

***Trichoderma harzianum* as a biocontrol for Dollar spot disease (*Sclerotinia homoeocarpa*) on creeping bentgrass turf - 2011 trial**

**K. Carey, A.J. Porter, E.M. Lyons and K.S. Jordan**

Department of Plant Agriculture and the Guelph Turfgrass Institute,  
University of Guelph, Ontario.

The objective of this research project was to determine the efficacy of formulations of *Trichoderma harzianum* on dollar spot 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 direct 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 “Penn A-4” creeping bentgrass turf maintained on a USGA sand rootzone 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. All treatments were applied with a compressed air sprayer (50 ml m<sup>-2</sup> spray volume; flat fan nozzles) according to the schedule given in Table 1.

Inoculum of the disease organism (*S. homoeocarpa*) was prepared by growing several strains of the fungus on autoclaved Kentucky bluegrass seed. Inoculum was applied to the turfgrass at 5 g m<sup>-2</sup> July 13, 2011, when dollar spot disease was beginning to be prevalent. Relative humidity in the inoculated turf was kept high by irrigation, to stimulate disease development.

Table 1. Treatments

1- <b>Negative control:</b> untreated
2- <b>Positive control:</b> preventive fungicide program (Daconil Ultrex 115 g 100 m <sup>-2</sup> biweekly)
3- <b>PlantClean/SolClean modified program</b> (without initial application of PlantClean): First application of SolClean (500 mL 100 m <sup>-2</sup> ) 3 weeks later: application of PlantClean (50 mL 100 m <sup>-2</sup> ) PlantClean (50 mL 100 m <sup>-2</sup> ) repeated every three weeks until mid-October
4- <b>PlantClean/SolClean intensive program</b> (with higher application rates of PlantClean in July and August): First application of SolClean (500 mL 100 m <sup>-2</sup> ) 3 weeks later: application of PlantClean (50 mL 100 m <sup>-2</sup> ) 3 weeks later: application of PlantClean (100 mL 100 m <sup>-2</sup> ) during the months of July and August, i.e. in the critical period for dollar spot establishment PlantClean (100 mL 100 m <sup>-2</sup> ) repeated every three weeks until mid-October
5- <b>Rootshield</b> label rate program: First application: 12 g 100 m <sup>-2</sup> incorporated into soil. 6 g 100 m <sup>-2</sup> incorporated biweekly
6- <b>Abnatura</b> label rate program: 20 mL 100 m <sup>-2</sup> weekly

Application dates<sup>1</sup>

Treatment	July				August				September				Oct		
	8	15	22	29	5	12	18	26	2	9	16	22	30	7	14
2	*		*		*		*		*		*		*		*
3	*			*			*			*			*		
4	*			*			*			*			*		
5	*			*	*		*		*		*		*		*
6	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*

<sup>1</sup>Dollar spot inoculum added to all plots in on July 13, 2011





Figure 1. Plot area on creeping bentgrass USGA green, July 14, 2011 (6 days after first treatment application; 1 day after dollar spot inoculation).

Disease incidence and severity was assessed by visual ratings of damage, point quadrat measurement of disease area, and analysis of digital images. 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 2012.

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

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

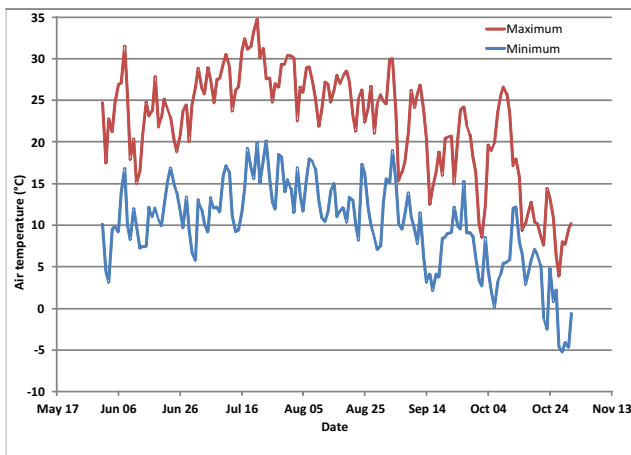


Figure 2. Daily air temperatures at GTI, summer 2011.

## RESULTS

*Environmental data.* Daily air temperatures for June – October 2011 are presented in Figure 2.

*Turf performance – canopy reflectance.* The canopy reflectance measurements (Table 2) were able to detect the decline in plots with visible dollar spot infection, which became significant towards the end of August (first visible DS lesions on Aug. 3; first consistent decline in NDVI in inoculated plots on Aug. 29).

*Visual ratings of dollar spot infection.* The first dollar spot lesions appeared in the plots 8 days after inoculation with *S. homoeocarpa* spores (July 21, Table 3). Significant disease presence developed in the inoculated half of the plots by 3 weeks after inoculation (August 3). Significant patterns in the uninoculated portion of the plots were not apparent until about 6 weeks after inoculation (August 24). Until 6 weeks after inoculation, disease pressure was assessed by counting lesions in plots. At this point lesions were too numerous to count in the most heavily affected plots, so area measures were used, first point-quadrat estimates of area, and then digital image analyses of diseased area (Table 3, Figure 3).

There was a significant correlation between lesion counts and percent area affected as estimated by point-quadrat methods (Figure 4),

Table 2. Canopy reflectance (NDVI) and change in canopy reflectance relative to negative control ( $\Delta$ NDVI) in treated plots.

Treatment	NDVI	Date								
		0629	0706	0713	0720	0720	0722	0722	0725	0725
		Uninoculated (0) / inoculated (1)								
		0	0	0	0	1	0	1	0	1
Fungicide		0.655 <sup>1</sup>	0.627	0.572	0.661	0.666 a	0.581 b	0.586	0.599	0.602
Abnatura		0.651	0.620	0.580	0.651	0.644 b	0.598 ab	0.597	0.582	0.596
PlantClean/SolClean intensive		0.653	0.627	0.581	0.656	0.659 ab	0.614 a	0.615	0.610	0.614
PlantClean/SolClean modified		0.654	0.619	0.584	0.658	0.653 ab	0.619 a	0.599	0.613	0.598
Rootshield		0.655	0.625	0.575	0.648	0.660 ab	0.602 ab	0.615	0.599	0.611
Control		0.651	0.623	0.582	0.649	0.648 ab	0.603 ab	0.599	0.602	0.587
	$\Delta$ NDVI									
Fungicide		0.004 <sup>2</sup>	0.004	-0.010	0.011	0.019 a	-0.023 b	-0.014	-0.004	0.015
Abnatura		0.000	-0.003	-0.001	0.002	-0.004 b	-0.006 ab	-0.002	-0.021	0.008
PlantClean/SolClean intensive		0.002	0.004	-0.001	0.007	0.012 ab	0.010 a	0.015	0.008	0.027
PlantClean/SolClean modified		0.003	-0.003	0.002	0.009	0.005 ab	0.016 a	0.000	0.011	0.011
Rootshield		0.004	0.002	-0.007	-0.001	0.012 ab	-0.001 ab	0.016	-0.003	0.024
msd p=0.05		NS	NS	NS	NS	0.019	0.032	NS	NS	NS
		0802	0802	0803	0803	0805	0805	0808	0808	0809
	NDVI	0	1	0	1	0	1	0	1	0
Fungicide		0.681	0.679	0.684	0.687	0.689	0.698 a	0.646	0.655	0.617
Abnatura		0.685	0.680	0.682	0.688	0.681	0.679 b	0.643	0.644	0.621
PlantClean/SolClean intensive		0.681	0.672	0.686	0.678	0.686	0.685 ab	0.654	0.652	0.621
PlantClean/SolClean modified		0.679	0.678	0.686	0.679	0.695	0.685 ab	0.659	0.645	0.637
Rootshield		0.677	0.679	0.686	0.681	0.686	0.693 ab	0.647	0.655	0.642
Control		0.680	0.675	0.684	0.678	0.688	0.676 b	0.653	0.645	0.642
	$\Delta$ NDVI									
Fungicide		0.000	0.004	-0.002	0.009	0.000	0.022 a	-0.007	0.010	-0.024
Abnatura		0.004	0.004	-0.003	0.011	-0.008	0.003 b	-0.009	-0.001	-0.020
PlantClean/SolClean intensive		0.000	-0.004	0.001	0.001	-0.002	0.009 ab	0.001	0.007	-0.021
PlantClean/SolClean modified		-0.002	0.002	0.001	0.002	0.007	0.009 ab	0.006	0.000	-0.005
Rootshield		-0.004	0.003	0.000	0.004	-0.003	0.017 ab	-0.005	0.010	0.001
msd p=0.05		NS	NS	NS	NS	NS	0.017	NS	NS	NS
		0809	0811	0811	0812	0812	0816	0816	0819	0819
	NDVI	1	0	1	0	1	0	1	0	1
Fungicide		0.634	0.571	0.583	0.561	0.580	0.586	0.602	0.593	0.601
Abnatura		0.629	0.569	0.580	0.557	0.582	0.584	0.586	0.602	0.590
PlantClean/SolClean intensive		0.628	0.569	0.580	0.571	0.578	0.578	0.582	0.601	0.602
PlantClean/SolClean modified		0.621	0.584	0.576	0.591	0.566	0.603	0.571	0.613	0.584
Rootshield		0.624	0.606	0.568	0.597	0.568	0.612	0.585	0.613	0.602
Control		0.633	0.597	0.582	0.591	0.587	0.601	0.587	0.610	0.602
	$\Delta$ NDVI									
Fungicide		0.002	-0.025	0.000	-0.029	-0.007	-0.015	0.014	-0.017	-0.002
Abnatura		-0.003	-0.028	-0.003	-0.033	-0.004	-0.017	-0.002	-0.008	-0.012
PlantClean/SolClean intensive		-0.004	-0.028	-0.003	-0.019	-0.008	-0.023	-0.006	-0.009	0.000
PlantClean/SolClean modified		-0.011	-0.013	-0.007	0.002	-0.020	0.001	-0.018	0.003	-0.018
Rootshield		-0.007	0.009	-0.015	0.007	-0.018	0.011	-0.003	0.003	-0.001
msd p=0.05		NS	NS	NS	NS	NS	NS	NS	NS	NS
		0822	0822	0823	0823	0825	0825	0829	0829	0831
	NDVI	0	1	0	1	0	1	0	1	0
Fungicide		0.604	0.612	0.601	0.605	0.618	0.623	0.631	0.637 a	0.624
Abnatura		0.602	0.588	0.592	0.570	0.603	0.584	0.606	0.581 b	0.600
PlantClean/SolClean intensive		0.597	0.594	0.586	0.585	0.599	0.591	0.605	0.593 b	0.597
PlantClean/SolClean modified		0.607	0.582	0.594	0.570	0.608	0.576	0.618	0.578 b	0.607
Rootshield		0.611	0.596	0.599	0.582	0.610	0.590	0.610	0.589 b	0.600
Control		0.609	0.590	0.602	0.581	0.610	0.586	0.614	0.580 b	0.608
	$\Delta$ NDVI									
Fungicide		-0.005	0.022	-0.001	0.025	0.008	0.038	0.016	0.057 a	0.015
Abnatura		-0.007	-0.002	-0.009	-0.010	-0.007	-0.002	-0.009	0.001 b	-0.009
PlantClean/SolClean intensive		-0.012	0.004	-0.016	0.004	-0.012	0.006	-0.011	0.013 b	-0.012
PlantClean/SolClean modified		-0.002	-0.008	-0.008	-0.010	-0.002	-0.009	0.003	-0.002 b	-0.001
Rootshield		0.002	0.006	-0.003	0.002	0.000	0.004	-0.005	0.009 b	-0.009
msd p=0.05		NS	NS	NS	NS	NS	NS	NS	0.042	NS

Table 2, continued.

Treatment	NDVI	Date								
		0831	0902	0902	0907	0907	0908	0908	0909	0909
		Uninoculated (0) / inoculated (1)								
		1	0	1	0	1	0	1	0	1
Fungicide		0.630 a	0.593	0.602	0.651	0.657 a	0.633	0.639 a	0.619	0.625 a
Abnatura		0.570 b	0.606	0.575	0.625	0.573 b	0.609	0.561 b	0.590	0.544 b
PlantClean/SolClean intensive		0.583 b	0.597	0.588	0.618	0.596 b	0.606	0.574 b	0.576	0.563 ab
PlantClean/SolClean modified		0.570 b	0.611	0.572	0.632	0.573 b	0.620	0.562 b	0.601	0.539 b
Rootshield		0.583 b	0.599	0.583	0.620	0.584 b	0.607	0.571 b	0.587	0.554 b
Control		0.572 b	0.607	0.572	0.627	0.571 b	0.608	0.557 b	0.587	0.539 b
	$\Delta$ NDVI									
Fungicide		0.059 a	-0.013	0.031	0.024	0.086 a	0.024	0.083 a	0.032	0.086 a
Abnatura		-0.001 b	0.000	0.004	-0.002	0.001 b	0.000	0.005 b	0.003	0.005 b
PlantClean/SolClean intensive		0.012 b	-0.009	0.017	-0.010	0.025 b	-0.003	0.018 b	-0.011	0.025 ab
PlantClean/SolClean modified		-0.001 b	0.004	0.000	0.005	0.002 b	0.011	0.006 b	0.014	0.001 b
Rootshield		0.012 b	-0.007	0.012	-0.007	0.013 b	-0.002	0.015 b	0.000	0.016 b
msd p=0.05		0.042	NS	NS	NS	0.059	NS	0.056	NS	0.063
		0912	0912	0914	0914	0919	0919	0921	0921	0922
	NDVI	0	1	0	1	0	1	0	1	0
Fungicide		0.613	0.619 a	0.602	0.607 a	0.589	0.596 a	0.549 a	0.551 a	0.583 a
Abnatura		0.574	0.524 b	0.549	0.504 b	0.544	0.490 b	0.497 b	0.450 b	0.521 ab
PlantClean/SolClean intensive		0.560	0.542 b	0.537	0.519 b	0.533	0.516 b	0.487 b	0.462 b	0.504 b
PlantClean/SolClean modified		0.586	0.523 b	0.567	0.502 b	0.565	0.497 b	0.514 ab	0.449 b	0.542 ab
Rootshield		0.570	0.536 b	0.547	0.516 b	0.549	0.504 b	0.505 ab	0.462 b	0.517 b
Control		0.574	0.519 b	0.547	0.491 b	0.540	0.492 b	0.502 ab	0.447 b	0.513 b
	$\Delta$ NDVI									
Fungicide		0.038	0.099 a	0.056	0.114 a	0.049	0.104 a	0.045 a	0.104 a	0.070 a
Abnatura		-0.001	0.003 b	0.003	0.012 b	0.004	-0.003 b	-0.006 b	0.004 b	0.007 ab
PlantClean/SolClean intensive		-0.015	0.021 b	-0.010	0.026 b	-0.007	0.023 b	-0.016 b	0.015 b	-0.010 b
PlantClean/SolClean modified		0.012	0.003 b	0.021	0.010 b	0.024	0.004 b	0.011 ab	0.003 b	0.028 ab
Rootshield		-0.005	0.016 b	0.001	0.023 b	0.009	0.012 b	0.002 ab	0.015 b	0.004 b
msd p=0.05		NS	0.063	NS	0.069	NS	0.068	0.048	0.062	0.066
		0922	0927	0927	0929	0929	1003	1003	1004	1004
	NDVI	1	0	1	0	1	0	1	0	1
Fungicide		0.583 a	0.603 a	0.602 a	0.599 a	0.596 a	0.584 a	0.581 a	0.586 a	0.577 a
Abnatura		0.462 b	0.512 b	0.443 b	0.495 b	0.420 b	0.474 b	0.408 b	0.460 b	0.394 b
PlantClean/SolClean intensive		0.474 b	0.502 b	0.452 b	0.491 b	0.435 b	0.476 b	0.428 b	0.458 b	0.401 b
PlantClean/SolClean modified		0.459 b	0.542 ab	0.436 b	0.529 ab	0.423 b	0.513 ab	0.411 b	0.502 ab	0.380 b
Rootshield		0.480 b	0.523 b	0.470 b	0.506 b	0.449 b	0.492 b	0.425 b	0.475 b	0.395 b
Control		0.457 b	0.521 b	0.425 b	0.495 b	0.412 b	0.481 b	0.394 b	0.469 b	0.370 b
	$\Delta$ NDVI									
Fungicide		0.126 a	0.081 a	0.177 a	0.103 a	0.184 a	0.101 a	0.189 a	0.117 a	0.206 a
Abnatura		0.005 b	-0.010 b	0.019 b	-0.001 b	0.008 b	-0.008 b	0.016 b	-0.009 b	0.023 b
PlantClean/SolClean intensive		0.017 b	-0.019 b	0.028 b	-0.006 b	0.023 b	-0.006 b	0.036 b	-0.010 b	0.029 b
PlantClean/SolClean modified		0.002 b	0.021 ab	0.012 b	0.033 ab	0.011 b	0.031 ab	0.018 b	0.033 ab	0.009 b
Rootshield		0.023 b	0.002 b	0.046 b	0.010 b	0.036 b	0.010 b	0.032 b	0.006 b	0.023 b
msd p=0.05		0.068	0.076	0.073	0.072	0.086	0.081	0.075	0.097	0.093
		1006	1006	1011	1011					
	NDVI	0	1	0	1					
Fungicide		0.577 a	0.574 a	0.560 a	0.556 a					
Abnatura		0.450 b	0.383 b	0.411 b	0.357 b					
PlantClean/SolClean intensive		0.451 b	0.377 b	0.409 b	0.341 b					
PlantClean/SolClean modified		0.493 ab	0.369 b	0.451 b	0.337 b					
Rootshield		0.472 b	0.393 b	0.423 b	0.355 b					
Control		0.456 b	0.367 b	0.412 b	0.321 b					
	$\Delta$ NDVI									
Fungicide		0.120 a	0.207 a	0.147 a	0.235 a					
Abnatura		-0.008 b	0.016 b	-0.002 b	0.036 b					
PlantClean/SolClean intensive		-0.006 b	0.010 b	-0.004 b	0.020 b					
PlantClean/SolClean modified		0.035 ab	0.002 b	0.038 b	0.017 b					
Rootshield		0.014 b	0.026 b	0.010 b	0.034 b					
msd p=0.05		0.097	0.087	0.104	0.095					

<sup>1</sup>Normalized-difference vegetation index: mean of 4 replicates; means within columns followed by the same letter are not significantly different (Tukey's HSD test, p=0.05).

<sup>2</sup>Normalized-difference vegetation index corrected against negative control mean (Control=0) ; means within columns followed by the same letter are not significantly different (Tukey's HSD test, p=0.05).



Table 3. Dollarspot disease estimation.

Treatment	Lesion counts						Percent area of plot					
							Point quadrat		Digital image analysis			
	0721	0802	0803	0810	0819	0824	0819	0824	0901	0907	0922	1006
	Uninoculated											
Fungicide	0.3 <sup>1</sup>	0.5	0.5	1.3	2.8	2.3 b				0.2 b	2.0 b	2.4 b
Abnatura	0.5	1.5	1.0	1.5	6.5	11.5 ab				1.6 ab	9.9 a	19.7 a
PlantClean/SolClean intensive	0.3	1.3	0.5	3.5	9.3	12.0 ab				1.8 ab	9.5 a	21.5 a
PlantClean/SolClean modified	0.0	1.0	1.5	3.5	8.5	13.8 a				1.9 ab	8.4 ab	17.4 a
Rootshield	0.8	1.8	5.0	6.3	9.3	14.5 a				2.4 a	8.7 ab	16.3 a
Control	0.5	2.8	4.3	4.8	8.0	12.3 ab				2.2 a	8.7 ab	17.9 a
msd p=0.05	NS	NS	NS	NS	NS	10.8				1.9	6.7	12.5
	Inoculated											
Fungicide	0.3	0.0	0.5 b	2.8 b	21.0 b	16.3 b	1.3 <sup>2</sup>	1.0	0.7 b <sup>3</sup>	1.0 b	2.6 b	4.3 b
Abnatura	0.5	4.5	29.3 a	27.5 ab	69.5 a	91.5 a	6.8	8.0	6.6 a	9.8 a	23.4 a	37.9 a
PlantClean/SolClean intensive	0.5	4.0	38.5 a	36.3 ab	66.5 a	80.5 a	4.8	8.3	6.9 a	10.0 a	23.8 a	41.1 a
PlantClean/SolClean modified	0.5	5.5	30.5 a	36.0 ab	69.5 a	90.0 a	7.3	9.5	6.9 a	9.7 a	23.8 a	41.0 a
Rootshield	1.3	6.3	23.8 ab	23.0 ab	55.0 ab	78.3 a	4.0	6.8	5.9 a	8.3 a	21.1 a	35.4 a
Control	1.8	3.5	23.8 ab	41.0 a	69.5 a	93.0 a	7.0	6.5	7.2 a	9.1 a	24.2 a	41.2 a
msd p=0.05	NS	NS	28.0	33.9	42.4	47.9	NS	NS	3.5	5.4	9.4	15.4

<sup>1</sup>Mean number of lesions per 1 m<sup>2</sup>; means within columns followed by the same letter are not significantly different (Tukey's HSD test, p=0.05).

<sup>2</sup>Percent area estimated by point-quadrat; 100 points per m<sup>2</sup>. Means of 4 replicates; means within columns followed by the same letter are not significantly different (Tukey's HSD test, p=0.05).

<sup>3</sup>Percent area estimated by digital image analysis. Means of 4 replicates; means within columns followed by the same letter are not significantly different (Tukey's HSD test, p=0.05).

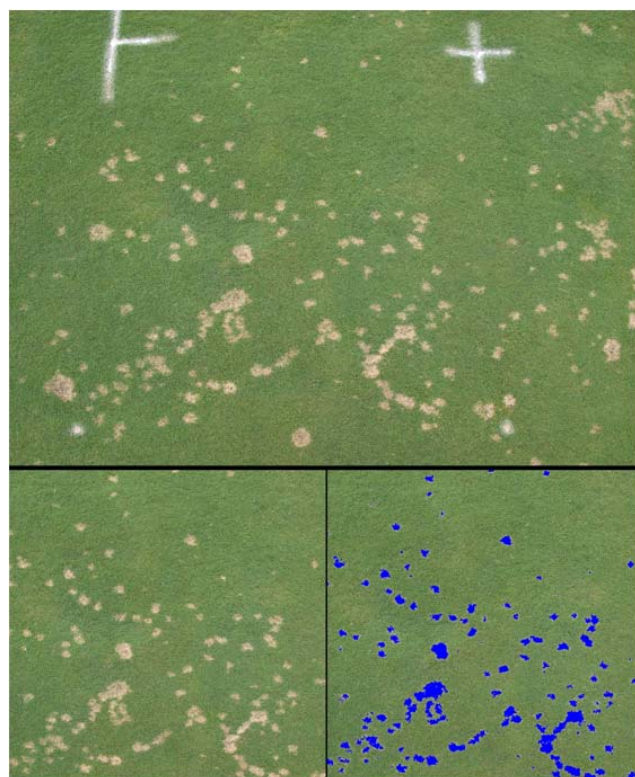


Figure 3. For digital image analyses, raw images of each 1x1 m subplot (top) were deskewed, cropped, and resized to 2000x2000 pixels in Adobe Photoshop (bottom left), and then analyzed with Sigma Scan software, which estimates dollar spot area based on color thresholds (bottom right). The actual estimate in the plot pictured was 5.8% area covered by dollar spot lesions.

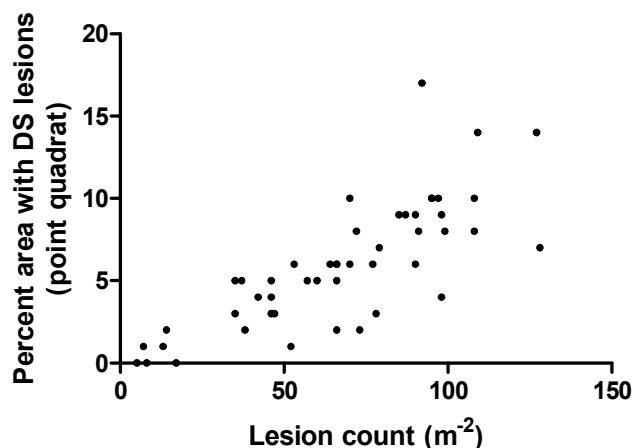


Figure 4. Association between counts of dollar spot infection centres and percent area of disease estimated by point quadrat (08/19 and 08/24). Correlation coefficient is 0.78.

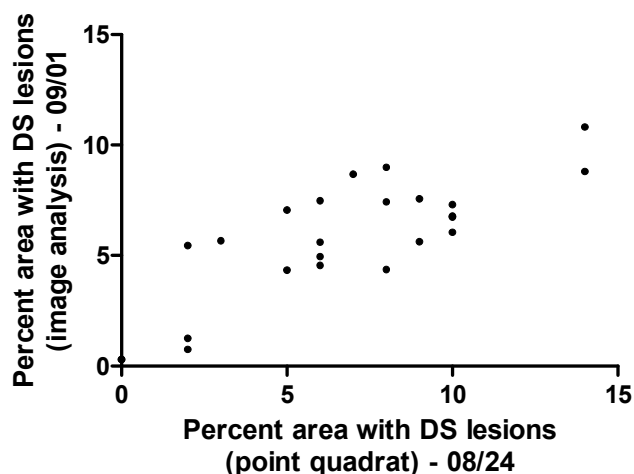


Figure 5. Association between percent area of disease estimated by point quadrat and by digital image analysis. Correlation coefficient is 0.81.

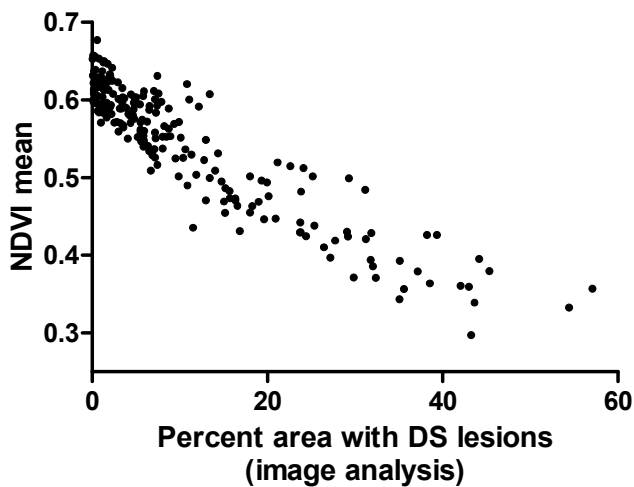


Figure 6. Association between canopy reflectance and percent area of disease estimated by digital image analysis. Correlation coefficient is -0.92.

and between point-quadrat and image analysis estimates of percent area (Figure 5). There was also a strong correlation between canopy reflectance and the area of dollar spot coverage estimated by image analysis (Figure 6).

### DISCUSSION AND CONCLUSIONS

Based on direct estimates of dollar spot disease, whether lesion counts, point-quadrat area estimates or image analysis, the fungicide treatment was the only one to give consistent control compared to the untreated plots. The canopy reflectance data, which appeared to be detecting differences based on dollar spot disease, showed a similar pattern. Because there are other factors that affect canopy reflectance (scalping and other stresses), the NDVI values should be taken as indirect estimates of dollar spot disease.

Sponsor: Biophytec/Olivier Besnard