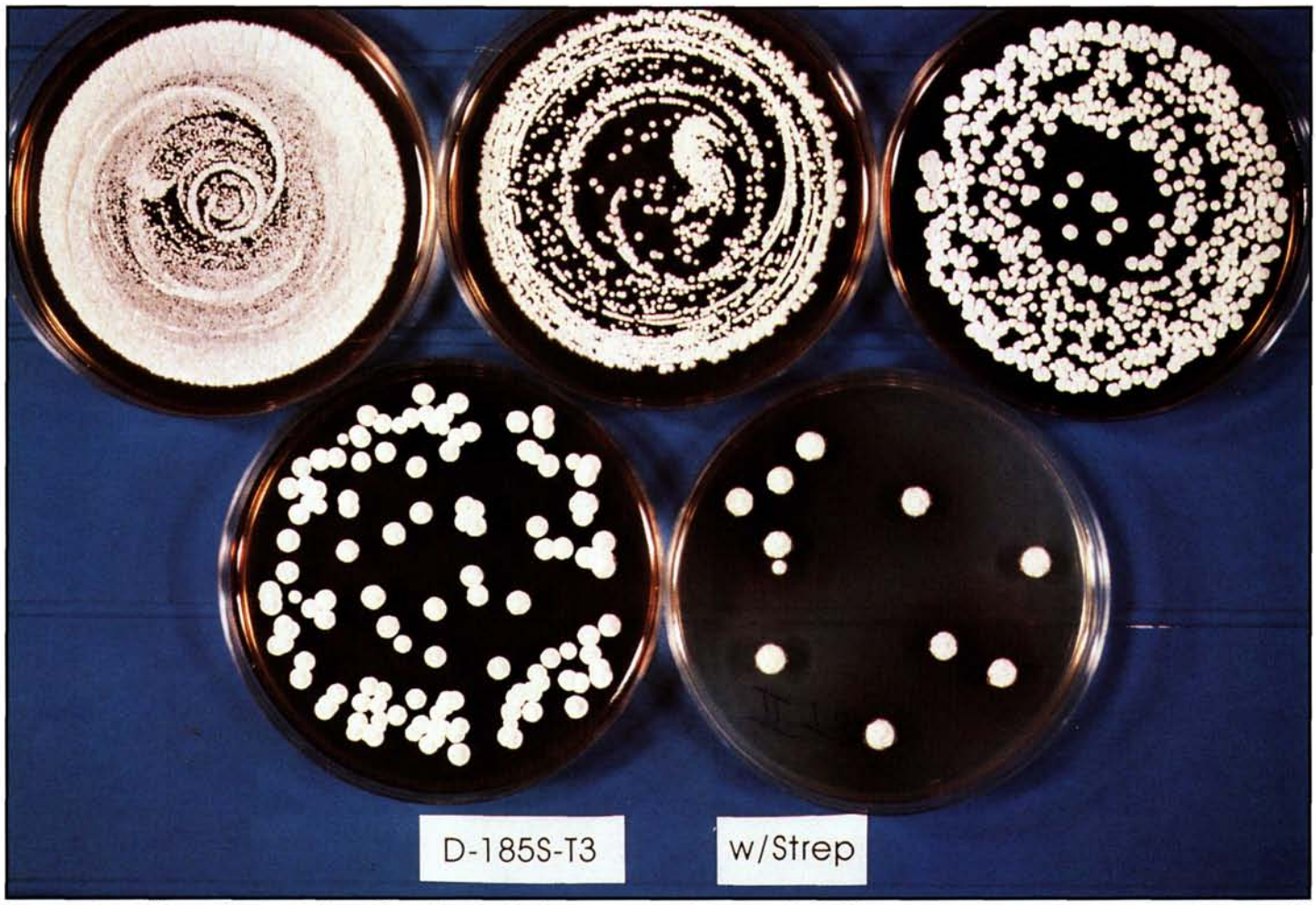



*In counting millions of bacteria from the soil, microbiologists use a technique called "dilution plating." A soil or root sample is mixed in a liquid and then diluted to a point that will allow the bacteria to grow as distinct colonies on selective or non-selective media.*

# BLACK BOX RESEARCH

*Seeking answers to the effectiveness of bacterial inoculents.*

**by MONICA L. ELLIOTT, Ph.D.**

THE PLANT rootzone, often referred to as the rhizosphere, is truly a black box of the microbial unknown (Bowen and Rovira, 1991). While extensive research has been conducted on the roots of annual crops such as corn, wheat, and cotton (Pankhurst et al., 1996), research on turfgrass roots and the soils or rootzone mixes they inhabit has only just begun. When so few facts are known, myths are bound to develop. A common myth is that golf course soils, especially putting greens, are sterile environments with few microbes. We have already learned that is not true (Mancino et al., 1993; Liu et al., 1995; Elliott and Des Jardin, 1999a). What we want to learn now, if possible, is how to manipulate microbial populations in the rootzone.

## Terminology

First, definitions of commonly used terms are in order. *Microbes* refers to bacteria and fungi. In this article, only bacteria will be discussed. *Bacteria* normally are single-cell organisms whose genetic material is not separated from the rest of the cell by a membrane. In contrast, fungi and plant cells do have their genetic material (nucleus) separated by a membrane. *Colony forming units* (CFU) is the measurement used to describe bacterial populations. In theory, each colony on a plate represents a single cell. Because bacterial numbers are so large, they are converted to log values. Instead of stating there are 2,700,000 CFU, we state there are $\log_{10}6.4$ CFU.

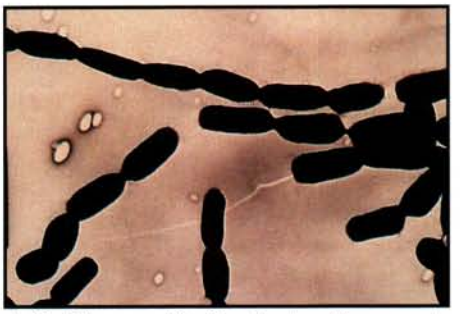
Common bacterial groups referred to in this article are: 1) fluorscent pseudomonads that glow in the dark with a UV light, 2) gram-negative bacteria, 3) gram-positive bacteria, 4) heat-tolerant bacteria that survive a 10-minute soak in a 176°F water bath, and 5) actinomycetes that look like fungi. Gram reactions (positive or negative) refer to a simple test that differentiates bacteria, based primarily on their cell wall components. Most heat-tolerant bacteria produce a specialized cell called an endospore. Actinomycetes also produce spores, but they are not tolerant of extreme heat. They are

often confused with fungi, but they are not related. The five groups listed above are detected using selective media.

A sixth group of bacteria is referred to as *total aerobic* bacteria. They represent all the bacteria that will grow on a non-selective medium. The counts for the other groups will *not* add up to the count for the "total" group. It is critical to understand that this technique of growing bacteria on media only accounts for approximately 10% of the bacteria in the soil (Olsen and Bakken, 1987). We have not learned how to grow most bacteria in the laboratory. This is just one of many techniques for examining bacterial populations. We use this technique because we want to save many of the bacteria we isolate for future research.

### Background Research

In a previous study we examined the effects of four natural organic nitrogen fertilizers on bacterial populations of an established (four years old) bermudagrass putting green (Elliott and Des Jardin, 1999a). For two years these products, plus a synthetic organic fertilizer, were applied to the putting green every two weeks at the normal South Florida annual rate of 18 pounds N per 1,000 square feet. Significant differences in bacterial populations due to fertilizer treatments were not observed. In other words, bacterial populations remained the same despite using natural organic fertilizers, even the two fertilizers that had bacteria added to them. There also were no consistent differences observed among the fertilizer treatments on turfgrass growth or quality (Elliott and Prevatte, 1997).



*A highly magnified look at actinomycete spores. They may look like fungi, but they are bacteria. This group is the source of most antibiotics.*

Every scientist wants to obtain dramatic, eureka!-type results, but we did not. Why? Two reasons may explain our results. First, since the putting green was maintained as a regular green, it was topdressed routinely (every two weeks). The topdressing (80/20 mix) did contain bacteria; in fact, more than the fertilizers. Second, since this was an established green, perhaps an optimum level of bacteria had already been obtained.

### New Research

For the next project, research started at the beginning — the very beginning of putting green construction. Questions to be considered were: Do brand-new greens have different bacterial populations? Would peat sources affect bacteria? Does fumigation kill all the bacteria in the rootzone mix? What bacteria rebound first after fumigation? What bacteria are associated with the bermudagrass sprigs?

At the research center, trenches were dug for placement of 100-gallon Lerio™ tree containers, 36 inches square and 18 inches deep, to build mini-greens. A 6-inch layer of non-calcareous, washed river gravel was placed in the bottom of each container. No intermediate layer was added, as the gravel and the two rootzone mixes evaluated complied with USGA recommendations. The rootzone mixes, matched for physical characteristics, contained either Canadian sphagnum peat (85/15 mix) or Dakota reed sedge peat (93/7 mix). The subplot or second

*Mini-greens were constructed in 36-inch square, 18-inch deep containers at the University of Florida Research Center in Fort Lauderdale. Soil and turfgrass samples were taken throughout the experiment to determine population sizes of six bacterial groups.*

**Table 1**
**Bacterial populations associated with individual components of the rootzone mixes and the bermudagrass sprigs planted into the mixes.**

| | log[10] colony forming unit of bacteria* | | | | | |
|---|---|---|---|---|---|---|
| | Fluorescent pseudomonads | Gram-positive | Gram-negative | Actinomycetes | Heat-tolerant | Total |
| Sand alone | 0 | 2.4 | 3.4 | 2.7 | 2.7 | 5.0 |
| Sphagnum peat alone | 0 | 1.2 | 5.8 | 2.3 | 4.1 | 6.6 |
| Reed sedge peat alone | 3.7 | 4.2 | 6.4 | 7.5 | 7.1 | 8.4 |
| Sand/sphagnum peat rootzone mix | 1.6 | 0.5 | 4.5 | 0 | 3.0 | 5.8 |
| Sand/reed sedge peat rootzone mix | 1.3 | 0 | 4.5 | 0 | 5.2 | 6.3 |
| Bermudagrass sprigs | 4.5 | 4.1 | 7.1 | 5.6 | 5.5 | 8.0 |

*Values are based on gram dry weight of either each component, rootzone mix, or plant material

factor in the experiment was fumigation type. The containers were either not fumigated (control) or were fumigated with methyl bromide (1 pound per cubic yard, injected) or metam sodium (2 gallons Metam 326 per 1,000 square feet).

Soil and/or turfgrass samples were obtained to determine population sizes of the six different bacterial groups for the individual rootzone components prior to blending and each rootzone mix after blending, but before placement in containers. Samples also were obtained from each container just prior to fumigation, 10 days after fumigation, 25 days after fumigation, and each month after planting of bermudagrass for five months. Samples also were obtained of the bermudagrass sprigs prior to planting. Protocol for the experiment was based on previous bermudagrass research (Elliott and Des Jardin, 1999b).

Bacterial populations associated with individual mix components, final mixes, and bermudagrass sprigs are presented in Table 1. The sand and sphagnum peat contained the lowest number of bacteria, with one group not detected at all (fluorescent pseudomonads). All bacterial groups were detected in the reed sedge peat. They were also all detected on the grass. This is important to remember since bermudagrass is vegetatively propagated and not seed propagated.

When the containers were sampled just prior to fumigation, there were

**Table 2**
**Effect of fumigation on bacterial populations in putting green rootzone mixes. Since significant differences were due to fumigations, results from the two mixes were combined for analysis.**

| | log[10] colony forming units of bacteria per gram dry weight of soil* | | | | | |
|---|---|---|---|---|---|---|
| | Fluorescent pseudomonads | Gram-positive | Gram-negative | Actinomycetes | Heat-tolerant | Total |
| **Pre-fumigation** | 2.2 | 2.4 | 4.9 | 4.4 | 4.1 | 6.8 |
| **10 days after fumigation** | | | | | | |
| Control (no fumigation) | 1.0[D] | 2.3 | 4.7 | 3.8 | 4.1 | 6.3[D] |
| Metam Sodium | 0.9[D] | 1.4[D] | 3.1[D] | 2.5[D] | 4.5 | 5.9[D] |
| Methyl Bromide | 0.1[D] | 5.0[I] | 5.5 | 2.6[D] | 5.2[I] | 7.0 |
| **25 days after fumigation** | | | | | | |
| Control (no fumigation) | 1.3[D] | 1.1[D] | 4.4 | 3.9 | 4.1 | 6.0[D] |
| Metam Sodium | 4.0[I] | 1.2[D] | 5.3 | 3.4 | 5.7[I] | 6.8 |
| Methyl Bromide | 0[D] | 4.9[I] | 5.2 | 3.0[D] | 5.2[I] | 7.0 |
| **50 days after fumigation** | | | | | | |
| Control (no fumigation) | 4.7[I] | 2.4 | 5.5[I] | 4.8 | 4.5 | 6.5 |
| Metam Sodium | 4.9[I] | 3.0 | 5.6[I] | 3.9 | 5.6[I] | 6.7 |
| Methyl Bromide | 4.4[I] | 4.6[I] | 5.6[I] | 4.4 | 4.8[I] | 6.9 |

*Values followed by a [D] are a significant decrease ($P = 0.05$) from the pre-fumigation values. Values followed by an [I] are a significant increase over the pre-fumigation values. If values have no [D] or [I], then they are not significantly different from the pre-fumigation values. Statistical comparison conducted using Dunnett's *t*-test, $P = 0.05$.

greater numbers of Gram-negative bacteria and total bacteria in the sand/sphagnum peat mix than in the sand/reed sedge peat mix. The actinomycetes and heat-tolerant bacteria were greatest in the reed sedge peat mix. After fumigation, there was no overall significant difference between rootzone mixes. Significant differences observed in bacterial populations were due to the fumigation method (Table 2). Ten days after fumigation, the least number of each bacterial population was often associated with the metam sodium fumigant. At 25 days after fumigation, the most interesting observation was that the fluorescent pseudomonads were no longer detected in any of the containers fumigated with methyl bromide. Although the bermudagrass had been planted for one month at 50 days after fumigation, the containers were less than 50% grown-in. Therefore, we conducted a third post-fumigation sampling of just the soil. By this time, all the bacterial populations had rebounded and were either equal to or greater than the pre-fumigation populations.

When the results from the four monthly post-planting turfgrass samples were evaluated, there were no significant differences due to type of rootzone mix or fumigation method. Differences were due to the month the samples were obtained. The highest counts were for four and five months post-plant, whereas the lowest counts were for two and three months post-plant (Table 3).

## Conclusions

As we have demonstrated, *bermudagrass putting greens are not devoid of bacteria.* Even newly constructed greens rapidly build up bacterial populations that include the most common groups associated with soils.

While the goal of research projects is to answer questions, new questions will also be raised. One concerns the application of general bacterial inoculants on the golf course. Some products claim to include 20 or more different bacteria. Because these products are not regulated and usually not evaluated, it is difficult to know what the consumer has actually purchased. If a product's only claim is to increase bacterial populations in the soil, this is probably a questionable benefit. Even if a product has proven to be effective on bentgrass, it does not mean it will be effective on bermudagrass. Also, some products contain more than just microbes. Often, nutrients or plant hormones are part of the formulation also.

The superintendent's best research tool for determining if a product will benefit a course is a piece of plywood. Simply place the plywood in the center of the green before applying the microbial inoculant product. Evaluate this control plot in comparison to the area treated with the product. Alternatively, apply the product to only one half of each green. Unwilling to take these chances? Then why are you taking a chance with the product in the first place?

## References


Bowen, G. D., and A. D. Rovira. 1991. The rhizosphere, the hidden half of the hidden half. Pages 641-649 *in* Plant Roots — The Hidden Half. Y. Waisel and U. Kafkafi, eds. Marcel Dekker, New York.

Elliott, M. L., and M. Prevatte. 1997. Effect of natural organic nitrogen fertilizers on hybrid bermudagrass growth. HortTechnology 7:289-292.

Elliott, M. L., and E. A. Des Jardin. 1999a. Effect of organic nitrogen fertilizers on microbial populations associated with bermudagrass putting greens. Biology and Fertility of Soils 28:431-435.

Elliott, M. L., and E. A. Des Jardin. 1999b. Comparison of media and diluents for enumeration of aerobic bacteria from bermudagrass golf course putting greens. Journal of Microbiological Methods 34:193-202.

Liu, L. X., T. Hsiang, K. Carey, and J. L. Eggens. 1995. Microbial populations and suppression of dollar spot disease in creeping bentgrass with inorganic and organic amendments. Plant Disease 79:144-147.

Mancino, C. F., M. Barakat, and A. Maricic. 1993. Soil and thatch microbial populations in an 80% sand:20% peat creeping bentgrass putting green. HortScience 28:189-191.

Olsen, R. A., and L. R. Bakken. 1987. Viability of soil bacteria: optimization of plate-counting technique and comparison between total counts and plate counts within different size groups. Microbial Ecology 13:59-74.

Pankurst, C. E., K. Ophel-Keller, B. M. Doube, and V.V.S.R. Gupta. 1996. Biodiversity of soil microbial communities in agricultural systems. Biodiversity and Conservation 5:197-209.



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**Table 3**
**Comparison of bacterial groups found on bermudagrass roots at two to five months after the bermudagrass was planted.**

| | log[10] colony forming units of bacteria per gram dry weight of roots* | | | | | |
|---|---|---|---|---|---|---|
| | Fluorescent pseudomonads | Gram-positive | Gram-negative | Actinomycetes | Heat-tolerant | Total |
| 2 months post-plant | 5.2c | 4.4b | 6.9b | 6.5a | 5.9c | 8.0c |
| 3 months post-plant | 5.8b | 3.8c | 7.2a | 6.4a | 6.1b | 8.2b |
| 4 months post-plant | 6.1a | 5.9a | 7.2a | 6.4a | 6.3a | 8.4a |
| 5 months post-plant | 5.9b | 6.2a | 7.0ab | 6.4a | 6.3a | 8.2ab |

*Values 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.