AN ABSTRACT OF THE THESIS OF

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Title: Cephalosporium Stripe of Wheat: Seedling-Based Resistance Screening and Pathogenic Variability

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Cephalosporium stripe of wheat (*Triticum aestivum*), caused by the soilborne fungus *Cephalosporium gramineum*, results in significant yield reductions in dryland winter wheat crops of the U.S. Pacific Northwest. The development of resistant cultivars offers the best hope for disease control. Breeding for resistance is hampered by the long trial times inherent in screening adult plants, and by cultivar x environment interactions in field tests. The principal objective of this research was to develop and test a procedure for screening wheat seedlings in controlled environments for resistance to Cephalosporium stripe.

Wheat seedlings were raised hydroponically in growth chambers, and the fungus was increased in large fermentation tanks. The seedlings were inoculated at about 12 days post-germination. Disease severity was assessed approximately seven days later using a

chlorophyll meter to measure the symptoms of chlorosis and striping.

In three trials, five soft white cultivars from the Pacific Northwest and four hard red cultivars from the Southern Great Plains with known levels of field resistance were tested with a Pacific Northwest fungal isolate. With one exception, chlorophyll readings ordered the cultivars appropriately, with moderately resistant cultivars ranking above susceptible cultivars. Three other moderately resistant cultivars from the Pacific Northwest also appeared in one or two trials, and were ranked properly by chlorophyll level.

Chlorophyll levels of uninoculated plants were assayed to determine if differences in chlorophyll content were innate in the cultivars. The chlorophyll levels of uninoculated and inoculated seedling treatments were only significantly correlated when the cultivar Madsen, which ranks high both in resistance and in chlorophyll content, was included. In adult plants, flag-leaf chlorophyll level corresponded to intensity of Cephalosporium stripe symptoms where disease was present, and was independent of known field resistance in undiseased cultivars.

The seedling screening technique was used to investigate pathogenic variability in *C. gramineum*. In two experiments, a total of eight cultivars from the Pacific Northwest and the Southern Great Plains were tested with three fungal isolates from each region. No

evidence of virulence/vertical resistance was found. There was also no significant adaptation of isolates to greater virulence on cultivars from the same region.

Cephalosporium Stripe of Wheat: Seedling-Based Resistance Screening and Pathogenic Variability

by

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I understand that my thesis will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my thesis to any reader upon request.

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Christina Cowger, Author

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CHAPTER I
INTRODUCTION

IMPORTANCE OF THE DISEASE

Cephalosporium stripe is a monocyclic, vascular wilt disease caused by *Cephalosporium gramineum* Nisikado & Ikata, a soilborne fungus. The pathogen infects wheat through the roots and colonizes the entire vascular system, causing stunting, leaf striping, and blighted heads with poor grain fill (4).

In the last 15 years, Cephalosporium stripe has become one of the most damaging diseases in Pacific Northwest winter wheat production. Under conditions favorable to disease, the yield of infected plants can be as little as 20% or less that of healthy plants (12,16). In areas conducive to Cephalosporium stripe, a generalized infection can reduce yield in a field by 50% or more (5). The dominant cultivar in Oregon, Stephens, is among the most susceptible to the disease.

PATHOGEN BIOLOGY

Life Cycle. In its parasitic phase, *C. gramineum* enters wheat roots in the fall and winter (4,32). It ramifies throughout the root cortex and then enters the xylem either by active penetration or passively through

breaks in vascular tissue (32). Once inside the xylem, the pathogen colonizes the entire vascular system. Its ability to infest wheat from the base to the tip of the rachis, creating a considerable reservoir of infected material upon death of the host, is key to its survival and future spread (9).

C. gramineum lives in host debris as a saprophyte, and can survive for up to three years between contacts with a living host (36). Its mycelium takes rapid and effective possession of the dead substrate, due to the high degree of initial vascular colonization (8). *C. gramineum* produces toxic metabolites which may serve to guarantee it sole access to the host substrate (9).

In the fall, with the onset of cooler, moister weather, *C. gramineum* enters its sporodochial stage (*Hymenula cerealis* El. & Ev.). The infested, superficial crop debris begins to support profuse sporulation (4). Sporodochia form on the outside of the wheat residue, and large quantities of conidia are produced in a mucilaginous matrix (4,38). The conidia, which wash down into the root zone, are the infective propagules for the next crop (19,37).

As with many soilborne pathogens, no sexual stage has been reported for *C. gramineum*. It reproduces asexually by means of sporodochia that produce copious phialospores,

phialides on mycelium, or blastogenously in host xylem vessels (38).

Conditions favoring disease. Low soil pH increases the severity of Cephalosporium stripe by mechanisms that are only partially understood. Survival of *C. gramineum* in infested straw is greater when soil in which the straw is buried is at pH 3.9-5.5 than at pH 7.6, possibly because the lower pH values enhance activity of the antibiotic produced by the fungus (7). Sporulation on artificially colonized oat kernels on soil, and survival of conidia in soil, are significantly greater at pH 4.7 than at 5.7-7.5 (31). Soil pH of 5.1-5.3 increases disease incidence over pH values above 6.0, particularly in years when root injury due to freezing is relatively minor; otherwise, the effect of the injury appears to overwhelm that of pH (25).

A number of researchers have observed that high soil moisture increases disease incidence and severity (1,5,28). Increased soil matric potential contributes to disease through improved saprophytic survival of *C. gramineum* in straw (6,7), increased sporulation (5,30), and probably through root damage from increased soil heaving due to frost (5,7). In Oregon's wheat-growing regions, prevalence of Cephalosporium stripe does not coincide with higher moisture (Mundt, *field observations*).

Evidently, moisture levels are not limiting where the disease is widespread and severe.

Early seeding results in extensive autumn root growth and, thus, more sites for potential infection when sporulation by *C. gramineum* is most profuse (27). Similarly, high soil temperatures following planting stimulate root growth and thus contribute to greater disease severity (26).

Infection mechanism and the role of freezing. For many years, it was thought that *C. gramineum* did not actively penetrate host roots, but rather relied for its entry on freeze-induced root breakage and the subsequent "vacuuming" effect of roots taking up water (19). A fungistatic factor operating in natural soil was believed to inhibit mycelial growth, sporulation, and germination (19). Root-wounding was held to be necessary for successful pathogenesis (5,24).

Subsequently, however, it was demonstrated that the conidia of *C. gramineum* are stimulated by freeze-induced wheat root exudates to germinate and produce mycelium that penetrates host roots (2). In addition, greenhouse studies indicated that severe disease can develop in the absence of freezing temperatures (1). Isolations from field-grown plants showed that the fungus becomes established in roots in the fall before the soil freezes (32). *C. gramineum* was rarely isolated from wheat roots

with intact tips or from young roots near intact tips, suggesting that the fungus may infect senescent root tissue (32).

It is therefore likely that *C. gramineum* is capable of active penetration, but generally requires weakened host tissue. Root breakage hastens colonization by allowing the fungus direct and immediate access to the xylem.

Role of toxin in pathogenesis. *C. gramineum* produces a wide-spectrum toxin, Graminin A, which inhibits the growth of many fungi and bacteria (10). The toxin helps *C. gramineum* maintain control of the wheat debris, which is its saprophytic substrate, by restricting other potential colonists (10). Some workers (14) have suggested that Graminin A may also be involved in pathogenesis. At low concentrations, the toxic compound produces chlorosis and browning of leaves and vascular tissues of wheat cuttings, but not cuttings of non-hosts (14). Other researchers (10,34), however, have shown that *C. gramineum* strains that do not produce the toxin are pathogenic.

POTENTIAL CONTROL MEASURES

Delayed seeding diminishes disease because plants have smaller root systems during the time of heavy sporulation by *C. gramineum*, and possibly because root

injury due to frost heaving or insect damage is minimized (27). Deep plowing and burning of debris also reduce inoculum loads; reduced tillage maintains higher levels of disease (3,15). However, delayed seeding, deep plowing, and burning are undesirable because they aggravate soil erosion (25).

Crop rotation helps reduce inoculum loads, but is not always economically feasible (25). Liming to raise soil pH reduces disease incidence in years when root injury from frost damage is relatively slight (25). Once plants are infected by *C. gramineum*, however, soil pH has no effect on subsequent colonization. Liming is unlikely to be economically feasible for most Oregon growers, since there is no ready and inexpensive source of lime.

No fungicide is registered for use against *C. gramineum*.

Resistance. In wheat, resistance to *C. gramineum* is quantitative and there are presently no cultivars with high levels of resistance (12,22,24). Nearly complete resistance to Cephalosporium stripe is present in wild wheat relatives, including *Agropyron elongatum* (20,21), and efforts are underway to locate and transfer the resistance genes to wheat (12).

Resistance phenotypes. The mechanism(s) of resistance to Cephalosporium stripe, and their genetic basis, are still not well understood. Various phenotypes

conferring resistance to the disease have been proposed. Martin *et al.* (18) identified three categories of resistance in cultivars they tested: (1) limited ingress to the host; (2) restricted pathogen movement within the host after infection; and (3) tolerance, indicated by minor symptoms despite high infection level.

One suggested resistance trait is reduced susceptibility to root breakage. No association between root tensile strength and resistance was found by Mathre and Johnston (20). However, root breakage and/or wound-healing may vary with acid tolerance (30).

The possibility of a crown barrier to *C. gramineum* was investigated by Mathre and Johnston (20). Movement of the fungus through root and especially through crown tissues was slowest in *Agropyron elongatum* and another wild relative. Movement was next slowest in Agrotritichum, and fastest in *Triticum aestivum* cultivars. Differences in crown morphology could therefore constitute one of the reasons for variation in susceptibility observed in wheat cultivars.

Breeding for resistance. Repeated planting of moderately resistant cultivars has proven an effective strategy for reducing Cephalosporium stripe over several seasons (11,29). In a three-year study in which cultivars were replanted each year in the same soil, moderately resistant cultivars showed substantial declines in disease

incidence and severity as compared to susceptible cultivars (29). In the central wheat region of Kansas, Cephalosporium stripe declined from a major disease in the mid-1970s to a minor one by the mid-1980s due to a shift from susceptible to moderately resistant wheat types (29).

To date, few cultivars are available in Oregon that combine high levels of resistance with other desirable qualities. Cephalosporium stripe resistance is therefore an important current breeding objective, and improved resistance screening procedures are needed.

Until now, resistance screening has been conducted in the field with adult, vernalized plants. This has presented several problems. Host reaction to Cephalosporium stripe is highly dependent on environment, and the ranking of cultivars can vary substantially from year to year (11). Distribution of the disease in a field can be patchy, making incidence difficult to quantify and complicating experiments. The use of adult plants also means that a single trial requires a year in the field or 5 to 6 months in the greenhouse (16). Moreover, given cultivar x environment interactions, multiple trials are necessary to properly evaluate new material.

For these reasons, there is a need for a rapid screening procedure under conditions that minimize environmental variation. The main objective of this research was to develop and test such a procedure.

GENETIC VARIATION IN THE PATHOGEN

Little is known about the genetics of *C. gramineum*. With the exception of one study from 1984 (35), which is discussed in Chapter III, there does not seem to be any literature on genetic variation, pathogenic or otherwise, in this fungus. Information about variation in soilborne fungi, while improving, is generally much scarcer than that on airborne pathogenic fungi.

Variability in *C. gramineum* is relevant to the strategy of breeding for resistance in at least two ways. First, it is important to know if there is variability in virulence *sensu* van der Plank (33) when designing a screening program. The existence of cultivar-specific races would condition the selection of isolates for screening experiments.

Second, information about pathogenic and other genetic variability in *C. gramineum*, will help predict the durability of resistance. Greater variation in the pathogen offers greater evolutionary potential to overcome resistance genes (23). If, as seems likely, the pathogenic variability of *C. gramineum* is low, the resistance of new varieties that are developed will likely be relatively long-lasting.

The research described here concerning pathogenic variability in *C. gramineum* merely constitutes a small step. Further investigation with a wider spectrum of

isolates and host material would be useful. It would also be interesting to complement these experiments by comparing the genotypes of isolates from diverse geographic regions at selected molecular markers.

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CHAPTER II

A HYDROPONIC, SEEDLING-BASED ASSAY FOR RESISTANCE TO CEPHALOSPORIUM STRIPE OF WHEAT

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ABSTRACT

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A procedure was developed to rate ≈20-day-old winter wheat seedlings for resistance to Cephalosporium stripe, a vascular wilt caused by the soilborne fungus *Cephalosporium gramineum*. Seedlings were inoculated after 12-15 days of growth in liquid culture in controlled-environment chambers, and then assessed for disease symptoms at 7-8 days post-inoculation. Disease severity was assayed by measuring chlorophyll in the youngest fully expanded leaf, using a chlorophyll meter. Four replicated trials included a total of 12 hard red winter cultivars from the U.S. Southern Plains and soft white winter cultivars from the U.S. Pacific Northwest. With one exception, the procedure consistently ranked cultivars correctly, according to field performance, as moderately resistant or susceptible. Jagger, a moderately resistant, hard red Kansas wheat, was ranked with susceptible cultivars in one of three trials.

Additional keywords: *Hymenula cerealis*, *Triticum aestivum*

INTRODUCTION

Cephalosporium stripe is a monocyclic, vascular wilt disease of winter wheat and other grasses (5). The causal agent, *Cephalosporium gramineum* Nisikado & Ikata (*Hymenula cerealis* Ell. & Ev.), is a soilborne, facultative fungal parasite (5). It enters wheat roots in the fall and winter (13,22), colonizes the vascular system, and causes leaf striping, stunting, and prematurely ripening heads (whiteheads) in the spring and summer (5,9). The fungus oversummers as a saprophyte in wheat debris and can remain viable in superficial wheat straw for up to three years (26).

Cephalosporium stripe inflicts substantial losses on winter wheat crops in the Pacific Northwest of the U.S. (6,17). Currently, no fungicide is known to be effective against the pathogen, and effective cultural practices (burning of stubble, deep tillage, and delayed seeding) result in increased soil erosion (25). Repeated planting of moderately resistant cultivars can reduce both the incidence and severity of Cephalosporium stripe (7,19). The development of host resistance currently offers the best hope for control of the disease in the Pacific Northwest.

Currently, breeding programs are hampered in identifying resistance to Cephalosporium stripe by long trial times and variability in symptom expression. Only

one field trial can be completed per year. Host reaction to the disease is highly dependent on environment, and the ranking of cultivars can vary substantially from year to year (7), rendering multiple trials both necessary and contradictory. Therefore, rapid initial screening of cultivars under controlled conditions is desirable.

Until now, most controlled-environment work with Cephalosporium stripe has been done with vernalized, adult wheat plants, requiring a total trial time of 5-6 mo (12; T. Murray, *personal communication*). In one study, winter wheat seedlings were assayed for Cephalosporium stripe resistance in controlled-environment chambers (24). That screening procedure distinguished highly susceptible from highly resistant lines, but was found less useful for varieties with intermediate susceptibility.

Our work shows that measurable symptoms can be produced in wheat seedlings grown in liquid culture in growth chambers, and that the severity of those symptoms corresponds to the resistance ratings of adult plants in the field. Our method distinguishes cultivars with moderate resistance from those that are susceptible, and allows characterization of resistance within one month after germination of seeds.

MATERIALS AND METHODS

Biological Materials. Wheat genotypes evaluated were eight soft white winter wheat cultivars from the Pacific Northwest (Oregon and Washington) and four hard red winter wheat cultivars from the U.S. Southern Plains (Kansas and Texas). The 12 cultivars vary in their field reactions to *C. gramineum* (Table 1). *C. gramineum* was isolated in July 1995 from a field trial in Pendleton, OR, that had been infested in 1993 with an isolate obtained from the Palouse region of eastern Washington.

Experimental Design. Cultivars were tested in four trials conducted in October 1995, November 1995, February 1996, and March 1996. The experiment was conducted in a randomized complete block design, with four blocks in the second and fourth trials and three blocks in the first and third trials. The experimental unit was a container of plants (described below); containers were placed in several growth chambers. There were one or two blocks per growth chamber, depending on chamber size.

In the second and third trials, each container was planted half with one cultivar and half with another. Cultivars were paired randomly, and randomization was conducted separately for each replication. In the first and fourth trials, each container held only one cultivar.

Plant Growth. Seed was surface-disinfested in 0.525% NaOCl solution for 15 min. Seeds were pre-germinated 1-2

Table 1. Chlorophyll readings from three trials of winter wheat seedlings inoculated with *Cephalosporium gramineum* in liquid culture

Cultivar	Origin[x]	Chlorophyll reading[v,w] Nov.	Feb.	Mar.	Comb.	Field Reaction[y]
Madsen	PNW	32.8a	30.5a	32.0a	31.7a	MR
Plainsman V	SGP	27.5b	24.6bc	27.0b	26.3b	MR
Newton	SGP	25.7bc	25.3b	27.0b	25.9bc	MR
Jagger	SGP	25.2bcd	24.8bc	21.8d	24.1cd	MR
Gene	PNW	23.0cde	22.4bc	22.4cd	22.6de	S
Stephens	PNW	23.1cde	21.8bc	22.3cd	22.4de	S
Sturdy	SGP	23.3cde	22.8bc	20.5d	22.4de	S
MacVicar	PNW	22.3de	22.5bc	20.5d	21.9e	S
Malcolm	PNW	21.5e	21.3c	20.5d	21.2e	S
Lewjain[z]	PNW	-	23.6bc	25.6bc	-	MR
Rod[z]	PNW	-	-	26.9b	-	MR
Lambert[z]	PNW	-	-	26.1b	-	MR
LSD		3.31	3.81	3.38	1.96	

[v]Readings taken with a chlorophyll meter in four places along the youngest fully expanded leaf of each plant and averaged to produce one measurement per plant. Lower values indicate greater disease severity. Values are means of four replicated treatments of ≈10 plants each in the first two trials, and three replicated treatments of ≈20 plants each in the third trial.

[w]Means within a column with the same letter are not significantly different ($P = 0.05$) according to Fisher's protected LSD test.

[x]PNW = Pacific Northwest soft white cultivar; SGP = Southern Great Plains hard red cultivar.

[y]MR = moderately resistant; S = susceptible.

[z]Cultivars tested in one or two trials only.

days in distilled water and continuously aerated with an aquarium bubbler. The seeds were then germinated over damp, sterile sand on plastic mesh in styrofoam holders that were covered with moist paper towels.

When roots protruded through the mesh into the sand, the seedlings were removed from the sand and the roots were rinsed in dH_2O. The seedlings were transferred in their holders to plastic containers measuring approximately 24.5 cm in height, 21 cm in width and 8.5 cm in depth (Tupperware Corp., P.O. Box 2353, Orlando, FL 32802-2353), and painted black to inhibit algal growth. The containers were filled with 4500 ml of a modified Hoaglund's solution (18) adjusted to pH 5.0 ± 0.1 [4 mM $Ca(NO_3)_2$, 2 mM $MgSO_4$, 4 mM KNO_3, 0.435 mM $(NH_4)_2SO_4$, 0.5 mM KH_2PO_4, 2 μM $MnSO_4$, 0.3 μM $CuSO_4$, 0.8 μM $ZnSO_4$, 30 μM NaCl, 0.0143 μM $(NH_4)_6Mo_7O_2$, 10 μM H_3BO_3, 10 μM Fe-EDTA].

Seedlings were then grown in growth chambers (≈ 3 x 10^8 erg $\cdot m^{-2} \cdot s^{-1}$) at 20° ± 2° C for 19-23 days, and the nutrient solutions were aerated with aquarium bubblers. After three days, each container was thinned to 20 seedlings.

Inoculation. *C. gramineum* was increased in 80%-strength (345 g/L) potato dextrose broth. The fungus was grown first in seed flasks with 1800 ml of broth each, which were then used to inoculate carboys each containing 18 L of broth. After about five days, the broth was

strained through cheesecloth to remove mycelium. Conidia were separated from the broth by centrifugation. The conidia were resuspended in a small volume of distilled water or nutrient solution, and their concentration was determined using a haemocytometer.

After 12-15 days' growth in liquid medium, each inoculated container of wheat seedlings received conidia and fresh nutrient solution in the proportions needed to generate a suspension of $\approx 1 \times 10^8$ conidia per ml. In three of the trials, uninoculated control treatments were included, and they received fresh nutrient solution only. The solution was adjusted to pH 5.0 ± 0.1 and roots were suspended in it by floating the styrofoam holders on the surface.

Germinability of the inoculum was determined by diluting it to $\approx 10^2$ conidia/ml, plating 0.5 ml on potato dextrose agar, and counting germinated spores after ≈7 days' incubation at 13° C. Germination averaged 45%.

Symptom Assessment. Plants were maintained in growth chambers with their roots bathed in the inoculum suspension for 7-8 days. Solution pH was returned to 5.0 ± 0.1 daily for 4 days after inoculation.

On day 7-8 after inoculation, yellowing and/or striping of leaves was assessed by measuring chlorophyll content with a SPAD-502 chlorophyll meter (Minolta Camera Co., Ltd., distributed by Spectrum Technologies, Inc.,

Plainfield, IL). The meter calculates values based on the ratio of light intensities transmitted by the leaf at two wavelengths which differ widely for absorption of light by chlorophyll. Values reported correspond to the amount of chlorophyll present in a leaf; lower values indicate less chlorophyll, or more severe yellowing. The youngest fully expanded leaf was measured in four places, spaced approximately equally from base to tip of the leaf, and the measurements were averaged to yield one reading per plant.

Infection by *C. gramineum* was confirmed by isolating the pathogen from infected plants following symptom assessment. Two plants from each of several randomly chosen treatments in each replication were sampled. Stem sections of approximately 6 cm were surface-disinfested in 0.525% NaOCl solution for 90 sec, allowed to air-dry under a hood, and cut into 1.5-cm segments. The segments were plated on *C. gramineum* selective medium (CGSM) as developed by Specht and Murray (21), and sequentially incubated 4 days at 5°, 4-5 days at 13° C, and 7-10 days at 5° C. On average, *C. gramineum* was recovered from 77% of treatments sampled. There was no association with host cultivar or replication among the segments from which *C. gramineum* was not recovered; most were overgrown by other fungi in the earlier experiments while the isolation technique was in development.

Comparison to Adult Plants in the Field. To test the relevance of chlorophyll measurements as predictors of infection severity in adult plants, readings were taken on Madsen (moderately resistant) and Stephens (susceptible) plots at the milk stage in plots at the Columbia Basin Agricultural Research Center in Moro, OR. The plots had been inoculated in fall 1993, and the inoculum present in fall 1995 was what remained in the debris from the 1993-94 winter wheat trial. Plots lay fallow during the 1994-95 season, and were replanted in fall 1995. In June 1996, 50 tillers of each cultivar were sampled in each of three plots in a randomized complete block design. Starting 1.5 m from the end of each plot, the first 50 tillers in a center row were selected. Chlorophyll was measured in flag leaves in the same manner as with seedlings, and the plants were tagged and numbered. Twelve days later, the same plants were surveyed for height and the presence of white heads.

Chlorophyll was also measured in the flag leaves of plants at the milk stage in June 1996 in an experiment planted in a naturally infested commercial field near Moro, OR. The susceptible cultivar Stephens and the three moderately resistant cultivars Lambert, Rod, and Madsen were sampled. Fifteen plants of each cultivar were selected blindly at 1-m intervals for measurement in each of four plots planted in a randomized complete block

design. In the same plots, the percentage of culms expressing white heads or incipient white heads was visually estimated at the late milk/early dough stage, 15 days after the chlorophyll readings.

Plants protected from foliar diseases in field plots at the Hyslop Field Research Laboratory in Corvallis, OR, were assayed for flag leaf chlorophyll in May 1996 and May 1997, at the heading stage. The purpose was to test whether chlorophyll content ranks healthy adult plants in the same order as diseased plants. Cephalosporium stripe does not occur in western Oregon, where this field is located. In the first year, one plot each of six cultivars and two plots each of two other cultivars were sampled. Eight to nine plants were selected randomly and flag leaf chlorophyll was measured in the same manner as in previously described experiments. In the second year, measurements were taken in three plots each of six cultivars.

Statistical Analyses. Chlorophyll data were subjected to analysis of variance for individual controlled-environment trials and across trials. Separate analyses were conducted for readings of the uninoculated plants. Data analyzed were the mean chlorophyll readings for all plants of the same cultivar in a container (the experimental unit). Cultivar means were separated using Fisher's protected least significant difference test. In

addition, correlation between inoculated and uninoculated means was evaluated for the October, November, and February trials.

Data on chlorophyll, plant height, and white head data from the field trials were first averaged over all plants in a plot (the experimental unit), and then subjected to analysis of variance and means separation using Fisher's protected LSD test.

RESULTS

Growth Chamber Trials. In each of the four trials, differences among inoculated cultivars were highly significant (respectively, $P \leq 0.0001$, $P \leq 0.0001$, $P \leq 0.0022$, and $P \leq 0.0001$). Three trials (November, February, and March) were analyzed in combination, since they included nine cultivars in common and the trial x cultivar interaction was not significant ($P = 0.7379$) in the combined analysis; cultivar differences were highly significant ($P \leq 0.0001$).

In each trial, cultivars usually ranked in accordance with their known field performance relative to each other (Tables 1 and 2). The five susceptible cultivars consistently ranked lowest, except where Jagger placed among them in the March trial. Jagger's field resistance is actually equal to or greater than that of Plainsman V (4 and W. W. Bockus, *personal communication*).

Table 2. Chlorophyll readings of month-old winter wheat cultivars inoculated and not inoculated with *Cephalosporium gramineum* in liquid culture

Cultivar[y]	Chlorophyll[w,x]		Field Reaction[z]
	Uninoc.	Inoc.	
Madsen	38.1a	37.2a	MR
Lambert	34.5b	33.1b	MR
MacVicar	32.4cd	28.6c	S
Stephens	34.0bc	27.6cd	S
Gene	31.5d	25.3d	S
LSD	1.91	3.08	

[w]Readings taken with a chlorophyll meter in four places along the youngest fully expanded leaf of each plant and averaged to produce one measurement per plant. Values are means of four replicated treatments of ≈10 plants each.

[x]Means within a column with the same letter are not significantly different ($P = 0.05$) according to Fisher's protected LSD test.

[y]All cultivars from Pacific Northwest.

[z]MR = moderately resistant; S = susceptible.

One of the four trials placed susceptible and moderately resistant cultivars in separate groups at the 0.05 probability level; the other three trials did not. In the combined analysis, the moderately resistant and susceptible groups were distinguished at the 0.05 probability level, except that Jagger (which ranked inconsistently among trials) bridged the two categories.

Chlorophyll differences among uninoculated plants in the October, November, and February trials were also highly significant ($P \leq 0.0004$), although much of the variation was accounted for by that between Madsen and the other cultivars (Tables 2 and 3). Chlorophyll content

Table 3. Chlorophyll readings of month-old winter wheat cultivars grown in liquid culture in the absence of *Cephalosporium gramineum*

Cultivar	Origin[y]	Chlorophyll reading[w,x]			Field Reaction[z]
		Nov.	Feb.	Comb.	
Madsen	PNW	36.3a	37.6a	37.0a	MR
Lewjain	PNW	-	34.9b	-	MR
Newton	SGP	31.7b	32.4c	32.0b	MR
Stephens	PNW	31.1bc	32.1c	31.6b	S
Plainsman	SGP	29.8bcd	32.5bc	31.2bc	MR
Gene	PNW	29.0cd	32.1c	30.6bcd	S
Jagger	SGP	28.8cd	31.1cd	30.0cd	MR
Sturdy	SGP	27.6d	30.9cd	29.2d	S
Malcolm	PNW	28.7cd	29.7d	29.2d	S
Macvicar	PNW	29.1cd	29.2d	29.2d	S
LSD		2.52	2.38	1.65	

[w]Readings taken with a chlorophyll meter in four places along youngest fully expanded leaf of each plant and averaged to produce one measurement per plant. Lower values indicate greater disease severity. Values are means of four replicated treatments of ≈10 plants each.

[x]Means within a column with the same letter are not significantly different ($P = 0.05$) according to Fisher's protected LSD test.

[y]PNW = Pacific Northwest soft white cultivar; SGP = Southern Great Plains hard red cultivar.

[z]MR = moderately resistant; S = susceptible.

ranked uninoculated and inoculated cultivars differently (Table 1 and 3). Correlation analysis of the three trials showed a positive correlation ($P = 0.05$) in the chlorophyll content of inoculated and uninoculated plants if the cultivar Madsen was included in the analysis, but the correlations were lower and not significant ($P = 0.05$) if Madsen was omitted (Table 4). Madsen is moderately resistant to Cephalosporium stripe and had a chlorophyll content in liquid culture distinctly higher than that of other cultivars.

Table 4. Correlation of chlorophyll readings on winter wheat cultivars inoculated and not inoculated with *Cephalosporium gramineum* in liquid culture

Trial		All cvs.[a]	No Madsen[b]
Oct.	*R*[c]	0.93	0.78
	P[d]	0.0229	0.2166
Nov.	*R*	0.84	0.37
	P	0.0049	0.3714
Feb.	*R*	0.79	0.41
	P	0.0063	0.2733

[a]Five cultivars in October, nine cultivars in November, and 10 cultivars in February.

[b]Correlations excluding the moderately resistant cultivar Madsen, which had a significantly greater chlorophyll rating than all other cultivars.

[c]Pearson correlation coefficient.

[d]Probability, with H_o: $R = 0$.

Comparison to Adult Plants in the Field. Flag leaf chlorophyll readings correlated with the field symptoms of Cephalosporium stripe (Table 5). In the artificially inoculated Moro field trial, a low chlorophyll reading in the flag leaf was highly correlated with the later development of a white head (P = 0.00005) and with short culm stature (P = 3 x 10^{-19}).

Table 5. Chlorophyll levels and plant height data read in June 1996 from a field trial of winter wheat cultivars inoculated with *Cephalosporium gramineum* in Moro, OR[x]

	Madsen (mod. res.)		Stephens (susc.)	
	Chlor[y]	Hght[z] (cm)	Chlor[y]	Hght[z] (cm)
White head	8.9a	58.3a	9.3a	64.3a
No white head	29.1b	79.4b	25.7b	77.0b

[x]Values are means of data collected on 50 culms of each cultivar in each of three replicated plots in a randomized, complete block experiment.

[y]Readings taken with chlorophyll meter in four places along flag leaf of each culm and averaged to produce one measurement per culm. Within a column, means with the same letter are not significantly different (P = 0.05) according to Fisher's protected LSD test.

[z]Culm height measured on same plants 12 days after chlorophyll measurement. Within a column, means with the same letter are not significantly different (P = 0.05) according to Fisher's protected LSD test.

Chlorophyll readings in the commercial wheat field with naturally-occurring inoculum clearly distinguished the susceptible cultivar from the three moderately resistant cultivars (Table 6).

By contrast, wheat free of Cephalosporium stripe in the field in Corvallis yielded flag-leaf chlorophyll readings at heading that did not rank plants according to susceptibility to Cephalosporium stripe (Table 7).

Table 6. Chlorophyll levels and white head incidence from a 1996 winter wheat trial in a commercial field with naturally-occurring *Cephalosporium gramineum* inoculum near Moro, OR

Cultivar	Chlorophyll[y]	% culms with white heads[z]
Lambert	36.9a	4.4c
Madsen	35.8a	0.1a
Rod	35.2a	1.2b
Stephens	30.9b	33.9d
LSD	3.10	2.42

[y]Readings taken with chlorophyll meter in four places along the flag leaf of each culm and averaged to produce one measurement per culm. Values are means of four plots in a randomized, complete block design, with 15 culms sampled at regular intervals in each plot. Measurements made 10 June (milk stage). Within a column, means with the same letter are not significantly different ($P = 0.05$) according to Fisher's protected LSD test.

[z]White heads assayed 25 June (late milk/early dough stage). Reported values are back-transformed from log-transformed data used in means separation. Within a column, means with the same letter are not significantly different ($P = 0.05$) according to Fisher's protected LSD test.

Table 7. Flag-leaf chlorophyll[w] levels of undiseased[x] winter wheat plants at heading stage in two years in Corvallis, OR

Cultivar	Year		Field Reaction[y]
	1996	1997	
Stephens	48.7	47.1a	S
Madsen	46.7	43.6b	MR
Macvicar	46.4	42.5bc	S
Rod	46.1	42.3bc	MR
Malcolm[z]	44.9	-	S
Gene	42.0	41.6c	S
Yamhill	42.9	39.3d	MR
Lewjain[z]	37.8	-	MR
LSD		1.92	

[w]Readings taken with chlorophyll meter in four places along the flag leaf of each culm selected blindly and averaged to produce one measurement per culm. Values for 1996 are means of two plots for Lewjain (39.0, 36.5) and Stephens (48.5, 48.8), and one plot for other cultivars. Values for 1997 are means of three replicated plots per cultivar. Means with the same letter are not significantly different ($P = 0.05$) according to Fisher's protected LSD test.

[x]Plants sprayed to protect from foliar disease in a field where Cephalosporium stripe does not occur.

[y]MR = moderately resistant; S = susceptible.

[z]Cultivars not present in 1997.

DISCUSSION

Wheat seedlings can be screened effectively under controlled conditions for resistance to Cephalosporium stripe. Chlorophyll level in the inoculated seedlings is

easily quantified and in general is an accurate predictor of the field resistance of adult plants.

Differences in greenness among diseased plants do not simply mirror the variation among healthy plants, either juvenile or adult. Although there is some correspondence in chlorophyll content between inoculated and uninoculated seedlings in liquid culture, one cultivar, Madsen, accounts for most of this correlation. Madsen coincidentally possesses both relatively low susceptibility to Cephalosporium stripe and a high native chlorophyll content. The chlorophyll levels of inoculated and uninoculated plants are significantly correlated when Madsen is included; without Madsen, the correlation is insignificant. Further, our observations of field-grown, adult wheat indicate that chlorophyll levels reflect Cephalosporium stripe susceptibility in diseased but not in undiseased plants.

Leaf chlorophyll content makes sense as a gauge of Cephalosporium stripe severity, since yellowing is one of the principal symptoms of a vascular wilt disease (3). Like other symptoms, such as flacidity and necrosis (which were also observed in the inoculated wheat seedlings), yellowing probably results from water shortage due to occlusion of the vascular elements by the host's defensive products (gums, tyloses and gels) (3). *C. gramineum* does

produce a toxin, Graminin A (10), but its role in pathogenesis is controversial (11,23).

Ranking cultivars by the difference in the chlorophyll readings of inoculated and uninoculated plants did not prove useful, probably because that difference is between two variables that are each subject to experimental error. Therefore, we omitted uninoculated treatments in the last trial in order to allow space for testing more cultivars. Various forms of visual estimation of disease severity were also attempted, such as a severity score from 1-11 based on leaf striping (16), a count of the proportion of striped leaves, and estimation of yellowing for each experimental unit. None of the visual estimates produced a consistently satisfactory ranking, possibly because these techniques were developed for use wih adult plants.

As a possible alternative to screening seedlings by assaying leaf chlorophyll, we are investigating the use of a GUS-transformed isolate of *C. gramineum* kindly supplied by Dr. Tim Murray, Washington State University. At issue is whether the GUS reporter gene (8) permits us to quantify the fungus present inside seedlings in a manner that can be correlated with resistance levels of cultivars, and whether the results would be more accurate than, and as early as, those from the chlorophyll technique.

Our screening method has three advantages. First, a trial can be completed in under 30 days, as opposed to the period of 5-6 mo needed for adult plants. Second, seedlings require less space than adult plants. These two factors will permit rapid initial screening of large numbers of new lines. Third, liquid culture allows inoculation without artificial root wounding, which is necessary to produce substantial disease when plants inoculated with *C. gramineum* are grown in pots (20,24), and ensures uniform distribution of inoculum around plant roots.

All cultivars were properly grouped in each trial reported here, except Jagger in the March trial. In subsequent controlled-environment trials using multiple inoculum types, Jagger also scored inappropriately as susceptible in three of eight trial x inoculum combinations (Cowger and Mundt, *in preparation*). Among the proposed phenotypes of resistance to Cephalosporium stripe (15) are those that limit ingress, such as resistance to rhizosphere acidity (20), and those that limit pathogen movement inside the host, such as crown morphology (14). Jagger's resistance may have a different physiological basis or may appear at a different developmental stage from that of the other cultivars we tested. It is interesting to note that the susceptible cultivar Stephens is a parent of Jagger.

The cultivar Madsen is moderately resistant and had a higher innate chlorophyll content than other cultivars in our controlled environment studies. Though we have not yet encountered the reverse case, i.e., a susceptible wheat genotype with high innate chlorophyll content, such a genotype could be falsely rated as moderately resistant to Cephalosporium stripe by our method. Thus, our procedures should be used only for initial screening, with the more promising genotypes being tested subsequently in the field.

Only one of the four individual trials provided demarcation between moderately resistant and susceptible cultivars with 95% confidence. While it would be helpful to draw a clearer line between moderately resistant and susceptible genotypes in single trials, ranking can be used to separate cultivars into two groups. With adequate replication and appropriate controls of known resistance, two trials should be sufficient to assign a new wheat line to the moderately resistant or susceptible class based on rank. Borderline cases, or inconsistent performers such as Jagger in our study, can be classed as moderately resistant. Moderately resistant lines must then be subjected to field tests to confirm growth chamber data.

We divided cultivars into two classes, susceptible and moderately resistant, based on consistent observations

in the field. There are also quantitative differences within these two classes, but field data are inadequate to provide a definitive ranking for all of the cultivars discussed here. However, the results of our seedling tests bear some similarities to field observations (Mundt, *unpublished*). Malcolm is consistently among the most susceptible of Pacific Northwest cultivars that we have tested in the field in Oregon, while Madsen is usually the most resistant of those we have tested. Further, both Stephens and Malcolm are very susceptible in the field, but Malcolm consistently shows a slightly higher disease rating. Among moderately resistant cultivars, Madsen is more resistant than Rod, and limited observations have shown Rod to be more resistant than Lambert.

Although these trials involved only one isolate of *C. gramineum*, subsequent trials using the same procedures compared fungal isolates from Oregon and Kansas. No significant difference was found among isolates in the way cultivars were ranked (Cowger and Mundt, *in preparation*).

Finally, our research addresses two aspects of the biology of Cephalosporium stripe. First, while it may contribute to disease, freezing of roots (2) is unnecessary for infection of wheat by *C. gramineum*, as has been noted by other workers (1,22). The fungus consistently penetrates the roots of seedlings maintained at about 20° C. Second, it would appear that juvenile and

adult plants share one or more traits conferring resistance. Thus, in wheat, resistance is perhaps at least partially a physiological phenomenon and not conditioned solely by the morphology of adult plants.

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CHAPTER III

INTERACTION OF WHEAT CULTIVARS AND *CEPHALOSPORIUM GRAMINEUM* ISOLATES FROM TWO DISTANT REGIONS

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ABSTRACT

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Winter wheat cultivars from the U.S. Southern Plains (hard red) and the Pacific Northwest (soft white) were inoculated with isolates of *Cephalosporium gramineum* from both regions, using a seedling-based resistance screening technique. No significant cultivar x inoculum-source interactions were found, suggesting low variability in virulence among the limited number of isolates surveyed. No significant difference was found in disease severity in homologous (same-region) and heterologous (different-region) combinations of host cultivar and pathogen isolate, indicating a lack of adaptation by *C. gramineum* to cultivars from its region of origin.

INTRODUCTION

Although genetic variability among fungal plant pathogens has received increasing attention (4,24,31), variability of soilborne pathogens has been much less studied than that of pathogens of aerial plant parts (17,31).

Soilborne plant-pathogenic fungi are thought to display less genetic variation than fungal leaf pathogens

(10,17,39). Airborne fungi more frequently include sexual reproduction in their life cycles, whereas many soilborne fungi are asexual (10). Further, root pathogens may experience less rapid change in gene frequencies than leaf pathogens because the forces of selection may operate more at the population level below ground and more on the individual above ground (17).

Cephalosporium gramineum Nisikado & Ikata (saprophytic, sporodochial stage = *Hymenula cerealis* Ell. & Ev.) is an important soilborne fungal parasite of winter wheat and other grasses (8). It enters wheat through the roots in the fall and winter (8,26,41) and causes the vascular wilt disease Cephalosporium stripe. The disease is responsible for substantial yield losses, particularly in the Pacific Northwest of the USA (9,36).

In wheat, resistance to *C. gramineum* is quantitatively inherited and moderate in expression (27,29,35). Various potential mechanisms of resistance have been investigated (25,27), but the phenotype(s) and genetic basis of resistance are still not well understood. Nevertheless, repeated planting of moderately resistant cultivars over several seasons has proven an effective strategy for reducing disease incidence (13,38). High levels of resistance to Cephalosporium stripe are present in wild wheat relatives, including *Agropyron elongatum*

(28), and efforts are underway to locate the genes and transfer them to wheat (14).

Very little is known about the extent of genetic polymorphism in *C. gramineum*. Van Wert et al. (43) noted apparently different virulence patterns produced by two wild-type Michigan field isolates of *C. gramineum* in 15 wheat lines. We are unaware of other published research on variation in either molecular characters or virulence patterns in *C. gramineum*.

Information about the extent of variation in the genes controlling interactions of *C. gramineum* with its hosts could be helpful for several reasons. First, it would shed light on one aspect of the population structure of this fungus (24). Second, it would improve the usefulness of resistance screening procedures, since the number and geographic diversity of isolates used for screening should depend on the variation among them. Knowing whether *C. gramineum* has distinct pathotypes or races would be helpful, since quantitative resistance may also be race-specific (20). Third, we might better predict the breadth and durability of host resistance, since greater variation in the pathogen offers greater evolutionary potential to overcome resistance genes (31).

Two aspects of the life cycle of *C. gramineum* suggest limited potential for variability. First, *C. gramineum* has no known sexual stage and is, therefore, likely to be

highly clonal with a limited spectrum of virulence within each lineage (4,31). Second, *C. gramineum* is a facultative parasite, surviving up to three years as a saprophyte (44) between contacts with living hosts. In the wheat-fallow-wheat system common to dryland cultivation in the Pacific Northwest, *C. gramineum* typically encounters a living host only in alternate years. Facultative parasites may develop less pathogenic variability than obligate parasites (17), perhaps because they are under less intense host selection pressure.

On the other hand, migration by *C. gramineum* among regions or population subunits is presumably limited. Little to no aerial migration is likely, since this fungus is confined to the vascular system of living grasses and the immediate vicinity of host debris (8,12). The conidia of *C. gramineum* are hyaline, lacking obvious protection against UV damage. They are produced in a slimy matrix that promotes rapid deposition (8,19,45). A low rate (0.33-7.3%) of seed transmission has been found (5,8). With low migration rates, we would expect little gene flow and, consequently, the opportunity for greater differentiation among population subunits (19,24,30).

An additional consideration is genetic drift, or random change in genotypic or gene frequencies, which tends to promote population substructuring (24). Genetic drift is greatest in small populations. *C. gramineum*

populations in the drier regions of the inland U.S. Pacific Northwest may be under severe stress during oversummering (11). When straw colonized by C. gramineum was incubated on soil at relevant relative humidities (86-90%), recovery rates after 270 days dropped to 19-37% (11). In eastern Washington, *C. gramineum* populations in soil decline to levels at which they are undetectable by dilution plating until moisture increases in late September (41).

Another factor that may promote variability among *C. gramineum* populations is differential host selection. Since the mid-1980s, Midwestern growers have significantly reduced Cephalosporium stripe by planting moderately resistant cultivars (38), while the susceptible cultivar Stephens dominated the Pacific Northwest wheat area from the early 1980s until very recently. Pathogens isolated from more resistant host populations may demonstrate a broader spectrum of virulence (virulence to more cultivars) than those isolated from susceptible populations (33).

A member of the same genus, *Phialophora gregata* (= *Cephalosporium gregatum*) (3,22), displays a very high level of uniformity among worldwide isolates at a specific genetic marker (16). *P. gregata* has at least two formae speciales; one infects soybeans and mung beans, the other adzuki beans. Restriction analysis of internal

transcribed spacer (ITS) sequences from the nuclear ribosomal DNA of 79 *P. gregata* isolates from the U.S., Brazil, and Japan revealed that all soybean isolates had identical ITS sequences, and their sequences were 98% similar to those of the adzuki bean isolates (16).

One type of genetic information relevant to control strategies is the geographic range over which differences among pathogen isolates can be detected (18). Our research made use of a recently developed seedling-based screening technique (Cowger and Mundt, *in preparation*) to determine whether variation in patterns of pathogenicity could be detected between isolates of *C. gramineum* collected in the U.S. Southern Great Plains region and in the U.S. Pacific Northwest. Resistance ratings resulting from this technique were found to correspond well with known reactions of cultivars to *C. gramineum* in the field.

In the *C. gramineum* populations we tested, we looked for evidence of virulence *sensu* van der Plank (42); i.e., the existence of races that interact differentially with a range of host cultivars. We were also interested in whether *C. gramineum* isolates cause more severe disease on cultivars from their geographic region of origin.

MATERIALS AND METHODS

Biological Materials. Wheat genotypes evaluated were four soft white winter wheat cultivars from the Pacific

Northwest (PNW) and four hard red winter wheat cultivars from the Southern Plains (SGP) states of Kansas and Texas. The eight cultivars vary in their field reactions to *C. gramineum* (Table 1).

Table 1. Eight winter wheat cultivars used in experiments on pathogenic variation in *Cephalosporium gramineum*

Cultivar	Market Class	Origin	Accession Number	Field React.[a]
Jagger	hard red	Kansas	PI 593688	MR
Plainsman V	hard red	Kansas	PVP 7500082	MR
Newton	hard red	Kansas	CI tr17715	MR
Sturdy	hard red	Texas	CI tr13684	S
Madsen	soft white	Wash.	PI 511673	MR
Gene	soft white	Oregon	PI 560129	S
Stephens	soft white	Oregon	CI tr17596	S
Malcolm	soft white	Ore./Ida.	PI 497672	S

[a]Known field reaction to *C. gramineum*

Six isolates of *C. gramineum* were used, three from each region. The PNW isolates were obtained in 1996 from Sherman and Gilliam counties, OR, and in 1995 from a Pendleton, OR, trial that had been inoculated in 1993 with an isolate from the Palouse region of eastern Washington. Cultures were isolated from single pieces of stem tissue. The three SGP cultures of *C. gramineum* were isolated in 1986 from Harvey County, Kansas, and in 1992 and 1995 from

Riley County, Kansas. They were kindly provided by Dr. William W. Bockus, Kansas State University.

Experimental Design. Two experiments were conducted. The first experiment was conducted three times, with trials in May, August, and December 1996. Six cultivars were each inoculated with a single SGP or a single PNW isolate. The second experiment was conducted twice, in February and March 1997. The same six cultivars, plus two additional ones, were tested with a mixture of two isolates each from the SGP and the PNW. These isolates were different from those used in the first experiment. Each trial of each experiment was conducted in a randomized complete block design, with three blocks in the May trial and four blocks in each of the other trials. Each block was placed in a different growth chamber.

Cultivars were paired in growth containers, so that each container was planted half with one cultivar and half with another. Cultivars were paired randomly, and randomization was conducted separately for each replication.

Plant Growth and Inoculation. Techniques for culturing wheat plants and inoculum were as described in Cowger and Mundt (*in preparation*). Seeds were germinated over damp, sterile sand on plastic mesh in styrofoam holders. Germlings were transferred in their holders to plastic containers painted black to inhibit algae and

filled with ≈4500 ml of a modified Hoaglund's solution (37) adjusted to pH 5.0 ± 0.1. Seedlings were raised in growth chambers (≈ 3 x 10^6 erg $\cdot m^{-2} \cdot s^{-1}$) at 20° ± 2° C for 19-23 days. After three days, each container was thinned to 20 seedlings (10 each of two cultivars).

C. gramineum was increased in 80%-strength (345 g/L) potato dextrose broth. The broth was strained through cheesecloth to remove mycelium, and conidia were separated from the broth by concentration and centrifugation.

After 12-15 days' growth in liquid medium, each container of wheat seedlings received conidia and fresh nutrient solution in the proportions needed to generate a suspension of ≈1 x 10^6 conidia per ml. The solution was adjusted to pH 5.0 ± 0.1 and roots were suspended in it by floating the styrofoam holders on the surface.

Germinability of the inoculum was determined by diluting it to ≈10^2 conidia/ml, plating 0.5 ml on potato dextrose agar, and counting germinated spores after ≈7 days' incubation at 13° C. Germination averaged 45%.

Plants were maintained in growth chambers with their roots bathed in the inoculum suspension for 7-8 days. Solution pH was returned to 5.0 ± 0.1 daily for 4 days after inoculation.

Symptom Assessment. On day 7-8 after inoculation, yellowing and/or striping of leaves was assessed by measuring chlorophyll content with a SPAD-502 chlorophyll

meter (Minolta Camera Co., Ltd., distributed by Spectrum Technologies, Inc., Plainfield, IL). Values reported by the meter correspond to the amount of chlorophyll present in a leaf. The meter calculates values based on the ratio of light intensities transmitted by the leaf at two wavelengths that differ widely for absorption of light by chlorophyll. Values reported correspond to the amount of chlorophyll present in a leaf; lower values indicate less chlorophyll, or more severe yellowing. The youngest fully expanded leaf was measured in four places, spaced approximately equally from base to tip of the leaf, and the measurements were averaged to yield one reading per plant.

Infection by *C. gramineum* was confirmed by isolating the pathogen from infected plants following symptom assessment. Two plants from each of several randomly chosen treatments in each replication were sampled. Surface-disinfested stem segments were plated on *C. gramineum* selective medium (CGSM) (40), and incubated approximately 2 wks.

Statistical analyses. Both experiments were subjected to analysis of variance, using block, cultivar, inoculum source (SGP or PNW), trial, and all possible interactions among cultivar, inoculum source, and trial as sources of variance. Chlorophyll means of cultivars were

separated using Fisher's protected least significant difference (LSD) test.

For both sets of combined trials, the mean of all homologous host-pathogen combinations (cultivars and isolate(s) from same geographic region) was compared to the mean of all heterologous combinations (cultivars and isolate(s) from different geographic regions). The means were compared using linear contrasts.

RESULTS

Virulence. Neither experiment resulted in a significant cultivar x inoculum-source interaction (Tables 2 and 3).

Table 2. Analysis of variance of leaf chlorophyll content in six winter wheat cultivars inoculated with a single Southern Great Plains or Pacific Northwest isolate of *Cephalosporium gramineum* and grown in liquid culture in growth chambers[a]

Source	df	Mean sq	*P* > F
Trial	2	30.51	0.0129
Block	8	12.73	0.0687
Cultivar	5	332.43	0.0001
Isolate	1	35.95	0.0226
Trial x isolate	2	328.55	0.0001
Trial x cultivar	10	6.35	0.4908
Cultivar x isolate	5	5.38	0.5481
Trial x cultivar x isolate	10	4.21	0.7840
Error	88	6.67	

[a]Experiment with three trials.

Table 3. Analysis of variance of leaf chlorophyll content in eight winter wheat cultivars inoculated with mixtures of either two Pacific Northwest or two Southern Great Plains isolates of *Cephalosporium gramineum*[a]

Source	df	Mean sq	*P* > F
Trial	1	207.60	0.0001
Block	6	24.96	0.0003
Cultivar	7	117.50	0.0001
Isolate mix	1	103.00	0.0001
Trial x isolate mix	1	5.50	0.3059
Trial x cultivar	7	7.42	0.2028
Cultivar x isolate mix	7	4.66	0.5121
Trial x cultivar x isolate mix	7	2.13	0.8929
Error	90	5.19	

[a]Experiment with two trials.

The two inoculum sources ranked cultivars in substantially the same order in both experiments. In the first experiment (Table 4), only the two susceptible cultivars Sturdy and Malcolm were reversed in rank by the two isolates, and their means were not significantly different.

In the second experiment (Table 4), small differences occurred in the ranking of the susceptible cultivars Stephens and Gene and the moderately resistant cultivar Jagger. Although Jagger's field resistance is equal to or greater than that of Plainsman (6 and W. W. Bockus, *personal communic.*), in one of the two trials it was ranked by SGP inoculum below the susceptible cultivar Stephens, as well as below the other three moderately

Table 4. Mean chlorophyll levels and resultant rankings from two experiments testing *Cephalosporium gramineum* isolates from the Southern Great Plains (SGP) and the Pacific Northwest (PNW) on winter wheat cultivars from both regions

			Experiment 1				Experiment 2			
			Source of isolate mix				Source of isolate mix			
			SGP		PNW		SGP		PNW	
Cultivar	Origin[v]	Field React.[w]	Rank	Chlor[x]	Rank	Chlor[x]	Rank	Chlor[x]	Rank	Chlor[x]
Madsen	PNW	MR	1	31.0a	1	31.8a	1	30.4a	1	29.4a
Plainsman	SGP	MR	2	25.6b	2	26.9b	2	27.6b	2	24.8b
Newton	SGP	MR	3	24.5b	3	24.1c	3	26.2bc	3	24.7b
Jagger	SGP	MR					5	25.1cd	4	24.5b
Gene	PNW	S	5	21.3c	5	21.9d	6	23.9de	5	23.1bc
Stephens	PNW	S					4	25.1cd	6	21.7cd
Sturdy	SGP	S	6	20.7c	4	22.1cd	7	23.0de	7	21.7cd
Malcolm	PNW	S	4	22.1c	6	21.1d	8	22.5e	8	19.6d
Mean[y]				23.8		24.9		25.5A		23.7B

(continued)

(Table 4, Continued)

	Exp 1	Exp 2
Mean of homologous[z] combinations	24.2	24.5
Mean of heterologous[z] combinations	24.6	24.7

[v]SGP = Southern Great Plains hard red wheat; PNW = Pacific Northwest soft white wheat.

[w]MR = moderately resistant; S = susceptible.

[x]Readings taken with a chlorophyll meter in four places along youngest fully expanded leaf in each plant and averaged to produce one measurement per plant. Lower values indicate greater disease severity. Values are means of three to four replications in each of three trials. Means within a column with the same letter are not significantly different ($P = 0.05$) according to Fisher's unprotected LSD test.

[y]Experiment 1 means not significantly different; Experiment 2 means different at $P \leq 0.0001$.

[z]Homologous = all cultivar-isolate combinations from the same geographic region (e.g., SGP host and SGP inoculum). Heterologous = all cultivar-isolate combinations from different geographic regions (e.g., PNW host and SGP inoculum). Means are not significantly different (Experiment 1: $P = 360$; Experiment 2: $P = 588$).

resistant cultivars. However, these differences were neither sufficiently great nor consistent to produce a significant cultivar x inoculum-source interaction.

Homologous/Heterologous Comparison. No significant difference in chlorophyll was detected between homologous and heterologous combinations of host and pathogen in either the first experiment ($P = 0.360$) or the second experiment ($P = 0.588$) (Table 4).

Inoculum-Source Effect. In the first experiment, the trial x isolate interaction was significant (Table 2) because the SGP and PNW isolates each produced more severe disease in one trial, and produced approximately equal disease levels in the third trial (data not shown). The significance of the main effect of isolate in this experiment reflected these differences.

In the second experiment, the trial x isolate-mix interaction was not significant (Table 3), and in both trials the PNW inoculum produced significantly more severe disease than did the SGP inoculum (Table 4).

Cultivar Differences. In both experiments, the main effect of cultivar was significant ($P \leq 0.0001$) (Tables 2 and 3). Chlorophyll level ranked cultivars in accordance with their field resistance, with moderately resistant cultivars ranking higher than susceptible cultivars. Neither experiment provided a clear line of demarcation between moderately resistant and susceptible cultivar means with ≥95% confidence.

DISCUSSION

Our experiments suggest low pathogenic variability in *C. gramineum*, although broad conclusions await further trials with more isolates and cultivars. Pathogen variability data in this and studies of other soilborne pathogens have been limited, in part, due to the technical

difficulties associated with testing such pathogens for variability.

The absence of interaction between cultivar and isolate(s) in both experiments suggests an absence of vertical resistance and virulence *sensu* van der Plank (42); i.e., no evidence of differentially interacting genotypes was found among the *C. gramineum* isolates and host cultivars used. Future studies with additional isolates and cultivars, and/or increased assay sensitivity, could uncover such variation, of course. Further, the use of isolate mixtures in the second experiment could mask isolate x cultivar interactions. We used mixtures to increase the chance of identifying regional effects that had been suggested by preliminary studies, but not confirmed by the experiments reported here.

Van Wert et al. (43) reported that two wild-type isolates of *C. gramineum* had differential interactions with Agrotritichum and several wheat lines. In that experiment, six plants were grown and inoculated in each pot, and the experiment was repeated once. The researchers considered individual plants within pots to be the experimental units, thus inflating the degrees of freedom available for error in the statistical analysis. Further, results were not always consistent between the two replications in time. As Kulkarni and Chopra have

noted (23), apparent race x cultivar interactions could in fact be race x environment, cultivar x environment, or race x cultivar x environment interactions.

The lack of significant difference between the means of homologous and heterologous cultivar-isolate combinations indicates that the isolates tested have not been selected for greater virulence on cultivars from the same geographic region. *C. gramineum* isolates that have co-existed with soft white wheats of the Pacific Northwest did not cause significantly more disease on those cultivars than on hard red wheats from the Southern Great Plains, nor vice versa.

Our findings can be contrasted to the unmistakable evidence of pathogenic variability in other pathosystems, for example, wheat and the airborne fungal pathogen *Mycosphaerella graminicola* (anamorph = *Septoria tritici*). Unlike *C. gramineum*, *M. graminicola* utilizes both sexual and asexual reproduction, and the ascospores appear to be the primary source of inoculum for the initiation of epidemics (32). Researchers have found high levels of genotypic variation in *M. graminicola* populations (7,15,34), and substantially differential interactions between fungal isolates and cultivars assayed both as field-grown adult plants and as seedlings grown in controlled environments (1,2,21). Further, there is also clear evidence of adaptation by *M. graminicola* to greater

aggressiveness on cultivars from the same region (1). These data stand in sharp contrast to the results reported here for *C. gramineum* and wheat.

Our experiments showed no clear tendency to greater aggressiveness by isolates from either of the two regions. In the first experiment, the relative aggressiveness of single isolates from the two regions was inconsistent among trials. In the second experiment, the Pacific Northwest isolate mixture caused more severe disease in both trials, but conclusions about regional differences are impossible due to the small number of isolates and the fact that both isolates from each region were mixed. Variability in apparent aggressiveness in these experiments may also be influenced by cultural conditions, since inocula were increased in large fermentation tanks prior to use.

With respect to the screening method itself, these trials confirm previous results (Cowger and Mundt, *in preparation*). Cultivars screened as seedlings in growth chambers are ranked by chlorophyll level in accordance with their known levels of field resistance. As an exception, the moderately resistant SGP cultivar Jagger varied in chlorophyll rank between moderately resistant and susceptible. This suggests that Jagger's resistance may have a different basis, either physiological or developmental, than that of other cultivars screened. It

is noteworthy that the susceptible PNW cultivar Stephens is a parent of Jagger.

If our evidence of low pathogenic variability in *C. gramineum* is corroborated by tests of other host cultivars and isolates, the screening procedure can be relied upon as robust even when only one or two isolates are used. Our findings also offer hope that resistance to Cephalosporium stripe incorporated into new wheat lines may be relatively durable.

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CHAPTER IV
CONCLUSIONS

The principal objective of this research was to develop and test a procedure for rapid screening, under controlled conditions, of new wheat lines for resistance to Cephalosporium stripe of wheat. In addition, the procedure was used for an initial examination of pathogenic variability in the causal agent of the disease, the soilborne fungus *Cephalosporium gramineum*.

Wheat seedlings were cultured in growth chambers, using a liquid, complete nutrient medium. *C. gramineum* was increased in large fermentation tanks and used to inoculate the seedlings' roots in a suspension of about 1 x 10^8 conidia per ml. Chlorosis, a principal symptom of this wilt disease, was assayed in seedlings' leaves with a chlorophyll meter at about seven days post-inoculation.

In four trials, 12 winter wheat cultivars from the Southern Great Plains (hard reds) and the Pacific Northwest (soft whites) were tested. Chlorophyll levels accurately ranked cultivars by their known field performance, with moderately resistant cultivars' chlorophyll means consistently above those of susceptible cultivars with one exception. Jagger, a hard red wheat from Kansas, has moderate resistance in the field, but performed inconsistently in the seedling trials.

Results of the procedure were consistent with field observations. Among moderately resistant Pacific Northwest cultivars, Madsen generally outperforms Rod, and Rod appears to be more resistant than Lambert. Of the susceptible Pacific Northwest cultivars, Malcolm is usually more susceptible than Stephens. These field rankings of adult plants were reflected in the growth chamber data for seedlings.

Jagger's inconsistent performance in the seedling trials highlights the need to use this procedure for initial screening, followed by field trials of the more promising material. Advantages of the screening procedure are short trial times (under 30 days), the small space required by seedlings, and the fact that the liquid medium permits even distribution of inoculum around the roots while removing the need for artificial wounding of roots.

This study confirmed that freezing is not necessary for infection of wheat roots by *C. gramineum*. It also demonstrated that at least some resistance characteristics are shared by juvenile and adult plants, and may thus be physiological traits rather than aspects of uniquely adult morphology.

The procedure was then applied to an initial investigation of pathogenic variability in the *C. gramineum* population. Little is known about the genetics of *C. gramineum*, and the accuracy of resistance screening

depends on whether the fungus has cultivar-specific pathotypes. Based on information about other soilborne pathogens and about the life cycle of *C. gramineum*, we hypothesized that this fungus would display low pathogenic variability.

Three fungal isolates each from Kansas and the Pacific Northwest were tested on a total of eight cultivars from both regions. No evidence of virulence/vertical resistance *sensu* van der Plank was found. There was also no evidence of adaptation by isolates to greater virulence on cultivars from the same region. In one of two experiments, Pacific Northwest isolates were more aggressive than Kansas isolates, but the experimental design did not allow any inferences about relative aggressiveness of isolates from the two regions overall. The variability experiments confirmed that the screening technique accurately assigns cultivars to either the moderately resistant or the susceptible group, although Jagger continued to perform inconsistently among trials.

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