Trichoderma harzianum as a biocontrol for Dollar spot disease (*Sclerotinia homoeocarpa*) on creeping bentgrass turf

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The objective of this research project was to determine the efficacy of formulations of *Trichoderma harzianum* on dollarspot disease and general performance of creeping bentgrass turf maintained as a putting green.

Data collected included the disease incidence and disease severity as well as any directs effects on health of turfgrass plants

MATERIALS/METHODS

The trial included five treatments (Table 1). An untreated check was included. Treatments were applied to 1 x 2 m plots of creeping bentgrass turf maintained as putting green at the Guelph Turfgrass Institute (mowing at 4 mm, irrigation to prevent stress – Figure 1). Treatments were replicated four times in a randomized complete block design.

Figure 1. Plot areas on creeping bentgrass soil green, September 28, 2010. Round 1 (top); Round 2 (bottom).

Inoculum of the disease organism (*S. homoeocarpa*) was prepared by growing several strains of the fungus on autoclaved Kentucky bluegrass seed. Inoculum was to be applied to the turfgrass at 5 g m^{-2} during June, July, and August, when dollarspot disease is prevalent. Relative humidity in the inoculated turf was kept high by irrigation, to stimulate disease development. Because of heavy natural infection of dollarspot on the first set of plots, inoculum was not added. Because of this, and because of some scalping that occurred on the first set, a second set of plots was set up in an adjacent area on the green, and the treatments applied with dollarspot inoculation.

Treatments in the first round of plots began July 13, 2010, and in the second round on September 9, 2010.

Disease incidence and severity was assessed by visual ratings of damage and point quadrat measurement of disease area. Response of the turf to treatments was assessed both visually and using instrumental color (canopy reflectance). Uniformity of the color response was assessed visually. Plots were rated regularly for turf quality, density and uniformity. Other stresses were measured as they occurred (disease, weed, drought). Winter survival and spring greenup will be assessed in April 2011.

All data was analysed statistically using the SAS package of statistical software.

An anecdotal photographic record was kept of the progress of the trial.

RESULTS

Environmental data. Rainfall and temperature data were recorded at the Environment Canada weather station in the research ranges at the GTI (Figures 2 and 3). The season was wetter than

Table 1. Treatments

1- Negative control: untreated

2- Positive control: preventive fungicide program (Daconil Ultrex 115 g 100 m^{-2} biweekly)

3- PlantClean/SolClean complete program:
First application of PlantClean (200mL/100m2)
2 weeks later: application of SolClean (500mL/100m2)
3 weeks later: application of PlantClean (50mL/100m2) This last application of PlantClean is repeated every three weeks until mid-October

4- PlantClean/SolClean modified program (without initial application of PlantClean):
First application of SolClean (500mL/100m2)
3 weeks later: application of PlantClean (50mL/100m2) This last application of PlantClean is repeated every three weeks until mid-October

5- PlantClean/SolClean intensive program (with higher application rates of PlantClean in July and August):
First application of PlantClean (200mL/100m2)
2 weeks later: application of SolClean (500mL/100m2)
3 weeks later: application of PlantClean (50mL/100m2)
3 weeks later: application of PlantClean (100mL/100m2) during the months of July and August, i.e. in the critical period for dollar spot establishment This last application of PlantClean is repeated every three weeks until mid-October

Application dates, round 1 and 2

Treatment	July		August			September			October		
Round 1	13	27	3	11	25	9	14	21	7	20	27
2	⋆	⋆		⋆	⋆	⋆		⋆	⋆	⋆	
3	⋆	⋆	⋆		⋆		⋆		⋆		⋆
4	⋆		⋆		⋆		⋆		⋆		⋆
5	⋆	⋆	⋆		⋆		⋆		⋆		⋆
Round 2[1]											
2						⋆	⋆		⋆	⋆	⋆
3						⋆	⋆	⋆		⋆	
4						⋆		⋆		⋆	
5						⋆	⋆	⋆		⋆	

[1]Dollar spot inoculum added to all plots in round 2 on September 9, 2010

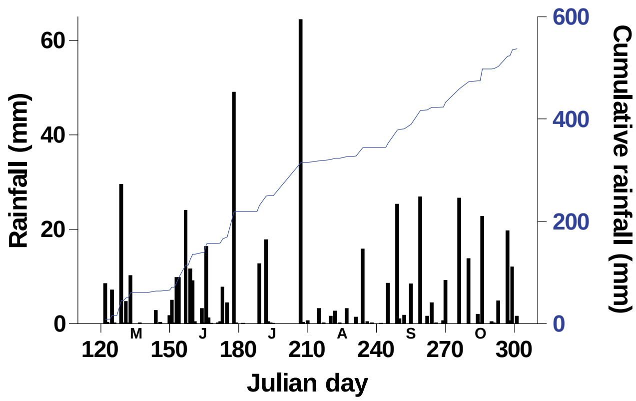


Figure 2. Daily and cumulative precipitation – summer 2010 Data are from the Environment Canada weather station at the GTI.

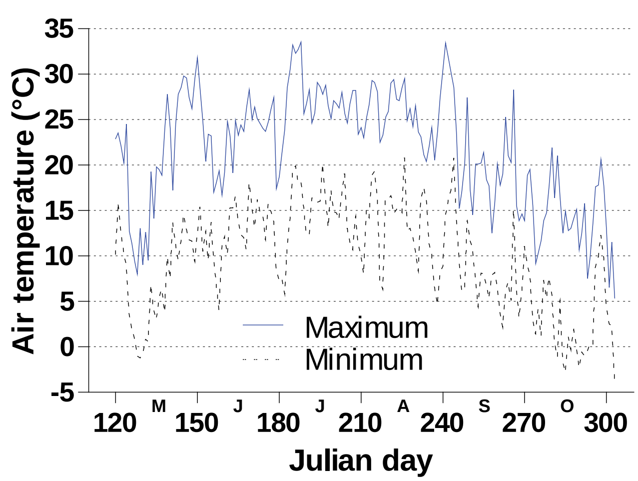


Figure 3. Daily maximum and minimum air temperatures – summer 2010. Data are from the Environment Canada weather station at the GTI.

average, with ~500 mm of rainfall during the course of the experiment. Temperatures were slightly below normal for summer in Guelph, with only four days above 30°C.

Turf performance – canopy reflectance. There were significant differences among the treatments for normalized-difference vegetation index on all measured dates both for Round 1 (Table 2) and Round 2 (Table 3). In Round 1 scalping that coincided with the beginning of natural dollarspot development in late July-early August caused a decline in canopy reflectance in all treatments. By late August the scalping had healed, but differences in dollarspot pressure continued to be reflected in the canopy reflectance values. The canopy reflectance values corrected for the performance of the untreated controls show strong differences among treatments until the end of the season, largely the result of dollarspot pressure (Figure 4). The differences among canopy reflectance in Round 2 were very slight, though statistically significant.

The relationship between NDVI and number of dollar spot infection centres (Figure 5), dollar spot disease severity ratings (Figure 6) and percent of plot area covered by dollar spot lesions (Figure 7) showed a fairly strong association, indicating that NDVI is a useful

Table 2. Canopy reflectance (normalized-difference vegetation index) in treated plots: Round 1.

Treatment	06/18	07/06	07/12	07/14	07/23	07/27	08/03	08/09
Fungicide	0.566 ab[1]	0.608 b	0.604 b	0.530 c	0.591 b	0.591 c	0.439 ab	0.450 c
Modified	0.570 a	0.604 b	0.600 b	0.539 b	0.595 b	0.602 ab	0.447 a	0.492 a
Intensive	0.566 ab	0.624 a	0.610 a	0.553 a	0.606 a	0.606 a	0.413 c	0.477 ab
Control	0.546 c	0.603 b	0.600 b	0.540 b	0.585 c	0.596 bc	0.421 bc	0.442 c
Complete	0.559 b	0.608 b	0.592 c	0.548 a	0.596 b	0.601 ab	0.436 ab	0.465 bc
msd p=0.05	0.009	0.006	0.006	0.008	0.005	0.008	0.020	0.024

	08/26	09/07	09/14	09/23	09/27	10/15	Season mean
Fungicide	0.543 c	0.569 a	0.556 a	0.612 a	0.639 a	0.627 a	0.563 a
Modified	0.591 a	0.544 b	0.550 a	0.564 b	0.577 b	0.545 b	0.558 b
Intensive	0.577 b	0.514 c	0.529 b	0.545 c	0.562 c	0.531 b	0.548 c
Control	0.540 c	0.498 d	0.518 b	0.543 c	0.562 c	0.538 b	0.535 d
Complete	0.539 c	0.475 e	0.476 c	0.490 d	0.516 d	0.486 c	0.524 e
msd p=0.05	0.010	0.012	0.012	0.013	0.013	0.015	0.005

[1] Normalized- difference vegetation index: mean of ~50 readings x 4 replicates; means within columns followed by the same letter are not significantly different (Tukey's HSD test, p=0.05)

Table 3. Canopy reflectance (normalized-difference vegetation index) in treated plots: Round 2.

Treatment	09/07	09/14	09/23	09/27	10/15
Complete	0.609 b[1]	0.616 d	0.641 ab	0.660 b	0.644 a
Control	0.611 b	0.625 a	0.644 a	0.656 b	0.644 a
Fungicide	0.610 b	0.619 cd	0.639 bc	0.657 b	0.643 a
Intensive	0.609 b	0.623 ab	0.638 c	0.649 c	0.633 b
Modified	0.616 a	0.621 bc	0.643 a	0.664 a	0.634 b
msd p=0.05	0.004	0.003	0.003	0.004	0.004

[1] Normalized- difference vegetation index: mean of ~50 readings x 4 replicates; means within columns followed by the same letter are not significantly different (Tukey's HSD test, p=0.05)

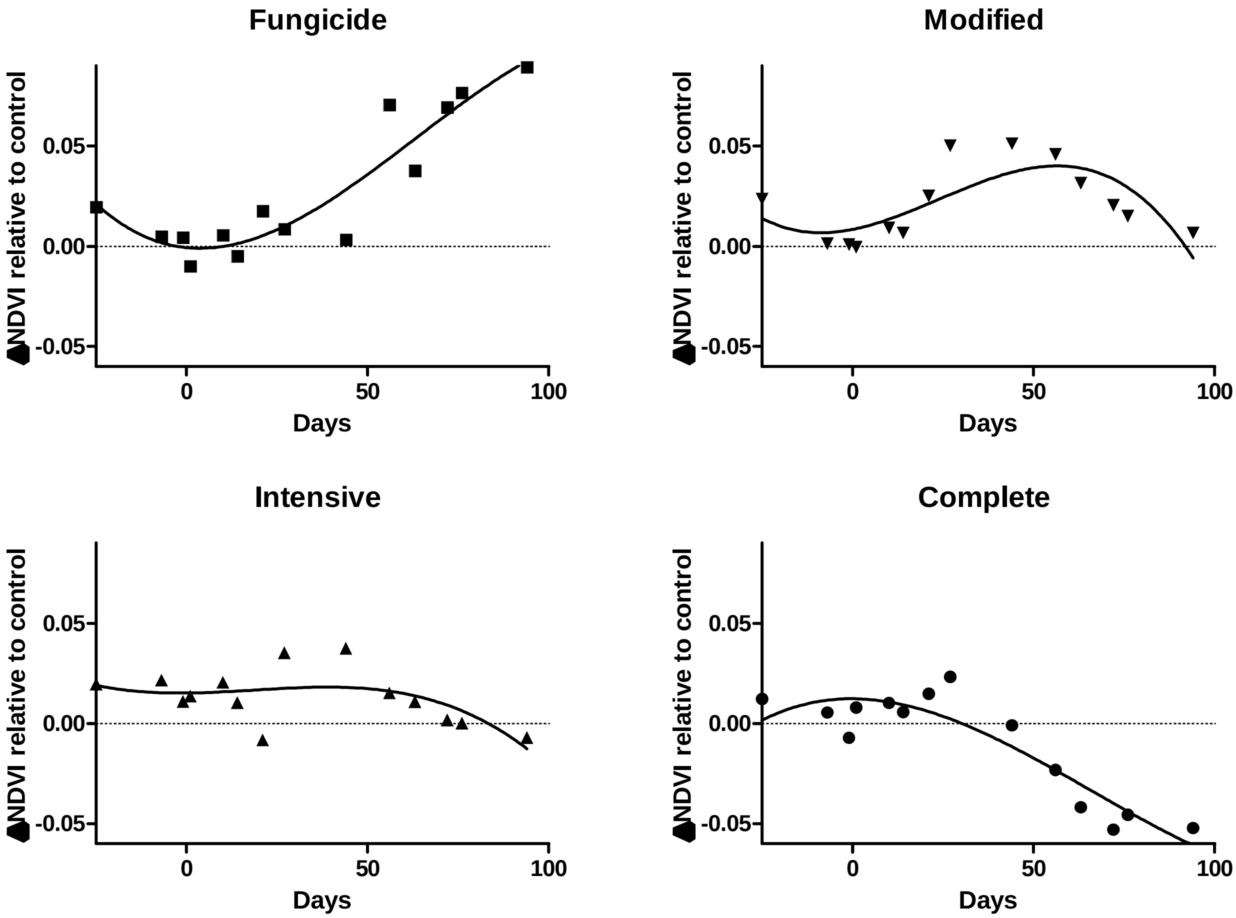


Figure 4. Change in canopy reflectance in Round 1 treated plots relative to untreated control (=0). Cubic polynomial lines are fitted to means. Day 0 is the first treatment application, July 13; scalping damage on the plots had disappeared by mid August (day 30).

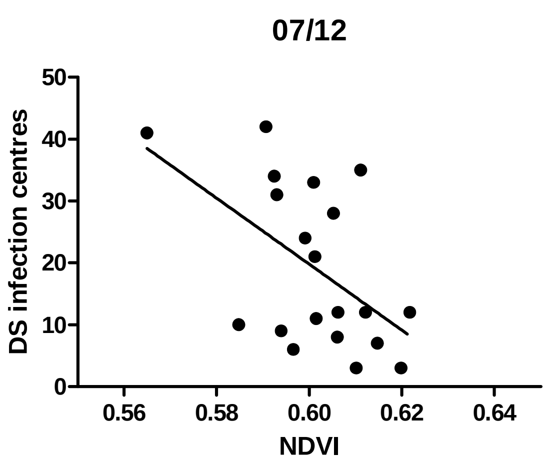


Figure 5. Association between NDVI and counts of dollar spot infection centres. Correlation coefficient is 0.53.

estimator of the effect of dollar spot on turf quality. The limited dollarspot development in Round 2 was detectable in the association between NDVI and infection centre count on October 6 (Figure 8).

Dollarspot disease. Natural dollarspot infection began in mid July in plots of Round 1. Dollarspot disease was assessed initially by counting number of infection centres in plots, then, when the number of centres were too numerous to count or started to overlap, by point quadrat estimation of percent cover, or by visual disease severity rating on a 0-10 scale. The relationship between the various estimates of disease development can be seen in Figure 9. All of the plots developed a heavy dollarspot infection by the end of July (Table 4). The natural infection was reasonably uniformly distributed over the plot area. Differences among the treatments began to appear by the end of July, but they were not

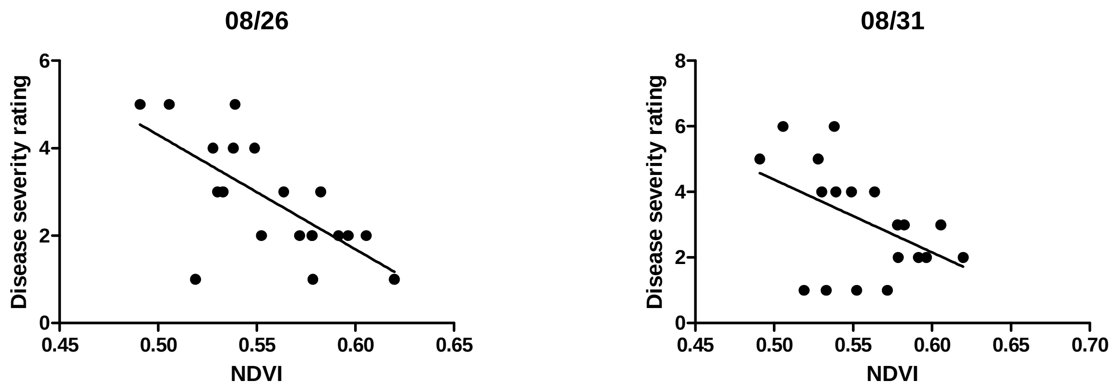


Figure 6. Association between NDVI and dollarspot disease severity ratings. Correlation coefficients are 0.68 (8/26) and 0.47 (08/31).

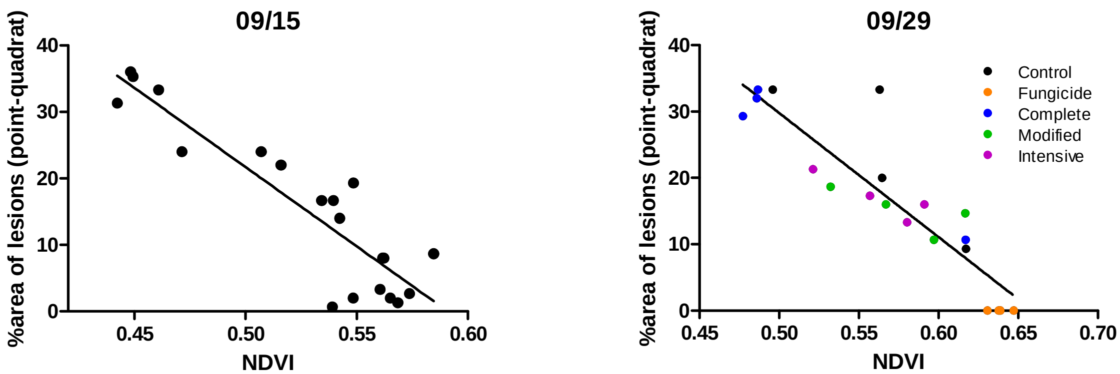


Figure 7. Association between NDVI and percent area covered by dollarspot lesions. Correlation coefficients are 0.91 (9/15) and 0.90 (09/29).

statistically significant until the end of August and early September. The fungicide treatment gave good control of new dollarspot infection; of the other treatments, the intensive and modified treatments showed some reduction relative to the control, but not statistically significant. The complete treatment was not different from the untreated control.

Plots in Round 2, which was inoculated with dollarspot inoculum on Sept. 9, only developed very slight symptoms of dollarspot disease. Infection centre counts were recorded on two dates (Table 5), and there were no significant differences among the treatments (although on the later date, when infection centres were more numerous, the mean number of centres on the fungicide treated plots was lower than the other treatments).

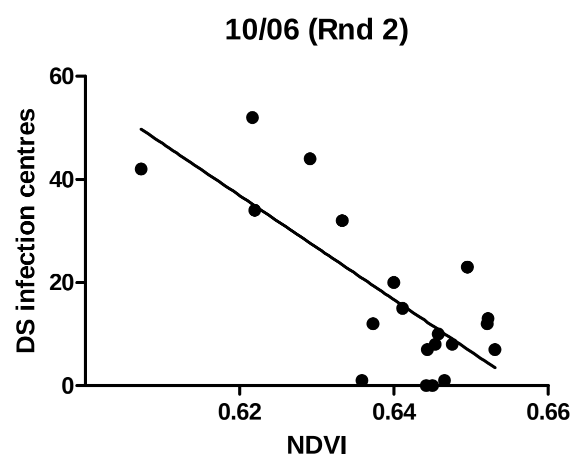


Figure 8. Association between NDVI and counts of dollar spot infection centres (Round 2). Correlation coefficient is 0.76.

Table 4. Dollarspot disease development on treated plots – Round 1.

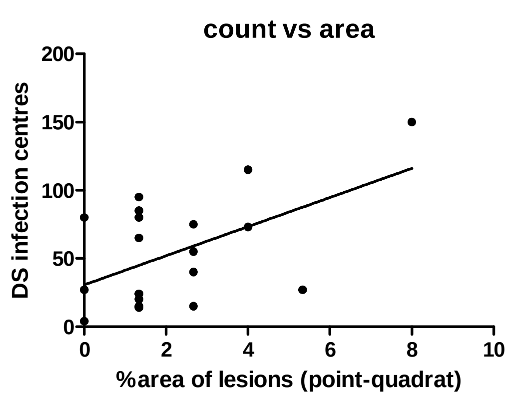
Treatment	Infection centre count 07/12	Infection centre count 07/26	% area (point quadrat 07/23	% area (point quadrat 09/15	% area (point quadrat 09/29	Disease severity 08/26	Disease severity 08/31 (total)	Disease severity 08/31 (active)
Complete	28.8[1]	70.0	2.33[2]	27.00 a	26.33 a	3.75[3]	4.50	3.25 a
Control	24.3	78.0	3.67	20.17 ab	24.00 ab	3.25	4.00	3.25 a
Fungicide	25.0	65.5	1.00	1.50 c	0.00 c	2.00	1.00	0.50 b
Intensive	9.8	37.5	2.33	15.67 b	17.00 ab	3.00	3.25	2.75 a
Modified	7.8	19.8	1.67	13.00 b	15.00 b	2.00	2.75	2.50 ab
msd p=0.05	NS	NS	NS	8.93	10.18	NS	NS	2.03

[1]Count of all separate infection centres in each plot.
[2]Percent area covered by dollar spot lesions (75 points per plot).
[3]Visual disease severity rating 0-10, 0=no disease, 10=full coverage of lesions. Plots were rated on October 31 for total lesions, and for lesions showing active mycelium.
All values are means of four replicates; means within columns followed by the same letter are not significantly different (p=0.05, Tukeys HSD test).

Table 5. Dollarspot disease development on treated plots – Round 2.

Treatment	Infection centre count 09/29	Infection centre count 10/06
Complete	0.3[1]	14.8
Control	0.0	21.0
Fungicide	0.0	0.5
Intensive	0.0	23.0
Modified	0.5	26.0
msd p=0.05	NS	NS

[1]Count of all separate infection centres in each plot. All values are means of four replicates; means within columns followed by the same letter are not significantly different (p=0.05, Tukeys HSD test).



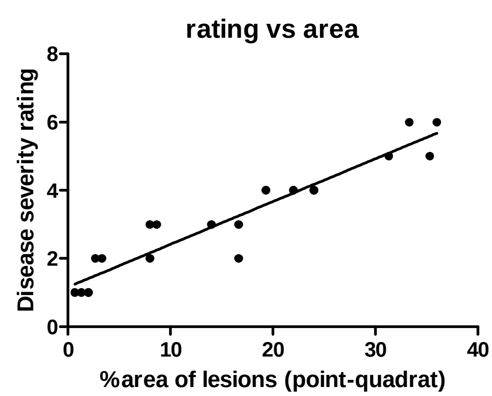


Figure 9. Association between various estimates of dollarspot disease development: (L) infection centre counts (07/26) *vs* percent area (07/23) (R=0.5); (R) severity rating (08/31) *vs* percent area (09/15) (R=0.95).

DISCUSSION AND CONCLUSIONS

There was a significant amount of natural dollarspot disease development on plots in the first round of this trial, and some treatment differences were evident by the end of the season. Based on direct estimates of dollarspot disease, particularly point-quadrat area estimates and severity ratings, the fungicide treatment was the only one to give consistent control compared to the untreated plots. Of the experimental treatments, the modified and intensive treatments were slightly better than the untreated control, which the complete treatment did not differ much from the untreated. The canopy reflectance data, which appeared to be detecting differences based on dollarspot disease, showed a similar pattern, but with statistically significant differences. Because there are other factors that affect canopy reflectance (scalping and other stresses), the NDVI values should be taken as indirect estimates of dollarspot disease.

The second round had only minimal development of dollarspot disease, because it was begun late in the season and after the environmental conditions conducive to dollarspot development had peaked.

Sponsor: Biophytec / Dr. Olivier Besnard