Resistance

Rate-Limiting Resistance to Pyrenophora Leaf Blotch in Spring Oats

J. A. Frank and B. J. Christ

Adjunct associate professor and assistant professor, Department of Plant Pathology, The Pennsylvania State University, University Park 16802.

Contribution 1638, Department of Plant Pathology, the Pennsylvania State Agricultural Experiment Station. Authorized for publication 6 November 1984, as Journal Series Paper 7659.

Accepted for publication 12 February 1988 (submitted for electronic processing).

ABSTRACT

Frank, J. A., and Christ, B. J. 1988. Rate-limiting resistance to Pyrenophora leaf blotch in spring oats. Phytopathology 78:957-960.

Several breeding lines of spring oats from the U.S. Department of Agriculture breeding program in Pennsylvania were identified as having a degree of resistance to Pyrenophora leaf blotch in 1979 and 1980 field trials. These lines were evaluated for components of rate-limiting resistance in greenhouse and field tests from 1981 to 1984. In comparison to susceptible cultivars, the relative infection efficiencies were similar. Resistant lines had smaller lesions, longer latent periods, and lower sporulation capacities. Rankings of resistance exhibited in the greenhouse tests were comparable to the disease severity rankings obtained in field trials. The field trials appeared to provide more precision in separating degrees of resistance among breeding lines.

Pyrenophora leaf blotch caused by *Pyrenophora avenae* Ito & Kurib. (anamorph: *Drechslera avenaceae* (Curt. ex Cke.) Shoemaker *Helminthosporium avenae* Eid.) is a major disease on oats in Pennsylvania (Frank, *unpublished*). Although losses attributed to this disease are generally minimal, significant losses have been reported (3,5,6,9,10). Some degree of resistance to this pathogen has been identified (4,5). In 1979, several spring oat breeding lines from the U.S. Department of Agriculture-Agricultural Research Service breeding program of H. G. Marshall were identified as resistant to Pyrenophora leaf blotch in field trials in Virginia (Frank, *unpublished*). Based on field observations, this resistance appeared to be rate limiting or horizontal in nature as described by Vanderplank (11). The purpose of this study was to compare several of these breeding lines with three oat cultivars in terms of the pathogen's infection efficiency, lesion size, latent period, and sporulation capacity on

them in the greenhouse and to evaluate these lines for disease reactions in field trials.

MATERIALS AND METHODS

Greenhouse studies. Tests were initiated in January 1981 and repeated in 1982 and 1983. Plants to be evaluated for rate-limiting resistance were grown in 10-cm-diameter plastic pots containing a mixture of soil-peat moss-perlite (1-1-1 by volume) in a greenhouse at approximately 23 C. Five seeds of each *Avena sativa* L. genotype were planted per pot with 10 single pot replications per trial. Six breeding lines (7930-6, 8172-2, 8174-20, 8184-5, 8184-14, 8184-18) and three commonly grown cultivars (Mariner, Noble, and Otee) were evaluated in a randomized complete block design. When the seedlings were in the three-leaf stage, they were fertilized with a solution containing 3 g of 20-20-20 (N-P-K) per liter at a rate of 40 ml/pot. Plants were inoculated with the pathogen when they were in the four-leaf stage. All plants were grown under 14 hr of supplemental cool-white fluorescent lamps.

The isolate used in the greenhouse studies was recovered from oat leaves collected in a field trial in 1980. This same isolate was used in all 3 yr of the greenhouse studies. The organism was maintained between testing periods on oat leaves stored at 4 C between paper towels. The isolate was inoculated onto oat plants and then recovered before each greenhouse experiment to assure that virulence was not lost through serial subculturing. A suspension of approximately 50,000 spores/ml was sprayed on susceptible oat plants (cv. Lang) in the six-leaf stage until runoff. These plants were placed in a mist chamber (approximately 90% RH) in the greenhouse for 24 hr after inoculation. They were then grown in the greenhouse at 23 C and misted until runoff twice a day (7:00 a.m. and p.m.) with a fine mist nozzle until lesions were detected on the leaves. These leaves were detached and placed in moist dishes until conidia were produced. These conidia were then transferred to low-sugar potato-dextrose agar (5 g/L of dextrose) and grown under continuous cool-white fluorescent light at 21 C to serve as the inoculum. When conidia were produced, an aqueous solution containing 5 drops of Tween-20/100 ml was added to the petri dish, and the cultures were scraped to release the conidia. Conidial suspensions were filtered through double layers of cheesecloth, and the spore concentrations were determined with a hemacytometer.

To estimate relative infection efficiency, a series of test plants were inoculated with a quantitative inoculator (7) that sprayed a 1-cm-diameter area in the center of the third leaf of each plant for 2 sec. After every pot of five plants was inoculated, a glass microscope slide coated with water agar was sprayed with the inoculum suspension. The average number of conidia per leaf inoculation site was estimated by counting the conidia on the same-sized area of the slide. The overall mean was nine conidia per inoculation area. Plants were grown in a plastic mist chamber (80% RH) and misted twice a day as described previously. Lesions that developed on the inoculated leaves were counted 14 days after inoculation, and the relative percentage infection efficiency was calculated as 100 times the number of lesions per estimated conidia per inoculation site.

Lesion length was measured on each of the five plants per pot at this same time as an estimate of lesion size (area). The mean lesion length for the five plants in each pot was used for statistical analysis.

Latent period was estimated by evaluating lesions daily, starting 10 days after inoculation, for conidial formation. Plants were grown in mist chambers as described above. The date of first appearance of mature lesions on each leaf was recorded, and the number of days from inoculation until sporulation was averaged for the five plants per pot.

Sporulation capacity was calculated as the number of conidia per leaf. When mature conidia were detected on a specific leaf, the leaf was detached and the areas with visible lesions were excised and placed into test tubes with 3 ml of water per tube. The tubes were agitated with a Vortex mixer, and two samples from each tube were examined in a hemacytometer to determine the conidial concentration. The mean from the two samples was determined.

All data were subjected to an analysis of variance, and means were separated with a Waller-Duncan Baysian least significant difference (LSD) test.

Field studies. The same nine lines and cultivars evaluated in greenhouse studies were planted in field plots in Rock Springs, PA, at The Pennsylvania State University Research Farm. The planting dates were 15, 28, and 24 April in 1982, 1983, and 1984, respectively. Seed was planted at a rate of 98 kg/ha in 5 rows spaced 18 cm with a 5-row cone-type planter. Every other drill strip between plots was planted with spring barley to serve as a buffer. Each plot was separated within the row by a 0.9-m alley. Fertilizer (NH_4NO_3, 67 kg/ha) was incorporated into the seedbed before planting. The test design was a randomized complete block with four replications.

When plants reached growth stage 5 (GS-5, Feekes scale) (2), plots were trimmed to a final size of 0.9 × 3.0 m. At GS-6, all plants in one of the outer rows of each plot were sprayed with a 50,000 conidia/ml suspension of the *P. avenae* isolate used in the greenhouse studies to help ensure a successful epidemic. The inoculum was prepared as previously described and applied with a hand-held pump sprayer. Twenty-five tillers from the center row of each plot were evaluated for disease severity (percent leaf area with lesions) at GS-10.54. The severities on the upper four leaves were averaged for each tiller, and the mean plot severity was calculated for the 25 tillers. All data were subjected to analysis of variance, and means were separated with a Waller-Duncan Baysian LSD test.

RESULTS

Greenhouse studies. There were no significant differences in relative infection efficiencies among oat lines and cultivars evaluated in all of the greenhouse trials. The relative infection efficiencies ranged from 22 to 29%.

There was a considerable range in lesion lengths in all three

TABLE 1. Lesion length, latent period, and sporulation capacity of six oat breeding lines and three cultivars inoculated with *Pyrenophora avenae* in 1981–1983[y]

Line or cultivar	Lesion length (mm)			Latent period[z] (days)			Conidia/leaf × 10^4		
	1981	1982	1983	1981	1982	1983	1981	1982	1983
7930-6	1.6 f	1.9 c	1.5 e	37 b	40 b	36 c	20 de	17 d	17 c
8172-2	2.7 ef	2.3 c	2.6 d	41 b	44 b	36 c	18 e	17 d	19 c
8174-20	2.9 ef	2.4 c	2.5 d	40 b	45 b	35 bc	17 e	19 d	25 c
8184-5	4.5 de	6.3 b	5.0 c	37 b	44 b	36 c	15 e	20 d	20 c
8184-14	4.5 cd	6.1 b	5.1 c	34 b	40 b	31 b	23 d	22 cd	19 c
8184-18	6.2 cd	5.9 b	6.1 b	35 b	41 b	31 b	18 e	20 d	21 c
Mariner	8.0 c	6.3 b	6.3 b	24 a	28 a	30 b	31 c	26 c	33 b
Noble	12.7 b	12.8 a	13.6 a	23 a	28 a	23 a	55 b	47 b	69 a
Otee	18.1 a	13.4 a	15.4 a	23 a	25 a	22 a	67 a	62 a	67 a

[y] All values presented are the means from the third leaf of plants inoculated in the four-leaf stage, five plants per pot, and 10 replications. Means within a column followed by the same letter are not significantly different (Waller-Duncan Bayesian least significant difference test, *k*-ratio = 100).

[z] Measured as number of days from inoculation until sporulation.

years, and the oat cultivars had the largest lesions (Table 1). Mariner had smaller lesions than Noble or Otee, and, in 1982, it had lesion lengths similar to those in three of the breeding lines. In all 3 yr, Mariner was comparable to line 8184-18 in lesion length.

The three cultivars had shorter latent periods than the breeding lines in all tests, except for Mariner in 1983 (Table 1). All of the breeding lines had comparable latent periods in 1981 and 1982.

The cultivars had the greatest number of spores per leaf, and Otee had significantly more spores than the other two cultivars in 1981 and 1982 (Table 1). Mariner had fewer spores per leaf than the other two cultivars in every test. The six breeding lines were very similar in sporulation capacity in all three tests.

Field studies. Disease severities ranged from 13 to 56% over the 3-yr period (Table 2). Otee and Noble were the most susceptible of the test lines. Mariner had a lower disease severity than the other two cultivars in 1982 and 1984. The disease epidemic was not as severe in 1983, with 33% as the greatest severity recorded. Lines 7930-6 and 8172-2 had the lowest disease severities among the six breeding lines tested.

When the greenhouse and field data were averaged over the 3 yr, several patterns emerged. There are two distinct groups within the breeding lines with respect to lesion length, but the lines did not differ in conidia production (Table 3). There was more variation among the lines and cultivars for latent period and severity, with the three cultivars having the shortest latent period and highest severity (Table 3). The combined data of all lines and cultivars showed that, as severity increased among lines or cultivars, latent period decreased (Fig. 1), lesion size increased (Fig. 2), and sporulation increased (Fig. 3).

TABLE 2. Severity of Pyrenophora leaf blotch on six oat breeding lines and three cultivars in field trials in central Pennsylvania[y]

Line or cultivar	Disease severity[z] (%)		
	1982	1983	1984
7930-6	13.0 f	13.5 f	17.4 f
8172-2	16.1 f	17.0 ef	22.3 ef
8174-20	21.7 e	17.4 ef	26.7 de
8184-5	26.5 de	21.3 de	34.0 cd
8184-14	32.7 c	24.1 cd	41.0 bc
8184-18	30.8 cd	26.1 bcd	36.5 c
Mariner	28.9 cd	26.8 bc	35.9 c
Noble	37.9 b	31.3 ab	48.6 ab
Otee	43.6 a	33.0 a	55.6 a

[y] Means within a column followed by the same letter are not significantly different (Waller-Duncan Bayesian least significant difference test, k-ratio = 100).

[z] Mean severity (percent leaf area infected) on the upper four leaves at growth stage 10.54 (Feekes scale). Each value is the average of 25 tillers and four replications.

TABLE 3. Mean lesion length, latent period, sporulation capacity, and disease severity of six oat breeding lines and three cultivars inoculated with *Pyrenophora avenae* over 3 yr[z]

Line or cultivar	Lesion length (mm)	Latent period (days)	Conidial leaf × 10^4	Disease severity (%)
7930-6	1.7 e	37.7 abc	18.0 d	14.6 e
8172-2	2.5 e	40.3 a	18.0 d	18.5 d
8174-20	2.6 e	40.0 a	20.3 d	21.9 d
8184-5	5.3 d	39.0 ab	18.3 d	27.3 c
8184-14	5.2 d	35.0 c	21.3 d	32.6 b
8184-18	6.1 cd	35.7 bc	19.7 d	31.1 bc
Mariner	6.9 c	27.3 d	30.0 c	30.5 bc
Noble	13.0 b	24.7 de	57.0 b	39.3 a
Otee	15.6 a	23.3 e	65.3 a	44.1 a

[z] Means within a column followed by the same letter are not significantly different (Waller-Duncan Bayesian least significant difference test, k-ratio = 100).

DISCUSSION

Disease control for Pyrenophora leaf blotch on oats is limited to crop rotation and seed treatment. In growing seasons with extended periods of wet weather, this may not be adequate. The incorporation of disease resistance into commercial cultivars may be necessary to provide additional protection (4).

All six of the breeding lines evaluated in this 3-yr study are potential sources of resistance, with 7930-6 and 8172-2 providing the highest levels of resistance. The type of resistance exhibited in these tests appears to be rate-limiting or horizontal. Although

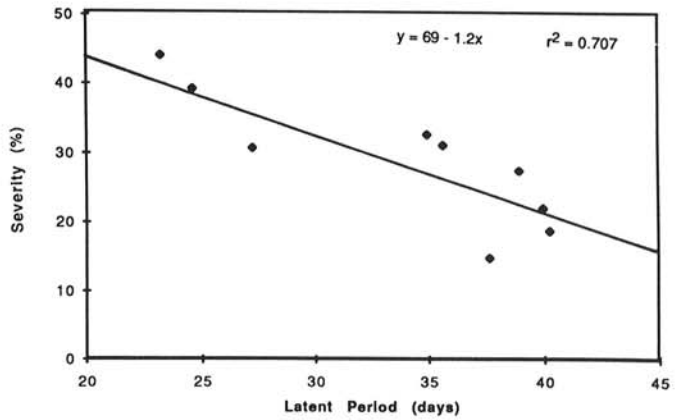

Fig. 1. Relationship between latent period and severity of *Pyrenophora avenae* on six oat lines and three cultivars. Points represent mean latent period and severity over 3 yr on individual lines or cultivars.

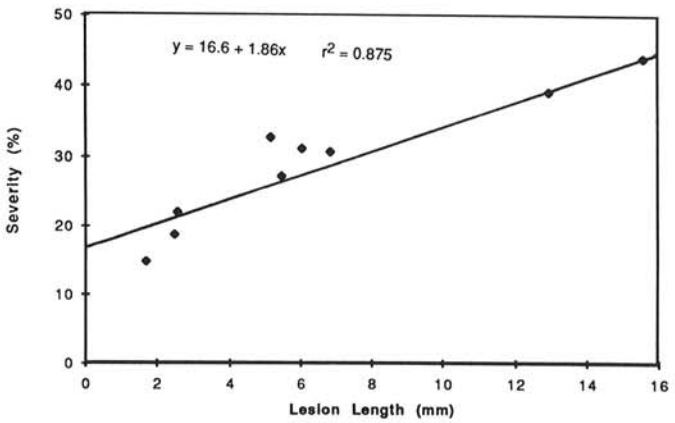

Fig. 2. Relationship between lesion size and severity of *Pyrenophora avenae* on six oat lines and three cultivars. Points represent mean lesion size and severity over 3 yr on individual lines or cultivars.

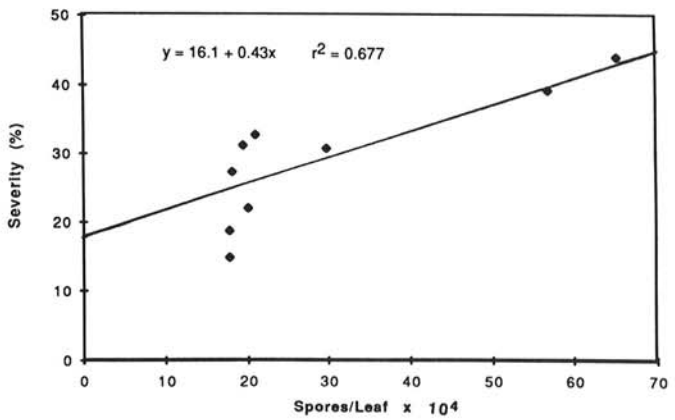

Fig. 3. Relationship between sporulation and severity of *Pyrenophora avenae* on six oat lines and three cultivars. Points represent mean sporulation and severity over 3 yr on individual lines or cultivars.

there were no differences in relative infection efficiencies, the breeding lines had reduced lesion lengths, longer latent periods, and reduced pathogen sporulation capacity.

Based on a preliminary evaluation of lesion area, there was a strong correlation between lesion length and total lesion area ($r = 0.87$, $P = 0.001$); therefore, only lesion length was measured. Although one might consider using lesion length as the sole criterion for measuring resistance in greenhouse tests, this could lead to inaccurate conclusions. As an example, although 7930-6 had the smallest lesion length in 1983, it allowed as much sporulation as the other five breeding lines. This might indicate that more spores may be produced in a given area of lesion on 7930-6. One would have to monitor disease progress over time to determine whether lesion length is a good predictor of disease resistance.

Even though latent periods were similar for all of the breeding lines, disease severity differed among these lines in field trials. Although a longer latent period could result in a reduced epidemic, leaf senescence could affect the latent period in the field. In contrast to some of the Pyrenophora leaf diseases, the oat pathogen does not sporulate until the leaf tissue is heavily colonized or until the leaf is dead (1,3,8,10). Therefore, premature leaf senescence caused by various environmental factors could lead to a shortened latent period and spore release. A more appropriate measure of latent period might be conducted under field conditions. The interval between GS-6 and the rating period averaged 32 days over the 3 yr.

Sporulation capacity was reduced significantly on the resistant lines in comparison to the cultivars evaluated. However, this component of rate-limiting resistance could be extremely variable, based on the nature of conidial formation on the leaf surface. Induction of sporulation is related to leaf senescence or death but the amount of conidiophore production on necrotic tissue can be extremely variable (3). Miscroscopic examination of lesion surfaces for conidial formation revealed that many areas were devoid of conidiophores, whereas other areas were heavily colonized with these structures. There was no apparent pattern to this conidial formation, which confirms the report of Muller (3).

Disease severity measured in field trials may provide the most accurate assessment of rate-limiting resistance with this disease. Even in 1983, when the disease severity was lower than in the other 2 yr, there was good separation among the six breeding lines. The precision of this evaluation may be enhanced further by calculating areas under disease progress curves. This would require several assessments of disease severity over time and would minimize effects of poor epidemiological conditions that might occur during any specific phase of the epidemic. Several of the current oat breeding lines with a degree of resistance are being evaluated in this manner.

LITERATURE CITED

1. Drechsler, C. 1923. Some graminicolous species of *Helminthosporium*. Int. J. Agric. Res. 24:641-739.
2. Large, E. C. 1954. Growth stages in cereals: Illustration of the Feekes scale. Plant Pathol. 3:128-129.
3. Muller, H. J. 1963. Untersuchungen uber Blattfleckenkrankheiten des Hafers. I. Symptomatologie von *Helminthosporium avenae* Eidam. Phytopathol. Z. 49:177-202.
4. Muller, H. J. 1963. Untersuchungen uber Blattflecken-krankheiten des Hafers. II. Pilzliche Blattflecken-erreger des Hafers: *Helminthosporium avenae* Eidam, *Septoria avena* Frank, *Helminthosporium sativum* P., K. et B., *Fusarium* spec. und *Heterosporium avenae* Oud. Phytopathol. Z. 49:266-290.
5. Rekola, O., Ruokola, A. L., and Kurtto, J. 1970. Damage caused by *Helminthosporium avenae* Eidam on the crop yield of oats in Finland. Acta Agric. Scand. 20:225-229.
6. Richardson, M. J. 1974. Response of oats to seed treatment and seed-borne inocula of *Pyrenophora avenae* and *Micronectriella nivalis*. Trans. Br. Mycol. Soc. 62:567-584.
7. Schein, R. D. 1964. Design, performance, and use of a quantitative inoculator. Phytopathology 54:509-513.
8. Shaner, G. E. 1981. Effect of environment on fungal leaf blights of small grains. Annu. Rev. Phytopathol. 19:273-296.
9. Sheridan, J. E., and Tan, P. E. 1973. Incidence and survival of *Pyrenophora avenae* in New Zealand seed oats. N. Z. J. Agric. Res. 16:251-253.
10. Turner, D. M., and Millard, W. A. 1931. Leaf-spot of oats *Helminthosporium avenae* (Bri. and Cav.) Eid. Ann. Appl. Biol. 18:535-558.
11. Vanderplank, J. E. 1968. Disease Resistance in Plants. Academic Press, New York. 206 pp.