Bacterial Populations and Diversity withing New USGA Putting Greens

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Goals:

- *Determine bacterial populations associated with putting green root-zone mix materials.*
- *Determine bacterial populations of the root-zone mixes before and after fumigation.*
- *Compare rhizosphere bacterial populations on two different turfgrasses, bentgrass and and South Carolina.*
- *Compare rhizosphere bacterial populations of bentgrass in two different locations, Alabam and South Carolina.*
- *Compare rhizosphere bacterial populations of bermudagrass in two differrent locations, southern Florida and notrthern Florida.*
- *Compare thatch development, rooting and bacterial population of bentgrass in relation to rootsone mix and nitrogen fertilization.*
- *Compare soil and rhizosphere bacterial populations of root-zone mixes containing various clay sources.*
- *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 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.

University of Florida

Trenches were dug at the Florida Research and Extension Center (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 of the bermudagrass sprigs also were obtained.

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 *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.

Table 15. 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 16. 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

Auburn University

Conducted in cooperation with Clemson University (H.D. Skipper) and the University of Florida (M.L. Elliott) this study evaluates bacterial species and their population fluxes in the soil and rhizosphere during the establishment and maintenance of putting greens. Treatments in this study include grass type (bent or bermudagrass), organic construction material (sphagnum vs. reed sedge), fumigants (methyl bromide, metam sodium or dazomet) and N fertility regimes (x vs 2x normal). At Auburn University, treatments are N rate (1 x or 2x normal rate) and construction materials (pure sand putting green or 80/20 sand/peat mix). Sixteen containerized greens were constructed at the Auburn University Turfgrass Research Unit, four replications of each fertility/soil mix combination. Greens were sodded in January 1997 with washed bentgrass sod (*CRENSHAW*). Greens are 1 m long x 0.5 m wide, and each drains to an individual collection chamber. Total leachate from each green is collected as needed, volume recorded and a subsample is analyzed for $N0_3$-N and NH_4-N concentration. In February, May, August and November of each year root and soil samples (0-4 inch depth) are collected from each green. These samples are shipped to the University of Florida, where they are subject to dilution plating and identification. Selected isolates are returned to Auburn University, where identification at the species level is conducted via GC FAME analysis. Nitrogen rates applied at the Auburn University site were originally 1 or 2 lbs N per 1000 ft^2 month (granular fertilizer source). Excessive loss of N through leachate and burning of turf at application resulted in a shift of application times and amounts to 1/5 or 1 /10 lb N per 1000 ft^2 per week applied via a $C0_2$-backpack sprayer. These N application rates were initiated on 25 August, 1997 and will likely continue for the rest of the study unless grass health indicates a need for more N.

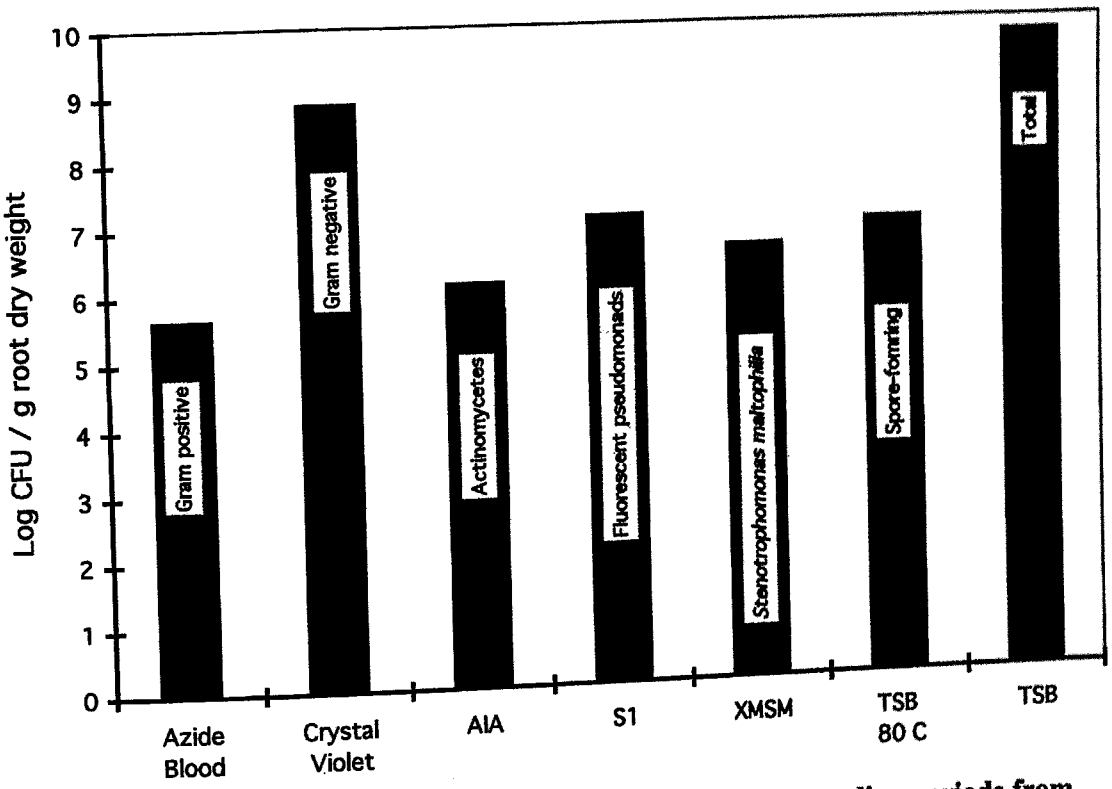

Figure 24. Rhizobacteria populations were averaged over four sampling periods from bentgrass greens. Samples were collected from December 1996 to September 1997 from Charlotte Country Club Golf Course, NC.

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 putting greens management is to understand the bacterial interactions in the rhizosphere of turfgrasses. Development of a database on turfgrass rhizobacteria from newly constructed bentgrass putting greens has been initiated. Each quarter, 160 randomly selected bacterial isolates on tryptic-soy broth agar (TSBA) were isolated. They now are being identified by FAME analyses. Broad classes of rhizobacteria populations were successfully separated on selective media. Preliminary numerical differences of rhizobacteria populations in bentgrass rhizosphere were observed (Figure 24). In the samples of December 1996, isolates identified from bentgrass rhizosphere belonged to 23 genera and 34 species and 76 percent were Gram negative. *Acidovorax, Burkholderia,* and *Pseudomonas* were the major genera. However, in the samples of Mar-1997, isolates identified from bentgrass rhizosphere belonged to 25 genera and 40 species and 78 percent were Gram negative. *Pseudomonas, Comanonas, Cytophaga,* and *Arthrobacter* were the major genera.