Resistance in Semihardy Winter Barley to Leaf and Glume Blotch Caused by *Stagonospora nodorum*

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ABSTRACT

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Leaf and glume blotch of barley (*Hordeum vulgare*) was found in Georgia for the first time in 1980. Breeding lines varied significantly in susceptibility to the barley biotype of *Stagonospora nodorum* (syn. *Septoria nodorum*). Elite lines and cultivars of semihardy winter barley adapted to the southern and mid-Atlantic regions of the United States were evaluated for resistance during 10 seasons in field and greenhouse tests. Among the 214 elite breeding lines tested, 13 resistant genotypes were identified. Among the 20 cultivars evaluated, Keowee and Miller were highly resistant. Anson, Barsoy, Dayton, Henry, Milton, Perry, Preamble, and Sussex were susceptible. Highly significant correlations were detected between results over years from field and greenhouse tests. Correlations between the response on seedlings in the greenhouse and adult plants in the field were significant but low. Resistance is partial, in that all genotypes are susceptible but differ in the rate of disease development. Diverse barley germ plasm lines adapted to southern and mid-Atlantic states with a high level of resistance to leaf and glume blotch are now available.

Stagonospora nodorum (Berk.) Castellani & Germano (syn. *Septoria nodorum* (Berk.) Berk. in Berk. & Broome) causes a leaf and glume blotch of barley (*Hordeum vulgare* L.) (2,6, 7,11). The disease was first observed in Georgia in barley breeding nurseries in April 1980, following a period of heavy rainfall. Subsequently the pathogen was identified at several locations in Georgia and North Carolina (2). Isolates adapted to barley have unique cultural characteristics that distinguish them as a distinctive biotype of *S. nodorum* (2,8). Rufty et al (9) previously reported the disease on barley in North Carolina but did not differentiate isolates of *S. nodorum* on barley from those that attacked wheat (*Triticum aestivum* L.). Because the reaction of cultivars varied greatly in the nurseries, cultivars and elite breeding lines adapted to the southern and mid-Atlantic regions of the United States were evaluated for resistance in field tests during 10 seasons and as seedlings in the greenhouse. The objectives were to identify barley germ plasm with superior resistance to the barley biotype of *S. nodorum* and to determine the reliability of seedling tests in the greenhouse compared with field tests of adult plants. The reaction of barley cultivars to the disease has been reported from northern Europe (7,10). No previous reports exist on the reaction of North American winter barleys to leaf and glume blotch.

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MATERIALS AND METHODS

Field tests. Winter barleys in the USDA Barley Disease Nursery, the USDA Uniform Winter Barley Yield Nursery, the USDA Uniform Barley Nursery–Semihardy Cultivars, the Georgia state barley nursery, and other selected cultivars and advanced breeding lines were evaluated for resistance from 1980 to 1990. A total of 53 lines and cultivars, evaluated during 1980–1983 in preliminary field trials, were chosen for replicated field tests in the 1983–1984 season. Each line was planted as a single row, 1 m long, at Griffin, Georgia. A replicated nursery consisting of new elite lines and the most resistant lines from previous field tests was planted each year from 1984 to 1990. Each year's nursery consisted of 30–70 entries in a randomized complete block with four replications. The 1990 nursery had five replications. The cultivars Dawn (1) and Milton were included in each trial as a moderately resistant and a susceptible control, respectively. Beginning in 1985, the line NC80-1 was included each year as an additional resistant control. In 1988 and again in 1990, the 13 most resistant lines from the previous 4 yr of replicated tests were included in the trial. During the 7 yr of replicated field tests, 20 cultivars and 214 elite breeding lines were evaluated.

Numerous single conidial isolates of the barley biotype of *S. nodorum* were collected from locations throughout Georgia in 1980 and subsequent years. Voucher specimens of barley leaves with typical lesions and pycnidia have been deposited in the mycological herbarium at the University of Georgia, Athens. A mixture of at least 10^6 conidia per milliliter from two virulent isolates was sprayed onto a portion of each field plot with a previously described procedure (2). Inoculations were made at decimal growth stages (GS) 31–37 and 37–47 (11) at approximately 2-wk intervals. Natural inoculum was also present each year. During dry periods between GS 51 and 83 the nurseries were irrigated by overhead sprinklers to promote disease development. Evaluations were made two to three times between flowering and late dough stage (GS 59–85). A 0–9 scale, in which 0 = healthy plants and 9 = >90% disease severity, was used to rate disease on the whole plant (5).

Greenhouse tests. Most entries from the replicated field trials were tested in the greenhouse from 1984 to 1991. Breeding lines and cultivars were planted in potting soil in Cone-Tainers (Ray Leach Co., Canby, OR). The cultivars Dawn and Milton were included as both inoculated and uninoculated controls. Beginning in 1985, NC80-1 was included as a resistant control in all subsequent trials. A randomized complete block design with four replications was used in each test. The plants were inoculated at the four- to six-leaf stage by spraying them with a conidial suspension prepared in the same manner as that used in the field. Plants were enclosed in moistened polyethylene bags for 48 hr at 18–24 C, then incubated in the greenhouse at the same temperature. Evaluations were made 9–14 days after inoculation when a large differential in symptoms occurred among the entries.

Statistical analyses. Data on disease severity from each experiment were subjected to analysis of variance using SAS (Statistical Analysis System, Cary, NC). After a significant F test, Duncan's new multiple range test was used for separation of means. Pearson correlation coefficients (r) were computed for the various data sets with cultivars and elite lines to compare responses between years and between field and greenhouse trials. PROC CORR of SAS was used to generate r values.

RESULTS

Field tests. Disease severity was moderate to severe each season and averaged 3.4–7.4 on the susceptible control Milton. Lesions caused by *S. nodorum* were evident on leaves by GS 77–85.

Susceptible entries also developed glume blotch symptoms. Typical pycnidia with conidia developed in the center of older lesions. Data from one evaluation date between GS 71 and 83 that gave the best differential among the entries were used for analysis of variance within each experiment.

Scald, caused by *Rhyncosporium secalis* (Oudem.) J. J. Davis, and spot blotch, caused by *Cochliobolus sativus* (Ito & Kuribayashi) Drechs. ex Dastur, occasionally interfered with evaluations for leaf and glume blotch. When a barley line was rated as 3 or higher (on a 0–9 scale) for scald or spot blotch, its rating for leaf and glume blotch was not included in subsequent data analyses. A few entries could not be evaluated during the 1984 season, because of scald and spot blotch. During the 1985–1990 seasons scald rarely interfered with evaluations. Powdery mildew and leaf rust were not observed at any time.

A total of 13 resistant lines were selected during the course of the yearly field tests (Tables 1 and 2). All lines except 84NB569 were evaluated together for 4 yr in replicated field tests. Data from 3 yr are reported for 84NB569. Disease severity on these lines was significantly less than on Milton. Among the GA series of lines (Table 1), GA-25 was more susceptible than Dawn. The other six lines of the GA series and the six lines evaluated between 1987 and 1990 (Table 2) were equal or more resistant than Dawn, and most were comparable to NC80-1.

Among the 19 cultivars evaluated in all replicated trials, Keowee and Miller were resistant, and Anson, Barsoy, Dayton, Henry, Milton, Perry, and Preamble were the most susceptible (Table 3). In several field and greenhouse tests in which some cultivars and the best elite lines were included, only Keowee and Miller were comparable in resistance to some of the best elite lines (data not shown). The cultivar Sussex, included in some of these tests and in several other trials, was also very susceptible (Cunfer, *unpublished*).

Greenhouse tests. The 13 most resistant elite lines were evaluated together in three tests. Milton had greater disease severity than all resistant lines in the 1990 and 1991 tests (Table 4). The range of disease severity was lowest in the 1988 test, and Milton's rating did not differ from ratings of some of the resistant lines. During two of the three years, 84NB569 and NC80-1 had the lowest disease ratings, which is consistent with their field ratings (Table 2). All cultivars were tested together in 1984 and 1985 (Table 3). The results were similar to those observed in the field. The most resistant cultivars were Boone, Dawn, and Miller. Henry and Perry had some of the highest disease-severity ratings in the greenhouse and the field.

Correlation of results between years and between field and greenhouse evaluations. Correlations were highest between years for field tests. The *r* values were 0.42 and 0.51 ($P = 0.01$) for 1984 versus 1990 and 1984 versus 1985, respectively, and ranged from 0.59 to 0.86 ($P = 0.0001$) for the other four comparisons for the Georgia series of elite lines (Table 1) during 1984, 1985, 1988, and 1990. For the second group of elite lines (Table 2), *r* values for

Table 1. Field evaluations of eight elite barley lines for resistance to leaf and glume blotch during 1984, 1985, 1988, and 1990 at Griffin, Georgia

	Disease severity[y]				
Cultivar or line	**1984**	**1985**	**1988**	**1990**	**Mean**
Milton	6.8 a[z]	4.9 a	6.3 a	5.6 a	5.8 a
Dawn	3.5 a	3.1 a–c	2.3 b	2.2 d	2.7 c
GA-25	2.8 b	4.5 ab	...	3.9 b	3.6 b
GA72003-6	1.0 c	1.5 cd	0.9 b	1.8 d	1.3 e
GA-26	1.0 c	1.5 cd	0.9 b	1.8 d	1.3 e
GA-24	1.0 c	1.6 cd	1.9 b	3.6 bc	2.3 cd
GA-27	0.6 c	2.0 cd	1.5 b	2.0 d	1.4 e
GA-28	0.5 c	1.4 cd	1.4 b	2.4 cd	1.5 de
GA-23	0.4 c	1.0 cd	1.1 b	2.2 d	1.3 e
NC80-1	...	0.5 d	1.3 b	1.9 d	1.4 e

[y]0–9 Scale, in which 0 = no disease and 9 = >90% disease severity.
[z]Means within columns followed by the same letter are not significantly different according to Duncan's new multiple range test ($P = 0.05$).

Table 2. Field evaluations of six elite barley lines for resistance to leaf and glume blotch during 1987–1990 at Griffin, Georgia

	Disease severity[y]				
Cultivar or line	**1987**	**1988**	**1989**	**1990**	**Mean**
Milton	3.4 a[z]	6.3 a	7.4 a	5.6 a	6.0 a
Dawn	1.6 b	2.3 b	3.3 bc	2.2 b	2.3 b
76574RA11	2.1 ab	1.5 bc	3.3 bc	2.0 b	2.2 bc
761823RA10	1.3 b	1.1 bc	3.3 bc	1.9 b	1.9 bc
76517RA3	1.0 b	1.8 bc	4.8 b	1.8 b	2.3 b
76567RA16	0.9 c	0.6 c	3.3 bc	0.7 c	1.3 bc
84NB569	...	0.9 bc	2.0 c	0.8 c	1.2 c
NC80-1	0.9 c	1.3 bc	2.8 c	1.9 b	1.8 bc

[y]0–9 Scale, in which 0 = no disease and 9 = >90% disease severity.
[z]Means within columns followed by the same letter are not significantly different according to Duncan's new multiple range test ($P = 0.05$).

Table 3. Field evaluations of 20 barley cultivars for resistance to leaf and glume blotch in the field and greenhouse during 1984 and 1985 at Griffin, Georgia

	Disease severity[y]			
	Field		Greenhouse	
Cultivar	**1984**	**1985**	**1984**	**1985**
Perry	7.5 a[z]	5.5 a	4.5 a–d	4.0 a–c
Henry	6.3 ab	5.5 a	5.7 ab	5.7 a
Sussex	...	...	4.1 b–e	3.6 b–e
Dayton	6.1 ab	3.0 a–f	3.2 c–h	3.2 b–f
Preamble	...	5.5 a	6.0 a	3.2 c–f
Barsoy	5.5 a–c	5.0 ab	3.6 c–g	3.6 b–e
Anson	5.3 b–d	4.8 a–c	3.8 c–g	3.2 b–f
Milton	6.8 ab	3.0 a–e	4.7 a–c	5.0 ab
Post	4.0 c–e	4.5 a–d	4.0 c–e	3.9 a–d
Volbar	4.0 c–e	4.0 a–e	3.3 c–j	3.8 b–d
Colonial 2	5.5 a–c	2.3 c–f	2.0 g–j	2.1 d–g
Pike	3.3 d–g	4.0 a–e	2.8 d–i	2.8 c–f
Venus	5.0 b–d	2.0 d–f	3.5 c–g	2.9 c–f
Boone	3.3 d–g	2.3 c–f	1.5 ij	1.3 fg
Dawn	3.5 c–f	1.8 ef	1.5 h–j	1.5 fg
Decatur	2.8 e–g	2.5 b–f	2.6 e–i	2.1 d–g
Kline	2.8 e–g	2.7 b–f	3.8 c–f	3.7 b–d
Redhill	2.8 e–g	1.8 ef	2.2 f–i	1.8 e–g
Keowee	1.8 fg	1.5 ef	2.9 d–i	2.1 d–g
Miller	1.3 g	0.8 f	1.6 h–j	1.4 fg
NC80-1	...	0.5 f	0.5 j	0.5 g

[y]0–9 Scale, in which 0 = no disease and 9 = >90% disease severity.
[z]All means within columns followed by the same letter do not differ according to Duncan's new multiple range test ($P = 0.05$).

1987–1990 ranged from 0.63 to 0.86 ($P = 0.0001$). The r value was 0.46 ($P = 0.01$) for 1987 versus 1989. Correlation coefficients for cultivars in 1984 versus 1985 and for all elite lines in 1988 versus 1990 were 0.52 ($P = 0.01$) and 0.78 ($P = 0.0001$), respectively.

Correlations between greenhouse tests for elite lines were significant ($P = 0.10$–0.001) for all three comparisons (Table 5), but r values were lower than those for field evaluations. The correlation coefficient between greenhouse evaluations for cultivars in 1984 versus 1985, 0.50 ($P = 0.001$), was comparable to the value for field evaluations.

Comparisons between field and greenhouse evaluations were significant ($P = 0.05$–0.0001) in four of five comparisons for elite barley lines (Table 5). The correlation between field results in 1990 and greenhouse results in 1988 for the elite lines was nonsignificant. Correlation coefficients between field and greenhouse tests for cultivars was significant at $P = 0.01$ in two comparisons but only significant at $P = 0.10$ in the other two comparisons (Table 5).

DISCUSSION

Semihardy winter barleys adapted to the southern and mid-Atlantic regions of the United States exhibited a wide range of response to *S. nodorum*. About 6% of the lines tested had a high level of resistance. Resistance to *S. nodorum* in barley appears to be of the incomplete or partial type of resistance (4). All genotypes have a susceptible response but differ in the rate of disease development.

Correlations between field and greenhouse results were lower than between field tests in different years. Correlations between greenhouse tests also were lower than those between field tests. Therefore, results of seedling evaluations in the greenhouse should be used cautiously. These results may indicate that a more precise method for conducting tests in the greenhouse is needed, or that there is greater variability of reaction in the seedling stage than at the adult stage. In preliminary tests, lesion development was reduced, and genotype reactions were not uniform, when greenhouse temperatures periodically reached 25–28 C. Therefore, it is important to conduct tests at temperatures between 18 and 24 C. Field evaluations may be more uniform because observations are based on more plants in each replicate than in the greenhouse. Because of these variations, suspected resistant lines should be evaluated more than one season in the field.

Magnus (7) found a high level of resistance in barley in Norway. Most of the six-rowed barleys he tested were resistant, whereas many of the two-rowed types were susceptible. A positive correlation existed between the results of greenhouse and field tests. All barleys tested in the current study were six-row types; therefore, no comparison between two-row and six-row types was possible.

Six lines, GA-23 to GA-28 (PI 537569 to PI 537574), with a high level of resistance and acceptable agronomic traits were released as germ plasm by the Georgia Agricultural Experiment Stations (3). The five additional lines listed in Table 2 and line GA72003-6 (Table 4), with resistance equal or superior to these lines, also will be released as germ plasm. NC80-1 will be made available by the North Carolina Agricultural Experiment Station.

Although *S. nodorum* has been recognized only recently as a pathogen of barley in the southeastern United States, it has probably been present many years at varying levels of severity. Several elite breeding lines from the Georgia program and the cultivar Dawn evaluated during the early phase of this work were resistant, although no conscious effort was made to select for resistance. Some lines with resistance to *S. nodorum* were probably selected during evaluation for resistance to scald and spot blotch, which are often severe in breeding nurseries in the Southeast. The elliptical lesions caused by *S. nodorum* and by *R. secalis* are similar when lesions are young (2). Spot blotch lesions are also similar in shape and size to those caused by *S. nodorum*, but they remain dark, whereas the centers of leaf blotch lesions become lighter with age and contain numerous dark brown pycnidia (2).

Leaf and glume blotch currently is a minor disease of barley in Georgia, but this may be due partly to the low acreage in the state, which results in long rotations between barley crops. The disease is as common and is sometimes as severe as scald in field nurseries where barley has been grown continuously for several years or in single-year rotations with wheat. In field trials in which plants were inoculated with *S. nodorum* and control treatments were maintained by fungicide applications, yield reduction exceeded 20% in susceptible cultivars (Cunfer, *unpublished*). *S. nodorum* is also seedborne on barley (2).

Winter barley breeders in humid areas, such as the eastern and southern United States, should be aware of the potential of leaf and glume blotch and include it among the complex of leaf spot diseases evaluated when selecting germ plasm for resistance. Resistant genotypes with acceptable agronomic characters and that are adapted to the mid-Atlantic and

Table 4. Evaluation of 13 elite barley lines in greenhouse tests for resistance to leaf and glume blotch during 1988, 1990, and 1991 at Griffin, Georgia

	Disease severity[y]		
Cultivar or line	**1988**	**1990**	**1991**
Milton	2.9 a[z]	7.0 a	5.0 a
Dawn	2.5 ab	1.6 e	3.0 b–d
GA-25	...	4.0 b–d	4.2 b
GA72003-6	3.5 a	1.0 e	2.4 b–d
GA-26	1.3 b–d	5.0 b	3.1 b–d
GA-24	1.0 cd	1.0 e	1.7 cd
GA-27	3.4 a	2.4 c–e	2.8 b–d
GA-28	2.0 a–c	1.2 e	1.4 cd
GA-23	1.2 b–d	1.0 e	1.6 cd
76574RA11	2.5 ab	4.2 bc	2.4 b–d
761823RA10	2.0 a–c	1.6 e	3.4 a–c
76517RA3	2.6 ab	4.4 bc	3.0 b–d
76567RA16	2.6 ab	1.8 e	3.8 b–g
84NB569	0.6 cd	2.2 de	1.2 d
NC80-1	0.4 d	2.0 e	1.2 d

[y] 0–9 Scale, in which 0 = no disease and 9 = >90% disease severity.
[z] Means within columns followed by the same letter are not significantly different according to Duncan's new multiple range test ($P = 0.05$).

Table 5. Pearson correlation coefficients for disease severity of leaf and glume blotch for barley cultivars in the field and greenhouse in 1984 and 1985 and for elite lines in the field during 1988 and 1990 and in the greenhouse during 1988, 1990, and 1991[x]

		Greenhouse		Greenhouse			Field
Location	**Year**	**1984**	**1985**	**1988**	**1990**	**1991**	**1990**
Field	1984	0.26[y]	0.25[y]				
Field	1985	0.44**[z]	0.47*				
Field	1988			0.28*	0.64****	0.47***	0.80****
Field	1990			0.12	0.46****	0.35**	
Greenhouse	1988				0.35**	0.22[y]	
Greenhouse	1990					0.38***	

[x] Includes the control cultivars Dawn and Milton.
[y] Significant at $P = 0.10$.
[z] Significant at * ($P = 0.05$), ** ($P = 0.01$), *** ($P = 0.001$), or **** ($P = 0.0001$).

southeastern United States are now available. The resistance of barley cultivars to *S. nodorum*, evaluated over a 10-yr period, and that of the best elite lines, evaluated for 4–9 yr, has remained stable. These results indicate that, as has been found in wheat (4), resistance to *S. nodorum* in barley appears to be durable for long periods of time.

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