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Editorial Board

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Editorial
Management of Wheat Diseases

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Diseases can severely reduce the economic return of growing wheat. Wheat diseases cause harvest losses, affect the quality of the harvest crop, and cause storage losses. Yield losses might depend on the genetically determined resistance and tolerance of the wheat cultivars to specific diseases, the diversity and level of the pathogen inoculum present, and the environmental conditions. This special issue includes two reviews articles and four research papers addressing the structure of the population of and the location of the resistance to some pathogens, the effect of fungal diseases affecting seedlings, leaves, and spikes on wheat yield and quality, and different strategies to manage them.

Two review articles on bacterial (*Pseudomonas syringae* pathovars) and fungal diseases of wheat (*Mycosphaerella graminicola* (Fückel) Schroeter in Cohn, anamorph *Septoria tritici* Rob. ex Desm.) causing septoria leaf blotch are included. The first one is written by A. J. Valencia-Botin and M. E. Cisneros-López and addresses recent advances in the characterization of the population of the bacterial pathogen by traditional and molecular techniques, the evaluation of its aggressiveness, the pattern of colonization in the wheat seeds and its effects on seed yield, yield components, and source-sink relationships during the postanthesis period. The second one is written by M. R. Simón et al. and describes the most recent efforts to investigate the structure of the population of the fungal pathogen and the location of genes for resistance, with special emphasis on the work carried out in Argentina during the last years.

In addition, M. C. Quincke et al. examine the incidence of *Cephalosporium stripe*, caused by *Cephalosporium gramineum* Nikisado and Ikata, a pathogen causing a serious disease of winter wheat in the Pacific Northwest of the USA, on yield loss, test weight, kernel weight, kernel diameter, and grain protein, whereas R. P. Marano et al. assess how supplementary irrigation affects yield and the incidence and severity of some foliar diseases mainly leaf rust caused by *Puccinia triticina* Eriks, tan spot caused by *Pyrenophora tritici-repentis* (Died.) Drechs., anamorph *Drechslera tritici-repentis* (Died.) Shoem., and septoria leaf blotch. Furthermore, I. M. Haigh and M. C. Hare describe the effect of freezing temperatures on *Monographella nivalis* (Schaffnit) Müller, anamorph *Microdochium nivale* (Fr.) Samuels, and Hallet and *Monographella majus* (Wollenw.) Glynn and Edwards, causing seeding blight of winter wheat, whereas M. Nicolau and J. M. C. Fernandes present a predictive model for daily inoculum levels of *Gibberella zeae* (Schwein.) Petch., anamorph *Fusarium graminearum* Schwabe in Passo Fundo, Brazil which combined with an infection process model might be useful to quantify the impact of Fusarium head blight epidemics on wheat yield and quality.

Together these papers give insight into the diversity of approaches in investigating the control of wheat diseases. We would like to thank all contributors for their interesting contributions and hope that this compilation will be instrumental in getting abreast of some recent advances on some of the main diseases of wheat.

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Research Article

Epidemiology of the Diseases of Wheat under Different Strategies of Supplementary Irrigation

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Wheat (*Triticum aestivum* L.) is one of the most important and highly productive crops grown under supplementary irrigation in the central region of Santa Fe. However, its production is limited by the presence of diseases in the main stages for yield definition. The objective of this work was to assess wheat health in response to different supplementary irrigation strategies under greenhouse and field conditions. The field experiment included three treatments: dry (D), controlled deficit irrigation (CDI), and total irrigation (TI) using the central pivot method. Disease incidence from stem elongation and severity in flag leaf and the leaf below the flag leaf were measured. Leaf area index (LAI), harvest index, air biomass, and yield components were determined. In greenhouse the treatments were TI and CDI, with evaluations similar to the field. The major leaf diseases observed were tan spot, leaf rust, and septoria leaf blotch. Significant differences in disease burden, LAI and yield components were observed in the different treatments. Under greenhouse conditions, only tan spot was observed. The results of this study indicated that the application of supplemental irrigation in wheat improved the yield, without increasing the incidence and severity of foliar diseases.

1. Introduction

The amount of water available for crops is defined by the balance between precipitation and evapotranspiration [1]. Wheat (*Triticum aestivum* L.) cultivated in the central region of Santa Fe is subjected to periods of water deficit that can significantly decrease yields [2].

Because most farmers are focused on grain yield potential, irrigation technology has become an important tool both to maximize production [3] and to reduce the interannual variability of yields [4]. Furthermore, wheat is one of the most important agricultural crops that are treated with supplementary irrigation in humid/subhumid regions [2]. Wheat is also important in crop rotation schemes, because its stubble has beneficial effects on soil structure and for diversify production [5].

Central pivot irrigation is the dominant technique used in this region, but it is not clear whether this technology affects disease susceptibility. This method wets the foliage, thus reducing its temperature while increasing the relative humidity and the length of time during which the leaves remain wet; both of them can promote foliar diseases.

Foliar diseases are the main biotic restrictions that reduce wheat yield in Argentina [6]. Photosynthesis, respiration, the translocation of water and nutrients, and reproduction are affected by pathogens. Any interference in these vital processes prevents the plant from taking advantage of the environmental factors necessary for their growth and development [7], resulting in decreased yield potential. This can be measured through the total amount of biomass generated and the proportion of it which is allocated to reproductive organs [6]. In wheat, the period from the beginning of stem elongation to flowering, during which the stalk and spike grow together and compete intensely, is crucial to determine the number of grains per unit area [6], the variable that is most closely associated with crop yield. Maintaining an adequate area of healthy and functional leaves during this period is essential to achieve higher rates of photosynthesis, allowing greater availability and partitioning of photoassimilates towards the ears and therefore a larger number of grains [6].

However, the negative effects of foliar diseases on wheat yield and quality have increased in Argentina over the last several years due to, among other things, the expansion of no-till, the dissemination of susceptible genotypes, and the use of infected seed [8]. Therefore, there has been an increase either in the prevalence of known foliar diseases like in the threat of the emergence of new diseases, according to Perelló and Moreno [8].

The major foliar fungal diseases caused by necrotrophic pathogens in Argentina have historically been tan spot (DTR) and septoria leaf blotch (SLB); the latter is caused by *Septoria tritici* Rob. ex Desm., teleomorph *Mycosphaerella graminicola* (Fuckel) J. Schröt. in Cohn. Together with some other pathogenic fungi (mainly *Bipolaris sorokiniana* (Sacc.) Schoem., teleomorph *Cochliobolus sativus* (Ito & Kuribayashi) Drechsler ex Dastur and *Alternaria* spp.), tan spot and septoria leaf blotch form a leaf spot disease complex in Argentina [9].

According to Fernández and Corro Molas [10], the most common and severe wheat diseases in Argentina are leaf rust (LR) (*Puccinia triticina* Eriks), SLB, DTR [*Pyrenophora tritici-repentis* (Died.) Drechs, anamorph *Drechslera tritici-repentis* (Died.) Shoemaker], and white blow or fusarium head blight (FHB) (*Fusarium graminearum*). Massaro et al. [11] conducted an experimental survey of wheat foliar diseases in the southern region of Santa Fe over seven consecutive years (2000 to 2006) and concluded that the most prevalent diseases were DTR and LR (71% and 86%, resp.); SLB was observed less frequently, in only one of the seven years studied (14%). Work carried out in the Santa Fe center since 2003 has shown that FHB is the most important disease of the year, with an erratic appearance that is highly dependent on the environmental conditions at the time of flowering [12].

Serrago et al., cited by Simón et al. [9], indicated that a complex of diseases formed by DTR, SLB, and LR reduced grain yield by 1020 kg ha^{-1} on average. Other authors have reported SLB yield losses of 2–50% [13–18] and as high as 75% [19]. In Argentina, yield losses from 21 to 37% [20, 21] and from 20 to 50% [21, 22] in high yielding cultivars have been found.

Additionally, in Argentina, the losses caused by the DTR can reach values as high as 14% in grain yield, as well as an 8 to 11% reduction in thousand grain weight and between 1.2 and 4.5% in hectoliter weight [23]. Globally, yield losses were reported up to 40% [24].

Wheat cultivars that are susceptible to LR regularly suffer yield reductions of 5–15% or greater, depending on the stage of crop development [25]. Reductions of 10–30% have also been reported [26, 27].

Seed quality is also essential; the health status of a seed lots is the main criterion for seed quality, together with purity, energy, and germinative power [28].

Few studies have investigated wheat diseases grown under supplementary central pivot irrigation in Argentina. Work carried out in southern Alberta (Canada) showed that wheat foliar diseases increased in the presence of sprinkler irrigation [29]. Crops cultivated under irrigation tend to be denser, and this modification of the microclimate influences not only the contraction of diseases but also the sporulation of pathogens and later spore dispersal [29]. The wetting of infested crops promotes the sporulation of pathogens, especially when the crop foliage is dense and the subhumid conditions produced by irrigation are prolonged. Pathogenic spores can be dispersed directly by irrigation water droplets or indirectly through the hydration of specialized fruiting bodies such as perithecia [29]. In southern Santa Fe, Andriani et al. [30] reported that wheat under central pivot irrigation developed a powdery mildew (*Blumeria graminis* f. sp. *tritici*) every year. In general, lower yields are closely related to the presence of diseases that affect the entire cycle of crop.

The concepts outlined above highlight the importance of obtaining local information about health problems in cultivated wheat and their possible effects on grain production, that is, comparing the yield maximization achieved through supplementary irrigation with the potential negative effects of irrigation on the evolution of diseases.

The objective of this work was to assess the relation between the health of a wheat crop (grown in the greenhouse or field) and the water management conditions used in the eastern/central region of Santa Fe.

2. Materials and Methods

2.1. General Procedures. The experiment was carried out over two successive growing seasons (2009-2010) in the "Miraflores" area (latitude 32°10′14″ S, longitude 60°59′57″ W), located in the eastern/central region of the Santa Fe province, with 800 ha under central pivot irrigation with water from the Coronda River. The system that they have has an intake in the river, which drives through channels, partly excavated and partly on an embankment, with four pumping stations. The central pivot covers an area of 32 ha (six towers, 325 m) with average irrigation flow and depth of 125 m^3 h^{-1} and 8 mm day^{-1}, respectively. The applied drops are between 1 and 2 mm, and the passage time on the leaves varies from a few minutes (extreme towers) to a few hours (central towers), depending on the applied depth.

The climate analysis considered historical information for the central region (Oliveros and Santa Fe), including rainfall, temperatures, pressure vapor, wind, radiation, and evaporation.

The soil is a Typic Argiudolls, which is suitable for agriculture (class I, INTA, 1992). Surface composite samples of soil (0–0.2 m) were extracted for chemical analysis (pH, total nitrogen, organic matter, phosphorous, sulfur) in order to calculate the fertilizer doses required.

2.2. Treatments. The treatments were as follows: D (rainfed, no irrigation) crops located outside the circle; TI, with irrigation managed according to the maximum expected yield and maximum demand for water; CDI, with irrigation managed strategically according to the water deficit. Three plots (replicates of 100 m^2 each) in each treatment area were selected for evaluation.

The Cronox cultivar was used for all treatments. Cronox is a short-intermediate cycle plant with moderate

susceptibility to DTR and LR, and moderate-to-low susceptibility to SLB, according to the information provided by their respective breeder. Seeding was carried out on June 10 with a density of 150 kg ha^{-1} seed, resulting in a density of 453 plants m^{-2}. Fertilizer was applied based on a prior analysis of the soil: 150 kg ha^{-1} urea (broadcast applied), 70 kg ha^{-1} of diammonium phosphate, and 50 kg ha^{-1} calcium sulfate, and the harvest was on November 12. In the 2010 season, Cronox was sowed on June 23 but at a higher density (160 kg ha^{-1}), 409 plants m^{-2}. Plants were fertilized with 120 kg ha^{-1} urea (broadcast applied), 100 kg ha^{-1} of ammonium phosphate, and 80 kg ha^{-1} calcium sulfate, and the harvest was on November 14. Management practices, which were usually carried out by the farmers, included the preventive treatment of seeds with an antifungal agent (25% carbendazim + 25% tiram).

2.3. Blotter Test. Seed samples with and without treatment (4 samples of 100 seeds each) were obtained and incubated to measure germination energy (GE) and germinative power (GP). Incubation was carried out using the top of paper method according to Peretti [31]. Seed health was measured in terms of pathogen burden as determined by "Blotter test" or, when it was necessary to isolate specific pathogens, through selective culture [32].

Incubation was carried out at 21 ± 1°C, a relative humidity of 80%, 12 h light, and 12 h of darkness [33] for four to ten days [31]. The protocol published by Peretti [31] was not used because the incubation temperature was inappropriate. The GE count was conducted after four days of incubation, and the final count to determine GP was conducted after eight days. Pathogen load was determined by visually observing incubated seeds for fungal colonies with a magnifying binocular, both from above and from below [31]. A stereoscopic microscope was used to diagnose fungal structures in specially made preparations.

2.4. Foliar Disease Incidence, Severity, and Biomass Determination. The Zadoks scale was used to monitor crop phenology [34]. Disease monitoring was conducted in the field, from stem elongation onwards because during tillering, new leaves quickly appear and there is a reduction in the intensity of disease [35]. Monitoring consisted of two weekly visits to evaluate LR and once per week to evaluate foliar spots caused by *Drechslera tritici-repentis*, *Septoria tritici*, or *Bipolaris sorokiniana*. The severity and incidence of these diseases were quantified as the percentage of affected leaf area on the flag leaf (FL) and the leaf below the flag leaf (FL-1).

Fusarium head blight (FHB) results from the development of a complex of pathogenic fungi. *Fusarium* consists of five main species (*Fusarium graminearum, Fusarium culmorum, Fusarium avenaceum, Fusarium poae,* and *Fusarium triticum*), with several strains per species. The most common of these species, which causes FHB, is *F. graminearum* [36]. To quantify FHB, we measured the percent incidence (sick spikes/assessed spikes × 100). We also monitored the cereal disease *Gaeumannomyces graminis*, which has become important in the wheat region within the last several years due to the increase of inoculum in soil [37], and a powdery mildew (*Blumeria graminis* f. sp. *tritici*) disease that was previously observed to develop upon irrigation [30]. Batch sampling was conducted by randomly selecting 50 tillers taken in a zigzag path of the sampling area. The Cobb scale was applied to evaluate the severity of LR and foliar diseases on FL and FL-1, and the Stack and McMullen scale was used to evaluate FHB [38, 39]. All scores were expressed as percentages [40]. The incidence (percentage of infected plants) and percentage of sick leaves (with respect to the total number of leaves) were calculated by separating green leaves and expanded bearers with symptoms from those that were healthy. Leaves that exhibited at least one lesion or leaf spots >2 mm [35] were considered to be infected with rust sheet. Because it is difficult to differentiate lesions caused by DTR and SLB, the accurate diagnosis was made based on the sign: Drechslera has long conidiophores and conidia, and those of septoria, pycnidia, and conidia are shorter, as observed at 40x with an optical microscope [41].

Distrain software was also used to estimate the severity of several diseases, including LR, powdery mildew, SLB, striated rust, stem rust, and DTR [42].

To estimate the total aboveground biomass (TAB), samples were taken from plants at three timepoints, Z 3.1, Z 6.5, and Z 9.2, according to Zadoks et al. [34]. Twenty stems were extracted from each of the first two samples, and leaf area index (LAI) was measured with a LIQUOR LI team index 3000.A instrument. The stem and leaf components were separated and dried to a constant weight at 65°C.

2.5. Yield and Yield Components. Yield was determined from two samples extracted at random from physiologically mature plants along one linear meter per plot. In the laboratory, plants and stems were counted for each sample, and subsamples (20 stems) were separated by components (stalk and spike); the number of spikelets per spike and fertile and infertile spikelets was counted. Each component and the rest of the sample were dried separately at 65°C to a constant weight. Each sub-sample of spikes was threshed manually, and the resulting grains were subsequently weighed and counted.

2.6. Statistical Analysis. The experiment was conducted in a random block design with three replicates, and analysis of variance (ANOVA) was used to evaluate the severity, impact, LAI, and yield parameters using the program INFOSTAT/professional-version 2009 [43]. Homogeneity of variance was tested by comparing the error mean squares for all dependent variables and Shapiro-Wilks modified [43]. Means were compared by Tukey ($P \leq 0.05$). The data on severity percentage and incidence percentage were arcsine square root transformed for analysis.

2.7. Greenhouse Experiment. In addition to epidemiological studies in the field, we evaluated plants grown in a greenhouse in order to compare the health and yield of this cultivar under different irrigation conditions.

The same variety of wheat was used (Cronox) with a sowing date of May 31, 2010, in furrows of 0.3 m and separated by 0.2 m. Greenhouse plants received either TI (irrigated

Table 1: Incidence (%) and genera of pathogens identified through blotter tests of seeds treated with fungicide or untreated in 2009 and 2010.

Treatment	Year	*Alternaria* spp.	*Aspergillus* spp.	*Penicillium* spp.	*Drechslera tritici-repentis*	*Bipolaris sorokiniana*	*Rhizopus* spp.
Seeds treated	2009	0	0	0	0	0	0
	2010	1.25	0	10.5	0	0	0.25
Seeds untreated	2009	22	5	0	2.5	0.5	0
	2010	26.5	0	28	0	6.25	11.25

at 100% field capacity) or CDI (75% of field capacity) treatments, but it was not feasible to use D (rainfed, no irrigation). Cultivation occurred normally with a density of 47 pl per treatment, equivalent to 400 plants m^{-2}. The treatments began with an initial moisture equivalent to field capacity.

The plants were kept in a greenhouse with a temperature of 22°C and a photoperiod of 16 : 8 (light and dark) [44] with high relative humidity (100% for the first four hours, followed by 80%) [45] and grown in plastic containers with a capacity of 84 dm^3 (approximately 0.6 m long × 0.4 m wide × 0.35 m deep). Each container was divided into two equal parts such that each pot contained both treatments. The soil was textured silt/clay which had been conditioned by grinding and sifting (2 mm mesh). The bulk density was 1200 kg m^{-3}, and P, N, and S fertilization was conducted according to a soil analysis.

Interval irrigation was initiated when 75% of the available water had been depleted. A 20 mm fixed dose was used, representing the estimate of useful water in the container, and a pressurized sprayer (Giber) was used to simulate sprinkler irrigation.

Given that wheat stubble constitutes a natural reservoir of many fungi that cause necrotrophic "leaf spots," such as *Drechslera tritici-repentis, Septoria tritici,* and *Bipolaris sorokiniana* [46], plants were inoculated using a non-quantitative method through a recreation of the stubble on the surface of an infected crop [47]. The plants remained in the greenhouse until harvest (October 19).

Nondestructive methods (i.e., weekly observation through manual magnifiers) were used to evaluate disease from the beginning of tillering to the filling of grains. LAI was estimated by subsampling 10 plants per treatment and was repeated at three phenological timepoints: *Z* 2.3, *Z* 4, and *Z* 7. We used a non-destructive method to measure the length and maximum width of the sheet and subsequently multiplied this product by a correction coefficient previously obtained with LAI (LIQUOR LI 3000.A) measurement equipment.

Yield was determined using the same methodologies that were used in the growing field. The trial was conducted in a randomized block design with four replicates, and severity, impact, LAI, and yield parameters were evaluated using analysis of variance (ANOVA).

3. Results

3.1. Seed Analysis and Blotter Test. The GE and GP values obtained for the seeds from the 2009 season were 100% and 99%, respectively, for untreated seeds and 99.5% and 97.5%, respectively, for treated seeds; in 2010, these values were 98.75%, 98.25%, 99.5%, and 99%, respectively. According to Peretti [31], all of these values are within the acceptable ranges for regulated wheat seed.

In the untreated seeds from 2009, the incidence of microorganisms was 30.5%, predominantly "black point" grains caused by *Alternaria* spp. and, to a lesser degree, *Aspergillus* spp., *Drechslera tritici-repentis,* and *Bipolaris sorokiniana*. In contrast, the incidence of microorganisms in treated seeds was 0%.

The conditions of high humidity and high temperatures that occurred towards the end of the growing season in 2009, coupled with poor storage conditions, increased the incidence of the pathogens that cause discoloration and deterioration of seeds. This result was verified in the analyses performed on seeds that were stored by the farmer and used for seeding in the 2010 season, which contained *Alternaria* spp., *Bipolaris sorokiniana*, *Penicillium* spp., and *Rhizopus* spp. The overall incidence of pathogens was 72% for untreated seeds and 12% for treated seeds. A higher incidence of pathogens (*Penicillium* spp., *Rhizopus* spp. and *Alternaria* spp.) was detected during storage (Table 1), while the presence of *Penicillium* spp. in the seeds treated by the farmer would indicate an incorrect dose of fungicide. However, GE and GP were not affected by this pathogen.

Exposure to fungi in the field and during storage affects germination, seedling stand, grain size and weight, and industrial quality. In the case of wheat, these fungi are associated with the grain spotting known as "black scutellum," or "blackpoint." This pathology is characterized by a black or brown coloration in the area of the embryo, which could also be extended to the surrounding area and the groove [33].

3.2. Field Trials (2009 Season). A total of 310 mm effective rainfall was received in 2009, which was greater than the historical average (Figure 1). Because of this heavy rain, only two irrigation treatments totaling 64 mm were applied during the growing season, and both the TI and CDI treatment received the same amount of water. The first irrigation was administered on August 15 during phenological state *Z* 2.3, and the second was administered on September 5 during phenological state *Z* 3.1.5.

The daily average air temperatures were lower than 16°C in June, July, and September, as well as in the first ten days of August and the second ten days of October. The lowest temperature was recorded on July 14 (−8°C). Three consecutive days with temperatures greater than 21°C were

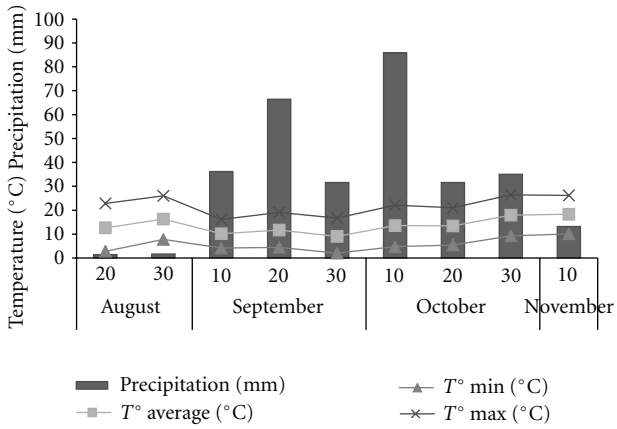

Figure 1: Average, minimum, and maximum air temperature values (°C) and precipitation during the 2009 growing season.

Table 2: Incidence (%) of foliar disease onset for total irrigation (TI), irrigation with controlled deficit (CDI) and dry (D) wheat at various phenological timepoints.

Treatment	Incidence (%)							
	10/09/2009		16/09/2009		30/09/2009		15/10/2009	
	Z 3.1		*Z* 3.9		*Z* 5.6		*Z* 7.05	
TI	17.1	A	19.6	A	14.2	A	27	A
CDI	18.8	A	13.5	A	15.7	A	16.8	A
D	51.3	B	31.3	B	46.7	B	59.5	B

Different letters indicate significant differences according to Tukey ($P \leq 0.05$).

recorded during August and October (last ten days) and two days in November (second ten days).

The ambient relative humidity remained above 60%, and wet leaves were still observed after 15 hours on two consecutive days during the second ten days of July and the first ten days of September.

The three most frequent leaf pathologies, LR, DTR, and SLB, were identified in all treatments.

The incidence of foliar diseases was higher in the D treatment than in the other treatments, although the severity remained below 1% in all treatments. The average disease incidence (percentage of sick leaves with respect to the total number of leaves), both in general and at different phenological stages, was significantly different (Table 2) between the D treatment and the two remaining treatments (CDI and TI).

This pattern was likely observed because the nonirrigated wheat did not achieve total coverage of furrows, even at advanced stages of development (*Z* 6, anthesis), suggesting that at the furrow minor coverage allowed the foliar disease to colonize the upper strata of the crop. This supposition is consistent with the LAI results, which were significantly different between D and the irrigation treatments at *Z* 4 (3.95 versus 4.99 and 5.56 for CDI and TI, resp.). In contrast, the length of leaf wetting caused by sprinkler irrigation was not significantly different than that from the normal wet period due to ambient humidity during the crop cycle.

The individual development of each foliar disease present during the crop cycle was analyzed. In general, epidemics of SLB is caused by a combination of favorable climatic conditions (usually characterized by long periods of light rain and moderate temperatures), certain cultivation practices, the availability of inoculum and the presence of susceptible varieties [48]. A relatively low intensity of foliar disease was observed during this growing season, and diseases were not identified in a uniform manner across treatments or sampling dates. The highest SLB incidence was just 10.5%, observed in samples from the D treatment analyzed on October 15. Injuries to FL-1 that corresponded with SLB were observed in a total of two leaves (of 50 analyzed) in the CDI treatment, but one of these exhibited a severity of less than 1%.

DTR was the most frequently observed disease throughout the analysis period, with an average incidence value of 20.07%. DTR also made up 31.58% of leaf injuries, together with LR. These injuries were observed on both FL and FL-1. The fungus survives in the stubble and, under humid conditions and adequate rainfall, releases spores that infect the lower leaves. From there, the disease advances to higher leaves by rain splashing or air circulation [49], which are conditions that occurred in the D treatment because the furrow was not completely covered.

There were significant differences between the D treatment and the irrigation treatments (CDI and TI), with the exception of the sampling on September 16, in which the differences were not significant (Table 3). This finding can be attributed to a dilution of the disease by an increase in leaf area; DTR was present in basal leaves initially, but these leaves had dried up at more advanced phenological stages.

The average incidence of LR was 11.52% over two sampling dates. LR was first identified in *Z* 3.9 (September 16) and was more frequent in the D treatment (11.73% versus 1.2 and 0.5 for CDI and TI, resp.). During the next week (September 22), an application of 18.7% trifloxystrobin (strobilurin) and 8% cyproconazole (triazole) was made to control the disease. This application remained active up to 60 days, which allowed a reduction in the number of active pustules of *Puccinia triticina* and the control of this disease. However, a second LR infection cycle followed. This likely happened because the urediniospores are relatively long lived and can survive in the field without being deposited on host plants for periods of several weeks [26]; this is why very early treatment, insufficient wetting of the basal leaves, or favorable environmental conditions may allow reinfection by this polycyclic pathogen.

On September 30 (the sampling that was conducted before the new LR attack), the conditions in the experimental area were highly favorable for pathogen development. According to INTA Gálvez, in the first ten days of October, the maximum, minimum, and average temperatures were 22.1°C, 4.7°C, and 13.5°C, respectively. Rainfall of 101 mm accumulated in just 15 days (for comparison, the historical average for October is 105 mm), and several days were misty

Table 3: Incidence (%) of tan spot (DTR) for total irrigation (TI), irrigation with controlled deficit (CDI), and dry (D) treatments at different sampling dates in 2009.

Treatment	Incidence (%) DTR							
	10/09/2009		16/09/2009		30/09/2009		15/10/2009	
	Z 3.1		*Z* 3.9		*Z* 5.6		*Z* 7.05	
TI	16.7	A	19.1	A	14.2	A	11	A
CDI	18.8	A	12.3	A	15.7	A	3.3	A
D	49.73	B	20.1	A	46.7	B	29.5	B

Different letters indicate significant differences according to Tukey ($P \leq 0.05$).

Table 4: Incidence (%) of (LR) for total irrigation (TI), irrigation with controlled deficit (CDI), and dry (D) treatments at different sampling dates in 2009.

Treatment	Incidence (%) LR			
	16/09/2009		15/10/2009	
	Z 3.9		*Z* 7.05	
TI	0.5	A	10.2	A
CDI	1.2	A	7.7	A
D	11.7	B	48.2	B

Different letters indicate significant differences according to Tukey ($P \leq 0.05$).

and foggy, which resulted in water accumulation on the leaves.

At *Z* 7.05 (October 15), this disease was identified in all three treatments. D exhibited the highest incidence (48.2%), while CDI and TI exhibited incidences of 7.67 and 10.17%, respectively. LR infection was found in FL-1 (38% in D, 6% in CDI and 6% in TI), but with severity levels of less than 1% in all treatments. Some FL was also infected, but only in D, with an incidence of 4%. Significant differences ($P \leq 0.05$) were found between D and the two irrigation treatments (Table 4). These differences likely occurred because D, as a result of not adequately covering the furrows, allowed a greater remobilization of spores by wind and rain and consequently higher levels of infection, peaking in FL and FL-1.

In addition to all of the observed foliar diseases of fungal origin, large, dry, grayish-green lesions corresponding to bacterial blight caused by *Pseudomonas syringae* were observed in FL at the last sampling date (October 15). This "leaf blight" is favored by relatively cool temperatures (14 to 23°C) and high relative humidity, which are conditions that were present in the experimental field.

Finally, at physiological maturity (November 12), spikes were analyzed using wet chamber method. The presence of stained glumes caused by the saprotroph fungus *Alternaria* spp. was detected, resulting in 100% incidence in D, 50% in TI, and 46% in CDI treatments. The presence of this fungus was also observed through blotter analyses, as described in the previous section. No frost damage or *Fusarium graminearum*, *Gaeumannomyces graminis,* and *Erysiphe graminis* were observed, but insect damage was present.

Although foliar diseases were common throughout the growing season, high yields were obtained in all treatments, as evaluated by the number of spikes. The only significant differences observed between D and irrigation (DIC and TI) were in the weight of 1000 grains (Table 5).

Table 5: 1000 grains weight, biomass of harvested grain (BHG) and index harvest (HI) measured during 2009 for total irrigation (TI), irrigation with controlled deficit (CDI) and dry (D) treatments.

Treatment	1000 grains weight (g)		BHG (kg ha^{-1})		HI	
TI	33.57	A	8057	A	0.5	A
CDI	33.95	A	8128	A	0.48	A
D	30.88	B	6919	B	0.42	B

Different letters indicate significant differences according to Tukey ($P \leq 0.05$).

The critical period for the main component of wheat yield (grains m^{-2}) ranges from 20 to 30 days before and 10 days after flowering. This is therefore the period during which leaf health is the most crucial for the plant to take advantage of incident radiation to maximize the growth and viability of the grains. Serious losses can also occur when the flag leaf is infected prior to anthesis. However, even the most prevalent diseases never exceeded 4% incidence or 1% severity in FL, so those were considered unlikely to have caused yield loss, regardless of the time of occurrence. Furthermore, crop health was generally very good, and yield differences between treatments were attributed to other causes (e.g., water availability differential, LAI achieved in each treatment).

3.3. Field Trials (2010 Season). During the wheat growing season, from implantation until the harvest, a total of 184 mm effective rainfall was received, well below the normal rainfall for the area of study. Due to the lack of rainfall, four irrigations were conducted, with a net sheet total of 180 mm. The first irrigation consisted of 40 mm conducted on August 7 (*Z* 2.2) with a blade, the second was 50 mm on September 28 (*Z* 3.9), the third was 40 mm on October 5 (*Z* 5.5), and the final irrigation was 50 mm on October 20 (*Z* 7).

The average daily temperature was below 16°C during the last third of June and during July, August, September, and October. In the first days of November, the daily average temperature exceeded 22°C (Figure 2). The lowest minimum temperature was recorded in the month of August at −7.7°C, and the highest maximum temperature was observed at the end of the growing season at 36.2°C. The average humidity remained above 57% over the whole growing season, and wet leaves were observed after 15 hours during the second ten days of July, the third ten days of August, and the first and third ten days of September.

Similar to the results from 2009, all three basic foliar diseases (LR, DTR and, to a lesser extent, SLB) were observed. Disease was significantly more prevalent in the D treatment than in either irrigation treatment ($P < 0,05$). Disease severity reached 15% on FL-1 and 10% on FL in the D treatment but only 5% and 1% on FL-1 in the CDI and TI treatment, respectively.

The first sampling was carried out in *Z* 2.2 (August 16), at which point some development of DTR could be observed

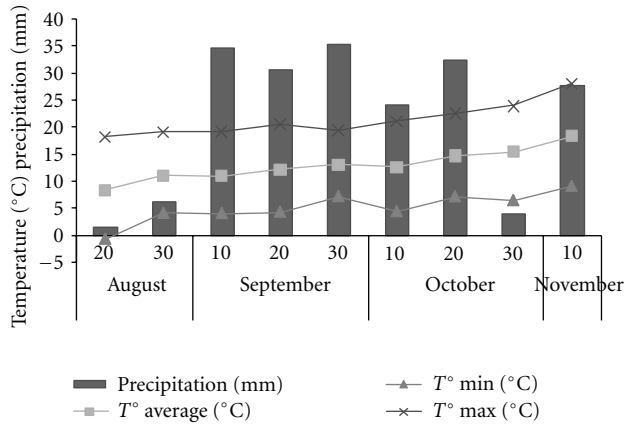

Figure 2: Average, minimum, and maximum air temperature (°C) and precipitation (mm) during the 2010 growing season.

Table 6: Leaf area index (LAI) at two sampling points for total irrigation (TI), irrigation with controlled deficit (CDI), and dry (D) treatments.

Treatment	LAI			
	16/09/2010		13/10/2010	
TI	6.39	A	7.95	A
CDI	6.38	A	6.15	AB
D	3.17	B	5.52	B

Different letters indicate significant differences according to Tukey ($P \leq 0.05$).

on the basal leaves in all three treatments. Later (September 16), the plants that had been irrigated and those that had not been irrigated exhibited different phenological states (Z 3.3 for D and Z 3.1 for TI and CDI). At this time, significant differences were observed between D and the two remaining treatments, both in terms of the variable incidence of diseases and in LAI (Table 6). Both DTR and, to a lesser degree, SLB were present. These diseases reached the upper strata in the D treatment but were restricted to the basal leaves in the irrigated treatments.

At the following sampling at Z 5 (September 30), only DTR was identified. This disease did remain confined to the lower strata in the irrigated treatments, in contrast to what was occurring upland, where DTR colonized the upper strata of the crop in the D treatment. This corresponded to an increased incidence of DTR: 52.7% in D compared to 23.7% and 20.1% for CDI and TI, respectively.

In the following sample, which was collected at Z 6.5 for D and Z 6.2 for TI and CDI (October 13), LR was observed in addition to DTR. Significant differences between irrigated and rainfed treatments were observed (Table 7). As suggested for the previous year, the differences in disease behavior could be due to the fact that plants in D did not totally cover the furrow, which is consistent with the measured LAI values (Table 6).

SLB infection levels were low due to the low rainfall and limited hours of wet leaves, which did not allow SLB establishment and dispersal. The registered incidence values

Table 7: Incidence (%) of foliar disease onset for individual phenological states under total irrigation (TI), irrigation with controlled deficit (CDI), and dry (D) treatments.

Treatment	Incidence (%)					
	16/09/2010		30/09/2010		13/10/2010	
	Z 3.2		Z 5		Z 6.5	
TI	10.3	A	20.1	A	50.2	A
CDI	11.7	A	23.7	A	48.2	A
D	21.6	B	52.7	B	74.3	B

Different letters indicate significant differences according to Tukey ($P \leq 0.05$).

Table 8: Incidence (%) of tan spot (DTR) in various phenological states for total irrigation (TI), irrigation with controlled deficit (CDI), and dry (D) treatments.

Treatment	Incidence of DTR (%)					
	16/09/2010		30/09/2010		13/10/2010	
	Z 3.2		Z 5		Z 6.5	
TI	6.8	A	20.1	A	46.77	A
CDI	9.3	A	23.7	A	45.2	A
D	20.4	B	52.7	B	71.2	B

Different letters indicate significant differences according to Tukey ($P \leq 0.05$).

were 1.22% in D, 3.14% in CDI, and 3.44% at TI. *Septoria tritici*, the causal organism of SLB, requires temperatures of 20 to 25°C [50] and water on leaves for 35 hours followed by 48 hours of high relative humidity, which favored heavy infection [51]. These conditions did not occur until November, and the disease was identified only in the first sampling.

DTR was present from the tillering stage to the end of the growing season. The stay of wheat straw at the soil surface, associated with moderately conducive weather conditions, favored the emergence and constant development of DTR throughout the entire crop cycle, with a variable but consistently increasing incidence according to phenological state. Significant differences were observed between D and the irrigated treatments (Table 8). DTR was observed more frequently on FL and FL-1 in D than in the irrigated treatments. For FL, the incidence of DTR was 24% in D versus 2% in CDI and 12% in TI; for FL-1, the incidence peaked at 86% in D versus 26% in CDI and 30% in TI.

The spread and infection of *Drechslera tritici-repentis* can occur under a wide range of environmental conditions; in general, temperatures between 10 and 30°C and 6- to 48-hour humid periods are sufficient [52–55]. Therefore, tan or DTR appears every year, in contrast to other diseases, such as FHB, which are strongly dependent on environmental conditions [41].

The onset of LR was significantly delayed in 2010 relative to 2009 and was first registered only at the beginning of flowering. According to INTA Galvez, the maximum, minimum, and average temperatures during the second ten days of September were 27.5°C, 8.1°C, and 16.8°C, respectively. A total of 56.4 mm of rainfall was recorded in

Table 9: 1000 grains weight, biomass of harvested grain (BHG) and harvest index (HI) measured during 2010 for total irrigation (TI), irrigation with controlled deficit (CDI) and dry (D) treatments.

Treatment	1000 grains weight (g)		BHG (kg ha^{-1})		HI	
TI	30.3	B	8898	B	0.49	A
CDI	27.2	BA	7820	BA	0.47	A
D	25.7	A	6899	A	0.48	A

Different letters indicate significant differences according to Tukey ($P \leq 0.05$).

the last two weeks of September and the first ten days of October, and leaves were wet for up to 17 consecutive hours for several days in the last third of September.

Statistical analysis showed significant differences in LR incidence between D and irrigation treatments (37.07% versus 8.73% in CDI and 9.5% in TI).

The disease reached FL-1 with an incidence of 40% in D, 2% in CDI, and 10% in TI. The severity reached levels of 15% in D but was less than 1% with only one to two pustules per leaf in the irrigation treatments. LR was observed in FL only in the D treatment, with an incidence of 6% and a maximum severity of 10%.

Finally, on November 11, samples were extracted to analyze the crop yield. Very good results were obtained in all treatments, although a significant difference ($P < 0.01$) in the weight of 1000 grains was observed between D and TI. The differences between CDI and D or TI were not significant (Table 9).

In terms of the health of the spikes and grains, *Fusarium* and *Alternaria* spp. were not identified because there were no environmental conditions that favor their appearance. The grain is susceptible to infection by *Alternaria* during filling or ripening stage, particularly in the states called milky, pasty ([56–58]). The sporulation of *Alternaria* range is between 0°C and 35°C, with optimum at 27°C, but is inhibited below 15°C or above 33°C [59]. Moschini et al. [57] determined that the severity of this disease in Argentina is favored by warmer temperatures, frequent rainfall, and days with relative humidities higher to 62%, in the grain development period spanning about 30 days after heading, but these conditions did not appear in the 2010 season.

Additionally, *Gaeumannomyces graminis* and *Erysiphe graminis* were not detected. On the other hand, agronomic frost did not generate the grains yield decrease, because it occurred in noncritical states for the crop.

3.4. Greenhouse Trials. The first irrigation was conducted during *Z* 2.2 (4 July) for both treatments. Over the entire growing season, TI received 340 mm, while CDI received 240 mm. The initial inoculum from the straw, accompanied by droplets of water from the first irrigation, led to the development of DTR and SLB.

The first symptoms were observed during full tillering (*Z* 2.2, 16 July), although differences became significant after September 14, when the TI treatment was in *Z* 7 and the CDI treatment was in *Z* 6. At this point, yield components had already been defined (Table 10).

DTR infection reached both FL-1 and FL. The maximum incidence in FL-1, observed at *Z* 7.0, was 65% in TI and 45% in CDI, and severity values reached 10% in both treatments. The incidence of DTR in FL reached 70% for TI and 25% for CDI, with a maximum severity of 5% for both treatments.

It should be noted that lower levels of incidence and severity were reported in CDI in the greenhouse trials than under field conditions.

LAI values were similar between treatments (Table 11), but significant differences ($P \leq 0.05$) in the weight of 1000 grains and BHG were observed between the two treatments (Table 12).

4. Discussion

The genera of fungi identified in this analysis correspond to those recognized by Can Xing et al. [60] and Ramirez et al. [61]. These results highlight the importance of using cured seed for seeding. The treatment of seeds with fungicide both eradicates the inoculum so that they do not constitute a primary or initial source of infection as well as protects the seed and seedlings from fungal infection in the soil, which indirectly leads to increased germination and ensures the implementation of cultivation [28].

During the two agricultural cycles evaluated, DTR and LR were the dominant foliar diseases. The cultivated plants remained healthy until advanced stages of development, and the severity of both foliar diseases was low in all of the treatments tested. In the 2009 season, 100% of plants in all treatments exhibited some degree of infection, although the severity was very low (less than 1%). Similarly, in the 2010 season, 100% of the experimental plants exhibited some degree of infection, again with relatively low severity (less than 15% in D, below 5% in CDI and 1% in TI).

Plants that received irrigation treatments exhibited lower levels of foliar diseases in both years. These results conflict with those of a previous study [29] conducted in southern Alberta, which suggested sprinkler irrigation to generate crops that are denser, thus modifying the local microclimate and creating optimal conditions for the development of diseases. However, these authors also suggested that irrigation influences the development of diseases not only through its impact on infection conditions but also through the sporulation of pathogens and later spore dispersal.

The lower disease burden of irrigated plants, observed during both years, may be attributed to the fact that better nourished plants (i.e., plants with greater water accessibility) are generally more tolerant of or less affected by foliar diseases. The work of Annone et al. [62] and that of Formento et al. [49] have shown that nitrogen fertilization at the right time may reduce the development of diseases such as DTR and increase the green tissue remaining in many leafy cultivars. The incidence of DTR in D was lower in 2010 than in the wet year 2009, despite the drier environmental conditions and thus limited water availability. In contrast, Annone and García [63] assert that any measure that directly or indirectly reduces the likelihood of secondary inoculum displacement, among plants both lower and higher levels of culture, reduces the final level of symptoms. Therefore,

Table 10: Incidence (%) of foliar disease onset at different times of measurement for total irrigation (TI) and irrigation with controlled deficit (CDI) treatments.

Treatments	Incidence of foliar disease (%)									
	13/08/2010		24/08/2010		02/09/2010		14/09/2010		28/09/2010	
	Z 3.1		*Z* 4		*Z* 6.5		*Z* 7		*Z* 7.5	
CDI	9.12	A	32.12	A	30.08	A	17.08	A	36.67	A
TI	9.63	A	31.38	A	33.21	A	57.38	B	70	B

Different letters indicate significant differences according to Tukey ($P \leq 0.05$).

Table 11: Leaf area index (LAI) measured during different phenological states under total irrigation (TI) and irrigation with controlled deficit (CDI) treatments.

Treatments	LAI							
	13/07/2010		24/08/2010		02/09/2010		14/09/2010	
	Z 2.3		*Z* 4		*Z* 6		*Z* 7	
CDI	6.57	A	6.16	A	5.63	A	3.56	A
TI	7.14	A	6.56	A	5.56	A	3.86	A

Different letters indicate significant differences according to Tukey ($P \leq 0.05$).

Table 12: 1000 grains weight, biomass of harvested grain (BHG) and harvest index (HI) measured for total irrigation (TI) and irrigation with controlled deficit (CDI) treatments.

Treatments	1000 grains weight (g)		BHG (kg ha^{-1})		HI	
TI	31.75	A	7328	A	0.39	A
CDI	28.59	B	4898	B	0.34	A

Different letters indicate significant differences according to Tukey ($P \leq 0.05$).

some management practices to obtain the highest possible density, such as the adjustment of seeding density based on grain weight, balanced fertilization to produce a compact cultivation structure, and the use of the lowest possible distance between lines and an appropriate cultivar for the desired sowing date, mitigate the development of "leaf spots." This is consistent with the higher incidence of DTR identified in D, which exhibited incomplete furrow coverage and low LAI, and therefore a less dense cultivation structure, which allowed higher levels of infection, even of FL and FL-1. The tests performed by Perello et al. [18] show that the disease incidence increased with the plant age and the severity increased with the growth stage when the evaluation was performed at 14 days compared to 7 days after inoculation. This coincides with the higher incidence found in more advanced stages of the crop at different treatments.

SLB was minimal (trace levels) in both years and was observed more frequently in D plants than in irrigated plants, especially during the more humid 2009 season. These results can be attributed to the density of plants generated in each treatment; as discussed above, plants in the D treatment did not fully cover the grooves, unlike the plants under irrigation, thus allowing the disease to develop further. This finding is consistent with the work of Massaro et al. [64], who emphasized that growing crops at an optimal density, without large spaces between plants, can reduce the epidemic development of "SLB of the road" through secondary infections from the sites of primary infection (basal leaves) into the upper leaves. In contrast, Klatt and Torres [48] have noted that tall varieties of wheat tend to be less affected by SLB than short or semidwarf varieties. In general, this is due to a morphological resistance as a result of the increased distance between the leaves, which tends to impede the upward progress of the pathogen through the splashing of raindrops. In semiannual wheat cultivars, the leaves are closer to each other and the foliage tends to be denser, facilitating the upward spread of disease.

The results of our greenhouse experiments should not override those obtained in the field; significant differences in the parameters severity and incidence for both irrigation systems have not been verified.

Finally, significant differences in productivity were observed between irrigation and rainfed treatments. These differences were due to the application of water during the stem elongation stage (*Z* 3.0), which allowed the survival of more tillers and therefore more spikes than in the D treatment [65].

5. Conclusions

Based on tests carried out over two consecutive years, supplementary sprinkler irrigation of cultivated wheat at opportune moments, even in small quantities, increases grain weight and thus yield without increasing the incidence of foliar disease. Two fundamental principles should be considered for the correct management of wheat diseases: (1) the initial health of the crop should be optimized by using seeds with a low pathogen load and (2) appropriate monitoring should be conducted to properly quantify the diseases present in the field.

References

[1] P. E. Abbate, J. L. Dardanelli, M. G. Cantarero, M. Maturano, R. J. M. Melchiori, and E. E. Suero, "Climatic and water availability effects on water-use efficiency in wheat," *Crop Science*, vol. 44, no. 2, pp. 474–483, 2004.

[2] G. Camussi and R. P. Marano, "Wheat response to supplemental irrigation in the central region of Santa Fe," *Revista FAVE Sección Ciencias Agrarias*, vol. 7, no. 1, pp. 7–21, 1990.

[3] N. Formento, "Productive and health behaviour of wheat cultivars in zero tillage, rainfed and irrigation, season 1999/2000," http://www.planetasoja.com.ar/index.php?sec=10&tra=29376&tit=29379.

[4] A. Salinas, E. Martellotto, P. Salas, J. Giubergia, S. Lingua, and E. Lovera, "Economic performance in supplementary irrigation production system with supplementary irrigation in continuous zero tillage," INTA Manfredi, 2003, http://anterior.inta.gob.ar/f/?url=http://anterior.inta.gob.ar/manfredi/info/documentos/docsuelos/rieresecono.htm.

[5] M. D'Onofrio, "Liquid fertilization in wheat under irrigation. SolUAN application (tillering) combined with FoliarSOL U (anthesis)," Communications- Valle Inferior, pp. 12–15, 2007, http://anterior.inta.gob.ar/f/?url=http://anterior.inta.gob.ar/valleinferior/info/r60/03.pdf.

[6] R. Carretero, R. Serrago, and D. Miralles, "Foliar diseases of wheat. An eco-physiological perspective," 2nd Meeting Wheat in the Central Region, pp. 17–23, 2007, http://www.bccba.com.ar/bcc/images/00001773_Actas%20de%20la%20Jornada%20vf.pdf.

[7] M. Carmona and E. Reis, *Potential Evaluation System for Yield in Wheat. Its Usefulness for Application of Fungicides for Economic Control of Diseases*, Imprenta Rago, Buenos Aires, Argentina, 1st edition, 2001.

[8] A. E. Perelló and M. Moreno, "Survey of foliar diseases of wheat and identification of causal agents," in *Proceedings of the 6th Congreso Nacional de Trigo. IV Simposio Nacional de Cultivos de siembra otoño-invernal*, p. 257, INTA, Bahia Blanca, Argentina, 2004.

[9] M. R. Simón, F. M. Ayala, S. I. Golik et al., "Integrated foliar disease management to prevent yield loss in Argentinian wheat production," *Agronomy Journal*, vol. 103, no. 5, 2011.

[10] J. Fernández and A. Corro Molas, "Disease management in wheat," 2003, http://anterior.inta.gob.ar/f/?url=http://anterior.inta.gob.ar/anguil/info/pdfs/boletines/bol76/cap7.pdf.

[11] R. Massaro, J. Castellarín, and J. Andriani, "Foliar diseases of wheat: frequency recorded during 6 years in southern Santa Fe and its relationship to climatic variables," pp. 55–58, 2007, http://www.elsitioagricola.com/articulos/massaro/enfermedadesFoliaresDelTrigo.pdf.

[12] M. Sillon, E. Weder, G. Gianinetto, and J. Albrech, "Phytosanitary analysis of the 2006 season and possible future scenarios," 2nd Meeting Wheat in the Central Region, pp. 25–30, 2007, http://www.bccba.com.ar/bcc/images/00001773_Actas%20de%20la%20Jornada%20vf.pdf.

[13] R. M. Hosford, "A form of Pyrenphora tricosthoma pathogenic to wheat and other grasses," *Phytopathology*, vol. 61, pp. 28–32, 1971.

[14] R. M. Hosford and R. H. Busch, "Losses in wheat caused by *Pyrenophora trichostoma* and *Leptosphaeria avenaria* f. sp. Triticea," *Phytopathology*, vol. 64, pp. 184–187, 1974.

[15] R. G. Rees, G. J. Platz, and R. J. Meyer, "Yield losses in wheat from yellow spot comparison of estimates derived from single tillers and plots," *Australian Journal of Agricultural Research*, vol. 33, pp. 899–908, 1982.

[16] R. G. Rees and G. J. Platz, "Effects of yellow spot on wheat: comparison of epidemic at different stages of crop development," *Australian Journal of Agricultural Research*, vol. 34, pp. 39–46, 1983.

[17] A. Tekauz and R. G. Platford, "Tan spot of wheat," *Manitoba Agronomy Proceedings*, pp. 60–65, 1982.

[18] A. E. Perelló, V. Moreno, M. R. Simón, and M. Sisterna, "Tan spot of wheat (*Triticum aestivum* L.) infection at different stages of crop development and inoculum type," *Crop Protection*, vol. 22, no. 1, pp. 157–169, 2003.

[19] R. G. Rees, R. J. Mayer, and G. J. Platz, "Yield losses in wheat from yellow spot: a disease-loss relationship derivated from single tillers," *Australian Journal of Agricultural Research*, vol. 32, pp. 851–859, 1981.

[20] G. Kraan and J. E. Nisi, "Septoria of wheat in Argentina. Status of the crop against the disease," in *Proceedings of the Septoria Tritici Workshop, CIMMYT*, L. Gilchrist et al., Ed., pp. 1–8, Mexico City, DF, Mexico, September 1993.

[21] M. R. Simón, A. E. Perelló, C. A. Cordo, and P. C. Struik, "Influence of Septoria tritici on yield, yield components, and test weight of wheat under two nitrogen fertilization conditions," *Crop Science*, vol. 42, no. 6, pp. 1974–1981, 2002.

[22] J. Annone, A. Calzolari, O. Polidoro, and H. Conta, "Effect of tan spot caused by Septoria tritici on yield," Informe 122, INTA EEA Pergamino, Pergamino, Argentina, 1991.

[23] E. Alberione, C. Bainotti, J. Fraschina et al., "Evaluation of seed treatment products for the control of yellow spots (*Drechslera tritici repentis*) in wheat," pp. 59–63, 2010, http://agrolluvia.com/wp-content/uploads/2011/05/EVALUACION-DE-PRODUCTOS-CURASEMILLAS-PARA-EL-CONTROL-DE-MANCHA-AMARILLA-DRECHSLERA-TRITICI-REPENTI-EN-TRIGO.pdf.

[24] N. Formento, J. de Souza, and J. C. Velázquez, "Yield losses by yellow spot in wheat (*Pyrenophora tritici-repentis, anamorph: Drechslera tritici-repentis*). Preliminary results," 2007, http://anterior.inta.gob.ar/f/?url=http://anterior.inta.gob.ar/parana/info/documentos/produccion_vegetal/trigo/enfermedades/20323_071221_perd.htm.

[25] J. A. Kolmer, "Genetics of resistance to wheat leaf rust," *Annual Review of Phytopathology*, vol. 34, pp. 435–455, 1996.

[26] A. P. Roelfs, R. P. Singh, and E. E. Saari, *Rust Diseases of Wheat: Concepts and Methods of Disease Management*, CIMMYT, Mexico City, Mexico, 1992.

[27] S. G. Layva Mir, E. E. Rangel, E. Villaseñor Mir, and J. H. Espino, "Effect of leaf rust (*Puccinia triticina.* Eriks) on wheat yield (*Triticum aestivum* L.) in dry conditions," Revista Mexicana de Fitopatología, pp. 40–45, ISSN: 0185-3309, 2003.

[28] N. Formento, "Health and aging of wheat seeds," 2002, http://crea30agomlqn.wikispaces.com/file/view/Sanidad+y+Curado+de+las+Semillas+de+Trig1.pdf.

[29] T. K. Turkington, A. Kuzyk, R. Dunn et al., "Irrigation and plant disease management," 2004, http://www1.agric.gov.ab.ca/$department/deptdocs.nsf/all/ind10759/$file/irrigation_and_plant_disease_management.pdf?OpenElement.

[30] J. Andriani, N. Huguet, and C. Regis, "Evaluation of wheat cultivars with supplemental irrigation," in *To Improve Production 16, Wheat Season 2000/01*, INTA EEA Oliveros, Santa Fe, Argentina, 2001.

[31] A. Peretti, *Seed Testing Manual*, Hemisferio Sur, Buenos Aires, Argentina, 1994.

[32] R. Arango Perearnau, R. Craviotto, and C. Gallo, "New container for analysis of germination and seed health: use in pathology," in *Proceedings of the 3rd Congreso de soja del MERCOSUR, CD*, pp. 166–169, 2006.

[33] M. Sisterna and D. Minhot, "The genus Marielliottia (hyphomycetes, Ascomycetes): a new taxon mycoflora associated with the grain of wheat in Argentina," *Boletín Sociedad Argentina de Botánica*, vol. 41, pp. 177–182, 2006.

[34] J. C. Zadoks, T. T. Chang, and C. F. Konzak, "A decimal code for growth stages of cereals," *Weed Research*, vol. 14, pp. 415–421, 1974.

[35] M. Carmona, "Chemical control of foliar diseases in wheat," 2007, http://www.concienciarural.com.ar/agricultura/control-quimico-de-enfermedades-foliares-en-el-cultivo-de-trigo_a226.

[36] A. Champeil, T. Doré, and J. F. Fourbet, "Fusarium head blight: Epidemiological origin of the effects of cultural practices on head blight attacks and the production of mycotoxins by Fusarium in wheat grains," *Plant Science*, vol. 166, no. 6, pp. 1389–1415, 2004.

[37] N. Formento, "Dose and timing of fungicides to control foliar diseases in wheat," Año 2001, 2002, http://dl.dropbox.com/u/12187844/Aguas/P%C3%A1gina/bibliografia%20adicional/Formento%2037.pdf/.

[38] R. F. Peterson, A. B. Campbell, and A. E. Hannah, "A diagrammatic scale for estimating rust intensity of leaves and stem of cereals," *Canadian Journal of Research Section C*, vol. 26, pp. 496–500, 1948.

[39] R. W. Stack and M. P. McMullen, "A visual scale to estimate severity of fusarium head blight in wheat," Extensión Serv. North Dakota State University, USA, 1995.

[40] N. Formento, "Effectiveness of tebuconazole and epoxiconazole more Carbendazim in the control of foliar diseases and ear of wheat," 2003, http://agrolluvia.com/wp-content/uploads/2011/05/Eficacia-del-Metconazole-y-Epoxiconazole-+-Carbendazim-en-el-Control-de-Enfermedades-Foliares-y-de-la-Espiga-del-Trigo.pdf.

[41] M. Carmona, E. M. Reis, and P. Cortese, *Leaf Spot of Wheat. Diagnosis, Epidemiology and New Approaches to the Management*, BASF, Buenos Aires, Argentina, 1999.

[42] M. Carmona, E. M. Reis, and P. Cortese, *Wheat Rusts: Symptoms, Epidemiology and Control Strategies*, Imprenta Commiso Industria Gráfica, Buenos Aires, Argentina, 1st edition, 2000.

[43] INFOSTAT, Universidad Nacional de Córdoba, Editorial Brujas, Córdoba Argentina, 1st edition, 2009.

[44] C. Jobet, J. Zúñiga, H. Campos de Quiroz, P. Rathgeb, and G. Marín, "Doubled haploid plants (DH) generated by intergeneric rosses of wheat x maize," 2002, http://www.inia.cl/medios/biblioteca/serieactas/NR29064.pdf.

[45] S. Leyva Mir, J. Terrones Rodriguez, J. Herrera Espino, and H. Villaseñor Mir, "Analysis of inductive resistance components of a slow development of wheat rust (*Puccinia triticina Eriks.*)," 2007, http://redalyc.uaemex.mx/redalyc/pdf/302/30211241007.pdf.

[46] N. Formento and Z. Burne, "Effectiveness of fungicides in the control of tan spot (*Drechslera tritici-repentis*) in wheat," 2002, https://dl.dropbox.com/u/12187844/Aguas/P%C3%A1gina/bibliografia%20adicional/Formento%2046.pdf.

[47] Z. Eyal, A. L. Scharen, J. M. Prescott, and M. Van Ginkel, *Wheat Disease Septoria: Concepts and Methods Related to the Management of these Diseases*, ClMMYT, Mexico City, Mexico, 1987.

[48] A. R. Klatt and E. Torres, "An overview of diseases of wheat caused by Septoria, Programa de Trigo del CIMMYT," in *Proceedings of the Conferencia Regional Sobre la Septoria Leaf Blotch del Trigo*, M. M. Kohli and L. T. Van Beuningen, Eds., CIMMYT, Mexico City, México, 1990.

[49] N. Formento, J. C. Velázquez, and J. de Souza, "Epidemiology of diseases in wheat," *Boletín Fitopatológico. Cultivo de Trigo*, vol. 1, no. 2, 2009.

[50] M. Díaz de Ackermann, "Leaf spot in wheat caused by *Mycosphaerella graminicola* (Fuckel) schroeter perfect state of *Septoria tritici Rob. Es Desm*," in *Curso Manejo de Enfermedades del Trigo*, M. M. Kohli, J. Annone, and R. García, Eds., pp. 100–117, INTA-CIMMYT, Pergamino, Argentina, 1995.

[51] G. Shaner and R. Finney, "Wheater and epidemics of septoria leaf blotch of wheat," *Phytopathology*, vol. 66, pp. 781–785, 1976.

[52] C. R. Larez, R. M. Hosford, and T. P. Freeman, "Infection of wheat and oats by *Pyrenophora tritici-repentis* and initial characterization of resistance," *Phytopathology*, vol. 76, pp. 931–938, 1986.

[53] R. M. Hosford, C. R. Larez, and J. J. Hammond, "Interaction of wheat period and temperature on *Pyrenophora tritici-repentis* infection and development in wheat of differing resistance," *Phytopathology*, vol. 77, pp. 1021–1027, 1987.

[54] D. N. Sah, "Effects of leaf wetness duration and inoculums level on resistance of wheat genotypes to *Pyrenophora tritici-repentis*," *Journal of Phytopathology*, vol. 142, pp. 324–330, 1994.

[55] J. Annone, "The tan spot or yellow wheat," in *Curso Manejo de Enfermedades del Trigo*, M. M. Kohli, J. Annone, and R. García, Eds., pp. 118–134, INTA-CIMMYT, Pergamino, Argentina, 1995.

[56] R. L. Conner, "Influence of irrigation and precipitation on incidence of blackpoint in soft white spring wheat," *Canadian Journal of Plant Pathology*, vol. 11, pp. 388–392, 1989.

[57] R. C. Moschini, M. N. Sisterna, and M. A. Carmona, "Modelling of wheat black point incidence based on meteorological variables in the southern Argentinean Pampas region," *Australian Journal of Agricultural Research*, vol. 57, no. 11, pp. 1151–1156, 2006.

[58] M. Miravalles, V. Beaufort, and F. Möckel, "Relative susceptibility to blackpoint in durum wheat varieties of Argentina," *Phyton*, vol. 77, pp. 263–273, 2008.

[59] L. Carrillo, "Fungi in Foods and Feeds," in *Alternaria*, pp. 81–86, Universidad Nacional de Salta, Tartagal, Salta, Argentina, 2003.

[60] D. Can Xing, W. Xiao Ming, Z. Zhen Dong, and W. Xao Fei, "Testing of seedborne fungi in wheat germplasm conserved in the national crop genebank of China," *Agricultural Sciences in China*, vol. 6, no. 6, pp. 682–687, 2007.

[61] N. A. Ramirez, M. Mezzalama, A. Carballo, and A. Livera, "Effect of fungicides on seed physiological quality of wheat miller (*Triticum aestivum* L.) and their effectiveness in controlling *fusarium graminearum* Schwabe," *Revista Mexicana de Fitopatología*, vol. 24, no. 2, pp. 115–121, 2006.

[62] J. Annone, R. García, O. Polidoro, and A. Calzolari, "Response of cultivars of wheat under different tillage combinations of nitrogen, supplemental foliar fungicide treatment," Trigo en Siembra Directa, pp. 61–67, AAPRESID, 2003.

[63] J. Annone and R. García, "The main wheat leaf spot. Importance, epidemiology and strategies to reduce their impact on production," 2003, http://www.inta.gov.ar/ediciones/idia/cereales/trigo10.pdf.

[64] R. A. Massaro, A. Gargicevich, M. González et al., "Association between cultural variables and severity of wheat leaf diseases," 2005, http://www.agrositio.com/vertext/vertext.asp?id=44901&se=12.

[65] R. A. Fischer, "Wheat physiology: a review of recent developments," *Crop and Pasture Science*, vol. 62, no. 2, pp. 95–114, 2011.

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Research Article

A Predictive Model for Daily Inoculum Levels of *Gibberella zeae* in Passo Fundo, Brazil

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The deposition of spores of *Gibberella zeae*, the causal agent of *Fusarium* head blight of wheat, was monitored during 2008–2011, in Passo Fundo, RS, Brazil. The sampling was carried out in a 31-day period around wheat flowering. The numbers of colonies formed were related to meteorological variables. In this study, a hierarchical autoregressive binary data model was used. The model relates a binary response variable to potential covariates while accounting for dependence over discrete time points. This paper proposes an approach for both model parameter inference and prediction at future time points using the Markov chain Monte Carlo (MCMC). The developed model appeared to have a high degree of accuracy and may have implications in the disease control and risk-management planning.

1. Introduction

Wheat (*Triticum aestivum* L.) is an important crop in Brazil especially in the South: 90% of the growing area is established in the states of Rio Grande do Sul, Santa Catarina, and Paraná. In this subtropical region, weather conditions during the growing season favor the occurrence of foliar and flowering diseases [1]. Usually, two to three fungicide applications may be needed to control these diseases, thus increasing production costs [2].

Among wheat diseases, *Fusarium* head blight (FHB) has increased its pressure on crops in many production regions. Apart from losses in grain yield and reductions in baking and seed quality, the major peril due to FHB is the contamination of grain with toxic fungal secondary metabolites known as mycotoxins. The most prevalent mycotoxins are trichothecenes such as deoxynivalenol (DON) and nivalenol (NIV). To protect consumers from mycotoxicosis, many countries, including Brazil, have established maximum allowed levels for the most prevalent *Fusarium* mycotoxins in cereals and cereal products [3].

The main causal agent of the disease is *Gibberella zeae* (Schwein.) Petch (anamorph *Fusarium graminearum* Schwabe) [4], a homothallic fungus that survives in host debris on the soil. Inoculum is made up of ascospores and macroconidia that are dispersed by rain splash and wind, landing on wheat heads and infecting the plant during flowering and grain-filling stages [5]. FHB has worldwide distribution although the severities of the outbreaks are influenced by local weather conditions [6]. The wider adoption of minimum and no tillage, short rotations with maize and global climate variability and change are central in the debate on the causes for the re-emergence and expansion of the disease worldwide [7].

In Brazil, similar to other parts of the world, an increasing frequency of severe FHB outbreaks has been reported over the last two decades (especially after 1990) resulting in severe yield losses [1, 8, 9]. No wheat varieties are immune to FHB and resistance is generally controlled by several genes of moderate/weak effect and they are defined genetically as quantitative trait loci (QTL). In addition to these, mycotoxins affect production throughout the world, the ability to predict FHB and DON and other mycotoxin contamination is important to reduce the year-to-year risk for producers. Owing to these dangerous consequences of reducing wheat yield and quality around the world, computer models, based

on weather variables (temperature, rainfall, and moisture level), have been developed to predict the likelihood of occurrence of FHB and DON contamination in wheat [10].

Inoculum quantification is an important step in process-based model development [11]. It has been shown that weather factors such as precipitation and temperature are highly related to inoculum density in the atmosphere [12–15]. Statistical models for this purpose have been made using techniques based on linear regression or other generalizations. When the response of the models is binary data, such as inoculum incidence, data fitting with generalized linear models based on logit link function [16] has proven to be the most appropriated. However, when the data are collected at successive time points such as daily or hourly, it may be correlated and under these circumstances an autoregressive structure, specially AR(1), can be used to solve the correlation in the data. Examples of this approach were proposed by [17–19].

This study examines the potential impact of climate variability on daily deposition levels of *G. zeae* propagules using hierarchical logistic model techniques. Our goal is to establish a statistical model of spore deposition that can be used to calculate probabilities of FHB infection as the wheat phenology advances from heading to soft dough stage. Within this framework, we aim, in the future, to relate the risk of FHB infection to the amount of inoculum within wheat fields, host phenology, susceptibility, and weather factors.

2. Materials and Methods

2.1. Study Area. Passo Fundo is located at the Planalto Médio Region, northern Rio Grande do Sul State, Brazil (latitude 28°15′00″S, longitude 52°25′12″W, altitude 684 m) (Figure 1). The region is one of the major wheat production areas in Brazil.

2.2. Data Collection. Patterns of spore deposition were monitored during 2008 through 2011. Each sample period is referred to as a wheat growing season environment. Consecutive sample periods covered the interval of 31 days starting from September 15th. Petri dishes (90 mm in diameter; surface area = 283 mm^2) containing *Fusarium* selective media (FSM) were used to sample viable spores of *G. zeae* from air. The FSM consisted of a modified Nash-Snyder formulation, prepared as described by [20]. The plates were mounted on a wind-driven sampler previously used by [12]. Two daily samplings performed at 9:00 and 21:00 h. were used with days deemed to begin and end at 09:00 hours GMT for consistency with the meteorological data. Plates were exposed in two periods of 12 h each, called night- and day-time sampling. After exposure to the environment, the plates were transported to the laboratory and incubated in a growth chamber (25°C and 12 h of darkness) in order to promote fungal growth. Colonies of *G. zeae* were identified according to color and morphology. Doubtful cases were transferred to Petri dishes containing PDA (potato dextrose agar) for comparison with confirmed true *G. zeae* colonies. The number of *G. zeae* colonies was recorded for each plate as CFU (colony forming units). Other *Fusarium* species were observed but not identified at the species level. Plates were placed, in the local weather station site, at 120 cm above a grass-covered ground.

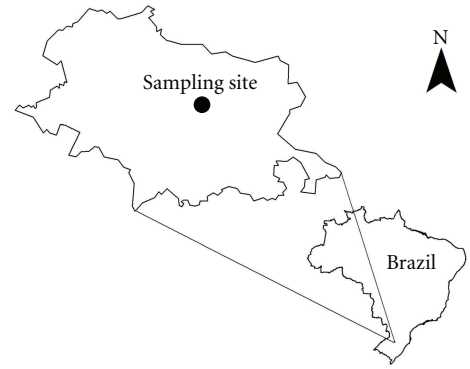

Figure 1: Location of the sampling site in Passo Fundo, Brazil.

Daily weather data comprised of maximum and minimum temperature (°C), total precipitation (mm), sunshine hours (h), and mean relative humidity (%). The data were provided by the National Institute of Meteorology (INMET).

2.3. Data Analysis. Records from the spore sampler were used as daily values (sum of two 12-hour periods), expressed as observed proportion of spores per day. The maximum colony count per Petri dish was fixed to 60 due to operation limitations (visual accuracy during colony identification) in this way the maximum count in each day was 120. The dataset consists of 93 observations where the variable of interest is a binary indicator $y_{[t]}$ with values in range 0-1 at time t. For the climate variables, each observation was centered on climatological normal representing the prevailing set of weather conditions calculated over a period of 30 years (1961–1990) in Passo Fundo this preprocessing step improved the simulation stability and accounted for strong serial correlation intrinsic to environmental data sequentially registered. For example in a given day the total precipitation observed is 25 mm, so the adjusted value is calculated like $25 - 6.2317 = 18.7683$ where the value 6.2317 corresponded to the precipitation mean observed in the months of September and October from the climatological normal in Passo Fundo.

We fitted an Hierarchical Autoregressive Binary Data Model (HARBDM) to the data. Model development was based on a combined approach from [19, 21, 22] using the free available software WinBUGS [23]. The statistical analysis and graphs were done in R [24] using the package R2WinBUGS [25]. We ran the simulation with 10000 interactions, in 3 chains, discarding the first 5000. The convergence of the chains was tested using Gelman-Rubin method [26]. We then took percentiles 5, 50, and 95% from the simulation results to get parameter estimates and credibility intervals. The density probability for the median spore incidence, by year, was fitted to a beta distribution.

MCMC (Markov chain Monte Carlo) methodology [27] is adopted to simulate from the full posterior distribution.

Updates were obtained by using the Gibbs sampler [28, 29]. The Gibbs sampler split the state vector into a number of components and updated each in turn by a series of Gibbs transitions. Posterior probability estimates for the incidence of spores in a given day were obtained in the context of the group (year).

The data of 2008–2010 was used to construct the model and the data of 2011 to validate the model.

2.3.1. Data Model. The functional form of the model is shown below:

$$\begin{aligned}
\text{logit}\left\{\Pr\left(Y_{[1,i]}=1\right)\right\} &= \pi_{[1,i]},\\
\pi_{[1,i]} &= \beta_{0[i]} + \sum_{k=1}^{4}\beta_{k[i]}X_{k[1,i]}\\
&\quad +\varepsilon_{[1,i]}, \quad \text{when } t=1,\\
\text{logit}\left\{\Pr\left(Y_{[t,i]}=1\right)\right\} &= \pi_{[t,i]},\\
\pi_{[t,i]} &= \beta_{0[i]} + \sum_{k=1}^{4}\beta_{k[i]}X_{k[t,i]} + \phi_{[i]}\pi_{[t-1,i]}\\
&\quad +\varepsilon_{[t,i]}, \quad \text{when } t\geq 2.
\end{aligned} \tag{1}$$

In (1) we used terms i for years (2008 to 2010) and t for days (1 to 31) after 15^{th} of September.

3. Results and Discussion

A total of 93 sampling days was included in the study. During the sampling time 2076 *G. zeae* colonies were accumulated. The lowest number of colonies (343) was recorded in 2010. Spores were present in 86 out of 93 days. Summary statistics for each dependent and independent variable are shown in Table 1. The number of rainy days by year were, respectively, 14, 15, and 13.

Visual observations in Figure 2 revealed that climate variability and the number of *G. zeae* spores present in the air appeared to be associated. Both relative humidity and rain were associated positively with spore incidence while sunshine hours were associated negatively. Temperature amplitude appeared to be weakly related to spore incidence.

The mean and median values of *G. zeae* incidence were very similar in 2008 and 2009 but contrasted to those observed in 2010 (Table 1).

The monitoring of deposition of *G. zeae* spores by means of Petri dishes containing selective media provided estimates of inoculum levels in the air of Passo Fundo area. Moderate-to-severe *Fusarium* head blight epidemics occurred during the study period. Thus, the strategy of monitoring spores of *G. zeae* through different wheat growing seasons was successful in obtaining data from *Fusarium* head blight epidemic and nonepidemic years. During each sampling period, *Fusarium* head blight incidence ranged from traces to about 100% of spikes affected. The wheat seasons of 2008, 2009, and 2010 in Passo Fundo area were categorized, respectively, as epidemic, highly epidemic, and nonepidemic.

Table 1: Summary statistics from raw data observed from September 15th to October 15th, during three years, in Passo Fundo, RS, Brazil.

	GZ	RH	SH	TA	RAIN
2008					
Mean	0.23	0.73	0.55	10.59	4.58
SD	0.28	0.13	0.38	4.20	9.62
MED	0.11	0.70	0.61	11.70	0.00
IQR	0.27	0.24	0.68	7.35	4.00
2009					
Mean	0.23	0.78	0.47	10.19	10.78
SD	0.32	0.11	0.37	4.12	22.98
MED	0.08	0.76	0.52	9.20	0.00
IQR	0.17	0.20	0.72	6.95	8.25
2010					
Mean	0.09	0.74	0.48	10.66	8.72
SD	0.12	0.12	0.35	4.23	25.28
MED	0.03	0.75	0.53	11.50	0.00
IQR	0.10	0.18	0.69	6.10	3.15

GZ: *Gibberella zeae*, RH: relative humidity, SH: sunshine hours, TA: temperature amplitude, RAIN: rainfall. SD: standard deviation, MED: median, IQR: interquartile range.

Coincidently, the ENSO phases in each period corresponded to "neutral," "warm," and "cold," respectively. This is in agreement with reports [9] that FHB epidemics are likely to be more severe in "neutral" and "warm" than in the "cold" phase, in this part of the world.

The model constructed with the data between September 15 and October 15 in 2008, 2009, and 2010, respectively, was used to predict the density of *G. zeae* spores in the air of Passo Fundo. In Table 2, the estimated parameters by group factor (year) are deviations from the climatological normal. In this context we can see that, for a day with no deviation from normal, the probability of incidence of FHB, expressed by $e^{\beta_0}/(1+e^{\beta_0})$ for each day, corresponds, by year, to 0.18, 0.20, and 0.06, respectively. The correlation index (ϕ) (Table 2) between days in 2008 and 2009 were negative and in 2010, positive.

In Table 3, the scale parameter β can be used to estimate the daily inoculum level for a specific year. For example in Passo Fundo, on years with β below a cutpoint (7.0), an alert for moderate-severe status could be set in a monitoring disease system. Otherwise, these parameters (α, β) could be used as priory information in Bayesian model framework.

Another measure of interest is the odds ratio (Table 4) that represents the increase in the incidence by each change in unit deviation from variables from the model.

The adjusted model is showed in (Figure 3) and was then validated by the actual observations (Figure 4). The validation analysis indicates that the model had reasonable accuracy over the predictive period, even though in day 9 the predicted spike was well behind that of the actual peak.

Mechanisms of spore deposition are gravity and scrubbing by rain drops which contribute in a random manner

Table 2: Estimated posterior medians and 95% credibility interval for the autoregressive model.

	2008			2009			2010		
	2.5%	50%	97.5%	2.5%	50%	97.5%	2.5%	50%	97.5%
β_0	−1.699	−1.511	−1.305	−1.586	−1.364	−1.186	−3.027	−2.826	−2.649
β_{TA}	−0.124	−0.064	−0.004	0.105	0.145	0.187	0.020	0.087	0.153
β_{RAIN}	0.028	0.043	0.057	0.014	0.017	0.021	0.005	0.009	0.013
β_{RH}	5.023	6.683	8.269	−0.927	1.180	3.561	−1.240	1.546	4.176
β_{SH}	−0.034	0.671	1.373	−3.427	−2.609	−1.626	−3.705	−2.691	−1.600
ϕ	−0.913	−0.429	0.141	−0.996	−0.880	−0.469	0.329	0.873	0.995

β_0: intercept, TA: iemperature implitude, RAIN: rainfall, RH: relative humidity, SH: sunshine hours, ϕ: correlation index [AR(1)].

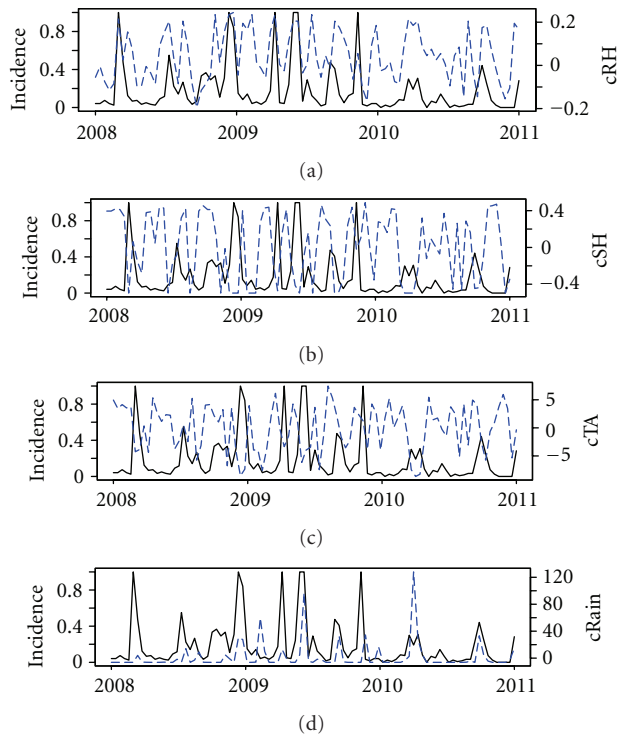

Figure 2: Relative daily incidence of *Gibberella zeae* spores (continuous line) and meteorological variables (dashed lines top to bottom graphs: relative humidity, sunshine hours, temperature amplitude, and rainfall), centered on climatological normal in Passo Fundo during 31-day period starting on September 15th of 2008, 2009, and 2010, respectively.

Table 3: Adjusted parameters for the beta distribution.

	2008	2009	2010
α	1.20	1.86	1.49
β	3.63	5.99	14.58

to spore deposition [30]. Wet deposition becomes relatively more important as the distance from a source of spores increases, because dry deposition tends to be limited to the removal of spores near the ground, whereas wet deposition can sweep spores from the entire depth of the spore cloud. For example, a significant portion of ascospores of *Venturia inaequalis* was collected during hours that rainfall rate was less than 0.25 mm h-1 [31].

Therefore, it is likely that the error range in predicting spore deposition, in our work, is due to the fact that we used total daily rainfall in the model in lieu of actual time courses of rainfall.

Another possible explanation would pertain to packets of air (a localized region of low air density or a descending air current) that settled those days containing higher spore populations due to an earlier massive spore release of some origin in an upwind direction, perhaps at a considerable distance.

The model we developed in this paper describes the deposition probability of airborne spores according to weather factors. In this study, a HARBD model was used in this attempt to develop a *G. zeae* spore density forecasting system for improving our capacity to predict FHB outbreaks. The developed model appeared to have a high degree of accuracy and may have implications in the disease control and risk-management planning.

The weaknesses of this study must be acknowledged. First, this is a broad assessment of the relationship between climate variability and the incidence of spores of *G. zeae* at one location. More detailed risk assessment at regional and farm levels may also be required if a comprehensive and systematic risk assessment is to be made. Inclusion of other information (e.g., crop management, stubble characteristics, and other fungal-relevant environmental information) may further improve the model. Second, the model may only be applicable to Passo Fundo and areas with a similar climate background, since only local data were used in the construction of the model.

4. Conclusions

The autoregressive model is a useful tool for interpreting and applying to local plant disease control measures. Once a satisfactory model has been obtained, it can be used to forecast expected numbers of cases for a given number of future time intervals. Since predictions from HARBD model have the capacity to forecast when an outbreak is likely to occur, it therefore has great potential to be used as a decision-support tool for both tactical and strategic recommendations for FHB management.

Table 4: Odds ratio and 95% interval for the autoregressive model.

	2008			2009			2010		
	2.5%	50%	97.5%	2.5%	50%	97.5%	2.5%	50%	97.5%
β_0	0.18	0.22	0.27	0.20	0.26	0.31	0.05	0.06	0.07
β_{TA}	0.88	0.94	1.00	1.11	1.16	1.21	1.02	1.09	1.17
β_{RAIN}	1.03	1.04	1.06	1.01	1.02	1.02	1.01	1.01	1.01
β_{RH}	151.87	798.71	3901.05	0.40	3.25	35.20	0.29	4.69	65.10
β_{SH}	0.97	1.96	3.95	0.03	0.07	0.20	0.02	0.07	0.20

β_0: intercept, TA: temperature amplitude, RAIN: rainfall, RH: relative humidity, SH: sunshine hours.

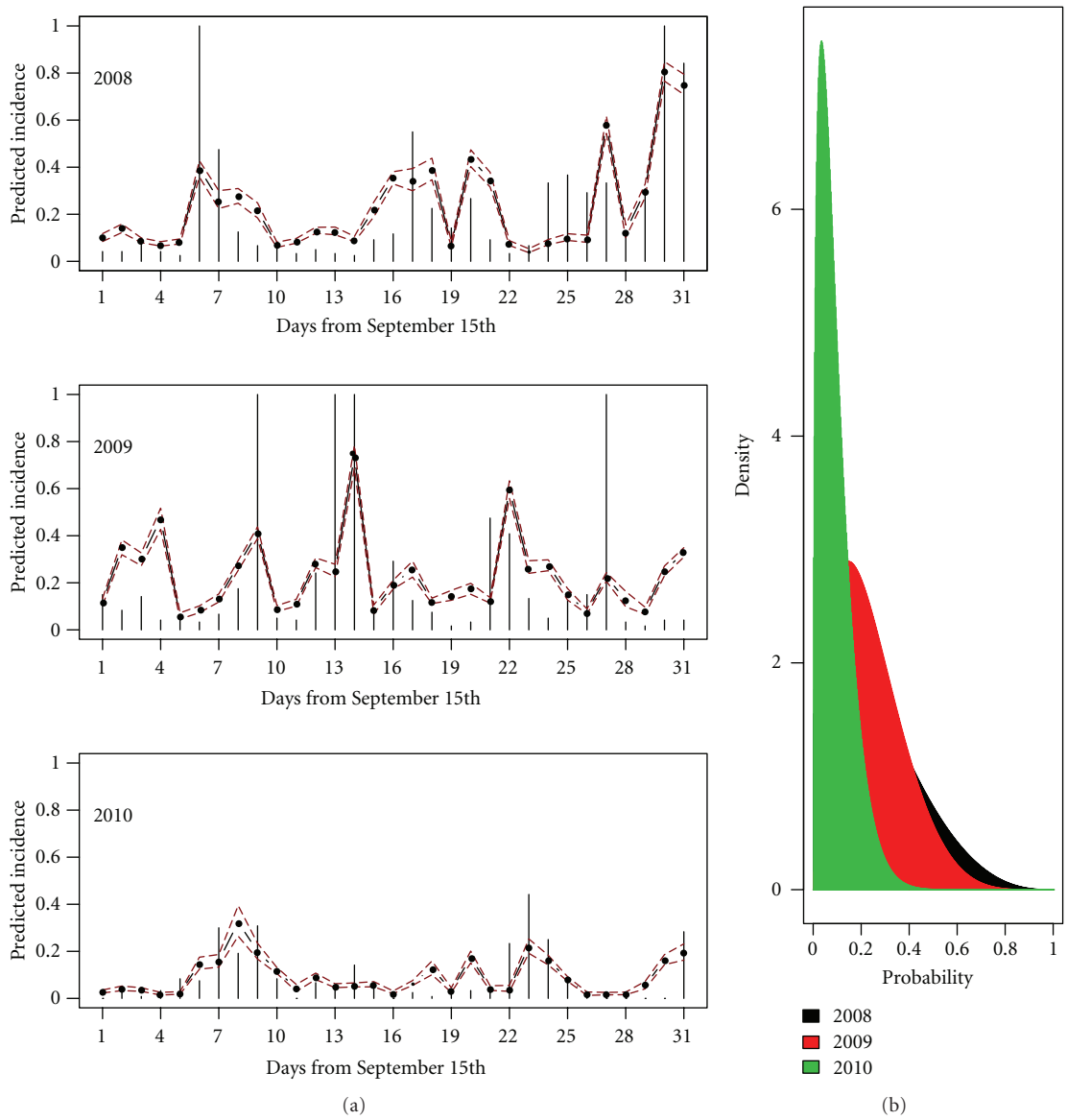

Figure 3: Predicted incidence of spores in a given day by year represented by the continuous line and simulated interval (5, 95 percents) dashed line. The vertical lines represent observed values (a). Estimated density for the beta probability distribution function in the different years (b).

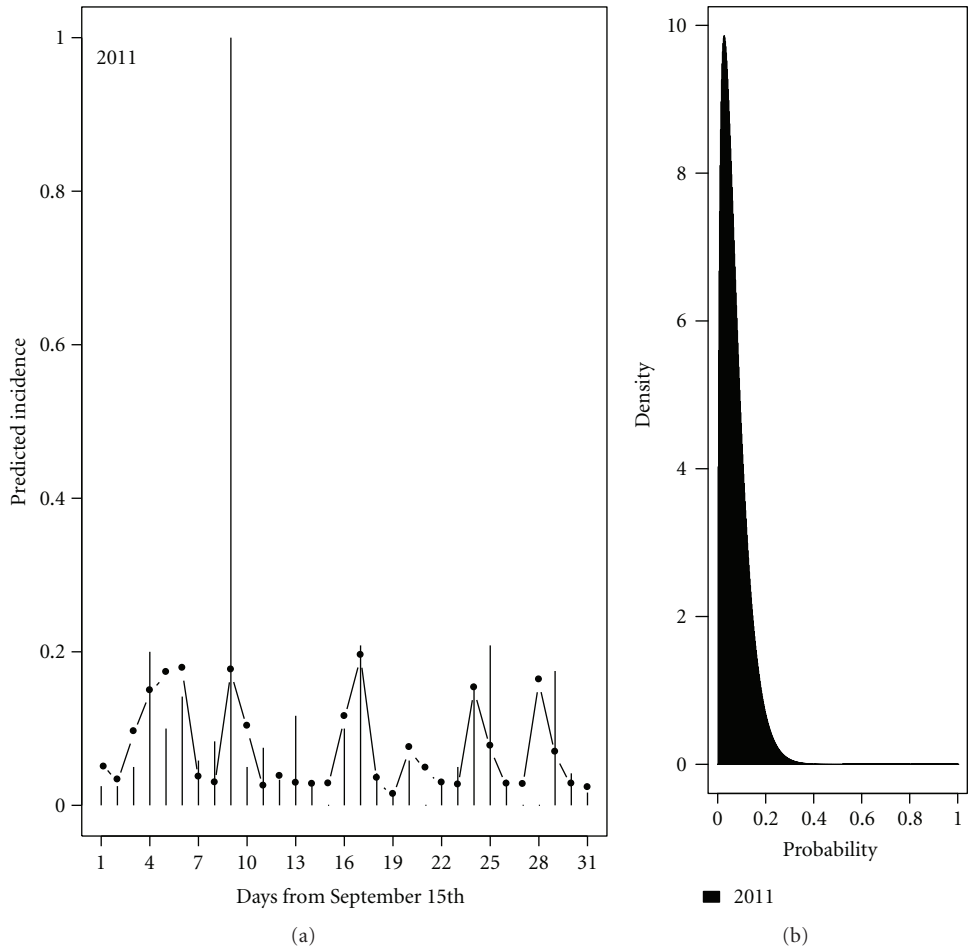

Figure 4: Validated model of spores incidence using climate variation in Passo Fundo, Brazil. The vertical lines represent the observed values and the continuous line the predicted values (a). Estimated density for the beta probability distribution function (b). The validation period was from September 15 to October 15, 2011.

Based on information from the model, we can establish a lower threshold of FHB probability incidence at 0.20.

In the future, combination of this model with an infection process model may result in a complex but more complete model. The combined model may be useful to quantify the impact of FHB epidemics on wheat yield and quality. The development of reliable epidemic forecasting systems should play an important role in FHB management, especially, if associated with expected advances in weather forecasting. Should an outbreak of FHB occur, a farm-scale intervention is usually required. Early warning based on forecasts from the model can assist in improving FHB control. Increasing fungicide spraying during high-risk periods and decreasing it during low-risk periods will improve cost effectiveness of operations. Crop advisers, if anticipating a higher FHB risk of occurrence, can increase vigilance, for example, by alerting farmers, planning for fungicide spraying and preparing for dealing with problem areas. These attempts, if successful, may have significant implications in wheat decision-making and practices, and may help farmers use resources more effectively and efficiently.

References

[1] J. M. C. Fernandes, "As doenças das plantas e o sistema plantio direto," *Revisão Anual de Patologia de Plantas*, vol. 5, pp. 317–352, 1997.

[2] E. C. Picinini, J. M. C. Fernandes, J.C. Ignaczak, and I. Ambrosi, "Impacto econômico do uso do fungicida propiconazole na cultura do trigo (*Triticum aestivum*)," *Fitopatologia Brasileira*, vol. 21, no. 3, pp. 362–368, 1996.

[3] H. P. Van Egmond, R. C. Schothorst, and M. A. Jonker, "Regulations relating to mycotoxins in food : perspectives in a global and European context," *Analytical and Bioanalytical Chemistry*, vol. 389, no. 1, pp. 147–157, 2007.

[4] F. Angelotti, D.J. Tessmann, T.C.A. Alves, J.B. Vida, D.S.J. Filho, and R. Harakava, "Caracterização morfológica e identificação molecular de isolados de *Fusarium graminearum* associados à giberela do trigo e triticale no sul do Brasil," *Summa Phytopathologica*, vol. 32, pp. 177–179, 2006.

[5] K. H. S. Peiris, M. O. Pumphrey, Y. Dong, E. B. Maghirang, W. Berzonsky, and F. E. Dowell, "Near-infrared spectroscopic method for identification of *Fusarium* head blight damage and prediction of deoxynivalenol in single wheat kernels," *Cereal Chemistry*, vol. 87, no. 6, pp. 511–517, 2010.

[6] D. S. Marshall, "Diseases which challenge global wheat production—powdery mildew, leaf, and head blights," in *Wheat: Science and Trade*, chapter 7, pp. 155–170, 2009.

[7] M. McMullen, R. Jones, and D. Gallenberg, "Scab of wheat and barley: a re-emerging disease of devastating impact," *Plant Disease*, vol. 81, no. 12, pp. 1340–1348, 1997.

[8] E. Panisson, E. M. Reis, and W. Boller, "Quantificação de danos causados pela giberela em cereais de inverno, na safra 2000, em Passo Fundo, RS," *Fitopatologia Brasileira*, vol. 28, no. 2, pp. 189–192, 2003.

[9] E. M. Del Ponte, J. M. C. Fernandes, W. Pavan, and W. E. Baethgen, "A model-based assessment of the impacts of climate variability on *Fusarium* head blight seasonal risk in Southern Brazil," *Journal of Phytopathology*, vol. 157, no. 11-12, pp. 675–681, 2009.

[10] A. Prandini, S. Sigolo, L. Filippi, P. Battilani, and G. Piva, "Review of predictive models for *Fusarium* head blight and related mycotoxin contamination in wheat," *Food and Chemical Toxicology*, vol. 47, no. 5, pp. 927–931, 2009.

[11] E. D. De Wolf and S. A. Isard, "Disease cycle approach to plant disease prediction," *Annual Review of Phytopathology*, vol. 45, pp. 203–220, 2008.

[12] E. M. Reis, "Quantificação de propágulos de *Gibberella zeae* no ar através de armadilhas de esporos," *Fitopatologia Brasileira*, vol. 13, no. 4, 1988.

[13] L. Francl, G. Shaner, G. Bergstrom et al., "Daily inoculum levels of *Gibberella zeae* on wheat spikes," *Plant Disease*, vol. 83, no. 7, pp. 662–666, 1999.

[14] E. M. Del Ponte, J. M. C. Fernandes, and C. R. Pierobom, "Factors affecting density of airborne *Gibberella zeae* inoculum," *Fitopatologia Brasileira*, vol. 30, no. 1, pp. 55–60, 2005.

[15] V. Rossi, L. Languasco, E. Pattori, and S. Giosue, "Dynamics of airborne *Fusarium* macroconidia in wheat fields naturally affected by head blight," *Journal of Plant Pathology*, vol. 84, no. 1, pp. 53–64, 2002.

[16] J. A. Nelder and R. W. M. Wedderburn, "Generalized linear models," *Journal of the Royal Statistical Society. Series A*, pp. 370–384, 1972.

[17] A. Azzalini, "Logistic regression for autocorrelated data with application to repeated measures," *Biometrika*, vol. 81, no. 4, pp. 767–775, 1994.

[18] K.Y. Liang and S.L. Zeger, "Longitudinal data analysis of continuous and discrete responses for pre-post designs," *Sankhyā: The Indian Journal of Statistics, Series B*, pp. 134–148, 2000.

[19] J. S. K. Chan, "Initial stage problem in autoregressive binary regression," *Journal of the Royal Statistical Society Series D*, vol. 49, no. 4, pp. 495–502, 2000.

[20] D. G. Schmale, J. F. Leslie, K. A. Zeller, A. A. Saleh, E. J. Shields, and G. C. Bergstrom, "Genetic structure of atmospheric populations of *Gibberella zeae*," *Phytopathology*, vol. 96, no. 9, pp. 1021–1026, 2006.

[21] S. L. Zeger and M. R. Karim, "Generalized Linear-models with random effects—a GIBBS sampling approach," *Journal of the American Statistical Association*, vol. 86, no. 413, pp. 79–86, 1991.

[22] S. G. Walker and B. K. Mallick, "Hierarchical generalized linear models and frailty models with bayesian nonparametric mixing," *Journal of the Royal Statistical Society. Series B*, vol. 59, no. 4, pp. 845–860, 1997.

[23] D. J. Lunn, A. Thomas, N. Best, and D. Spiegelhalter, "WinBUGS - A Bayesian modelling framework: concepts, structure, and extensibility," *Statistics and Computing*, vol. 10, no. 4, pp. 325–337, 2000.

[24] R. Development Core Team, *R: A Language and Environment for Statistical Computing*, R Foundation for Statistical Computing, Vienna, Austria, 2011.

[25] S. Sturtz, U. Ligges, and A. Gelman, "R2WinBUGS: a package for running WinBUGS from R," *Journal of Statistical Software*, vol. 12, article no. 3, pp. 1–16, 2005.

[26] A. Gelman and D. B. Rubin, "Inference from iterative simulation using multiple sequences," *Statistical Science*, vol. 7, no. 4, pp. 457–472, 1992.

[27] W. K. Hastings, "Monte carlo sampling methods using Markov chains and their applications," *Biometrika*, vol. 57, no. 1, pp. 97–109, 1970.

[28] S. Geman and D. Geman, "Stochastic relaxation, GIBBS distributions, and the bayesian restoration of images," *IEEE Transactions on Pattern Analysis and Machine Intelligence*, vol. 6, no. 6, pp. 721–741, 1984.

[29] A. E. Gelfand and A. F. M. Smith, "Sampling-based approaches to calculating marginal densities," *Journal of the American Statistical Association*, vol. 85, no. 410, pp. 398–409, 1990.

[30] D. E. Aylor and R. K. Kiyomoto, "Relationship between aerial concentration of Venturia inaequalis ascospores and development of apple scab," *Agricultural and Forest Meteorology*, vol. 63, no. 3-4, pp. 133–147, 1993.

[31] D. E. Aylor, T. B. Sutton et al., "Release of venturia inaequalis ascospores during unsteady rain: relationship to spore transport and deposition," *Phytopathology*, vol. 82, no. 5, pp. 532–540, 1992.

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Review Article

Population Structure of *Mycosphaerella graminicola* and Location of Genes for Resistance to the Pathogen: Recent Advances in Argentina

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Leaf blotch of wheat (*Septoria tritici* Rob. ex Desm., teleomorph *Mycosphaerella graminicola* (Fückel) Schröt. in Cohn) causes significant losses in wheat. During the last decades studies about the genetic variability of the pathogen and location of the resistance have been intensive around the world. The knowledge about the genetic variation of *M. graminicola* is very important because it could allow us to determine which genotypes predominate within a geographic area. It also can be used to evaluate the germplasm resistance of wheat cultivars with isolates with high genetic differences. In addition, the knowledge of the genes conditioning resistance in different genotypes allows getting precise combination in new germplasm. The incorporation of the known genes in new cultivars could contribute to broadening the resistance to the pathogen. A paper about genetic variability of the pathogen and location of the resistance, with special emphasis in the work carried out in Argentina, is presented.

1. Importance and Biology of the Disease

Leaf blotch of wheat (*Septoria tritici* Rob. ex Desm., teleomorph *Mycosphaerella graminicola* (Fückel) Schröt. in Cohn) causes significant losses in wheat. In Argentina, yield losses from 21 to 37% [1], from 20 to 50% [2], and from 16 to 45% [3] have been found. In some other countries, yield reductions range from 31 to 54% [4], from 10 to 45% [5], and even reductions >60% have been reported [6].

Mycosphaerella graminicola is a hemibiotrophic pathogen; early infection is biotrophic, followed by a switch to necrotrophic growth just prior to symptom expression. The sexual stage is known to play a role in the disease cycle. It has been reported to cause most of the initial infection of winter wheat crops during the autumn in the UK [7] and in the USA [8]. An increase in ascospores at harvest time has been reported, suggesting that the sexual stage may be important to initiate the infection in the next growing season [9]. In Argentina, the sexual stage was also found [10].

Unburied crop residue is the major source or primary inoculum for *Septoria tritici* infecting wheat [8]. Ascospores are produced and released on this substrate [11]. Pseudothecia mature during winter and remain viable until early spring. Only 30 min of moistening stubble are necessary for ascospore release and dispersal [12, 13].

Different studies [9, 14] have confirmed that during spring and the beginning of summer, the severity of the epidemic was conditioned by pycnidiospores produced in the

crop; nevertheless, ascospores were present from the time the first basal leaves were infected [9]. Following stem elongation, infection of the upper leaves of a crop has been thought to be due entirely to the asexual stage of the fungus, in which pycnidia give rise to pycnidiospores, which are splash dispersed from infected basal tissue to the upper leaves by rain drops. However, more recent work has shown that upward movement of inoculum can occur in the absence of splashy rainfall, being influenced by the position of developing leaves in relation to infected leaf layers [15]. Another possible means of spread within a crop during summer is by air-borne ascospores, which may play a more important role than previously recognised [16]. It has been suggested [16] that airborne ascospores play a major role in the epidemiology of the disease during the growing season and, together with splash-dispersed spores, both have implications for the forecasting of the disease.

Recently [17], the relative abundance of *M. graminicola* ascospores and *S. tritici* conidia during two growing seasons have been quantified weekly in Argentina, establishing its relationship with weather variables (including rainfall, air temperature, relative humidity, and radiation). Pycnidiospores and ascospores were released during the entire growing cycle (June to December) in both growing seasons. However, their relative abundances depended on the time of the year and the weather conditions. Coincidentally with previous reports around the world, pycnidiospores were predominant during an important period of the crop cycle (from October or November until December) and ascospores predominated in an important part of the period after harvest when the stubble was lying on the ground and at the beginning of the crop cycle (June to September). However, there were some peaks, during which the predominance of the sexual or the asexual form was related to the environmental conditions, ascospores being less dependent on rainfall than pycnidiospores. Some other researchers [15, 16] also mentioned that ascospores are released at two peak times of the year: the first one establishes primary infections in newly sown wheat crops and the second one approximately coincides with the emergence of the upper two leaves. This makes it possible that infection occurs in the upper leaves without rainfall [15, 16].

2. Genetic Variability of the *Mycosphaerella graminicola* Population and Pathogenicity Test

The pathogen has a high variability, partially caused by the presence of both asexual and sexual reproduction. Genetic evidences [18–21] showed that sexual fruit bodies of *M. graminicola* undergo recombination both during and between growing seasons. Therefore, ascospores served as primary inoculum to initiate the epidemic of *S. tritici* leaf blotch, and they also contributed to secondary infection on the upper leaves during the growing season [18].

Consideration of genetic variation of *M. graminicola* population is essential to understand the virulence on the different cultivars. Differences in the population around the world could be attributed to variation in regular recombination, different migration patterns, and presence and importance of the sexual form. Sexual reproduction creates large numbers of genetically diverse isolates. Populations in this fungus are in genetic equilibrium as well as in drift migration equilibrium [22] attributed to a high rate of sexual recombination.

Genetic structure of *M. graminicola* populations have been studied over the last decade all around the world [21, 23]. Many studies using different molecular markers showed that there was a high level of genetic variability within them and that populations were composed of many different genotypes.

Czembor and Arseniuk [24] studied different species of *Septoria* (*S. avenae f. sp. triticea; S. nodorum,* and *S. tritici*) and found that SSR and ISSR markers were the most sensitive techniques for the detection of DNA polymorphisms. Extensive population genetic analyses of *M. graminicola* have also been conducted with RFLP markers [1]. Schnieder et al. [23] used AFLP markers to analyse one population of *M. graminicola* from Germany. They observed high within-population diversity, and the significant migration between populations prevented genetic isolation and differentiation of putative geographically separated populations. Razavi and Hughes [25] worked on a total of 90 isolates of *M. graminicola* from western Canada using RAPD and they detected a high degree of DNA polymorphism with many different molecular phenotypes in this population. The genetic structure of the Kansas populations of *M. graminicola* was evaluated at different spatial scales (microplot, macroplot, and statewide) using AFLP. Genetic identities among populations were >98%. Tests for population subdivision revealed that 98% of the genetic variability occurred within populations [26]. In addition, Gurung et al. [27] determined that there was a small but statistically significant level of genetic differentiation between populations from spring versus winter wheat. Spring and winter wheat are exposed to differences in environmental conditions and resistance sources used in wheat breeding programs; however, most of the genetic variation (>98%) occurred within spring and winter wheat regions, while <2% was due to genetic differentiation between these regions. The authors assumed that those results indicated that sexual recombination occurs frequently in the *M. graminicola* populations and that most populations are genetically differentiated over the major spring and winter wheat growing regions of USA.

Medini and Hamza [28], using AFLP analysis, revealed a high level of genetic diversity in populations of *M. graminicola* isolates, no clones were obtained and each isolate showed a unique haplotype. Abrinbana et al. [29] found that five populations from five major wheat-growing provinces in Iran showed intermediate to high genotypic diversity. Low levels of gene flow and high genetic differentiation were observed among populations and different clustering methods revealed five genetically distinct groups in accordance with the sampling areas, indicating a population structure of the pathogen contrasting to that of most other countries studied.

Recently, Goodwin et al. [30] analysed a database of 30,137 EST (expressed sequence tag) sequences from *M.*

graminicola and identified 38 di- and 71 trinucleotide microsatellites with repeat numbers of six or more. Microsatellites that showed polymorphism between the parents of the *M. graminicola* mapping population were integrated into the existing genetic linkage map [31]. The EST database provided an excellent source of new, highly polymorphic microsatellite markers that can be multiplexed for high-throughput genetic analyses of *M. graminicola* and related species. The complete genome of *Mycosphaerella graminicola* was recently sequenced. It contains 21 chromosomes, eight of which could be lost with no visible effect on the fungus and thus are dispensable. This eight-chromosome dispensome is dynamic in field and progeny isolates, is different from the core genome in gene and repeat content, and appears to have originated by ancient horizontal transfer from an unknown donor [32].

In Argentina, a first study of the *M. graminicola* population was conducted with a limited set of isolates of the pathogen from some areas using RFLP. This study showed that the pathogen has a high virulence degree variation [33]. Jürgens et al. [34], using RFLP, also compared five populations from Argentina (Los Hornos, Balcarce, and Barrow) and determined that the populations from uninoculated fields in Argentina had higher gene and genotype diversities compared to those from inoculated fields.

A new study using ISSR molecular markers was carried out with isolates from several locations of the Argentinean wheat region: subregion IV (SE of Buenos Aires Province) and II South (central part of Buenos Aires Province). Samples were taken from different bread wheat (*Triticum aestivum* L.) cultivars. A total of 126 isolates were subjected to molecular analysis to compare the genetic structure of the isolates from both wheat subregions. Ten ISSR primers were used: (GACA)4; (AAC)7; (ATC)7; (AC)9; (AAG)7; (AG)9; (AGC)5; (CAG)5; (GTG)5; (GACAC)3. Eighty-four bands ranging from 200 bp to 8,000 bp were amplified. Eighty-one distinct haplotypes were identified and 43 isolates did not generate any amplification products. The highest number of polymorphic DNA fragments was produced using ISSR primers (ATC)7 and (GTG)5, which detected bands in 38 isolates. The molecular analysis revealed the existence of 81 different haplotypes among the 126 isolates studied [35]. These results revealed a high degree of genotypic diversity in the *M. graminicola* population in Argentina (100% in the subregion IV, and 94.3% in the subregion II South). Furthermore a high gene flow was found between both subregions without significant genetic differences between populations. Although the asexual pycnidiospores are dispersed by rain-splash [7], the sexual ascospores of *M. graminicola* have the potential to move at least several hundred meters, perhaps even over ten kilometers [36] indicating their potential as a source of genetic exchange between spatially distant populations.

In addition, virulence tests were conducted on nine selected Argentinean wheat cultivars and 14 foreign cultivars with some level of resistance to the pathogen inoculated with 16 different isolates molecularly characterized in the previous work, and with genetic differences, in two environments. Significant differences among isolates, cultivars, and isolates × cultivar interactions were observed. Cultivars with good levels of partial and complete resistance to some isolates were detected. From the Argentinean cultivars, "Klein Dragón", "Buck 75 Aniversario" and "Klein Volcán" showed resistance or moderate resistance to most of the isolates probed, which could indicate the presence of partial resistance in seedlings. "Klein Volcán" and "Buck 75 Aniversario", showed partial resistance in the adult stage. From the foreign lines tested "Tonic", "Oasis", "IAS 20", "TE 9111", and "Oasis" showed the best levels of resistance in seedlings and "TE 9111" and "IAS 20" in the adult stage [37].

These recent studies about the structure of the population in Argentina were the first step to locate *Stb* genes and QTL in Argentinean cultivars. We are actually starting the studies to determine which of the known genes are present in Argentinean wheat cultivars and also developing double haploid populations with some genotypes to identify new genes.

3. Location of the Resistance

During the last decade, 18 major genes conferring resistance to the pathogen have been identified. They were: *Stb1* [38], *Stb2* [39], *Stb3* [39], *Stb4* [40], *Stb5* [41], *Stb6* [42], *Stb7* [43], *Stb8* [44], *Stb9* [45], *Stb10* [46], *Stb11* [47], *Stb12* [46], *Stb13* [48], *Stb14* [48], *Stb15* [49], *Stb16* [50], *Stb17* [51], and *Stb18* [52]. The known genes, chromosomal location, sources of resistance, and closest molecular markers are indicated in Table 1.

In addition, several QTL were also found. Eriksen et al. [53] mentioned some QTL on chromosomes 2BL, 3AS, 3BL, 6B, and 7B in a doubled-haploid (DH) population of a cross between the susceptible winter wheat cultivar Savannah and the resistant cultivar Senat. Risser et al. [54] also detected QTL on chromosomes 3B and 6D from "Floret" and 4B and 6B from "Tuareg". Furthermore, Kelm et al. [55] found that cv "Solitär" conferred resistance to a specific isolate goberned by *Stb6* on chromosome 3A as well as to some other isolates by a QTL on chromosome 1BS, possibly corresponding to *Stb11* and minor QTL on chromosomes 1B, 3D, 6B, and 7D. Resistance of Marzuka to some isolates was caused by a QTL located in a region on 4AL which harbours *Stb7* or *Stb12*. Miedaner et al. [56] detected five QTL in each of two populations (Arina/Forno, History/Rubens) amounting to an explained genotypic variance of 45–63%. Zwart et al. [57] in a double haploid population derived from the cross between the synthetic hexaploid CP1133872 and the bread wheat cultivar Janz identified a cluster of foliar disease resistance QTL in chromosome 3DL. Major QTL each for resistance to *Septoria tritici* blotch and yellow leaf spot were contributed by the synthetic hexaploid parent and linked in repulsion with the coincident Lr24/Sr24 locus carried by parent Janz. Raman et al. [58] assessing three double haploid populations derived from Chara/WW2449, Whistler/WW1842, and Krichauff/WW2451 found that resistance to the pathogen was conditioned in the three populations by a single major gene designated as *Stb*WW2449, *Stb*WW1842, and *Stb*WW2451 located on the short arm of chromosome 1B.

Table 1: Major genes conditioning resistance to *Mycosphaerella graminicola* identified in hexaploid wheat.

Stb genes	Cultivars source	Chromosomal location	Closest (flanking) markers
Stb1	Bulgaria 88	5BL	*Xgwm335*
Stb2	Veranopolis	3BS	*Xgwn389*
Stb3	Israel 493	7AS	Not published yet
Stb4	Tadinia	7DS	*Xgwm111*
Stb5	Synthetic 6x	7DS	*Xgwm44*
Stb6	Shafir	3AS	*Xgwm369*
Stb7	Estanzuela Federal	4AL	*Xwmc219, Xwmc313*
Stb8	W7984	7BL	*Xgwm146, Xgwm577*
Stb9	Courtot	2B	*XksuF1, Xfbb226*
Stb10	KK4500	1D	*Xgwm848, Xgwm603*
Stb11	TE9111	1BS	*Xbarc008*
Stb12	KK4500	4AL	*Xwmc219, Xwmc313*
Stb13	Salamouni	7BL	*Xwmc396*
Stb14	Salamouni	3BS	*Xwmc500*
Stb15	Arina	6AS	*Xpsr904*
Stb16	Synthetic hexaploid M3	3DL	*Xgwm494*
Stb17	Synthetic hexaploid M3	5AL	*Xhbg247*
Stb18	Balance	6DS	*Xgpw5176*, *Xgpw3087*

Although during the last decade, several genes have been identified and several molecular markers have been developed, the analysis of resistance gene expression and utility for plant improvement programs would be increased if the resistance genes were isolated in a common susceptible background. To address that problem Goodwin and Thompson [59] started a program to backcross resistance genes *Stb1–8* into two susceptible wheat cultivars. Their work with genes *Stb2*, *Stb3*, *Stb6,* and *Stb8* has proceeded the farthest. They are also validating molecular markers linked to the resistance genes in the backcross progeny, which would provide the materials for efficient introgression of those genes into elite germplasm. They also determined that *Stb3* is dominant, while *Stb2* may be recessive.

Our group determined the chromosomal location of the resistance to the pathogen in substitution lines of a "Synthetic 6x" (*T. dicoccoides* × *T. tauschii*), *T. spelta* and the wheat cultivars "Cheyenne" and "Cappelle-Desprez". Several minor gene effects were detected at the seedling stage. Only chromosome 7D of "Synthetic 6x" was found having a major effect against the two isolates inoculated (IPO 92067 and IPO 93014). When tested in the adult stage, the line carrying chromosome 7D of "Synthetic 6x" showed resistance to isolate IPO 92067 but not for isolate IPO 93014. Major gene effects effective against both isolates were found on chromosomes 5A and 5D of "Synthetic 6x". Lines carrying chromosomes 1B, 5D, or 6D from "Cheyenne" showed major effects against isolate IPO 92064 [60, 61].

On the basis of these results, a series of chromosome 7D introgression lines in the background of the susceptible recipient landrace "Chinese Spring" and the resistant donor (Synthetic 7D) was inoculated with the isolates IPO 92067 and IPO 93014. The resistance was effective at both the seedling and the adult stage against both isolates and the resistance locus mapped to the centromeric region of chromosome arm 7DS. On the basis of its relationship with the microsatellite marker *Xgwm44*, it is likely that the gene involved was *Stb5*, which proved to be effective against *M. graminicola* isolates originating from both Europe and South America [62].

In addition, a source of resistance has been mapped on chromosome 7D of spelt wheat, *Triticum aestivum* L. subsp. *spelta* (L.) Thell. The microsatellite-based genetic map was constructed from a set of 87 single-chromosome recombinant doubledhaploid lines bred from the cross between the landrace "Chinese Spring" and a "Chinese Spring-" based line carrying chromosome 7D from spelt wheat. Two regions of the chromosome were associated with isolate-specific QTL, one expressed at the seedling and another at the adult plant stage. The seedling resistance locus *QStb.ipk-7D1* was found in the centromeric region of chromosome 7D, which corresponds to the location of the major resistance gene *Stb4* originated from bread wheat cultivar "Tadinia" and *Stb5* originated from *Triticum tauschii.* The adult resistance locus *QStb.ipk-7D2* was found on the short arm of chromosome 7D in a similar position to the locus *Lr34/Yr18* known to be effective against multiple pathogens. Composite interval mapping confirmed *QStb.ipk-7D1* and *QStb.ipk-7D2* to be two distinct loci [63].

Furthermore, using a mapping population of the International Triticeae Mapping Initiative (W7984 × Opata 85), three loci were discovered on the short arms of chromosomes 1D, 2D, and 6B at the seedling stage effective to isolates IPO 92067 and IPO 93014. At the adult plant stage, two isolate-specific QTL were found. The loci specific for isolates IPO 92067 and IPO 93014 were mapped on the long arms of chromosomes 3D and 7B, respectively [51].

Furthermore, one of the most confounding factors in selecting for resistance to *Septoria tritici* blotch could be the reported interaction between resistance and plant height or heading date. Miedaner et al. [56] found moderate and negative correlations between disease ratings and heading date in two populations, whereas correlation between disease rating and plant height was higher and negative. In our recent work, the effects of the plant height and heading date on the expression of the resistance were investigated in wheat near isogenic lines in the Mercia and Cappelle-Desprez backgrounds and differing in dwarfing genes (*Rht*) or in genes for insensitivity to photoperiod (*Ppd*). Strong associations between susceptibility and reduced height were only found in very short wheats indicating that moderately short wheats are not necessarily more susceptible to *Septoria tritici* blotch. The association between heading date and resistance was due to weather conditions [64]. In addition, experiments with 50 Argentinean wheat cultivars demonstrated no evidence of genetic associations between plant height, heading date, and resistance, indicating that selection of early and short lines

with high levels of quantitative resistance is possible. In these materials, the association between those traits was mainly caused by environmental and epidemiological factors which indicates that management of cultivars should be optimized to minimize these association [64]. In addition, when the location of the resistance on chromosome 7D of spelt wheat, *Triticum aestivum* L. subsp. *spelta* was investigated, there was variation for flowering date within the mapping population, but none of this was associated with the resistance QTLs on chromosome 7D, showing that neither linkage nor pleiotropy is involved between this particular resistance and flowering date. This findings indicated that while some *septoria tritici* blotch resistance factors do suffer from this complication (association between resistance and heading date or plant height), others, like the present one, do not [51, 64, 65]. Risser et al. [54] also determined that all correlations between *Septoria tritici* blotch and heading date as well as between *Septoria tritici* blotch and plant height were low. Such a lack of correlation is encouraging from the breeding point of view, since it allows for the improvement in *septoria tritici* blotch resistance independently of flowering time.

4. Conclusions

Important and recent advances have been made on the population structure and location of the resistance to the pathogen. However, in Argentina little is known about genes conditioning resistance and how they are effective against the local population of the pathogen. In addition, there still much work to do in relation to the incorporation of the genes in new cultivars broadening the resistance to the pathogen.

References

[1] G. Kraan and J. E. Nisi, "Septoriosis of wheat in Argentina. Situation of the crop against the disease. P1-8," in *Proceedings of the Septoria tritici Workshop*, L. Gilchrist et al., Ed., pp. 20–24, CIMMYT, Mexico City, Mexico, 1993.

[2] J. G. Anonne, A. Calzolari, O. Polidoro, and H. Conta, *Effect of the Leaf Blotch Caused by Septoria tritici on the Yield of Wheat*, Booklet 122, INTA EEA Pergamino, Pergamino, Argentina, 1991.

[3] M. R. Simón, A. E. Perelló, C. A. Cordo, and P. C. Struik, "Influence of *Septoria tritici* on yield, yield components, and test weight of wheat under two nitrogen fertilization conditions," *Crop Science*, vol. 42, no. 6, pp. 1974–1981, 2002.

[4] Z. Eyal, A. L. Scharen, J. M. Prescott, and M. van Ginkel, *The Septoria Diseases of Wheat. Concepts and Methods of Disease Management*, CIMMYT, Mexico City, Mexico, 1987.

[5] Caldwell R. M. and Narvaez I., "Losses of winter wheat from infection by *Septoria tritici*," *Phytopathology*, vol. 50, p. 630, 1960.

[6] W. A. Shipton, W. J. R. Boyd, and A. A. Rosielle, "The common Septoria diseases of wheat," *Botanical Review*, vol. 37, pp. 231–262, 1971.

[7] M. W. Shaw, "Assessment of upward movement of rain splash using a fluorescent tracer method and its application to the epidemiology of cereal pathogens," *Plant Pathology*, vol. 38, pp. 35–43, 1987.

[8] W. Schuh, "Influence of tillage systems on disease intensity and spatial pattern of Septoria leaf blotch," *Phytopathology*, vol. 80, pp. 1337–1340, 1990.

[9] C. A. Cordo, M. R. Simón, A. E. Perelló, and H. E. Alippi, "Spore dispersal of leaf blotch of wheat *Mycosphaerella graminicola* and *Septoria tritici*," in *Septoria and Stagonospora Diseases of Cereals. A Compilation of Global Research*, M. van Ginkel, A. McNab, and J. Krupinsky, Eds., pp. 98–101, 1999.

[10] C. A. Cordo, A. E. Perelló, H. E. Alippi, and H. O. Arriaga, "Presence of *Mycosphaerella graminicola* (Fuckel) Schroeter teleomorph *Septoria tritici* Rob. ex Desm. in maturity wheat of Argentina," *Revista de la Facultad de Agronomía*, vol. 66-67, pp. 49–55, 1990.

[11] G. Shaner, "Effect of environment of fungal leaf blights of small grains," *Annual Review of Phytopathology*, vol. 19, pp. 273–296, 1981.

[12] F. R. Sanderson, "Mycosphaerella species as the ascogenous state of Septoriatritici Rob. and Desm. N. Z.," *Journal of Botany*, vol. 10, pp. 707–709, 1972.

[13] F. R. Sanderson and J. G. Hampton, "Role of the perfect states in the epidemiology of the common Septoria diseases of wheat," *New Zealand Journal of Agricultural Research*, vol. 21, pp. 277–281, 1978.

[14] L. Eriksen and L. Munk, "The occurrence of *Mycosphaerella graminicola* and its anamorph *Septoria tritici* in winter wheat during the growing season," *European Journal of Plant Pathology*, vol. 109, no. 3, pp. 253–259, 2003.

[15] D. J. Lovell, S. R. Parker, T. Hunter, D. J. Royle, and R. R. Coker, "Influence of crop growth and structure on the risk of epidemics by *Mycosphaerella graminicola* (*Septoria tritici*) in winter wheat," *Plant Pathology*, vol. 46, no. 1, pp. 126–138, 1997.

[16] T. Hunter, R. R. Coker, and D. J. Royle, "The teleomorph stage, *Mycosphaerella graminicola*, in epidemics of *Septoria tritici* blotch on winter wheat in the UK," *Plant Pathology*, vol. 48, no. 1, pp. 51–57, 1999.

[17] C. A. Cordo, M. R. Simón, C. I. Mónaco et al., "Model for predictingthe release of spores of *Mycosphaerella graminicola*".

[18] R. S. Chen and B. A. McDonald, "Sexual reproduction plays a major role in the genetic structure of populations of the fungus *Mycosphaerella graminicola*," *Genetics*, vol. 142, no. 4, pp. 1119–1127, 1996.

[19] J. Zhan, C. C. Mundt, and B. A. McDonald, "Estimation of rates of recombination and migration in populations of plant pathogens: a reply," *Phytopathology*, vol. 90, no. 4, pp. 324–326, 2000.

[20] J. Zhan, C. C. Mundt, M. E. Hoffer, and B. A. McDonald, "Local adaptation and effect of host genotype on the rate of pathogen evolution: an experimental test in a plant pathosystem," *Journal of Evolutionary Biology*, vol. 15, no. 4, pp. 634–647, 2002.

[21] J. Zhan, R. E. Pettway, and B. A. McDonald, "The global genetic structure of the wheat pathogen *Mycosphaerella graminicola* is characterized by high nuclear diversity, low mitochondrial diversity, regular recombination, and gene flow," *Fungal Genetics and Biology*, vol. 38, no. 3, pp. 286–297, 2003.

[22] R. S. Chen, J. M. Boeger, and B. A. McDonald, "Genetic stability in a population of a plant pathogenic fungus over time," *Molecular Ecology*, vol. 3, no. 3, pp. 209–218, 1994.

[23] F. Schnieder, G. Koch, C. Jung, and J. A. Verreet, "Genotypic diversity of the wheat leaf blotch pathogen *Mycosphaerella graminicola* (anamorph) *Septoria tritici* in Germany," *European Journal of Plant Pathology*, vol. 107, no. 3, pp. 285–290, 2001.

[24] P. C. Czembor and E. Arseniuk, "Study of genetic variability among monopycnidial and monopycnidiospore isolates derived from single pycnidia of Stagonospora ssp. and *Septoria tritici* with the use of RAPD-PCR, MP-PCR and rep-PCR techniques," *Journal of Phytopathology*, vol. 147, no. 9, pp. 539–546, 1999.

[25] M. Razavi and G. R. Hughes, "Molecular variability of *Mycosphaerella graminicola* as detected by RAPD markers," *Journal of Phytopathology*, vol. 152, no. 10, pp. 543–548, 2004.

[26] M. Kabbage, J. F. Leslie, K. A. Zeller, S. H. Hulbert, and W. W. Bockus, "Genetic diversity of *Mycosphaerella graminicola*, the causal agent of *Septoria tritici* blotch, in Kansas winter wheat," *Journal of Agricultural, Food, and Environmental Sciences*, vol. 2, no. 1, pp. 1–9, 2008.

[27] S. Gurung, S. B. Goodwin, M. Kabbage, W. W. Bockus, and T. B. Adhikari, "Genetic differentiation at microsatellite loci among populations of *Mycosphaerella graminicola* from California, Indiana, Kansas, and North Dakota," *Phytopathology*, vol. 101, no. 10, pp. 1251–1259, 2011.

[28] M. Medini and S. Hamza, "Pathotype and molecular characterization of *Mycosphaerella graminicola* isolates collected from Tunisia, Algeria, and Canada," *Journal of Plant Pathology*, vol. 90, pp. 65–73, 2008.

[29] M. Abrinbana, J. Mozafari, M. Shams-bakhsh, and R. Mehrabi, "Genetic structure of *Mycosphaerella graminicola* populations in Iran," *Plant Pathology*, vol. 59, no. 5, pp. 829–838, 2010.

[30] S. B. Goodwin, T. A. J. van der Lee, J. R. Cavaletto, B. te Lintel Hekkert, C. F. Crane, and G. H. J. Kema, "Identification and genetic mapping of highly polymorphic microsatellite loci from an EST database of the *Septoria tritici* blotch pathogen *Mycosphaerella graminicola*," *Fungal Genetics and Biology*, vol. 44, no. 5, pp. 398–414, 2007.

[31] G. H. J. Kema, S. B. Goodwin, S. Hamza et al., "A combined amplified fragment length polymorphism and randomly amplified polymorphism DNA genetic linkage map of *Mycosphaerella graminicola*, the *Septoria tritici* leaf blotch pathogen of wheat," *Genetics*, vol. 161, no. 4, pp. 1497–1505, 2002.

[32] S. B. Goodwin, S. B. M'Barek, B. Dhillon et al., "Finished genome of the fungal wheat pathogen *Mycosphaerella graminicola* reveals dispensome structure, chromosome plasticity, and stealth pathogenesis," *PLoS Genetics*, vol. 7, no. 6, article 1002070, 2011.

[33] C. A. Cordo, C. C. Linde, J. Zhan, and B. MCDonald, "Genotypic diversity of the wheat leaf blotch pathogen (*Septoria tritici*) in Buenos Aires Province," *Sociedad Argentina Botánica*, vol. 41, pp. 293–305, 2006.

[34] T. Jürgens, C. C. Linde, and B. A. McDonald, "Genetic structure of *Mycosphaerella graminicola* populations from Iran, Argentina and Australia," *European Journal of Plant Pathology*, vol. 115, no. 2, pp. 223–233, 2006.

[35] N. Castillo, C. Cordo, and M. R. Simón, "Molecular variability among isolates of *Mycosphaerella graminicola*, the causal agent of *Septoria tritici* blotch, in Argentina," *Phytoparasitica*, vol. 38, no. 4, pp. 379–389, 2010.

[36] M. W. Shaw and D. J. Royle, "Airborne inoculum as a major source of *Septoria tritici* (*Mycosphaerella graminicola*) infections in winter wheat crops in the UK," *Plant Pathology*, vol. 38, pp. 35–43, 1989.

[37] Castillo N., *Characterization of isolates of* Mycosphaerella graminicola *and identification of molecular markers associated to genes of resistance to the pathogen*, Ph.D. thesis, Faculty of Natural Sciences and Museum, La Plata, Argentina, 2010.

[38] T. B. Adhikari, X. Yang, J. R. Cavaletto et al., "Molecular mapping of Stb1, a potentially durable gene for resistance to *Septoria tritici* blotch in wheat," *Theoretical and Applied Genetics*, vol. 109, no. 5, pp. 944–953, 2004.

[39] T. B. Adhikari, H. Wallwork, and S. B. Goodwin, "Microsatellite markers linked to the Stb2 and Stb3 genes for resistance to *Septoria tritici* blotch in wheat," *Crop Science*, vol. 44, no. 4, pp. 1403–1411, 2004.

[40] T. B. Adhikari, J. R. Cavaletto, J. Dubcovsky, J. O. Gieco, A. R. Schlatter, and S. B. Goodwin, "Molecular mapping of the Stb4 gene for resistance to *Septoria tritici* blotch in wheat," *Phytopathology*, vol. 94, no. 11, pp. 1198–1206, 2004.

[41] L. S. Arraiano, A. J. Worland, C. Ellerbrook, and J. K. M. Brown, "Chromosomal location of a gene for resistance to *Septoria tritici* blotch (*Mycosphaerella graminicola*) in the hexaploid wheat 'Synthetic 6x'," *Theoretical and Applied Genetics*, vol. 103, no. 5, pp. 758–764, 2001.

[42] P. A. Brading, E. C. P. Verstappen, G. H. J. Kema, and J. K. M. Brown, "A gene-for-gene relationship between wheat and *Mycosphaerella graminicola*, the *Septoria tritici* blotch pathogen," *Phytopathology*, vol. 92, no. 4, pp. 439–445, 2002.

[43] C. A. McCartney, A. L. Brûlé-Babel, L. Lamari, and D. J. Somers, "Chromosomal location of a race-specific resistance gene to *Mycosphaerella graminicola* in the spring wheat ST6," *Theoretical and Applied Genetics*, vol. 107, no. 7, pp. 1181–1186, 2003.

[44] T. B. Adhikari, J. M. Anderson, and S. B. Goodwin, "Identification and molecular mapping of a gene in wheat conferring resistance to *Mycosphaerella graminicola*," *Phytopathology*, vol. 93, no. 9, pp. 1158–1164, 2003.

[45] L. Chartrain, P. Sourdille, M. Bernard, and J. K. M. Brown, "Identification and location of Stb9, a gene for resistance to *Septoria tritici* blotch in wheat cultivars courtot and tonic," *Plant Pathology*, vol. 58, no. 3, pp. 547–555, 2009.

[46] L. Chartrain, S. T. Berry, and J. K. M. Brown, "Resistance of wheat line Kavkaz-K4500 L.6.A.4 to *Septoria tritici* blotch controlled by isolate-specific resistance genes," *Phytopathology*, vol. 95, no. 6, pp. 664–671, 2005.

[47] L. Chartrain, P. Joaquim, S. T. Berry, L. S. Arraiano, F. Azanza, and J. K. M. Brown, "Genetics of resistance to *Septoria tritici* blotch in the Portuguese wheat breeding line TE 9111," *Theoretical and Applied Genetics*, vol. 110, no. 6, pp. 1138–1144, 2005.

[48] R. A. McIntosh, K. M. Devos, J. Dubcovsky et al., "V Catalogue of gene symbols for wheat Supplement," 2007, http://wheat.pw.usda.gov/ggpages/wgc/2007upd.html

[49] L. S. Arraiano, L. Chartrain, E. Bossolini, H. N. Slatter, B. Keller, and J. K. M. Brown, "A gene in European wheat cultivars for resistance to an African isolate of *Mycosphaerella graminicola*," *Plant Pathology*, vol. 56, no. 1, pp. 73–78, 2007.

[50] S. M. T. Ghaffari, J. D. Faris, T. L. Friesen et al., "New broad-spectrum resistance to *Septoria tritici* blotch derived from synthetic hexaploid wheat," *TAG Theoretical and Applied Genetics*, vol. 124, no. 1, pp. 125–142, 2011.

[51] M. R. Simón, F. M. Ayala, C. A. Cordo, M. S. Röder, and A. Börner, "Molecular mapping of quantitative trait loci determining resistance to *Septoria tritici* blotch caused by *Mycosphaerella graminicola* in wheat," *Euphytica*, vol. 138, no. 1, pp. 41–48, 2004.

[52] S. M. T. Ghaffari, O. Robert, V. Laurent et al., "Genetic analysis of resistance to *Septoria tritici* blotch in the French winter wheat cultivars Balance and Apache," *Theoretical and Applied Genetics*, vol. 123, no. 5, pp. 741–754, 2011.

[53] L. Eriksen, F. Borum, and A. Jahoor, "Inheritance and localisation of resistance to *Mycosphaerella graminicola* causing *Septoria tritici* blotch and plant height in the wheat (*Triticum aestivum* L.) genome with DNA markers," *Theoretical and Applied Genetics*, vol. 107, no. 3, pp. 515–527, 2003.

[54] P. Risser, E. Ebmeyer, V. Korzun, L. Hartl, and T. Miedaner, "Quantitative trait loci for adult-plant resistance to *Mycosphaerella graminicola* in two winter wheat populations," *Phytopathology*, vol. 101, no. 10, pp. 1209–1216, 2011.

[55] C. Kelm, S. M. T. Ghaffary, H. Bruelheide et al., "The genetic architecture of seedling resistance to *Septoria tritici* blotch in the winter wheat doubled-haploid population Solitär × Mazurka," *Molecular Breeding*. In press.

[56] T. Miedaner, P. Risser, S. Paillard et al., "Broad-spectrum resistance loci for three quantitatively inherited diseases in two winter wheat populations," *Molecular Breeding*. In press.

[57] R. S. Zwart, J. P. Thompson, A. W. Milgate et al., "QTL mapping of multiple foliar disease and root-lesion nematode resistances in wheat," *Molecular Breeding*, vol. 26, no. 1, pp. 107–124, 2010.

[58] R. Raman, A. W. Milgate, M. Imtiaz et al., "Molecular mapping and physical location of major gene conferring seedling resistance to *Septoria tritici* blotch in wheat," *Molecular Breeding*, vol. 24, no. 2, pp. 153–164, 2009.

[59] S. B. Goodwin and I. Thompson, "Development of isogenic lines for resistance to *Septoria tritici* blotch in wheat," *Czech Journal of Genetics and Plant Breeding*, vol. 47, no. 1, pp. S98–S101, 2011.

[60] M. R. Simón, A. J. Worland, C. A. Cordo, and P. C. Struik, "Chromosomal location of resistance to *Septoria tritici* in seedlings of a synthetic hexaploid wheat, Triticum spelta and two cultivars of *Triticum aestivum*," *Euphytica*, vol. 119, no. 1-2, pp. 151–155, 2001.

[61] M. R. Simón, A. J. Worland, and P. C. Struik, "Chromosomal location of genes encoding for resistance to *Septoria tritici* blotch (*Mycosphaerella graminicola*) in substitution lines of wheat," *Netherlands Journal of Agricultural Science*, vol. 53, no. 2, pp. 113–129, 2005.

[62] M. R. Simón, F. M. Ayala, C. A. Cordo, M. S. Röder, and A. Börner, "The use of wheat/goatgrass introgression lines for the detection of gene(s) determining resistance to *Septoria tritici* blotch (*Mycosphaerella graminicola*)," *Euphytica*, vol. 154, no. 1-2, pp. 249–254, 2007.

[63] M. R. Simon, E. K. Khlestkina, N. S. Castillo, and A. Börner, "Mapping quantitative resistance to *Septoria tritici* blotch in spelt wheat," *European Journal of Plant Pathology*, vol. 128, no. 3, pp. 317–324, 2010.

[64] M. R. Simón, A. J. Worland, and P. C. Struik, "Influence of plant height and heading date on the expression of the resistance to *Septoria tritici* blotch in near isogenic lines of wheat," *Crop Science*, vol. 44, no. 6, pp. 2078–2085, 2004.

[65] M. R. Simón, A. E. Perelló, C. A. Cordo, S. Larrán, P. E. L. Van Der Putten, and P. C. Struik, "Association between *Septoria tritici* blotch, plant height, and heading date in wheat," *Agronomy Journal*, vol. 97, no. 4, pp. 1072–1081, 2005.

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Research Article

The Effect of Freezing Temperatures on *Microdochium majus* and *M. nivale* Seedling Blight of Winter Wheat

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Exposure to pre-emergent freezing temperatures significantly delayed the rate of seedling emergence ($P < 0.05$) from an infected and a non-infected winter wheat cv. Equinox seed lot, but significant effects for timing of freezing and duration of freezing on final emergence were only seen for the *Microdochium*-infested seed lot. Freezing temperatures of −5°C at post-emergence caused most disease on emerged seedlings. Duration of freezing (12 hours or 24 hours) had little effect on disease index but exposure to pre-emergent freezing for 24 hours significantly delayed rate of seedling emergence and reduced final emergence from the infected seed lot. In plate experiments, the calculated base temperature for growth of *M. nivale* and *M. majus* was −6.3°C and −2.2°C, respectively. These are the first set of experiments to demonstrate the effects of pre-emergent and post-emergent freezing on the severity of *Microdochium* seedling blight.

1. Introduction

Microdochium nivale (Fr.) Samuels and Hallett (teleomorph *Monographella nivalis* (Schaffnit) E. Müller) and *Microdochium majus* (Wollenw.) Glynn and S.G. Edwards (teleomorph *Monographella)* can cause seedling blight of cereals in the UK. *Microdochium nivale* var. *majus* and *M. nivale* var. *nivale* were reclassified as species by Glynn et al. [1]. Before this, mention of *M. nivale* refers to both subspecies unless stated. *Microdochium spp*. may be soil or seed borne; however, seed-borne inoculum is considered to be the predominant cause of seedling blight in the UK [2]. *Microdochium* seedling blight can cause death of cereal plants at the pre-emergent and post-emergence stages of development and surviving seedlings exhibit brown lesions on the coleoptile and roots [3]. Seedling death can result in significant yield losses when surviving plants cannot compensate for large reductions in establishment [4]. In addition, *M. majus* and *M. nivale* inoculum from coleoptile and root lesions has been demonstrated to be able to cause foot rot disease and stem colonisation in glasshouse experiments [5].

Microdochium seedling blight is more severe at cold temperatures and low soil moisture contents [6]. Hare et al. [7] described a strong correlation between the rate of seedling emergence from a wheat seed lot naturally infected with 72% *M. nivale*-infection and final emergence over a range of temperatures and soil moisture contents. In many situations, winter wheat seedlings are likely to be exposed to air temperatures below 0°C. However, the only published work at near freezing temperatures is that by Bateman [8] who reported that maintaining newly emerged wheat seedlings from *M. nivale*-infected seeds at 0-1°C for several weeks increased disease severity.

Despite the lack of evidence for the effect of temperatures below 0°C on the severity of seedling blight from naturally infected wheat seeds, freezing has been observed to increase both the incidence and severity of *Microdochium* seedling blight on oats and barley from surface-inoculated seeds and soil-borne *M. nivale* inoculum. Rawlinson and Colhoun [9] described 4-hour freezing (−6°C) on 4 occasions at weekly intervals beginning 1 month after planting increased the incidence of isolation of *M. nivale* from oat seedling

mesocotyls and roots grown from untreated seeds in *M. nivale*-infected soil.

When surface inoculated barley seeds with conidia of *M. nivale* (1×10^6 conidia mL^{-1}) were frozen 10 days after planting at $-2°C$ for 48 hours, coleoptiles lesion index increased to 100% compared to 5% on seedlings exposed to 2°C, and 39% on seedlings maintained at 10°C [10]. It is possible that temperatures below 0°C stop winter wheat seedling growth [11] giving *M. majus* and *M. nivale* increased opportunity for infection. However, there is a lack of information for the effects of temperatures below 0°C on the development of seedling blight from seed-borne *Microdochium spp.* in wheat and on *M. majus* and *M. nivale* growth.

A series of controlled environment experiments were designed to test the following hypotheses: (i) timing, duration, and severity of freezing does not affect seedling blight from seeds naturally infected with *M. majus* and *M. nivale;* (ii) timing, duration, and severity of freezing does not affect seedling emergence from non-infected seeds; (iii) *in vitro* growth of *M. majus* and *M. nivale* does not occur below 0°C.

2. Materials and Methods

2.1. In Vitro Growth of Microdochium majus and M. nivale. Five *M. majus* and 5 *M. nivale* isolates from the Harper Adams culture collection were cultured on potato dextrose agar (PDA) at 15°C for 8 days. Plugs of 5 mm diameter from the edges of actively growing colonies were transferred to Petri dishes containing 20 mL wheat flour agar (5% (w/w) winter wheat cv. Equinox flour; 2% (w/w) No. 1 agar (Oxoid Ltd, Basingtoke, UK)). Four dishes of each isolate were incubated in darkness at 5, 10, 15, and 20°C. Fungus colony diameters were measured in 2 directions at 90° angles at 2 day intervals and fungus growth rates (mm day^{-1}) calculated. Base temperatures for growth of *M. majus* and *M. nivale* were calculated by extrapolation following simple regression of the growth rate of each isolate. Data was analysed using *t*-test.

2.2. Effect of Freezing on the Rate of Seedling Emergence, Final Seedling Emergence, and Severity of Microdochium Seedling Blight. Two winter wheat seed lots cv. Equinox (88% *Microdochium* infection; 95% germination potential (infected seed lot) and 0% *Microdochium* infection; 98% germination potential (non-infected seed lot)) were used in this experiment. Due to a lack of incubator space, experiments for each seed lot were conducted separately. Experiments were conducted testing exposure to temperatures of 0°C or −5°C for 12 hours or 24 hours. Each seed lot was surface-sterilised by immersion in 10% NaOCl solution (1% available chlorine) for 3 minutes, rinsed 3 times in sterile distilled water, placed on sterile filter paper, and dried in a flow of sterile air. The severity of *Microdochium spp*. infection was determined by plating 200 surface-sterilised seeds of each seed lot onto PDA amended with 130 $\mu g\,mL^{-1}$ streptomycin sulfate (Sigma-Aldrich Company Ltd., Dorset, UK) and 25 $\mu g\,mL^{-1}$ Bavistin DF (carbendazim 50% w/w; BASF, Bury St. Edmunds, UK). The germination potential of each seed lot was assessed by the tetrazolium biochemical test [12]. PCR analysis [13] confirmed both *M. majus* and *M. nivale* to be present in the infected seed lot and not present in the non-infected seed lot.

John Innes No. 2 compost was passed through a 5 mm sieve and autoclaved (121°C; 1.08 bar) for 1 hour on 3 consecutive days and adjusted to 40% w/w soil water content. For each seed lot, 100 seeds were planted crease-down 20 mm deep in 45 seed trays. Trays were watered every 3 days to maintain constant 40% w/w soil water content. Trays were placed in an incubator set at 12 hours light (11°C) and 12 hours darkness (7°C) according to a fully randomised design and re-randomised daily. Freezing (12 hours or 24 hours at 0°C or −5°C) was applied 7 days (pre-emergent) or 28 days (post-emergent) after planting to 5 trays in a separate incubator. After freezing, trays were returned to their original incubator. Seedlings not exposed to freezing were used as controls.

Rate of seedling emergence (seedlings $days^{-1}$) was calculated from daily plant counts [14]. Final emergence and disease severity were measured at GS 12. A disease index on emerged seedlings was calculated (1), where *a* is the number of seedlings with category 0 symptoms (no symptoms), *b* is the number of seedlings with category 1 symptoms (≤2 lesions on coleoptile), *c* is the number with category 2 symptoms (>2 lesions on coleoptile), *d* is the number with category 3 symptoms (total necrosis of coleoptile), *e* is the number with category 4 symptoms (total necrosis of coleoptile and deformed seedling growth) and *N* is the number of seedlings assessed [15],

$$\frac{(a \times 0) + (b \times 1) + (c \times 2) + (d \times 3) + (e \times 4)}{N \times 4} \times 100. \quad (1)$$

Diseased cotyledons were surface sterilised and plated onto PDA amended with 130 $\mu g\,mL^{-1}$ streptomycin sulfate to confirm *Microdochium spp*. were the causal agents of disease.

Each experiment was repeated twice and the data combined prior to analysis. Analysis of each seed lot was conducted separately. A factorial analysis of variance was conducted with rate of seedling emergence, final emergence and disease index as variables, and exposure to freezing, timing, severity and duration of freezing as factors using Genstat 5.0 (Rothamsted Experimental Station, Hertfordshire, UK). Significant probabilities are given as $P < 0.05$. Data for the infected and non-infected seed lots were square-root transformed prior to analysis to ensure normality. Disease index values for the infected seed lot could not be transformed to a normal distribution, therefore standard error values are presented.

3. Results

3.1. In Vitro Growth of Microdochium majus and M. nivale. There were no significant differences between growth rates of the 5 *M. nivale* and 5 *M. majus* isolates so data was pooled for each species. The calculated base temperature for growth of *M. nivale* was significantly lower than *M. majus*. The growth rate for *M. majus* was significantly faster than *M. nivale* (Figure 1).

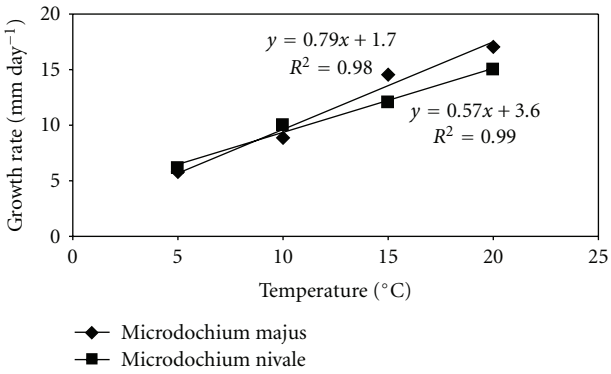

Figure 1: *In vitro* growth of 5 *Microdochium majus* and 5 *Microdochium nivale* isolates on winter wheat cv. Equinox flour agar.

3.2. Effect of Freezing on the Rate of Seedling Emergence and Final Seedling Emergence. For the infected and non-infected seed lots, timing and duration of freezing had a significant effect on rate of seedling emergence. Only pre-emergent freezing and exposure to freezing for 24 hours signifi- cantly delayed the rate of seedling emergence for the infected and non-infected seed lots (Table 1). The timing of freezing∗ duration of freezing interaction only significantly affected rate of seedling emergence from the non-infected seed lot. Exposure to pre-emergent freezing for 24 hours significantly delayed rate of seedling emergence (Table 1).

For the infected seed lot, exposure to freezing, duration and timing of freezing, and the timing of freezing∗duration of freezing and freezing temperature∗timing of freezing interactions had a significant effect on final seedling emergence. Exposure to freezing significantly reduced final seedling emergence compared to non-frozen seedlings. Only exposure to pre-emergent freezing for 24 hours and pre-emergent freezing to −5°C significantly reduced final seedling emergence (Table 2). Timing, duration, and severity of freezing had no significant effect on final seedling emergence from the non-infected seed lot (data not shown).

3.3. Effect of Freezing on the Severity of Microdochium Seedling Blight. Disease symptoms did not occur on seedlings grown from the non-infected seed lot. Isolations from diseased coleoptiles of seedlings grown from the infected seed lot confirmed *Microdochium spp.* were the causal agents of disease. Seedlings exposed to 0°C had significantly less disease than non-frozen seedlings. Seedlings exposed to −5°C generally had significantly more disease than seedlings frozen to 0°C, but only exposure to −5°C for 24 hours post-emergence significantly increased disease above non-frozen seedlings (Figure 2). Timing and duration of freezing had no significant effect on disease index when seedlings were exposed to 0°C or −5°C. Post-emergent freezing caused more severe seedling blight than pre-emergent freezing.

Table 1: Effect of timing (a) and duration (b) of freezing on rate of seedling emergence (seedlings day^{-1}) from a winter wheat cv. Equinox seed lot naturally infected (88%) with *Microdochium spp.* and a non-infected seed lot, and the timing of freezing∗duration of freezing interaction (c) on rate of seedling emergence (seedlings day^{-1}) from a non-infected winter wheat cv. Equinox seed lot.

(a)

Timing of freezing	Infected seedlot	Non-infected seedlot
None	0.055	0.253
Pre-emergent	0.046	0.221
Post-emergent	0.058	0.267
LSD ($P < 0.05$)	0.0267	0.0246
SEM	0.0120	0.0111
DF	79	80
CV (%)	16.6	7.1

Data are back transformed, statistical analysis performed on square root transformed data.

(b)

Duration of freezing	Infected seedlot	Non-infected seedlot
None	0.055	0.253
12 hours	0.056	0.258
24 hours	0.048	0.229
LSD ($P < 0.05$)	0.0267	0.0246
SEM	0.0120	0.0111
DF	79	80
CV (%)	16.6	7.1

Data are back transformed, statistical analysis performed on square root transformed data.

(c)

	Duration of freezing					
Timing of freezing	12 hours	24 hours	LSD	SED	DF	CV (%)
Pre-emergent	0.250	0.193	0.0269	0.0111	80	7.1
Post-emergent	0.267	0.267				

Rate of emergence of non-frozen seedlings 0.253 seedlings day^{-1}.
Data are back transformed, statistical analysis performed on square root transformed data.

4. Discussion

This is the first study to demonstrate that exposure to zero and sub-zero temperatures can affect the severity of *Microdochium* seedling blight on winter wheat from naturally infected seeds. For the infected and non-infected seed lots, freezing for 24 hours was required to significantly delay rate of seedling emergence. Freezing may increase the opportunity for seedling infection as *M. majus* and *M. nivale* could continue to grow *in vitro* at temperatures below the minimum air temperatures for growth of winter wheat seedlings [11]. Lowest emergence from the infected seed lot was caused by pre-emergent freezing (−5°C) for 24 hours 7 days after planting. This is in line with the results obtained

Table 2: Effect of exposure to freezing temperatures (a), timing and duration of freezing (b) and the timing of freezing∗duration of freezing and freezing temperature∗timing of freezing interactions (c) on final emergence (%) of seedlings from a winter wheat cv. Equinox seed lot naturally infected (88%) with *Microdochium spp.*

(a)

Exposure to freezing	Final emergence (%)
Yes	71
No	56
LSD ($P < 0.05$)	0.9
SEM	0.4
DF	79
CV (%)	17.8

Data are back transformed, statistical analysis performed on square root transformed data.

(b)

Timing of freezing	Final emergence (%)	Duration of freezing	Final emergence (%)
None	71	None	71
Pre-emergent	46	12 hours	64
Post-emergent	67	24 hours	48
LSD ($P < 0.05$)	1.0		1.0
SEM	0.4		0.4
DF	79		79
CV (%)	17.8		17.8

Data are back transformed, statistical analysis performed on square root transformed data.

(c)

	Timing of freezing					
Duration of freezing	Pre-emergent	Post-emergent	LSD	SED	DF	CV (%)
12 hours	65	60	0.9	0.4	79	17.8
24 hours	34	69				
Freezing temperature	Pre-emergent	Post-emergent				
0°C	52	62	0.9	0.4	79	17.8
−5°C	40	72				

Final emergence of non-frozen seedlings 71%.
Data are back transformed, statistical analysis performed on square root transformed data.

by Perry [10] when emergence was lowest from barley seeds surface-inoculated with *M. nivale* and exposed to −2°C for 48 hours 10 days after planting.

Post-emergent freezing (0°C and −5°C) increased the disease index compared to pre-emergent freezing. This is possibly because pre-emergent freezing resulted in heavily diseased seedlings not emerging. These results suggest that freezing increases the severity of *Microdochium* seedling blight rather than directly damaging the winter wheat seedlings as in line with previous observations [16, 17] no damage was seen on the coleoptiles of frozen seedlings from the non-infected seed lot. A similar trend for zero and sub-zero post-emergent temperatures increasing seedling blight

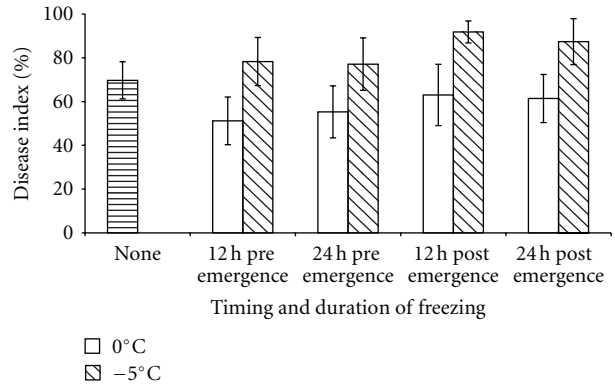

Figure 2: Disease index on seedlings grown from a winter wheat cv. Equinox seed lot naturally infected (88%) with *Microdochium spp.* exposed to different freezing temperatures at pre- and post-emergence. Vertical bars represent standard error.

severity has been reported for soil-borne *M. nivale* infecting ryegrass [18] and oats [9] and *Fusarium avenaceum* infecting barley from artificial soil inoculation [19].

Throughout this investigation no attempt was made to distinguish between *M. majus* and *M. nivale*. *Microdochium majus* had a faster *in vitro* growth rate than *M. nivale* which could confer a competitive advantage upon it but Glynn et al. [20] in *in vivo* experiments found no differences in pathogenicity. The effect of freezing temperatures on seed- lings growing in a range of soil moisture conditions is a further avenue for research. The results of this investigation may be used to more accurately target the use of fungicide seed treatments for the control of *Microdochium* seedling blight to planting conditions where seedling blight is likely to occur.

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References

[1] N. C. Glynn, M. C. Hare, D. W. Parry, and S. G. Edwards, "Phylogenetic analysis of EF-1 alpha gene sequences from isolates of *Microdochium nivale* leads to elevation of varieties *majus* and *nivale* to species status," *Mycological Research*, vol. 109, no. 8, pp. 872–880, 2005.

[2] D. W. Parry, T. R. Pettitt, P. Jenkinson, and A. K. Lees, "The cereal *Fusarium* complex," in *Ecology of Plant Pathogens*, P. Blakeman and B. Williamson, Eds., pp. 301–320, CAB International, Wallingford, UK, 1993.

[3] C. S. Millar and J. Colhoun, "*Fusarium* diseases of cereals—IV. Observations on *Fusarium nivale* on wheat," *Transactions of the British Mycological Society*, vol. 52, pp. 57–66, 1969.

[4] J. Humphreys, B. M. Cooke, and T. Storey, "Effects of seed-borne *Microdochium nivale* on establishment and grain yield of winter-sown wheat," *Plant Varieties and Seeds*, vol. 8, no. 2, pp. 107–117, 1995.

[5] I. M. Haigh, P. Jenkinson, and M. C. Hare, "The effect of a mixture of seed-borne *Microdochium nivale* var. *majus* and

Microdochium nivale var. *nivale* infection on *Fusarium* seedling blight severity and subsequent stem colonisation and growth of winter wheat in pot experiments," *European Journal of Plant Pathology*, vol. 124, no. 1, pp. 65–73, 2009.

[6] C. S. Millar and J. Colhoun, "*Fusarium* diseases of cereals—VI. Epidemiology of *Fusarium nivale* on wheat," *Transactions of the British Mycological Society*, vol. 52, pp. 195–204, 1969.

[7] M. C. Hare, D. W. Parry, and R. A. Noon, "Towards the prediction of *Fusarium* seedling blight of wheat," in *A Vital Role for Fungicides in Cereal Production*, Hewitt et al., Ed., pp. 211–220, Bios Scientific, Oxford, UK, 1995.

[8] G. L. Bateman, "Control of seed-borne *Fusarium nivale* on wheat and barley by organomercury seed treatment," *Annals of Applied Biology*, vol. 83, pp. 245–250, 1976.

[9] C. J. Rawlinson and J. Colhoun, "The occurrence of *Fusarium nivale* in soil," *Plant Pathology*, vol. 18, pp. 41–45, 1969.

[10] D. A. Perry, "Pathogenicity of *Monographella nivalis* to spring barley," *Transactions of the British Mycological Society*, vol. 86, pp. 287–293, 1986.

[11] J. R. Porter and M. Gawith, "Temperatures and the growth and development of wheat: a review," *European Journal of Agronomy*, vol. 10, no. 1, pp. 23–36, 1999.

[12] "International rules for seed testing," *Seed Science & Technology*, vol. 13, pp. 464–480, 1985.

[13] N. C. Glynn, R. Ray, S. G. Edwards et al., "Quantitative *Fusarium* spp. and *Microdochium* spp. PCR assays to evaluate seed treatments for the control of *Fusarium* seedling blight of wheat," *Journal of Applied Microbiology*, vol. 102, no. 6, pp. 1645–1653, 2007.

[14] E. M. Khah, R. H. Ellis, and E. H. Roberts, "Effects of laboratory germination, soil temperature and moisture content on the emergence of spring wheat," *Journal of Agriculture and Science*, vol. 107, pp. 431–438, 1986.

[15] I. M. Haigh, *The effects of temperature and soil water on Fusarium seedling blight of winter wheat and its effective control by fungicide seed treatments*, Ph.D. thesis, Harper Adams University College, 2003.

[16] M. K. Pomeroy, C. J. Andrews, K. P. Stanley, and J. Y. Gao, "Physiological and metabolic responses of winter wheat to prolonged freezing stress," *Plant Physiology*, vol. 78, pp. 207–210, 1985.

[17] C. W. Windt and P. R. Van Hasselt, "Development of frost tolerance in winter wheat as modulated by differential root and shoot temperature," *Plant Biology*, vol. 1, no. 5, pp. 573–580, 1999.

[18] S. J. I. Holmes and A. G. Channon, "Glasshouse studies on the effect of low temperature on infection of perennial ryegrass seedlings by *Fusarium nivale*," *Annals of Applied Biology*, vol. 79, pp. 43–48, 1975.

[19] M. N. Smith and C. R. Olien, "Pathological factors affecting survival of winter barley following controlled freeze tests," *Phytopathology*, vol. 68, pp. 773–777, 1978.

[20] N. C. Glynn, M. C. Hare, and S. G. Edwards, "Fungicide seed treatment efficacy against *Microdochium nivale* and *M. majus in vitro* and *in vivo*," *Pest Management Science*, vol. 64, no. 8, pp. 793–799, 2008.

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Review Article

A Review of the Studies and Interactions of *Pseudomonas syringae* Pathovars on Wheat

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Wheat is affected by some pathovars of *Pseudomonas syringae* and by other *Pseudomonas* species. Of these, *P. syringae* pv. *syringae* is the major one responsible for reduction. Recent studies have been made to characterize and identify the pathogen and to determine its aggressiveness and the pattern of colonization in seed and its effects on seed yield, yield components, and source-sink relationships during postanthesis. It was found that the reduction in the aerial biomass production is the best way to evaluate the aggressiveness of this bacterium, and the spray inoculation is good tool to make evaluations at seedling stage. The characterization of bacteria fingerprintings with molecular markers such as RAPD-PCR, ERIC, and REP-PCR is available. Genomic evolution has been elucidated with next-generation genome sequencing. Also, the colonization pattern shows that, early on, microcolonies are frequently detected in the aleurone layer, later in the endosperm and finally close to the crease and even in some cells of the embryo itself. In the wheat cultivars Seri M82 and Rebeca F2000 seed yield and its components are negatively affected. In general, *P. syringae* pv. *syringae* reduces the plant height, seed yield, and yield components, as well as the growth of most organs. When this bacterium attacks, the stems are the predominant sink organs and the leaf laminae and panicles are the predominant source organs.

1. Introduction

1.1. Global Importance of Wheat. Wheat (*Triticum* spp.) is one of the four major staple foods for human consumption [1, 2], ranking the first above the others for cultivated land area and yield and also providing the highest percentage of protein and carbohydrate [3–5]. While considered primarily a human food and much valued for its baking characteristics, wheat is also grown for animal feed and for industrial processing [4]. International demand for wheat is estimated to be growing at about 2% per year [4, 6]. World production exceeds 689 million tons from 25 high-producer countries [4, 7].

1.2. Importance of Wheat in Mexico. Mexico produces about 3.7 million tonnes of wheat on 0.71 million ha situated mainly in the north, southwest, central, and southeast regions. The major part (85%) of Mexico production is in the states of Sonora (35%), Guanajuato (17.5%), Baja California (11.5%), Sinaloa (9.2%), Michoacán (6.4%), and Jalisco (4.4%) where it is the fourth most important crop in terms of land area, being exceeded only by maize (*Zea mays* L.), beans (*Phaseolus vulgaris* L.), and sorghum (*Sorghum bicolor* [Linn.] Moench) [5]. National wheat production in Mexico in terms of the area planted and the yield per unit area has increased over the 60-year period 1925–1985 but there have been small annual declines since 1985. This has made it necessary to import 1.35 million tonnes per year to meet domestic demand with a *per capita* annual consumption of 54 kg [4, 8].

2. Importance of Wheat Diseases Caused by *Pseudomonas Syringae* and Other Pseudomonads

2.1. Generalities. There are about 50 pathovars described for *P. syringae* and at least nine different species or

genomospecies based on DNA homology [9, 10]. The *Pseudomonas* strains causing the wheat disease "basal glume blotch" area are classified as *Pseudomonas syringae* pv. *atrofaciens* (McCulloch) [11] while those causing leaf blight are grouped under *Pseudomonas syringae* pv. *syringae* van Hall 1902. In many countries including Argentina, Australia, New Zealand, Italy, USA, Canada, South Africa, and Pakistan the occurrence of *P. syringae* pv. *atrofaciens* and/or pv. *syringae* has been reported only once [12, 13]. Diseases caused by *P. syringae* pv. *syringae* on wheat are known as dieback or leaf blight. This bacterium induces water-soaked spots on expanding leaves which become necrotic and turn from grey-green to tan-white. The entire leaves may become necrotic. During periods of high rainfall, white droplets containing cells of *P. syringae* pv. *atrofaciens* (McCulloch) may be visible causing basal glume blotch [13]. *P. syringae* pv. *japonica* (McCulloch) can cause dull, brownish-black discolored areas at the base of each glume covering the kernel, as well as blight or striated areas on the nodes. At last, *Pseudomonas cichorii* causes stem or shank melanosis and *Pseudomonas fuscovaginae* induces a black rot in the wheat sheath. In wheat, although major emphasis has been placed towards the study of diseases caused by fungus, studies of bacterial diseases such as *P. syringae* pv. *syringae* are scarce. This has delayed the development of specific information on the impact of this group of pathogens. These bacteria are considered important in cereals for their broad host range and because some of them are transmitted in the seed. The incidence of *Pseudomonas syringae* pv. *syringae* tends to be sporadic and their geographical distribution is limited [14–16]. Studies on the bacterial diseases of wheat are scarce, so only limited quantitative information on, for example, yield loss and disease epidemiology is available, especially under field conditions. *P. syringae* subp. *syringae* is considered unique among *P. syringae* pathovars for its ability to cause disease in at least 180 plant species from several unrelated genera [17]. Diseases caused by this bacterium, although classified as of low importance [15], have decreased yield by over 50% in Germany [18]. Maximum damage is usually associated with conditions of high humidity and low temperature [15]. The bacterium first infects the glumes, lemma, palea, and caryopsis and then invades and multiplies in the intercellular spaces of the seed tissues [19].

2.2. Losses in Grain Yield. In the field, losses in grain yield caused by *Pseudomonas syringae* depend on many factors including the incidence and severity of the disease, the aggressiveness of the pathogen, environmental conditions (especially temperature and humidity), the resistance or susceptibility of the host, and the phenological stage in which the infection occurs. *P. syringae* pv. *syringae* populations are almost always present epiphytically on the surfaces of wheat plants and other hosts, which indicates that weather conditions are more relevant to disease outbreaks than the presence of the inocula [13]. Internationally, yield losses range from 5 to 50% [15, 20] and, in Mexico, from 5 to 20% [21]. Moreover, it has been shown that infestation of the seed is very important in the epidemiology of the disease [12, 22]. For example, seeds inoculated with *P. syringae* pv. *atrofaciens* and *P. syringae* pv. *syringae* have been sown and these bacteria have subsequently been found in the leaf blades of the resulting seedlings, which suggests transmission by seed [23]. This demonstrates the need to ensure that wheat seed is free of plant pathogenic bacteria because, although it is unusual for all the conditions needed for extreme damage to occur in the field, the shipment of contaminated seed could spread the disease to regions where it has not previously been reported. Many seedling inoculation methods have been evaluated, and several indicators have been developed to estimate the aggressiveness of the strains of *P. syringae* pv. *syringae*. The production of aerial biomass (dry weight) at 34 days after emergence seems to be the best indicator to detect differences among the *Pseudomonas* strains, inoculation methods and their interactions [22], so this would seem to be the alternative method for evaluating aggressiveness when the seed is sprayed or vacuum infiltrated with the bacterium. In general, *P. syringae* pv. *syringae* reduces the wheat plant height, seed yield, and yield components, as well as the growth of most organs in wheat cultivars Seri M82 and Rebeca F2000. When this bacterium attacks, the stems are the predominant sink organs and the leaf laminae and panicles are the predominant source organs. In conclusion, it was suggested that wheat disease records caused by this bacterium should complement crop physiological variables to evaluate and to explain bacterial disease effects [24].

2.3. Study of the Pattern of Spikelet and Seed Colonization. The studies conducted by Fukuda et al. [19] on the histology of wheat seeds invasion by *Pseudomonas syringae* pv. *japonica* indicate that the bacteria colonize first the lemma and palea, then continue to invade the funiculus caryopsis, and then multiply in the intercellular spaces. The sequence and the timing with which the seed tissues are infected have not been explained in detail. Reporter genes can be used to detect changes in plant tissues. The principle of their use is that the amount of protein quantified is a measure of gene expression. The reporter gene for the enzyme β-glucuronidase (GUS) has been used in various plant species but its use has not been reported for determining patterns of colonization of seeds by pathogenic bacteria [25]. Experiments with the GUS gene require destructive sampling, making it impossible to carry out multiple analyses on the same piece of tissue [26]. The use of green fluorescent protein (GFP) overcomes some of the disadvantages of GUS gene detection. The GFP was first discovered in 1962 [27] and was isolated in its natural form from the jellyfish (*Aequorea victoria*). It is characterized as a polypeptide of 27 kDa, which converts the blue chemiluminescence of the Ca^{2+} plus aequorin (a naturally luminescent protein) into blue light [28]. The main advantage in using GFP over GUS is that it can be detected nondestructively in living tissues and even in individual cells, whereas the GUS enzyme activity is often expressed as patches around the tissue under observation, as well as in transformed cells [29]. In plant pathology, the use of the *gfp* gene is increasing. For example, Sexton and Howlett [30] determined that the fungus *Leptosphaeria maculans* can colonize and cause symptoms specifically in the cotyledons and stems of *Brassica* spp., and Du et al. [31] were able to

identify and quantify the accumulation of aflatoxins in corn caused by *Aspergillus flavus*.

In wheat seeds inoculated with *Streptomyces* sp. transformed with the *gfp* gene, Coombs and Franco [32] determined that pathogen causes infection in the tissue of the embryo, endosperm, and radicle. Similarly, studies with pathogens marked with the *gfp* gene in yeast have allowed visualization of expression and confirm the usefulness of the *gfp* gene as a reporter in the study of plant diseases [33, 34]. We have used the *gfp* reporter genes to detect microorganisms and to study biological properties [23]. The pattern of colonization by *P. syringae* pv. *syringae* in wheat was recently elucidated [23]. One strain of this bacterium was transformed to express the GFP in wheat. The *gfp*-tagged bacteria showed strong GFP expression when visualized under green light. After 6 h it was detected in the aleurone layer and, 24 h later, in the endosperm cells. By 36 h it was close to the crease and by 48 h it appeared as patches in some cells of the embryo tissue.

3. Taxonomy of Wheat Pathogenic *Pseudomonas*

The genus *Pseudomonas sensu lato* has been subdivided based on rRNA-DNA hybridization, 16S rRNA sequence comparisons, and multilocus sequence typing [35–38]. Phytopathogenic fluorescent *Pseudomonas* representatives are grouped in the genus *Pseudomonas sensu stricto*, within rRNA similarity group I for γ-Proteobacteria subclass [39]. Most members of this group are saprophytic *Pseudomonas* and are metabolically and physiologically versatile [40]. Revisions of the genus *Pseudomonas* differentiation is provided in detail by Kersters et al., Silva-Rojas, and Anzai et al. who reported five rRNA homology groups, highlighting their importance in phytopathogenic groups I, II, III, and V [35, 41, 42]. *Pseudomonas syringae* is genetically diverse and is subclassified into approximately 50 pathovars and at least nine genomospecies based on pathogenicity and host range and DNA homology [10, 43, 44]. It is a bacillary bacterium, negative, mobile with a polar flagella, and strictly aerobic. In solid King B medium it produces a green fluorescent pigment under UV irradiation resulting in a former case of grouping [45]. The characterization and identification of this species, in addition to biochemical tests, can be accomplished with commercial systems such as API-50CH, 50AO, and 50AA, BIOLOG (Biolog Inc., Hayward, USA), and Biotype 100 (bioMérieux, La Balme Les Grottes, France) has been used to determine the ability of isolates to assimilate or oxidize a wide range of organic compounds in the presence of tetrazolium salt. However, with the application of such tools it has still not been possible to distinguish between *P. syringae* pv. *syringae* and *P. syringae* pv. *japonica*, making it necessary to rely on results of molecular tests.

In this sense, to identify pathogenic bacteria, particularly of the genus *Xanthomonas* and, the technique of repeated sequences (REP-PCR) has been used successfully to identify *X. translucens* [46] and other species of this genus [47, 48]. Fatty acid methyl ester (FAME) analysis has been applied to distinguish *Pseudomonas syringae* but it was not conclusive for this genus [45]. At this moment, no research using repeated sequences like ERIC and REP or FAME analysis has been used to distinguish *Pseudomonas syringae* pathovars affecting wheat worldwide. Other molecular tools, like DNA genomic restriction fragment length polymorphism (RFLP) analysis of DNA by pulsed field gel electrophoresis and ELISA-PCR, have also been used for genomic characterization of bacteria on wheat [15]. Direct sequencing of the amplification product of the small subunit ribosomal 16S rRNA is a discriminative method that identifies strains of prokaryotes rapidly [49, 50]. This subunit is a characteristic universally distributed among all prokaryotic species [51]. The respective sequence is compared with the GenBank database (National Center for Biotechnology Information, NCBI) which establishes phylogenetic relationships and results in a prompt identification. Our understanding of the evolution of plant pathogenesis in *Pseudomonas syringae* strains has further improved by next-generation genome sequencing [52]. It is possible to identify *P. syringae* pv. *syringae* on the basis of amplifying and sequencing the small 16 rRNA subunit. However, results are unavailable for the application of molecular markers like RAPD-PCR to characterize and identify plant pathogenic bacterial strains in wheat [53].

4. Conclusion

The main control measures for plant diseases caused by pathogens are crop rotations with nonhost species, inoculation with antagonistic bacteria (e.g., fluorescent *Pseudomonas* genus), seed production in areas free of the disease, and plant breeding for resistance. In Mexico there are not yet any effective treatments for the control of basal glume blotch in wheat caused by *Pseudomonas syringae* pv. *syringae.* In experiments in Egypt and Russia, which assess the resistance of wheat genotypes to diseases caused by *P. syringae* pv. *syringae* and *P. syringae* pv. *atrofaciens*, sources of genetic resistance have been found in the varieties Sakha 69, Len, Marchal, Nowesta, Red River 68, Bounty 208, Bonanza and Alex as well as in species of the *Aegilops* grasses. The evaluation of alternative controls using physical and chemical measures has been shown not to be feasible for large amounts of seed and is anyway considered likely to impair its viability or not to have sufficient efficacy to be worthwhile. Therefore, at this stage it is important to use healthy seed, from an uninfected crop, grown in an upland area.

References

[1] D. J. D. Ávila, H. V. C. Santoyo, R. R. Schwentesius, and V. H. M. Palacio, *El Mercado del Trigo en Mexico ante el TLCAN*, Centro de Investigaciones Económicas, Sociales y Tecnológicas de la Agroindustria y la Agricultura Mundial (CIESTAAM-PIAI), Universidad Autónoma Chapingo, Chapingo, Mexico, 2001.

[2] P. K. Anderson, A. A. Cunningham, N. G. Patel, F. J. Morales, P. R. Epstein, and P. Daszak, "Emerging infectious diseases of

plants: pathogen pollution, climate change and agrotechnology drivers," *Trends in Ecology & Evolution*, vol. 19, no. 10, pp. 535–544, 2004.

[3] S. C. Wayne, *Crop Production. Evolution, History and Technology*, John Wiley & Sons, New York, NY, USA, 1995.

[4] H. E. Villaseñor, "Importancia del trigo," in *El Trigo de Temporal en Mexico*, H. E. Villaseñor and E. Espitia, Eds., pp. 7–24, INIFAP, CIR-CENTRO, Centro, Mexico, 2000.

[5] FAOSTAT, "Production, crops," 2010, http://faostat.fao.org/site/339/default.aspx.

[6] K. D. Sayre, S. Rajaram, and R. A. Fischer, "Yield potential progress in short bread wheats in North West Mexico," *Crop Science*, vol. 37, no. 1, pp. 36–42, 1997.

[7] United States Department of Agriculture, "World agricultural supply and demand estimates," WASDE, 501, 2011, http://www.usda.gov/oce/commodity/wasde/latest.pdf.

[8] P. L. Pingali and S. Rajaram, "Global wheat research in a changing world: options for sustaining growth in wheat productivity," in *1998-99 World Facts and Trends, Global Wheat Research in a Changing World: Challenges and Achievements*, P. L. Pingali, Ed., pp. 1–18, Centro Internacional de Mejoramiento de Maíz y Trigo, El Batán, Mexico, 1999.

[9] L. Cardan, H. Shafik, S. Belouin, R. Broch, F. Grimont, and P. A. Grimont, "DNA relatedness among the pathovars of *Pseudomonas syringae* and description of *Pseudomonas tremae* sp. nov. and *Pseudomonas cannabina* sp. nov. (ex Sutic and Dowson 1959)," *International Journal of Systematic Bacteriology*, vol. 49, no. 2, pp. 469–478, 1999.

[10] J. L. Vanneste, D. A. Cornish, J. Yu, and C. E. Morris, "The application of polymerase chain reaction for characterising strains of *Pseudomonas syringae* isolated from New Zealand rivers," *New Zealand Plant Protection*, vol. 62, pp. 256–261, 2009.

[11] J. M. Young, D. W. Dye, J. F. Bradbury, C. G. Panagopoulus, and C. F. Robbs, "A proposed nomenclature and classification for plant pathogenic bacteria," *Journal of Agricultural Research*, vol. 21, no. 1, pp. 153–177, 1978.

[12] J. von Kietzell and K. Rudolph, "Epiphytic occurrence of *Pseudomonas syringae* pv. *atrofaciens*," in *Pseudomonas syringae Pathovars and Related Pathogens*, K. Rudolph, T. J. Burr, J. Mansfield, D. Stead, A. Vivian, and J. von Kietzell, Eds., pp. 29–34, Kluwer Academic, Dodrecht, The Netherlands, 1997.

[13] M. N. Kazempour, M. Kheyrgoo, H. Pedramfar, and H. Rahimian, "Isolation and identification of bacterial glum blotch and leaf blight on wheat (*Triticum aestivum* L.) in Iran," *African Journal of Biotechnology*, vol. 9, no. 20, pp. 2860–2865, 2010.

[14] M. Diekmann and C. A. J. Putter, Eds., *Technical Guidelines for the Safe Movement of Germoplasm. Small Grain Temperate Cereals. No. 14*, Food and Agriculture Organization of the United Nations, Rome/International Plant Genetic Resources Institute, Rome, Italy, 1995.

[15] J. von Kietzell and K. Rudolph, "Wheat diseases caused by *Pseudomonas syringae* pathovars," in *The Bacterial Diseases of Wheat. Concepts and Methods of Disease Management*, E. Duveiller, L. Fucikovsky, and K. Rudolph, Eds., pp. 49–57, Centro Internacional de Mejoramiento de Maíz y Trigo, El Batán, Mexico, 1997.

[16] P. D. Hernández, *Enfermedades de maíz (Zea mays L.), trigo (Triticum aestivum L.) y cebada (Hordeum vulgare L.) presentes en Mexico*, Tesis de Licenciatura, Universidad Autónoma Chapingo, Chapingo, Mexico, 1998.

[17] E. L. Little, R. M. Bostock, and B. C. Kirkpatrick, "Genetic characterization of *Pseudomonas syringae* pv. *syringae* strains from stone fruits in California," *Applied and Environmental Microbiology*, vol. 64, no. 10, pp. 3818–3823, 1998.

[18] H. Toben, A. Mavridis, and K. W. E. Rudolph, "On the occurrence of basal glume root wheat and barley caused by *Pseudomonas syringae* pv. *atrofaciens* in West Germany," *Journal of Plant Disease and Protection*, vol. 98, no. 3, pp. 225–235, 1991.

[19] T. Fukuda, K. Azegami, and H. Tabel, "Histological studies on bacterial black node of barley and wheat caused by *Pseudomonas syringae* pv. *japonica*," *Annals of the Phytopathological Society of Japan*, vol. 56, no. 2, pp. 252–256, 1990.

[20] R. L. Forster and N. W. Schaad, "Control of black chaff of wheat with seed treatment and a foundation seed health program," *Plant Disease*, vol. 72, pp. 935–938, 1988.

[21] E. Duveiller and H. Maraite, "Study of yield loss due to *Xanthomonas campestris* pv. *undulosa* in wheat under high rainfall temperate conditions," *Journal of Plant Disease and Protection*, vol. 100, no. 5, pp. 453–459, 1993.

[22] A. J. Valencia-Botín, L. E. Mendoza-Onofre, H. V. Silva-Rojas et al., "Indicadores de agresividad y métodos de inoculación con bacterias fitopatógenas en plántulas y semillas de trigo 'Seri M82'," *Revista Fitotecnia Mexicana*, vol. 30, no. 3, pp. 255–259, 2007.

[23] H. A. Zavaleta-Mancera, A. J. Valencia-Botín, L. E. Mendoza-Onofre, H. V. Silva-Rojas, and E. Valadez-Moctezuma, "Use of green fluorescent protein to monitor the colononization of *Pseudomonas syringae* subsp. *syringae* on wheat seeds," *Microscopy and Microanalysis Journal*, vol. 13, no. S02, pp. 298–299, 2007.

[24] A. J. Valencia-Botín, L. E. Mendoza-Onofre, H. V. Silva-Rojas, E. Valadez-Moctezuma, L. Cordova-Tellez, and H. E. Villaseñor-Mir, "Effect of *Pseudomonas syringae* subsp. *syringae* on yield and biomass distribution in wheat," *Spanish Journal of Agricultural Research*, vol. 9, no. 4, pp. 1287–1297, 2011.

[25] R. A. Jefferson, T. A. Kavanagh, and M. W. Bevan, "GUS fusions: β-glucuronidase as a sensitive and versatile gene fusion marker in higher plants," *The EMBO Journal*, vol. 6, no. 13, pp. 3901–3907, 1987.

[26] M. T. Buenrostro-Nava, *Characterization of GFP gene expression using an automated image collection system and image analysis*, Ph.D. thesis, The Ohio State University, Columbus, Ohio, USA, 2002.

[27] O. Shimomura, F. H. Johnson, and Y. Saiga, "Extraction, purification and properties of aequorin, a bioluminescent protein from the luminous hydromedusan Aequorea," *Journal of Cellular and Comparative Physiology*, vol. 59, pp. 223–239, 1962.

[28] M. Chalfie, Y. Tu, G. Euskirchen, W. W. Ward, and D. C. Prasher, "Green fluorescent protein as a marker for gene expression," *Science*, vol. 263, no. 5148, pp. 802–805, 1994.

[29] B. Bohanec, Z. Luthar, and K. Rudolf, "A protocol for quantitative analysis of green fluorescent protein-transformed plants, using multiparameter flow cytometry with cluster analysis," *Acta Biologica Cracoviensia Series Botanica*, vol. 44, pp. 145–153, 2002.

[30] A. C. Sexton and B. J. Howlett, "Green fluorescent protein as a reporter in the Brassica-Leptosphaeria maculans interaction," *Physiological and Molecular Plant Pathology*, vol. 58, no. 1, pp. 13–21, 2001.

[31] W. Du, Z. Huang, J. E. Flaherty, K. Wells, and G. A. Payne, "Green fluorescent protein as a reporter to monitor gene expression and food colonization by *Aspergillus flavus*," *Applied and Environmental Microbiology*, vol. 65, no. 2, pp. 834–836, 1999.

[32] J. T. Coombs and C. M. Franco, "Visualization of an endophytic *Streptomyces* species in wheat seed," *Applied and Environmental Microbiology*, vol. 69, no. 7, pp. 4260–4262, 2003.

[33] N. Chen, T. Hsiang, and P. H. Goodwin, "Use of green fluorescent protein to quantify the growth of *Colletotrichum* during infection of tobacco," *Journal of Microbiological Methods*, vol. 53, no. 1, pp. 113–122, 2003.

[34] L. Oren, S. Ezrati, D. Cohen, and A. Sharon, "Early events in the *Fusarium verticillioides*-maize interaction characterized by using a green fluorescent protein-expressing transgenic isolate," *Applied and Environmental Microbiology*, vol. 69, no. 3, pp. 1695–1701, 2003.

[35] K. Kersters, W. Ludwig, M. Vancanneyt, P. de Vos, M. Gillis, and K. H. Schleifer, "Recent changes in the classification of the *Pseudomonas*: an overview," *Systematic and Applied Microbiology*, vol. 19, no. 4, pp. 465–477, 1996.

[36] N. J. Palleroni, R. W. Ballard, E. Ralston, and M. Doudoroff, "Deoxyribonucleic acid homologies among some *Pseudomonas* species," *Journal of Bacteriology*, vol. 110, no. 1, pp. 1–11, 1972.

[37] A. Willems, P. de Vos, M. Gillis, and K. Kersters, "Towards an improved classification of *Pseudomonas* sp.," *Society for Applied Bacteriology Technical Series*, vol. 29, pp. 21–43, 1992.

[38] C. T. Bull, C. R. Clarke, R. Cai, B. A. Vinatzer, T. M. Jardini, and S. T. Koike, "Multilocus sequence typing of *Pseudomonas syringae* sensu lato confirms previously described genomospecies and permits rapid identification of *P. syringae* pv. *coriandricola* and *P. syringae* pv. *apii* causing bacterial leaf spot on parsley," *Phytopathology*, vol. 101, no. 7, pp. 847–858, 2011.

[39] N. J. Palleroni, "Genus I. *Pseudomonas* Migula 1984," in *Bergey's Manual of Systematic Bacteriology*, N. R. Krieg and J. G. Holt, Eds., pp. 141–149, Williams & Wilkins, Baltimore, Md, USA, 1984.

[40] C. Weinel, *Comparative and functional genome analysis of Pseudomonas putida KT2440*, Ph.D. thesis, University of Hannover, Hanover, Germany, 2003.

[41] H. V. Silva-Rojas, *Caracterización de bacterias fitopatógenas causantes de manchas foliares en el cultivo de frijol (Phaseolus vulgaris L.) en Mexico*, Tesis de Doctor en Ciencias, Colegio de Postgraduados, Montecillo, Mexico, 2000.

[42] Y. Anzai, H. Kim, J. Park, H. Wakabayashi, and H. Oyaizu, "Phylogenetic affiliation of the pseudomonads based on 16S rRNA sequence," *International Journal of Systematic and Evolutionary Microbiology*, vol. 50, no. 4, pp. 1563–1589, 2000.

[43] K. W. E. Rudolph, "*Pseudomonas syringae* pathovars," in *Pathogenesis and Host Specificity in Plant Diseases*, R. P. Singh and K. Kohmoto, Eds., vol. I of *Prokaryotes*, pp. 47–138, Elsevier Science, Oxford, UK, 1995.

[44] H. Sawada, F. Suzuki, I. Matsuda, and N. Saitou, "Phylogenetic analysis of *Pseudomonas syringae* pathovars suggests the horizontal gene transfer of *argK* and the evolutionary stability of *hrp* gene cluster," *Journal of Molecular Evolution*, vol. 49, no. 5, pp. 627–644, 1999.

[45] B. Slabbinck, B. de Baets, P. Dawyndt, and P. de Vos, "Analysis of plant-pathogenic *Pseudomonas* species using intelligent learning methods: a general focus on taxonomy and fatty acid analysis within the genus *Pseudomonas*," *Revista Mexicana de Fitopatología*, vol. 28, pp. 1–16, 2010.

[46] M. Maes and P. Garbeva, "Detection of bacterial phytopathogens based on nucleic acid technology," *Parasitica*, vol. 50, no. 1-2, pp. 75–80, 1994.

[47] F. J. Louws, J. L. W. Rademaker, and F. J. de Bruijn, "The three DS of PCR-based genomic analysis of phytobacteria: diversity detection, and disease diagnosis," *Annual Review of Phytopathology*, vol. 37, pp. 81–125, 1999.

[48] C. Bragard, *From immunological detection and identification to phenotypic and genotypic characterization of Xanthomonads pathogenic for small grains*, Ph.D. thesis, Faculté des Sciences Agronomiques, Université Catholique de Louvain, Louvain-la-Neuve, Belgium, 1996.

[49] B. Alberts, D. Bray, J. Lewis, M. Raff, K. Roberts, and J. D. Watson, *Biología Molecular de la Célula*, Ediciones Omega S. A., Barcelona, Spain, 3rd edition, 1996.

[50] M. D. R. Rodicio and M. D. C. Mendoza, "Identificación bacteriana mediante secuenciación del ARNr 16S: fundamento, metodología y aplicaciones en microbiología clínica," *Enfermedades Infecciosas y Microbiologia Clinica*, vol. 22, no. 4, pp. 238–245, 2004.

[51] D. Gevers, F. M. Cohan, J. G. Lawrence et al., "Re-evaluating prokaryotic species," *Nature Reviews*, vol. 3, no. 9, pp. 733–739, 2005.

[52] H. E. O'Brien, S. Thakur, and D. S. Guttman, "Evolution of plant pathogenesis in *Pseudomonas syringae*: a genomics perspective," *Annual Review of Phytopathology*, vol. 49, pp. 269–289, 2011.

[53] A. J. Valencia-Botín, *Caracterización, identificación, colonización y repercusión agronómica de bacterias fitopatógenas en trigo*, Tesis de Doctor en Ciencias, Colegio de Postgraduados, Montecillo, Mexico, 2007.

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Research Article

Relationship between Incidence of Cephalosporium Stripe and Yield Loss in Winter Wheat

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Cephalosporium stripe (caused by *Cephalosporium gramineum*) can be a serious disease of winter wheat (*Triticum aestivum* L.) in the Pacific Northwest of the USA. Effects of Cephalosporium stripe on yield, test weight, protein, and kernel characteristics were examined using 12 winter wheat varieties in field plots inoculated and not inoculated with the pathogen. Averaged over varieties, inoculation decreased yield, test weight, kernel weight, and kernel diameter; grain protein and the standard deviations of kernel weight and kernel diameter were increased by inoculation. Grain yield of the susceptible check was reduced by as much as 41% with addition of inoculum. The most resistant and the most susceptible varieties performed similarly for yield in the two environments, while varieties with intermediate levels of resistance were sometimes inconsistent. There was a linear relationship between yield and % whiteheads (sterile heads caused by disease) in one environment and a curvilinear relation in the other.

1. Introduction

Cephalosporium stripe of wheat is caused by the soil-borne fungal pathogen *Cephalosporium gramineum* Nisikado and Ikata (syn. *Hymenula cerealis* Ellis & Everh.) [1–3]. The fungus has a wide range of hosts, mainly among winter cereals [4, 5]. Cephalosporium stripe is of economic importance only in winter wheat, however. The disease is an important, limiting factor in many winter wheat production areas [6–9]. It is widespread throughout the Pacific Northwest of the USA, where wheat growers in erosion-prone areas are particularly affected when early plantings and reduced or no tillage are practiced [4, 10–12].

C. gramineum survives between host crops saprophytically as mycelium and conidia in association with host residues on or near the soil surface [13]. Infested crop residue is the primary source of inoculum. Conidia produced in the top layer of soil on crop stubble and released during cool and moist weather conditions during fall and winter are washed down into the root zone to infect the next crop [14, 15]. Once inside the roots, the fungus invades the vascular system and has the potential to colonize the entire plant. Successful establishment of *C. gramineum* inside the host is enhanced by the production of toxic metabolites that block the vascular system, thus preventing normal movement of water and nutrients [4, 11, 16].

The most typical and recognizable symptom, chlorotic leaf striping, is apparent on the younger, upper leaves during jointing and heading. Severely infected stems are stunted and prematurely ripen, producing a white and usually sterile head, containing sometimes just a few shriveled seeds. It is at this level of infection where the greatest amount of yield loss is observed [3, 11, 17–19]. In areas conducive to Cephalosporium stripe (i.e., Kansas and Montana in the USA, and Scotland), up to 80% yield reduction from a generalized infection on a susceptible cultivar can occur [17, 20–23]. Precise information on the impact of Cephalosporium stripe on grain yield for Pacific Northwest conditions is not available. Yield losses caused by this fungus appear to be the product of a combination of reduced seed number and

reduced seed weight [17, 24]. Impacts on grain protein, test weight, and end-product quality also may occur [17, 21, 22].

Reducing incidence of Cephalosporium stripe has generally been accomplished by reducing inoculum in the soil via cultural controls such as crop rotation, management of crop residues, altering soil pH with lime applications, and fertilizer management [14, 25–30]. However, these practices are only partially effective in reducing the incidence and severity of the disease [31] and often are practically or economically unfeasible. Additionally, Cephalosporium stripe cannot be controlled with fungicides. Although variation in the degree of resistance among cultivars has been confirmed, genotypes with complete resistance to *C. gramineum* have not been found [21, 24, 32, 33]. However, repeated planting of moderately resistant cultivars has been reported to reduce both the incidence and severity of Cephalosporium stripe over years [34].

The goals of this study were to estimate the magnitude of potential grain yield loss caused by Cephalosporium stripe under Oregon production conditions, its association with changes in test weight and kernel characteristics, and to estimate the level of host plant resistance required to attain minimal yield loss.

2. Materials and Methods

2.1. Plant Material. Varieties were included in the experiments based on commercial importance, performance in previous Cephalosporium stripe screening nurseries, and their range in disease response. Ten varieties were evaluated in the Pendleton trials. Stephens (CI 017596), Madsen (PI 511673), and Tubbs (PI 629114) are major cultivars grown in the region. The European cultivar Rossini and two derived breeding lines (OR9800919 and OR9800924, Rossini/Ysatis//Oracle) were included, as these were previously shown to have moderate-to-high levels of disease resistance. Three experimental lines with varying levels of resistance were also included. These originated from crosses between the Rossini-derived lines and adapted Oregon material (OR02F-B-46 (Tubbs//OR9800924/Weatherford), OR02F-C-169 (Tubbs//OR9800924/OR9900553) and OR02F-D-27 (OR9800924/Weatherford)). A highly resistant club winter wheat with an alien source of resistance (WA 7437, PI 561033) was included as a resistant check. At Moro, two new releases were added to the previous list of varieties to verify their performance to the disease (Skiles and ORSS-1757). Skiles had previously shown moderate-to-high levels of resistance, while ORSS-1757 (PVP 200500336) was considered moderately susceptible to the disease.

2.2. Field Trials. Field trials were conducted at the Columbia Basin Agricultural Research Center field stations near Pendleton, OR, during the 2005-2006 winter wheat season and in Moro, OR in 2005–2007. Both locations are in semiarid wheat-producing areas of the Columbia Plateau, with mean annual precipitation of 406 mm in Pendleton and 279 mm in Moro. These sites are representative of eastern Oregon winter wheat production areas where Cephalosporium stripe is frequent. A randomized complete block design with four replications was used at each location. Treatments consisted of a factorial of two levels of disease (inoculated and noninoculated), with 10 varieties in Pendleton and 12 in Moro; 10 varieties were common to both trials.

Differential disease levels were obtained by sowing autoclaved oat kernels that were previously infested with *C. gramineum*. Autoclaved oat kernels not infested with the fungus were added to the noninoculated plots. Inoculum was produced following the description by Mathre and Johnston [18] and was added to the seed envelopes before planting at a dose equal in volume to the wheat seed.

Trials were sown into stubble mulch on 12 September 2005 in Pendleton and on 12 September 2006 in Moro. Early September sowing dates increase severity of Cephalosporium stripe at these sites. Each plot was four rows (1.5 m) × 6.1 m long. Border plots were included around each trial. A Hege 500 series plot drill (H&N Manufacturing, Colwich, KS) with deep furrow openers was used to place seed into moist soil. Fertilization and weed control practices were appropriate to commercial winter wheat production at the two sites. Hand weeding was necessary in Pendleton at postanthesis to keep weed pressure low. A spring application of fungicide (Bumper 41.8EC, propiconazole) was applied to avoid infection by *Pseudocercosporella herpotrichoides*, which can mask symptoms of Cephalosporium stripe. Plots were mowed to approximately 4.5 m in length postheading and prior to collecting disease data. Plot lengths were recorded before harvest to adjust yield estimations. Trials were harvested during July after maturity and once an adequate level of grain moisture was reached. Entire plots were harvested with a plot combine, adjusted to maximize the retention of shriveled kernels.

Cephalosporium stripe incidence was recorded on a plot basis through visual estimation of the percentage of tillers that were ripening prematurely, and which usually expressed complete or partial reduction of grainfill (whiteheads) [18, 24]. Evaluation of known check varieties and random examination of lower stems and roots provided confidence that whiteheads were caused predominately by Cephalosporium stripe. Disease notes were taken at each location about 3 wks after heading. Developmental stage of the entries ranged from early milk to early dough at this time. Plant height and physiological maturity were also recorded to study possible association with Cephalosporium stripe resistance.

2.3. Grain Analyses. Harvested grain was carefully cleaned using airflow to remove nongrain contamination. Grain weight per plot was measured with a precision digital scale. A 1 kg sample was taken from each bag to determine test weight (hectoliter weight), grain protein concentration (%), and grain moisture content (%). Test weight and grain moisture were measured with a Grain Analysis Computer (GAC) model 2100b (DICKEY-john Corporation, Auburn, IL). Protein content was measured with an Infratec 1241 Grain Analyzer (Foss, Eden Prairie, MN) with appropriate settings for soft white or hard red winter wheat varieties.

A 300-seed subsample was randomly taken from each bulk and analyzed for kernel weight (mg) and diameter (mm), using a Single-Kernel Characterization System (SKCS) model 4100 (Perten Instruments, Springfield, IL).

Table 1: Analysis of variance for % whiteheads (square root transformed) and grain parameters for wheat genotypes grown in plots inoculated or not inoculated with *Cephalosporium gramineum* in Pendleton 2006 and Moro 2007.

Environment Source of variation	DF	Whiteheads	Yield	Test weight	Protein	Kernel weight		Kernel diameter	
						avg	SD	avg	SD
Pendleton, 2006									
Block	3	0.31	3.12**	8.88**	0.714	9.40**	0.677	0.030**	0.0002
Inoculation	1	113.34**	21.65**	212.23**	5.274**	77.15**	42.506**	0.143**	0.0590**
Genotype	9	15.08**	1.21**	36.27**	0.546	139.08**	20.803**	0.332**	0.0277**
Inoculation x genotype	9	4.33**	0.63**	6.61**	0.678*	4.52**	0.781*	0.010**	0.0017**
Error	57	0.20	0.20	1.34	0.320	1.61	0.330	0.003	0.0006
CV (%)		22.0	9.3	1.6	5.3	3.6	5.9	2.3	4.9
Moro, 2007									
Block	3	0.11	0.83**	10.63**	0.371	25.50**	0.659	0.056**	0.0011
Inoculation	1	198.27**	25.06**	33.36**	0.004	12.80*	5.880**	0.028*	0.0093**
Genotype	11	7.25**	1.37**	30.81**	2.318**	96.50**	8.978**	0.160**	0.0109**
Inoculation x genotype	11	2.37**	0.35**	1.34	1.179*	3.33	1.209**	0.006	0.0022**
Error	69	0.46	0.11	0.81	0.520	2.53	0.250	0.005	0.0005
CV (%)		16.4	9.4	1.2	8.4	4.7	5.7	2.9	4.5

* Significant at the 0.05 probability level.
** Significant at the 0.01 probability level.

For each sample, the SKCS integrated computer software (Perten Instruments, Springfield, IL) provided the means and standard deviations of the 300 individual kernel determinations.

2.4. Statistical Analyses. Statistical analyses were performed with the Statistical Analysis System (SAS) (SAS v9.1, SAS Institute Inc., Cary, NC, USA). Analyses of variance (ANOVA) for disease response, yield, test weight, and kernel related traits were conducted with PROC GLM to determine the level of variation between blocks and to test the significance of both treatment factors (disease and varieties) and their interaction. Type III F statistics were used to test the significance of variance sources. For ANOVA, whitehead percentages were square-root-transformed to meet the assumptions of normality and homogeneity of variance. The significance of the disease treatment on individual varieties was determined with the SLICE option in the LSMEANS statement.

Yield loss for genotypes was estimated as the reduction in grain yield between noninoculated and inoculated plots expressed as percentage relative to the yield in noninoculated plots. Similar calculations were done to estimate loss or change in test weight and kernel traits due to disease.

Pearson correlation coefficients among traits were estimated from genotype least square means using the PROC CORR procedure in SAS, pooling means from inoculated and noninoculated treatments together.

Linear regressions were fitted to estimate the relationship of grain yield loss and test weight loss to the susceptibility of genotypes to Cephalosporium stripe. The level of susceptibility was measured as the difference in whiteheads between inoculated and noninoculated plots. Grain yield loss was calculated for each cultivar as (grain yield inoculated − grain yield noninoculated) * 100/grain yield inoculated, test weight loss was estimated as well and was calculated similarly. Linear and quadratic regressions of yield and test weight loss on disease response differential were fitted using the PROC REG procedure in SAS.

3. Results

Averaged over varieties, inoculation significantly decreased yield, test weight, kernel weight, and kernel diameter; grain protein at Pendleton and the standard deviations of kernel weight and kernel diameter at both locations were significantly increased by inoculation (Table 1). Main effects were significant for all variables in both experiments, with the exception of grain protein, which showed no significant effect of genotypes in either location (Table 1). However, all variables showed a significant interaction ($P = 0.05$) between the inoculation treatment and genotype in Pendleton. For the Moro trial the interaction term was also significant ($P = 0.05$) for most of the variables, with the exceptions of test weight, kernel weight, and kernel diameter.

Cephalosporium stripe occurred in noninoculated plots of both trials, but more so at Moro than at Pendleton (Tables 2 and 4). Nonetheless, the mean disease scores (% whiteheads) for genotypes differed significantly ($P < 0.01$) under inoculated versus noninoculated conditions for all varieties, with the exception of WA 7437 and OR9800924 in Pendleton (Table 2) and WA 7437 in Moro (Table 4). Stephens, a highly susceptible cultivar, showed the biggest difference in percentage whiteheads between inoculated and noninoculated plots (41.3 and 42.5% for Pendleton and Moro, resp.) and the greatest yield loss (32.0 and 41.2%, resp.).

Table 2: Percent whiteheads, grain yield, test weight, and grain protein of 10 wheat genotypes noninoculated (U) and inoculated (I) with *Cephalosporium gramineum* in field plots in Pendleton, Oregon, 2006.

	Whiteheads (%)			Grain yield (t ha^{-1})			Test weight (kg hl^{-1})			Grain protein (%)		
	U	I	Change[a]	U	I	Loss (%)[b]	U	I	Loss	U	I	Change
Stephens	5.8	47.0	41.3**	5.35	3.64	32.0**	77.15	71.73	5.43**	10.31	11.46	1.15**
Madsen	0.5	13.3	12.8**	5.58	4.20	24.8**	78.05	73.68	4.38**	10.40	10.97	0.57
Tubbs	2.9	35.8	32.9**	5.66	4.03	28.9**	76.95	72.10	4.85**	10.17	10.92	0.75
OR9800919	0.6	9.0	8.4**	5.77	4.95	14.2*	74.28	71.10	3.18**	10.47	10.88	0.42
OR9800924	0.2	1.1	0.9	4.90	4.82	1.7	75.80	74.38	1.43	10.74	10.92	0.18
Rossini	1.1	17.5	16.4**	5.91	4.50	23.8**	77.63	73.93	3.70**	10.02	10.63	0.61
OR02F-B-46	0.4	4.8	4.3**	5.71	4.54	20.6**	72.83	70.30	2.53**	10.31	10.69	0.38
OR02F-C-169	0.5	11.8	11.2**	4.70	3.38	28.1**	78.30	72.88	5.43**	10.29	11.59	1.31**
OR02F-D-27	0.1	5.0	4.9**	5.01	4.59	8.3	71.78	70.33	1.45	9.86	10.47	0.62
WA 7437	0.0	0.0	0.0	4.72	4.27	9.5	78.08	77.85	0.23	11.32	10.49	−0.83*
LSD (0.05)	3.7	3.7		0.63	0.63		1.64	1.64		0.80	0.80	

* Significant at the 0.05 probability level. ** Significant at the 0.01 probability level.
[a]Significance is based on percent whiteheads square root transformed.
[b]Grain yield loss (%) = (noninoculated − inoculated)/noninoculated * 100.

Table 3: Kernel parameters of 10 wheat genotypes grown noninoculated (U) and inoculated (I) with *Cephalosporium gramineum* in field plots in Pendleton, Oregon, 2006.

	Kernel weight avg (mg)			Kernel weight SD			Kernel diameter avg (mm)			Kernel diameter SD		
	U	I	Change[a]	U	I	Change	U	I	Change	U	I	Change
Stephens	40.59	37.42	−3.17**	10.93	13.41	2.48**	2.784	2.650	−0.135**	0.582	0.667	0.085**
Madsen	33.18	30.96	−2.22*	8.62	9.94	1.32**	2.437	2.355	−0.082	0.444	0.509	0.065**
Tubbs	37.88	36.36	−1.51	10.28	12.49	2.21**	2.673	2.594	−0.078	0.525	0.619	0.094**
OR9800919	38.67	36.63	−2.05*	8.89	9.97	1.08**	2.791	2.704	−0.087*	0.530	0.560	0.031
OR9800924	37.28	35.65	−1.63	8.77	9.52	0.75	2.704	2.641	−0.063	0.496	0.516	0.020
Rossini	44.08	42.74	−1.34	9.74	11.28	1.54**	2.991	2.970	−0.021	0.517	0.588	0.071**
OR02F-B-46	34.04	31.38	−2.66**	8.90	10.61	1.71**	2.523	2.390	−0.134**	0.474	0.514	0.040*
OR02F-C-169	35.39	30.34	−5.05**	9.51	11.18	1.67**	2.576	2.337	−0.239**	0.485	0.572	0.088**
OR02F-D-27	30.64	31.07	0.42	7.59	8.99	1.40**	2.382	2.388	0.006	0.460	0.487	0.027
WA 7437	30.62	30.19	−0.43	6.21	6.61	0.40	2.359	2.346	−0.013	0.405	0.426	0.022
LSD (0.05)	1.80	1.80		0.81	0.81		0.078	0.078		0.035	0.035	

* Significant at the 0.05 probability level. ** Significant at the 0.01 probability level.
[a]Change = (inoculated − noninoculated).

Genotypes with significant grain yield loss also had significant reductions in test weight in Pendleton. The reduction in test weight ranged from 0.23 to 5.43 kg hl^{-1}. SD of kernel weight and kernel diameter also were affected by increased disease levels. The same eight genotypes that showed an increase in whitehead scores had a significant increase in kernel weight SD, and six of these genotypes also showed an increase in kernel diameter SD, reflecting an increase in kernel size variability due to higher disease incidence (Tables 3 and 5). Differences in test weight among varieties at Moro were small and nonsignificant for many genotypes. None of the other variables studied at this location presented consistent changes among inoculated and noninoculated treatments. Significant changes were observed but were always genotype dependant.

There was a negative correlation of disease scores with grain yield, with r-values of −0.62 at Pendleton and −0.81 at Moro (Table 6). Similar correlations, but lower in magnitude, were observed between disease and test weight, with $r = -0.52$ at Pendleton and $r = -0.44$ at Moro. Overall, grain yield was independent of test weight, with a nonsignificant correlation ($P > 0.05$). In Pendleton 2006, Cephalosporium stripe response was positively correlated ($P < 0.001$) with kernel weight SD and kernel diameter SD, as would be expected from differences observed among genotypes at contrasting disease levels. These correlations were not significant

Table 4: Percent whiteheads, grain yield, test weight and grain protein of 12 wheat genotypes noninoculated (U) and inoculated (I) with *Cephalosporium gramineum* in field plots in Moro, Oregon, 2007.

	Whiteheads (%)			Grain yield (t ha^{-1})			Test weight (kg hl^{-1})			Grain protein (%)		
	U	I	Change[a]	U	I	Loss (%)[b]	U	I	Loss	U	I	Change
Stephens	10.0	52.5	42.5**	4.26	2.51	41.2**	77.62	74.81	2.81**	9.03	8.70	−0.33
Madsen	6.8	18.3	11.5**	4.10	3.57	13.1*	78.17	77.33	0.84	9.88	8.25	−1.63**
Tubbs	11.8	45.0	33.3**	3.82	2.88	24.6**	76.03	74.16	1.87**	9.00	8.75	−0.25
OR9800919	2.8	31.8	29.0**	5.01	3.51	30.0**	74.19	72.45	1.74**	7.43	8.15	0.73
OR9800924	4.3	26.3	22.0**	4.44	3.41	23.2**	74.87	74.68	0.19	7.80	9.15	1.35**
Rossini	1.5	14.5	13.0**	4.21	3.58	14.8*	77.94	76.07	1.87**	8.83	8.18	−0.65
OR02F-B-46	22.5	44.3	21.8**	3.41	2.71	20.7**	72.71	72.77	−0.06	8.80	9.23	0.42
OR02F-C-169	7.8	42.5	34.8**	3.80	2.43	36.0**	77.97	76.87	1.10	9.85	9.30	−0.55
OR02F-D-27	14.0	34.3	20.3**	4.12	2.81	31.6**	73.58	73.19	0.39	7.68	8.15	0.48
WA 7437	7.5	12.8	5.3	3.15	2.76	12.3	76.94	76.00	0.94	8.53	8.50	−0.03
Skiles	6.8	35.0	28.3**	4.65	3.41	26.6**	79.43	78.00	1.42*	8.38	8.80	0.43
ORSS-1757	5.0	37.5	32.5**	4.07	3.10	23.6**	77.91	76.87	1.03	7.93	7.80	−0.13
LSD (0.05)	8.6	8.6		0.47	0.47		1.27	1.27		1.02	1.02	

* Significant at the 0.05 probability level. ** Significant at the 0.01 probability level.
[a]Significance is based on percent whiteheads square root-transformed.
[b]Grain yield loss (%) = (noninoculated − inoculated)/noninoculated * 100.

Table 5: Kernel parameters of 12 wheat genotypes grown noninoculated (U) and inoculated (I) with *Cephalosporium gramineum* in field plots in Moro, Oregon, 2007.

	Kernel weight avg (mg)			Kernel weight SD			Kernel diameter avg (mm)			Kernel diameter SD		
	U	I	Change	U	I	Change	U	I	Change	U	I	Change
Stephens	37.34	35.22	−2.12	8.42	10.77	2.35**	2.628	2.534	−0.094	0.473	0.563	0.090**
Madsen	31.26	31.31	0.05	7.63	8.17	0.53	2.396	2.441	0.045	0.420	0.468	0.048**
Tubbs	33.75	32.76	−0.99	8.94	9.90	0.96**	2.471	2.413	−0.058	0.463	0.500	0.037*
OR9800919	37.06	33.30	−3.75**	7.80	8.07	0.27	2.610	2.458	−0.152**	0.475	0.473	−0.002
OR9800924	34.61	33.77	−0.84	8.69	8.10	−0.58	2.555	2.508	−0.047	0.501	0.474	−0.027
Rossini	41.39	41.15	−0.24	10.67	11.68	1.00**	2.811	2.784	−0.027	0.558	0.582	0.024
OR02F-B-46	30.55	32.01	1.46	8.05	8.86	0.80*	2.360	2.397	0.037	0.437	0.468	0.032*
OR02F-C-169	33.17	33.04	−0.13	8.69	9.14	0.46	2.477	2.465	−0.012	0.466	0.496	0.030
OR02F-D-27	32.43	32.49	0.06	8.40	8.53	0.13	2.425	2.429	0.004	0.471	0.497	0.026
WA 7437	27.36	26.97	−0.39	7.12	6.66	−0.46	2.207	2.173	−0.034	0.450	0.422	−0.028
Skiles	37.43	36.08	−1.35	9.15	9.04	−0.11	2.538	2.482	−0.056	0.526	0.513	−0.013
ORSS-1757	34.99	34.48	−0.52	8.62	9.19	0.58	2.517	2.499	−0.018	0.483	0.503	0.019
LSD (0.05)	2.24	2.24		0.71	0.71		0.100	0.100		0.032	0.032	

* Significant at the 0.05 probability level. ** Significant at the 0.01 probability level.
[a]Change = (inoculated − noninoculated).

at Moro. There was no significant correlation between disease and mean kernel weight or mean kernel diameter at either location.

Regressions were fitted to estimate % grain yield and test weight loss as a function of increasing susceptibility to Cephalosporium stripe. The response of grain yield to whiteheads difference between inoculated and noninoculated plots in Pendleton followed a polynomial regression that included a quadratic term (Figure 1). The regression model was highly significant ($P < 0.001$) with a coefficient of determination (r^2) of 0.76. The intercept was estimated to be 6.44%, but was not significant ($P = 0.11$). In Moro, the data were best represented with a simple linear regression with coefficient of determination (r^2) of 0.74 (Figure 1). The intercept (7.49%) was significant at $P = 0.06$.

The relationship between whiteheads and reductions in test weight was nonlinear for both locations. The best fit was a polynomial regression with significant quadratic terms ($P < 0.05$) (Figure 2). The intercept was significant for Moro ($P < 0.05$); however, it was not statistically different from zero at Pendleton ($P = 0.19$). The linear coefficients were highly significant ($P < 0.01$) for Pendleton but not for

Table 6: Pearson correlation coefficients among Cephalosporium stripe rating, grain yield, test weight, and several kernel traits of 10 varieties tested in Pendleton 2006 (below diagonal) and 12 varieties tested in Moro 2007 (above diagonal) under inoculated and noninoculated conditions.

	Whiteheads	Whiteheads SQRT	Grain yield	Test weight	Grain protein	Kernel weight (avg)	Kernel weight (SD)	Kernel diameter (avg)	Kernel diameter (SD)
Whiteheads	—	0.986 ***	−0.809 ***	−0.435 *	0.134 ns	−0.155 ns	0.290 ns	−0.190 ns	0.217 ns
Whiteheads SQRT	0.952 ***	—	−0.820 ***	−0.447 *	0.142 ns	−0.217 ns	0.228 ns	−0.249 ns	0.145 ns
Grain yield	−0.626 **	−0.618 **	—	0.304 ns	−0.271 ns	0.461 *	−0.096 ns	0.464 *	−0.011 ns
Test weight	−0.433 +	−0.517 *	0.389 +	—	0.273 ns	0.263 ns	0.093 ns	0.210 ns	0.140 ns
Grain protein	0.540 *	0.526 *	−0.749 ***	−0.262 ns	—	−0.161 ns	−0.001 ns	−0.141 ns	−0.235 ns
Kernel weight (avg)	0.176 ns	0.211 ns	0.419 +	0.229 ns	−0.266 ns	—	0.747 ***	0.971 ***	0.794 ***
Kernel weight (SD)	0.793 ***	0.881 ***	−0.378 ns	−0.413 +	0.332 ns	0.442 *	—	0.730 ***	0.910 ***
Kernel diam. (avg)	0.123 ns	0.161 ns	0.428 +	0.183 ns	−0.262 ns	0.988 ***	0.382 +	—	0.751 ***
Kernel diam. (SD)	0.812 ***	0.879 ***	−0.376 ns	−0.429 +	0.367 ns	0.520 *	0.946 ***	0.486 *	—

+Significant at the 0.10 probability level. *Significant at the 0.05 probability level. **Significant at the 0.01 probability level. ***Significant at the 0.001 probability level.

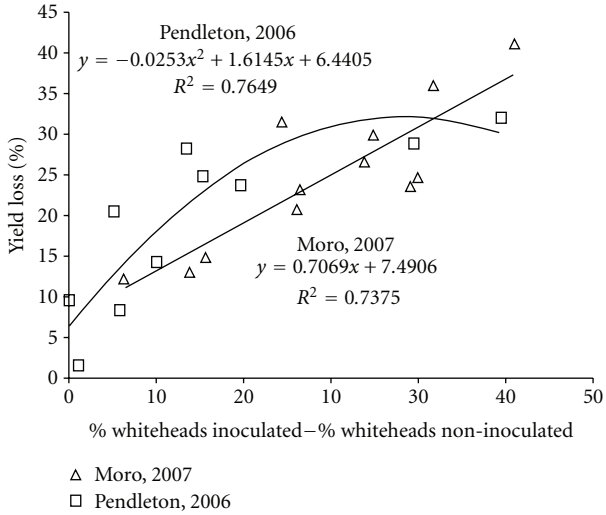

Figure 1: Yield loss caused by inoculation of wheat genotypes with *Cephalosporium gramineum* in Pendleton 2006 (10 wheat genotypes) and Moro 2007 (12 wheat genotypes).

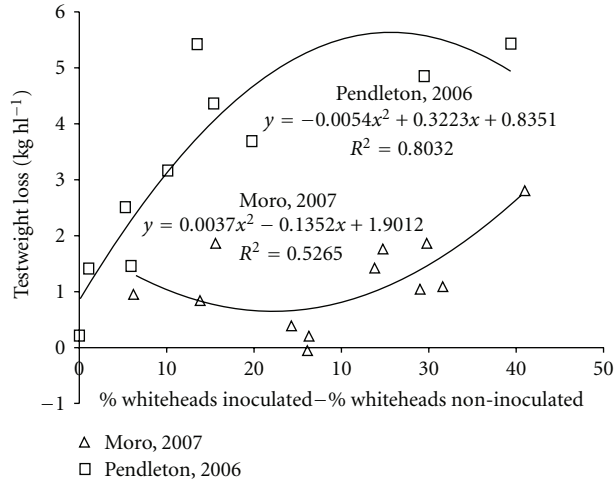

Figure 2: Reduction of grain test weight caused by inoculation of wheat genotypes with *Cephalosporium gramineum* in Pendleton 2006 (10 wheat genotypes) and Moro 2007 (12 wheat genotypes.

the Moro trial ($P = 0.10$). Coefficients of determination were 0.80 and 0.53 for Pendleton and Moro, respectively.

4. Discussion

The general objective of yield loss studies is to provide quantitative estimates regarding effect of disease on its host crop [35]. In many host-pathogen systems, the assessment of crop damage is done using comparisons with a fungicide-protected control [36, 37]. Trials often include artificial inoculation of the pathogen to ensure high and uniform disease pressure. For Cephalosporium stripe, such studies are limited to treatments with artificial inoculation only [17, 20, 22, 38] as no fungicides are yet available for the control of

C. gramineum. Because low levels of whiteheads occurred in noninoculated plots in Pendleton, and even higher levels occurred in Moro, we evaluated yield loss based on differences in whiteheads between inoculated and noninoculated plots.

Despite major differences in rainfall, soil fertility, and environmental stress, susceptible and resistant varieties performed similarly at both Pendleton and Moro. The range of differences in whiteheads between inoculated and noninoculated plots was also similar among locations, with a maximum increase of 40%. Lines with intermediate levels of Cephalosporium stripe showed more variability between locations, however. OR9800924 performed similarly to WA 7437 (resistant check), in Pendleton. In Moro, however, the same variety had a mean 22.0% increase in whiteheads and 23.2% grain yield loss under inoculation. This is not surprising, as significant year × treatment interaction has been reported for Cephalosporium stripe response and associated yield losses by Bockus et al. [20]. Roberts and Allan [39] found no significant genotypic differences for response to *C. gramineum* among 20 varieties in one trial, but important differences among the same set of varieties were found in two other experiments. In our study, some varieties performed differently at each location. Madsen, for example, which is considered to have acceptable levels of field resistance, showed at Pendleton 12.8% whiteheads difference with a mean 24.8% yield loss, close to the level of yield reduction exhibited by the susceptible cultivars Stephens and Tubbs. In Moro, however, with similar whiteheads difference (11.5%), yield loss was only 13.0% and was similar to the reduction observed for the resistant check (WA 7437). All Rossini-derived genotypes, selected in Pendleton, showed less whiteheads under inoculation than their resistant parent in the Pendleton trial, with yield losses ranging from 1.7% to 28.1%. In Moro, however, these lines had higher whiteheads differences than Rossini and yield losses between 21.0 and 36.0%. This indicates that selection for resistance to Cephalosporium stripe should be performed under inoculation in both environments for best results.

Regressions of grain yield loss on whitehead change were similar for both environments, although fitted models were not the same. At Pendleton, a quadratic polynomial provided the best fit, while at Moro the relationship was linear. Intercepts of the two models indicated similar yield loss at zero whitehead change (6.44 and 7.49% at Pendleton and Moro, resp.), though statistical support for the intercepts was not high in either case ($P = 0.06$ and $P = 0.11$ at Pendleton and Moro, resp.). It is noticeable that the intercepts were positive, indicating that even for highly resistant varieties some level of yield loss may occur under high disease pressure. As an example, the highly resistant selection WA 7437 showed around 10% grain yield loss in both environments, yet not significant, with 5% whiteheads or less. Thus, infection by *C. gramineum* probably induces sufficient damage to cause yield loss even in absence of whiteheads. In fact, leaf symptoms are commonly seen on infected plants in absence of whiteheads. Another possibility is that resistance mechanisms induced by the pathogen result in physiological "costs" to the host [40, 41]. "Cost of resistance" has been observed for resistance genes in several host-pathogen systems [37, 42]. When a plant is attacked by a pathogen it induces defense mechanisms that are energy demanding, implying an extra cost for the plant. However, there are no reports as to whether resistance to Cephalosporium stripe involves such a defense mechanism.

At-low-to intermediate disease levels, the relationship between disease and yield loss was linear for both environments. Regression coefficients for Pendleton (0.6) and Moro (0.7) suggest that for each additional unit increase in disease pressure, there is a loss of 0.6 to 0.7% in grain yield (Figure 1). Maximum yield losses estimated by the regressions were around 30 to 35% for Pendleton and Moro, though higher yield losses are certainly possible under more severe disease pressure. Bockus et al. [20] reported yield loss to Cephalosporium stripe ranged from 26 to 65% on a single susceptible cultivar, depending on the year. Earlier, Richardson and Rennie [23] and Johnston and Mathre [17] had reported estimates of potential yield loss on individual plants of up to 70 and 78% respectively.

Analysis of test weight is often included in yield-loss studies to evaluate the effect of disease on grain quality, which can be an important component of the monetary value of the crop. The inclusion of a quadratic effect increased the overall fit of the regressions however, shapes of the curves differed between the two sites. For Pendleton, the function was parabolic while, for Moro, there was a hyperbolic relationship between loss of test weight and whitehead increase. Maximum reduction in test weight was recorded for Stephens and OR02F-C-169 at Pendleton and was $5.43\,\mathrm{kg\,hl^{-1}}$.

Test weight loss increased linearly at a rate of $0.32\,\mathrm{kg\,hl^{-1}}$ for each unit increase in whiteheads in Pendleton. As whiteheads increased to 15 to 20%, the slope decreased, meaning the rate of change in test weight was less at higher disease levels. In contrast, results from Moro indicated that test weight losses were not substantial until more than 25% whiteheads change was observed. The maximum loss observed at Moro was about half of that observed in Pendleton. In studies on take-all, which is another soil-borne pathogen that affects wheat and also produces whiteheads, test weight was usually inversely related to disease severity and responded to take-all intensity similarly to grain yield [38].

In Pendleton, Cephalosporium stripe not only affected grain yield and test weight, but also had a significant impact on uniformity of kernel size and weight. Morton and Mathre [22], investigating the physiological effects of *C. gramineum* on winter wheat, determined that pathogenesis was most damaging after anthesis during the grain filling period. The disease had little impact on the number of seeds per spike, but had large impact on kernel weight. Richardson and Rennie [23] also attributed grain yield losses to the effects of the pathogen that occur later in the life of plants, meaning grain filling. Johnston and Mathre [17] reported that decreased yield of infected plants was related to both decreased weight and number of seeds formed per head. In the Moro trial, although test weight decreased, kernel attributes did not change in relation to increasing disease. Perhaps the disease contributed to subtle changes in kernel conformation, or shape, unrelated to weight or size, that

impacted test weight. The significant yield losses at Moro, without corresponding changes in kernel weight, suggest the disease either reduced tillering or kernel number, as was suggested by Johnston and Mathre [17].

The role and impact of plant pathogens are not static, but change in relation to varieties, environments, management, and cropping systems. Understanding potential damage, risk, and vulnerability from pathogens is important to prioritizing breeding objectives and allocating resources to crossing, selection, and screening of germplasm. It also has direct impact on release decisions, in that new cultivars should have low risk of yield loss from major diseases that occur in the target region. For producers, risk of losses from pathogens are important considerations in many management decisions, including choice of tillage practices, planting date, crop rotations, and choice of varieties. For example, potential yield gains from early fall seeding dates can be far outweighed by increased risk of damage from soil diseases.

Cephalosporium stripe is known to cause significant damage to wheat grown in the Pacific Northwest. Economic damage has been erratic, often inconsistent within fields, and generally reduced by avoiding early plantings. Cephalosporium stripe often is lumped into the category of "chronic diseases," for which modest resources have been allocated for prevention and breeding for resistance. In this study, there was evidence for yield reduction in presence of the disease before whitehead symptoms were significant. Yield losses of nearly 50% were found in the most susceptible varieties. Intermediate levels of resistance were shown to be valuable in reducing economic damage from the pathogen. Varieties with intermediate resistances should be sufficient for most production situations, especially as the disease is generally not highly aggressive. However, higher levels of resistance, as observed in WA 7437, are needed to avoid losses with high inoculum levels and favorable environmental conditions.

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References

[1] G. W. Bruehl, "Cephalosporium stripe disease of wheat in Washington," *Phytopathology*, vol. 46, pp. 178–180, 1956.

[2] J. B. Ellis and B. M. Everhart, "New species of fungi from various localities," *Proceedings of the Academy of Natural Sciences of Philadelphia*, vol. 46, pp. 322–386, 894.

[3] Y. Nisikado, H. Matsumoto, and K. Yamuti, "Studies on a new Cephalosporium, which causes the stripe disease of wheat," *Bericht des Ohara Instituts fur Landwirtschaftliche Forschungen*, vol. 6, pp. 275–306, 1934.

[4] G. W. Bruehl, "Cephalosporium stripe disease of wheat," *Phytopathology*, vol. 47, pp. 641–649, 1957.

[5] W. G. Willis and O. D. Shively, "Cephalosporium stripe of winter wheat and barley in Kansas," *Plant Disease Reporter*, vol. 58, pp. 566–567, 1974.

[6] E. G. Gray and M. Noble, "Cephalosporium stripe in cereals in Scotland," *FAO Plant Protection Bulletin*, vol. 8, p. 46, 1960.

[7] D. L. Hawksworth and J. M. Waller, "*Hymenula cerealis*," Descriptions of Fungi and Bacteria, CAB International, UK, Sheet 501, 1976.

[8] K. Kobayashi and T. Ui, "Phytotoxicity and antimicrobial activity of graminin A, produced by *Cephalosporium gramineum*, the causal agent of Cephalosporium stripe disease of wheat," *Physiological Plant Pathology*, vol. 14, no. 1, pp. 129–133, 1979.

[9] D. B. Slope and R. Bardner, "Cephalosporium stripe of wheat and root damage by insects," *Plant Pathology*, vol. 14, pp. 187–187, 1965.

[10] C. C. Mundt, "Performance of wheat cultivars and cultivar mixtures in the presence of Cephalosporium stripe," *Crop Protection*, vol. 21, no. 2, pp. 93–99, 2002.

[11] C. C. Mundt, "Cephalosporium stripe," in *Compendium of Wheat Diseases and Pests*, W. W. Bockus, R. L. Bowden, R. M. Hunger, W. L. Morrill, T. D. Murray, and R. W. Smiley, Eds., pp. 23–26, APS Press, St. Paul, MN, USA, 3rd edition, 2010.

[12] T. D. Murray, "Seed transmission of *Cephalosporium gramineum* in winter wheat," *Plant Disease*, vol. 90, no. 6, pp. 803–806, 2006.

[13] P. Lai and G. W. Bruehl, "Survival of *C. gramineum* in naturally infested wheat straws in soil in the field and in the laboratory," *Phytopathology*, vol. 56, pp. 213–218, 1966.

[14] D. E. Mathre and R. H. Johnston, "Cephalosporium stripe of winter wheat: infection processes and host response," *Phytopathology*, vol. 65, pp. 1244–1249, 1975.

[15] M. V. Wiese and A. V. Ravenscroft, "Quantitative detection of propagules of *Cephalosporium gramineum* in soil," *Phytopathology*, vol. 63, pp. 1198–1201, 1973.

[16] D. H. Spalding, G. W. Bruehl, and R. J. Foster, "Possible role of pectinolytic enzymes and polysaccharide in pathogenesis by *Cephalosporium gramineum* in wheat," *Phytopathology*, vol. 51, pp. 227–235, 1961.

[17] R. H. Johnston and D. E. Mathre, "Effect of infection by *Cephalosporium gramineum* on winter wheat," *Crop Science*, vol. 12, pp. 817–819, 1972.

[18] D. E. Mathre and R. H. Johnston, "Cephalosporium stripe of winter wheat—procedures for determining host response," *Crop Science*, vol. 15, pp. 591–594, 1975.

[19] J. B. Morton, D. E. Mathre, and R. H. Johnston, "Relation between foliar symptoms and systemic advance of *Cephalosporium gramineum* during winter wheat development," *Phytopathology*, vol. 70, pp. 802–807, 1980.

[20] W. W. Bockus, M. A. Davis, and T. C. Todd, "Grain yield responses of winter wheat coinoculated with *Cephalosporium gramineum* and *Gaeumannomyces graminis* var. *tritici*," *Plant Disease*, vol. 78, no. 1, pp. 11–14, 1994.

[21] D. E. Mathre, R. H. Johnston, and C. F. McGuire, "Cephalosporium stripe of winter wheat—pathogen virulence, sources of resistance, and effect on grain quality," *Phytopathology*, vol. 67, pp. 1142–1148, 1977.

[22] J. B. Morton and D. E. Mathre, "Physiological-effects of *Cephalosporium gramineum* on growth and yield of winter-wheat cultivars," *Phytopathology*, vol. 70, pp. 807–811, 1980.

[23] M. J. Richardson and W. J. Rennie, "An estimate of the loss of yield caused by *Cephalosporium gramineum* in wheat," *Plant Pathology*, vol. 19, pp. 138–140, 1970.

[24] J. B. Morton and D. E. Mathre, "Identification of resistance to Cephalosporium stripe in winter wheat," *Phytopathology*, vol. 70, pp. 812–817, 1980.

[25] W. W. Bockus, J. P. O'Connor, and P. J. Raymond, "Effect of residue management method on incidence of Cephalosporium stripe under continuous winter wheat production," *Plant Disease*, vol. 67, pp. 1323–1324, 1983.

[26] R. X. Latin, R. W. Harder, and M. V. Wiese, "Incidence of Cephalosporium stripe as influenced by winter wheat management practices," *Plant Disease*, vol. 66, pp. 229–230, 1982.
[27] J. M. Martin, R. H. Johnston, and D. E. Mathre, "Factors affecting the severity of Cephalosporium stripe of winter wheat," *Canadian Journal of Plant Pathology*, vol. 11, pp. 361–367, 1989.
[28] T. D. Murray, C. C. Walter, and J. C. Anderegg, "Control of Cephalosporium stripe of winter wheat by liming," *Plant Disease*, vol. 76, pp. 282–286, 1992.
[29] R. A. F. Pool and E. L. Sharp, "Some environmental and cultural factors affecting Cephalosporium stripe of winter wheat," *Plant Disease Reporter*, vol. 53, pp. 898–902, 1969.
[30] P. J. Raymond and W. W. Bockus, "Effect of seeding date of winter wheat on incidence, severity, and yield loss caused by Cephalosporium stripe in Kansas," *Plant Disease*, vol. 68, pp. 665–667, 1984.
[31] H. Li, R. L. Conner, and T. D. Murray, "Resistance to soilborne diseases of wheat: contributions from the wheatgrasses *Thinopyrum intermedium* and *Th. ponticum*," *Canadian Journal of Plant Science*, vol. 88, no. 1, pp. 195–205, 2008.
[32] G. W. Bruehl, T. D. Murray, and R. E. Allan, "Resistance of winter wheats to Cephalosporium stripe in the field," *Plant Disease*, vol. 70, pp. 314–316, 1986.
[33] J. M. Martin, D. E. Mathre, and R. H. Johnston, "Genetic variation for reaction to *Cephalosporium gramineum* in 4 crosses of winter wheat," *Canadian Journal of Plant Science*, vol. 63, pp. 623–630, 1983.
[34] P. A. Shefelbine and W. W. Bockus, "Decline of Cephalosporium stripe by monoculture of moderately resistant winter wheat cultivars," *Phytopathology*, vol. 79, pp. 1127–1131, 1989.
[35] S. Savary, P. S. Teng, L. Willocquet, and F. W. Nutter, "Quantification and modeling of crop losses: a review of purposes," *Annual Review of Phytopathology*, vol. 44, pp. 89–112, 2006.
[36] C. A. Griffey, M. K. Das, and E. L. Stromberg, "Effectiveness of adult-plant resistance in reducing grain yield loss to powdery mildew in winter wheat," *Plant Disease*, vol. 77, pp. 618–622, 1993.
[37] S. A. Herrera-Foessel, J. Huerta-Espino, J. Crossa, J. Yuen, and A. Djurle, "Effect of leaf rust on grain yield and yield traits of durum wheats with race-specific and slow-rusting resistance to leaf rust," *Plant Disease*, vol. 90, no. 8, pp. 1065–1072, 2006.
[38] R. J. Gutteridge, G. L. Bateman, and A. D. Todd, "Variation in the effects of take-all disease on grain yield and quality of winter cereals in field experiments," *Pest Management Science*, vol. 59, no. 2, pp. 215–224, 2003.
[39] D. E. Roberts and R. E. Allan, "Cephalosporium stripe resistance and grain yield potential of wheat lines with strawbreaker resistance derived from *Aegilops ventricosa*," *Plant Disease*, vol. 74, pp. 852–857, 1990.
[40] J. K. M. Brown, "Yield penalties of disease resistance in crops," *Current Opinion in Plant Biology*, vol. 5, no. 4, pp. 339–344, 2002.
[41] C. B. Purrington, "Costs of resistance," *Current Opinion in Plant Biology*, vol. 3, no. 4, pp. 305–308, 2000.
[42] J. Bergelson and C. B. Purrington, "Surveying patterns in the cost of resistance in plants," *American Naturalist*, vol. 148, no. 3, pp. 536–558, 1996.