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 data base 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 putting greens management is to understand the bacterial interactions in the rhizosphere of turfgrasses. A data base on turfgrass rhizobacteria from newly constructed bentgrass putting 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 eight sampling periods were observed (Figure 1). In the samples of Dec-1996, isolates identified from bentgrass rhizosphere belonged to 23 genera and 34 species. *Acidovorax, Burkholderia,* and *Pseudomonas* were the major genera. However, in the samples of Jun-1998, isolates identified from bentgrass rhizosphere belonged to 23 genera and 43 species. *Pseudomonas* and *Arthrobacter* were the major genera. Based on the KOH method, 83% of the bentgrass isolates were Gram-negative over eight sampling periods.

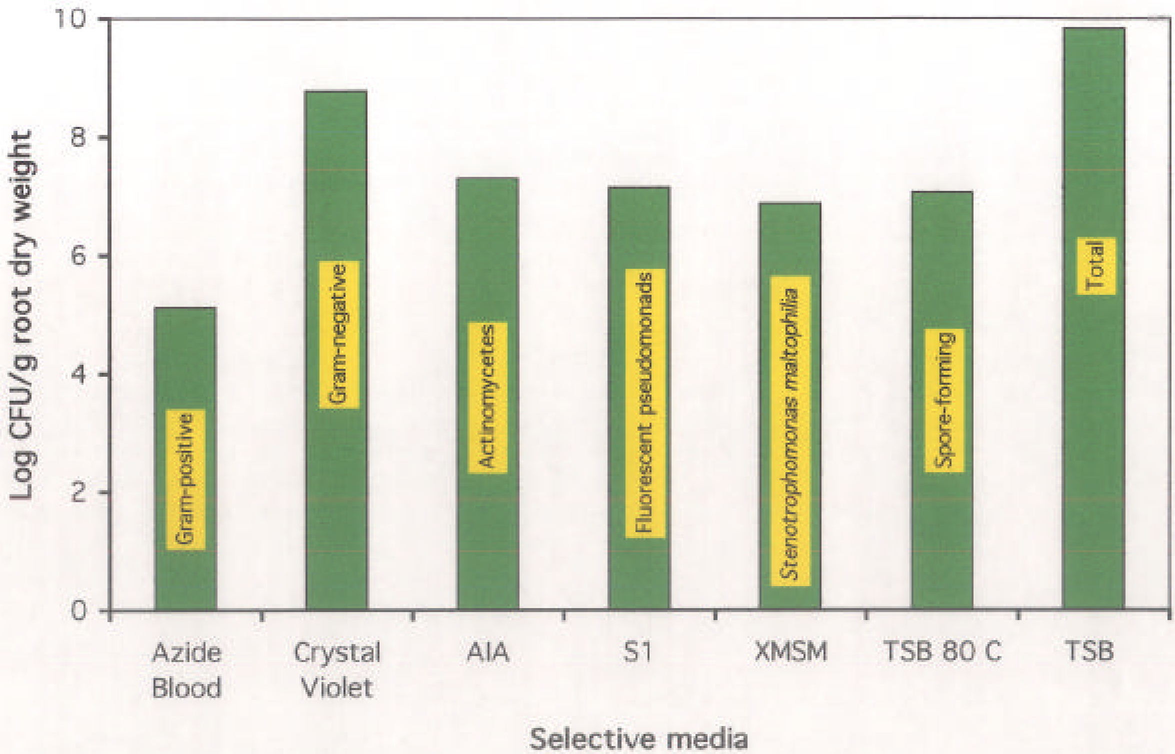

Figure 1. Rhizobacterial populations were averaged over eight sampling periods from bentgrass greens. Samples were collected from Dec-96 to Sep-98 from Charlotte Country Club Golf Course, NC.

BACTERIAL POPULATIONS AND DIVERSITY WITHIN NEW USGA PUTTING GREENS.

Sponsored by:

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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 data base for rhizobacteria diversity of bentgrass.

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 September 1998. 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 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 rotatory 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 1,280 bacteria isolates from the collected samples. The FAME analyses of these isolates are under way. Over the 4-year project period, an estimated 2,000 bacterial isolates will be selected for identification from the Charlotte Country Club Golf Course. The other selective media will generate a data base 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 June 1998 samples have been identified. Bacterial isolates from September 1998 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 June, 1996. Since then, we have sampled eight 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 eight 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.

Isolates from bentgrass rhizosphere in Dec-1996 samples belonged to 23 genera (Table 1) and 34 species (Table 2). *Acidovorax, Burkholderia,* and *Pseudomonas* were major genera in bentgrass in Dec-1996 samples. However, isolates from Jun-1998 samples belonged to 23 genera (Table 1) and 43 species (Table 2). *Pseudomonas* and *Arthrobacter* were major genera in Jun-1998 samples with Pseudomonas representing 48.5% of the genera identified.

Shifts of major species associated with bentgrass were observed from Dec-1996 to Jun-1998 samples (Table 2). Some dominant species in Dec-1996 samples, *Acidovorax delafieldii* and *Burkholderia pickettii* for instance, were not major species in Jun-1998 samples. 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, 1,280 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 data base has been generated for bermudagrass greens and will be reported under the Clemson University Turfgrass Initiative grant.

Acknowledgments

Thanks to Mr. Mark Stoddard, CGCS, Superintendent of the Charlotte Country Club Golf Course, Charlotte, NC for his cooperation.

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Presentations

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Table 1 Percentage of genera present in bentgrass samples collected from Dec-1996 to Jun-1998. Blank indicates either the genus was not detected or the percentage was below 8.0%.

PERCENTAGE OF DOMINANT GENERA PRESENT IN BENTGRASS GREENS

Genus	Dec-96	Mar-97	Jun-97	Sep-97	Dec-97	Mar-98	Jun-98
Acidovorax	17.3%						
Agrobacterium					8.2%		
Arthrobacter		12.0%				20.0%	9.7%
Burkholderia	13.3%						
Clavibacter	8.0%				8.2%		
Comamonas		15.8%					
Cytophaga	10.0%	15.8%				11.9%	
Hydrogenophaga	10.0%						
Mehtylobacterium			9.9%				
Pseudomonas	13.3%	28.5%	12.7%	9.1%	14.4%	39.3%	48.5%
Rhodococcus			12.7%				
Xanthomonas					14.4%		
Other Genera	17 (28.0%)	21 (27.8%)	24 (64.6%)	23 (90.9%)	23 (54.8%)	14 (28.8%)	23 (41.8%)
Total Genera	23	25	27	24	27	18	23

Table 2 Percentage of dominant species present in bentgrass samples collected from Dec-1996 to Jun-1998. Blank indicates either the species was not detected or the percentage was below 6.0%.

PERCENTAGE OF DOMINANT SPECIES PRESENT IN BENTGRASS GREENS

Species	Dec-96	Apr-97	Jun-97	Sep-97	Dec-97	Mar-98	Jun-98
Acidovorax delafieldii	17.3%		7.0%				
Agrobacterium radiobacter					8.2%		
Arthrobacter ilicis						8.9%	6.7%
Aureobacterium esteraromaticum					6.2%		
Burkholderia pickettii	12.7%						
Clavibacter michiganensis	8.0%			6.1%	8.2%		
Comamonas acidovorans		15.8%		6.1%			
Cytophaga johnsonae	10.0%	15.8%				11.9	
Hydrogenophaga pseudoflava	8.7%						
Pseudomonas fluorescens		6.3%				17.0%	
Pseudomonas putida	6.7%	14.6%	8.5%	6.1%	10.3%	18.5%	35.8%
Stenotrophomonas maltophilia				6.1%			
Xanthomonas campestris				6.1%	12.4%		
Other Species	28(36.6%)	36(47.5%)	37(91.5%)	30(69.5%)	34(54.7%)	24(43.7%)	41(57.5%)
Total Species	34	40	38	35	39	28	43

BIBLIOGRAPHIES for CLEMSON SCIENTISTS

Horace D. Skipper is a professor in the Department of Crop and Soil Environmental Science. He joined Clemson University in 1974. Skip's research program emphasizes enhancement of plant growth through selection of superior plant host: rhizobacteria: bradyrhizobia: mycorrhizal fungi combinations. Research projects involve rhizosphere biology/ecology of rhizobacteria on bentgrass, bermudagrass, bradyrhizobia, field tracking of genetically engineered microorganisms, survival of rhizobacteria for biocontrol of weeds, and biodegradation of pesticides and other xenobiotics. Skip teaches courses in soil microbiology and can be reached at skipper@clemson.edu.

Landon C. Miller is a professor in the Department of Horticulture. He joined the Clemson University faculty in 1965, spent 3 years at Auburn University from 1967 through 1970, and returned to Clemson in 1971. He had a dual appointment of research and extension education, both in turfgrass science. His present research includes turf type tall fescues, buffalograsses, and design of a turfgrass education information site on the World Wide Web. His present extension education covers all commercial turf including golf courses, athletic fields, sod production, lawn services, and rights of way. Landon is presently co-advisor to the student Turf Club and can be reached at lmiller@clemson.edu.

A. Robert Mazur is a professor in the Department of Horticulture. He joined Clemson University in 1973. Bob's research program focuses in the areas of turfgrass breeding, stress physiology, and soil fertility. Research projects including developing turf type tall fescue and bermudagrass cultivars with improved stress tolerance and disease resistance, investigating the environmental and cultural factors associated with stress on turfgrasses in the transition zone, and studying the potential in turf for minimizing nitrogen losses into ground water. Bob teaches courses in turfgrass management and can be reached at amazur@clemson.edu.

N. Dwight Camper is a professor of plant Pathology and Physiology with joint appointments in the Departments of Biological Sciences, and Microbiology and Molecular Medicine. He joined Clemson University in 1966. Dwight's research program involves the environmental fate of pesticides in water, sediment and soils, and aspects of plant physiology including plant medicine and toxicology and plant growth regulator responses. Recently, laboratory studies have focused on rhizosphere studies with containerized plants and the interaction of the plant/microbe/pesticide system, and on plant medicine and toxicology. Teaching responsibilities include graduate plant physiology courses and undergraduate courses in plant medicine and toxicology. Dwight can be reached at dcamper@clemson.edu.

Jung H. Kim is a postdoctoral fellow in soil microbiology and ecology in the Department of Crop and Soil Environmental Science. He earned his Ph.D. and M.S. in microbiology from Clemson University, and his B.S. in chemistry in Korea. His research interests include environmental fate of xenobiotics, biocontrol, microbial ecology, rhizosphere ecology, microbial community analysis, modeling, and database construction. His current research projects include: 1) diversity of rhizobacteria in new USGA putting greens; 2) community structure analysis and modeling of rhizosphere of tobacco, peanut, soybean, and weeds; 3) phytoremediation of pyrene. Jung can be reached at kim@clemson.edu.

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