

//BEST BERMUDAS

Infection and colonization of bermudagrass by spring dead spot

By Francisco Flores and Nathan Walker, Ph.D.

Spring dead spot is the most damaging and important disease of bermudagrass grown in locations where the grass undergoes cold temperature induced dormancy (Smiley et al., 2005). The disease is caused by one of three fungal species in the genus *Ophiosphaerella* (spp. *herpotricha*, *korrae* or *narmari*). The disease results in the appearance of unsightly, dead patches on fairways, tees and greens in late spring and early summer. The patches may persist into summer months and result in increased

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management inputs to eliminate opportunistic weeds and encourage regrowth of bermudagrass into the dead areas. The diseased patches are often sunken, resulting in an uneven playing surface that can interfere with ball roll or lie. In the transition zone, the weather conditions associated with late spring and early summer, are often some of the most desirable to play golf. These coincide with the poor turf appearance and reduced quality of playing surfaces associated with the disease.

Management approaches for spring dead spot can include cultural or chemical control measures or host resistance. Cultural methods can include



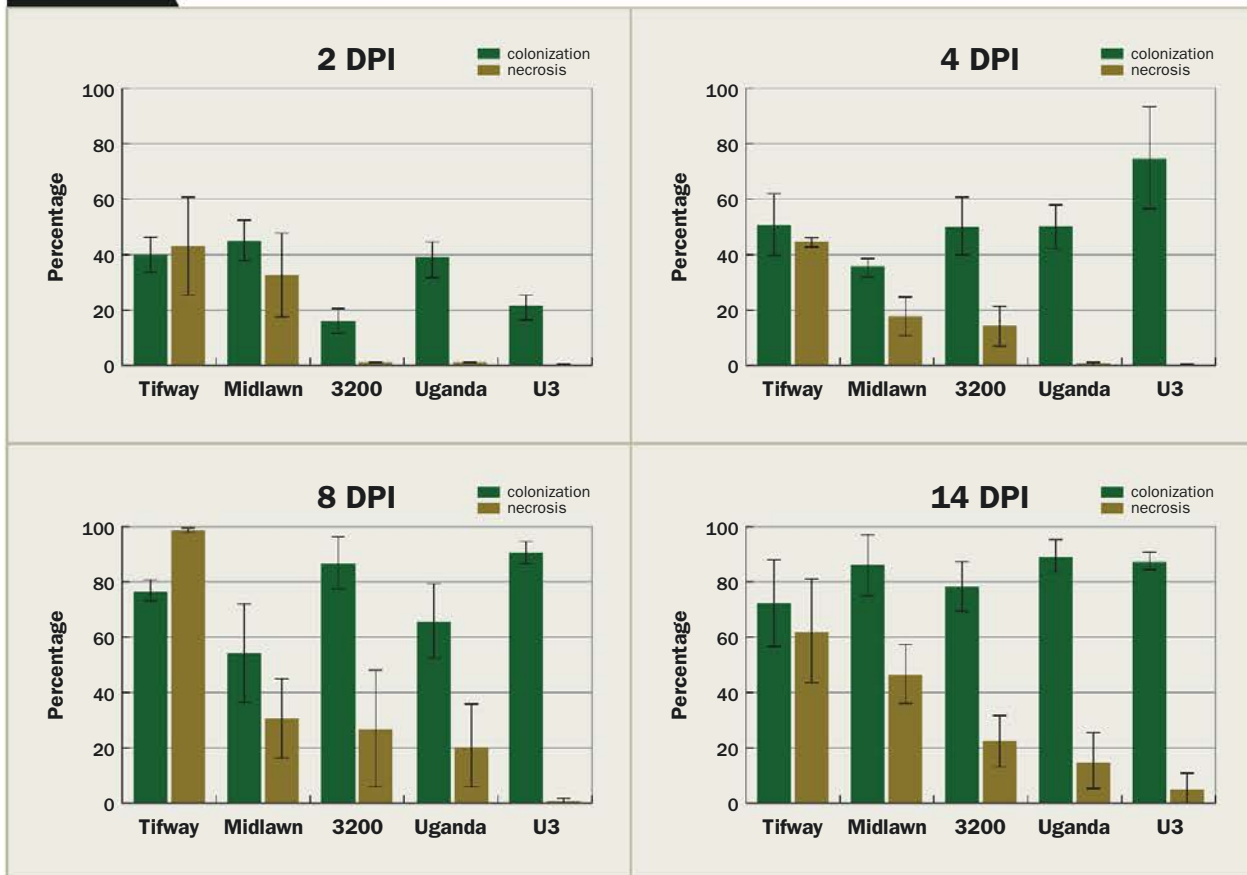
soil aeration or disturbance, to reduce compaction, and raising mowing heights prior to dormancy (Lucas 1980; Smiley et al 2005). Additional management efforts have addressed nitrogen fertility including reducing application rates and avoiding applications late in the growing season that may delay normal plant dormancy. Many studies have also examined the use of fungicides for the control of spring dead spot. Fungicide applications can be expensive and are typically made in the fall. Following application, irrigation water is used to move the fungicide into the rootzone to target the fungus-plant association. A second fungicide application is usually recommended approximately 28 days later.

Both cultural and fungicide management approaches have not been entirely effective in suppression of the disease. Long-term, durable management of plant diseases can be achieved through host genetic resistance to the pathogen. Through host resistance, the plant can recognize the pathogen early in the infection process and take a variety of steps to prevent successful infection and establishment of disease. These include induced plant cell death that prevents the pathogens from having access to living cells and acts like a wall, stopping the pathogen. Other actions plant cells can

utilize are manufacturing of toxins or other compounds that inhibit or kill the pathogen, or the formation of barriers inside cells.

In previous studies that examined host resistance to spring dead spot, isolates of *O. herpotricha* were genetically transformed to express fluorescent visualization proteins which permitted the study of the fungus-plant interaction. This interaction was studied on several different cultivars that varied in sensitivity to spring dead spot, including the interspecific hybrid (*Cynodon dactylon* × *C. transvaalensis*) bermudagrass cultivars, Tifway 419 and Midlawn, and an African bermudagrass accession (*C. transvaalensis*). Tifway 419 and Midlawn root cortical cells were rapidly colonized, while the vascular tissues remained uncolonized. Infection of Tifway 419 roots almost always resulted in necrosis whereas colonization of Midlawn roots exhibited very little necrosis. For *C. transvaalensis* roots, the cortical cells were sparsely colonized, while the vascular tissues were extensively colonized and very little root necrosis was observed. In general, Tifway 419 roots exhibited greater colonization and necrosis than the more tolerant cultivar Midlawn and *C. transvaalensis*.

On stolon surfaces, Tifway 419 appeared to be colonized more than the stolons of either Midlawn or *C. transvaalensis*. Colonization of stolons of all three bermudagrasses appeared limited to the surface and no fungal ingress into stolon cortical tissues was observed. Internal colonization of stolons was observed when *O. herpotricha* grew into the cut end of the stolons. For Tifway 419, internal stolon infection resulted in

FIGURE 1


Colonization and necrosis caused by *O. korrae* on bermudagrass roots at 2, 4, 8 and 14 days post inoculation of different cultivars. Quantitative data were obtained using ImageMagick. Bars represent standard error.

necrotic tissue, while in Midlawn stolons, similar internal infection resulted in less severe necrosis. For *C. transvaalensis* stolon tissues were internally colonized without any apparent necrosis.

Results of these studies permitted the formulation of several ideas about host recognition and response to fungal infection. The purpose of this study was to continue efforts to identify bermudagrasses that may have better and more durable resistance to spring dead spot.

HYPOTHESIS AND RESEARCH OBJECTIVES

Given that several species of *Ophiophtherella* cause spring dead spot, we wanted to test the hypothesis that these fungi have the same interactions with their various hosts. The objective of this study was to describe the interaction of *Ophiophtherella korrae* with

various bermudagrass hosts (e.g. how different host tissues and organs react to infection) and to provide a rational basis for the development of strategies for more effective disease control based on host genetic resistance.

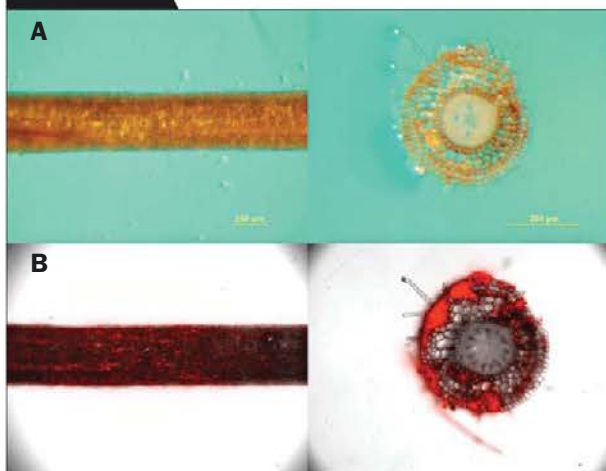
EXPERIMENT AND METHODS

A transformation technique described by Caasi et al. (2010) was used to transform an isolate of *Ophiophtherella korrae* to express tdTomato (tdTom)

Continued on page 34

**“In one year, one
sedge tuber can become
6,900 new tubers.”**

FIGURE 2



Colonization and infection of a Tifway root by *Ophiostphaerella korrae* red fluorescent transformant, 8 days post infection. (A) Bright field (visible) image of root having dark discoloration indicating necrosis and (B) same image with red fluorescence corresponding to the fungus.

FIGURE 3



Colonization and infection of the roots of U-3 by *Ophiostphaerella korrae* expressing red fluorescence genes, 8 days post infection. (A) Bright field (visible) image of root lacking dark necrosis and (B) same image with red fluorescence corresponding to the fungus.

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fluorescent protein. The hybrid bermudagrass cultivars (*Cynodon dactylon* × *C. transvaalensis*) Tifway and Midlawn, a common bermudagrass (*Cynodon dactylon*) U-3, and two *C. transvaalensis* cultivars Uganda and 3200 were evaluated for their response to infection by *O. korrae*. Stolon segments were surface sterilized using bleach, and incubated for up to seven days at 77° F to permit root growth. Rooted stolons that were free of contamination were selected and inoculated with *O. korrae* either on a root or on the stolon internode with a 1/64 inch diam. agar plug from the margin of an *O. korrae* culture. Inoculated plants were incubated at 63° F, which is conducive for fungal infection, and were exposed to a 12-hour simulated daylight photoperiod. One non-inoculated plant for every three inoculated replicates was used as a non-inoculated control.

Whole plant organs or thin sections through roots or stolons were observed from one day post inoculation to 28 days post inoculation using an epifluorescent (ultraviolet light) microscope. Digital images were obtained using a camera mounted on the microscope at various wavelengths in the ultraviolet

spectrum. Multiple single-plain images within plant organs were stacked as layers and combined as one image. Additional images in the full ultraviolet spectrum (which permits visualization of cellular necrosis) were also obtained. To assess potential differences in fungal colonization and root necrosis, pictures were transformed to an eight-color image and the number of pixels corresponding to each color were counted using ImageMagick. Red pixels correspond to fungal colonization and black pixels corresponded to necrotic host plant tissues.

RESULTS

Agrobacterium mediated transformation was successful in producing an *O. korrae* isolate that contained a fluorescent gene. The transformed *O. korrae* was similar to the wild-type isolate in respect to its ability to infect and cause necrosis in plants. Fluorescent microscopy allowed detecting superficial and deep fungal colonization. In addition, the use of digital photography and image manipulation software allowed for quantitative disease severity ratings for the different cultivars tested.

Ophiostphaerella korrae colonized roots of all cultivars tested at a similar rate

with necrosis evident as early as 2 days post inoculation on Tifway and Midlawn, while on 3200 and Uganda necrosis appeared at 8 days post inoculation. The most severe necrotic response in roots was observed in Tifway, the most susceptible cultivar to SDS. After colonizing the surface of the roots the fungus penetrated the epidermal (outermost) layer of cells by direct penetration and rapidly invaded the cortex (cell layer just beneath the epidermis) on all bermudagrass cultivars.

In the cultivars Midlawn and Tifway, the fungal hyphae completely colonized the cortex of the roots moving between and through cells but would rarely extend into the vascular tissues, since hyphal growth was arrested at the endodermis (cell layer enclosing the vascular tissues) (Fig. 2). In the rare cases where the fungus did grow into the vascular tissues of these two cultivars, it appeared to do so by penetrating through the root tip where young tissues are not defined.

In more tolerant cultivars, Uganda, 3200 and U-3, vascular colonization was more common and was observed as early as 4 days post inoculation. Root colonization of U-3 was very different from colonization observed for the other cultivars. In U-3, the fungus locally colonized the epidermis and cortex of



the root and then it would penetrate the vascular tissues and colonized it extensively (Fig. 3). For 3200 and Uganda, the fungus grew through the endodermis of the root but vascular colonization was rarely observed before the surface of the whole root was colonized. Root colonization of the cultivars Midlawn and Tifway (Fig. 2) by *O. korrae* corresponded with necrosis (Fig. 1). However, colonization did not correspond with necrosis of the roots for U-3 (Fig. 3), and the *C. transvaalensis* cultivars 3200 and Uganda (Fig. 1).

For intact stolons, necrotic spots were evident on Midlawn and Tifway at 4 days post inoculation while for 3200 and Uganda, stolons had light discoloration but not necrosis up to 22 days post inoculation. The fungus was not observed in the vasculature of intact stolons of any cultivar up to 22 days post inoculation and did not penetrate beyond the epidermis of intact stolons. For wounded stolons, localized necrosis started to appear from seven to 15 days post inoculation. The fungus colonized the cortex of these stolons but it did not penetrate into vascular tissues unless the injury continued into these tissues. Once in the vascular tissues, the fungus caused extensive necrosis and decay.

CONCLUSIONS

The use of transformed fungi with the expression visualization proteins permitted the study of the infection and colonization of various hosts. Furthermore, along with the use of imaging software, provided a method to quantitatively assess disease severity in the different hosts. Colonization of the roots of the susceptible bermudagrass cultivars by *O. korrae* can be correlated to necrosis, while partially resistant cultivars were less necrotic despite heavy colonization. The most severe necrotic response and strongest correlation between colonization and necrosis was observed for Tifway, a cultivar which is highly susceptible to SDS.

Vascular colonization was rarely

observed on susceptible cultivars while it was common in more tolerant cultivars, especially for U-3, which typically has less disease. These findings are consistent with the study of Caasi et al. where *O. herpotricha* only colonized the vasculature of partially resistant cultivars. It appears that the endodermis forms a barrier that restricts access to the vascular tissues for SDS-causing fungi in susceptible bermudagrass cultivars. When fungal growth into the vascular tissues does not occur, the fungus can cause significant damage to cortical cells and this may be one component for the greater susceptibility to the disease. Finally, the differences between susceptible and resistant cultivars in vascular colonization and the correlation between colonization and necrosis correlation can be detected as early as 14 days post inoculation, which could provide a powerful

tool for the early assessment of disease resistance for new cultivars.

Acknowledgment

We thank the USGA's Turfgrass and Environmental Research Program and Oklahoma State University Agricultural Experiment Station for support of this research program.

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