Evaluation of Resistance to *Septoria nodorum* in Wheat

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ABSTRACT

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A technique for screening wheat germ plasm for resistance to *Septoria nodorum* was evaluated. Disease was evaluated in inoculated seedlings in a growth chamber and in mature plants in a 3-yr field study. The growth chamber technique was adequate for detecting differences in varietal reactions to glume blotch because determination of susceptibility at the seedling stage was highly correlated with ratings of adult plants in the field ($R^2 = 0.64$, $P < 0.01$). A rank correlation of 0.86 was observed between the two tests. Spreading infested straw in the field was an effective inoculation method, particularly in the spring.

Differences in susceptibility of wheat cultivars to *Septoria nodorum* (Berk.) Berk., perfect stage *Leptosphaeria nodorum* Müller, have been reported in many cases (2,5,8,14,15,17), and cultivars reported as resistant in one part of the world may appear susceptible in another. Factors that may account for such inconsistencies include variability in the fungus, influence of environmental conditions (1,2,18), plant stage at the time of inoculation (3,4,10,11,15), differences in inoculation technique, and measurements of resistance that range from severity of symptoms to estimates of yield loss (2,3,5,6,8,12,15,19,20).

The major objective of this study was to evaluate differential cultivar susceptibility at the seedling stage and to compare these results with observations on mature plants under field conditions. We also investigated the use of infested straw as an inoculation method. Straw remaining near the soil surface after harvest harbors pycnidia capable of producing viable spores for as long as 2 yr (9,16,22).

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MATERIALS AND METHODS

Screening. We modified Eyal and Scharen's technique (7) for screening seedlings for resistance to glume blotch as follows: For every treatment, 12–15 seeds per cultivar were sown into 10-cm plastic pots containing artificial growing medium (Terra Lite Metro Mix, W. R. Grace Co., Cambridge, MA 02140). After emergence, seedlings were thinned to 10 and inoculated at the third leaf stage or stage 1 of the Feeke's scale (13). Four pots were inoculated simultaneously on a phonograph turntable (as described by Eyal and Scharen [7]) using a No. 15 DeVilbiss atomizer attached to a pressure pump at 1.05 kg. m^{-2}. After inoculation, plants were covered with clear polyethylene bags for 72 hr to provide a water-saturated atmosphere conducive to infection.

Visual readings were made when lesions became distinct—6–10 days after inoculation, depending on disease development. Host susceptibility was expressed as percent leaf area covered by lesions with percent values transformed using an arc-sin transformation.

All isolates used were collected in North Carolina and 10 were selected at random to avoid cultivar/isolate interactions. Equal amounts of each isolate were composited, and this mixture was used in all growth chamber and field tests. Fungal isolates were grown on potato-dextrose agar (PDA) in 9-cm petri dishes for 7 days at 20 C under constant fluorescent light. Pycnidiospores were collected by flooding plates with 2–3 ml sterile tap water and scraping the agar surface with a rubber policeman. The resulting suspension was filtered through two layers of cheesecloth and adjusted to a concentration of 1.0×10^6 spores per milliliter using a hemacytometer. Volume was standardized to 10 ml per four pots (40 seedlings).

The three parts of the investigation were two growth chamber experiments and a 3-yr field study.

The first growth chamber experiment used a randomized complete block design

to evaluate the susceptibility of four wheat cultivars and to determine whether cultivar ranking was consistent in a series of seven tests with from six to 16 replications. The cultivars were Blueboy (CI 14031), Anderson (CI 12536), Coker 68-15 (CI 15291), and Hadden (CI 13488).

Treatments consisted of simultaneously inoculating a set of four cultivars with a spore suspension made from a mixture of isolates. Appropriate controls were included in each set.

The second growth chamber experiment used a randomized complete block design with four replications. The 20 winter and spring wheat lines evaluated were: winter—Hadden, Redhart (CI 8898), Red Chief (CI 12109), Anderson, Arthur 71 (CI 15282), Oasis (CI 15929), Bulgaria 88 (PI 94407), Chalk, Coker 68-15, Blueboy II, and Blueboy; spring—Fortuna (CI 13596), Newana (CI 17430), Lew (CI 17429), Ciguena, Ariana "S," Klein Toledo, Md × Mc-Exch, IAS 55, and Pel 13737-62. These lines were selected from the USDA Uniform Septoria Nursery. Four pots selected at random were simultaneously inoculated as above.

In both experiments plants were grown, as previously described, with constant day/night temperatures of 23/18 C and 10 hr of fluorescent and incandescent lights (395 $\mu E \cdot m^{-2} \cdot sec^{-1}$). Disease was assessed on the second leaf, with percent infection being the mean of 10 observations.

For the field study, the same 20 lines as in the second growth chamber experiment were evaluated at the Central Crops Research Station at Clayton, NC, to compare field versus growth chamber results. Entries were planted in mid-October in 2.4-m rows spaced 30 cm apart. Fertilization was applied before planting at 600 kg/ha of 12-24-24 and a 225 kg/ha (33% N) topdressing was applied in March. The field entries were not replicated, but they were evaluated for 3 yr.

Field inoculations were made with a backpack sprayer using a spore suspension prepared by grinding the entire contents of culture dishes (fungus plus agar) in a Waring blender and filtering the slurry through several layers of cheesecloth. Concentration and volume were not quantified. Humidity was provided by an overhead sprinkle irrigation system turned on intermittently through the day and delivering approximately 25 mm of water per week. Inoculation was initiated at the boot stage or stage 10 of the Feeke's scale (13) and repeated two to four times during the season until levels of disease were considered adequate.

Disease assessments were based on percent glume area covered by lesions (row average) measured at the soft dough stage or growth stage 11.2 of the Feeke's scale (13). Results were subjected to analysis of variance and regression analysis; pertinent correlation coefficients were calculated. Rank correlations were based on Spearman's (21) rank-order correlation coefficient.

Wheat debris as inoculum. Wheat straw from infected plots was collected after harvest and applied to the subsequent crop at various times in order to evaluate the role of debris on disease development.

The experiment was conducted for 2 yr at the Central Crops Research Station at Clayton, NC (1977-1978 and 1978-1979) and consisted of planting a wheat field and dividing it into sections measuring approximately 100 m^2. Only the center 4 m^2 of each section received treatments in order to have a sufficient barrier between plots. The following treatments were arranged in a completely randomized design and replicated four times: straw applied in November, March, and April of each year and control plots that received no treatment.

Arthur 71 was the host cultivar for the first year and Coker 747 for the second. They were planted about October 20 each year at 180 kg/ha. Fertilizer applications consisted of 600 kg of 12-24-24 per hectare before planting and a topdressing of 100 kg/ha of 33.5% N applied in March each year.

Infested straw from previously inoculated plots was saved after threshing and stored dry in burlap bags until needed for inoculation. About 0.085 m^3 of straw per plot was loosely scattered over plants.

RESULTS AND DISCUSSION

Screening. Differences among cultivars were highly significant in every test of the first growth chamber experiment (Table 1). Blueboy was the most susceptible cultivar in all seven tests, averaging 32.7% infection. In all but one test the greater susceptibility of Blueboy was statistically significant. Coker 68-15 was the most resistant in six of seven tests with an average infection of 17.4%. In the seventh test Coker 68-15 ranked second to Anderson but the difference was not statistically significant. Anderson and Hadden were intermediate in susceptibility with average infection levels of 22.9% and 25.7%, respectively. Differences in the level of infection between these two cultivars were usually not significant.

A pooled analysis of variance over all tests showed the test × cultivar interaction to be nonsignificant. This indicates that cultivars are consistent from test to test in their relationship to one another.

After the reliability of the technique was established, 20 winter and spring wheat lines were evaluated in the second growth chamber experiment (Table 2), and the results were compared with the average performance of these lines in the field. Differences among lines were highly significant. In general, winter wheats were more resistant than spring wheats. Redhart was the most resistant, with an average of 2% infection. Newana, Ciguena, Pel 13737-62, and Fortuna were the most susceptible, with an average infection of 90%.

In the field study, the same 20 lines used in the second growth chamber experiment were evaluated at maturity for three consecutive years and their average reactions are also shown in Table 2. Infection was very slight in 1977, extremely heavy in 1978, and moderate in 1979. Mean differences among the lines were significant at the 1% level. Again, winter wheats were more resistant than spring wheats. Overall, Chalk was the most resistant with an average of 15% infection, and Ciguena and Fortuna were the most susceptible with an average of 80%.

A regression analysis was conducted using the average degree of infection in the growth chamber test as the independent variable X and the average degree of infection in the field test as the dependent variable Y. A linear model was highly significant and gave the following regression equation: $Y = 35.65 + 0.41X$. The R^2 was 0.64 ($P < 0.01$) with a correlation (r) of 0.80. Correlations between individual years were: 1977 and 1978 = 0.63, 1978 and 1979 = 0.70, and 1977 and 1979 = 0.61.

The wheat lines were ranked in order of resistance in both the growth chamber and field tests. Field ranks were again averaged over 3 yr. Ties in ranking were corrected as defined by Spearman's rank-correlation coefficient (21) and a

Table 1. Resistance of four wheat cultivars to *Septoria nodorum* in seven growth chamber tests

Test	No. of replications	LSD 0.05	Percent leaf area diseased			
			Blueboy	Anderson	Hadden	Coker 68-15
1	16	2.4	24.0	11.0	14.3	7.1
2	16	4.6	46.8	35.0	33.9	22.1
3	7	2.8	23.3	12.5	18.5	10.8
4	6	3.8	19.6	16.0	18.1	12.1
5	16	4.4	26.2	21.3	18.8	11.3
6	16	4.6	47.9	37.0	42.0	27.6
7	9	3.2	40.6	27.7	33.9	30.9
Mean			32.7	22.9	25.7	17.4
Overall LSD	0.05	= 3.6				
	0.01	= 4.9				

Table 2. Resistance to *Septoria nodorum* of winter and spring wheat seedlings in growth chambers and mature plants in the field

Cultivar	Mean percent diseased tissue		Rank	
	Growth chamber[a]	Field[b]	Growth chamber	Field
Redhart	2.0	38.3	1	3
Coker 68-15	5.0	45.0	2	5
Chalk	10.0	15.0	3	1
Bulgaria 88	16.3	20.0	4	2
Red Chief	17.5	40.0	5.5	4
Hadden	17.5	46.7	5.5	6
Anderson	20.0	50.0	7	7
Oasis	27.5	63.3	8	10.5
Klein Toledo	37.5	63.3	9	10.5
Arthur 71	38.8	65.0	10	13
IAS 55	78.8	63.3	11.5	10.5
Lew	78.8	70.0	11.5	16.5
Md × Mc-Exch	86.3	63.3	14	10.5
Blueboy II	86.3	68.3	14	14.5
Blueboy	86.3	68.3	14	14.5
Ariana S	87.5	70.0	16	16.5
Pel 13737-62	90.0	58.5	18.5	8
Newana	90.0	73.3	18.5	18
Ciguena	90.0	80.0	18.5	19.5
Fortuna	90.0	80.0	18.5	19.5
LSD 0.05	9.2	11.3	Spearman's correlation	
0.01	12.6	15.5	(rho) = .86	

[a] Average of 40 seedlings.
[b] Average of 3 yr.

correlation of 0.86 ($P < 0.01$) was obtained for the rank order of the varieties between the two tests (Table 2). Ranking correlations between any two years were calculated as follows: 1977 and 1978 = 0.78, 1978 and 1979 = 0.77, and 1977 and 1979 = 0.70. Rankings were fairly consistent from year to year with most lines varying from one to five ranking points.

Highly significant correlations in degree of infection and in ranking among cultivars under the two sets of conditions demonstrates the efficacy of the method in detecting differences in varietal reactions. Because agreement was good between seedling reaction to artificial inoculation and field rating of adult plants, the technique would permit the screening of many lines in a short period with minimum space requirements. Nevertheless, differences among varieties based on numbers of lesions are not necessarily correlated with field resistance or tolerance. As Scharen (19) indicated, reduction in yield is not always correlated with symptoms on which disease is assessed. Tolerance, he reported, "is subtle and cannot be readily identified on the basis of symptomatology." Thus, the technique cannot unequivocally establish resistance, but it would be satisfactory for identifying highly susceptible material.

Lines such as Chalk and Bulgaria 88, which were resistant under natural and artificial conditions, are late in maturing. Because resistance is sometimes associated with undesirable agronomic traits, lines that are selected under artificial conditions must still be evaluated in the field, not only for symptoms but also for yield reduction and agronomic quality.

Wheat debris as inoculum. Addition of infested straw at different times of the year had no effect on disease development when glume blotch reached epidemic proportions in the spring of 1978. All plots showed 90% infection at maturity. Lesions caused by *S. nodorum* were widespread as early as January, even among plots not yet treated. Thus, before any artificial inoculation, inoculum levels were already sufficiently high to initiate infection, and the addition of straw was insignificant in terms of its contribution to disease development.

Secondary infection from the fall application of straw in the field would have been rare because of the cold temperatures in North Carolina in the winter of 1977-1978. The field had not been planted to wheat the year before, so previous residue was also an unlikely source of inoculum. Seedborne infection could have accounted for the widespread distribution of glume blotch so early in the season. This aspect of the disease should be investigated further.

Disease levels were much lower the following year, and straw applications at different times significantly affected the levels of disease at maturity. The November application of straw gave no more infection than the check, so the observed 10% infection at the end of the season was probably due to secondary infection from adjacent plots. Application of infested straw late in the season resulted in the highest levels of disease, as plants increase in susceptibility as they mature. When straw was applied in March, plants were in the boot stage and infection reached 13.8% at the end of the season. In April, plants were at the heading stage and infection levels reached 27.5%. These findings support those of Bronnimann (3), Holmes and Colhoun (10), and Jones and Rowling (11) who found that fall infection resulted in weak manifestation of the disease in the spring but that inoculation at ear emergence resulted in the most severe disease development. Sanitation and crop rotation are advisable to help reduce inoculum potential.

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