EXECUTIVE SUMMARY

Project Title: Bacterial Populations and Diversity within New USGA Putting Greens.

Principal Investigators: Horace D. Skipper, Jung H. Kim, Kun Xiong, Landon C. Miller, A. Robert Mazur, and N. Dwight Camper, Clemson University.

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.

Progress Report: Rhizobacteria are being evaluated for promotion of plant growth and for biological control of weeds, insects, diseases, and nematodes in a number of ecosystems. A critical research need in greens management is to understand the bacterial interactions in the rhizosphere of turfgrasses. A database on turfgrass rhizobacteria from newly constructed bentgrass greens was initiated in Dec-1996. Each quarter, 160 randomly selected bacterial isolates on tryptic soy broth agar (TSBA) were isolated and are being identified by FAME analyses. Broad classes of rhizobacterial populations were successfully separated on selective media. Numerical differences of rhizobacterial populations in bentgrass rhizosphere over ten sampling periods were observed (Figure 1). In the samples of Dec-1996, the major genera from bentgrass isolates belonged to *Acidovorax, Burkholderia, Pseudomonas*, *Cytophaga*, *Hydrogenophaga*, and *Clavibacter*. However, in the samples of Dec-1998, the major genera were *Bacillus*, *Arthrobacter*, *Pseudomonas,* and *Cytophaga*. From the beginning of this study, *Pseudomonas* has been a major genera; however, *Bacillus* has been a key group only since Jun-1998. The appearance of *Bacillus* as a major genera after 21 months suggest a shift in carbon sources available for growth of rhizobacteria from bentgrass roots.

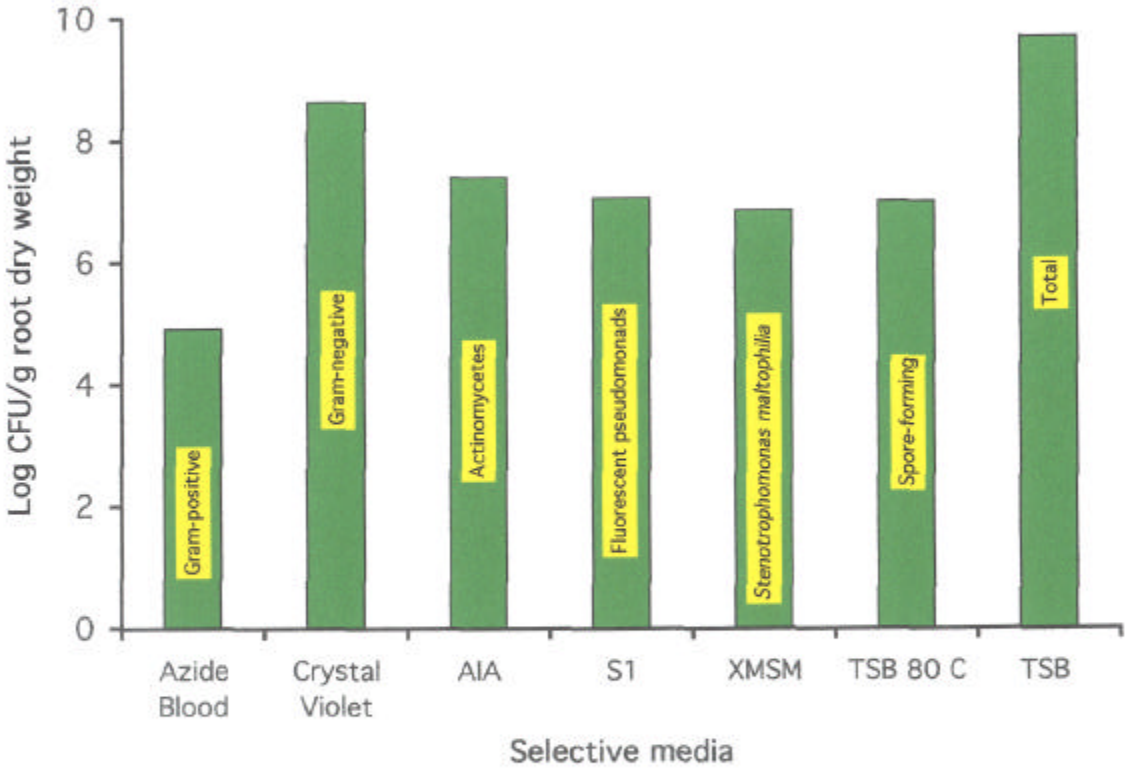

Figure 1. Rhizobacterial populations were averaged over ten sampling periods from bentgrass greens with an exception of *S. maltophilia*. *S. maltophilia* was averaged over seven sampling periods from Dec-96 to Jun-98. Samples were collected from Dec-96 to Mar-99 from Charlotte Country Club Golf Course, NC.

BACTERIAL POPULATIONS AND DIVERSITY WITHIN NEW USGA PUTTING GREENS.

Sponsored by:

United States Golf Association, Green Section Research.

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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.
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Experimental Procedures

Root-zone Mix. The Charlotte Country Club Golf Course, Charlotte, NC was selected for this project with Mr. Mark Stoddard, CGCS, as the Superintendent. 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 March 19989. A 3/8 inch probe was used and ten 4-inch deep 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 will be obtained four times each year.

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. We have selected and stored 1,600 bacteria isolates from the collected samples. 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 1998 samples have been identified. Bacterial isolates from 1999 samples are being identified.

Data Base 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 data base for microbial population profile of bentgrass is being constructed.

Summary of Research Progress

The Charlotte Country Club Golf Course was chosen for this study because it was reconstructed in Jun-1996. Since then, we have sampled 12 times from four greens (#15, #17, #18, and Big Putt).

Broad classes of rhizobacterial populations were successfully separated by the selective media (Figure 1). Average total rhizobacteria were 6.76×10^9 CFU/g root dry weight in bentgrass. Most rhizobacterial populations and total populations were relatively stable over 10 sampling periods. Percentage of Gram-positive and Gram-negative bacteria based on the KOH method were relatively stable over eight sampling times with approximately 83% of the bacteria being Gram-negative. However, shifts of rhizobacterial populations were observed. For instance, Gram positive and fluorescent pseudomonad populations were changed at different magnitudes.

In the samples of Dec-1996, the major genera from bentgrass isolates belonged to *Acidovorax, Burkholderia, Pseudomonas*, *Cytophaga*, *Hydrogenophaga*, and *Clavibacter*.(Table 1). However, in the samples of Dec-1998, the major genera were *Bacillus*, *Arthrobacter*, *Pseudomonas,* and *Cytophaga*. From the beginning of this study, *Pseudomonas* has been a major genera; however, *Bacillus* has been a key group only since Jun-1998. The appearance of *Bacillus* as a major genera after 21 months suggest a shift in carbon sources available for growth of rhizobacteria from bentgrass roots.

Shifts of major species associated with bentgrass were observed from Dec-1996 to Dec-1998 samples. Some dominant species in Dec-1996 samples, *Acidovorax delafieldii* and *Burkholderia pickettii* for instance, were not major species in Dec-1998 samples (Figure 2). At this latter date, the major species were *Bacillus cereus*, *Arthrobacter ilicis*, *Pseudomonas putida, Bacillus*

coagulans, *Bacillus thuringiensis*, and *Cytophaga johnsonae*. The management practice and other abiotic conditions such as nutrient, pH, aeration etc. may have contributed to the change. The functions of the species associated with turfgrass remain to be investigated.

To date, 1,920 bacterial isolates have been selected and stored for FAME analysis. We have established standard method for FAME extraction procedures in our lab to continue this phase.

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

Acknowledgments

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Presentations

J.H. Kim, K. Xiong, H.D. Skipper, L.C. Miller, A.R. Mazur, M.L. Elliott, E.A. Guertal, J.A. McInroy, and J.W. Kloepper. 1999. Seasonal Shifts of Rhizobacteria in New USGA Greens. ASA-CSSA-SSSA Annual Meeting, Salt Lake City, UT.

H.D. Skipper, R.O. Ankumah, J.H. Kim, L.C. Miller, A.R. Mazur, and J.A. McInroy. 1999 Rapid biodegradation of Nemacur and microbial ecology in new USGA greens. Clemson University, Tuskegee University, and Auburn university. Clemson University Turfgrass Field Day, Florence, SC.

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Table 1. Percentage of genera present in bentgrass samples collected from Dec-1996 to Dec-1998. Blank indicates either the genus was not detected or the percentage was below 4.0%.

PERCENTAGE OF DOMINANT GENERA PRESENT IN BENTGRASS GREENS

Genus	Dec-96	Mar-97	Jun-97	Sep-97	Dec-97	Mar-98	Jun-98	Sep-98	Dec-98
Acidovorax	17.3%		7.0%				5.8%	6.8%	
Agrobacterium					8.2%				
Arthrobacter		12.0%	7.0%			20.0%	10.2%		17.2%
Aureobacterium					7.2%				
Bacillus				7.6%			6.6%	14.3%	23.8%
Burkholderia	13.3%		5.6%						
Clavibacter	8.0%			6.1%	8.2%				
Comamonas		15.8%		6.1%	4.1%				
Cytophaga	10.0%	15.8%				11.9%			5.70%
Hydrogenophaga	10.0%								
Methylobacterium			9.9%						
Micrococcus				10.6%		8.2%			
Paracoccus			5.6%						
Pseudomonas	13.3%	28.5%	12.7%	9.1%	14.4%	39.3%	48.9%	27.1%	9.8%
Rhodococcus			12.7%						
Sphingobacterium					4.1%				
Stenotrophomonas				6.1%					
Xanthobacter								7.50%	
Xanthomonas				6.1%	14.4%				
Other Genera	28.1%	27.9%	39.5%	48.3%	39.4%	20.6%	28.5%	44.4%	43.5%

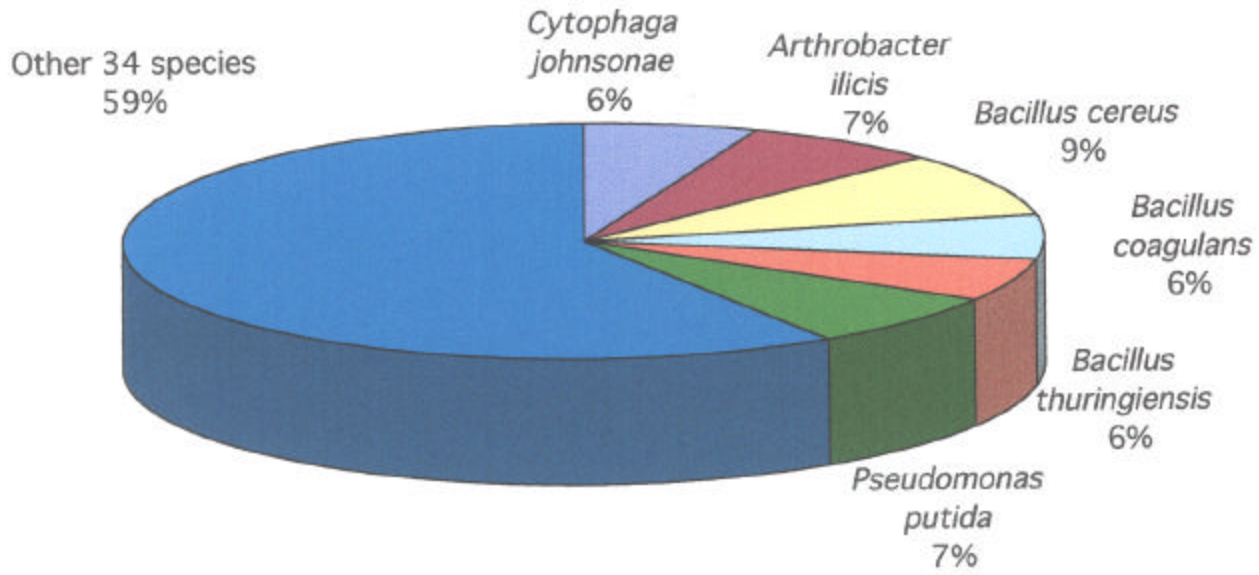

Fig. 2. Distribution of rhizobacteria by species from bentgrass greens. Samples were collected in Dec-1998.