AN ABSTRACT OF THE THESIS OF

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Abstract approved:

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The specificity of quantitative host resistance to plant disease has long been a controversial issue. We examined interactions between wheat (*Triticum aestivum*) and *Mycosphaerella graminicola*, causal agent of Septoria tritici blotch, to determine whether specific interactions occur between host and pathogen genotypes that could be involved in eroding quantitatively expressed resistance. Pathogen isolates were collected from two moderately resistant wheat cultivars, Madsen and Foote, in the field in 2004 and 2005 and tested on each cultivar in a factorial design in growth chamber and greenhouse experiments. The resistance of Madsen has eroded significantly in Willamette Valley, OR wheat fields, and Foote is a replacement cultivar expressing a higher level of resistance. In all of the experiments, there was a significant isolate source by cultivar interaction, with isolates generally causing more disease on their cultivar of origin. The cultivar Madsen reacted to isolates in a manner typical of quantitative interactions, while the cultivar Foote demonstrated qualitative reactions more typical of the breakdown of a major resistance gene. The two cultivars may have had similar levels of quantitatively expressed resistance in the field upon commercial release, but it appears that the pattern

of reaction to pathogen isolates is different. Pathogen populations may have the ability to adapt to both types of genetic backgrounds, suggesting that specific interactions in quantitative systems can lead to the erosion of moderate resistance in the field.

Specificity of Quantitatively Expressed Host Resistance to *Mycosphaerella graminicola*

by
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APPROVED:

Major Professor, representing Botany and Plant Pathology

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Dean of the Graduate School

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.

Jennifer E. Krenz, Author

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Introduction

The host specificity of quantitatively expressed resistance to plant disease has been controversial for decades (22,50,51,54). More recently, quantitative trait loci (QTL) conferring quantitative disease resistance have been found to often times be associated with the same chromosomal region as major resistance genes (e.g. 43,52). This has led to the suggestion that QTLs for resistance are defeated or allelic variations of major genes (55). In some cases, a single partially expressed major resistance gene conferring quantitative resistance may be interpreted as the effect of several genes (30). Because quantitative resistance is thought to be durable, it is crucial to understand the mechanisms behind quantitatively expressed resistance.

The wheat (*Triticum aestivum*)-*Mycosphaerella graminicola* pathosystem, which results in the foliar disease, Septoria tritici blotch, is extensively researched with well documented quantitative interactions, making it a very useful candidate for exploring the impact of quantitatively expressed host resistance on pathogen population evolution (3,8,12,35,56). Moreover, the disease is economically important worldwide, and research findings can be applied for use in breeding programs for durable resistance (33).

Both quantitative and qualitative resistance reactions to *M. graminicola* isolates have been observed in the same wheat genotype (12). Over 12 major

genes conferring high levels of host resistance have been identified, and most have been mapped to specific regions of the wheat genome (1,2,6,10,14,45). The pathogen, *M. graminicola*, is an ascomycete fungus with a heterothallic, bipolar mating system that undergoes more than one sexual cycle per season (19). Sexual reproduction is followed by cycles of asexual reproduction of splash-dispersed pycnidiospores, which allows for the amplification of more fit genotypes and possible selection for traits such as increased virulence and aggressiveness (19). Gene-for-gene interactions have been demonstrated between wheat and *M. graminicola* (7,38).

The Willamette Valley of Oregon is an ideal location to study this pathosystem because favorable environmental conditions allow for the development of Septoria tritici blotch epidemics, large off-season pathogen populations prevent genetic bottlenecks, geographic isolation prevents immigration of outside populations, and extensive sexual recombination occurs (49). In addition, ascospores are wind-dispersed throughout the Willamette Valley at the beginning of the season, suggesting that within population gene flow is an important evolutionary force shaping the population structure of *M. graminicola* (C.C. Mundt, unpublished). All of these conditions increase the probability of detecting changes of quantitative variation in *M. graminicola* populations in response to changes in the deployment of host resistance genes.

Opposing experimental results have occurred in the Willamette Valley regarding adaptation of *M. graminicola* populations. Cowger and Mundt (21) found little evidence of pathogen adaptation to host cultivar. In another study, isolates from Oregon cultivars Madsen and Stephens tested on both cultivars indicated evidence of adaptation but with no distinct preference for their cultivar of origin (58). Other research has found that isolates are better adapted to the host cultivar they originate from than other wheat cultivars (3,4). This raises the question of whether quantitative host resistance has a selective influence on the pathogen population.

Host plant resistance to Septoria tritici blotch has been unstable in the Willamette Valley. The cultivar Gene contains at least one major gene, *Stb6* (11), and possibly another major resistance gene, *Stb4* (18). Gene was released in the Willamette Valley of Oregon, and resistance broke down after two years of significant production (18). The quantitatively expressed, moderate resistance of the cultivar Madsen has eroded in the field as well, though at a slower rate than that of Gene (48). Another moderately resistant cultivar, Foote, was commercially released in the Willamette Valley in 2000, and monitoring efforts began to determine if similar patterns of resistance erosion would occur. If the pattern of Foote follows that of Madsen, it will further support the idea that adaptation in the field leads to erosion of quantitatively expressed resistance.

The objective of this study was to explore specific interactions potentially involved in the erosion of quantitatively expressed resistance in the field using isolates of *M. graminicola* and the wheat cultivars Madsen and Foote. It is difficult to detect frequency changes in *M. graminicola* genotypes because high rates of recombination disperse pathogenicity alleles to many isolates, and it appears that commonly used neutral molecular markers are not linked to those under selection (47). Thus, the most reliable way to track the evolution of pathogen populations is through inoculations and phenotypic disease assessments. Significant host cultivar x isolate source interactions were used as indicators of specificity. Furthermore, if isolates of *M. graminicola* caused more disease on their cultivar of origin, then adaptation to host of origin was suggested. In addition, the robustness of interactions was tested using different methodologies. Both individual isolates and bulk populations were tested, and the amount of disease caused by bulk populations of isolates was compared to the mean of individual isolates from which the bulk populations were composed. The performance of isolates in different environments (growth chamber versus greenhouse) was assessed to determine the importance of genotype x environment interactions.

Literature Review

Host-Pathogen Interactions

The total disease causing ability of a pathogen, termed pathogenicity, can be subdivided into two categories according to Vanderplank (54). Virulence is the genetic variation among pathogen isolates that acts differentially with host genotypes. Variation in virulence allows a pathogen to "breakdown" host resistance. Aggressiveness is attributed to the genetic variation among pathogen isolates that does not act differentially with host genotypes and is generally considered stable. When evaluating resistance, the ranking of hosts will not change when tested against different pathogen genotypes that vary only in aggressiveness. In host-pathogen interactions, both aggressiveness and virulence may contribute towards the level of disease in a given host genotype. On the host side, resistance can be divided into specific, which is effective against some genotypes of the pathogen, and nonspecific, which is effective against all genotypes of the pathogen.

Differential reactions associated with virulence have often been described with the gene-for-gene model proposed by H.H. Flor (27). According to this model, for each gene conditioning resistance in the host there is a specific gene conditioning avirulence in the pathogen. Studies at the molecular level have demonstrated that pathogen avirulence effectors trigger a resistance response either

directly by interacting with a host resistance protein or indirectly by interacting with an intermediate host protein that activates the resistance protein (15). With many obligate plant parasites the presence or absence of disease indicates whether or not a resistance gene is present. Since resistant and susceptible host genotypes are categorized based on differential reactions conditioned by a gene of major effect, this type of host resistance is deemed qualitative (54). Distinct phenotypic reactions in addition to Mendelian inheritance have made qualitative interactions relatively straightforward to study.

Conversely, quantitative resistance is expressed as a range of reactions, with host genotypes falling at various levels between susceptible and resistant. The mechanisms by which quantitative resistance functions are not fully understood and could involve both specific interactions between host and pathogen gene products and nonspecific, or general, resistance responses. Different theories have been formulated, some or all of which possibly play a role in quantitatively expressed resistance. Nelson (50) proposed that genes responsible for both qualitative and quantitative resistance were the same genes, and the type of expression depended on the genetic background. Parlevliet and Zadoks (51) described a different model for quantitative resistance in which the genes involved have smaller effects than those involved with qualitative resistance, but both have mechanisms in accordance with the gene-for-gene model. In a study looking at disease levels in the barley (*Hordeum vulgare*)-*Puccinia hordei* pathosystem, a

quantitative system resulting in barley leaf rust, specific interactions were reported dependant on the combination of isolate and cultivar (51).

A review by Jones and Takemoto (34) discussing possible links between host-specific and non-host specific resistance found that although they are activated by different pathogenicity factors, many of the signaling pathways are shared, suggesting overlap at some level.

Association Between QTL and Major Resistance Gene Loci

Molecular genetics have identified quantitative trait loci (QTL) regions in plants that are often located in the same chromosomal region as known qualitative resistance genes. Work investigating QTLs in rice for race specificity against the rice blast pathogen, *Magnaporthe grisea*, identified 18 QTLs, 10 of which were located in the same region as major resistance genes (52). This has led to the idea that QTLs for resistance are defeated versions, or allelic variations, of genes for complete resistance (55). Research on the quantitatively expressed resistance of potato to *Phytophthora infestans* suggested that allelic variations at resistance loci determined the type of resistance expressed (43).

Epistasis between resistance QTLs can make it difficult to clarify the molecular basis of resistance (40). Furthermore, population size has been shown to influence the number of QTLs identified, suggesting that smaller populations are adequate to identify QTLs with large effects, but larger populations are needed

to identify QTLs of smaller effect (53). Previous studies may have overestimated the magnitude of effect of identified QTLs (29). Although identification of QTLs is helpful, genetic analyses are still essential to confirm molecular findings.

The Wheat-*M. graminicola* Pathosystem

The wheat (*Triticum aestivum*)-*Mycosphaerella graminicola* pathosystem, which results in the foliar disease Septoria tritici blotch, is extensively researched with well documented quantitative interactions, making it a very useful candidate for exploring the impact of quantitatively expressed host resistance on pathogen population evolution (3,8,12,35,56). Moreover, the disease is economically important worldwide, and research findings can be applied for use in breeding programs for durable resistance (33).

M. graminicola is a hemibiotrophic, ascomycete fungus that hinders grain development when upper leaves of wheat cultivars are infected. The pathogen overseasons as pseudothecia in stubble from the previous season or on volunteer wheat. When environmental conditions are conducive (moderate temperatures with high moisture levels), pseudothecia burst and the ascospores are wind-dispersed to new wheat seedlings. The leaves are infected, and the pathogen grows intercellularly. Host tissues become necrotic and pycnidia can be seen in the lesions. Pycnidiospores, which serve as secondary inoculum, are splash-

dispersed to neighboring leaves. It is also thought that pycnidiospores in pycnidia can overseason and serve as primary inoculum. The pycnidiospores then germinate on leaves, form germ tubes and extensive hyphae, then penetrate through stomata and other openings in the epidermis (26).

M. graminicola has a heterothallic, bipolar mating system, and a previous study has shown that more than one sexual cycle within a season can contribute to inoculum (19). Furthermore, sexual reproduction is followed by cycles of asexual reproduction, which allows for the amplification of more fit genotypes and possible selection for traits such as increased virulence and aggressiveness (19).

Resistance to *M. graminicola*

Both quantitative and qualitative resistance reactions to *M. graminicola* isolates have been observed in the same wheat genotype (12). Over 12 major genes conferring high levels of host resistance have been identified, and most have been mapped to specific regions of the wheat genome (1,2,6,10,14,45). Other studies have found QTLs to be in the same chromosomal region as qualitative resistance genes, suggesting they are the same source of resistance (10,23). A gene-for-gene interaction conferred by a single resistance gene with incomplete dominance has been demonstrated genetically (7). When crosses of *M. graminicola* isolates were made, avirulence segregated in a 1:1 ratio, which is indicative of a monogenic response or that of cosegregating avirulence genes for this haploid pathogen (38).

A linkage map of *M. graminicola* was created using AFLP and RAPD markers that confirmed avirulence to some host cultivars is under single gene control (36).

Wheat cultivars have shown moderate to high levels of narrow-sense heritability for resistance, which suggests that there is a strong genetic basis to this trait (5,23,59). Whether interaction or additive effects are more important is not clear. Resistance of F1 doubled-haploid progeny of a cross between a susceptible and resistant cultivar demonstrated a significant isolate by line interaction, indicating specificity, but upon further analysis it was suggested that additive effects conferring non-specific resistance were primarily responsible for overall resistance (13).

Thus, although pathogen aggressiveness and non-specific resistance cannot be completely discounted, a component of resistance in this pathosystem is specific.

Adaptation of *M. graminicola* Populations to Host Cultivars

A number of studies have investigated the ability of *M. graminicola* populations to adapt to host cultivars with quantitative resistance, as measured by increasing disease levels. When using RFLPs to explore the effect of different host populations on the genetic structure of *M. graminicola*, there was no evidence of directional selection or adaptation to the cultivar from which the isolate

originated (47). It was concluded that the RFLP markers were selectively neutral and not linked to genes under selection. Other studies have demonstrated specificity in host-pathogen interactions without adaptation to the cultivar of origin or geographical origin (6,35).

Impact of Evolutionary Forces on *M. graminicola*

Evaluation of evolutionary forces that influence *M. graminicola* population dynamics indicated that gene flow and sexual recombination were important in maintaining variation within populations, while selection influenced by host cultivar resistance decreased variation within populations (57). The study demonstrated that individual populations may evolve locally, but the global population is at equilibrium because of the impact of opposing evolutionary forces (57).

The Willamette Valley of Oregon as a Field Laboratory

The Willamette Valley of Oregon is a favorable location to study this pathosystem (49). There are yearly epidemics of Septoria tritici blotch on winter wheat that range from moderate to severe, and both qualitative and quantitative interactions have been documented (3,18). Generally, environmental conditions are conducive to the rapid evolution of *M. graminicola* isolates, and the moderate climate allows pathogen populations to survive year-round throughout the valley

(49). These large off-season populations prevent genetic bottlenecks and supply high genetic variation for the next season of selection (49). The geographic isolation of the valley from other areas with high incidence of *M. graminicola* ensures that between population gene flow is not a factor in shaping the genetics of pathogen populations (C.C. Mundt, unpublished). Research has shown that there is gene flow on a global scale, but efficient natural selection locally against fungal immigrants circumvents influence from outside populations (57). Additionally, a previous study using neutral RFLP markers showed that most genetic diversity is found locally in an area of 1 to 9 m^2, and population differences among regions and continents are not as significant (44). Thus, if ascospores did arrive in the Willamette Valley via long-distance transport, they are not likely to influence pathogen population dynamics.

Opposing experimental results have occurred in the Willamette Valley regarding adaptation of *M. graminicola* populations. Cowger and Mundt (21) found little evidence of adaptation to host cultivar. Isolates from Oregon cultivars Madsen and Stephens tested on both cultivars indicated evidence of adaptation but with no distinct preference for their cultivar of origin (58). Other research has found that isolates are better adapted to the host cultivar they originate from (3,4). This raises the question of whether quantitative host resistance has a selective influence on the pathogen population.

Host plant resistance to Septoria tritici blotch has been unstable in the Willamette Valley. The cultivar Gene was thought to have the major resistance gene *Stb4*, but more recently has been shown to contain *Stb6* instead of or in addition to *Stb4* (11,18). Gene was released in the Willamette Valley of Oregon, and resistance broke down after two years of significant production, which is not unusual for a major resistance gene (18). However, the quantitatively expressed, moderate resistance of the cultivar Madsen has eroded in the field as well, though at a slower rate than the resistance of Gene (48). Another moderately resistant cultivar, Foote, was commercially released in the Willamette Valley in 2000, and monitoring efforts began to determine if similar patterns of resistance erosion would occur. The reactions of *M. graminicola* isolates on both Foote and Madsen need to be examined to discern patterns of specificity and adaptation.

Methodology Influences Identification of Host-Pathogen Specificity

Whether bulk populations or individual isolates are more suitable for experiments is debatable and depends on the research focus. Examining individual isolates is informative because it excludes competition or compensation effects that could be taking place. Testing bulk populations of isolates is advantageous because it allows for the evaluation of a larger number of isolates and is more representative of field conditions (4). There is a great deal of recombination in *M. graminicola* populations, making it difficult to track individual genotypes. In fact,

the concept of physiologic race as seen in obligate pathogens is nearly impossible with *M. graminicola* because many isolates are unique, and different genotypes can even be identified within a lesion (44). On the other hand, it was not possible to use bulk populations to evaluate the breakdown of major gene resistance in the cultivar Gene, most likely owing to induced resistance in that major gene system (C.C. Mundt, unpublished). Other research has yielded different responses from bulk populations and individual isolates, and theoretical models suggest that specificity is difficult to prove when using bulk populations (37).

The environment in which experiments are being conducted can also be influential. Ellingboe (22) found that disease levels of powdery mildew on wheat (caused by *Blumeria graminis*) differed depending on whether the experiment was performed in the greenhouse or growth chamber. Slow mildewing appeared to be quantitative in the greenhouse under more environmental variability and qualitative in the growth chamber under more controlled conditions. Higher temperatures have been shown to reduce latent periods, leading to more pycnidial formation of *M. graminicola* in susceptible cultivars of wheat, which indicates varied results can be obtained if disease development is temperature-dependant (16). Thus, it is important to examine responses in different environments to evaluate the importance of genotype x environment interactions.

Materials and Methods

Host Cultivars

Two commercially important cultivars that have been grown in the Willamette Valley of Oregon were chosen for this study (Fig. 1). The cultivar Madsen was released in 1990, and upon release exhibited moderate levels of resistance. Growers and researchers reported that this partial resistance has eroded substantially over time (48). The cultivar Foote was released in 2000 and demonstrated high levels of quantitative resistance (Fig. 2), which are still observed in the field (C.C. Mundt, unpublished).

Pathogen Collection

Flag leaves of Madsen and Foote with visible lesions and pycnidia of *M. graminicola* were collected in late June 2004 and 2005. By this time multiple rain events had splash-dispersed conidia to upper canopy levels, maximizing the number of generations and, as a result, selection among isolates. It is probable that ascospores are widely distributed throughout wheat fields in the Willamette Valley at the beginning of each season (4). Thus, each field was thought to represent the genetic diversity within the Willamette Valley, and flag leaves were collected from a single field each year. In 2004 leaves were collected at the Hyslop Crop Science Field Research Laboratory in Corvallis, OR. Leaves were randomly chosen from a

plot of each cultivar. In 2005 leaves were collected at Oregon State University's Botany and Plant Pathology Field Laboratory in Corvallis, OR. Due to an epidemic of stripe rust (caused by *Puccinia striiformis*) that affected Foote, flag leaves from both cultivars were collected in plots that had received a single fungicide application of Stratego (Bayer CropScience, Research Triangle Park, NC) at a rate of 0.725 L/ha 42 days earlier. This fungicide application controlled stripe rust sufficiently, which allowed Septoria tritici blotch to establish and increase. Twenty flag leaves were randomly chosen from each of four plots of the same cultivar and bagged separately. In both years, leaves were dried by leaving them uncovered and spread out on a lab bench for approximately 5 days. They were then grouped in bags according to cultivar and plot and placed in cold storage until processed.

Pathogen Isolation

In 2004, all collected leaves were used to obtain isolates while, in 2005, five randomly chosen leaves from each plot were used. These leaves were cut into sections approximately 5 to 7 cm in length, clipped to glass microscope slides, placed in moist chambers constructed from glass Petri dishes with wet filter paper, and left overnight. These conditions promoted the sporulation of pycnidia in the leaf sections. The next day leaves were examined under a dissecting scope in a hood. Cirrhi that were not in contact with neighboring cirrhi, in addition to being

in separate lesions, were plucked off with a dental pick or tweezers and placed on yeast malt agar plates amended with 10mg/L of gentamicin. Isolates from a single leaf section were put on the same plate. These isolates were allowed to grow for 3 to 5 days. Isolates that were not contaminated were then transferred to new plates, each plate being used for a single isolate. Approximately one month later isolates were transferred to yeast malt agar slants amended with 10mg/L of gentamicin, sealed with parafilm for long-term storage, and refrigerated.

Isolate Selection and Experimental Design

Six experiments were conducted (Table 1). Isolates used for the experiments were randomly chosen from those that were successfully grown on artificial media, with each isolate originating from a different leaf to avoid sampling the same genotype. The first trial in the greenhouse (Experiment 1) used bulk populations of 14 isolates collected from both Foote and Madsen. The number of isolates used in these bulk populations was limited due to difficulties finding isolates from the cultivar Foote. Because this trial indicated adaptation to Foote, we decided to also evaluate individual isolates in growth chamber studies (insufficient temperature control precludes greenhouse studies from April to October). Initially, seven isolates from each 2004 bulk population were randomly chosen to evaluate the individual isolates separately. Space constraints led to some contamination among pots in the growth chamber, and the data were not analyzed.

However, the number of isolates from each cultivar was narrowed from seven to four based on isolate rankings in this trial (see below).

From the preliminary run with the seven randomly chosen isolates from each cultivar, the 2004 isolates that caused the most disease, the least amount of disease, and two intermediate levels of disease on the cultivar of origin were chosen for evaluation. To attain consistency between years, an identical sampling procedure was used to choose four isolates per cultivar for the 2005 collection. The eight isolates from each year were tested on each cultivar in a factorial design. The bulk populations were composed of a mixture of the four isolates from the same cultivar and year, which made a total of two bulk populations from each season. These were also tested on the two cultivars in a factorial design. For growth chamber experiments, individual isolate testing was conducted separately from bulk population testing due to space limitations. Both individual isolates and bulk populations were tested simultaneously in the greenhouse for the 2005 isolates.

In both the growth chamber and greenhouse experiments, precaution against contamination allowed only two replications per inoculation event. In the growth chamber, there were three inoculation events for both individual isolates and bulk populations each year, for a total of six replications for each growth chamber experiment. The greenhouse experiment with 2005 isolates was repeated once, for a total of four replications. The first 2004 trial using bulk populations in

the greenhouse was an exception, with five replications but only one inoculation event.

Inoculation Procedure

Seeds were planted in 10 cm square plastic pots and bottom watered in trays as needed. Thirteen seeds were planted per pot and thinned to 10 seedlings per pot prior to inoculation. Sunshine SB40 potting soil (Sun Gro Horticulture Canada Ltd.) was used and amended with 2.5 grams per pot of Osmocote 18-6-12 extended time-release fertilizer (Scott-Sierra Horticultural Products Co., Marysville, Ohio).

Isolates stored on refrigerated slants were transferred to fresh yeast malt agar slants supplemented with gentamicin 3 days before inoculation and grown at room temperature. On the day of inoculation, conidia were mixed with distilled water and the concentration was adjusted to 10^6 conidia per milliliter using a hemacytometer. A drop of surfactant, Tween 20 (polyoxyethylene sorbitan monolaurate), was added to the conidial suspensions. In the bulk population inoculations each isolate was represented in equal frequency. The control "inoculum" was prepared simultaneously and consisted of distilled water and Tween 20.

Inoculations took place when seedlings were 21 days old. Using a handheld sprayer, four pots of seedlings were sprayed at a time with 25 ml of

suspension, which was sufficient to inoculate to runoff. The seedlings were placed on a turntable set at 16 rpm to ensure an equal distribution of conidial suspension. The seedlings were then placed in a randomized complete block design in a mist chamber. The turntable, surrounding area, and our hands were cleaned with 95% ethanol after each conidial suspension was used in order to prevent cross-contamination. The mist chamber was constructed of a wooden frame covered with plastic sheeting. Ultrasonic humidifiers were attached to a PVC pipe running the length of the chamber with equally spaced holes drilled in the pipe to disperse the mist evenly. The humidifiers were set at medium moisture levels with timed intervals to prevent over saturation. Seedlings were kept in the mist chamber for 96 hours to initiate infection. In the growth chamber, the humidifier was then shut off and the plastic cover removed. The seedlings remained in the same location. In the greenhouse, seedlings were transferred to a neighboring bench and placed in the same randomized complete block design. All seedlings were in trays and bottom watered as needed.

The growth chamber (Conviron CMP 3023, Controlled Environments Inc., Pembina, North Dakota) was set at 15ºC, 50 % relative humidity, and 16-hour days provided by white fluorescent lighting supplemented with incandescent bulbs. The greenhouse was set for 21.1ºC days and 18.3ºC nights. Daylight was supplemented with halogen lights, which were set for 16-hour days.

Disease Assessments

Disease assessments were performed 21 days after inoculation. The third leaf from the bottom of each seedling was chosen for assessment. The leaf was measured from ligule to blade tip to 1/10 cm accuracy, and then the length of necrotic tissue was estimated by measuring necrotic tissue beginning after the ligule to an average ending point of lesions, disregarding pycnidial density. Due to difficulties in discerning natural tip senescence from diseased tissue in some of the infected plants, the entire necrotic length was measured. The length of necrotic tissue divided by the entire length of the leaf blade multiplied by 100 gave an estimate of percent disease severity. Percent disease severity was used as an indicator of how adapted the isolates were to the cultivar, with more necrosis indicating higher levels of adaptation.

Statistical Analyses

The six experiments (Table 1) were analyzed separately. For all of the experiments, homogeneity of variance was checked with the Fmax test (41) using the mean square errors of each trial. The results indicated homogeneity of variance among trials of each experiment. In addition, all of the data sets had insignificant ($P \geq 0.10$) trial by cultivar, trial by isolate source, and trial by isolate interaction effects, thus trials for each experiment were combined. Residual plots

for individual isolate data from 2004 and 2005 indicated log transformations were suitable. The other data sets were analyzed without transformation.

The data were analyzed with the PROC MIXED procedure in SAS version 9.1 (SAS Institute, Cary, NC). Each individual pot was considered an experimental unit, and the seedlings within the pots were considered subsamples. Cultivar, isolate source, isolate, and interactions between cultivar and isolate or isolate source were all considered fixed effects. Trial (each inoculation event), block, and interactions between these and cultivar, isolate source, or isolate were all considered random effects. Significant main effects were not interpreted when they were part of significant interaction terms. The water controls were excluded from the analyses because the amount of disease was low, and to include the values would possibly underestimate variability of the other treatments. In order to compare treatments to water controls, Dunnett's one-tailed t-test was used in a separate analysis of each experiment.

Pre-planned linear contrasts were made to further evaluate significant cultivar by isolate source or cultivar by isolate within source interaction terms. For individual isolate experiments, we compared how Foote isolates performed on Foote versus Madsen, Madsen isolates performed on Foote versus Madsen, the cultivar Foote performed when inoculated with Foote versus Madsen isolates, and how the cultivar Madsen performed when inoculated with Foote versus Madsen isolates. With bulk population experiments, the degrees of freedom allowed for

one contrast. The contrast tested was how the bulk population from Madsen performed on the cultivar Madsen in comparison to the cultivar Foote. This contrast was chosen because it was expected that Madsen isolates would perform well on Madsen but poorly on Foote. In addition, for the greenhouse experiment with 2005 isolates, contrasts were made to compare the mean of the individual isolates from the same source to the bulk populations that were composed of those individuals across both cultivars.

Table 1. Description of six experiments performed to explore specificity in quantitatively expressed resistance of wheat to *Mycosphaerella graminicola*

Experiment	Year of Isolate Collection	Environment	Inoculum	Total Number of Isolates	Block(Trial)= Total Number of Replications
1	2004	Greenhouse	Bulk Populations	28	5(1) = 5
2	2004	Growth Chamber	Individual Isolates	8	2(3) = 6
3	2004	Growth Chamber	Bulk Populations	8	4(3) = 12
4	2005	Greenhouse	Individual Isolates and Bulk Populations	8	2(2) = 4
5	2005	Growth Chamber	Individual Isolates	8	2(3) = 6
6	2005	Growth Chamber	Bulk Populations	8	4(3) = 12

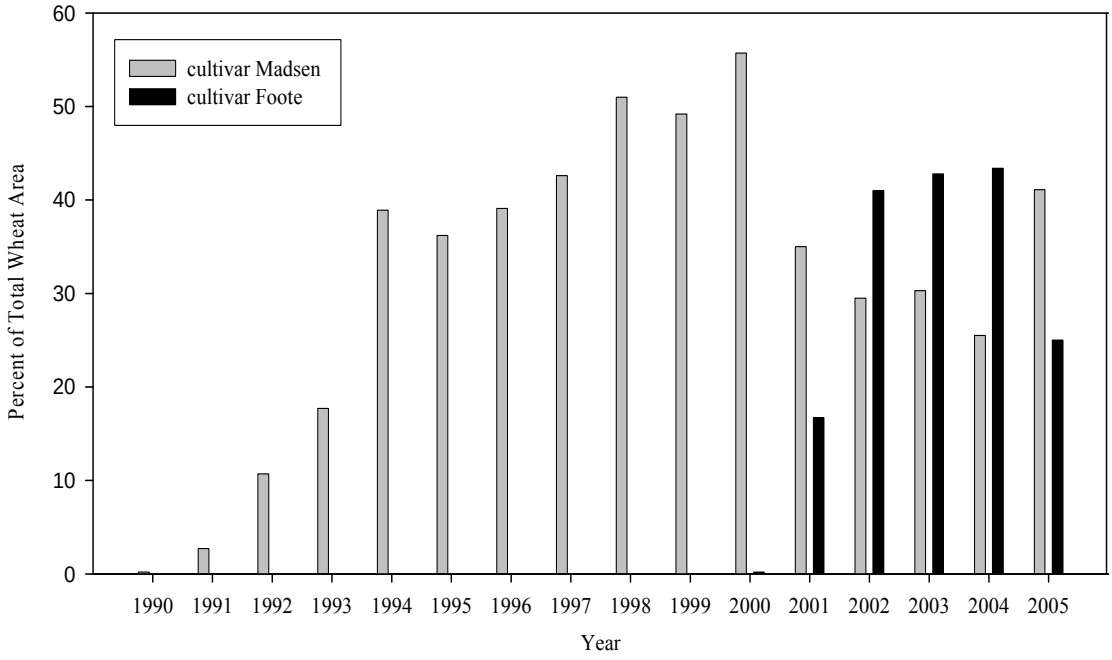


Fig. 1. Percent of total wheat area in Willamette Valley of Oregon planted with cultivars Madsen and Foote from 1990 to 2005.

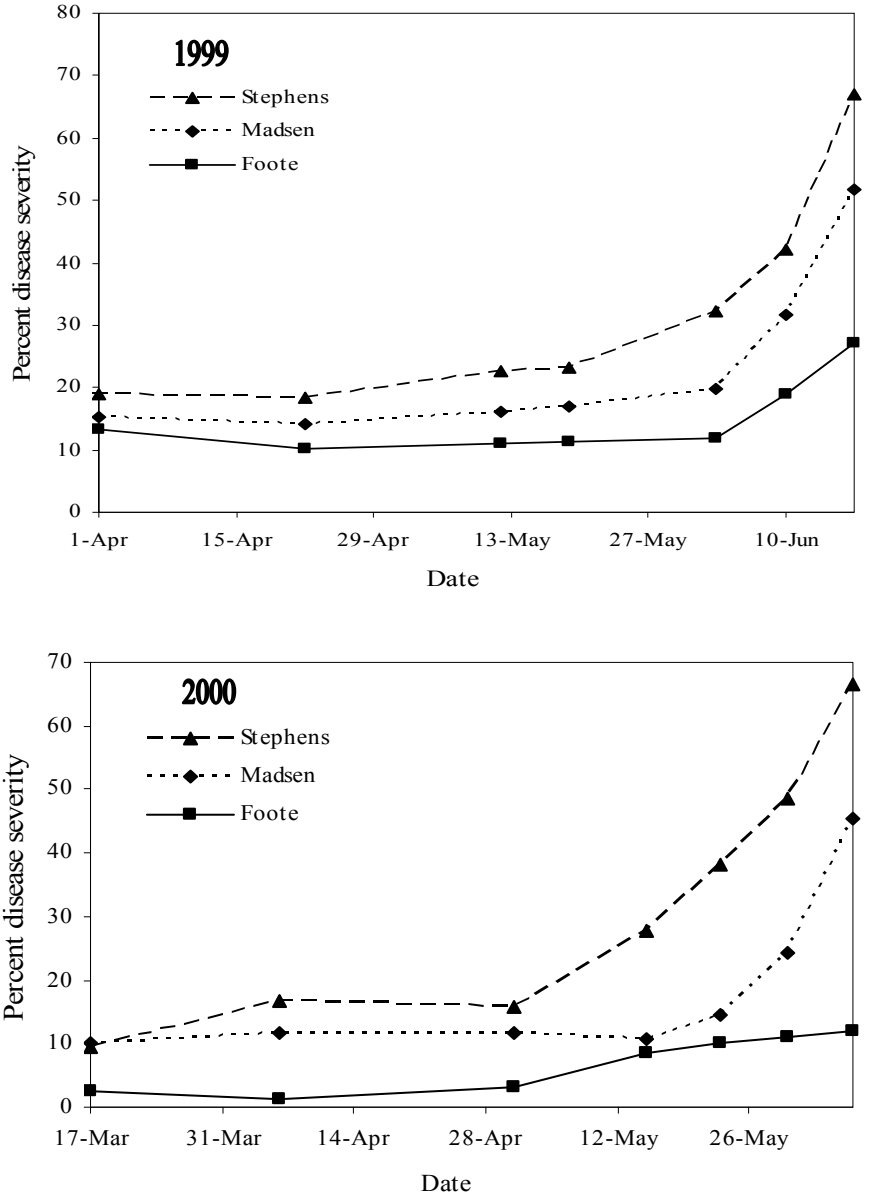


Fig. 2. Disease progress curves for Septoria tritici blotch in experimental field plots in the Willamette Valley of Oregon in 1999 and 2000. Percent disease severity was measured by visual assessments of whole plots. Stephens, a known susceptible cultivar, was used as a standard for comparison because of differences in disease pressure among years. (Source: C. Cowger, unpublished)

Results

Individual Isolates in Growth Chamber

Cultivar and isolate source interacted in both 2004 and 2005 (Table 2). Individual isolates caused more disease on their cultivar of origin, with the exception of one isolate from Madsen collected in 2004 (Fig. 3). In 2004, both isolate sources caused similar amounts of disease on the cultivar Madsen, whereas isolates from Foote caused significantly more disease on Foote than on Madsen ($P<0.0001$). In contrast, in 2005 there was a significant difference in the performance of isolate sources when inoculated on the cultivar Madsen ($P=0.0036$). In both years, isolates originating on Foote and tested on Foote demonstrated higher levels of disease when compared to all other treatment combinations. Overall, disease on the cultivar Madsen was more consistent across isolates.

The interaction between cultivar and isolate within source was significant in 2004, but not in 2005 (Table 2). This was likely due to the Madsen isolate M5b-5, which was highly virulent on Foote.

Bulk Populations in Growth Chamber

There were significant interactions between cultivar and isolate source in 2004 and 2005 (Table 3). Bulk populations composed of isolates from the cultivar

Foote and inoculated on Foote caused more disease than any other treatment (Fig. 4). In 2004, Madsen isolates caused more disease on Foote than on the cultivar of origin, which is in contrast to the general pattern seen with individual isolates in 2004. This likely was caused by the Madsen isolate M5b-5, which has high virulence towards Foote and may mask effects of the other isolates. Bulk populations from Madsen in 2005 caused more disease on Madsen (P=0.0009), which is in agreement with results from individual isolate testing.

Greenhouse Trials

In the 2004 trial with bulk populations, there was a significant interaction between cultivar and isolate source (Table 4). All treatments were similar except Madsen isolates tested on Foote, which had the lowest amount of disease (Fig. 5; Table 4). Madsen isolates caused significantly more disease on Madsen than on Foote (P=0.0003).

In the 2005 greenhouse trials there was a significant interaction between cultivar and isolate source (Table 4). Generally, both bulk populations and individual isolates caused more disease on the cultivar of origin (Fig. 6), although there was no significant difference between performance of Foote isolates on either cultivar (P=0.9537). All Madsen isolates caused low amounts of disease on Foote in comparison to Foote isolates (P<0.0001). Disease on the cultivar Madsen was more consistent across isolates.

Mean of Individual Isolates Versus Bulk Populations

Individual isolates and bulk populations were tested in different experiments in growth chamber trials due to space constraints. However, the ranking of bulk treatments in growth chamber trials were similar to that of means for individual isolates of the same treatment (Figs. 3 and 4).

Space in the greenhouse allowed for individual isolates and bulk populations to be tested simultaneously with 2005 isolates. There were no significant differences between bulk populations and the mean of individual isolates for either source (Fig. 7, Table 4).

Growth Chamber Versus Greenhouse

Isolates acted differently in the greenhouse when compared to the growth chamber in both years. Most notably, in the growth chamber Foote was more susceptible to compatible isolates than Madsen (Figs. 3 and 4). In the greenhouse, all treatments caused similar levels of disease, except for Madsen isolates inoculated on Foote (Figs. 5 and 6).

Table 2. Analysis of variance for percent disease severity caused by eight isolates of *Mycosphaerella graminicola* collected in the field, four from the wheat cultivar Madsen and four from the wheat cultivar Foote. Each isolate was inoculated on both cultivars and placed in the growth chamber in a randomized complete block design with two blocks per inoculation event conducted in three separate trials. Disease severity on the third leaf of each seedling was evaluated three weeks after inoculation.

2004				
Type of Effect	**Source of Variation**	**DF**	**Z-Value**[c]	**P-Value**
Random	Trial	na	0.78	0.2185
	Block(Trial)	na	0.68	0.2493
	Trial x Cultivar	na	0.79	0.2134
	Trial x Isolate(Source)	na	0.60	0.2729
	Source of Variation	**DF**	**F-Value**[c]	**P-Value**
Fixed	Cultivar	1	2.71	0.2416
	Source	1	81.99	<0.0001
	Isolate (Source)	6	10.06	0.0002
	Cultivar x Source[a]	1	141.36	<0.0001
	Cultivar x Isolate(Source) [b]	6	17.73	<0.0001
	Foote Isolates on Foote vs. Madsen	1	42.07	<0.0001
	Madsen Isolates on Foote vs. Madsen	1	12.04	0.0010
	Foote inoculated with Foote vs. Madsen isolates	1	211.32	<0.0001
	Madsen inoculated with Foote vs. Madsen isolates	1	0.94	0.3375
2005				
Type of Effect	**Source of Variation**	**DF**	**Z-Value**[c]	**P-Value**
Random	Trial	Na	0.69	0.2466
	Block(Trial)	Na	1.07	0.1419
	Trial x Cultivar	Na	0.85	0.1989
	Trial x Isolate(Source)	Na	1.28	0.0997
	Source of Variation	**DF**	**F-Value**[c]	**P-Value**
Fixed	Cultivar	1	3.24	0.2139
	Source	1	77.11	<0.0001
	Isolate (Source)	6	1.33	0.3067
	Cultivar x Source[a]	1	311.64	<0.0001
	Cultivar x Isolate(Source) [b]	6	0.22	0.9687
	Foote Isolates on Foote vs. Madsen	1	22.81	<0.0001
	Madsen Isolates on Foote vs. Madsen	1	66.01	<0.0001
	Foote inoculated with Foote vs. Madsen isolates	1	301.57	<0.0001
	Madsen inoculated with Foote vs. Madsen isolates	1	9.18	0.0036

[a]Represents interaction between cultivar and isolate source.

[b]Represents interaction between cultivar and individual isolate.

[c]For random effects, Z-values were calculated from the Wald test for significance using covariance parameter estimates (Pr > |Z|). For fixed effects, F-values were used to determine significance (Pr>F).

Table 3. Analysis of variance for percent disease severity caused by bulk populations of *Mycosphaerella graminicola* collected in the field, with one population composed of four isolates from the wheat cultivar Madsen and one population composed of four isolates from the wheat cultivar Foote. Each bulk population was inoculated on both of the cultivars and placed in the growth chamber in a randomized complete block design, with four blocks per inoculation event conducted in three separate trials. Disease severity on the third leaf of each seedling was evaluated three weeks after inoculation.

2004				
	Source of Variation	**DF**	**Z-Value**[b]	**P-Value**
Random Effects	Trial	na	0.64	0.2611
	Block(Trial)	na	1.54	0.0621
	Trial x Cultivar	na	0.84	0.1999
	Trial x Source	na	0.84	0.2018
	Source of Variation	**DF**	**F-Value**[b]	**P-Value**
Fixed Effects	Cultivar	1	13.42	0.0671
	Source	1	1.47	0.3487
	Cultivar x Source[a]	1	13.51	0.0010
	Madsen Isolates on Foote vs. Madsen	1	4.21	0.0494
2005				
	Source of Variation	**DF**	**Z-Value**[b]	**P-Value**
Random Effects	Trial	na	0.92	0.3570
	Block(Trial)	na	-0.47	0.6374
	Trial x Cultivar	na	0.71	0.4806
	Trial x Source	na	0.88	0.3782
	Source of Variation	**DF**	**F-Value**[b]	**P-Value**
Fixed Effects	Cultivar	1	1.07	0.4104
	Source	1	2.49	0.2555
	Cultivar x Source[a]	1	34.17	<0.0001
	Madsen Isolates on Foote vs. Madsen	1	13.60	0.0009

[a]Represents interaction between cultivar and isolate source.

[b]For random effects, Z-values were calculated from the Wald test for significance using covariance parameter estimates ($Pr > |Z|$). For fixed effects, F-values were used to determine significance ($Pr>F$).

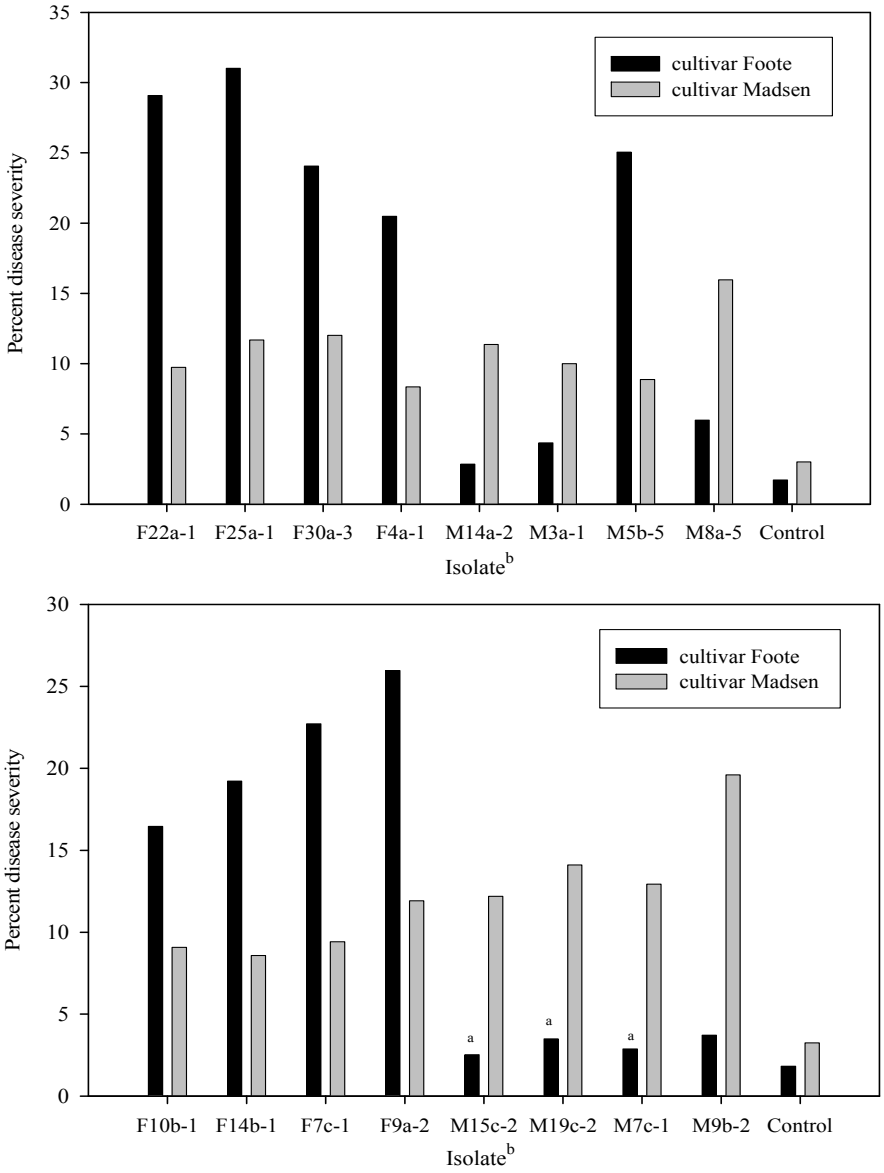
Table 4. Analysis of variance for percent disease severity caused by individual isolates and bulk populations of *Mycosphaerella graminicola* collected in the field. For isolates collected in June of 2004 two bulk populations, one composed of 14 isolates from the wheat cultivar Madsen and one composed of 14 isolates from the wheat cultivar Foote, were inoculated on each cultivar and placed in the greenhouse in a randomized complete block design, with a total of five blocks. For isolates collected in June of 2005, four isolates from the wheat cultivar Madsen and four from the wheat cultivar Foote along with bulk populations composed of the four isolates from each cultivar were inoculated on both cultivars and placed in the greenhouse in a complete randomized block design, with two blocks per inoculation event conducted in two separate trials. Disease severity on the third leaf of each seedling was evaluated three weeks after inoculation.

2004				
	Source of Variation	**DF**	**Z-Value**[c]	**P-Value**
Random Effects	Block	na	0.87	0.3848
	Source of Variation	**DF**	**F-Value**[c]	**P-Value**
Fixed Effects	Cultivar	1	10.08	0.0080
	Source	1	25.00	0.0003
	Cultivar x Source[a]	1	15.16	0.0021
	Madsen Isolates on Foote vs. Madsen	1	24.98	0.0003
2005				
	Source of Variation	**DF**	**Z-Value**[c]	**P-Value**
Random Effects	Trial	na	0.38	0.3520
	Block(Trial)	na	0.60	0.2726
	Trial x Cultivar	na	0.65	0.2583
	Trial x Isolate (Source)	na	1.17	0.1206
	Source of Variation	**DF**	**F-Value**[c]	**P-Value**
Fixed Effects	Cultivar	1	10.12	0.1939
	Source	1	13.50	0.0079
	Isolate (Source)	6	2.13	0.1723
	Cultivar x Source[a]	1	11.42	<0.0001
	Cultivar x Isolate(Source)[b]	6	3.58	0.0067
	Foote Isolates on Foote vs. Madsen	1	0.00	0.9537
	Madsen Isolates on Foote vs. Madsen	1	36.52	<0.0001
	Foote inoculated with Foote vs. Madsen isolates	1	74.16	<0.0001
	Madsen inoculated with Foote vs. Madsen isolates	1	5.41	0.0256
	Foote isolates: Bulk vs.Individual Mean	1	0.10	0.7587
	Madsen isolates: Bulk vs. Individual Mean	1	0.41	0.5387

[a]Represents interaction between cultivar and isolate source.

[b]Represents interaction between cultivar and individual isolate.

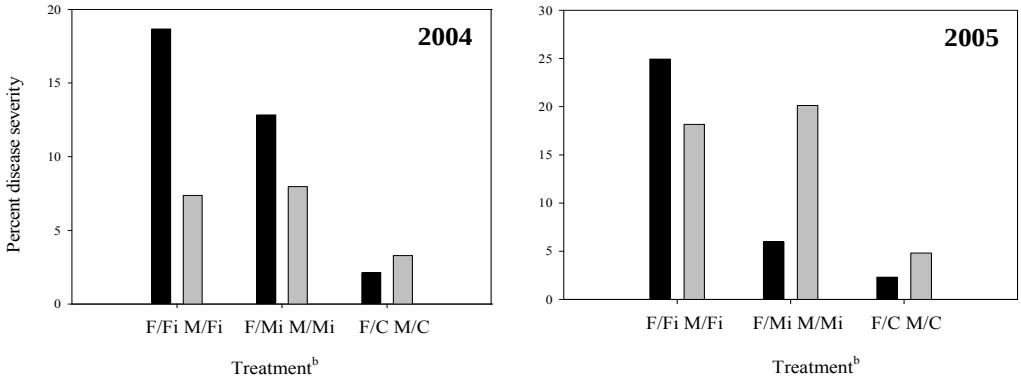
[c]For random effects, Z-values were calculated from the Wald test for significance using covariance parameter estimates ($Pr > |Z|$). For fixed effects, F-values were used to determine significance ($Pr>F$).



[a]Indicates that disease severity measured as percent necrotic leaf area was not significantly different from that recorded on control of the same cultivar based on a one-tailed Dunnett's test (P=.05). All other treatments were significantly different from the water control of the same cultivar.

[b]Isolates preceded with F originated on Foote, isolates preceded with M originated on Madsen

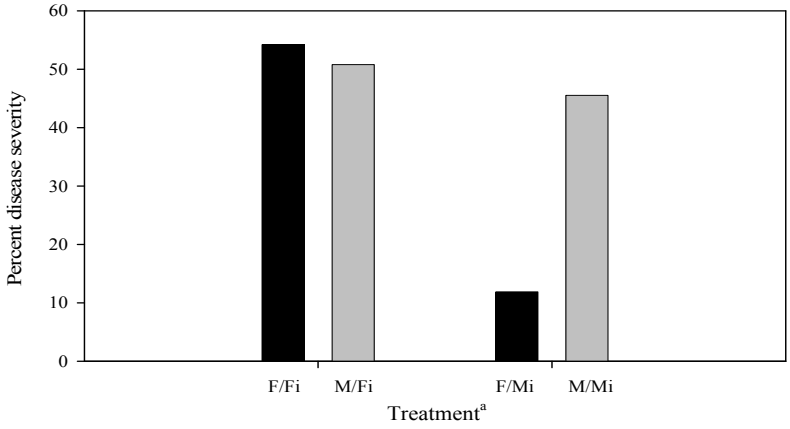
Fig. 3. Percent disease severity measured as the percent of necrotic leaf area on third leaf of seedlings of Madsen and Foote wheat cultivars after inoculation with individual isolates of *Mycosphaerella graminicola* collected from the field in 2004 and 2005. Inoculated plants were placed in the growth chamber and disease assessments occurred three weeks after inoculation. Values are the means of six replications.



[a]Indicates that disease severity measured as percent necrotic leaf area was not significantly different from that recorded on control of the same cultivar based on a one-tailed Dunnett's test (P=0.05). All other treatments are significantly different from the water control.

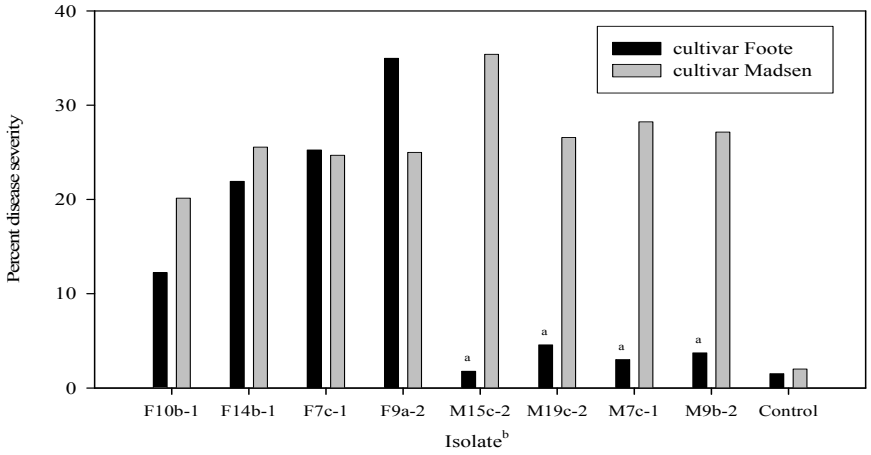
[b]The first letter indicates the cultivar with F representing Foote and M representing Madsen. The label after the slash indicates the isolate source with Fi representing Foote, Mi representing Madsen, and C representing the water control.

Fig. 4. Percent disease severity measured as the percent of necrotic leaf area on third leaf of seedlings of Madsen and Foote wheat cultivars after inoculation with bulk populations of *Mycosphaerella graminicola* collected from the field in 2004 and 2005. Inoculated plants were placed in the growth chamber and disease assessments occurred three weeks after inoculation. Values are the means of 12 replications.



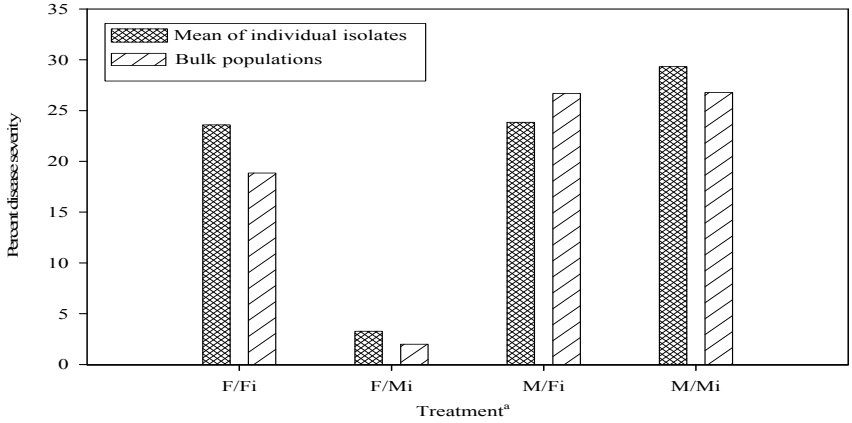
[a]The first letter indicates the cultivar with F representing Foote and M representing Madsen. The letters after the slash indicate the isolate source, with Fi representing Foote and Mi representing Madsen.

Fig. 5. Percent disease severity measured as the percent of necrotic leaf area on third leaf of seedlings of Madsen and Foote wheat cultivars after inoculation with bulk populations of *Mycosphaerella graminicola* collected from the field in 2004, with one population composed of 14 isolates from the wheat cultivar Madsen and one population composed of 14 isolates from the wheat cultivar Foote. Inoculated plants were placed in the greenhouse and disease assessments occurred three weeks after inoculation. Values are the means of five replications.



[a]Indicates that disease severity was not significantly different from that recorded on control of the same cultivar based on a one-tailed Dunnett's test (P=0.05). All other treatments are significantly different from the water control of the same cultivar.
[b]Isolates preceded with F originated on Foote, isolates preceded with M originated on Madsen.

Fig. 6. Percent disease severity measured as the percent of necrotic leaf area on third leaf of seedlings of Madsen and Foote wheat cultivars after inoculation with individual isolates of *Mycosphaerella graminicola* collected from the field in 2005. Inoculated plants were placed in the greenhouse and disease assessments occurred three weeks after inoculation. Values are the means of four replications.



[a]The first letter represents the cultivar with F representing Foote and M representing Madsen. The letters after the slash indicate the isolate source, with Fi representing Foote and Mi representing Madsen.

Fig. 7. Percent disease severity measured as the percent of necrotic leaf area on third leaf of seedlings of Madsen and Foote wheat cultivars after inoculation with isolates of *Mycosphaerella graminicola* collected from both Madsen and Foote in the field in 2005. Inoculated plants were placed in the greenhouse and disease assessments occurred three weeks after inoculation. Bulk populations were composed of the four isolates used to calculate means of individual isolates. Values are the means of four replications.

Conclusion

The nature of resistance of wheat to *M. graminicola* has been controversial because of difficulties in identifying races of the pathogen that interact differentially with cultivars (24). Subsequent research established specificity and adaptation of isolates to host cultivars (3,4,24,25). In addition, gene-for-gene relationships have been confirmed with wheat cultivars and pathogen isolates (7,38). Our study focused on quantitatively expressed host resistance. We detected significant cultivar x isolate source interactions in all six experiments. Furthermore, both individual isolates and bulk populations generally caused more disease on their cultivar of origin. We thus demonstrated both host-pathogen specificity and pathogen adaptation in quantitative interactions. Although specificity has been demonstrated in several quantitative systems (9,17,32,42,43,52), there is little evidence of natural pathogen populations in the field eroding host resistance. Observations in the Netherlands suggested a slow erosion of wheat resistance to Septoria tritici blotch (39). The gradual deterioration of resistance of Madsen is another documented example of quantitatively expressed resistance eroding in the field (48).

Our results were very robust, as the same trends in specificity and adaptation were detected using different methodologies. Bulk populations performed similarly to the mean of individual isolates from which the bulk populations were composed. In 2004 bulk population trials in the growth

chamber, however, the population from Madsen caused more disease on Foote than on Madsen (Fig. 4), which was generally not the case when isolates were tested individually. Bulk populations that were composed of 14 isolates collected in 2004, including the isolates used in the 2004 growth chamber experiments, showed a different pattern when tested in the greenhouse. It is possible that the influence of isolate M5b-5 was diluted by the larger total number of isolates. Similar patterns of specificity and adaptation were also detected in growth chamber and greenhouse experiments. It is not known why Foote isolates tested on Foote caused more disease than other treatments in the growth chamber but caused comparable amounts of disease to other treatments in the greenhouse. However, this discrepancy did not change the overall patterns of specificity and adaptation.

Although Foote and Madsen both express quantitative disease resistance, patterns of pathogen adaptation differ. The reaction of isolates to Foote showed a qualitative pattern in which isolates expressed either high virulence or low virulence. It is possible that resistance to Foote is controlled by a single major gene with incomplete expression. Segregation ratios of progeny from a cross between Foote and a known susceptible could elucidate whether a single gene is responsible for the resistance. The source of resistance of Foote is unknown, as it

was developed from a complex cross with both winter and spring wheat cultivars: Heima//Kalyansona/Bluebird/3/WWP7147, F1/4/D6301/HeinesVII//ERA/3/ Buckbuck. Disease on the cultivar Madsen was more consistent across isolates, possibly because the cultivar has been grown in the Willamette Valley for over a decade, and the pathogen has had significant opportunity to become adapted.

Reactions of Willamette Valley wheat cultivars to *M. graminicola* have been monitored for over 15 years. The cultivar Stephens dominated the crop area from the 1980s through the mid-1990s. Although we have no information as to the resistance level of Stephens at the time of release, its decline in popularity in the mid-1990s was due almost entirely to susceptibility to Septoria tritici blotch (C.C. Mundt, unpublished). Furthermore, *M. graminicola* isolates collected from Stephens in the early 1990s showed strong adaptation to Stephens and its sister cultivar, Malcolm (4). The area planted to Madsen increased significantly after a devastating Septoria tritici blotch epidemic in 1993 that affected Stephens severely. Madsen replaced Stephens by the mid/late-1990s and originally showed considerable quantitative resistance. This quantitative resistance has gradually eroded and to a substantial degree (48), though it currently maintains some resistance (C.C. Mundt, unpublished). Major gene resistance in the cultivar Gene was nearly complete at the time of release, but broke down catastrophically due to selection of virulent pathogen genotypes (18,48). The current study shows clear

adaptation to the cultivar Foote, which has dominated over Madsen in recent years, even though resistance appears to be holding up in the field (C.C. Mundt, unpublished). There clearly will be strong selection during the growing season for pathogen genotypes adapted to a given cultivar. We collected isolates late in the season, when the maximum number of generations of selection would have occurred. However, the high frequency of sexual recombination in *M. graminicola* at the end of the season would reassort genes and make the process of selection for high virulence more gradual (39). Eventually, we would expect alleles for virulence to Foote to reach proportions sufficient to erode resistance. Unfortunately, the durability of Foote against Septoria tritici blotch will never be known since the cultivar is being abandoned owing to extreme susceptibility to a new race of *P. striiformis* (C.C. Mundt, unpublished). Regardless, the quantitative resistance of both Madsen and Foote were much more durable than the complete resistance of Gene.

Host plant resistance to Septoria tritici blotch, including quantitative resistance, appears to be more ephemeral in the Willamette Valley of Oregon than has been reported in other areas. The Willamette Valley is conducive to disease development owing to extensive sexual recombination, favorable conditions for epidemic development, lack of genetic bottlenecks, and absence of significant immigration (49). These factors likely allow for rapid selection of pathogen genotypes that can evade host plant resistance. Another possibility, however, is

that there simply has been more effort devoted to studying adaptation of *M. graminicola* to host resistance in the Willamette Valley as compared to other locations.

Although pathogen adaptation to quantitative resistance seems important for *M. graminicola* populations in the Willamette Valley, we expect this process to be of little or no importance in many other cases. Most plant pathogens undergo little or no recombination and experience substantial genetic bottlenecks, which would likely slow pathogen adaptation to the point where a cultivar would be replaced for other reasons (e.g., replaced by a higher yielding cultivar) before any significant loss of resistance would occur. Furthermore, despite extensive recombination in the pathogen population, there appears to be durable forms of resistance to potato late blight in the Toluca Valley of Mexico (31).

This study also has implications for disease management. Although both qualitatively and quantitatively expressed resistance may not be permanent, quantitative resistance may be a more appropriate goal for breeding programs. McDonald (46) evaluated stability of host plant resistance based on the genetic structure of the pathogen population. Under this scheme, *M. graminicola* was ranked 7 on a 1-9 scale, where 9 ranks as the most unstable. This rating for *M. graminicola* is owing to its mixed mating system (i.e., both sexual and asexual) and large effective population size, which would favor pathogen adaptation, but tempered by moderate dispersal capabilities (asexual spores of *M. graminicola* are

splash-dispersed). McDonald (46) suggested that the best genetic strategy for control of *M. graminicola* would be to focus on quantitative resistance, with major genes being deployed regionally. Although major gene resistance broke down rapidly in the Willamette Valley, the major gene *Stb1* has remained durable in the Midwestern U.S. (2). It is not known whether this is due to less selection pressure or the nature of the gene.

Arguments have also been made for the value of major gene pyramids to achieve stable resistance to *M. graminicola* (10). Cultivar mixtures are an additional possibility for disease management, although in practice they have led to mixed results (20). This could be the result of disruptive selection favoring different alleles on different host genotypes and making it difficult to discern patterns at the population level (46).

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