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In the zone

A look at the diversity of rhizosphere bacteria in USGA putting greens

The soil environment immediately around a root frequently has a larger number of microorganisms compared to soil just a few millimeters away from a root. This zone of influence is called the rhizosphere (Rovira, 1991), which is composed of many groups of organisms that are capable of affecting plant health beneficially and deleteriously (Schippers et al., 1987).

Putting greens are artificially constructed soils, built from a predetermined mixture usually composed of sand and organic matter (USGA Green Section staff, 1993). In the Southeastern U.S., newly built putting greens are often fumigated before planted. However, previous research shows microbial populations present before fumigation rebound quickly after fumigation (Elliott and Des Jardin, 2001; Elliott et al., 2004). Additionally, as the putting greens mature, thatch, root and shoot production will cause significant increases in organic matter (Gaussoin et al., 2006), which will promote microbial growth.

Natural materials, organic materials and microbial inoculants are used by the golf course industry because there's an assumption few microbes are present in the turfgrass system or the "wrong" microbes are present. However, recent studies indicate turfgrass systems have extensive microbial populations (e.g., Bigelow et al., 2002; Elliott and Des Jardin, 1999; Elliott et al., 2004; Feng et al., 2002; Mercier, 2006) and diverse microbial communities (e.g., Mueller and Kussow, 2005; Sigler et al., 2001; Yao et al., 2006). Also, it's unclear whether introduced bacteria can influence bacterial populations in the phyllosphere, thatch, rhizosphere soil or bulk soil (Hodges et al., 1993; Lynch, 2002; Mercier, 2006; Mueller and Kussow, 2005; Sigler et al., 2001).

The emphasis of the project described herein was on culturable bacteria because it's culturable bacteria that are being exploited by the golf course industry. In other words, if you can't grow bacteria in large quantities (by a company or directly on the golf course in fermentation tanks), they aren't useful as products. While we know there's a diverse microbial community present in turfgrass root systems, it's not known which culturable fluorescent pseudomonad species or culturable bacilli species are present.

A joint project was undertaken by Auburn University, Clemson University and the University of Florida to examine bacterial populations and diversity in USGA putting greens during a three-year period after the greens were established. We've reported on the flux of the extensive bacterial populations present in putting greens (Elliott et al., 2004), the

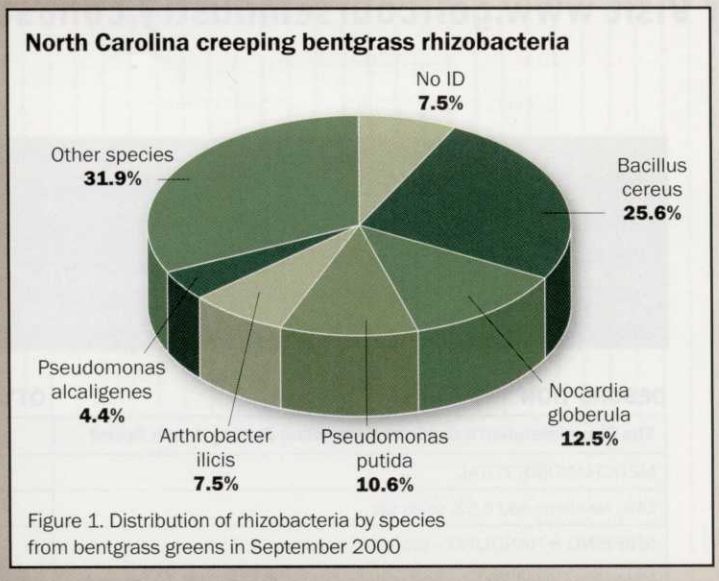

Figure 1. Distribution of rhizobacteria by species from bentgrass greens in September 2000

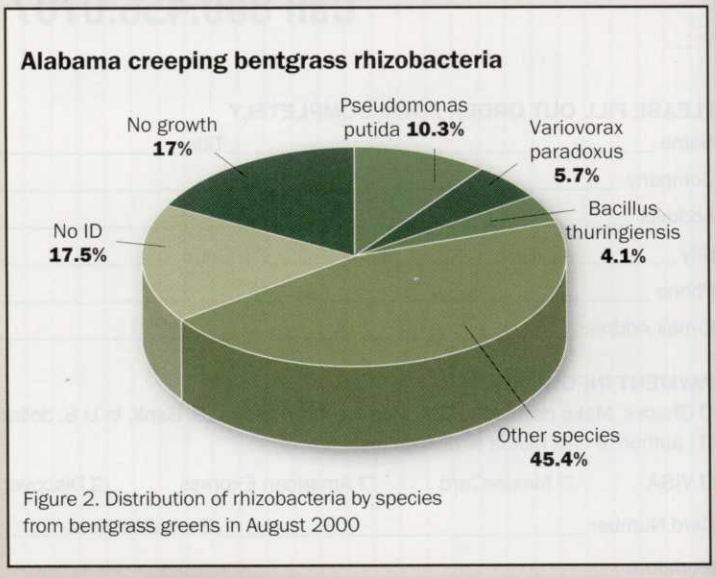

Figure 2. Distribution of rhizobacteria by species from bentgrass greens in August 2000

effect of nitrogen rate and root-zone mix on rhizosphere bacterial populations (Elliott et al., 2003), and the identification of a diverse group of denitrifying bacteria from putting greens (Wang and Skipper, 2004). This report summarizes which culturable bacterial genera and species were present and dominant in bentgrass and Bermudagrass putting greens in the Southeastern U.S. (Elliott et al., submitted).

STUDY SITES

The bentgrass (Crenshaw) putting greens are located at the Charlotte (N.C.) Country Club Golf Course and Auburn (Ala.) University. The hybrid Bermudagrass (Tifdwarf) putting greens are located at the Cougar Point Golf Course in Kiawah Island, S.C., and the University of Florida in Fort Lauderdale. All four sites were fumigated with methyl bromide before planting the turfgrass. Putting greens at university sites (Alabama and Florida) are miniature versions of those on golf courses. All greens were managed in a manner typical for the region.

RHIZOSPHERE SAMPLE

Four putting greens from each location were sampled four times a year (about every three months) for a minimum of three years in 1997 to 2000. Ten cores (0.40 inch by 4 inches)

Summary

Taxonomic diversity of bacteria associated with turfgrass roots hasn't been widely explored. The purpose of this project was to isolate and identify culturable bacteria from the rhizosphere of creeping bentgrass and hybrid Bermudagrass in the Southeastern U.S. Almost 10,000 randomly selected bacterial isolates were analyzed using gas chromatography fatty acid methyl ester (GC-FAME).

- The two dominant genera in bentgrass and Bermudagrass rhizospheres were *Bacillus* and *Pseudomonas*, with *Bacillus* dominant in Bermudagrass and *Pseudomonas* dominant or equal to *Bacillus* in bentgrass.
- Other genera that composed at least 1 percent of the isolates at all four sites were *Clavibacter*, *Flavobacterium*, and *Microbacterium*.
- *Arthrobacter* also composed a significant portion of the bacterial isolates in the bentgrass rhizosphere but not the Bermudagrass rhizosphere. Overall, there were 40 genera common to all four sites.
- At the species level, there were five that composed at least 1 percent of the isolates at each location – *B. cereus*, *B. megaterium*, *C. michiganensis*, *F. johnsoniae*, and *P. putida*.
- This project demonstrates there's considerable taxonomic diversity of bacteria present in the rhizosphere of putting greens.

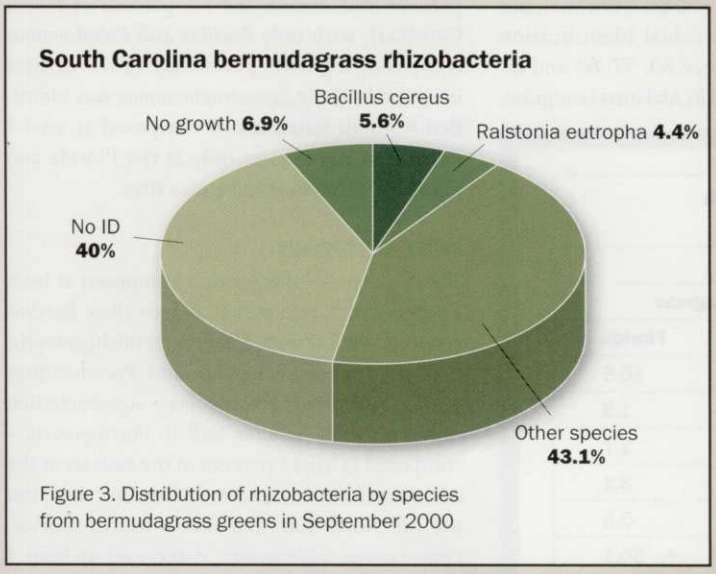

Figure 3. Distribution of rhizobacteria by species from bermudagrass greens in September 2000

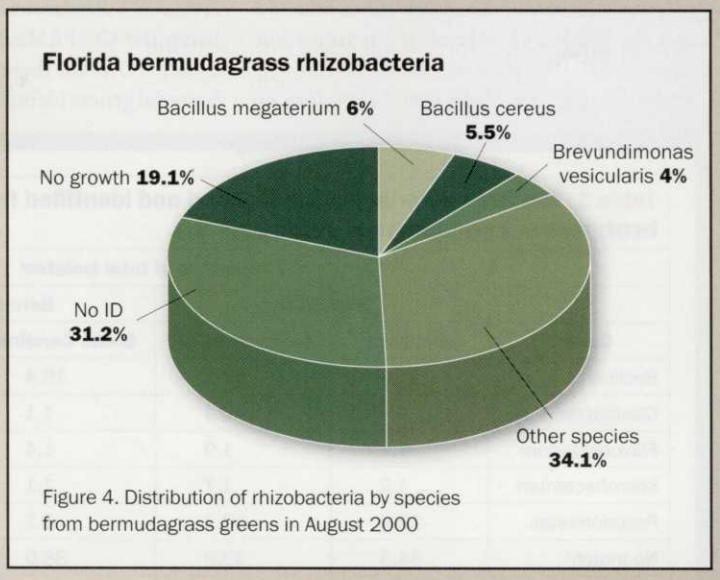

Figure 4. Distribution of rhizobacteria by species from bermudagrass greens in August 2000

Scientists' research demonstrates there's a considerable taxonomic diversity of bacteria in the rhizosphere of putting greens.

were collected per putting green to constitute a sample. Green tissue was removed from each core with a sterile razor blade. For each sample, turfgrass roots were separated from the root-zone mix, and all root material and rhizosphere soil was subjected to shaking in a sterile diluent. Aliquots of dilutions were spread plated onto duplicate plates of selective and nonselective media (Elliott et al., 2004). For enumeration of total aerobic bacteria and selection of bacteria for identification with GC-FAME, solidified 10 percent tryptic soy broth (10 percent TSBA), amended with 100 µg mL-1 cycloheximide to inhibit fungi, was used. For each sampling date, 40 bacterial isolates per green sampled were randomly selected from the 10 percent TSBA for future identification. An estimated 10,000 bacterial isolates were selected for identification during the course of this study.

ID BACTERIA ISOLATES

Analysis of the bacterial isolates was conducted using the GC-FAME/Microbial Identification System (MIDI in Newark, Del.) at Auburn University or at the Multi-user Laboratory at Clemson University. Isolates were processed according to the protocol for aerobic bacteria of environmental origin (Sasser, 2006). Fatty acid peak profiles were analyzed using the Sherlock Standard Aerobe Libraries (MIS version 4.0, Microbial ID, www.midi-inc.com). According to literature provided by MIDI, strains with a similarity index of 0.50 or greater are considered a good match at the species level, whereas strains with a similarity index between 0.30 and 0.49 are considered a good match at the species level but indicates an atypical strain (Anonymous, 2005a). Because the bacterial species present in putting greens were largely unknown when this study was initiated, a similarity index of 0.30 or greater was used as the basis for identifying bacterial isolates.

BACTERIAL GENERA

A total of 9,216 bacterial isolates were analyzed using the GC-FAME/Microbial Identification System. Overall, there were 50, 57, 64 and 64 bacterial genera identified in Alabama bentgrass, North Carolina bentgrass, Florida Bermudagrass and Sout Carolina Bermudagrass, respectively. There were 76 genera identified at both Bermudagrass sites, with 13 unique to Florida, 13 unique to South Carolina and 50 common to both. There were 59 genera identified at both bentgrass sites, with three unique to Alabama, nine unique to North Carolina and 47 common to both. Forty genera were common to all four sites.

There were five genera that composed at least 1 percent of the isolates at all four sites (*Bacillus*, *Clavibacter*, *Flavobacterium*, *Microbacterium* and *Pseudomonas*, with *Bacillus* and *Pseudomonas* the dominant bacterial genera at each location. However, the percentage of isolates identified as *Bacillus* in the Bermudagrass sites was almost twice the number of isolates identified as *Pseudomonas*. At the bentgrass sites, *Pseudomonas* was either the dominant genus (North Carolina) or was equal to *Bacillus* (Alabama). This is consistent with the previously reported enumeration data that *Bacillus* is the dominant genus over *Pseudomonas* in the Bermudagrass rhizosphere, and that significantly greater numbers of fluorescent pseudomonads are found in the bentgrass rhizosphere than in the Bermudagrass rhizosphere (Elliott et al., 2004).

Arthrobacter composed a significant portion of the bacterial isolates at the bentgrass sites (9.1 percent at Alabama and 7.5 percent at North Carolina), with only *Bacillus* and *Pseudomonas* composing a greater percentage of the isolates identified. While *Stenotrophomonas* was identified from all four sites, it composed at least 1 percent of the isolates only at the Florida and South Carolina Bermudagrass sites.

Table 1. Top five bacterial genera isolated and identified from bentgrass or bermudagrass putting greens.

	Percentage of total isolates[y]			
	Bentgrass		Bermudagrass	
Genera	Alabama	North Carolina	South Carolina	Florida
Bacillus	13.9	12.5	19.4	10.5
Clavibacter	2.0	2.4	1.1	1.3
Flavobacterium	1.5	1.9	1.4	1.7
Microbacterium	1.2	1.7	1.1	3.1
Pseudomonas	13.6	18.7	9.1	5.8
No match[z]	34.3	32.0	38.0	50.1

[y] Total isolates analyzed is 1,896 for Alabama, 2,832 for North Carolina, 2,617 for South Carolina and 1,871 for Florida.

[z] No isolate for that site had a match to a genus in the FAME database.

BACTERIAL SPECIES

There were five species that composed at least 1 percent of the isolates at all four sites: *Bacillus cereus*, *B. megaterium*, *Clavibacter michiganensis*, *Flavobacterium johnsoniae* and *Pseudomonas* putida. Another three species – *Agrobacterium radiobacter*, *B. pumilus* and *B. thuringiensis* – composed at least 1 percent of the isolates at the Alabama, Florida and South Carolina sites, but not the North Carolina site. A fourth species, *Comamonas acidovorans*, composed at least 1 percent of the isolates at the NC, AL and Florida sites but not the South Carolina site. One species was common at the 1-percent level only to the Bermudagrass locations: *Stenotrophomonas maltophilia*. Four species were common at the

1-percent level only to the bentgrass locations: *Arthrobacter ilicis*, *P. chlororaphis*, *P. fluorescens* and *P. syringae*. Figures 1 to 4 illustrate examples of species composition for single dates at each study site.

UNIDENTIFIABLE ISOLATES

The number of unidentifiable isolates (similarity index of less than 0.30) was 50.1 percent for Florida Bermudagrass, 38 percent for South Carolina Bermudagrass, 34.3 percent for Alabama bentgrass and 32 percenty for North Carolina bentgrass (Table 1). These values fall within the range of unidentifiable isolates obtained in other studies using GC-FAME for identification purposes (Germida and Siciliano, 2001; Gooden et al., 2004; Kim et al., 2001/2002; Mahaffee and Kloepper, 1997; Poonguzhali et al., 2006; Siciliano and Germida, 1999). Thus, the number of unidentified isolates in this study, obtained from an artificially constructed soil, would appear to be similar to the number from field soils in the same states using the same identification system.

Why are some bacterial isolates not identified? The MIDI aerobe bacteria library includes fatty acid profiles for 695 environmental species, with usually 20 or more strains representing each species or subspecies (Anonymous, 2005b; Sasser, 2006). Our results and those of others illustrate that a significant number of bacteria isolated from bulk or rhizosphere soils aren't part of the bacterial collection that's the basis of the MIDI environmental species library. Any database is only as good as the data – in this case, fatty acid methyl ester profiles of bacterial isolates – accumulated within it. The unidentifiable isolates aren't necessarily new species per se but simply might be species not represented in the MIDI database.

TAXONOMIC DIVERSITY

This is the first study to survey for a portion of the culturable, aerobic bacterial genera and species common to golf course putting greens in the southeastern U.S. It demonstrates there's considerable taxonomic diversity present in the rhizosphere of putting greens, despite their intense management. Obviously, while we have identified some of the bacteria genera and species present in golf course putting greens, there still are many unidentified bacteria. Even less information is known regarding what these bacteria do in the turfgrass system. **GCI**

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Editor's note: Literature cited for this article can be found online at www.golfcourseindustry.com posted with this article.

IMPACT ON THE BUSINESS

Back to basics

BY PAT JONES

Much of the research in the golf and turf academic community is directed to determine highly specific results. Which Bermudagrass performs the best under saline conditions? Which fungicide was most effective on *Poa annua*/bentgrass greens? Which wetting agent was best suited for highly compacted soils?

This article separates itself from the pack because it looks at a very basic question: Which tiny critters (bacteria) are hanging out in the soil of different types of putting greens? Ultimately, it gets back to the age-old question of what is the right soil ecosystem for creating greens that are viable and manageable.

WHY?

Soil microbial action has become increasingly recognized as an important indicator of turf health (or lack thereof) throughout the past decade. Yet, the presence or nonpresence of these diverse microscopic thingies raises a bunch of questions that turf scientists are just starting to answer. Those questions include:

- Which microbes are present?
- Are they beneficial or harmful?
- What is the right balance for your soils?
- How can they be promoted or eliminated to achieve that balance?

This article addresses the first question: What do you typically find in different kinds of greens structures.

WHAT'S NEXT?

The real question is how do you manage the microbial populations that inevitably live under every golf putting surface. Once again, soil testing is a big part of the answer. According to experts in the field, certain elements (calcium, potassium, etc.) probably promote a healthy microbial mix. Regular and relatively extensive soil testing offers a chance to benchmark soil mineral composition against microbial activity.

COSTS AND BENEFITS

As noted previously, soil testing costs can range from zero (the free services offered by chemical companies) to thousands of dollars annually for sophisticated testing provided by independent labs. Unfortunately, course owners and green committees sometimes fail to understand the value of independent testing and will look at this "consulting" fee as one of the first things to be axed when budgets get tight.

That short-sighted view can result in much higher costs later when the soil ecosystem gets out of balance and much more expensive cures are needed. In short, testing almost always pays for itself. **GCI**