Biological Control of Turfgrass Diseases

Application, Efficacy and Commercialization

A Healthy Ecosystem

Studies have demonstrated that more effective control of dollar spot is achieved when higher populations of inoculants are introduced or when higher populations are maintained between application intervals.

New technology for applying microbial inoculants to turfgrasses was introduced in the United States in 1999 with the registration of the BioJect® Fermentation and Injection System to deliver *Pseudomonas aureofaciens* strain Tx-1 for control of dollar spot. This system is also used to deliver inoculants in other agricultural settings. Much of the rationale for such a device is that it provides a means of making daily or continuous applications, thus facilitating the maintenance of high populations of the introduced inoculant.

Numerous studies have demonstrated that more effective control of dollar spot on creeping bentgrass is achieved when higher populations of inoculants are introduced or when higher populations are maintained between application intervals. For example, *Pseudomonas aureofaciens* strain Tx-1 is more effective in controlling dollar spot when applied at a level of 10^7 cells/ml than when applied at a level of 10^5 cells/ml. Repeated applications of this strain at densities of 10^6 cells/ml allow it to become well established in turfgrass systems and to overwinter 1-2 cm below the turfgrass surface. Other inoculants behave similarly. For example, *T. harzianum* strain 1295-22 was more effective in controlling dollar spot, brown patch, and Pythium root rot when applied at cell densities $>10^7$ conidia/ml than when applied at lower concentrations. In field studies, populations persisted at levels $>10^5$ cfu/g dry wt soil following applications of granular or spray formulations of strain 1295-2. Similar observations have been made with *Stenotrophomonas maltophilia* strain C3 for control of leaf spot, *Typhula phacorrhiza* for control of Typhula blight. Immediately following the application of most inoculants, populations begin to decline. Although populations of *E. cloacae* could be introduced into golf putting greens at levels between 10^7 and 10^9 cfu/g soil, populations declined to 10^4 to 10^5 cfu/g 13 wk after application but overwintered at extremely low populations.

Suppression of summer patch of KBG was more effective when *S. maltophilia* strain 21C6 or *Serratia marcescens* strain 9M5 were applied 1-4 wk after planting than when applied at the time of sowing. Greater suppression was observed with both strains when applied at cell densities of 10^{10} cfu/ml than when applied at 10^8 cells/ml. Thirty five weeks after inoculation, populations declined to between 10^4 and 10^5 cfu/g soil.

Aside from application frequency, the time of day at which inoculants are applied may influence efficacy. In a study by Craft *et al* the timing (day vs. night) and frequency (daily vs weekly) of application of microbial inoculants was studied in field trials to determine their impacts on the efficacy of control of dollar spot. Over a two-year period the efficacy of all microbial inoculants was unaffected by applica-

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tion frequency. Some inoculants, however, were strongly affected by application timing. In each of the two years of the study, *Enterobacter cloacae* strain EcCT-501 and *Pseudomonas aureofaciens* strain Tx-1 were significantly more effective in controlling dollar spot following nighttime applications than when applied during the daytime. *Azospirillum brasiliense* strain Cd-1 was inconsistent in dollar spot control but unaffected by application timing and frequency. *Trichoderma harzianum* strain 1295-22 was also unaffected by application timing and frequency.

Populations of *E. cloacae* and *P. aureofaciens* were introduced at ~10^7 cells/g dry wt rhizosphere soil. When applications ceased, populations declined, independent of the application frequency or timing, one to two orders of magnitude after a three-week period. These results indicate that the time of day in which some microbial inoculants are applied may strongly influence their efficacy. Such improvement in efficacy is most likely due to the synchronization of biological control activities with the activities of the pathogen.

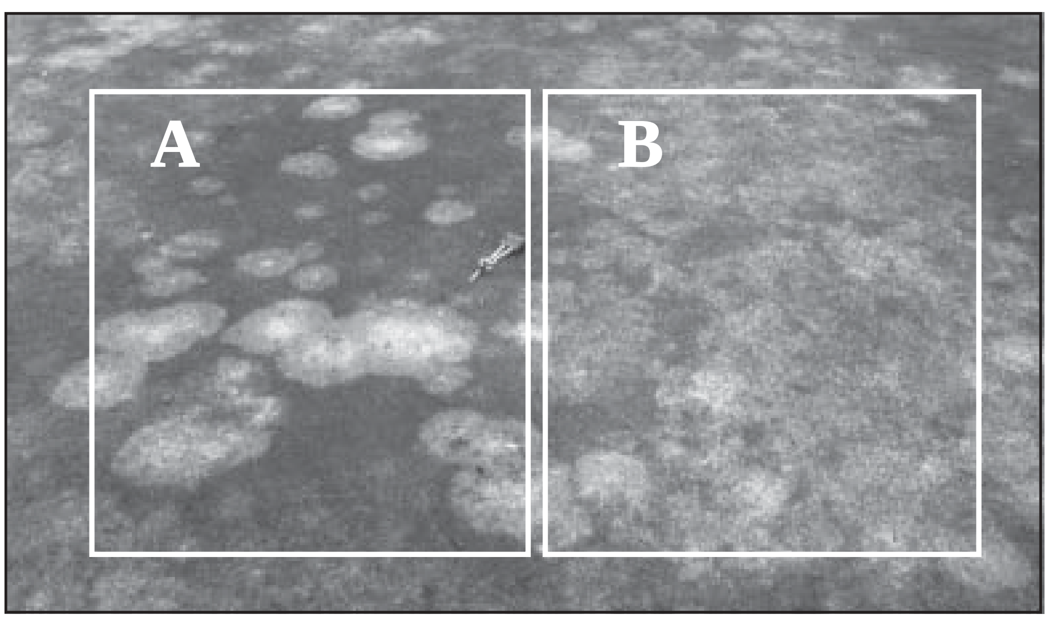
Mechanisms of Biological Control

Inconsistent performance of microbial inoculants may be related to inconsistencies in the expression of specific traits critical to pathogen and disease suppression. Knowledge of the manner in which pathogens and diseases are suppressed by microorganisms and how the expression of these mechanisms is regulated is critical to the development of strategies for ensuring consistent and efficacious performance. Nearly all of the studies on biological control mechanisms have come from studies with crop plants other than turfgrasses. However, many of the same processes may also play important roles in turfgrass ecosystems.

Of the traits common to soil microbes, there are at least three that have been consistently linked with biological control processes. These include 1) antibiotic biosynthesis, 2) resource competition, and 3) hyperparasitism. Some microorganisms may express various combinations of these mechanisms for successful biological control.

Antibiotic biosynthesis. Antibiotic biosynthesis, particularly in bacterial systems, has been the most commonly studied trait related to biological control. Antibiotic compounds produced by species of *Pseudomonas*, *Bacillus*, and *Streptomyces* are known to play key roles in the biological control of *Pythium*, *Rhizoctonia*, and *Gaeumannomyces* diseases and, more recently, have been shown to play roles in the suppression of *Sclerotinia homoeocarpa* and *Drechslera poae* in bentgrass and Kentucky bluegrass, respectively.

A number of ecologically important antibi-



Differential suppression of snow mold diseases on creeping bentgrass putting greens with topdressing applications of a turkey litter compost (Sustane). A) compost treated plot. B) nontreated plot. Plots were treated with compost at the rate of 20 lbs./1000 ft^2 at monthly intervals the entire previous season. The photo was taken the following April.

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otics have been described in biocontrol systems, including pyrollnitrin, pyoluteorin, 2,4-diacetylphloroglucinol, phenazine-1-carboxylic acid, and oomycin A. New antibiotics are constantly being described that could potentially play important roles in the biological control of plant diseases. The majority of studies on antibiotic biosynthesis and its relation to biological control have been limited primarily to fluorescent *Pseudomonas* species; the majority of research encompassing only 5 to 10 strains of *Pseudomonas fluorescens*, a few strains of *P. aureofaciens*, and a couple of strains of *P. putida*. Compelling evidence now exists for the link between production of antibiotics in the rhizosphere and the role of these antibiotics in suppressing root rot.

Resource competition. Microbial competition often occurs when two or more microorganisms compete for the same nutrient resource. Despite the fact that competition has been proposed as a mechanism of biological control, no definitive evidence to support this hypothesis currently exists. Although intuitively, competition should be an important mechanism of biological control, the complexities of the soil environment make the proof of competition mechanisms difficult to obtain. The best current examples of competition in biological control are competition for iron and seed exudate fatty acids.

Siderophore-mediated iron competition. Siderophores are low molecular weight iron chelates that are produced by many soil microbes under iron-limiting conditions. They chelate ferric iron and serve as a major vehicle for iron transport into microbial cells. Nearly all organisms produce siderophores, but those produced by species of *Pseudomonas* and enteric bacteria generally have higher affinities for iron than do other fungal siderophores. In a number of specific pathosystems, the biological control of soilborne pathogens has been attributed to the production of siderophores, whereas in other cases no role for siderophores in biological control processes can be found. Although siderophore competition has generally been considered a direct form of biological control, it is possible that some siderophores may be acting indirectly by enhancing natural plant defense mechanisms under iron-limiting conditions. More work is needed to resolve the functional relationships between siderophore production and biological control.

Fatty acid competition. Seed and root exudates play an important role in the initiation of soilborne plant diseases by serving as stimulants of fungal propagules. Without the release of stimulatory molecules in these exudates, pathogenic relationships between plants and soil pathogens do not occur. Recently, there has been interest in understanding the interaction of biocontrol organisms with stimulatory components of seed and root exudates. Since soilborne fungal pathogens are highly dependent on exudate molecules to initiate plant infec-

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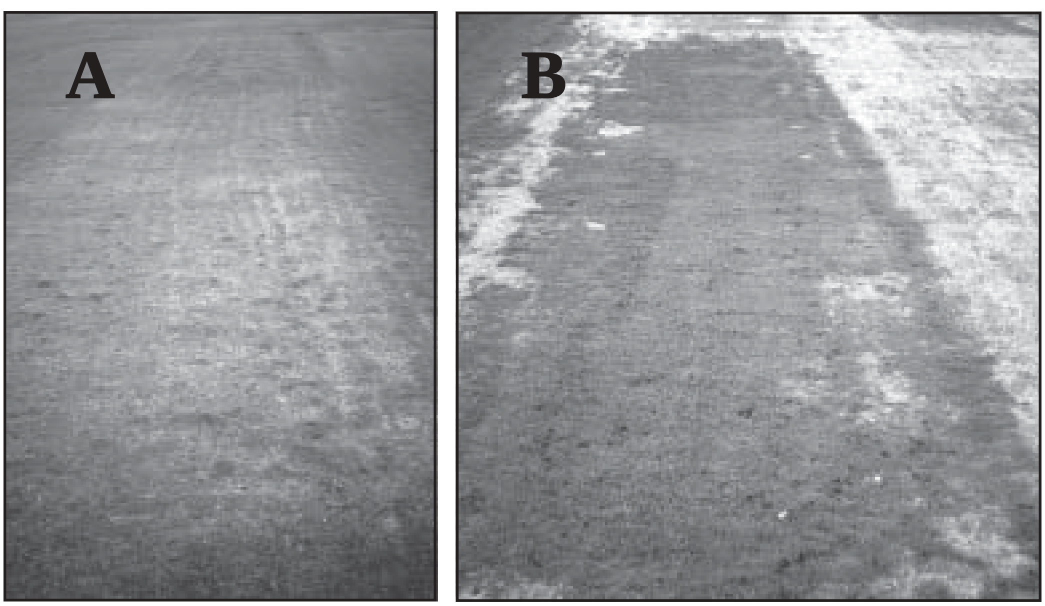

Establishment of creeping bentgrass on unamended or compost-amended sand-based root zones. A) seedling stands 6 weeks after seeding into 100% silica sand root zone. B) seedling stands 6 weeks after seeding into silica sand root zone amended with composted brewery sludge at the rate of 20% by volume.

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One of the greatest disappointments of biological control over the past decade is that more potentially useful inoculants have not been commercialized, despite the diversity, efficacy, and availability of various microorganisms from various research laboratories around the world. As mentioned before, two inoculants are currently registered for control of turfgrass diseases in the United States with only four others that are currently in the registration pipeline.

tions, microbial interference with the production and activity of exudate stimulants could be an effective mechanism of biological control among seed-applied spermosphere bacteria.

Recent studies have shown that *Enterobacter cloacae* strain EcCT-501 and other seed-associated bacteria that are effective in suppressing *Pythium* seed rot and damping-off of various plant species can also reduce the stimulatory activity of plant seed exudates to sporangia of the seed-rotting Oomycete, *Pythium ultimum*. Many of the seed-associated bacterial strains tested could reduce the stimulatory activity within 24 hours to levels supporting germination of less than 20% of *Pythium sporangia*. In particular, strains of *E. cloacae* can rapidly metabolize linoleic acid, a major *Pythium*-stimulatory molecule found in seed exudates, making it unavailable for the stimulation of *Pythium* pathogenesis. Strains of *E. cloacae* deficient in fatty acid metabolism fail to protect bentgrass seedlings from infection by *P. ultimum*, *P. graminicola*, and *P. aphanidermatum*.

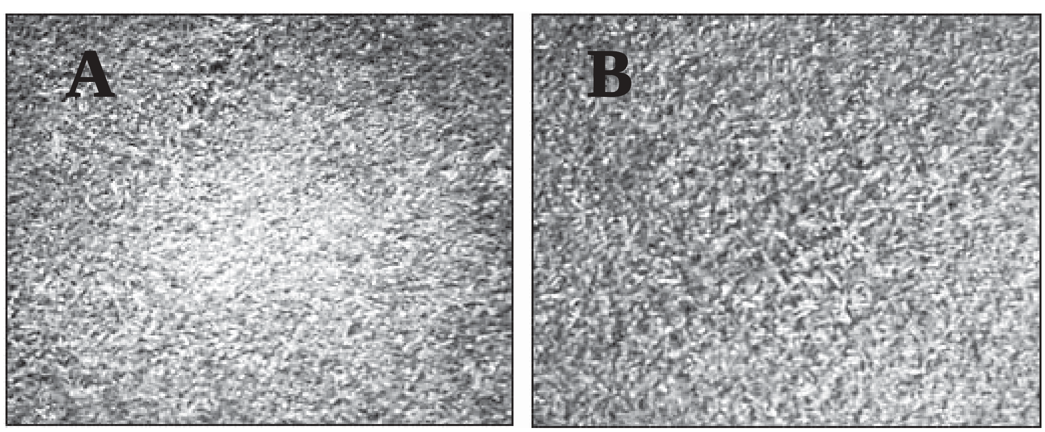
Hyperparasitism. Hyperparasitism is a complex process by which microorganisms parasitize other microorganisms. This process is generally mediated by the attachment and production of cell wall degrading enzymes, such as chitinases, glucanases, and proteinases, by the biocontrol strain that destroys the host (i.e., turfgrass pathogen) cell. Species of the biocontrol fungus *Trichoderma* are well known for this property. However, to date, there is no proof that this process plays a significant role in the biological control of turfgrass diseases by *Trichoderma* and other mycoparasitic fungi.

Bacteria may also be parasitic to pathogenic fungi. In these cases biocontrol efficacy is mediated by the production of chitinase enzymes. *Serratia marcescens* is known not only for its chitinase production, but also for its activity as a biological control agent for soilborne diseases, including summer patch on KBG caused by *Magnaporthe poae*. Much of the success of *S. marcescens* as a biological control agent is believed to result from its production of chitinolytic enzymes inhibitory to chitin-containing soilborne fungi. Similarly, strains of *Stenotrophomonas maltophilia* that produce chitinase are also effective in suppressing *Magnaporthe poae Bipolaris sorokiniana*, and *Rhizoctonia solani*.

Commercialization of Microbial Inoculants

One of the greatest disappointments of biological control over the past decade is that more potentially useful inoculants have not been commercialized, despite the diversity, efficacy, and availability of various microorganisms from various research laboratories around the world. As mentioned before, two inoculants are currently registered for control of turfgrass diseases in the United States with only four others that are currently in the registration pipeline.

The turfgrass industry has been particularly reluctant to embrace biological control technologies for a number of reasons. First, fungicides have been the mainstay of turfgrass disease management since the early 1900s primarily because they have been effective, proven, and nothing else was as effective. Breaking into such a fungicide dependent industry with rela-



Pythium *root rot suppression in root zones amended with a brewery sludge compost at the rate of 20% by volume. A) non-amended plot (100% sand root zone) 14 days after innoculation with* Pythium graminicola. *B) compost-amended plot 14 days after innoculation.*

tively unproven biological technologies that require a greater level of knowledge and skill for their effective use, cannot be accomplished easily. Second, fungicides are generally cheaper, easier to store, and easier to apply than microbial inoculants. Turfgrass managers are not particularly interested in complicating their jobs by introducing what is perceived to be difficult-to-implement technologies. Third, fungicides generally have a broader spectrum of activity and are effective over a broader range of environmental conditions than many inoculants, making the decision making process of which approach to use easier for the turfgrass manager. Fourth, there is very little financial incentive for companies to develop biological control products when the market potential is relatively small and uncertain.

Harman has argued for developing different product concepts for microbial inoculants in which niche markets are found where these organisms can provide advantages that go beyond those provided by fungicides. These would include: 1) situations where fungicides are lost due to regulatory action. This is occurring at a higher frequency today in the turfgrass industry; 2) replacements or reductions of fungicides in environmentally-sensitive area, many of which are found on or near golf courses; 3) applications where inoculants accomplish tasks not possible with chemical fungicides, such as plant growth promotion and enhanced nutrient uptake; and 4) applications for organic turf management programs. There is a growing interest in management programs characterized by reduced or no applications of chemical fungicides. It is likely that biological control inoculants may serve as important tools for disease management in this important area in the future.

Another major problem with commercialization of microbial inoculants for the turfgrass industry is that many small companies are developing microbial-based products for use on turfgrasses. These companies generally lack the capital to support the licensing and development efforts required for the successful registration, development, and implementation of microbial inoculants. Literally dozens of new microbial-based products for use in turfgrass management show up in the marketplace every year. Various microbial concoctions are used in these products with little rationale as to the choice of strains or the application strategy used. Product claims are often outrageous and in many cases disease control claims may not be based on sound science. With many of these products, there is little or no research to back up the claims that are made and, in many cases,

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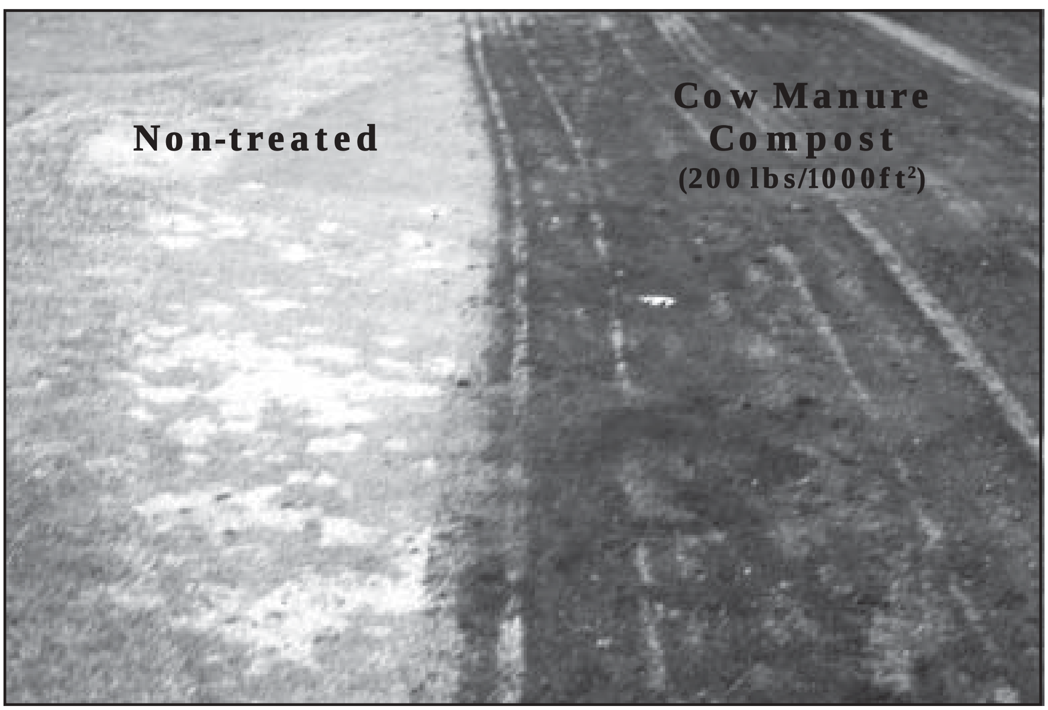

Suppression of Typhula *blight on a creeping bentgrass putting green with heavy dormant applications of composted cow manure applied at 200 lbs./1000 ft^2. Typical symptoms of* Typhula *blight in nontreated plot. Compost-treated plot remained disease free.*

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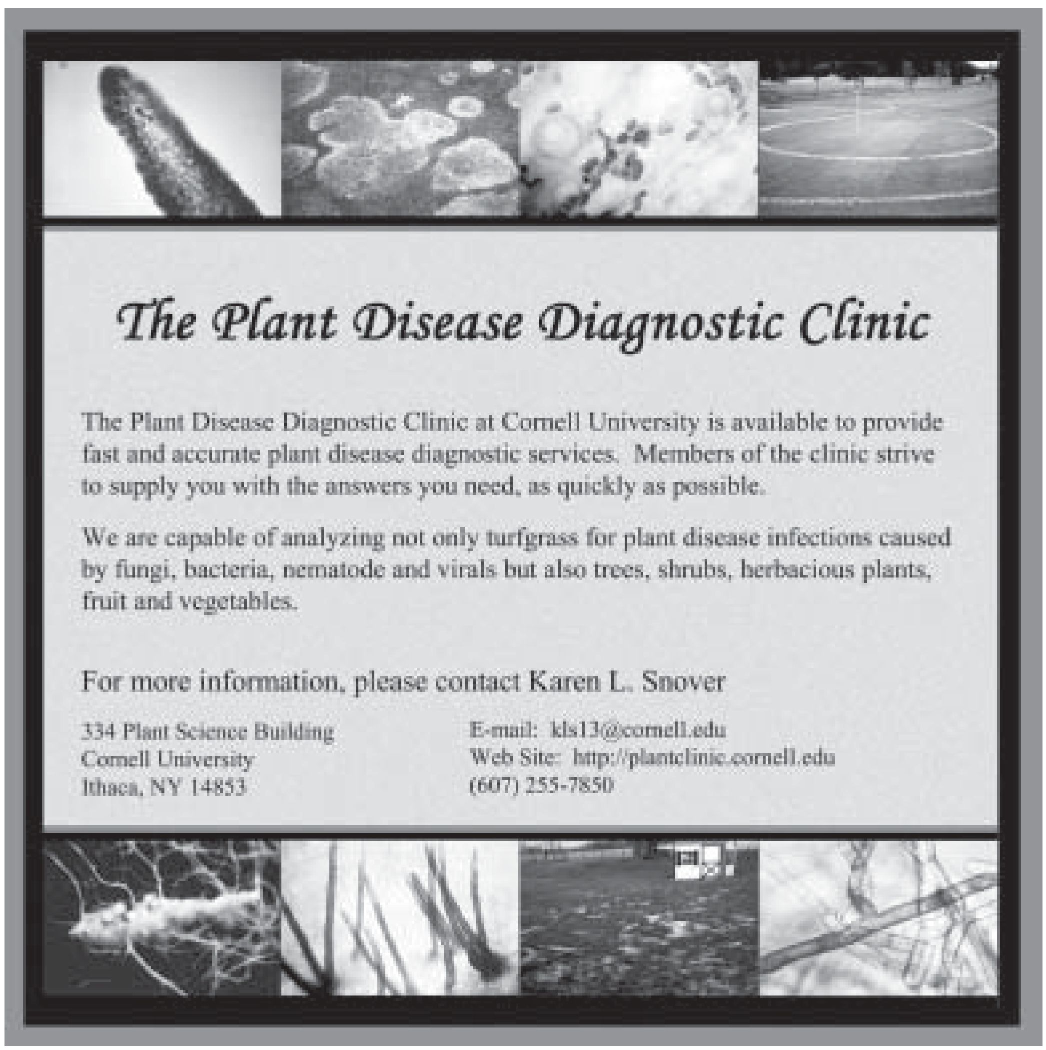
the products are ineffective in controlling diseases. This has become such a problem that some scientists are calling for turfgrass managers to exercise more caution and common sense in deciding whether to use such products.

Unfortunately most turfgrass managers develop an impression of a technology based on the commercial products that represent that technology. This is one reason that fungicide products have been so widely accepted and used. A fear with microbial inoculants is that turfgrass managers will reject the entire technology because the products currently on the market are faulty and fail to match the claims made on the label. Clearly, these types of products need to be replaced by those based on strains and application technologies developed from numerous years of research. Only then will we begin to see the effective use of microbial inoculants in the turfgrass industry for the biological control of turfgrass diseases.

Eric B. Nelson

Table 1. Commercial Turf Products in the United States

Organism	Strain	Trade Name	Status
Trichoderma harzianum	1295-22	Turf Shield (BioTrek)	Registered 1996
Gliocladium catenulatum	J1446	Primastop	Registration Application 5/97
Pseudomonas aureofaciens	Tx-1	SpotLess	Registered 1999
Streptomyces lydicus	WYEC 108	Actinovate	Registration Application 10/00
Bacillus subtilis	GB03	Companion	EUP Application 12/01
Bacillus licheniformis	SB3086	GR 710-140	Registration Application 06/02



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