Evaluation of *Pseudomonas aureofaciens* Strain Tx-1 for Disease Control in Fairways

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Objective

This two-year study focused on determining the efficacy of *P. aureofaciens* Tx-1 applied via the BioJect System for dollar spot and brown patch control and if control is affected by fungicide regime and/or nitrogen regime.

Rationale

The use of biological disease control products is a popular idea due to the public's ongoing concern for safety and the environment. The integration of a "natural", biological disease control could double as a public relations tool as well as a disease control tool. *Pseudomonas aureofaciens* strain Tx-1 may be one such biological control. It was isolated at Michigan State University and was found to be antagonistic to fungal turfgrass pathogens in laboratory plate bioassays. Field studies conducted at Michigan State have also shown effective reduction of dollar spot when *P. aureofaciens* was applied three times per week at a rate of 1.3×10^8 cfu per sq. in. Frequent application over a broad area has become possible with the advent of a bioreactor/injection system. This type of system allows the daily culture of antagonistic bacteria followed by injection into an irrigation system. Our project is designed to evaluate the efficacy of *P. aureofaciens* applied through an irrigation system in combination with various fertility and fungicidal management strategies to reduce dollar spot and brown patch.

How It Was Done

The study was located at the Wm. H. Daniel Turfgrass Research and Diagnostic Center at Purdue University, West Lafayette IN. The soil type was Starks-Fincastle silt loam with pH of 7.0, 163 lbs P/A, and 534 lbs K/A. Separate but adjacent areas were used to evaluate dollar spot and brown patch. The area was previously in corn and this was the initial turf planting on the site established during August and September of 1997. 'Crenshaw' creeping bentgrass was used for the dollar spot evaluation and 'Astoria' colonial bentgrass and 'Fiesta' perennial ryegrass were use for the brown patch evaluation. The bentgrasses were mowed at 5/8 inch with the clippings returned and the ryegrass was mowed at 3/4 inch with the clippings returned.

Treatments included a 2 X 2 X 4 factorial with two levels of *P. aureofaciens* application (none and nightly), two nitrogen fertility regimes and four fungicide programs (Table 1). Experimental design was a split block with three replications. Main plots differentiated *P. aureofaciens* application while the combinations of fungicide strategies and fertility rates made up the subplots (6.5 ft x 6.5 ft).

***P. aureofaciens* strain Tx-1** was incubated in the BioJect system each day and then injected into the irrigation system each evening after dusk during a two-minute/zone syringe cycle (0.05 inch of water). Additional irrigation was done in the morning only as needed to prevent drought stress. Bacterial injection was initiated in mid-May 1998 and ran through mid-Sep 1998 and repeated during the same time in 1999. To reduce bacterial application to the control plots, injected

and non-injected irrigation zones were supplied through separate piping and tarps were used to cover the non-injected plots while the injected irrigation system was running.

Nitrogen treatments consisted of two regimes representing either a typical golf course fertilization program or a program to encourage dollar spot or brown patch. For dollar spot, the low nitrogen regime included 0.5 lbs N per 1000 sq. ft. applied mid-May and 1.0 lbs N per 1000 sq. ft. applied early Sep. and early Nov. The high nitrogen regime included 0.5 lbs N per 1000 sq. ft. applied mid-May, -June, and -July, and 1.5 lbs N per 1000 sq. ft. applied early Sep. early Nov. For brown patch, the low nitrogen regime included 0.5 lbs N per 1000 sq. ft. applied mid-May, -June, and –July, and 1.0 lbs N per 1000 sq. ft. applied early Sep. and early Nov. The high nitrogen regime consisted of 1.0 lbs N per 1000 sq. ft. applied mid-May, mid-June, early July, early Sep., and early Nov. Nitrogen treatments were applied with shaker bottles through the summer in the form of sulfur coated urea with a final November application of urea.

Fungicide treatments consisted of a preventative program, a curative program, another biocontrol and none (Table 1). Fungicide applications were made with a CO_2 backpack sprayer in 4 gal H_2O/1000 sq. ft.. The preventative program consisted of Daconil Weatherstik (4 oz per 1000 sq. ft.) and Banner Maxx (2 oz per 1000 sq. ft.) for each disease, which were alternately applied on a set schedule. Preventative fungicidal applications began in mid- of each year for dollar spot and late June in each year for brown patch. The curative program consisted of Daconil Weatherstik (4 oz per 1000 sq. ft.) applied across both injected and non-injected plots after each dollar spot epidemic or, for brown patch, when the disease severity rating reached 1.5 on the Horsfall-Barratt scale. Trichoderma, BioTrek 22G, was applied with shaker bottles as two spring applications of 1.5 lbs of product/1000 sq. ft.

Samples were collected weekly for Tx-1 enumeration. A sample was collected directly from the bioreactor just prior to the injection cycle. Irrigation water was collected from the injected irrigation zones by placing an autoclaved catch pan on the turf surface immediately before the injection/irrigation cycle. Samples were stored at 35 ± 2^0F for 10 hours before enumeration. *P. aureofaciens* concentrations were determined through plate counts with three replicate plates per sample utilizing $1/10^{th}$ trypticase soy agar as the plating medium supplemented with rifampicin and cycloheximide each at 50 micrograms/ml. Dilution blanks consisted of $1/10^{th}$ Ringer solution. Visual quality of plots was rated on a scale of 1 to 9 where 1=dead turf, 5=acceptable as a fairway, and 9=excellent. Color was rated visually on a scale of 1 to 9 where 1=dead turf, 5=acceptable green for a fairway, and 9=dark green. Disease incidence for brown patch was rated using the Horsfall-Barratt system consisting of 1 to 10 where 1=1 to 3% affected turf, 2=3 to 6%,3=6 to 12%, 4=12 to 25%, 5=25 to 50%, 6=50 to 75%, 7=75 to 87%, 8=87 to 93%, 9=93 to 96, 10=96 to 100%. This disease severity rating was then converted back to a percentage before statistical analysis. Disease incidence for dollar spot was rated either as number of infection centers per plot or a percent affected turf. Data was analyzed with SAS.

Results

Reactor bacterial counts ranged from low 10^7cfu/ml to low 10^9 cfu/ml. Bacterial counts in the irrigation were generally low 10^6 to mid 10^7 cfu/ml and were fairly uniform across the area on most sampling dates. Of the 258 nightly applications in 1998 and 1999, 25 nights were missed due to windy conditions, irrigation supply, and/or human error whereas 15 nights were missed due to the bioreactor, most shortly after initiation of the bioreactor in 1998.

Dollar Spot on 'Crenshaw' Creeping Bentgrass. The application of *P. aureofaciens* when averaged across all N and fungicide treatments reduced dollar spot on two of the 27 rating dates in 1998 and 1999. *P. aureofaciens* is much more effective when N fertility is high. In 1998, *P. aureofaciens* reduced dollar spot in the high N regime compared to the low N regime on four dates. In 1999, *P. aureofaciens* increased the dollar spot severity at the low N rate on five dates in 1999. There were no significant interactions in disease severity between fungicide strategies and the application of *P. aureofaciens.* However, there was a general trend toward a reduced dollar spot severity due to *P. aureofaciens* when curative fungicide applications were made. BioTrek had no effect on dollar spot.

Brown Patch on 'Astoria' Colonial Bentgrass or 'Feista' Perennial Ryegrass. *P. aureofaciens* applications had little and/or inconsistent effect on brown patch severity regardless of N regime and/or fungicide control strategy. BioTrek had no effect on brown patch.

Summary

Two years of disease control studies were completed at Purdue University evaluating the efficacy of *P. aureofaciens* applied through the BioJect System (via irrigation) in combination with various fertility and fungicidal management strategies to reduce dollar spot and brown patch. Our results indicate that *Pseudomonas aureofaciens* applied through the BioJect System will reduce dollar spot to a minimal extent, only during low disease pressure. However, nitrogen regime plays a significant role in the efficacy of *P. aureofaciens* as a control for dollar spot as increased nitrogen improves the efficacy. *P. aureofaciens* did not increase or decrease the efficacy of fungicide control during either season. The application of *P. aureofaciens* applied through the BioJect System had little and/or inconsistent effects on brown patch severity with no consistent trend throughout the studies on colonial bentgrass or perennial ryegrass.

Table 1. Treatments used in Study 1 to evaluate *P. aureofaciens* for control of dollar spot and brown patch in 1998 and 1999.

Disease	*P. aureofaciens* Application	Nitrogen Fertility (lbs N per 1000 sq. ft. per yr)	Fungicide Programs
Dollar Spot	Nightly	2.5	None
	None	4.5	Curative Daconil Weatherstik 4 oz per 1000 sq. ft. Preventative Daconil Weatherstik 4 oz per 1000 sq. ft. (14 days) Banner 2 oz per 1000 sq. ft. (21 days) BioTrek 22G - 1.5 lbs per 1000 sq. ft. mid-April and start of May
Brown Patch	Nightly	3	None
	None	5	Curative Daconil Weatherstik 4 oz. per 1000 sq. ft. Preventative Daconil Weatherstik 4 oz per 1000 sq. ft. (14 days) Banner 2 oz per 1000 sq. ft. (14 days) BioTrek 22G - 1.5 lbs per 1000 sq. ft. mid-April and start of May