

Bacterial Populations and Diversity within New USGA Putting Greens

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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 in hybrid bermudagrass putting greens were determined. These have been incorporated into the cooperative research project being conducted in Florida, Alabama and South Carolina.

Miniature putting greens were constructed using USGA specifications at the Auburn University turfgrass research site. Crenshaw bentgrass was planted as washed sod. The grow-in and general maintenance conformed to normal putting green maintenance practices.

Beginning May 1997, samples from each replicate minigreen have been obtained and shipped to Florida 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*.

With only a few exceptions, there was less than two log units difference in bacterial counts, for all bacterial groups enumerated, over all dates from May 1997 through August 2000 (Figure 1). For all groups except the gram-positive bacteria, there was a significant drop in bacterial counts in February 1999. This can probably be attributed to very cold temperatures that occurred just prior to sampling. If one ignores the February 1999 drop, the difference over time for total aerobic bacteria, heat tolerant bacteria, and gram-negative bacteria counts was one or less than one log unit difference. For reasons we cannot explain, no actinomycetes were detected on the first sampling date.

Across all sampling dates, the greatest colony counts were associated with the medium used to enumerate total bacteria (Figure 2). The gram-negative bacteria were the next greatest in number followed by actinomycetes, heat-tolerant bacteria and fluorescent pseudomonads. The heat-tolerant bacteria include gram-positive bacteria such as *Bacillus* species. The bacterial groups with the least number present were the gram-positive bacteria and *S. maltophilia*.

While the project is not complete, it is safe to state that new USGA putting greens certainly are not sterile environments with few bacteria present. So far, there would appear to be no universal cyclic trends. Except for the drop in all bacterial numbers for February 1999, there was no discernable pattern that developed. This project has two more samplings before it is completed. At that time, comparisons can be made among all sites.

Figure 1. Comparison of bacterial groups in USGA miniature bentgrass putting greens in Alabama from May 1997 - August 2000.

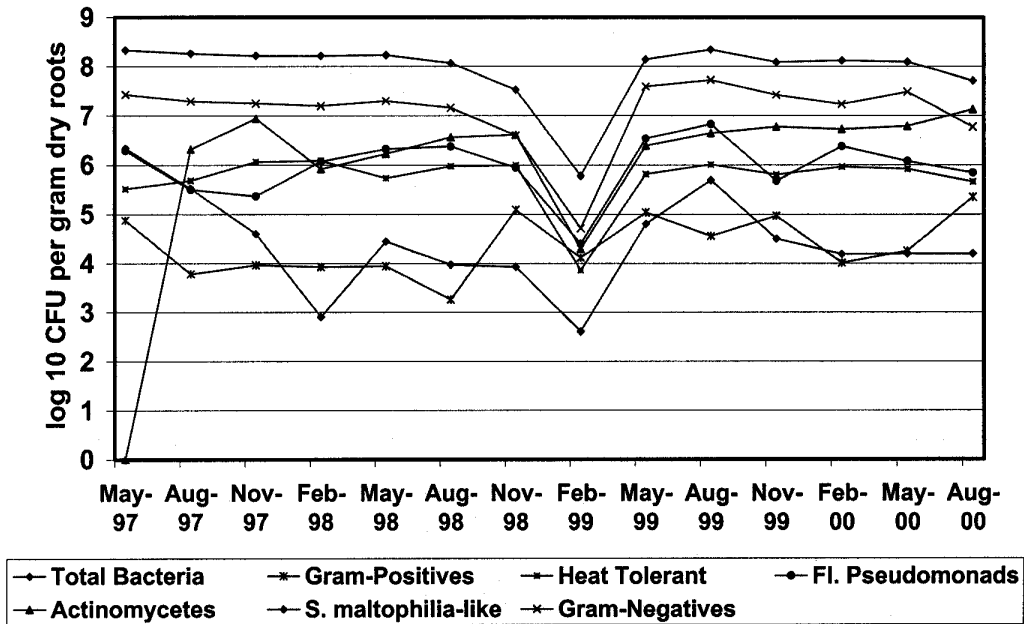
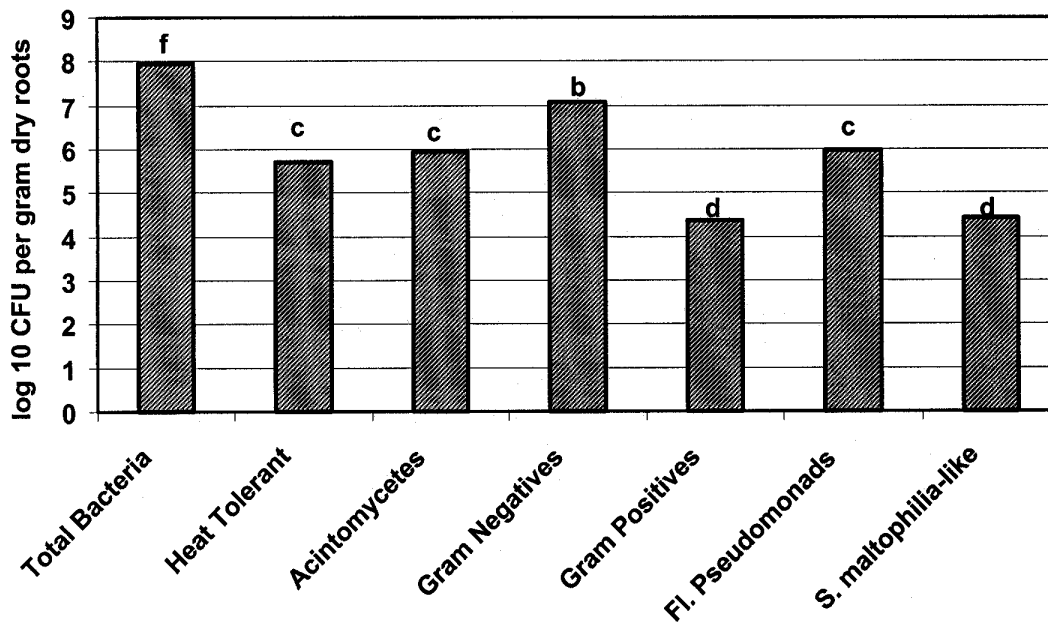


Figure 2. Comparison of bacterial groups across all sampling dates in USGA miniature bentgrass putting greens, May 1997- August 2000. (Columns with different letters indicate significant differences at $P < 0.05$.)



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Introduction

Unlike agricultural row crops, very little information is known about the bacteria associated with turfgrass roots, often called rhizosphere bacteria. Since golf course putting greens are not normally built from native soil, much of the information from agriculture may not even be applicable to putting greens. 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.

The project described below is just one portion of a larger tri-state cooperative project that includes research being conducted at Clemson University (project leader, Dr. Horace Skipper) and Auburn University (project leader, Dr. Elizabeth Guertal). During 1996, the best methods for enumerating specific groups of bacteria from turfgrass roots were determined. In late 1996 and early 1997, putting greens were established in South Carolina (bentgrass and bermudagrass), Alabama (bentgrass) and Florida (bermudagrass). Beginning in 1997, samples have been obtained from these greens every three months to determine the number and type of bacteria associated with the turfgrass roots. Last year, preliminary results from the Florida portion of this project were presented. The following is a summary of preliminary results obtained from the Alabama portion of this project.

Materials and Methods

Information concerning the building of the bentgrass putting greens at Auburn University have been described previously by Dr. Guertal. Beginning in May 1997, samples were obtained for enumeration of seven different bacterial groups on a quarterly interval. Protocol for sampling from the mini-greens is as follows.

1. From each container, ten cores (3/8-in diameter x 4-in deep) are removed.
2. Green leaves, just below verdure, are removed using a sterile razor blade and discarded.
3. The remaining portion of the cores are placed into containers for transport to laboratory. Samples are shipped overnight on ice to the Florida laboratory.
4. Roots are separated from the root-zone mix using sterile tweezers, and the roots placed into a sterile plastic 250-ml flask.
5. Sterile diluent (95-ml) is added to the roots in the flask, and the flasks placed on a rotary shaker (200 rpm) for 30 min.
6. A 10-fold dilution series is completed using the flask suspension and the sterile diluent. Contents of the flask are filtered onto preweighed filter papers, and then placed in an 80 C oven for 48 hours. The dry weight is then recorded.

7. Aliquots (0.1 ml) of the dilutions are spread on the appropriate media. Each dilution is duplicate plated. The bacterial groups enumerated are:
 - total aerobic bacteria using solidified 1/0 strength tryptic soy broth (1/10 TSBA)
 - actinomycetes using humic acid vitamin agar
 - gram-negative bacteria using 1/10 TSBA amended with crystal violet
 - gram-positive bacteria using azide blood agar base
 - fluorescent pseudomonads using selective medium S1
 - *Stenotrophomonas maltophilia* using a selective medium XMSM
8. The appropriate dilutions are then placed in an 80 C water bath for 10 minutes. Aliquots of the dilutions are spread on 1/10 TSBA to enumerate heat-tolerant bacteria.
9. Plates are incubated at 28 C and microbial colonies counted after the appropriate interval, 36 hours for fluorescent pseudomonads to 14 days for actinomycetes.
10. For each replicate green, approximately 50 colonies are selected from the total aerobic bacterial dilution plates. These are streaked on new 1/10 TSBA plates to obtain pure cultures. The pure cultures are placed in sterile deionized water in cryogenic vials, sealed with Parafilm and stored at room temperature until shipped to Dr. Joe Kloepper at Auburn University. Dr. Kloepper's laboratory will conduct fatty acid analysis on the cultures to determine tentative colony identification.

Results

With only a few exceptions, there was less than two log units difference in bacterial counts, for all bacterial groups enumerated, over all dates from May 1997 through August 2000 (Figure 1 and Figures 3-9). For all groups except the gram-positive bacteria, there was a significant drop in bacterial counts in February 1999. This can probably be attributed to very cold temperatures that occurred just prior to sampling. If one ignores the February 1999 drop, the difference over time for total aerobic bacteria, heat tolerant bacteria, and gram-negative bacteria counts was one or less than one log unit difference. For reasons we cannot explain, no actinomycetes were detected on the first sampling date.

Across all sampling dates, the greatest colony counts were associated with the medium used to enumerate total bacteria (Figure 2). The gram-negative bacteria were the next greatest in number followed by actinomycetes, heat-tolerant bacteria and fluorescent pseudomonads. The heat-tolerant bacteria include gram-positive bacteria such as *Bacillus* species. The bacterial groups with the least number present were the gram-positive bacteria and *S. maltophilia*.

Conclusions

While the project is not complete, it is safe to state that new USGA putting greens certainly are not sterile environments with few bacteria present. So far, there would appear to be no universal cyclic trends. Except for the drop in all bacterial numbers for February 1999, there was no discernable pattern that developed. This project has two more samplings before it is completed. At that time, comparisons can be made among all sites.

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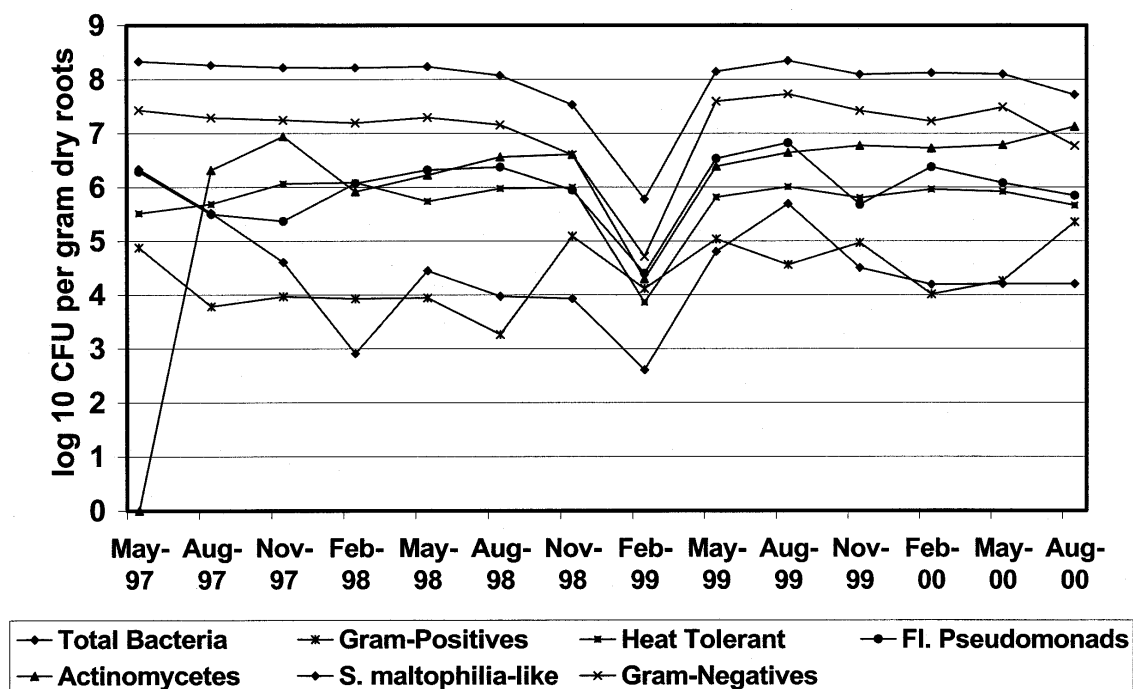


Figure 2. Comparison of bacterial groups across all sampling dates in USGA miniature bentgrass putting greens, May 1997- August 2000. (Columns with different letters indicate significant differences at $P<0.05$.)

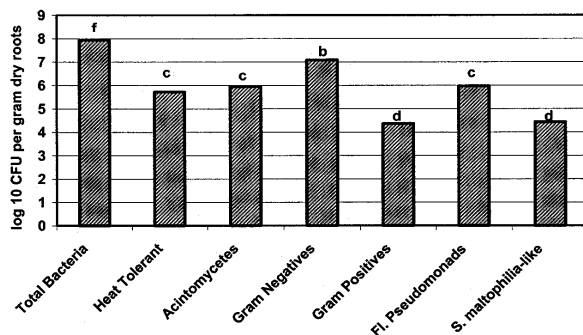


Figure 3. Comparison of total aerobic bacterial group in USGA miniature bentgrass putting greens from May 1997 - August 2000. (Different letters indicate significant differences at $P<0.05$.)

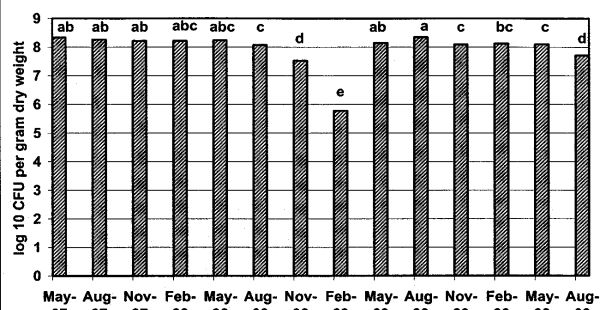


Figure 4. Comparison of heat tolerant bacterial group in USGA miniature bentgrass putting greens from May 1997 - August 2000. (Different letters indicate significant differences at $P<0.05$.)

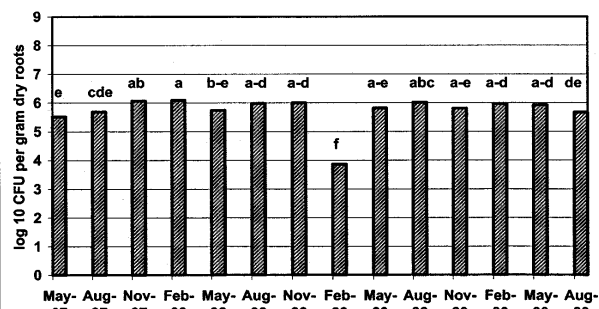


Figure 5. Comparison of actinomycete bacterial group in USGA miniature bentgrass putting greens from May 1997- August 2000. (Different letters indicate significant differences at $P<0.05$.)

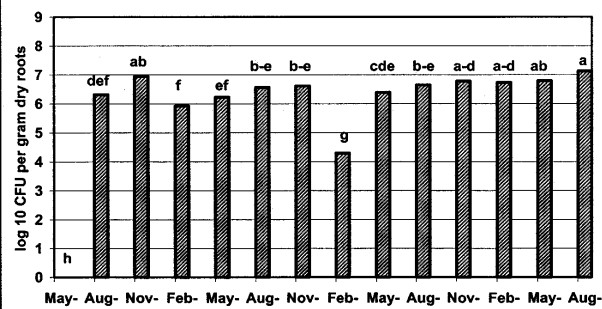


Figure 6. Comparison of gram-positive bacterial group in USGA miniature bentgrass putting greens from May 1997 - August 2000. (Different letters indicate significant differences at $P < 0.05$.)

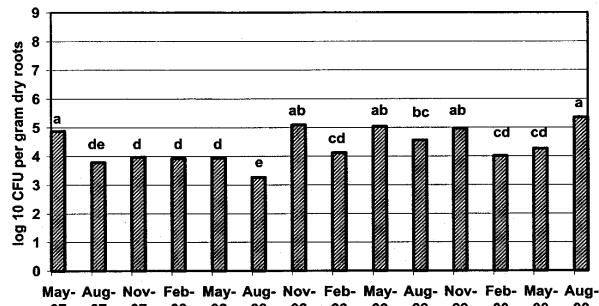


Figure 7. Comparison of gram-negative bacterial group in USGA miniature bentgrass putting greens from May 1997 - August 2000. (Different letters indicate significant differences at $P < 0.05$.)

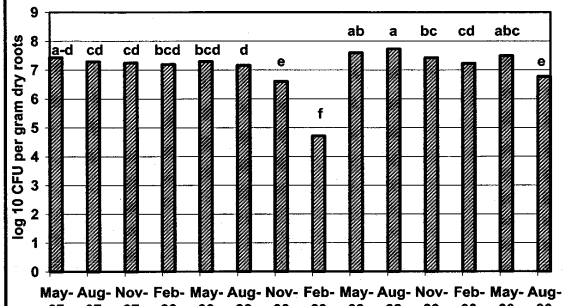


Figure 8. Comparison of fluorescent pseudomonad bacterial group in USGA bentgrass putting greens from May 1997 - August 2000. (Different letters indicate significant differences at $P < 0.05$.)

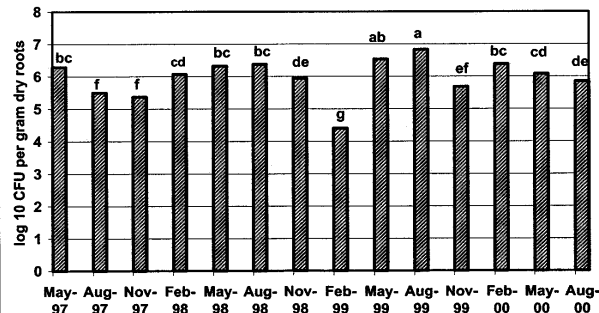


Figure 9. Comparison of *S. maltophilia*-like bacterial group in USGA miniature bentgrass putting greens from May 1997 - August 2000. (Different letters indicate significant differences at $P < 0.05$.)

