Biological control of dollar spot of golf courses using *Pseudomonas aureofaciens* TX-1 delivered via the BioJect

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Biological control of foliar diseases presents several problems. First and foremost among these is the fact that the leaf surface is generally much less hospitable to biocontrol agents than is the thatch, rootzone or bulk soil. Leaf surfaces dry out during the day and are exposed to UV radiation from the sun. Additionally, leaf surfaces often have limited energy reserves available for supporting or sustaining microbes. Pathogens bypass these difficulties by entering leaves and feeding on plant tissue, but this obviously is not an option for a biocontrol agent. For these reasons, the long-term survival of beneficial microbes sprayed or otherwise introduced on leaves is low. Secondly, even when microbes do survive, they sometimes enter a dormant state. Biocontrol agents usually must be in an active state to suppress disease. For historical reasons, most of the biological control agents presently available and under development were originally isolated from thatch, roots or soil. Very few are natural inhabitants of the foliage. This leads to a need for frequent (at least 3-5 times / week) applications of biocontrol agents to obtain acceptable levels of disease suppression. Such frequent applications, however, are not practical for most real-world turf care applications.

Since we have yet to discover a biocontrol agent that can survive and maintain disease suppressive activity on leaves for an extended period, the short-term future of biological control in the foliage must involve repeated applications of microbes. One method for making such applications on golf courses is the BioJect. The BioJect was developed by Eco Soil Systems of San Diego, CA, as a way to grow a fresh culture of a biocontrol agent daily and distribute it to a golf course through the irrigation water. Typically, a BioJect unit consisting of a tank for growing the biocontrol agent, inoculum and media storage, control units and a pump for injecting the biocontrol agent into the irrigation line will be located in the pumphouse. It is an automated unit which is designed to grow a 25-gallon culture daily, inject it into the irrigation water, then clean and sterilize itself in preparation for the next day's growth cycle. This design is intended to eliminate the need for employees and spray equipment to apply the biocontrol agents and allow frequent (up to daily) applications of microbes.

Currently, the biocontrol agent used for dollar spot control in the BioJect is a bacterium, *Pseudomonas aureofaciens* strain TX-1. TX-1 was isolated from bentgrass thatch and roots and is able to inhibit the growth of the dollar spot fungus, *Sclerotinia homoeocarpa*, in the laboratory. Dwyer and Vargas, who initially isolated TX-1, found that it can also suppress

dollar spot in small-plot studies when applied at least 5 days / week, but not when applied only once / week. This makes TX-1 a good candidate for use in a system like the BioJect, if the BioJect can grow high populations of TX-1 and the irrigation system can deliver it evenly and predictably to turf.

Field testing TX-1 for suppression of dollar spot

A cooperative research project was initiated in 1999 to study the efficacy of TX-1 for dollar spot suppression, both on golf courses and on research plots. Participants in the study included The Ohio State University, Turf Partners / Eco Soil Systems (distributor and manufacturer of the BioJect), and three Ohio golf courses: Double Eagle Club, Sawmill Creek Golf Course and Scioto Country Club. The BioJect system had been in place for at least one season prior to the start of the project at all three golf courses. We established plots to study the efficacy of TX-1 for dollar spot control at the three golf courses and at The Ohio State University / OTF Research and Education Center. In addition, we established plots at the research center to evaluate the effects of varying N fertility and TX-1 concentration on the efficacy of TX-1.

We set out the following four treatments at each golf course and at the OSU research plots: 1) A non treated (no TX-1 or fungicides) control; 2) a fungicide control (no TX-1); 3) a hand-applied, concentrated TX-1 suspension offloaded directly from the BioJect; and, 4) an irrigation system-delivered TX-1 treatment (at the OSU research plots, this was a dilute TX-1 suspension sprayed by hand). Polystyrene sheets, set out every night by the golf course staff / interns, covered the control plots (#1 and #2) to keep irrigation water containing TX-1 off the turf. Plots #1 and #2 were hand-watered as needed during the day. Plots #1, #3 and #4 were covered during fungicide treatments.

The plots were moved to different locations on the same fairways in mid-July to both provide a replication of the experiment in time and to save the turf from loss due to dollar spot. At the OSU research plots, the same effect was accomplished by spraying the plots with a rescue application of iprodione at the curative label rate. Dollar spot severity was assessed by placing a string grid over plots and counting the number of symptomatic squares. The percent diseased turf was then calculated.

Results of field tests

Similar results were obtained from the trials at Double Eagle, Scioto and the OSU research plots. Unfortunately, dollar spot did not develop in the plots at Sawmill Creek. At both Double Eagle and Scioto, treatment with hand-sprayed TX-1 did - on some rating dates - reduce the amount of dollar spot observed. This reduction was much more pronounced at Scioto than at Double Eagle. At Scioto, dollar spot severity was reduced from about 60% to

20-30% of turf diseased. At Double Eagle, dollar spot was more severe both in the control plots and in the TX-1 plots. There was also suppression of dollar spot by irrigation-applied TX-1 on one rating date at Double Eagle and though the early summer at Scioto. There was no significant difference between hand-sprayed and irrigation-applied TX-1 at Scioto or at Double Eagle for most of the study. At both golf courses, fungicide sprays provided much greater suppression of dollar spot than TX-1, reducing the diseased area to 0-5%. These trends were observed both before and after the plots were moved at Double Eagle. At Scioto, very little dollar spot developed in the plots after they were moved.

Fungicide also provided the best dollar spot control at the OSU research plots. However, TX-1 did reduce the severity of dollar spot under certain conditions. Nitrogen fertilizer application also affected efficacy of TX-1 at the research plots. TX-1 had no effect on dollar spot severity in June and July on plots fertilized with 1 or 2 lbs N / 1000 ft^2. However, TX-1 did reduce dollar spot severity slightly on plots fertilized with 4 lbs N / 1000 ft^2. After the cleanup fungicide application on the plots, dollar spot did redevelop on the 1 lb N / 1000 ft^2 plots and TX-1 (both concentrated and dilute sprays) was observed to reduce dollar spot. Dollar spot pressure was much less in September than in June and July due to environmental conditions that were more favorable to grass growth and recovery. In June and July, high temperatures over 85 F limited grass growth and night temperatures in the 60s F provided good growing conditions for the dollar spot fungus. In September, daytime highs were cool enough (60s F) to allow good bentgrass growth.

Finally, we tested four different concentrations of TX-1 at the research plots to see if there would be any effect on dollar spot suppression. However, at the concentrations we used (10^5 - 10^8 bacteria / ml), there was no difference in dollar spot severity among the varying concentrations.

More questions than answers

We observed some suppression of dollar spot with the use of TX-1, but this suppression was never as complete as that provided by traditional fungicides. Whether a reduction in turf affected by dollar spot from, for example, 60% down to 20-30% is an acceptable level of control will depend on an individual course's considerations. Many golf courses have a "threshold" level of dollar spot which is acceptable. If this threshold is exceeded, then fairways must be sprayed with fungicide. The key question is whether TX-1 reduces dollar spot below the spray threshold. Although TX-1 delivered through the BioJect may be able to reduce dollar spot compared to no treatment, it is not a useful management tool if it cannot reduce dollar spot to an acceptable level.

Other factors which could affect the efficacy of TX-1 include the populations of TX-1

grown in the BioJect and the distribution of the bacteria through irrigation water. Since the BioJect tank contents are injected into a very large volume of water, the highest possible population of TX-1 in the BioJect is desired. The population of TX-1 in the BioJect tank at Double Eagle averaged 5 x 10^6 CFU / ml (CFU = colony forming units, a single cell or small group of cells which land on an agar plate and give rise to a colony large enough to be counted). The population at Scioto averaged 2 x 10^6 CFU / ml. These populations are not very large for liquid cultures of bacteria. In contrast, the population of TX-1 in the BioJect at the OSU research plots was 500 x 10^6 CFU / ml. We don't know what the effects of dilution might have been, but we do know that the irrigation events used to deliver TX-1 at Double Eagle and Scioto used approximately 80,000 and 30,000 gallons of water, respectively. This led to a large dilution factor, as evidenced by counts of TX-1 in the irrigation water at Double Eagle, which ranged from none found to a maximum of 1.1 x 10^4 CFU / ml, with water at most of the irrigation heads tested having populations in the 5-10 x 10^2 CFU / ml range.

Additionally, there is the challenge of distributing TX-1 evenly through a golf course. To address this question, we added Blazon, a spray pattern indicator, to irrigation water and ran the BioJect irrigation cycle to visually track the blue color as it came out of the irrigation heads. At Double Eagle, the two Blazon tests run by the staff covered the back nine holes only. However, they indicated that the color indicated that material injected into the water at the pumphouse could be spread through at least half of the golf course, although it took several minutes for Blazon to reach some fairways. In contrast, three holes at Scioto never received Blazon in two tests there, and the intensity and timing of the blue color on other fairways was variable. Clearly, we are a long way from understanding how water and bacteria move through golf course irrigation systems.

Summary and Conclusions

- TX-1 is not a stand-alone dollar spot control or replacement for proper fungicide use. Although TX-1 could reduce the severity of dollar spot compared to no treatment, its suppression of dollar spot was limited. Fungicides provided complete or near-complete control.
- Under conditions of high disease pressure and poor potential grass recovery, TX-1 was not an effective dollar spot management tool. At both Double Eagle and the OSU research plots, when dollar spot severity on the controls reached very high levels (70 - 80% of the turf area diseased), TX-1 treatments failed to suppress dollar spot as well as when pressure was mild.
- TX-1 was more effective on turf treated with 4 lbs N / 1000 ft^2 than with 1 or 2 lbs N / 1000 ft^2. Fertility requirements should be considered when deciding whether to use

TX-1.

- TX-1 and the BioJect should be tools to consider when designing a dollar spot management program, but not the only management used. Each superintendent will need to weigh the possible benefits of TX-1 and the BioJect against cost, expected disease suppression and acceptable disease thresholds before deciding whether or not to make it a part of an integrated turf health management program.

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