Variation in Virulence in Isolates of *Septoria nodorum*

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ABSTRACT

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Nine isolates of *S. nodorum* differed significantly in pathogenicity on four wheat cultivars. Seven isolates were more virulent on Blueboy than on Coker 68-15 and two isolates were more virulent on Coker 68-15 than on Blueboy. The cultivars Anderson and Hadden were intermediate in reaction to the nine isolates. A significant cultivar × isolate interaction indicated the presence of specific resistance. Passage of an isolate of *S. nodorum* from wheat through three cycles of inoculation and reisolation on barley decreased the disease (percent leaf area covered by lesions) from 75 to 35% on wheat and increased the disease on barley from 5 to 50%. Similarly, passage of a barley isolate through three cycles on wheat decreased the disease on barley from 75 to 25% and increased the disease on wheat from 5 to 60%. Both isolates infected species of *Agropyron*, *Elymus*, *Festuca*, *Hordeum*, *Hystrix*, *Lolium*, and *Poa*. Neither isolate infected oats.

Additional key words: Triticum aestivum, glume blotch.

Septoria nodorum Berk. (*Leptosphaeria nodorum* Müller) causes the glume blotch of wheat, *Triticum aestivum* L. em Thell., reducing yield in many parts of the world (2,5,7,11,13,18). The fungus has been reported on a wide range of gramineous hosts (6,8,15,19) and we have recently observed a similar organism on barley in North Carolina. A high degree of variability has been reported in the fungal population (1,10,12), but no designation of races or biotypes has been proposed.

This investigation was conducted to study the variability in pathogenicity and the host range of *S. nodorum* in North Carolina.

MATERIALS AND METHODS

Pathogenic variability. Nine isolates of *S. nodorum*, eight from North Carolina and one from Montana (isolate #4), were tested for pathogenicity on four wheat cultivars: Blueboy, CI 14301; Anderson, CI 12536; Coker 68-15, CI 15291; and Hadden CI 13488. For every treatment, 12–15 seeds per cultivar were sown in 10-cm-diameter plastic pots containing artificial growing medium (Terra-Lite-Metro-Mix™, W. R. Grace and Co., Cambridge, MA 02140). After emergence, seedlings were thinned to 10 and grown in the greenhouse at temperatures ranging from 20 to 30 C under natural light conditions.

Single-pycnidium isolates (labeled 1–9) were grown on potato-dextrose agar (PDA) in 9-cm-diameter petri dishes for 7 days at 20 C under constant 4200 lux fluorescent light (Sylvania F400W). Pycnidiospores were collected by flooding plates with 2–3 ml of 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 by using a hemacytometer. Volume was standardized to 10 ml per isolate. Hereafter, this method will be referred to as the standard procedure.

Inoculation was performed at the third-leaf stage by using Eyal and Scharen's (4) turntable technique with the following modifications: four pots, one of each cultivar, were inoculated simultaneously by using a No. 15 De Vilbiss atomizer attached to a pressure pump, 1.05 kg/cm^2 (15 psi). After inoculation, plants were covered with clear polyethylene bags for 76 hr to provide a water-saturated atmosphere conducive to infection.

Visual readings were made based on James' scales (9) when lesions became distinct 6–10 days after inoculation, depending on the rate of disease development. Pathogenicity was evaluated by host susceptibility expressed as a percentage (mean of 10 observations) of the area of the second leaf covered by lesions; the percentage values were arc sine transformed prior to analysis according to a split-plot design with isolates as main plots and cultivars as subplots. Treatments were replicated twice and consisted of simultaneously inoculating the four cultivars with a given isolate. A noninoculated set served as the control. The experiment was repeated four times and data were pooled into a combined analysis over all experiments.

Host range. A host range study was made of two single-pycnidium isolates of *Septoria, S. nodorum* from wheat and a morphologically similar *Septoria* sp. from barley, both collected in North Carolina. The isolate from barley produced symptoms on barley similar to those produced by *S. nodorum* on wheat. The study was made to compare the pathogenicity of the two isolates on selected hosts and to test the susceptibility of grasses that might play a role in the multiplication and survival of *S. nodorum*. These grasses were selected on the basis of taxonomic relationship to small grains and prevalence in North Carolina. The species inoculated were: *Agropyron repens* L., *Elymus virginicus* L., *Festuca arundinacea* Schreb., *F. elatior* L., *F. rubra* L., *Hordeum bulbosum* L., *H. marinum* Huds., *H. pusillum* Nutt., *Hystrix patula* Moench., *Lolium multiflorum* Lam., *L. perenne* L., *Poa compressa* L., *P. diversifolia* (Boiss. and Bal.) Hack ex Boiss., *P. pratensis* L., *Triticum aestivum* L. em Thell. 'Blueboy' and 'Coker 68-15,' *Avena sativa* L. 'Carolee' and 'Salem,' and *Hordeum vulgare* L. 'Boone' and 'Clayton.' Seeds of the gramineous species were sown in 10-cm-diameter clay pots containing a mixture of sterilized sandy loam soil, sand, and peat moss in a ratio of 2:1:1, respectively. Due to the small size of the seeds, no attempt was made to quantify the number of seeds per pot. Small grains were planted 12 seeds per pot 8 wk after the other species (to compensate for their faster growth). A small amount of slow-release fertilizer (Osmocote™) was applied to each pot after the seeds had germinated. Seedlings were grown in the greenhouse at temperatures ranging from 20 to 30 C under natural light conditions.

The isolates were grown by the standard procedure and inoculations were made as previously described, except that plants

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were inoculated directly on the greenhouse bench with spore suspension applied by spraying until runoff. The small grains were at the second-leaf stage and the other gramineous species were approximately 10 wk old (three-to-six-leaf stage) when first inoculated (spore concentration 2.05×10^7 spores per milliliter). Inoculation was repeated 2 wk later (spore concentration, 2.75×10^7 spores per milliliter). Visual readings were taken the following week and lesions were plated on PDA to determine if they were caused by *S. nodorum*. Cultures reisolated from each set were inoculated to Blueboy wheat and Boone barley to determine whether the ability to reinfect their original hosts was retained after passage through other species. Ratings of host susceptibility were based on the development of lesions and reisolation of the fungus from them. The experiment was conducted in a randomized complete block design with two replications per isolate plus an uninoculated set that served as a control.

RESULTS

Pathogenic variability. Analysis of variance indicated highly significant differences among isolates and cultivars (Table 1). Differences due to experiments also were highly significant. Seven isolates were more virulent on Blueboy than on Coker 68-15, but isolates 7 and 8 were more virulent on Coker 68-15 than on Blueboy (Table 1). Seven isolates were more virulent on Hadden than Anderson, but isolates 4 and 5 were more virulent on Anderson. These differences in ranking account for a significant cultivar × isolate interaction in the analysis of variance.

TABLE 1. Susceptibility of four wheat cultivars to nine isolates of *Septoria nodorum*

Isolate	Percent leaf area covered by lesions[a]				Mean
	Blueboy	Hadden	Anderson	Coker 68-15	
1	74.2 ± 6.4[b]	46.2 ± 5.3	46.1 ± 8.3	34.6 ± 5.5	50.3
2	70.2 ± 6.9	54.2 ± 5.1	53.4 ± 7.2	40.6 ± 5.8	54.7
3	57.2 ± 7.2	46.0 ± 7.5	44.0 ± 7.1	34.6 ± 6.4	45.5
4	54.8 ± 4.8	45.0 ± 6.0	49.1 ± 5.3	28.9 ± 5.4	42.2
5	85.8 ± 8.8	58.1 ± 9.3	66.7 ± 7.8	50.8 ± 9.6	65.4
6	70.9 ± 6.9	58.5 ± 7.9	45.7 ± 7.4	41.4 ± 7.8	54.1
7	33.1 ± 6.1	60.7 ± 6.1	57.1 ± 9.0	60.8 ± 6.7	52.9
8	35.6 ± 5.9	46.2 ± 6.2	44.7 ± 6.2	73.4 ± 8.2	49.9
9	58.8 ± 7.6	53.5 ± 6.7	42.9 ± 5.4	32.4 ± 6.4	46.9
Mean	60.1	52.1	48.9	44.2	
	Cultivar LSD .05 = 2.6 .01 = 3.5		Isolate LSD .05 = 6.1 .01 = 8.2		

[a] Means of four experiments.
[b] Standard error of the mean.

TABLE 2. Host range and pathogenicity of two isolates of *Septoria* sp. on gramineous species

Host species / Cultivars	Leaf area covered (%)		Organism reisolated	
	WI[a]	BI[b]	WI[a]	BI[b]
Agropyron repens	5	10	+[c]	+
Elymus virginicus	5	10	+	+
Festuca elatior	5	1	+	−
F. arundinacea	1	0	+	−
F. rubra	0	0	−	−
F. pratensis	1	5	+	−
Hordeum pusillum	10	75	+	+
H. bulbosum	1	35	+	+
H. marinum	10	25	+	+
Hystrix patula	15	25	+	−
Lolium multiflorum	10	5	−	−
L. perenne	5	10	+	−
Poa compressa	5	10	−	−
P. diversifolia	5	0	+	−
P. pratensis	5	0	+	−
Triticum aestivum				
'Blueboy'	75	5	+	+
'Coker 68-15'	50	5	+	+
Avena sativa				
'Salem'	0	0	−	−
'Carolee'	0	0	−	−
Hordeum vulgare				
'Boone'	5	75	+	+
'Clayton'	5	75	+	+

[a] WI = wheat isolate.
[b] BI = barley isolate.
[c] + = yes, − = no.

Host range. The response of several grasses to inoculation with wheat and barley isolates of *Septoria* spp. is shown in Table 2. The wheat isolate produced lesions on all grasses except *Festuca rubra* and *Avena sativa*. Compared to wheat, infection levels were relatively low, but symptoms—necrotic lesions with yellow halos—were typical of *S. nodorum* on all hosts. *S. nodorum* was reisolated from most of the lesions, except for those on *Festuca rubra*, *Lolium multiflorum*, and *Poa compressa*. The barley isolate produced lesions on all but the following grasses: *Festuca arundinacea*, *F. rubra*, *Poa diversifolia*, *P. pratensis*, and *Avena sativa*. Symptoms were similar to those produced by the wheat isolate except that lesions produced by the barley isolate were almost twice the size of those typically produced by wheat isolates of *S. nodorum* in the greenhouse. The barley isolate could not be reisolated from lesions on many of the grasses; only those on *Agropyron repens*, *Elymus virginicus*, *Triticum aestivum*, and *Hordeum* species, yielded cultures of *Septoria* spp. On most species, the barley isolate was more virulent than the wheat isolate, as indicated by the higher percent leaf area covered by lesions. The barley isolate produced abundant lesions on the *Hordeum* species and very few on wheat. Conversely, the wheat isolate was strongly virulent on both wheat cultivars but only weakly so on *Hordeum* species.

All reisolated cultures obtained from lesions on both wheat and barley were strongly virulent on Blueboy wheat and Boone barley, respectively. However, after three cycles of inoculation in a growth chamber and reisolation on barley, the wheat isolate lost some of its virulence for wheat (decreasing from 75 to 35% leaf infection from a standard inoculation dose) and became more virulent on barley (increasing from 5 to 50% leaf infection). Similarly, the original barley isolate acquired some specificity to infect wheat (increasing from 5 to 60% leaf infection) after three passages through wheat and lost its strong virulence for barley (decreasing from 75 to 25% leaf infection).

The high degree of specificity observed among the isolates for their original hosts suggested the need to closely examine the morphological traits of the two isolates both under field conditions and in culture. These studies (Table 3) indicated that spores of the wheat isolate were shorter and more cylindrical than those of the barley isolate. Spores of the barley isolate were slightly longer, more elliptical, and guttula were more clearly seen. These differences were not statistically significant. After passage of the wheat isolate through barley, its spores were longer than those

TABLE 3. Comparison of morphological characterisitics of wheat and barley isolates of *Septoria* sp.

	Wheat isolate on wheat[a]	Barley isolate on barley[a]
Spore length (μm)		
Under field conditions	18.7	24.9 NS[b]
In culture	22.8	27.7 NS
Spore length after passage through opposite host (μm)		
In culture	19.6	17.3 NS
Pycnidial diameter (μm)		
Under field conditions	119.9	128.1 NS
In culture	170.0	299.2 **
Pycnidial diameter after passage through opposite host (μm)		
In culture	163.2	152.0 NS

[a] Entries are means of 100 observations.
[b] NS = difference between isolates not significantly different based on Student's *t*-tests. ** = Differences significant, $P = 0.01$.

found in nature but shorter than those produced in culture on PDA; on the other hand, spores of the barley isolate were shorter after passage through wheat. Under natural conditions, pycnidia of the wheat isolate were smaller than those of the barley isolate, but this difference also was not significant. Pycnidia produced by the barley isolate on PDA were almost twice the diameter of those formed under identical conditions by the wheat isolate. After passage through the other host, both isolates produced pycnidia on PDA that were smaller than those of the original isolates in culture but larger than those produced in nature.

DISCUSSION

Isolates of *S. nodorum* differed significantly in ability to infect wheat cultivars. These findings are in agreement with previous reports by Bronnimann (1) and Scharen and Krupinsky (12) who found a wide range in the cultural and pathogenic characteristics of the fungus. Since the sexual stage is not common in the United States, this variability is probably due to mutation. Scharen found that single-spore isolates transferred for several generations rarely gave stable cultures of the fungus. There also were highly significant differences among cultivars in their resistance to colonization by the fungus. Coker 68-15 was the cultivar least susceptible to all but two isolates and Blueboy was the most susceptible. However, with isolates 7 and 8, Blueboy was the least susceptible and Coker 68-15 was the most susceptible cultivar. According to Vanderplank (16), significant isolate × variety interactions in the analysis of variance indicate the presence of specific resistance; ie, pathogen variants are differentially adapted to specific host cultivars. In this study, the isolate × cultivar interaction was significant ($P = 0.05$), indicating that specific resistance to this pathogen occurs in wheat, but the magnitude of this specificity is not high. All isolates of the fungus infected all tested cultivars, but differentially.

Resistance to *S. nodorum* has been reported to be nonspecific (2,3,12) although Frecha (5) reported resistance in cultivar Atlas 66 to be conditioned by a single gene. The existence of specific resistance does not exclude the presence of horizontal resistance in the same cultivar; in fact, horizontal resistance is probably always present along with vertical resistance (16). Without a high degree of host-pathogen specificity, vertical and horizontal resistance are epidemiologically similar in that both may reduce the rate at which an epidemic proceeds (17). The importance of distinguishing between the two forms of resistance is that in vertical resistance the correlated variation between host and pathogen implies that when the host changes, selective pressure is exerted and the pathogen population tends to adapt to the host. Thus, resistance may be lost, whereas in horizontal resistance, the variation in host and pathogen are not correlated and resistance is not lost through adaptation by the pathogen (17). Yet, with vertical resistance of low specificity, Vanderplank (17) says that variation in virulence of the pathogen may be expected to lag behind variation in host resistance, so this type of resistance would probably be longer lived than highly specific vertical resistance.

The origin of the isolates used in this study is not known so it is not possible to determine if, for example, isolates 7 and 8 originated from Coker 68-15 and are thus adapted to it. Some specialization probably occurs with this fungus as pointed out elsewhere in this paper. It is of interest that Blueboy is used as a susceptible check in North Carolina and Coker 68-15 generally appears to have some resistance. Conversely, in Arkansas, Blueboy exhibits a high degree of tolerance and Coker 68-15 is highly susceptible (F. C. Collins, *personal communication*). These differences could be due to the pathogen adapting to the host.

We do not recommend classification of isolates into separate races because the magnitude of the differences in host response is not great and these differences may be greatly influenced by environmental conditions. Nevertheless, isolate × cultivar interactions are important in breeding for disease resistance as a cultivar resistant in one location will not necessarily be resistant in another.

Most of the grasses inoculated with the wheat isolate developed lesions, which confirms previous reports that the host range of *S. nodorum* is quite broad. The barley isolate infected fewer hosts as it was not pathogenic on most of the *Festuca* and *Poa* species. The relatively low infection levels observed on most grasses may have been influenced by greenhouse conditions, host characteristics like leaf size, leaf texture, etc., and the fact that inoculation was performed during the vegetative state and *S. nodorum* causes greater damage at the heading to soft dough stage. Although differences were observed between the wheat and barley isolates in the degree of pathogenicity on specific hosts (ie, each isolate was more virulent on the host from which it was isolated, and the barley isolate was generally more virulent on other hosts), the two isolates are similar in their ability to infect many diverse species. *Avena sativa* and *Festuca rubra* were resistant to both isolates. Although some lesions were observed on *Lolium multiflorum* and *Poa compressa*, the fungus could not be reisolated from them.

Of particular interest is the host-passage effect. After passing through barley three times, the wheat isolate exhibited increased virulence for barley; similarly, after passing through wheat three times, the barley isolate became more virulent on wheat. Passage through a host evidently induces some kind of adaptation in the fungus. Specificity of isolates of *S. nodorum* obtained from grass hosts was also found by Holmes and Colhoun (8), Shearer and Zadoks (14), and Harrower (6).

These data, coupled with the parallelism in host range, suggest that the two isolates are strains of *S. nodorum* rather than separate species. This contention is supported by data on morphological traits (Table 3), which show very little difference between the two isolates. It is important to note, however, that the barley isolate is quite difficult to isolate from leaf tissue and has a slower growth rate on PDA than all other isolates of *S. nodorum* grown in the course of these studies. This may explain the difficulty in reisolating the organism from many of the lesions produced by this isolate. Furthermore, the barley isolate also produces black and red pigments on PDA that were not observed with wheat isolates.

Because *S. nodorum* cultures reisolated from all the susceptible grasses were capable of reinfecting wheat and barley, the role of these grasses in the overwintering and oversummering of *S. nodorum* may be important. These grasses, particularly the perennial species, could serve as sources of primary inoculum. We have found "little barley" (*Hordeum pusillum*) infected with *S. nodorum* in the field. Farmers may be able to reduce losses from this disease by eliminating these grasses (many of which are common weeds in North Carolina) from areas near their wheat fields.

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