Symptoms, Association, and Pathogenicity of *Discula campestris*, a Cause of Sugar Maple Seedling Anthracnose

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ABSTRACT

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Mortality of sugar maple (*Acer saccharum*) seedlings during their first season of growth occurred in 1989 and 1990 in northern Pennsylvania stands infested with pear thrips (*Taeniothrips inconsequens*). Leaves became spotted and water-soaked, then collapsed or dropped, and upper portions of stems became necrotic. Ten samples, each consisting of five symptomatic seedlings, were collected in June from each of five stands (one in 1989, four in 1990) and incubated 2–4 days in moist chambers. Acervular conidiomata and conidia of a fungus identified as *Discula campestris* were observed on leaves of 247 of 250 seedlings. The fungus was isolated from one seedling of each sample (50 of 50 attempts). Wounding of leaves followed by inoculation with a sprayed conidial suspension resulted in collapse of leaves and mortality of laboratory-grown seedlings. Unwounded inoculated seedlings did not die, and leaf symptoms were less frequent and less severe. *D. campestris* should be considered among the causes of foliar symptoms and mortality of sugar maple seedlings, especially those injured by pear thrips.

Sugar maple (*Acer saccharum* Marsh.) seedlings are shade tolerant and can persist in the understory of northern hardwood stands (8). Seedlings can accumulate in the understory to compose advance regeneration that can contribute to an increase in proportion of sugar maple during succession to a climax (4, 7). Advance regeneration can be abundant and well established in the understory in portions of the natural range of sugar maple (23). In stands on the Allegheny Plateau of north-central and northwestern Pennsylvania, however, advance regeneration of this species is often lacking.

Failure of sugar maple seedling establishment in these stands cannot be fully explained by lack of viable seeds or poor initial seedling emergence. Although seed production in this region may be more irregular than has been reported elsewhere (8), it can be abundant every several years (2,9). In a single year, more than 1 million seeds per hectare were produced in stands on the Allegheny Plateau (2). Large seed crops can be followed by emergence of large numbers of seedlings. In spring 1990, the year following a very large seed crop, the average number of seedlings initially present in four stands exceeded 900,000/ha (21). However, losses of very large numbers of these new sugar maple seedlings have been documented on the Allegheny Plateau. In two separate

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studies, seedling mortality exceeded 99% in the first year following emergence (2,21), and similar observations have been made elsewhere on the Allegheny Plateau (O. Lynn Frank and Paul Lilja, *personal communication*).

Little information about the effects of biotic factors on emergence and survival of sugar maple seedlings is available. In an investigation done during 1985–1990 in New York State, however, seedling damage and mortality were monitored and attributed to a variety of insects, pathogens, and other agents (5). Pear thrips (*Taeniothrips inconsequens* (Uzel), Thysanoptera: Thripidae) was considered the primary cause of mortality. This introduced insect recently has been implicated as a cause of widespread and severe damage to foliage of overstory sugar maples in forests of the eastern United States.

Mortality of the 1989 and 1990 cohorts of sugar maple seedlings occurred rapidly in spring and early summer in northern Pennsylvania stands (21; Paul Lilja, *personal communication*). Pear thrips adults and larvae initially were present and symptoms included those attributed to thrips injury (5). Symptom development and mortality, however, continued even after larvae had entered the soil to continue their development.

Preliminary examination of seedlings revealed fruiting structures, similar to those formed by anthracnose fungi, on necrotic areas of leaves. The objectives of this study were to describe symptoms on seedlings, establish association of the fungus with symptomatic seedlings, identify the fungus, and demonstrate its pathogenicity. Preliminary reports of this work have been presented (19,20).

MATERIALS AND METHODS

Symptoms and association. Collections and observations were made in five stands of the black cherry–sugar maple type (11) located at elevations of 670–760 m in the Susquehannock Forest District, Potter County, Pennsylvania. One stand was sampled in June 1989 following chance observation of symptoms on seedlings of the 1989 cohort. The other four stands were sampled in June 1990 but had been selected earlier that spring before symptom development on the basis of abundance of seedlings of the 1990 cohort. All stands were in an area generally infested with pear thrips. Symptoms were observed in these stands throughout the growing season of the year that each was sampled.

To determine the association of the fungus with symptoms, 10 samples were collected in each stand at intervals of approximately 10 m along transects. A sample consisted of five symptomatic seedlings, each with two leaves which were >50% expanded. Seedlings were placed individually into plastic bags and kept in an ice chest until arrival at the laboratory (within 24 hr of collection), where they were stored at 4 C. The next day, after addition of a moist paper towel to each bag, the seedlings were incubated in darkness at 24 C. After 2–4 days of incubation, leaves were examined at 50× for conidiomata. Conidia were mounted in lactophenol-cotton blue and examined at 430× for consistency with the characteristics described below. Isolation of the fungus was attempted from one seedling of each sample by streaking conidial masses or placing minute pieces of leaf that often bore conidiomata onto Difco potato-dextrose agar (PDA). Petri dishes were incubated in darkness at 24 C. Subcultures were obtained by making transfers from colony margins to PDA slants and observed after at least 2 wk of growth for consistency with characteristics described below.

Identification. Identification of the fungus was based on examination of conidiomata and conidia from leaves and on cultural characteristics. Ten conidia from a single conidioma on each of five symptomatic seedlings collected from the forest were mounted in lactophenol-cotton blue and measured to 0.1 μm at 970×. Ten conidiomata from each of five symptomatic leaves collected from the forest were measured to 10 μm at 100×. Squash mounts of conidiomata in water and lactophenol-cotton blue were observed at 970×. Cultures on PDA were examined after 2 wk of incubation at 24 C either in the dark or exposed to light provided by two cool-white fluorescent tubes in the incubator door (also the source of light for methods described below). Conidiomata on leaves of symptomatic seedlings collected from the forest and on leaves of seedlings inoculated in the laboratory also were examined by Scott Redlin, USDA, Beltsville, Maryland; John Bissett, Biosystematics Research Centre, Agriculture Canada, Ottawa, Ontario; and B. C. Sutton, CAB International Mycological Institute, Kew, England. A single conidial isolate designated 89-32 was examined by Scott Redlin and John Bissett.

Pathogenicity. Sugar maple seedlings were grown from seeds collected in early spring 1990 in Potter and Tioga counties. Seeds were germinated on moist paper towels at 2 C with a 16-hr photoperiod. Germinated seeds were planted individually in a 1:1:1 mix of nonsterile forest soil:peat:perlite in 150-ml paper cups and watered as needed. Seedlings were allowed to develop at 24 C with a 16-hr photoperiod to the two-leaf stage with leaves approximately 50% expanded (1.5–2 cm long) before use.

Isolate 89-32 was used to produce inoculum. Three 5-mm plugs from margins of growing colonies were spaced equally on approximately 15 ml of PDA in 8.5-cm petri dishes. Then, 0.2 g of air-dried, autoclaved, crushed sugar maple leaf was distributed on the surface of the medium. After 3–4 wk of incubation at 24 C with a 16-hr photoperiod, conidiomata were abundant on the leaf fragments. Petri dishes were flooded with approximately 10 ml of sterile water, and the leaf fragments and agar surface were rubbed vigorously with a glass rod. The resulting suspension was decanted, and the concentration of conidia was quantified with a hemacytometer and adjusted by addition of sterile water to 10^6 conidia per milliliter.

Twenty seedlings were randomly assigned to five replicates of four treatments in each of two separate runs. Treatments were unwounded and uninoculated, wounded and uninoculated, unwounded and inoculated, and wounded and inoculated. Seedlings were wounded by puncturing each cotyledon

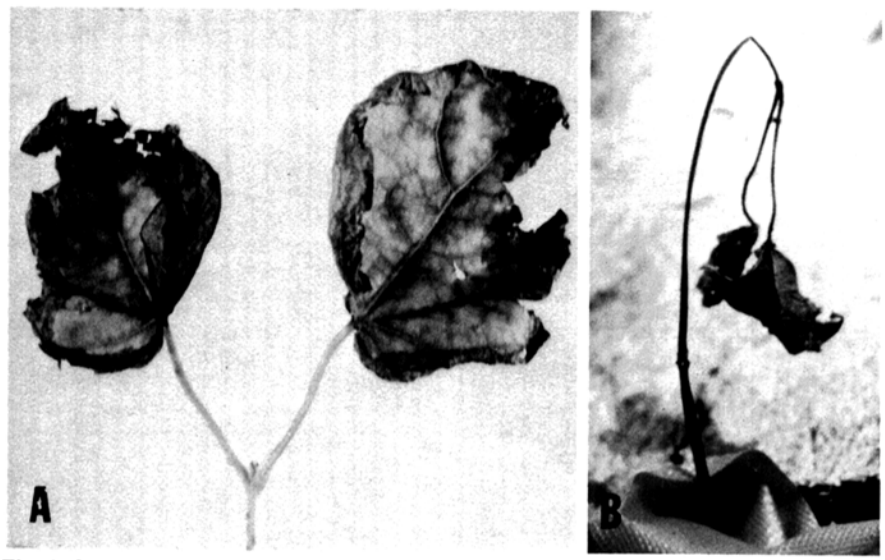

Fig. 1. Sugar maple seedlings with symptoms of anthracnose caused by *Discula campestris*: **(A)** Water-soaked, necrotic leaf margins and **(B)** collapsed leaves and "shepherd's crook" of stem.

(three times) and each leaf (three times on each side of the midvein) with a hot dissecting needle immediately before inoculation. Conidial suspension was applied to the drip point using an atomizer. Uninoculated seedlings were sprayed with sterile water. Each seedling was covered with a clear, 1.5-mil polyethylene bag to maintain a moist environment and incubated at 24 C with a 16-hr photoperiod. After 4 days, bags were removed, and after an additional 6 days (run 1) or 7 days (run 2), seedling condition was recorded and isolation of the fungus from both control and inoculated seedlings was attempted as described above. At the time of inoculations, the conidial suspension also was sprayed onto PDA in two petri dishes per trial. After 24 hr of incubation with the seedlings, 100 conidia per dish were examined to quantify germination.

RESULTS

Symptoms and association. Symptoms on seedlings observed in the forest included leaf abnormalities and necrosis. Leaves exhibited chlorosis and often appeared wrinkled or puckered. The leaf margin was sometimes absent, resulting in tattered or irregularly shaped leaves. Leaf margins and spots, along and between veins, became dark and necrotic and appeared water-soaked when moist (Fig. 1A). Necrotic areas were irregular, with indistinct margins, and were not limited by veins. Necrosis was progressive and sometimes involved entire leaf laminae, petioles, and upper stems. Necrotic leaves were often shed, but dying and recently killed seedlings sometimes exhibited a "shepherd's crook" of the upper stem with leaves attached (Fig. 1B).

The fungus being investigated was associated consistently with symptomatic seedlings in all five stands. Conidiomata and conidia consistent with the characteristics described below were found on 247 of 250 seedlings. The fungus was isolated in 50 of 50 attempts.

Identification. Conidiomata on symptomatic leaves of seedlings collected from forest stands were subcuticular, acervular, and consistent with the genus *Discula* (23). Conidiomata were pale brown when moist and brown to black when dried. They usually were hypophyllous and most frequent on interveinal tissue but also occurred on veins, petioles, and occasionally the upper stems of seedlings. Conidiomata were circular to angular, 80–210 μm ($\bar{x}$ = 133 μm) in diameter, or sometimes elliptical (up to 230 μm) on or along veins. White masses of conidia exuded under conditions of high humidity (Fig. 2A). Conidia were ellipsoidal, 6.1–9.0 μm ($\bar{x}$ = 7.7 μm) long × 1.8–2.8 μm ($\bar{x}$ = 2.3 μm) wide and usually contained polar oil droplets (Fig. 2B).

Colonies on PDA grew at 24 C to cover the entire surface of the medium in 8.5-cm diameter petri dishes in 10–14 days (Fig. 3). Hyphae at colony margins were appressed or submerged. Aerial hyphae appeared white and were more abundant and flocculent toward the center of colonies. Colonies darkened with age, sometimes in sectors, to gray or black as submerged hyphae became pigmented. Black, spherical conidiomata formed on and below the surface of the medium. Pigmentation and conidiomata were more prevalent in colonies grown in the light. Conidiomata ruptured, and conidia identical to those produced in foliar conidiomata exuded in white masses.

A tentative identification of the fungus as *Discula campestris* (Pass.) von Arx by Scott Redlin was confirmed by John Bissett and B. C. Sutton. Dried leaves bearing conidiomata and cultures of isolate 89-32 have been deposited at the National Mycological Herbarium, Biosystematics Research Centre, and its associated culture collection as DAOM 213564 and 213565, respectively. Leaves bearing conidiomata also have been deposited in the CAB International

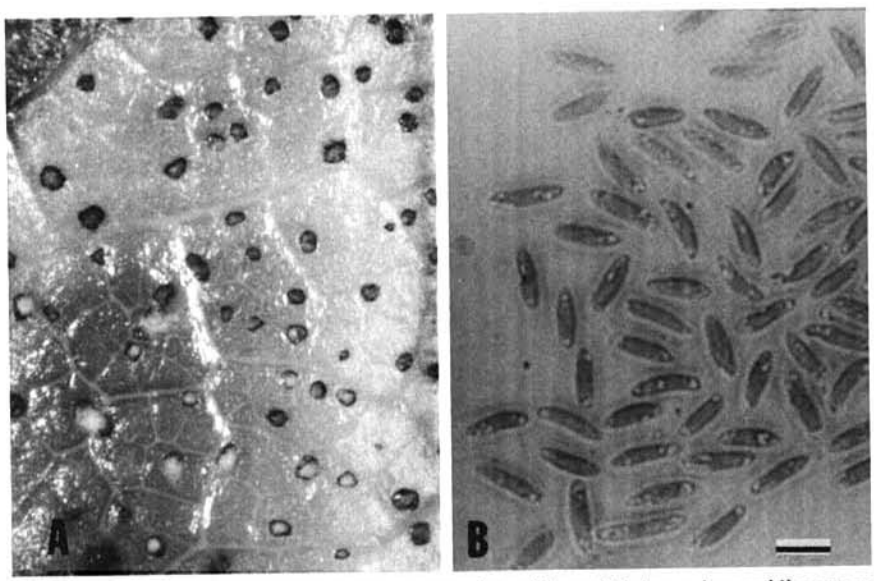

Fig. 2. Signs of *Discula campestris* on sugar maple seedlings: **(A)** Acervular conidiomata on lower surface of incubated leaf and **(B)** conidia. Scale bar = 5 μm.

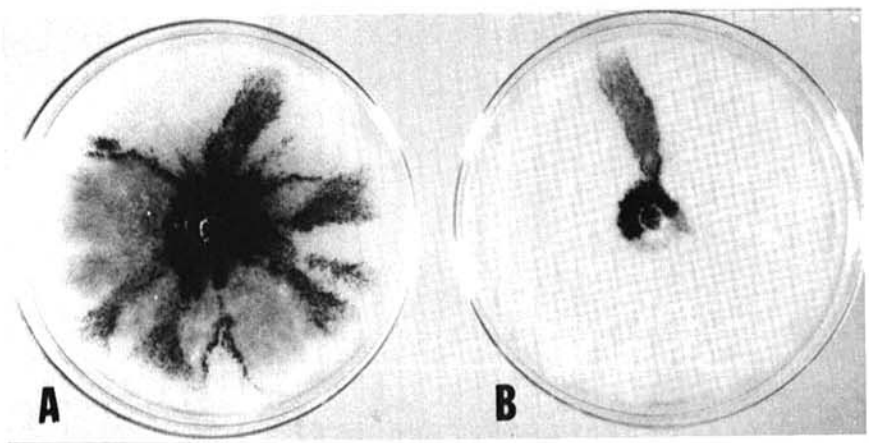

Fig. 3. Cultures of *Discula campestris* grown on PDA in **(A)** light and **(B)** darkness.

Table 1. Results of inoculation of sugar maple seedlings with *Discula campestris*

Treatment[a]	No. of seedlings/10[b] With leaf necrosis	Killed	Fungus isolated
Unwounded			
Uninoculated	0	0	0
Inoculated	3[c]	0	4
Wounded			
Uninoculated	10[d]	0	0
Inoculated	10[e]	10	7

[a] A conidial suspension or water was applied to seedlings at the two-leaf stage with leaves approximately 50% expanded. Leaves were wounded by puncturing with a hot dissecting needle.
[b] Results are pooled from two separate runs with five seedlings per treatment per run.
[c] Necrosis was limited to small, discrete spots.
[d] Necrosis was limited to a narrow, discrete zone around each wound.
[e] Necrosis was complete.

Mycological Institute herbarium as IMI 343469.

Pathogenicity. Seedling responses varied among treatments (Table 1). Unwounded and uninoculated seedlings continued development without symptoms (Fig. 4A). Leaves on wounded and uninoculated seedlings became distorted as they continued to expand around very narrow, discrete zones of necrotic tissue surrounding each wound (Fig. 4B and C). Similar leaf distortion was associated with small, discrete necrotic areas on leaves of some unwounded and inoculated seedlings (Fig. 4D and E). On wounded and inoculated seedlings, parts of leaves beyond the narrow zone killed by wounding darkened and appeared water-soaked as quickly as 4 days after inoculation (Fig. 4F). The necrotic areas on these seedlings expanded, coalesced, and eventually included petioles and stems to result in seedling death. Conidiomata of *D. campestris* were observed on the lower surfaces of these leaves. The fungus was isolated only from inoculated seedlings. Germination of conidia on PDA exceeded 90% in each trial.

DISCUSSION

Numerous fungi once classified in the genus *Gloeosporium*, including *Discula* species, are associated with leaf spots and anthracnose diseases of woody plants (18,24). *D. campestris* previously has been identified as the cause of anthracnose of sugar maple in Ontario (13), where damage to overstory foliage can be locally severe (1). The host range of *D. campestris* and the relationship of this fungus to unidentified *Discula* species on maples (18) and to similar fungi on other hosts is unclear.

Samples of overstory foliage from some of the same stands in which diseased seedlings were collected also have yielded *D. campestris* (14). Conidia produced in conidiomata on canopy leaves and washed from foliage by spring rainfall might be the inoculum for seedling infection. Pear thrips larvae also might transport spores to seedlings. These larvae develop in and emerge from overstory foliage in spring and early summer when conidiomata and conidia are present. When larvae fall to the forest floor, they might carry inoculum to the seedlings on which they feed.

Enhanced penetration or colonization of wounded sugar maple leaves by *D. campestris* is consistent with reports for fungi that cause anthracnose of ash, dogwood, oak, and sycamore (15–17). Ash anthracnose, caused by *Gnomoniella fraxini* Redlin & Stack, was associated with feeding by the ash plant bug (*Tropidosteptes amoenus* Reuter) (10, 16). The occurrence of symptoms on sugar maple seedlings in forest stands infested with pear thrips may be more than coincidence. Feeding and oviposition by this insect (5) may produce wounds that allow frequent infection and severe disease.

A related species, *D. umbrinella* (Berk. & Broome) Sutton (syn. *D. quercina*) occurs as an endophyte of *Castanea, Fagus*, and *Quercus* (22) and causes anthracnose of oak (15) and beech (3,12). Also, proliferation of endophytic *D. umbrinella* along the cut edge of surface-sterilized oak leaf pieces has been observed (Dennis Wilson, *personal communication*). The potential for *D. campestris* to maintain itself endophytically without causing detectable leaf damage is unknown. However, if *D. campestris* also is endophytic, anthracnose on sugar maple seedlings and overstory trees might result from proliferation in response to damage by thrips or other agents.

The significance of seedling anthracnose caused by *D. campestris* on sugar maple seedlings will depend on its effect, with other pests, on regeneration of affected stands. Activities of pathogens, insects, and herbivores on seedlings may result in a lack of diversity, regeneration failure, and alteration of successional patterns. The association of *D. campestris* with sugar maple and pear thrips and the potential consequences of seedling disease to the sugar maple component of forest stands should be investigated further.

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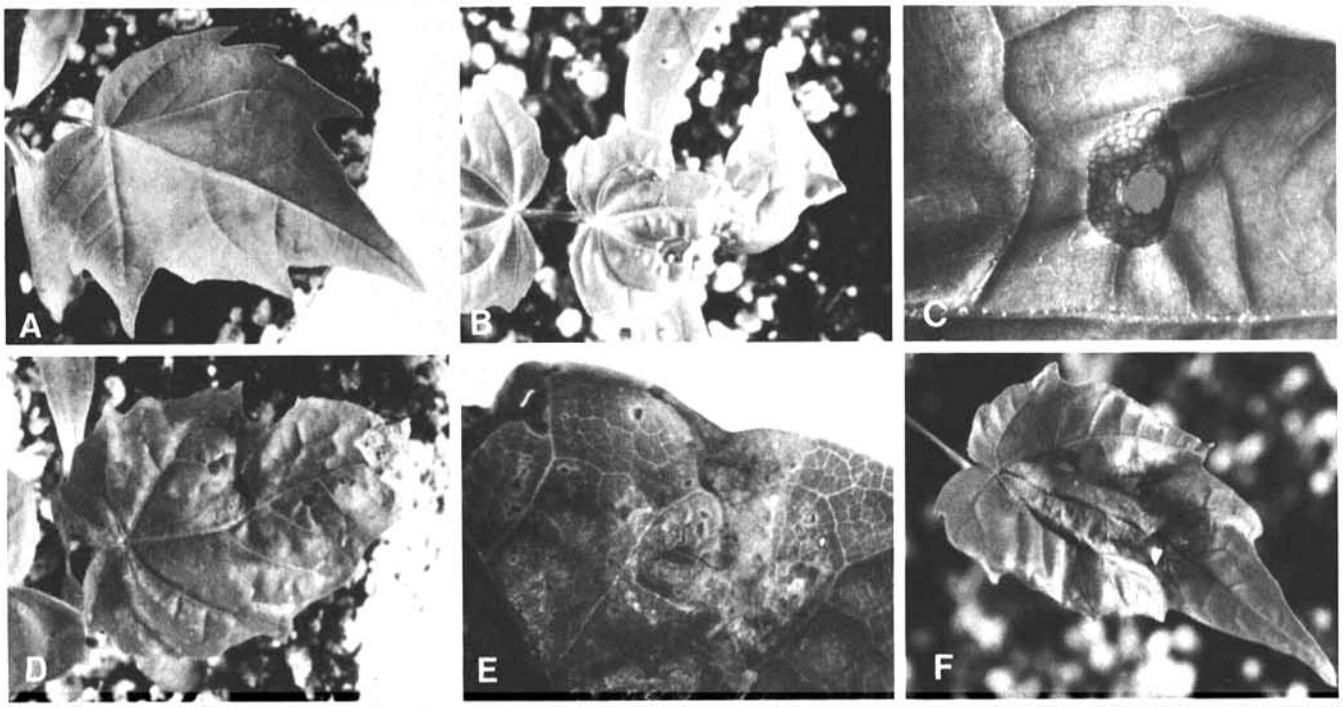

Fig. 4. Symptoms induced on leaves of sugar maple seedlings by *Discula campestris* in pathogenicity tests: **(A)** Nonsymptomatic unwounded and uninoculated seedling; **(B)** wounded and uninoculated seedling with **(C)** narrow, discrete zone of necrotic tissue around hole produced by hot dissecting needle; **(D)** unwounded and inoculated seedling with **(E)** small, discrete necrotic areas; and **(F)** wounded and inoculated seedling with darkened, water-soaked area. Time after treatment was 10 or 11 days for A–E and 4 days for F. Approximate magnification: ×2 for A, B, D, and F and ×5 for C and E.

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