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Research at Clemson University continues to investigate *Rhizoctonia* leaf and sheath spot on bermudagrass. In recent years in South Carolina, North Carolina, Georgia, Mississippi, Alabama, Louisiana and Texas symptoms have been observed on bermudagrass putting greens. Shown above are typical field symptoms of *Rhizoctonia* leaf and sheath spot on bermudagrass putting greens where patches and rings typically are about 6 inches up to 2 feet in diameter.

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PURPOSE

The purpose of *USGA Turfgrass and Environmental Research Online* is to effectively communicate the results of research projects funded under USGA's Turfgrass and Environmental Research Program to all who can benefit from such knowledge. Since 1983, the USGA has funded more than 400 projects at a cost of $34 million. The private, non-profit research program provides funding opportunities to university faculty interested in working on environmental and turf management problems affecting golf courses. The outstanding playing conditions of today's golf courses are a direct result of ***using science to benefit golf***.

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Identification, Pathogenicity, and Control of *Rhizoctonia* Leaf and Sheath Spot of Bermudagrass Putting Greens.

Bruce Martin

SUMMARY

Research at Clemson University continues to investigate *Rhizoctonia* leaf and sheath spot on bermudagrass. Their observations include:

- *Rhizoctonia* leaf and sheath spot has increased in frequency and importance on bermudagrass putting greens.
- Fungi identified as *Rhizoctonia zeae* have most consistently been associated with symptoms of the disease, although related fungi such as *R. oryzae* and *R. circinata* var. *circinata* may also be involved.
- Curative control with fungicides or masking of symptoms with fertilizers has been largely ineffective.
- Early curative control or preventive control with several fungicides with good efficacy against *Rhizoctonia* diseases has been successful.

There is no more interesting group of pathogens of plants than those in the genus *Rhizoctonia*. This group of fungi, as they say, is "older than dirt" and the best known representatives among *Rhizoctonia solani* were described originally and coincident with the dawn of the discipline of plant pathology. In order to understand modern terminology and the context of turf diseases caused by different *Rhizoctonia*, including 'leaf and sheath spot' and related diseases, we need to review a bit of history.

Rhizoctonia solani was first described by Kühn in 1858 as a pathogen of potato (*Solanum tuberosum*), and since then has continued to be the most studied species group within the genus. We know now as a result of volumes of research, that *Rhizoctonia solani* is actually a group of separate and distinct biological species which we identify as sub-specific groups.

In turfgrass, the disease 'large brown patch' was first observed on a bentgrass putting green in a golf course near Philadelphia, PA in 1913. But it was not until experiments were conducted in 1917 by C. V. Piper and H. S. Coe that the suspected causal agent was consistently isolated from 'large brown patch' in a red fescue turf garden in Philadelphia, PA. and identified as *R. solani*. Members of the species group *R. solani* affect all of the major warm- and cool-season turf species. But, what are the characteristics of the genus *Rhizoctonia*?

Most fungi produce spores that are the result of sexual or asexual reproduction, but members of *Rhizoctonia* do not produce a spore in the asexual form. They are composed of mycelia and sclerotia only in the asexual state, so characteristics are few that can be used taxonomically.

Typical mycelia characteristics of *Rhizoctonia* fungi as viewed through the microscope. No spores or clamp connections are produced. Secondary hyphae tend to emerge from primary runner hyphae at right-angle branches, and septa are formed in secondary hyphae near the point of origin of the secondary branch. These characteristics only provide some information to tentatively place the fungus in the genus *Rhizoctonia*.

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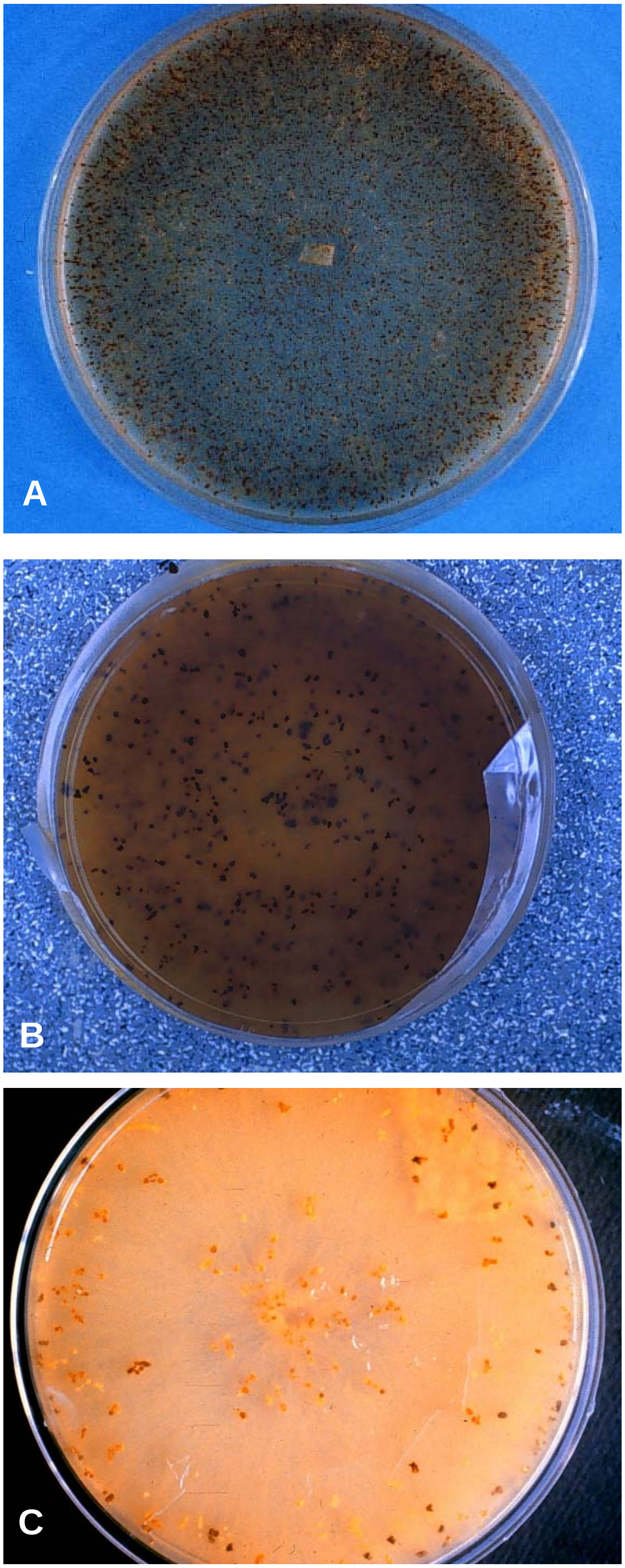
Some of the characteristics that place a sterile fungus into *Rhizoctonia* include: lack of spores, septate hyphal strands, lack of clamp connections (a microscopically visible bridge across septa in hypha present in mushroom fungi), and sclerotia of varying form and organization. Not much to go on, so it's no wonder many fungi previously described as species of *Rhizoctonia* are not related at all. Many, however, were shown to belong to the Basidiomycete class of true fungi (which includes mushroom fungi, puffballs, and jelly fungi, as well as rusts and smuts) and that is where 'true' *Rhizoctonia* are presently placed taxonomically.

To be placed in the 'species' *R. solani*, the fungi posses the following characteristics: mycelia are tan to brown color, with right-angled branching (although young hyphae can produce acute angular branching), septa near the origin of hyphal branching, constriction of hyphal branches near their origin, multinucleate hyphal cells, and formation of dolipore septa. Conidia, clamp connections, and rhizomorphs are absent, and sclerotia are not differentiated into a rind and medulla (like an orange). *Rhizoctonia solani* fungi have a rapid growth rate and pathogenicity may or may not be present.

Different forms of *R. solani* have been identified based on physiological and genetic differences that determine host range, pathogenicity, ecological niche, etc. We refer to ‘AG’ which stands for 'anastomosis group' followed by numbers and letters to identify specific forms. In turf, at least 4 AGs have been identified, with specific subgroups beyond AG. These are: AG 1, AG 2, AG 4, and AG 5. To be more specific, *R. solani* AG 1, 1A is a major cause of brown patch in tall fescue and perennial ryegrass. It also causes a major disease of rice, called 'sheath blight'. AG 2-2IIIB is also found on tall fescue and ryegrass, but is the most frequently identified form on creeping bentgrass, causing brown patch.

Another subgroup of AG 2, is *R. solani* AG 2-2 LP, which causes large patch disease on warm-season grasses. We now distinguish the diseases and pathogen associations as 'brown patch' on cool-season turf and 'large patch' on warm-season turf. These are distinct diseases with distinct causal agents. AG 4 isolates are occasionally isolated, and are typically found on juvenile turf causing damping off, or seedling disease. AG 5 isolates have been infrequently isolated from tall fescue and other grasses, but are not major turf pathogens as far as we know now.

Typical field symptoms of *Rhizoctonia* leaf and sheath spot on bermudagrass putting greens. Patches and rings typically are about 6 inches up to 2 feet in diameter.



Typical culture characteristics of *Rhizoctonia zeae* (A), *Rhizoctonia circinata* var. *circinata* (B), and *Rhizoctonia oryzae* (C). Cultures may be obtained that overlap across the typical characteristics of color and sclerotial size and morphology shown here.

Other *Rhizoctonia*

From the original identification of *R. solani* on red fescue in the 1920s until the mid-1970s, *R. solani* was the only fungus associated with turf diseases, which were called 'brown patch'. In the mid-70s, however, several researchers began to pay attention to 'cool season' brown patch which was shown to be caused by a binucleate species of *Rhizoctonia* that obviously was not *R. solani* (it is white to buff in color, binucleate, and disease occurs in cold weather). The 'cool season' brown patch was observed on bentgrass and *Poa*, and zoysia originally. Eventually, Lee Burpee (now at the University of Georgia) identified the fungus as *R. cerealis*, and named the disease it causes 'yellow patch'.

In the late 1970s and early 1980s, I studied under Leon Lucas at NC State and worked to identify specific *Rhizoctonia* associated with many turfgrasses. We were the first to associate *Rhizoctonia zeae* with turfgrasses in the United States, although the fungus had been associated with Gramineae since it's original description as a corn pathogen in 1932. We also identified *R. oryzae* on turf which was identified originally in 1938 as a pathogen of rice, and the disease in rice was called 'leaf and sheath spot' of rice.

Do you begin to see where names come from? *Rhizoctonia zeae* and *Rhizoctonia oryzae* are the names given to the asexual state (no spores), but both *R. zeae* and *R. oryzae* have been shown to form a sexual state, which is classified as *Waitea circinata*. It should be noted that the sexual state of turf isolates has not been identified in turf in nature and only once in the United States in the laboratory by Dr. Alan Windham, University of Tennessee.

Waitea circinata was described by Warcup as a soil-borne fungus and was not associated at that time as a pathogen of plants (19). Warcup worked at the Waite Agricultural Research Institute near Adelaide, South Australia, hence the name 'Waitea'. In 1979, Christensen identified *Waitea circinata* as a '*Rhizoctonia*' disease on turfgrasses in New Zealand, but noted that the sexual state was not formed with his isolates.

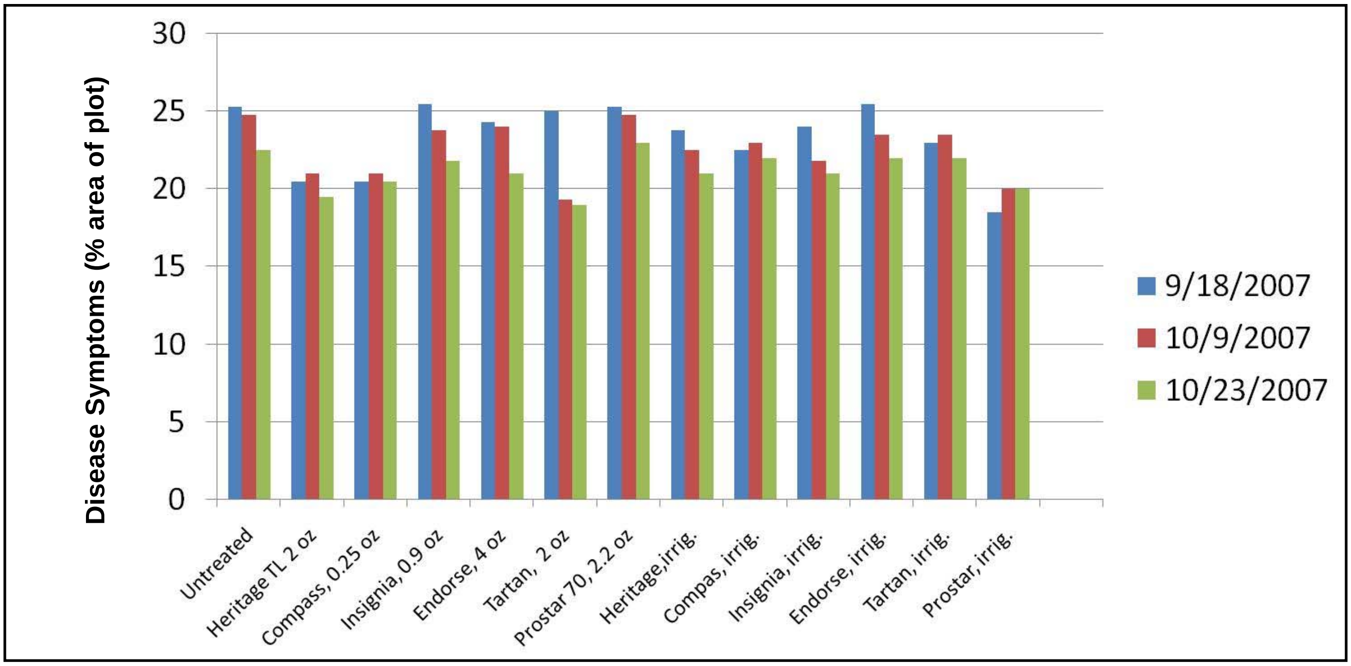


Figure 1. Curative fungicides applied with and without post-application irrigation. Fungicides were applied on September 18, 2007 and again on October 9, 2007. Fungicides were not effective in controlling symptoms curatively.

Interestingly, he also reported his *Waitea circinata* isolates to be immune to benomyl. A couple of years later, our isolates of *R. zeae* from the United States were also shown to be immune to benomyl, and after many experiments by others, it is now accepted that *R. zeae*, *R. oryzae*, and *R. circinata* var. *circinata* are tolerant of benzimidazole fungicides, including benomyl and thiophanate methyl.

Getting More Specific with Molecular Biology

In Japan recently, researchers led by Takeshi Toda distinguished fungi causing a 'brown ring patch' disease in bentgrass to be caused by *Waitea circinata* var. *circinata*. Isolates were distinguished from *R. zeae* and *R. oryzae* based on DNA sequences from ribosomal DNA which has become a standard tool to aid in identification of fungi and other organisms. In the United States, Dr. Frank Wong, University of California-Riverside has identified similar fungi, identified as *Rhizoctonia circinata* var. *circinata*, distinct from *R. zeae* and *R. oryzae*, as a causal agent of brown ring patch in *Poa annua*. Dr. Phil Harmon, University of Florida, has identified *R. circinata* var. *circinata* as a cause of brown ring patch in *Poa trivialis* overseedings. To be proper and prudent, since the *Waitea* sexual stage has not been found or induced in the lab, the fungus should be called *Rhizoctonia circinata* var. *circinata*, and *R. zeae* could be called either *R. zeae* or as proposed by Carling: *R. circinata* var. *zeae*, and *R. oryzae* could be called R. oryzae or as proposed by Carling: *R. circinata* var. *oryzae*. These fungi and the diseases they cause are related, but appear to be distinct.

What About Warm-season Grasses?

Much less work has been done with warm-season grasses, except for the important disease called large patch, caused by *R. solani* AG 2-2 LP, which has been reported on virtually all warm-season grasses. However, outbreaks of a different disease on bermudagrass putting greens began to be more frequent in 2002 up to recent years. Symptoms were distinct rings of a few inches up to greater than a foot in diameter that occurred in spring and fall on non-overseeded putting greens. These symptoms were initially prominent in the southeastern United States, gulf coastal region, but have been noted in many locations throughout the world on bermudagrass putting greens.

The disease became known as 'mini-ring'

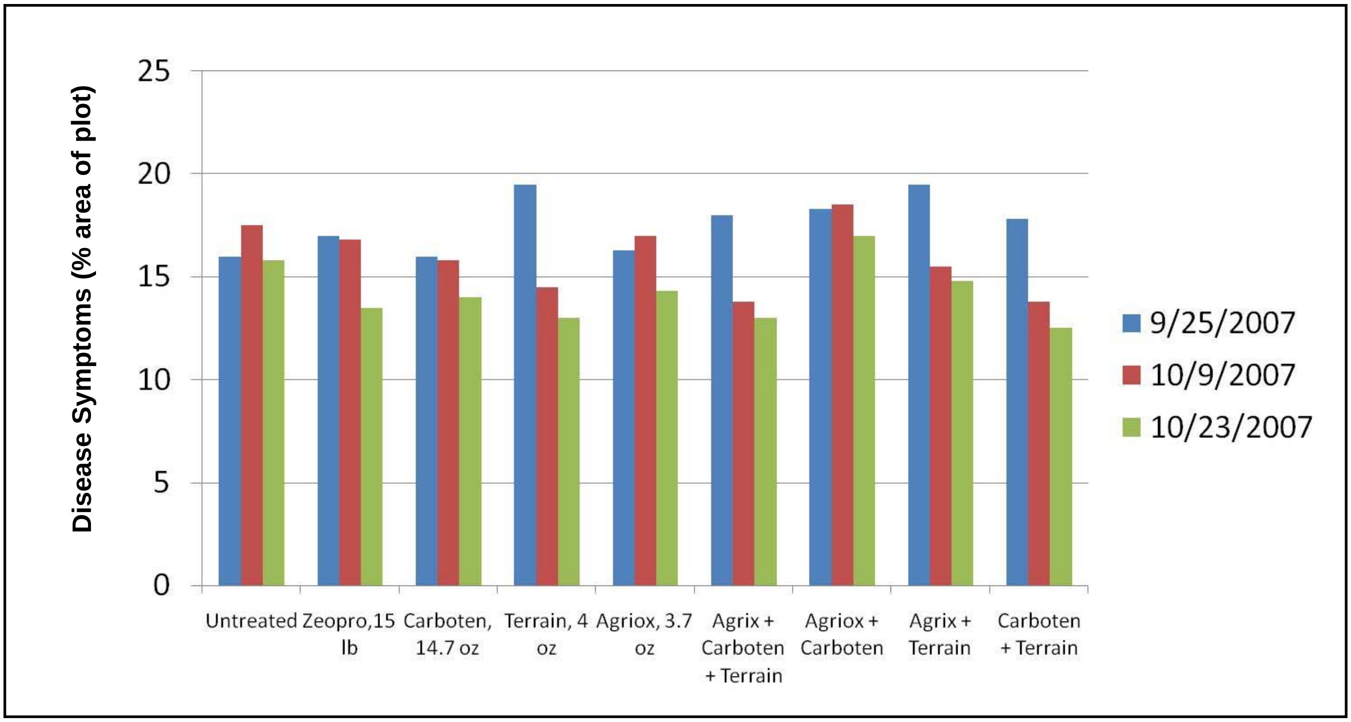


Figure 2. Fertilizer effects on symptoms of *Rhizoctonia* leaf and sheath spot. Terrain 16-1-2-7 Fe reduced symptoms temporarily but effects were not prolonged greater than 2 weeks post application.

and a causal agent was unknown initially. However, symptoms of the disease corresponded exactly with leaf and sheath spot, caused by *R. zeae*, as described by Dr. Monica Elliott, University of Florida, in the late 1990s on bermudagrass in Florida and identified at about the same time by me in South Carolina. The diseases Dr. Elliott identified initially occurred on 'Tifdwarf'bermudagrass.

Leaf and Sheath Spot on Bermudagrass

In recent years in South Carolina, North Carolina, Georgia, Mississippi, Alabama, Louisiana, and Texas (and in Argentina and Uruguay), similar symptoms have been observed on bermudagrass putting greens. In South Carolina, *R. zeae* is a well-documented pathogen of creeping bentgrass putting greens, causing brown-patch like symptoms in the heat of summer. It has also been seen frequently causing similar symptoms to overseedings (primarily *Poa trivialis*) in fall and spring when temperatures are warm and humidity is high and prolonged, although *R. circinata* var. *circinata* has recently been identified on *Poa trivialis* in Florida (Harmon). The fungus has been identified and pathogenicity documented as well on St. Augustinegrass, centipedegrass, and seashore paspalum (Canegallo and Martin, unpublished). In all cases, the disease has not been controlled with benzimidazole fungicides, as *R. zeae*, *R. oryzae*, and *R. circinata* var. *circinata* are essentially immune to that chemistry.

Recent outbreaks have occurred on all of the common ultradwarf bermudagrass cultivars on putting greens ('TifEagle', 'Champion', and 'Mini-Verde') as well as 'TifDwarf' and even 'TifGreen' in the southeastern United States. Although timing varies, generally the disease is first noticed in late August to early fall months. In Florida, spring symptoms have been described. Initial symptoms are bronze patches of a few inches up to a foot or more in diameter. If not controlled quickly with fungicides, the pathogen blights and bleaches the lower leaves which results in a persistent distinct patch symptom that is very hard to heal when days are becoming shorter and night temperatures cooler.

This difficulty in healing the disease likely is more problematic in transition zone climates than in more tropical or subtropical environments

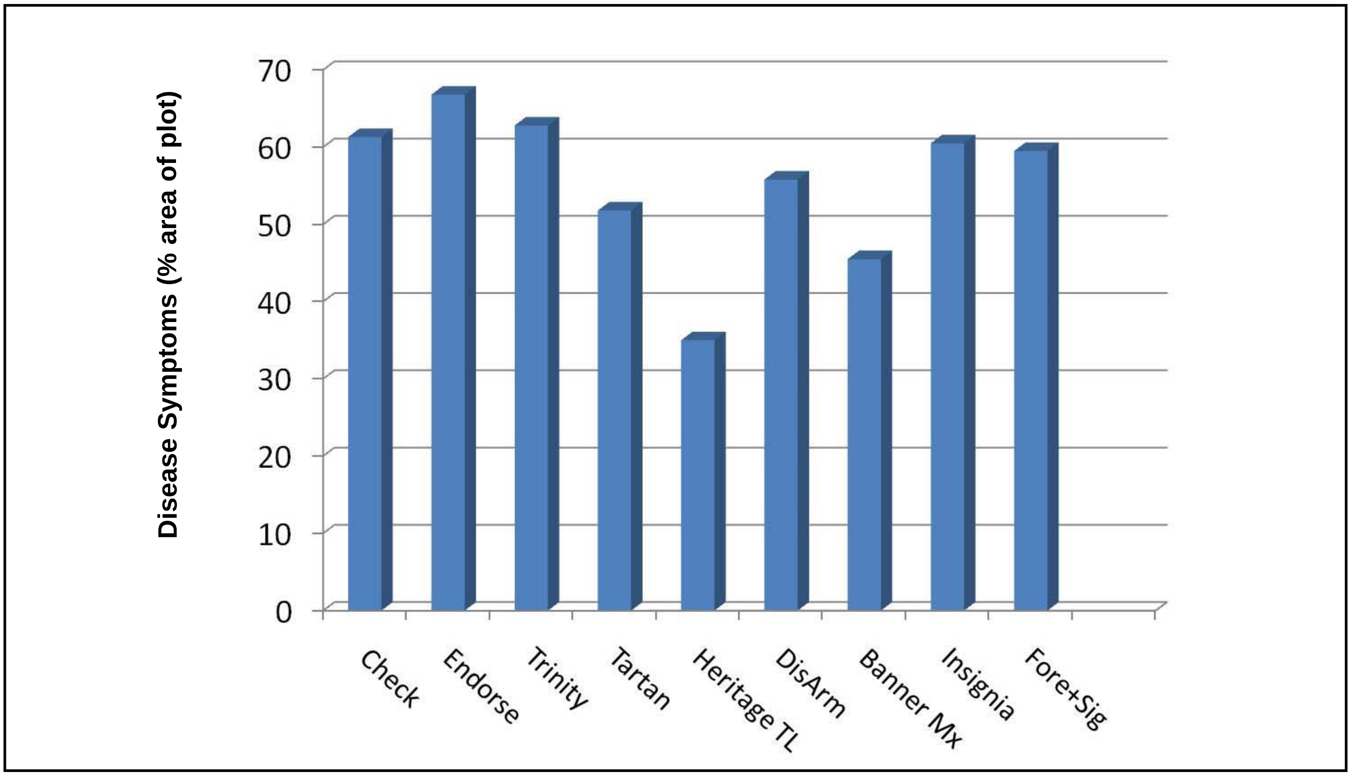


Figure 3. Early curative effects of single component fungicides applied 3 times at 2 week intervals, beginning July 15, 2008. Only Heritage TL and Banner Maxx significantly reduced symptoms of severe disease.

where turf recuperative potential is prolonged. Disease has been known to recur in spring months after turf emerges from dormancy, and presumably can detrimentally affect spring transition in overseeded systems.

Why are these new outbreaks increasing in frequency? Possible reasons include: a) reduced fertility b) nutrient deficiency due to increased irrigation of sand-based greens planted to shallow rooted ultradwarf bermudagrass cultivars c) increased use of thiophanate methyl in summer for bermudagrass decline, thereby inducing an increase in this non-target disease. In several instances, 'stress' induced by aggressive verticutting has induced severe outbreaks of persistent disease symptoms. Low nutrition or reduced recuperative potential by any of the reasons outlined would be expected to increase disease severity and prolong healing.

Research at Clemson University has focused on the following objectives:

1. Collect isolates of *Rhizoctonia* from symptomatic bermudagrass and complete Koch's postulates in greenhouse tests. Identify isolates to subgroup of the *Waitea circinata* complex .
2. Measure disease severity in greenhouse-inoculated pots of ‘Champion’ and ‘TifEagle’ bermudagrass treated with various rates and ratios of N and K. Proposed treatments would be 0.5, 1 and 2 lb N/1000 sq.ft./ 14 days supplemented with 0, 1 or 2 lb K in all combinations. Equal amounts of other nutrients would be applied to all treatments.
3. Induce the disease in the field.
4. In ‘TifEagle’ and ‘Champion’ bermudagrass USGA putting greens at the Pee Dee Research and Education Center, determine the influence of Primo and thiophanate-methyl and Heritage treatments on disease severity in plots treated with two levels of fertility (0.125 lb N/1000 ft^2/ wk and 0.25 lb N/1000 ft^2/wk).

Results to Date

Many isolates of *Rhizoctonia* have been collected from bermudagrass over the past three years and fall into the *R. circinata* complex. These fungi have been identified as *R. zeae*, *R.. oryzae*, and *R. circinata* var. *circinata*. At this time, it has

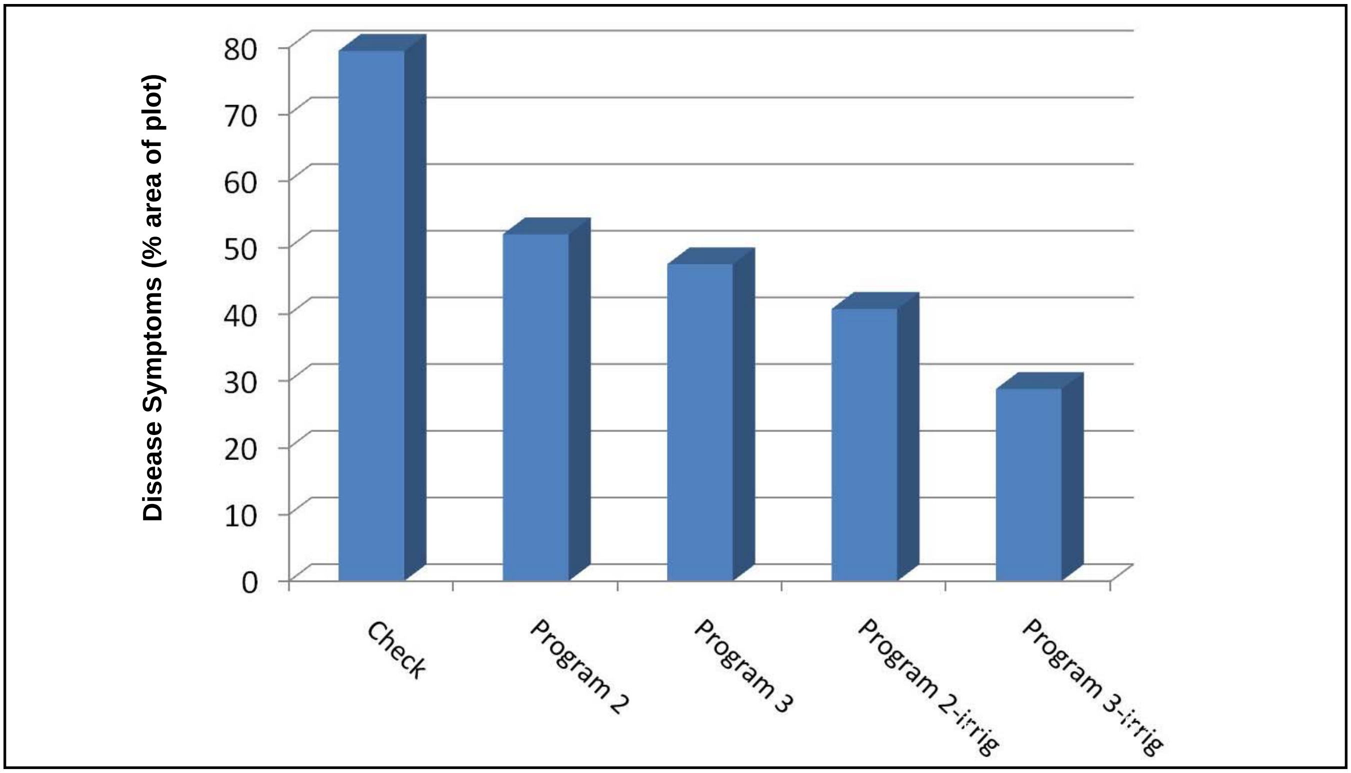


Figure 4. Early curative effects of fungicides in tank-mix or rotation on *Rhizoctonia* leaf and sheath spot. Program 2 was: spray 1- Heritage + Medallion (1 +0.5 oz), spray 2 - Headway (1.5 oz) and spray 3 - Renown + Medallion (4.5 + 0.5 oz). Program 3 was: spray 1 - Heritage TL (1 oz), spray 2 - Medallion (0.5 oz), and spray 3 - Headway (1.5 oz). Programs 2 and 3 were applied with or without post-application irrigation at 0.5 inches water.

not been determined if one or all can be responsible for the symptoms consistently observed on golf course bermudagrass putting greens.

In the fall of 2007, the disease occurred on several golf courses in South Carolina and throughout portions of the southeastern United States. Cultivars were 'TifEagle', 'Champion', 'TifDwarf' and in one case, 'MiniVerde'. Fungi most closely resembling *Rhizoctonia zeae* or related fungi were isolated from some samples when they were submitted to the lab before treatment with fungicides.

In one case, only thiophanate methyl had been used, and *Rhizoctonia* was easily and readily isolated from all leaf tissues that were plated. In many cases, after multiple fungicides had been applied, *R. zeae* or other potential causal agents could not be isolated. So, superintendents that wish to get the best diagnosis of submitted samples are encouraged to simply pull the sample before spraying, as a number of broad-spectrum fungicides complicate the isolation and identification of a purported causal agent.

Identification of *R. zeae* has been based previously solely on cultural characteristics, based primarily on sclerotia size, color, and abundance: *R. zeae* typically has multiple very small sclerotia (< 1 mm) which are produced both submerged and on culture media surfaces. *Rhizoctonia circinata* has been described to have somewhat larger sclerotia which typically turn brown in culture after a few days. However, these fungi are obviously closely related and cultural characteristics invariably will overlap, so final identification and association to a variety of *R. circinata* (*zeae*, *circinata*, *oryzae*) depends on separation using DNA technology.

Chemical Control Trials

During initiation of this project, severe disease outbreaks in South Carolina made it necessary to concentrate on field control of symptoms to develop better recommendations for control. Field trials were initiated on golf courses with severe disease outbreaks to attempt curative control and provide additional information on fungicide efficacy or the influence of fertilizers.

In a separate trial, some fertilizer products from Geoponix company were applied in September and October, 2007 to determine if fertilizers could assist in healing or in masking of symptoms. In all cases where control was attempted for existing disease that had developed in early fall, results were disappointing as symptoms persisted throughout the dormancy period, but some slight improvement was noted in one experiment from Tartan applications (Figure 1).

In Experiment 2 with fertilizers from Geoponix, only treatments with Terrain (16-1-2-7Fe) reduced symptoms slightly and temporarily, as might be expected with iron applications (Figure 2). Trials were conducted in late September when symptoms were already severe and day length and temperatures were not favorable for bermudagrass growth.

In 2008, trials were initiated to look at preventive treatments of fungicides to determine if preventive control was more efficacious (Figure 3 and 4). Data shown in Figure 3 represent another trial to examine the effects of fungicides with known efficacy against *Rhizoctonia*-induced diseases. Data shown in Figure 4 represent a simple experiment to look at tank-mixes and rotation of 3 treatments as 'programs' and determine if post-application irrigation assisted in control.

In these experiments in 2008, even though the first application of fungicides was placed on July 15, symptoms had already occurred at a severe level. In Experiment 3, only Heritage TL and Banner Maxx reduced the symptoms in early curative applications (Figure 3). Although Banner Maxx and other DMI fungicides can induced detrimental growth regulation of bermuda in summer, detrimental effects noted in these trials were negligible.

It should be noted however that we can't rely on multiple applications of DMI fungicides for control due to unwanted growth regulation problems that will result. Best results were obtained with rotations and post application irrigation experiment (Figure 4), where we looked at tank-mixes or rotations of some fungicides that previously had shown some efficacy. The best treatment was a simple rotation of Heritage TL (1 fl oz) followed by Medallion (0.5 oz) after 2 weeks, and then a final application of Headway (a premix of azoyxystrobin and propiconazole) at 1.5 fl oz. Efficacy was boosted in both programs by applying irrigation immediately after sprays were applied with 0.5 inches of water.

In 2009 to date, the disease has been much less severe overall, although a few notable epidemics have occurred. Courses with a history of this disease have adopted a strategy of increased, judicious fertilization throughout summer, as well as preventive fungicide applications. In trials shown here and in other trials, fungicides with excellent activity against *Rhizoctonia*-induced diseases have been beneficial, but applications must be applied preventively.

It is suggested that superintendents be aware of the potential for this disease to develop and scout greens routinely beginning in early summer. If off-color (bronze) rings begin to develop, take a sample and submit to a lab with a quick turn-around time before treating. *Rhizoctonia* can develop quickly in a sample that has not been treated with fungicides, so an answer should be forthcoming and can give some information that may be valuable in determining if fungicide treatments are warranted.

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