Bacterial Populations and Diversity within New USGA Putting Greens
1998 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 were determined. These were incorporated into the research accomplished during the past two years. For the project solely associated with Florida, the work completed thus far in 1998 was a repeat of the experiments carried out in 1997, with some modifications. Bacterial groups associated with putting green construction materials, prior to and after fumigation, and the bermudagrass sprigs used at planting were enumerated. These same groups were enumerated on a monthly basis after the bermudagrass was planted.

Materials and Methods

Trenches were dug at the FLREC for placement of 24 100-gallon size Lerio^{TM} tree containers. These containers are 36-in square and 18-in deep. All 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. No intermediate layer was added as the gravel and root-zone mixes described later met USGA specifications.

The sand used in the root-zone mixes was again obtained from Golf Agronomics (Sarasota, FL), but it came from a different sand mine. Two peat materials were used to make the mixes, sphagnum peat and reed sedge peat. The Canadian sphagnum peat was obtained from Sun-Gro and was mixed with the sand to obtain an 85/15 mix. The Dakota reed sedge peat was obtained from Dakota Peat and Blenders and was mixed with the sand to obtain a 93/7 mix.

The experimental design is a split plot with root-zone mix as the main treatment arranged as a randomized complete block. There are two main treatments, 85/15 root-zone mix made with sphagnum peat and 93/7 root-zone mix made with reed sedge peat. The subplots or second factor is fumigation. The root-zone mixes are either not fumigated at all or fumigated with either methyl bromide (gas) or metam sodium (liquid). The methyl bromide was applied at the rate of one pound per cubic yard of root-zone mix (46 grams per container) via injection from a methyl bromide gas cylinder. The metam sodium was applied at 85 gallons Metam 326 per acre (66.5 ml per container). The metam sodium was mixed with tap water (7.6 liters per container) and applied with a fine-nozzle sprinkler can. Containers were immediately covered with 4-ml black plastic after the fumigant was applied. Non-fumigated containers were not covered with plastic.

- Protocol for sampling of root-zone mix materials prior to mixing and after mixing:
  1. From each material or mix, remove 4 random samples of ~25 g each.
  2. Randomly remove 10 grams (wet weight) from each sample and place in a 250 ml flask with 90 ml of sterile diluent. Place flask on a rotary shaker (200 rpm) for 30 min.
  3. Place 10 grams (wet weight) into container to be dried at 80 C for 48 hours and then weigh to determine dry weight.
  4. Complete a 10-fold dilution series using the suspension and the sterile diluent (0.1% $Na_2P_2O_7$ with 1.0% glycerol).
  5. Spread 0.1 ml aliquots of the dilutions on the following media for enumeration:
     a) S-1 medium for fluorescent pseudomonads
     b) selective medium for *Stenotrophomonas maltophilia* (XMSM)
     c) humic acid vitamin agar (HAVA) for actinomycetes
     d) Azide Blood Base Agar for gram-positive bacterial counts
     e) Crystal Violet Agar for gram-negative bacterial counts
     f) 1/10 TSBA for "total" aerobic bacterial counts
  6. The dilutions are then placed in an 80 C water bath for 10 minutes. Aliquots (0.1 ml) of the dilutions are spread on 1/10 TSBA to enumerate heat-tolerant bacteria, e.g. *Bacillus*.
  7. Plates are incubated at 28 C and microbial colonies counted at the appropriate time.

- Protocol for sampling root-zone mixes after placed in containers but prior to fumigation, after fumigation when plastic first removed and 14 days after the plastic was removed:
  1. From each container, remove 4 cores (3/4 in diameter x 12-in deep).
  2. Remove the top 6-in section of the 4 cores from each container and place into a plastic beaker. Thoroughly mix each core section together in the beaker.
  3. Remove the bottom 6-in section of the 4 cores from each container and place into a plastic beaker. Thoroughly mix each core section together in the beaker.
  4. Randomly remove 10 grams (wet weight) from a beaker and place in a 250-ml flask with 90 ml of sterile diluent. Place flask on a rotary shaker (200 rpm) for 30 min.
  5. Randomly remove 10 grams (wet weight) from a beaker and place in a preweighed beaker. Place in and 80 C oven for 48 hours and record weight.
  6. Complete a 10-fold dilution series using the flask suspension and the sterile diluent.
  7. Spread 0.1 ml aliquots of the dilutions on the media listed previously for enumeration:
  8. The dilutions are then placed in an 80 C water bath for 10 minutes. Aliquots (0.1 ml) 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 at the appropriate time

- Protocol for sampling bermudagrass plants at delivery.
  1. Bermudagrass sod is washed clean of soil. Grass is torn into small pieces to mimic sprigs.
  2. Eight grass samples of 3 grams (wet weight) each are placed into sterile plastic 250-ml flasks.
  3. Add 100-ml of sterile diluent to the grass material. Place flasks on a rotary shaker (200 rpm) for 30 min.
  4. 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.

2

5. Spread 0.1 ml aliquots of the dilutions on the media listed previously for enumeration:
6. 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.
7. Plates are incubated at 28 C and microbial colonies counted at the appropriate time.

- Protocol for sampling from containers at two, three, four and five months after planting the bermudagrass sprigs:
  1. From each container, remove 4 cores (3/4 in diameter x 4-in deep).
  2. Place all 4 cores from each pot into a plastic beaker and take into the lab.
  3. Dump all the cores from each pot onto a paper towel. Remove, with ethanol-treated tweezers, all plant material. Discard leaf tissue. Place remaining plant material into a sterile plastic 250-ml flask. Place the root-zone mix left behind back in the beaker and thoroughly mix. Randomly remove 10 grams (wet weight) from the beaker and place in a 250-ml flask.
  4. Add 90-ml of sterile diluent to the root-zone mix material flask. Add 98-ml sterile diluent to the plant material flask. Place flasks on a rotary shaker (200 rpm) for 30 min.
  5. Randomly remove 10 grams (wet weight) of the root-zone mix from the beaker and place in a preweighed beaker. Place in 80 C oven for 48-hours and record weight.
  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 media listed previously for enumeration:
  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 at the appropriate time.

Results

The individual root-zone mix components were obtained at the mixing site, placed in self seal plastic bags and removed to the laboratory for sampling. The two root-zone mixes were delivered to the FLREC in trucks, and samples were obtained within two hours of delivery. The sand and sphagnum peat contained the lowest number of bacteria, with two groups not detected at all (fluorescent pseudomonads and *S. maltophilia*) (Table 1). All bacterial groups were detected in the reed sedge peat. Only actinomycetes were not detected in the two root-zone mixes.

The plastic was removed from the containers at 9 days post-fumigation with samples obtained immediately after the plastic was removed. Samples were obtained again 14 days later. Results are summarized in Tables 2 and 3 for the sphagnum peat root-zone mix and Tables 4 and 5 for the reed-sedge peat root-zone mix.

For the sphagnum peat root-zone mix, there were significant differences within each fumigation treatment among the different bacterial groups at both sampling periods. Within each bacterial group, significant differences among fumigation treatments were not obtained for the fluorescent pseudomonads, *S. maltophilia* or actinomycetes when sampled immediately after

plastic was removed (Table 2). Fourteen days later, only the actinomycetes did not result in significant differences among fumigation treatments (Table 3). Except for the actinomycetes and fluorescent pseudomonads, the lowest number of bacteria within a bacterial group were associated with the control (non-fumigated) treatment. There were no fluorescent pseudomonads detected in the methyl bromide fumigation treatment containers.

For the reed-sedge peat root-zone mix, there were significant differences within each fumigation treatment among the different bacterial groups at both sampling periods. Within each bacterial group, significant differences among fumigation treatments were obtained for all bacterial groups, except for *S. maltophilia*, immediately after the plastic was removed (Table 4). Fourteen days later, only the actinomycetes did not result in significant differences among fumigation treatments (Table 5). As with the sphagnum peat root-zone mix, no fluorescent pseudomonads were detected in the methyl bromide fumigation treatment containers.

All the bacterial groups were present when the 'Tifdwarf' bermudagrass was sampled prior to planting (Table 6). All groups continued to be detected on plant material and in the root-zone mix throughout the next three months of sampling. Data have not been analyzed to date as to differences among root-zone mixes or fumigation treatments after planting.

Conclusions

It is still too early in the study to make any sweeping conclusions. Results this year indicate that bacterial numbers for most of the bacterial groups enumerated are actually increased by fumigation with either methyl bromide or metam sodium. The only consistent results obtained were: 1) the inability to detect fluorescent pseudomonads in the methyl bromide treated containers 14 days after the plastic was removed; and 2) the lack of effect of the fumigants on actinomycete numbers 14 days after the plastic was removed. Overall, the study would thus far indicate that bacteria certainly are not absent from root-zone mixes, even after fumigation, and that planting of the bermudagrass will introduce even more bacteria into the putting green.

Plan of Work: October 1998 through September 1999

- Two more monthly samples (October and November) will be obtained from the study described above.
- Samples will continue to be processed on a quarterly basis for bermudagrass from Florida and bentgrass from Alabama (long-term continuous study not described). This is in coordination with sampling in South Carolina.
- Effects of different types of clay on bacterial populations in a sphagnum peat root-zone mix will be determined (see original project proposal).

Table 1. Bacterial groups present when materials delivered to UF/FLREC.

	colony forming units per gram dry weight[y]						
Bacterial group	Sand	SP[z]	RSP[z]	SP-mix	RSP-mix	P > F	F value
Total	5.0 A e	6.6 A b	8.4 A a	5.8 A d	6.3 A c	0.0001	398.1
Fl. pseudomonads	0 E c	0 E c	3.7 D a	1.6 D b	1.3 D b	0.0001	14.1
S. maltophilia	0 E c	0 E c	1.3 E b	2.1 CD ab	2.4 C a	0.0003	10.6
Gram positive	2.4 D b	1.2 D c	4.2 D a	0.5 E cd	0 E d	0.0001	18.1
Gram negative	3.4 B d	5.8 A b	6.4 C a	4.5 B c	4.5 B c	0.0001	293.4
Actinomycetes	2.7 C b	2.3 C b	7.5 B a	0 E c	0 E c	0.0001	75.3
Heat tolerant	2.7 C d	4.1 B c	7.1 BC a	3.0 C d	5.2 B b	0.0001	42.6
P > F	0.0001	0.0001	0.0001	0.0001	0.0001		
F value	456.2	43.6	70.2	37.4	75.3		

[x]Values are means of four replicate samples. Means in the same column followed by the same capital letter or means in the same row followed by the same small letter are not significantly different ($P \leq 0.05$), according to the Waller Duncan *k*-ratio *t* test.
[z]SP, sphagnum peat; RSP, reed sedge peat

Table 2. Bacterial groups present prior to fumigation and 9 days after fumigation (immediately after plastic removed) for sphagnum peat root-zone mix.

	colony forming units per gram dry weight					
Bacterial Group	Pre-fumigation[y]	Control[z]	Metam sodium	Methyl bromide	P > F	F value
Total	6.9 A	6.4 A b	6.0 A c	7.0 A a	0.0001	33.2
Fl. pseudomonads	2.5 D	0.3 A	1.9 C	0.2 D	0.0768	2.9
S. maltophilia	2.6 D	0.5 E	1.6 C	1.9 C	0.3309	1.2
Gram positive	2.3 D	1.7 D b	2.0 C b	4.8 B a	0.0001	14.2
Gram negative	5.1 B	4.9 B b	4.4 B b	5.7 B a	0.0022	8.3
Actinomycetes	3.2 C	2.2 CD	0.7 C	1.6 C	0.2155	1.7.
Heat tolerant	3.3 C	3.1 C b	4.5 B b	5.2 B a	0.0001	17.0
P > F	0.0001	0.0001	0.0001	0.0001		
F Value	121.2	42.1	13.6	31.5		

[y]Values are mean of twelve replicate samples. Means in the same column followed by the same letter are not significantly different ($P \leq 0.05$), according to the Waller Duncan *k*-ratio *t* test.
[z]Values are means of four replicate samples. Means in the same column followed by the same capital letter or means in the same row followed by the same small letter are not significantly different ($P \leq 0.05$), according to the Waller Duncan *k*-ratio *t* test.

Table 3. Bacterial groups present prior to fumigation and 23 days after fumigation (14 days after plastic removed) for sphagnum peat root-zone mix.

	colony forming units per gram dry weight					
Bacterial Group	Pre-fumigation[y]	Control[z]	Metam sodium	Methyl bromide	P > F	F value
Total	6.9 A	6.6 A c	6.8 A b	7.1 A a	0.0015	9.1
Fl. pseudomonads	2.5 D	1.6 D b	4.5 C a	0 E c	0.0001	46.8
S. maltophilia	2.6 D	0.8 D b	2.9 D a	3.1 C a	0.0008	10.5
Gram positive	2.3 D	0.7 D b	1.6 E b	5.2 B a	0.0001	17.1
Gram negative	5.1 B	4.6 B c	5.5 B a	5.3 B b	0.0001	35.4
Actinomycetes	3.2 C	3.0 C	2.8 D	1.9 D	0.0846	2.8
Heat tolerant	3.3 C	3.4 Cc	5.9 B a	5.1 B b	0.0001	142.6
P > F	0.0001	0.0001	0.0001	0.0001		
F Value	121.2	38.0	27.8	84.9		

[y]Values are mean of twelve replicate samples. Means in the same column followed by the same letter are not significantly different ($P \leq 0.05$), according to the Waller Duncan *k*-ratio *t* test.
[z]Values are means of four replicate samples. Means in the same column followed by the same capital letter or means in the same row followed by the same small letter are not significantly different ($P \leq 0.05$), according to the Waller Duncan *k*-ratio *t* test.

Table 4. Bacterial groups present prior to fumigation and 9 days after fumigation (immediately after plastic removed) for reed sedge peat root-zone mix.

	colony forming units per gram dry weight					
Bacterial Group	Pre-fumigation[y]	Control[z]	Metam sodium	Methyl bromide	P > F	F value
Total	6.7 A	6.1 A b	5.7 A c	7.0 A a	0.0001	78.4
Fl. pseudomonads	1.9 E	1.6 E a	0 E b	0 E b	0.0004	11.5
S. maltophilia	2.4 D	1.1 E	0 E	1.5 D	0.1020	2.6
Gram positive	2.5 D	2.9 D b	0.8 D c	5.2 B a	0.0001	46.6
Gram negative	4.8 C	4.6 C a	1.8 C b	5.3 B a	0.0001	31.3
Actinomycetes	5.6 B	5.4 AB a	4.4 B b	3.6 C c	0.0001	20.5
Heat tolerant	4.8 C	5.0 BC a	4.6 B b	5.1 B a	0.0050	6.9
P > F	0.0001	0.0001	0.0001	0.0001		
F Value	87.7	46.6	75.4	68.8		

[y]Values are mean of twelve replicate samples. Means in the same column followed by the same letter are not significantly different ($P \leq 0.05$), according to the Waller Duncan *k*-ratio *t* test.
[z]Values are means of four replicate samples. Means in the same column followed by the same capital letter or means in the same row followed by the same small letter are not significantly different ($P \leq 0.05$), according to the Waller Duncan *k*-ratio *t* test.

6

Table 5. Bacterial groups present prior to fumigation and 23 days after fumigation (14 days after plastic removed) for reed sedge peat root-zone mix.

	colony forming units per gram dry weight					
Bacterial Group	Pre-fumigation[y]	Control[z]	Metam sodium	Methyl bromide	P > F	F value
Total	6.7 A	5.5 A b	6.9 A a	6.9 A a	0.0562	3.3
Fl. pseudomonads	1.9 E	1.0 CD b	3.4 C a	0 F c	0.0001	35.4
S. maltophilia	2.4 D	0.3 D c	2.0 D b	3.0 E a	0.0001	17.1
Gram positive	2.5 D	1.5 C b	0.8 E b	4.6 C a	0.0001	30.9
Gram negative	4.8 C	4.2 B b	5.1 B a	5.1 B a	0.0001	26.0
Actinomycetes	5.6 B	4.7 AB	3.8 C	4.1 D	0.3278	1.2
Heat tolerant	4.8 C	4.8 AB c	5.5 B a	5.2 B b	0.0001	44.8
P > F	0.0001	0.0001	0.0001	0.0001		
F Value	87.7	20.7	58.6	288.7		

[y]Values are mean of twelve replicate samples. Means in the same column followed by the same letter are not significantly different ($P \leq 0.05$), according to the Waller Duncan *k*-ratio *t* test.

[z]Values are means of four replicate samples. Means in the same column followed by the same capital letter or means in the same row followed by the same small letter are not significantly different ($P \leq 0.05$), according to the Waller Duncan *k*-ratio *t* test.

Table 6. Bacterial groups present when 'Tifdwarf' bermudagrass delivered to UF/FLREC.

Bacterial group	colony forming units per gram dry weight[z]
Total	8.1 a
Fluorescent pseudomonads	4.5 e
Stenotrophomonas maltophilia	6.1 c
Gram positive	4.1 f
Gram negative	7.1 b
Actinomycetes	5.6 d
Heat tolerant	5.5 d

[z]Values are mean of eight replicate samples. Means in the same column followed by the same letter are not significantly different ($P \leq 0.05$), according to the Waller Duncan *k*-ratio *t* test.