Pythium root dysfunction of creeping bentgrass greens

Pythium root dysfunction differs from other *Pythium* diseases and therefore requires a unique management approach.

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Pythium root dysfunction is a stress-induced disease that has recently become a common problem on creeping bentgrass greens throughout the southeastern U.S. Even though similar fungi cause Pythium root dysfunction, Pythium blight and Pythium root rot, Pythium root dysfunction is very different from the other diseases and requires a unique management approach. In this article, we review recent advances in our understanding of the biology and management of Pythium root dysfunction, with emphasis on our experiences in North Carolina and other southeastern states.

History

Discovering the disease

Although it has received increased attention over the last five years, Pythium root dysfunction is not a new disease. In 1985, Clint Hodges, Ph.D., at Iowa State University was the first to describe the disease (2). He observed rapid decline of creeping bentgrass foliage in discrete patches on newly constructed putting greens in summer, but he was unable to find a fungal pathogen in the declining foliage.

Hodges did, however, note several abnormalities in the roots: They were tan in color, had bulbous root tips and lacked root hairs. Microscopic examination of roots with these symptoms revealed an abundance of hyphae and oospores characteristic of *Pythium* species. Hodges and his co-author L.W. Coleman demonstrated that *P. aristosporum* and *P. arrhenonmanes* were the culprits behind the disease (2), which they named "Pythium-induced root dysfunction" because the roots were not severely necrotic or destroyed, but were obviously impaired. Hodges speculated that nutrient and water uptake was hindered in plants infected with these two *Pythium* species, which ultimately led to dieback of the foliage.

Mid-Atlantic Pythium *species*

Pythium root dysfunction did not appear in the literature again until 1999, when similar symptoms were described in the Mid-Atlantic U.S. (1). Researchers at the University of Maryland collected 25 *Pythium* isolates from creeping bentgrass exhibiting symptoms of Pythium root dysfunction and identified eight different species: *P. aristosporum, P. aphanidermatum, P. catenulatum, P. graminicola, P. torulosum, P. vanterpoolii, P. volutum and P. ultimum* var. *ultimum* (1). The authors conducted pathogenicity assays on creeping bentgrass seedlings at 64 F (18 C) and 82 F (28 C). They concluded that *P. aristosporum* was most likely the dominant pathogen causing Pythium root dysfunction in the Mid-Atlantic because it was frequently isolated from affected root tissue and it was highly aggressive at both temperatures (1). They also obtained one isolate of a relatively obscure *Pythium* species, *P. volutum*, and found it to be very aggressive to creeping bentgrass seedlings at 64 F.

Diagnosis in North Carolina

In 2003, we began to observe dieback of creeping bentgrass putting greens in North Carolina and surrounding states during the summer. Symptoms appeared in circular to irregular patches,

from several inches to several feet in diameter, and mild necrosis of the roots and crowns was observed.

The disease was initially diagnosed as take-all patch based on these symptoms. However, *Gaeumannomyces graminis* could not be isolated from the affected turf, and traditional management programs for take-all patch were ineffective. Closer inspection of roots from affected areas in fall and spring revealed an abundance of *Pythium* hyphae and oospores and symptoms of Pythium root dysfunction similar to those originally described by Hodges (2). We consistently isolated a new Pythium root dysfunction pathogen, *Pythium volutum*, from the infected roots and demonstrated its pathogenicity toward mature creeping bentgrass plants in the growth chamber (6).

Symptoms and signs

Turfgrass stand symptoms

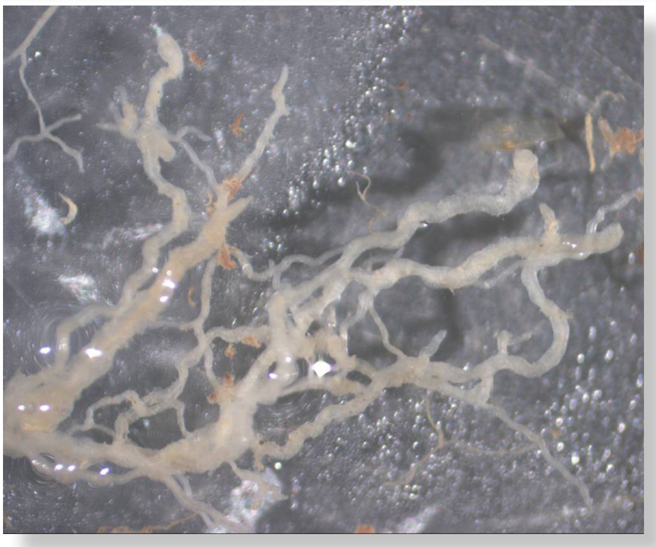
Pythium root dysfunction is a disease of relatively young stands (≤8 years old) of creeping bentgrass (*Agrostis stolonifera* L.) (3,4,6). The disease is most severe in high sand-content root zones with excellent infiltration and percolation (3,4,6). Symptoms of Pythium root dysfunction typically appear in summer when creeping bentgrass is subjected to heat and drought stress, but patches may also develop in the spring, fall and winter during warm and dry weather or other periods of stress.

Initially symptoms appear as circular areas of wilt or chlorosis that range in size from 4 to 8 inches (10 to 20 centimeters) in diameter. These areas progress to larger, irregular patches of yellow to orange foliar decline ranging in size from 8 to 20 inches (20 to 50 centimeters). Eventually, if the affected areas are left untreated, large areas of turf can be killed.

(**Top**) Initial symptoms of Pythium root dysfunction include distinct patches that are 4 to 8 inches in diameter and can resemble drought stress. Symptoms are most severe during the summer, but can appear at any time of year. **Photos by L. Tredway**

(**Middle**) Typical Pythium root dysfunction symptoms observed during the first onset of heat and drought stress. Note the yellow-orange foliar decline appearing in distinct, irregularly shaped patches.

(**Bottom**) If left untreated, Pythium root dysfunction can cause widespread turf loss over a period of one to two weeks.



(**Top**) Comparison of root depth and density in creeping bentgrass that is affected (left) and unaffected (right) by Pythium root dysfunction.

(**Bottom**) Roots affected by Pythium root dysfunction typically maintain a relatively white appearance, but have a slight tan coloration, bulbous root tips and completely lack root hairs.

(**Right**) Soil profiles comparing the root system in creeping bentgrass affected by Pythium root dysfunction (left) and healthy turf (right). Note the majority of the sand has fallen off the affected roots, indicating a lack of root hairs.

Symptoms of Pythium root dysfunction typically appear first on mounds, along slopes or other areas that are most prone to drought stress. In addition, symptoms are generally most severe in greens that have good air circulation with full sun exposure. Superintendents often will report symptoms characteristic of Pythium root dysfunction after a tournament or other event, especially if the greens were mowed lower or allowed to dry out.

Roots

Root depth within affected areas may be significantly reduced when compared to unaffected turf areas. Although root depth may not be affected within the patch early in the season, significant reductions in root depth are very noticeable in mid- to late-summer as infected roots die back more rapidly upon heat exposure.

Regardless of their depth, affected creeping bentgrass roots will have bulbous root tips and a light tan color and they will lack root hairs, which is a useful diagnostic feature. In the field, sand will not cling to roots affected by *P. volutum* as it does to healthy roots, presumably because of the lack of root hairs.

Difficult diagnosis

The rapid dieback of infected roots upon exposure to heat often complicates diagnosis of Pythium root dysfunction. The disease becomes very difficult to diagnose in mid- to late summer because the most heavily infected roots are no longer attached or are easily torn away from the affected bentgrass plants, enabling the pathogen to escape detection. If oospores and hyphae characteristic of *Pythium* species cannot be observed in the lab, the disease can only be tentatively diagnosed based on field symptoms.

To increase the chances of detecting the pathogen, we encourage superintendents to submit samples to a diagnostic laboratory immediately after

symptoms resembling Pythium root dysfunction appear. When collecting the sample, superintendents should carefully avoid detaching the infected roots.

Biology

Background

Hodges and Coleman (2) demonstrated that two pathogens, *Pythium arrhenomanes* and *P. aristosporum*, caused Pythium root dysfunction in the Midwestern U.S. At the University of Maryland, other researchers (1) concluded that, in the Mid-Atlantic area, *P. aristosporum* was likely the most important species associated with Pythium root dysfunction because of its aggressiveness and frequency of isolation.

Our research

In the southeastern U.S., 75 *Pythium* isolates were obtained from creeping bentgrass roots in North Carolina, Virginia, South Carolina and Georgia. Isolates were identified using a combination of morphological and molecular techniques (6). The dimensions and characteristics of the oogonia, oospore, antheridia and sporangia (sexual and asexual spores and structures) were used to identify each isolate to the species level. Of these 75 isolates, 59 were identified as *P. volutum* and 16 were *P. torulosum*. Molecular characterization using DNA sequence data confirmed these morphological identifications.

Mature creeping bentgrass plants were inoculated with *P. volutum* or *P. torulosum* in the greenhouse to determine the pathogenicity of these species (6). Penn A-1 creeping bentgrass was seeded into pots containing sand meeting USGA specifications. The plants were grown in a greenhouse at 75 F (24 C)/72 F (22 C) (day/night) for six weeks. Once the plants were 6 weeks old, the roots were cut off at 2 inches (5 centimeters), and fresh sand was placed into the pots. Grass blades infested with *P. volutum* or *P. torulosum* were placed on the surface of the fresh sand, and the turf plug was replaced.

The pots were transferred to growth chambers set at 75 F (24 C)/61 F (16 C) (day/night) for four weeks to simulate cool temperatures in the fall and spring when these pathogens are most active. After four weeks, the temperature in the growth chambers was elevated to 90 F (32 C)/79 F (26 C) (day/night) to simulate the stressful conditions of summer and induce the expression of symptoms in infected plants (6).

Within 14 days of the initiation of heat stress, the plants inoculated with *P. volutum* became chlorotic or yellow. After four weeks at high temperatures, the same plants exhibited severe chlorosis and foliar dieback (data not shown). Root depth and root mass were severely reduced in these inoculated plants when compared to non-inoculated plants (6).

Plants inoculated with *P. torulosum* did not develop foliar symptoms, and root depth or mass were not negatively affected. Pots that were inoculated with a mixture of both species were similar to non-inoculated plants. The roots of plants inoculated with *P. volutum* were tan in color, lacked root hairs and possessed bulbous root tips.

Pythium volutum was readily re-isolated from this symptomatic root tissue, proving that this fungus is pathogenic toward mature creeping bentgrass plants (6). This work demonstrated that *P. volutum* is the predominate pathogen inducing Pythium root dysfunction in the southeastern U.S.

Epidemiology

Although Pythium root dysfunction symptoms are most common and severe in summer, our observations indicated that infection occurred at lower soil temperatures in the fall and spring. Our pathogenicity assay demonstrated that *P. volutum* infects creeping bentgrass roots when soil temperatures are between 61 F and 75 F but does not induce foliar symptoms under these conditions (5,6). This is in agreement with the results of researchers at the University of Maryland (1), who demonstrated that *P. volutum* was more aggressive toward creeping bentgrass seedlings at 64 F than at 82 F. Hodges and Coleman (2) also observed that colonization of roots by *P. aristosporum* and *P. arrhenomanes* was most rapid at temperatures between 64 F (18 C) and 72 F (22 C).

Favorable infection temperatures

The specific soil temperatures favorable for infection of creeping bentgrass roots by *P. volutum* were not discernible from the research reviewed above. Therefore, experiments were conducted to determine the optimal temperatures for infection and pathogen growth. First, the pathogen was placed on agar medium and incubated at temperatures ranging from 54 F (12 C) to 93 F (34 C). Radial growth of the fungus was measured 48 hours later, and it was discovered that *P. volutum* grew optimally when temperatures were between 64 F and 79 F (26 C) (5).

Temperature and pathogen aggressiveness

A series of growth chamber tests were also conducted to determine the influence of temperature on *P. volutum* aggressiveness. Creeping bentgrass plants were established, maintained and inocu-

Creeping bentgrass root depth

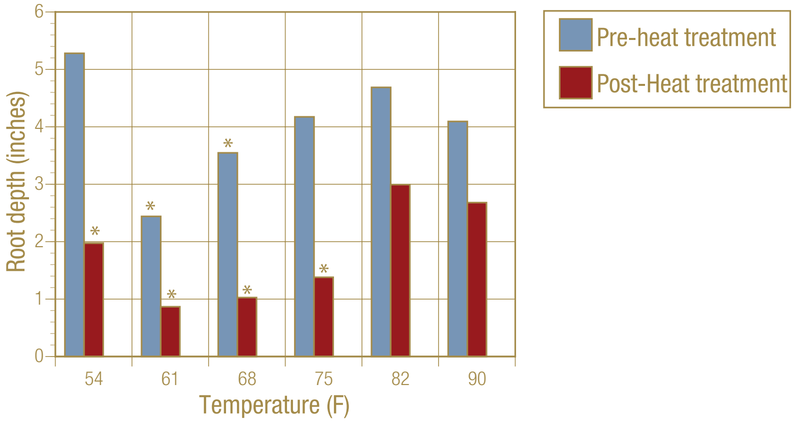


Figure 1. Effect of infection temperature treatments on creeping bentgrass root depth as measured pre- and post-heat treatment. Root depth was measured before initiation of the heat treatment and four weeks after initiation of heat treatment. Asterisks indicate a significant reduction in root depth as compared to the non-inoculated pots, which are not shown in this graph.

lated following procedures similar to those mentioned above. Following inoculation, the plants were placed in growth chambers set at a range of infection temperatures (54 F, 61 F, 68 F, 75 F, 82 F or 90 F [12 C, 16 C, 20 C, 24 C, 28 C, or 32 C]). After four weeks at these infection temperatures, the temperature in each chamber was increased to 90 F/79 F (day/night) to impose heat stress and induce the expression of symptoms (5).

Following heat treatment, extensive foliar dieback of creeping bentgrass was observed when infection temperatures were between 54 F and 75 F (5). Root depth before heat treatment was negatively affected only at infection temperatures of 61 F and 68 F (Figure 1). However, after four weeks of heat treatment, rooting depth was also reduced in the 54 F and 75 F infection temperature treatments (Figure 1). No foliar symptoms or reductions in root growth were detected before or after heat treatment in the 82 F or 90 F infection temperature treatments.

Fungicide effects

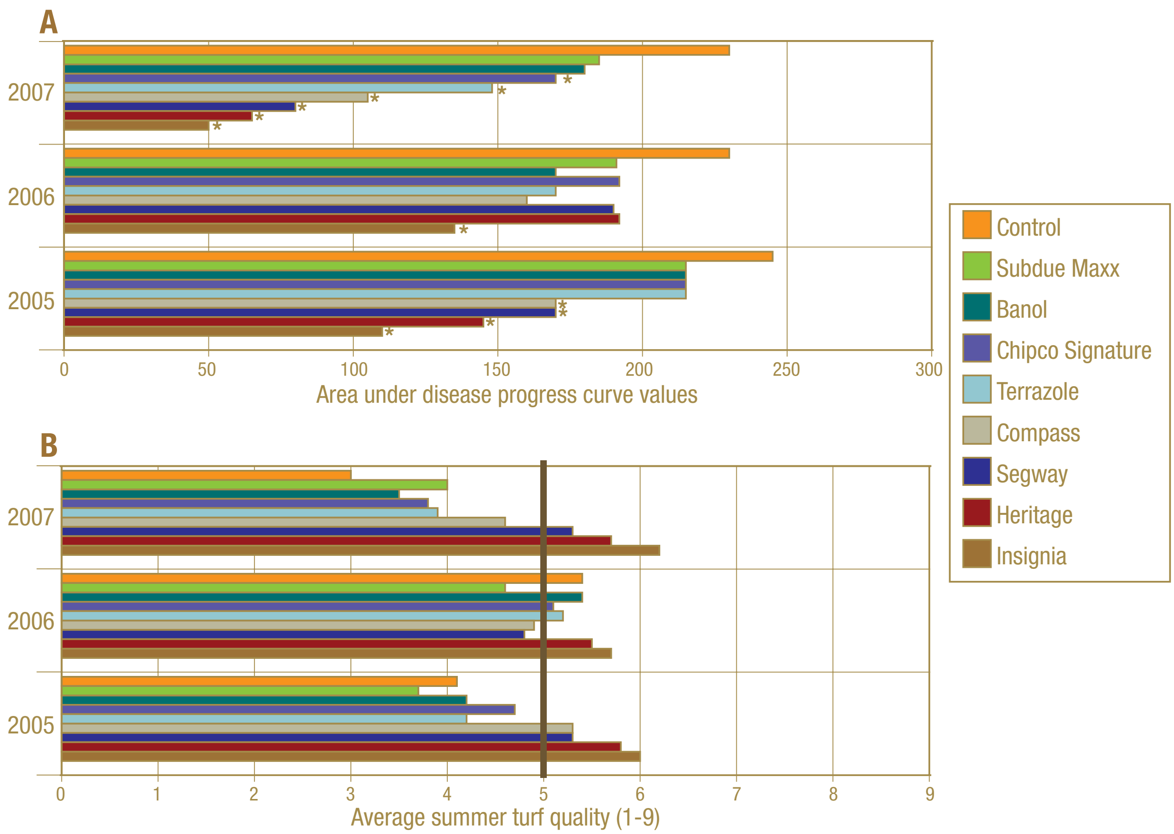


Figure 2. Impact of preventive fungicide applications on Pythium root dysfunction severity (A) and turfgrass quality (B) on Penn G-2 creeping bentgrass at Pinehurst (N.C.) Resort. Fungicides were applied twice in the fall when soil temperatures fell to 75 F and three times in the spring when soil temperatures reached 54 F. Fungicides were applied at full label rates and, except for Chipco Signature, were watered-in with 0.125 inch of irrigation. Asterisks denote a significant difference from the non-treated controls. Turfgrass quality was visually rated on a scale of 1 to 9. The vertical line represents acceptable turfgrass quality.

Based on these results, we concluded that pathogen activity and infection occurs when soil temperatures are between 54 F and 75 F, and preventive fungicides should be applied when soil temperatures are within this range (5).

Management

Once we defined the range of soil temperatures at which *Pythium volutum* is most active, we needed to determine which fungicides were most effective in protecting roots from infection by this pathogen.

Field experiments

A three-year field trial was established at the Pinehurst (N.C.) Golf Resort on a Penn G-2 creeping bentgrass green that was severely affected with Pythium root dysfunction. The green was constructed in 1993 according to USGA recommendations with an 85% sand and 15% sphagnum peat moss root zone.

Fungicide treatments were applied at the highest label rates and included Subdue Maxx (Syngenta, mefenoxam), Banol (Bayer, propamocarb), Chipco Signature (Bayer, fosetyl-Al), Terrazole (OHP, ethazole), Heritage (Syngenta, azoxystrobin), Insignia (BASF, pyraclostrobin), Compass (Bayer, trifloxystrobin) and Segway (FMC, cyazofamid). Treatments were applied on a monthly interval, twice in the fall and three times in the spring. Fall applications were initiated when average daily soil temperatures declined to approximately 75 F (24 C), and spring applications were conducted once soil temperatures increased to 54 F (12 C). All treatments except for Chipco Signature were watered-in with 0.125 inch (3.2 millimeters) of irrigation immediately after application. Disease severity was visually rated biweekly throughout the summer months of 2005, 2006 and 2007.

Insignia was the only fungicide that suppressed Pythium root dysfunction development in all three years of the experiment (Figure 2). Heritage, Compass and Segway reduced Pythium root dysfunction severity in 2005 and 2007, but not in 2006. Applications of Compass did reduce Pythium root dysfunction severity in two years of the experiment, but acceptable levels of turfgrass quality were observed only in 2007 (Figure 2). Although Pythium root dysfunction severity was reduced in 2007 with applications of Terrazole and Signature, acceptable turfgrass quality was not observed in plots receiving these treatments (Figure 2). In general, the specific oomycete fungicides, such as Terrazole, Banol, Subdue Maxx and Chipco Signature, were not effective for Pythium root dysfunction suppression.

Resistance to *P. volutum*

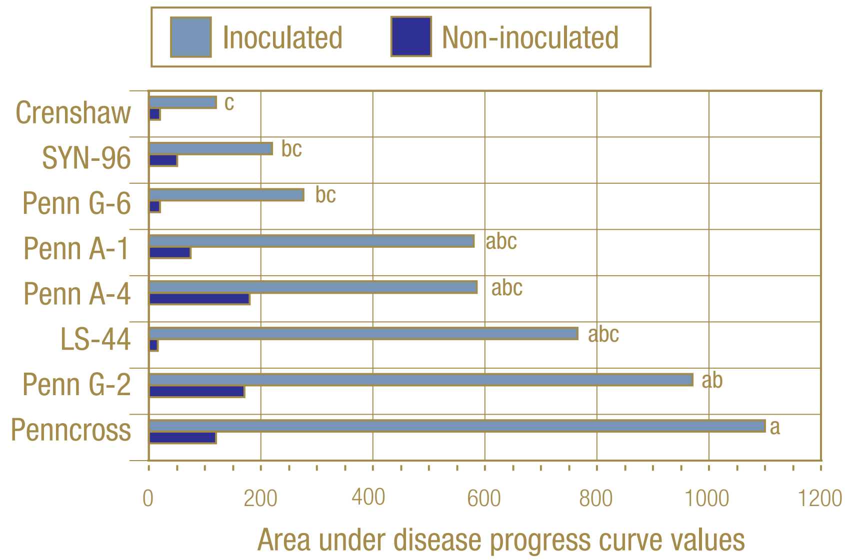


Figure 3. Relative resistance of eight creeping bentgrass cultivars to *Pythium volutum.* Bars followed by the same lowercase letter indicate values are not statistically different.

Laboratory experiments

In conjunction with our field experiment, we evaluated the sensitivity of 11 *P. volutum* isolates to azoxystrobin, fluoxastrobin, pyraclostrobin, cyazofamid, mefenoxam, propamocarb and fluopicolide (a component of Stellar [Valent]). Fluoxastrobin and fluopicolide were not included in the field experiments described above because they were not commercially available when the experiment was initiated in 2005. Only active ingredients are listed here because formulated product was not always used in the laboratory experiments.

To perform this experiment, potato dextrose agar was amended with six concentrations (0, 0.001, 0.01, 0.1, 1, and 10 ppm) of the above fungicides. The diameter of the fungal colony was measured after three days' growth on the amended media. Fungal colony diameter measurements were converted to EC_{50} values. EC_{50} values refer to the effective concentration required to cause a 50% reduction in growth.

On average, *P. volutum* isolates were very sensitive to pyraclostrobin, cyazofamid and fluoxastrobin; moderately sensitive to azoxystrobin; and the least sensitive to mefenoxam (data not shown). A dose response was not detected for propamocarb and fluopicolide, indicating that these active ingredients have very little activity against *P. volutum*. For comparison, an isolate of *P. aphanidermatum*, a causal agent of Pythium blight, was most sensitive to pyraclostrobin, cyazofamid and mefenoxam; moderately sensitive to azoxystrobin and fluopicolide; and least sensitive to fluoxastrobin (data not shown). The *P. aphanidermatum* isolate was not sensitive to propamocarb.

These results helped to confirm and explain the results of our early experiences with Pythium root dysfunction control. Even though it is a *Pythium* species, *P. volutum* is not very sensitive to some of the traditional Pythium fungicides like ethazole, mefenoxam and propamocarb. Instead, this species is most sensitive to the QoI fungicides azoxystrobin, fluoxastrobin and pyraclostrobin and the new QiI fungicide cyazofamid. As a result, products containing these active ingredients have been most effective for Pythium root dysfunction prevention. In addition, tank-mixtures of Chipco Signature with either Banol or Subdue Maxx have provided good suppression of the disease in our field studies, and we encourage superintendents to employ these tank-mixtures to slow the development of fungicide resistance to the QoI and QiI classes of chemistry.

Cultivar susceptibility

The vast majority of confirmed cases of Pythium root dysfunction between 2001 and 2005 were on greens planted with the creeping bentgrass varieties Penn A-1 or Penn A-4, or a mixture of the two. Consequently, it was reasonable to speculate that these varieties were more susceptible to Pythium root dysfunction, and emergence of Pythium root dysfunction was related to the recent wave of conversion to these varieties. To address this issue, we tested the relative resistance of eight creeping bentgrass cultivars (Penncross, Penn A-1, Penn A-4, LS-44, SYN-96, Crenshaw, Penn G-2 and Penn G-6) to *P. volutum* using the growth chamber methods described above.

Crenshaw, SYN-96 and Penn G-6 were the least susceptible varieties, Penn A-1 and Penn A-4 were moderately susceptible, and LS-44, Penn G-2 and Penncross were the most susceptible varieties (Figure 3). Although no varieties were completely resistant to Pythium root dysfunction, clearly Penn A-1 and Penn A-4 are not the most susceptible varieties on the market. So why was Pythium root dysfunction so widespread on greens that were recently established with Penn A-1 and/or Penn A-4? Maybe the answer lies in the soil, and not in the grass growing on top.

A final set of growth chamber experiments were conducted to look at the effect of organic matter content and irrigation frequency on Pythium root dysfunction development. For these experiments, we used large cylindrical pots that were 10 inches (25 centimeters) in diameter and 12 inches (30.5 centimeters) deep to more closely simulate the profile in a USGA putting green. For the organic matter study, we incorporated sphagnum peat moss with sand meeting USGA recommendations at ratios of 100:0, 90:10, 80:20, 70:30 and 50:50 sand to peat moss. Pots were irrigated six, four, three, two and one times per week during the infection temperature period with 13.5 ounces (400 milliliters) of water added to each pot at each irrigation.

A root zone of 70% sand and 30% peat moss at establishment was the only root zone that significantly limited Pythium root dysfunction development, demonstrating that increasing soil organic matter levels acts to suppress Pythium root dysfunction. Although a 70:30 sand:peat ratio would not meet USGA putting green recommendations with respect to infiltration rates, this result may explain why Pythium root dysfunction is most severe in newly constructed putting greens and tends to become less severe as the greens mature and accumulate more organic matter.

Irrigating only three times per week, when compared to irrigating four or six times per week, significantly limited Pythium root dysfunction development with little effect on turfgrass quality. Irrigating only once or twice per week in this experiment yielded very poor turf quality during the infection period. Thus, superintendents should also limit irrigation frequency during periods favorable for growth and infection by Pythium root dysfunction pathogens.

Conclusion

Pythium root dysfunction is a stress-induced disease that can be caused by three pathogens: *P. aristosporum, P. arrhenomanes* and *P. volutum*. Earlier research in the Midwest and Mid-Atlantic U.S. implicated *P. aristosporum* and *P. arrhenomanes* as the most common causes of Pythium root dysfunction, but our work demonstrates that *P. volutum* is the dominant pathogen causing Pythium root dysfunction in the southeastern U.S.

Symptoms of Pythium root dysfunction appear in irregular patches that range in size from 6 to 20 inches (15 to 50 centimeters) in diameter. Because of the lack of pathogen activity during summer and rapid dieback of infected roots during heat exposure, Pythium root dysfunction is difficult to diagnose and is often confused with take-all patch or other diseases. *Pythium volutum* grows and infects creeping bentgrass roots when soil temperatures are between 54 F and 75 F, yet symptoms are not expressed until affected plants are exposed to heat and/or drought stress, typically during the summer.

Pythium root dysfunction can effectively be managed with certain fungicides applied when soil temperatures are favorable for pathogen growth and infection. Creeping bentgrass cultivars vary in response to *P. volutum* infections, yet none of the eight varieties we tested were completely resistant.

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Literature cited

1. Feng, Y., and P.H. Dernoeden. 1999. *Pythium* species associated with root dysfunction of creeping bentgrass in Maryland. *Plant Disease* 83:516-520.
2. Hodges, C.F., and L.W. Coleman. 1985. *Pythium*-induced root dysfunction of secondary roots of *Agrostis palustris*. *Plant Disease* 69:336-340.
3. Kerns, J.P., H.D. Shew, D.M. Benson and L.P. Tredway. 2009. Impact of irrigation frequency, organic matter content, and creeping bentgrass cultivar on the development of Pythium root dysfunction. *International Turfgrass Society Research Journal* 11:1-13.
4. Kerns, J.P., M.D. Sokia and L.P. Tredway. 2009. Preventive control of Pythium root dysfunction in creeping bentgrass putting greens and sensitivity of *Pythium volutum* to fungicides. *Plant Disease* 93:1275-1280.
5. Kerns, J.P., and L.P. Tredway. 2008. Influence of temperature on pathogenicity of *Pythium volutum* toward creeping bentgrass. *Plant Disease* 92:1669-1673.
6. Kerns, J.P., and L.P. Tredway. 2008. Pathogenicity of *Pythium* species associated with Pythium root dysfunction of creeping bentgrass and their impact on root growth and survival. *Plant Disease* 92:862-869.

GCM

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The research says

→ Pythium root dysfunction is a stress-induced disease caused by three pathogens, but *Pythium volutum* is the dominant pathogen in the southeastern U.S.

→ Pythium root dysfunction causes irregular patches, 6 to 20 inches in diameter, in the turfgrass.

→ Because the pathogens are not active in summer and infected roots rapidly die back during heat exposure, the disease is difficult to diagnose and is often confused with take-all patch or other diseases.

→ Pythium root dysfunction can be managed effectively by applying certain fungicides when soil temperatures are favorable for pathogen growth and infection.

→ Creeping bentgrass cultivars vary in response to *P. volutum* infections, yet none of the eight varieties we tested was completely resistant.