A Form of Pyrenophora trichostoma Pathogenic to Wheat and Other Grasses

Robert M. Hosford, Jr.

Department of Plant Pathology, North Dakota State University, Fargo 58102.

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ABSTRACT

A form of *Pyrenophora trichostoma* was severely pathogenic on wheat in North Dakota in 1968 and 1969. It caused light-brown lesions with distinct yellow halos on leaves, and formed abundant ascostromata on stubble. In the glasshouse, the fungus was pathogenic on common and durum wheats, other *Triticum* species, *Agropyron* species, brome grass, and rye; slightly pathogenic on barley; and not pathogenic on *Elymus junceus*, oats, corn, alfalfa, and flax. *Triticum turanicum* P.I. 184526 was highly resistant to all isolates. North Dakota spring wheat line 495 was very susceptible. Wheat cultivars varied in resistance, and isolates of the fungus varied in pathogenicity. Resistance in wheats was related to duration of free moisture on the leaves. At 23 C, the fungus produced immature ascostromata and characteristic dark mycelial columns on several media. At 16 C, it formed mature ascostromata on autoclaved wheat seed. Observations were used to outline a disease cycle. Phytopathology 61:28-32.

Severe leaf spot damage has occurred on the foliage of wheat growing in North Dakota, particularly in wet years. This damage was attributed to adverse weather and the fungi, *Helminthosporium sativum* and *Septoria* species. In studies begun in 1967, it was found that *H. sativum* and *Septoria avenae* f. sp. *triticea* were causing slight to severe loss of leaf surface (5). Beginning in 1968, however, it became apparent that the most prevalent foliar damage was related to a light-brown leaf spot with a distinct yellow border. A correlation was observed between the sexual fruiting bodies of a fungus on wheat stubble and these spots on growing wheat. The present study shows that the fungus on the stubble causes the spots, identifies the fungus, indicates sources of resistance, characterizes the growth and reproduction of the fungus in culture, examines potential host range and variable pathogenicity, and outlines a disease cycle.

MATERIALS AND METHODS.—Diseased wheat plants were collected throughout the growing seasons from farms and branch experiment stations in North Dakota in 1968 and 1969. Fungal cultures were isolated from leaf lesions by washing green leaves in running tap water for 15-30 min and placing lesion-containing sections of the leaves on agar culture media in petri plates. The media used were potato dextrose, malt, oatmeal, cornmeal, lima bean, V-8 juice, Trappe, Hagen's, and mycophil. Cultures also were obtained by single sporing ascospores from ascostromata on wheat stubble. Cultures and subcultures were maintained in covered plastic boxes at approx 23 C to retard drying of the media. To promote fructification, subcultures from leaf lesions were grown at 16 C on autoclaved wheat seed on water agar.

For pathogenicity tests, cultures of four leaf spot isolates from three different areas of the state and nineteen single ascospore isolates from six different areas of the state were grown on media for 20-25 days. Individual cultures were then blended for 20 sec with 125 ml of distilled water, and the suspension was mixed with one drop of Tween 20 (polyoxyethylene sorbitan monolaurate) in an Erlenmeyer flask.

Two-leaf stage to heading plants were dipped in a mycelial suspension. The number of plants dipped in a given inoculum ranged from eight larger plants to 120 smaller plants. Tests were repeated at least once with each plant variety and fungal isolate. Inoculated plants were placed in a mist chamber in which free water was maintained on the leaves for 6 to 48 hr or 2 to 5 days. Temp in the chamber was 23 ± 5 C. After incubation in the mist chamber, all plants were transferred to an open bench in the glasshouse. Reisolations of the fungus were made from lesions appearing on susceptible plants.

Plants were rated for disease incidence and severity 5 days after inoculation. Similar ratings were made of disease severity from natural inoculum in the field. The system developed to rate disease severity on leaves in both glasshouse and field is outlined in Table 1.

When the plants in glasshouse tests had turned brown and dry, lesioned and unlesioned leaves were placed on moist paper towels in petri plates in covered plastic boxes to promote fructification. These leaves were maintained at 23 C for 1-2 months and examined periodically for fruiting bodies.

RESULTS.—*Culture and identification.*—Cultures of similar morphology on potato-dextrose agar (PDA) were obtained from approx 400/450 fungal isolates and reisolates from lesions on wheat leaves and from 21/21 single spore isolates from ascostromata on wheat stubble. When grown for 20 or more days at 23 C on PDA, malt agar, or mycophil, the isolates produced thick, tufted, gray to grayish-white mycelia. Characteristic large, thick, black columns of mycelium were formed on the inner glass walls of the petri plates (Fig. 4). These columns became a useful diagnostic tool for identifying the fungus on these media. On oatmeal, cornmeal, lima bean, and V-8 juice agar, the isolates produced a sparser, gray to grayish-white mycelium, infrequent black columns, and numerous immature ascostromata in and on the media. Similar black immature ascostromata were produced in lesioned wheat leaves after incubation for 30 to 60 days at 23 C in a moist chamber. Ascostromata did not form on noninfected leaves in the moist chamber. On Trappe medium,

TABLE 1. Ratings for disease caused by *Pyrenophora trichostoma*

Reaction	Numerical rating	Lesion			
		Type	Size, mm	No./leaf	% Foliage damaged
Immune	1	No lesions			
Resistant	2A	Brown fleck	Less than 1	2-8	Under 1
Resistant	2	Light brown with yellow halo	1-2	0.2-1	Under 1
Moderately resistant	3	Light brown with yellow halo	2-3	2-5	2-5
Moderately susceptible	4	Light brown with yellow halo	3-6	6-15	10-20
Susceptible	5	Light brown with yellow halo and coalescing	Greater than 6	Over 10	30-75

the isolates produced a dense, fluffy, grayish-white mycelium with no black columns or ascostromata. On Hagen's medium, the isolates produced flattened, slow-growing, brownish-gray colonies composed of short cells. Hyphal anastomoses and the most abundant production of immature ascostromata were observed on oatmeal agar. Following transfer, subcultures maintained on PDA frequently changed into white, reddish-white or bright orange, often slower-growing, colonies (Fig. 4). Subcultures grown on autoclaved wheat seed at 16 C for 47 days produced immature ascostromata containing white cells and mature ascostromata containing asci and ascospores. The appearance and dimensions of the ascostromata, asci, and ascospores on wheat stubble in the fields and on wheat seed in culture were similar to Wehmeyer's (12) description of *Pyrenophora trichostoma* (Fr.) Fckl. One ascus did contain four unusually large spores (75-82 × 30-33 μ). All other fertile asci contained 2-8 spores of sizes within the described size range. No asexual reproductive stage was found in nature or culture in association with the ascostromata, leaf lesions, or isolates.

Host range and pathogenicity in the glasshouse.— Inoculations with mycelial cultures from two leaf lesions and from six single ascospores grown on oatmeal, mycophil, malt, or PDA resulted in severe leaf lesions (rating 5) on 14 wheat cultivars and breeding lines. No difference in pathogenicity among the isolates grown on the different media was found, and PDA was selected as the standard medium for subsequent pathogenicity studies.

Oat, barley, and wheat cultivars and breeding lines were inoculated with 2 virulent fungal isolates (W2 and W20) to determine the length of time required in the mist chamber for disease development. These plants were maintained in the mist chamber for 6, 12, 18, 24, 30, 36, 42, or 48 hr, and the trials were repeated 4 times. In additional tests, plants were held in the mist chamber for 2, 3, 4, or 5 days. The disease became severe (Fig. 1) on North Dakota spring wheat breeding line 495 after only 6-hr incubation in mist following inoculation. Waldron wheat required 12 hr in mist for severe disease development, Chris wheat required 18, and Leeds durum, 36 hr. The time required for disease development varied with the fungal isolates and the tests. In all tests, however, the relative varietal reaction stayed within the order outlined above. Small brown flecks (rating 2A) developed on Larker barley inoculated with isolate W2 after 18 hr in the mist. No lesions developed on Larker inoculated with isolate W20 and maintained for up to 48 hr in the mist. Small brown flecks (rating 2A) developed in Dickson barley inoculated with isolate W20 after 36 hr in mist, but did not develop with isolate W2. No lesions developed on Lodi oats inoculated with either isolate or on check plants (Fig. 2) inoculated with blended PDA. In all tests, the leaf lesions began to appear 2 days after inoculation and were fully expressed in 5 days. Disease severity did not appear to increase when the plants were held for longer than 48 hours in the mist chamber.

In 48-hr mist incubation, 23/23 fungal isolates were virulent (rating 5) on 10 or more wheats. Isolates Py1, W1, W2, and W20 were virulent on all tested wheats and on some other cereals and grasses (Table 2). Isolate PyM12 was virulent (rating 5) on the cross *T. araraticum* Jakulz.-*T. monoccum* L., but isolates W2, W20, PyW2-2, and PyW4 were not (rating 1-2). Re-

TABLE 2. Reaction of plants to inoculation with *Pyrenophora trichostoma* followed by 48-hour mist incubation

Plant	Rating[a]
Avena sativa, Elymus junceus, Linum usitatissimum, Medicago sativa, Zea mays	1
Hordeum vulgare—Dickson, Larker	2A
Triticum turanicum P.I. 184526	3
T. turanicum P.I. 113392, *T. turgidum* v. *lusitanicum, T. monococcum*	4
T. aestivum—Bulgaria, Chris, C306 (India), C518 (Pakistan), Lerma 50, Manitou, Marquis, North Dakota 487 and 495, Polk, Red River 68, Selkirk, Thatcher, Waldron	5
T. durum—Hercules, Lakota, Langdon, Leeds, Mindum, North Dakota D6750, Wells	
T. araraticum, T. carthlicum, T. dicoccoides, T. macha, T. orientale, T. palecolchicum, T. pyramicale, Agropyron desertorum, A. intermedium, A. repens, A. smithii, Bromus inermis, Secale cereale.	

[a] = 1-Immune; 2,3-resistant; 4,5-susceptible; A-fleck reaction.

sistance was present in *T. turanicum* Jakulz. P.I. 184526, which rated 1 with isolates PyW2 and PyW2-9, 2 with Py1 and PyP18, and 3 with W2 and PyD10. Extreme susceptibility was found in North Dakota spring wheat breeding line 495, which had three-fourths of its foliage destroyed by 10|14 isolates.

Field observations.—Ascostromata were found on the prior year's wheat stubble (Fig. 3) throughout North Dakota in the spring and summer of 1968 and 1969. They were most prevalent wherever continuous wheat cultivation and stubble-mulch fallow were practiced. No leaf lesions caused by this fungus were found on wheat seedlings from emergence to the two-leaf stage. Mature asci were present in ascostromata by early June 1969, and lesions began to appear on the leaves of three-leaf-stage wheat seedlings. Lesions continued to appear on new leaves as they developed, and lesions increased in number and size until they coalesced on the leaves. On young seedlings, the frequency of lesions coincided with prevalence of ascostromata on adjacent stubble. In 1968 and 1969, at Williston, N. D., the disease steadily progressed on growing wheat plants, and by the early milk stage of kernel development, the lesions were causing considerable damage to all leaves, including the flag leaf.

During the summer of 1969, severity of this disease was rated at several locations in North Dakota. Many wheat cultivars at the Williston Experiment Station developed lesions (disease rating 4-5) on the leaves of plants from the three-leaf-stage to heading. This contrasted with low ratings, 1-3, prior to heading at the Fargo, Carrington, Minot, and Dickinson Experiment Stations. In this wet year, the disease was severe throughout the state during the period of early heading, destroying over half the foliage of some wheat cultivars. The North Dakota spring wheat breeding line ND495 had three-fourths of its foliage destroyed (rating 5) by early heading at Williston, Dickinson, and Langdon. At early heading at Williston and Langdon, most spring wheat and durum cultivars and breeding lines had disease ratings between 3-5. Waldron rated 4-5, Chris 3-4, Leeds 3-4, and Manitou and Wells rated 1 at Williston and 4 at Langdon. Following early heading in the field, damage caused by this fungus increased through the late dough stage of heading, but in many areas became difficult to distinguish because of the appearance of similar damage related to the *Septoria* stage of *Leptosphaeria avenaria* Weber f. sp. *triticea* T. Johnson (5). Dickson barley and wild oats, *Avena fatua* L., were rated 1 when observed growing near or in severely damaged wheat fields.

Discussion.—Several different fungi have been called *Pyrenophora trichostoma.* Wehmeyer (12) grouped 10 species of fungi of similar sexual morphology but differing conidial stages and grass hosts (10) in the species *P. trichostoma.* Shoemaker (7) maintained these fungi as separate species. Where temp requirements were studied, it was found that a cold period lasting 2-4 months was required for maturation of their ascostromata (6). Grey (4) reported the mating of two of these fungi, *P. teres* and *P. avenae.*

Little is known about the members of this group which parasitize wheat. *Pyrenophora tritici-repentis* was reported to cause a severe disease of wheat called "yellow leaf blotch" in Canada and India (2). In the Northern Great Plains of the USA, it was thought to be saprophytic on wheat (10). A similar fungus, *P. tritici-vulgaris,* was reported to cause leaf spots on wheat in eastern and central USA, Japan, and Germany (2, 3). Conners (2) and Shoemaker (8) considered these two fungi to be the same fungus. Simmons (9) and Wehmeyer (11) reported a homothallic strain of *P. trichostoma* on wheat straw in Kansas that did not produce conidia.

The *P. trichostoma* of this study is similar to several reported fungi. Isolates sent to W. C. McDonald have produced conidia of the *Helminthosporium* imperfect stage of *P. tritici-repentis* or *P. tritici-vulgaris* when grown on V-8 juice agar in a 12-hr photo period (W. C. McDonald, *personal communication*). The fungus is pathogenic to *Agropyron* species, as is the similar *P. tritici-repentis* (10); to rye, as is *P. secalis* (13); and to brome grass, as is *P. bromi* (1, 10). Unlike *P. tritici-repentis* (10), it is not pathogenic to *Elymus junceus.* The similar *P. secalis* has been reported only on rye (13). *Pyrenophora bromi* has been reported to attack brome grass but not to attack wheat, rye, barley, or *Agropyron* (1). Because of the similarity in descriptions, it is very probable that the fungus of this study has been labeled *P. trichostoma, P. tritici-repentis,* and *P. tritici-vulgaris,* and should be called the earliest applied name, *P. trichostoma.* The fungus described herein is designated as a strain of *Pyrenophora trichostoma* pathogenic on wheat, *Agropyron* species, brome grass, and rye, slightly pathogenic on barley, but not pathogenic on oats, *Elymus junceus,* corn, alfalfa, or flax.

The results of this study show that this fungus is an aggressive parasite of *T. aestivum* and *T. durum* in North Dakota, causing moderate to severe damage to foliage of these species. Substrain differences for pathogenicity exist within its population, and differences in resistance exist among susceptible host plants.

Varietal reactions to *P. trichostoma* correlated well between glasshouse and field. ND495 was the most susceptible cultivar tested and required the shortest incubation period, under 6 hr with free water on the leaves, for severe disease development. Other cultivars, such as Chris, required longer incubation periods for

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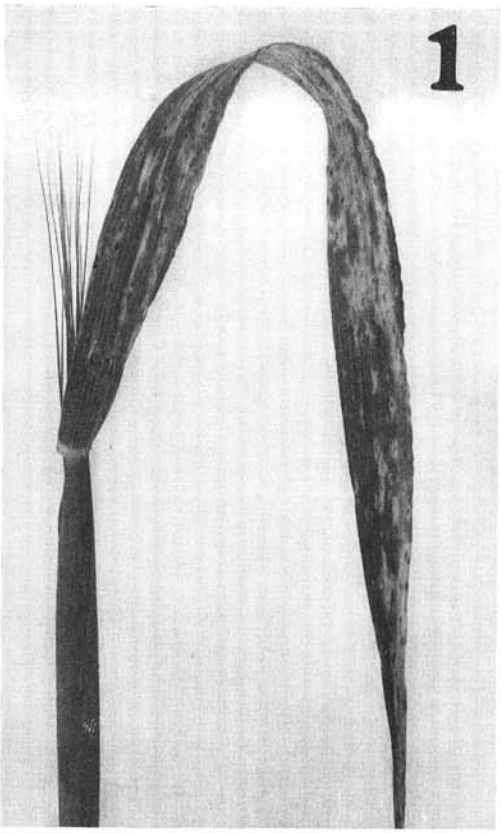
Fig. 1-4. 1) Lesions on wheat flag leaf inoculated with aqueous suspension containing *Pyrenophora trichostoma.* **2)** Healthy wheat; flag leaf inoculated with aqueous suspension not containing *Pyrenophora trichostoma.* **3)** Ascostromata of *Pyrenophora trichostoma* on wheat stubble. **4)** Upper plate: typical gray and grayish-white fluffy mycelial growth of *Pyrenophora trichostoma* on potato-dextrose agar (PDA) with characteristic dark mycelial columns developing on inner glass wall. Lower plate: white prostrate colony that frequently develops from subcultures on PDA. Photographs by J. M. Olson and J. M. Wayman.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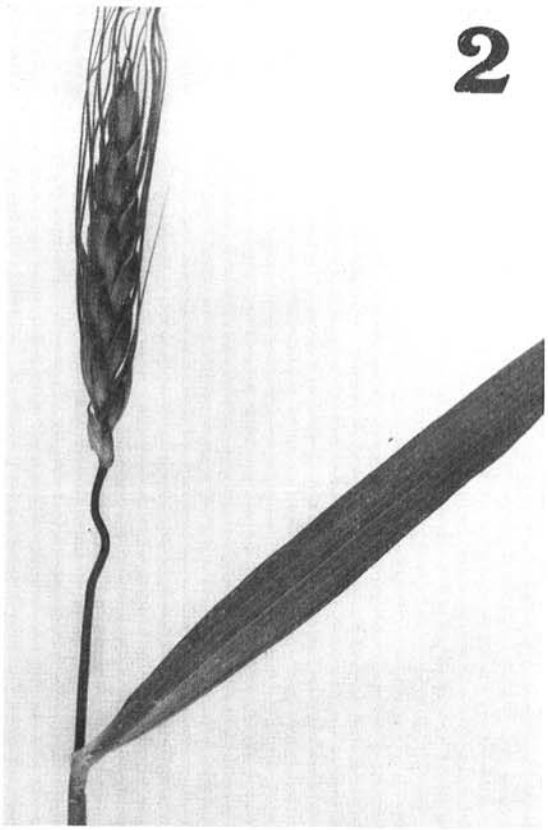
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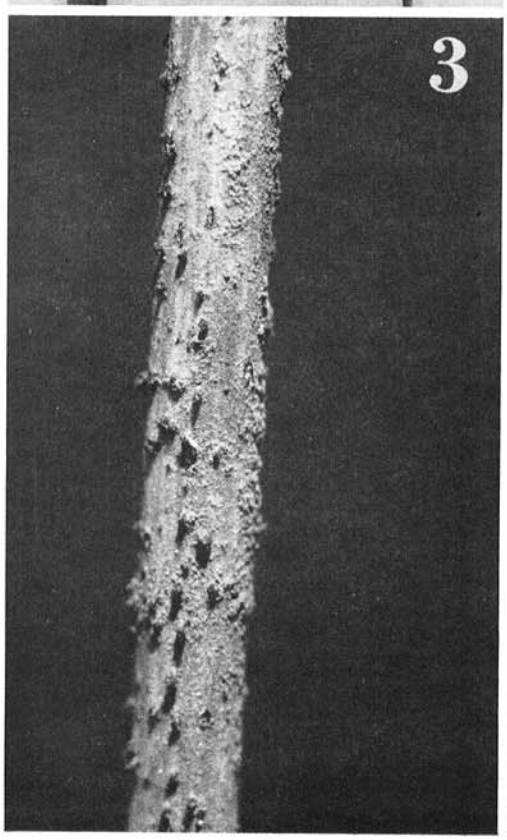
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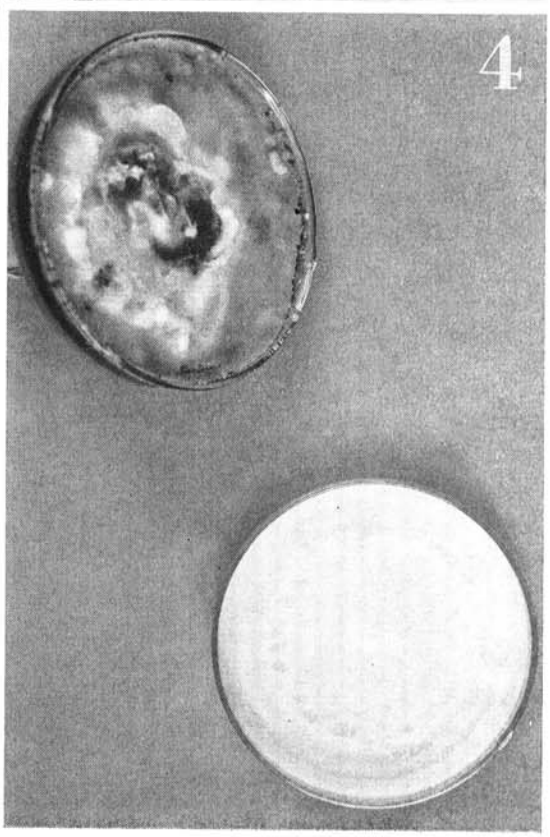
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disease development. This indicated a varietal difference in resistance among wheats, expressed under different incubation periods. This difference in incubation period would explain the high disease ratings on ND495 at all locations in the field and the variable ratings on Chris, Leeds, and other less susceptible wheats.

In contrast to ND495, *T. turanicum* P.I. 184526 appeared to be the most resistant of the *Triticum* species, maintaining a high degree of resistance throughout the 48-hr incubation period. Other *Triticum* species and wheat cultivars appear to be intermediate between these two extremes.

The severe pathogenicity of the fungus on *Triticum* species other than wheat, *Agropyron* species, brome grass, and rye in the glasshouse suggests that these plants may also be hosts of the fungus in nature. Barley was resistant to this parasite and is, therefore, a questionable host. Wild and cultivated oats, corn, *Elymus junceus*, alfalfa, and flax were not affected by this fungus.

The epidemic which occurred in North Dakota in 1969 was probably due not only to the wet weather but also to the increase in wheat stubble on the ground. Lush growth during recent wet years and the development of cultural practices that leave stubble on the soil surface have favored high levels of inoculum.

The following disease cycle is proposed. In spring and summer, the ascostromata mature on wheat stubble and discharge ascospores that infect wheat leaves. In both this study and that of Conners (2) in Canada, this was found to begin in June. Number and size of leaf lesions are related to duration of available moisture from rain and dew and to susceptibility of the host. This would account for the severe foliar leaf spotting observed during or following periods of wet weather and during wet years. The fungus invades the culm and overwinters in the wheat stubble. A cool period appears to be required for maturation of ascostromata which form on leaves and culms. During spring and summer, ascostromata discharge ascospores that infect the new wheat crop. The *Helminthosporium* conidial stage may be a source of inoculum. While it has not been observed with the disease lesions or sexual fruiting bodies in North Dakota, it has been periodically observed on wheat and in the air. Conners (2) found it occurring sparsely in old diseased lesions in Canada. Rye, *Agropyron* species, brome grass, *Triticum* species other than wheat, and other wild grasses may be additional hosts.

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