# Leaf Spots of Big Bluestem, Little Bluestem, and Indiangrass Caused by *Ascochyta brachypodii*

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**ABSTRACT**

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Ascochyta leaf spot was the most important disease on big bluestem (*Andropogon gerardi*), little bluestem (*A. scoparius*), and indiangrass (*Sorghastrum nutans*) in Pennsylvania and New York from 1976 through 1980. A *Didymella* sp., which was shown for the first time to be the ascigerous state of *A. brachypodii*, was also associated with the disease on these species. The morphology and cultural characteristics of *A. brachypodii* and its *Didymella* state are described. In greenhouse inoculations, isolates of *A. brachypodii* from big bluestem, little bluestem, and indiangrass were not equally pathogenic on the three hosts. Three other warm-season grasses—caucasian bluestem (*Bothriochloa caucasica*), old world bluestem (*B. ischaemum*), and switchgrass (*Panicum virgatum*)—and eight cool-season grasses were not susceptible or were highly resistant. Corn (*Zea mays*) and sudangrass (*Sorghum sudanense*) were moderately susceptible to *A. brachypodii* and are reported as new hosts. In field plots, leaf spot was consistently less severe on big bluestem cultivar NY-1145 than on Kaw and Pawnee. Disease severity was related to duration of high relative humidity at two locations. The diseases caused by *A. brachypodii* on susceptible warm-season grasses can be important in the humid eastern part of their range.

Additional key words: disease resistance, environmental plant pathology, forage crops

Currently there is considerable interest in perennial warm-season grasses to supplement cool-season species for grazing and hay production during the dry summer months in the midwestern and northeastern United States (5). Big bluestem (*Andropogon gerardi* Vitman), little bluestem (*Andropogon scoparius* Michx.), and indiangrass (*Sorghastrum nutans* (L.) Nash) are among the species being evaluated for use on marginal lands and for soil conservation at northeast and midwest agricultural experiment stations. The foliage of these species is attacked by several pycnidial fungi that cause leaf spots, chiefly species of *Ascochyta, Stagonospora*, and *Septoria* (10). *Ascochyta brachypodii* (Sydow) Sprague and A. G. Johnson, the incitant of the leaf spot disease reported here, has been reported on big bluestem and indiangrass only in North Dakota and on little bluestem only in Arizona (8). The *Didymella* state of *A. brachypodii* is sometimes associated with the disease but has not been reported on these grasses. No reports of artificial inoculations with *A. brachypodii* were found in the literature.

Because there is little information on diseases of warm-season grasses, studies were initiated to determine the prevalence and relative importance of diseases of these species in several counties in Pennsylvania. This paper describes an important disease of big bluestem, little bluestem, and indiangrass that was prevalent in field plots and production fields in Pennsylvania during five growing seasons and the cultural, host range, and resistance studies with the pathogen.

## MATERIALS AND METHODS

Leaves and stems of big bluestem, little bluestem, and indiangrass with mild to severe leaf spot symptoms were collected from experimental plots or production fields in five counties in Pennsylvania and from seed production plots at Big Flats, NY. Collections were made from July to mid-October each year from 1976 through 1980. The fungi were isolated on potato-dextrose agar (PDA) and cultured on 20% V-8 juice agar (V-8A).

Plants of 20 gramineous species listed under "Cross inoculations and host range" were grown from seed in a 1:1 commercial mixture of peat moss and vermiculite in 10-cm pots in the greenhouse at 25–30 C. Plants were 6 to 7 wk old when inoculated, except for corn, oats, wheat, barley, and sudangrass, which were 4 wk old. Rice plants were 10 wk old.

**Inoculations**. Conidial suspensions of *A. brachypodii* isolates from big bluestem, little bluestem, and indiangrass collected in Pennsylvania and New York were prepared by blending sporulating pycnidia and some mycelia from 6- to 7-day-old cultures in distilled water with one drop of Tween 20 per liter. Conidial concentration was about $10^4$ spores per milliliter. Of the 11 isolates tested, seven were from big bluestem from three Pennsylvania counties and Big Flats, NY, two were from indiangrass in Westmoreland County, PA, and two were from little bluestem in Huntingdon County, PA. No attempt was made to inoculate plants using ascospores of the *Didymella* state.

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The seven greenhouse inoculation tests conducted during 1977–1979 used isolates collected during 1976–1978. In each test, three pots of each species were inoculated with each isolate of *A. brachypodii* being tested. Inoculum was sprayed on leaves of plants until they were thoroughly wet. Control plants were sprayed with distilled water. Plants were incubated in a large dark chamber at 19–21 C, where moist conditions were maintained by periodic misting with distilled water, and then transferred to the greenhouse after 48 hr of incubation.

Disease severity ratings were made 8–18 days after inoculation. A 1–9 scale was used where 1 = no disease, 2 = trace, 3 = slight, 5 = moderate, 7 = severe, and 9 = very severe, sometimes killing the plant. Ratings were based on size and number of lesions, but lesion size was considered most important in evaluating the susceptibility of species. The same rating scale was used to assess disease severity in the field.

Temperature and relative humidity were monitored with hygrothermographs at one experimental site in Centre County and at one site in Huntingdon County in central Pennsylvania from 9 June to 25 August 1980. The duration of leaf wetness was measured in Centre County with a recording dew meter (Model AI-101B, Ag Tech Instrument Co., Savannah, GA) and in Huntingdon County with a Taylor dew meter (9) from 24 June to 11 August. In early May 1980, the dead stems and leaves from the 1979 growth of the grasses at both sites were cut and burned or removed.

In cultural, morphological, and ascigerous state studies, the fungi were grown on V-8A in an incubator at 22 C under a daily regime of 12 hr of fluorescent light and 12 hr of darkness.

## RESULTS

**Symptoms and prevalence.** The disease caused by *A. brachypodii* is characterized by reddish brown, elongated spots or blotches with tan centers, 2–6 × 1–1.5 mm (Fig. 1). In advanced stages, symptoms are sometimes streaklike. Leaves and leaf sheaths are affected. *A. brachypodii* was first isolated from big bluestem in seed increase rows at the USDA Plant Materials Center, Big Flats, NY, and subsequently from research plots and hay fields in five Pennsylvania counties. Moderate to severe leaf spot was observed on big bluestem in research plots during 1976 through 1980. A *Didymella* sp., which was shown to be the ascigerous state of *A. brachypodii*, was also isolated from reddish brown lesions on big bluestem, indiangrass, and little bluestem at several locations. Sometimes the conidial and ascigerous states were isolated from the same lesion.

**Culture and morphology.** *A. brachypodii* and the associated *Didymella* state grew and sporulated well on V-8A and PDA, but V-8A was used for most cultural and inoculation studies. *A. brachypodii* in culture produced globose, beaked pycnidia that were initially hyaline to light brown, becoming dark brown after 5–6 days. Most pycnidia developed on the surface of the medium, but some were submerged. The size range of 25 pycnidia was 122–291 × 85–188 μm (avg. 190 × 130 μm). The colonies, which were covered with scant growth of grayish white aerial mycelium, averaged 34 mm in diameter after 96 hr and 54 mm after 144 hr of incubation.

Conidia were hyaline, oval, slightly curved, mostly one-celled, but sometimes two-celled, with four to eight round guttulae per conidium (Fig. 2). The size range of 25 conidia was 16.2–21.6 × 5.4 μm (avg. 18.8 × 5.4 μm). The shape and size of conidia are similar to those of *A. brachypodii* given by Sprague (8). Isolates from big bluestem, little bluestem, and indiangrass were not culturally or morphologically different.

Pseudothecia, asci, and ascospores of the *Didymella* state were often present in the same cultures with *A. brachypodii*. In 8-day-old cultures, pycnidia of *A. brachypodii* were usually concentrated near the center, with pseudothecia of *Didymella* near the margin of the colony. This production of pycnidia and pseudothecia occurred in cultures from big bluestem and indiangrass. Isolates originally identified as *Didymella* later produced only pycnidia and conidia of *A. brachypodii* in culture. One isolate from big bluestem that originally produced some two- or even three-celled conidia later produced only one-celled conidia. The *Didymella* state produced hyaline, thin-walled, saccate, cylindric asci in parallel arrangement in the pseudothecium, each ascus bearing eight hyaline ascospores (Fig. 3). Ascospores were two-celled, markedly constricted at the central septum, with one end more pointed than the other, and had one to three guttulae per cell. Ascospores were usually in

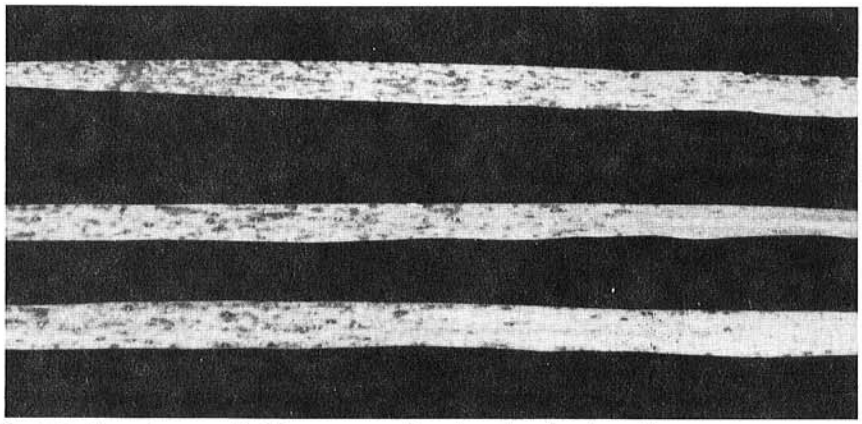

**Fig. 1.** Leaf symptoms on big bluestem caused by *Ascochyta brachypodii*.

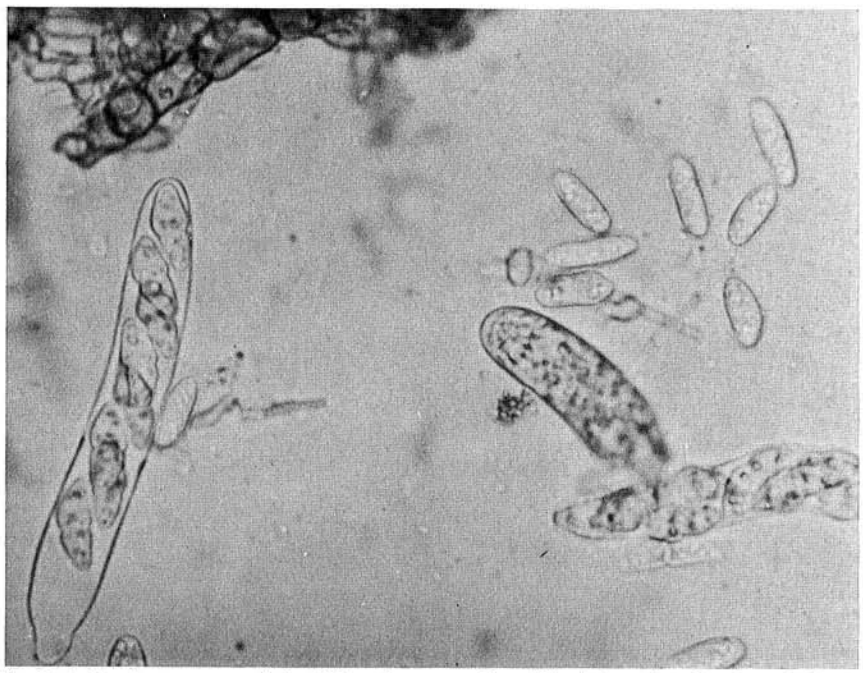

**Fig. 2.** Asci and ascospores of the *Didymella* state and conidia of *Ascochyta brachypodii* from a culture on V-8 juice agar (×400).

biseriate arrangement but were sometimes uniseriate or a combination of both. Based on 25 measurements, asci were 59–127 × 13–15 μm (avg. 78 × 14 μm); ascospores were 16.2–21.6 × 6.7–8.1 μm (avg. 19.4 × 8.0 μm).

**Ascigerous state.** Fifteen isolates of *A. brachypodii* were taken from storage at 3 C, transferred to V-8A, and incubated at 22 C. After 12 days, no ascocarps were found. The cultures were stored for 19 days at 3 C and then transferred to fresh V-8A. After 7 days of incubation, pseudothecia, asci, and ascospores of the *Didymella* sp. were present in two of the cultures along with pycnidia of *A. brachypodii*. This result suggested that the cold period stimulated development of the *Didymella* sp., the presumed ascigerous state of *A. brachypodii*.

In a further test, 10 single ascospores were isolated from these colonies. Each ascospore gave rise to a colony containing mature sporocarps of both states without cold storage. However, most of the sporocarps were pycnidia, with abundant production of conidia. The pseudothecia were very difficult to distinguish from pycnidia in the colonies under the dissecting microscope. Sporocarps near the center yielded asci and ascospores of *Didymella* in five of the 10 colonies. In three colonies, there were numerous dark sporocarps in the aerial mycelium after 9 days of incubation. Some of these were pseudothecia that contained asci and ascospores, and some were pycnidia that contained conidia. The *Didymella* sp. did not grow in culture except in association with *A. brachypodii*.

**Table 1.** Susceptibility of some gramineous species to isolates of *Ascochyta brachypodii* from big bluestem, little bluestem, and indiangrass[a]

| Species inoculated | Source of isolates | | |
|---|---|---|---|
| | Big bluestem | Little bluestem | Indiangrass |
| Warm-season grasses | | | |
| Big bluestem | M–H | Sl–M | M–H |
| Little bluestem | Sl | Sl–M | Sl–M |
| Indiangrass | N–Sl | N–Sl | Sl |
| Caucasian bluestem | N | N | N |
| Old world bluestem | N | N | N |
| Switchgrass | N | N | N |
| Sudangrass (annual) | Sl–M | Sl | Sl–M |
| Grain crops | | | |
| Corn | Sl–M | Sl | Sl–M |
| Wheat | N | ··· | ··· |
| Barley | N | ··· | ··· |
| Oat | Sl | ··· | ··· |
| Rice | N | ··· | ··· |

[a] Disease severity was rated on a 1–9 scale. N = not susceptible or highly resistant (mean rating, 1 or 2). Sl = slightly susceptible (mean rating, 3 or 4). M = moderately susceptible (mean rating, 5 or 6). H = highly susceptible (mean rating, 7 or 8). ··· = not tested.

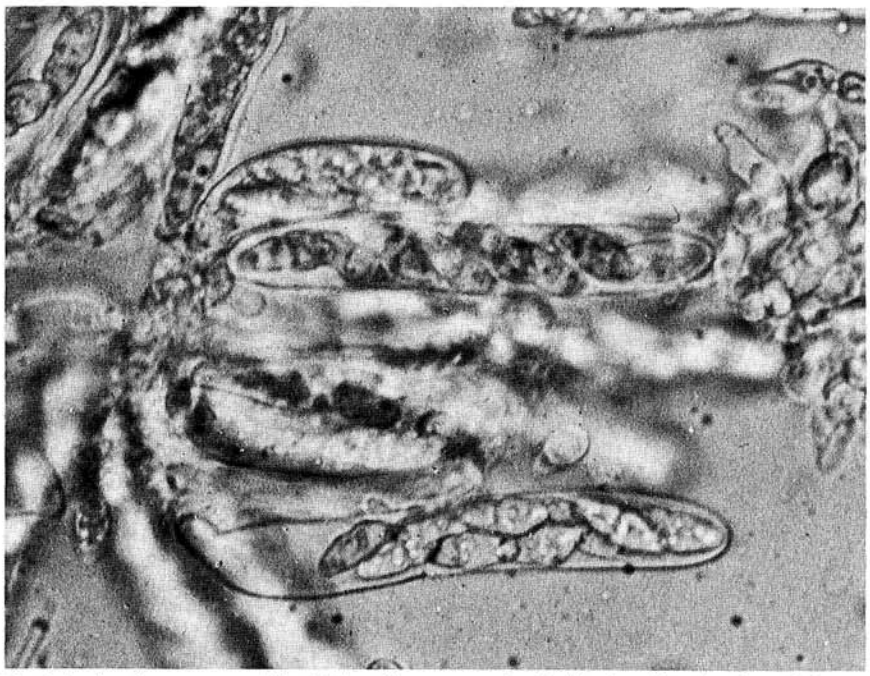

**Fig. 3.** Asci and ascospores of the *Didymella* state associated with *Ascochyta brachypodii* from a culture on V-8 juice agar. Note biseriate arrangement and septation of ascospores (×400).

These findings showed that the *Didymella* sp. was the ascigerous state of *A. brachypodii* and that the fungus was homothallic. Results also showed that a cold period is not essential for induction of the ascigerous state.

**Cross inoculations and host range.** Seven warm-season grasses, eight cool-season grasses, and five grain species were screened for susceptibility to isolates of *A. brachypodii* from big bluestem, little bluestem, and indiangrass. The cool-season species *Bromus inermis* Leyss, *Phleum pratense* L., *Phalaris arundinacea* L., *Lolium perenne* L., *Poa pratensis* L., *Festuca arundinacea* Schreb., *F. rubra* L., and *Panicum clandestinum* L. were highly resistant or immune to *A. brachypodii* isolates.

The susceptibility ratings of the warm-season grasses and grain crops (Table 1) indicated a certain degree of pathogenic specialization by isolates of *A. brachypodii* from big bluestem, which were moderately to highly pathogenic on that species but only slightly so on little bluestem and indiangrass. Isolates from indiangrass were only mildly pathogenic on indiangrass but were moderately to highly pathogenic on big bluestem; little bluestem isolates were no more than moderately pathogenic on any species. Caucasian bluestem (*Bothriochloa caucasica* C.E. Hubb.), old world bluestem (*B. ischaemum* Keng.), and switchgrass (*Panicum virgatum* L.) were not susceptible or were highly resistant to *A. brachypodii* isolates from all three host species. Corn (*Zea mays* L.) and sudangrass (*Sorghum sudanense* (Piper) Stapf.) were slightly to moderately susceptible, oat (*Avena sativa* L.) was slightly susceptible, and wheat (*Triticum aestivum* L.), barley (*Hordeum vulgare* L.), and rice (*Oryza sativa* L.) were not susceptible to isolates from big bluestem.

**Field disease observations.** Susceptibility to *A. brachypodii* differed among cultivars of big bluestem at several locations in Pennsylvania over a 5-yr period. The adapted cultivar NY-1145 (selected in West Virginia) consistently had less severe leaf spot in field plots than did cultivars Kaw and Pawnee, which were developed in Kansas and Nebraska, respectively (3). Differences among varieties of little bluestem and indiangrass were not consistent.

The severity of disease incited by *A. brachypodii* varied from year to year and among locations each year. Data on the duration of high relative humidity indicated a close relationship between the longer periods of damp weather and much greater severity of disease on big bluestem, little bluestem, and indiangrass in Centre County, compared with the shorter periods and much lower disease ratings in Huntingdon County, PA (Table 2). From 9 June to 4 August 1980, the hours of 98–100% RH in Centre County exceeded that in Huntingdon

**Table 2.** Severity of leaf spot disease caused by *Ascochyta brachypodii* on three warm-season grasses and hours of high relative humidity in two Pennsylvania counties in 1980

| Species Location (county) | No. of varieties rated | Date[a] 1 July | 6 Aug. | 1 Oct. |
|---|---|---|---|---|
| Big bluestem | | | | |
| Centre | 4 | 6.4 | 6.8 | 7.1 |
| Huntingdon | 3 | 2.1 | 4.2 | 4.3 |
| Little bluestem | | | | |
| Centre | 1 | 5.5 | 7.0 | 7.5 |
| Huntingdon | 4 | 2.1 | 3.5 | 4.1 |
| Indiangrass | | | | |
| Centre | 5 | 3.6 | 5.7 | 7.0 |
| Huntingdon | 2 | 2.0 | 2.1 | 2.1 |
| Location average | | | | |
| Centre | (10) | 5.2 | 6.5 | 7.2 |
| Huntingdon | (9) | 2.1 | 3.3 | 3.5 |

| | Hours of 98–100% relative humidity 9–30 June | 1 July–4 Aug. |
|---|---|---|
| Centre | 230 | 485 |
| Huntingdon | 180 | 415 |

[a]Values given are averages of the mean ratings of four replications for Centre County and five replications for Huntingdon County. Disease severity scale: 1 = no disease, 2 = trace, 3 = slight, 5 = moderate, 7 = severe, 9 = very severe.

County for each of the 8 wk. The excess ranged from 4 to 38 hr/wk, and the total excess was 120 hr. During 4 wk for which comparable data were available (24–30 June and 8–28 July), the duration of dew at Centre County exceeded that in Huntingdon County every week. Differences ranged from 2 to 18 hr or a total of 45 hr. Day and night temperatures at the two sites were similar.

## DISCUSSION

Big bluestem, little bluestem, and indiangrass are major native grasses of North America that are distributed throughout the eastern and central United States to the eastern edge of the Great Plains. Hardison (4) reported that leaf spots occur on the bluestems from North Dakota to New Mexico but that these diseases are generally more severe in the southern humid part of their range. According to my observations, the leaf spot diseases caused by *A. brachypodii* on big bluestem, little bluestem, and indiangrass are the most prevalent and important on these species in the humid eastern part of their range. Diseases usually began to appear about mid-July and continued to increase in severity until about 1 October. Disease incidence and severity increased if stands were not cut or grazed.

According to Sprague (8), *A. brachypodii* has been reported on 14 gramineous species in the western United States, including *Andropogon gerardi, A. scoparius, Sorghastrum nutans, Avena sativa, Festuca rubra* L., *Lolium perenne*, and *Phalaris arundinacea*. The latter three species, which are cool-season grasses, were not susceptible in inoculation tests. Three other warm-season grasses, caucasian bluestem, old world bluestem, and switchgrass were not susceptible, and corn and sudangrass are reported here as new hosts of *A. brachypodii*. The fact that eight cool-season grasses were not susceptible to *A. brachypodii* indicates that the fungus may be confined to warm-season species.

A *Didymella* sp. was shown for the first time to be the ascigerous state of *A. brachypodii*. Ascocarps (pseudothecia) of the *Didymella* sp. were produced in culture but only in association with *A. brachypodii*. Smith (6) showed that *Didymella festucae* (Weg.) Holm is the ascigerous state of *Phleospora idahoensis* Sprague, but Smith and Shoemaker (7) were unable to induce ascocarp formation by *D. festucae* in culture. *Didymella* is very similar to *Mycosphaerella*, according to Corbaz (1), who considered *Ascochyta* Lib. to be the typical pycnidial form of *Didymella*, as did Wehmeyer (11). Features of the *Didymella* sp. reported here that are in agreement with those reported by Wehmeyer include the more parallel arrangement of the asci in the pseudothecium compared with the fascicular arrangement in *Mycosphaerella*, two-celled ascospores that are sharply constricted at the septum, and conidia borne in pycnidia similar to the ascostromata.

There are no reports of a *Didymella* sp. on big bluestem, little bluestem, or indiangrass and no reports of an ascigerous state of *A. brachypodii*. *Didymella andropogonis* Ell. & Ev. was described as a new species from dead leaves of *Andropogon muricatus* Retz. (2), a nonforage species of the sedge family from India. This species is also reported on *Vetiveria zizanioides* (L.) Nash (10), a name synonymous with *A. muricatus*. No conidial state of *D. andropogonis* was mentioned. Because the description of the *Didymella* sp. reported here is not similar to that given for *D. andropogonis* (2), it is concluded that this is a different species of *Didymella* not previously reported.


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