Bacterial Populations and Diversity withing New USGA Putting Greens

University of Florida **Auburn University**
Dr. M. Elliott *Dr. E. Guertal*

Clemson Universtiy
Dr. H. Skipper

Start Date: 1996
Number of Years: 5
Total Funding: $66,667

Objectives:

1. *Determine bacterial populations associated with putting green root-zone mix materials.*
2. *Determine bacterial populations of the root-zone mixes before and after fumigation.*
3. *Compare rhizosphere bacterial populations on two different turfgrasses, bentgrass and bermudagrass.*
4. *Compare rhizosphere bacterial populations of bentgrass in two different locations, Alabama and South Carolina.*
5. *Compare rhizosphere bacterial populations of bermudagrass in two differrent locations, southern Florida and northern Florida.*
6. *Compare thatch development, rooting and bacterial population of bentgrass in relation to rootzone mix and nitrogen fertilization.*
7. *Compare soil and rhizosphere bacterial populations of root-zone mixes containing various clay sources.*
8. *Document rhizosphere bacterial population dynamics on bentgrass and bermudagrass over a four year time period.*

The overall objective of this project is to develop baseline data concerning bacterial composition (populations and diversity) of new USGA putting greens, both during and after construction. During 1996, the best methods for enumerating specific groups of bacteria were determined. These were incorporated into the research accomplished during the past two years.

University of Florida. Trenches were dug in a research green at the FLREC for placement of four 100-gallon size Lerio ™ tree containers to represent miniature putting greens. Greens were constructed using USGA specifications with a 6-in layer of noncalcareous washed river gravel on the bottom followed by a root-zone mix composed of 80% sand and 20% peat, by volume. The research area containing the mini-greens was fumigated with methyl bromide. Certified 'Tifdwarf' hybrid bermudagrass was planted as sprigs on 3 May 1997. The grow-in and general maintenance conformed to normal putting green maintenance practices,

Beginning August 1997, samples from each replicate mini-green obtained for enumeration of seven different bacterial groups on a quarterly interval. The bacterial groups enumerated are: total aerobic bacteria, heat tolerant bacteria, actinomycetes, gram-negative bacteria, gram-positive bacteria, fluorescent pseudomonads, and *Stenotrophomonas maltophilia*.

For all bacterial groups enumerated, there was less than two log units difference in bacterial counts over all nine sampling dates from August 1997 through August 1999. For total aerobic bacteria, heat tolerant bacteria, fluorescent pseudomonads and gram-negative bacteria, the difference over time was one or less than one log unit

difference. For five of the seven bacterial groups, the greatest number of bacteria were obtained in the last sampling in August 1997. For four of these five groups, the values were significantly greater than for any other sampling date. In general, the lowest bacterial counts were associated with the first two sampling dates after planting. Across all sampling dates, the greatest colony counts were associated with the medium used for total bacteria. The gram-negative bacteria were the next greatest in number followed by actinomycetes and heat-tolerant bacteria. The heat-tolerant bacteria include grampositive bacteria such as *Bacillus* species. The bacterial group with the least number present were the gram-positive bacteria. Exactly what bacterial species are associated with this group is currently unknown.

It is still too early in the study to make any sweeping conclusions. However, it is safe to state that new USGA bermudagrass putting greens certainly are not sterile environments with few bacteria present. So far, there would appear to be no universal cyclic trends, but this may only be true in southern Florida where our soil temperatures seldom drop below 50 F. Additional sampling dates will help to determine if trends will be observed.

Auburn. Treatments in this study include grass type (bent or bermuda), organic construction material (reed peat moss vs. sphagnum), fumigants (methyl bromide, metam sodium or dazomet) and N fertility regimes (1/10 or 1/5 lb N/1,000 ft^2 /week). At Auburn University, treatments are N rate and construction materials (100% sand or 80/20 sand/peat). Sixteen containerized greens were constructed at the Auburn University Turfgrass Research Unit, four replications of each fertility/soil mix combination. Each green is 1 m long and 0.5 m wide, and each drains to a leachate collection chamber. Total leachate is collected from each green each week, and a subsample collected for NO_3- and NH_4-N analysis.

In February, May, August and November of each year, root and soil samples (0-4 in. depth) are collected from each green. An additional soil sample is also collected for $N0_3$-N and NH_4-N analysis. Root samples are shipped to the University of Florida where they are subjected to dilution plating and identification. Selected isolates are returned to Auburn University, where identification at the species level is conducted via GC FAME analysis.

Results of 3 years of leachate collection have revealed that, once grow-in and application of higher rates of N were completed (a rate of 1 or 2 lb N/1,000 ft^2/month) was used for the first three months of the study), little $N0_3$-N or NH_4-N leaches through the rooting profile of the putting greens. During the past two years no more than 2 ug NO_3- of leachate has been collected at any one sampling, indicating the frequent application of low rates of N has maintained quality turf and reduced $N0_3$-N leaching. Preliminary evaluation of 8 dilution platings (from Univ. FL data) indicates that populations of selected bacterial species were affected by both N rate and putting green mix. There were no strong trends in bacterial populations over time

Clemson. 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 December 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. In the samples of December 1996, the major genera from bentgrass isolates belonged to *Acidovorax*, *Burkholderia*, *Pseudomonas*, *Cytophaga*, *Hydrogenophaga*, and *Clavibacter*. However, in the samples of December 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 June 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