The First Registered Biological Control Product For Turf Disease: Bio-Trek 22G

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Diseases of golf green turfgrasses cause unsightly spots and discolorations that are undesirable and unacceptable to golf course managers and the golfing public. The development of highly effective turfgrass fungicides has revolutionized disease management of turf, especially on golf courses. However, high levels of fungicides are required. Fungicide usage on golf courses, and greens especially, is probably the most intense large-scale application per unit area in the United States. Fungicide sales are about $400 million annually in the United States, and about $100 million is spent on turf applications, with 90% of this used on golf courses.

There are disadvantages to this heavy use. The most obvious of these is the frequent exposure of workers and users of managed turf areas to fungicides. In addition, there is the possibility of contamination of soil and water in and around golf courses and other areas of managed turf. This possibility of contamination is a matter of concern because much of the treated turf is in urban areas with high adjacent human populations. Wildlife may also be affected by contamination of soil or water.

In addition, a large and diverse population of soil microorganisms is important to plant health. Typically, populations of fungi and bacteria (including actinomycetes) predominate in soils. Generally, the greater the diversity and activity of these soil microorganisms, the greater the overall health and fertility of the soil. However, repeated fungicide applications can severely impair microbial diversity and activity in soils of golf courses and other intensively managed turf areas. In our preliminary studies, we found one area of golf course turf in which no fungi could be detected in soil or roots. This is very unusual, and aside from golf turf ecosystems, we know of no other situation where such a drastic reduction in fungi populations has occurred. Specific undesirable consequences of this alteration of soil microflora are as follows:

- It is not uncommon (nearly 100 examples can be documented) to see increases in certain diseases following fungicide application for control of other disease. This increase is due primarily to detrimental effects on nontarget organisms.
- In soils where fungicides have not been applied at high rates, the diverse microbial communities present frequently provide a substantial measure of biological control. The increase in disease noted in the preceding paragraph probably is due to destruction of nontarget beneficial microorganisms.
- Heavy fungicide use encourages the development of resistant populations of plant pathogens. There are numerous reports on the development of pathogen populations that are resistant to chemical fungicides, including cases where resistance was observed on golf courses. This would be expected, given the heavy fungicide applications made to greens.

The Development of Bio-Trek 22G

Clearly, alternatives to chemical pesticides are needed for turf disease management and other applications. The authors, in conjunction with Dr. E. B. Nelson, have been developing biological alternatives to chemical pesticides for turf disease management for several years, especially beneficial fungi in the genus Trichoderma. These fungi are present in nearly all soils and no doubt contribute to a lessening of disease where they occur. However, their numbers and physiological types are normally insufficient to give high levels of disease controls. About 10 years ago, we produced strain 1295-22 (also known as KRL-AG2) of *T. harzianum*, and this organism seemed to have a number of useful attributes. Not only did it have the ability to control disease when properly used, but it also was extremely efficient in colonizing roots. Once established on roots, it persisted for the lifetime of annual crops, and continued to colonize plant roots as they grew. Therefore, all parts of the root system were colonized. On perennial plants, such as turfgrass, the fungus survived on roots even over the cold winters of upstate New York. As a consequence, application of the fungus may substantially increase crop yield and increase root growth.

This fungus and formulations based upon it recently were registered with the U.S. Environmental Protection Agency for plant disease control. It has received an exemption from tolerance for use on food crops, since toxicology testing has shown no observable toxi or pathogenic effects upon plants, mammals or birds. Products based on *Trichoderma harzianum* are manufactured by TGT Inc., Geneva, New York and marketed for tuf applications as Bio-Trek 22G by Wilbur-Ellis Co., Fresno, CA. Bio-Trek is the first EPA-registered biological disease control agent that is available commercially for turfgrass disease control in the United States.

The following is a description of tests and uses of Bio-Trek 22G, and an assessment of our expectations for

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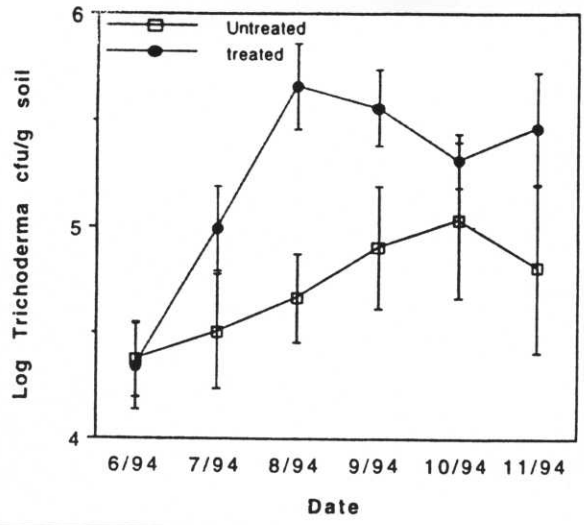

Fig. 1. Colonization of roots and soil of creeping bentgrass in a replicated trial on a creeping bentgrass golf green. Bio-Trek 22G was applied on the dates indicated, and root/soil samples were taken at monthly intervals. Note that *Trichoderma* persisted at a high level throughout the year. The y axis is a log scale, so each number given represents a 10-fold increase in *Trichoderma* levels. For example, log 5 represents 100,000 viable propagules of the fungus, and log 6 represents 1,000,000. The increase in *Trichoderma* levels in the nontreated plots over time probably is a consequence of spread of *T. harzianum* from adjacent treated plots. Data is from Lo, C-T, Nelson, E. B., and Harman, G. E. 1996. Improving the biocontrol efficacy of Trichoderma harzianum 1295-22 for controlling foliar phases of turf diseases by spray applications. Plant Dis. (in preparation)

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future development. It is also a story of the translation of basic biological findings at a university into commercial applications.

We first tested a granular formulation very similar to Bio-Trek 22G in 1990 and found that it reduced dollar spot incidence. Trials since that date in the Northeast, Midwest and Far West have demonstrated continued efficacy. A critical aspect of this product's efficacy is the ability of *T. harzianum* to establish itself at effective levels on turfgrass roots. We expect that it can be established by one or two applications in the spring and that it will then persist over the following growing season. Data on establishment from our 1994 trials are given in **Fig. 1** Two applications early in the year were sufficient to provide high levels of colonization of soil and creeping bentgrass roots that persisted at high levels throughout the year. We sampled this same area in August, 1995, and found *T. harzanium* at levels about 10 times higher than in adjacent turf areas. This indicates survival times of the biocontrol agent on turf roots for more than a year. However, in 1995, *T. harzanium* populations had dropped to levels necessitating another application. The cultivars used in these field trials were Penncross and Cobra.

This establishment of a biocontrol fungus resulted in reduced severity of several diseases. Dollar spot data are shown for 1993 and 1994 **(Fig. 2)**, while control of brown patch and Pythium root rot are shown for 1994 **(Fig. 3)**. In 1993, brown patch and Pythium were not evident.

Another benefit of Bio-Trek 22G was also evident in our trials. The product was applied in June and July, but in November the treated areas were easily recognizable; the plots that had received the granular formulation of *T. harzanium* were greener than adjacent plots. This turf had not been fertilized after mid-summer, so the enhanced color may have reheated the ability of a more vigorous root system to provide better uptake of nutrients. This improved color persisted into 1995, and was still evident in August of that year, even though no additional *T. harzanium* had been applied to those areas.

These data indicate that Bio-Trek 22G can be a useful product for turf disease management. Its advantages are:

■ It provides a means of establishing *T. harzanium* in soil and on roots, thereby providing a means of restoring beneficial microbes in turf soils.

■ It reduces the level of disease organisms in soil, and so initial disease levels will be lower once the biocontrol fungus is established.

■ It is nontoxic and nonpolluting, but has good persistence, so its beneficial effects can persist over an extended time period.

Based on our experience with both turf and other crops, it can enhance root health and growth. However, there are some things that this biological agent cannot do, and these limitations must be recognized as well. These are:

■ Bio-Trek 22G is applied to soil, and the beneficial fungus becomes established in roots and soil. Therefore, it cannot control foliar diseases, or foliar diseases spread rapidly and are favored by frequent mowing and watering, so chemical fungicide sprays will have to be used to control them.

■ *T. harzanium* is a living organism that must become established in soil and on roots of turf to be effective. However, some chemical fungicides are lethal to *T. harzanium*, and if possible they should not be used in conjunction with Bio-Trek 22G. A list of fungicides and their compatibility/incompatibility with *T. harzanium* is given in **Table 1.**

■ In addition, like all living organisms, *T. harzanium* will be more active under some conditions than others. In particular, it will not be effective when soil temperatures are below 50°F. However, it survives on roots and becomes active when soils warm. It is most effective at soil temp-

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eratures between 70°F and 90°F.

The first commercial *T. harzanium* products were available for sale in 1995. These were granular formulations designed for broadcast application. Efforts to evaluate these products in our lab were primarily concerned with assessing the level of establishment of the fungus on roots. Roots from sites around the USA indicated that establishment did occur.

There were some problems, however. First, the product was formulated for multiple uses and was quite dusty. This made broadcast application difficult. Second, while the product was quite effective for many applications, we found that transfer of the fungus from the granule to the roots was not as effective with broadcast application as it was when the granules were directly incorporated into soil. As a consequence, even though the fungus did become established, in some cases its population level remained at suboptimal levels.

Therefore, in 1996, TGT Inc. will formulate Bio-Trek 22G specifically for broadcast application to turf, and its properties will be different from the general use material. The turf product will have a larger particle size to facilitate broadcast application, the dust level will be substantially reduced and its concentration of *T. harzanium* will be higher to enhance root and soil colonization. We expect that this product will be effective for its intended uses.

The Development of Future Technologies

Bio-Trek 22G is highly useful but, as noted above, it has limitations. Most notably, since the product is applied to the soil and the fungus is located in the root-soil zone, it cannot protect against foliar pathogens. With this factor in mind, we have begun testing a spray formulation that consists primarily of conidia (spores of the fungus). The first trials, conducted in 1994, were successful. Levels of control were equivalent to standard chemical fungicides for brown patch, dollar spot, and Pythium root rot and blight (**Fig. 4**) when a surfacant (Triton X-100) was included in the spray mixture.

When disease pressure was light, a monthly spray schedule sufficed, but applications had to be increased to once a week when disease was more severe. As a bonus, this spray application resulted in root colonization that was nearly as effective as the granular product. These results give promise of a largely biological turf management option, but problems remain.

Difficulties were evident when we attempted to apply the 1994 findings to commercial golf course trials in 1995. Little or no efficacy was obtained; this problem appears at least in part to be related to toxic fungicide residues in the spray tank. As the biocontrol agent was suspended in tanks that have been used repeatedly to apply fungicides, some factor, probably low levels of residual pesticides, prevented spores of the fungus from germinating.

Other problems also remain. Technologies for large-scale manufacture of sprayable biological formulations at a reasonable cost are not full developed, and so only prototype preparations are available now. Further, *T. harzanium* is useful only as a preventative application and cannot cure existing disease. Of course, like all materials available to golf course managers, this fungus will not be effective against all diseases. These last two factors indicate a need for the development of integrated biological-chemical control systems that reduce the need for chemical fungicides.

Research efforts at Cornell University will focus on the development of spray formulations for commercial golf

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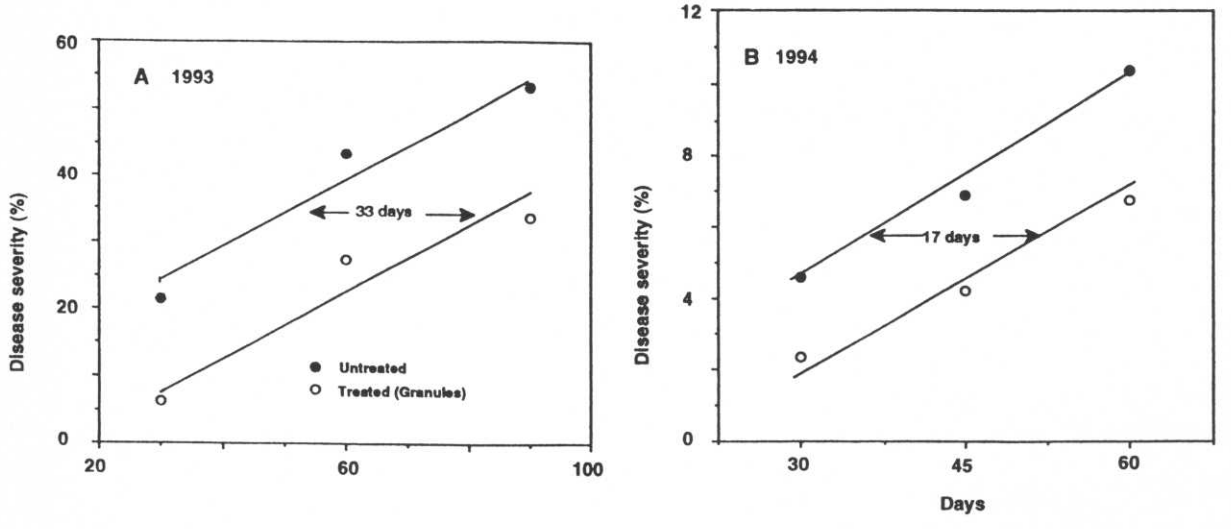

Fig. 2. Disease progress of dollar spot in replicated trials in 1993 and 1994 in the presence and absence of Bio-Trek 22G. Lines were fitted using the general models program (SAS, Cary, NC). Disease severity is defined as the percentage of total plot area with diseased turf. The differences between treatments and time required to reach specific disease levels were significantly different. These data are used with permission of the American Phytopathological Society, and are from Lo, C-T, Nelson, E. B., and Harman, G. E. 1996. Control of turfgrass diseases with a rhizosphere competent strain of *Trichoderma harzianum*. Plant Dis. (accepted for publication with revision).

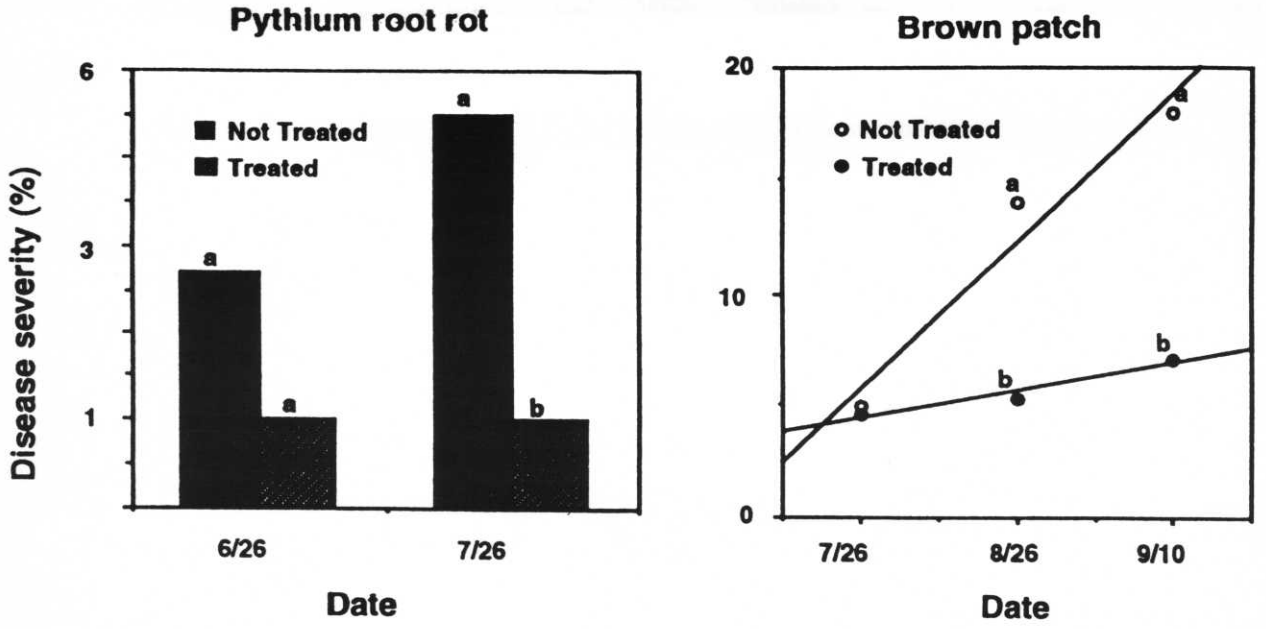

Fig. 3. Incidence of Pythium root rot and brown patch in replicated plots on a creeping bentgrass green after no treatment and treatment with Bio-Trek 22G. Disease severity is defined as the percentage of total plot area with diseased turf. Numbers followed by dissimilar letters are significantly different for the date shown. Data is from Lo, C-T, Nelson, E. B., and Harman, G. E. 1996. Improving the biocontrol efficacy of *Trichoderma harzianum* 1295-22 for controlling foliar phases of turf diseases by spray applications. Plant Dis. (in preparation).

Table 1. Fungicides compatible or incompatible with Bio-Trek 22G

Compatible fungicides	Incompatible fungicides
Chloroneb(e.g. Chloroneb, Terreneb)	Benomyl (e.g. Tersan 1991)
Etridiazole(e.g. Koban, Terrazole)	Propiconazole (Banner)
Iprodione (e.g. Chipco 26019)	
Mancozeb (e.g. Fore)	Questionable, or no data
Metalaxyl (e.g. Subdue)	Anilazene (e.g. Dryene)
Quitozene(e.g. PCNB, Terrachlor)	Chlorothalonil (e.g. Daconil 2787)
Triadimefon (e.g. Bayleton)	Fenarimol (e.g. Rubigan, Lesco Twosome)
Vinclozolin (e.g. Vorlan, Touche, Curalan)	Thiram (e.g. Spotrete)
Fosetyl Al (e.g. (Aliette)	Thiophanate methyl (e.g. Clearys 3336,Fungo)

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courses. We will determine which chemicals cannot be used in sprayers employed for *T. harzanium* application, and attempt to devise methods for removal of the most important toxic materials. We anticipate that only a few of the incompatible materials in **Table 1** will cause most of the problems. At least, we should be able to make recommendations regarding fungicides to be avoided.

We will also test prototype commercial products and develop full dosage information for them. And we will determine spray adjuvants, primarily spreader/sticker materials, that provide the best results with *T. harzanium*.

With this information, we will develop recommendations for using *T. harzanium* that will be tested on golf courses and other commercial sites. In addition, we will investigate develoment of integrated sprays that combine reduced rates of a compatible fungicide with the beneficial fungus. An integrated biological-chemical system may lessen fungicidal use, provide some of the curative ability of fungicides, result in root colonization of *T. harzanium*, establish diverse microbial soil populations that promote

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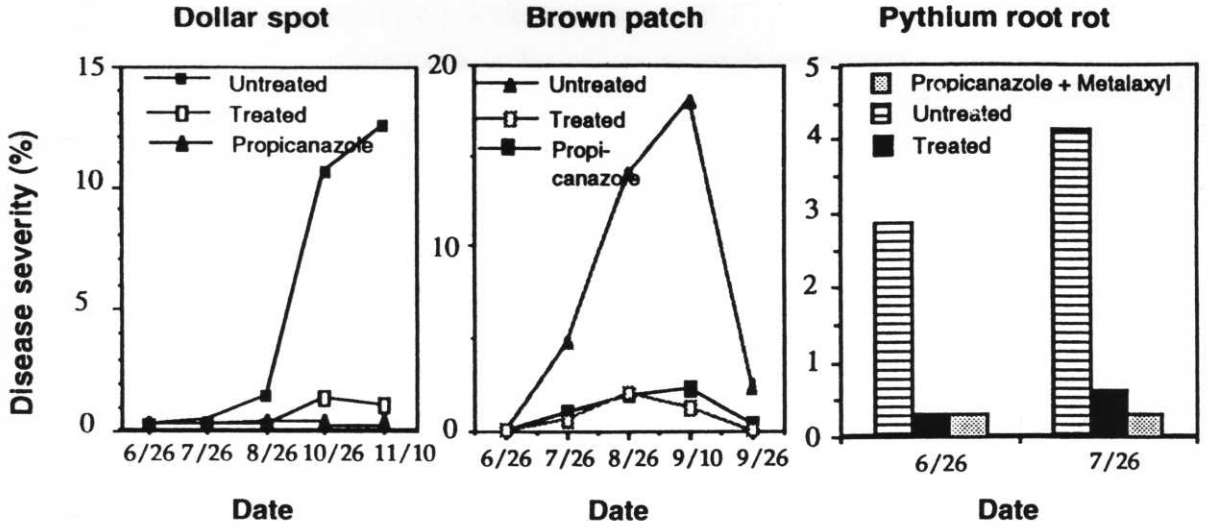

Fig. 4. Severity of dollar spot, brown patch, or Pythium root rot in replicated plots on a creeping bentgrass green after no treatment, treatment with a standard fungicide, or treatment with sprays containing spores of *T. harzianum* and Triton X-100. Disease severity is defined as the percentage of total plot area with diseased turf. *T. harzianum* treatments were applied monthly until July 26, and thereafter weekly. In all cases, the disease severity in nontreated plots was significantly different from treated plots, but fungicide and *T. harzianum* treatments were not significantly different. Data is from Lo, C-T, Nelson, E. B., and Harman, G. E. 1996. Improving the biocontrol efficacy of *Trichoderma harzianum* 1295-22 for controlling foliar phases of turf diseases by spray applications. Plant Dis. (in preparation).

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plant health, and be competitively priced. We hope to begin research scale trials of both full biological and biolgocial-chemical control systems this summer.

In Summary

The first registered biological control product, Bio-Trek 22G, for the control of turf diseases is now available. This product contains a strain of the beneficial soil fungus, *Trichoderma harzanium*, and is designed for broadcast application to turf. The fungus becomes established on the roots and in the soil of turf and persists for months after application. Once establishement occurs, it can become a component of a healthy soil microbial community and reduce soilborne disease. It cannot control foliar diseases, however, and therefore must be used in conjunction with compatible fungicides. We anticipate that Bio-Trek 22G will be the first of several biological products for turf disease control. Other biological and integrated biological chemical control products products will be manufactured by TGT that will extend the usefulness of Bio-Trek 22G.

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