Effect of Cold Acclimation and Freezing on Spring Dead Spot Severity in Bermudagrass

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Abstract. Spring dead spot (SDS), caused by three root-infecting species of *Ophiosphaerella*, is a destructive disease of bermudagrass (*Cynodon spp.* L.C. Rich). We tested the effects of incubation temperature and duration, and exposure to decreasing freezing temperatures on bermudagrass shoot survival following inoculation with SDS pathogens. Inoculated plants exposed to freezing temperatures as high as -2°C following a two month incubation exhibited extensive shoot mortality and had SDS symptoms consistent with those observed in the field. Lowering the freezing temperature from -2 to -8°C increased disease severity and shoot mortality on noninoculated bermudagrass. Inoculated bermudagrass incubated for 1 month in the greenhouse, then for an additional month at 4°C had greater shoot mortality following freezing than plants incubated at 25°C . Although cold acclimation and freezing intensified SDS symptoms, the technique did not reliably distinguish between resistant and susceptible cultivars.

Spring dead spot, caused by the three closely related soil-borne fungi *Ophiosphaerella korrae* (J. Walker & A. M. Smith) Shoemaker & C. E. Babcock, *O. narmari* (J. Walker & A. M. Smith) Wetzel, Hulbert & Tisserat, and *O. herpotricha* (Fr.: Fr.) J. Walker, is a disease of common bermudagrass (*Cynodon dactylon* (L.) Pers.) and its hybrids (*C. dactylon* x *C. transvaalensis* Burt-Davy) in the United States (Crahaay, et al., 1988; Endo et al. 1985; Tisserat et al., 1989; Wetzel et al., 1999) and Australia (Hawkes, 1987; Smith, 1971). SDS results in the formation of circular dead spots in spring as the turfgrass breaks winter dormancy. These spots are usually less than 1 m in diameter, but they may coalesce to blight larger areas of turfgrass. Roots and stolons within patches are severely rotted and bermudagrass recovery during the summer is slow. Dead spots may reappear in the same location each year.

Control of SDS has been difficult to achieve by cultural or chemical practices. Reducing thatch by aerification and vertical mowing, using ammonium-based fertilizers or sulfur to reduce soil pH, and increasing potassium levels in the soil, have provided only moderate reductions in SDS severity (Derneoden et al., 1991; Tisserat and Fry, 1997). Similarly, preventive fungicide applications in fall or spring generally have not

provided consistent control of SDS (Tisserat and Fry, 1997)

Another method of managing SDS is the use of moderately resistant cultivars. Pair et al. (1986) observed that the bermudagrass hybrid 'Midiron' exhibited increased winter hardiness and less SDS severity when compared to other common bermudagrass selections and to 'Tifway' hybrid bermudagrass. This observation was corroborated by others (Tisserat et al., 1989; Baird et al., 1998) in subsequent field trials. Several other vegetative and seeded bermudagrass cultivars with improved turf quality, cold tolerance and increased resistance to SDS have been identified (Baird et al., 1998; Martin et al., 2001a, 2001b).

Current techniques for screening new bermudagrass selections for resistance to SDS are slow and relatively labor intensive. Typically, SDS symptoms do not develop with consistency until 2 years after field inoculation (Baird et al., 1998; Tisserat et al., 1989). This requires that a relatively large number of bermudagrass selections must be managed in the field for 3 or more years to assess accurately SDS resistance. Early attempts to develop a more rapid greenhouse-based screening procedure for SDS involved inoculating plants, incubating them for several months and then rating roots for discoloration and rotting (Baird et al., 1998; Tisserat et al., 1989). All selections exhibited root discoloration but none developed shoot mortality typical of SDS and no differences among selections were detected. Nus and Shashikumar (1993) found that inoculation of bermudagrass cultivars with

O. korrae increased the temperature at which freeze injury to roots occurred, and that the effect was more pronounced on the susceptible cultivar. Thus, exposure to freezing temperatures following fungal infection of roots might be a useful technique for screening bermudagrass selections for SDS resistance.

The objective of our research was to develop techniques for consistently reproducing SDS symptoms on bermudagrass in a controlled environment. Specifically, we tested the effects of incubation temperature and duration, and exposure to decreasing freezing temperatures on bermudagrass shoot survival following inoculation with SDS pathogens.

Materials and Methods

Incubation duration. An experiment was conducted to determine the effect of two inoculum levels of four *Ophiosphaerella* isolates, and the duration of incubation at 4°C , on shoot survival of 'Midlawn' and 'Tifgreen' bermudagrasses. Soil-free stolons of each cultivar were placed in 6.4×6.4 -cm plastic pots containing a steamed 1 soil : 1 perlite : 1 peat mixture (by volume) and plants were grown in the greenhouse for 4 months. Plants were watered, fertilized, and trimmed with scissors as needed. Soil in four pots of each cultivar was amended with two or four sterilized oats or oats infested with *O. herpotricha* isolate KS-1, *O. narmari* isolate KS 370, *O. korrae* isolate A16-C4 representing an amplified fragment length polymorphism group (AFLP group I) widely distributed on kentucky bluegrass and bermudagrass in the northern United States (Iriarte, 2003; Wetzel et al. 1999), or *O. korrae* isolate MS-73 representing an AFLP group (group II) distributed in the southern United States (Wetzel et al. 1999). Infested oat seeds, prepared as previously described (Tisserat et al., 1989), were inserted into the potting mix about 2 cm below the surface and 0.5 cm deep into the side of the root-soil plug. The grass was then repotted. The process was repeated on four additional pots of each cultivar after 15 and 30 d. All pots were placed in the greenhouse at 21 to 28°C for 1 month as previously described, then moved to a growth chamber at a constant 4°C with a 12-h photoperiod (light intensity $200 \mu\text{mol m}^{-2}\text{s}^{-1}$). The staggered inoculation dates resulted in the three sets of plants being incubated at 4°C for 15, 30, or 45 d. All plants were then placed in a programmable freezer (Tenney Engineering, Union, N.J.) at 0°C . Ice was placed on top of the plants to speed the cooling process to 0°C . Soil temperature at a depth of 1.5 cm was measured with a thermocouple embedded in a pot located in the center of the chamber. The chamber temperature was lowered by 1°C h^{-1} until the soil temperature reached and was maintained at -4°C for 1 h. Pots then were held at 4°C for 24 h to allow the soil to thaw slowly. Bermudagrass leaves in all pots were killed following freezing and it was not possible to immediately assess treatment effects on shoot mortality. Therefore, pots were placed in the greenhouse for 1 month at temperatures ranging from 21 to 28°C to allow for bermudagrass regrowth. Percentage shoot

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Fig. 1. Shoot survival in two bermudagrass cultivars after inoculation with *Ophiostoma* spp. Plants were incubated for one month in the greenhouse and then at 4 °C for up to 45 d before freezing at -4 °C for 1 h. Vertical bars represent 95% confidence intervals.

was determined after 1 month as previously described. Acclimated and nonacclimated plants were analyzed separately as 4 (freezing regime) × 4 (cultivar) × 5 (pathogen) factorial designs with 2 replications. The experiment was repeated and data from the two experiments were combined for analysis using SAS.

Results

Inoculum level and incubation duration. Doubling the inoculum level of *O. herpotricha*, but not *O. korrae* or *O. narmari*, decreased shoot

survival (7.8% vs. 23.0 % for *O. herpotricha* inoculum, $P \leq 0.05$) when averaged over all cultivars. Shoot survival was affected by interactions ($P = 0.03$) of SDS pathogens and the duration of incubation at 4 °C on individual cultivars (Fig. 1). Shoot survival in noninoculated Tifgreen and Midlawn was >90% following cold acclimation for 15 and 30 d respectively, but dropped to <60% on Midlawn at 45 d. Generally, inoculations with *Ophiostoma* further reduced shoot survival on both cultivars. *O. herpotricha* caused the most disease severity, with almost no survival of Midlawn after 30 d of incubation at 4 °C and Tifgreen after 45 d at 4 °C. Shoot survival on Tifgreen inoculated with *O. herpotricha*, but not with *O. narmari*

or the two *O. korrae* isolates, decreased with increased duration of incubation.

Cold acclimation and freezing. Disease severity in bermudagrass cultivars was affected by the interactions ($P < 0.0001$) of incubation and freezing temperatures following inoculation with *Ophiostoma* (Figs. 2 and 3). Shoot survival was >60% in noninoculated cultivars incubated at 25 °C before freezing at temperatures as low as -8 °C, with the highest survival rates in Guymon and Tifgreen (Fig. 2). Shoot survival was further reduced by inoculation with some *Ophiostoma*, but generally only after plants were exposed to freezing temperatures less than -4 °C. Aggressiveness of SDS pathogens varied with cultivar. *O. korrae* group I was the most aggressive on Tifgreen, whereas *O. korrae* group II was more damaging to Guymon.

Freezing temperature injury and SDS severity were more severe on all cultivars when they were incubated at 4 °C compared to 25 °C before freezing. Shoot survival on noninoculated cultivars incubated at 4 °C decreased from >95% after freezing at -2 °C to <25% following exposure to -8 °C (Fig. 3). Inoculations with SDS pathogens further reduced shoot survival, even at the highest freezing temperature. In general, *O. herpotricha* was more aggressive than either *O. korrae* I or II on 'Midlawn' and 'Tifgreen'.

Discussion

Increased resistance to spring dead spot has been noted in bermudagrass cultivars that also exhibited increased freezing resistance (Baird et al., 1998, Martin et al., 2001). We hypothesized that the less winter hardy cultivars 'Champion' and 'Tifgreen' would have more shoot mortality than Midlawn and Guymon. However inoculation with *O. herpotricha*, and in some cases with *O. korrae*, caused such extensive damage to cold-acclimated cultivars that differences

survival was determined by dividing the number of live shoots by the total number of shoots in each pot. Data for each cultivar were analyzed separately as a 2 (inoculum level) × 3 (incubation period) × 5 (pathogen) factorial design with 4 replications using SAS (version 8.11 for Windows; SAS Institute, Cary, N.C.).

Cold acclimation and freezing. Bermudagrass cultivars 'Tifgreen', 'Champion', 'Midlawn' and 'Guymon' were grown as previously described. Four sterile oats or oats infested with a mixture of three *O. korrae* isolates (A16-C4, A11-84A, and A16-3-2) representing AFLP group I, three *O. korrae* isolates (AR155, MS73, and AL-387-6) representing an AFLP group II or a single isolate of *O. herpotricha* (KS-1) were inserted into the potting mix as previously described. Inoculated plants were placed in the greenhouse at 21 to 28 °C for 1 month. Eight pots of each treatment combination (cultivar × inoculant) were then placed in growth chambers at a constant 25 °C (nonacclimated) or 4 °C (cold-acclimated). After 1 month, cold-acclimated plants were placed in the freezing chamber at 0 °C, and the temperature was lowered 1 °C·h⁻¹ as previously described. Two pots of each cultivar × SDS pathogen combination were removed when soil temperatures reached -2, -4, -6, or -8 °C. The same procedure was followed the next day for the nonacclimated plants. Percentage shoot survival

Fig. 2. Shoot survival in four bermudagrass cultivars after inoculation with *O. herpotricha* and *O. korrae* (isolates representing AFLP groups I and II). Plants were incubated in the greenhouse for 1 month and then in a growth chamber at 25 °C for an additional month before freezing at either -2, -4, -6, or -8 °C. Vertical bars represent 95% confidence intervals.

Fig. 3. Shoot survival in four bermudagrass cultivars after inoculation with *O. herpotricha* and *O. korrae* (isolates representing AFLP groups I and II). Plants were incubated in the greenhouse for one month and then in a growth chamber at 4 °C for an additional month before freezing at either -2, -4, -6, or -8 °C. Vertical bars represent 95% confidence intervals.

were not discernable. Thus, our technique cannot currently be used to screen bermudagrass selections for SDS resistance. A more reliable technique may be to screen bermudagrass genotypes for SDS resistance in field trials after they have been tested for freeze tolerance under controlled conditions (Anderson and Taliaferro 2002; Anderson et al., 1993, 2003).

In the first experiment, inoculation with a single *O. korrae* isolate representing AFLP group I and II caused only a slight reduction in shoot survival when compared to noninoculated plants. Iriarte et al. (2004) reported differences in aggressiveness among *O. korrae* isolates to bermudagrass. In the second experiment we inoculated with a mixture of *O. korrae* isolates from each AFLP group to enhance disease development. This resulted in increased disease severity. We did not note any consistent differences in aggressiveness of isolates representing the two AFLP groups. These results are similar to those reported by Iriarte et al. (2004).

We initially incubated plants at 4 °C for 30 days to reduce detrimental effects of freezing nonacclimated bermudagrass (Nus and Shashikumar, 1993; Rajashekar, et al. 1983). However, SDS severity was greater in inoculated bermudagrass incubated at 4 °C compared to 25 °C. Crahay et al. (1988) postulated that cool soil temperatures inhibit bermudagrass root growth while favoring root colonization by *O. korrae*. Smith (1971) observed that soil temperatures ranging from 10 to 20 °C were most favorable for SDS development. Endo et al. (1985) reported severe damage to bermudagrass plants inoculated with *O. korrae* and incubated at 13 °C, but only slight injury in plants maintained at 24 °C. Conversely, Tisserat et al. (1989) found no differences in bermudagrass root discoloration caused by *O. herpotricha* following incubation at 15 and 25 °C. Furthermore, in

vitro growth of *O. korrae* and *O. herpotricha* was inhibited at temperatures below 10 °C (Tisserat et al., 1989; Crahay et al., 1988). Thus it is unlikely that the enhanced SDS severity we observed in cold-acclimated plants can be fully explained by increased root colonization by *Ophiophora* spp. at 4 °C. Alternatively cold acclimation may have further depleted carbohydrate reserves in crowns and roots that had already been colonized and damaged by SDS pathogens during incubation at 25 °C. Long exposures to low temperatures can deplete carbohydrate reserves in crown and roots and increase susceptibility to freeze damage (Dunn et al., 1980) and this may explain the decreased shoot survival in noninoculated, cold-acclimated plants we observed at freezing temperatures below -4 °C. We observed that 'Midlawn' had similar or lower survival rates than 'Tifgreen' bermudagrass following exposure to freezing temperatures in the absence of disease. This was unexpected since previous studies have shown that 'Midlawn' is more freeze tolerant (Anderson et al., 2002). We noted that 'Tifgreen' grew more vigorously in the greenhouse and resulted in a more dense root system. This could have increased carbohydrate reserves and influence shoot survival following freezing. McCarty et al. (1991) found that carbohydrate reserves were lower in SDS-affected bermudagrass shoots following low temperature exposure in the fall, and that the diseased bermudagrass was more susceptible to freezing. Thus, SDS severity apparently is a function of reduced freeze tolerance caused by colonization and damage by SDS pathogens in late summer and fall at temperatures above 10 °C, and by carbohydrate depletion during winter.

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