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BACTERIAL POPULATIONS AND DIVERSITY WITHIN NEW USGA PUTTING GREENS.

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Introduction

The soil environment immediately around the root frequently has a larger number of microorganisms than soil just a few millimeters away from the root. This zone of influence is called the rhizosphere. Methods for studying the rhizosphere have been well established (Rovira, 1991; Kloepper & Beauchamp, 1992; Bolton et al., 1993). After approximately 20 years of intense research on the rhizosphere, Rovira (1991) indicated that there are over 2,000 publications on the this topic and stated "prospects are bright for improving our understanding of rhizosphere biology and managing the rhizosphere microflora to increase plant growth". However, he indicated our frustrations would continue unless more thought and effort are put into the microbial ecology of the rhizosphere.

The rhizosphere is composed of many groups of organisms that are capable of affecting plant health, both beneficially (Nelson and Craft, 1991; Hodges et al., 1993) and deleteriously (Elliott and Lynch, 1985; Schippers et al., 1987; Suslow and Schroth, 1982). A critical research need for bentgrass greens is to understand the bacterial interactions in the rhizosphere.

Research on microbial populations associated with turfgrass has been limited (Cole and Turgeon, 1978; Smiley and Craven, 1979; Mancino et al., 1993; Liu et al., 1995). Knowledge gained from this research will help to:

(1) Assess impacts on environmental quality as reflected by microbial diversity and function in the rhizosphere of bentgrass;
(2) Improve potential for biological management of pests in turfgrass;
(3) Assess seasonal nitrogen concentrations in bentgrass greens; and
(4) Improve turfgrass productivity by enhancing nutrient uptake efficiency and plant growth.

Objectives

The overall objective is to develop baseline data concerning bacterial composition (population and diversity) of new USGA bentgrass putting greens after construction. Specific objectives are:

1. Determine bacterial populations associated with new bentgrass putting greens via selective media and identification of bacteria by FAME.
2. Compare rhizosphere bacterial populations on two different turfgrasses, bentgrass and bermudagrass. The bermudagrass work is part of a Clemson University Turfgrass Initiative project.
3. Document rhizosphere bacterial population dynamics on bentgrass over a four-year time period.
4. Construct a database for rhizobacteria diversity of bentgrass.

Experimental Procedures

Golf Course and Superintendent. The Charlotte Country Club Golf Course, Charlotte, NC was selected for this project with Mr. Mark Stoddard, CGCS, as the Superintendent. Mr. Mike Pilo is now the Superintendent.

Root-zone Mix and Planting. The new bentgrass greens were constructed in the summer of 1996 with an 85:15 root-zone mix composed of quartz sand and Canadian sphagnum peat moss. Greens were seeded with Crenshaw bentgrass on August 14, 1996.

Sampling Schedule. Samples were collected from four greens (#15, #17, #18, and Big Putt) of the Charlotte Country Club Golf Course every three months from December 1996 to October 2000. A 3/8 inch probe was used and ten 4-inch cores/green (randomly selected) were collected at each sampling time. The probe was disinfected using 70% ethanol before sampling each green. The samples were kept on blue ice until being processed within 48 hours. Samples were obtained four times each year for four years.

Experimental Protocol. Plant roots were separated from the soil mix, placed in 90-mL dilution buffer, and shaken for 30 min at 200 rpm on a rotary shaker. The resulting suspensions were subjected to serial dilution and plating using standardized techniques and media.

Six kinds of media were used in this study: 1/10 tryptic soy broth agar (TSBA) for total bacteria and spore-forming bacteria (after treatment at 80°C for 10 min), azide blood agar for gram positive bacteria, crystal violet agar for gram negative bacteria, S1 for fluorescent pseudomonads, Actinomycete isolation agar (AIA) for actinomycetes, and *Xanthomonas maltophilia* selective medium (XMSM, Juhnke and Des Jardin, 1989) for *Stenotrophomonas maltophilia* , the new name for *X. maltophilia* .

From the 1/10 TSBA plates for total bacterial populations, we randomly selected 40 isolates/green to be identified using the GC/FAME analysis. The FAME analyses of these isolates are under way. Over the 4-year project period, an estimated 2,560 bacterial isolates will be selected for identification from the Charlotte Country Club Golf Course. The other selective media will generate a database on broad classes of bacteria.

FAME Analysis. Identification of the bacterial isolates will be determined using the gas chromatography/MIDI Microbial Identification System MIS (Microbial ID, Newark, DE) in Dr. Joe Kleopper's lab at Auburn University. To date, bacterial isolates from December 1996 to December 1999 samples have been identified. Bacterial isolates from March, June, and October 2000 samples are being identified.

Database of Microbial Population Profile. The results obtained from selective media and FAME analyses by Clemson University, Auburn University and University of Florida will be statistically analyzed and stored electronically. The database for microbial population profile of bentgrass is being constructed.

Results and Discussion

The Charlotte Country Club Golf Course was chosen for this study because it was reconstructed in June 1996. Since December 1996, we have sampled sixteen times from four greens (#15, #17, #18, and Big Putt).

Broad classes of rhizobacterial populations were successfully separated by the selective media. Total rhizobacteria ranged from 6 to 7 $\times$ 10^9 CFU/g root dry weight in bentgrass. Most rhizobacterial populations and total populations were relatively stable over these sampling periods. Percentage of Gram-positive and Gram-negative bacteria based on the KOH method were relatively stable over eight sampling times with approximately 75+% of the bacteria being Gram-negative (data not presented in this report).

For rhizobacteria from bentgrass in December 1996, there were 32 species with seven species above 4% each (Figure 1). In December 1999, 31 species were identified and six species accounted for $\geq$ 4% each (Figure 2). *Flavobacterium johnsoniae* was present in both years; whereas, the other species shifted from Dec-1996 to Dec-1999.

More than 25 genera were identified by GC-FAME for bentgrass (Table 1). Shifts of major genera associated with bentgrass were observed from December 1996, December 1997, December 1998, and December 1999 samples. Some dominant genera in December 1996 samples, Acidovorax, Comamonas, and Ralstonia in bentgrass for instance, were not major genera in December 1997, December 1998, or December 1999 samples. Also, some dominant genera in December 1998 and 1999 samples, Arthrobacter and Bacillus, were not major genera in December 1996 or 1997 (Table 1).

In addition to competition among species, the management practice and other abiotic conditions such as nutrient, pH, aeration etc. may have contributed to the shifts in genera or species. The functions of the species associated with both turfgrasses remain to be investigated.

A similar database has been generated for bermudagrass greens and will be reported under the Clemson University Turfgrass Initiative grant.

Summary

From the results of December 1996 to December 1999, major shifts in rhizobacteria were noted in genera and species for bentgrass. Prior to June 1998, Pseudomonas was the dominant genus for bentgrass followed by Arthrobacter. However, after 21 months, Bacillus also began to be a dominant genus on bentgrass. This shift may be related to root exudates or physical/chemical changes in the turfgrass rhizosphere.

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Presentations

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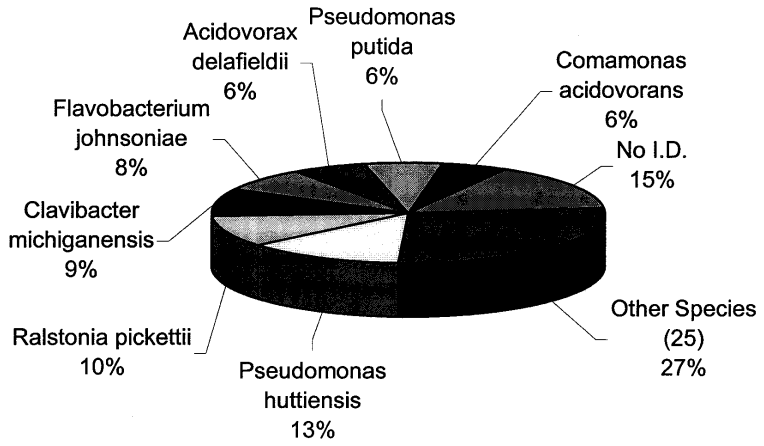

Figure 1. Distribution of rhizobacteria by species from Bentgrass greens. Samples were collected in Dec-1996.

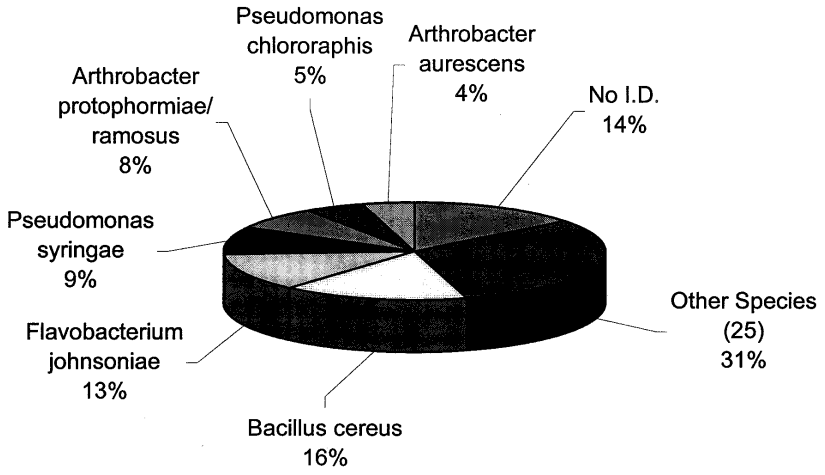

Figure 2. Distribution of rhizobacteria by species from Bentgrass greens. Samples were collected in Dec-1999.

Table 1 : Percentage of genera present in Bentgrass samples collected from December-1996 to December-1999. Blank indicates either the genus was not detected or the percentage was below 4.0%.

PERCENTAGE OF DOMINANT GENERA PRESENT IN BENTGRASS GREENS

Genus	12-96	3-97	6-97	9-97	12-97	3-98	6-98	9-98	12-98	3-99	6-99	9-99	12-99
Acidovorax	6.9						5.0	5.6					
Arthrobacter		8.8	4.4			18.8	7.5		13.1	5.6	16.3		18.1
Aureobacterium													
Bacillus							5.6	11.9	16.3		15.6	21.9	17.5
Chryseobacterium											6.9		
Clavibacter	8.8				5.0					8.1		6.3	
Comamonas	5.6	18.1											
Cytophaga									4.4				
Flavobacterium	7.5	8.8				10.6				7.5	16.3		13.1
Microbacterium												8.8	
Pseudomonas	25.0	22.5	5.6	4.4	7.5	34.4	38.8	22.5	7.5	8.1	23.1	15.6	21.9
Ralstonia	13.1											5.6	
Rhodococcus			5.6										
Xanthobacter								6.3					
Xanthomonas					9.4					13.8			
No ID	15.0	18.8	58.8	68.1	41.9	16.3	14.4	16.9	24.4	30.6	6.3	22.5	15.0
Other Genera	18.1	23.1	25.6	27.5	36.2	20.0	28.8	36.9	34.4	26.3	15.6	19.4	14.4