Characterization and Host Range of *Drechslera catenaria*, the Pathogen of Leaf Blight and Crown Rot of Creeping Bentgrass

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ABSTRACT

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Drechslera catenaria, the pathogen of leaf blight and crown rot of Toronto creeping bentgrass, was found extremely pathogenic on five other cultivars of bentgrass (*Agrostis palustris* 'Cohansey,' 'Emerald,' 'Penncross,' and 'Seaside'; *A. tenuis* 'Highland') and a forage grass (*Phalaris arundinacea*). Necrotic local lesions formed on *Festuca* spp. and *Lolium* spp. after inoculation with *D. catenaria*. Four Kentucky bluegrass cultivars were unaffected. Similar to other *Drechslera* spp., conidial germination of this fungal isolate was found to be amphigenous. Conidiogenesis was holosporus, but the first septum delimited the basal cell.

Additional key words: *Helminthosporium catenarium*, turfgrass

The pathogen of leaf blight and crown rot of Toronto creeping bentgrass (*Agrostis palustris* Huds.) has been previously identified as *Drechslera catenaria* (Drechs.) Ito (syn. *Helminthosporium catenarium*) (5). Since 1977, *D. catenaria* has been isolated from both stolons and leaves of diseased plants, and conidia have been separated from the thatch of several Toronto creeping bentgrass greens and commercially available sod (10). *D. catenaria* has yet to be isolated, however, from any other cultivar of bentgrass or any other turfgrass species. *D. catenaria* has been commonly reported as a pathogen of forage grasses (3,6,9,11,12). This study confirms the original report of *D. catenaria* as a pathogen of creeping bentgrass and provides additional information regarding the host range and morphological characters of this fungal isolate.

MATERIALS AND METHODS

Isolation and characterization. The fungus was isolated from Toronto creeping bentgrass showing typical symptoms of leaf blight and crown rot (5). All isolations were made using the technique described by Larsen et al (5). Mycelium and conidia of *D. catenaria* were established as pure cultures on potato-dextrose agar (PDA), lactose casein hydrolysate medium (LCH) (8), and V-8 juice agar (V-8JA) (2); all were adjusted to pH 6.0 with sodium hydroxide or hydrochloric acid. Cultures were incubated at 20 C with fluorescent lighting (5.5 klux) for 12 hr daily.

Fifty mature representative conidia and 50 conidiophores were harvested from incubated diseased host tissue after 48 hr. Conidia and conidiophores were mounted in lactophenol (85% lactic acid, glycerine, phenol, water [1:1:1:0.5, v/v]) and measured with a stage micrometer. Measurements were reported as means and ranges with sum totals of measurements used to calculate the length-to-width ratio (L/W), the width of the apical septum to width of the basal septum ratio (AS/BS), the ratio of the distance of the widest part from the conidial base to conidial length (WP), and the ratio of conidial length to number of cells (the average cell length).

The mode of conidial germination was observed from conidia mounted in sterile distilled water on glass slides and incubated at 20 C under fluorescent lighting. A conidium was considered germinated when the germ tube was twice as long as it was wide.

Origin of conidia. An agar block technique was used to observe conidiogenesis. Water agar (2%) blocks 1 × 1 × 0.4 cm thick were placed in the centers of sterile glass slides. A small amount of mycelium was placed on the sides of the blocks, and each block was covered with a sterile coverglass (22 × 22 mm). The slides were incubated in a moisture chamber at 20 C with fluorescent lighting (5.5 klux) for 12 hr daily. Each moisture chamber consisted of a covered glass petri dish partially filled with sterile distilled water. The slides were supported above the water in the dishes by bent glass rods. After 4 days, the slides were removed from the moisture chambers and observed periodically during a 10-hr period.

Sclerotiumlike body formation. Disks of a 10-day-old culture of *D. catenaria* on LCH were placed on bentgrass-extract agar (BEA) or on autoclaved bentgrass leaf clippings that had been arranged on 2% water agar in petri plates. Bentgrass-extract agar was prepared by adding 20 g of water agar to 1 L of extract water from the autoclaved bentgrass leaf clippings. The BEA and the leaf-clipping media, previously seeded with the fungus, were incubated at 20 C with fluorescent lighting (5.5 klux) for 12 hr daily. The presence of sclerotiumlike bodies in the leaf tissue or in the BEA medium was recorded after 14 days.

Pathogenicity tests. Six bentgrasses (*Agrostis tenuis* Sibth. 'Highland,' *A. palustris* Huds. 'Cohansey,' 'Emerald,' 'Penncross,' 'Seaside,' and 'Toronto'), four Kentucky bluegrasses (*Poa pratensis* L. 'Adelphi,' 'Delta,' 'Merion,' and 'Park'), two fescues (*Festuca arundinacea* Schreb. 'Kentucky-31' and *F. rubra* L. 'Pennlawn'), and two forage grasses (*Lolium perenne* L. 'Manhattan' and *Phalaris arundinacea* L.) were evaluated for susceptibility to this isolate of *D. catenaria*. All grasses were established from seed except Cohansey and Toronto creeping bentgrasses. These two grasses were established from commercially available stolons. Plants were grown in 8.8-cm-diameter Styrofoam cups in a soil, peat, and perlite medium (1:1:1, v/v) in full sun in the greenhouse and fertilized at each watering with a water-soluble 20-20-20 fertilizer containing 150 μg/ml of nitrogen.

Inoculum was prepared by flooding 14-day-old LCH cultures with distilled water and gently dislodging conidia with a glass rod. A drop of Tween 20 was added per 100 ml of conidial suspension. An artist's airbrush (Badger Airbrush Co., Franklin Park, IL) was used to inoculate each pot of plants with 1 ml of the conidial suspension (about 10,000 conidia per milliliter). The pots were then sealed in polyethylene bags and incubated for 60 hr in growth chambers at 25 C with fluorescent and incandescent lighting for 12 hr daily. After incubation, the plants were uncovered and maintained at 25 C for 2 wk, then rated for disease severity.

Disease severity was rated on the basis of the percentage of diseased leaf tissue per pot on a scale of 1–6, where 1 = no

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disease, 2 = 1–5%, 3 = 6–25%, 4 = 26–50%, 5 = 51–75%, and 6 = 76–100%. The experiment was replicated three times with three subsamples per replicate. A subsample consisted of a single pot.

RESULTS

The pathogen. Fungal hyphae grew from the diseased plant tissue on LCH medium within 24–36 hr of incubation. Conidiophores were yellowish brown, simple, cylindrical, and arose singly or in small clusters, especially from leaf veinal regions. Solitary conidia (Fig. 1) formed acropetally on the conidiophores. A short (2–3 μm) secondary conidiophore formed at the apex of the primary conidium and bore a secondary conidium. Chains of three or four conidia were not uncommon. An apical scar remained on the primary conidium where the secondary conidiophore detached. Primary conidiophores measured 58–94 × 5.6–8.1 μm (av. 76.3 × 6.9 μm), 2–3 septate, with short enlarged foot cells and swollen apical cells.

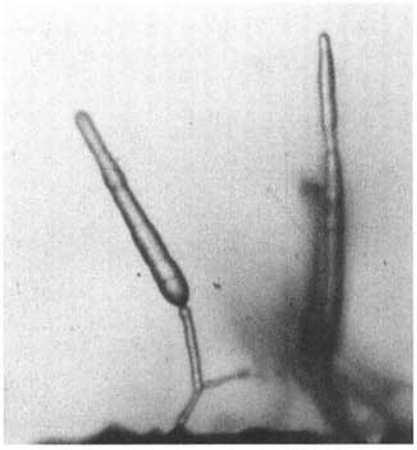

Fig. 1. Conidium and conidiophore of *Drechslera catenaria* on incubated Toronto creeping bentgrass leaf tissue (×100).

Conidia were hyaline to light yellow with thin walls and dark hila, obclavate, and tapered toward a narrower apex (AS/BS 0.50–0.71, av. 0.62). Conidia measured 73.6–126.5 × 13.8–96.6 μm (av. 110.0 × 16.2 μm), with 3–6 septa (av. 4.9), L/W 4.4–8.8 (av. 6.8), and an average cell length of 18.7 μm. The widest point (WP) of the conidium was at 0.15–0.25 (av. 0.17), which corresponded to the first septum or the second cell from the base. Basal cells of the conidia were 15.0–20.7 μm (av. 18.4 μm) long and basal septa were 13.8–19.6 μm (av. 16.1 μm) wide, giving the basal cell a hemiellipsoidal shape. Basal scars were within the contour of the basal cells.

More than 90% of the conidia germinated in distilled water within 2 hr when maintained at 20 C. Germ tubes emerged from end cells, from one or more intercalary cells, or from a combination of both. Germ tubes grew laterally or semilaterally from the basal and intercalary cells but emerged terminally from the apical cells. Multiple germ tubes emerged from any cell, but especially from the basal cell. No germ tubes emerged through the hila.

The colony characteristics of this fungus differed according to the culture medium composition. On LCH media, colonies were moderately erumpent, initially white to tan, and later olive-gray. Colonies were dense, erumpent, slate-gray to black on PDA and pale gray, moderately erumpent on V-8JA. Concentric bands of white aerial hyphae on the V-8JA colonies corresponded approximately with diurnal dark periods. Sporulation occurred on all media but only in the presence of light.

No sclerotiumlike bodies were produced when the fungus was cultured on LCH, PDA, or V-8JA under this light and temperature regime. Numerous dark, globose, sclerotiumlike bodies were found embedded in both the autoclaved leaf tissue and in the BEA plates after a 2-wk incubation period. These sclerotiumlike bodies formed from thickened mycelia and were found at or near the bottom of the BEA plates.

Conidiogenesis. The conidiogenic process could be observed on the incubated agar block cultures at ×100. The conidiophores attained their ultimate length before conidiogenesis started. The conidium appeared to have originated as a protrusion through a pore in the conidiophore apex wall (Fig. 2A). The immature conidium then expanded to a cylindrical form (Fig. 2B). The first septum delimited the basal cell (Fig. 2C), whereas the remainder of the immature conidium continued to elongate to a narrower apex (Fig. 2D,E). The next septum formed near the middle of the cell (Fig. 2F), and additional septa formed in the apical (Fig. 2G) and basal (Fig. 2H) regions. This sequence occurred within a 5-hr period.

Host range. All creeping and colonial bentgrass cultivars were susceptible to this isolate (Table 1). Disease severity was significantly higher for the bentgrasses than for most other grasses. Bentgrass cultivars Toronto, Emerald, Highland, and Penncross rated the highest in disease severity; cultivars Seaside and Cohansey were less severely affected. The pathogen was reisolated from 95–100% of the diseased leaves sampled from each bentgrass cultivar.

First symptoms on bentgrass were small reddish tan lesions or bands on the leaf blades. After several weeks, there was a tip dieback and extensive blighting of entire leaf blades. Only a tip dieback was observed on reed canarygrass (*Phalaris arundinacea*) after inoculation with this pathogen. Inoculation of K-31 tall fescue, Pennlawn red fescue, and Manhattan perennial ryegrass with *D. catenaria* resulted in reddish brown necrotic local lesions less than 1 mm in diameter. The lesions did not expand or coalesce with time and were commonly without a chlorotic halo. The fungus was reisolated from fewer than 50% of leaves with these symptoms. No symptoms were observed on any of the Kentucky bluegrass cultivars, and the pathogen could not be reisolated. No symptoms were observed on any uninoculated control plants.

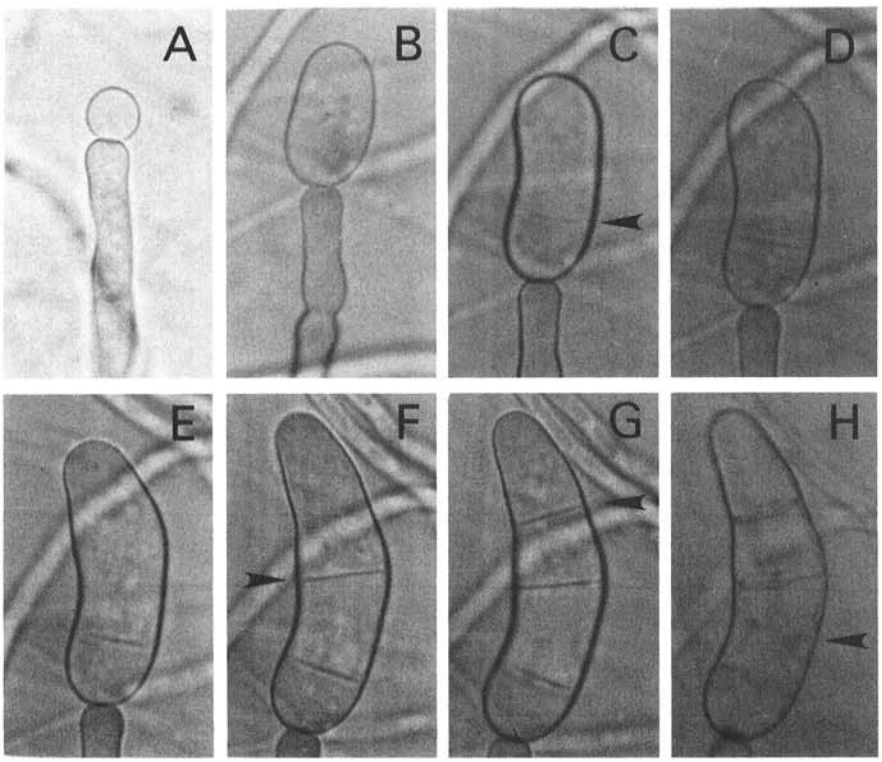

Fig. 2. Development of a conidium of *Drechslera catenaria* (×200). **(A and B)** Origin of a conidium. **(C–H)** Stages in septation (arrows) of a conidium.

Table 1. Pathogenicity of *Drechslera catenaria* on various greenhouse-grown turf and forage grasses[x]

Host	Disease severity[y]
Agrostis palustris 'Toronto'	5.0 a[z]
A. palustris 'Emerald'	3.4 b
A. tenuis 'Highland'	3.2 bc
A. palustris 'Penncross'	3.1 bcd
Phalaris arundinacea	2.7 cde
A. palustris 'Seaside'	2.4 de
A. palustris 'Cohansey'	2.4 de
Festuca arundinacea 'K-31'	2.0 ef
Lolium perenne 'Manhattan'	2.0 ef
F. rubra 'Pennlawn'	1.7 fg
Poa pratensis 'Adelphi'	1.0 g
P. pratensis 'Delta'	1.0 g
P. pratensis 'Merion'	1.0 g
P. pratensis 'Park'	1.0 g

[x] Pots of grass were inoculated with a conidial suspension (about 10,000 conidia per milliliter), incubated for 60 hr under high humidity, then maintained at 25 C with 12 hr of illumination per day throughout the test.

[y] Disease severity was rated 2 wk after inoculation on the basis of the percentage of diseased leaf tissue per pot on a scale of 1–6, where 1 = no disease, 2 = 1–5%, 3 = 6–25%, 4 = 26–50%, 5 = 51–75%, and 6 = 76–100%.

[z] Means followed by the same letter are not significantly different ($P = 0.05$) according to Duncan's new multiple range test. Means are the averages of three replicates of three subsamples each.

DISCUSSION

Conidia of the *D. catenaria* isolated from blighted creeping bentgrass were similar in morphology to conidia described by Drechsler (3) for *H. catenarium*. Conidiogenesis was found to be holosporous (sensu Luttrell [7]), with the basal cell delimited first and the conidium maturing as a whole after the remaining septa had formed. This process is similar to that reported for *D. avenacea* (syn. *H. avenaceum* Curt. ex Cke.) by Luttrell (7).

In pure culture on potato-glucose agar, Drechsler (3) reported that *H. catenarium* produced a white to dirty yellow aerial mycelium. This is in contrast to that of the isolate from bentgrass, which was initially white but later dark gray on potato-dextrose agar. In the original description of this fungus, Drechsler (3) did not report the production of sclerotiumlike bodies in culture media; neither did Braverman and Graham (1). Zeiders (12) reported the production of these structures by *H. catenarium* isolated from reed canarygrass when cultured on V-8 JA. The bentgrass isolate produced sclerotiumlike bodies on host-tissue media and in bentgrass-extract agar but not in PDA, V-8JA, or LCH media.

The bentgrass isolate of *D. catenaria* caused only necrotic local lesions on artificially inoculated *Festuca* and *Lolium* spp. Zeiders (12) found no symptoms on these two genera, but Wilkins (11) reported a similar flecking response on resistant genotypes of *L. perenne* when inoculated with *D. catenaria*. Artificial inoculation of reed canarygrass with the bentgrass isolate resulted in symptoms similar to those first reported on this host (12) in that distal portions of the leaves became necrotic.

Successful inoculation of creeping and colonial bentgrass cultivars with a *D. catenaria* isolate had not been demonstrated before the first report by Larsen et al (5). Previous inoculations of several grass species with *D. catenaria* from *Cinna arundinacea* (1,4) did not result in disease. However, *D. catenaria* was capable of causing severe damage to the bentgrass cultivars Emerald, Highland, Penncross, and Toronto, but little or no damage to the fescues, perennial ryegrass, or Kentucky bluegrass. The blighting symptoms on the inoculated bentgrass plants were similar to those observed on golf course greens with leaf blight and crown rot. The damage observed on golf course greens and the severe damage to inoculated cultivars of bentgrass show that *D. catenaria* can be a destructive pathogen and a potential threat to midwestern golf courses.

A representative isolate of *D. catenaria* from creeping bentgrass has been deposited with the American Type Culture Collection as ATCC 44224. Herbarium specimens have also been deposited with the Farlow Herbarium of the Harvard University, the National Fungus Collections, and the Ohio State University Herbarium.

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