Application of *Pseudomonas aureofaciens* Tx-1 through Irrigation for Control of Dollar Spot and Brown Patch on Fairway-height Turf

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Abstract. *Pseudomonas aureofaciens* strain Tx-1 is suggested as a biological control for *Sclerotinia homoeocarpa* (E.T. Bennett) and brown patch (*Rhizoctonia solani* Kuhn) on golf courses. To overcome application difficulties, a field bioreactor is used to grow Tx-1 daily and then inject into nightly irrigation on the golf course. Though Tx-1 shows some promise for disease control in vitro, it is relatively untested under field conditions. We conducted three field experiments to 1) evaluate the efficacy of Tx-1 when applied through an irrigation system for the control of dollar spot and brown patch; 2) determine if there is an interaction between nitrogen fertility or fungicides on efficacy of Tx-1; and 3) determine if Tx-1 can extend the duration of dollar spot control by a single application of fungicide. Nightly applications of Tx-1 through irrigation did not affect brown patch on ‘Astoria’ colonial bentgrass (*Agrostis capillaris* Sibth.) during 2 years of our study. Tx-1 reduced dollar spot in ‘Crenshaw’ creeping bentgrass (*Agrostis palustris* Huds.) by 37% in 1998 compared to non-Tx-1 treatments, but Tx-1 had no effect on dollar spot in 1999. Under low disease pressure, Tx-1 increased the dollar spot control of fungicides by 32% and increased the duration of control up to 2.6 days. However, Tx-1 had no effect on fungicide efficacy or duration of control later in the summer when dollar spot pressure was high. Fungicides did not negatively affect Tx-1’s control of brown patch or dollar spot, nor did fertilizer regime affect brown patch or dollar spot control by Tx-1. Although delivery of Tx-1 in our studies was optimized, disease control was marginal and occurred only under low disease pressure. Therefore, we conclude Tx-1 has limited practical value for turfgrass disease control on golf courses.

Dollar spot (caused by *Sclerotinia homoeocarpa* F.T. Bennett) and brown patch (caused by *Rhizoctonia solani* Kuhn) are destructive diseases of both cool- and warm-season turfgrasses (Burpee and Martin, 1992; Walsh et al., 1999). These diseases are controlled culturally and with fungicides (Fidanza, and Dernoeden, 1996a; Fidanza and Dernoeden, 1996b; Settle et al., 2001; Walsh et al., 1999). However, over-reliance on fungicides may lead to evolution of insensitive strains (Golembiewski et al., 1995). Biological disease control may help minimize reliance on fungicides by turfgrass managers.

Biological disease control is attempted by introducing disease suppressive antagonists or by manipulating the activity of antagonistic organisms already present in soils or on plant parts (Smiley et al., 1992). Disease suppressive organisms have been identified and isolated in a variety of crops including turfgrass (Cruz et al., 1992; Hodges et al., 1994; Lo and Nelson, 1996; Nelson and Craft, 1991; Qing and Shipp, 2000; Thomspson et al., 1996). *Trichoderma harzianum* is one biological control that has been identified and used for control of disease in turfgrass (Harmon, 2000). Although many disease-suppressive microorganisms have been isolated, their ability to suppress disease relates to colonization and persistence in sufficient numbers (Bull et al., 1991; Parke, 1990; Paulitz and Baker, 1987). Efficacy of an introduced organism suffers unless it can occupy a specific niche, proliferate, and produce an antagonistic response (Nelson et al., 1994). Colonization and persistence problems may be overcome by repeated applications of the antagonistic organism. This is the basis of the BioJect system (Eco Soil Systems Inc., San Diego, Calif.), which is a field bioreactor that grows a population of bacteria daily and then injects the culture into nightly irrigation. This delivery system reduces the labor demand of repeated sprays, supplies freshly grown bacteria, and allows nighttime application of the bacteria, which reduces viability losses due to desiccation and UV radiation. However, delivery and efficacy of this system is relatively untested.

*Pseudomonas aureofaciens* strain Tx-1 (Tx-1) is a commercially available biofungicide marketed under the trade name Spot-less Biofungicide (Eco Soil Systems Inc.) and is used in conjunction with the BioJect system. Tx-1 is known to produce the phenazine antibiotic phenazine-1-carboxylic acid (PCA), which is primarily responsible for disease suppressive activity (Pierson and Thomashow, 1992; Powell and Vargas, 2000). PCA inhibits growth of several turfgrass pathogens in vitro, including *R. solani* and *S. homoeocarpa* and suppressed dollar spot development in greenhouse and field trials (Powell and Vargas, 2000).

Three separate experiments were initiated to 1) evaluate the efficacy of Tx-1 when applied through an irrigation system for the control of dollar spot and brown patch; 2) determine if there is an interaction between nitrogen fertility or fungicides on efficacy of Tx-1; and 3) determine if Tx-1 can extend dollar spot control by a single application of fungicide.

Materials and Methods

Tx-1, fertilization regime, and fungicides for control of brown patch or dollar spot. Field studies were conducted at the Wm. H. Daniel Turfgrass Research and Diagnostic Center at Purdue University, West Lafayette Ind., during 1998 and 1999. Soil type was Starks-Fincastle silt loam (fine-silty, mesic Aeric Ochraqualf) with pH of 7.0, 183 kg ha⁻¹ P, and 598 kg ha⁻¹ K. The area was established in Aug. 1997. The experimental area was mowed at 1.3 cm three times per week with clippings returned. A fresh culture of Tx-1 was grown in a field bioreactor (Eco Soil Systems, San Diego, Calif.) daily and injected into the irrigation system each evening beginning at 2100 h during a two-minute watering cycle delivering 0.13 cm of water. The small-pilot irrigation system was plumbed separately into 11 m x 11 m zones to eliminate cross contamination of Tx-1 into non-injected water. To further reduce contamination, plots not receiving Tx-1 were covered with vinyl tarps during irrigation of the plots receiving Tx-1 injected water. Tarps were immediately removed and plots not receiving Tx-1 were irrigated with fresh water. Additional morning irrigation was applied to all plots as needed to prevent drought stress. In 1998, Tx-1 was applied from 14 May through 20 Sept. with reactor production averaging 5.8 x 10⁸ colony forming units (CFU)/mL and injected plot irrigation averaging 1.6 x 10⁷ CFU/mL. In 1999, Tx-1 was applied from 15 May through 19 Sept. with reactor production averaging 6.9 x 10⁹ CFU/mL and injected plot irrigation averaging 2.6 x 10⁸ CFU/mL. Tx-1 concentrations were determined by sampling the field reactor and irrigation weekly. Samples were taken directly from the bioreactor immediately before the irrigation cycle whereas irrigation water was sampled from each main plot with an autoclaved catch pan on the turf surface during the entire irrigation cycle. Samples were stored at 2 ± 1 °C for 10 h before enumeration. Tx-1 concentrations were determined through plate counts with three replicate plates per sample using 1/10th trypticase soy agar as the plating medium supplemented with rifampicin and cycloheximide each at 50 µg mL⁻¹. Dilution blanks consisted of 25 % Ringer solution (Weaver et al., 1994).

Brown patch. Colonial bentgrass (*Agrostis capillaris* Sibth.) ‘Astoria’ was used for this study. Treatments were arranged in a 2 x 2 x 4 factorial with two Tx-1 treatments (nighly and none),
two fertilizer regimes (high N and low N), and four fungicide programs (none, preventative, curative and *Trichoderma harzianum*). The low N regime included 24 kg·ha⁻¹ N applied 14 May and 11 June and 49 kg·ha⁻¹ N applied 3 Sept. and 12 Nov. each year. The high N regime included 49 kg N/ha applied 14 May, 11 June, 9 July, 3 Sept. and 12 Nov. in both years. Nitrogen was applied manually with shaker bottles as sulfur coated urea except in November when urea was applied. The preventative fungicide treatment began 25 June during both years and consisted of chlorothalonil (tetrachloroisophthalonitrile) at 9.1 kg·ha⁻¹ alternated with propiconazole (1-[2-[2,4-dichlorophenyl]-4-propyl-1,3-di-oxolan-2-yl]-1H-1,2,4-triazole) at 1.0 kg·ha⁻¹ applied every two weeks. A post-symptom fungicide treatment (curative) consisted of chlorothalonil at 9.1 kg·ha⁻¹ when the disease severity rating reached 1.5 on the Horsfall-Barratt scale, which was equivalent to three percent infection (Campbell and Madden, 1990). Curative applications were made on 8 July, 30 July, and 20 Aug. during 1998 and 20 July during 1999. All fungicide applications were made with a CO₂ backpack sprayer in 1630 L·ha⁻¹ water. A commercial preparation of *Trichoderma harzianum* (Bio-Trek 22G, Wilbur-Ellis, Fresno, Calif.) was applied manually with shaker bottles as two May applications per year with 73 kg·ha⁻¹ product per label recommendations.

Experimental design was a split plot with Tx-1 treatments as main plots while subplot 566 spots per plot was greater than 15. The preventative fungicide treatment applied when the average number of spots per plot was >15. The preventative applications were made on 8 July, 30 July, 12 Aug. and 2 Sept. during 1998 and 20 June, 31 June and 17 Aug. during 1999. The site was inoculated with a sorghum culture of a local isolate of *Sclerotinia homeocarpa* on 29 June 1998 as described for the brown patch experiments. Disease severity during 1998 was recorded weekly as the number of dollar spots per plot. Due to rapid increase of disease in 1999, dollar spot severity was recorded twice weekly as a visual percentage of affected turf. Disease severity data were used to calculate AUDPC and then standardized by dividing by the length of the epidemic in days (Campbell and Madden 1990). AUDPC data were square root transformed as suggested by Box-Cox evaluation (Box et al., 1978). Statistical analyses were performed using procedures by SAS.

### Table 1. Effects of Tx-1, nitrogen, and fungicide application on brown patch in ‘Astoria’ colonial bentgrass.

| Fungicide program          | 1998 (Mean AUDPC) | 1999 (Mean AUDPC) |
|----------------------------|------------------|------------------|
|                            | +Tx-1 | -Tx-1 | Mean | +Tx-1 | -Tx-1 | Mean |
| Preventive⁴                 | 2.7   | 2.2   | 2.4  | 1.9   | 1.9   | 1.9  |
| Curative⁴                  | 3.6   | 2.3   | 2.9  | 2.6   | 3.5   | 3.0  |
| *T. harzianum*             | 9.0   | 7.7   | 8.3  | 6.5   | 7.1   | 6.8  |
| None                       | 6.5   | 8.5   | 7.4  | 5.6   | 7.9   | 6.7  |
| Mean                       | 4.9   | 4.3   | 4.4  | 3.7   | 4.4   | 4.4  |
| N treatment (kg·ha⁻¹·yr⁻¹) |       |       |      |       |       |      |
| 146                        | 5.5   | 3.5   | 4.4  | 4.2   | 5.2   | 4.7  |
| 244                        | 4.3   | 6.2   | 4.7  | 3.2   | 3.7   | 3.4  |
| Mean                       | 4.9   | 4.3   | 4.4  | 3.7   | 4.4   | 4.4  |

### Table 2. Effects of Tx-1, nitrogen and fungicide application on dollar spot in ‘Crenshaw’ creeping bentgrass.

| Fungicide program          | 1998 (Mean AUDPC) | 1999 (Mean AUDPC) |
|----------------------------|------------------|------------------|
|                            | +Tx-1 | -Tx-1 | Mean | +Tx-1 | -Tx-1 | Mean |
| Preventive⁴                | 7.3 ab | 3.2 a | 5.1 a | 0.0 | 0.0 | 0.0 a  |
| Curative⁴                 | 10.2 ab | 16.2 b | 13.0 a | 15.4 | 19.6 | 17.5 b |
| *T. harzianum*             | 56.0 c | 92.7 d | 73.3 b | 61.9 | 60.5 | 61.2 d |
| None                       | 53.6 c | 109.2 d | 78.9 b | 52.6 | 54.0 | 53.3 c |
| Mean                       | 26.8 a | 42.0 c | 32.7 b | 22.7 | 24.2 |      |
| N treatment (kg·ha⁻¹·yr⁻¹) |       |       |      |       |       |      |
| 122                        | 43.0  | 50.8  | 47.3 | 27.9 | 27.2 | 27.6 |
| 220                        | 13.8  | 33.9  | 22.8 | 18.1 | 21.3 | 19.6 |
| Mean                       | 26.8  | 42.0  | 32.7 | 22.7 | 24.2 |      |

### Notes:

- AUDPC values were standardized by dividing by the duration of the disease epidemic in days and then transformed by log10(AUDPC). Backtransformed values are presented.
- The preventative fungicide treatment began 25 June during both years and consisted of chlorothalonil at 9.1 kg·ha⁻¹ and propiconazole at 1.0 kg·ha⁻¹ applied alternately on a 2-week schedule.
- The curative treatment consisted of chlorothalonil at 9.1 kg·ha⁻¹ and was scheduled when the disease severity rating reached 1.5 on the Horsfall-Barratt scale.
- *Trichoderma harzianum* (Bio-Trek 22G, Wilbur-Ellis, Fresno, Calif.) at 73 kg·ha⁻¹ product applied twice in May each year.

### Statistical Analyses

- *p* = 0.05, 0.01, or 0.001 not significant, respectively.
- NS, *, **, *** Nonsignificant or significant at *p* = 0.05, 0.01, or 0.001 not significant, respectively.
Efficacy of Tx-1 for extending dollar spot control by various fungicides. The experimental site was adjacent to the previously described studies and was established with ‘Penneagle’ creeping bentgrass in August 1997. The area was maintained at 1.3 cm with clippings returned and fertilized with 24 kg·ha⁻¹ N in May, 49 kg·ha⁻¹ N in Sept., and 73 kg·ha⁻¹ N in Nov. Treatments were in a 2 × 8 factorial with two levels of P. aureofaciens application (none and nightly) and eight fungicide treatments. Fungicide treatments were applied once with a CO₂ backpack sprayer in 1630 L·ha⁻¹ H₂O. Treatments included an untreated check, propiconazole at 0.3 kg·ha⁻¹, iprodione (3-[3,5-dichloro-phenyl]-N-[1-methylthyl]-2,4-dioxo-1-imidazolidine-carboxamide) at 2.3 kg·ha⁻¹, thiophanate-methyl (dimethyl[[1,2-phenylenebis[iminocarbonothioyl]]biscarbamate] at 3.1 kg·ha⁻¹, mancozeb (Mn + Zn + ethylene bisthiocarbamate) at 14.6 kg·ha⁻¹, chlorothalonil at 6.8 kg·ha⁻¹, trifloxystrobin ([E,E]-alpha-[methoxyimino]-2,2′-[1-[3-trifluoro-1H-phenyl]phenyl]ethyldieneaminooxy]methyl thyl]methyl ester) at 0.3 kg·ha⁻¹ and a tank mix of propiconazole at 0.3 kg·ha⁻¹ plus 0.3 kg·ha⁻¹ trifloxystrobin .

Experimental design was a split block with Tx-1 treatment as main plots and fungicide treatments as subplots (1.5 m × 1.5 m). Main plots were replicated three times and the experiment was conducted four times during the summer of 1999 beginning on 2 June, 23 June, 14 July, and 4 Aug. All four trials were conducted on separate but adjacent areas, and dollar spot was minimized with chlorothalonil at 9.1 kg·ha⁻¹ applied seven days before the start of each trial. Culture, application, and sampling of Tx-1 as well as plot size and irrigation zones were consistent with that described previously. The application of Tx-1 began on 2 June across all trial areas and continued throughout the study. Bioreactor production averaged 7.5 × 10⁸ CFU·mL⁻¹ and injected plot irrigation averaged 2.2 × 10⁸ CFU·mL⁻¹.

Dollar spot disease severity was recorded twice weekly as the number of dollar spots per plot up to 300. AUDPC data were calculated using eight ratings in all trials. Days to 50 infection centers/plot threshold were determined by interpolating disease severity data for each plot, and this threshold would be considered commercially unacceptable on fairway height turf. AUDPC data were transformed to log₁₀(Y + 1) for further analysis with one being added to the raw Y value to prevent the occurrence of log₁₀(0) (Box et al., 1978). Analysis of variance was performed on the transformed AUDPC data with PROC ANOVA, and threshold data was analyzed using PROC GLM.

### Results and Discussion

Tx-1, fertilization regime, and fungicides for control of brown patch. Brown patch symptoms were present in 1998 from 13 July to 27 July and again from 17 Aug. through 31 Aug. During 1999, brown patch symptoms were present 20 July through 26 July and recurred on 30 Aug. Tx-1 did not significantly affect AUDPC in either year (Table 1). This was similar to our preliminary studies where Tx-1 at concentrations of 2 × 10⁴, 2 × 10⁶ and 2 × 10⁸ CFU·mL⁻¹ did not suppress brown patch development in a growth chamber (Hardebeck, 2001). Although Tx-1 produces PCA which inhibits R. solani in vitro (Powell and Vargas, 2000), our studies indicate Tx-1 has little effect on brown patch development in the field. Previous work in our laboratory had shown occurrence of PCA in the irrigation water (Sigler et al., 1999), thus the Tx-1 was active in our studies. Davis and Dernoeden (2001) also

### Table 3. Effect of Tx-1 and fungicides on dollar spot in ‘Penneagle’ creeping bentgrass.

| Fungicide (F) | Early June | Late June | July | August |
|--------------|------------|-----------|------|--------|
|              | +Tx-1      | -Tx-1 Mean| +Tx-1 | -Tx-1 Mean| +Tx-1 | -Tx-1 Mean| +Tx-1 | -Tx-1 Mean|
| Untreated control | 1036 | 1818 | 1372 | 3009 | 3244 | 3124 | 5687 | 5703 | 5695 | 3446 | 3520 | 3483 |
| Propiconazole | 83 | 228 | 138 b | 415 | 364 | 388 ab | 532 | 712 | 615 b | 1065 | 1361 | 1204 |
| Iprodione | 120 | 210 | 159 b | 505 | 351 | 421 ab | 1951 | 1618 | 1777 c | 985 | 1084 | 1033 |
| Thiophanate-methyl | 10 | 19 | 14 a | 558 | 108 | 246 a | 197 | 241 | 218 a | 295 | 373 | 332 a |
| Mancozeb | 1054 | 1292 | 1167 c | 549 | 2603 | 1166 bc | 5654 | 5093 | 5366 d | 3190 | 3407 | 3297 d |
| Chlorothalonil | 626 | 904 | 752 cd | 1404 | 2663 | 1934 c | 3611 | 4036 | 3817 d | 2450 | 2499 | 2474 d |
| Trifloxystrobin | 586 | 650 | 617 c | 2879 | 2248 | 2544 c | 4970 | 4693 | 4829 d | 2273 | 3023 | 2622 d |
| Trifloxystrobin + propiconazole | 83 | 106 | 94 b | 344 | 335 | 339 a | 996 | 2014 | 1416 c | 594 | 660 | 626 b |

| Mean | 208 | 330 | 848 | 835 | 1814 | 2047 | 1353 | 1548 |

ANOVA

| Fungicide (F) | * | NS | *** | *** | *** | NS | NS |
| Tx-1 | * | NS | *** | *** | *** | NS | NS |

*Means are backtransformed from log₁₀(AUDPC + 1). Backtransformed values are presented.

**Means within each column followed by the same letter are not significantly different at P = 0.05.

### Table 4. Effect of Tx-1 and fungicides on days to reach 50 dollar spot infection centers/plot in ‘Penneagle’ creeping bentgrass.

| Fungicide (F) | Early June | Late June | July | August |
|--------------|------------|-----------|------|--------|
|              | +Tx-1      | -Tx-1 Mean| +Tx-1 | -Tx-1 Mean| +Tx-1 | -Tx-1 Mean| +Tx-1 | -Tx-1 Mean|
| Untreated control | 16.3 | 13.7 | 15.0 a | 6.3 | 5.7 | 6.0 a | 4.3 | 3.0 | 3.7 a | 11.3 | 10.0 | 10.7 a |
| Propiconazole | NA | NA | NA | 23.3 | 23.3 | 23.3 c | 20.7 | 18.7 | 19.7 c | 19.7 | 18.7 | 19.2 c |
| Iprodione | 30.3 | 25.3 | 27.8 c | 22.3 | 23.7 | 25.0 c | 14.7 | 16.0 | 15.3 c | 20.3 | 20.0 | 20.2 cd |
| Thiophanate-methyl | NA | NA | NA | 19.3 | 24.4 | 21.9 c | 30.7 | 27.7 | 29.2 d | 23.7 | 24.0 | 23.8 e |
| Mancozeb | 17.0 | 18.3 | 17.7 ab | 6.9 | 9.0 | 8.0 ab | 5.0 | 5.7 | 5.3 ab | 12.7 | 10.7 | 11.7 a |
| Chlorothalonil | 21.0 | 19.7 | 20.3 b | 14.7 | 9.6 | 12.2 b | 9.3 | 7.7 | 8.5 b | 15.3 | 14.3 | 14.8 b |
| Trifloxystrobin | 19.3 | 19.7 | 19.5 b | 10.3 | 11.0 | 10.7 b | 6.0 | 6.7 | 6.3 ab | 16.0 | 14.3 | 15.2 b |
| Trifloxystrobin + propiconazole | 33.5 | 25.0 | 29.3 c | 24.0 | 23.7 | 23.8 c | 18.0 | 14.3 | 16.3 c | 21.3 | 21.3 | 21.3 d |

| Mean | 22.9 | 20.3 | 15.9 | 16.3 | 13.6 | 12.5 | 17.5 | 16.7 |

ANOVA

| Fungicide (F) | * | NS | *** | *** | *** | NS | NS |
| Tx-1 | * | NS | *** | *** | *** | NS | NS |

*Means within each column followed by the same letter are not significantly different at P = 0.05.

NA = not applicable; 50 infection centers/plot threshold was not reached during study.

**Means within each column followed by the same letter are not significantly different at P = 0.05, 0.01, or 0.001 not significant, respectively.
reported sporadic results with Tx-1 on brown patch in their field studies where Tx-1 reduced brown patch on two rating dates in the first year of their study under low disease pressure, only to increase brown patch the following year under high disease pressure. Nitrogen main effects were not significant on AUDPC in either year of this study, even though higher N regimes reportedly increase brown patch severity (Smiley et al., 1992). Preventative and curative fungicide treatments reduced AUDPC in both years compared to both Trichoderma harzianum and no fungicides.

**Tx-1, fertilization regime, and fungicides for control of dollar spot**. Dollar spot symptoms appeared on 22 June 1998 and developed steadily through 14 Sept. In 1999, symptoms appeared 20 July, progressed rapidly and remained through 6 Sept. The main effect of Tx-1 was significant in 1998 when AUDPC was reduced 37% in Tx-1-treated plots compared to Tx-1 non-treated plots (Table 2). However, Tx-1 had no effect on AUDPC in 1999. As expected, the preventative and curative fungicide treatments provided lowest AUDPC during both years, reducing AUDPC up to 94% compared to the no fungicide treatment. Trichoderma harzianum application resulted in 13% higher AUDPC than the no fungicide treatment in 1999. Tx-1 × fungicide interaction occurred only in 1998 where AUDPC was reduced up to 51% on plots receiving Tx-1 plus either Trichoderma harzianum or no fungicide, compared to the same treatments without Tx-1.

As expected, higher N regime reduced AUDPC during both years of our study (Smiley et al., 1992; Williams et al., 1996). However, there was no interaction between Tx-1 and nitrogen levels. This agrees with Davis and Dernoeden (2001) who found nutrient supplements did not increase dollar spot control with Tx-1. Fidanza, M.A. and P.H. Dernoeden (2001) who found nutrient supplementation did not increase dollar spot control with Trichoderma harzianum plus either fungicide, and no fungicide compared to the same treatments without Tx-1. Our studies indicate Tx-1 applied via the Bioject through an irrigation system is capable of marginally reducing dollar spot severity and extending fungicidal control to a minimal extent only when disease pressure is low. Our field research was done under ideal Tx-1 delivery conditions. We irrigated less than 0.3 ha with a new, well-functioning irrigation system with irrigation heads a maximum of 70 m from the Tx-1 source. However, golf courses will irrigate 12 ha or more with irrigation systems complicates Tx-1 delivery, dilutes Tx-1 concentration delivered to the turf, and likely decreases efficacy. Therefore, we conclude Tx-1 has limited practical value for disease control on golf course turf.

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