *Haynaldia villosa,* with the highest level of spot blotch resistance, showed sparse necrotic spots on leaves; the next most resistant species belonged to the *Elytrigia, Elymus,* and *Secale* genera. *Aegilops* was an important genus, comprising many species with higher spot blotch resistance levels such as *T. tauschii, Ae. longissima* Chw. et Musch, and *Ae. speltoides* Tausch.

Field-resistance selection results suggest that there are plenty of resistance traits for spot blotch in each genus of wheat relatives, except for *Agropyron desertorum,* which was susceptible. Resistance in the wild relatives included diverse characters, since the genes controlling resistance belonged to different genomes. The results also show that wheat relatives have valuable sources of resistance that could be exploited for spot blotch control (Table 1).

Lesion types on excised wheat leaves

To study disease reaction change, the flag and penultimate leaves excised from wheat plants at heading were inoculated with drops of a conidial suspension of *B. sorokiniana.* Five spring wheat varieties were used. After drop inoculation of the leaves in petri dishes, light chlorosis appeared at the site of the drops after about 24 h. After 48 h, brown and black necrotic spots were obvious, occasionally surrounded by water soaked chlorosis. At 72 h, the lesion coalesced and expanded

along the veins with an area of obvious yellowish chlorosis. After 140 h, the necrosis and chlorosis joined together between the drop inoculation sites, causing leaf death. The control leaves not only stayed alive and green at 24, 48, 72, and 120 h, but there was no sign of chlorosis or wilting in that time. Rate of formation and area of necrosis with chlorosis between the two uppermost leaves differed between the flag and penultimate leaf. Prior to 72 h in the petri dish, the disease area on the penultimate leaf expanded faster than that on the flag leaf. By 120 h after inoculation, lesion types on both leaves reached the same score, and the lesions joined together, causing the leaves to wilt.

After inoculation, lesion development was similar on both the flag and penultimate leaves, and after 120 h the disease reaction was equal for the five spring wheat varieties. Compared with the results of adult plant resistance evaluated in the field, the five spring wheat varieties had the same disease scores (Figure 1).

Table 1. Adult plant resistance of wheat relatives in the field.

Species	Resistance type	Species	Resistance type
Aegilops		*T. tauschii*	S
Ae. umbellulata	S	*T. tauschii*	R
Ae. umbellulata	S	*T. tauschii*	R
Ae. umbellulata	S	*T. tauschii*	S
Ae. ovata	R	*T. tauschii*	S
Ae. triaristata	R	*T. tauschii*	S
Ae. recta	R	*T. tauschii*	S
Ae. recta	R	*T. tauschii*	R
Ae. variabilis	MS	*T. tauschii*	R
Ae. triuncialis	S	*T. tauschii*	R
Ae. triuncialis	MR	*T. tauschii*	R
Ae. triuncialis	S	*Roegneria*	
Ae. triuncialis	R	*tsuckushienesis*	MR
Ae. triuncialis	S	*Elytrigia*	
Ae. cylindrica	R	*Et. intermedia*	R
Ae. cylindrica	R	*Et. elongata*	R
T. tauschii	R	*Haynaldia villosa*	R
T. tauschii	R	*Leymus racemosus*	R
T. tauschii	MR	*Agropyron desertorum*	S
Ae. crassa	S	*Secale*	
Ae. crassa	S	*S. cereale*	S
Ae. ventricosa	S	(spring rye)	
Ae. ventricosa	S	*S. cereale*	R
Ae. juvenalis	S	(Xinjiang rye)	
Ae. varilovii	S	*S. cereale*	MR
Ae. varilovii	S	*S. cereale*	MR
Ae. speltoides	R	*S. cereale*	MR
Ae. speltoides	R	(winter rye)	
Ae. speltoides	R	*S. cereale*	S
Ae. longissima	S	(winter rye)	
Ae. longissima	S	*S. cereale*	MR
Ae. longissima	MS	(winter rye)	

Effect of wheat growth stage on lesion type

The flag and penultimate leaves of the five wheat varieties were inoculated with drops of conidiospore suspension *in vitro* at elongation, boot, early heading, and heading. Disease reaction and type on the leaves were recorded and compared (Figure 2). Results showed that reaction type on leaves at early heading differed with growth stage and leaf position at 48 hours after drop inoculation. At 120 h, lesion type differed with growth stage

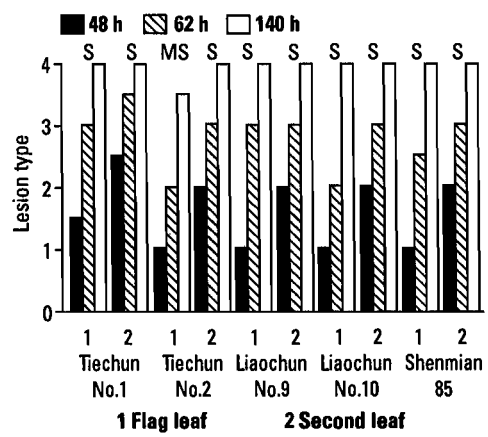

Figure 1. Change in lesion type among wheat cultivars.

and leaf position, but reaction type differed only with growth stage and leaf position until 120 h. These results suggest that there were variations in spot blotch resistance of wheat, depending on growth stage and leaf position, yet the terminal reaction was only affected by growth stage. Therefore, to select for spot blotch resistance in wheat by drop inoculation, the flag leaf excised at early heading is most practical for recording lesion type for evaluation of resistance level.

Lesion type and special features in wheat relatives

Six species of wheat relatives were selected to study spot blotch resistance after drop inoculation. The excised flag and penultimate leaves were inoculated and disease scores were recorded (Figure 3).

Among the six wheat relatives, two species of *H. villosa* with the highest resistance levels and *A. desertorum* with

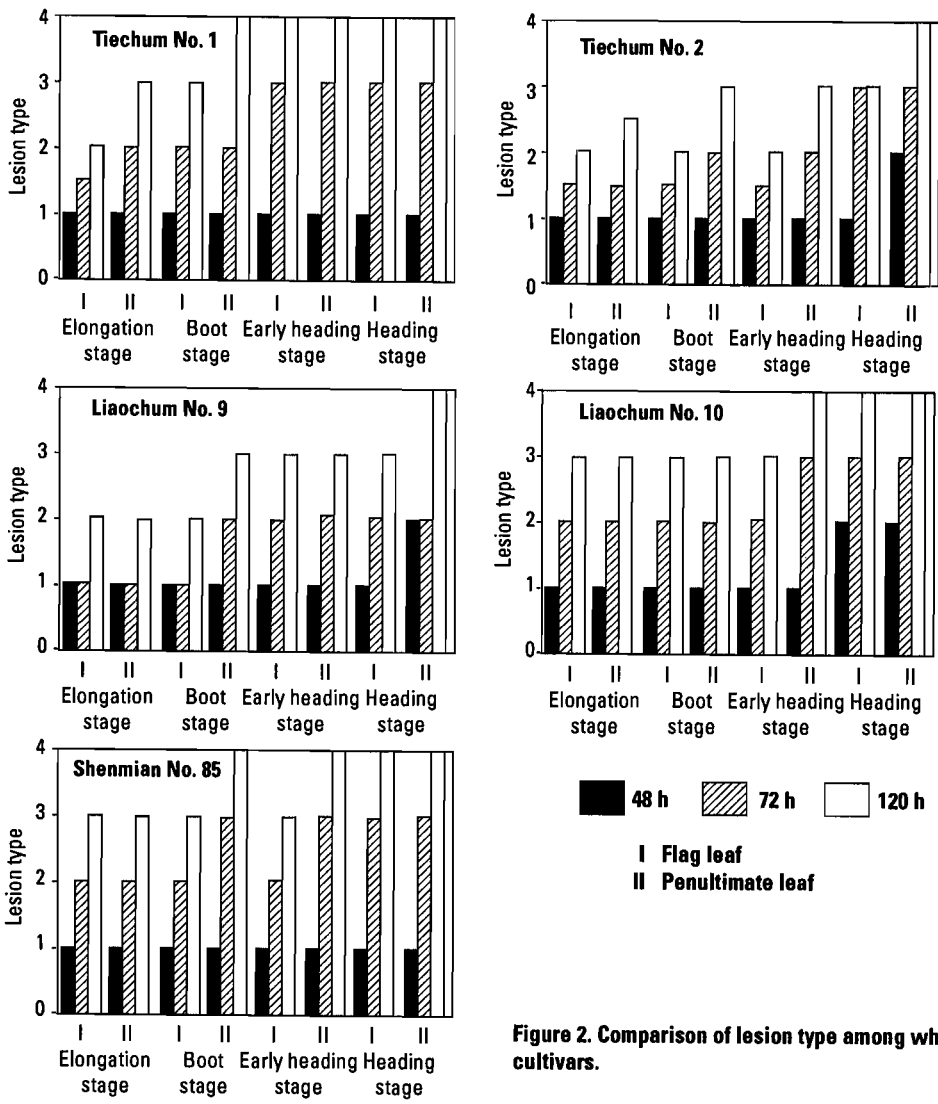

Figure 2. Comparison of lesion type among wheat cultivars.

the lowest resistance level produced results equal to those attained in the field. Results for the other four species with moderate disease resistance scores [*Et. elongata, Et. intermedia, T. tauschii* Coss (D genome), and *Leymus racemosus*] differed from the resistance levels they showed in the field. In contrast to wheat, disease reactions on the leaves of wild species (except *A. desertorum*) did not clearly appear before 120 h after drop inoculation. Stable disease reactions with low expanding rates occurred at 144 h, and disease reactions finished at 216 hours. This was a special feature of wheat relatives. Resistance traits were stable over time and high levels of disease resistance were observed in wheat relatives after drop inoculation. Resistance levels were not easy to determine and would otherwise be achieved through multilocation tests over a series of years. Moreover, differences in disease reaction between the flag and penultimate leaves were not obvious among wheat relatives; only a few species had larger necrotic spots on the penultimate leaf than on the flag leaf during lesion development.

Discussion

Gilchrist (1984) and Large and Doling (1962) reported on selection methods for spot blotch resistance. In China, both natural occurrence (Wu Wei *et al.* 1989) and conidiospore inoculation in the field (Zhang Jingchun and Zhu Zinting 1991) evaluation methods have been used for resistance selection. The methods cited above for selection for spot blotch resistance depend on percent necrosis and associated chlorosis on the surfaces of the two uppermost leaves. Since these are easily affected by environmental factors, disease scores of wheat varieties recorded in the field often change over years at the same location. Therefore, multilocation testing over several years is needed to determine whether resistance levels of the species or varieties are stable.

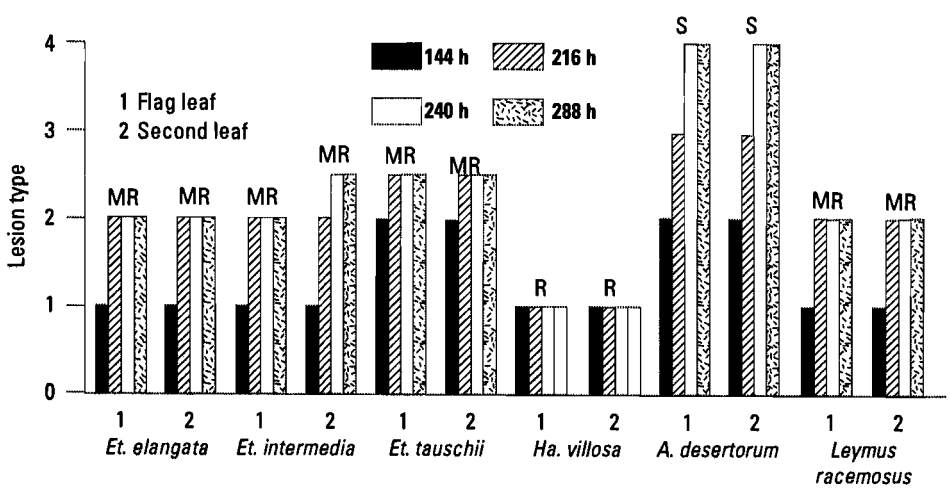

Figure 3. Evaluation of spot blotch resistance in wheat relatives by drop-inoculation method.

Bailey *et al.* (1988) reported that spot blotch resistance was polygenic. Conner (1990) considered that each part of the wheat plant had its own resistance to *B. sorokiniana* that was independently effected. For scoring lesion type, the drop inoculation method was easy to perform and repeat. The method could decrease environmental effects and produce comparable, stable disease scores over years and locations. It was found that while percent diseased area and lesion type methods for selecting spot blotch resistant traits could compensate for each other, the lesion type method was more sensitive for reflecting resistance change with growth stage. This method showed stable resistance traits that expressed high levels of spot blotch resistance in wheat relatives, and would be useful in future breeding programs. To date, no resistance selection method is able to precisely and completely demonstrate spot blotch resistance traits in wheat and its relatives due to the complicated nature and diversity of the resistant traits.

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In vitro Selection for Spot Blotch Resistance in Wheat

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Abstract

Spot blotch of wheat, caused by Bipolaris sorokiniana *(syn.* Helminthosporium sativum*), is an important wheat disease in China. The main varieties currently grown in China are susceptible to the disease, and sources of resistance are limited. A research program was undertaken in 1988-92 to improve resistance by developing a practical procedure for somaclonal selection for spot blotch resistance. Resistance enhanced mutants were obtained from four spring wheats. After field testing the regenerated plantlets for four successive generations, resistance was found to be stable. Resistance evaluation in the field suggested that* in vitro *selection of embryonic calli using* H. sativum *toxins was effective. The mutants obtained expressed higher levels of stable, heritable resistance.*

Introduction

Somaclonal variation is known to cause wide genetic changes and has been used in breeding for disease resistance, salt and drought tolerance, and increased protein levels since 1970. Carlson (1973) was the first to successfully use *in vitro* somaclonal screening for tobacco black foot disease (*Pseudomonas tabaci*) resistance by the toxin-like material to obtain a disease resistant mutant. Since then, there have been reports on somaclonal *in vitro* selection for resistance to helminthosporium leaf spot (*Helminthosporium maydis*), sugarcane eye spot disease (*H. sacchari*), mustard family black foot disease (*Phoma lingam*), and rice bacterial blight disease (*Xanthomonas oryzae*).

Spot blotch, caused by *Bipolaris sorokiniana* (syn. *H. sativum*) of wheat is an important disease in China. There are limited sources of spot blotch resistance available; the principal varieties currently grown in the epidemic regions are susceptible to the disease. In 1988-1992, a research program was undertaken to develop a practical procedure for somaclonal selection for spot blotch resistance. Resistance enhanced mutants from four spring wheats were obtained. The regenerated plantlets were evaluated for spot blotch resistance in the field for four successive generations, and resistance was found to be stable. The cell membrane resistance value technique was used to detect tissue sensitivity to HS (*H. sativum*) toxins.

Materials and Methods

Varieties

Four spring wheat varieties were studied including A211 (MS), Tiechun No. 1 (S), Shenmian 85-9 (S), and Shenmian 85-14 (S). Varietal resistance level was evaluated in the field for two growing seasons (Zhang Jingchun *et al.* 1991).

Purification and toxicity of HS toxins

Strains of *B. sorokiniana* were isolated from diseased wheat plants in the field, transferred to PDA medium, and maintained at 4°C. Toxins (helminthosporal) were extracted and purified according to the method of Wu Wei *et al.* (1989). Toxicity of the HS toxins was tested by the resistance value and bioassay method. The partially purified toxin was kept at 4°C and utilized to screen for resistance (somaclonal) variation in the embryonic calli.

Induction and plantlet regeneration of embryonic callus

Young embryos 17-20 days post-anthesis were selected as explants to induce calli from donor plants. The basic medium used was MS medium. Callus-inducing medium consisted of 2.0 mg L^{-1} 2,4,5-T, 0.2 mg L^{-1} KT, and 0.4 mg L^{-1} IAA in MS medium. Plantlet regeneration medium contained no hormone. Callus induction, and subculture, screening of HS toxins, and plantlet regeneration from the HS toxin-screened calli were done on different media in a 100 ml flask at 25±1°C under 12 h light of 2000 lux.

Choosing suitable HS toxin concentration for resistant mutant selection

While toxicity was being measured, the partially purified HS toxin was applied to the research work and concentration was set at 0.1, 0.5, 1.0, 1.5, 2.0, 2.5, and 3.0 treatment units (1 treatment unit = 1 ml HS toxin in 30 ml MS medium). Controls were kept. According to callus size and the interval between the two HS toxin subculture selections, survival rate and plantlet regeneration rate of the callus were calculated at each HS toxin concentration according to the following formulae:

$$\text{Survival rate (\%)} = \frac{\text{Live calli after HS toxin selection}}{\text{Total number of calli inoculated}} \times 100$$

$$\text{Plantlet regeneration rate (\%)} = \frac{\text{Regenerated calli screened by HS toxins}}{\text{Live calli after HS toxin selection}} \times 100$$

Measuring resistance in the callus

The method used for measuring resistance level in the calli was that of Zhang Xianzheng *et al.* (1986). An improved needle-like electrode was used to measure and compare the change in resistance of the cell membrane during HS toxin selection. The electrode was connected parallel to an electric conductance meter (Model DDS-11). The procedure took place under conditions of saturated humidity and was repeated six times for each test.

Evaluating resistance in regenerated plants

Plants regenerated from HS toxin selected calli were evaluated for spot blotch resistance in adult plants using the field method mentioned above in the nursery at Shenyang Agricultural University. Morphological changes in the regenerated plants were recorded at the same time. Both donor varieties and non-toxin screened plants were maintained in the nursery as controls. Resistance evaluation was recorded over four successive growing seasons to test the heredity of resistance stability in the mutants.

Results

Callus survival and regeneration rate

Immature wheat embryos were inoculated on induction medium repeatedly for 5-6 weeks until the calli were 2-4 mm long. Calli were then transferred to HS toxin screening medium. Survival and regeneration rates of the screened calli were compared over HS toxin concentrations and used as double inspection standards to determine practical HS toxin concentrations for selection. All seven HS toxin concentration treatments were toxic on calli and HS toxins were observed to inhibit callus growth and differentiation (Figures 1 and 2). At 3.0 toxic units, most inoculated calli turned brownish or hydrated and died within two weeks, while some calli survived but lost the ability to regenerate. Although sensitivity to HS toxins differed among calli induced from the four cultivars, the effect of toxins on plantlet regeneration rates showed a similar changing tendency among the four cultivars. In addition, the subculture time on toxic medium affected plantlet regeneration rate of calli, i.e., an increase in toxic subculture time decreased the plantlet regeneration rate. Resuming subculture immediately after toxic medium subculture increased callus regeneration rate.

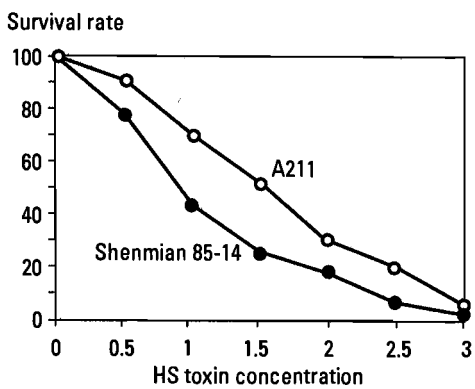

Figure 1. Correlation between *Helminthosporium sativum* (HS) toxin concentration and calli survival rate.

Note: The unit of HS toxin concentration was ml 30 ml^{-1} medium solution. Calli were cultured on toxic medium for two weeks.

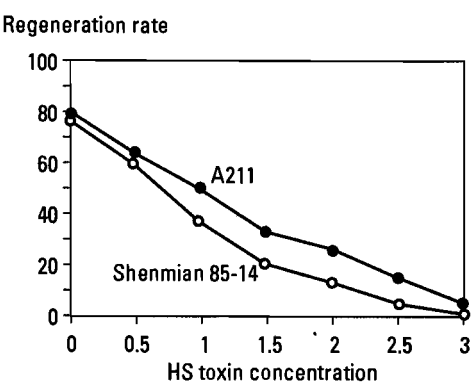

Figure 2. Correlation between *Helminthosporium sativum* (HS) toxin concentration and plantlet regeneration rate.

Note: The unit of HS toxin concentration was ml 30 ml^{-1} medium solution. Calli were cultured on toxic medium for two weeks.

Results showed that callus survival and plantlet regeneration rates ranged between 20-30% and 15-40%, at 1.5 and 2.0 toxic units, respectively. Accordingly, 1.5 (for cultivars with low regeneration rates) and 2.0 toxic units were chosen as HS toxin selection pressure. The toxin selection cycle involved two weeks of toxic subculture repeated twice, and a three-week interval during which subculture was resumed between the two toxic subcultures. When HS toxin selection had finished, the surviving calli were transferred to differentiation media to induce plantlet regeneration. Plantlets were then grown in pots in the greenhouse. HS toxin selection results and callus regeneration rates of the four cultivars are listed in Table 1. After the second toxic medium subculture, callus survival rates ranged between 3.8 and 10.3% and callus regeneration rates were relatively stable at about 40%.

Cell membrane resistance with HS toxin selection

Plant cell membrane resistance value method is an electric physiological method used to inspect both cell membrane stability and ability to endure external stress. It was found that membrane resistance varied among cultivars and their development status. For a cultivar, the resistance value of calli after HS toxin selection was much higher than that of non-toxin screened calli (Figure 3), suggesting that HS toxin selection may increase cell membrane stability and reduce tissue sensitivity to HS toxins.

Heredity stability of spot blotch resistance in regenerated plants

Plantlets regenerated (Ro generation) from HS toxin screened calli were recorded and harvested in the greenhouse. Progeny of Ro generation plants were continuously evaluated for spot blotch resistance in the field at milk stage for four years (Table 2). Regenerated plants from toxin selected calli were evaluated for spot blotch resistance and compared with donor and

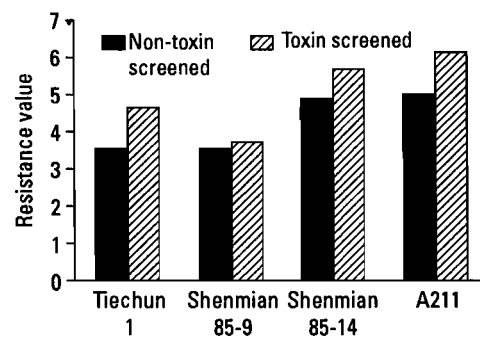

Figure 3. Comparison of cell membrane resistance change in toxin-screened calli and unscreened varieties.

Table 1. *In vitro* screening of wheat cultivars and comparison of plantlet regenerating rates.

Donor variety	No. of calli	No. of surviving calli after toxin screening		Toxin screening rate (%)	Plantlet regeneration rate (%)
		1st screening cycle	2nd screening cycle		
Tiechun 1	958	327	71	7.4	34
Shenmian 85-9	654	125	45	6.9	37
Shenmian 85-14	3214	627	124	3.8	42
A211	657	230	68	10.3	40

regenerated plants from non-toxin selected calli. Individual screened plants with plant height and prolonged heading stage variation in the Ro generation showed stability in spot blotch resistance from the R1 to R4 generation without restoration. Some Ro plants with varying leaf and spike morphology, and spikelet number regained normalcy before R2 generations; other plants in the R3 or R4 generation showed variation in susceptibility to powdery mildew (*Erysiphe graminis*) and leaf rust (*Puccinia recondita*). There was also some variation in susceptibility to spot blotch in the R3 and R4 generations. The head line numbers were stable in the R3 and R4 generation.

Resistance evaluation in the field suggested that *in vitro* selection of embryonic calli for spot blotch resistance using HS toxins was effective, and the mutants obtained expressed a higher level of spot blotch resistance with stable resistance heredity.

Discussion

Chalwa *et al.* (1987) and Guo Lijuan *et al.* (1991) studied somaclonal selection for *B. sorokiniana* resistance in wheat; however, research has not yet been undertaken on the toxicity of HS toxins, establishment of HS toxin screening pressure and resistance assessment methods, and the stability of resistance hereditary in plants regenerated from toxin screened calli. In contrast with donor plants, the number and structure of chromosomes for plant height and prolonged heading stage variation in regenerated plants were found to be normal. Since plant height, heading, and spot blotch resistance are polygenically controlled traits in wheat, it was not clear that morphological changes were due to resistance variation.

It was also found that HS toxin screened tissue with higher HS toxin resistance levels expressed higher cell membrane resistance. Also, activity of enzymes such as PAL (phenylalaninase), PO (peroxidase), and SOD (superoxide dismutase) increased in the resistant mutant (data not shown) and reached higher levels in regenerated plants infected by *B. sorokiniana*. The reasons for resistance variation in the biochemistry reaction will be studied further.

In somaclonal selection for disease resistance, the key step was to set up the screening procedure according to disease characteristics. Since difficulties exist in

Table 2. Spot blotch resistance of regenerating plantlets in the field.

Donor variety	Resistance type	Regenerated plantlets	R3 generation			R4 generation		
			Head line	Plant (no.)	Resistant plant (no.)	Head line	Plant (no.)	Resistant plant (no.)
A211	MS	491	3	578	344	3	320	100
Shenmian 85-9	S	28	3	64	57	3	46	27
Shenmian 85-14	S	144	6	154	226	6	199	107
Tiechun 1	S	1372	16	1728	962	16	557	364
Total	4	2035	28	2524	1589	28	1122	618

using genetic engineering for crop improvement, more attention should be paid to improving *in vitro* somaclonal selection technique and developing new germplasm for use in wheat resistance breeding programs. Other theoretic and technical problems need to be studied further.

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Identification and Inheritance of Resistance to Foliar Blight of Wheat

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Abstract

A total of 102 wheat genotypes were tested for resistance to Bipolaris sorokiniana *and* Alternaria triticina *under artificial epiphytotics for three consecutive crop years. Among these, 13 genotypes (PATO (R)/3/TZPP/SN64//NAR, VEE"S", BOW"S", KVZ/BON, BJY"S"// TOB/CHA, PF 70354/YACOS"S", HD2206/HORK"S", DL 153-2, HS 284, NI 8289, BW 1052, BW 11259, and CPAN 1910) constantly showed resistance throughout the years of testing. Twenty-one genotypes were found to be moderately resistant; 48, moderately susceptible; 15, susceptible; and 5, highly susceptible. No genotype was found to be free from foliar blight. In inheritance studies, F1 progenies of all crosses (NI 8289/UP 262, CPAN 1910/ UP 262, and NI 8289/HUW 234) showed high susceptibility to foliar blight and were comparable to parents UP 262 and HUW 234. In the F2 generation, the segregation in all crosses followed a 1 (resistant):15 (susceptible) ratio, indicating that inheritance of resistance to foliar blight is controlled by two major recessive genes.*

Although foliar blight of wheat was reported in India as early as 1924 (Kulkarni 1924; McRae 1924), it was not of much consequence until recently. With the changes in cropping system, cropping intensity, crop management, and varietal spread, the foliar blight complex has now become a serious disease, causing high yield losses in eastern India. A number of pathogens causing leaf spots, blights, and blotches on wheat have been reported in India. Among them, *Bipolaris sorokiniana* (Sacc. in Sorok.) (syn. *Helminthosporium sativum* Pamm., King, & Bakke) and *Alternaria triticina* (Prasada & Prabhu) are considered important, and it has been shown that *B. sorokiniana* is more important than *A. triticina* (Singh *et al.* 1992).

The main concern today is disease management to achieve sustainable wheat yields. Breeding varieties with leaf blight/ blotch resistance provides the cheapest and easiest means of increasing productivity; however, very few resistant sources have been identified.

This paper describes our investigations into the identification of resistant sources to both pathogens, and the elucidation of the inheritance pattern of *B. sorokiniana* for further use in breeding.

Materials and Methods

Seeds of 102 wheat genotypes were obtained from the Directorate of Wheat Research, Karnal, India. Each genotype

was sown in a 1 m long row with 25 cm between rows and 5 cm between plants under irrigated and high fertility conditions for three years (1988/89 to 1990/91). Pure cultures of *B. sorokiniana* and *A. triticina* were obtained from the Department of Plant Pathology, N.D. University of Agriculture and Technology, Kumarganj, Faizabad, India. These inocula were multiplied separately on PDA medium. Spore suspensions of both pathogens were prepared in distilled water. The final mixed spore suspension contained 8-10 spores per microscopic field (10x). Three days prior to each inoculation, the crop was irrigated. Using an atomizer, the mixed inoculum was sprayed thoroughly onto each genotypes, during the evening at growth stages DC 37 (flag leaf initiation) and DC 52 (heading initiation). The field was irrigated just after inoculation, followed by frequent irrigations to create favorable conditions for rapid disease development with high disease pressure. Disease intensity was noted 10 days after inoculation according to the 0-9 Saari Prescott scale (Saari and Prescott 1975). Using this scale, a score of 1-3 was considered resistant; 4, moderately resistant; 5 and 6, moderately susceptible; 7 and 8, susceptible; and 9, highly susceptible.

During 1991/92, the following crosses were also made: NI 8289/UP 262, CPAN 1910/UP 262, and NI 8289/HUW 234. Lines NI 8289 and CPAN 1910 were used as resistant sources, and UP 262 and HUW 234 as agronomic parents susceptible to leaf blight. The F1 progeny was grown during 1992/93, inoculated with a spore suspension of *B. sorokiniana*, and disease observations recorded. The F1s, F2s, and their parents were grown again during 1993/94 to study the mode of inheritance of resistance. All were inoculated with a spore suspension of *B. sorokiniana* and disease development observations were recorded. Based on observations of individual F2 plants, the mode of segregation was tested using the Chi-square test:

$$\chi^2 = \Sigma \frac{(O-E)^2}{E}$$

where O is the observed frequency, and E is the expected frequency in any particular distribution class.

Results and Discussion

Results presented in Table 1 indicate that the 13 genotypes (PATO(R)/3/TZPP/SN64//NAR, VEE"S", BOW"S", KVZ/BON, BJY"S"//TOB/CHA, PF 70354/YACO"S", HD 2206/HORK"S", DL 153-2, HS 284, NI 8289, BW 1052, BW 11259, and CPAN 1910) were found to be consistently resistant during the three years of testing. Among the remaining genotypes, 21 were moderately resistant; 48, moderately susceptible; 15, susceptible; and 5, highly susceptible. No entry was found to be free from foliar blight during the testing.

Observations shown in Table 2 indicate that all F1 plants in the three crosses (NI 8289/UP 262, CPAN 1910/UP 262, and NI 8289/HUW 234) were highly susceptible to leaf blight and were comparable to the susceptible parents UP 262 and HUW 234.

Data shown in Table 3 indicate that in the F2 generation, the segregation in all three crosses followed a 1 (resistant):15 (susceptible) ratio. This suggests that inheritance of blight is controlled by two major gene loci. Further, it was evident that resistance was controlled by two pairs of recessive genes.

Screening under artificial conditions provides the best means for identifying resistance sources, as it ensures sufficient disease pressure to distinguish between resistant and susceptible types. Furthermore, if the testing continues over years and disease response remains constant, results can be confirmed. In the present case, testing was performed for three years under artificial epiphytotics which helped to clearly categorize the 102 genotypes into resistance grades. Genotypes such as PATO (R)/3/TZPP/SN 64//NAR, VEE"S", BOW"S", KVK/BON, BJY"S"//TOB/CHA, PF 70354/YACO"S", HD 2206/HORK"S", DL 153-2, HS 284, NI8289, BW 1052, BW 11259, and CPAN 1910, which constantly scored 1-3 on the Saari Prescott scale, are clearly resistant and could be utilized as parental lines harboring blight resistance.

Table 1. Foliar blight resistance reaction of 102 wheat genotypes over three years.

Category	Score[1] (0-9)	No. of entries	Genotypes number/name
Resistant	1-3	13	PATO(R)/3/TZPP SN 64 X NAR, VEE"S", BOW"S", KVZ/BON, BJY"S"//TOB/CHA, PF 70354/YACO"S", HD 2206//HORK"S", DL 153-2, HS 284, NI 8289, BW 1052, BW 11259, and CPAN1910
Moderately resistant	4	21	
Moderately susceptible	5 and 6	48	
Susceptible	7 and 8	15	
Highly susceptible	9	5	

[1] Saari Prescott scale.

Table 2. Disease scores observed on F1 progenies of three crosses using the 0-9 Saari Prescott scale for assessment of foliar blight intensity.

Cross	Before inoculation	After inoculation	
		I observation	II observation
NI 8289/UP 262	3	7	8
CPAN 1910/UP 262	4	8	9
NI 8289/HUW 234	4	7	9

Table 3. Distribution of resistant/susceptible plants in the F2 generation.

Crosses	Resistant	Susceptible	P value (χ^2)	Mode of inheritance (ratio)
NI 8289 / UP 262	24	292	0.50-0.30	1:15
CPAN 1910/UP 262	19	269	0.90-0.80	1:15
NI 8289/HUW 234	21	272	0.70-0.50	1:15

Similarly, highly susceptible genotypes such as HD 2329, Sonalika, Bansi, A-9-30-1, and HD 2285 could be used as susceptible checks.

The identification of resistance donors is only the first step in breeding for resistant varieties. The most important aspect is the knowledge of the inheritance of resistance, and unfortunately little is known about the inheritance of foliar blight caused by *B. sorokiniana*. The current study, involving three different crosses, indicated that two major recessive genes are responsible for resistance to foliar blight caused by *B. sorokiniana*. Earlier studies on resistance to foliar blight caused by *A. triticina* also indicated a similar nature of inheritance (Narula and Srivastava 1971; Sokhi 1971).

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Root Rot of Wheat: Inoculation and Screening Techniques, Yield Loss Assessment, and Germplasm Evaluation

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Abstract

The effects of root rot inoculation on the agro-physiological traits of wheat were evaluated in laboratory/greenhouse and field experiments in Colorado and Morocco during 1986-1990. Under laboratory/greenhouse conditions, inoculated adult plants had significantly lower leaf relative water content, reduced plant height, fewer tillers, and higher cell ion leakage compared with non-inoculated plants. Wheat genotypes differed significantly for most traits studied. Genotypes CO840186 and Sandy were the most susceptible and tolerant to root rot, respectively. At Colorado, in early planting trials, root rot severity, measured by average root rot score (ARRS) and white heads (WH), was significantly higher compared with normal planting. Genotypes significantly differed for grain yield, test weight, and reaction to root rot. Seeding rates and inoculation with Fusarium acuminatum *and* Cochliobolus sativus *did not significantly affect disease development; CO840186 was most affected. Seed treatment with imazalil and* Trichoderma harzianum *did not inhibit disease development. WH due to root rot was, in general, a more accurate measure of root rot severity than ARRS to differentiate between genotypes under Colorado field conditions. Under Moroccan conditions, N level had no significant effect on disease epidemics. From February to April, 1989/90, high root rot incidence (WH percentage) after seed inoculation with a spore suspension coincided with severe drought. Inoculation significantly reduced grain yield and kernel weight, and increased WH percentage of both durum and bread wheat cultivars. Durums were generally more affected by inoculation than bread wheats for all studied traits.*

Root rot, foot rot, crown rot, pink root, culm rot, and stalk rot are all terms used to describe a group of wheat diseases caused by one or more organisms, alone or in association (Burgess *et al.* 1975). Common root rot has been reported in most cereal growing areas of the world. Root and foot rots can be important diseases worldwide; however, the occurrence and frequency of causal agents vary regionally (Fernandez *et al.* 1985; Lyamani 1988). Damage caused by root rot pathogens depends on the amount of inoculum present, cultural practices, and soil and climatic factors (Statler and Darlington 1972; Tinline 1986; Wildermuth and McNamara 1991). Typical disease symptoms include discoloration, and necrosis or rotting of roots, subcrown internodes, crown and/

or the stem base (Sallans 1965; Watkins and Kerr 1980; Stack 1982). Under severe disease pressure, symptoms such as stunting, late death tillers, and premature ripening and bleaching of spikes, known as "white heads" (WH) or "dead heads", may occur (Scardaci and Webster 1982; Satck 1982; Lyamani 1988; Mergoum 1990, 1991).

Root rot can be caused by one or several pathogens, alone or in combination (Statler and Darlington 1972): *Cochliobolus sativus* (Ito & Kuribayashi) Drechsler ex Dastur (conidial stage = *Helminthosporium sativum* Pammel, King & Bakke, syn. *Bipolaris sorokiniana* (Sacc. in Sorokin Shoemaker)) (Verma *et al.* 1976; Specht and Rush 1988), *Fusarium culmorum* (W.G. Smith) Sacc., *Fusarium graminearum* Schwabe, and *Fusarium avenacum* (Cook 1980; Saari and Wilcoxon 1984; Dodman and Wildermuth 1987).

Root rot is a persistent and inconspicuous disease that reduces seedling vigor and impairs the functioning of roots and crowns, resulting in stand loss, reduced yield, and lower grain quality (Fenster *et al.* 1972; Mergoum 1990). Number of tillers per plant and number of kernels per spike appear to be the yield components most affected by root rot. Protein content seems to be only slightly affected by root rot (Ismail and Michalik 1980). Plants can recover from initial stunting due to early infection by root rot pathogens if favorable environmental conditions prevail later (Sallans 1959). Recovery from *F. culmorum* infection is less marked than from *C. sativus* infection and is cultivar dependent. Simultaneous infection of wheat seedlings by both *F. culmorum* and *C. sativus* causes more initial stunting and is followed by a stronger recovery than with either pathogen alone (Sallans, 1965). When severe damage occurs in the crown, plants will not recover, even under optimum environmental conditions. Recovery depends on damage of crown tissue (Fenster *et al.* 1972).

This paper summarizes the work that has been conducted on root rot diseases under field conditions and in the laboratory and greenhouse in Morocco and Colorado (USA) during the last decade. The main results reported in this paper relate to: 1) inoculation and evaluation techniques of root rot, 2) effects of the disease on wheat under different water regimes and N levels, and 3) wheat germplasm evaluation in Morocco.

Inoculation

Selection of resistant cultivars to root rot pathogens has been hindered by inadequate and inconsistent inoculation methods, and lack of accurate and suitable techniques for measurement of disease response. In this paper, two (seed and seed/soil inoculations) and one (seedling inoculation with a single germinated macroconidia) inoculation methods were used for field and greenhouse experiments, respectively.

Fusarium spp. (*F. acuminatum* or *F. culmorum*) were isolated on potato

dextrose agar (PDA). Isolates were then grown on water agar containing carnation leaves to induce macroconidium production. After six days, a piece of carnation leaf was placed in a test tube containing sterile water and then shaken. Two ml of conidial suspension were added to a test tube containing 10 ml of sterile water, thoroughly mixed, and 2 ml were placed in petri dishes containing water agar. The dilution procedure was repeated three to five times. Petri dishes were incubated at room temperature under continuous light for one to two days to allow spore germination. Single, isolated and germinated macroconidia were cut out of the agar with a sterile needle under a binocular microscope (100x) and used to inoculate seedlings. Seedling inoculation was performed by placing the macroconidia on the largest root of each vernalized seedling. One-half of the seedlings were inoculated. After four days, all seedlings were transplanted to 15 x 20 cm pots containing steamed soil, peat, and vermiculite in a ratio of 2:2:1 (three plants per pot) and incubated in the greenhouse.

One *Fusarium* spp. isolate and one *C. sativus* isolate were separately cultured on PDA for 15 days at room temperature (20°C). After 15 days, distilled sterile water was added to the petri dishes, spores and mycelia were gently scraped off the plates into a 250 ml flask containing 200 ml distilled water, and flasks were shaken. The solution was filtered through sterilized cheesecloth. The spore suspension was adjusted to 10^6 spores per ml and used for wheat seed inoculation in the field trial. The fungal suspension (200 ml) and 2 kg of seeds were mixed in a plastic bag for 4 to 5 min. Seeds were dried at room temperature for 48 h, placed in paper bags, and planted one week later.

In addition, one *Fusarium* spp. isolate grown on PDA medium was used to infest autoclaved barley or oat seeds. Barley or oat seeds (200 g) were immersed in sterile water in a flask and autoclaved for 30 minutes at 103°C. Barley or oat substrate, inoculated with *Fusarium*, was incubated at room temperature. After three weeks incubation, barley seeds were removed from the flasks, dried at room temperature for four days, chopped into 1-3 mm chunks, sprayed with a *C. sativus* spore suspension (10^6 spores per ml), and mixed with wheat seed (200 g kg^{-1}).

Disease Evaluation

Many scoring techniques have been used for root rot diseases (Dodman and Wildermuth 1985, 1987; Lyamani 1988; Kommedahl and Patel 1966; Harding 1971; Hill *et al.* 1987; Mergoum 1991; Armitage 1984; Ismail and Michalik 1980), but are not effective for differentiating small quantitative differences in disease resistance among genotypes. Disease incidence and severity and subcrown internode discoloration have also been used for disease assessment since the above ground symptoms are seldom observed (Ledingham *et al.* 1973; Piening 1973; Mergoum 1991).

In our studies, root rot severity was evaluated by assessing percent premature ripening and "white heads" (WH) or

"dead heads" and average root rot score (ARRS). ARRS is based on subcrown internode discoloration according to a 0-4 scale. ARRS was used to evaluate 20 plants in the 1987/88 experiments. WH percentage was used in 1988/89 in Colorado, and in 1988/89 and 1989/90 in Morocco. In Colorado experiments, treatments were seed density, planting date, inoculation, chemical (imazlil) and biological control (*Trichoderma*); in Morocco treatments included inoculation, water regime, and N level.

Average root rot score

In Colorado experiments (1987/88), ARRS was significantly higher in early planting than normal planting trials. ARRS was significantly different among cultivars for both early and normal planting dates (Table 1). Significant ARRS differences between treatments were also detected within cultivars. In early planting, the lowest ARRS was attributed to *Trichoderma* for both CO840186 and Sandy. In the normal planting date experiment, ARRS was very low (<1), but treatments were also significantly different for both C0840186 and Sandy. The lowest ARRS was consistently recorded on the *Trichoderma* treatment for both cultivars in addition to imazalil treatment for C0840186.

Although significant differences between cultivars and treatments were detected, correlation coefficients between ARRS, grain yield, and grain test weight were, in general, not significant. Thus, root rot evaluation based on ARRS is not considered very reliable under our conditions. Previous studies also showed variable correlations between yield reduction and similar root rot disease evaluation methods (Piening 1973; Tinline and Ledingham 1979). Other disease scoring techniques also yielded inconsistent results (Kommedahl and Patel 1966; Harding 1971; Armitage 1984; Ismail and Michalik 1980). The percentage

Table 1. Grain yield (GY), volume weight (VW), and root rot scores (ARRS) of wheat cultivars Sandy and C0840186 grown at Julesburg, Colorado, USA, 1987/88.

		Early planting			Normal planting		
Genotype	Treatment[1]	GY (kg ha^{-1})	VW (kg m^{-3})	ARRS (0-5)	GY (kg ha^{-1})	VW (kg m^{-3})	ARRS (0-5)
C0840186	Im.	155	196	1.3	1945	762	0.1
"	Tr.	0	0	0.5	2160	807	0.1
"	Im.+Tr.	0	0	1.1	2160	790	0.3
"	In.	0	0	0.9	2302	802	0.5
"	80G	332	190	0.9	2641	823	0.3
"	40G	0	0	1.2	1821	756	0.2
Sandy	Im	5182	877	0.7	5970	883	0.4
"	Tr.	4652	876	0.5	5741	878	0.1
"	Im.+Tr.	5950	882	0.9	5706	904	0.2
"	In.	5614	894	1.7	5674	888	0.7
"	80G	6446	892	1.5	6202	901	0.6
"	40G	5245	889	0.9	5661	881	0.2
Means		2798	475	1.0	3999	840	0.3
LSD (P<0.05)		1103	134	0.7	905	40	0.2

[1] Im. = Imazalil; Ir. = *Trichoderma*; In. = Inoculated (seed/soil inoculation); 40G = 40 g seed per plot (check); 80G = 80 g seed per plot (double normal seed rate).

of diseased plants or tillers, however, was successfully used to evaluate crown rot of wheat caused by *F. graminearum* Group 1 (Dodman and Wildermuth 1987, 1985; Liddell *et al.* 1986; Klein *et al.* 1985).

White heads

In 1988/89, root rot disease estimates in Colorado experiments were based on WH percentage. Wheat cultivars were significantly different but treatments within cultivars did not differ significantly at either planting date (Table 2). In the early planting experiment, WH percentage varied from 2.8 (control = 0) to 5.5% (IG, inoculated with infected ground barley; and 80G, 80 g seed per plot - double normal seed rate) for Sandy. All C0840186 plants were killed before heading in all treatments. At normal planting date, WH percentage was generally lower than when scored at the early planting date for Sandy, but ranged from 13 (IG) to 17.5% (40G, 40 g seed per plot - check) for C0840186. This suggests that if C0840186 had survived in the early planting dates, higher WH scores could have been observed. The WH percentage method used at Julesburg in 1988/89 showed a clear difference between cultivars but not between treatments within cultivars (Table 2). Therefore, this method is better than ARRS to separate cultivars under Colorado field conditions. Also, correlations between WH percentage and grain volume weight (-0.5) in early planting, and between WH and grain yield (-0.8) in normal planting were significant.

Effects of Root Rot Inoculation on Agronomic Performance of Wheat

Greenhouse and laboratory experiments

Five laboratory and greenhouse experiments were conducted from 1986 to 1988. These experiments included two winter wheat cultivars (Sandy and

Table 2. Grain yield (GY), volume weight of grains (VW), plant height (HT), and white head percentage (WH) of wheat cultivars Sandy and C0840186 grown at Julesburg, Colorado, USA, 1988/89.

		Early planting				Normal planting			
Entry	Treatment[1]	GY (kg ha^{-1})	VW (kg m^{-3})	HT (m)	WH (%)	GY (kg ha^{-1})	VW (kg m^{-3})	HT (m)	WH (%)
C0 84016	IG	0	0	0	0	5016	894	0.70	13.0
"	IW	0	0	0	0	5699	900	0.71	13.0
"	0	0	0	0	0	5334	887	0.70	14.3
"	40G	0	0	0	0	5052	886	0.70	17.5
"	80G	0	0	0	0	5551	906	0.71	15.5
Sandy	IG	9441	898	0.80	5.5	8581	954	0.81	1.3
"	IW	10318	905	0.80	3.5	9294	954	0.81	1.0
"	0	11059	930	0.80	2.8	8828	953	0.82	1.2
"	40G	10762	920	0.80	3.5	8883	954	0.80	1.2
"	80G	11454	915	0.79	5.5	8862	941	0.80	1.2
Means		5303	457	0.40	2.1	7110	923	0.76	7.9
LSD (P<0.05)		1063	18	0.02	4.5	750	20	0.02	5.0

[1] IG = Inoculated with infected ground barley (seed/soil inoculation); IW = Inoculated with infected whole barley (seed/soil inoculation); 0 = Not inoculated; 40G = 40 g seed per plot (check); 80G = 80 g seed per plot (double normal seed rate).

CO840186) considered resistant and susceptible, respectively, to common dryland root rot under Colorado field conditions (Quick 1986, personal communication), two inoculation treatments (inoculated vs. non-inoculated), and two water stress treatments (stressed vs. irrigated). The water stress treatment was similar in all experiments, except one, where all plants were grown under full irrigation until tillering (6, Feekes' scale) (Large 1954) before irrigation was stopped in stressed plots. Samples of three plants per treatment were collected at 3-day intervals.

Seedling survival was assessed one week after transplanting plants to pots; plant infection and height were recorded at heading (10, Feekes' scale), relative water content (RWC) of the flag leaf was scored, and ion leakage (IL) was measured based on electrical conductivity.

Plant tiller number and height— Seedling inoculation with a single germinated *F. acuminatum* macroconidium significantly reduced number of tillers per plant and height, particularly under water stress (Table 3).

Under full irrigation, number of tillers per plant was significantly reduced by *F. acuminatum* inoculation from 4.2 to 3.9 and 3.9 to 2.9 for CO840186 and Sandy, respectively. Under water stress, tiller number was reduced more drastically from 4.0 to 3.7 and 3.4 to 1.9 for Sandy and C0840186, respectively. This is in agreement with other results (Piening 1973; Ledingham *et al.* 1973; Verma *et al.* 1976; Grey *et al.* 1991). Similarly, root

Table 3. Trait means of two wheat cultivars grown in the greenhouse under two root rot inoculation treatments (*Fusarium acuminatum*), 1986-1988.

		Sandy			CO 84018		
Trait	Inoculation	Full irrigation	Stressed	LSD (P<0.05)	Full irrigation	Stressed	LSD (P<0.05)
Seedling survival (%)	Inoculated	70	-	-	35	-	-
	Control	75	-	-	74	-	-
	LSD (P<0.05)	15	-	-	15	-	-
Plant infection (%)	Inoculated	56	83	14	73	97	14
	Control	5	7	14	4	9	14
	LSD (P<0.05)	11	11	11	11		
Plant height (m)	Inoculated	0.60	0.43	0.07	0.62	0.39	0.07
	Control	0.65	0.47	0.07	0.66	0.45	0.07
	LSD (P<0.05)	0.03	0.03	0.03	0.03		
Tiller number/plant	Inoculated	3.9	3.7	0.2	2.9	1.9	0.2
	Control	4.2	4.0	0.2	3.9	3.4	0.2
	LSD (P<0.05)	0.4	0.4	0.4	0.4		
Relative water content (%)	Inoculated	52	44	6	41	29	6
	Control	60	55	6	53	50	6
	LSD (0.05)	4	4	4	4		
Electric conductivity (micromho)	Inoculated	1.57	2.84	0.35	1.67	3.24	0.35
	Control	0.98	1.40	0.35	1.32	1.32	0.35
	LSD (P<0.05)	0.30	0.30	0.30	0.30		

inoculation and water stress resulted in significant plant height reduction (Table 3).

Relative water content (RWC)—The relative water content (RWC) of the flag leaf was measured to determine the effect of root inoculation on the wheat plant's water status. RWC of Sandy and C0840186 was significantly reduced from 60 to 52% and 53 to 41%, respectively, without water stress, and from 55 to 44% and 50 to 29%, respectively, under water stressed conditions (Table 3). These results showed that CO840186 was more affected by root rot inoculation than Sandy, particularly under water stress. RWC reduction for CO840186 due to inoculation was 12 and 21% for non stressed and stressed treatments, respectively. RWC of Sandy was reduced only by 8 and 11% for the same treatments. RWC of the flag leaves decreased drastically with water stress severity. Beyond nine days after the last irrigation, however, RWC reduction due to inoculation was less marked and comparable to low stress levels (11 and 12%). However, RWC reduction was less marked in Sandy than CO840186 and varied from 8-13% only (Table 4). These results are in agreement with field observations in Colorado, suggesting that CO840186 is more susceptible than Sandy. Root rot disease may inhibit the movement of water from the soil to the flag leaf, especially under water stress conditions. Therefore, RWC is another character that may be useful for screening wheat for root rot tolerance.

Ion leakage—EC is proportional to the number of free ions in solution and indicates the amount of cell injury caused by stress (Shanahan *et al.* 1990). In our experiments, the stresses were inoculation and soil water availability. Results showed the significant effect of cultivar, water stress, and inoculation on EC of flag leaf solutions (Tables 3 and 4). Without water stress, EC of both Sandy and CO840186 were significantly increased from 0.98 to 1.57 and 1.32 to 1.67 micromhos, respectively. The effect of inoculation on flag leaf EC was drastically higher for plants grown under continuous

Table 4. Effects of water stress and *Fusarium acuminatum* inoculation on relative water content (RWC) and electric conductivity (EC) of flag leaves of plants grown in the greenhouse in Colorado.

Water stress level (days from last irrigation)	Inoculation	Trait			
		RWC (%)		EC (micromhos)	
		Sandy	CO 840186	Sandy	CO84018
3	Inoculated	75	68	0.97	1.11
	Control	83	79	0.95	1.01
6	Inoculated	65	51	1.18	1.44
	Control	78	76	1.14	1.18
9	Inoculated	43	26	1.40	2.89
	Control	56	47	1.23	1.24
12	Inoculated	33	23	1.54	3.96
	Control	41	34	1.43	1.53
15	Inoculated	31	21	1.64	3.90
	Control	40	33	1.65	1.75
LSD (P<0.05)		4	4	0.47	0.47

water stress, particularly for CO840186. With varying water stress, significant EC differences between both cultivars were observed as a result of inoculation (Table 4). Under moderate water stress, inoculation by the root rot pathogen did not significantly affect EC of both cultivars. Severe water stress (>9 days since last irrigation), however, caused significant EC differences between inoculated and control plants for CO840186 only (Table 4). These results show another aspect of root rot tolerance in cultivar Sandy and suggest that EC is possibly useful to separate wheat genotypes with respect to root disease tolerance.

Field Experiments

Colorado (USA)

Field experiments on root rot were conducted in 1987/88 and 1988/89 (Julesburg, Colorado, USA) to determine the effect of *F. acuminatum* inoculation on several characteristics of wheat, as well as the role of seeding rate, sowing date, and seed treatment on disease development.

Grain yield—Genotypes significantly differed in grain yield in all field experiments (Tables 1 and 2). In the early planting experiments, CO840186 did not produce any grain because of root rot, except in 1987/88 when treated with fungicide or when seed rate was doubled (Tables 1 and 2).

In 1987/88, the double seed rate produced higher yields than other treatments at both planting dates. For the normal sowing time, grain yield significantly differed between cultivars; differences between treatments were only found for CO840186.

In 1988/89, wheat grain yield was significantly different at both planting dates. In the early planting trial, yield was significantly different for Sandy, and C0840186 was not harvested due to total destruction by root rot.

Although significant differences occurred between both cultivars for grain yield, the effect of treatments on grain yield, including inoculation, were variable at both planting dates in both years. Using a doubled seed rate, however, produced significantly higher grain yields. The effects of *F. acuminatum* inoculation in the experiments were not supported by previous works in the USA (Hill and Fernandez 1983; Stack 1982; Grey *et al.* 1991), Canada (Ledingham *et al.* 1973; Piening *et al.* 1976; Verma *et al.* 1976; Tinline and Ledingham 1979), Australia (Dodman and Wildermuth 1985; Wearing and Burgess 1977), and Brazil (Luz 1984).

Volume weight of grain—Wheat genotypes significantly differed in grain volume weight (VW) for both planting dates and seasons (Tables 1 and 2). In 1987/88, only C0840186 showed significant differences in grain VW at both planting dates. At normal planting, VW was 756 and 823 kg m^{-3} for normal and double seed rate treatments, respectively. VW of Sandy was significantly higher in most cases, but was not significant at both planting dates.

In 1988/89, although the two cultivars significantly differed for VW at both planting dates, significant differences for treatments were only observed for Sandy at the early planting date.

Results indicated, in general, no significant effect of *F. acuminatum* inoculation on VW. This is probably due to the presence of root rot pathogens in the soil which infected plants in non-inoculated plots. Other studies on the effect of root rot on VW, however, reported negative effects of the disease on VW (Sims *et al.* 1961; Ledingham *et al.* 1973; Verma *et al.* 1976; Weise 1977).

Plant height—Plant height was examined in 1988/89. Significant differences between genotype were observed (Table 2); however, treatments within genotypes did not significantly affect plant height. Field results contrasted with those from the greenhouse (Table 3).

Chemical and biological control—The effects of imazalil and *Trichoderma harzianum* on *F. acuminatum* were examined in the field during 1987/88 only (Table 1). Compared to the checks and other treatments, imazalil and *Trichoderma* did not reduce disease severity and did not significantly affect grain yield or VW (Table 1). These results confirmed that chemical seed treatments have limited value since infection from soilborne pathogens can occur after the protective period of the fungicide treatment has ended (Watkins and Kerr 1980; Stack 1982); however, positive results have been obtained in controlling root rot in spring wheat with fungicides including imazalil (Verma 1983; Verma *et al.* 1986) and other systemic fungicides (Cassini 1967, 1970; Lyamani 1988). In other studies, *Tr. harzianum* was reported to have strong control over both *F. culmorum* and *C. sativus* (Anwar 1949; Uoti 1976).

Morocco

In Morocco, root rot is caused mainly by *C. sativus* and *F. culmorum* (Baye 1984; Lyamani 1988). Previous studies have shown the importance of water deficit and N fertilization effects on disease development (Baye 1984; Mergoum 1991). Although Moroccan plant breeders and pathologists believe that root rot is a serious problem for cereals, no accurate information is available to document the effects of root rot pathogens on wheat under water stress and low N. Several experiments were conducted at Sidi El Aydi and Tassaout experiment stations during 1988/89 and 1989/90 growing seasons to study the effect of root rot inoculation by *C. sativus* and *F. culmorum* on several traits of wheat.

Root rot evaluation—Percent WH was recorded in 1988/89 and 1989/90 (Tables 5 and 6). WH scores were much lower in 1988/89 compared to 1989/90. WH percentage significantly varied only for irrigation regimes and cultivars.

During 1989/90, however, WH percentage varied with irrigation, cultivar, and inoculation; there was a cultivar x inoculation interaction. Cultivars showed consistently higher WH percentages under stressed conditions than under irrigation in all 1989/90 field experiments

at both locations (Tables 5 and 6). Under stressed conditions, WH percentages were as high as 20% for durum cultivar Cocorit at Tassaout and 31% at Sidi El Aydi in inoculated plots (Table 5). Inoculation significantly increased WH percentages of cultivars Jouda, Marzak, Kyperouda, and Cocorit at Tassaout and Sidi El Aydi under stressed conditions. Under irrigation, however, inoculation did not significantly effect WH percentage (Table 5).

Cocorit and Marzak had the highest WH levels. These results are in agreement with those reported in the same regions by Lyamani (1988)

Inoculation techniques—Although the seed/soil inoculation technique has produced satisfactory results with *F. graminearum* in several previous works (Dodman and Widermuth 1987), low WH percentages resulted from this technique in 1988/89 (Tables 5 and 6). The failure to

Table 5. White head percentage (%) caused by root rot pathogens of six wheat cultivars grown under two water regimes at Tassaout and Sidi El Aydi, Morocco, 1989/90.

	Tassaout				Sidi El Aydi			
	Irrigated		Non-irrigated		Irrigated		Non-irrigated	
Cultivar	Control	Inoc.[1]	Control	Inoc.	Control	Inoc.	Control	Inoc.
Marchouch	0	0	4	8	0	0	4	6
Jouda	0	3	4	9*	0	0	4	7*
Teguey-32	0	0	2	5	0	0	1	2
Marzak	1	6	5	28**	0	3	4	18**
Kyperouda	0	2	3	9*	0	0	3	9*
Cocorit	0	5	6	31**	0	1	4	20**
Mean	0	3	5	15	0	1	3	10
LSD (P<0.05)	4	4	5	5	3	3	4	4

[1] Inoc. = Inoculated with root rot.
*, ** Significantly different to control at P<0.05 and P<0.01.

Table 6. Means of kernel weight, kernel yield, and white head percentage of six wheat cultivars affected by root rot pathogens, grown under two water regimes at Tassaout and Sidi El Aydi, Morocco, 1988/89 and 1989/90.

	Tassaout				Sidi El Aydi			
	Irrigated		Non irrigated		Irrigated		Non irrigated	
Trait[1]	Non-inoc.[2]	Inoc.	Non-inoc.	Inoc.	Non-inoc.	Inoc.	Non-inoc.	Inoc.
1989/90 (inoculation with a spore suspension)								
Kernel weight	49	43*	27	20*	39	32**	36	33*
Grain yield	4526	3864**	1850	1180**	3140	2311**	3536	2236**
White heads	2	3	5	15**	0	2	3	10**
1988/89 (inoculation with infected barley seed)								
Kernel weight	36	35	34	33	44	43	47	46
Grain yield	2691	2579	2462	2423	4096	4016	3784	3852
White heads	2	3	3	4	2	4	1	3

[1] Kernel weight = 1000-kernel weight (g); grain yield = Grain yield (kg ha^{-1}), and white heads = White heads percentage (%).
[2] Non-inoc. = Non-inoculated and Inoc. = Inoculated with root rot pathogens.
*, ** Significantly different to non-inoculated treatments at P<0.05 and P<0.01, respectively.

obtain adequate levels of root rot symptoms could be due to environmental conditions rather than the inoculation technique. At Sidi El Aydi, average air temperature was similar in both years and total rainfall was 341 and 326 mm in 1988/89 and 1989/90, respectively. However, rainfall distribution drastically differed, particularly during February to April; whereas 168 mm of rainfall occurred during this period in 1988/89, only 84 mm were recorded in 1989/90. At Tassaout, average air temperature and total rainfall were comparable and had similar distribution patterns to that of Sidi El Aydi in both seasons. The inoculation technique was modified in 1989/90 by treating wheat seed with a spore suspension of the pathogens. This enabled an average WH percentage of 31% to be obtained on durum wheat Cocorit at Tassaout under the non-irrigated regime (Table 5).

Effects of N—In general, N fertilization did not effect root rot development. At Sidi El Aydi, this was possibly due to the high level of residual N in the soil from heavy applications in previous years. At Tassaout, however, the soils were relatively N deficient. These results are in agreement with some previous reports (Sims *et al.* 1961; Broscious and Frank 1986) but conflict with others that demonstrate that root rot severity is enhanced by high N levels (Baye 1984; Smiley *et al.* 1972). Excess N normally promotes growth and increases transpiration of plants, resulting in depletion of soil moisture (Papendick and Cook 1974; Cook 1980).

Effects on grain—Inoculation treatments did not significantly affect yield in 1988/89. In 1989/90, however, inoculation, cultivar, and inoculation x cultivar interaction effects were significant for grain yield.

Grain yield reduction caused by inoculation was higher under the limited water regime than under irrigated conditions (Tables 6 and 7). Under water stress in 1989/90, inoculation significantly reduced average grain yield of all cultivars by 37% (Table 7); however,

Table 7. Grain yield reduction (%) caused by root rot pathogens in six wheat cultivars grown under two water regimes at Tassaout and Sidi El Aydi, Morocco, 1988/89 and 1989/90.

	1988/89				1989/90			
	Tassaout		Sidi el Aydi		Tassaout		Sidi el Aydi	
Cultivars	Irr.[1]	Non-irr.	Irr.	Non-irr.	Irr.	Non-irr.	Irr.	Non-irr.
Marchouch	1	3	2	2	10*	26**	12**	25**
Jouda	3	2	0	3	1	14**	23**	20**
Teguey-32	0	4	1	2	19**	31**	36**	48**
Marzak	3	6	5	4	33**	50**	40**	51**
Kyperouda	4	4	3	5	12*	60**	27**	40**
Cocorit	2	5	4	6	11*	43**	20**	41a**
Mean	2	4	2	4	14	37	26	38
LSD (P<0.05)	4	6	6	7	8	9	8	10

[1] Irr. = Irrigated, Non-irr. = Non-irrigated.
*, ** Significantly different to non-inoculated treatments at P<0.05 and P<0.01, respectively.

under irrigation, inoculation reduced yield by only 26 and 14%. Inoculation greatly reduced grain yield of all cultivars without regard to water stress at both locations in all field experiments in 1989/90. Yield losses as high as 51 and 60% were recorded for durum wheats Marzak and Kyperounda, respectively.

Large yield losses resulting from inoculation by *F. culmorum* and *C. sativus* in 1989/90 (Tables 6 and 7) showed that durum wheat cultivars Marzak, Cocorit, and Kyperounda were more affected than bread wheat cultivars Jouda, Marchouch, and Teguey-32. This is in agreement with previous works conducted in the USA (Hill and Fernandez 1983; Stack 1982), Canada (Ledingham *et al.* 1973; Verma *et al.* 1976), and Australia (Wearing and Burgess 1977).

Cultivar and N level showed significant differences for kernel weight under all environments in both seasons. Inoculation had no effect on kernel weight of cultivars. In 1989/90, however, inoculation and the interaction with irrigation showed significant effects on kernel weight. Cultivars significantly differed for kernel weight in all field experiments at both locations (Table 8). Reductions of up to 34% of kernel weight were recorded on durum wheat Marzak, and, in general, kernel weight of durum wheat cultivars was reduced more than bread wheat.

Table 8. Kernel weight reduction (%) caused by root rot pathogens in six wheat cultivars grown under two water regimes at Tassaout and Sidi El Aydi, Morocco, 1989/90.

	Tassaout		Sidi el Aydi	
Cultivars	Non Irrigated	Irrigated	Non Irrigated	Irrigated
Marchouch	1	20**	1	2
Jouda	2	12*	0	2
Teguey-32	4	8	3	9
Marzak	8*	34**	10*	14**
Kyperouda	2	28**	3	12**
Cocorit	2	29**	11*	17**
Mean	3	26	4	11
LSD (P<0.05)	7	11	6	10

*, ** Significantly different to non-inoculated treatments at P<0.05 and P<0.01, respectively.

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Transformation Technologies Available for Enhancing Fungal Resistance in Wheat

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Abstract

The range of sources of disease resistance genes for a given plant species encompasses particular alleles at one or more loci in its primary gene pool, alleles whose origin might be more or less removed from that pool (thus requiring wide crosses for their introgression) and, finally, completely foreign genes from unrelated species that would require the use of transformation techniques for their transfer. In the case of helminthosporium diseases of wheat, sources of resistance genes have been identified both in hexaploid wheat itself and in its wild relatives. Breeding efforts using both sources have yielded satisfactory products and the prospects for further resistance improvement by combining diverse sources are good since resistance appears to be controlled by only one to three or four genes. With the publication of comprehensive molecular marker maps of the wheat genome over the last four years, and the availability of new marker techniques for mapping the complex wheat genome more effectively and efficiently, it should be possible to more precisely dissect the genetic factors contributing to helminthosporium disease resistance in wheat. However, molecular markers should prove most effective in identifying the genes in alien sources of resistance and in accelerating their transfer to modern wheats. Ultimately, agronomically viable levels of durable resistance in crop plants against a relatively broad range of fungi might be achieved using transformation approaches. Such an approach could include insertion and expression of genes encoding inhibitors of fungal enzymes or known antifungal proteins such as chitinases and ß1-3 glucanases. These hydrolytic enzymes catalyze the degradation of chitin and ß1-3 glucan and, since these compounds are abundantly present in the cell wall of many filamentous fungi, they are thought to be capable of inhibiting fungal growth in planta. *Other genes that could be used include ribosome inhibiting proteins (RIP), which inhibit protein synthesis in target cells, and osmotin, which forms pores in fungal membranes. Insertion of such genes into wheat, when combined with the corresponding plant resistance genes, provides plant breeders with additional sources of resistance.*

Enhanced levels of durable resistance in wheat against a relatively broad range of fungi, including *Helminthosporium*, might be achieved using transformation approaches. Transformation is the insertion of a specific gene or genes from a related or completely unrelated species into the genome of a target organism. One of the main advantages of transformation techniques over those used in conventional breeding is that you are not limited to the pool of genes available in the crop species or even closely related species; it is possible to use genes from

other plants, bacteria, animals etc. In addition, it is also possible to introduce very specific genes without extra genetic information being "carried over", as in the case of conventional backcrossing, therefore leading very rapidly to production of elite lines. However, we are at present still limited by the number of genes which have been cloned, so it is not always possible to target the desired trait. On the other hand, the number of new genes being cloned is always increasing. Another limiting factor is that cereal transformation (especially wheat) is still in its infancy and there is a need to improve the efficiency of transformation by optimizing transformation parameters using biolistics or by using *Agrobacterium*-mediated transformation systems.

Plants respond to pathogen attack or abiotic stresses by activating a complex network of defense mechanisms. These include modifications designed to strengthen the plant cell wall, for example, by deposition of lignin and (hydroxy) proline-rich structural (glyco) proteins, thereby inhibiting, or at least restricting, access of the pathogen to the plant cell. In addition, the plant may synthesise toxic antimicrobial compounds (phytoalexins) as well as inducing and accumulating proteinase inhibitors and lytic enzymes such as chitinase and ß-1,3-glucanase (Broglie *et al.* 1991). These chitinases and ß1-3 glucanases are hydrolytic enzymes which catalyze the degradation of chitin and ß1-3 glucan, respectively, and, since these compounds are abundant in the cell wall of many filamentous fungi (Table 1), they are thought to be capable of inhibiting fungal growth *in planta*. One transformation strategy is to express, in a constitutive manner, defense genes that are normally only induced as a result of pathogen attack. Some of the above-mentioned lytic enzymes have been shown to be active *in vitro* against various pathogens (Mauch *et al.* 1988) and since the active antimicrobial agents are individually encoded by single genes, these defense systems should be highly amenable to manipulation by gene transfer. The first report of success with such a strategy was the expression of a bean vacuolar chitinase gene in tobacco and *Brassica napus*, which resulted in decreased symptom formation of *Rhizoctonia solani* (Broglie *et al.* 1991). Significant reduction in fungal growth and delay in disease development were observed, and in the case of *B. napus*, protection approached potentially useful levels with respect to reduced crop damage at inoculum densities likely to be encountered in the field. Since this initial work was done, several other groups have found similar results by

Table1. Cell wall taxonomy of fungi showing the potential range of fungi which may be affected by chitinase and glucanase transgenes.

Chemical category	Taxonomic group
I. Cellulose-glycogen	Acrasiales
II. Cellulose-**glucan**	Oomycetes
III. Cellulose-**chitin**	Hyphochytridiomycetes
IV. Chitosan-**chitin**	Zygomycetes
V. **Chitin-glucan**	Chytridiomycetes
	Ascomycetes
	Basidiomycetes
	Deuteromycetes
VI. Mannan-**glucan**	Saccharomycetaceae
	Cryptococcaceae
VII. Mannan-**chitin**	Sporobolomycetaceae
	Rhodotorulaceae
VIII. Polygalactosamine-galactan	Trichomycetes

Source: J. Webster 1980.

transforming various antifungal genes into a range of crop plants including tobacco, tomato, canola, and rice which have given enhanced resistance to a number of fungal pathogens including *Rhizoctonia solani*, *Fusarium oxysporum*, *Cercospora nicotianae*, and *Cladosporum fulvum* (Table 2).

Genes encoding ribosome-inactivating proteins (RIP) are also candidates as defense transgenes as they inhibit protein synthesis by specific RNA N-glycosidase modification of 28S rRNA (Endo *et al.* 1988). RIPs do not activate 'self' ribosomes but show activity towards ribosomes from distantly related species including fungi (Endo *et al.* 1988; Stirpe *et al.* 1992). Thus, Logemann *et al.* (1992) have shown that expression of a barley endosperm RIP under the control of an inducible promoter resulted in an increased protection of transgenic tobacco plants against *Rhizoctonia solani* without influencing plant growth.

A notable feature of plant response to pathogen attack is the coordinative nature of the complex network of defense, suggesting that different protective mechanisms may have complementary roles in the overall expression of resistance (Zhu *et al.* 1994). For hydrolytic enzymes such as chitinases or ß-1,3-glucanases, synergistic antifungal properties, leading to enhanced mycelial cell wall destructions, were demonstrated by combining these proteins in *in vitro* assays (Leah *et al.* 1991; Mauch *et al.* 1988). *In vitro* synergistically enhanced antifungal activity of other antifungal proteins such as the barley endosperm RIP combined with the barley class II ß-1,3-glucanase was also observed (Leah *et al.* 1991). The first evidence for enhanced protection *in planta* was reported by Zhu *et al.* (1994) where co-expression of a rice basic chitinase and an acidic ß-1,3-glucanase derived from alfalfa gave substantially greater protection against the pathogen *Cercospora nicotianae* than either transgene alone. Similar results were obtained in tomato (Jongedijk *et al.* 1995) where the synergistic effects of chitinase and ß-1,3-glucanase genes gave enhanced resistance to *Fusarium oxysporum* f.sp. *lycopersici*. Jach *et al.* (1995) showed that the performance of tobacco plants co-expressing the barley transgenes GLU/CHI (ß-1,3-glucanase and chitinase) or CHI/RIP (chitinase, ribosome inhibiting protein) in a *Rhizoctonia solani* infection assay were enhanced, in terms of fungal attack, when

Table 2. Crop plants transformed with anti-fungal genes which gave enhanced resistance to a number of fungal pathogens.

Crop plant transformed	Gene(s)	Fungal pathogen attacked	Reference
Tobacco	Chitinase, ß1-3 glucanase, RIP	*Rhizoctonia solani*	Jach *et al.* 1995
Tobacco	Barley RIP	*Rhizoctonia solani*	Logemann *et al.* 1992
Tobacco, canola	Bean chitinase	*Rhizoctonia solani*	Broglie *et al.* 1991
Rice	Chitinase	*Rhizoctonia solani*	Lin *et al.* 1995
Tomato	Chitinase, ß1-3 glucanases	*Fusarium oxysporium*	Jongedijk *et al.* 1995
Tomato	Chitinase, ß1-3 glucanases	*Cladosporum fulvum*	Wubben *et al.* 1996
Tobacco	Chitinase, ß1-3 glucanase	*Cercospora nicotianae*	Zhu *et al.* 1994

compared with the protection levels obtained with corresponding isogenic lines expressing a single barley transgene to a similar level. Preliminary infection assays with other phytopathogenic fungi such as *Alternaria alternata* and *Botrytis cinerea* also revealed significantly enhanced protection against fungal attack of GLU/CHI or CHI/RIP transgenic tobacco lines. These results indicate that the combinatorial expression of different antifungal proteins can lead to improved protection against a broad range of phytopathogenic fungi. The strategy of producing different antifungal proteins in the same plant is not limited to the combination of different hydrolytic enzymes attacking fungal cell walls. It is possible that the hydrolytic activity of chitinase or ß-1,3-glucanase could result in an increased uptake of RIP into fungal cells and therefore drastically enhance the inhibition of the growth of invading fungi (Jach *et al.* 1995). It is likely that this battery of inducible defenses represents a series of complementary mechanisms for protection against both the initial attack and possibly secondary, opportunistic infections.

Other candidates as defense transgenes are osmotin, which forms pores in fungal membranes, and the antifungal peptide (AFP) from the mold *Aspergillus giganteus* (Nakaya *et al.* 1990). Preliminary data on this AFP revealed protection against fungal attack *in planta* as well as dramatically enhanced inhibition of fungal growth *in vitro* when combined with RIP (Görnhardt *et al.* 1994), suggesting further interesting combinations of antifungal transgenes.

Defense responses such as phytoalexin biosynthesis or lignin deposition in the cell wall require the action of many genes (Lamb *et al.* 1989). Prospects for enhancing the expression of such multigenic defenses depend on either the identification of a rate determining step or the identification of regulatory genes that play a part in the coordinate expression of groups of functionally interdependent defense genes (Lamb *et al.* 1992). In addition, confrontation of pathogens with unfamiliar phytoalexins is potentially an attractive strategy for enhancing resistance. Advances are being made in this area, but our current strategy at CIMMYT has involved insertion of single defense genes in combination (GLU/CHI or CHI/RIP) to give synergistic effects which we hope will lead to enhanced resistance to a range of pathogenic fungi. Early strategies for breeding for fungal resistance were based on single genes; however, such a single gene strategy favored the rapid co-evolution of resistance-breaking pathogen strains. As a result, more recent breeding programs have tried to achieve more durable resistance using multigene combinations (Rajaram *et al.* 1988; Singh and Rajaram 1992). Similarly, breeding for fungal resistance using transgenic plants with multigene combinations seems to be a wise approach and may result in a reduction of the probability of the emergence of resistance-breaking strains of phytopathogenic fungi. Insertion of antifungal transgenes into wheat, when combined with the corresponding host plant resistance genes, provides plant breeders with additional sources of

resistance, and such a concerted approach should give rise to cultivars with a broad and durable field resistance.

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Molecular Analyses of Toxin(s) Produced by *Pyrenophora tritici-repentis*

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Abstract

We are interested in the key events that regulate specificity in the Pyrenophora-*wheat interaction. The host-specific toxin(s) (HST) produced by* Pyrenophora tritici-repentis *is a protein. In the continuing effort to assess the role of toxin production in the pathogenesis of* P. tritici-repentis, *we have purified a major necrosis toxin produced by our fungal isolates, designated ToxA. Analysis of purified toxin by mass spectroscopy indicated a molecular weight of 13.2 kD. Western analysis indicated that antibodies raised against purified toxin reacted with and are specific to a 13.2 kD band associated with toxic activity. cDNA libraries were constructed from toxin-producing isolates of the fungus, and ToxA antibodies were used to identify and subclone cDNAs for the ToxA protein. A subgenomic library was prepared from a toxin-producing isolate of the fungus, and a cDNA insert was used to select a genomic copy of the* ToxA *gene that included its endogenous promoter region. A tox- isolate of* P. tritici-repentis *was transformed with the* ToxA *gene. All tox+ transformants tested produced wildtype levels of the active ToxA protein in culture. Additionally, tox+ transformants were shown to be fully pathogenic when inoculated onto susceptible wheat cultivars. Present efforts are directed toward the elucidation of possible regulation of the* ToxA *gene* in planta, *and to determine whether multiple forms or toxins produced by* P. tritici-repentis *are due to post-translational modification of the* ToxA *gene product.*

Pyrenophora tritici-repentis (Died.) Drechs. (syn. *P. trichostoma* (Fr.) Fckl.), anamorph: *Helminthosporium tritici-repentis* (syn. *Drechslera tritici-repentis* (Died.) Shoem.) is the causal agent of tan spot of wheat (*Triticum aestivum* L.). Tan spot is a destructive disease that has been identified throughout the world (Hosford 1982). Yield losses attributed to this disease have ranged from 2 to 50% (Shabeer *et al.* 1991; Sykes and Bernier 1991). Changes from conventional to minimum tillage have increased the possibility of severe yield losses (Kohli *et al.* 1992). In Australia, the change from stubble burning to retention and zero-tillage practices is believed to be the cause of the increase in tan spot incidence (Brennen and Murray 1988; Lamari and Bernier 1989a). Foliar fungicides are effective for disease control but costs are prohibitive.

Certain isolates of *P. tritici-repentis* in culture were shown to produce a host-specific toxin (HST) that induced typical tan spot necrosis upon infiltration into tissue of susceptible wheat cultivars. Host sensitivity to the toxin was shown to correlate with disease susceptibility

(Tomas and Bockus 1987; Lamari and Bernier 1989b), indicating causality of the toxin in pathogenesis. Because toxin production appears causal in disease development, this information could be exploited to control this wide-ranging and serious disease.

Our approach to investigating molecular mechanisms in the *P. tritici-repentis*-wheat interaction is firstly to isolate and clone the gene(s) responsible for pathogenesis. We then determine how the expression of these genes impacts on host function such that a successful interaction can occur. Ptr toxin has provided a unique opportunity for the isolation of an HST gene (a pathogenicity gene) because the toxin is a protein and, consequently, the direct product of a single gene. Cloning of the gene for toxin production has lead to a direct analysis of the toxin's role as a primary determinant of pathogenicity, and further analysis should indicate how its production impacts on host function. Because a single gene appears responsible for pathogenesis and host sensitivity is conditioned by a single dominant gene (Faris *et al.* 1996; Stock *et al.* 1996), the wheat-*P. tritici-repentis* interaction presents an ideal system for the investigation of plant/microbe interactions. We have been able to take a straightforward approach to the isolation of the gene responsible for toxin production because the gene product is available. Our most recent goal was to directly evaluate the effect of toxin production on pathogenicity.

Molecular Analysis of ToxA Produced by *Pyrenophora tritici-repentis*

A necrosis-inducing toxin was purified (Tuori *et al.* 1995) by a modification of the procedures published by Tomas *et al.* (1990) and Ballance *et al.* (1989). The purified toxin appeared homogeneous by analyses of both SDS and native gel electrophoresis. The toxin is heat stable and an average minimum concentration of 60 nM toxin is required to induce visible necrosis in susceptible wheat cultivars. Analysis of purified toxin by mass spectroscopy indicated a molecular weight of 13.2 kD (Tuori *et al.* 1995). Our purified toxin was designated ToxA.

The detection of necrosis-inducing components distinct from the 13.2 kD ToxA protein was reported by Tuori *et al.* (1995). These components elute in two fractions during HPLC chromatography: 1) in the flow-through, i.e., material which did not bind to the cation exchange resin (anionic toxic component or AI); and 2) immediately following elution of the ToxA protein (cationic toxic component or CI). These other toxic components appear to be chromatographically and immunologically distinct from ToxA or are much more active than the ToxA protein. Interestingly, the CIs appear to be heat-labile. The loss of most activity following a heat treatment indicates that these fractions contain a toxin different from ToxA.

Polyclonal and monoclonal antibodies were raised against purified ToxA. Western analysis indicated that the antibodies against the toxin reacted with and are specific to the 13.2 kD band associated with toxin activity. Indirect immunoprecipitations of crude toxin preparations confirmed the specificity of the antibodies for the 13.2 kD toxin band and indicated that the 13.2 kD protein was the major toxic agent. Electrophoretic analysis of immunoprecipitated products revealed a unique 13.2 kD protein band present only when anti-ToxA antisera were used for the reaction. This unique protein band co-migrated with purified toxin on polyacrylamide gels. Bioassay of the immunoprecipitated 13.2 kD band demonstrated specific necrosis-inducing activity when infiltrated into susceptible (toxin sensitive) wheat (Tuori *et al.* 1995).

Cloning the ToxA gene from *P. tritici-repentis*

Time-course studies were performed comparing toxin production and toxic activity in culture filtrates. Toxin production was determined by western blotting, and toxic activity was assessed by symptom development following leaf infiltration of culture filtrates into sensitive wheat cultivars. Toxin was detected by western analysis as early as two days and appeared to reach a maximum level at 10-12 days post-inoculation. The appearance of toxic activity was directly correlated with the appearance of the ToxA protein (Ciuffetti *et al.* 1997).

Poly(A)$^+$ RNA was prepared from mycelia from 6-, 7-, and 10-day-old cultures. *In vitro* translations of the isolated mRNA were performed to confirm the integrity of the mRNA. Further, indirect immunoprecipitations of total translation products with anti-ToxA antibody were performed to confirm the presence of mRNA for the toxin. Immunoprecipitation results indicated the presence of a *ca.* 19,500 D translation product that reacted with anti-ToxA antibody. The translation product had a molecular weight larger than the mature toxin. This result supports the contention that multiple proteolytic processing is involved in the formation of mature ToxA. Additionally, the results indicated that the translation product was present in relatively high abundance, suggesting a high relative abundance of the mRNA for toxin. Immunoprecipitation results also further established that the antibody reaction is specific, as indicated by the precipitation of a single translation product.

A lambda cDNA library was prepared from mRNA isolated from the fungus during maximum rate of toxin production. This library was screened with anti-ToxA antibody, and antibody-positive recombinants were identified at a high frequency that reacted with anti-ToxA antibodies. Initially we subcloned and conducted nucleotide sequence analysis on five cDNA inserts. Amino acid sequence information deduced from nucleotide sequence was compared with amino acid sequence obtained from the protein. These data confirmed the identity

of the cloned cDNAs (Ciuffetti *et al.* 1997). Southern analysis was performed with a labeled cDNA clone. Digested genomic DNA of toxin producing (pathogenic) and non-toxin producing (non-pathogenic) isolates were probed with a ^{32}P-labeled cDNA insert, encoding ToxA, to establish the presence or absence of the toxin gene in these isolates. Southern analysis of independent fungal isolates indicated that the gene for ToxA is present only in toxin-producing isolates of the fungus. These data support the causal role of the toxin in successful disease interactions.

A genomic clone for the *ToxA* gene was identified by hybridization with the *ToxA* cDNA insert. Restriction analysis identified a 1.3-kb fragment that contained the *ToxA* gene and its putative endogenous promoter. This 1.3-kb subcloned fragment was sequenced (Ciuffetti *et al.* 1997). The deduced open reading frame from the *ToxA* gene encodes a protein with a calculated mass of 19,707 D. This is very close to that predicted from immunoprecipitation of *in vitro* translation products. The first 22 amino acids of the N-terminal domain comprise the signal peptide necessary for secretion of the ToxA protein. The putative signal peptide is followed by a 38-39 amino acid region with a theoretically determined anionic isoelectric point of 4.55. The C-terminal domain encodes the 117-118 amino acid mature ToxA region, as deduced by mass spectral analysis and amino-acid sequence analysis. Based on the determined mass of 13,208 D (Tuori *et al.* 1995) and the assumption that the inferred signal sequence of 22 amino acids is proteolytically cleaved following import into the endomembrane system, additional proteolytic processing is apparently involved in the production of the mature ToxA. The 278-nucleotide region 5′ to the transcribed region of the gene functions as a promoter both in culture and *in planta*. Two introns have been identified: intron 1 has typical eukaryotic splice junctions, whereas intron 2 lacks this consensus (Ciuffetti *et al.* 1997).

Transformation of a Tox- Isolate to a Tox+ Phenotype

A tox- isolate of *P. tritici-repentis* was transformed (Ciuffetti *et al.* 1997) with a vector containing the genomic copy of the *ToxA* gene and its putative endogenous promoter and the hygromycin B resistance gene for use as a selectable marker (*P. tritici-repentis* is sensitive to hygromycin). DNA was prepared from the tox+ control isolate, the tox- recipient, the vector-only control transformant, and three putative tox+ transformants, and analyzed by polymerase chain reaction (PCR). PCR analysis confirmed that the tox+ transformants contained the *ToxA* gene. The PCR products were sequenced and nucleotide sequence analysis of the PCR products from the tox+ transformants confirmed that the products were generated from the *ToxA* gene.

To determine the presence of the ToxA protein, total protein precipitated from samples of culture filtrate were evaluated by western analysis. All tox+ transformants shown to contain the *ToxA* gene produced ToxA in culture. Culture filtrates from the tox+ transformants were infiltrated into sensitive and insensitive wheat cultivars. Culture filtrate from all tox+ transformants tested caused typical tan spot necrosis in the sensitive but not in the insensitive wheat cultivars. Culture filtrates from the tox- recipient and the tox- recipient transformed with the vector only did not induce necrosis in either sensitive or insensitive wheat cultivars (Ciuffetti *et al.* 1997).

To determine whether ToxA is responsible for pathogenicity, the tox+ transformants were inoculated onto susceptible and resistant cultivars of wheat. The tox+ transformants produced typical tan spot lesions on susceptible wheat (TAM 105), but not on resistant wheat (Auburn). The tox- recipient and the tox- recipient transformed with the vector only did not develop typical tan spot symptoms in susceptible or resistant wheat cultivars.

Conclusions

This host-pathogen interaction provided a unique opportunity to isolate a single gene for the production of a HST and transformation of a tox- isolate to the tox+ phenotype. Our recent studies demonstrate that the *ToxA* gene is both necessary and apparently sufficient for the pathogenicity of *P. tritici-repentis* on the toxin-sensitive wheat genotypes tested. Our previous work indicated the detection of necrosis-inducing activity distinct from the 13.2 kD ToxA protein. These other toxic components appear to be distinct from the ToxA protein or are much more active than the ToxA protein. A possible explanation for the appearance of multiple toxins in pathogenic isolates of *P. tritici-repentis* is that these toxins are all encoded by the *ToxA* gene. This possibility gains plausibility based on the analysis of the *ToxA* gene. With the combined possibility of differential proteolytic processing and/or post-translational modification, the *ToxA* gene could account for all forms of the proteinaceous host-selective toxins found in the pathogenic isolates of the fungus. Future studies will be directed to determine if one or multiple genes exist for the production of the various toxic components. The long-term goal of our research program is a complete molecular description of the *P. tritici-repentis*-wheat interaction. With toxin purification and gene cloning for *ToxA* production, we are now in a position to extend our studies to elucidate the site- and mode-of-action of this proteinaceous host-specific toxin.

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Role of Host Metabolism in Action of Necrosis Toxin from *Pyrenophora tritici-repentis*

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Abstract

Ptr necrosis toxin, a cultivar-specific toxin produced by Pyrenophora tritici-repentis, *has been closely associated with the symptomatology of tan spot of wheat. We have developed a rapid quantitative bioassay for Ptr necrosis toxin based on electrolyte leakage. All toxin-sensitive wheat lines (based on necrosis development during a 72-h incubation with toxin) showed enhanced electrolyte leakage in response to toxin relative to water controls, whereas all toxin-insensitive wheats did not. The magnitude of leakage increased with toxin concentration under standard assay conditions. A 4-h toxin exposure permitted completion of the assay in one day. The electrolyte leakage bioassay has been used to demonstrate that toxin action is temperature sensitive in the host. Other experiments with the assay indicate that toxin action requires host metabolism, including ATP-utilizing proton pumps, protein synthesis, and putative signaling mechanisms.*

Most North American isolates of *Pyrenophora tritici-repentis*, the causal agent of tan spot of wheat, produce lesions with a necrotic center (Lamari and Bernier 1989a). Necrosis development has been genetically linked to production of a toxin in the pathogen (Ciuffetti *et al.* 1997) and to toxin sensitivity in the host (Lamari and Bernier 1989b, 1991; Faris *et al.* 1996). The toxin produced by the fungus is a 13.2-kD protein that has been designated by different groups as Ptr necrosis toxin (Ballance *et al.* 1989) and ToxA (Tuori *et al.* 1995). (My laboratory uses the fungal strain described by Lamari and associates, so I will use the Ptr necrosis toxin nomenclature.) In recent years, impressive advances in our knowledge have been made with regards to toxin biochemistry (Ballance *et al.* 1989; Tuori *et al.* 1995; Zhang *et al.* 1997), host genetics (Lamari and Bernier 1989b, 1991; Faris *et al.* 1996), and toxin molecular biology (Ciuffetti *et al.* 1997). However, to date, relatively little is known of the mechanisms by which necrosis develops in the host in response to toxin. This is the area in which my laboratory has been working in recent years.

When this work was initiated, the only bioassay for toxin described in the literature was based on necrosis development 48-72 hours after infiltration into the host. This "necrosis bioassay" is fairly easy to perform, gives reproducible

results, and requires no special equipment; however, it is not well-suited for mode-of-action studies because necrosis is slow to develop and difficult to quantify. Our first step, then, was to develop a more rapid quantitative bioassay for Ptr necrosis toxin. The bioassay developed, which was recently published (Kwon *et al.* 1996), is based on electrolyte leakage. Important attributes of this bioassay are that it can be completed in one day, gives quantitative data, and requires no special equipment other than a conductivity meter.

In developing the bioassay, it was found that toxin exposures of approximately four hours were required for reliable development of electrolyte leakage (Kwon *et al.* 1996). This was interpreted to mean that electrolyte leakage develops fairly late in toxin action, and possibly is preceded by other processes or events in the host.

Materials and Methods

Biological material

All experiments used ND495, a hard red spring wheat sensitive to Ptr necrosis toxin. Plants were grown in the greenhouse or in growth chambers for approximately 14-17 days until the third leaf was approximately one-half expanded. Ptr necrosis toxin was obtained from *P. tritici-repentis* (strain 86-124), as described by Zhang *et al.* (1997). All experiments used pure toxin quantified by the method of Bradford (1976) using BSA (bovine serum albumin) as a standard.

Electrolyte leakage

Electrolyte leakage bioassays were as described by Kwon *et al.* (1996) with only slight modification as needed. The second true leaf of intact seedlings was infiltrated with water (controls), pure toxin, or toxin combined with one of the metabolic inhibitors listed below. After a defined exposure period (generally 4-8 h), a single 2.5 cm leaf section was obtained from the infiltrated region of each leaf. Five of these leaf sections were combined to form a single replicate and there were two replicates per treatment. Each replicate was placed in 15 ml of distilled water and vacuum infiltrated. Conductivity of the ambient water was measured periodically with a conductivity meter.

Metabolic inhibitors

Sodium vanadate, cobalt chloride, lanthanum chloride, cycloheximide, and actinomycin D were purchased from Sigma Chemical (St. Louis, MO, USA). All inhibitors were dissolved in water except actinomycin D, which was dissolved in dimethyl sulfoxide.

Temperature experiments

One experiment examined the effect of cold temperature on toxin action, as determined by electrolyte leakage. In this experiment, ND495 seedlings were held in a Percival growth chamber at 4°C for 24 h prior to infiltration. The second true leaf was infiltrated as described above, then the seedlings were returned to the growth chamber for incubation at 4°C. Following exposure to toxin or water, leaf sections were leached at 4°C in distilled water previously equilibrated at 4°C. As a

positive control, electrolyte leakage was determined for seedlings in a growth chamber at 20°C.

Results and Discussion

Effect of cold temperature on electrolyte leakage

Cold temperature (4°C) completely eliminated the action of Ptr necrosis toxin, as determined by electrolyte leakage, relative to controls at 20°C. Conductivity of ND495 seedlings treated with water and toxin for 8 h was 6.6 and 6.5 μS, respectively, after 6 h leaching. By comparison, leachate from seedlings at 20°C at the same time had conductivity measurements of 6.2 and 23.8 μS for water and toxin treatments, respectively. The data indicate that toxin action is inhibited by cold temperatures, and this suggests that toxin action requires active metabolism from the host. This hypothesis was tested in subsequent experiments by determining the effect of various metabolic inhibitors on the action of Ptr necrosis toxin.

Effect of sodium vanadate on electrolyte leakage

Sodium vanadate significantly reduced the amount of electrolyte leakage when co-applied to seedlings with toxin, relative to toxin treated controls. Conductivity measurements after 8 hours exposure and 6 hours of leaching were 5.5, 32.2, and 9.0 μS for water controls, toxin only controls, and toxin + vanadate, respectively. Vanadate is a well-known inhibitor of H^+-ATPases, which are membrane-bound enzymes that utilize ATP to pump hydrogen ions out of the cell. This creates a pH gradient across the membrane that is then used by the cell for various metabolic functions such as uptake of solutes (Serrano 1989). This experiment clearly demonstrates that wheat plants need an active H^+-ATPase in order to develop electrolyte leakage in response to toxin, but the mechanism of this requirement is not yet clear. Plants require an active H^+-ATPase to undergo the hypersensitive response (HR) to bacteria (Atkinson *et al.* 1990), and this suggests, at least superficially, that necrosis development in response to toxin may involve processes similar to those activated by the HR. Further, the action of certain elicitors of the defense response involve H^+-ATPase (Vera-Estrella *et al.* 1994; Xing *et al.* 1996).

Effect of calcium channel blockers on toxin action

The inorganic calcium channel blockers cobalt chloride and lanthanum chloride significantly reduced electrolyte leakage when co-infiltrated with toxin. For example, conductivity measurements after 8 h exposure to toxin and 6 h leaching were 6.1, 27.5, and 10.2 μS, for water controls, toxin only controls, and toxin + cobalt chloride, respectively. Similar results were obtained for lanthanum chloride. These data suggest that calcium may be part of the signal transduction pathway that acts between toxin perception and necrosis development. Calcium is known to act as a secondary messenger for many plant responses (Poovaiah and Reddy 1993), including the HR (Atkinson and Baker 1989; Goodman and Novacky 1994).

Effect of staurosporine on electrolyte leakage

Staurosporine, a potent inhibitor of protein kinases, including those protein kinases responsive to calcium, reduced electrolyte leakage by approximately 50% when applied to leaves with toxin. Conductivity after 4 h exposure and 6 h leaching was 4.1, 20.1, and 11.9 μS for water controls, toxin only controls, and toxin + staurosporine, respectively. This experiment implicates protein kinases in the signal transduction for necrosis development. It is possible that the protein kinase(s) involved are activated by calcium.

Effect of cycloheximide and actinomycin D on electrolyte leakage

Cycloheximide, but not actinomycin D, blocked the action of Ptr necrosis toxin. Cycloheximide provided approximately 90% protection relative to controls in experiments with an 8 h exposure and 6 h leaching period. Conductivity measurements were 5.1, 30.2, and 8.2 μS for water controls, toxin only controls, and toxin + cycloheximide, respectively; however, no protection was obtained with actinomycin D. Cycloheximide is a known inhibitor of translation, and these experiments suggest that Ptr necrosis toxin triggers the synthesis of at least one new protein required for cell death in wheat. The identification of the protein(s) synthesized is required for a thorough understanding of the mode-of-action of Ptr necrosis toxin. It is most plausible that the protein(s) synthesized by the host in response to toxin are under transcriptional control, but this was not supported by the actinomycin D experiments. Actinomycin D is a known inhibitor of transcription, but it is notorious for being slow to enter cells. Thus, available data do not support the hypothesis that transcription is required, but this needs to be examined much more thoroughly with other inhibitors and approaches.

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Fungi Associated with Foliar Blight of Wheat in Warm Areas

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Abstract

As part of a CIMMYT-UCL collaborative research project, 360 wheat leaf samples with blight symptoms were collected during 1993-1996 from Bangladesh, China, India, Nepal, Vietnam, Morocco, South Africa, Mexico, Bolivia, and Argentina, and analyzed in Belgium. Bipolaris sorokiniana *was detected in 81% of the samples and was the only fungus associated with leaf blight in hot and humid areas, while* Drechslera tritici-repentis, *detected on 29% of the samples, prevailed in the colder regions. In the Gangetic Plain, both pathogens were associated with indistinguishable blight spots, sometimes combined on a single leaf, with proportions varying according to local climatic conditions.* Drechslera gigantea *was detected in Nayarit state, Mexico. Occasionally* Alternaria *spp. were found associated with various types of lesions. Differences in colony appearance were noted among* B. sorokiniana *strains. The radial growth rate on minimal medium at 20°C ranged from 2 to 9 mm/day among a set of 29 strains, with no relation to origin or pathogenicity. Inoculation of Ciano, Kanchan, BH1146, and Mayoor with a selection of strains also revealed wide variation in aggressiveness, with some specific and nonspecific interactions, the latter especially for the most aggressive strains inducing extensive blight lesions even on the highly resistant genotype Mayoor. No specific relationship between pathogenicity and country or genotype of origin was evident.*

A survey carried out by Dubin and van Ginkel (1991) on the perception among wheat researchers of the economic importance of wheat diseases in the warmer areas of the developing world highlighted fungal foliar blights as a major production constraint. Leaf spot associated with the anamorph state *Bipolaris sorokiniana* (Sacc.) Shoem. of *Cochliobolus sativus* (Ito & Kurib.) Drechsler ex Dastur was identified as the most important disease in all environmental zones. Tan spot with sporulation of the anamorph *Drechslera tritici-repentis* (Died.) Shoem. of *Pyrenophora tritici-repentis* (Died.) Drechsler was reported mostly from the coolest zones of the warm areas, while alternaria leaf blight, associated with *Alternaria triticina* Prasada & Prabhu, was mostly identified in the hottest zones. Nevertheless, the symptoms caused by these fungi are similar, which can cause diagnostic confusion.

Crop management practices and soil fertility are considered to significantly influence disease occurrence, and sources of resistance to leaf spot and tan spot have been identified; however, genotypes

resistant in one location sometimes show severe disease symptoms in others (Raemaekers 1988). The exact cause, differences in the pathogen population and/or effect of the environment on resistance expression are generally not clearly established. Through a collaborative research project set up by Drs. E. Saari and J. Dubin with our group at UCL, and funded by the Belgian Agency for Development Cooperation, CIMMYT has extended research into leaf blight resistance of wheat and the evaluation of cropping systems on the development of this pathosystem. As part of this project, diseased samples collected during field trips, and surveys of CIMMYT scientists or partners in various warm areas, were sent to our laboratory at Louvain-la-Neuve, Belgium, for identification and isolation of pathogens, and for assessment of pathogen diversity. This paper presents some of the data obtained from samples collected between 1993-1996, as well as initial studies on the pathogenic diversity among *C. sativus* strains. Data on diversity of *P. tritici-repentis* isolates are presented elsewhere (Di Zinno *et al.*, this proceedings).

Materials and Methods

Diseased leaf samples were collected at random from farmers fields and trials with the main purpose to identify the fungus associated with the lesion, not to make a systematic quantitative survey on the incidence of the various fungi. A sample generally comprised up to five leaves collected from one plot. Each sample was dried separately in a paper bag and stored and mailed at room temperature. In the laboratory, lesions were analyzed under a stereo-microscope to detect fungal sporulation. Conidia and conidiophores were removed with Scotch tape and mounted on cotton blue lactophenol for precise identification at higher magnification.

To isolate the causal fungus, leaf sections were surface sterilised with NaClO (0.5 % in distilled water containing two drops of Tween 20 per 100 ml) for 3 min, rinsed three times with sterile water, and transferred to petri dishes containing water agar (1.5 %) amended with streptomycin (150 mg L^{-1}). Plates were incubated in the dark at 20°C until fungal growth appeared on the agar. The plates were then placed at room temperature under continuous white and black fluorescent light to stimulate conidiophore production, before being returned to darkness at 20°C to induce sporulation. These conditions appeared critical for isolation of *P. tritici-repentis.* Conidia were spread on fresh water agar plates and after germination were singly transferred to V8-PDA plates (150 ml V8 juice, 10 g Difco PDA, 3 g $CaCO_3$, 10 g Difco bacto agar, and distilled water up to a total volume of 1 L). Single-spore cultures were stored on V8 slants under oil.

Colony morphology and growth rate were analysed on minimal medium plates (100ml of solution 1, 100ml of solution 2, 1 ml of solution 3, 5 g sucrose, 15 g agar, 800 ml distilled water; solution 1: 31.2 g KNO_3, 7.5 g K_2HPO_4, 7.5 g

KH_2PO_4, 1g NaCl, 1L H_20; solution 2: 5g $MgSO_4$.7H2O, 1g $CaCl_2$, 1L H_20; solution 3: 0.198 g $ZnSO_4.7H_2O$, 0.04 g $CuSO_4.5H_2O$, 0.016 g $MnSO_4.H_2O$, 0.009 g MoO_3, 0.268g $FeC_6H_5O_7.3H_2O$, 0.019 g $Na_2B_4O_7.10H_2O$, 0.5 L H_2O).

For production of *B. sorokiniana* conidia, subcultures were grown on V8-PDA plates at 20°C either in the dark or under continuous light for 7-10 days, depending on the trial. Conidia were harvested by flooding the colony with sterile distilled water amended with two drops of Tween 20 per 100 ml, followed by scraping the surface with a glass rod. The resulting suspension was filtered through cheesecloth (mesh: 200 mm) and the conidial suspension adjusted to 10000 conidia ml^{-1}.

Pathogenicity tests were performed on the following differentials: BH1146 with intermediate resistance; CianoT79 as the susceptible check; Kanchan as the local check for the South Asian region; and Mayoor, a resistant wide cross of bread wheat with *Thinopyrum curvifolium*. Ten seeds were sown in each pot containing a 1:1 mixture of loam soil and compost previously autoclaved twice for 2 h. Seedlings were grown in a greenhouse at 22/17°C day/night temperature with a 16-h photoperiod, thinned to five vigorous plantlets per pot, and inoculated at the three-leaf stage by spraying the conidial suspension until runoff. Pots were then covered for 12 h with a plastic bag and placed in the shade before returning them to normal growing conditions. Two pots per strain and genotype were inoculated. The test was repeated twice for some strains. The percentage of diseased area on the third leaf was assessed one week after inoculation using the scale and the codes described by Hetzler (1992), with the following conversion of severity code to percentage of lesion area (x): 0 = 0<x<3%, 1 = 3<x<8.75%, 2 = 8.75<x< 20%, 3 = 20<x<38.75%, 4 = 38.75<x<87.5%, 5 = x>87.5%.

Results and Discussion

Leaf samples with fungal lesions were received from the Gangetic Plain in India (77), Nepal (81) and Bangladesh (105), and also from Mexico (39), Bolivia (24), Argentina (9), Morocco (2), South Africa (6), China (13), and Vietnam (4) during field trips performed each year between 1993 and 1996 (Figure 1). The anamorph *B. sorokiniana* was found associated with 81% of the analyzed samples and was the only fungus identified from hot and humid areas such as Bolivia, Vietnam, and Poza Rica, Mexico. *Drechslera tritici-repentis* sporulation was detected on 29% of all analyzed samples and was the only leaf blight fungus detected on samples from the central highland of Mexico and Pergamino, Argentina; however, this does not exclude the possible occurrence of *B. sorokiniana* in these areas. *Alternaria* spp., *Curvularia* spp., and *D. gigantea* (Heald & Wolf) Ito were occasionally observed on various types of lesions ranging from restricted necrotic spots to tan spots with well defined edges or even diffuse yellow blotches. *Drechslera gigantea* was detected only in samples from Nayarit state of

western Mexico. The *Alternaria* spp. associated with blight lesions, sometimes as the only fungus, such as in Obregon, Mexico, appeared very heterogeneous. It was difficult to precisely identify *A. triticina* because of its similarity to *A. tenuissima* (Kunze ex Pers.) Wiltshire. Of a world-wide selection of 12 strains including *A. triticina* reference strains, none were pathogenic on Bansi, HD2329, Sonalika, and UP262 plants inoculated at the three-leaf or heading stage (Maraite *et al.*, unpublished data). It was often difficult to make a definite distinction between spot blotch and tan spot lesions with the naked eye or even with help of a magnifying glass because of the range of variation in symptoms induced by both pathogens. Lesions associated with *B. sorokiniana* or *D. tritici-repentis* sporulation sometimes occurred on the same leaf and were so similar that they could only be differentiated under the microscope.

Breakdown of the data according to areas in the Indian subcontinent revealed the highest occurrence of *D. tritici-repentis* in cooler areas: the Haryana and Punjab states in India, the midland valleys in Nepal, and the northern part of Bangladesh (Figure 2). These basic

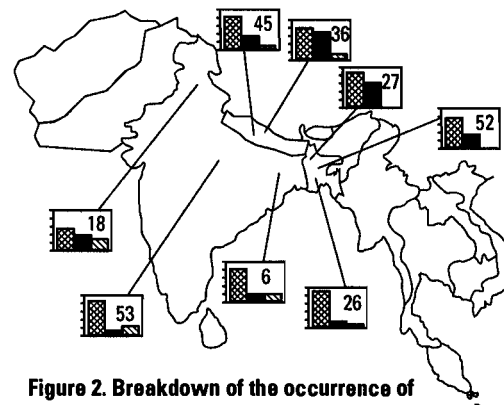

Figure 2. Breakdown of the occurrence of *Bipolaris sorokiniana* (left column), *Drechslera tritici-repentis* (central column), and sporulation of other fungi (*Alternaria* spp., *Curvularia* spp.; right column) on leaf blight lesions collected in India [Punjab-Haryana (18 samples), Uttar Pradesh (53), Bihar (6)], Nepal [Midlands (36), Terai (45)] and Bangladesh [north (27), central (52), south (26)]. (Y axis: % of detection on analyzed samples, maximum = 100; figures are the number of samples analyzed).

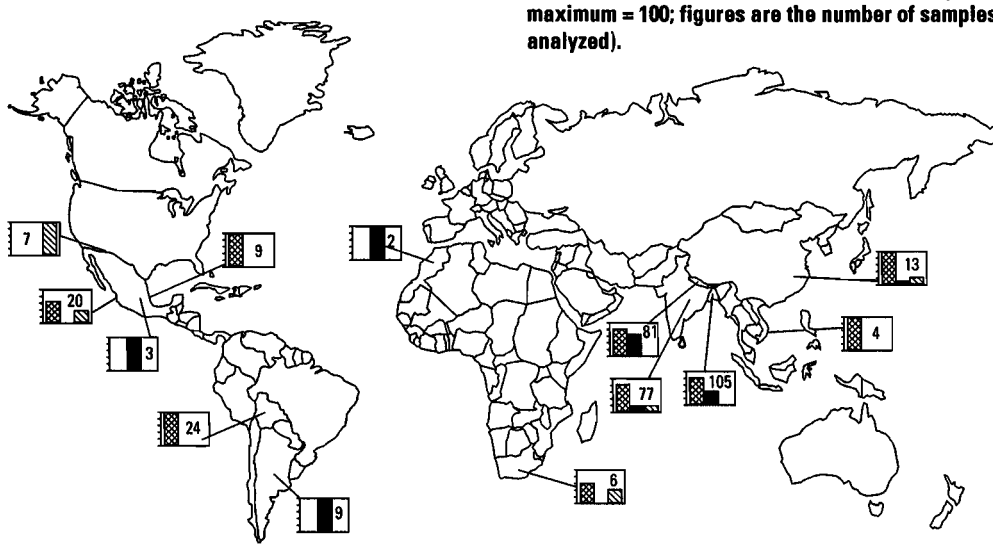

Figure 1. Frequency of *Bipolaris sorokiniana* (left column), *Drechslera tritici-repentis* (central column), and sporulation of other fungi (*Alternaria* spp., *Curvularia* spp., *D. gigantea*; right column) from a worldwide collection of leaf blight lesions on wheat. (Y axis: % of detection on analyzed samples, maximum = 100; figures are the number of samples analyzed).

differences were consistently observed over the various sampling dates each year between February 15 and March 15, although minor variations in relative importance were observed from one date to another, but without any specific trend. This suggests variation in occurrence due to differences in growth stage as well as climate. From the National Wheat Research Experiment Station, Bhairahawa, Nepal, Dubin and Bimb (1994) reported higher levels of conidial dispersal and blight by *D. tritici-repentis* compared to *B. sorokiniana* in February; *B. sorokiniana* became predominant later in the season with a temperature increase and wheat maturation. Our data do not claim to give a complete evaluation of the relative importance of the various leaf blight fungi; however, they are in line with previous and more recent in depth surveys, e.g., from India (Singh *et al.* 1986; Nagarajan, this proceedings). Tan spot was considered a new problem in Bangladesh, linked to the introduction of high yielding varieties (Alam and Saha 1991); however, *D. tritici-repentis* was already reported by McRae (1932) as more common on wheat than *B. sorokiniana* in Pusa (Bihar). This highlights the possible fluctuation in time of the relative importance of the various fungi associated with leaf blight and the necessity to adapt control strategies to the complex of pathogens which may occur in a specific area.

Comparison of the detection of *B. sorokiniana* and *D. tritici-repentis* sporulation under the microscope and subsequent isolation frequencies (Table 1) revealed *B. sorokiniana* growth from lesions where this fungus was not detected before. Furthermore, there was a systematic reduction of *D. tritici-repentis* isolation frequency. This suggests a higher saprophytic capacity of *B. sorokiniana* under the experimental conditions as well as an underestimation of *D. tritici-repentis* when incidence is assessed only by isolation frequency.

From this world-wide collection of leaf blight symptoms, a collection of fungal isolates was established, composed until now of *Alternaria* spp. (34 strains), *Cochliobolus sativus* (247), *Curvularia* spp. (4), *D. gigantea* (10), and *Pyrenophora tritici-repentis* (73). The aim was to use this collection for pathogen diversity and toxin production studies. In a first series of trials, a selection of 27 *Cochliobolus sativus* strains (Table 2) were tested. The color of the colonies on minimal medium varied from white (strain CF 02-03) to light pink (J 1-15 and

Table 1. Comparative detection of *Bipolaris sorokiniana* (BS) and *Drechslera tritici-repentis* (DTR) on wheat leaf samples received from various origins in 1994 and subsequent isolation frequencies.

	Detection				Isolation			
Origin of samples	No. of samples	BS	DTR	Others	No. of strains	BS	DTR	Others
		%				%		
Bangladesh	24	65	30	5	24	91	9	9
India	34	44	18	38	31	63	10	10
Nepal	28	42	48	10	27	59	30	30

K 3) and dark green (the remaining strains). Maximum radial growth rate also appeared very variable and ranged from 2 mm/day (D8) up to 9 mm/day (CS 12-06); most strains ranged between 4-7 mm/day.

When these 27 strains were used to inoculate four genotypes with different resistance backgrounds, a wide and continuous range of aggressiveness was revealed, with some strains inducing only very restricted lesions on the most susceptible Ciano, while others caused severe symptoms even on the most resistant Mayoor (Figure 3). The regular progression of mean disease severity suggests the occurrence of quantitative differences in aggressiveness. On the whole, spot blotch severity decreased as follows: Ciano [a] Kanchan > BH1146 > Mayoor. This order differed for some strains with medium aggressiveness, suggesting specific interactions. Nevertheless, there appeared to be no specific link between aggressiveness and the genotype from which the strain was isolated, e.g., strain CS 11-20 isolated from Kanchan showed a low aggressiveness on this cultivar but a comparatively high aggressiveness on BH1146. These most aggressive strains

Table 2. Origin of *Cochliobolus sativus* strains used in pathogenicity studies.

Strain code[1]	Origin of sample			
	Country	Location	Genotype	Date
A18	Canada	Saskatoon	Unknown	01/11/94
CF 02-01	South Africa	Kimberley	Durum	27/10/93
CF 02-03	South Africa	Kimberley	Unknown	27/10/93
CF 02-05	South Africa		Durum	27/10/93
CM 01-02	Mexico	Poza Rica	Pavon	21/01/93
CM 01-05	Mexico	Poza Rica	Alondra	21/01/93
CM 03-01	Bolivia	Okinawa 1	Guapay	22/08/94
CM 03-02	Bolivia	Okinawa 1	Guenda	22/08/94
CM 03-04	Bolivia	Okinawa 2	Guapay	18/08/94
CM 03-08	Bolivia	Okinawa 2r	Guenda	18/08/94
CS 10-03	Vietnam	Hanoi	Barley	24/02/94
CS 10-04	Vietnam	Van Dien	Zebra	24/02/94
CS 10-05	Vietnam	Duyën Thai	Unknown	24/02/94
CS 11-01	Bangladesh	Joydepur	Pavon76	28/02/94
CS 11-10	Bangladesh	Ranpur	Kanchan	02/03/94
CS 11-13	Bangladesh	Rajshahi	Kanchan	02/03/94
CS 11-20	Bangladesh	Jessore	Kanchan	03/03/94
CS 12-06	Nepal	Rampur	Unknown	06/03/94
CS 12-13	Nepal	Bhairahawa	Sonalika	07/03/94
CS 13-05	Nepal	Hardinath	UP 262	01/03/94
CS 14-06	India	Basti-Faizab.	Unknown	08/03/94
CS 14-07	India	Basti-Faizab.	Barley	08/03/94
CS 14-17	India	Luknow	Sonalika	10/03/94
CS 14-31	India	Sarhand	Unknown	05/04/94
D8[1]	Canada	Saskatoon	Unknown	01/11/94
J1-15[1]	Canada	Saskatoon	Unknown	01/11/94
K3[1]	Canada	Saskatoon	Unknown	01/11/94

[1] Reference strains of *C. sativus* mating type provided in Nov. 1994 by K. Bailey, Agricultural Canada, Saskatoon, Saskatchewan.

occurred in South Asia but also in South Africa. Both very high and low aggressive strains were isolated in one country, and even in one area. The strains tested from the spot blotch hot spot in Poza Rica, Mexico, appeared very aggressive on Ciano but not on Mayoor, and intermediate on BH 1146, which corresponds to field ratings. No relation between *in vitro* growth rate and aggressiveness was noted.

The observed differences in pathogenicity among the tested strains confirm the large variability in virulence already reported by Mehta (1981), who identified 32 races among 98 isolates in southern Brazil. They are also entirely in line with Hetzler's (1992) conclusions concerning the operation of both vertical and horizontal systems of resistance in this pathosystem, and of the difficulty in defining races because of the large number of possible gene-for-gene interactions. The apparent wide-spread occurrence of strains able to overcome promising sources of resistance under the tested inoculation conditions raises the question of the possible increase in importance of these strains once the area sown to resistant cultivars increases.

Systematic monitoring could provide data on possible pathogenicity shifts in *B. sorokiniana* populations. Due to this wide pathogen diversity, it is recommended that integrated leaf blight control be developed to sustain resistant cultivars through the reduction of inoculum source as developed by Reis (1990), and by adequate fertilization. Deficiency in N, K and/or P, depending on location and induced by soil depletion or root health problems, appears to greatly affect leaf blight incidence in the Gangetic Plain (Dubin and Bimb 1994).

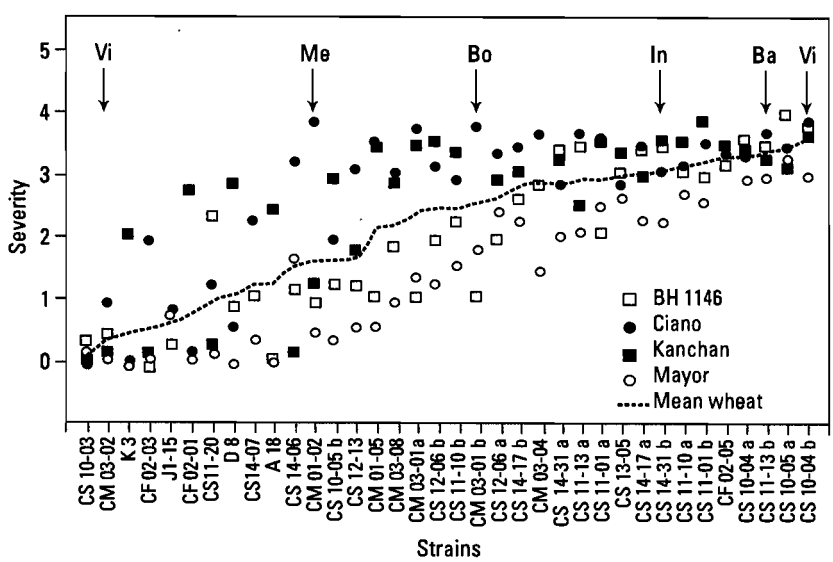

Figure 3. Spot blotch severity induced by 27 strains of *Cochliobolus sativus* on four bread wheat genotypes. Mean of 10 plants, standard deviation generally under one severity code. Nine strains were tested twice; a and b suffixes correspond to the repetitions. Arrows point to strains from Vietnam (Vi), Mexico (Me), Bolivia (Bo), India (In), and Bangladesh (Ba).

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Characterization of the *Pyrenophora tritici-repentis* Necrosis Toxin and a Folding Precursor

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Abstract

A Pyrenophora tritici-repentis *necrosis-inducing toxin was purified from crude extracts from isolate 86-124. The purified toxin was characterized by SDS-PAGE, isoelectric focusing, and circular dichroism (CD) spectroscopy. Analysis of the CD spectrum indicated a composition of 13% alpha helix, 36% antiparallel beta sheet, 25% turn, and 25% other structure. Enzymatic proteolysis and subsequent gas-phase amino acid sequencing resulted in sufficient overlapping fragments to produce a continuous sequence of 101 amino acids. The amino acid sequence information was used to synthesize degenerate polymerase chain reaction (PCR) primers and the DNA sequence of the toxin was obtained from cDNA. Secondary structure prediction based on the amino acid sequence indicated a beta sheet protein with little alpha helix, in agreement with the CD measurement. During toxin purification two additional peaks of toxin activity were observed. All peaks contained a protein that has a similar molecular weight to the toxin and is antigenically related to the necrosis toxin. One peak undergoes conversion to a molecule essentially indistinguishable from the necrosis toxin as measured by ion exchange and reversed phase HPLC chromatography, CD spectroscopy, and mass spectrometry. CD spectroscopy of this protein before conversion indicated incomplete formation of secondary structure when compared to the necrosis toxin. This evidence suggests that this protein is a folding precursor of the toxin.*

Diversity of *Pyrenophora tritici-repentis* Isolates from Warm Wheat Growing Areas: Pathogenicity, Toxin Production, and RAPD Analysis

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Abstract

The diversity of Pyrenophora tritici-repentis *isolates collected from warm wheat growing areas around the world was studied. Four cultivars (Ciano, Hartog, Vicam, and BH1146) at the three-leaf stage were inoculated with a conidial suspension under controlled conditions. Vicam appeared resistant to all* P. tritici-repentis *isolates, while Ciano, BH1146, and Hartog showed varying susceptibility levels. Interactions were observed between cultivars and isolates, especially between Hartog and Ciano and the most aggressive isolates. Using a single conidium inoculation method on detached leaves, all* P. tritici-repentis *isolates failed to produce symptoms on Vicam, whereas tan necrosis was induced by some isolates on Ciano, Hartog, and BH1146. Strains and cultivars were differentiated by induction and expression of necrosis, respectively. Strains that induced lesions were observed to produce necrosis-inducing toxins on all susceptible cultivars; however, ND/VG9144//KAL/BB/3/YA was the exception. It failed to develop symptoms when infiltrated by the toxin, even though it is susceptible to tan spot under both field and controlled conditions. Preliminary results from random amplified polymorphic DNA (RAPD) analyses indicated the presence of polymorphism between strains, but no correlation could be established between these differences and geographic origin, pathogenicity, and toxin production.*

Tan spot of wheat, caused by *Pyrenophora tritici-repentis*, is widespread in temperate areas such as the Great Plains of USA, Canada, Australia, and, less frequently, in western Europe (Maraite and Weyns 1982). It is also present in warmer areas of the world, including South Asia (Bangladesh, Nepal, India), Latin America, and Africa. The host range covers wheat, other cereals such as barley and rye, and also grasses (Kuprinsky 1992). Typical symptoms are necrotic elliptical spots with a black point at the center, surrounded by a chlorotic ring. Tan spot is considered a major disease in all warm wheat growing areas, causing severe yield losses worldwide (Dubin and van Ginkel 1991). This situation is partly due to the recent progress in breeding for rust resistance, which has emphasized diseases previously less apparent, but also to the expansion of wheat cultivation into nontraditional environments and to

changes in agricultural practices, including minimum tillage and stubble management (Duveiller 1994).

Resistant cultivars offer an economically effective means of keeping disease losses to a minimum; however, resistance varies with wheat cultivar, leaf position, and fungal isolate (Hosford *et al.* 1990). Physiological specialization, i.e., differential adaptation of pathogen isolates to certain host cultivars, could complicate screening strategies in the development of disease-resistant varieties. Isolate x cultivar interaction specificity should be a consideration not only in systems where resistance is governed by major genes, but also in quantitative host-pathogen systems. In the latter, physiological specialization may be less obvious and based on quantitative differences in disease expression (Schilder and Bergstrom 1990). *Pyrenophora tritici-repentis* has been reported to exhibit isolate x cultivar interactions in a number of studies (Schilder and Bergstrom 1990; Lamari and Bernier 1989a) but not in others (Diaz de Ackermann *et al.* 1988; Hosford *et al.* 1990). These discrepancies could be due, in part, to differences in methods for rating host reaction among researchers, as well as to possible differences in the virulence of *P. tritici-repentis* isolates used in each study (Lamari and Bernier 1989a). Therefore, the refinement of methods and ratings systems that are both informative and reliable is important in the development of tan spot resistant cultivars (Francl and Jordahl 1994).

Pyrenophora tritici-repentis isolates have been grouped into pathotypes based on their ability to induce tan necrosis and extensive chlorosis (nec+, chl+), tan necrosis only (nec+, chl-), extensive chlorosis only (nec-, chl+), and neither necrosis nor chlorosis (nec-, chl-) (Lamari and Bernier 1989b). Tan necrosis-inducing isolates release a host-selective toxin that induces necrosis in culture (Lamari and Bernier 1989b; Tomas *et al.* 1990; Tuori *et al.* 1995). The toxin exhibits the same host specificity as the isolates from which it is produced, and is assumed to be the primary pathogenicity factor for tan necrosis development in susceptible wheats (Lamari and Bernier 1989b). Therefore, characterization of toxin production and analysis of isolate pathogenicity are of primary importance in understanding the role of toxin in host-pathogen interactions and in determining how to efficiently use the toxin in screening for tan spot resistant wheat.

The use of random amplified polymorphic DNA (RAPD) techniques for identification and differentiation of many microorganisms has greatly expanded during the last few years. For plant pathogenic fungi, RAPD analysis has been used to analyze genetic variation or distinguish races in several *Fusarium* spp. (Manulis *et al.* 1994), and in *Colletotrichum graminicola* (Guthrie *et al.* 1992), *Cochliobolus carbonum* (Jones and Dunkle 1993), and *Pyrenophora teres* (Peltonen *et al.* 1996). Using this technique, a study was undertaken to explore the possibility of a correlation between DNA polymorphism and pathogenicity and

toxin production of *P. tritici-repentis* isolates. Preliminary results are presented in this paper.

Materials and Methods

The pathogen

Pyrenophora tritici-repentis isolates were obtained from naturally infected leaves collected from warm wheat growing areas. A further four isolates were acquired from L. Lamari (University of Manitoba, Winnipeg, Canada): ASC1, which induces necrosis and chlorosis on cultivars; 86-124, which induces necrosis on cultivars; 331-9, which induces chlorosis on cultivars; and 90-2, which is unable to induce neither necrosis nor chlorosis (L. Lamari, personal communication). The origins of the monoconidial isolates used in this study are listed in Table 1. Mycelial plugs from single-spore cultures were transferred to PDA plates and conserved on V8 slants under oil. Conidia used for inoculation were obtained by transferring mycelial plugs from PDA plates to V8 agar plates, and kept in the dark at 20°C for 6 days. Aerial mycelia were flattened and the cultures placed at room temperature under near UV light for 48 h, followed by 18 h in the dark at 20°C. Conidia were harvested by flooding the plates with sterile water plus Tween 20 (3 drops 100

Table 1. Identity of isolates used in the characterization of *Pyrenophora tritici-repentis* populations on wheat from warm areas.

Isolate	Location	Year of isolation-Original collection[1]
ASC1	Manitoba, Canada	Lamari-Manitoba
86-124	Manitoba, Canada	Lamari-Manitoba
331-9	Manitoba, Canada	Lamari-Manitoba
90-2	Manitoba, Canada	Lamari-Manitoba
CF04-01	Ouezzane, Morocco	1994, NSFP-UCL'
CM02-02 (= PTR2M1)	El Batan, Mexico	CIMMYT
CM02-03 (= PTR3)	Chalco, Mexico	CIMMYT
CM04-01	Pergamino, Argentina	1994, NSFP-UCL
CM04-03	Pergamino, Argentina	1994, NSFP-UCL
CM04-04	Pergamino, Argentina	1994, NSFP-UCL
CM04-07	Pergamino, Argentina	1994, NSFP-UCL
CM04-09	Pergamino, Argentina	1994, NSFP-UCL
CM05-01 (= PTR9M1)	Oaxaca, Mexico	CIMMYT
CM05-02 (= PTR10M1)	Oaxaca, Mexico	CIMMYT
CS02-04	Chakia, India	1993, NSFP-UCL
CS03-01	Alampur, Bangladesh	1993, NSFP-UCL
CS04-06	Dinajpur, Bangladesh	1993, NSFP-UCL
CS11-26	Jessore, Bangladesh	1993, NSFP-UCL
CS12-11	Jhurjhure, Nepal	1994, NSFP-UCL
CS12-12	Bhairahawa, Nepal	1994, NSFP-UCL
CS12-18	Gairwa, Nepal	1994, NSFP-UCL
CS12-19	Rampur, Nepal	1994, NSFP-UCL
CS12-20	Rampur, Nepal	1994, NSFP-UCL
CS14-05	Basti, Nepal	1994, NSFP-UCL
CS19-08	Rising Patan, Nepal	1996, NSFP-UCL
CB05-01	Merbe-le-Château, Belgium	1996, NSFP-UCL

[1] CIMMYT, Lamari-Manitoba, and NSFP-UCL are collections maintained at CIMMYT by E. Duveiller and colleagues, the University of Manitoba by L. Lamari and colleagues, and UCL in the Non-Specific Foliar Pathogen of Wheat Project (NSFP), respectively.

ml^{-1}) and scraping the agar surface with the edge of a glass slide. The suspension was filtered through cheesecloth and adjusted to a concentration of 2000 conidia ml^{-1}.

Plant material

The host differential comprised four hexaploid wheat cultivars (Ciano T79, BH1146, Vicam, and Hartog) provided by CIMMYT. Plants were grown in pots containing a 1:1 mixture of soil and compost previously twice sterilized at 120°C for 2 h. Approximately 10 kernels per cultivar were sown in each pot. Seedlings were grown in the greenhouse (22/17°C day/night with a 16 h photoperiod) and were fertilized at the one- and three-leaf stages. At this time, before inoculation, plants where thinned to five plants per pot. Plants were sprayed with fungicide (Corbel, BASF), as necessary, to control powdery mildew.

Inoculation

Seedlings were spray inoculated with the conidial suspension at the three-leaf stage using an atomizer until run-off. Pots were then covered with a plastic bag for 24 h to maintain high humidity in the greenhouse (22/17°C day/night, with a 16 h photoperiod). Plants were evaluated after one week according to the following qualitative scale adapted from Lamari and Bernier (1989a): 1) small dark brown to black spots without any surrounding chlorosis or tan necrosis; 2) small dark brown to black spots with very little chlorosis or tan necrosis; 3) small dark brown to black spots completely surrounded by a distinct chlorotic or tan necrotic ring, lesions generally not coalescing; 4) small dark brown to black spots completely surrounded by a distinct chlorotic or tan necrotic ring, some of the lesions coalescing; 5) dark brown or black centers may or may not be distinguishable, most lesions consist of coalescing chlorotic or tan necrotic zones; 6) extensive chlorosis; 7) extensive chlorosis with visible black point infection; and 8) all lesions coalesce, resulting in leaf death. In a second experiment, percentage diseased leaf area (%DLA) was used to assess fungal infection level. In both experiments, a randomized complete block design was used. Ten replicate plants were inoculated by isolates for the first experiment, and five for the second.

Detached leaf assays were performed by placing wheat leaf sections (a 6-cm center section of the third leaf) on water agar (0.5%) containing 150 ppm of benzimidazole in square plastic dishes (120 x 15 mm). Leaves were inoculated either by depositing a 10 µl drop of conidial suspension (as described above) in the center of the leaf section or by single conidia inoculation. Conidia for the latter method were obtained by spreading conidia suspension on a water agar plate and transferring the conidia one by one onto the detached leaves. Inoculated dishes were incubated in a controlled temperature chamber at 20°C under a 16-h photoperiod. Presence or absence of necrosis was scored after one week.

Toxin production and bioassays

Toxin was produced in liquid Fries medium (No. 66; Dhingra and Sinclair 1985) inoculated with 2 ml of a conidial

suspension (2000 conidia ml^{-1}) per 100 ml of medium. After three weeks in the dark at 20°C the medium was filtered. Proteins were precipitated from the filtrate using ammonium sulfate at 75% saturation at 4°C overnight, and then pelletized by centrifugation for 20 min at 8000 g. Pellets were re-suspended in sodium acetate (20 mM, pH 4.8) and dialyzed using the same buffer for two days at 4°C. To evaluate toxin effect on wheat, the third leaf of a three-week-old seedling was infiltrated with 30-40 µl of the proteinic solution using a syringe without a needle. Infiltration area was outlined with a black pencil. Necrosis production was noted after one week in the greenhouse (22/17°C day/night, with a 16 h photoperiod).

RAPD analysis

Strains were cultivated in liquid V8 medium and shaken for two days at 28°C. DNA was extracted from lyophilized, ground, and filtered mycelium by the CTAB method (Rogers and Bendich 1985) and quantified by measuring absorbance at 260 nm. DNA was amplified using a Perkin Elmer/Cetus DNA Thermal Cycler programmed for one cycle of denaturation at 94°C for 3 min, followed by 45 cycles of denaturation at 94°C for 1 min, annealing at 35°C for 1 min, and extension at 72°C for 3 min. Each amplification mix (25 µl) contained template DNA (20 ng), Taq polymerase (0.8 units, Dynazyme, FMC, USA), primers (1.5 µl ml^{-1} Operon Technologies Inc., USA), nucleotides (200 µM, Pharmacia, Netherlands), 10X PCR buffer (2.5 µl), and sterile water. The 20 primers of the Operon kit A (A01-A20) were tested. Polymorphic bands were recorded to calculate a matrix of distances (Sorensen-Dice coefficient) which was then analyzed using the PHYLIP program (Phylogeny Inference Package, version 3.2; Felsenstein, 1989) to construct a tree by the UPGMA method (phenogram form).

Results

Pathogenicity tests

In the seedling inoculation tests where lesion type was scored, a different ranking based on cultivar susceptibility and isolate pathogenicity was evident. On Vicam, lesion type ranged between 0-7, with a predominance of types 1 and 2 (Figure 1). On BH1146, lesion type varied between 2-8 but were mostly type 2. On Hartog, lesion type ranged between 1-8, but the cultivar mostly expressed extensive chlorosis. Ciano showed lesion types between 3-8, but mostly expressed tan necrosis lesions surrounded by a chlorosis ring. Vicam appeared to be the most resistant cultivar, while Ciano and Hartog were most susceptible. BH1146 showed intermediate reactions. A continuum in

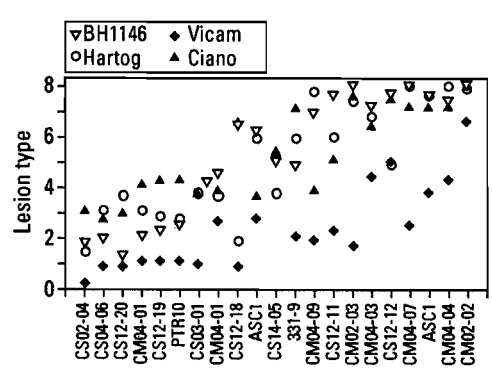

Figure 1. Lesion type induced by *Pyrenophora tritici-repentis* isolates from warm areas on four wheat cultivars. Each point is the mean of 10 plants.

pathogenicity from low to highly aggressive isolates seems to exist in the population tested. However, differences in reaction from one inoculation to another were noted: ASC1 was classified as mildly aggressive in a first inoculation and, in a second, strongly aggressive (Figure 1). This difference is due to a change in the reaction of the isolate on Ciano from 3 to 7. This shift toward higher severity classes can be explained by the scores in classes equal or higher than 3, which are more variable than in category 1 or 2 (Francl and Jordahl 1994). On the other hand, CM04-01, also inoculated twice, caused different reactions for the more resistant cultivars Vicam and BH1146. In this case, the different reactions could not be explained by the nature of the rating scale. Two problems appeared during this experiment. Firstly, it was possible for lesion types to mix on a leaf, thus leading to difficulties in categorizing the isolates/cultivars (Francl and Jordahl 1994). In addition, the reaction caused by a given isolate x cultivar interaction differed from one inoculation to the other. Finally, there seems to be a cultivar-specific lesion type, such as in the case of Hartog, which almost always shows extensive chlorosis.

Table 2. Analysis of variance for the percent diseased lesion area on four wheat cultivars by isolates of *Pyrenophora tritici-repentis*.

Source of variation	Sum of squares	df	Mean square	F test
Cultivar	117938	3	39312.7	176.4*
Isolate	48351	12	4029.3	18.1*
Cultivar x isolate	123135.4	36	3420.4	15.3*
Residual	46363.6	208	222.9	
Total	335788.4	259	1296.5	

* Highly significant (P<0.01).

In seedling tests where %DLA was calculated, all isolates were pathogenic, inducing at least necrotic point lesions on the four wheat cultivars tested, but differences in virulence were observed. Analysis of variance of disease severity showed highly significant effects of cultivar, isolate, and cultivar x isolate on disease expression (Table 2). Differences among the virulence patterns of isolates on different cultivars are apparent (Figure 2), especially when severity on Ciano and Hartog are compared. For example, isolate CB05-01 caused 100% DLA on Hartog and 31% on Ciano, while isolate CS12-18 was highly pathogenic on Ciano and weakly pathogenic on Hartog. Another point to be stressed is the weak pathogenicity of isolate 86-124 on Hartog and of isolate 331-9 on Ciano. These results are in agreement with those of Lamari and Bernier (1989a, 1989b) who defined these isolates as chl- and nec-pathotypes, respectively. Finally, as observed in the former test and expected for Vicam, which is resistant to all the isolates, susceptibility of the three other cultivars ranged: Hartog (11-100 %),

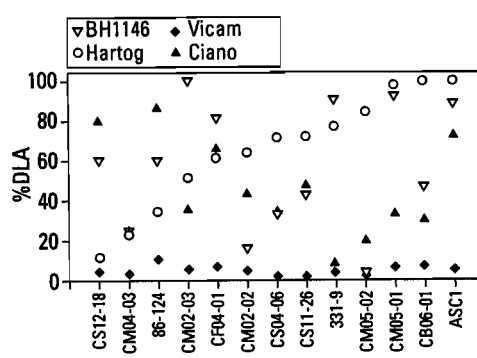

Figure 2. Percentage diseased leaf area (%DLA) induced by isolates of *Pyrenophora tritici-repentis* from warm areas on four wheat cultivars in glasshouse seedlings inoculations. Each point is the mean of five plants.

BH1146 (2-99%), and Ciano (8-87%) (Figure 2).

In the detached-leaf assay, chlorosis development was observed for all isolate x cultivar combinations tested, but this does not fit the description of extensive chlorosis described by Lamari and Bernier (1989a). The tested cultivars thus appear unable to express extensive chlorosis under our conditions. Isolates ASC1 and 86124 caused necrosis development beyond the area of inoculation on all cultivars except Vicam. Isolate 331-9 produced necrosis but not beyond the area where the drop of inoculum was deposited on the leaf. This is probably due to the combined effect of all fungal infection sites (Table 3). Results from the single conidia inoculation confirmed those of the conidia suspension inoculation. Necrosis was again observed on genotypes Ciano, BH1146, and Hartog for isolates ASC1 and 86124, but not for isolates 331-9 and 90-2. The resistant Vicam did not display any necrotic symptoms.

A set of isolates originating from different countries was tested on the four cultivars (BH1146, Hartog, Vicam, and Ciano) using the drop and single spore inoculation techniques in a detached leaf assay. Results of tan necrosis production, similar for the two inoculation types, are presented in Table 4. For all isolates, Vicam did not express any necrosis, while necrosis was induced on BH1146, Hartog, and Ciano by all isolates except 331-9 and 90-2 (nec-), two isolates isolated by E. Duveiller in Oaxaca, Mexico, and one from Bangladesh.

Toxin production

BH1146, Hartog, and Ciano, but not Vicam, showed necrosis after leaf infiltration with a proteinic solution obtained by partial purification of culture filtrates (Table 5). Four additional cultivars were tested using this protocol (Table 5): GUAMUCHIL, considered susceptible in field tests (E. Duveiller, personal communication), showed necrosis, while the two resistant wheat genotypes ALD/COC//URES and

Table 3. Reactions of *Pyrenophora tritici-repentis* isolates inoculated as conidial droplets or single conidium on four wheat cultivars in detached-leaf assays.

Isolate	Origin	BH1146	Hartog	Vicam	Ciano
ASC1	Manitoba, Canada	N[1]	N	0	N
86124	Manitoba, Canada	N	N	0	N
331-9	Manitoba, Canada	0 (N)	0 (N)	0 (N)	0 (N)
90-2	Manitoba, Canada	0	0	0	0
CF 04-01	Ouezzane-Morocco	N	N	0	N
CM 04-03	Pergamino-Argentina	N	N	0	N
CM 02-02	El Batan-Mexico	N	N	0	N
CM 02-03	Chalco-Mexico	N	N	0	N
CM 05-01	Oaxaca-Mexico	0	0	0	0
CM 05-02	Oaxaca-Mexico	0	0	0	0
CS 04-06	Dinajpur-Bangladesh	0	0	0	0
CS 11-26	Jessore-Bangladesh	N	N	0	N

[1] N = Induction of necrosis; (N) = Scattered necrotic spots with droplets of conidial suspension; 0 = No induction.

SHA8//PRL/VEE#6 and susceptible ND/VG9144//KAL/BB/3/YACO/4/CHIL did not. This last genotype needs to be further studied to explain the unusual combination of fungal susceptibility and toxin resistance.

Infiltration of the partially purified toxin of the nec+ isolates of Lamari and colleagues, ASC1 and 86-124, resulted in necrotic development on the susceptible wheats, while the nec- isolate 331-9 did not produce any necrosis on these cultivars. Among the isolates from our collection, all isolates except CM05-01, CM05-2, and CB05-01 induced necrosis on the susceptible genotypes (Table 4).

Table 4. Reactions of susceptible wheat varieties (Ciano, Hartog, and BH1146) to infiltrations of partially purified culture filtrates from *Pyrenophora tritici-repentis* isolates.

Isolate	Origin	Necrosis induction[1]
ASC1	Manitoba, Canada	+
86-124	Manitoba, Canada	+
331-9	Manitoba, Canada	0
CF04-01	Ouezzane, Morocco	+
CM04-03	Pergamino, Argentina	+
CM02-02	El Batan, Mexico	+
CM02-03	Chalco, Mexico	+
CM05-01	Oaxaca, Mexlco	0
CM05-02	Oaxaca, Mexico	0
CS04-06	Dinajpur, Bangladesh	+
CS11-26	Jessore, Bangladesh	+
CS19-08	Rising Patan, Nepal	+
CS12-18	Gairwa, Nepal	+
CB05-01	Merbe-le-Château, Belgium	0

[1] + Necrosis induction; 0 Absence of necrosis.

RAPD analysis

For Canadian isolates ASC1, 86-124, 331-9, and 90-2, 15 primers allowed the amplification of polymorphic bands. In all cases, the pattern of isolate 90-2 was clearly different to the other isolates. Primers A05, A07, A10, A12, A14, and A18 (6 of 20) distinguished between isolates able to produce and isolates unable to produce necrosis; however, no polymorphic band was able to differentiate between isolates inducing or not inducing necrosis. Without further studies, a strong relationship between the production of the necrosis-inducing toxin and this polymorphism could not be identified; however, the preliminary results encouraged us to select promising primers and to test our collection of isolates to confirm these observations. A phylogenetic tree was observed from the results of an extended collection of

Table 5. Comparison of the susceptibility of nine bread wheats to *Pyrenophora tritici-repentis* in the field[1] or in greenhouse experiments and their reaction to necrosis toxin infiltration.

Cultivars	*P. tritici-repentis* resistance in field and greenhouse	Reaction to necrosis toxin infiltration[2]
CIANO	Susceptible	+
GUAMUCHIL	Susceptible	+
HARTOG	Susceptible	+
ND/VG9144//KAL/BB/3/YACO/4/CHIL	Susceptible	0
BH1146	Semi-resistant	+
ALD/COC//URES	Resistant	0
SHA8//PRL/VEE#6	Resistant	0
VICAM	Resistant	0

[1] Source: E. Duveiller, personal communication.
[2] + Necrosis expression; 0 Absence of necrosis.

isolates (5 primers and 16 polymorphic bands), but in this case we could not associate the observed diversity using RAPD markers with diversity in pathogenicity, geographic origin, or toxin production (Figure 3); however, isolate 90-2 was observed to be distinctly separate from the others isolates.

Discussion and Conclusion

The various pathogenicity tests demonstrated the wide diversity among *P. tritici-repentis* isolates studied, not only from the view point of types of symptoms induced, as suggested by Lamari and Bernier (1989), but also the quantitative aspect of host pathogen interaction, as illustrated by disease severity tests. Due to the difficulties and problems encountered during the scoring of lesion type (i.e., different lesion types coexisting on the same leaf and repeatability problems), we are further standardizing pathogenicity tests by inoculation with conidia on detached leaves in petri dishes to improve control of environmental conditions. This last test has proved very useful for isolate characterization with regard to their ability to induce necrosis on wheat cultivars. Nevertheless, the lack of information on host x pathogen interaction is too important and the test must be completed by a more detailed analysis of the pathogenicity of these isolates such as the %DLA test. Interactions between isolates and cultivars were also detected in the seedlings test where the ranking of isolate aggressiveness depended on the cultivars examined. For example, as in the detached-leaf assay, the cultivar Vicam was resistant to all isolates whereas the susceptibility of the other cultivars allows a separation between isolates that can or cannot induce tan necrosis.

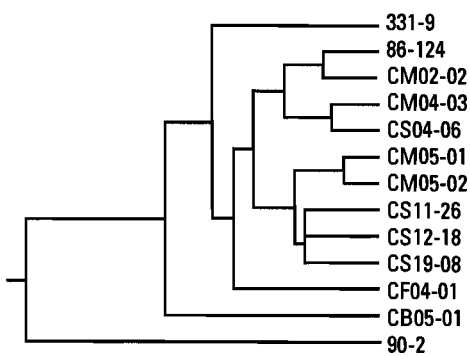

Figure 3. Relationships among 13 *Pyrenophora tritici-repentis* isolates on wheat as estimated by RAPD.

Leaf infiltration with partially purified culture filtrates allowed the rapid characterization of *P. tritici-repentis* isolates and wheat cultivars according to necrosis toxin production and necrosis expression, respectively. This characterization can be directly correlated to results of the detached leaf assay. Necrosis is observed only in the interaction between isolates able to produce necrosis toxin and cultivars able to express this symptom. However, the reaction of cultivar ND/VG9144//KAL/BB/3/YACO/4/CHIL raises questions: it is susceptible (expresses tan necrosis) in field and greenhouses experiments but, when infiltrated by necrosis-inducing toxin, it does not show any symptoms. A more detailed study into the possible reasons behind this lack of reaction will help to understand the role of the necrosis toxin in host-pathogen interactions.

Finally, correlations among pathogenicity, toxin production, and geographic origin were not evident through clustering of the isolates by RAPDs. Molecular differentiation based on sequences related more to pathogenicity, such as genes for toxin production, should permit a faster characterization of isolates.

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Role of Root Exudates and Toxins in Susceptibility of Yemeni Wheat Varieties to *Cochliobolus sativus*

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Abstract

The pathogenicity of eight isolates of Cochliobolus sativus (Bipolaris sorokiniana) *(six from Scottish barley, one from Yemeni barley, and one from Yemeni wheat) was tested on roots and leaves of eight Yemeni wheat cultivars. There were significant differences in resistance among cultivars and in pathogenicity among isolates, but no evidence of strong host specialization. The most resistant cultivar, Sonalica, and the most susceptible, Mohtar, were selected to study possible resistance mechanisms. Root exudates may influence how successfully the pathogen grows before infection takes place. Compared to deionized water, root exudates of 10-day-old seedlings of Mohtar promoted spore germination and growth of* C. sativus, *whereas root exudates of Sonalica were inhibitory. The Mohtar exudates contained significantly higher levels of water-soluble carbohydrate and total nitrogen than corresponding Sonalica exudates, with different sugar and amino acid composition. When seedlings of Mohtar and Sonalica were grown in water culture with* C. sativus, *the Mohtar seedlings became chlorotic and were killed much more quickly than the Sonalica seedlings, suggesting that they were more sensitive to toxins produced by the pathogen. We compared the effects of culture filtrates from two pathogen isolates on seed germination, seedling growth, and leaves of Mohtar and Sonalica. Filtrates from both isolates were much more toxic to Mohtar than to Sonalica, although toxicity was reduced by neutralization of the filtrate.*

Characterization of *Cochliobolus sativus* Isolates from the UK and Yemen

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Abstract

The properties of six isolates of Cochliobolus sativus (Bipolaris sorokiniana) *from barley in Scotland, one isolate from Yemeni barley with black point disease, and one from Yemeni wheat with black point disease were compared. The eight isolates differ in pathogenicity, conidia size, growth rate, and response to temperature; only the isolate from Yemeni wheat was able to grow well at 35°C. All isolates were all able to produce xylanase when grown in culture. To compare genes coding for cell-wall-degrading enzymes in the different isolates, total DNA was digested with several restriction enzymes and probed with XYL1 and PGN1 cDNAs of C. carbonum. Southern hybridization patterns were similar for all isolates except the one from Yemeni wheat, and were distinct from those obtained with DNA of the related species C. heterostrophus. DNA of all eight isolates and of* C. heterostrophus *could be amplified by the polymerase chain reaction (PCR) with sequences from the* C. sativus *XYL2 gene as primers. DNA of most isolates gave two bands; sequence analysis showed that these correspond to the XYL1 gene (with one intron) and the XYL2 gene (with two introns) of C. carbonum. DNA of the isolate from Yemeni wheat, however, also gave a third, smaller band. DNA of C. heterostrophus gave two bands, but one corresponds to XYL1 and the other co-migrates with the third band of the Yemeni wheat isolate of* C. sativus.

A Xylanase Gene from *Cochliobolus sativus*

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Abstract

Cell-wall-degrading enzymes are expected to be important in pathogenesis by Cochliobolus sativus *(*Bipolaris sorokiniana*). The xylanase activity of an isolate from barley was assayed in five different culture media. The highest activity was in Fries' medium containing 2% oat spelt xylan, 3% cellulose, and 4% peptone. To investigate genes coding for cell-wall-degrading enzymes in* C. sativus, *total DNA was digested with restriction enzymes and probed with* C. carbonum XYL1 *and* PGN1 *cDNAs, coding for a xylanase and an endo-polygalacturonase. The hybridization patterns indicate that* C. sativus *has genes resembling both. A cDNA library was constructed from mRNA of* C. sativus *grown on xylanase-inducing medium, and screened with* C. carbonum XYL1 *cDNA. All xylanase-encoding clones identified in this way were more similar to a second* C. carbonum *xylanase gene,* XYL2, *than to* XYL1. *Part of the* C. sativus *xylanase gene corresponding to the clones was amplified by the polymerase chain reaction (PCR), and found to have two introns like* C. carbonum XYL2. *Amplification with reduced stringency gave a second PCR product; DNA sequencing showed that it is derived from the* C. sativus *homologue of the* C. carbonum XYL1 *gene, with a single intron. The 5¢ and 3¢ flanking regions of the* C. sativus XYL2 *homologue were amplified by inverse PCR. The resemblance between the* C. sativus *and* C. carbonum XYL2 *sequences extends to the ends of the reported* C. carbonum *sequence.*

Most microbial plant pathogens can produce extracellular enzymes that degrade plant cell wall carbohydrates (Bateman 1976). Although the role of such cell-wall-degrading enzymes (CWDEs) in pathogenesis is not yet fully understood, initial penetration of plants and tissue maceration through host cell wall degradation are two phenomena that can be attributed to their production. Penetration of cell walls may also occur through mechanical pressure, and CWDEs may assist in this process. In some instances, CWDEs have been shown to have an additional effect, acting as elicitors of plant defence responses. CWDEs of plant pathogens seem to be a suitable model system for molecular manipulation approaches to understanding host-pathogen interactions (Yoder 1985).

It is estimated that xylans make up one-third of all renewable organic carbon available on earth (Prade 1995). Not surprisingly, therefore, xylanase production is widespread among saprotrophic organisms. Besides

saprotrophs, plant pathogens may produce xylanases (Manners 1993); because b-1,4-xylans comprise up to 40% of the content of monocot primary cell walls (Apel *et al.* 1993), these enzymes are likely to be particularly important for pathogenicity of organisms that attack monocots. Indeed, xylanases are among the first CWDEs produced by cereal pathogens during early stages of infection (Keon *et al.* 1987). Thus, our investigations have focused mainly on xylanase genes.

Cochliobolus sativus ((Ito & Kurib.) Drechs. ex Dent., anamorph *Bipolaris sorokiniana* (Sacc.) Shoemaker), has been shown to produce several CWDEs including xylanase (Karjalainen *et al.* 1992), pectolytic enzymes (Muse *et al.* 1972), and cellulase (Gamal-El-Din *et al.* 1987), but it is not known whether these enzymes have a role in pathogenesis, and no *C. sativus* genes coding for CWDEs have been reported. Genes for xylanase (Apel *et al.* 1993), polygalacturonase (Scott-Craig *et al.* 1990), and cellulase (Sposato *et al.* 1995) have, however, been described in the related maize pathogen *C. carbonum*. In that organism, the role of CWDEs in pathogenesis has been analyzed by gene disruption experiments; disruption of individual genes did not make any difference to the symptoms produced, but the effects of disrupting multiple genes have not been reported. As a first step in analyzing the role of CWDEs in pathogenesis by *C. sativus*, we characterize here a xylanase cDNA and gene from an isolate of *C. sativus* from barley grown in Scotland (isolate 5; see Hassan and Hack, this proceedings).

Results

Detection of CWDE activity

Qualitative assay of CWDEs showed production of cellulase, xylanase, polygalacturonase, and pectate lyase by all isolates of *C. sativus* tested (not shown). For isolation of mRNA and subsequent cloning of xylanase cDNA, it was important to identify a medium supporting the production of large amounts of xylanase. Modified Fries' medium (Pringle and Braun 1957), supplemented with 2% oat spelt xylan, induced xylanase activity. When 3% cellulose and 4% peptone were added to the medium, as suggested by Haltrich *et al.* (1994) for induction of *Sclerotium rolfsii* xylanase, nine-day-old cultures contained high enzyme activity (Table 1). Accordingly, this medium was chosen for isolation of mRNA for cDNA preparation.

cDNA library construction and screening

Several methods for RNA extraction were tried, but only the method of Yoder (1988), designed for *C. heterostrophus*,

Table 1. Effect of culture medium on xylanase activity of *Cochliobolus sativus* after nine days of growth.

Medium	Xylanase activity (μmol min^{-1} ml^{-1})
2% xylan	0.02
2% xylan + 3% cellulose	0.04
2% xylan + 4% peptone	0.13
2% xylan + 3% cellulose + 4% peptone	0.20

Note: Fries' medium was supplemented with different C and/or N sources. Xylanase activity was assayed by measuring the liberation of reducing sugar (Miller 1959.) from 0.2% oat spelt xylan at 37°C in 50 mM potassium phosphate - citrate buffer (pH 6.4). (Ferreira 1991.) Activity is expressed in μmol min^{-1} per ml of culture filtrate.

gave good quality RNA. The mRNA was purified by oligo(dT)-cellulose column chromatography (Pharmacia mRNA purification kit). cDNA was synthesized (Stratagene cDNA synthesis kit) and cloned in the *Eco*RI and *Xho*I cloning sites of the l ZAP vector (Stratagene Zap-cDNA Gigapack III Gold Cloning Kit).

In order to identify xylanase clones, the resulting library was probed with radiolabeled *C. carbonum XYL1* cDNA, since this probe had been shown to hybridize to *C. sativus* DNA on Southern blots (Hack *et al.*, this proceedings). Several strong positive signals were observed. For further analysis, the phage identified in this way were converted into plasmids (Short *et al.* 1988) in *E. coli* strain SOLR' (Stratagene).

Xylanase production by transformed bacteria

Plate assays were carried out to determine whether there was any xylanase activity in transformed *E. coli* cells containing putative *C. sativus* xylanase cDNA. Transformants were grown overnight on LB medium containing 25mg ml^{-1} ampicillin, at 37°C and 200 rpm. Cell-free extracts of the bacteria were placed in wells in agar plates containing 20 ml of 0.1% solubilized oat spelt xylan, 0.8% agar. For some transformants, Congo red staining of the plates and subsequent destaining with 1 M NaCl revealed xylanase activity as clear zones against a red background, showing that these transformants did indeed contain xylanase activity.

Sequencing of xylanase cDNA

Plasmids were isolated from positively transformed *E. coli* cells, digested with *Eco*RI and *Xho*I, analyzed by agarose gel electrophoresis, and screened again by Southern hybridization with *XYL1* cDNA of *C. carbonum*, in order to confirm the identity of the inserts and estimate their size. Six clones were sequenced (Perkin Elmer Sequencer). The longest of these had an 861-bp insert. Although not all inserts were the same length, all corresponded to a single gene product, which contains an open reading frame of 693 bp (Figure 1). The cDNA sequence is similar to *C. carbonum XYL1* (Apel *et al.* 1993), as expected, but it shows greater similarity to another *C. carbonum* xylanase gene, *XYL2* (P.C. Apel-Birkhold and J.D. Walton, unpublished, GenBank/EMBL accession number U58915, submitted 1996) - it is 96% identical to *XYL2*, but only 59% identical to *XYL1*[1] - suggesting that it is the *C. sativus* counterpart of *C. carbonum XYL2*. Consistent with this suggestion, the predicted translation product is 98% identical to the *C. carbonum XYL2* gene product (Figure 2), but only 66% identical to the *C. carbonum XYL1* gene product.

Introns

In *C. carbonum*, the *XYL1* gene has a single intron, whereas the *XYL2* gene has two. To identify introns in *C. sativus* xylanase genes, two primers were designed to correspond to sequences common to the *C. sativus* cDNA and both the *XYL1* and *XYL2* genes of *C. carbonum*[2]. These primers were used to amplify *C. sativus* genomic DNA by the

```
   1 ATATTTTGGG CAGGACCGTG TTCCAACCGG TGGTTAACTG AAAGATTGCC CGAGATTTCC CGCCTAGAGC CTTCTGTGTC
  81 CTGTTCACGT GTGATACTAC CCCTGATCGA TTCCTCGTCT ATGCATGTTT TAGCCCCGAT TGAAGTCCCT TCTTGGGCTG
 161 TGAAAGCATA TGGGGAGTGT GGGGTAGGGT GTCTGCACTT ATTTCTGTGG CGTTCCCTTT ATATTAACGT CCTCGAAACC
 241 CGGTTCAAAC AGCGCGTAAA CCCGGGTAGA GCGATGTTCT GTTAATTATG TTATGCAGAT TTACGC.AGG CTACGGATCT
                       .......... ..........  ......... .......... ...... ... .... .....
        C. carbonum 1 CCCGGGTAGA GCGATGTTCT TTTAATTATG TTATGCAGAT TTACGCAAGG CTACAGATCT

 321 TTTTGATCAC CCCGTGGTGG CTGTTGCTAC CGAAACTGGA TGCCGATCGG TGCATGTTCC CTATGTGTCT CGACTTGGTC
     .......... ..... .... . ........ .......... .......... ....... .. .......... ..........
  62 TTTTGATCAC CCCGTCGTGG CCGTTGCTAC CGAAACTGGA TGCCGATCGG TGCATGTCCC CTATGTGTCT CGACTTGGTC

 401 ATTGTGGCCG CCGCTTAGGG CGAGGTTGTC GAAGTGAGAG GCTCTAACCT ACCACCAATG TGTACACCAT CACGAATAGA
     .......... ..........  ... ..... .......... ..  ...... .......... .......... ..........
 142 ATTGTGGCCG CCGCTTAGGG TGAGCTTGTC GAAGTGAGAG GCCTTAACCT ACCACCAATG TGTACACCAT CACGAATAGA

 481 GACAACACGT GAGACGAGAC CTCATGACCC CCCAGTATCA TTTCATCCTG CGATTCAGGC TCAACTATTA CTCGGCGTAG
     .... ....  .... ..... ....... ..  ..... ... .......... .......... .......... ..........
 222 GACAGCACGC GAGATGAGAC CTCATGA.CC ACCAGTGTCA TTTCATCCTG CGATTCAGGC TCAACTATTA CTCGGCGTAG

 561 AGATATAAGT TGATAGCTGC TCCCAGTCAG TAGCATCACT GCATCCAACA CAGATCAGCA ACACTCAACA CAAGATGGTT
     ..........  ......... .......... .......... .......... .......... .......... ..........
 301 AGATATAAGT CGATAGCTGC TCCCAGTCAG TAGCATCACT GCATCCAACA CAGATCAGCA ACACTCAACA CAAGATGGTT

 641 TCCTTCAAGT CTCTGCTTCT CGCCGCTGTG GCTACCACCA GTGTCCTCGC TGCTCCCTTC GACTTCCTTC GTGAGCGCGA
     .......... .......... .......... .......... . ........ .......... .. ....... ..........
 381 TCCTTCAAGT CTCTGCTTCT CGCCGCTGTG GCTACCACCA GCGTCCTCGC TGCTCCCTTC GATTTCCTTC GTGAGCGCGA

 721 CGATGGCAAC GCGACTGCTC TCCTTGAGAA GCGTCAGTCT ACTCCCAGCT CTGAGGGATA CCACAACGGA TACTTCTACT
     ..... .... .......... .......... .......... .........  . ........ ...... ... ..........
 461 CGATGTCAAC GCGACTGCTC TCCTTGAGAA GCGTCAGTCT ACTCCCAGCG CCGAGGGATA CCACAATGGA TACTTCTACT

 801 CGTGGTGGAC TGATGGCGGC GGCTCTGCCC AGTACACCAT GGGTGAGGGC AGCAGGTACT CCGTGACCTG GAGGAACACT
     .......... .........  .......... ....... .. .......... .......... . ........ ..........
 541 CGTGGTGGAC TGATGGCGGT GGCTCTGCCC AGTACACTAT GGGTGAGGGC AGCAGGTACT CTGTGACCTG GAGGAACACT

 881 GGCAACTTTG TTGGTGGCAA GGGATGGAAC CCTGGAACCG GCCGGTAGGT TGCGAAGACT GGTTGCTGAT TAGAATTTTA
     .......... ....... .. ... ...... ....... .. .......... .......... . ... ....  ..... ...
 621 GGCAACTTTG TTGGTGGAAA GGGGTGGAAC CCTGGAAGCG GCCGGTAGGT TGCGAAGACT GTTTGGTGAT GAGAATCTTA

 961 CTAATGTGAC TTCGTAGTGT CATCAACTAC GGCGGAGCTT TCAACCCCCA GGGCAACGGA TACCTCGCTG TGTACGGATG
     ........   .......... .......... .... ... . .......... .......... .......... ..........
 701 CTAATGTGGA TTCGTAGTGT CATCAACTAC GGCGCAGCCT TCAACCCCCA GGGCAACGGA TACCTCGCTG TGTACGGATG

1041 GACCCGCAAC CCGCTTGTCG AGTACTACGT AATTGAATCC TACGGAACCT ACAACCCCAG CAGTGGAGCC CAGGTCAAGG
     .......... .......... ..........  ......... .......... .......... .......... ..  ......
 781 GACCCGCAAC CCGCTTGTCG AGTACTACGT GATTGAATCC TACGGAACCT ACAACCCCAG CAGTGGAGCC CAAATCAAGG

1121 GAAGCTTCCA GACTGACGGT GGTACTTACA ACGTTGCCGT CTCCACCCGT TACAACCAGC CCTCCATTGA CGGAACAAGG
     . ........ ... ...... .......... .......... .......... .......... .......... ..........
 861 GCAGCTTCCA GACCGACGGT GGTACTTACA ACGTTGCCGT CTCCACCCGT TACAACCAGC CCTCCATTGA CGGAACAAGG

1201 ACCTTTCAGC AGTATTGGTA AGTCATTTTG GTGTTTGATT CAACAGAAGC GTAGATTCTG ACTATTGTGA TAGGTCTGTC
     .......... .... ..... ........ . ...... ..    ........ ......  .. ... .. ... ..........
 941 ACCTTTCAGC AGTACTGGTA AGTCATTTCG GTGTTTAATC AGACAGAAGC GTAGATGTTG ACTGTTATGA TAGGTCTGTC

1281 CGCCAACAGA AGCGTGTCGG TGGAAGCGTG AACATGCAGA ACCACTTCAA CGCTTGGTCT CGCTATGGCT TGAACCTTGG
     ...   .... .......... .......... .......... .......... .......... .......... ..........
1021 CGCACCCAGA AGCGTGTCGG TGGAAGCGTG AACATGCAGA ACCACTTCAA CGCTTGGTCT CGCTATGGCT TGAACCTTGG

1361 TCAACACTAC TACCAGATCG TCGCCACTGA GGGTTACCAG TCTTCCGGAA GCTCTGACAT CTACGTGCAG ACTCAGTAGA
     .......... .......... .......... .......... .. .. .... .......... ... ...... ..........
1101 TCAACACTAC TACCAGATCG TCGCCACTGA GGGTTACCAG TCCTCTGGAA GCTCTGACAT CTATGTGCAG ACTCAGTAGA

1441 GGAGTTGTTG TCTGTGGAGC GAGCAACTAG CAGACGAGAT CAGACAAGAA TAGTGCCTCG GATAGTTGTG AGCAAATTGG
     . ........ ...... ... ..........  .........  ......... ..... .... .........  ....  ....
1181 GTAGTTGTTG TCTGTGAAGC GAGCAACTAG TAGACGAGAT TAGACAAGAA TAGTGTCTCG GATAGTTGTA AGCAGGTTGG

1521 AGAAAGAGCG ATGCGTTCGA GTTACTGTAC CTAGAAACAT GTCAATTCAC TCGCTATAAA ATATTCCGTT CTCGAATTTG
     .......... ... ......  .. ......  ......... .......... .......... .  ......  ....... ..
1261 AGAAAGAGCG ATGTGTTCGA TTTCCTGTAC ATAGAAACAT GTCAATTCAC TCGCTATAAA ACCTTCCGTC CTCGAATGTG

1601 TTCTTTTTTT TTTGGAGTAT TTGAGCATGG TATCATGCTG CAGCAAAAGC AGCTAGAATC TAAGTGAGGT AGAAAACGAA
     ...    . . .... . ..   ....... . .... .. .  . ........ .. .. . .  .. ......          . 
1341 TTC....TCT TTTGTATTAG CTGAGCATTG TATCGTGGTT CGGCAAAAGC AGGTAAAGTT TAGGTGAGGG CATTTGTTAT

1681 AATCATGAAG TAGCTAGCAT GATGATGGCG CAATAGCACT TTGCCCCAGT AGGCGCACTA ATGTGTTGAT TTCACAGGGG
     .. ..   .     .. . .           . .    .         .
1417 AAACAGTCAT AGTCTCGTAG CCATCGAATG CTGGTGTGTG AATTC

1761 TGCCTTGACG ATCGTTTTGT GTATAGCTGA CTCATGGAGA CTTGTATTGC AGACCGTCTT AGTCCAGTAG CCATAGAATG
1841 CTGGTATGAG TTCGACGTAG CTAACACATG TGTGCTGACT GAGCGGTTTT CTCCTCTCAT CTACTAACCA GCAACAGCAT
1921 ACTACTCTTG TTTGGCAACT TTACATTTTG TTAAAGACAT TCGTTCCAGT CATATATTGT TCCGTGCCCT CTCTCATCCT
2001 GCCTTCAGTC TCCATGTTAT AACTGTCCTC GGTGACATGA ACTCATCAGT TAGTCCTTCC AATAATACGC TATTTTGGCG
2081 CCTCCAGCCT CGGCCATGAG GTCGAAGCAT TCGCCAGACT CACTGCCTGG CCTTTTCCAA AACGGCCAAG ACCACCATCG
2161 AACCAACGTG CCTGCTCCCT TCGAACTTCC CTAAAGCCGG AATTCCCAGC A
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Figure 1. Nucleotide sequence of the *Cochliobolus sativus XYL2* gene, aligned with the *C. carbonum XYL2* gene sequence; dots indicate identical residues. Italics show the introns. The start and stop codons are in bold. The cloned *C. sativus* cDNA sequence is underlined. Single overlines indicate positions of primers for amplifying the middle part of the gene, including the introns; double overlines indicate positions of primers for inverse PCR.

polymerase chain reaction (PCR). The products depended on the primer annealing temperature used. When annealing was carried out at 62°C, electrophoresis of the PCR products on a 2% agarose gel gave a single band of 530 bp, but reducing the stringency of annealing by lowering the temperature to 55°C resulted in the appearance of an additional prominent band of 480 bp. PCR products were cloned in a 'TA' cloning vector (pCR' 2.1; Invitrogen) and clones were sequenced. The results showed that the 530-bp band comes from the gene coding for the previously sequenced *C. sativus* cDNA. It contains two introns which are similar in sequence and identical in location to those of *C. carbonum XYL2* (Figure 3). Thus, the intron sequences confirm that the gene coding for the sequenced cDNA clones is a counterpart of *C. carbonum XYL2*. For this reason, we refer to this gene as *C. sativus XYL2*. The sequence of the 480-bp band has not yet been definitively determined, but preliminary evidence indicates that it comes from a gene corresponding to *C. carbonum XYL1*, with a single intron.

Upstream and downstream sequencing of the gene

To find out the sequences of the 5' and 3' regions of the *C. sativus XYL2* gene, inverse PCR (IPCR) was carried out by the method of Silver (1991). Primers corresponding to residues 700-680 and 1462-1483 (Figure 1) were used to amplify the DNA outwards from the known sequence. Southern blotting of total fungal DNA digested with several different restriction enzymes showed that the *C. sativus XYL2* cDNA clone hybridizes to fragments of approximately 2800 bp in DNA digested with *Sal*I and 3000 bp in DNA digested with *Bam*HI, suitable for amplification by IPCR. Total DNA was digested with *Sal*I or *Bam*HI,

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                 ↓            .↓
CS   1  MVSFKSLLLAAVATTSVLAAPFDFLRERDDGNATALLEKRQSTPSSEGYH  50
        ||||||||||||||||||||||||||||||.||||||||||||||.||||
CC   1  MVSFKSLLLAAVATTSVLAAPFDFLRERDDVNATALLEKRQSTPSAEGYH  50

CS  51  NGYFYSWWTDGGGSAQYTMGEGSRYSVTWRNTGNFVGGKGWNPGTGRVIN  100
        ||||||||||||||||||||||||||||||||||||||||||||||.|||||
CC  51  NGYFYSWWTDGGGSAQYTMGEGSRYSVTWRNTGNFVGGKGWNPGSGRVIN  100

CS 101  YGGAFNPQGNGYLAVYGWTRNPLVEYYVIESYGTYNPSSGAQVKGSFQTD  150
        ||||||||||||||||||||||||||||||||||||||||||:|||||||
CC 101  YGGAFNPQGNGYLAVYGWTRNPLVEYYVIESYGTYNPSSGAQIKGSFQTD  150

CS 151  GGTYNVAVSTRYNQPSIDGTRTFQQYRSVRQQKRVGGSVNMQNHFNAWSR  200
        ||||||||||||||||||||||||:||| ||||||||||||||||||||
CC 151  GGTYNVAVSTRYNQPSIDGTRTFQQYWSVRTQKRVGGSVNMQNHFNAWSR  200

CS 201  YGLNLGQHYYQIVATEGYQSSGSSDIYVQTQ                    231
        |||||||||||||||||||||||||||||||
CC 201  YGLNLGQHYYQIVATEGYQSSGSSDIYVQTQ                    231
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Figure 2. Alignment of *Cochliobolus sativus* (CS) and *C. carbonum* (CC) *XYL2* gene products. 'I' marks identical amino acids, ':' marks conservative substitutions, '.' marks less conservative substitutions. Arrows denote predicted amino acid after site of signal peptide cleavage (expected to occur between residues 19 and 20) and predicted start of the mature protein (residue 40).

then self-ligated to give a circular template. Both templates gave products when used for IPCR. DNA digested with *Sal*I was chosen for further analysis; further cutting of the ligated DNA with *Sca*I gave a template that was more efficient for amplification than circular DNA. The >2000-bp product of the IPCR was cloned in pCR™2.1 and sequenced, giving another 612 base pairs of the 5′ and 629 base pairs of the 3′ flanking regions of the *C. sativus XYL2* gene (Figure 1).

Discussion

We have determined the sequence of a total of 2211 bp of a xylanase gene from *C. sativus*. This gene codes for a xylanase of glycosyl hydrolase family 11 (Henrissat 1991). Although the gene was originally detected with a *C. carbonum XYL1* probe, it is more similar to *XYL2* (91% identity at the genomic DNA level; Figure 2) than to *XYL1* of that fungus (50% identity at the genomic DNA level). On the 5¢ side, *XYL2* of *C. carbonum* and *C. sativus* are very similar for as far as the *C. carbonum* sequence has been reported; on the 3¢ side, the sequences become completely different about 90 bp beyond the polyadenylation site. The high level of similarity between *XYL2* of *C. carbonum* and *C. sativus* is consistent with other evidence that these species are closely related (Jones and Dunkle 1993). *Cochliobolus sativus XYL2* can, however, be distinguished from *C. carbonum XYL2* by its possession of a single internal *Hin*dIII restriction site (AAGCTT at position 1122).

When the translated coding sequence was compared to protein sequences in the OWL database (Bleasby *et al.* 1994), the most similar sequence was *C. carbonum*

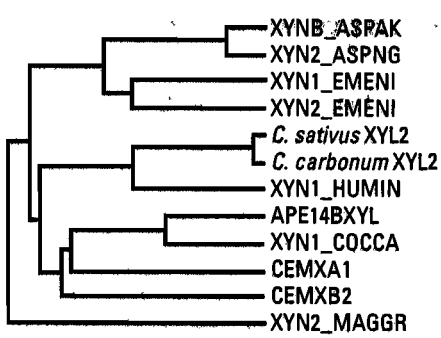

Figure 3. Dendrogram of xylanase sequences similar to *Cochliobolus sativus* XYL2, produced by the GCG program PILEUP. Branch lengths are proportional to distances between sequences. Except for *C. sativus* and *C. carbonum* XYL2, all abbreviations are as in the OWL composite protein sequence database (Bleasby *et al.* 1994). The xylanases are from: XYNB_ASPAK, *Aspergillus awamori* (var. *kawachi*); XYN2_ASPNG, *Aspergillus niger*; XYN1_EMENI and XYN2_EMENI, *Emericella nidulans* (*Aspergillus nidulans*); XYN1_HUMIN, *Humicola insolens*, APE14BXYL, *Ascochyta pisi*; XYN1_COCCA, *Cochliobolus carbonum* (xylanase I); CEMXA1 and CEMXB2, *Chaetomium gracile*; XYN2_MAGGR, *Magnaporthe grisea*.

[1] Sequence comparisons were carried out with the GCG program GAP (Devereux *et al.* 1984; Program Manual for the Wisconsin Package, Version 8, August 1994, Genetics Computer Group, 575 Science Drive, Madison, Wisconsin 53711, USA). The calculation of percentage identity ignores insertions and deletions in the sequences.

[2] Forward, 5¢-TGTAAAACGACGGCCAGT-GGCAAGGGATGGAACC-3¢
Reverse, 5¢-CAGGAAACAGCTATGACC-GCGACGATCTGGTAGTAGTG-3¢
The 5¢ 18 nucleotides of each primer are derived from bacteriophage M13, for use in direct sequencing of PCR products. Direct sequencing did not, however, give reliable results. The last 16 nucleotides of the forward primer correspond to residues 896-911 of the genomic sequence (Figure 2), the last 20 nucleotides of the reverse primer to residues 1384-1365.

XYL2. The next most similar sequence was not *C. carbonum* XYL1 but the XYL1 xylanase of *Humicola insolens* (Dalbøge and Heldt-Hansen 1994). Multiple comparison among xylanase sequences (Figure 3) shows that *C. sativus* XYL2, *C. carbonum* XYL2, and *H. insolens* XYL1 form a group of sequences resembling one another more closely than any other xylanase in the database. *Cochliobolus carbonum* XYL1, in contrast, is most similar to a xylanase of *Ascochyta pisi* (P.S. Lubeck *et al.*, unpublished, GenBank/EMBL accession number Z68891, submitted 1996). That XYL1 and XYL2 of *C. carbonum* resemble xylanases of other fungi more than each other suggests that the *XYL1*-type and *XYL2*-type xylanase genes diverged in a common ancestor of all these fungi. The *XYL2* genes of *C. carbonum* and *C. sativus* are the only reported fungal genes coding for family-11 xylanases that have two introns: all others have a single intron in approximately the same position as the first intron of *XYL2*. For *H. insolens XYL1*, only the cDNA sequence has been reported, but the similarity of the protein to *Cochliobolus* XYL2 suggests that this gene, too, may have two introns. Since *H. insolens* and *Cochliobolus* species are probably not closely related, xylanase genes of this sub-family may be widespread among fungi.

Karjaleinen *et al.* (1992) purified and determined the amino-terminal sequence of a xylanase produced by *C. sativus* in liquid culture. This enzyme is in a different family of glycosyl hydrolases from XYL2, probably family 10. Thus, *C. sativus* has the ability to synthesize at least two different kinds of xylanase. Apel *et al.* (1993) reported that in *C. carbonum* cultures, *XYL1* is the most highly expressed xylanase gene. *Cochliobolus sativus* probably possesses a *XYL1*-type gene, since a genomic sequence with one intron was amplified by PCR. Our results suggest, however, that in *C. sativus*, *XYL2* is more strongly expressed than *XYL1*, at least under the culture conditions we used: all six cDNA clones we identified using *XYL1* as probe were of the *XYL2* type, not the *XYL1* type. In the future, it will be important to examine the roles of the various xylanase gene products in degradation of cell walls in infected plants. Since *H. insolens* is not a plant pathogen, there does not appear to be a specific association between genes of the *XYL2* type and pathogenicity. Preliminary results indicate, however, that *XYL2* is expressed in infected plants, suggesting that it plays a part in pathogenesis.

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Leaf Spot Diseases of Wheat in a Conservation Tillage Study

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Abstract

A conservation tillage cropping systems project was initiated 12 years ago to study the influence of crop rotation, tillage practice, fertility level, and crop cultivar on the severity of leaf spot diseases. The experiment included the following experimental variables in all combinations with three replicates: 1) two cropping rotations (spring wheat-fallow and spring wheat-winter wheat-sunflowers); 2) three tillage treatments (conventional till, minimum till, and zero till); 3) three nitrogen (N) fertilizer rates; and 4) two cultivars of each crop grown. Pyrenophora tritici-repentis *and* Stagonospora nodorum *(syn.* Septoria nodorum*) were the main causal agents of a leaf spot disease complex. Higher levels of necrosis and chlorosis were associated with the no additional N (crop-fallow) and the low N (continuous cropping) treatments compared to higher N levels. When significant differences were evident among tillage treatments, in general higher levels of necrosis and chlorosis were associated with wheat leaves from zero till plots than those from minimum or conventional till plots. The winter wheat cultivar Roughrider had higher levels of necrosis and chlorosis compared to Norstar; however, the differences between the spring wheat cultivars Stoa and Butte 86 were not consistent. With some disease ratings, the effect of tillage on leaf spot diseases varied depending on N level. With the no additional N and low N treatments, leaf spot severity was higher under zero tillage than conventional tillage, but at the higher N treatments the differences in leaf spot severity among tillage treatments was greatly reduced or eliminated.*

In the USA, the Conservation Technology Information Center (1995) indicated that the use of conservation tillage (i.e., over 30% residue on the surface) increased from 26% in 1989 to 35% in 1995. Use of zero tillage, alone, increased from 5% in 1989 to 15% in 1995 in the USA. The increase in conservation tillage methods has created a need for more information on plant diseases in conservation tillage systems. Recent literature reviews are available (Bailey 1996; Bailey and Duczek 1996; Burton *et al.* 1994; Conway 1996; McMullen and Lamey 1994; Rothrock 1992; Scott *et al.* 1992; Watkins and Boosalis 1994).

[1] USDA-ARS, Northern Plains Area, is an equal opportunity/affirmative action employer and all agency services are available without discrimination. 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.

Conservation tillage may influence the incidence and severity of plant diseases. Disease response to conservation tillage practices may also depend on the soils, the region, prevailing environment, and biology of the disease organism. Management decisions for minimizing diseases in cereal crops include: using crop rotation; using adapted tolerant or disease resistant varieties; using pathogen-free seed with high germination; eliminating volunteer plants that can harbor diseases; planting at the proper time and at seeding rates recommended for the area, weather permitting; using a proper balance of fertilizers; monitoring fields; and using fungicides, if foliar diseases are present and yield potential is high (Krupinsky *et al.* 1997b).

A comprehensive conservation tillage-cropping systems research project, which included the variables of tillage, crop rotation, N fertilization, and cultivar selection, was used to determine the influence of these factors on the severity of leaf spot diseases. Preliminary reports on disease results from individual years have been made (Krupinsky *et. al.* 1995, 1997a). This report provides a brief summary of the overall results and interpretation of results from the past 12 years.

Materials and Methods

A conservation tillage-cropping systems research project was conducted from 1984-1996 on a 25 ha site of Temvik-Wilton silt loam. The experimental variables used in all combinations with three replicates were as follows: 1) two cropping rotations (spring wheat-fallow and spring wheat-winter wheat-sunflowers); 2) three residue treatments (conventional till with <30% surface residue cover, minimum till with 30-60% surface residue cover, and zero-till with >60% surface residue cover); 3) three N fertilizer rates [0, 22, 45 kg ha^{-1} N (0, 20, and 40 lb ac^{-1} N)] for spring wheat fallow, and 34, 67, 101 kg ha^{-1} N (30, 60, and 90 lb N ac^{-1}) for continuous cropping; and 4) two cultivars of each crop grown (Black and Tanaka 1997; Halvorson *et al.* 1996). The winter wheat cultivars were Roughrider and Norstar and the spring wheat cultivars were Stoa and Butte 86. In this semi-arid region, precipitation averaged 41 cm (16 in) per year with 30 cm (12 in) received as rain during the growing season (April, May, June, July, and August) for the last 13 years (Figure 1).

Three wheat crops were evaluated for leaf spot diseases each year: winter wheat in the continuous cropping system

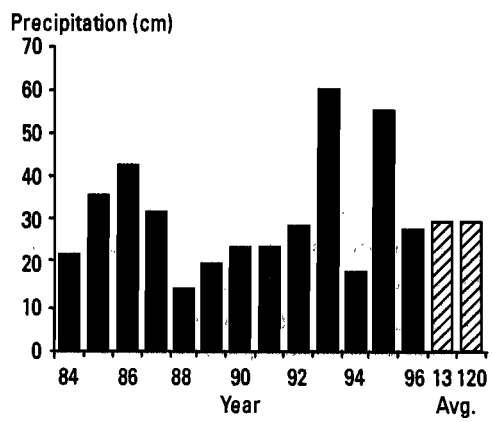

Figure 1. Growing season precipitation from April to August 1984-96. Field plots, Mandan, North Dakota.

(WWCC), spring wheat in the continuous cropping system (SWCC), and spring wheat in the spring wheat-fallow system (SWF). The total percentage of necrosis and chlorosis on wheat leaves was used as an indicator of the amount of damage caused by leaf spot diseases. Twenty leaves of the same leaf type (e.g., flag leaf) from plants at the same development stage were collected at random from each plot (18 plots per replicate) each time a crop was rated. Plots were rated several times each year during the growing season. The leaves most commonly rated were the flag leaf (F), F-1, and F-2.

To confirm the identity of the most common leaf spot pathogens present under natural field conditions, infected wheat leaves with lesions were collected from one replication of each study while being rated. Leaves were pressed, allowed to dry, and stored in a refrigerator at 3-5°C until they were processed, one to six months after collection. Leaf sections about 3 cm long were surface-sterilized for 3 min in a 1% sodium hypochlorite solution containing a surfactant, rinsed in sterile distilled water, plated on water agar in plastic petri dishes, and incubated under a 12-h photoperiod (cool-white fluorescent tubes) at 20°C. After seven days, leaf sections were examined for fungi. Pycnidiospores from pycnidia on the leaf sections were identified microscopically. The number of leaf sections infected with a particular fungus was used as an indicator of the relative importance of that fungus in causing leaf spot diseases in the field.

An analysis of variance was conducted using the arcsin-transformed percentage necrosis data (SAS version 6.08, SAS Institute, Cary, NC) from each rating. Statistical comparisons within each study were made with Student-Newman-Keuls' test (SAS).

Results and Discussion

Leaf spot disease complex

Based on annual isolations during 1987-1996, the major causal agents of the leaf spot disease complex present in the field plots were *Pyrenophora tritici-repentis* (Died.) Drechs. (anamorph *Drechslera tritici-repentis* (Died.) Shoemaker) and *Phaeosphaeria nodorum* (E. Müller) Hedjaroude (= *Leptosphaeria nodorum* E. Müller), anamorph: *Stagonospora nodorum* (Berk.) Cast. et Germ. (= *Septoria nodorum* (Berk.) Berk. in Berk. & Broome). Minor components of the leaf spot disease complex were *Cochliobolus sativus* (Ito & Kurib.) Drechs. ex Dastur, and *Phaeosphaeria avenaria* (G. F. Weber) O. Eriksson f. sp. *triticea* T. Johnson, anamorph: *Stagonospora avenae* Bissett f. sp. *triticea* T. Johnson (= *Septoria avenae* A.B. Frank f. sp. *triticea* T. Johnson).

Leaf spot diseases under the different treatments—During 1986-1987 and 1989-1996, there were 50 ratings of WWCC, 51 ratings of SWCC, and 61 ratings of SWF. Ratings from 1988, a low precipitation year (Figure 1), were not included because of low disease levels.

Differences in severity of leaf spot diseases among N treatments were significant for 56% of the WWCC ratings,

for 45% of the SWCC ratings, and for 10% of the SWF ratings. When significant differences were evident for N treatments, higher levels of necrosis and chlorosis were associated with the no additional N (crop-fallow) and the low nitrogen (continuous cropping) treatments compared to the higher N levels (Figure 2). This indicates that N level had an influence on leaf spot disease, and emphasizes the importance of providing adequate N, particularly in a continuous cropping system. One can speculate that N had a lesser impact on the SWF rotation compared to a very short fallow period in the continuous cropping system, because of the higher level of available soil N. This higher level was caused by mineralized N from the organic matter that became available due to a long fallow period in the SWF rotation.

Differences in leaf spot severity among tillage treatments were significant for 20% of the WWCC ratings, for 47% of the SWCC ratings, and for 16% of the SWF ratings. In general, higher levels of necrosis and chlorosis were associated with wheat leaves from the zero till plots compared to those from minimum or conventional till plots. Occasionally exceptions occurred, e.g., in 1987 the severity of leaf spot diseases were higher under conventional tillage compared to zero till.

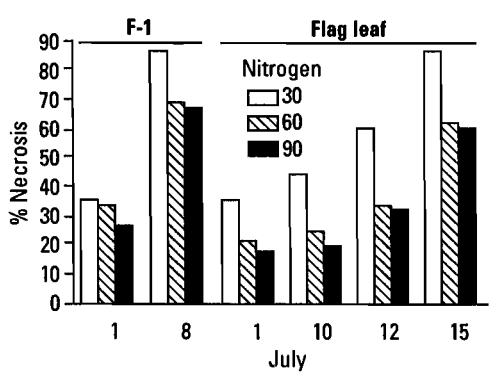

Figure 2. Leaf spot diseases on winter wheat after spring wheat as related to nitrogen, averaged over tillage and cultivars, Mandan, North Dakota, 1996.

Differences in severity of leaf spot diseases among cultivar treatments were significant for 32% of the WWCC ratings, for 20% of the SWCC ratings, and for 21% of the SWF ratings. When significant differences were evident for cultivar treatments, higher levels of necrosis and chlorosis were generally associated with the cultivar Roughrider compared with Norstar. Differences between Stoa and Butte 86 were not consistent.

The N x tillage interaction was significant for 12% of the WWCC ratings, for 15% of the SWCC ratings, and for 21% of the SWF ratings. Thus, at times, the tillage effect varied depending on the N level. With the no additional N and low N treatments, leaf spot severity was higher

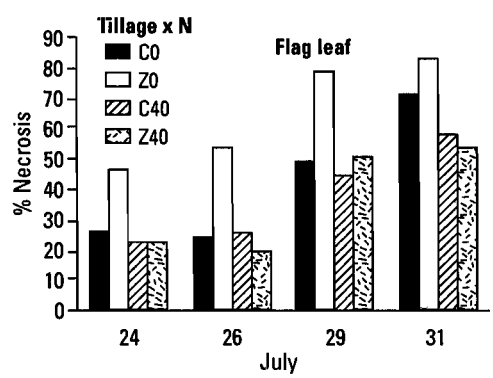

Figure 3. Leaf spot diseases on spring wheat after fallow as related to nitrogen and tillage, Mandan, North Dakota, 1996.
CT = Conventional tillage.
ZT = Zero tillage.
C0 = CT at 0N; Z0 = ZT at 0N;
C40 = CT at 40N; Z40 = ZT at 40N.

for zero tillage than conventional tillage, but at higher N levels, the difference in leaf spot severity for the tillage treatments was greatly reduced or eliminated, e.g., leaf spot diseases for SWF for 1996 (Figure 3).

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Control of Leaf Blights of Wheat by Elimination of the Inoculum Source

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Abstract

*The main inoculum sources of wheat leaf blights are infected seed and crop residues. Infected seeds introduce the pathogens into newly cultivated areas or to areas where the inoculum was eliminated through crop rotation with non-host crops. Disease transmission rate from seeds is high and very efficient. High sporulation on coleoptile tips confirm the potential of seed inoculum for causing epidemics, depending on climatic conditions during emergence to tillering. Such findings highlighted the need for eradication of seedborne inoculum. Once inoculum is introduced by seed, disease develops on above-ground plant parts where pathogens sporulate. Conidia are transported to new infection sites and to the soil by wind and rain splashes. Inoculum in the soil, as free dormant conidia (*Bipolaris sorokiniana*), increases with the amount of necrotic tissue. Sporulation continues in the residue until its complete decomposition. Dormant conidia may survive free in the soil for up to 37 months. Crop rotation may eliminate pathogens or reduce their inoculum to such an extent that they cannot cause an epidemic, even under favorable weather. Spot blotch may be economically controlled by treating seeds with fungicides and by rotating crops. In this case, epidemics do not reach the economical damage threshold level of 70-80% foliar incidence.*

The most important leaf blights of wheat in Brazil are spot blotch (*Bipolaris sorokiniana* Sacc. in Sorok.), yellow spot (*Drechslera tritici-repentis* (Died) Shoemaker), leaf, node, and glume blotch (*Septoria nodorum* (Berk.) Berk.), and speckled leaf blotch (*Septoria tritici* Rob. in Desm.). The latter has become of secondary importance after the generalized use of seed treatment and crop rotation. This group of necrotrophic pathogens are all introduced into new areas through infected seed and maintained indefinitely by monoculture (the succession of similar crops in the field). These diseases have increased in intensity under monoculture and no-till farming.

The development of wheat cultivars with good levels of genetic resistance to leaf blights has been difficult, hence the introduction of an alternative disease control strategy: the use of systemic fungicides (mainly propiconazole and tebuconazole) sprayed on the foliage at a cost of US $31.00 ha^{-1} per application.

Disease Control Based on Reduction of Inoculum Source

Seeds as inoculum source

According to Shaner (1981), infected seed is the main source of inoculum of leaf blight pathogens. Surveys carried out

in the state of Paraná showed a seed incidence of *B. sorokiniana* as high as 91% (Mehta and Igarashi 1981). In this warm region, wheat is cultivated mainly under monoculture and disease intensity has been very high, as has incidence in the seed. Seed disease incidence relates to the disease level on the above-ground plant parts. Hence, the more severe the disease, the higher the seed incidence.

The importance of seed inoculum has been illustrated by several works, which demonstrated that pathogen transmission from infected seeds to plumules and coleoptile tips may reach an efficiency of 87% for *B. sorokiniana* (Reis and Forcelini 1993). These authors also quantified sporulation in a greenhouse experiment. On coleoptile tips, number of conidia was as high as 1,509 conidia per coleoptile. Taking into consideration that wheat is seeded at a rate of 320 viable seeds m^{-2}, one may expect a spore density of 250,000 m^{-2}on the 49th day after crop emergence. When climatic conditions are favorable for infection, disease may reach epidemic levels. Couture and Sutton (1978) reported that spot blotch infection will occur when leaves remain wet for more than 18 h at a mean temperature >18°C. Such conditions very often prevail in most wheat growing areas in the states of Paraná, Mato Grosso do Sul, and São Paulo, Brazil, and in Paraguay.

For *S. nodorum* and *D. tritici-repentis*, transmission rates from seeds to coleoptiles of 34% and 38%, respectively, have been reported by Reis and Forcelini (1994).

These findings have led to the approach of controlling spot blotch and other leaf blights by the elimination of inoculum from seed (Cunfer 1983). Questions have been raised: What is the epidemiological meaning of high seed incidence? Can this inoculum lead to an epidemic? What is the real meaning of seed inoculum? Is it of real importance? Can we reduce the disease severity on leaves by reducing or eliminating seed inoculum? Such questions prompted us to investigate seed treatment with a new approach — eradication of *B. sorokiniana* and other pathogens from the seeds. A 100% control efficiency was pursued, taking into consideration the high transmission efficiency, the potential of sporulation, and climatic conditions conducive to disease outbreak.

Eradication is not easily achieved with commercial fungicides (iprodione + thiram and guazatine). Only in seed lots with low disease incidence (<30%) can 100% control be obtained. Nevertheless, the fungicide iminoctadine (a good research tool) eradicates *B. sorokinina* from infected seed independently of the incidence level.

Once leaf blight pathogens have been introduced into new wheat growing areas through infected seed, they will remain as saprophytes in the area as long as wheat residues remain on the field. Monoculture ensures the presence of stubble indefinitely. Pathogens will continue to sporulate on the residue and *B. sorokiniana* will add more inoculum to the soil as free dormant conidia; thus, the presence of residue also indicates the presence of leaf

blight pathogens. It is well known that under monoculture, residue, as a source of inoculum, is more important than seed inoculum because it is more abundant; hence, the opportunity for epidemics is greater with residue than with seed inoculum.

Seed disease incidence is directly related to disease intensity on foliage. In southern Brazil, a higher disease incidence is always found on farms under monoculture, where infected crop residue is the main inoculum source, and a lower incidence is found on farms under crop rotation.

Crop residue as the inoculum source

Following seed transmission in the field, several secondary cycles lead to infection of the green plant parts. These necrotrophic fungi will sporulate on necrotic tissues throughout the growing season (Figure 1) and will reach the heads, finally returning to the seed, which ensures the most efficient mechanism for pathogen survival. After harvesting, multiplication continues saprophytically on the residue until the straw breaks down completely (Figure 2). The presence of infected residues indicates the presence of inoculum, as shown in Figures 2 and 3.

Would seed inoculum alone be sufficient to cause an epidemic under

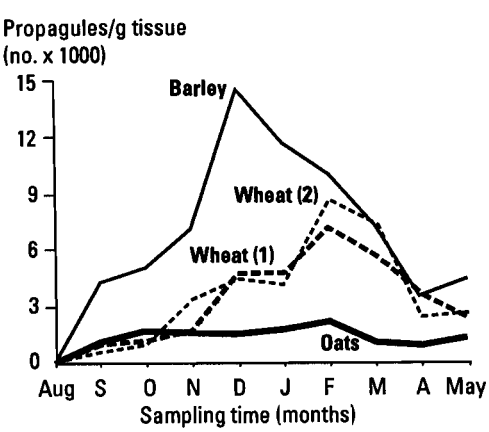

Figure 1. Monthly fluctuation of *Cochliobolus sativus* propagules on above-ground tissue of barley, oats, and wheat.
[1] 1 year rotation.
[2] 2 year rotation.
Source: Reis and Santos 1987.

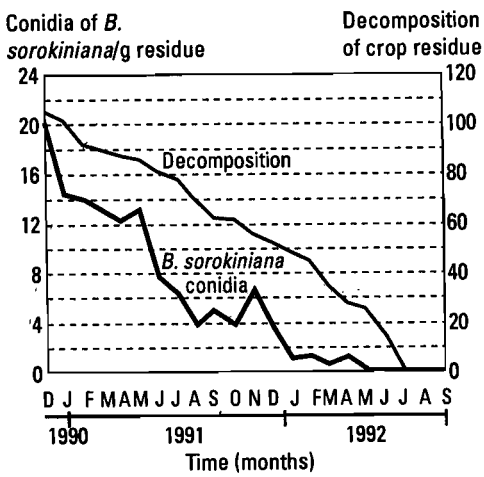

Figure 2. Decomposition of wheat residue and sporulation of *Bipolaris sorokiniana*.
Source: Reis *et al.* 1994.

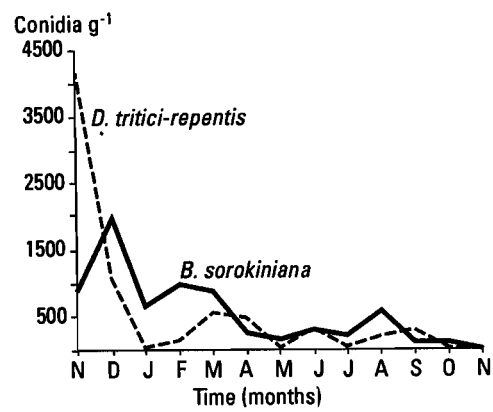

Figure 3. Density of *Bipolaris sorokiniana* and *Drechslera tritici-repentis* conidia on wheat residue on the soil.
Source: Reis 1990.

favorable environmental conditions following seed emergence? We still do not know, but, based on field observations, it is likely that at least two to three monoculture cycles are required for adequate inoculum buildup to cause disease levels that reach the economical damage threshold. For a disease such as net blotch of barley, caused by *D. teres*, it has been shown by Reis *et al.* (1995) that seed inoculum is sufficient to cause an epidemic within a season, depending only on climate.

Soil as the inoculum source

Bipolaris sorokiniana may also survive in soil as free dormant conidia (Chinn and Tinline 1963). Soil inoculum originates from the multiplication of the parasite either on growing plants or on residue (Figure 4). Spores are transported to the soil by wind or rain drops.

Soil inoculum may infect roots, causing common root rot. Seed inoculum may also cause the same symptoms. Conidia in the soil may infect lower leaf sheaths and lower leaves in contact with the soil. Free conidia in the soil can remain viable for up to 37 months (Reis and Santos 1987; Figure 5). In Brazil, the numerical threshold for soil inoculum is still unable to be determined. Nevertheless, we know that this inoculum may be reduced by crop rotation with non-host crops such as oats, vetch, and rape. Oats is the main alternative crop and it is being cultivated as a cover crop in monoculture on no-till farms. This has led to the increase of oat helminthosporiosis, caused by *D. avenae* (Eidan) Scharif (anamorph of *Pyrenophora avenae* Ito & Kurib), presently the most important leaf blight of oats in Brazil.

Under southern Brazilian conditions, residue decomposition takes place over a period of 12-17 months, depending on the weather (Reis 1990; Reis *et al.* 1994; Figures 2 and 3). When should wheat be cultivated once again on such a farm? The answer is after 18 months or one

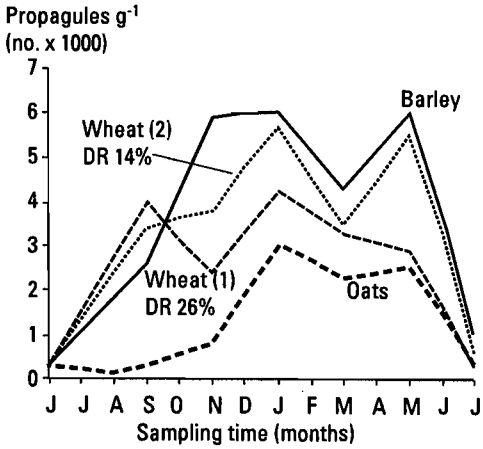

Figure 4. Fluctuation of *Cochliobolus sativus* propagules in the soil with barley, oats, and wheat.
[1] 1 year rotation.
[2] 2 year rotation. DR = Disease rating in roots.
Source: Reis and Santos 1987.

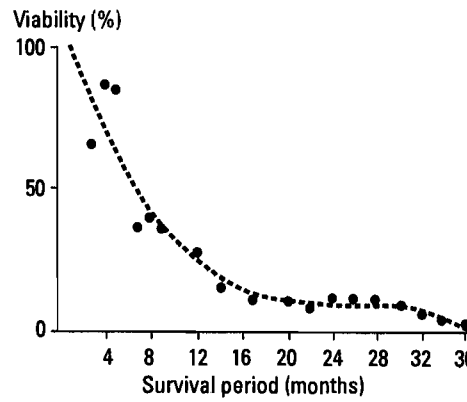

Figure 5. Longevity of *Cochliobolus sativus* propagules at the soil surface under field conditions.
Source: Reis 1989.

season of winter rotation, even under a no-till regime (Reis *et al.* 1994).

Based on these findings, we may infer that inoculum of wheat leaf blight pathogens may be reduced by rotation. The characteristics of a pathogen able to be controlled by this practice are: large spores (such those of *B. sorokiniana* and *D. tritici-repentis*), or small spores carried in rain drops, which makes them heavy; a narrow host range (such as *S. nodorum* and *S. tritici*); and absence of resting structures such as chlamydospores and sclerotia. Wheat leaf blight pathogens fulfill these requirements (Reis and Medeiros 1995).

From the plant pathologist's point of view, monoculture means farm management that favors pathogen survival by ensuring the presence of crop debris, and thus inoculum, on the cultivated area. Conversely, crop rotation means farming so that a plant species is not cultivated where its own residue is present; residues are eliminated naturally through soil microbial activity. In the case of no-till farming, soil is always covered with the plant debris of an alternative crop (one not susceptible to wheat pathogens).

The last question is: Will efficient seed treatment plus a one-season wheat rotation keep disease intensity below the economical damage threshold? Experimental data shown in Table 1 may help to answer this question. Also, several farms using seed treatment and crop rotation have been monitored to validate the experimental data. From Tables 2 and

Table 1. Effect of soil management and crop rotation on leaf blight[1] severity (%) at flowering stage of cv. Trigo BR-23, Passo Fundo, CNPT/EMBRAPA, 1991.

Crop rotation	Soil management systems[2]				Mean[3]
	DD	MT	DP	MBP	
Monoculture of wheat	8.0 aA	1.0 aB	0.7 aB	0.7 aB	2.6
1 year rotation (vetch-wheat)	0.9 bA	1.4 aA	2.0 aA	1.7 aA	1.5
2 year rotation (oats-vetch-wheat)	1.7 bA	1.8 aA	1.5 aA	1.5 aA	1.6
Mean	3.5 A	1.4B	1.4 B	1.3 B	

[1] Leaf blotches: *Drechslera tritici-repentis* (65%), *Septoria nodorum* (16%), and *Bipolaris sorokiniana* (6%).
[2] DD = Direct drilling; MT = Minimum tillage; DP = Conventional drilling with disc plow + disc harrow; MBP = Conventional drilling with moldboard plow + disc harrow.
[3] Means followed by the same capital letter in horizontal and by the small letter in vertical are similar according to Duncan's multiple range test at 1% of probability.
Note: CV (%) for crop rotations and soil management systems = 32.41%.

Table 2. Wheat disease incidence on farms surveyed in Panambi, RS, Brazil, 1994.

Diseases	Farms (no.)	Wheat cultivar	Incidence (%)	EDT[1] (%)	Fungicide application
Leaf rust	23	BR 34[2]	61.5	35-45	+
Leaf blights[3]	22	BR 35[4]	40.3	70-80	-
Leaf blights[3]	23	BR 36[4]	40.8	70-80	-

[1] Economic damage threshold.
[2] Susceptible to leaf rust.
[3] *Bipolaris sorokiniana, Drechslera tritici-repentis, Septoria nodorum.*
[4] Resistant to leaf rust.

3, one may see the effect of correct crop management on the incidence of leaf blights of wheat in Southern Brazil during the 1994 growing season. It was clearly shown that under climatic conditions conducive to disease, many growers produced wheat without applying fungicides. Crop sustainability may thus be achieved through integrated disease management.

This is a contribution to the sustainability of wheat to feed the poor.

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Table 3. Effect of seed treatment and crop rotation on leaf blight incidence (%) at anthesis on cv. BR-23, Passo Fundo, RS, Brazil, 1997.

Seed treatment	Monoculture	Crop rotation
Without	-	52.3 (4)
With	88.2 (3)[1]	33 (22)

[1] Number of farms.

Note: Economic damage threshold: incidence of 70-80%. No-till farming.

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Incidence and Severity of Leaf-Spotting Diseases of Spring Wheat in Southern Manitoba

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Abstract

From 1989 to 1993, annual surveys were carried out to monitor incidence and severity of septoria nodorum blotch (SNB), tan spot, and spot blotch. Between 123-185 fields of bread, Canada Prairie Spring (CPS), and durum wheat types were surveyed each year. Infected leaf tissue was collected, surface sterilized, and placed in moisture chambers for 5-7 days to facilitate disease identification based on conidiation of pathogenic species. Environmental data were collected from the weather station closest to each field. In durum wheat, incidence and severity of SNB were lower, and of tan spot higher, than in the other two wheat types. Incidence of spot blotch was similar in all classes, but severity was higher in CPS wheat than in the other two wheat types. All wheat types showed a positive correlation between spot blotch severity and minimum and maximum temperatures, and a negative correlation for tan spot severity and amount of rainfall. Higher levels of SNB were associated with lower temperatures and with rain. In part, the environmental data help to explain the variability in annual incidence and severity of leaf-spotting diseases of wheat in Manitoba. A study to monitor these disease levels in conventional and conservation tilled fields was initiated in Manitoba in 1993. Infected leaf tissue was collected at growth stages (GS) 39, 65, and 75. Variation between years was highly significant for all diseases except spot blotch. Tan spot and septoria diseases increased as plants matured, but spot blotch was most severe at GS 61 and least at GS 39. Effect of tillage system on disease levels was not significant in this study.

In southern Manitoba, leaf-spotting diseases of wheat have the potential to cause estimated annual losses of up to 20% due to kernel shriveling (Tekauz *et al.* 1982; Gilbert and Tekauz 1993). The predominant leaf-spotting pathogens are *Pyrenophora tritici-repentis* (Died.) Drechs. (anamorph, *Drechslera tritici-repentis* (Died.) Shoemaker), *Cochliobolus sativus* (Ito & Kuribayashi) Drechs. Ex Dastur (anamorph, *Bipolaris sorokiniana* (Sacc.) Shoemaker), *Phaeosphaeria nodorum* (E. Müller) Hedjaroude (anamorph, *Stagonospora nodorum* (Berk.) Castellani and E.G. Germano), and *Mycosphaerella graminicola* (Fuckel) J. Schröt. in Cohn (anamorph, *Septoria tritici* Roberge in Desmaz.), causing tan spot, spot blotch, septoria nodorum blotch (SNB), and septoria tritici blotch (STB), respectively. The importance of these diseases has increased in the last few decades (Fernandez *et al.* 1993) as genetic control of rusts and smuts has been incorporated into adapted cultivars, leaving tissue available for colonization by facultative fungi.

The data presented here are based on collections of infested tissue made as part of the annual disease surveys during 1989-1993. Preliminary results have been published in the Canadian Plant Disease Survey (Gilbert and Tekauz 1990, 1992; Gilbert *et al.* 1991, 1992, 1993, 1994). The objectives of the survey study were to relate environmental variables, namely temperature and precipitation, with incidence and severity of leaf-spotting diseases, and to determine if wheat types vary in reaction to the predominant leaf-spotting pathogens. The objective of the second study was to examine levels of leaf-spotting diseases in conservation and conventional tilled fields.

Materials and Methods

Annual surveys were carried out in July and August of each year (Table 1). Wheat fields were selected at random every 20-30 km along the survey routes, and wheat type, location, and growth stage were recorded. Fields sampled between ear emergence and soft dough stage of ripeness were included in the analysis. Infected leaf tissue from several plants was collected at a single location approximately 30 m from the field edge. To examine the effects of tillage system on levels of leaf-spotting diseases, farms practising conservation tillage were paired with neighboring farms that used conventional tillage systems. Leaf tissue was collected from these fields at GS 39, 65, and 75. In the laboratory, two pieces of diseased leaf tissue (approximately 1 cm^2) from each of five leaves were surface sterilized in 1% sodium hypochlorite for 30 seconds and washed in sterile distilled water for 1 minute. Surface sterilized leaf tissue was placed in moisture chambers made of glass petri dishes with dry filter paper on the lower surface and moistened filter paper in the lid. Moisture chambers were incubated at 20°C in a chamber with a 12 h photoperiod, and examined after 5-7 days for presence of sporulation on leaf pieces. Pathogens present were identified according to conidial morphology. Incidence was based on presence or absence of a disease in a field, while a measure of severity was obtained by summing the number of leaf pieces from which a pathogen grew. Often two or more pathogens were found on the same piece of leaf tissue. Incidence and severity data were recorded for commonly isolated leaf spot pathogens.

Table 1. Wheat fields sampled for leaf-spotting diseases in southern Manitoba, 1989-1993.

	Wheat type			
Year	Bread	CPS[1]	Durum	Total
1989	99	12	12	123
1990	102	14	22	138
1991	112	57	16	185
1992	126	38	13	177
1993	107	24	18	149
Total	546 (70.7%)	145 (18.8%)	81 (10.5%)	772 (100%)

[1] Canada Prairie Spring wheat.

Environmental variables, including daily minimum and maximum temperatures and precipitation, were obtained for 37 stations in southern Manitoba from the Atmospheric Environment Service of Environment Canada. Environmental variables computed for each field site over the 14 days before the field was surveyed were: mean daily high and low temperatures, cube root of total rainfall, and number of days with rain based on the closest

weather station. The annual means are shown in Table 2. For the survey study, a full factorial model on year and wheat class was fitted to the five years' incidence data using the SAS procedure Catmod (SAS Inst., Cary, NC), and the fitted means by wheat class were compared using contrasts. Only incidence data were collected in 1989. The relationship between severity and year and wheat class was investigated by fitting a full factorial logistic regression model to four years of severity data using the SAS procedure Genmod (SAS Inst., Cary, NC), and the adjusted means were compared using contrasts. The proportion of fields in each wheat class varied from year to year, and there appeared to be differences in infection levels between wheat classes. Accordingly, Pearson correlation coefficients between annual means of disease incidence and severity, and environmental factors were computed using weighted means, with weights based on the total number of fields in each wheat class over the five years. An analysis of variance was performed for the tillage study.

Results

Incidence of the major leaf spotting pathogens varied from year to year (Figure 1). *Cochliobolus sativus* was predominant in 1989 and 1991, *P. tritici-repentis* in 1990 and 1992, and *P. nodorum* in 1993. Incidence of STB was nil or low during 1989-1991, but increased in 1992 and 1993. *Septoria tritici* was omitted from the analysis because there were only two years in which it was found at more than 5% incidence. Incidence of tan spot ranged from 52-83 %, SNB increased annually from 33% in 1989 to 85% in 1993, and spot blotch decreased from a high of 88% in 1991 to 48% in 1993.

Incidence of SNB, tan spot, and spot blotch was similar in CPS and bread wheat, but in durum wheat, tan spot levels were higher and SNB levels lower (Figure 2). Spot blotch was found at similar levels in all three wheat types.

There were significant differences in overall disease severities in the different wheat types (Table 3). SNB was found at a

Table 2. Mean daily high and low temperatures, number of days with rain, and total rainfall for the two-week period prior to sampling of each southern Manitoba wheat field, 1989-1993.

	Tmax[1]	Tmin[1]	Rain (mm)	Rday[2]	R3RN[3]
1989	29.2	14.6	11.7	2.5	2.02
1990	24.3	12.0	29.6	4.5	2.98
1991	26.1	13.9	30.5	6.2	3.00
1992	22.5	10.0	26.3	6.7	2.85
1993	21.6	11.1	65.9	8.2	3.84

[1] Maximum and minimum temperatures.
[2] Number of days with rain.
[3] Cube root of rainfall averaged for two weeks prior to survey.

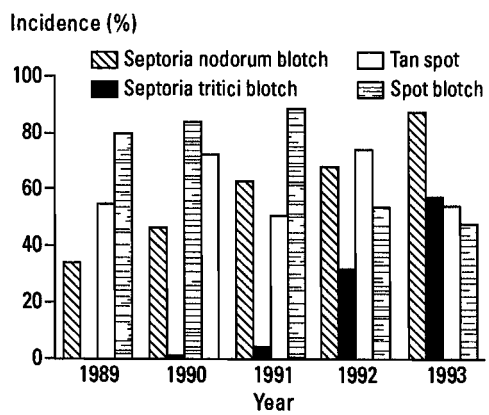

Figure 1. Incidence of septoria nodorum blotch, septoria tritici blotch, tan spot, and spot blotch in spring wheat fields in southern Manitoba, 1989 - 1993.

lower severity on durum than on bread and CPS wheat types; spot blotch was more severe on CPS wheat than on bread and durum types; and tan spot levels differed for the three wheat types with CPS least, and durum most affected.

Averaged over four years and all wheat classes, severity of SNB, tan spot, and spot blotch did not differ; however, each disease could be correlated with a different set of environmental factors (Table 4). SNB was negatively correlated with maximum temperatures and positively correlated with rain. Severity of tan spot was negatively correlated with rain, but the correlation was not significant. Spot blotch severity was positively correlated with maximum and minimum temperatures.

Cochliobolus sativus was the predominant pathogen in 1989 and 1991, when the warmest mean maximum and minimum temperatures for the five year period were recorded (Table 2). When all years were combined, incidence and severity of *C. sativus* were negatively correlated with rain (Table 4). Occurrence of *S. nodorum* was positively correlated with rain and peaked in 1993 when Manitoba experienced one of the wettest summers on record. Incidence was also negatively correlated with maximum temperature. The same trend, although not significant, occurred for SNB severity. Significant correlations were not found between the environmental variables measured and tan spot occurrence. The highest levels of tan spot were recorded in

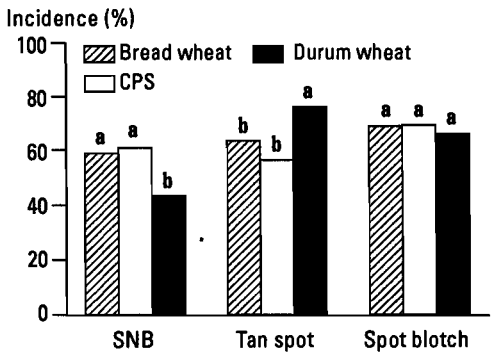

Figure 2. Incidence of septoria nodorum blotch (SNB), tan spot, and spot blotch in bread, Canada Prairie Spring (CPS) and durum wheat in Manitoba from 1989 to 1993. Within groups, bars with the same letter are not significantly different (P > 0.05 on a comparison-wise basis).

Table 3. Mean percent severity of leaf-spotting diseases on bread, Canada Prairie Spring (CPS), and durum spring wheat types in southern Manitoba, 1990-1993.

Wheat types	SNB[1]		Tan spot		Spot blotch	
	Raw	Adjusted	Raw	Adjusted	Raw	Adjusted
Bread	23.3	22.1 a	23.1	22.1 b	18.9	16.6 b
CPS	23.8	22.5 a	14.7	16.0 c	28.0	21.7 a
Durum	14.1	13.1 b	35.9	34.7 a	16.2	14.8 b

[1] Septoria nodorum blotch.
Note: Adjusted means based on logistic regression of severity on year, wheat, and interaction. Within columns, means with the same letter are not significantly different (P> 0.05).

Table 4. Correlations between environmental variables and yearly means of incidence and severity of leaf-spotting diseases of wheat in southern Manitoba.

	Tmax[1]	Tmin[1]	R3RN[2]	Rday[3]
Incidence 1989-93 (n = 5)				
Spot blotch	0.796	0.845+	-0.563	-0.656
Tan spot	-0.280	-0.501	-0.129	-0.118
SNB	-0.851*	-0.660	0.893*	0.991***
Severity 1990-93 (n = 4)				
Spot blotch	0.973**	0.952**	-0.392	-0.460
Tan spot	0.098	-0.300	-0.731	-0.780
SNB[4]	-0.597	-0.193	0.929+	0.930*

[1] Average maximum and minimum temperatures for two weeks prior to survey.
[2] Cube root of rainfall averaged for two weeks prior to survey.
[3] Number of days with rain for two weeks prior to survey.
[4] Septoria nodorum blotch.
*** P = 0.01, **P = 0.05, *P = 0.10

1990 and 1992, which were relatively cool, and with fewer rainy days and less rainfall than either 1991 or 1993. Negative correlations for moisture occurred, but were not significant (Table 4).

In the tillage study, significant differences were found for disease levels in each year, except for spot blotch. SNB levels did not differ over the growing season, but spot blotch levels were highest at GS 65, while STB and tan spot increased with crop maturity (Figure 3). There were no significant differences in disease levels in conservation and conventional tilled fields.

Discussion

The results of the tillage study do not confirm the findings of other researchers in which higher disease levels were found in conservation and zero-tilled fields as opposed to those under conventional till (Sutton and Vyn 1990). This is a preliminary analysis and other differences may emerge when weather variables and rotations are incorporated.

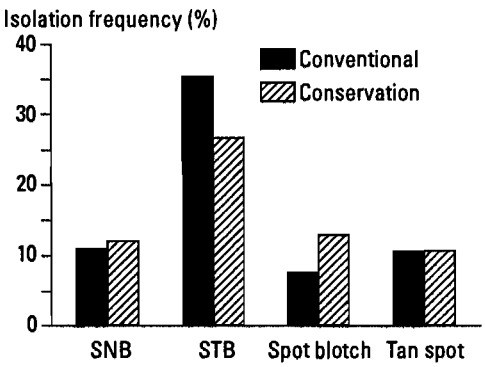

Figure 3. Severity of septoria nodorum blotch (SNB), septoria tritici blotch (STB), spot blotch and tan spot in conventional and conservation tilled spring wheat fields in southern Manitoba, 1993 - 1996.

The survey study data help to explain the variation in incidence and severity of individual leaf-spotting diseases of wheat observed from year to year. Each disease is favored by certain environmental conditions. In general, the data support the reported observations of other researchers. However, while Eyal (1981) reported that severe outbreaks of septoria diseases can occur in regions having either high or low levels of rainfall, our results, plus those of Djurle *et al.* (1996) and Leath *et al.* (1993), indicate that high precipitation levels during the growing season are an important factor influencing incidence and severity of SNB. Such conditions occurred in the two weeks prior to field sampling in much of southern Manitoba in 1990-1993, a period when SNB incidence and severity increased annually.

The increase in tan spot occurrence reported in recent years has been attributed, in part, to conservation tillage practices (Fernandez *et al.* 1993). Zhang and Pfender's (1992) work indicated that pseudothecial formation and longer periods of moisture (>12 h) were dependent on straw position. A greater number of long periods of moisture occurred in straw on or close to the soil surface than in straw that was not in contact with the ground. In treatments where above-surface residues were retained, a negative correlation was found between the number of pseudothecia produced and the number of long moisture period events. In our study, tan spot levels generally were negatively correlated with rain. High levels of tan spot have been observed in

Saskatchewan in years of generally dry weather, and a greater prevalence of the disease on durum cultivars was likewise observed (Bailey *et al.* 1993).

In 1991, warm temperatures and high levels of rainfall in southern Manitoba provided a humid environment that promoted spot blotch development. In 1993, a wet but cool year, spot blotch levels were low. In Nepal, Dubin (personal communication) attributed a change in predominance from tan spot to spot blotch during 1990-1991 to foggy weather and higher than normal night temperatures.

The results of this study indicate that the pathogens which contribute to the leaf spotting complex on wheat in Manitoba require rather specific conditions and inhabit unique ecological niches. This has important ramifications on the focus and prioritization needed in wheat breeding programs. While it is convenient to consider the leaf spotting pathogens individually, it is evident that because of variable conditions in the Manitoba environment, one or more members of the complex can predominate. Therefore, breeding programs should aim for resistance, even if moderate, to all members of the complex, to ensure yield stability in all years.

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Tan Spot of Wheat in Argentina: Importance and Disease Management Strategies

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Abstract

Tan spot of wheat, caused by Pyrenophora tritici-repentis, *was first observed in Argentina in the early 1980s and has now been detected in most wheat producing areas. The disease is currently recognized as one of the most important leaf spots of wheat. The north-central area of Argentina's wheat producing region has become a disease hot spot due to favorable environmental conditions and a steady increase in the area under minimum tillage. Loss appraisals obtained by comparing fungicide treated plots with non-protected checks have demonstrated yield reductions of 10-20%. Current disease management is far from optimum. Most commercial wheat cultivars are susceptible to highly susceptible to* P. tritici-repentis *under high inoculum pressure; however, cultivars derived from Bobwhite, Veery, Alondra, IAS55, and 58 germplasm, and old Argentine cultivars, such as Klein Rendidor and Buck Cimarrón, have shown partial resistance., Several entries in cooperative nurseries screened under naturally induced epidemics were reported as showing partial resistance to the fungus. These entries included germplasm of Bobwhite, Kavkaz, Veery, Kauz, and Attila, as well as crosses involving lines from Brazil, Chile, Mexico, Uruguay, and some Argentine cultivars. Fungicides applied as foliar treatments have shown potential to reduce disease development under conditions of moderate to low inoculum pressure, but inconsistent results were obtained under high inoculum pressure. Among fungicides tested under field conditions, tebuconazole and propiconazole achieved the highest levels of control, though results varied widely (30-80 %). Recently released Azoxystrobin was considerably effective (50-75 %) in one year of testing. Tan spot symptoms have been suppressed in farmers' fields where adequate soil fertility, normal row spacing (17.5 cm), and normal to high crop densities (300-500 plants m^{-2}) promoted a dense crop canopy.*

Tan spot of wheat, caused by *Pyrenophora tritici-repentis* (Died.) Drechsler (anamorph: *Drechslera tritici-repentis* (Died.) Shoem.), has become one of the most important foliar diseases of wheat in Argentina. The disease began to noticeably affect wheat crops in the north-central region of the Buenos Aires province in the early 1980s (Annone 1985, 1996). Since then, tan spot symptoms have been detected in most wheat growing areas in the country. The disease is particularly prevalent and intense in the northern area of the Argentine wheat producing region (central and northern Buenos Aires, southern Santa Fe, southeastern Cordoba, and Entre Rios

provinces), where highly conducive environmental conditions and increasing use of minimum tillage have created a disease hot spot.

In the region, pseudothecia of *P. tritici-repentis* are found on wheat residue left on the soil surface at crop sowing and/or early growth stages. Conidia are formed and released soon after the development of the first symptoms on leaves (Annone *et al.* 1994).

Importance

Tan spot is frequently observed in most farmers' fields in the northern region, often affecting the upper leaves at flowering to early grain filling stages. Severity levels of over 50% are not uncommon, with up to 80% of the leaf tissue affected.

Estimates of losses (10-20%) caused by the disease have been made by comparing fungicide protected with non-protected wheat plots (Table 1). Such estimates are considered conservative since completely disease-free checks were never attained. Similar results were obtained by Galich and Galich (1994) in Marcos Juarez, Cordoba province, who determined that losses due to tan spot associated with septoria tritici blotch ranged between 6-13.5%.

Disease Management Strategies

Until recently, tan spot was frequently confused with septoria diseases, bacterial blotches, or even with natural or early abiotic leaf aging. In the last three years, farmers have become increasingly concerned about wheat diseases, mainly due to an important fusarium head blight epidemic in 1993 and the relatively high price for wheat during that period. Farmers are demanding information on cultivar disease reaction and effectiveness of fungicide treatments.

At present, the status of tan spot management is far from optimum since

Table 1. Estimates of wheat yield losses due to tan spot: a comparison of fungicide protected and non protected plots.

Cultivar	Yield (kg ha^{-1})			Severity of tan spot on upper leaves (%)	
	Protected[3]	Non protected	Loss (%)	Protected[3]	Non protected
Klein Cacique[1]	2854	2804	1.7	4.4	14
ProINTA Quintal[1]	2657	2447	7.9	29.5	36
ProINTA Isla Verde[1]	2998	2683	10.5	18.7	46
Granero INTA[1]	2316	2069	10.7	28.9	56
ProINTA Federal[1]	3312	2867	13.4	9.8	36
Coop. Calquín[2]	1690	1410	16.6	18.0	73

[1] Yield differences among cultivars and between protected and non protected plots were statistically significant. The cultivar-protected/non protected interaction was not significant. Source: Annone, unpublished data.

[2] Yield difference between protected and non-protected plots was statistically significant at $P<0.05$. Source: Annone *et al.* 1994.

[3] Protected plots were periodically treated with the systemic fungicide tebuconazole (125 g ai ha^{-1}).

Note: Results from trials carried out at Pergamino, Buenos Aires, Argentina, 1994 and 1996.

several commercial wheat cultivars are susceptible to highly susceptible to the disease, many chemical treatments show moderate to low effectiveness under field conditions, and the need for soil conservation requires an increasing adoption of minimum tillage practices.

Genetic resistance

Available tan spot resistance in wheat is partial/incomplete (Rees and Platz 1992). These researchers reported that of more than 60 cultivars screened, only a few displayed detectable *P. tritici-repentis* resistance. The situation is similar in the north-central area of Argentina's wheat producing region. Most commercial wheat cultivars are susceptible to highly susceptible to tan spot under conditions conducive to the disease. Some cultivars, however, display a partially resistant response compared to the high susceptibility shown by many varieties under similar inoculum loads/ environmental conditions. Examples of such behavior are presented in Table 2, where severity readings from 19 trials conducted in the central-northern region of the Buenos Aires province highlighted 11 cultivars that were consistently less affected than the most susceptible entries in every situation. The pedigree of the partially resistant cultivars involves widely recognized germplasm such as Bobwhite, Veery (Ures=Veery), Alondra, IAS55 and 58, and some old Argentine cultivars such as Klein Rendidor (Aldan includes K. Rendidor in its pedigree) and Buck Cimarrón (Table 2).

Screening advanced bread wheat lines from the 14th (1994), 15th (1995), and 16th (1996) LACOS Nursery (Advanced Wheat Lines from the Southern Cone) has also highlighted lines partially resistant to tan spot (Table 3). Pedigrees of these

Table 2. Commercial bread wheat cultivars showing partial tan spot resistance under naturally induced epidemics.

Cultivar	Pedigree	Tan spot severity range (00-99)[1]	Tan spot severity range of the most affected cultivars (00-99)
Buck Charrúa	RAP/RE//TRAP/LOVRIN/3/RAP/RE//IRAP	31-81	84-86
Buck Guaraní	URES/JUNCO	74-85	82-86
Coop. Calquín	BOBWHITE"S"	73-84	82-86
Coop. Maipún	DONATA/3/FLN/ACC/4/ALD"S"/COC	72-83	83-86
Don Ernesto	INTA BOBWHITE"S"	31-82	73-86
Klein Cacique	BCIM/25348//VEE"S"	52-81	83-85
Klein Centauro	KLLUC/Y53//KLSEND/4/CNO/NO66//SON64	31-82	73-85
Klein Estrella[2]	COCHICO INTA/CHAT"S"//BPONCHO	53-82	84-86
ProINTA Federal	BOBWHITE"S"	32-83	73-86
ProINTA Guazú	JUP/ZP"S"//COC/3/ALDAN"S"	55-83	73-85
ProINTA Real	VI/SNB"S"/5/IAS58/IAS55//ALD"S"/3/ MRNG/4/ALD"S"/IAS58//ALD"S"	82-85	85-86

[1] Double digit scale.
[2] One-year observations.
Note: Results for each cultivar are compared with results obtained by the most severely diseased entries in the same trial. Data from 19 trials/demonstration plots in the central-northern region of Buenos Aires province, 1994, 1995, and 1996.
Source: Annone, unpublished.

selected lines also involve Bobwhite, Veery (which includes Kavkaz), Kavkaz, Mexican lines (Myna/Vulture, Parula/Bobwhite, Ures/Bobwhite), Brazilian lines (BR..., PF..., CEP..., IAS58), Chilean lines (TEMU...), Uruguayan lines (LE2150 and LI 105), and some old Argentine cultivars (Buck Cimarrón and Buck Namuncurá).

Chemical protection

Most fungicide treatments for wheat have been developed to reduce fusarium head blight epidemics. The complex of foliar diseases is now beginning to be considered a production constraint, so foliar treatments have only been recently adopted. Most treatments carried out in farmers' fields are evaluated by crop appearance (how green it looks), some are evaluated by yield increment alone, and only a few are evaluated by reduction in disease severity combined with yield increment.

Effectiveness of chemical treatments in reducing tan spot severity has not yet reached satisfactory levels (around 50% control in the best situations). Reasons for this are the use of inadequate volumes of spray, inappropriate timing of application, and the relatively low effect

Table 3. Bread wheat lines showing partial tan spot resistance under naturally induced epidemics.

Entry	Tan spot severity (00-99)[1]	
	Entry score	Highest score in trial
ATTILA	82[2]+82[3]	85+87
BCIM/7C//FCT/4/LIB/BB//BNAM/3/BCIM	81+73	84+87
BOW"S"	82	85
BOW"S"/NKT"S"	81	85
BR14/CEP847	81	85
BR32/CEP21//CNO79	76	87
CMH79A210/PIFED	73	87
CHAT"S"/CEP7780//PRL"S"/BOW	81	85
E.CAL/LI 105	75	85
E.FED/LE2150	81	84
ENC/PF79768//PF80284	81	87
GAA"S"/KAUZ"S"	81	84
GLL/CUCKOO//KVZ/SX	81	85
IAS58/4/KAL/BB//CJ"S"/3/ALD"S"/5/BOW	81	85
IAS58/4/KAL/BB//CJ/3/ALD/5/VEE	82	87
KLT"S"/BOW"S"	82	85
MJI//PAK/CHAP70/3/DEI	82	85
MJI//PAK/CHAP70/3/PRL"S"/ALD"S"	81	85
MYNA"S"/VUL"S"	82	85
P.DALB/4/CNO67/MED//MON"S"/3/BOW	72	84
PRL"S"/BOW"S"	82	85
PRL"S"/VEE#6/MYNA/VUL=PRINIA	81+76	84+87
RFN*2//980/FN/3/KVZ/5/BR23/6/CEP8466	81	85
TEMU 49-82	82	85
TEMU193-83/TEMU233-85	81	85
URES"S"/BOW"S"	81	85

[1] Double digit scale [2] Readings from year 1. [3] Readings from year 2.

Note: Data from the 14th (1994), 15th (1995), and 16th (1996) LACOS Nursery, Pergamino Experiment Station of INTA, Buenos Aires, Argentina.

Source: Annone, unpublished.

achieved by most chemicals available for tan spot control.

A series of trials conducted at Pergamino, Buenos Aires, in 1994 (Annone *et al.* 1995), 1995 (Annone and García 1996), and 1996 (Annone and García, unpublished) confirmed the effectiveness of some fungicides for reducing tan spot severity and enabled the efficacy of control and related yield increments to be compared (Table 4). Fungicide was applied using a hand-operated knapsack sprayer with conic projection nozzles, delivering a volume of 170-190 L ha^{-1} at a pressure of 20-30 psi.

Table 4 summarizes cases where the differences in tan spot severity between treatments and checks were significant ($P<0.05$) according to an LSD test. These differences were converted to levels of control efficacy according to Abbot (Puntener 1981). Yield increments were recorded as 'observed' and 'statistically significant'.

The highest levels of control efficacy based on severity were achieved by tebuconazole and propiconazole (systemic fungicides from the group of triazoles and ergosterol biosynthesis inhibitors) and by azoxystrobin (a recently released fungicide produced by higher fungi that inhibits mitochondrial respiration). Prochloraz (imidazole inhibitor of ergosterol biosynthesis) and flutriafol (triazole inhibitor of ergosterol biosynthesis) were also found to be effective, but to a lesser extent. Most treatments were associated with a yield increment not greater than 10%. Yield increments were statistically significant in a few cases (tebuconazole, flutriafol and azoxystrobin), with values around 15-30%.

Adjustment of cultural practices

It is widely recognized that tillage practice has a strong influence on tan spot development in many of the world's wheat producing areas (Mehta and Gaudencio 1991). The need to maintain

Table 4. Effect of foliar fungicide treatment on tan spot severity and wheat yield under naturally induced epidemics.

Fungicide	Range of control[1]		Yield increase	
	(%)	Statistical significance	(%)	Statistically significant
Propiconazole	26-67	P<0.05	3.9-7.3	NS
Flutriafol	7-54	P<0.05	0-18.6	NS
			14.9	P<0.05
Prochloraz	7-50	P<0.05	0-13.4	NS
Tebuconozole	32-82	P<0.05	0-12.9	NS
			15.7	P<0.05
			29	P<0.05
Azoxistrobin[2]	53-75	P<0.05	6.2	NS
			14.5	P<0.05
			18	P<0.05

[1] According to Abbot. Source: Puntener 1981.
[2] One-year observations.
Note: Data extracted from nine trials conducted at Pergamino, Buenos Aires, Argentina, 1994, 1995, and 1996.
Sources: Annone *et al.* 1995; Annone and García 1996; Annone and García, unpublished.

wheat residues on the soil surface to reduce erosion has dramatically increased the importance of tan spot in countries including Australia (Rees and Platz 1979), Brazil and Paraguay (Mehta and Gaudencio 1991; Kohli and Reis 1994), and Argentina (Annone and Kohli 1996). Under minimum tillage, the onset of a tan spot epidemic occurs earlier than under conventional tillage, and higher levels of disease severity occur during grain filling (Mehta and Gaudencio 1991; Kohli *et al.* 1992). Annone and Kohli (1996) reported high tan spot levels (50-60%) on wheat grown under minimum or zero tillage, compared to situations where residues were partially or totally buried (20%).

Observations carried out in farmers' fields in north-central Buenos Aires province during the last two years suggest that severe tan spot development is not only related to the amount of wheat residue on the soil surface, but also to plant vigor and density. Severely affected crops in this region are generally associated with reduced stands and plants of low vigor. Wider spacing between rows than normally adopted (17.5 cm) seems to provide a more conducive microenvironment for tan spot development. Conversely, dense (300-500 plants m^{-2}) and vigorous crops, and fertility "spots" on poorly developed crops, are less affected by tan spot, even under minimum or zero tillage. This could be due to a lower possibility of conidia spreading from primary infection sources to surrounding plants and from lower to upper leaves, and/or physiological modifications that limit the pathogenesis process.

To date, no observations have been made in the region on the possibility of alternate hosts such as gramineous weeds and pastures, as reported by Kohli *et al.* (1992), Mehta and Gaudencio (1991), Kohli and Reis (1994), and Krupinsky (1987).

Considerable reduction in disease development has been achieved by sowing wheat in fields that are free from surface crop residues and by breaking the wheat-wheat sequence by crop rotation; however, these practices are limited by the urgent need to protect soils from erosion through the use of minimum tillage, and by the lack of profitable alternative winter crops, respectively.

Conclusions

Tan spot has become one of the most important foliar diseases of wheat in Argentina, where effect on yield has been estimated at 10-20%. Most commercial wheat cultivars in current use are susceptible to highly susceptible to tan spot, though some show partial resistance. Some advanced wheat lines involving well-known germplasm, as well as lines from Brazil, Chile, Mexico, and Argentina, have shown partial tan spot resistance under regional conditions. Some fungicide treatments may reduce tan spot severity by up to 70-80%; however, efficacies of control vary widely (30-80 %). Cultural practices that induce a vigorous and dense crop canopy appear to restrict the spread of secondary inoculum; hence, disease severity on the upper leaves is reduced by the time of grain development.

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Influence of Agronomic Practice on Foliar Blight, and Identification of Alternate Hosts in the Rice-Wheat Cropping System

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Abstract

Foliar blight of wheat, caused mainly by Bipolaris sorokiniana *and* Alternaria triticina, *adversely affects the crop, particularly under late sown conditions. Due to the prevalence of the rice-wheat cropping system and late rice harvest in eastern Uttar Pradesh, wheat is sown after mid December. The crop sown during late December coincides with foliar blight infection during late February and March, resulting in substantial yield losses. The influence of agronomic practice on foliar blight, and the identification of alternate hosts in the rice-wheat cropping system are presented. With less space between rows (15 cm), a high seed rate (125 kg* ha^{-1}*), and full fertilizer dose (120:60:60 N:P:K), foliar blight intensity increased in comparison to wider spaced rows (23 cm), a lower seed rate (100 kg* ha^{-1}*), and half the fertilizer dose (60:30:30 N:P:K). An increased number of irrigation applications (6) and delayed sowing (after 30th November) increased foliar blight intensity compared with fewer irrigation applications (3) and timely sowing. Isolating fungi from potential hosts indicated that* Phalaris minor *harbors* B. sorokiniana *and* A. alternata, *while only* A. alternata *was isolated from* Anagallis arvensis *and* Cannabis sativa. Alternaria alternata *and* A. triticina *were isolated from* Chenopodium album *and* Circium arvense. *Only* B. sorokiniana *was pathogenic on both wheat and* Phalaris minor, *indicating that* P. minor *serves as an alternate host.*

Foliar blight of wheat, caused mainly by *Bipolaris sorokiniana* and *Alternaria triticina*, adversely affects the crop, particularly under late sown conditions. Although foliar blight was reported in India as early as 1924 (Kulkarni 1924; McRae 1924), it was of little consequence until very recently. With changes in the cropping system, cropping intensity, crop management, and varietal spread, the foliar blight complex is causing serious wheat yield losses in eastern Uttar Pradesh compared with other wheat diseases. Ojha and Mehta (1970) and Sokhi (1971) reported that susceptibility of wheat to *A. triticina* increased with increasing N levels. Singh *et al.* (1995) found that high fertilizer and irrigation levels favored the incidence and severity of foliar blight of wheat. Further, there is the possibility of alternate hosts of the foliar blight pathogens. The present study was undertaken to observe the influence of agronomic practice on foliar blight, and to identify alternate hosts, if any, of foliar blight pathogens.

Materials and Methods

Trials were conducted using a susceptible wheat variety (Sonalika) and were replicated four times. Treatments including seed rate (100 and 125 kg ha^{-1}), row spacing (15 and 23 cm), fertilizer dose (120:60:60 and 60:30:30, N:P:K), number of irrigations (3 and 6), and date of sowing (30th November and 20th December) were tested. Disease observations were recorded at full disease development according to the percent disease coverage on the flag leaf.

Weed leaves showing foliar blight in wheat fields and surrounding areas were collected. From these leaves, fungi were isolated and identified. All isolated fungi were maintained in culture to confirm pathogenicity in the next season (confirmation of pathogenicity in the same season is not possible due to the rise in temperature after March). To test pathogenicity, five-week old seedlings of wheat cultivar UP 262 and the weed from which the fungus was isolated were inoculated with the individual fungal cultures in the greenhouse in February. Uninoculated checks were also considered. All conditions favorable for disease expression (humidity etc.) were maintained. Final observations were recorded 10-15 days after inoculation.

Results and Discussion

Table 1 shows that a higher seed rate (125 kg ha^{-1}), lower row spacing (15 cm), full fertilizer dose (120:60:60 N:P:K), higher number of irrigation applications (6), and late sowing (20th December) increased foliar blight intensity compared with a lower seed rate (100 kg ha^{-1}), wider spaced rows (23 cm), half fertilizer dose (60:30:30 N:P:K), a lower number of irrigation applications (3), and timely sowing (30th November).

The data presented in Table 2 indicates that *P. minor* harbors *B. sorokiniana* and *A. alternata*, while only *A. alternata* was isolated from *Anagallis arvensis* and *Canabis sativa*. *Alternaria alternata* and *A. triticina* were isolated from *Chenopodium album* and *Circium arvense*. Of these fungi, only isolates of *B. sorokiniana* were pathogenic on wheat and *P. minor*. These findings indicate that a weed such as *P. minor* serves as an alternate host for *B. sorokiniana*.

Similarly, Sokhi (1971) reported that higher levels of N, P, and K resulted in an increase in foliar blight intensity. Singh *et al.* (1995) reported that high levels of N, P, and K and increased irrigation applications promoted disease incidence. High plant populations may create a

Table 1. Effect of agronomic practice on foliar blight intensity.

Treatment	Application	Foliar blight intensity (% on flag leaf)
Seed rate (kg ha^{-1})	100	60
	125	75
Row spacing (cm)	15	70
	23	60
Fertilizer (N:P:K)	60:30:30	65
	120:60:60	80
Irrigations	3	60
	6	75
Sowing date	30th November	60
	20th December	80

better micro-environment for pathogen multiplication and dispersal, and result in increased foliar blight severity.

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Table 2. Pathogenicity of fungi isolated from weeds.

Weeds	Fungi isolated	Pathogenicity[1]	
		Wheat	Weed
Phalaris minor	*Bipolaris sorokiniana*	+	+
	Alternaria alternata	-	-
Anagallis arvensis	*A. alternata*	-	-
Chenopodium album	*A. triticina*	-	-
	A. alternata	-	-
Canabis sativa	*A. alternata*	-	-
Circium arvense	*A. alternata*	-	-
	A. triticina	-	-

[1] + Pathogenic; - Nonpathogenic.

Evaluation of Tan Spot Research in Morocco

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Abstract

Tan spot of wheat, caused by Pyrenophora tritici-repentis, *has become important in Morocco in recent years. Surveys conducted during the last decade revealed that the disease affected durum wheat more severely than bread wheat. Yield losses from field experiments, using current moderately susceptible cultivars, were calculated at 12-18% under moderate infestation levels. Number of grains per spike and 1000-kernel weight were generally not affected. Comparisons of field inoculation methods showed that spraying was more effective than mulching with infected straw under humid conditions prevailing in northern Morocco, while mulching was more reliable under dry conditions. Several laboratory and greenhouse studies were conducted to refine screening and evaluation methodologies. Lesion size on the upper leaves best showed the variation in tan spot expression related to genotype. Disease severity on both upper and lower leaves was found to be a useful indicator. According to several race identification studies, virulence of different tan spot isolates varied significantly; however, there was little physiological specialization. Screening for disease resistance showed large variation in genotype response, with moderately to highly resistant accessions identified. Resistance was found to be linked to maturity date and plant height. Breeding for resistance to tan spot using the available tall, late maturing, resistant parents in crosses with adapted material, coupled with selection under semi-arid conditions, was not effective due to the linkage of resistance with undesired traits. The current breeding approach is to search for materials with flexible maturity date and plant height that could produce acceptable levels of tan spot resistance.*

Tan spot was unknown in Morocco prior to 1980. The old cultivars, widely grown by farmers because of grain quality, were mostly tall and late maturing and did not suffer from tan spot early in the season. Disease levels were severe only shortly before maturity and did not cause concern. When early varieties became popular, it was realized that the leaf spots believed to be caused by septoria were in fact tan spot. The change in varieties may not be the only factor causing an increase in tan spot occurrence; however, at the present time, most of the new, early maturing, high yielding varieties are moderately to highly susceptible to the disease.

Importance of Tan Spot

Prior to 1989, several cereal disease surveys highlighted the importance of tan spot on durum wheat which, until this time, was mistakenly reported as

septoria disease. During 1989-1995, mid-season disease surveys consistently showed that tan spot was always present on durum wheat. Furthermore, in the numerous growing seasons when leaf rust was not severe, tan spot was rated the most important disease on durum, whereas it was reported as negligible on bread wheat. The disease appeared earlier in the southern and drier growing areas than in the northern, more humid areas. Most of the attacks were observed around the time of heading.

Yield Loss

Yield losses caused by tan spot were evaluated during the 1990/91 to 1992/93 growing seasons at the experiment stations of Jemaat Shaim, Sidi El Aydi, and Annoceur, which represent the different durum growing regions of Morocco. The experiments were laid out in a split plot design with three replicates. The main plot comprised three treatments: 1) chemical protection at heading growth stage; 2) spray and straw mulch inoculation with propagules of *Pyrenophora tritici-repentis*; and 3) natural epidemics. Sub-plots consisted of 10 durum wheat and 5 bread wheat cultivars. Observations were made on grain yield, number of grains per spike, 1000-grain weight, and black point severity.

The tan spot epidemics achieved through inoculation with propagules of the fungus were no more severe than most natural epidemics in Morocco. Some of the stations/years were not considered due to low disease severity resulting from drought, but data from at least one station per year was available. Yields of the protected plots were highest, followed by yield under natural conditions and in inoculated plots; however, yields under natural conditions and in the inoculated plots were comparable in some cases. Yield loss, calculated by the difference between inoculated and chemically protected plots, ranged between 12-18% over stations and years. It is believed, however, that yield loss is underestimated since the tested seasons were all partly dry, which did not favor disease development. Number of grains per spike and 1000-kernel weight were generally not affected by tan spot inoculation; however, results varied among cultivars. Black point severity was not influenced by tan spot inoculation, possibly because wet conditions never occurred at the end of the growing season.

Evaluation of Field Inoculation Methods

During the 1989/90 and 1990/1991 growing seasons, a study was conducted to compare three field inoculation procedures at two experiment stations, Sidi El Aydi and Annoceur. Field inoculation procedures were: 1) early spray (before heading), 2) late spray (10 days after heading), and 3) application of straw mulch at the three-leaf stage.

The experiments were conducted on 100 advanced lines sown in six completely randomized blocks for each inoculation procedure in hill plots. The

objective was to compare the variation in tan spot expression under each inoculation method. Data from the three experiments was compared to results obtained under greenhouse conditions where the trial was repeated.

Results showed that inoculation success depended on growing conditions. Tan spot expression was higher after spray inoculation under favorable conditions similar to those prevailing in the north of Morocco. An early spray induced more contrasting responses between genotypes than a late spray of inoculum. Straw mulch inoculation induced the best infection levels under dry climatic conditions.

Field and Greenhouse Disease Evaluation Methodologies

Two studies were carried out to compare disease evaluation methods in the field (Sidi El Aydi, 1989-90; Annoceur, 1990-91) and the greenhouse (Settat). In the field, 100 advanced lines were inoculated with tan spot and the resulting disease severity was evaluated using four methods: lesion size on the upper and the lower leaves, and disease severity (percentage leaf area covered by lesions) on the upper and the lower leaves. In the greenhouse, a replicate of the field trial was planted with the addition of 11 known checks. Lesion size was evaluated on each leaf. Results from the field and the greenhouse were compared.

Lesion size on the upper leaves was the most useful method to distinguish differences in tan spot resistance among genotypes. Lesion size on the lower leaves was not a reliable indicator of disease level. Assessment of disease severity on both the upper and the lower leaves was a good disease evaluation tool. In the greenhouse, a comparison of lesion size on the different leaves showed that the first and second uppermost leaves were the most suitable for estimating differences in tan spot resistance between genotypes.

Another study of disease assessment on separate leaves was carried out in the field. Results showed that the second uppermost leaf was most suitable for evaluating differences between genotypes.

Heritability and Correlation of Resistance to Other Traits

The F5 progenies of a cross between two resistant parents (PI166308 and PI188526) and genotypes Calvin and Edmore were tested at the Sidi El Aydi and Annoceur experiment stations during two growing seasons, 1989/90 and 1990/91. Broad sense heritability was estimated based on the analysis of variance components. Heritability of resistance ranged from 52% (disease severity) to 79% (lesion size), depending on the disease evaluation method used in the field. A highly significant correlation was observed between tan spot resistance,

number of days to heading, and the decimal growth stage at the time of scoring. Plant height was positively correlated to tan spot resistance.

Physiological Specialization of the Pathogen

The evaluation of *P. tritici-repentis* pathotypes in Morocco was conducted in three separate studies (1989-1993). Three groups of differential wheat genotypes were used to study the reaction of 12, 19, and 20 *P. tritici-repentis* isolates occurring in Morocco. All three studies showed a small but significant host-pathogen interaction. Virulence of the different isolates significantly differed but differences between host genotypes were more significant.

Screening and Breeding Trials

Several separate studies on screening for tan spot resistance have been conducted since 1990 on durum wheat collections from the USA, Canada, CIMMYT, and ICARDA, including germplasm from international and national collections. All studies showed a large variation in genotype response to the disease and a correlation between disease resistance and late and tall plant types. No complete resistance was observed. The most resistant parents identified to date are PI166308 and PI188526, followed by several moderately resistant cultivars from Tunisia and Algeria.

Crosses of early, adapted Moroccan durum wheat varieties with late maturing, tall, and resistant PI166308 and PI188526 lines, followed by field selection at Sidi El Aydi and Jemaat Shaim experiment stations, yielded no early maturing, resistant progeny. Furthermore, no germplasm selected for tan spot resistance under other environments was found to have satisfactory resistance in Morocco.

The current objective of breeding for tan spot resistance in adapted germplasm is to search for germplasm with flexible earliness and plant height; these traits are present in some drought tolerant lines that may harbor satisfactory tan spot resistance. Due to the difficulty of combining tan spot resistance with drought escape or drought tolerance related traits, durum wheat breeding should aim to combine tan spot resistance with adaptability and yield potential. Adaptability and flexibility could be the starting point to which improved resistance to tan spot could be added.

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Controlling Leaf Spot of Wheat through Nutrient Management

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Abstract

Leaf spot of wheat, caused by Bipolaris sorokiniana, *is a major problem for wheat production in Bangladesh. During a study on the status of leaf blight of wheat, it appeared that none of the currently cultivated wheat varieties in the country are free from the disease. More than 600 wheat materials of exotic and national origin were screened in the field under natural epiphytotics and artificial inoculation. It was observed that only a few showed resistance to* B. sorokiniana *but none showed immunity. A study into the effect of* B. sorokiniana *on wheat yield revealed that the artificial inoculation of plants at the flag leaf stage reduced the number of grains per ear and 1000-grain weight by 7-100% and 12-100%, respectively, compared with the control. Nutrient elements, especially micro-elements Cu, B, and Mo, were used as an alternate method of leaf spot control with the least possible disturbance of the overall natural soil fertility balance. Cu, B, and Mo, applied either as a foliar spray or to the soil, did not significantly affect leaf blight incidence; however, B application increased grain formation and wheat yields. An* in vitro *study revealed that B has a strong inhibitory effect on* B. sorokiniana.

Wheat is an important cereal after rice in Bangladesh. Of the many diseases affecting wheat, spot blotch, caused by *Bipolaris sorokiniana*, is considered the most important and common in the country (Hossain and Azad 1992; Alam *et al.* 1994). Under field conditions, the disease may be recorded on the wheat plant from the seedling stage, but severity increases with plant age. All commercial varieties/cultivars in the country are either moderately susceptible or susceptible to spot blotch. Under field conditions, cultivars showed seedling blight, leaf spot, leaf blight, head blight, and black pointed grains. Furthermore, if infection is severe, the disease may cause sterile spikes. Considering the importance of the disease, research into 1) sources of spot blotch resistance, 2) the effect of disease on wheat yield, and 3) reducing yield loss through nutrient application is being conducted as discussed in this paper.

Reaction to *B. sorokiniana* of Local and Exotic Wheat Materials

Field study under natural epiphytotics

In the search for sources of *B. sorokiniana* resistance, 619 wheat lines of local and exotic origins were screened in the field under natural epiphytotic conditions during 1990-1995. Wheat

genotypes were received from Brazil (Dr. Y.R. Mehta, Instituto Agronomico do Parana, Brazil), Japan (Dr. Shoji Ohta, Plant Germplasm Institute, Faculty of Agriculture, Kyoto University, Japan), India, Nepal, and Bangladesh (Dr. A. Islam, Department of Genetics and Plant Breeding , Bangladesh Agricultural University, Mymensingh, Bangladesh). Moreover, wheat materials from CIMMYT, obtained through Prof. Dr. A. Islam, were included in the study.

The 619 genotypes showed different reactions to *B. sorokiniana*. Disease evaluations were made at seedling, maximum tillering, flag leaf, and flowering stages. Combined disease severity was taken into account to assess the entries. The rating scale of Gilchrist (1984) and Alam and Gustafson (1988) were modified and used according to Hossain and Azad (1992). Only two entries, one from Brazil and one from Japan, were recorded as highly resistant to leaf spot. It was observed that 28, 10, 1, 1, and 71 materials collected from Brazil, Japan, India, Nepal and Bangladesh, respectively, were recorded as resistant, i.e., only 17.9% of the materials showed resistance under natural infection in the field. Another 15.8% materials were rated as moderately resistant, while the rest were found moderately susceptible, susceptible, and highly susceptible. Variation in disease reaction among the wheat materials may be due to: 1) genetic variation among the test wheat materials, 2) genetic variation in the strains/races of *B. sorokiniana*, and 3) variation in climatic conditions.

Artificial inoculation study

To further evaluate the promising wheat genotypes, seed of the resistant to highly resistant materials was grown and tested under artificial inoculation conditions in the susbsequent growing seasons. Pathogen isolation, and culture and inoculum preparations were performed according to a CIMMYT method (Gilchrist 1984), and reactions of the wheat materials to *B. sorokiniana* were scored according to the scale of Alam and Gustafson (1988). In total 113 wheat materials were tested.

Wheat plants were found to be more vulnerable to *B. sorokiniana* at the seedling stage. Some materials showed resistance to the pathogen at one growth stage, and susceptibility at others. Only one material was rated as highly resistant at both seedling and maximum tillering stages, but not at the flag leaf stage. Only 15 of 113 (13.2%) wheat materials were recorded as resistant.

Effect of Spot Blotch on Wheat Yield

A study was undertaken to determine the relationship between leaf spot severity caused by *B. sorokiniana* under artificial epiphytotics and wheat yield. Isolation and culture of the pathogen were performed following the method of Gilchrist (1984). Inoculum preparation, growing of the test plants, and inoculation were carried out using the methods of Hossain *et al.* (1992). Only the flag leaves of the plants were inoculated. Disease reaction was estimated according

to the same scale used in the artificial inoculation study described above. Of the 24 wheat materials tested, only three were rated as resistant, while 16, 2, 2, and 1 were recorded as moderately resistant, moderately susceptible, susceptible, and highly susceptible, respectively. The pathogen was observed to cause marked yield reductions. Reduction in grains/ear and 1000-grain weight over the control ranged between 7-100% and 12-100%, respectively.

Nutrient Effects on Leaf Spot and Yield of Wheat

At the present time, researchers are aiming to control plant diseases with minimum possible impact on the natural balance. According to this objective, a study was designed to control leaf spot of wheat caused by *B. sorokiniana* while maximizing yield by using nutrient elements, in particular micronutrients Cu, B, and Mo, as a source of plant nutrition. The study was initially carried out in the field at Rangpur (northern Bangladesh) following a randomized complete block design with three replications. In addition to a basal dose of 100 kg N ha^{-1}, 60 kg P_2O_5 ha^{-1}, 40 kg K_2O ha^{-1}, and 20 kg S ha^{-1}, Cu, B, and Mo were applied either as a basal dose at 3 kg ha^{-1} or a foliar spray of 100, 200, and 400 ppm solution. Sources of B, Cu, Mo, N, P_2O_5, K_2O, and S were boric acid (H_3BO_3), $CuSO_4.5H_2O$, $Na_2MoO_4.2H_2O$, $Co(NH_2)_2$, $Ca(H_2PO_4)_2$, KCL, and $CaSO_4.2H_2O$, respectively. Where a single foliar spray (fs) was used, spraying was performed 35 days after sowing. The treatments and effect of Cu, B, and Mo on disease reaction and yield of wheat are presented in Table 1. Leaf spot severity was recorded following the scale used by Hossain and Azad (1992). Disease severity of Kanchan under different treatments was observed to range from moderately resistant to moderately susceptible. Boron applied to the soil (3 kg ha^{-1}) and as a foliar spray

Table 1. Effect of Cu, B, and Mo on spot blotch reaction and yield of wheat cv. Kanchan in Rangpur.

Treatment	Disease reaction	Weight of grains/ear (g)	Yield (kg ha^{-1})	Yield increase/decrease over the control (%)
1. Control	MS	1.34	2080	
2. Cu (Sa)[1]	MS	1.04	2048	-1.6
3. B (Sa)	MS	1.68	2720	+23.5
4. Mo (Sa)	MR	1.13	2272	+8.4
5. Cu100 (fs)	MR	1.22	2200	+5.5
6. Cu200 (fs)	MS	1.22	2016	-3.1
7. Cu400 (fs)	MS	1.00	2256	+7.8
8. B100 (fs)	MR	1.63	2296	+9.4
9. B200 (fs)	MS	1.63	2344	+11.2
10. B400 (fs)	MR	1.66	2632	+20.9
11. Mo100 (fs)	MR	1.30	2240	+7.1
12. Mo200 (fs)	MS	1.40	2264	+8.1
13. Mo400 (fs)	MS	1.20	2072	-0.4
LSD (P = 0.05)		0.19	103.5	

[1] Sa = Soil application: 3 kg ha^{-1}, fs = Foliar spray: 100, 200, and 400 ppm.

(400 ppm solution) caused increases in wheat yield of 23.5% and 20.9%, respectively, over the control treatment.

Based on these results and the findings of others (Chatterjee *et al.* 1980; Rahman 1989; Jahiruddin *et al.* 1992; Mondal 1993), investigations with respect to B were carried to: 1) evaluate the effects of B on grain formation (healthy and diseased), 2) determine the effect of B on yield (t ha^{-1}), 3) determine the effects of B on wheat cultivars/varieties, and 4) evaluate the effect of B on mycelial growth of *B. sorokiniana*, *Fusarium graminearum*, *F. culmorum*, and *Curvularia lunata*.

Treatment combinations tested in the two field studies in Mymensingh are shown in Tables 2 and 3. The experiments were conducted using a complete randomized block design with three replications, where N, P, K, and S were applied as a basal dose at 100 kg ha^{-1} N, 60 kg ha^{-1} P_2O_5, 40 kg ha^{-1} K_2O, and 20 kg ha^{-1} S. Nutrient applications were as for the field study in Rangpur. Foliar sprays were applied at 15-day intervals. The field experiments revealed that B has strong effect on grain formation and yield. In the first experiment with cv. Kanchan (Table 2), it was observed that B (3 kg ha^{-1}) applied to the soil resulted in a 200.98% increase in the formation of healthy grains/ear compared with the control treatment. Moreover, this treatment reduced the formation of diseased grains/ear by 43.37%. The highest yield (2.98 t ha^{-1}) was found using 5 kg ha^{-1} B as a soil application; a yield increase of 129.5% compared with the

Table 2. Effect of boron on grain/ear formation and yield of wheat cv. Kanchan in field studies conducted at Mymensingh.

Treatment	No. of grains/ear	Number of grains/ear		Yield (kg ha^{-1})	Yield increase over the control (%)
		Healthy	Diseased		
1. Control	18.5	10.2	8.3	1300.0	
2. 3 kg ha^{-1} (Sa)[1]	35.4	30.7	4.7	2867.0	120.5
3. 4 kg ha^{-1} (Sa)	31.3	28.4	2.9	2946.0	126.6
4. 5 kg ha^{-1} (Sa)	30.6	26.8	3.8	2983.5	129.5
5. 100 ppm (fs1)	19.0	11.1	7.9	1340.0	3.1
6. 100 ppm (fs2)	19.6	15.6	4.0	1451.5	11.6
7. 100 ppm (fs3)	18.7	12.9	5.8	1463.0	12.5
8. 200 ppm (fs1)	18.7	10.7	8.0	1479.0	13.7
9. 200 ppm (fs2)	23.8	16.9	6.9	1716.0	32.0
10. 200 ppm (fs3)	27.0	20.1	6.9	2116.0	62.7
11. 400 ppm (fs1)	23.9	18.1	5.8	1575.0	21.2
12. 400 ppm (fs2)	26.9	20.7	6.2	1883.0	44.8
13. 400 ppm (fs3)	31.7	26.3	5.4	1747.0	34.4
14. 500 ppm (fs1)	32.2	26.1	6.1	2073.0	59.5
15. 500 ppm (fs2)	32.2	26.2	6.0	2151.0	65.5
16. 500 ppm (fs3)	33.3	28.4	4.9	2686.5	106.6
17. 1000 ppm (fs1)	33.6	29.7	3.9	2841.5	118.5
18. 1000 ppm (fs2)	35.2	30.3	4.9	2849.5	119.2
19. 1000 ppm (fs3)	32.4	26.9	5.5	2857.0	119.7
LSD (P = 0.05)	5.06	5.36	2.37		

[1] Sa = Soil application, fs1 = one foliar spray, fs2 = two foliar sprays, fs3 = three foliar sprays.

control. Boron at 3 kg ha^{-1} produced yields of 2.87 t ha^{-1} which was 120.5% higher than the control. Three foliar sprays of B at 1000 ppm also resulted in significantly higher results and there was no significant difference with respect to production of healthy grains/ear among treatments 2, 3, 4, 13, 14, 15, 16, 17, 18, and 19 (See Table 2).

Boron was found to always significantly affect yields of the cultivars tested (Table 3). It is interesting to note that B produced similar yield effects for cultivars Kanchan, Agrani, and Sonalika.

In vitro study

An *in vitro* test revealed that mycelial growth of *B. sorokiniana* was completely inhibited at 6000 ppm B on PDA at 25+1°C; however, growth of *F. graminearum*, *F. culmorum*, and *C. lunata* were not evident at 5000 ppm of B.

Conclusion

Leaf spot of wheat is common in Bangladesh and is ranked the most important disease with respect to prevalence and reduction in wheat yield and grain quality. Since no wheat cultivars are free from leaf spot, sources of resistance need to be identified under Bangladesh conditions. In reality, it is difficult and time consuming to develop disease resistant wheat cultivars; therefore, an integrated approach for leaf spot control needs to be implemented.

Table 3. Effect of boron and wheat variety on number of grains/ear (healthy and diseased) and yield of wheat.

	No. of grains/ear								
	Healthy			Diseased			Yield (t ha^{-1})		
Treatment	V1[1]	V2	V3	V1	V2	V3	V1	V2	V3
B0 (Control)	6.4	8.2	16.2	4.8	5.9	7.1	1.3	1.4	1.9
B3 (Sa)[2]	32.7	43.1	29.1	4.8	2.9	2.7	2.5	2.3	2.3
LSD (P = 0.05)		9.39			ns			ns	

[1] V1 = Kanchan, V2 = Agrani, and V3 = Sonalika.
[2] B3 (Sa) = 3 kg ha^{-1} B applied to soil.

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Phytosanitary Effect of the Combined Application of Green Manure and Antagonistic Bacterium *Bacillus subtilis* on *Bipolaris sorokiniana*

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Abstract

A study was carried out to assess the potential of antagonistic bacterium Bacillus subtilis *for biological control of* Bipolaris sorokiniana, *which causes common root and black point of cereals. In* in vitro *experiments,* Bacillus subtilis *was found to negatively affect germ tube length, hyphal development, and radial mycelial growth of* B. sorokiniana. *A pre-sowing treatment of barley seed with a cell suspension of* Bacillus subtilis *was found to improve crop yield. Inoculation with* Bacillus subtilis, *after covering soil with green manure, increased the phytosanitary effect of the soil, increased plant height, and improved seed quality. These improvements led to a decrease in ratings of common root rot to 6% and disease severity to 32%. Crop yield increased by 35% and 1000-grain weight by 45%. Furthermore, the amount of seed infected by black point (*B. sorokiniana*) fell below significant levels.*

A bacterium antagonistic to most soilborne cereal pathogenic fungi was selected by the biological agriculture group of the All-Russia Institute of Agricultural Microbiology. The bacterial strain was found to produce an antibiotic in pure culture that inhibits the growth of pathogenic fungi, a group B vitamin, and indole-acetic acid. Based on cultural, physiological, and biochemical tests, the bacterium was identified as *Bacillus subtilis*.

The aim of the study was to assess the potential of *Bacillus subtilis* for biological control of cereal pathogenic fungi. To achieve this, the interaction mechanisms between antagonist, soil, plant, and phytopathogen had to be determined.

Materials and Methods

The fungus used in the experiment was *Bipolaris sorokiniana* (Sacc.) Shoemaker. The number of *B. sorokiniana* spores in the soil was calculated by the flotation method according to Ledingham and Chinn (1955). Soil microflora were identified by the dilution-plate technique. *Bacillus subtilis* was selected from the soil, characterized by its gram-positive, spore-forming, and rod-shaped properties. A bacterial cell suspension containing metabolites was cultured in a liquid meat-peptone broth with 0.5% $CaCO_3$. A cell suspension of *Bacillus subtilis* was extracted from the culture by centrifugation at 40,000 revolutions per minute. *Bacillus subtilis* infection on seeds was 1×10^8 cells per seed.

In the greenhouse and in the field, soil was artificially inoculated prior to sowing with dry *B. sorokiniana* conidia at 90 propagules per gram of soil. This quantity of conidia corresponds to an infection load typical of temperate climates. Green manure and *B. sorokiniana* were applied to the soil 15-20 days before sowing. This decomposition period is crucial when using green manure so that growing plants are not poisoned by volatile substances. Disease ratings were based on lesion size on the subcrown internode. Disease severity was calculated as the percentage of infected plants. The test plant was spring barley (susceptible cultivar Pirkka).

Results

The effect of *Bacillus subtilis* on the germination of *B. sorokiniana* was investigated in *in vitro* laboratory studies. Pathogen propagules and cell suspensions were simultaneously deposited on membrane filters. The filters were placed in petri dishes and incubated at 27°C. The control was a filter without bacteria. Every seven days, the filters were fixed and observed under a light and electron microscope at x200 and x900. Results are illustrated in Figures 1 and 2.

Morphological changes were noticed in the fungi after incubation of the *Bacillus subtilis* metabolites and *B. sorokiniana* conidia including short germ tubes, delay in hyphae germination, and inhibition of radial mycelia development. These changes, in our opinion, lead to a partial reduction in pathogenic aggressiveness. When the effect of the cell suspension on fungal germination was investigated, lysis in an external part of the mycelia and a microcycle fungal development was observed (conidia-germ tube-conidia). This indicated unfavorable conditions for fungal development. Also, the prolonged inhibition of mycelial growth induced autolysis at the expense of endogenic nutritive substances (Arora *et al.* 1983).

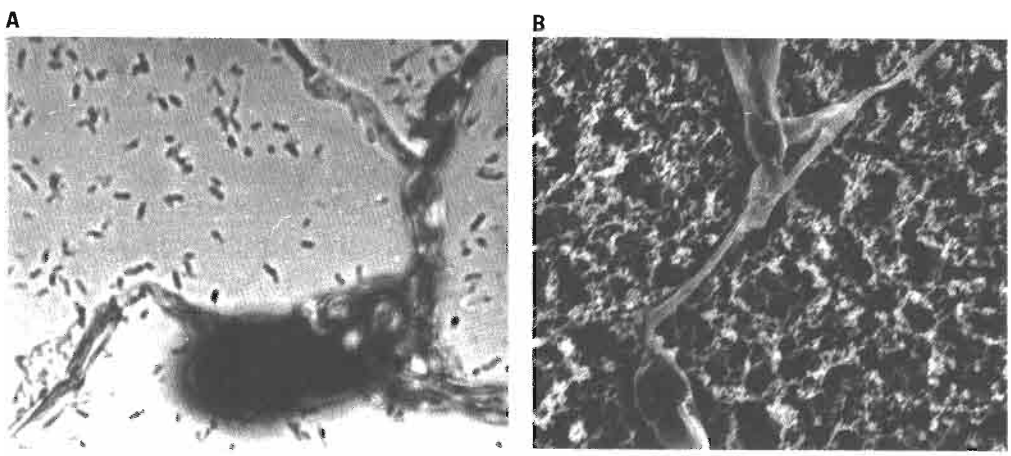

Figure 1. Morphological changes in *Bipolaris sorokiniana* under incubation with metabolites of *Bacillus subtilis*. A = Limitation of germ tube. B = Reduction of radial mycelia development.

To study the potential of *Bacillus subtilis* for biological control on barley seedlings, soil was sterilized twice under 2 at 30 min. Seeds were surface sterilized with $HgCl_2$ (1:1000), repeatedly washed with distilled water, and placed on a meat-peptone agar prior to seedling shooting.

It was established that metabolites increased root length by 39.4%, and infection of seeds by spores increased seedling weight by 34.8% (Table 1). This was obviously due to stimulation of growth by metabolites produced by the *Bacillus subtilis* culture during development in the soil. To investigate further, the cell suspension culture and metabolites of *Bacillus subtilis* were combined in other experiments.

It was previously demonstrated that green fertilizers activate soil microflora and the production of antibiotics by *Bacillus subtilis* (Voznyakovskaya and Nikonorova 1992). Using this knowledge, the effect of a combined application of green organic matter and antagonistic *Bacillus subtilis* culture on *B. sorokiniana* survival in the soil was tested.

In the greenhouse, different types of organic matter were analyzed including common winter cress, blue lupine, and green oat. Two seed inoculation methods were used: at the time of sowing, and after covering with green manure. Results showed that: 1) inoculation of seeds with *Bacillus subtilis* after covering with green manure (independent of the green manure quality) improved the phytosanitary effect of the culture; and 2) the positive effect of the combined application of green manure and antagonistic bacterium was independent of the inoculation method.

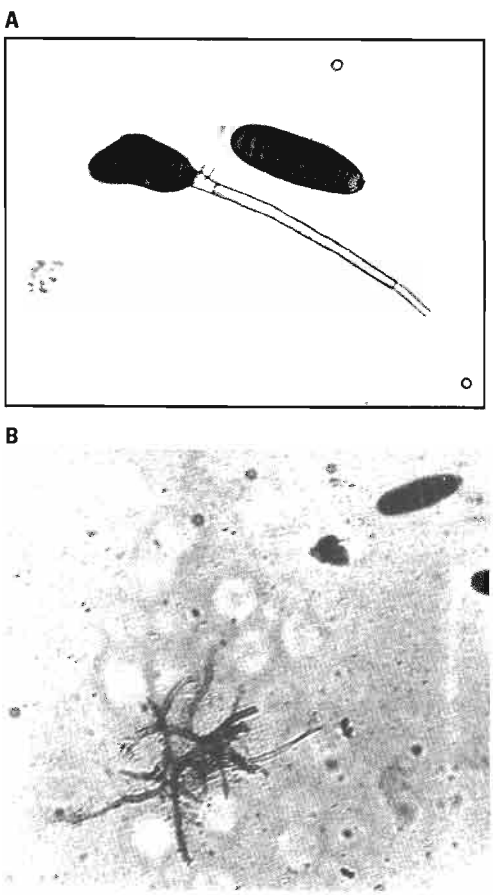

Figure 2. Conidia of *Bipolaris sorokiniana* incubated with a cell suspension of *Bacillus subtilis*. A = Lysis of mycelia. B = Microcycle.

Table 1. Effect of *Bacillus subtilis* on the development of barley seedlings. A comparison of different formulations of the antagonist.

	Root length		Seedling height		Dry weight	
Treatment	**(cm)**	**(%)**	**(cm)**	**(%)**	**(g)**	**(%)**
Liquid media	9.9	0.0	24.4	0.0	0.23	0.0
Spores	10.8	9.1	25.9	6.1	0.31	34.8
Metabolites	13.8	39.4	25.5	4.5	0.25	8.7
P<0.05 (LSD)	0.7		0.5		0.01	

In the field, the bacterium culture can be used in the form of dry spores or liquid culture (cell suspension with metabolites). Pre-inoculation of seeds or at the time of seeding is recommended.

Soil in the field sites was artificially inoculated with *B. sorokiniana* and soil samples were taken after harvesting. Microbiological analysis of samples revealed that for seeds inoculated with antagonistic bacteria after coverage with green manure, there was an increase in non-sporic, spore forming, and mycolytic bacteria up to 30.0×10^3 cells per g soil compared with 1.3×10^3 per g soil in the control. At the same time, the total population of fungal species decreased, with the number of *B. sorokiniana* conidia falling below the noxious threshold (10 propagules per g soil, compared with 63 propagules per g soil in the control). In addition, an increase in soil fungistasis to 80% was observed. This ensured the maintenance of the decreased pathogen population in the soil (Table 2). The level of *Bacillus subtilis* compared to the total number of spore forming bacteria in the soil was calculated at 93% in treatments with green manure and 61% without. The culture probably obtains nutrition from the green manure to aid its development process in the soil.

Heights of the barley plants from the combined treatment (green manure + seed inoculation) were greater than those from the treatment lacking green manure, i.e., 109 cm and 57 cm, respectively. This was due to the stimulating effect of the culture on plant growth and to the decrease in *B. sorokiniana* infection levels during early plant growth stages (Duczek and Peining 1982.) In other words, the increase in *Bacillus subtilis* population density prevented severe *B. sorokiniana* infection on the plants during early growth.

The improvement in soil-microbiological conditions, plant growth, and the creation of phytosanitary soil conditions led to a decrease in the disease ratings of common root rot to 6% and disease severity to 32% (Table 3). Hence, a combined application of green manure and seed inoculation in extreme disease conditions can improve plant height, straw weight, seed weight, and 1000 kernel weight. Yield increased by 35.1%, whereas 1000-kernel weight increased by 45.3% (Table 4). It should be pointed out that the amount of seed infected by *B. sorokiniana* (black point) fell below significant levels, and this was positively reflected in the yield of the following year.

Table 2. Effect of the combined application of green manure and antagonistic *Bacillus subtilis* on soil microflora.

	Bacteria (10/g soil)				*B. sorokiniana*	
		Spore forming			Propagules/	Fungistasis
Treatment	Nonsporic	Total	(%)	Mycolytic	g soil	(%)
Control	15400	510	0	1.3	63	50
Seed inoculation	24400	1200	61	9.5	20	81
Green manure	31100	910	0	14.0	28	71
Green manure + inoculation	37000	3410	93	30.0	10	80

Discussion and Conclusions

Analysis of results show that a pre-sowing treatment of seed with a cultural cell suspension of *Bacillus subtilis* is recommended to improve spring barley yields. To increase the phytosanitary effect of soil and improve seed quality, it is advantageous to inoculate with *Bacillus subtilis* after covering with green manure. The process is as follows: lightly-hydrolyzed organic matter promotes the growth of phytopathogenic fungal propagules, and thereby decreases the infection potential of the soil and reduces soil fungistasis. The growing hyphae become the target of antagonistic bacteria, including the introduced *Bacillus subtilis* culture, which limit germination of fungi mycelia and form an obstacle for new conidia. An increase in the number of mycolytic and antagonistic microflora at the end of the vegetation period leads to an increase in soil fungistasis. This ensures the intensification of soil fungistasis, resulting in a decrease in fungal infection and further maintenance of the low infection potential of the soil. Furthermore, lightly hydrolyzed organic matter intensifies reproduction and metabolic activity of antagonistic microorganisms, and stimulates growth.

Table 3. Effect of combined application of green manure and antagonistic *Bacillus subtilis* on the phytosanitary conditions of barley development.

Treatment	Height (cm)	Disease rating (%)	Disease severity (%)
Control	57	62	90
Seed inoculation	82	26	50
Green manure	88	18	42
Green manure + inoculation	109	6	32
P<0.05 (LSD)	7	-	-

Table 4. Barley improvement after green manure coverage and seed inoculation with *Bacillus subtilis*.

Treatment	Dry weight of seed		1000-kernel weight	
	($g\ m^{-2}$)	(%)	(g)	(%)
Control	166.2	0.0	23.0	0.0
Seed inoculation	188.8	16.4	26.2	11.0
Green manure	201.9	24.5	9.2	23.7
Green manure + inoculation	20.7	35.1	34.3	45.3
P<0.05 (LSD)	4.7		1.7	

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Seed Pathology of Tan Spot

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Abstract

Pyrenophora tritici-repentis *is a common component of the internal flora of wheat seed. A model of seed infection and transmission of* P. tritici-repentis *was developed based primarily on research with soft white winter wheat. Spores of the pathogen germinate on the wheat floret. The fungus initially colonizes the surfaces of the lemma, palea, or glume, and reaches the seed via mycelial proliferation. Penetration of the kernel pericarp is direct via appressoria. The pathogen ramifies throughout the pericarp, where it remains viable for at least three years. Maximal seed infection results from spike inoculation at kernel milk stage. In naturally infected wheat seeds,* P. tritici-repentis *is found throughout the pericarp but seldom in the seed coat or embryo. Seed transmission occurs in a non-systemic manner. The emerging coleoptile is externally infected by hyphal growth from the infected pericarp, and the first and second seedling leaves may be infected by external hyphal growth from the coleoptile. Transmission efficiency varies with seed lot and ranges from 0 to 92%. Factors affecting seed transmission are not well understood; they include soil temperature and may also include age of seed, wheat cultivar, developmental stage when seed infection occurred, and soil moisture during emergence. Infected seed may provide inoculum for tan spot epidemics and for dispersal of fungal strains to new geographic locations.*

Pyrenophora tritici-repentis is documented worldwide as a component of the fungal flora of wheat seed (Schilder and Bergstrom 1993). Seeds infected by *P. tritici-repentis* often show a salmon-pink discoloration at the embryo end or elsewhere on the seed surface (Valder 1954; Vanterpool 1963), but may otherwise look healthy. Schilder and Bergstrom (1993) reviewed practical procedures for assessing seed lots for the presence of the fungus. Seed infection does not affect germination, but does reduce seedling vigor (Hyltén-Cavallius 1984; Schilder and Bergstrom 1995). The fungus also may be transmitted to seedlings. Symptoms on seedlings grown from infected seed on water agar consist of numerous, tiny dark brown spots on the lower coleoptile, whereas seedlings grown in soil show elongated brown lesions up to 2 mm in length on the coleoptile and occasionally on the first leaf (Hyltén-Cavallius 1984; Obst 1989). Infected seedlings are smaller than their healthy counterparts and sometimes distorted (Hyltén-Cavallius 1984; Schilder and Bergstrom 1995). Transmission efficiency has been reported to range between 18 and 92% (Schilder and Bergstrom 1995; Hyltén-Cavallius 1984; Obst 1989). Bergstrom (unpublished) has also examined lots with a high incidence of infected seed, but with seedling transmission efficiencies of 0-10%. The relative contribution of seedborne inoculum to tan spot epidemics has not been established.

Highlighted findings are reported here from studies on seed pathology of *P. tritici-repentis* in wheat at Cornell University, and the reader is referred to the original references (Schilder 1993; Schilder and Bergstrom 1993, 1994a, b, 1995) for more details on procedure and reviews of other pertinent literature. Models are presented of the processes of seed infection and seed transmission as derived from experiments performed primarily with soft white winter wheat.

The Seed Infection Process

Schilder and Bergstrom (1993) employed targeted inoculation of flower parts followed by microscopic examination to elucidate the infection process. *Pyrenophora tritici-repentis* initially colonized the outer and sometimes the inner surfaces of the glume, lemma, and palea (Figure 1), and reached the seed primarily via mycelial proliferation. The thick-walled epidermides of the glume, lemma, and palea were initially sufficiently resistant to penetration to permit invasion only at the thin edges. Small dark lesions observed on these parts were also indicative of a resistant reaction. Only at the onset of senescence did *P. tritici-repentis* ramify through the internal cell layers of the glume, lemma, and palea.

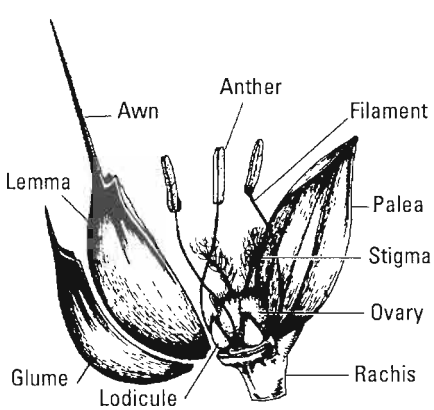

Figure 1. A floret of wheat.

Pyrenophora tritici-repentis penetrated the pericarp (Figure 2) of the wheat kernel directly via appressoria. Hyphal density quickly increased below the upper pericarp, and the fungus also established itself readily in the lower pericarp, where it can remain viable for at least three years in refrigerated storage. The glume by itself apparently contributed less to seed infection than did the lemma and palea. Seed infection after inoculation of the glume *per se* was attributed to invasion and colonization of the glume at senescence and subsequent penetration of the various layers at the base of the floret. Anthers appeared to slightly stimulate seed infection by *P. tritici-repentis*, possibly by serving as a source of nutrients or as a

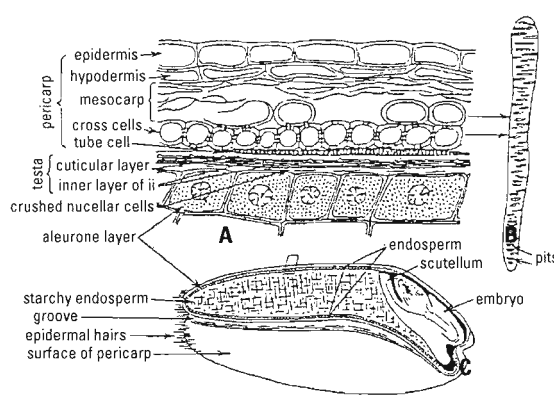

Figure 2. The caryopsis (C) of wheat and its pericarp (A, B). The caryopsis was cut longitudinally parallel with the groove. The small rectangle in C, above, indicates the location of the section in A. B shows a surface view of the pitted wall of a cross cell. Letters ii signify inner integument.
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port of entry, as many of the dehisced anthers partly extruded from between the lemma and palea.

Most seed infection events occurred between 5-14 days after inoculation, but infection took place as early as 3 and up to 21 days after inoculation. The time frame of seed infection may also be influenced by humidity, temperature, inoculum density, and seed development stage. Wheat seeds were susceptible to infection by *P. tritici-repentis* throughout most of their development, from the end of anthesis through the soft dough stage and possibly beyond. An increase in the number of *P. tritici-repentis*-infected seeds was noted when inoculation took place at the milk stage. This may be explained by the large seed size and high moisture content favoring access to and infection of the seeds at this stage. The lower incidence of infected seeds after inoculation at the dough stage may also be due to the reduced time available for infection before harvest. A field experiment showed that, under natural conditions, seed infection can take place at very late stages of development, and that the incidence of seed infection is likely to be affected by the amount of inoculum available at the time of ripening.

Although incidence of seed infection by *P. tritici-repentis* was affected by genotype, foliar susceptibility was generally not a good predictor of susceptibility to seed infection. Fernandez *et al.* (1992) reported that durum wheat varieties differed in pink smudge incidence which was not correlated with flag leaf resistance to *P. tritici-repentis*. Resistance to seed infection may be explained by incompatibility with the fungus, an unfavorable microenvironment of the florets, or restriction of fungal invasion of floral and seed tissues. The latter may have been a factor in the apparent resistance of genotype BR 8 to seed infection at early and late stages of seed development, as the palea was observed to have broader margins and the seed was more tightly enclosed. Perhaps BR 8 also supported less hyphal colonization of the floret surfaces. Since even BR 8 could attain high levels of infected seeds when inoculated at the appropriate stage, resistance to seed infection may only be partially effective; however, no information is available on the efficiency of transmission of the fungus by infected seed of resistant cultivars.

The Seed Transmission Process

Schilder and Bergstrom (1995) studied seed transmission *in vitro* and in soil. Seed transmission rates of *P. tritici-repentis* from infected seeds to seedling coleoptiles were as high as 92% *in vitro*, and as high as 60% in potting soil outdoors. Transmission rates to seedling leaves were lower at 14% *in vitro* and 17% in potting soil. Hyltén-Cavallius (1984) reported a 59% transmission rate in seedlings grown in potting mix at 6°C for 10 days, followed by 20°C. A transmission rate of 13% for seedlings grown at 8-9°C at high humidity was reported by Obst (1989). The humid environment created by water agar in test tubes was very conducive to

both growth and transmission of *P. tritici-repentis*. Seed infection by *P. tritici-repentis* reduced emergence only slightly, but resulted in a marked and long-lasting reduction in plant vigor. This could affect winter survival, susceptibility to other pathogens, and possibly even yield. Activity of *P. tritici-repentis* within the seed remnants was evident by the formation of immature pseudothecia in the pericarp. The general stunting observed in plants grown from infected seed may be explained by competition with the seedling for the nutrients stored in the endosperm and aleurone layer.

Microscopic examination of infected seedlings provided information regarding the probable path followed by the fungus during seed-to-seedling transmission (Schilder and Bergstrom 1995). *Pyrenophora tritici-repentis* externally infected the coleoptile as it emerged during germination by hyphal growth from the pericarp. The first leaf apparently became infected by contact with hyphae from an infected coleoptile as the leaf pushed through its tip.

Seed transmission efficiency of *P. tritici-repentis* was reduced by an increase in soil temperature. The fungus may have been at a relative advantage due to slow growth of the seedling at lower temperatures. A delay in seed germination by means of an osmotic sugar solution enhanced symptom development on coleoptiles of seedlings grown from *P. tritici-repentis*-infected wheat seed (Schilder 1993). These findings imply that seedborne inoculum of *P. tritici-repentis* may be managed by adjusting the planting date. In contrast to seed transmission efficiency, recovery of *P. tritici-repentis* from symptomatic coleoptiles and leaves improved with an increase in soil temperature. More thorough colonization of plant tissues probably occurred at warmer temperatures. In general, the fungus was more frequently isolated from large lesions than from small lesions.

The incidence of lesions on the upper coleoptile, which may be of epidemiological importance, was much lower than the incidence of lesions on the entire coleoptile, and also exhibited a negative linear response to an increase in temperature. Proximity of the fungus within the pericarp to the emerging coleoptile may determine the location of lesions on the coleoptile, and indirectly the incidence of foliar lesions, which apparently resulted from hyphae on lesions at the coleoptile tip or along cracks in the coleoptile.

Further Research

Research is needed to determine the relative contribution of infected seed to tan spot development and to subsequent yield losses under growing conditions in various parts of the world. Important epidemiological factors that will determine the impact of seedborne inoculum on subsequent disease development in the field are incidence of seed infection, the efficiency of seed-to-seedling transmission, and the rate of disease development from the initial focus of inoculum. The effects of soil moisture, planting depth, wheat cultivar,

fungus isolate, and even degree of seed colonization on seed transmission of *P. tritici-repentis* should be investigated further. Perhaps high transmission rates occur only with 'deep-seated' infections associated with infection at early stages of seed development. A preliminary experiment showed differences in seed transmission of *P. tritici-repentis*, unrelated to foliar susceptibility, among wheat cultivars (Schilder, unpublished). This question needs to be explored. Both seed applied fungicides and microbial antagonists need to be evaluated for control of seedborne inoculum of *P. tritici-repentis*.

Even if seedborne inoculum turns out to play a minor role in the development of tan spot epidemics, it may still provide additional inoculum, possibly with a different array of virulence phenotypes than already exists in a field. Fungal populations that are normally isolated in space and time may get a chance to interact through the planting of infected seed and may constitute, in effect, a single, large population. Seed infection by *P. tritici-repentis* has consequences for both wheat production and trade of wheat seed at a national and international level. In 1964, de Tempe postulated that movement of infected seed was responsible for the worldwide occurrence of tan spot. Different pathotypes of the fungus may also spread via seed to areas where they did not previously exist. Among international cooperators, seed health testing and fungicidal seed treatment should be practiced to reduce risk associated with seed exchange.

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Wheat Reaction to Kernel Infection by *Pyrenophora tritici-repentis* and Effect on the Subsequent Crop

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Abstract

Infection of wheat kernels by Pyrenophora tritici-repentis *(Ptr) causes different types of discoloration. The symptom most commonly associated with kernel infection by this fungus is a reddish/pinkish discoloration known as red smudge, but dark smudge/specks and black point are also common. Other fungi, mostly* Alternaria *spp. and* Cochliobolus sativus, *have been more frequently isolated than Ptr from black-pointed durum wheat kernels in southern Saskatchewan. All wheat cultivars currently registered for use in western Canada are susceptible to Ptr-kernel infection. There is no correlation between susceptibility of leaves and of kernels to Ptr. There are differences among cultivars in incidence and severity of infection, and in the prevalent type of symptom developed. The intensity, incidence, and extent of kernel discoloration is partially related to seed color. The reddish discoloration was more apparent in white than in red seeds, and percent and severity of Ptr infection were generally higher in white seeded wheats than in red seeded wheat cultivars. Black point in Ptr-infected kernels was observed in all wheat classes. Its development was genotype-dependent, although it also appeared to be related to infection severity. The effect of red smudge infection of durum wheat seed on the health of the subsequent plants was examined in controlled-environment and field studies. Percent germination was lower for naturally infected red smudged seed than for uninfected seed, whereas germination rate was higher for artificially infected red smudged seed than for uninfected seed. The observation that red smudge speeds up germination of seeds was confirmed in common wheat. Growth of the coleoptile and primary roots, and number of roots were also depressed in red smudged seeds, indicating that Ptr-infection has a negative effect on seed vigor. A field study designed to examine the effect of red smudge on seedling emergence, plant growth, and yield using three red smudge treatments (0%, 50%, and 100%) showed that percent emergence of seedlings declined with increasing proportion of red smudged seeds. This resulted in poor stands and lower yields per unit area in the field. Rate of seedling emergence and time to heading were also lower for red smudge treatments than controls; however, grain weight and yield per plant were not significantly affected. In none of the experiments was transmission of Ptr to durum wheat seedlings observed, but only to the coleoptile under controlled conditions.*

Infection of wheat (*Triticum aestivum* L.) kernels by *Pyrenophora tritici-repentis* (Died.) Drechs. (anamorph *Drechslera tritici-repentis* (Died.) Shoemaker) (Ptr) causes different types of discoloration. The most common symptom is a pink/red discoloration commonly known as red smudge. Ptr infection also results in dark discoloration including black point (Fernandez *et al.* 1994b; Francl and Jordhal 1992). Fernandez *et al.* (1994b) reported that Ptr was the only fungus isolated from red smudged durum wheat kernels, whereas other fungi were more commonly isolated from kernels with black point/dark smudge (Table 1). Ptr has been found primarily in the pericarp of red smudged winter wheat seed (Schilder and Bergstrom 1994).

Discoloration of grain causes downgrading (Canadian Grain Commission 1991). Tolerance levels for red smudge are quite low. For the Canada Western Amber Durum (CWAD) wheat class, 0.25% of red smudge will cause a drop from grade #1 to #2, and 1% will cause a drop to grade #3. Downgrading of durum wheat from CWAD #1 to #2 represents an average loss of CDN $12 per ton. Considering that 4-5 million t of durum wheat are produced in western Canada each year, this potential downgrading can mean a loss of millions of dollars to producers.

Kernel Reaction to Ptr

In durum wheat, no correlation was found between foliage susceptibility (tan spot), either at the seedling or adult stage, and kernel susceptibility to Ptr (Fernandez *et al.* 1994a). In addition, there was no association between discoloration of glumes and kernels in durum wheat genotypes inoculated with Ptr at the milk stage. These observations suggest that different resistance mechanisms to Ptr might be operating in different plant organs, and that therefore the reaction of kernels to Ptr should not be inferred from that of vegetative tissue.

Currently registered Canadian wheat cultivars were evaluated for kernel reaction to Ptr using artificial inoculation of spikes at the milk stage (GS 74-77; Zadoks *et al.* 1974). The cultivars tested

Table 1. Percentage of organisms isolated from black-pointed kernels of 14 durum wheat genotypes grown at Swift Current and Outlook, Saskatchewan, 1991 and 1992.

Year/location	*Alternaria* spp.	*Cochliobolus sativus*	*Pyrenophora tritici-repentis*	Other
1991				
Swift Current	73.9[1]	7.8	8.8	10.0
Outlook	74.5	13.2	4.9	7.5
1992				
Swift Current	29.5	0.6	3.5	38.6
Outlook	55.6	1.2	3.6	31.4

[1] Mean incidence (%) of organisms isolated from surface-disinfested black-pointed kernels plated on water agar.
Source: Fernandez *et al.* 1994.

belonged to different quality classes: 15 Canada Western Red Spring, 5 Canada Prairie Spring (2 red and 3 white), 2 Canada Western Soft White Spring, and 6 Canada Western Amber Durum wheat cultivars. All cultivars tested were found to be susceptible to kernel infection by Ptr. They all developed red to dark discoloration, including black point, although they differed in the prevalent type of symptom developed. Some showed mostly red discoloration, while others developed primarily dark specks/smudge. In many cases, symptoms also developed with time, from harvest of the inoculated seed at maturity to several weeks later.

There were differences among classes/cultivars tested in the incidence and severity (>50% of kernel area discolored) of the infection. The intensity, incidence, and extent of kernel discoloration was partially related to seed color. Red discoloration was more apparent in white than in red seeds, and percent incidence of all Ptr-discolored kernels and infection severity were, on average, higher in the white than in the red wheats (Table 2); however, there was also variation among cultivars within classes. For example, the incidence and severity of infection for some hard red spring cultivars was as high as for the white-seeded durum cultivars (data not shown).

Black point in Ptr-infected kernels was observed in all cultivars and the surface area of many of these black-pointed kernels was more than 50% discolored. There were differences among cultivars in whether black point was often associated with red discoloration extending beyond the black germ end or not. Some differences in black point incidence among cultivars suggested that this type of discoloration was mostly genotype-dependent. For example, among the durum wheat cultivars tested, Plenty and Sceptre had similar high percent incidence of Ptr-discolored kernels (average of 82%), but the average number of black-pointed kernels among those were lower for Sceptre (23%) than for Plenty (38%).

Effect of Kernel Infection on the Following Crop

The effect of red smudge on seed germination, seedling vigor, seedling emergence, plant development, and grain yield of durum wheat was examined in controlled-environment and field studies. Naturally and artificially infected red smudged seeds of the durum wheat cultivars Medora, Sceptre, Wakooma, and Plenty were used (Fernandez *et al.* 1996, 1997).

Table 2. Percent incidence and severity of *Pyrenophora tritici-repentis* infection of kernels of red- and white-seeded wheat cultivars.

	P. tritici-repentis infection	
Seed color	**Incidence (%)**	**Severity[1] (%)**
Red (n = 18)	55.7 b[2]	24.8 b
White (n = 10)	78.1 a	37.9 a

[1] >50% of kernel area discolored.
[2] Values followed by different letters are significantly different (P<0.05), according to an LSD test.

Percent germination at 8°C and high humidity was lower for naturally infected red smudged than for uninfected seed, whereas the time to germination of all seeds (T100) was lower for artificially infected red smudged than for uninfected seed (Table 3). Red smudge could also speed up germination in common wheat. Fox *et al.* (unpublished) observed that Ptr-infected seed of some common wheat genotypes had significantly lower mean germination time than healthy seed when incubated under high humidity at 20°C. These observations suggest that, under field conditions, kernel infection by Ptr might contribute to sprouting.

Growth of the coleoptile and primary roots, and number of roots, measured at 12 days of incubation at 8°C under high humidity, were depressed in red smudged seeds (Table 4), indicating that Ptr infection has a negative effect on seed vigor. Transmission of Ptr to the coleoptile, but not to the true leaves, was observed under these controlled conditions (Fernandez *et al.* 1996).

Seedling emergence, plant growth, and grain yield were determined in a replicated field trial conducted in 1993 and 1994 near Swift Current, Sask. There were three treatments: 0 (control), 50, and 100% red smudge, randomized in a factorial, complete block design with four replicates. Each replicate consisted of 50 space-planted seeds in 3-m single rows, with a 30 cm space between rows. Seedling emergence was recorded daily after seeding, and rate of emergence and total percent emergence calculated. Measurements of plant growth parameters and grain yield were taken during plant growth and after harvest.

The lower the percent emergence of seedlings, the higher the proportion of red smudged seeds. The control treatment (0% red smudge) had 14% and 22% greater emergence than the 50% and 100% red smudge treatments, respectively. Red smudge also had an effect on T100. It took, on average, 2.4 more days for all seedlings in the 50% and 100% red smudge treatments to

Table 3. Percent germination and time to complete germination of all seeds (T100) of naturally or artificially infected red smudged and healthy seeds of four durum wheat cultivars, incubated under high humidity at 8°C.

Test/treatment	Germination (%)	T100 (h)
Naturally infected		
Red smudge	90.5 (0.9)[1]	152.9 (6.3)
Control (healthy)	97.6 (0.4)	149.7 (6.1)
Artificially infected		
Red smudge	99.7 (0.1)	161.0 (4.4)
Control (healthy)	99.6 (0.1)	196.5 (6.1)

[1] Standard errors.
Source: Fernandez *et al.* 1996.

Table 4. Number of seminal roots and length of longest root and coleoptile of naturally or artificially infected red smudged seeds and controls of four durum wheat cultivars, at 12 days of incubation at 8°C and under high humidity.

Test/treatment	Number of roots	Length of roots (cm)	Length of coleoptile (cm)
Naturally infected			
Red smudge	4.2 (<0.1)[1]	2.2 (<0.1)	1.5 (<0.1)
Control (healthy)	4.5 (<0.1)	3.4 (<0.1)	2.0 (<0.1)
Artificially infected			
Red smudge	3.3(<0.1)	2.0 (<0.1)	0.8 (<0.1)
Control (healthy)	3.1(<0.1)	3.2 (<0.1)	1.1 (<0.1)

[1] Standard errors.
Source: Fernandez *et al.* 1996.

emerge than those in the control. Based on the controlled-environment study discussed above, the lower percent seedling emergence could be attributed to reduced germination of naturally infected seeds, whereas increased time to emergence could be mostly attributed to reduced seedling vigor.

The lower emergence of seedlings in the red smudge treatments resulted in poor stands in the field. Number of spikes, above-ground dry weight, and grain yield per plot were 20%, 18%, and 17% lower, respectively, in the 100% red smudge treatment than in the control. There was no treatment effect for 1000-kernel weight or number of kernels per spike.

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