Evaluation of Entomopathogenic Nematodes *Heterorhabditis bacteriophora* 'Oswego' and *Steinernema carpocapsae* 'NY001' for the suppression of red imported fire ants, *Solenopsis invicta* Buren (Hymenoptera:Formicidae)

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Fire ants continue to be a pest of medical, aesthetic, and economic importance. Effective means of control using biological control agents to manage fire ants may be an important IPM strategy. Entomopathogenic nematodes in the genus *Steinernema* and *Heterorhabditis* have been previously used in the biological control of soil-inhabiting insect pests. This research project used the species strains *Heterorhabditis bacteriophora* 'Oswego' and *Steinernema carpocapsae* 'NY001' to determine their efficacy on the control of fire ant mounds when used as soil drenches. Both nematode strains have been shown to produce positive results for the control of other soil dwelling insects such as Alfalfa Snout Beetle and they can persist in the soil for up to seven years after application (Neumann & Shields 2008; Shields et al 1999).

Materials and Methods

Two sites were utilized during this research project: one in Clarksville, TX (Site 1) and the other in a pasture in Elemendorf, TX (Site 2)

At each site, five treatment groups were utilized (See Table 1). Nematodes were reared by Dr. Pat Porter and placed in vials with a medium and mailed overnight. The shipments were kept cool by ice packs in a cooler. Each vial contained 3000 infective juvenile nematodes. Once received, they were placed in the refrigerator at 40°F until use.

Table 1. Number of mounds per treatment group by site.

	Treatment Group				
	Untreated Control	Water Treated Control	Species NY001*	Species Oswego **	Species Combination of NY001 and Oswego***
Site 1	40	40	40	40	40
Site 2	20	28	28	28	28

* *Steinernema carpocapsae* 'NY001'
** *Heterorhabditis bacteriophora* 'Oswego'
*** Combination *Steinernema carpocapsae* 'NY001' & *Heterorhabditis bacteriophora* 'Oswego'

At Site 1a 100 acre hay meadow near Clarksville, TX was divided into two strips measuring 50-feet wide. The temperature at application was 77° F with winds 5-10 mph. Two surveying flags were placed on either side of the strip after locating and flagging 10 active mounds. Flags were also placed along both edges of the plots to delineate the boundaries of each plot. A 10 foot wide buffer was placed between strips to prevent effects of treatments

from affecting fire ant colonies in adjacent plots. A total of 20 plots were measured (Figure 1). Plot lengths were arrayed from shortest to longest, then divided into 4 blocks containing 4 treatment plots each. This allowed the total length of plots for all the treatment plots to be roughly equal, so colony migration into and out of the plot areas was similar for all treatments. Within each treatment block, treatments were assigned to plots at random and to minimize pre-treatment differences in total plot length (Table 1).

At Site 2, an area of approximately 20 acres of grazing pasture land in Elemendorf, TX was scouted for fire ant mounds. Once mounds were counted, plots were determined. Treatment groups were randomly assigned to a plot and mounds were marked. Each plot contained 7 mounds, with four replications per treatment group. Untreated control plots were the exception, with 5 mounds per plot.

Fire ant activity was determined prior to treatments by minimally disturbing mounds. At Site 1 evaluations were made using the minimal disturbance method: If many fire ant (dozens) workers were observed 15 seconds after lightly disturbing the mound with a stick, then the fire ant colony was deemed active. At Site 2, mound activity was determined using a Lickert Scale. After disturbing mounds, a rating of 0-4 was designated. A rating of 0 indicated that no ants emerged within 10 seconds, at rating of 1 was assigned with -25 ants emerged, 2 meant 26-50 ants emerged, 3 meant 51-100 emerged, and 4 meant over 100 emerged when disturbed.

Nematodes were in pre-measured quantities and mixed in 1 gallon of water and drenched directly on fire ant mounds. There were approximately 3000 infective juvenile nematodes per vial, therefore the rate of application was 3000 infective juvenile nematodes per mound. Both sites initiated treatments on October 3, 2008. At Site 1, skies were clear and the temperature was 70° F with winds 0-5 mph. At Site 2, skies were partly sunny, with a temperature range of 75-89° F. Also, 4 plots were used to test Safer® Brand Fire Ant Killer (78.2% d-limonene) at 4 tablespoons per gallon of water to determine compare efficacy. Evaluations for Site 1 were made at 7, 14, 21, 30 and 60 days post-treatment. Evaluations for Site 2 were made at 7, 14, 30, and 71 days post-treatment. Data were analyzed using SPSS Analysis of Variance (ANOVA) test with means separated using Duncan's Multiple Range Test at $P \leq 0.05$ (SPSS for Windows, Lead Technologies, Version 13.0).

Along with post treatment mound activity, the presence of dead piles was also noted. When found, the dead pile was collected and evaluated for the presence of nematodes.

Results and Discussion

At Site 1, d-limonene treated mounds showed the most immediate decrease in mound activity, with a significant decrease when compared to all other treatment groups at throughout the study (Table 1). There was no significant difference between untreated controls, water treated controls and all nematode treated groups at 7, 14, and 21 days post treatment. At 28 days post treatment, the NY001/Oswego combination treated groups had significantly less mound activity compared to the untreated controls and Oswego treated groups. At 60 days post treatment, NY001 and NY001/Oswego combination treated groups had significantly less mound activity than Oswego alone and untreated control groups. However, those treatments were not significantly different than water treated controls, indicating that disturbing mounds with any drench may cause enough agitation to move the mound from the initial spot.

At Site 2, there was no significant difference in mound activity between treatment groups at pre treatment and at 7 or 14 days post treatment (Table 2). At 30 days post treatment, there was a significant difference in the *Steinernema carpocapsae* 'NY001' and the untreated control groups. However, there was significantly less activity in the untreated control group when compared to the NY001 treated group. At 71 days post treatment, there were not significant differences between treatment groups. Throughout the duration of the study, overall activity did decrease among all treatment groups; however this was not correlated with nematode infestation. The decrease in mound activity is possibly due to the lack of rain during this time frame and drop in temperatures, which caused the fire ants to move deeper in the soil and become less active on the surface. Although mound activity was not significantly decreased among nematode treated groups at Site 2, there was evidence that *Steinernema carpocapsae* NY001 parasitized fire ants. Infective juvenile nematodes were discovered from fire ant cadavers collected from a dead pile of the NY001 treated group (Figures 1 & 2). This indicates that parasitism did occur, however, it did not result in a reduction of fire ant activity. The *Heterorhabditis bacteriophora* 'Oswego' strain is appropriate for long term insect suppression within stable ecosystems (Sheilds et al 1999). Fire ants thrive in unstable ecosystems, which may explain why this strain did not show any positive results in decreasing mound activity.

An individual mound treatment using infective juvenile nematodes is only one method of application. Perhaps, applying the nematodes over an entire area and then letting them increase naturally in the soil would be a more effective means for control. If the nematodes were present in the soil, then they could infect newly colonized mounds and foraging workers for possibly more effective control.

Table 1. Number of active fire ant mounds 7, 14, 21, 28 and 60 days post treatment in Clarksville, TX.

Treatment	**7 Days**	**14 Days**	**21 Days**	**28 Days**	**60 Days**
d-Limonene	5.75**a**	3.50**a**	3.75**a**	4.00**a**	3.00**a**
NY001	9.75b	9.25b	8.25b	7.50bc	7.00b
Oswego	9.75b	9.50b	9.00b	8.75c	8.25c
NY001/ Oswego	9.25b	9.00b	9.00b	7.00b	6.25b
Water	9.75b	9.50b	8.75b	8.50bc	8.00bc
Dry	10.00b	10.00b	9.25b	9.25c	9.00c

[a]Means followed by the same letter within the same column were not significantly different using Analysis of Variance (ANOVA) and means separated using Duncan's Multiple Range Test at $p \leq 0.05$ (SPSS, Windows 11.5).

Table 2. Mean mound activity as determined by a Lickert Scale in Elmendorf, TX.

Treatment	Pre-treat	7 Days	14 Days	30 Days	71 Days
NY001	3.15a	2.68a	3.07a	2.50b	1.86a
Oswego	3.29a	2.61a	2.71a	2.00ab	1.79a
Combination	3.39a	2.36a	2.43a	2.25ab	1.75a
Water	3.32a	2.14a	2.61a	2.14ab	2.11a
Dry	3.40a	2.25a	2.45a	1.75a	1.95a

[a]Means followed by the same letter within the same column were not significantly different using Analysis of Variance (ANOVA) and means separated using Duncan's Multiple Range Test at $p \leq 0.05$ (SPSS, Windows 11.5).

Figure 1. Fire ant cadaver head parasitized by *Steinernema carpocapsae* NY001.

Figure 2. Fire ant cadaver head parasitized by *Steinernema carpocapsae* NY001.

Literature Cited

Neumann, G and E. Shields. 2008. Multiple Species Natural Enemy Approach for Biological control of Alfalfa Snout Beetle (Coleoptera: Curculionidae) Using Entomopathogenic Nematodes. Environ. Entomol. 101(5): 1533-1539.

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