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.

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 non-calcareous 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 have been 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 (Figure 1). 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 (Figure 2). The gram-negative bacteria were the next greatest in number followed by actinomycetes and heat-tolerant bacteria. The heat-tolerant bacteria include gram-positive 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.

Figure 1. Comparison of bacterial groups in USGA miniature putting greens from August 1997 through August 1999.

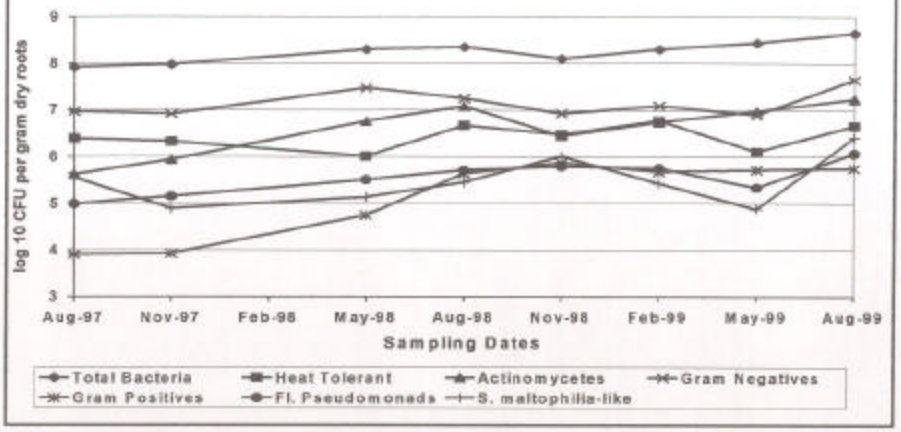
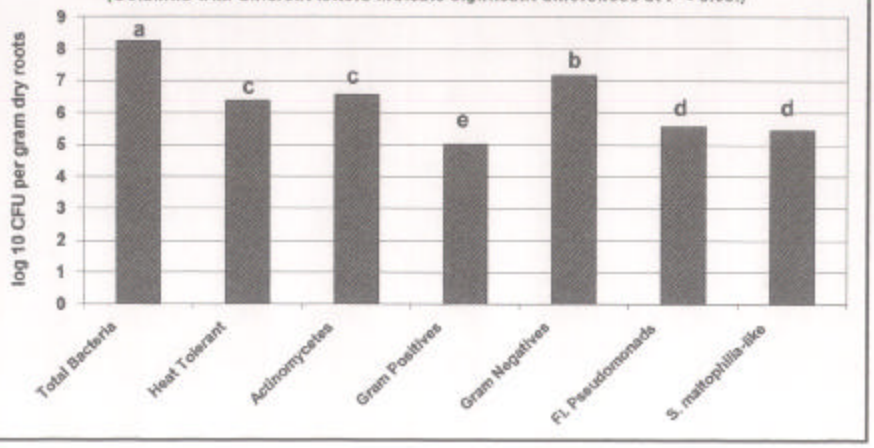


Figure 2. Comparison of bacterial groups across all sampling dates from a USGA miniature putting green, August 1997 through August 1999. (Columns with different letters indicate signficant differences at P < 0.05.)



Bacterial Populations and Diversity within New USGA Putting Greens
1999 Annual Report

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Introduction

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.

Materials and Methods

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. These containers are 36-in square and 18-in deep. All construction materials were evaluated by Dr. Norman Hummel (Hummel & Co., Inc., Trumansburg, NY). Non-calcareous washed river gravel was obtained from Conrad Yelvington Distributors, Inc. A 6-in layer was placed in the bottom of each container. The sand used in the root-zone mix was obtained from Golf Agronomics (Sarasota, FL). It was mixed with Canadian sphagnum peat obtained from Sun-Gro. The mix contained 80% sand and 20% peat, by volume. No intermediate layer was added as the gravel and root-zone mix met USGA specifications. 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 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, remove ten cores (3/8-in diameter x 4-in deep).
2. Remove green leaves just below verdue, using a sterile razor blade and discard
3. Place remaining portion of the cores into a plastic bag for transport to laboratory.
4. Separate roots from root-zone mix using sterile tweezers, and place roots into a sterile plastic 250-ml flask.
5. Add 95-ml of sterile diluent to the roots in the flask. Place flasks on a rotary shaker (200 rpm) for 30 min.
6. Complete a 10-fold dilution series using the flask suspension and the sterile diluent. Filter flask contents onto preweighed filter papers. Place in 80 C oven for 48 hours and record weight.

7. Spread 0.1 ml aliquots of the dilutions 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
    - selective medium for *Stenotrophomonas maltophilia*
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

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 (Figure 1 and Figures 3-9). 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 (Figure 2). The gram-negative bacteria were the next greatest in number followed by actinomycetes and heat-tolerant bacteria. The heat-tolerant bacteria include gram-positive 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.

Conclusions

It is still too early in the study to make any sweeping conclusions. However, 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, 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.

Plan of Work: November 1999 through October 2000

- Samples will continue to be processed on a quarterly basis for bermudagrass from Florida and bentgrass from Alabama, as part of the long-term study being conducted in each state.

- I am still undecided as to the next project to pursue in Florida as related to this grant. Originally, the next project would have examined the effects of different types of clay on bacterial populations. However, when that was proposed, I was making an assumption that bacterial numbers would not be as great as have been encountered in new greens. Since it is highly unlikely that we would ever convince anyone to put clays in their greens, this may not be a worthwhile project. It may be better to examine other aspects of new greens and their maintenance as to influence on microbial populations. Ideas would include:
  1) differences in populations based on bermudagrass cultivars
  2) pesticide influences, especially insecticides and the nematicide Nemacur; the latter is especially interesting since we do know it influences microbial populations if used too often and the fact it is routinely used, even if there is no good reason to do so
  3) nitrogen source influences; does an application of acidic ammonium sulfate drop the populations?

Figure 1. Comparison of bacterial groups in USGA miniature putting greens from August 1997 through August 1999.

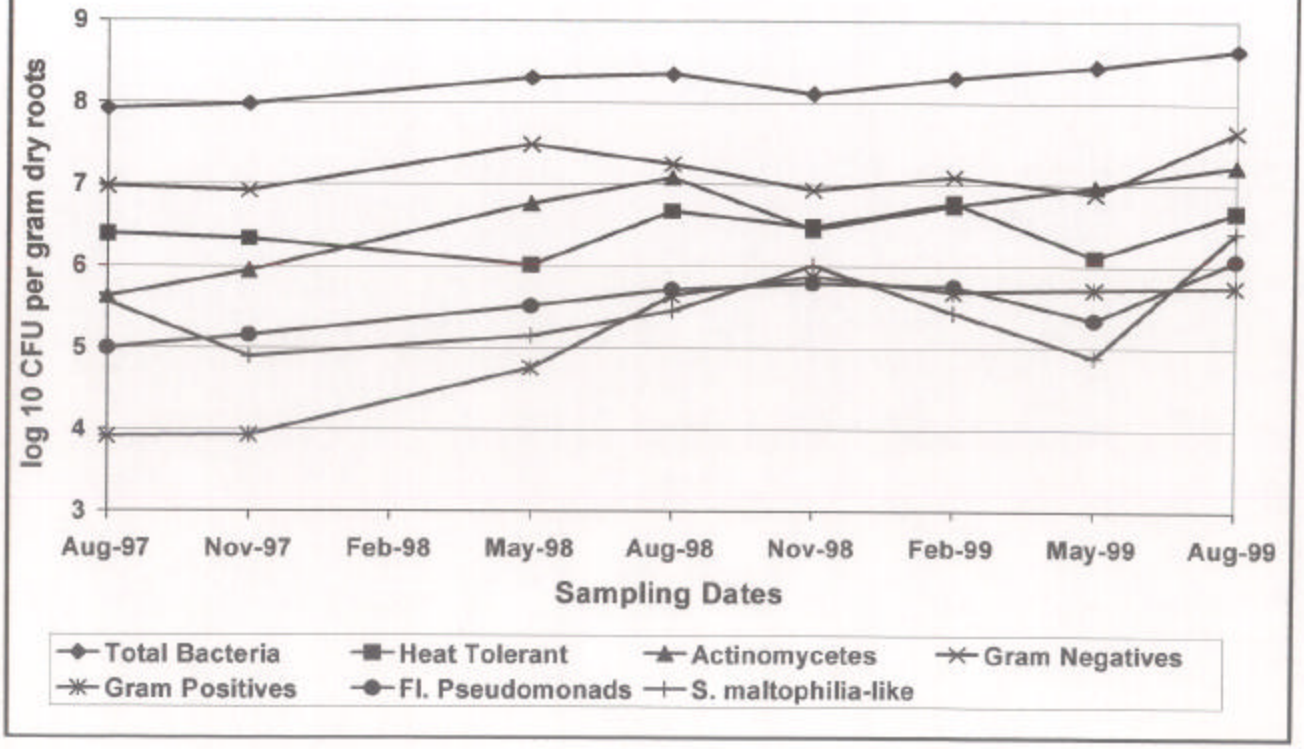
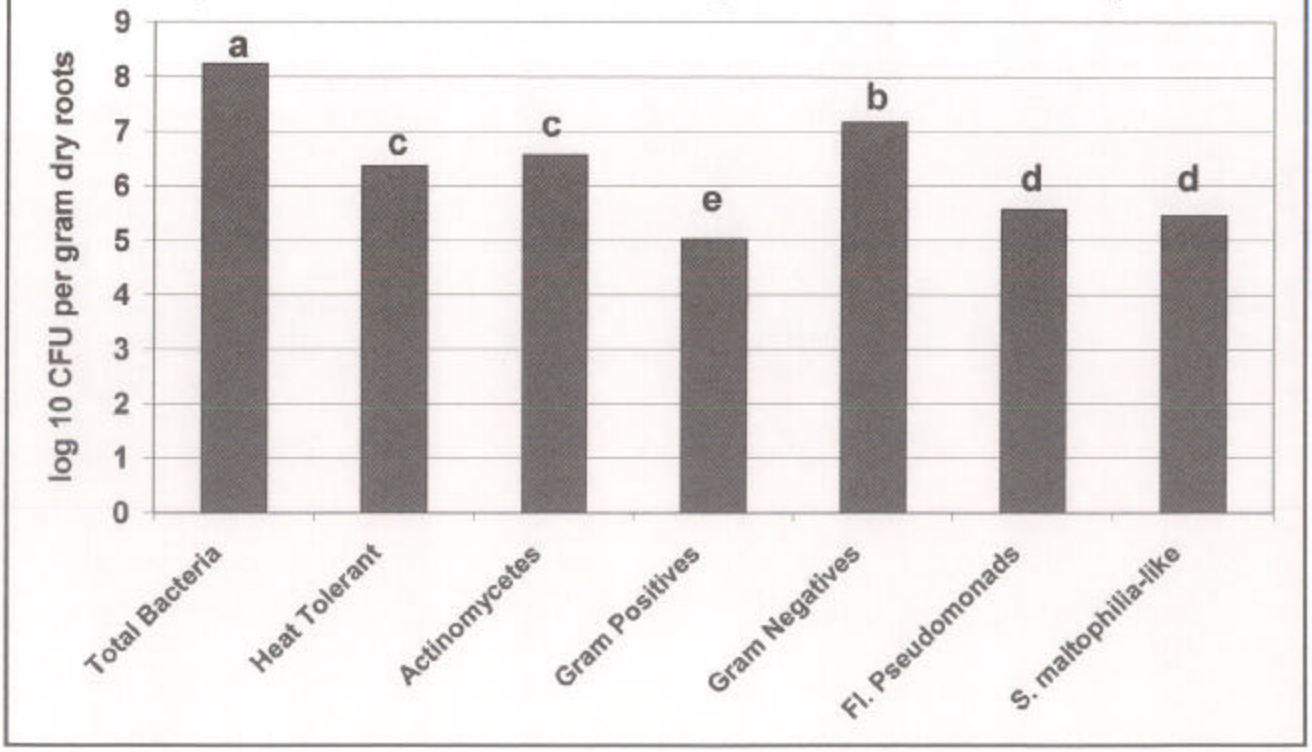


Figure 2. Comparison of bacterial groups across all sampling dates from a USGA miniature putting green, August 1997 through August 1999. (Columns with different letters indicate signficant differences at P < 0.05.)



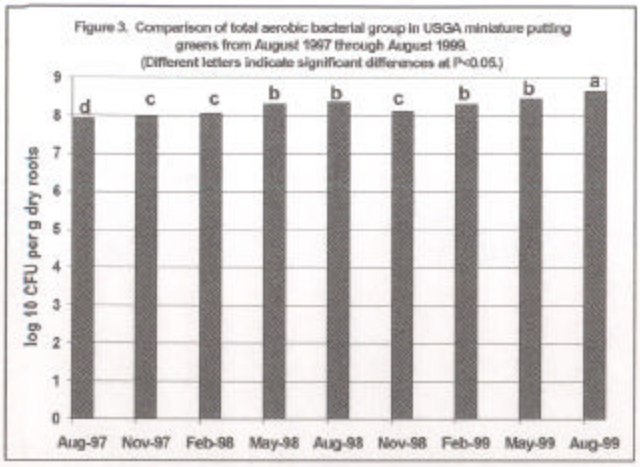


Figure 3. Comparison of total aerobic bacterial group in USGA miniature putting greens from August 1997 through August 1999.
(Different letters indicate significant differences at P<0.05.)
log 10 CFU per g dry roots
d c c b b c b b a
Aug-97 Nov-97 Feb-98 May-98 Aug-98 Nov-98 Feb-99 May-99 Aug-99

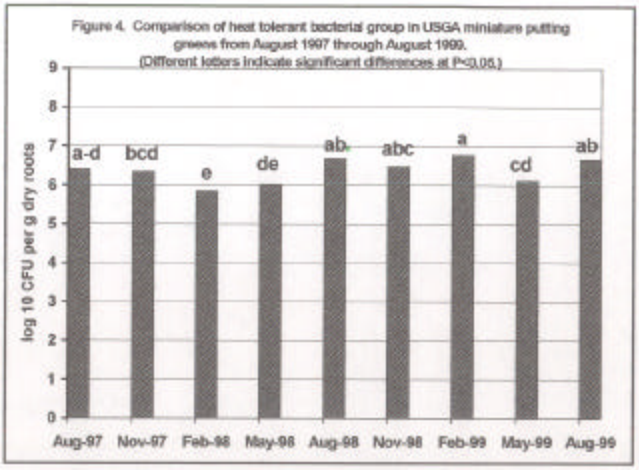


Figure 4. Comparison of heat tolerant bacterial group in USGA miniature putting greens from August 1997 through August 1999.
(Different letters indicate significant differences at P<0.05.)
log 10 CFU per g dry roots
a-d bcd e de ab abc a cd ab
Aug-97 Nov-97 Feb-98 May-98 Aug-98 Nov-98 Feb-99 May-99 Aug-99

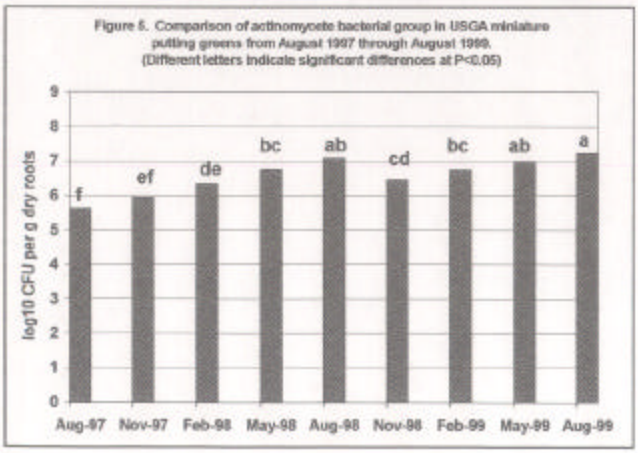


Figure 5. Comparison of actinomycete bacterial group in USGA miniature putting greens from August 1997 through August 1999.
(Different letters indicate significant differences at P<0.05)
log10 CFU per g dry roots
f ef de bc ab cd bc ab a
Aug-97 Nov-97 Feb-98 May-98 Aug-98 Nov-98 Feb-99 May-99 Aug-99

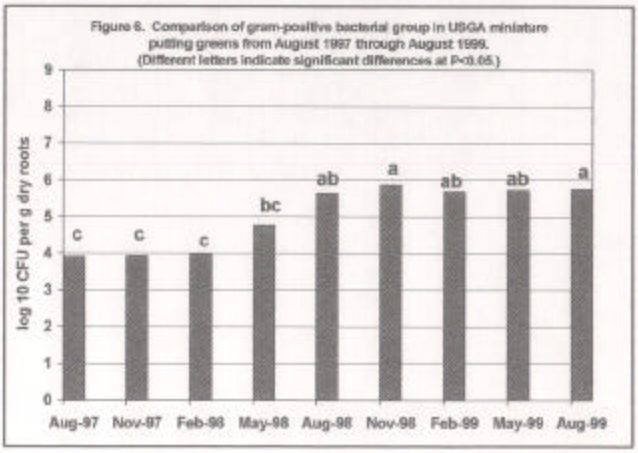


Figure 6. Comparison of gram-positive bacterial group in USGA miniature putting greens from August 1997 through August 1999.
(Different letters indicate significant differences at P<0.05.)
log 10 CFU per g dry roots
c c c bc ab a ab ab a
Aug-97 Nov-97 Feb-98 May-98 Aug-98 Nov-98 Feb-99 May-99 Aug-99

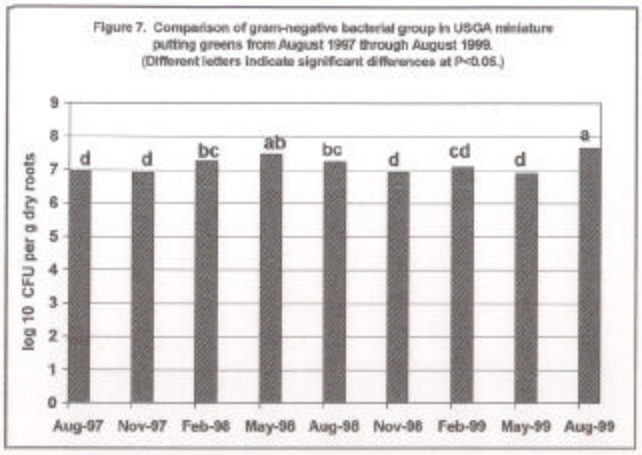


Figure 7. Comparison of gram-negative bacterial group in USGA miniature putting greens from August 1997 through August 1999. (Different letters indicate significant differences at P<0.05.)
log 10 CFU per g dry roots
d d bc ab bc d cd d a
Aug-97 Nov-97 Feb-98 May-98 Aug-98 Nov-98 Feb-99 May-99 Aug-99

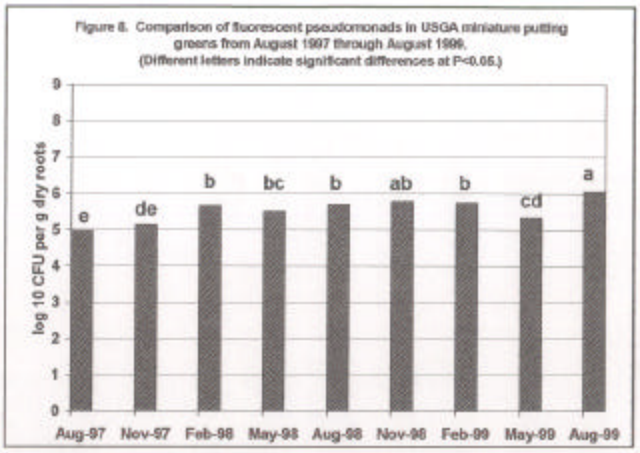


Figure 8. Comparison of fluorescent pseudomonads in USGA miniature putting greens from August 1997 through August 1999. (Different letters indicate significant differences at P<0.05.)
log 10 CFU per g dry roots
e de b bc b ab b cd a
Aug-97 Nov-97 Feb-98 May-98 Aug-98 Nov-98 Feb-99 May-99 Aug-99

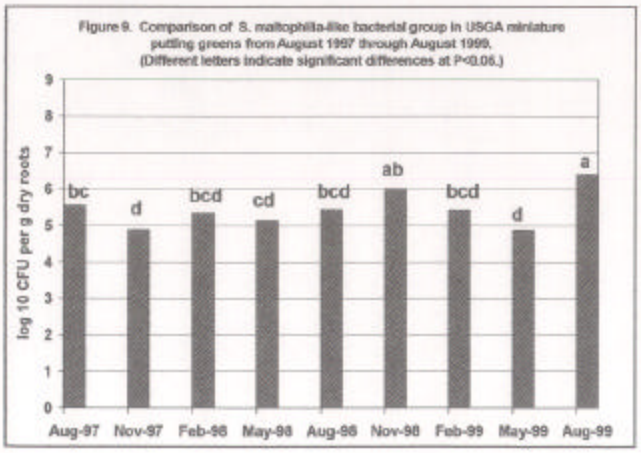


Figure 9. Comparison of S. maltophilia-like bacterial group in USGA miniature putting greens from August 1997 through August 1999. (Different letters indicate significant differences at P<0.05.)
log 10 CFU per g dry roots
bc d bcd cd bcd ab bcd d a
Aug-97 Nov-97 Feb-98 May-98 Aug-98 Nov-98 Feb-99 May-99 Aug-99

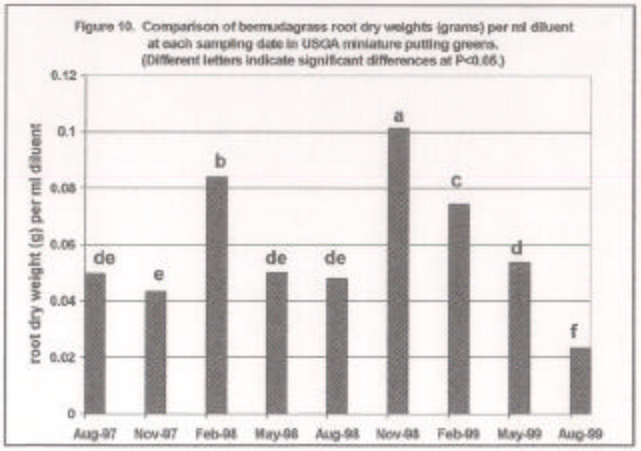


Figure 10. Comparison of bermudagrass root dry weights (grams) per ml diluent at each sampling date in USGA miniature putting greens. (Different letters indicate significant differences at P<0.05.)
root dry weight (g) per ml diluent
de e b de de a c d f
Aug-97 Nov-97 Feb-98 May-98 Aug-98 Nov-98 Feb-99 May-99 Aug-99