Bacterial Populations and Diversity within New USGA Putting Greens

Monica L. Elliott, Ph.D.
University of Florida
Fort Lauderdale Research and Education Center

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 1997. This past year was our first attempt to enumerate bacterial groups associated with putting green construction materials, prior to and after fumigating and planting of bermudagrass sprigs.

Trenches were dug at the FLREC for placement of 100-gallon size Lerio™ tree containers. These containers are 36-in square and 18-in deep. A 6-in layer of non-calcareous washed river gravel was placed in the bottom of each container. No intermediate layer was added as the gravel and root-zone mixes met USGA specifications. Two peat materials were used to make the mixes, either sphagnum peat or reed sedge peat. The Canadian sphagnum peat was mixed with the sand to obtain an 80/20 mix. The Dakota reed sedge peat was mixed with the sand to obtain a 90/10 mix. The root-zone mixes are the two main treatments. The subplots or second factor are three fumigants, methyl bromide (gas), metam sodium (liquid) and dazomet (granule). The active ingredient for both metam sodium and dazomet is MITC.

Samples were obtained for enumeration of seven different bacterial groups from: 1) individual root-zone components prior to mixing, 2) each root-zone mix after blending, 3) prior to fumigation, 4) 10 days post-fumigation, 5) 25 days post-fumigation, 6) each month after planting of bermudagrass for five months total. Samples were also obtained of the bermudagrass sprigs.

At delivery of the individual root-zone components, the sand was essentially devoid of bacteria. All bacterial groups were detected in the reed sedge peat. The fluorescent pseudomonads and *S. maltophilia* were not detected in the sphagnum peat. Similar results were obtained in the two root-zone mixes.

At 10 days post-fumigation. No or minimal fluorescent pseudomonads or *S. maltophilia* were detected from containers treated with dazomet or metam sodium. Dazomet and metam sodium break down to the same active ingredient (MITC). These bacterial groups were detected in containers treated with methyl bromide, but actinomycetes were not detected.

The bacterial groups detected 15 days after the plastic was removed were different from those detected immediately after the plastic was removed.. The fluorescent pseudomonads were now isolated from all containers, regardless of fumigant used. However, actinomycetes were still not detected in containers fumigated with dazomet and metam sodium nor were they now detected in containers fumigated with methyl bromide.

All the bacterial groups were present when the 'Tifdwarf' bermudagrass was sampled prior to planting). All groups continued to be detected on plant material and in the root-zone mix throughout the next five months of sampling. By the fourth month, there appeared to be few differences among treatments.

Bacterial groups present prior to fumigation and 10 days after fumigation.

	colony forming units per gram dry weight[x]							
	Pre-fumigation		Dazomet		Metam sodium		Methyl bromide	
Bacterial Group	S.P.[y]	R.S.P.[z]	S.P.	R.S.P.	S.P.	R.S.P.	S.P.	R.S.P.
Total	6.4	7.4	4.0	5.5	3.4	5.1	4.6	5.6
Fl. pseudomonads	<2.0	3.7	ND	ND	ND	ND	2.7	3.0
S. maltophilia	2.8	4.0	ND	ND	<2.0	ND	<2.0	<2.0
Gram positive	4.1	5.5	<2.0	2.8	ND	<2.0	3.4	3.2
Gram negative	4.8	5.9	ND	3.1	<2.0	<2.0	<2.0	3.8
Actinomycetes	3.0	5.9	2.8	4.8	2.7	4.8	ND	ND
Heat tolerant	3.2	5.4	3.3	5.1	3.2	4.2	2.5	2.8

[x]Values are mean of twelve replicate samples for pre-fumigation and four replicate samples for post-fumigation.
ND, not detected
[y]S.P., sphagnum peat root-zone mix
[z]R.S.P., reed sedge peat root-zone mix

Bacterial groups present prior to fumigation and 25 days after fumigation.

	colony forming units per gram dry weight[x]							
	Pre-fumigation		Dazomet		Metam sodium		Methyl bromide	
Bacterial Group	S.P.[y]	R.S.P.[z]	S.P.	R.S.P.	S.P.	R.S.P.	S.P.	R.S.P.
Total	6.4	7.4	4.0	4.5	7.0	7.4	6.6	6.9
Fl. pseudomonads	<2.0	3.7	<2.0	3.5	5.5	4.8	5.7	5.4
S. maltophilia	2.8	4.0	ND	<2.0	5.7	4.3	4.8	4.2
Gram positive	4.1	5.5	<2.0	<2.0	2.6	5.4	5.1	5.4
Gram negative	4.8	5.9	<2.0	4.1	6.4	6.5	6.2	6.4
Actinomycetes	3.0	5.9	ND	ND	ND	<2.0	ND	ND
Heat tolerant	3.2	5.4	2.3	3.6	6.0	5.6	5.7	5.4

[x]Values are mean of twelve replicate samples for pre-fumigation and four replicate samples for post-fumigation.
ND, not detected
[y]S.P., sphagnum peat root-zone mix
[z]R.S.P., reed sedge peat root-zone mix

Bacterial Populations and Diversity within New USGA Putting Greens
1997 Annual Report

Monica L. Elliott, Ph.D.
University of Florida
Fort Lauderdale Research and Education Center

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 1997. This past year was our first attempt to enumerate bacterial groups associated with putting green construction materials, prior to and after fumigating and planting of bermudagrass sprigs.

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 obtained from Golf Agronomics (Sarasota, FL) and is called FM 200 sand. Two peat materials were used to make the mixes, sphagnum peat and reed sedge peat. The Canadian sphagnum peat moss was obtained from Sun-Gro and was mixed with the FM 200 sand at the rate of 43.0 lb peat @ 48% moisture per yard of sand to obtain an 80/20 mix. The Dakota reed sedge peat was obtained from Dakota Peat and Blenders and was mixed with the FM 200 sand at 36.7 lb peat @ 36% moisture per yard of sand to obtain a 90/10 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, 80/20 root-zone mix made with sphagnum peat and 90/10 root-zone mix made with reed sedge peat. The subplots or second factor are the three fumigants, methyl bromide (gas), metam sodium (liquid) and dazomet (granule). The active ingredient for both metam sodium and dazomet is MITC.

- 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 and place in ziploc bag.
2. Randomly remove 10 grams (wet weight) from a each bag 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*
    c) RASS medium or 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 15 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 80C 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 following media for enumeration:
    a) S-1 medium for fluorescent pseudomonads
    b) Azide Blood Base Agar for gram-positive bacterial counts
    c) Crystal Violet Agar for gram-negative bacterial counts
    d) selective medium for *Stenotrophomonas maltophilia*
    e) HAVA medium for actinomycetes
    f) 1/10 TSBA for total aerobic bacterial counts
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 and root-zone mix at one, 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 etoh-treated tweezers, all plant material (i.e., all leaves and roots). Remember, these are new plants. Place plant

material into a sterile plastic 250-ml flask. For the fourth and fifth month after planting, roots were separated from leaves and stolons and only roots were sampled. 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 99-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 80C 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 80C oven for 48 hours and record weight.
7. Spread 0.1 ml aliquots of the dilutions on the following media for enumeration:
    a) S-1 medium for fluorescent pseudomonads
    b) Azide Blood Base Agar for gram-positive bacterial counts
    c) Crystal Violet Agar for gram-negative bacterial counts
    d) selective medium for *Stenotrophomonas maltophilia*
    e) RASS or HAVA medium for actinomycetes
    f) 1/10 TSBA for total aerobic bacterial counts
8. The appropriate dilutions are then placed in an 80^{o}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^{o}C and microbial colonies counted at the appropriate time.

Results

At delivery of the individual root-zone components, the sand was essentially devoid of bacteria. All bacterial groups were detected in the reed sedge peat. The fluorescent pseudomonads and *S. maltophilia* were not detected in the sphagnum peat. Similar results were obtained in the two root-zone mixes. (Table 1)

The plastic was removed from the containers at 10 days post-fumigation with samples obtained immediately after the plastic was removed. Results are summarized in Table 2. Dazomet and metam sodium break down to the same active ingredient (MITC). The results were indeed similar. No or minimal fluorescent pseudomonads or *S. maltophilia* were detected from containers treated with dazomet or metam sodium. These bacterial groups were detected in containers treated with methyl bromide, but actinomycetes were not detected in these containers.

The bacterial groups detected 15 days after the plastic was removed were different from those detected immediately after the plastic was removed (Table 3). The fluorescent pseudomonads were now isolated from all containers, regardless of fumigant used. However, actinomycetes were still not detected in containers fumigated with dazomet and metam sodium nor were they now detected in containers fumigated with methyl bromide.

All the bacterial groups were present when the 'Tifdwarf' bermudagrass was sampled prior to planting (Table 4). All groups continued to be detected on plant material and in the root-

zone mix throughout the next five months of sampling. By the fourth month, there appeared to be few differences among treatments.

Conclusions

No conclusions can be made at this time since this was the first year of field work. Adjustments will be made in sampling procedures as required. These will include the elimination of one of the MITC products (probably the dazomet) so a non-fumigated treatment can be included. If possible, standard amounts of the methyl bromide fumigant will be used rather than the approximate 10X rate used this year when the 1.5-lb can of Brom-O-Gas was the treatment.

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

	colony forming units per gram dry weight[x]				
Bacterial group	Sand	S. P.[y]	R. S. P.[z]	Sand/S.P.	Sand/R.S.P.
Total	3.6	6.9	7.8	5.0	7.1
Fluorescent pseudomonads	ND	ND	3.3	ND	5.1
Stenotrophomonas maltophilia	ND	ND	3.3	ND	3.9
Gram positive	ND	4.0	5.3	4.6	5.2
Gram negative	ND	5.7	3.3	4.2	6.2
Actinomycetes	ND	6.0	7.2	2.6	4.6
Heat tolerant	ND	5.0	5.9	2.9	4.4

[x]Values are mean of four replicate samples. ND, not detected
[y]S.P., sphagnum peat
[z]R.S.P., reed sedge peat

Table 2. Bacterial groups present prior to fumigation and 10 days after fumigation.

	colony forming units per gram dry weight[x]							
	Pre-fumigation		Dazomet		Metam sodium		Methyl bromide	
Bacterial Group	S.P.[y]	R.S.P.[z]	S.P.	R.S.P.	S.P.	R.S.P.	S.P.	R.S.P.
Total	6.4	7.4	4.0	5.5	3.4	5.1	4.6	5.6
Fl. pseudomonads	<2.0	3.7	ND	ND	ND	ND	2.7	3.0
S. maltophilia	2.8	4.0	ND	ND	<2.0	ND	<2.0	<2.0
Gram positive	4.1	5.5	<2.0	2.8	ND	<2.0	3.4	3.2
Gram negative	4.8	5.9	ND	3.1	<2.0	<2.0	<2.0	3.8
Actinomycetes	3.0	5.9	2.8	4.8	2.7	4.8	ND	ND
Heat tolerant	3.2	5.4	3.3	5.1	3.2	4.2	2.5	2.8

[x]Values are mean of twelve replicate samples for pre-fumigation and four replicate samples for post-fumigation. ND, not detected
[y]S.P., sphagnum peat root-zone mix
[z]R.S.P., reed sedge peat root-zone mix

Table 3. Bacterial groups present prior to fumigation and 25 days after fumigation.

	colony forming units per gram dry weight[x]							
	Pre-fumigation		Dazomet		Metam sodium		Methyl bromide	
Bacterial Group	S.P.[y]	R.S.P.[z]	S.P.	R.S.P.	S.P.	R.S.P.	S.P.	R.S.P.
Total	6.4	7.4	4.0	4.5	7.0	7.4	6.6	6.9
Fl. pseudomonads	<2.0	3.7	<2.0	3.5	5.5	4.8	5.7	5.4
S. maltophilia	2.8	4.0	ND	<2.0	5.7	4.3	4.8	4.2
Gram positive	4.1	5.5	<2.0	<2.0	2.6	5.4	5.1	5.4
Gram negative	4.8	5.9	<2.0	4.1	6.4	6.5	6.2	6.4
Actinomycetes	3.0	5.9	ND	ND	ND	<2.0	ND	ND
Heat tolerant	3.2	5.4	2.3	3.6	6.0	5.6	5.7	5.4

[x]Values are mean of twelve replicate samples for pre-fumigation and four replicate samples for post-fumigation.
ND, not detected
[y]S.P., sphagnum peat root-zone mix
[z]R.S.P., reed sedge peat root-zone mix

Table 4. Bacterial groups present when 'Tifdwarf' bermudagrass delivered to UF/FLREC.

Bacterial group	colony forming units per gram dry weight[z]
Total	8.7
Fluorescent pseudomonads	5.5
Stenotrophomonas maltophilia	5.7
Gram positive	5.8
Gram negative	7.9
Actinomycetes	7.0
Heat tolerant	7.4

[z]Values are mean of four replicate samples.

Monica L. Elliott
Associate Professor, Plant Pathology
University of Florida, Fort Lauderdale Research and Education Center
70% Research/30% Extension

B.S.- Environmental Biology at Eastern Illinois University, 1978
B.S.- Botany at Eastern Illinois University, 1978
B.A.- History at Eastern Illinois University, 1978
M.S.- Plant Pathology at Montana State University, 1983
Ph.D.- Plant Pathology at Montana State University, 1987

Extension responsibility is to develop and disseminate an education program on integrated disease management techniques for turfgrass, bermudagrass and St. Augustinegrass. Research is focused on: 1) turfgrass diseases in general, with specialized interest in the soilborne pathogen *Gaeumannomyces graminis*, 2) microbial ecology of putting green root-zone mixes, 3) golf course management, 4) Ganoderma butt rot of palms.

Selected Publications:

Elliott, M. L., and E. A. Des Jardin. 1997. Effect of organic nitrogen fertilizers on microbial populations associated with bermudagrass putting greens. Biology and Fertility of Soils (in press).

Elliott, M. L., and M. Prevatte. 1996. Response of `Tifdwarf' bermudagrass to seaweed-derived biostimulants. HortTechnology 6:261-263.

Elliott, M. L. 1995. Disease response of bermudagrass to *Gaeumannomyces graminis* var. *graminis*. Plant Disease 79:699-702.

Elliott, M. L. 1995. Effect of systemic fungicides on a bermudagrass putting green infested with *Gaeumannomyces graminis* var. *graminis*. Plant Disease 79:945- 949.

Elliott, M. L. 1995. Effect of melanin biosynthesis inhibiting compounds on *Gaeumannomyces* species. Mycologia 87:370-374.

Elliott, M. L., and M. Prevatte. 1995. Comparison of damage to `Tifgreen' bermudagrass by petroleum and vegetable oil hydraulic fluids. HortTechnology 5:50-51.

Elliott, M. L., E. A. Des Jardens, and R. Di Bonito. 1994. Reisolation and enumeration of transformed *Streptomyces* strains from plant roots. Journal of Applied Bacteriology 77:80-87.

Elliott, M. L., E. A. Des Jardens, and J. M. Henson. 1993. Use of a polymerase chain reaction assay to aid in identification of *Gaeumannomyces graminis* var. *graminis* from different grass hosts. Phytopathology 83:414-418.