NEW HERBICIDE RESISTANCE TOOL

The Herbicide Resistance Education Committee of the Weed Science Society of America has released a new series of training modules on herbicide resistance management developed specifically for turf. The training modules are in PowerPoint format and can be accessed at: http://wssa.net/weed/resistance/

Each training module is brief, well-illustrated and easy to understand. The specific modules are:

- Lesson 1: Current Status of Herbicide Resistance in Weeds
- Lesson 2: How Herbicides Work
- Lesson 3: What is Herbicide Resistance?
- Lesson 4: Scouting After a Herbicide Application and Confirming Herbicide Resistance
- Lesson 5: Principles of Managing Herbicide Resistance

Cases of herbicide-resistant weeds worldwide are becoming increasingly common since the first reports of their occurrence in the 1950s. These biotypes survive herbicide application at doses that usually give effective control of the species. Resistant weed biotypes are a consequence of basic evolutionary processes. Individuals within a species that are best adapted to a particular practice are selected for and will increase in the population. Once a weed population is exposed to a herbicide to which one or more plants are naturally resistant, the herbicide kills susceptible individuals, but allows resistant individuals to survive and reproduce. With repeated herbicide use, resistant weeds that initially appear as isolated plants or patches in a field can quickly spread to dominate the population and the soil seed bank.

An annual bluegrass plant survives while surrounding annual bluegrass plants were controlled by a herbicide application.
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 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 aerification 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 florescent 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...
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 *Ophiophysphaerella* 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 *Ophiophysphaerella 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 *Ophiophysphaerella korrae* to express tdTomato (tdTom) Continued on page 34

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

**Ophiosphaerella 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 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**

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Francisco Flores is a Ph.D. candidate and Nathan Walker, Ph.D., is a professor in the Department of Entomology and Plant Pathology at Oklahoma State University. Dr. Walker can be contacted at nathan.walker@okstate.edu.

**References**


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The struggles of science

One of the most exciting times of the year is early October when the Nobel Prize announcements are made. In science there is no higher award. My first realization of the importance of a Nobel Prize came when I was in my early teens while caddying at Champaign (Ill.) Country Club.

Caddying at that time was pretty much like it was portrayed in the movie “Caddyshack.” We would be herded together in a decrepit shack at the end of the driving range until some of us were called to come to the clubhouse where we would sit on a bench until we were needed. One Saturday morning as I was sitting on the bench listening to my transistor radio, whispering started among the older caddies and the caddy master. I was then told to stand up straight. Soon this older gentleman in plaid shorts walks by, says hello and asks how we are doing. He then proceeds to the practice putting green.

My first question was, “Who is this guy?”

“Dr. Bardeen, the Nobel Laureate,” was the reply. It was a surprising scene to see the genuine respect shown by a bunch of caddies to this man. Dr. John Bardeen, Professor at the University of Illinois, is the only person to win two Nobel Prizes in Physics, the first for co-invention of the transistor (1956) and the second for the fundamental theory of superconductivity (1972).

This year the 2013 Nobel Prize in Physics went to Dr. Peter Higgs and François Englert, who in 1964 independently proposed how subatomic particles acquire mass, which is known as the Higgs particle or the God particle. It has taken almost 50 years for the Higgs particle existence to be confirmed. At the same time that this Nobel Prize was being announced, two previous Nobel Prize winners in physics (2001, 2012) were being furloughed during the government shutdown.

The 2013 Nobel Prize in Physiology and Medicine was shared among three individuals. James Rothman, one of those who shared the award, had recently lost his National Institute of Health (NIH) funding.

I believe a concerted effort in the area of turfgrass research funding should be focused on young faculty and graduate students. The ideas and research conducted by these individuals will lead the advancement and health of our industry.

Why is this so important? Returning to Dr. James Bardeen, he was 36 years old when he submitted the first paper leading to the creation of the transistor. His first doctoral student was Nick Holonyak who, after graduating, invented the first useful light-emitting diode (LED).

Karl Danneberger, Ph.D., Golfdom’s science editor and a professor at The Ohio State University, can be reached at danneberger.1@osu.edu.
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Who will carry the torch?

Two of the pioneers of the golf course industry, Dr. Joe Duich and Dr. Jim Watson, passed away recently. Both men had a great impact on our industry including scientific advances, educating students and practicing professionals, instilling pride in the golf course industry, and creating an atmosphere of “we are all in this together” which carries on to this day.

One of many debts of gratitude that we all owe Drs. Duich and Watson was their commitment to developing the next generation of turfgrass scientists. So when people ask “Who are the next leaders in our industry?” thanks in part to Drs. Duich and Watson; the answer is right in front of us at universities and industries all over the country.

There are many fine turfgrass scientists at all stages of their careers working in academia and industry. This in itself is a tribute to Drs. Duich and Watson. When they started their careers you could count the number of turfgrass scientists on two hands. Today, the number of university educated turfgrass scientists numbers in the hundreds, and they are hard at work all across the country dedicated to improving turfgrass performance and advancing turfgrass science.

A few of the many talented and dedicated early or mid-career turfgrass scientists that are carrying the torch forward and keeping the turfgrass scientific community and the turfgrass industry thriving are mentioned briefly below. As with all lists, there is not space to recognize everyone who deserves a share of the spotlight. My apologies to the many fine scientists who are not listed in this column.

Michelle DeCosta, Ph.D., University of Massachusetts. If your golf course is in the north, chances are you have lost annual bluegrass to winter injury. Michelle is working to improve our understanding of winter injury of annual bluegrass greens and hopefully how to minimize damage. Winter injury is a difficult research problem and Michelle is taking on this problem head-on.

Brian Horgan, Ph.D., University of Minnesota. Brian was a voice of reason for the turfgrass industry during the efforts to ban phosphorus from turfgrass fertilizers in Minnesota. Thanks to his efforts, reasonable legislation was passed that all can make work. Brian is also very active in nitrogen management research that will cause those in northern states to rethink their nitrogen fertility programs.

Doug Karcher, Ph.D., University of Arkansas. If you want to learn more about wetting agents, look for Doug’s research results on the subject. He has closely examined many aspects of wetting agent performance. Doug and colleagues have also developed a number of research techniques that lead to a more objective evaluation of turfgrass performance.

Scott McElroy, Ph.D., Auburn University. Scott is a leading turfgrass weed scientist responsible for developing strategies to control some of the toughest weeds like goosegrass and annual bluegrass. Scott and others are leading the charge to understand herbicide resistant annual bluegrass and what can be done to cope with this emerging problem.

Doug Soldat, Ph.D., University of Wisconsin. When it comes to turfgrass soil problems, chances are Doug has devoted time and effort to solving the problem. Doug’s research on phosphorus behavior in soil, turfgrass response to nitrogen applied in fall and late fall and wetting agents has changed how we maintain turfgrass and benefitted us all.

Drs. Stacy Bonos, Jim Brosnan, Kevin Frank, Dave Gardner, John Kaminski, Kevin Kenworthy, Jim Kerns, Aaron Patton, John Sorochan, Brian Schwartz and Eric Watkins all deserve a share of the spotlight as well for their current and future contributions to the turfgrass industry.

I think Drs. Duich and Watson would be proud of the state of the turfgrass research community and they deserve a large portion of the credit for the research community and turf industry they helped shape.
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LET IT SNOW

BY CURT HARLER // Contributing Editor

WITH APOLOGIES TO ALL OUR READERS DOWN SOUTH, it’s time to talk snow (or “white gold” as the snow removal equipment companies like to call it). While at the recent GIE+Expo show in Louisville, we saw plenty of equipment designed with the operator in mind. If the course is already put to bed and you’re working on getting your equipment geared up for next season, this equipment will get you back to finishing that task as quickly as possible.

1. **F-90**
Heavy-duty implement options for specialized applications on KUBOTA’S F-90 series include a snow blower as well as debris blower, front blade, grass catcher, mulching kit and rotary sweeper. The F3990, pictured, has the highest ever horsepower among all Kubota front-mount mowers. Each model has a 16-gallon fuel tank and single speed pedal hydrostatic transmission for quick response and increased working speed, while the auto-assist 4WD forward and reverse ensures greater efficiency with appropriate traction.

kubota.com

2. **Path-Pro**
New for this snow season, the ARIENS Path-Pro is a single-stage snowthrower that is lightweight, compact and maneuverable. Although small in size, it is built to be durable enough for heavy weight use by professional crews. Powered by either an Ariens AX 136cc or a 208cc motor, it has a 21-inch clearing width. It has a throwing distance of up to 35 feet. Chute rotation option allows snow to be tossed left or right. Commercial-duty housing cuts through packed snow and reinforced rubber augers move snow fast. Five model options include recoil or electric start models.

ariens.com

3. **9000 V-Plow**
The new 9000 series VF trip-edge V-Plows from HINIKER feature deep-curl flared wings to toss deep snow farther and higher, while providing more scoop capacity than ever. Twelve laser-cut ribs are incorporated into the high-tensile steel superstructure of the plow, providing exceptional strength for heavy-duty applications. V-Plows come with super-bright quad halogen lights and double-acting hydraulic power. Also available in a conventional level-top configuration, VFs come in 8.5- and 9.5-foot widths. Pinch-free pivot point is located nine inches above ground level, for improved protection from higher obstacles such as curbs and parking barriers.

hiniker.com

4. **XV2**
The XV2 features extreme flared wings, FISHER ENGINEERING’S proven trip-edge design, super-fast hydraulics to quickly change blade positions and standard InstaLock double-acting cylinders to securely hold the wings in place for windrowing, back-dragging and straight blade operation. Optional bolt-on shoe kit protects plows where surfaces are rocky and/or abrasive. This extends the life of the cutting edge and/or base angle.

fisherplows.com

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