

## Ascochyta tritici on Wheat

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### ABSTRACT

*Ascochyta tritici* was identified on wheat leaves collected in 11 localities of Pennsylvania and New York in 1969. Nine cultures of the fungus were compared with six isolates collected in Ohio and Pennsylvania in 1968. All were designated "atypical *Septoria nodorum*" at that time. All isolates were stable in cultural characters, with a strong tendency to produce papillate, warty pycnidia. *Ascochyta tritici* isolates varied in pathogenicity when inoculated on 12 lines of wheat. *Ascochyta tritici* was a weaker pathogen than *Septoria nodorum*, producing

*Additional key words:* *Triticum aestivum*.

less severe symptoms and fewer spores under similar conditions. Oats, barley, rye, corn, and triticale were inoculated with *A. tritici* and *S. nodorum* under greenhouse conditions. All hosts, except triticale, were less severely infected than was wheat, based on symptoms and numbers of spores produced. Redcoat wheat grown in soil collected from the same fields where leaf samples were taken failed to exhibit any symptoms of disease. *Phytopathology* 61:675-680.

An unusual fungus pathogen of wheat, *Triticum aestivum* L., was isolated from wheat leaves collected in Pennsylvania and Ohio in 1968. The causal agent was tentatively identified as an atypical strain of *Septoria nodorum* (Berk.) Berk. (6, 8), but its true identity was later determined to be *Ascochyta tritici* Hori & Enjoji (3). Examination of the literature revealed that *A. tritici* had been described in Japan (3) and briefly referred to by Sprague (11), but insofar as we were able to determine, this is the first confirmed report of *A. tritici* on wheat in North America.

Several leaf collections from New York and Indiana, also made in 1968, were examined but yielded only *S. nodorum* and *Septoria tritici* Rob. & Desm. Despite these negative results, symptoms on wheat made us suspect that the disease now known to be caused by *A. tritici* was probably widespread in the area. In June 1969, wheat leaves and soil samples were collected from wheat fields in several parts of Pennsylvania and New York. Although symptoms were mild compared with the previous year, several cultures of *A. tritici* as well as *S. nodorum* were isolated from wheat leaves. Isolates of *A. tritici* and *S. nodorum* collected in both 1968 and 1969 were included in experiments designed to compare the cultural and pathogenic variability and host range of the two pathogens.

**MATERIALS AND METHODS.**—Sixteen collections of wheat leaves were made near the following locations: East Bloomfield, Ithaca, and Buffalo, N. Y.; Landisville and University Park, Pa. Eight soil samples were collected near University Park and State College, Pa.; Ithaca, Bloomfield, and Avon, N. Y.

Leaf samples from each location were surface-sterilized with 1:1,000 mercuric chloride and plated on water agar at 20 C. *Ascochyta tritici* was identified by its characteristic pycnidia and spores. After identification of the pathogen on leaf samples, spores were photo-

graphed and measured. Isolates were taken from several samples for further study.

As soon as the 1969 soil samples were returned to Beltsville, they were placed in 6-inch clay pots. Six soil samples from 1968, collected near State College and Centre Hall, Pa., were also included in the group. The 1968 samples had been used to grow wheat previously.

Redcoat, C.I. 13170, wheat was planted, 5 seeds/pot, in all the soil samples. Seeds were planted in sterile greenhouse soil as a control. Pots were placed in a growth room maintained at 10 C, with an 8-hr day, 1,200-1,500 ft-c at plant height. Two months after planting, the temperature was raised to 15 C. Three months after planting, the light period was lengthened to 10 hr, and plants were fertilized. Three and one-half months after planting, the light period was lengthened to 12 hr and the temperature raised to 20-21 C. This scheme was suggested by N. C. Stoskopf (Scharen, *personal communication*) as having been successful in producing symptoms of what was then called wheat variegation.

**Fungus stability.**—Except for the use of yeast-extract agar instead of PDA, the cultural conditions for fungus growth were as described previously (8).

Cultures were maintained and kept actively growing with mass spore transfers approx every 2 weeks. When cultures were not being used, they were stored in the dark at 4-5 C.

Two 1968 isolates, one from Pennsylvania and one from Ohio, were used to check stability of the fungus in culture. From each culture, beaked and nonbeaked pycnidia were selected. Twelve selections, 20 single-spore isolates/selection, were made from the Pennsylvania isolate and 13 from the Ohio isolate, making a total of approx 500 cultures.

Beaked and nonbeaked pycnidia were found on one

leaf of Genesee, C.I. 12653, wheat from Ithaca, N. Y. collected in 1969. Isolates were made of each type. Single-spore subcultures were made from each original isolate and carried through four culture generations, 12 single spore isolates/generation, giving a total of approx 96 isolates. The method for determining the number of spores per slant culture was reported previously (9, 10).

Six inoculations were made with a 1968 Pennsylvania isolate (P-6) on seven wheat cultivars: Asosan, C.I. 12665; Wisc. Selection, C.I. 12632; Hadden, C.I. 13488; Little Club, C.I. 4066; Nainari 60, C.I. 13747; Gaines, C.I. 13448; and Redcoat. From each inoculation, leaf samples were selected at random from each variety and plated on water agar. Twenty-five isolates were made from each group of leaf samples. Over a period of 2 months, 150 isolates were made; 25 each from Asosan, Hadden, Little Club, and Nainari 60, and 50 from Redcoat.

*Pathogenicity.*—Plants were inoculated with spore suspensions as described previously (8). Inoculum (18-48 million spores/ml) was sprayed on plants until it dripped from the leaves.

To check the pathogenicity of the different isolates of *A. tritici*, five isolates were used: (i) P-6, Penna., 1968; (ii) P-5, Ohio, 1969; (iii) P-11, New York, 1969; (iv) P-12, Penna., 1969; and (v) P-13, Penna., 1969, to inoculate 12 cultivars of wheat. Wheat cultivars were the seven mentioned above, plus Avon, C.I. 13477; Pennoll, C.I. 12755; Genesee; Yorkwin, C. I. 11855; and Yorkstar, C.I. 14026. One *S. nodorum* isolate was used for comparison. All plants were inoculated twice with each isolate. Forty seedlings of each cultivar were used for each inoculation; 20 control and 20 inoculated. If poor infection was obtained on the first inoculation with an isolate, the length of incubation and spore load in the inoculum were increased on the second inoculation.

*Host range.*—The following wheat, rye, barley, and oats species and lines were inoculated in the adult plant stage with culture P-6 of *A. tritici*: Wheat-Wisc. Sel. and Asosan; wheat-rye hybrids, Triticale 6TA202, C.I. 14498 and Triticale 6TA204, C.I. 14499; oats, Ascencao, C.I. 7146, and Florida 500, C.I. 8023; barley, Kindred, C.I. 6969, and B112, C.I. 11531. For seedling inoculations, the following were used in addition to those listed above: wheat, Hadden, Little Club, Gaines, Nainari 60, Redcoat, and Reed, C.I. 13513; rye, Tenn. 4063, C.I. 29; Triticale, 6TA131, C.I. 14501; and one unidentified experimental line of corn.

Adult plants were inoculated as previously described (8). After 15 days, leaf samples from among the three youngest diseased leaves, 3 cm<sup>2</sup> each, were surface-sterilized and plated on water agar. After incubation for 10 days, the leaf pieces were shaken in 10 ml water for 30 min, then allowed to stand for 2 hr. The leaf pieces were again shaken briefly before an aliquot was removed for counting of spores in a hemocytometer.

The amount of damage to seedlings was determined as necrosis of the first and second leaves of inoculated plants in comparison with controls.

*Other inoculation methods.*—Bits of agar containing

*A. tritici* were placed on sterile wheat seeds on moist filter paper in petri dishes. After an incubation period of 1 week, the fungus usually covered the seed but normally had not interfered with germination and seedling development. Seedlings were then planted in sterile and nonsterile soil in the greenhouse. Also, the fungus was grown on sterile chopped green leaves and stems of wheat, then mixed with sterile soil. Redcoat seed was planted in this soil, and plants were grown at 15-22 C, and 12 hr day.

*RESULTS.*—As a basis for comparison with our material, the original description of *A. tritici* as translated from the Japanese (3) is as follows: [*Ascochyta tritici* Hori & Enjoji, sp. nov. Infected spot: ellipsoidal, round to spindle-shaped, 1-5 mm long, 0.5-3 mm wide, at first purplish brown, the central part changing from ashy brown to ashy white, concentric circles lacking. Pycnidia: spherical to flattened, 105-200 × 118-240 μ, light yellow brown to dark brown to black, buried beneath the epidermis and opening at the surface by a warty or papillate projection, clustered or scattered. Conidiospores: long ellipsoidal, cylinder or cocoon-shaped, 3-6.5 × 14-21 μ, averaging 4.79 × 18.36 μ, with a septum in the central part, colorless or at length yellow, with oil bodies in each spore (3).]

In our material, the purplish-brown color of the lesion was not common, but the spindle-shaped ashy-brown to ashy-white lesions were usual. Pycnidia were 103-320 × 124-172 μ, averaging 157 × 142 μ (Fig. 1). The warty or papillate projections were viewed at first as beaks in our materials, and clusters of pycnidia were very common in culture and on leaves (Fig. 2). Pycnosporos (conidiospores) of our material were 13.5-27 × 3-6 μ, averaging 18.45 × 4.52 μ and normally having one central septum, although two and even three septa were seen rarely. Oil droplets were always present, usually at the tips and on either side of the septum (Fig. 3).

*Fungus stability.*—From the 16 leaf collections made in 1969, *A. tritici* was identified from 11 and *S. nodorum* from 3 samples. Nonbeaked pycnidia were rare in slant cultures, and when isolated their spores often failed to grow, or were slower in starting to grow than spores from the papillate or beaked pycnidia. Even though spherical pycnidia were selected, beaked pycnidia predominated in succeeding generations. No stable culture of *A. tritici* having nonbeaked pycnidia was obtained in some 250 attempts.

In the case of the isolates having beaked pycnidia from a single wheat leaf (Ithaca, N. Y. 1969), a great difference in spore production was seen in the first cultural generation. The slant cultures with beaked pycnidia produced an average of 1,372,000 spores/ml, compared to an average of 346,000 spores/ml from those having nonbeaked pycnidia. By the fourth generation, this difference was considerably reduced because the selection that originally had nonbeaked pycnidia had mostly beaked pycnidia and an increased spore production.

Only *A. tritici* was observed in 150 slant cultures isolated from the five wheat varieties which were inoculated with P-6, a 1968 Pennsylvania isolate.

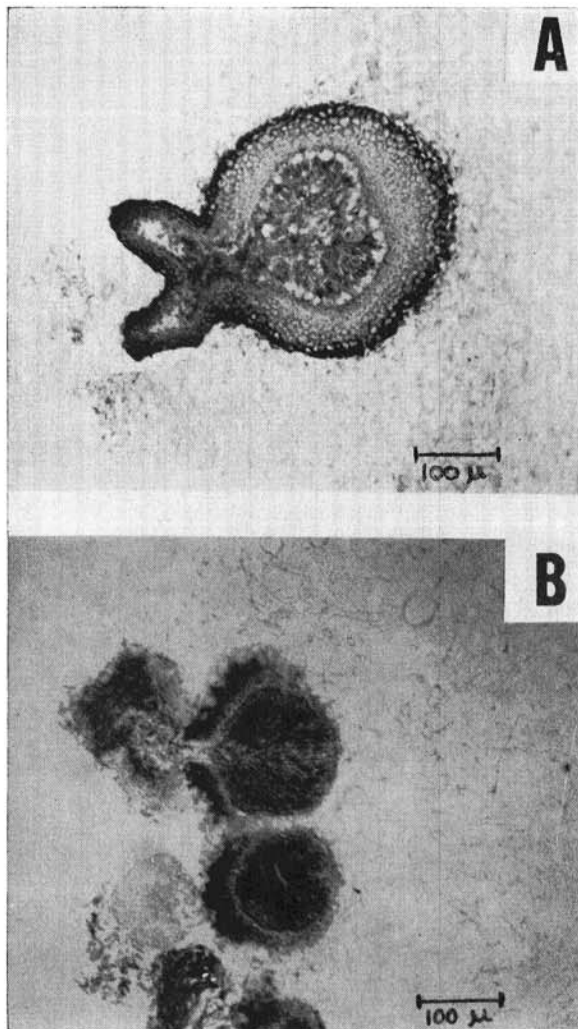


Fig. 1. Cross sections of pycnidia of *Ascochyta tritici* (A) and *Septoria nodorum* (B).

**Pathogenicity.**—All cultivars inoculated were susceptible to *S. nodorum*, but exhibited much variation in reaction to *A. tritici* (Table 1). For example, Little Club wheat was susceptible to P-6 and P-11, but tolerant or resistant to P-5, P-12, and P-13.

**Host range.**—All the cereal species and cultivars inoculated were infected to some degree by both *S. nodorum* (N-38) and *A. tritici* (P-6). Spore counts were made on six leaf samples of each species of adult plants. Sporulation was most abundant on triticale and wheat, but significant numbers of spores were recovered from all the cereals inoculated. *Ascochyta tritici* sporulates more abundantly than *S. nodorum* on triticale (500,000 to 30,000, respectively), but produces about 8-fold fewer spores than *S. nodorum* on wheat and barley. Both pathogens produce about the same number of spores on oats.

**Soil experiments.**—About 2 months after planting, symptoms appeared on the Redcoat wheat plants growing in the soil samples. Chlorotic leaf tips, chlorotic

mottle, and yellow spots were observed on plants in several soils from different locations. Symptoms continued to intensify for about 1 month, during which time "fleck-streaks" developed on plants growing in two of the soils. Leaves showing symptoms were removed periodically and examined for the presence of fungi or virus. Leaf pieces were surface-sterilized and plated on water agar or moist filter paper, then incubated at 20 C. Neither *A. tritici* nor *S. nodorum* was found. Other leaf pieces were ground with Carborundum, and healthy wheat seedlings were wiped with the juice to test for transfer of a virus. No evidence of virus was found. After the plants were fertilized, the day lengthened, and the temperature raised, these symptoms disappeared. The plants headed approximately 6 months after planting without symptoms of disease. We do not know what caused the early symptoms.

**Other methods of inoculation.**—Application of *A. tritici* to germinating wheat seed slowed plant growth, but when the seed was planted in soil in the greenhouse, the plants recovered and the fungus seemed to have no long-term effect. After infested plant material was incorporated into the soil, a few infected leaves were found in two pots containing different concentrations of the inoculum. Results were not consistent, and more work would have to be done to obtain reliable results with these methods.

**DISCUSSION.**—We found only two reports of *Ascochyta* on wheat in North America (11). The Canadian report by T. Johnson (1) refers to *A. graminicola*. The spore size given,  $18.1 \times 5.8 \mu$ , would fit better the present description of *A. tritici*. The maximum spore diam reported for *A. graminicola* was  $4.0 \mu$ . The report by Preston (7) from Oklahoma also referred to *A. graminicola*, but no spore sizes or other details were given. The *Ascochyta* sp. reported on *Triticum* by Diedicke (2) had spores too narrow to be assigned to *A. tritici*. *Ascochyta tritici* is close to *A. hordei* Hara, as discussed by Sprague (11), Sprague & Johnson (12), and Ideta (4), differing mainly in the symptoms on barley. *Ascochyta hordei* infection is characterized by lesions having 5 or 6 concentric dark brown rings, while lesions incited by *A. tritici* have no such rings. No other reports of *Ascochyta* on *Triticum* were found.

Cultural characteristics, morphology of the fruiting structures and spores, and host reactions to inoculation all emphasize the distinction between *A. tritici* and *S. nodorum*. *Ascochyta tritici* is much more stable than we had originally thought (8). When we tried to select away from the papillate, beaked, or warty pycnidia, we failed. Spherical pycnidia were usually immature stages of the warty, older structures.

Isolates of *A. tritici* produced a wide range of reactions on wheat and other cereals. Since its host range is so broad, it probably could damage any of the cereals when environmental conditions are favorable.

Above-normal precipitation in 1967, and high rainfall with overcast much of the time in 1968 (5) created ideal conditions for increase and spread of inoculum. Widespread occurrence of *A. tritici* was observed in

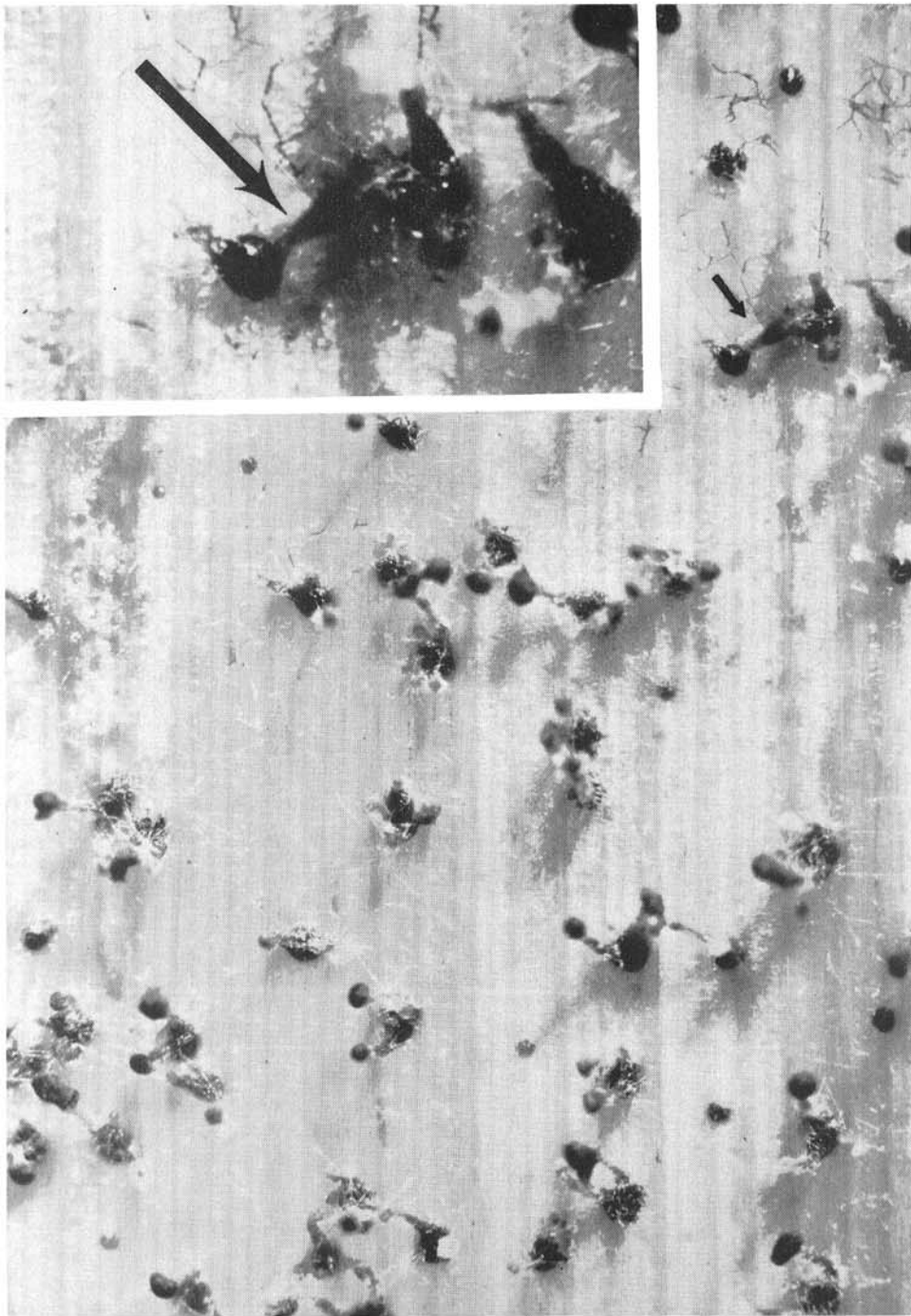


Fig. 2. Clusters of warty, papillate pycnidia of *Ascochyta tritici* on a wheat leaf. Arrows indicate a beak or papillate extension of an ostiole with a droplet of pycnospores in liquid matrix at the tip. ( $\times 15$ ; insert,  $\times 50$ )

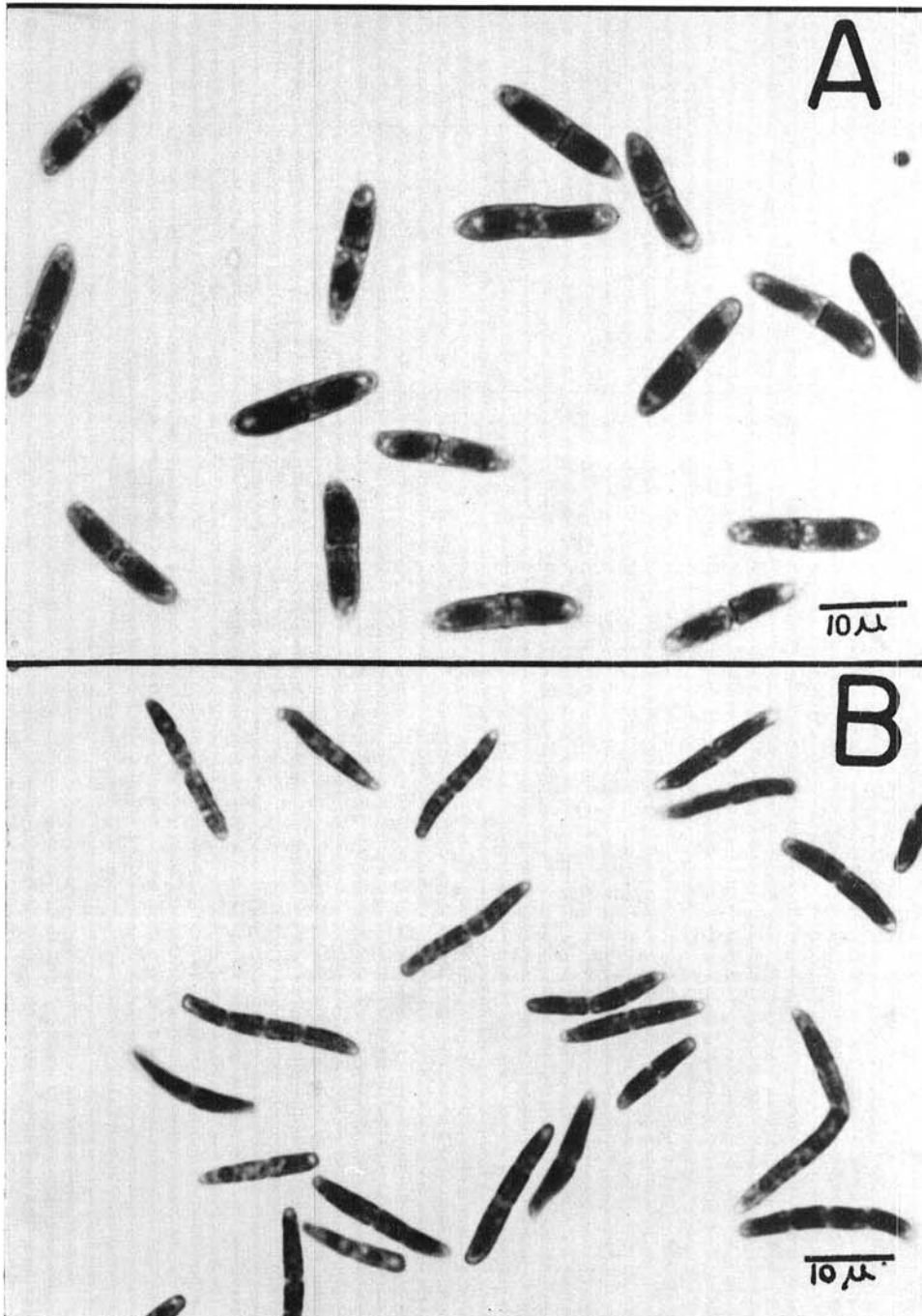


Fig. 3. Pycnospores of A) *Ascochyta tritici*; and B) *Septoria nodorum*.

TABLE 1. Major symptoms produced on cultivars of wheat when inoculated with isolates of *Ascochyta tritici*

Wheat cultivar	Symptoms					
	Necrosis (on primary leaf)		Chlorosis (on primary leaf)	Necrosis (on primary leaf and flecks on 2nd and 3rd leaves)	Chlorosis (on primary and flecks on 2nd and 3rd leaves)	Flecks (on 2nd and 3rd leaves)
Asosan	P-6 <sup>a</sup>	P-11	P-12	P-13		
Wisc. Selection	P-6	P-11	P-12	P-13		
Hadden	P-6	P-11	P-12		P-13	
Little Club	P-6	P-11				
Gaines	P-6	P-11	P-12			
Nainari 60	P-6		P-12		P-11	P-13
Redcoat	P-6		P-12			P-11
Avon	P-6			P-13		
Pennoll	P-6			P-11	P-12	P-13
Genesee	P-6			P-11	P-12	P-13
Yorkwin	P-6			P-11	P-12	P-13
Yorkstar	P-6			P-11	P-12	P-13

<sup>a</sup> Isolates of *A. tritici* giving the specified symptoms on inoculated plants.

June 1968. In 1969, higher average temperature and fewer extended periods of cloudy, wet weather were unfavorable for a serious outbreak of either *A. tritici* or *S. nodorum*.

Although *A. tritici* is a less aggressive parasite than *S. nodorum*, it is known to be present over a wide area, and probably could cause or contribute to significant losses of cereals in the northeastern USA in cool, wet years.

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