Effects of Wetting Period on Resistance to Leaf Spotting of Wheat by Leptosphaeria microscopica with Conidial Stage Phaeoseptoria urvilleana

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The cooperation of H. B. Caldwell in reproducing photographs, that of S. J. Gregor, R. L. Haspel, and R. Kloberdanz in manuscript typing, and that of Lillian Hjelmstad, K. Knutson,and P. Nolte in glasshouse and laboratory assistance is gratefully acknowledged.

Published with the approval of the Director of the North Dakota Agricultural Experiment Station as Journal Series Article No. 787.

Accepted for publication 29 November 1977.

ABSTRACT

HOSFORD, R. M., JR. 1978. Effects of Wetting period on resistance to leaf spotting of wheat by Leptosphaeria microscopica with conidial stage Phaeoseptoria urvilleana. Phytopathology 68:908-912.

A fungus was isolated from leaf spots on wheat and found producing conidia resembling those of *Phaeoseptoria urvilleana* on wheat stubble in western North Dakota. On potato-dextrose agar it produced ascospores resembling *Leptosphaeria microscopica* and conidia resembling *Phaeoseptoria urvilleana*. This fungus was incubated on all of the following cultivars in all of the following sunlit wet periods. It caused no leaf spotting on any cultivar following postinoculation wet (mist) periods of 24, 36, or 42 hr. Following wet periods of 48 hr or longer it caused severe leaf spotting on spring wheat cultivars Waldron and Red River 68. Following 72- and 91-hr wet periods it caused severe spotting on ND495 spring wheat and slight spotting on Ward durum; no leaf spotting developed on Chris and Duri spring wheats, Wells and Hercules durum, WW8 awned winter wheat, Larker barley, Caribou rye, or Lodi oats. This appeared to be the first report of leaf spotting caused by *L. microscopica*. The fungus required a long postinoculation wet period to cause spotting. Expression of varietal resistance to leaf spotting was associated with the duration of wet period. This phenomenon of varietal resistance related to the duration of postinoculation wet period now has been detected with seven wheat leaf-spotting fungi. Compilation of the literature suggests that among four wheat leaf-spotting *Leptosphaeria* spp. with similar three-septate ascospores, increasing pathogenicity to more wheat cultivars is coupled to decreasing pycnidiospore size, septation, and pigmentation.

Additional key words: *Hendersonia* species, *Leptosphaeria avenaria* Weber f. sp. *triticea* T. Johnson, *L. eustomoides* Sacc., *L. nodorum* Müller, *L. tritici* (Gar.) Pass., *Phaeosphaeria* Speg., *Septoria avenae* Frank f. sp. *triticea* T. Johnson, *S. nodorum* (Berk.) Berk., *Phaeoseptoria airae* (Grove) Sprague, *P. festucae* Sprague.

Leptosphaeria microscopica Karst. has been found reproducing on wheat in Great Britain (20). Either it or the asexual stage, *Phaeoseptoria urvilleana* (Speg.) Sprague, reproduces on other members of the Gramineae in Argentina, Europe, Great Britain, and the United States (2, 12, 18, 20). Little is known about the nature or distribution of this fungus.

The objective of this investigation was to determine if this fungus causes leaf spotting of wheat; and if so, whether duration of postinoculation wet period-related cultivar resistance, previously detected with six other wheat leaf-spotting fungi, occurs with wheat cultivars and this fungus.

MATERIALS AND METHODS

Irregular leaf spots were observed on Centurk winter wheat at Williston in western North Dakota in early July 1975. Beneath the leaf-spotted wheat on the previous year's winter wheat stubble were pycnidia containing pycnidiospores resembling *P. urvilleana*. A fungus was isolated from the leaf spots. On potato-dextrose agar (PDA) it produced ascospores resembling *L. microscopica* and pycnidiospores resembling *P. urvilleana*. In preliminary pathogenicity tests 27- to 115-day-old PDA cultures of leaf-spot isolates of this fungus caused leaf spotting on wheat. For this study, isolates 1 and 3 were selected from the above and grown for 80 days in petri plates on PDA, until the mycelium containing ascospores and conidia covered most of the medium surface. Four petri plate cultures of isolate 1 or 3 were then homogenized for 20 sec with 500 ml of distilled water and four drops of Tween-20 (polyoxyethylene sorbitan monolaurate). The suspension contained approximately 5.5×10^6 spores and mycelial fragments per milliliter. The leaves of one-hundred eighty cereal plants, 15 plants per cultivar, in the three- to five-leaf stage were inoculated by dipping them into the fungal suspension. The plants were planted (five per pot) in 15.2-cm (6-inch) diameter clay pots. The inoculated plants and an equal number of noninoculated check plants were incubated in a sunlit mist chamber at 23 ± 5 C for 24, 36, 42, 48, 72, or 91 hr. Supplemental incandescent bulbs were used during winter trials. The mist chamber consisted of mist nozzles mounted above a glasshouse bench which was enclosed by a thin, transparent, plastic film suspended on a 120-cm (4-foot) tall aluminum scaffolding. Following the wet periods, the plants were dried with a fan and placed on a

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glasshouse bench at 23 ± 5C. Six days after inoculation any spots appearing on the plants were fully developed, and the plants were rated for percentage of leaf surface spotted.

All cereal selections were tested two or more times in each wet period for a total of 16 trials. The cereals tested were: the spring wheats (*Triticum aestivum* L.) Waldron (C.I. 13958), Red River 68 (C.I. 14193), ND495, Chris (C.I. 13751), and Duri (P.I. 106301); the durums (*T. turgidum* L.) Ward (C.I. 15892), Wells (C.I. 13333), and Hercules (C.I. 14559); one awned winter wheat (*T. aestivum*) cultivar WW8; barley (*Hordeum vulgare* L.) cultivar Larker (C.I. 10648); rye (*Secale cereale* L.) cultivar Caribou (C.I. 14005); and oat (*Avena sativa* L.) cultivar Lodi (C.I. 7561).

RESULTS AND DISCUSSION

In western North Dakota the fungus caused leaf spots on green wheat leaves of plants in the late-milk stage of development and produced conidia on wheat stubble. On PDA the fungus formed dense, prostrate, grey colonies, which sometimes developed small white mycelial tufts on the surface as they aged. Starting 41 to 50 days after placement on PDA the fungus produced small, scattered clumps of dark pycnidia with diameters ranging 132-235 ($\bar{X}$=196) μm, in the grey superficial mycelium and in the mycelium in the PDA beneath. The pycnidia were filled with pycnidiospores which were at first hyaline then changed to light brown [Table 1, Fig. 1-(D to I)]. On wheat stubble in the field the fungus produced scattered dark pycnidia with 3- to 7-septate pycnidiospores of dimensions corresponding with those described above and in Table 1. Starting 62 days after placement on PDA, some cultures of the fungus (including 1 and 3 used in pathogenicity tests) produced in the small scattered clumps, dark pseudothecia that ranged 98-224 ($\bar{X}$=162) μm in diameter. Each pseudothecium contained numerous asci with dimensions that ranged 34-106 ($\bar{X}$=71) × 11-13 ($\bar{X}$=12) μm. Each mature ascus usually contained eight ascospores [Table 1, Fig. 1-(A to C)].

The conidial (pycnidiospore) stage of this fungus most closely resembled *Phaeoseptoria urvilleana* which was distinguished from other *Phaeoseptoria* species in particular by its wider spores (18, 19, Table 1).

The sexual (3-septate ascospore) stage of this fungus resembled those of several fungi with very similar ascospores placed in the genera *Leptosphaeria* Cesati & de Notaris or *Phaeosphaeria* and found on cereals and grasses (1, 2, 11, 12, 13, 14, 15, 20, 21, Table 1). For these ascomycetes, Webster (20) found that only *Leptosphaeria microscopica* Karst. (Table 1) produces a *Phaeoseptoria*

TABLE 1. Ascospores and pycnidiospores of related *Leptosphaeria* spp. associated with wheat leaf spotting

Ascospores (3-septate unless otherwise described)	Pycnidiospores	Sources of information
20-26 × 4 μm, yellowish (*L. nodorum* Müller)	15-32 × 2.4 μm, hyaline, 0-to 3-septate [*Septoria nodorum* ((Berk.)) Berk.]	1,15,19
(rarely 16) 19-25 (rarely 28) × 4-6 μm, light yellow (*L. avenaria* Weber f. sp. *triticea* T. Johnson)	(rarely 18) 26-42 × (rarely 2.3) 2.8 - 3.5 (rarely 4.2) μm, hyaline, 3- (rarely 4-) septate (*S. avenae* Frank f. sp. *triticea* T. Johnson)	11
18-27 × 3-6 μm[a] [*L. eustomoides* Sacc. Syn. *L. tritici* ((Gar.)) Pass.]	13-44 × 2-3.5 μm[a], pale-brown, 3- to 7-septate[a] (Hendersonia types)	1,14,21
19-32 × 5-8.5 μm, pale-yellow to golden, 3-, (occasionally 4-, rarely 5-) septate (*L. microscopica* Karst.)	30-96 × 3.5-6 μm, yellow to brown, 3- to 15-septate, usually 7 (*Phaeoseptoria* species)	20
	50-85 × 2.8-4.8 μm, yellow to light brown, 8- to 11-septate (*P. festucae* Sprague)	18,19,20
	51-56 × 3.0-3.5 μm (later 60-75 × 2.5-3 μm), yellow to light-brown, 9- to 10-septate [*P. airae* ((Grove)) Sprague]	18,19,20
	20-90 × 4.5-6 μm, light brown 5- to 7-septate [*P. urvilleana* ((Speg.)) Sprague]	18,19
21-26 ($\bar{X}$=25) × 5-7 ($\bar{X}$=6) μm, hyaline to faintly colored (*Leptosphaeria microscopica*-like fungus causing leaf spots on wheat in North Dakota)	38-57 ($\bar{X}$=50) × 3-6 ($\bar{X}$=4) μm, light-brown, 0- to 9- ($\bar{X}$=6.4-) septate (*Phaeoseptoria urvilleana*-like fungus produced by *L. microscopica*-like fungus in North Dakota)	(This article)

[a]Composite of spore dimensions from several collections.

asexual stage (Table 1). He (20) found *L. microscopica* producing ascospores on many grasses and on cultivated wheat. He sent conidia of *L. microscopica* on *Festuca rubra* L. to R. Sprague, who identified the conidia as *Phaeoseptoria festucae* Sprague (18, 19, 20, Table 1). Webster noted that some pycnidiospores of *L. microscopica* resembled *Phaeoseptoria airae* (Grove) Sprague (20, Table 1). He suggested that some of the species of *Phaeoseptoria* distinguished by Sprague might represent conidia of *L. microscopica* on different hosts (20). Until these differences in conidia are resolved by host-range and conidial morphology studies, I am identifying the fungus causing leaf spots on wheat in western North Dakota as *L. microscopica* Karst., with the imperfect stage *P. urvilleana* (Speg.) Sprague.

Among *Leptosphaeria* sp. with similar 3-septate ascospores and found on cereals and grasses (1, 2, 11, 12, 13, 14, 15) there appears to be a group that causes leaf spots on wheat. Compilation of the literature suggests that in this group, increasing pathogenicity to more cultivars is related to decreasing pycnidiospore size, pigmentation, and septation. The member of this group with the smallest, hyaline, least-septate pycnidiospores, *Septoria nodorum* (Berk.) Berk. (19, Table 1) of *L. nodorum* Müller (1, 15, Table 1), causes the most damage to many wheat cultivars (17). The longer, hyaline, more-septate pycnidiospore type, *S. avenae* Frank f. sp. *triticea* T. Johnson of *L. avenaria* Weber f. sp. *triticea* T. Johnson (11), has damaged fewer cultivars (9, 11, 16, 17, Table 1). Similar, but more septate pale brown Hendersonia-type pycnidiospores have been related to *L. eustomoides* Sacc. *L. tritici* ((Gar.)) Pass.] (21, Table 1), which has been associated once with to leaf spotting (10). The larger, more septate, pale brown to brown *Phaeoseptoria*-type pycnidiospores (*P. festucae*, *P. airae* and *P. urvilleana*) have been related to *L. microscopica* (18, 19, 20, and this article, Table 1), which has been reported to cause leaf spotting on wheat only once (this article).

The leaf spots caused by *L. microscopica* were irregular, diffuse, first yellow then tan, and started to become visible to the naked eye 4 days after inoculation, two days subsequent to the shortest postinoculation wet

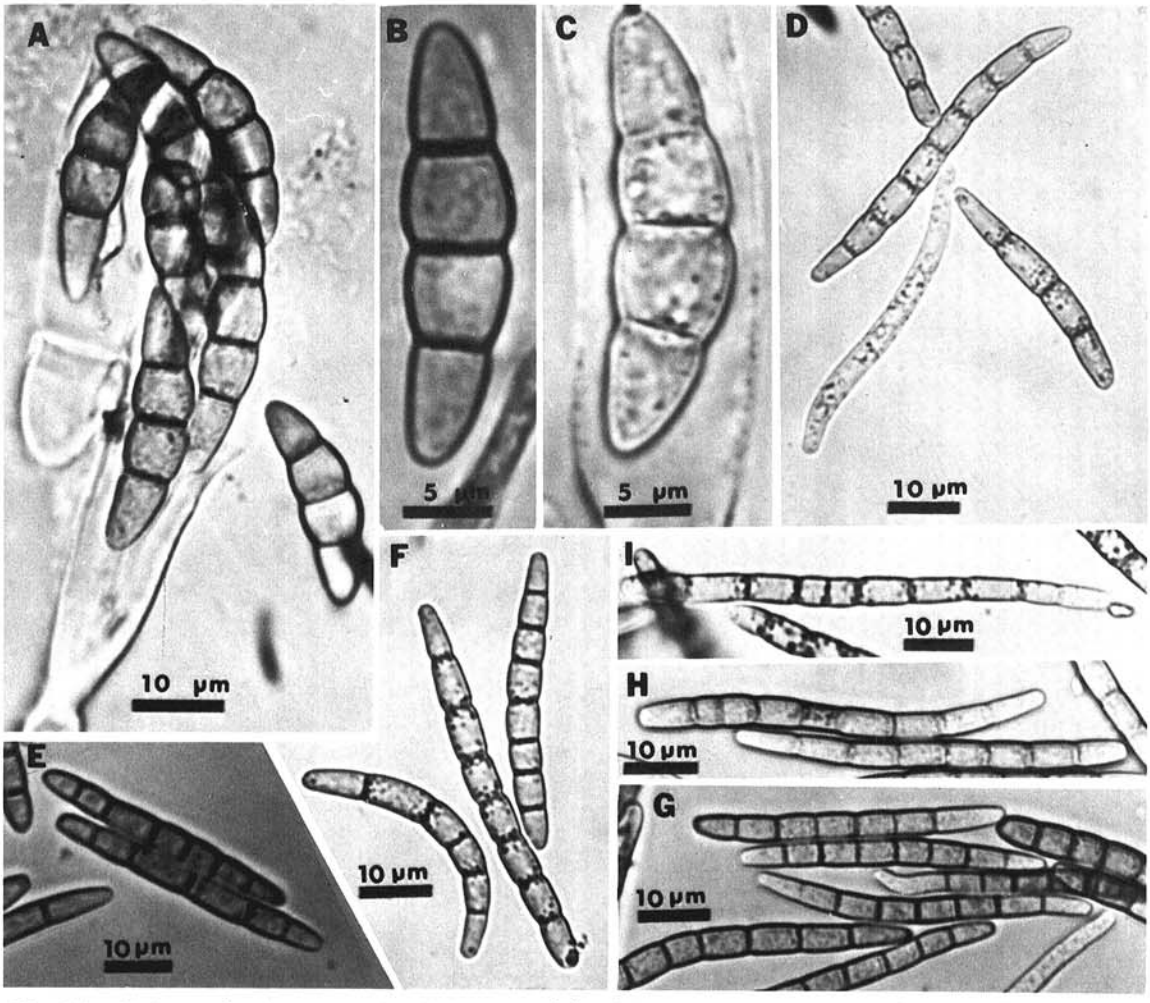

Fig. 1-(A to I). *Leptosphaeria microscopica.* **A)** Ascus containing eight spores. **B to C)** Ascospores. **D to I)** Aseptate to nine-septate pycnidiospores of imperfect stage *Phaeoseptoria urvilleana.*

period (48-hr) which resulted in leaf spotting. By 6 days after inoculation the spots had developed to their maximum expansion (Fig. 2). No differences were detected in spotting caused by the fungal isolates. Noninoculated check plants produced no spots. *Leptosphaeria microscopica* consistently was reisolated from the spots but not from nonspotted inoculated plants nor from check plants.

None of the cereals tested developed leaf spots following postinoculation wet (mist) periods of 24, 36, and 42 hr. After a 48-hr postinoculation wet period only Waldron and Red River 68 spring wheats became severely damaged with 30 to 50% of their leaf surface spotted. Following a 72-hr or longer wet period the ND495 spring wheat was severely spotted. Ward durum was slightly spotted with 1 to 5% of its leaf surface damaged. After a 91-hr wet period Ward continued to exhibit only slight spotting; no leaf spotting developed on Chris or Duri spring wheats, WW8 awned wheat (a selection out of Winalta winter wheat), Wells, and Hercules durums, Larker barley, Lodi oats, or Caribou rye. All of the twelve cultivars or selections were tested in all of the wet periods.

The change from no macroscopic leaf spotting after a long postinoculation wet period to severe leaf spotting after a longer wet period suggests a threshold effect of some type. This change occurs with several wheat leaf spotting fungi (5, 6, 7, 8). Preliminary investigations indicate that with one of these fungi, *Pyrenophora trichostoma* Fr. Fckl. (5), microscopic lesions occur after short wet periods on some wheat cultivars that do not show macroscopic spots until subjected to longer postinoculation wet periods (F. S. Holbrook, *personal communication*).

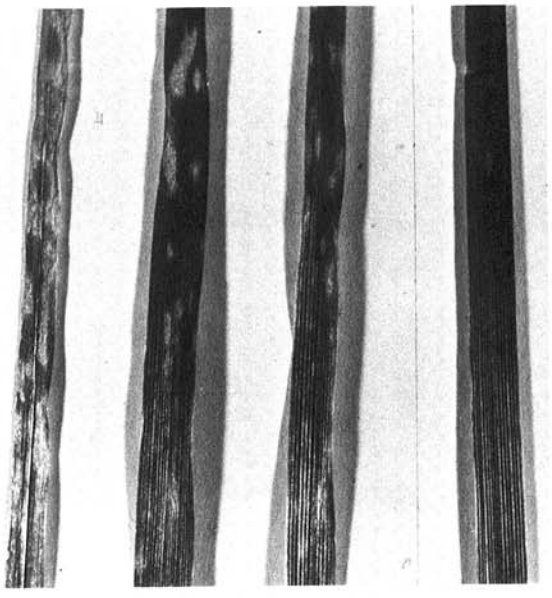

Fig. 2. Leaf spotting of wheat caused by *Leptosphaeria microscopica* following a 48-hr, postinoculation wet period. Left to right: three inoculated, spotted leaves and one noninoculated nonspotted leaf.

Leptosphaeria microscopica is present in several parts of the world (2, 12, 18, 20) but currently it appears to be only a minor pest. Its requirement of long periods of free water on the leaves to cause leaf spotting suggests that it might become a problem only on more susceptible cultivars in wetter seasons or in irrigated areas or in wetter wheat-growing areas. It is the seventh leaf-spotting fungus for which the expression of cultivar resistance in wheat has been related to the duration of the postinoculation wet period (3, 4, 5, 6, 7,8). It is the fifth fungus with which severe leaf spotting occurs after a long postinoculation wet period, indicating a threshold effect (5, 6, 7, 8, and this article). Differing resistance among wheat cultivars and other cereals to some of these fungi suggests that different genes for resistance are operative in the various hosts (5, 6, 7, 8).

Compilation of data from the literature suggests that among four wheat leaf-spotting *Leptosphaeria* spp. with similar three-septate ascospores (Table 1) increasing pathogenicity to more wheat cultivars (3, 4, 9, 10, 11, 16, 17, 19) is coupled to decreasing pycnidiospore size, septation, and pigmentation (1, 11, 14, 15, 18, 19, 20, 21, and this article).

Isolates 1 and 3 of *L. microscopica* will be deposited with the American Type Culture Collection.

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