Bipolaris Leaf Blight of Panicum fasciculatum: Effects of Host Age and Photoperiod on Susceptibility

E. S. Luttrell, H. B. Harris, and H. D. Wells

Professor, Department of Plant Pathology, University of Georgia, Athens 30602; Professor, Department of Agronomy, Georgia Experiment Station, Experiment 30212; and Plant Pathologist, USDA, Georgia Coastal Plain Experiment Station, Tifton 31794.

Accepted for publication 15 October 1973.

ABSTRACT

Browntop millet, *Panicum fasciculatum* Swartz, is highly susceptible to leaf blight caused by *Bipolaris (Helminthosporium) setariae* (Saw.) Shoemaker in the early seedling stage and in the maturation stage. Resistance in young, as well as old, leaves increases from the seedling stage onward until plants 5-6 wk old are essentially immune. Resistance decreases sharply after plants begin to head at 6-7 wk, and blight is severe by the time seeds reach the hard dough stage at 8-9 wk. Vegetative growth and resistance may be prolonged indefinitely in plants grown under a 16-h light/8-h dark cycle. Hay is normally harvested before blight causes extensive damage. The disease appears too late to cause appreciable seed losses, and seedling infection in the field is not important, except as a bridge between seed-borne inoculum and mature plants.

Phytopathology 64:476-480.

Many plant diseases are caused by pathogens capable of attacking only senescent tissues. Although no sharp dividing line can be drawn, these diseases of senescence probably should be distinguished from diseases of maturation in which the onset of disease in the entire plant is delayed until the setting of fruit (2, 16) or vegetative storage organs (1). In some diseases, such as Diplodia seedling blight and Diplodia stalk and ear rot of corn (2, pp. 91-95), the host may have two periods of susceptibility, and the same pathogen may cause essentially distinct diseases at different growth stages of the host. Field observations and greenhouse inoculations have demonstrated that a previously unrecorded blight of browntop millet (*Panicum fasciculatum* Swartz) caused by *Bipolaris (Helminthosporium) setariae* (Saw.) Shoemaker occurs on the leaves in two phases, one in the juvenile and one in the maturing stages of the host and, further, that the loss of resistance in older plants may be controlled through the effects of photoperiod on heading of the millet.

MATERIALS AND METHODS.—*Bipolaris setariae* was isolated from single lesions on leaves of *Panicum fasciculatum* and other grasses in Georgia. The fungus was grown on plates of V-8 agar (180 ml Campbell's V-8 juice/liter) at 22.5 C under 2,152 lx (200 ft-c) of constant light from 15 W cool-white, fluorescent tubes. Inoculum was prepared by scraping conidia from one 7-day-old plate culture into 100 ml of water. The suspension was filtered through cheese cloth, and applied to plants to run-off with an electric paint sprayer. Inoculated plants were held in a mist chamber at approximately 25 C for 48 h before they were returned to greenhouse benches. Percentage of leaf area infected (Fig. 8) was estimated 6 days after inoculation.

Greenhouse tests were made during July-September and December-April. Those concerned with photoperiod were made during December-February when plants grown in one section with normal day-lengths of less than 12 h (short day) could be compared with plants in another section under supplementary illumination from incandescent bulbs to extend day-length to 16 h (long day). Browntop millet seed, surface-sterilized in 0.5% sodium hypochlorite for 3 min, was planted in 15-cm diam pots of soil mix fumigated with methyl bromide, and seedlings were thinned to five plants/pot. Pots were seeded at successive weekly intervals to produce plants varying in age from 1-9 wk at time of inoculation (Fig. 8-A). Inoculations were made also on four groups of 9-wk-old plants, all of which were started at the same time under the long-day cycle. One group was grown for the entire period in the long-day section; the other three groups were successively transferred to the short-day section after initial growth periods of 2, 4, and 6 wk under long days (Fig. 8-B).

OBSERVATIONS AND RESULTS.—*The disease.*—Browntop millet, originally a wildlife food crop, was adopted for hay production in the 1950's. Because of its short maturation period it may be planted as a catch crop in June to produce hay in 7-8 wk and seed in 10-11 wk. As new millets were introduced, acreage in Georgia declined from 98,564 in 1955 to 45,200 in 1972, when it represented 13% of the acreage planted to millets and Sudan grasses (6). Browntop millet is subject to only one major disease, Bipolaris leaf blight. The plants remain nearly free of disease until they begin to head at 6-7 wk. At this time reddish-brown flecks and short streaks, often surrounded by chlorotic areas, appear in the leaves. The lesions rapidly increase in number and elongate, and infected leaves die back from the tips and turn brown (Fig. 1). The entire dead area may be covered with conidiophores of the pathogen. Brown spots, often with tan centers, appear on the glumes, and ultimately the glumes and exposed surfaces of the grain become brown and bear conidiophores.

Only traces of infection have been found on seedlings in field searches. Occasional lesions occur as small (1 to 2 mm in diam), oval, buff-colored spots surrounded by narrow reddish-brown borders. When such leaves near the base of the culms die in thick stands, they become covered with conidiophores and conidia of the pathogen. Thus, an adequate, though inconspicuous, source of inoculum is available at heading time of the host.

The fungus.—The pathogen is typical of the genus *Bipolaris*, one of two genera *(Drechslera, Bipolaris)* made up mostly of graminicolous species segregated from *Helminthosporium* (8, 13). Conidia produced on the host (Fig. 2-4) were ellipsoid to fusoid, obclavate-fusoid, or linear-fusoid, straight to slightly curved, thin to moderately thick walled, pale to dark brown, 39-126 × 11-17 μm, and 4 to 11-septate. The basal cell, especially in the long, narrow spore forms, often was narrowed and had parallel lateral walls (Fig. 4). Mean dimensions for the collections illustrated were 62.8 × 14.1 μm (Fig. 2), 66.9 × 15.1 μm (Fig. 3), and 73.6 × 14.2 μm (Fig. 4). In cultures of single-lesion isolates grown on V-8 agar under continuous illumination conidia were larger and more uniform within populations but showed an even greater range of interstrain variation. The extremes are shown in Fig. 5 and Fig. 7. Conidia in the strain illustrated in Fig. 5 (short-spored) were 61-102 × 14-19 μm (mean 81.9 × 16.6 μm), 6 to 10-septate (mean 8.2-septate), ellipsoid fusiform to decidedly obclavate-fusoid, dark brown, and relatively thick walled. Conidia in the strain illustrated in Fig. 7 (long-spored) were 80-175 × 12-16 μm (mean 135.0 × 14.8 μm), 7 to 14-septate (mean 9.7-septate), linear-fusoid with the basal cell often narrowed, thin-walled, and pale to moderately dark brown. Figure 6 represents one of the many intermediate types. In this strain conidia were 74-122 × 13-16 μm (mean 101.4 × 13.8 μm), 7 to 10-septate (mean 8.8-septate), obclavate-fusoid with the basal cell sometimes narrowed, thin- to moderately thick-walled, and moderately dark brown.

Effect of photoperiod on plant development.—Plants of browntop millet grown under normal day lengths in periods from December-April and July-September had similar courses of development as follows: week 1–plants 4-7 cm tall to the tip of the longest leaf, first leaf expanded, second leaf 25% expanded; week 2–12-23 cm, three to four leaves expanded, some plants beginning to tiller; week 3–17-38 cm, fifth leaf showing, one to three tillers with two to three leaves each; week 4–30-46 cm, five leaves, three to six tillers with up to four leaves each; week 5–44-60 cm, sixth leaf showing, many tillers and branches; week 6–44-59 cm, six to seven leaves, beginning to head; week 7—blooming to green grain in milk stage; week 8—grain in milk to hard dough stage; week 9—grain in hard dough stage to mature; week 10—grain fully mature.

Plants grown under long-day conditions failed to head and by week 10 were up to 60 cm tall and had produced 12-14 leaves on the primary culm. Plants transferred to short day after an initial long day period of 2 wk headed normally at week 6 when they were 49-53 cm tall and had six to seven leaves on the primary culm. Initial growth periods under long days of 4 and 6 wk delayed heading until week 7 and week 9, approximately 3 wk being required after transfer to short days before heads began to appear.

Effect of age on susceptibility.—Data on effect of age on susceptibility of plants grown under normal day-lengths in Fig. 8-A (short day) are drawn from several tests, and each point represents the mean of 7-13 replications. Under severe conditions of inoculation (48 h in mist chamber), susceptibility in terms of leaf area infected declined steadily from 54% in plants inoculated at week 1 to less than 10% in plants inoculated at weeks 5-7. A striking increase in susceptibility occurred after week 7, and 75% of the foliage was infected in plants inoculated at week 9.

Related changes in lesion type occurred. At weeks 1-2 lesions were irregular, unbordered, buff-colored or pale tan areas usually 1-11 × 0.8-2.5 mm, but occasionally up to 50 × 2.5 mm. In some plants all leaf blade tissue was

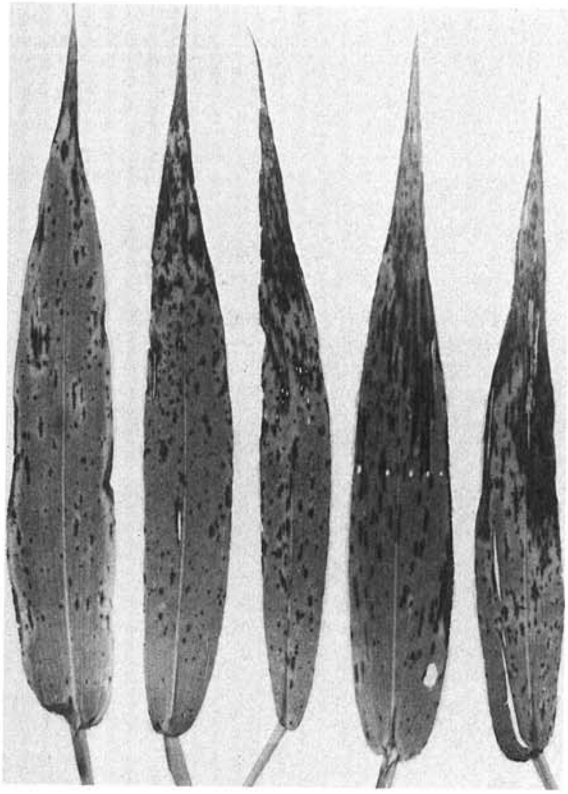


Fig. 1. Symptoms of Bipolaris leaf blight on leaves from mature plants of browntop millet.

blighted. In plants inoculated at week 3, similar lesions 1-17 × 0.5-4 mm occurred, but there were many smaller spots 2-3 × 0.5-1 mm with thin to conspicuous brown borders. In plants inoculated at week 4 most lesions were small brown-bordered or uniformly brown spots with only a few unbordered tan spots 2-5 × 1-1.5 mm. In plants inoculated at weeks 5-6, the slight infection was entirely in the form of small bordered spots and brown flecks. In plants inoculated at week 7, lesions were flecks and short brown stripes with some brown-bordered tan spots up to 4 × 2 mm on the leaves and up to 2 × 2 mm on the glumes. In plants inoculated at weeks 8-9, lesions were brown flecks and stripes 3-15 × 1.5-3.5 mm, often surrounded by chlorotic areas.

In one test, a comparison was made between a short-spored (Fig. 5) isolate of the fungus (No. 8008), and a long-spored (Fig. 7) isolate (No. 7862) from browntop millet. Inoculations were made on plants 1 to 9 wk old. Two pots of plants in each age group were inoculated with each isolate, and plants in one pot served as controls. Percentages of infection and lesion types produced on plants of all ages by the two isolates were similar.

Effect of photoperiod on susceptibility.—Data on effect of age on susceptibility of plants grown continuously with supplementary light to furnish a 16-h light/8-h dark cycle in Fig. 8-A (long day) represent the means of 3 to 10 replications. Amounts of leaf area infected declined from 28% in plants inoculated at weeks

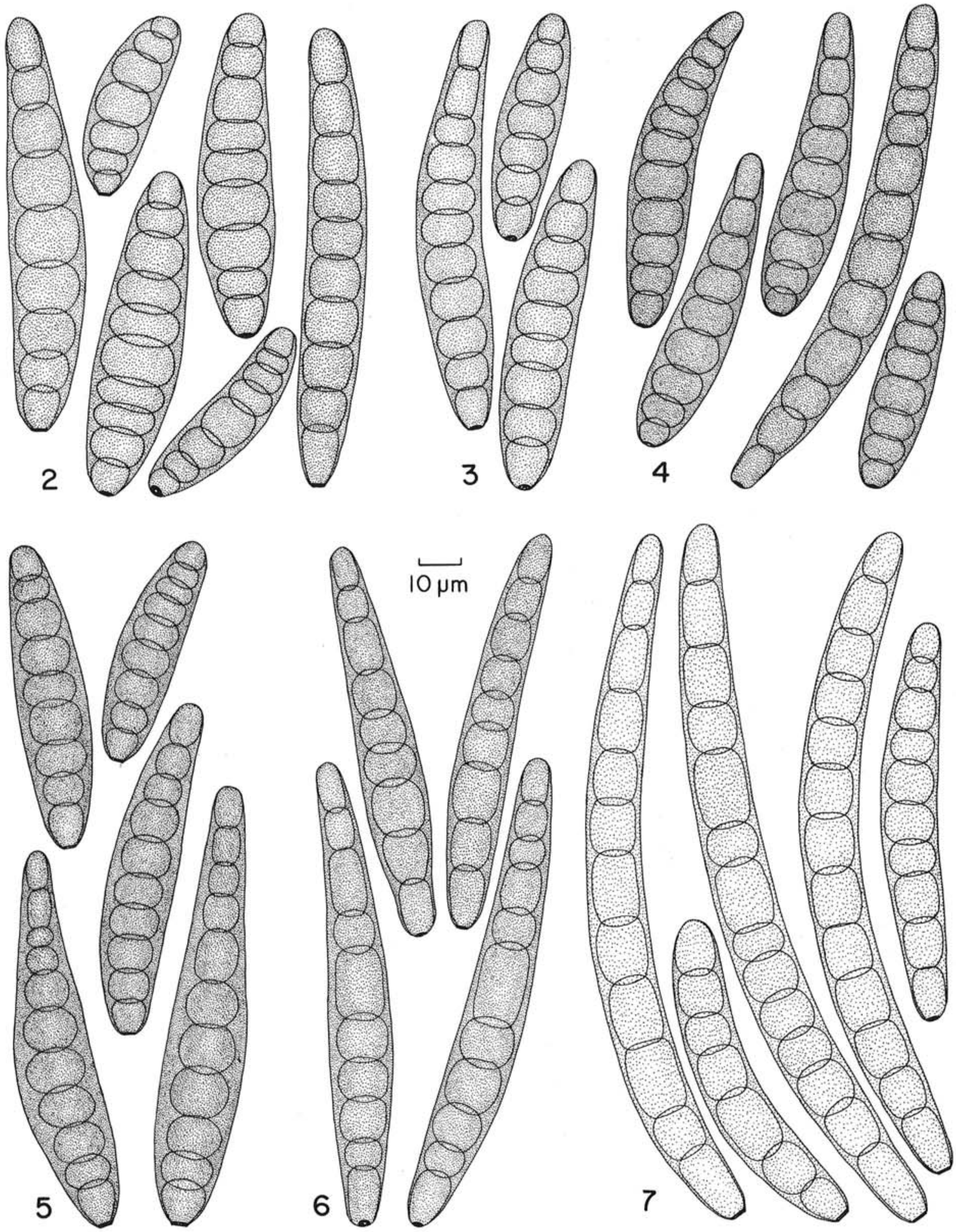


Fig. 2-7. Conidia of *Bipolaris setariae* from browntop millet. **2-4)** Produced on host in three collections. **5-7)** Produced by three isolates in culture on V-8 agar, under continuous illumination.

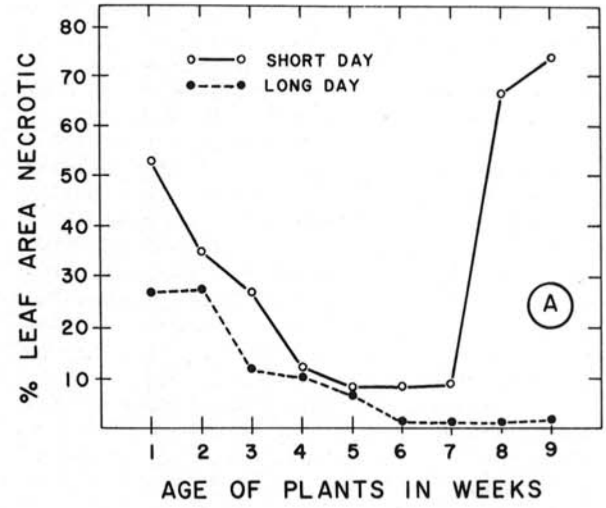
1-2, to a trace in plants inoculated at week 6 and later.

Figure 8-B shows mean percentages of infection obtained from inoculations with nine isolates of *Bipolaris setariae* at week 9 in the growth of four groups of plants whose development had been controlled by varying initial growth periods under long days. Stages of development of the plants were as follows: long day 2 wk–mature, hard dough stage, most grain ripe; long day 4 wk–hard dough stage, 10% of grain ripe; long day 6 wk–heading; long day continuous–no heads. Infection decreased from 68% on plants with maturing seed to 3% on plants that had not started to head. Infection obtained on mature plants with five isolates from browntop millet varied from 60-95% (mean 80%); infection with four isolates from *Cynodon* and *Paspalum* varied from 25-80% (mean 54%). Of the isolates from browntop millet, two short-spored isolates (Fig. 5) produced infection of 90-95% (mean 93%); three long-spored isolates (Fig. 7) produced infection of 60-95% (mean 72%).

Effect of disease on seedling growth.—A comparison was made of plant height (to tip of longest leaf) between 15 seedlings in three pots inoculated at week 1 and the same number of noninoculated controls. At week 2, control seedlings were 13-17 cm tall (mean, 14.6 cm); at week 3, they were 33-36 cm tall (mean, 34 cm). Inoculated seedlings at week 2 showed infection of 18-65% (mean, 44%) and were 6-12 cm tall (mean, 10.1 cm); at week 3 they were 23-36 cm tall (mean, 31.7 cm). Reduction in mean height resulting from inoculation was 30.8% at week 2, but only 6.8% at week 3.

DISCUSSION.—The two-phase susceptibility to Bipolaris leaf blight observed in browntop millet is similar to that reported (16) in pearl millet [*Pennisetum glaucum* (L.) R. Br.]. The increasing resistance, following the susceptible seedling stage, that develops in browntop millet from the first to the fourth week of growth and results in near immunity by the fifth and sixth weeks is whole-plant resistance, as in mature plant resistance to Dothistroma needle blight developed in *Pinus radiata* D. Don. (5). The young expanding leaves at the tips of the primary culms and on tillers are as resistant as the older leaves. This is strikingly apparent in plants under long day cycles that continue to grow vegetatively but maintain the same high level of resistance. The progressive changes in lesion type on plants inoculated in later stages of growth also indicate that changes occur in the physiological condition of young leaves on older plants. Lesions on 1- to 2-wk-old plants appear to be unrestricted under conditions of high moisture and low light. Under the same conditions, lesions on older plants develop distinct borders and are restricted, ultimately to small spots and flecks. Second-phase susceptibility is a function of stage of maturation rather than absolute age as is demonstrated by the indirect control of resistance through the effect of photoperiod on heading. Prevention of tuber initiation in potato by growth under long-day cycles, likewise maintains resistance to Verticillium wilt (1).

Although seeds of browntop millet almost invariably carry the pathogen and inoculations have demonstrated that seedlings are susceptible, the disease causes little damage to seedlings in the field, and seed treatments produced no significant increases in stands (9). The slight amount of infection in the crop prior to heading probably is most significant as a bridge between seed-borne inoculum and maturing plants. Since, however, isolates of *Bipolaris setariae* from other grasses have been demonstrated to infect browntop millet, exogenous sources of inoculum for maturing plants may exist. Despite the fact that leaf blight is common and conspicuous on maturing plants, damage to the crop probably is low. The disease develops so late that reduction in the seed crop is insignificant, and the crop normally is harvested for hay before serious damage is done to the foliage.



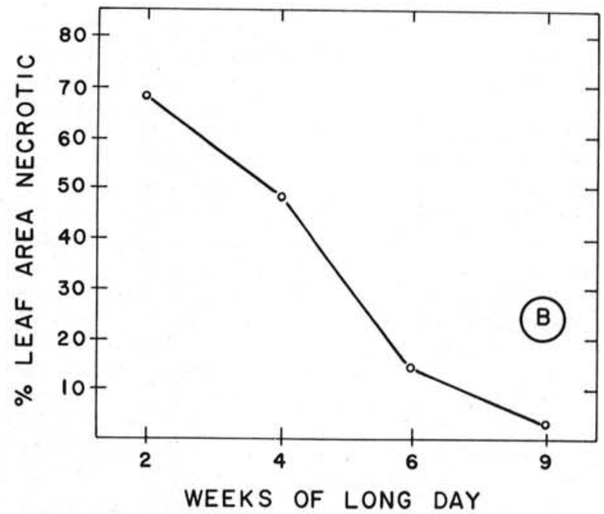


Fig. 8. Results of inoculations of browntop millet with *Bipolaris setariae*. **A)** Effect of host age and photoperiod on susceptibility. **B)** Relative susceptibility of 9-wk-old plants in different stages of maturity, controlled by growth under long days for 9 wk compared with return to short days after initial growth periods of 2, 4, and 6 wk under long days.

Assignment of the pathogen to *Bipolaris setariae* is only provisional. A wide range of biotypes is associated with leaf blight of browntop millet, and strains differing greatly in morphology may be isolated from leaf lesions in the field and may produce similar symptoms in greenhouse inoculations. These strains belong to the *setariae* group of *Bipolaris*. This group comprises a bewildering array of variable and intergrading strains commonly encountered as the cause of nonspecific lesions in the form of small spots and stripes on millets, Bermudagrass, corn, and many other cultivated and wild grasses. A few, such as *B. victoriae* (Meehan & Murphy) Shoemaker (7, 13), *B. sacchari* (Butl. in Butl. & Hafiz) Shoemaker (11, 13), and *Helminthosporium (Bipolaris) carbonum* Ullstrup race I (14), may be distinguished by the production of specific lesions and differential pathogenicity on cultivars of crop plants. At one morphological extreme, strains of the *setariae* group must be distinguished from *B. sorokiniana* (Sacc. in Sorok.) Shoemaker (7, 13), which has much broader (commonly more than 20 μm in diam), usually ellipsoid, very thick-walled, dark brown conidia. At the other extreme they must be distinguished from *B. maydis* (Nisi.) Shoemaker (13), which has paler-colored, more strongly curved, and frequently arcuate, conidia. A number of species names are available within the *setariae* group. Short-spored strains on browntop millet like those in Fig. 2-3 are within the range of *B. setariae, B. victoriae, B. bicolor* (Mitra) Shoemaker (13), and *H. carbonum*. Conidia in most strains tend to be longer and narrower and correspond more nearly to *H. (Bipolaris) panici* v. Overeem (15) [not *H. panici* F. L. Stevens = *Pleurophragmium dorycarpum* (Mont.) Hughes (4, p. 797)] or *B. stenospila* (Drechs.) Shoemaker (3, 11, 13). *B. zizaniae* (Nisi.) Shoemaker with obclavate conidia as in Fig. 5 and *B. panici-milacei* (Nisi.) Shoemaker and *B. yamadai* (Nisi.) Shoemaker with fusoid conidia similar to those in Fig. 6 also must be considered, but for all three of these long-spored species Nisikado (10) recorded diam ranging above 26 μm. The most distinctive strains are those with long, linear-fusoid, relatively thin-walled and paler-colored conidia with narrowed basal cells. (Fig. 7). These correspond closely with the description of *B. urochloae* (Putterill) Shoemaker (12, 13). Until the studies correlating morphology, pathogenicity, and mating behavior necessary to resolve problems of classification in the *setariae* group are accomplished, the strains causing leaf blight of browntop millet are referred to *B. setariae*, the earliest described species in the group.

LITERATURE CITED

1. BUSCH, L. V., and L. V. EDGINGTON. 1967. Correlation of photoperiod with tuberization and susceptibility of potato to Verticillium albo-atrum. Can. J. Bot. 45:691-693.
2. DICKSON, J. G. 1956. Diseases of field crops. (2nd ed.) McGraw-Hill, New York. 517 p.
3. DRECHSLER, C. 1928. A species of Helminthosporium distinct from Helminthosporium sacchari, causing brown stripe of sugar cane. Phytopathology 18:135-136.
4. HUGHES, S. J. 1958. Revisiones hyphomycetum aliquot cum appendice de nominibus rejiciendis. Can. J. Bot. 36:727-836.
5. IVORY, M. H. 1972. Resistance to Dothistroma needle blight induced in Pinus radiata by maturity and shade. Trans. Br. Myc. Soc. 59:205-212.
6. JACKSON, J. E. 1973. Agronomy summary – 1940-1972. Univ. Georgia Coop. Ext. Serv. Mimeo, Agronomy 1-2. 6 p.
7. LUTTRELL, E. S. 1955. A taxonomic revision of Helminthosporium sativum and related species. Am. J. Bot. 42:57-68.
8. LUTTRELL, E. S. 1969. Curvularia coicis and the nodulosa group of Bipolaris. Mycologia 61:1031-1040.
9. LUTTRELL, E. S., L. V. CROWDER, and H. D. WELLS. 1955. Seed treatment tests with pearl millet, Sudan grass, and browntop millet. Plant Dis. Rep. 39:756-761.
10. NISIKADO, Y. 1928. Studies on the Helminthosporium diseases of Gramineae in Japan. Ohara Inst. Agric. Res. Special Rept. 4:1-394. (in Japanese; English summary in Ber. Ohara Inst. Landwirtsch. Forsch. Kurashiki. Japan 4:111-126. 1929).
11. PARRIS, G. K. 1950. The Helminthosporia that attack sugar cane. Phytopathology 40:90-103.
12. PUTTERILL, K. M. 1954. Some graminicolous species of Helminthosporium and Curvularia occurring in South Africa. Bothalia 6:347-378.
13. SHOEMAKER, R. A. 1959. Nomenclature of Drechslera and Bipolaris, grass parasites segregated from 'Helminthosporium'. Can. J. Bot. 37:879-887.
14. ULLSTRUP, A. J. 1944. Further studies on a species of Helminthosporium parasitizing corn. Phytopathology 34:214-222.
15. VAN OVEREEM, C. 1925. Beitrage zur Pilzflora von Niederlandisch Indien. II. 11. Ueber eine verheerende Helminthosporiose des bengalischen Grases (Panicum maximum Jacq.). Bull. Jard. Bot. Buitenzorg, Ser. III, 7:431-434.
16. WELLS, H. D., and G. W. BURTON. 1967. Helminthosporium setariae on pearl millet, Pennisetum typhoides, as affected by age of host and host differences. Crop Sci. 7:621-622.