

## Effects of Wetting Period on Resistance to Leaf Spotting of Wheat, Barley, and Rye by *Leptosphaeria herpotrichoides*

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The cooperation of H. B. Caldwell in reproducing photographs, R. L. Haspel in manuscript typing, Lillian Hjelmstad, K. Knutson, and P. Nolte in glasshouse and laboratory assistance, and R. A. Moreno and A. P. Roelfs in supplying infested wheat stubble for this study is gratefully acknowledged.

Published with the approval of the Director of the North Dakota Agricultural Experiment Station as Journal Series Article No. 779.

Accepted for publication 19 September 1977.

### ABSTRACT

HOSFORD, R. M., Jr. 1978. Effects of wetting period on resistance to leaf spotting of wheat, barley, and rye by *Leptosphaeria herpotrichoides*. *Phytopathology* 68: 591-594.

*Leptosphaeria herpotrichoides* was tested on plants of 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 and 36 hr. Following wet periods of 48 hr it caused severe leaf spotting on Waldron, Red River 68, ND 495, and Marquis spring wheats and on Larker barley and Caribou rye. Chris spring wheat was less severely spotted and Leeds durum was slightly spotted. After 72-hr wet periods Chris and Leeds were severely spotted and Hercules durum was moderately spotted. Following 96-hr wet periods Hercules was still only

moderately spotted. After 121-hr wet periods Hercules, Wells durum, and C306 spring wheat were severely spotted; Lodi oats was not spotted. This appears to be the first report of leaf spotting caused by *L. herpotrichoides*. The fungus requires a long postinoculation wet period to cause spotting. Expression of varietal resistance to leaf spotting was associated with the duration of the wet period. This phenomenon of varietal resistance, which is related to the duration of the postinoculation wet period, has now been detected with six leaf spotting fungal pathogens of wheat.

*Additional key words:* synonyms: *Phaeosphaeria herpotrichoides*, (de Not.) L. Holm, *Leptosphaeria culmifraga* (Fries) Cesati & de Notaris.

In the early 1900's *Leptosphaeria herpotrichoides* de Notaris commonly was related to foot rot of wheat and rye, especially in Europe. However, findings beginning in the 1930's indicated that it was a saprophyte or weak parasite and that *Cercospora herpotrichoides* Fron. was responsible for most of the foot rot previously attributed to *L. herpotrichoides* (18, 19, 20). Since the 1930's *L. herpotrichoides* occasionally has been reported to cause foot rot (14, 17), but continued definitive studies corroborating this have not been published. The fungus itself has been found reproducing on wheat, rye, numerous grasses, and related herbs (1, 2, 3, 4, 13, 15, 16, 21). It overwinters in the mycelial stage and as a few surviving ascospores (7).

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 relates to varietal resistance.

### MATERIALS AND METHODS

Pseudothecia of *L. herpotrichoides* containing clumps of immature and mature asci with ascospores (Fig. 1) were found beneath the surface of the culms of spring and winter wheat stubble in North Dakota and Minnesota since 1970. For pathogenicity tests, isolates 3, 6, and 10

were derived from clumps of asci containing ascospores from pseudothecia on straw of Cheyenne hard red winter wheat from Ramsey County, Minnesota. Leaf spotting was produced by 17- to 79-day-old isolates cultured on potato-dextrose agar (PDA). In most tests, four 56-day-old cultures of mycelium and ascospores covering the surface of PDA in petri plates were homogenized for 20 sec with 500 ml of distilled water and four drops of Tween-20 (polyoxyethylene sorbitan monolaurate). The suspension contained approximately  $4 \times 10^6$  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 of development were inoculated by dipping them into the fungal suspension. The plants were in 15-cm (6-inch) diameter clay pots, five plants per pot. The inoculated plants and an equal number of noninoculated check plants were incubated in a sunlit mist chamber at  $23 \pm 5$  C for 24, 36, 48, 72, 96, or 121 hr. Supplemental incandescent bulbs were used during part of the winter. The mist chamber consisted of misters (mist nozzles) on a glasshouse bench, which was enclosed by a thin, transparent, plastic film suspended on a 120-cm-tall aluminium scaffolding. Following the wet periods, the plants were dried with a fan and placed on a glasshouse bench at  $23 \pm 5$  C. Six days after inoculation, spots on the plants were fully developed, and the plants were rated for disease severity using a rating system developed for other leaf spotting fungi (9, 10, 11).

All cereal selections were tested two or more times in

each wet period for a total of 22 trials. The cereals were: the spring wheats (*Triticum aestivum* L.) Waldron (C.I. 13958), Red River 68 (C.I. 14193), ND 495, Marquis (C.I. 3641), Chris (C.I. 13751) and C 306 (P.I. 322275); the durums (*Triticum turgidum* L.) Leeds (C.I. 13768), Hercules (C.I. 14559), and Wells (C.I. 13333); Larker (C.I. 10648), barley (*Hordeum vulgare* L.); Caribou (C.I. 14005), rye (*Secale cereale* L.); and Lodi (C.I. 7561), oats (*Avena sativa* L.).

#### RESULTS AND DISCUSSION

On wheat stubble in the fields, the pseudothecia of *L. herpotrichoides* were individually scattered beneath the surface of the culm and contained ascospores with 2-9 septations. Five-septate ascospores, from field specimens, used to produce isolates 3, 6, and 10 for pathogenicity studies, produced ascospores with 4-8 septations on PDA. On this medium the fungus produced dense, prostrate, yellow-gray colonies that quickly turned gray, then dark gray. Approximately 34 days after placement on PDA, the fungus produced tiny scattered clumps of pseudothecia which by 60 days had expanded to irregular clumps 1-2 mm in diameter. Pseudothecia on wheat and

on PDA were golden brown to dark brown, subglobose, ostiolate, and contained both immature and mature asci. Pseudothecia ranged from 140 to 396  $\mu\text{m}$  in diameter and averaged 207  $\mu\text{m}$ . Mature asci (Fig. 1-A) ranged from 44-101  $\times$  6.2-9.9  $\mu\text{m}$  and averaged 73.3  $\times$  8.4  $\mu\text{m}$ . Ascospores ranged from 21-41  $\times$  3.3-7.6  $\mu\text{m}$  and averaged 29.6  $\times$  4.4  $\mu\text{m}$  and were 2-9 septate, with an average of 5.3 septations (Fig. 1-B to J). Ascospores often were enlarged at the second or third cells from the apical end (Fig. 1-B,C). Ascospores readily anastomosed using very short or longer germ tubes (Fig. 1-G,H). Most spores from nature and culture were shaped like those illustrated in Fig. 1-B to H. In culture, a few ascospores were shaped like those shown in Fig. 1-I, J. On the basis of the above characters, it was concluded that the fungus was *Leptosphaeria herpotrichoides* de Notaris (1, 3, 4, 5, 20).

The leaf spots caused by *L. herpotrichoides* were irregular, diffuse, yellow-to-tan, and started to become visible to the naked eye 4 days after inoculation, 2 days subsequent to the shortest postinoculation wet period (48 hr) which resulted in leaf spotting. By 6 days after inoculation the spots developed to maximum expansion (Fig. 2). No differences were detected in spotting caused by the three fungal isolates. Noninoculated check plants

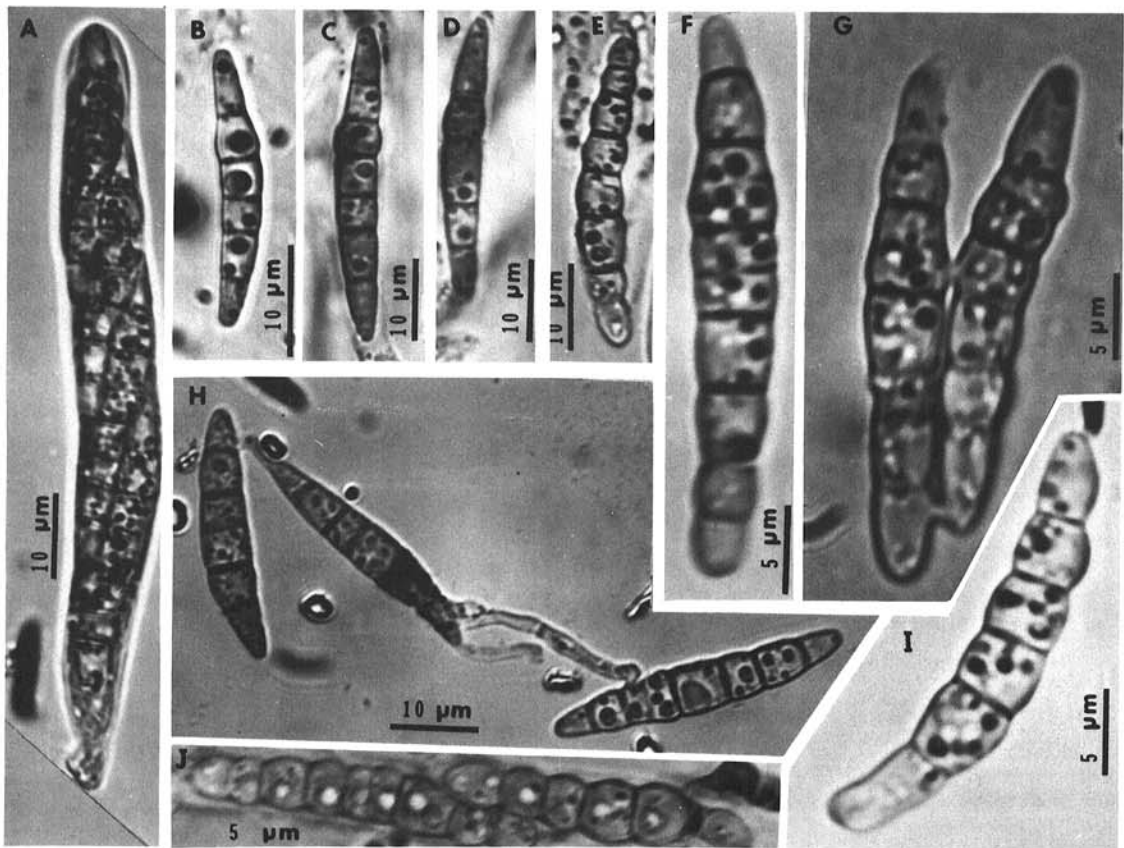


Fig. 1-(A to J). Asci and ascospores of *Leptosphaeria herpotrichoides*. A) Ascus containing eight spores. B to J) Ascospores, 4-8 septate, second or third cell from apical end often enlarged. G and H) Ascospore anastomosis. I and J) Atypical ascospores, produced occasionally in culture.

produced no spots. *Leptosphaeria herpotrichoides* 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 and 36 hr. After a 48-hr postinoculation wet period only Waldron, Red River 68, ND 495, and Marquis spring wheats, Larker barley, and Caribou rye became severely damaged, with 30 to 50% of their leaf surfaces spotted. The spring wheat cultivar Chris was less severely damaged with 20-25% of the leaf surface spotted. The durum cultivar Leeds was slightly spotted with only 1-5% of the foliage covered by spots. Following a 72-hr wet period Chris and Leeds were severely (30-50%) spotted and Hercules durum was moderately spotted with 10-15% of its leaf surface damaged. After 96 hr in mist Hercules continued to exhibit only moderate spotting. After 121 hr in the mist chamber Hercules, Wells durum, and C306 spring wheat were severely spotted (30-50%) but Lodi oat plants were not spotted. All of the twelve cultivars or selections were tested in all of the wet periods. Some of the

above results represent reductions of earlier reported (12) exposure to mist required for leaf spotting to develop on particular cultivars.

The requirement of this pathogen for long periods of free water on the leaves to cause leaf spotting suggests that it might become a problem only in wetter seasons and wetter, wheat-growing areas on more susceptible cultivars. It is present in many parts of the world (1, 2, 3, 4, 5, 13, 14, 17, 20, 21) but currently appears to be a minor pathogen. It is the sixth leaf-spotting fungus for which the expression of cultivar resistance in wheat has been related to the duration of the postinoculation wet period. Individual cultivars displayed differential resistances to leaf spotting from each of these six fungi (6, 8, 9, 10, 11). This suggests that different genes for resistance to these leaf spotting fungi are operative in the various cereals and wheat cultivars.

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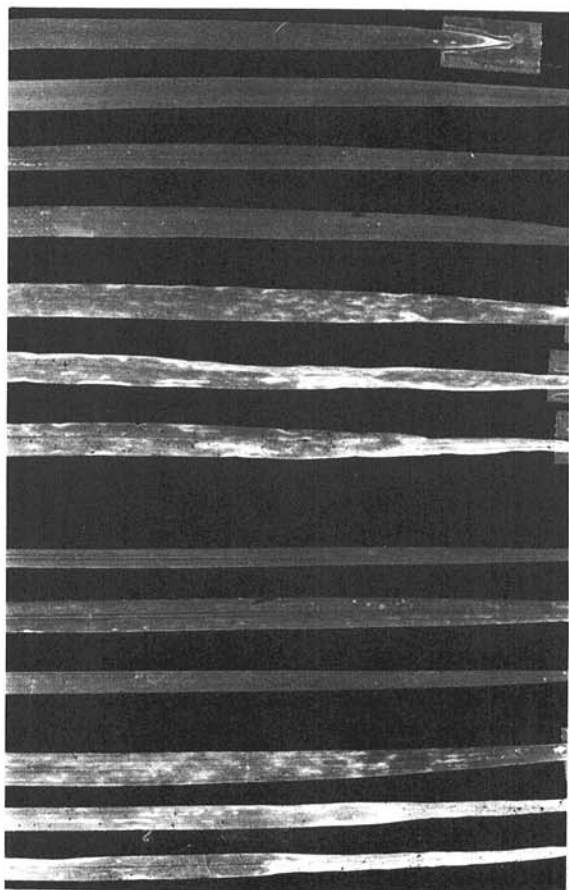


Fig. 2. Leaf spotting of wheat caused by *Leptosphaeria herpotrichoides* following a 48-hr, postinoculation wet period. Top to bottom: Four noninoculated leaves and three inoculated leaves of Red River 68 spring wheat and three noninoculated leaves and three inoculated leaves of Waldron spring wheat.

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