Growth and Pathogenicity of *Leptosphaeria korrae* in Bermudagrass

J. N. CRAHAY, Graduate Student, and P. H. DERNOEDEN, Associate Professor, Department of Agronomy, The University of Maryland, College Park 20742, and N. R. O’NEILL, Research Plant Pathologist, USDA-ARS, Beltsville, MD 20705

ABSTRACT


Maryland isolates of *Leptosphaeria korrae* from bermudagrass (*Cynodon dactylon* 'Tufcote') affected with spring dead spot (SDS) were compared with a New York and a Rhode Island isolate of *L. korrae* from Kentucky bluegrass (*Poa pratensis*). Pseudothecia produced on inoculated bermudagrass roots were identical and contained ascospores of similar length (mean range, 168–189 μm) for all three isolates, confirming a common identity. The three isolates grew similarly at temperatures ranging from 15 to 30 C, with most rapid growth occurring at 25 C. Tufcote bermudagrass plants inoculated with a Maryland isolate were severely damaged at 15 C. Mortality at 20 C was 44%, but at 25 and 30 C, no disease symptoms were visible. The fungus was reisolated from diseased or necrotic plants. Greenhouse studies revealed significant differences in isolate virulence and susceptibility of bermudagrass cultivars to disease. The greenhouse inoculation methods employed, however, did not provide a reliable means for assessing cultivar susceptibility to *L. korrae*. Isolation of *L. korrae* from SDS-affected bermudagrass in Maryland and subsequent pathogenicity tests provide documentation that this fungus is an incitant of SDS in the eastern United States.

Additional keywords: Midiron, Tifway, Vamont

Spring dead spot (SDS) is a destructive disease of bermudagrass (*Cynodon dactylon* (L.) Pers.) and bermudagrass hybrids in North America and Australia (6). Wadsworth and Young (13) first documented the symptomatology of SDS in 1960 but were unable to determine its cause. Later it was speculated that *Helminthosporium spiciferum* (Bain.) Nicot. was the possible incitant of SDS (12).

In 1965, Smith (9) described a patch disease of bermudagrass in Australia with symptoms similar to those of SDS and reported the incitant to be *Ophiobolus herpotrichus* (Fr.) Sacc. Smith (10) later showed that an undescribed species of *Leptosphaeria* was a more common incitant of the patch disease than *O. herpotrichus*. Reexamination of the original collection of *O. herpotrichus*, however, revealed that this fungus should have been placed in the genus *Leptosphaeria* (14). Walker and Smith (14) redescribed *O. herpotrichus* and renamed the species *Leptosphaeria korrae* Walker & Smith and described a second, more common incitant of the disease in Australia, *L. narmari* Walker & Smith.

In 1984, Smiley and Craven-Fowler (7) isolated *L. korrae* from diseased roots of Kentucky bluegrass (*Poa pratensis* L.) showing symptoms of Fusarium blight syndrome. Pathogenicity tests confirmed that *L. korrae* could cause a patch disease of Kentucky bluegrass. Also in 1984, Jackson (3) reported the in vitro production of *L. korrae* ascospores from an isolate obtained from diseased Kentucky bluegrass patches. In 1986, Worf et al. (15) reported their pathogenicity tests with *L. korrae* and Kentucky bluegrass and renamed the new disease necrotic ring spot.

The cause of SDS of bermudagrass in the United States remained uncertain until 1985, when Endo et al. (2) reported *L. korrae* inciting typical SDS disease symptoms in California. The disease first appears in spring (2, 13), following winter dormancy. Symptoms appear as well-defined, circular patches of dead or dying turf that vary in size from a few centimeters to 0.5 m or greater in diameter. Endo et al. (2) observed blackening and rotted stems and buds, and roots. Primary and secondary roots were invaded by gray mycelium within and between cortical cells. Sclerotia, hyphae, and a “brown oozing substance” occurred in the xylem of roots, but infection of the stelar was uncommon and occurred late in disease development. Brown, elongate lesions were also observed on stolons and basal portions of stems.

A single ascosporangial isolate of the pathogen was made from one pseudothecium produced on an SDS-affected stolon (2). J. Walker verified this isolate to be *L. korrae*. In 1986, N. Jackson isolated *L. korrae* from SDS-affected Tufcote bermudagrass collected in Denton, Maryland (personal communication). The in vitro production of pseudothecia from diseased bermudagrass tissue in the United States has not been reported.

The purpose of this study was to establish the etiology of SDS of bermudagrass in the eastern United States. The objectives were to: 1) fulfill Koch’s postulates using a Maryland isolate of *L. korrae* from SDS-affected bermudagrass, 2) induce, in vitro, the production of *L. korrae* pseudothecia from diseased bermudagrass roots, 3) determine the growth rate and pathogenicity of isolates of *L. korrae* at various temperatures, and 4) determine the susceptibility of five bermudagrass cultivars to three isolates of *L. korrae*.

MATERIALS AND METHODS

Source of isolates. Isolates of *L. korrae* were obtained from New York (NY), Rhode Island (RI), and Maryland (MD). The NY isolate, received from M. Craven-Fowler of Cornell University, was isolated from roots of Kentucky bluegrass. The RI isolate, received from N. Jackson of the University of Rhode Island, was also isolated from roots of Kentucky bluegrass. The MD isolate, designated in this study as MDI, was cultured by N. Jackson from roots of SDS-affected Tufcote bermudagrass obtained from a golf course fairway in Denton, Maryland. We also isolated *L. korrae* from roots of SDS-affected Tufcote bermudagrass from the same Denton golf course and designated this isolate MDII. The technique described by Worf et al. (15) was used for isolating MDII. Briefly, roots of living plants from diseased patches were washed in running tap water for 1 hr. Roots were soaked 1 hr in sterile water and surface-sterilized for 30 sec in 1:95% ethanol and 1% NaOCl. Roots were then soaked in sterile water for 1 hr, dried on sterile filter paper for 24 hr, and transferred to a growth medium. Although several media were tested, PDA plus 100 mg of ampicillin per liter was most often associated with successful isolation of *L. korrae* and became the standard medium for resolation of the fungus from diseased roots.

In vitro production of pseudothecia. Pseudothecia of *L. korrae* were produced using the technique described by Smiley.
and Fowler (8). Roots with runner hyphae were selected from infected plants obtained from the cultivar susceptibility study (described below). Briefly, roots were washed in running tap water for 30 min, then placed in sterile, cotton-plugged test tubes on a piece of moistened gauze (100 × 40 mm). Test tubes were incubated at 22 ± 3 C on a laboratory bench and inverted twice a week to keep the gauze moist. After 2 wk, 3-5 ml of distilled water was added weekly to each tube to moisten the gauze. After 6 wk, pseudeohcia were observed and collected from roots. Ascocarps were placed on a glass slide in water and crushed under a coverslip to liberate ascospores and ascosporas. At least 10 ascospores from at least three pseudeohcia were obtained for each isolate and were measured at 400X magnification.

**Growth of L. korrae in response to temperature.** Four isolates of *L. korrae* (NY, RI, MD1, and MDII) were compared for growth rate at five temperatures. A 3-mm disk of mycelium and PDA was removed aseptically from an actively growing colony on PDA and placed in the center of a sterile petri dish containing 15-20 ml of PDA. The dishes were incubated in the dark at 10, 15, 20, 25, and 30 C. Colony diameters were measured in two directions to obtain a mean every 48 hr for 2 wk. The experimental design was a completely randomized plan with four replications.

**Temperature study.** Plants of Tufcote bermudagrass were grown about 2 wk in sand with overhead mist irrigation and transplanted to soil (described below). In this study, a 5% inoculum of the MDII isolate and soil was prepared as follows: Seeds of tall fescue (*Festuca arundinacea* Schreb.) were soaked overnight in tap water, rinsed with tap water, and mixed with wheat bran at a ratio of 1:1 (v/v). The mix was placed in plastic boxes and autoclaved twice at 120 C for 110 min. The medium was infested by placing 20 3-mm disks of mycelium and agar removed from an actively growing colony on top of the tall fescue/bran mix. The boxes were maintained in the dark at 23 C in a growth chamber for 6 wk. The inoculum was removed, spread on paper, and air-dried for 48 hr on a laboratory bench. The soil (pH 6.7) was a mixture of 53% sand, 20% silt, and 28% clay (v/v) with 2.9% organic matter. Before being mixed with inoculum, soil was autoclaved at 120 C for 90 min. Pots containing soil without inoculum served as the control. Ten plants of Tufcote were separately placed in each pot, and pots were immediately placed in growth chambers maintained at 15, 20, 25, or 30 C with 12 hr of light (150 μE·m⁻²·s⁻¹) provided daily. Each pot received 50 ml of distilled water three times per week. Plants were observed for disease symptoms and mortality counts were recorded every 3 days for 6 wk. The experiment was designed as a randomized complete block with five replications per treatment.

Seventy-one days after inoculation, five plants were taken out from each pot and thoroughly washed under tap water to remove soil debris. Tillers were counted and the length of the majority (i.e., >80%) of the roots of each plant was measured. Roots and crowns were removed, oven-dried for 48 hr at 60 C, and weighed. The mean of five plants per replication was analyzed by analysis of variance, and significant means were separated for each parameter by the Duncan multiple range test.

**Cultivar susceptibility.** Inoculum was prepared for each of the NY, RI, and MDI isolates of *L. korrae* as previously described. Mature, greenhouse-grown plants of five bermudagrass cultivars—Tifgreen, Tifway, Tufcote, Vamont, and Midiron—were evaluated for disease reaction. Tifgreen and Tifway were hybrids (*C. dactylon × C. transvaalensis* Burtt-Davy) and the other cultivars were *C. dactylon*. Stolon pieces 2-3 cm long from each cultivar with one node and two to four leaflets were surface-sterilized for 30 sec in a 5% solution of NaOCl and transplanted into sand (pH 6.5). Plants were maintained at 20-25 C and watered for 1 min every hour by mist. In subsequent studies, because of NaOCl phytotoxicity, stolons were only washed in running tap water for 2 hr. After 2 wk, plants were 2-5 cm high, had three to five leaves, and had developed roots 1-2 cm long.

The first cultivar susceptibility experiment was initiated 5 December 1986. Seed-braun inoculum (50 ml) was mixed with 450 ml of autoclaved soil to provide a 10% (v/v) inoculum. Ten plants with at least three leaves were transplanted to plastic pots (10 × 10 cm) containing 500 ml of infested soil. Five pots of 10 plants each without inoculum served as the control. Pots were equally spaced on a greenhouse bench and received 50 ml of distilled water two times a week. The temperature in the greenhouse was maintained at 22 ± 3 C. Plants were not fertilized during the test period.

Plants were observed at 3-day intervals for symptom development for 3 mo. Cultivar susceptibility and virulence of the isolates were determined by plant mortality. Plants were considered dead if all leaf and sheath tissues were necrotic. The experiment was repeated 18 February 1987 under similar test conditions. In April and May, the temperature in the greenhouse increased to 25-30 C and the plants were given additional water as needed. In each study, pots were arranged as a randomized complete block with five replications per treatment, and data were analyzed as a factorial over time.

**RESULTS AND DISCUSSION**

Pseudeohcia and ascospores. The

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**Table 1. Mean and range of ascospore length of three isolates of Leptosphaeria korrae**

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Mean</th>
<th>Range</th>
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<tbody>
<tr>
<td>NY</td>
<td>168</td>
<td>115–215</td>
</tr>
<tr>
<td>RI</td>
<td>189</td>
<td>130–235</td>
</tr>
<tr>
<td>MDI</td>
<td>172</td>
<td>100–230</td>
</tr>
</tbody>
</table>

*Mean of 10 ascospores from three pseudeohcia of NY, five pseudeohcia of RI, and 10 pseudeohcia of MDI.*

![Fig. 1. Growth of four isolates of Leptosphaeria korrae on PDA at five temperatures after 14 days. Points marked by uncommon letters are significantly different at the 5% level according to Duncan's multiple range test.](image-url)
pseudothecia produced on inoculated roots were black and flask-shaped with a thick neck. The morphology of pseudothecia originating from the three isolates was similar. On roots in test tubes, the NY isolate produced only a few pseudothecia, whereas RI and MDI were more prolific.

There were slight differences in ascospore length among the three isolates (Table 1). Ascospore length ranged from 100 to 235 μm, and mean length ranged from 168 to 189 μm. Ascospores were light brown and had six or seven septa (seven typical). No other morphological differences in ascospores among the three isolates were observed. Walker and Smith (14) described ascospores of L. korrae as filamentous and slightly twisted in the ascus, with a mean length of 140–170 μm and a range of 120–180 μm.

Smiley and Craven-Fowler (7) reported that L. korrae ascospores from pseudothecia formed on P. pratensis measured 113–138 μm. Worf et al. (15) reported ascospores ranging from 105 to 163 μm. The mean ascospore length of L. korrae from pseudothecia produced on bermudagrass roots in this study matched those reported by others (7,14,15); however, the range (100–235 μm) was much wider than those reported elsewhere.

**Growth of L. korrae in response to temperature.** The optimum temperature for growth of each of the four isolates on PDA was 25 C (Fig. 1). No growth occurred at 30 C and very little growth occurred at 10 C. These results are in agreement with those of Worf et al. (15) and Walker and Smith (14). There was no significant difference (P=0.05) in growth rate between MDI and MDII at 10, 20, or 25 C; at 15 C, however, MDI grew slightly faster than MDII. The RI isolate grew slower than MDI at 15, 20, and 25 C and slower than MDII at 20 and 25 C. The NY isolate grew slower than the three other isolates at 10, 15, 20, and 25 C. There were few differences in colony morphology or color among the isolates.

**Temperature study.** The mortality of Tufcote plants inoculated with MDII isolate was greatest at 15 C (Table 2). Most of the inoculated plants survived when inoculated at 25 or 30 C. A significant reduction in plant numbers was observed 45 days after inoculation at both 15 and 20 C. Mortality at 15 and 20 C plateaued by 49 days after inoculation and remained constant until the study ended at 57 days. All plants maintained at 15 C became necrotic 57 days after inoculation. At 20 C, 56% of the plants had survived. Nearly all of the noninoculated plants survived at each of the four growth temperatures.

Because of the 100% mortality of plants maintained at 15 C, tiller, root length, and root plus crown dry weight data were obtained only for plants maintained at 20, 25, and 30 C (Table 3). The greatest number of tillers produced after 71 days was observed in noninoculated plants maintained at 30 C. Except at 30 C, tiller number did not vary significantly between inoculated and noninoculated plants. Tiller number data show that temperature rather than presence of the pathogen was a greater limiting factor in the growth of Tufcote plants.

Root length was reduced significantly in inoculated plants at 20 C (Table 3). While runner hyphae and infection mats were observed on roots of inoculated plants at 25 and 30 C, no reduction in root length was observed between inoculated and noninoculated plants. Furthermore, the dry weight of roots plus crowns was similar for inoculated and noninoculated plants at all three temperatures. At 20 C, root plus crown dry weight was significantly reduced for inoculated and noninoculated plants when compared with plants maintained at 25 and 30 C.

Plants maintained at 15 C did not go dormant. Inoculated plants initially showed a tip dieback and chlorosis followed by death of leaves and sheaths. Roots of plants maintained at 15 C appeared dark brown to black and stunted, with only few secondary roots. Often, there was a large buildup of hyphae at the juncture between primary and secondary roots. Secondary roots were invariably necrotic, and primary roots were shorter and appeared functional.

A microscopic examination of root sections of inoculated plants revealed numerous coarse brown threads of runner hyphae at the root surface fusing into large dark, oblong infection mats or

<table>
<thead>
<tr>
<th>Temperature (C)</th>
<th>Mean number of living inoculated plants per pot</th>
<th>Mean number of living noninoculated plants per pot</th>
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<tr>
<td></td>
<td>Days after inoculation:</td>
<td>Days after initiation of study:</td>
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<tr>
<td></td>
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</tr>
<tr>
<td>15</td>
<td>10 a</td>
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*Means in columns and rows followed by different letters are significantly different at the 5% level according to Duncan’s multiple range test.

<table>
<thead>
<tr>
<th>Temperature (C)</th>
<th>Mean number of tillers</th>
<th>Root length (cm)</th>
<th>Root plus crown dry weight (mg)</th>
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<tr>
<td></td>
<td>Inoculated</td>
<td>Nominoculated</td>
<td>Inoculated</td>
</tr>
<tr>
<td>20</td>
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<td>4.9 de</td>
<td>3.1 c</td>
</tr>
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<td>25</td>
<td>7.9 bc</td>
<td>6.4 cd</td>
<td>8.5 a</td>
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<tr>
<td>30</td>
<td>9.3 b</td>
<td>11.7 a</td>
<td>8.7 a</td>
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</tbody>
</table>

*Data omitted for 15 C because all plants died.
*Tillers of five plants from each of five replicates were counted.
*Root lengths of five plants from each of five replicates were measured with a ruler and averaged.
*Roots plus crowns of five plants from each of five replicates were oven-dried at 60 C for 48 hr.
*Means in columns and rows followed by the same letter are not significantly different at the 5% level according to Duncan’s multiple range test.
infection cushions. Infection of the cortex by finer hyphae was also frequently noted in plants maintained at 15°C. Endo et al (2) reported that the hyphae can appear in and between the cortical cells and noted the presence of sclerotia in the cortex of the primary roots. They also found sclerotia produced externally on leaf sheaths, stolons, and culms. Sclerotia-like bodies were not observed in this study, but they might have developed if the incubation period had been longer. Endo et al (2) also described the presence of a brown, oozing substance in the xylem of primary and secondary roots and noted that infection of the stele was infrequent but may occur in the last stages of the disease.

Examination of roots from inoculated plants revealed the presence of dark brown runner hyphae and root injury of plants maintained at 20, 25, and 30°C. At 30°C, roots of inoculated plants were longer, white, and healthy appearing and tended to have fewer runner hyphae and infection cushions than roots of plants maintained at 20 or 25°C. Examination of root sections of control plants also revealed the presence of runner hyphae, although these hyphae generally appeared wider than L. korrae and did not produce infection cushions.

Data presented in Tables 2 and 3 show that injury caused by L. korrae was most severe at 15°C, but 44% mortality occurred at 20°C. Endo et al (2) reported severe damage to nondormant bermudagrass plants inoculated with L. korrae and maintained at 13°C, but only slight injury was observed in plants maintained at 24°C. Low temperatures have been long recognized as an important factor in SDS causation. In 1960, Wadsworth and Young (13) concluded that a cool-weather root rot pathogen was probably responsible for SDS, because disease symptoms appeared in the spring, and that damage probably occurred during the winter dormancy period. Smith (11) observed that temperatures ranging from 10 to 20°C were most favorable for SDS development in Australia. Lucas (4) suggested the disease may also occur in the fall prior to winter dormancy in North Carolina. In Maryland, circular depressions in the thatch, induced by the SDS pathogen, generally appear in March and April before spring green-up in early to mid-May. Cool temperatures are invariably associated with SDS, presumably because the pathogen is able to grow at soil temperatures that slow the growth of bermudagrass (6). Hence, low temperature retards the capacity of bermudagrass roots to resist or escape invasion by L. korrae.

Bermudagrass root growth is optimum in 35°C but extremely slow in 15°C (6). Whereas the optimum growth of L. korrae is at 25°C, it appears that root growth of bermudagrass at this temperature is faster than the ability of the pathogen to injure roots significantly. It is also possible that bermudagrass defense mechanisms are more effective at temperatures above 25°C, as compared with 15 and 20°C. Warm-season grasses such as bermudagrass begin to enter winter dormancy when temperatures fall below 12°C (1). When soil temperatures rise to 17°C in spring, however, bermudagrass spring root decline may result (5). It is probable that the pathogen is capable of actively invading roots in spring or fall when soil temperatures are above 10°C. It therefore appears possible that pathogen invasion concomitant with spring root decline may exacerbate SDS. This may explain the sometimes divergent field symptoms of SDS, which often include both circular patches and diffuse areas of injured or dead turf.

Cultivar susceptibility. The results of the first cultivar susceptibility study are presented in Table 4. Because there were significant differences (P = 0.05) in mortality rate of the plants among the three isolates, results are presented separately. The data in Table 4 show the mean number of living plants for each cultivar-isolate combination 72 days after inoculation for study I. There was no significant difference between the control plants and Tifgreen or Vamont plants inoculated with the NY isolate. Survival of Tufcote, Tifway, and Midiron plants inoculated with the NY isolate, however, was significantly reduced. When inoculated with the RI or MDI isolate, all cultivars had significantly reduced plant counts. Vamont was most severely damaged by the RI isolate, whereas Tufcote was severely injured by MDI.

In the second host range study, data were combined because there was no interaction in plant mortality among the three isolates within a cultivar. Seventy days after inoculation, only Tifgreen plant counts had not been reduced significantly (data not shown). Inoculated Midiron, Tifway, Tufcote, and Vamont plant counts were reduced significantly and showed a similar level of survivability (mean range of surviving inoculated plants was 4.4–7.0 plants per pot and that of noninoculated plants, 9.4–10.0 plants per pot). Plant survival was greater in study II than in study I. This was attributed to the higher greenhouse temperatures (25–30°C) in study II than in study I (22 ± 3°C). In both cultivar studies, however, Tifgreen showed higher plant survival when inoculated with the NY isolate. There were no obvious differences in the level of virulence of the RI and MDI isolates among the five cultivars in either study.

Results of the cultivar susceptibility studies were not consistent with field observations on the relative susceptibility of the cultivars to SDS. For example, Vamont and Midiron are generally considered to have good to excellent SDS resistance, respectively, whereas Tifgreen, Tifway, and Tufcote are extremely susceptible to the disease. Data therefore indicated that the greenhouse environment and/or inoculation techniques used in this study do not provide a reliable means for assessing bermudagrass cultivar susceptibility to L. korrae.

The significance of the cultivar susceptibility study was, however, twofold. Pseudothecia of the three isolates were produced on roots taken from plants at the conclusion of study I. Second, the MDI isolate of L. korrae was reisolated from roots of infected plants in study I and used in study II to fulfill Koch's postulates. Hence, production of pseudothecia and ascospores of L. korrae and the reisolation of the MDI isolate from study I followed by infection and death of plants in study II establish that L. korrae is associated with SDS in the eastern United States.

ACKNOWLEDGMENTS

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LITERATURE CITED


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</table>

*a* Mean greenhouse temperature ranged from 20 to 24°C.

*b* There were 10 plants per pot and five replications per treatment and five replications of noninoculated control pots for each cultivar.

*c* Means in columns and rows followed by the same letter are not significantly different at the 5% level according to Duncan's multiple range test.

Table 4. Mean number of living plants of five bermudagrass cultivars 72 days (study I) after inoculation with three isolates of Leptosphaeria korrae.


