EXECUTIVE SUMMARY

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

Principal Investigators: Horace D. Skipper, 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 new project sponsored by the Clemson University Turfgrass Initiative.
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. Development of a data base on turfgrass rhizobacteria from newly constructed bentgrass putting greens has been initiated . 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. Preliminary numerical differences of rhizobacterial populations in bentgrass rhizosphere were observed (Figure 1). In the samples of Dec-1996, isolates identified from bentgrass rhizosphere belonged to 23 genera and 34 species and 76% were Gram negative. *Acidovorax, Burkholderia,* and *Pseudomonas* were the major genera. However, in the samples of Mar-1997, isolates identified from bentgrass rhizosphere belonged to 25 genera and 40 species and 78% were Gram negative. *Pseudomonas, Comanonas, Cytophaga*, and *Arthrobacter* were the major genera.

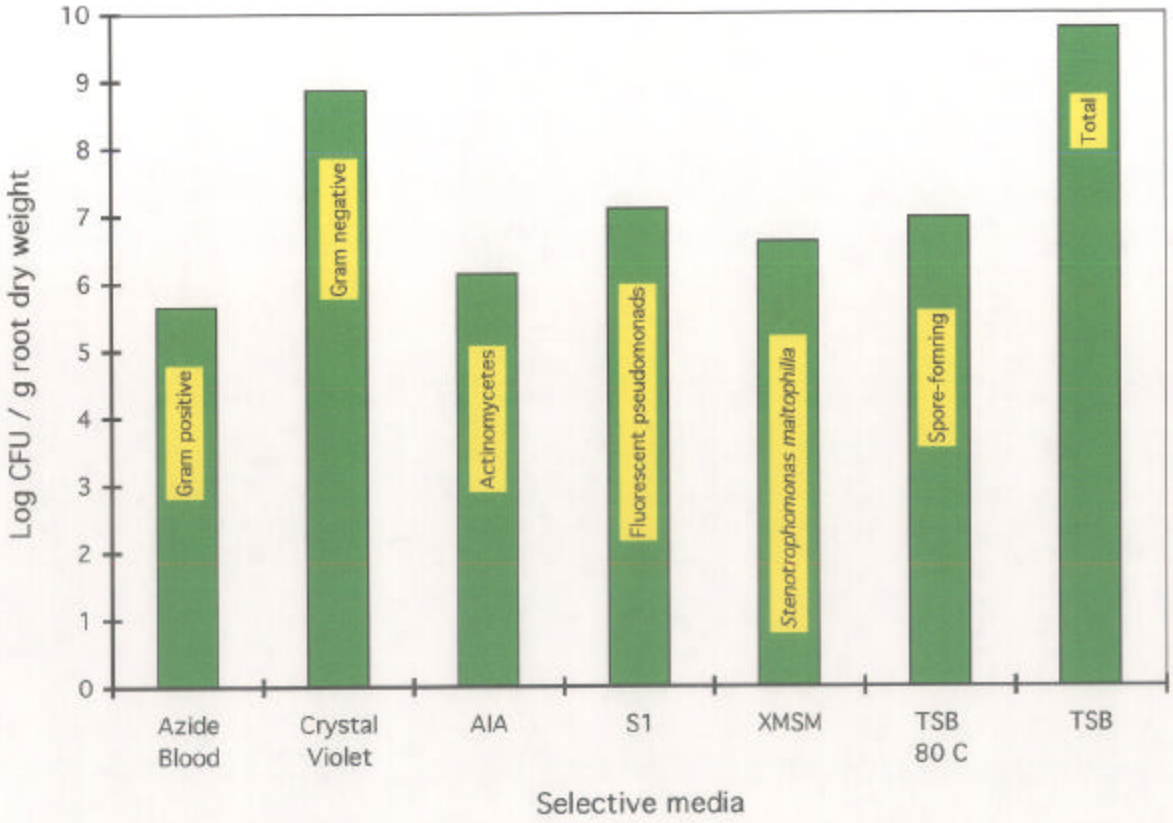

Figure 1. Rhizobacterial populations were averaged over four sampling periods from bentgrass greens. Samples were collected from Dec-96 to Sep-97 from Charlotte Country Club Golf Course, NC.

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 new 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. Four bacterial populations samples were collected from four greens (#15, #17, #18, and Big Putt) of the Charlotte Country Club Golf Course on December 13, 1996, March 25, 1997, June 24, 1997, and September 22, 1997, respectively. 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 dilution 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 640 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 and/or

Dr. Lissa Riley's analytical lab at Clemson University. To date, bacterial isolates from on December 13, 1996 and March 25, 1997 samples have been identified. Bacterial isolates from June 24, 1997 and September 22, 1997 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 will be constructed.

Summary of Research Progress

The Charlotte Country Club Golf Course was chosen for this study because it was reconstructed in June, 1996. Greens were seeded with Crenshaw bentgrass on August 14, 1996. Since then, we have sampled four 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 6.09×10^9 CFU/g root dry weight in bentgrass. Most rhizobacterial populations and total populations were relatively stable over the four sample dates. Percentage of Gram-positive and Gram-negative bacteria based on the KOH method were relatively stable over four sampling times with approximately 75% of the bacteria being Gram-negative. However, shifts of rhizobacterial populations were observed. For instance, Gram positive and fluorescent pseudomonad populations showed considerable variation over time.

Isolates from bentgrass rhizosphere in Dec-1997 samples belonged to 23 genera and 34 species (Figure 2A and 3A). *Acidovorax, Burkholderia,* and *Pseudomonas* were major genera in bentgrass in Dec-1997 samples (Figure 2A). However, isolates from Mar-1997 samples belonged to 25 genera and 40 species (Figure 2B and 3B). *Pseudomonas, Comamonas, Cytophaga*, and *Arthrobacter* were major genera in Mar-1997 samples (Figure 2B).

Shifts of major species associated with bentgrass were observed in Nov-1996 and Mar-1997 samples (Figure 3). Some dominant species in Nov-1996 samples, *Acidovorax delafieldii* and *Burkholderia pickettii* in bentgrass for instance (Figure 3), were not major species in Mar-1997 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 bentgrass remain to be investigated

To date, 640 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

Kun Xiong, H.D. Skipper, L.C. Miller, A.R. Mazur, M.L. Elliott, E.A. Guertal, J.A. McInroy, and J.W. Kloepper. 1997. Diversity of rhizobacteria in new USGA putting greens. ASA-CSSA-SSSA Annual Meetings, Anaheim, CA

Kun Xiong, H.D. Skipper, L.C. Miller, A.R. Mazur, N.D. Camper, J.A. McInroy, and J.W. Kloepper. 1997. Rhizobacterial diversity from new USGA putting greens in SC and NC. Clemson University Turfgrass Fielday, Florence, SC.

Literature Cited

Bolton, H., Jr., J. K. Fredrickson, and L. F. Elliott. 1993. Microbial ecology of the rhizosphere. p.63 in F. B. Metting, Jr., ed. Soil Microbial Ecology. Marcel Dekker, Inc., New York.

Cole, M. A., and A. J. Turgeon. 1978. Microbial activity in soil and litter underlying bandane- and calcium arsenate-treated turfgrass. Soil Biol. Biochem. 10:181-186.

Hodges, C. F., D. A. Campbell, and N. Christians. 1993. Evaluation of *Streptomyces* for biocontrol of *Bipolaris sorokiniana* and *Sclerotinia homoeocarpa* on the phylloplane of *Poa pratensis*. J. Phytopathology 139:103-109.

Juhnke, M. E., and E. Des Jardin. 1989. Selective medium for isolation of *Xanthomonas maltophilia* from soil and rhizosphere environments. Appl. Environm. Microbiol. 55:747-750.

Kloepper, J. W., and C. J. Beauchamp. 1992. A review of issues related to measuring colonization of plant roots by bacteria. Can. J. Microbiol. 38:1219-1232.

Liu, L. X., T. Hsiang, K. Carey, and J. L. Eggens. 1995. Microbial populations and suppression of dollar spot in creeping bentgrass with inorganic and organic amendments. Plant Dis. 79:144-147.

Mancino, C. F., M. Barakat, and A. Maricic. 1993. Soil and thatch microbial populations in an 80% sand:20% peat creeping bentgrass putting green. HortScience 28:189-191.

MIDI, Inc. Cherlock microbial identification system, Operation manual, version 5.

Nelson, E. B., and C. M. Craft. 1991. Introduction and establishment of strains of *Enterobacter cloacae* in golf course turf for the biological control of dollar spot. Plant Dis. 75:510-514.

Rovira, A. D. 1991. Rhizosphere research - 85 years of progress and frustration. p. 3-13 in D. S. Keister and P. B. Cregan, eds. The Rhizosphere and Plant Growth. Kluwer Academic Publishers, Boston.

Schippers, B., A. W. Bakker, and P. A. Bakker. 1987. Interaction of deleterious and beneficial rhizosphere microorganisms and the effect of cropping practices. Annu. Rev. Phytopathol. 25:339-358.

Smiley, R. W., and M. M. Craven. 1979. Microflora of turfgrass treated with fungicides. Soil Biol. Biochem. 11:349-353.

Suslow, T. V., and M. N. Schroth. 1982. Role of deleterious rhizobacteria as minor pathogens in reducing crop growth. Phytopathology 72:111-115.

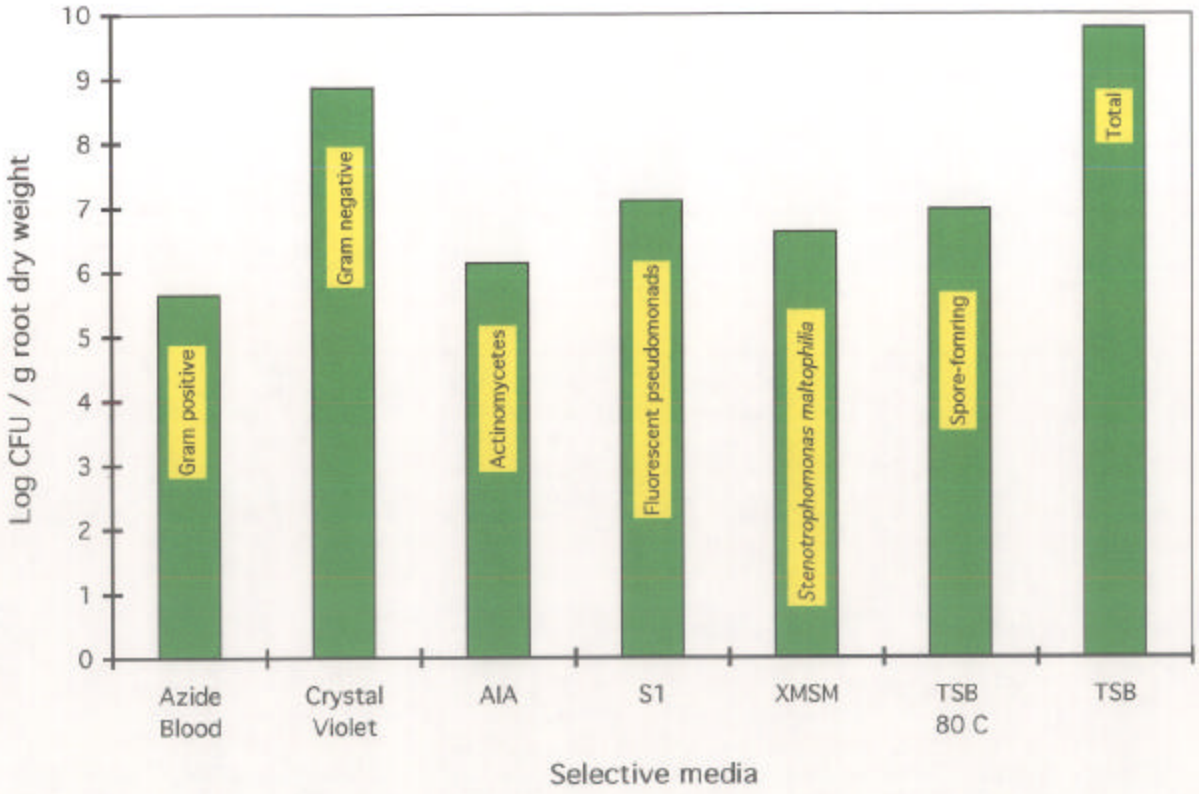
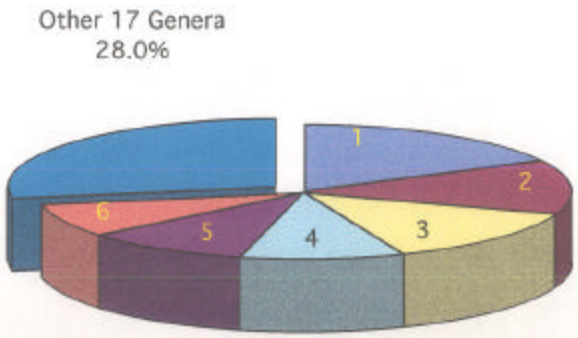
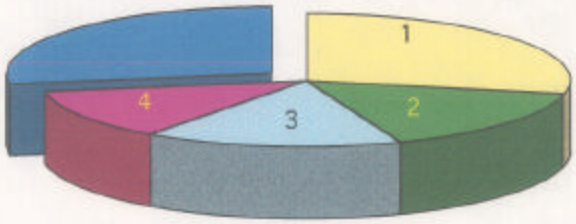


Figure 1. Rhizobacterial populations were averaged over four sampling periods from bentgrass greens. Samples were collected from Dec-96 to Sep-97 from Charlotte Country Club Golf Course, NC.



1.	*Acidovorax*	17.3%
2.	*Burkholderia*	13.3%
3.	*Pseudomonas*	13.3%
4.	*Cytophaga*	10.0%
5.	*Hydrogenophaga*	10.0%
6.	*Clavibacter*	8.0%

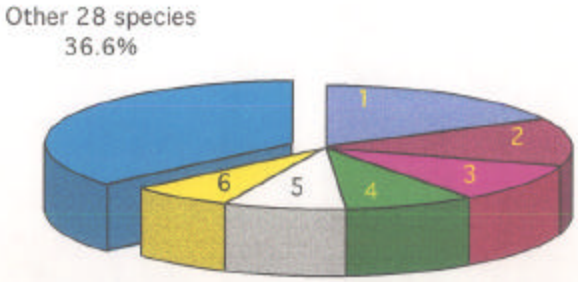
A. Total 23 genera in Dec-1996



1.	*Pseudomonas*	28.5%
2.	*Comamonas*	15.8%
3.	*Cytophaga*	15.8%
4.	*Arthrobacter*	12.0%

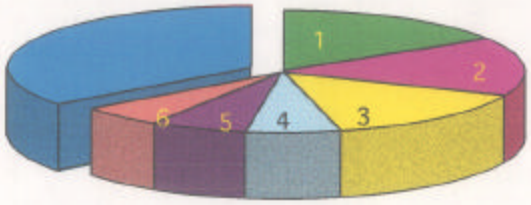
B. Total 25 genera in Mar-1997

Figure 2. Distribution of rhizobacteria by genus from bentgrass greens. Samples were collected in Dec-1996 and Mar-1997.



1.	*Acidovorax delafieldii*	17.3%
2.	*Burkholderia pickettii*	12.7%
3.	*Cytophaga johnsonae*	10.0%
4.	*Hydrogenophaga pseudoflava*	8.7%
5.	*Clavibacter michiganensis*	8.0%
6.	*Pseudomonas putida*	6.7%

A. Total 34 species in Dec-1996



1.	*Comamonas acidovorans*	15.8%
2.	*Cytophaga johnsonae*	15.8%
3.	*Pseudomonas putida*	14.6%
4.	*Pseudomonas fluorescens*	6.3%
5.	*Pseudomonas syringae*	6.3%
6.	*Arthrobacter ilicis*	5.7%

B. Total 40 species in Mar-1997

Figure 3. Distribution of rhizobacteria by species from bentgrass greens. Samples were collected in Dec-1996 and Mar-1997.

BIBLIOGRAPHIES for CLEMSON SCIENTISTS

Horace D. Skipper is a professor in the Department of Agronomy. 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 has 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.

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