

Advances in the Biological Control of Turfgrass Diseases

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In July 2001, the International Turfgrass Society (ITS) met in Toronto, Ontario. Numerous research papers involving turfgrass pathology were presented and published in the ITS Research Journal. In this issue of Turfax, a summary of two ITS papers dealing with biological control are reviewed.

Dr. Gary Yuen and coworkers at the University of Nebraska reported on the mechanism of leaf spot (*Bipolaris sorokiniana*) control with the bacterium *Stenotrophomonas maltophilia* strain C-3. **Biological agents suppress diseases either by (a) antagonism and competition, (b) parasitism of the pathogen, or (c) triggering natural host defenses.**

Antagonism involves the production of antibiotics by an agent that inhibit growth or outright kills sensitive microbes (sometimes called antibiosis) or they outcompete pathogens for nutrients and space. **Antagonism is perhaps the most common mechanism of control by biological agents. C-3 was shown to provide control of leaf spot in tall fescue (*Festuca arundinacea*) by both antagonism and induction of host resistance.** C-3 produces an enzyme called chitinase. Most fungi have cell walls composed of chitin (see Polyoxin D article in Turfax 9(4)). In the case of C-3, the chitinase inhibits cell wall production in germinating spores. The Nebraska researchers also found strong evidence that C-3 triggers the plants' own natural defense mechanism on leaves, but not roots. They worked with both live and heat-killed C-3 cells. Live cells provided better disease control than heat-killed cells. The application of C-3 cells to any portion of a tall fescue leaf inhibited the germination of *B. sorokiniana* spores on the entire surface of the same leaf. Hence, placement of C-3 cells on just the leaf tips resulted in a reduction of spore germination on lower segments of the same leaf that was not treated with C-3. The phenomenon, however, only occurred on leaves treated with C-3 and therefore, the effect was not systemic. The nature of induced host resistance is imperfectly understood. Evidently, the presence of C-3 cells on a leaf sends a signal to the plant that the leaf is about to be infected by a pathogen. The signal is believed to be elicited by substances exuded by a microbe on the surface of the plant. The cells in the leaf respond by producing specialized cells that inhibit infection or the cells may produce antifungal chemicals known as phy-

toalexins. Phytoalexins are phenolic compounds that are toxic to or inhibit the growth of a pathogen. These compounds may be preformed or induced as a result of infection by specialized cells. Other lab and field studies conducted by Yuen and coworkers showed that C-3 suspensions reduced the level of blighting by *Rhizoctonia solani* (i.e., brown patch) in both tall fescue and perennial ryegrass (*Lolium perenne*). Hence, unlike most biological agents, which often target one pathogen, C-3 shows promise for controlling at least two diseases.

Davis and Dernoeden reported on their four-year study involving the Bioject Biological Management System[®] (Bioject) (Eco-Soil Systems, San Diego, CA). The Bioject automatically ferments and distributes disease suppressive bacteria onto turf and soil through the irrigation system. **The bacterium evaluated was *Pseudomonas aureofaciens* strain Tx-1 (Tx-1).** Tx-1 was developed by Dr. Joseph Vargas Jr. and coworkers at Michigan State University. Vargas and coworkers showed that Tx-1 effectively suppressed dollar spot (*Sclerotinia homoeocarpa*) in lab and field studies (Powell and Vargas, 2001). They found that the mechanism of disease suppression was the production of phenazine-1 carboxylic acid (PCA) by Tx-1. The PCA is an inhibitor (i.e., antibiosis) of fungal growth and hence, the mechanism of action of Tx-1 is antagonism.

The Bioject first appeared on golf courses in large numbers around 1995. The initial system consisted of a plastic fermentation tank, which was hooked-up to the irrigation system in the pump house. Tx-1 was added to the tank only a few times per month and the tank had to be manually cleaned each time new Tx-1 was added. In 1996, a prototype of the Bioject was evaluated at the University of Maryland. The prototype failed to ferment and deliver high populations of Tx-1. It was shown that Tx-1 could live only a few days in the plastic tank and it was clear that potable water was required. Use of pond water introduces countless numbers of other bacteria, which outcompete Tx-1 for nutrients. **There appears to be no possibility of using pond water in the Bioject for delivering high populations of a bacterial biological agent.**

In 1997, Eco-Soil Systems introduced a much more sophisticated system. Tx-1 in the new system is stored in

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a refrigerated unit. Tx-1 and nutrients are automatically dispensed into a stainless steel fermentation tank on a daily basis. This newer unit is equipped with a daily cleaning mechanism and a UV light filter to kill most bacteria entering the system from a potable water source. This new unit was tested in Maryland in 1997. It was a tremendous improvement in fermentation, but populations of Tx-1 delivered again were too low to provide any suppression of dollar spot. During the 1997–1998 winter, scientists from Eco-Soil Systems worked with us to improve the fermentation capabilities of the Bioject. The amounts of nutrients and Tx-1 metered into the fermentation tank were adjusted and high populations of Tx-1 were produced.

In 1998 and 1999, the Bioject again was field-tested. The research involved monitoring the number of colony forming units (cfu) of Tx-1 fermented and delivered through the irrigation heads. The number of Tx-1 cfu's found in the foliage plus thatch and soil were quantified. The Tx-1 was delivered to four blocks of turf daily at 8:00 P.M. (20:00 h). Each block was split in half, with Crenshaw creeping bentgrass (*Agrostis stolonifera*) grown on one-half and SR 7000 (*A. tenuis*) grown on the other half. The blocks were divided into seven subplots consisting of three fungicides, three nutrient supplement treatments and a control. There were four identical control blocks, which received irrigation water without Tx-1. **The Crenshaw is very susceptible to dollar spot and the SR 7200 is very susceptible to brown patch.** Both diseases developed naturally and uniformly across the study areas.

Results from 1998 and 1999 showed that the Bioject effectively fermented and delivered high populations of Tx-1 to the foliage plus thatch (average = 40 million cfu's per gram of tissue) and soil (average = 340,000 cfu's per gram soil). The Tx-1 also was shown to survive the winter in low populations in the foliage plus thatch and the soil. **Dollar spot was suppressed on average of 30% over both years.** There were dates in June and July of each year when Tx-1 was shown to provide dramatic reductions in dollar spot during high disease pressure periods. The level of dollar spot suppression provided by Tx-1 was not improved in subplots receiving ammonium sulfate (0.2 lb N/1000 ft² or 0.1 kg•100 m⁻², applied on a 2-week interval throughout the test period) or the 2 other nutrient supplements that were evaluated. **In 1998, but not 1999, Tx-1 was shown to increase the residual effectiveness of Chipco 26GT® (iprodione) and Banner MAXX® (propiconazole) by 7 to 10 days.** These fungicides and Daconil Ultrex® (chlorothalonil) had little effect on the levels of Tx-1 recovered from the turf on soil. **Although some brown patch suppression was noted under low disease pressure in 1998, Tx-1-treated plots**

were more severely blighted than nontreated control plots during moderate to high disease pressure in 1999.

This study showed that the Bioject fermentation and delivery technology works well and demonstrated that Tx-1 could significantly reduce dollar spot. Although the reduction in dollar spot was only 30%, it should be noted that Crenshaw is among the most susceptible cultivars to infection by *S. homoeocarpa*. Ohio researchers observed 50 to 60% dollar spot suppression in Penncross (Han et al., 2000). Hence, where cultivars with greater dollar spot resistance are grown, there can be a significant benefit from using Tx-1. Unfortunately, interest in the Bioject has declined in recent years. The company in a rush to earn revenue, placed unproven technology in the market. Golf course superintendents, as a result, may be hesitant to utilize the system due to some early failures. Eco-Soil Systems provided the Bioject, technical support and some funding for the study. The University of Maryland, however, invested over \$25,000 of its own money to complete this research project. This should be a warning to other scientists not to get involved with costly research projects without up-front funding. **This research provided more evidence that it is unlikely that biological agents will replace the need for fungicides on golf courses. The use of Tx-1 and other biological agents, however, may extend the residual effectiveness of some fungicides and reduce the potential for *S. homoeocarpa* resistant biotypes from developing.** The search for more effective biological agents continues. Extensive field testing should be performed in order to inform end-users the best possible information on their target disease(s) and the levels of control that can be realistically expected. 

References

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