Project:	Biorational products for nematode control
Principal investigators:	Larry Stowell, Ph.D., CPAg and Wendy Gelernter, Ph.D., PACE Turf LLC and Michael McClure, Ph.D. University of Arizona
Cooperator:	Troy Mullane, Rancho Santa Fe Farms Golf Club, Rancho Santa Fe ,CA
Sponsor:	PACE Turf LLC

Summary

Nematodes are causing increasing problems on golf course greens, and, with the cancellation of Nemacur (fenamiphos), there are few effective products available for their control.

A field study was conducted on a bentgrass practice green to evaluate the efficacy of four biorational products for control of the plant parasitic nematodes *Meloidogyne* (root knot nematode), *Helicotylenchus* (spiral nematode), and *Criconemella* (ring nematode). Key findings were:

- Two treatments – *Bacillus firmus* and methionine – resulted in significant reductions in populations of root knot nematode. Since this nematode has in the past been associated with turf damage at this, and other locations, this finding has important implications for California golf courses.
- The *Bacillus firmus* product showed this response only when applied twice, at a rate of 35 lb/A. A single application of 70 lb/A was not effective for reasons that are not clear at this point.
- The impact of the methionine treatment was more problematic, due to significant phytotoxicity produced by this treatment. In addition to unacceptable changes in turf quality, it is possible that the observed reduction in nematode populations may be a result of a reduced root system (due to phytotoxicity) and not a nematicidal effect. Further work is needed to determine whether lower rates and more frequent applications of methionine can reduce nematode populations without damaging turf.
- The same two treatments – *Bacillus firmus* and methionine – may have had some impact on ring nematode populations as well. While numbers of ring nematodes were significantly increased in all other treatments, there were no increases observed for either the *B. firmus* or methionine treatments. While ring nematode does not appear to cause significant turf damage, this observation is worth following up on in future tests.
- Turf quality data did not correlate with trends in nematode populations.

Materials and Methods

I. Treatments

Treatment Number	Product	Company	Rate/A	Application Dates
1	Non-treated		---	---
2	D/L methionine	ScienceLab.	250 lb	8/20
3	BioFence (ground mustard plant)	BioFence	871 lb	8/20, 9/16
4	*Bacillus firmus* (strain I-1582)	Bayer Environmental Science	70 lb	8/20
5	*Bacillus firmus* (strain I-1582)	Bayer Environmental Science	35 lb	8/20, 9/16
6	Inoculaid VSC plus Inoculaid Light	Applied and Experimental Biology	1 gallon plus 1 gallon	8/20, 9/16

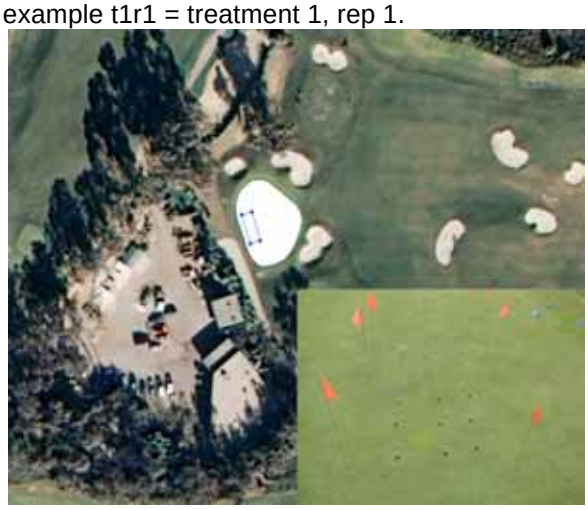
II. Experimental Design and Sampling

- Plots were located on a bentgrass (Dominant Plus) practice putting green. Each treatment consisted of 4 replicates (randomized design) with each plot measuring 3x3 ft (9 sq ft/plot).
- D/L and Biofence were applied using shakers. *Bacillus firmus* and Inoculaid were applied by suspending each product in 2 liters of water and using a watering can to evenly apply the suspension over the plot area. All treatments were watered in for 10 minutes (first application date) and 5 minutes (2nd application date) with the irrigation system.
- On the first application date, 8/20/09, the BioFence material did not move into the canopy easily. Following 10 min irrigation, the material was rubbed using the smooth side of a bunker rake to aid in moving the material into the canopy and then hand watered with a watering can that delivered approximately 2 gal of water to encourage the product to disperse. On the second application date (9/16), after BioFence was shaken out, it was lightly brushed to improve uniformity of distribution.
- Original plans had called for multiple applications of methione. However, significant phytoxicity was observed two weeks after the first application (see below). As a result, additional methione applications were not made.
- Turf quality evaluations were made on four dates. A pre-treatment evaluation was made on 8/20/09, and follow up evaluations were made on 9/4, 9/16 and 10/6. Turf quality was rated two different ways: visually, using a 1 – 9 scale, and analytically, using the Spectrum Technologies CM1000 chlorophyll meter (average of 3 readings per plot).
- Nematode counts were made only on 8/10 (pre-treatment) and 10/6 (47 days after the first treatment).
- Nematode populations were determined by collecting nine one-inch soil cores to a depth of 4 inches and placing the cores into a ziploc bag for each plot. 250 cc of soil from each plot were extracted under an intermittent mist for 72 hours. Nematodes recovered were collected on a 500-mesh sieve and re-suspended in 20 cc of water for observation and counting. One cc of the suspension was counted at magnifications of 60-80X.

III. Plot plan

1 t1r1	9 t3r2	17 t4r3
2 t2r1	10 t6r2	18 t1r3
3 t3r1	11 t5r2	19 t4r4
4 t4r1	12 t1r2	20 t5r4
5 t5r1	13 t2r3	21 t6r4
6 t6r1	14 t3r3	22 t1r4
7 t2r2	15 t6r3	23 t3r4
8 t4r2	16 t5r3	24 t2r4

Photograph is oriented with north to the top of the image. Plot No. 1 is located in the northwest corner of the plot area and plot 24 is located at the southeast corner. Plots are 3'x3' with 1' buffers between the plots. Plot designations are t=treatment number and r=replicate number. For example t1r1 = treatment 1, rep 1.



Results

I. Nematode control

Table 1. Nematode counts were taken prior to the first treatment, on 8/10/09 ("pre-treat evaluation"), and 57 days later, at the completion of test, on 10/6/09 ("final evaluation"). An asterisk (*) denotes a significant change (P<0.05, Fisher's LSD) in readings between the pre-treat vs. the final evaluation for each nematode and for each treatment. Values shaded in green indicate a significant, beneficial change during the course of the trial, while treatments shaded in red indicate a significant, detrimental change that occurred during the course of the trial.

	Root knot nematode/cc		Ring nematode		Spiral nematode	
Trt #	Pre-treat evaluation	Final evaluation	Pre-treat evaluation	Final evaluation	Pre-treat evaluation	Final evaluation
1	7.80	3.84	14.04	33.768*	2.56	2.08
2	7.40	3.20*	14.40	15.52	0.06	0.02
3	9.16	8.16	14.32	33.44*	0.16	0.96
4	9.60	5.44	13.84	38.40*	0.72	1.60
5	11.00	4.64*	19.20	36.16	4.00	1.00
6	10.76	7.68	15.48	37.92*	0.18	1.60

Figure 1. Root knot nematode counts indicate that treatments 2 and 5 caused significant reductions in this nematode (as noted by asterisks).

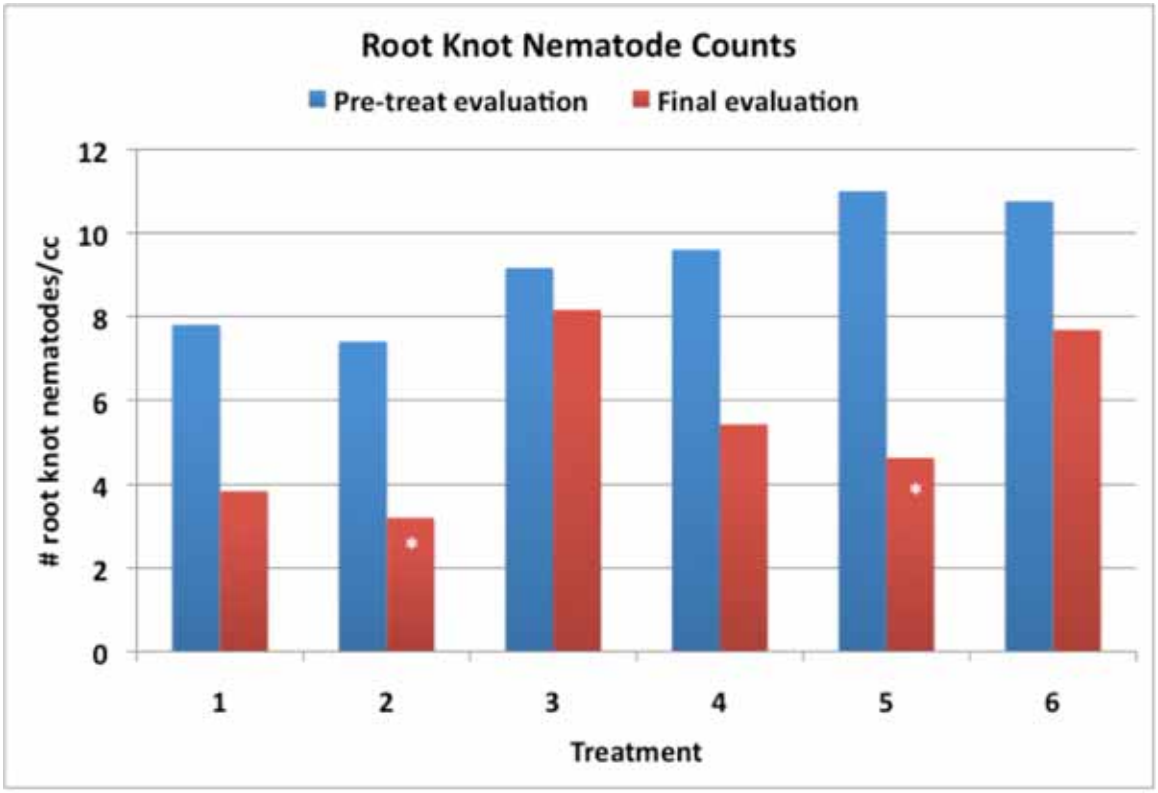


Figure 2. Spiral nematode counts indicate that none of the treatments tested had any significant impact on this nematode.

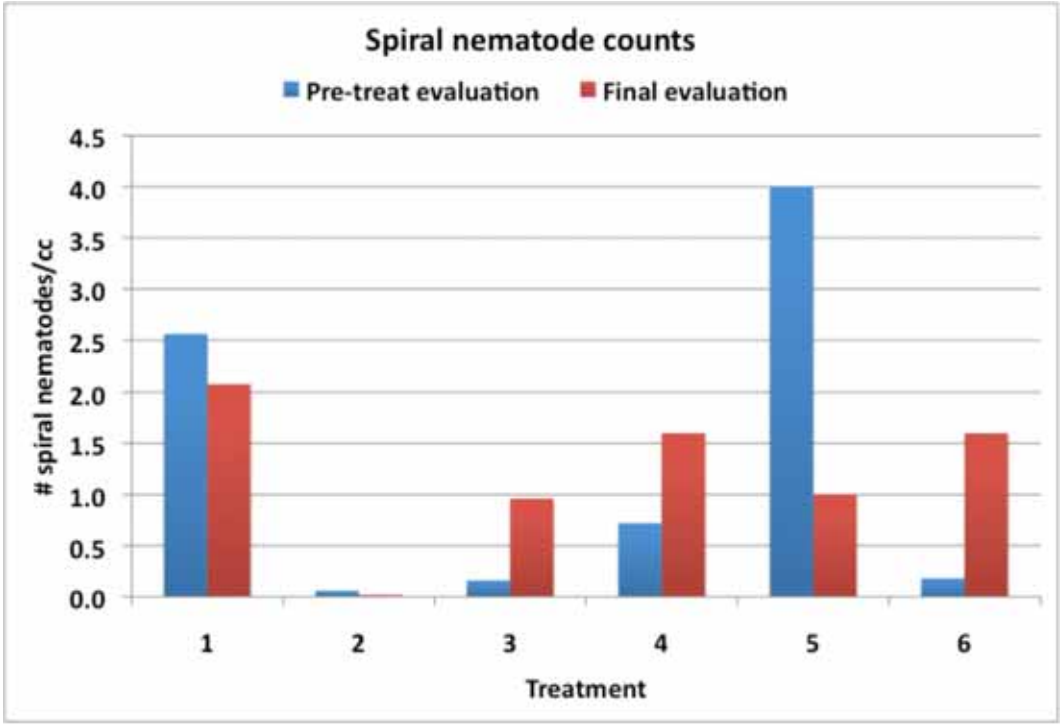
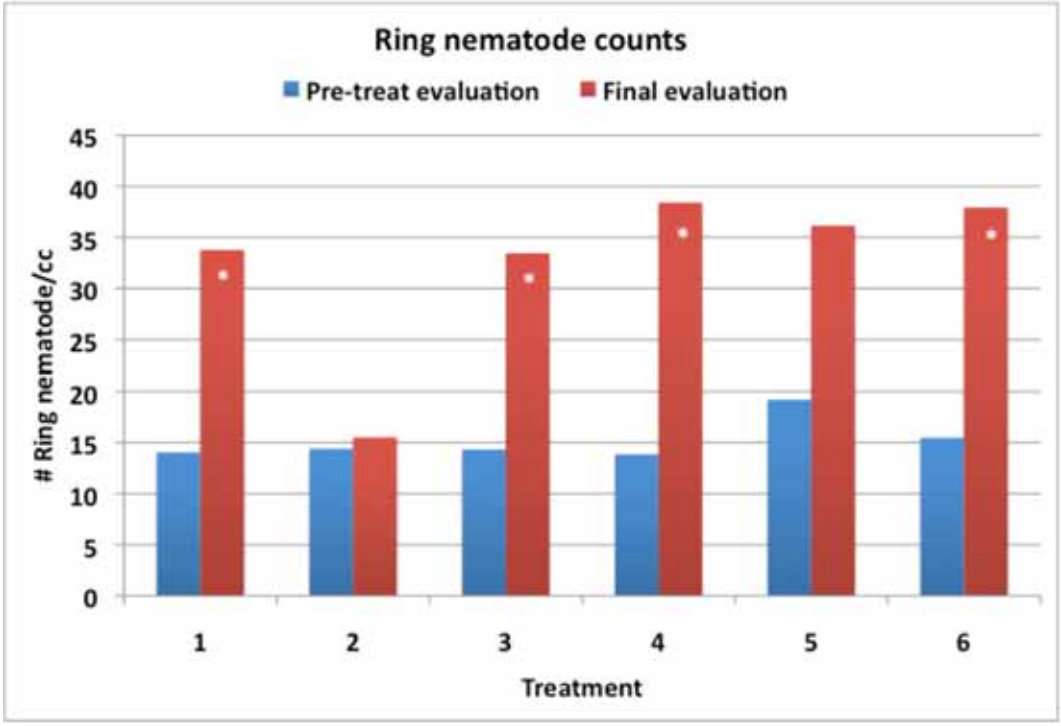


Figure 3. Ring nematode counts indicate that all treatments *except* treatments 2 and 5 showed a significant increase in populations of this ectoparasitic nematode (as noted by asterisks).



II. Turf quality

Changes in turf quality over the course of the trial were not correlated with changes in nematode populations, but instead with golf course cultural practices and/or secondary effects of the test products. For example, turf quality was lower in all plots, including the non-treated check, at the conclusion of the trial (Figure 4) due to inadvertent scalping of the entire practice putting green, approximately 1 week prior to the final evaluation. In addition, the presence of nitrogen in the Biofence treatment (treatment 3), produced a short-lived increase in turf quality (see 9/4 rating in Table 2 and Figure 5 below), and the phytotoxic effect of methionine (treatment 2) caused a decrease in turf quality, also on 9/4 (Table 2, Figure 5).

Figure 4. Turf quality, as measured with the CM1000 chlorophyll meter, declined in all treatments over the course of the trial, including the non-treated check (as noted by asterisks). This was the result of a non-treatment related effect, due to inadvertent scalping of the entire practice putting green approximately 1 week prior to the final evaluation.

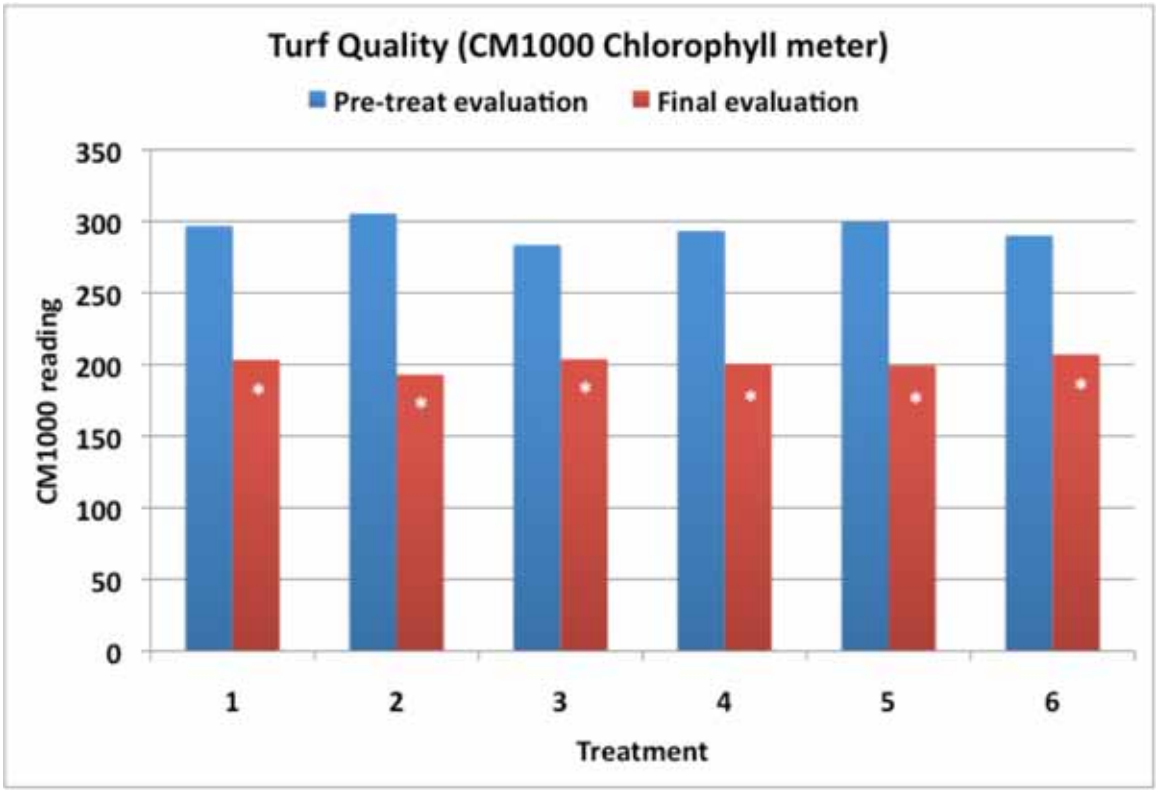


Table 2. CM1000 readings. Reading down a column for a given date, treatments sharing the same letter are not significantly different (Fisher's LSD, P<0.05). Treatments with significantly better quality than the non-treated check are shaded in green. Treatments with significantly worse quality than the check are shaded in red.

Treatment	20-Aug	4-Sep	16-Sep	6-Oct
1	296.75 a	277.50 b	288.00 ab	203.25 a
2	305.25 a	259.25 c	267.00 b	193.00 a
3	283.50 a	300.75 a	300.25 a	203.75 a
4	293.25 a	297.50 ab	284.50 ab	200.50 a
5	299.75 a	285.25 b	282.50 ab	199.25 a
6	290.00 a	273.75 bc	277.00 b	207.00 a

Figure 5. Phytotoxicity of methione treatment (treatment 2). While turf quality in all treatments declined towards the end of the trial due to inadvertent scalping of the entire test area, the methionine treatment (treatment 2) showed a significant decline in turf quality almost immediately after the initial treatment. For this reason, planned follow-up applications of methionine were cancelled. Treatment 3 (Biofence) showed significantly improved turf quality on 9/4. This is probably the result of the nitrogen present in the Biofence formulation.