Septoria nodorum on Barley and Relationships Among Isolates from Several Hosts

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ABSTRACT

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The causal agent of a leaf spot and glume blotch of barley and little barley (*Hordeum pusillum*) in Georgia was identified as *Septoria nodorum*. Symptoms are described. Conidial morphology of isolates from both hosts was identical to that of isolates from wheat and triticale. Isolates from barley and wheat were highly virulent to their original host but weakly virulent to the opposite crop in reciprocal inoculations. Isolates from *H. pusillum* were pathogenic to all four plant species. Isolates from triticale were similar to those from wheat by all criteria tested. *S. nodorum* was isolated from seeds of all four hosts. Isolates from wheat, triticale, and *H. pusillum* were fluorescent under near-ultraviolet light on unrefined media and when grown on autoclaved wheat and barley seeds placed on oxgall agar whereas most isolates from barley were nonfluorescent. Fluorescent isolates from barley were identical to isolates from wheat in cultural characters on six media and in pathogenicity. Isolates from wheat and barley with differing characters are considered to be biotypes of *S. nodorum*. Colony characters must be noted carefully during assays from barley seed on oxgall agar since most isolates are nonfluorescent.

A distinctive leaf spot, sometimes causing defoliation, was observed in commercial fields and on breeding lines of barley (*Hordeum vulgare* L.) near Griffin, GA. The lesions contained pycnidia with conidia similar to those of *Septoria nodorum* (Berk.) Berk. (asexual stage of *Leptosphaeria nodorum* Müller). The symptoms were unlike those of other barley leaf spot diseases commonly found in Georgia, but similar to those reported by European workers (1,4,16) as being caused by *S. nodorum*. Septoria glume blotch of wheat is often severe in Georgia, but its occurrence on barley has not been reported. Pycnidia bearing conidia resembling *S. nodorum* were also found on little barley, *Hordeum pusillum* Nutt., growing near fields of small grains. Isolates from these plants were also included in the study.

Experiments were conducted to identify the disease and pathogen and to determine the relationship to Septoria glume blotch of wheat. Preliminary results have been published (2).

MATERIALS AND METHODS

Lesion measurement. Infected leaves were collected from six barley lines and placed in a plant press. Within 1 wk after collection, 75–150 lesions from each collection were measured. A comparison with fresh samples showed that shrinkage due to drying during this period was negligible.

Conidial measurements. Thirty to 50 conidia were measured from pycnidia on plants collected in the field or on seedlings germinated in the laboratory. Collections included 15 from barley, three from *H. pusillum*, and two each from wheat and triticale. The conidia were mounted in water and measured at ×400 with an ocular micrometer.

Colony morphology. Eighteen isolates from barley, two from *H. pusillum*, 18 from wheat, and eight from triticale were grown on Czapek-Dox V-8 agar. Plugs of mycelium were cut from the actively growing margin of the colony and placed on oatmeal, wheatmeal, yeast extract, Czapek-Dox, minimal media prepared according to Krüger and Hoffmann (9), and on oxgall agar (13). Cultures were also grown on autoclaved wheat and barley seeds that were then transferred to oxgall agar. All cultures were incubated for 3 days in the dark then moved to a 12-hr daily photoperiod under cool-white fluorescent lamps (2,700 lux) at 20 C. Data were recorded for growth rate, sporulation, colony type, and fluorescence under near-ultraviolet light (blacklight).

Cross inoculations. All isolates were inoculated onto wheat, barley, and triticale seedlings, and *H. pusillum* at the heading stage in the greenhouse. In another test, two resistant and two susceptible cultivars or lines each of barley, wheat, and triticale, as determined by field reaction, were selected for inoculation. The cereal lines were planted in greenhouse flats in a randomized complete block design with four replications. Two isolates from barley and one each from wheat and triticale were grown on Czapek-Dox V-8 agar. When cultures were 10–14 days old, suspensions of 10^6 conidia per milliliter were prepared. Tween-20 (polyoxyethylene sorbitan monolaurate) was added as a surfactant. Seedlings at the three- to five-leaf stage were atomized to run off with the conidial suspension and incubated in a mist chamber for 48 hr. The plants were then incubated for 2–3 wk at 22–25 C. Ten leaves selected at random from each replication were rated visually for leaf spot using the assessment key of James (6). The experiment was conducted twice.

Isolations from seed. Seeds were harvested from plants of barley and *H. pusillum* exhibiting leaf lesions and glume blotching in the field. Seeds were germinated in vermiculite as previously described (5) for observation of symptoms on coleoptiles or formation of pycnidia on seeds. Following removal from vermiculite, the seedlings were incubated at 20 C on moist blotters for observation of pycnidia and conidia.

Spikelets from barley and *H. pusillum* infected in the field and from heads inoculated in the greenhouse were dissected into outer glumes, sterile florets, lemma and palea, and seed. Seeds were surface sterilized for 3–4 min in 0.5% sodium hypochlorite, then rinsed twice in sterile water and blotted dry. Other spikelet parts were not surface sterilized. Parts of at least 10 spikelets were plated on oxgall agar. Suspected colonies of *Septoria* were transferred to Czapek-Dox V-8 agar and examined for sporulation. Conidia from representative colonies were inoculated onto barley, wheat, and *H. pusillum*.

RESULTS

Symptoms on barley. The lesions are elliptical, up to 35 mm long and 10 mm wide, with a chlorotic margin 1- to 2-mm wide (Fig. 1A). Inside the chlorotic edge the lesions are buff with narrow chocolate brown areas that extend through the lesions; such dark necrotic areas may encompass a large portion of some lesions. As the lesions become older, they become ash gray in the center, but the brown streaks along the veins and through the center of the spots remain prominent. Numerous dark pycnidia scattered throughout the

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center of older lesions give the lesions a speckled appearance. Lesions merge if initiated within a few millimeters of one another, but many remain separate. Chlorosis sometimes extends from the lesion to the tip of the leaf. If there are numerous lesions the tissue between them often becomes chlorotic, then collapses and turns buff-colored (Fig. 1A). Small buff lesions with dark-brown margins are also observed on the lemma and palea (Fig. 1B). The entire head may become brown and produce shrivelled seed. Pycnidia on the leaves contained great numbers of septate, hyaline, filiform conidia typical of *S. nodorum*.

The fungus was identified and isolated from barley in breeding nurseries at Griffin, Athens, and Tifton, GA, and Clayton and Laurinburg, NC. The disease was also found in five commercial barley fields in Georgia. The perfect stage was not found on any plant material during the three seasons that observations were made.

Symptoms on *Hordeum pusillum*. Lesions begin as light-brown to chocolate-brown flecks on leaf blades. The flecks elongate up to 5 mm but remain less than 1 mm wide. The tissue surrounding the spots becomes chlorotic and quickly bleaches to tan. Irregular lesions form from the coalescence of the smaller spots. Leaf ends turn tan and die back rapidly, joining the expanding lesion on the blade below the tip. There are no pycnidia in the lesions, but they develop within 2–4 days after leaves are placed in moist chambers.

Spots on glumes are initially dark chocolate brown. Older portions of the lesions become tan. Spotting is greatest on the lemma and often most severe near the base of the awn. However, spotting develops on all portions of the floral tissues. Infection of the long tapering awns and the long narrow bracts outside the lemma results in rapid browning of these tissues, which become tan in contrast to the darker lesions on the lemma. Severe head infection results in premature drying of the seed. *Septoria* is readily isolated from nearly all glume, sterile floret, lemma and palea fragments, but only rarely from seeds.

Fig. 1. *Septoria nodorum* on barley: **A,** Elliptical lesions on a leaf. **B,** Glume blotch symptoms on heads.

Lesion size. Lesions were the same shape and appearance on six barley lines regardless of susceptibility. The average lesion size was $12.2 \pm 0.5 \times 4.2 \pm 0.1$ mm.

Conidial morphology. Conidial morphology was similar among isolates on all hosts (Table 1). The range in size of conidia was $12–32 \times 2.2–4.4$ μm for all hosts. Conidia from triticale were identical to those from wheat. The maximum length of conidia from these hosts was 25 μm. With one exception the number of septations ranged from one to three. One isolate from *H. pusillum* had a few conidia with four to five septa (average 2.9) but this isolate did not differ from the other isolate from *H. pusillum* in cultural characters or pathogenicity.

Cultural characteristics. Most isolates of *Septoria* from wheat and triticale and both isolates from *H. pusillum* fluoresced pale green to yellow-green on media containing unrefined plant material (eg, oatmeal and wheatmeal agars) (Table 2). Fluorescence was most intense when cultures were grown on autoclaved seeds on oxgall agar. All isolates from wheat, barley, and *H. pusillum* fluoresced on this medium. Thirteen isolates from barley were nonfluorescent; one was weakly fluorescent on wheatmeal agar only. These typical isolates were pathogenic to barley. The four fluorescent atypical isolates from barley were pathogenic to wheat and triticale only.

Colonies on oatmeal and wheatmeal agar of many isolates from wheat and triticale had gray aerial mycelium and olive to brown mycelium in the agar, whereas typical isolates from barley had pink to light orange colonies. Colonies of atypical isolates from barley were olive like the isolates from wheat. Colonies on yeast extract, minimal, and Czapek-Dox agars of typical isolates from barley were pink to violet, whereas isolates from the other hosts varied from orange to olive or olive brown. Atypical isolates from barley varied from pink to violet or olive. None of the isolates varied much on oxgall agar, producing scant gray-white aerial mycelium and orange-brown mycelium in the medium. On Czapek-Dox V-8 agar typical isolates from barley were distinctly dark violet with black pycnidia and little aerial mycelium except at the edge of the colony. Isolates from wheat and triticale varied from pink to yellow and often had abundant aerial mycelium. As typical isolates from barley became older there was pronounced folding of the mycelium on most media. Atypical isolates from barley and isolates from wheat did not exhibit this folding.

The typical isolates from barley grew less than one-third as fast as the atypical isolates on minimal and Czapek-Dox agars. Growth rates on all other media were comparable regardless of host of origin. Only two isolates from barley and one from wheat sporulated on oxgall agar.

Host range. Most isolates from barley were pathogenic to barley and nonpathogenic to wheat and triticale. Alternatively, isolates from wheat and triticale were pathogenic to both of these hosts but not to barley. Four isolates from barley, similar to isolates from wheat in other characters, were also pathogenic to wheat. Isolates

TABLE 1. Mean conidial size of *Septoria nodorum* from four hosts

Host	Number of isolates	Dimensions (μm ± SE)		Number of septations + SE
		Length	Width	
Barley	15	19.3 ± 0.1	2.7 ± 0.1	1.32 ± 0.1
Hordeum pusillum	3	18.7 ± 0.4	2.6 ± 0.1	1.95 ± 0.1
Wheat	2	17.8 ± 0.3	2.5 ± 0.1	1.72 ± 0.1
Wheat (published)		22.7	3.0	···
Triticale	2	17.6 ± 0.3	2.5 ± 0.1	1.37 ± 0.1

TABLE 2. Frequency of fluorescence under near ultraviolet light and sporulation on six agar media by *Septoria nodorum* isolates from four hosts

Host	No. isolates	No. isolates fluorescent (F) and sporulating (S) on each medium												Autoclaved seeds[a] on oxgall agar
		Oatmeal		Wheatmeal		Yeast extract		Minimal		Czapek-Dox		Oxgall		
		F	S	F	S	F	S	F	S	F	S	F	S	F
Barley	18	4[b]	18	5[b]	18	2	14	1	11	1	10	1	2	4
Hordeum pusillum	2	2	2	2	2	1	2	0	1	0	2	0	0	2
Wheat	18	13	16	13	18	1	11	3	12	1	11	0	1	18
Triticale	8	7	8	8	8	0	3	1	4	1	4	0	0	8

[a]Cultures were grown on autoclaved wheat and barley seeds for four days, then seeds were plated on oxgall agar.
[b]Four of these isolates were also pathogenic to wheat.

from *H. pusillum* were intermediate in reaction, being moderately virulent to all suscepts. Isolates from the other three cereals were pathogenic to *H. pusillum*.

Isolations from seed. Nonfluorescent colonies typical of *S. nodorum* grew from a few barley seeds and lemma and palea tissue on oxgall agar. All isolates sporulated when subcultured on Czapek-Dox V-8 agar. *S. nodorum* was isolated from several lots of triticale seed. Seed infection ranged from 0 to 29%. No symptoms were observed on coleoptiles of barley and *H. pusillum* in the germination tests.

DISCUSSION

The disease symptoms we observed differed markedly from the description of leaf blotch caused by *S. passerinii*. Lesions caused by *S. passerinii* are indefinite in shape, yellow, and blend into the healthy tissue (10,17,18). Macroconidia are up to twice as long as those of *S. nodorum* (9,17), and cultures are yeastlike on agar media (15). No microconidia were produced by our isolates.

An isolate of *S. avenae triticea* from wheat in North Dakota (from R. M. Hosford) produced conidia that were mostly three-septate and >30 μm long. This isolate produced abundant ascostromata with ascospores after a month on oatmeal and wheatmeal agar and was fluorescent on autoclaved seeds on oxgall agar.

The symptoms we observed on the barley cultivars are like those caused by *S. avenae triticea* (7), but the conidia are much shorter (12–32 μm versus 18–53 μm) and many conidia have fewer than three septa. Richardson and Noble (14) noted that pycnidia of *S. nodorum* and *S. avenae triticea* were found in identical lesions on wheat. Johnson (7) also commented on the difficulty in separating some isolates of *S. avenae triticea* from *S. nodorum* because of similarities of pycnidial morphology and size of conidia.

The distinct chlorotic margin and the prominent pycnidia distinguish the lesions we observed from those caused by *Cochliobolus sativus*, the most common leaf spot pathogen of barley in Georgia. Young lesions are similar to those of scald caused by *Rhyncosporium secalis*. However, as scald lesions become older, dark-brown margins develop and the center turns white. The fungus produces no pycnidia.

The size of the conidia of the isolates from barley and *H. pusillum* are comparable to *S. nodorum* on wheat. Our measurements of conidia from barley are nearly identical to those reported by Smedegard-Petersen (16) for *S. nodorum* on barley in Denmark.

Several features differentiate the barley pathogen from the one attacking wheat. With a few exceptions isolates from wheat and barley were virulent to their original hosts, but avirulent to the opposite host. Isolates from the two hosts differ in fluorescence, colony color, and growth rate on minimal and Czapek-Dox agar. Therefore, we consider the isolates from barley and wheat to be two biotypes of *S. nodorum*. The relation of these differences to host specificity is unclear. Several European workers have noted the specificity of isolates of *S. nodorum* for barley or wheat, but no cultural differences among the isolates were reported (1,12,16). Kietreiber (8) noted that colonies of *S. nodorum* growing on barley coleoptiles fluoresced under near-ultraviolet light. The host range and cultural characters of these isolates were not reported.

TABLE 3. Pathogenicity of *Septoria nodorum* isolates on barley, wheat, and triticale[a]

Crop	Cultivar or line	Leaf area with symptoms (%)			
		Barley isolate		Wheat isolate	Triticale isolate
		603	611	505	401
Barley	Volbar	15	26	0	0
	Milton	17	23	0	0
	Redhill	5	14	0	0
	Barsoy	3	15	0	0
Triticale	Omni	0	0	3	13
	Ark 2094	0	0	15	17
	AM 2873	7	7	5	15
	OK 77842	0	0	6	24
Wheat	Holley	0	1	33	33
	Omega 78	1	0	24	24
	Oasis	0	0	8	17
	Arthur 71	0	0	7	15

[a]Inoculations on seedlings at 8–10 leaf stage.

Comparative studies are needed on isolates from barley from a wide geographical area to determine if the differences in cultural characters also reflect pathogenicity differences.

Because colony fluorescence is important in the oxgall agar seed assay (13), additional care is needed to identify *S. nodorum* from barley. The significance of isolating wheat biotypes from barley is unresolved. Some wheat biotypes are weakly virulent to barley or colonization may result from the invasion of weakened tissue by these biotypes.

Machacek (11) reported considerable seed infection of barley in western Canada, but also noted an absence of symptoms on seedlings. *S. nodorum* was common in barley seed in Britain (3). Plant debris and infected seeds of *H. pusillum* may serve as reservoirs of inoculum. Because triticale seed may also harbor *S. nodorum*, there exist several seedborne inoculum sources to perpetuate the fungus in the field.

Damage from Septoria leaf spot and glume blotch on barley appears to be sporadic. We noted severe damage only after 1 mo of high rainfall and mild weather early in the season. There is considerable variation in susceptibility among barleys and several cultivars are resistant (*unpublished*).

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