

Selective Influence of Wheat Cultivars on Pathogenicity of *Mycosphaerella graminicola* (Anamorph *Septoria tritici*)

Hafiz U. Ahmed, Christopher C. Mundt, Molly E. Hoffer, and Stella M. Coakley

Department of Botany and Plant Pathology, 2082 Cordley Hall, Oregon State University, Corvallis 97331-2902.

Current address of H. U. Ahmed: Plant Pathology Division, Bangladesh Rice Research Institute, Joydepur, Gazipur 1701, Bangladesh.

Paper 10,416 of the journal series of the Oregon Agricultural Experiment Station.

Supported by the Bangladesh Rice Research Institute-International Rice Research Institute Collaborative project and by the National Science Foundation grant DEB 9307163.

Accepted for publication 15 January 1996.

ABSTRACT

Ahmed, H. U., Mundt, C. C., Hoffer, M. E., and Coakley, S. M. 1996. Selective influence of wheat cultivars on pathogenicity of *Mycosphaerella graminicola* (anamorph *Septoria tritici*). *Phytopathology* 86:454-458.

Populations of *Mycosphaerella graminicola* isolated from winter wheat cultivars near crop maturity were subsequently evaluated on seedlings of the same wheat cultivars in the greenhouse. Significant pathogen population effects, cultivar effects, and pathogen population \times cultivar interactions were demonstrated. Pathogen populations isolated from

susceptible cultivars produced higher levels of disease, averaged over cultivars, than did populations isolated from moderately resistant or resistant cultivars, indicating that susceptible cultivars selected for higher levels of pathogen aggressiveness in the field. In addition, pathogen populations were more virulent on the cultivars they were isolated from in the field than on other cultivars, indicating selection for cultivar-specific virulence.

Additional keywords: pathogenicity, resistance, *Septoria tritici* blotch, *Triticum aestivum*.

In many host/pathogen systems, resistance genes have been only temporarily effective because of increases in the frequency of matching virulence in pathogen populations (25). Further, even in the absence of clear gene-for-gene interactions, pathogen populations may be selected for increased virulence (2,5,12,18), potentially reducing the stability of quantitatively inherited resistance (7,18,22).

Septoria tritici blotch of wheat (*Triticum aestivum* L.), caused by the fungus *Mycosphaerella graminicola* (Fuckel) J. Schröt. in Cohn (anamorph *Septoria tritici* Roberge in Desmaz.), is a serious disease in all wheat growing areas of the world and causes severe yield losses (15). Variation in virulence (1,15) and host specialization (8,15,26) have been documented in populations of *M. graminicola*. Existence of specific virulence genes has been suggested within *M. graminicola*, and putative virulence frequencies varied considerably among regions and within countries (9).

A high level of genetic variation has been reported within *M. graminicola* populations based on restriction fragment length polymorphisms (RFLPs), suggesting the potential for the selection of individuals with increased virulence to resistant wheat cultivars (20). Location-specific adaptation has been demonstrated among isolates of *M. graminicola*, indicating that isolates were more virulent to wheat cultivars to which they had been previously exposed (1,9). It is also important to know if there is the potential for adaptation of *M. graminicola* to its host within a given location. Thus, the present study was undertaken to determine pathogenic variation among *M. graminicola* populations isolated from winter wheat cultivars, and to determine if there is selection for virulence of *M. graminicola* populations to the cultivars from which they were isolated.

MATERIALS AND METHODS

Two experiments were performed in the greenhouse. Experiment 1 was conducted during the period from January to March 1993. Experiment 2 was done during October to December 1994, and was repeated during January to February 1995.

Host. Four winter wheat cultivars were used in experiment 1: Gene, PI 560129; Madsen, PI 511673; Malcolm, PI 497672; and Stephens, CI 017594. Only 'Madsen' and 'Stephens' were used in experiment 2. The four cultivars are commonly grown in western Oregon and have been observed extensively for their degree of susceptibility to *M. graminicola*. 'Malcolm' and 'Stephens' are both highly susceptible, with 'Malcolm' usually being somewhat more susceptible than 'Stephens'. 'Madsen' is moderately resistant and consistently demonstrates half or less of the level of *Septoria tritici* blotch as compared with 'Stephens'. 'Gene' was highly resistant to *M. graminicola* when initially released for commercial production in 1992, though increased levels of *Septoria tritici* blotch have been observed on 'Gene' in recent years.

Plants were raised in 10-cm plastic pots filled with a 1:1:2 mixture of peat/sand/loam/#8 pumice. Approximately 15 seeds of a cultivar were sown in each pot. At 7 days after seeding, plants were thinned to 10 per pot. For experiment 1, the plants were fertilized twice per pot with 50 ml of a solution containing 15 g of fertilizer (ammoniacal nitrogen, 6.8%; urea nitrogen, 8.2%; available phosphoric acid, P₂O₅, 30%; soluble potash, K₂O, 15%; boron, 0.02%; copper, 0.07%; iron, 0.15%; manganese, 0.05%; molybdenum, 0.0005%; and zinc, 0.06%) per liter. Plants were fertilized at 2 and 5 weeks after seeding. Experiment 2 plants were fertilized once, at sowing, with 0.6 g of fertilizer (14% N, 14% P, and 14% K) per pot. Plants were sprayed and/or fumigated with insecticide (insecticidal soap [15 ml/liter] and/or nicotine smoke) as necessary to control aphids. To control powdery mildew (caused by *Erysiphe graminis*), 2 ml of fenarimol (12.5% a.i.) in 10 ml of water was volatilized inside the greenhouse once

Corresponding author: C. C. Mundt; E-mail address: mundtc@bcc.orst.edu

Publication no. P-1996-0215-01R

© 1996 The American Phytopathological Society

per week during experiment 1. For experiment 2, plants were treated at 3 weeks after sowing with 50 ml of a soil drench of ethirimol (0.11 g a.i./liter). Greenhouse temperature was maintained from 20 to 25°C, and sodium-halide lights were used to extend day length to 16 h.

Pathogen. For experiment 1, *M. graminicola* isolates were obtained from the four winter wheat cultivars Gene, Madsen, Malcolm, and Stephens grown in a randomized complete-block design at the Oregon State University Botany and Plant Pathology Field Laboratory during the 1991 to 1992 winter wheat season (21). Two leaves (usually flag leaves) of each cultivar were collected randomly from each of the four replicates. *M. graminicola* isolates for experiment 2 were secured from flag or F-1 leaves of the two wheat cultivars Madsen and Stephens, which were grown in experimental plots in a commercial wheat field in Hillsboro, Oregon, during the 1993 to 1994 wheat season. In this case, the isolates were obtained from diseased leaf samples from a single replication and were collected in a uniform pattern. Each leaf was collected from a different plant within the two plots. For both experiments, leaf samples were collected in June when the crop was approaching maturity and after the pathogen was exposed to repeated generations of selection on the host cultivars. At this time, disease severity was greater than 50%, on a whole canopy basis, for susceptible cultivars.

For experiment 1, leaf sections of about 5 cm with distinct lesion(s) were washed with sterilized water, and then surface-sterilized for 30 s with a 20% solution of 5% sodium hypochlorite. After sterilization, the leaf sections were pressed with blotting paper to remove excess water. Two sterilized leaf sections were then taped on a sterilized glass slide. The slide with leaf sections was placed inside a petri dish containing moistened blotting paper and then placed on a laboratory bench overnight at room temperature. On the following day, cirrhi from individual pycnidia were transferred with a needle under a stereoscope onto a potato-dextrose agar (PDA) plate fortified with 50 mg/liter of streptomycin solution. At 2 days after transfer, a loop of sterile water was added to the spore mass with a bacterial transfer loop and streaked onto a fresh PDA plate. After 3 to 4 days, isolates were obtained from an individual colony and transferred onto PDA slants. Twenty isolates were obtained from different pycnidia on the eight leaves collected from each cultivar. Each isolate was maintained on silica gel in a refrigerated cryovial. Isolates for experiment 2 were originally isolated in the laboratory of B. A. McDonald of Texas A & M University, using methodology similar to that described above. Five isolates arising from different pycnidia on different leaves were obtained from each of the two cultivars.

Inoculation. For experiment 1, crystals of silica gel from cryovials were plated on PDA containing 50 mg/liter of streptomycin. After 3 or 4 days, blastospores that had developed were streaked over the same plates. Two days after streaking, each isolate was transferred to a separate PDA slant and spread uniformly with a bacterial transfer loop. The isolates were used for inoculations after an additional 3 days of growth. To do so, 10 ml of sterile water was added to a PDA slant of each of the isolates. Suspensions of the 20 isolates from each cultivar were combined and then adjusted to 10^6 spores/ml.

For experiment 2, isolates were maintained on yeast-malt agar (YMA) slants with 50 mg/liter of gentamicin. Equal numbers of spores of the five isolates from each cultivar were combined before inoculation. All other procedures for pathogen multiplication and inoculum preparation were the same as experiment 1.

For experiment 1, a set of 16 pots of plants (four cultivars \times four replications) was inoculated at 21 days after seeding with 75 ml of spore suspension of each of the four pathogen populations, using a hand sprayer. Similarly, for each of the two trials of experiment 2, eight pots of plants (two cultivars \times four replications) were inoculated with 50 ml of spore suspension for each patho-

gen population. These amounts were sufficient to inoculate the plants to runoff. After inoculation, the plants were kept in a moist chamber (wooden frame covered with a polyethylene sheet) for 96 h. High humidity (95% or above) was maintained using a humidifier (ultrasonic, cool mist type) inside the moist chamber. Fluorescent tube lights above the moist chamber provided supplemental illumination when necessary. For experiment 1, the pots were subsequently returned to a greenhouse bench in a randomized complete-block design (16 treatment combinations of four spore populations and four cultivars in each block) until disease scoring. For both trials of experiment 2, a completely randomized design was used. Four additional pots of both cultivars were sprayed with water only and included in both trials of experiment 2; otherwise, they were treated in the same manner as inoculated plants.

Disease assessments. For experiment 1, visual estimates of percent leaf area covered by lesions, averaged over all leaves in each pot, were estimated at 2-day intervals beginning at 13 days after inoculation, when the first visible lesions occurred. Only lesions obviously caused by *M. graminicola* were included. For example, chlorosis of leaf margins not associated with pycnidia or pycnidial initials was excluded from the assessments, as such symptoms are often caused by natural senescence. Area under the disease progress curve (AUDPC) was then calculated following the method described by Shaner and Finney (29). For experiment 2, percent lesion area on the second leaf from the base of each of 10 plants in each pot was estimated visually at 3 weeks after inoculation. For both experiments, a single observer conducted all disease assessments, and pots were assessed randomly with regards to treatment.

Statistical analyses. All analyses were performed using the general linear models procedure of SAS (27). Exploratory analyses of variance (ANOVAs) indicated that the square root and natural logarithm transformations provided homogeneous variances for experiments 1 and 2, respectively. Square root transformed data of experiment 1 were treated with a factorial ANOVA for a randomized complete-block design. For experiment 2, data of the two trials were pooled in a combined ANOVA for a completely randomized design after applying a natural logarithm transformation. As lesion areas on the noninoculated controls of experiment 2 were very low (often zero), these data were not included in the statistical analyses.

Fisher's protected least significant difference ($P = 0.05$) was used for making multiple comparisons among pathogen population and cultivar means. Expected values of AUDPC (experiment 1) and percent lesion area (experiment 2) were calculated for each population \times cultivar combination, under the assumption of no population \times cultivar interaction. This was done by using the marginal means of each combination's pathogen population and cultivar, i.e., expected disease = (population mean over all cultivars \times cultivar mean over all populations)/grand mean. If pathogen populations were selected for greater virulence on their cultivar of origin in the field, homologous combinations of population and cultivar should show higher than expected disease severity when tested in the greenhouse. To determine if each population \times cultivar combination was different from its expected value, t tests ($P = 0.05$) were used. To do so, the mean of each population \times cultivar combination (each mean was calculated from transformed values) was compared with the square root of its predicted value for experiment 1 and the natural logarithm of its predicted value for experiment 2. In addition, a test of the hypothesis that pathogen populations were selected for increased virulence on their host of origin was conducted over all population \times cultivar combinations within each experiment through use of a linear contrast of all homologous (source of population and tester cultivar were the same) versus all heterologous (source of population and tester cultivar were different) population \times cultivar combinations. For experiment 2, this contrast was identical to the F test of the

population × cultivar interaction, as this interaction had only one degree of freedom for that experiment.

RESULTS

In both experiments, there were highly significant effects of population, cultivar, and population × cultivar interaction (Tables 1 and 2). For experiment 2, none of the interaction terms involving trial were statistically significant (Table 2), indicating that it was appropriate to conduct an ANOVA combined across the two trials of experiment 2. Mean lesion area of the uninoculated controls in experiment 2 was 0.2% for both 'Madsen' and 'Stephens'.

Mean comparisons showed significant differences in susceptibility among all cultivars tested, as indicated by tester cultivar means over all pathogen populations (Tables 3 and 4). In addition, pathogen populations derived from the two susceptible cultivars were more aggressive than populations originally isolated from the resistant or moderately resistant cultivar, as indicated by pathogen population means over all tester cultivars (Tables 3 and 4). Two of sixteen population × cultivar combinations in experiment 1 and two of four population × cultivar combinations in experiment 2 differed significantly from expected values (Tables 3 and 4). For all cultivars, and in both experiments, the observed amount of disease was numerically higher than the expected amount when population and cultivar were homologous. Overall, disease was 32 and 68% higher for homologous than for the heterologous combinations in experiments 1 and 2, respectively (Tables 3 and 4). The comparison of all homologous versus all heterologous population × cultivar combinations was highly significant for both experiments (Tables 1 and 2).

DISCUSSION

Results were qualitatively similar between the two experiments, despite the fact that the two study populations were sampled 2 years and 110 km apart. Though questions could reasonably be raised regarding the relevance of greenhouse studies to the field,

TABLE 1. Analysis of variance^y of area under disease progress curve (percent days) caused by populations of *Mycosphaerella graminicola* isolated from four winter wheat cultivars in the field and tested on the same wheat cultivars in the greenhouse

Source	df	Mean square	P > F
Block	3	3.9	0.0122
Population	3	15.5	0.0001
Cultivar	3	265.2	0.0001
Population × cultivar	9	4.1	0.0001
Homologous vs. heterologous ^z	1	10.9	0.0015
Error	45	0.9	

^y Analysis performed after applying square root transformation.

^z Linear contrast of all homologous versus all heterologous pathogen population × cultivar combinations.

TABLE 2. Analysis of variance^z of percent leaf area covered by lesions of *Mycosphaerella graminicola* for pathogen populations isolated from two winter wheat cultivars in the field and tested on the same wheat cultivars in the greenhouse

Source	df	Mean square	P > F
Trial	1	8.47	0.0001
Population	1	1.10	0.0096
Cultivar	1	0.77	0.0274
Trial × population	1	0.11	0.3836
Trial × cultivar	1	0.07	0.4979
Population × cultivar	1	1.48	0.0034
Trial × population × cultivar	1	0.02	0.7427
Error	22	0.14	

^z Analysis conducted after applying natural logarithm transformation.

it is significant that relative susceptibilities of the four cultivars that we measured in the greenhouse were the same as those we had observed consistently in the field. For example, Septoria blotch severity at grain-filling averaged over three seasons in Corvallis, Oregon, was 53, 39, 10, and 1% for 'Malcolm', 'Stephens', 'Madsen', and 'Gene', respectively (21). Further, Septoria tritici blotch epidemics on winter wheat in western Oregon are active from December through June. Thus, seedling data and adult plant data are equally relevant to field epidemics.

Highly significant pathogen population, cultivar, and population × cultivar interaction in both experiments of this study indicated that the *M. graminicola*/wheat pathosystem varied for both vertical and horizontal resistance and for both virulence and aggressiveness (sensu Vanderplank [33]). Most studies of this type have tested individual isolates of a pathogen on different cultivars. However, several other studies have also utilized bulk populations of pathogens (2,19,34) or insects (11) to test for host adaptation. We chose to test bulk populations collected from different cultivars, because, given finite resources, a larger number of isolates can be tested as bulk populations than as single isolates. Further, inoculation with multiple isolates is more similar to the natural life cycle of *M. graminicola* in the field. Populations of only five isolates per cultivar were used in experiment 2, primarily to meet the objectives of an allied study not reported here. Clearly, five isolates is fewer than optimum to adequately sample a population. Nevertheless, results from experiment 2 were very similar to those of experiment 1, in which 20 isolates per cultivar were sampled. Thus, our results seem robust despite the limited sampling utilized in experiment 2.

It is of particular interest that *M. graminicola* populations isolated from the susceptible cultivars Malcolm and Stephens were more aggressive as compared with the populations obtained from the resistant cultivar Gene and the moderately resistant cultivar Madsen. Similar results have been noted for *M. graminicola* isolates and wheat cultivars collected from different regions of the United States (1), and for isolates of *Xanthomonas oryzae* collected from and tested on both a susceptible and a moderately resistant rice cultivar in the Philippines (H. U. Ahmed, M. R. Finckh, R. F. Alfonso, and C. C. Mundt; unpublished data). Knott and Mundt (16) found different levels of aggressiveness of wheat leaf rust (*Puccinia recondita*) isolated from different wheat cultivars, but there was not a clear relationship between susceptibility of a source cultivar and aggressiveness of its corresponding pathogen population in that study.

Increased selection for aggressiveness on susceptible cultivars as compared with less susceptible ones can be explained by differences in variances of aggressiveness when the pathogen is on different host genotypes. There is a greater potential range in disease severity on a susceptible cultivar as compared with moderately resistant or resistant ones. As variances are commonly observed to be positively correlated with their corresponding means, and gain due to selection is directly proportional to genetic variance, one might expect greater gains in cultivar selection for aggressiveness on susceptible cultivars than on resistant or moderately resistant ones. In support of this hypothesis, Falconer (10) postulated that the positive correlation between mean and variance may explain why there is often a greater rate of gain due to selection for quantitative traits in the upward than in the downward direction in reciprocal selection experiments. Analysis of individual isolates of experiment 1 confirmed a larger variance among individual isolates of *M. graminicola* when tested on susceptible wheat cultivars than when tested on a resistant or moderately resistant cultivar (M. E. Hoffer and C. C. Mundt, unpublished data).

It is also possible that shorter pathogen generation times allowed for more generations of selection on susceptible than on moderately resistant or resistant cultivars. However, recent analyses of greenhouse data (C. C. Mundt, unpublished data) sug-

gested little or no variation for the latent period of *M. graminicola* on different wheat cultivars grown in Oregon. Similarly, Shaw (30) found only slight differences in latent period among 14 cultivars when using controlled inoculations of plants in an outdoor environment.

The presence of pathogen × host interaction indicates the potential for cultivar-specific adaptation (18), though this is certainly not a necessary result of the presence of such an interaction. However, the design of our study allowed us to test whether pathogen adaptation had actually occurred in the field, since we evaluated all combinations of pathogen population and cultivar in a factorial set of treatments.

The presence of a significant main effect of pathogen population in this study made the analysis of cultivar-specific selection of *M. graminicola* complex. For example, in experiment 1, *M. graminicola* populations obtained from the susceptible cultivars Malcolm and Stephens had high levels of aggressiveness, and, thus, produced more disease on the cultivar Madsen than the spore population secured from 'Madsen' itself. Calculation of expected values, under the assumption of no population × cultivar interaction, made such comparisons clearer (Tables 3 and 4). The highly significant homologous versus heterologous linear contrasts and the fact that observed disease severity was, without exception, numerically larger than expected disease severity for homologous combinations indicated clear cultivar-specific selection in this study. Similarly, Ahmed et al. (1) found that *M. graminicola* isolates from California were more virulent on California cultivars than on Oregon cultivars, while Oregon isolates were more virulent on Oregon than on California cultivars. Eyal et al. (9) reported that *M. graminicola* isolates obtained from tetraploid wheats were more virulent to tetraploid wheats than hexaploid wheats.

Detecting statistically significant host influences on quantitative variation of pathogens can be difficult, especially when such differences are small. In experiment 1, in which four replications per treatment were used, only 2 of the 16 pathogen population × cultivar combinations deviated significantly from expected values, based on *t* tests. In experiment 2, in which use of eight replications per treatment increased statistical power, half of the pathogen population × cultivar combinations deviated significantly from expected values. For both experiments, the more powerful approach of using a preplanned, linear contrast to compare all homologous versus all heterologous combinations provided very high levels of statistical significance (Tables 1 and 2). Thus, statistical power should be a prime consideration when designing studies to detect quantitative variation of plant pathogens.

Many studies have addressed the question of whether selection for increased virulence of a pathogen occurs during cycles of asexual reproduction on the same host (2,3,5,7,12-15,18,22-24), but results have varied considerably. Expression of pathogen adaptation to a particular host depends, in part, on the starting genetic diversity in the pathogen population (12). As in other parts of the world (28,31), field observations in western Oregon implicated ascospores as the source of initial inoculum for Septoria tritici blotch epidemics (C. C. Mundt, unpublished data). Further, RFLP analyses indicate patterns of genetic diversity in western Oregon populations of *M. graminicola* that could occur only through sexual recombination (4,6), and the number of initial infections resulting from fall ascospore showers was very large (on the order of hundreds per square meter; C. C. Mundt, unpublished data). Analysis of infections on trap plants have shown that ascospore showers usually peak in late fall/early winter in both the United Kingdom (31) and western Oregon (M. E. Hoffer and S. M. Coakley, unpublished data). Subsequent progression of disease is highly correlated with rain events favorable to splash dispersal of pycnidiospores (32; M. E. Hoffer and S. M. Coakley, unpublished data), and such rain events occur throughout the

winter and spring in western Oregon. In addition, *M. graminicola* strains of known RFLP pattern inoculated once in December 1994 (to competitively exclude peak ascospore infections) dominated experimental plots when sampled in June 1995 in western Oregon (C. C. Mundt and B. A. McDonald, unpublished data). Splash dispersal of conidia after the initial ascospore infections also greatly restricts asexual dispersal of *M. graminicola* (4,20) and the potential for migration of pathogen genotypes among different cultivars. Thus, we studied a very large and diverse pathogen population that had significant opportunity to be selected for increased pathogenicity in the field before being collected and tested in the greenhouse.

In experiment 1, heterologous population × cultivar combinations showed disease levels both higher and lower than the calculated, expected values. Though lack of sufficient statistical power

TABLE 3. Area under disease progress curve (percent days) caused by populations of *Mycosphaerella graminicola* collected from four winter wheat cultivars in the field and tested on the same wheat cultivars in the greenhouse

Tester cultivar	Pathogen population derived from				Mean ^y
	'Gene'	'Madsen'	'Malcolm'	'Stephens'	
Gene	2.6	2.7	1.3	2.7	2.3 d
	<u>2.0^w</u>	<u>1.5</u>	<u>3.0</u>	<u>2.7</u>	
Madsen	55.9	43.5	86.8	52.5**	59.7 c
	<u>52.7</u>	<u>39.5</u>	<u>77.3</u>	<u>69.2</u>	
Malcolm	100.5	75.6	149.2	123.0	112.1 a
	<u>99.0</u>	<u>74.2</u>	<u>145.2</u>	<u>130.0</u>	
Stephens	79.0	56.5	111.5	134.0*	95.3 b
	<u>84.2</u>	<u>63.1</u>	<u>123.4</u>	<u>110.4</u>	
Mean ^y	59.5B	44.6C	87.2A	78.1A	

Mean of homologous^y combinations = 82.3

Mean of heterologous^z combinations = 62.3

^y Means followed by the same letter are not significantly different at *P* = 0.05 according to Fisher's protected least significant difference.

^w Underlined figures are expected values under the assumption of no pathogen population × cultivar interaction, and were calculated as follows: expected disease = (pathogen population mean over all cultivars × cultivar mean over all pathogen populations)/grand mean.

^x An asterisk indicates that the observed value is significantly different from its expected value at *P* = 0.05, based on a *t* test.

^y "Homologous" indicates disease reaction of *M. graminicola* populations on cultivars from which the isolates were originally obtained in the field.

^z "Heterologous" indicates disease reactions of *M. graminicola* populations on the cultivars from which the isolates were not obtained in the field.

TABLE 4. Percent leaf area covered by lesions of *Mycosphaerella graminicola* for pathogen populations collected from two winter wheat cultivars in the field and tested on the same wheat cultivars in the greenhouse

Tester cultivar	Population derived from		Mean ^y
	'Madsen'	'Stephens'	
Madsen	12.6	12.4**	12.5 b
	<u>9.4^x</u>	<u>15.6</u>	
Stephens	11.4*	27.4	19.4 a
	<u>14.6</u>	<u>24.2</u>	
Mean ^y	12.0A	19.9B	

Mean of homologous^y combinations = 20.0

Mean of heterologous^z combinations = 11.9

^y Means followed by the same letter are not significantly different at *P* = 0.05 according to Fisher's protected least significant difference.

^w An asterisk indicates that the observed value is significantly different from its expected value at *P* = 0.05, based on a *t* test.

^x Underlined figures are expected values under the assumption of no pathogen population × cultivar interaction, and were calculated as follows: expected disease = (pathogen population mean over all cultivars × cultivar mean over all pathogen populations)/grand mean.

^y "Homologous" indicates disease reaction of *M. graminicola* populations on cultivars from which the isolates were originally obtained in the field.

^z "Heterologous" indicates disease reactions of *M. graminicola* populations on the cultivars from which the isolates were not obtained in the field.

prevents definitive conclusions, this result suggests that there may have been pathogen × host interactions caused by mechanisms other than cultivar-specific selection. In fact, unexplained pathogen × cultivar interactions have been reported previously in the *M. graminicola*/wheat system. Ahmed et al. (1) found that California and Texas isolates of *M. graminicola* caused very low levels of disease on Oregon cultivars with the exception of the cultivar Madsen, on which California and Texas isolates sometimes caused more disease than Oregon isolates.

Our data indicated simultaneous selection for both increased aggressiveness and increased virulence in *M. graminicola*. Simultaneous selection for both virulence and aggressiveness was detected by Kolmer and Leonard (17) for *Cochliobolus heterostrophus* on maize (*Zea mays*). They used the sexual state of *Cochliobolus heterostrophus* to conduct recurrent selection for increased lesion size on a single maize genotype over three generations. This selection resulted in increased aggressiveness of the pathogen on all maize cultivars tested, as well as increased virulence for the cultivar on which the selection for increased lesion size selection was carried out.

As our study indicated adaptation of *M. graminicola* to its cultivars of origin, the long-term use of a resistant cultivar might be endangered. Thus, gene rotation, cultivar mixtures, and other resistance deployment strategies might be useful to sustain the stability of resistance to *Septoria tritici* blotch. The simultaneous influence of the host on both virulence and aggressiveness of *M. graminicola* may, however, make predictions of the effectiveness of different deployment strategies very complex for this host/pathogen system.

LITERATURE CITED

- Ahmed, H. U., Mundt, C. C., and Coakley, S. M. 1995. Host-pathogen relationship of geographically diverse isolates of *Septoria tritici* and wheat cultivars. *Plant Pathol.* 44:838-847.
- Alexander, H. M., Groth, J. V., and Roelfs, A. P. 1985. Virulence changes in *Uromyces appendiculatus* after five asexual generations on a partially resistant cultivar of *Phaseolus vulgaris*. *Phytopathology* 75:449-453.
- Ao, H. C., and Griffiths, E. 1976. Change in virulence of *Septoria nodorum* and *Septoria tritici* after passage through alternative hosts. *Trans. Br. Mycol. Soc.* 66:219-224.
- Boeger, J. M., Chen, R. S., and McDonald, B. A. 1993. Gene flow between geographic populations of *Mycosphaerella graminicola* (anamorph *Septoria tritici*) detected with restriction fragment length polymorphism markers. *Phytopathology* 83:1148-1154.
- Caten, C. E. 1974. Intra-racial variation in *Phytophthora infestans* and adaptation to field resistance for potato blight. *Ann. Appl. Biol.* 77:259-270.
- Chen, R.-S., Boeger, J. M., and McDonald, B. A. 1994. Genetic stability in a population of a plant pathogenic fungus over time. *Mol. Ecol.* 3:535-546.
- Clifford, B. C., and Clothier, R. B. 1974. Physiologic specialization of *Puccinia hordei* on barley hosts with nonhypersensitive resistance. *Trans. Br. Mycol. Soc.* 63:421-430.
- Eyal, Z., Amiri, Z., and Wahl, I. 1973. Physiologic specialization of *Septoria tritici*. *Phytopathology* 63:1087-1091.
- Eyal, Z., Scharen, A. L., Huffman, M. D., and Prescott, J. M. 1985. Global insights into virulence frequencies of *Mycosphaerella graminicola*. *Phytopathology* 75:1456-1462.
- Falconer, D. S. 1960. Introduction To Quantitative Genetics. Ronald Press, New York.
- Gould, F. 1979. Rapid host evolution in a population of the phytophagous mite *Tetranychus urticae*. *Evolution* 33:791-802.
- James, R. V., and Fry, W. E. 1983. Potential for *Phytophthora infestans* populations to adapt to potato cultivars with rate-reducing resistance. *Phytopathology* 73:984-988.
- Jefferey, S. I. B., Jinks, J. L., and Grindle, M. 1962. Intraracial variation in *Phytophthora infestans* and field resistance to potato blight. *Genetica* 32:323-338.
- Jinks, J. L., and Grindle, M. 1963. Changes induced by training in *Phytophthora infestans*. *Heredity* 8:245-264.
- King, J. E., Cook, R. J., and Melville, S. C. 1983. A review of *Septoria* diseases of wheat and barley. *Ann. Appl. Biol.* 103:345-373.
- Knott, E. A., and Mundt, C. C. 1991. Latent period and infection efficiency of *Puccinia recondita* f. sp. *tritici* populations isolated from different wheat cultivars. *Phytopathology* 81:435-439.
- Kolmer, J. A., and Leonard, K. J. 1986. Genetic selection and adaptation of *Cochliobolus heterostrophus* to corn hosts with partial resistance. *Phytopathology* 76:774-777.
- Latin, R. X., MacKenzie, D. R., and Cole, H., Jr. 1981. The influence of host and pathogen genotypes on the apparent infection rates of potato late blight epidemics. *Phytopathology* 71:82-85.
- Leonard, K. J. 1969. Selection in heterogeneous populations of *Puccinia graminis* f. sp. *avenae*. *Phytopathology* 59:1851-1857.
- McDonald, B. A., and Martinez, J. P. 1990. DNA restriction fragment length polymorphisms among *Mycosphaerella graminicola* (anamorph *Septoria tritici*) isolates collected from a single wheat field. *Phytopathology* 80:1368-1373.
- Mundt, C. C., Brophy, L. S., and Schmitt, M. E. 1995. Choosing crop cultivars and mixtures under high versus low disease pressure: A case study with wheat. *Crop Prot.* 14:509-515.
- Newton, A. C. 1989. Genetic adaptation of *Erysiphe graminis* f. sp. *hordei* to barley with partial resistance. *J. Phytopathol.* 126:133-148.
- Osbourn, A. E., Caten, C. E., and Scott, P. R. 1987. Adaptation of *Septoria nodorum* to wheat or barley on detached leaves. *Plant Pathol.* 36:565-576.
- Osbourn, A. E., Scott, R., and Caten, C. E. 1986. The effect of host passaging on the adaptation of *Septoria nodorum* to wheat or barley. *Plant Pathol.* 35:135-145.
- Parlevliet, J. E. 1977. Evidence of differential interaction in the polygenic *Hordeum vulgare*-*Puccinia hordei* relation during epidemic development. *Phytopathology* 67:776-778.
- Saadaoui, E. M. 1987. Physiologic specialization of *Septoria tritici* in Morocco. *Plant Dis.* 71:153-155.
- SAS Institute. 1988. SAS User's Guide: Statistics. Release 6.03 ed. SAS Institute, Inc., Cary, NC.
- Schuh, W. 1990. Influence of tillage systems on disease intensity and spatial pattern of *Septoria* leaf blotch. *Phytopathology* 80:1337-1340.
- Shaner, G., and Finney, R. E. 1977. The effect of nitrogen fertilization on the expression of slow-mildewing resistance in Knox wheat. *Phytopathology* 67:1051-1056.
- Shaw, M. W. 1990. Effects of temperature, leaf wetness and cultivar on the latent period of *Mycosphaerella graminicola* on winter wheat. *Plant Pathol.* 39:255-268.
- Shaw, M. W., and Royle, D. J. 1989. Airborne inoculum as a major source of *Septoria tritici* (*Mycosphaerella graminicola*) infections in winter wheat crops in the UK. *Plant Pathol.* 38:35-43.
- Shaw, M. W., and Royle, D. J. 1993. Factors determining the severity of epidemics of *Mycosphaerella graminicola* (*Septoria tritici*) on winter wheat in the UK. *Plant Pathol.* 42:882-899.
- Vanderplank, J. E. 1968. Disease Resistance in Plants. Academic Press, Inc., New York.
- Wolfe, M. S., and Knott, D. R. 1982. Populations of plant pathogens: Some constraints on analysis of variation in pathogenicity. *Plant Pathol.* 31:79-90.