Seeking Alternatives to Nemacur:
Evaluation of Natural Suppression and Testing New Products

Summary Report, 15 January 2006

Robert L. Wick Dept. Plant Soil and Insect Sciences
Fernald Hall University of Massachusetts

Acknowledgements: I thank the New England Regional Turfgrass Foundation for supporting this research over the last three years. Thanks are also due to Eric Abbot and Shawn Massoni who collected samples, made extractions and identified nematodes over the course of this project.

Effectiveness of Nemacur-altematives for reducing plant parasitic nematode in golf greens- 2003

Evaluation of BioNem L@

A putting green in Amherst, MA consisting of a mixture of annual bluegrass and creeping bentgrass was chosen for this study. Top-dressing over the years has resulted in approximately 4 in. of sandy soil. Mechanical analysis of the top 4 in. showed it to be 84.6% sand, 11.4% silt, and 3.9% clay. USDA criteria classifY this soil as a loamy sand based on the sand subfractions. BioNem L is a formulation of *Bacillus firmus* and seaweed extract. Treatments and controls were replicated six times (plots were 6 ft x 6 ft) and completely randomized. BioNem L was applied at 3 oz and 6 oz/l,OOO sq ft. in approximately 55 gal water on 21 May, and again on 19 June. Nemacur lOG was applied once at the rate of2.3 Ib/1,000 sq ft on 21 May. Following application of treatments, the plots received approximately y.. in. of water. For nematode assays, ten subsamples per plot were collected with a 1 in. soil sampling tube to a depth of 4 in. and bulked. Nematodes were recovered by wet sieving/sugar flotation and identified to genus. *Helicotylenchus* (spiral) and *Tylenchorhynchus* (stunt) nematodes were assessed. The data were subjected to analysis of variance and a test of LSD.

There were no differences in turf color or thinning among the treatments. Nemacur resulted in a decrease in both populations of nematodes but it was only statistically different on the 7 Aug collection date for each population.. Neither the 3 oz or 6 oz BioNem L treatments significantly reduced nematodes in this trial. The summer of2003 had considerable rainfall and several inches of rain occurred following the 16 May treatments. It is possible that the excessive rainfall reduced the efficacy of the BioNem L treatments.

Effectiveness of Nemacur-alternatives for reducing plant parasitic nematode in golf greens- 2003

Evaluation of BioNem L@

A putting green in Amherst, MA consisting of a mixture of annual bluegrass and creeping bentgrass was chosen for this study. Top-dressing over the years has resulted in approximately 4 in. of sandy soil. Mechanical analysis of the top 4 in. showed it to be 84.6% sand, 11.4% silt, and 3.9% clay. USDA criteria classify this soil as a loamy sand based on the sand subfractions. BioNem L is a formulation of *Bacillus firmus* and seaweed extract. Treatments and controls were replicated six times (plots were 6 ft x 6 ft) and completely randomized. BioNem L was applied at 3 oz and 6 *0:zJ1,000* sq ft. in approximately 55 gal water on 21 May, and again on 19 June. Nemacur lOG was applied once at the rate of2.3 Ib/1,000 sq ft on 21 May. Following application of treatments, the plots received approximately Y-i in. of water. For nematode assays, ten subsamples per plot were collected with a 1 in. soil sampling tube to a depth of 4 in. and bulked. Nematodes were recovered by wet sieving/sugar flotation and identified to genus. *Helicotylenchus* (spiral) and *Tylenchorhynchus* (stunt) nematodes were assessed. The data were subjected to analysis of variance and a test of LSD.

There were no differences in turf color or thinning among the treatments. Nemacur resulted in a decrease in both populations of nematodes but it was only statistically different on the 7 Aug collection date for each population.. Neither the 3 oz or 6 oz BioNem L treatments significantly reduced nematodes in this trial. The summer of2003 had considerable rainfall and several inches of rain occurred following the 16 May treatments. It is possible that the excessive rainfall reduced the efficacy of the BioNem L treatments.

Evaluation of Dragonfire@ and Rutopia@

A putting green in Ardsley-on-Hudson, NY consisting of a mixture of annual bluegrass and creeping bentgrass was chosen for this study. Top-dressing over the years has resulted in approximately 4 in. of sandy soil. Mechanical analysis of the top 4 in. showed it to be 95.9% sand, 1.8% silt, and 2.3% clay. USDA criteria classify this soil as a fine sand based on the sand subftactions. Dragonfire CPP is 100% sesame oil and was mixed with 7509 Emulsifier (coconut alkanolamide) at the rate of5:1 just before application. Rutopia is a mixture of natural sugars, sugar extracts, humectants, *Trichoderma*, "beneficial microorganisms" and other natural substances, and was mixed with JetWet@ and the rate of 1.33:1. Treatments and controls were replicated six times (plots were 6 ft x 6 ft) and completely randomized. Dragonfire CPP was applied at 14.7 oz and Eutopia at 17.8 *o:zJl,OOO* sq ft. in approximately 55 gal water on 5 Jun and again on 7 Jul. Eutopia was mixed with 13.2 oz JetWet@ wetting agent just before application. Nematodes were recovered by wet sieving/sugar flotation and identified to genus. *Hoplolaimus* (lance) and *Tylenchorhynchus* (stunt) nematodes were assessed. The data were subjected to analysis of variance and a test of LSD.

There were no differences in turf color or thinning among the treatments. The summer of2003 had considerable rainfall and it is possible that the excessive rainfall reduced the efficacy of the materials.

	Hovlolaimus(lance)/6.2 cu in. (100 cc) soil			
Treatment and ratell.OOO so ft	16 Mav 03	19 Jun 03	7 Aut!: 03	9 SeD 03
Control	772	851	10068	8798
Dragonfire, 14.7 oz + 7509 Emulsifier, 2.94 oz..	9418	13278	11898	8198
RutoPIa. 17.8 oz + JetWet 13.20z.....	7748	9378	8558	10778

*Means in a column followed by the same letter are not significantly different at $P = 0.05$ level according to LSD test.

	Tvlenchorhvnchus (stunt)/6.2 cu in. (100 cc)			
Treatment and rate/l.000 so ft	16 Mav 03	19 Jun 03	7 Aut!: 03	9 SeD 03
Control	2198	1638	1478	1128
Dragonfire,14.70z+7509Emulsifier,2.940z..	4698	3798	2538	2688
Rut0P-m.. 17.8 oz + JetWet 13.20z.....	5098	3658	2078	2568

Means in a column followed by the same letter are not significantly different at $P = 0.05$ level according to LSD test

Evaluation of Neo- Tec@

A putting green in Bolton, MA consisting of a mixture of annual bluegrass and creeping bentgrass was chosen for this study. Top-dressing over the years has resulted in approximately 4 in. of sandy soil. Mechanical analysis of the top 4 in. showed it to be 89.1 % sand, 8% silt, and 2.9% clay. USDA criteria classify this soil as a coarse sand based on the sand subtractions. Neo- Tec is 50% sesame oil mixed in 50% lecithin and water. The treatment and control were replicated six times (plots were 6 ft x 6 ft) and completely randomized. Neo- Tec was applied at the rate of 2.94 *oz/I,OOO* sq ft in approximately 55 gal water on 3 Jun and again on 3 Jul. Nematodes were recovered by wet sieving/sugar flotation and identified to genus. *Criconemella* (ring) and *Tylenchorhynchus* (stunt) nematodes were assessed. The data were subjected to analysis of variance and a test of LSD.

There were no differences in turf color or thinning among the treatments. The summer of 2003 had considerable rainfall and it is possible that the excessive rainfall reduced the efficacy of the materials.

	Criconemella (rin!!V6.2 cu in. noo cc) soil			
Treatment and rate/l.OOO sa ft	27 May 03	3 Jul 03	15 Aug 03	11 Sep 03
Control	98 a	141 a	125 a	189 a
Neo- Tec. 2.94 oz	76a	85a	164a	133 a

*Means in a column followed by the same letter are not significantly different at $P = 0.05$ level
according to LSD test.

	Tvlenchorhvnchus (stuntV6.2 cu in. noo cc) soil			
Treatment and rate/l.OOO sa ft	27Mav 03	3 Jul 03	15 Au!! 03	11 SeD 03
Control	681 a	462a	423 a	258a
Neo- Tec. 2.94 oz	781 a	645 a	567a	293 a

.Means in a column followed by the same letter are not significantly different at $P = 0.05$ level according to LSD test.

Summary of Nemacur-altemative products, 2003

BioNem, Dragonfire, Rutopia and Neo- Tec were tested for their effectiveness in reducing or suppressing plant parasitic nematodes in turfgrasses. Products were used at labeled rates, twice during the growing season. Nemacur was the standard industry control. The Nemacur alternatives did not suppress plant parasitic nematodes but the Nemacur significantly reduced nematode populations.

Effectiveness of Nemacur-altematives for reducing plant parasitic nematode in golf greens- 2004

Plant parasitic nematodes have been documented as important pathogens of plants including many important grass and turfgrass species. Surveys and nematode assays carried out for diagnostic purposes from golf courses throughout the New England region have demonstrated that at least 10 different genera of plant parasitic nematodes occur. In many cases, high numbers of nematodes have been associated with decline of turf health, most particularly on golf greens.

Nemacur@ is the only effective nematicide registered for use on turfgrasses but it will not be available after 2007. There are no other conventional nematicides registered for use on golf greens. Several biological control agents and other Nemacur-alternatives have been commercially-available over the past several years. Many of these have not performed well in university trials, and some are no longer available. Every year several new or reformulated products are introduced into the market. It is important that they be tested in an unbiased, systematic fashion.

Evaluation ofNeo-Tec@ (Repeat of previous trial with increase in rate)

A golf green in Bolton, MA was chosen for the evaluation ofNeo- Tec, a sesame oil product (Figures I and 2). In 2004, a 2X rate ofNeo-Tec was applied three times; the previous year the same plots received a IX rate twice. The green consisted of a mixture of annual bluegrass and creeping bentgrass. Neo- Tec is 50% sesame oil mixed in 50% lecithin and water.

A golf green in Ardsley NY was chosen for the Dragonme and Neo- Tec study (Figures 3 and 4). Dragonme is another formulation of sesame oil. In each case, the treatments and control were replicated six times (plots were 6 ft x 6 ft) and completely randomized. In Bolton, Neo-Tec was applied at the rate of5.88 oz/I,OOO sq ft in approximately 55 gal water on 25 May, and again on 8 and 23 JUD. In Ardsley, the treatments were applied once at the beginning of the season at the labeled rate. Nematodes were recovered by wet sieving/sugar flotation and identified to genus. *Criconemella* (ring), *Tylenchorhynchus* (stunt) and *Hoplolaimus* (lance) nematodes were assessed. The data were subjected to analysis of variance and a test of LSD.

At the Bolton green, ring nematodes appeared to have been suppressed by NeoTec, however the data between the control and the treatment were not statistically different from each other. This commonly happens when working with ring nematodes because they are very unequally distributed in the soil. Unequal distribution between plots makes it difficult to demonstrate differences between treatments. There were no statistical differences between the control and the treatment for stunt nematodes.

The Ardsley plots also showed no statistical differences even though it appeared that the two Nemacur-alternatives actually increased the nematode population over the control.

Summary of Nemacur-alternative **products, 2004**
Although NeoTec was applied three times at twice the labeled rate, it had no effect on ring, stunt or lance nematodes. In addition, these higher rates were applied on the same plots that received the labeled rate the previous year. A single application ofNeo- Tec at the labeled rate did not significantly reduce nematode populations.

Effectiveness of N emacur-alternatives for reducing plant parasitic nematode in golf greens- 2005

Evaluation of *Bacillus firmus*

A private golf course in South Hadley, MA was selected for this study. The green was approximately 83 years old but rebuilt, largely with the existing soil, about 15 years ago. The grass species consist of a mixture of annual bluegrass and creeping bentgrass. During the course of this study, the green was maintained with conventional practices.

Treatments were as follows:
1. Untreated control
2. Bacillusjirmus liquid formulation 6 oz. Per 1,000 sq ft.
3. Bacillusjirmus liquid formulation 6 oz. Per 1,000 sq ft., two applications *4. Bacillusjirmus* dry formulation 4.11bs per 1,000 sq ft.

Application dates. 2005
May 31, first application of liquid and dry formulation June 21, second application of liquid formulation

Treatments and controls were replicated six times (plots were 6 ft x 6 ft) and completely randomized. The treatments were applied in approximately 55 gal water per 1,000 sq ft. Following application of treatments, the plots received approximately 0.10 in. of water. For nematode assays, ten subsamples per plot were collected with a 1 in. soil sampling tube to a depth of 4 in. and bulked. Nematodes were recovered by wet sieving/sugar flotation and identified to genus. Data for *Tylenchorhynchus* (stunt) and *Criconemella* (ring) nematodes were collected.

No injury to the turfgrass was evident trom the treatments. Nematode populations were relatively low this year throughout southern New England. This was unexpected considering this site had nearly 1,000 stunt nematodes by the first week of June in 2004. Also, a very hot early summer apparently suppressed nematode populations, and they did not begin to rebound until later in the season. No difference in grass health was noted among the treatments and controls. Statistical analysis showed no differences in nematode populations among treatments.

Evaluation of AgroNeem@

A golf green in Greenwich CT was chosen for the evaluation of AgroNeem. The green was approximately 80 years old and consisted of a mixture of annual bluegrass and creeping bentgrass. Top-dressing over the years resulted in approximately 4 in. of sandy soil. Mechanical analysis of the top 4 in. showed it to be 95% sand, 3.4% silt, and 1.6% clay. USDA criteria classify this soil as a sand. AgroNeem contains 15% neem-biomass; 0.15% azadirachtin. The treatment and control were replicated six times (plots were 6 ft x 6 ft) and completely randomized. AgroNeem was applied at the rate of32 oz/I,OOO sq ft in approximately 55 gal water. FP-747 (Floratine Products Group, TN), a phosphate buffer, was added to bring the AgroNeem solution to pH 5.5. Two applications were made in 2004; 21 Jun and 30 Aug. Three applications were made in 2006; Jun, 14 Jul and 29 Sep. Nematodes were recovered by wet sieving/sugar flotation and identified to genus. *Criconemella* (ring), *Tylenchorhynchus* (stunt) and *Helicotylenchus* (spiral) nematodes were assessed. The data were subjected to analysis of variance and a test of LSD.

There were no differences in turf color or thinning among the treatments. *Criconemella* in the AgroNeem treatment was significantly lower than the control on 28 Sept 05. There were no significant differences between AgroNeem and the control for *Tylenchorhynchus* or *Helicotylenchus.* Ring nematodes showed a significant reduction with the AgroNeem treatment on 28 Sep; applications will continue in 2006 to determine if this treatment will continue to suppress ring nematodes.

	Criconemella 16.2 cu in. (100 cc) soil					
Treatment and rate/l,OOO sq ft	13 Jun 04	19 Jul 04	16 Aug 04	18 May 05	13 Jul 05	28 Sep 05
ControL	1289 a*	1307 a	837 a	466 a	229 a	805 a
AgroNeem 32 oz	1115 a	1095 a	1105 a	223 a	348 a	286 b

*Means in a column followed by the same letter are not significantly different at $P = 0.05$ level according to LSD test.

	Tylenchorhynchus 16.2 cu in. (100 cc) soil					
Treatment and rate/l,OOO sq ft	13 Jun 04	19 Jul 04	16 Aug 04	18 May 05	13 Jul 05	28 Sep 05
ControL	504 a*	752 a	729 a	381 a	520 a	901 a
AgroNeem 32 oz	528 a	1024 a	1225 a	305 a	1173 a	622 a

*Means in a column followed by the same letter are not significantly different at $P = 0.05$ level according to LSD test.

Helicotylenchus /6.2 cu in. (100 cc) soil

Treatment and rate/1,000 sq ft	13 Jun 04	19 Jul 04	16 Aug 04	18 May 05	13 Jul 05	28 Sep 05
ControL	646 a*	656 a	433 a	163 a	72 a	153 a
AgroNeem 32 oz	727 a	523 a	505 a	115 a	958 a	121 a

*Means in a column followed by the same letter are not significantly different at $P = 0.05$ level according to LSD test.

Summary of Nemacur-altemative **products, 2005**

Bacillusfirmus used at the IX or 2X rate did not suppress plant parasitic nematodes. However, this is not a labeled product; it is under experimental review by a private company. AgroNeem showed some activity against ring nematodes at the end of the 2005 season. This is the second consecutive season that applications have been made to the same experimental plots. Additional applications will be made in 2006. It would be premature to suggest that AgroNeem has activity against ring nematodes.

Effectiveness of *Pasteuria* infections for reducing plant parasitic nematode in golf greens

Summary of three years evaluation of *Pasteuria* infection of nematodes

	Total Nematodes			Percent Infected			Disease Index		
I.D.	2003	2004	2005	2003	2004	2005	2003	2004	2005
1	Nr	383	Nr	13	28	Nr	0.29	Nr	Nr
2	204	483	Nr	11	63	Nr	0.61	Nr	Nr
3	183	418	Nr	5	19	Nr	0.41	Nr	Nr
4	271	306	Nr	12	12	Nr	0.74	Nr	Nr
5	4200	31	14	12	0.9	7.17	0.18	0.23*	0.43*
6	Nr	148	119	33	42	51	1.1	3.71	2.72
7	Nr	Nr	77	24	2	23		0.04	0.66
8	Nr	Nr	319	2	1	8	0.02	0.01	0.17
9	Nr	223	47	80	23	79	6.94	1.27	7.0*
10	47	71	1	76.5	25.4	100	10.1*	1.6	10*
11	244	140	72	75	27	82	5.41	1.2	7.74
12	97	95	181	49.5	6	9	2.7	0.55	0.2
13	233	35	67	80	1.14	43	10.22	0.46*	4.12*
14	22	8	3	95.5	37.5	100	21.7*	1.375*	8*
15	109	Nr	-	73	62	-	5.76	3.48	-
16	772	9	32	2	-	9	0.04	-*	0.28*
17	700	Nr	698	5	1	15	0.34	0.02	0.15
18	383	Nr	904	6	1	1	0.10	0.02	0.01
19	87	190	74	74.7	21	52	11.45	0.76	0.70
20		92	96		17.28	62		0.83	0.65

* Less than 70 nematodes evaluated for this data field
Nr= not recorded, Total nematodes=total per 100 cc of soil; Percent infected=the percent of nematodes with at least on bacterium; Disease Index=the total number of bacterial cells counted divided by the number of nematodes evaluated.

Summary of Three Years of Testing Nemacur-Alternatives for Suppression of Plant Parasitic Nematodes.

Bionem L@, Neo- Tec@, Dragonfire CPP@, Rutopia@ and AgroNeem are commercially available, biorational products designed to manage or suppress plant parasitic nematodes in turf grasses. Each of these products was trialed at labeled rate, or higher than labeled rate. Several products were trialed more than once. Each product was replicated six times on 6 x 6 foot plots on established golf greens. Nemacur (fenamiphos)

was used as a standard industry control in some trials. Six untreated controls were also evaluated. In each plot, 10 subsamples of soil were collected, bulked and assayed for plant parasitic nematodes. Nematode assays were carried out four times during the course of the growing season. Nematodes were identified to genus and counted.

AgroNeem was the only product that significantly reduced nematode populations, and that occurred only after two years of applications and only on the last assay date. AgroNeem treatments will continue in 2006 to determine if the suppression was an artifact, or a promising trend. Nemacur resulted in a significant reduction of nematodes. No phytotoxicity occurred to the grass by any of the treatments.

While I do not believe that any of the Nemacur-alternatives tested here have any value for suppression or reduction of nematodes in golf greens, I remain optimistic that safe products can be and will be found for treatment of nematodes. Conventional nematicides rely on poisons to kill the nematodes; safe alternatives could simply block nematode receptors necessary for mating or finding the host. An analogy would be deet used to control mosquitoes ITom biting.

I am encouraged that several entrepreneurs are trying to fill the void that will be left when Nemacur is no longer on the market but I am disappointed at the lack of critical testing that the current set of products have received before being brought to the market place. Lack of critical testing means that golf course superintendents have to rely on anecdotal information, or advice ITom sales people for help in deciding if a product is effective. The use of ineffective materials results in a waste of time, money and other resources. I hope that the golf course associations continue to fund trials of new products that find their way to the market place.

Summary of Three Years of Testing Evaluating *Pasteuria* infections For Suppression of Plant Parasitic Nematodes.

Thousands of nematodes were examined individually to asses the incidence and extent of infection by *Pasteuria*, a bacterial parasite. Unfortunately, the unequal distribution of nematodes and *Pasteuria* in the soil made it impossible to get consistent results. In some cases, the rate of infections went up and in others they went down. In either case these trends may have reflected the natural population shift over time. However, several plots showed extreme variability ITom one year to the next. No conclusions can be drawn ITom the *Pasteuria* study.

The contribution of *Pasteuria* to changes in nematode populations cannot be ascertained ITom the data collected here. However *Pasteuria* has been shown to significantly suppress the population of root-knot nematodes in Florida. Future studies on *Pasteuria* should limit the number of collection sites to four or five, increase the number of subsamples per site, and be carried out for a longer period of time, perhaps 10 years.