Entomopathogenic Nematode Drenches for Control of Fungus Gnats during Summer Florida Conditions

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Table of Contents

Executive Summary

Fungus gnats (*Bradysia spp*.) are a common insect pest problem of greenhouse crops. This study evaluated the efficacy of nine entomopathogenic nematode (EPN) products and concentrations during summer in a Florida research greenhouse, compared with dinotefuran (Safari) and an untreated control. The entomopathogenic nematode products tested included three species: *Steinernema kraussei* (Nemasys-L); *Steinernema feltiae* (Nemasys, SF Max, Nemashield, Scanmask, and Entonem) and *Steinernema riobrave* (BioVector).

In this trial, bacopa liners were drenched with EPN products in a University of Florida research greenhouse during June-July 2007 (soil temperatures 82°F day/73°F night). Compared with the number of adult fungus gnats that emerged from untreated control trays, fungus gnat levels in trays treated with dinotefuran (Safari, 1% of the control) or Entonem (67% of the control) differed significantly from the control trays. Although treatment with the other EPN products resulted in lower average fungus gnat counts than from untreated trays, fungus gnat levels with these EPN products were not significantly different from the control. Dinotefuran (Safari) provided greater mortality of fungus gnat larvae than the EPN products.

Efficacy of EPN was lower in this trial compared with our previous studies. For example, a trial in poinsettia crops in four locations (three commercial sites, University of New Hampshire) in October 2005 found that the number of emerging fungus gnats per 6-in azalea pot following a single drench of Nemasys (*Steinernema feltiae*) was between 12 and 39% of control pots. In a summer trial with poinsettia liners at the University of New Hampshire in 2006, with very similar soil temperatures (83°F day/73°F night) to the Florida trial, the number of emerging fungus gnats per tray from a peat/perlite medium treated once with Nemasys was 49% of the control. A single application of Nemasys to phlox liners grown in peat/perlite during a Feb to Mar trial at the University of New Hampshire resulted in only 6% of the emerging adults per tray compared with untreated control trays.

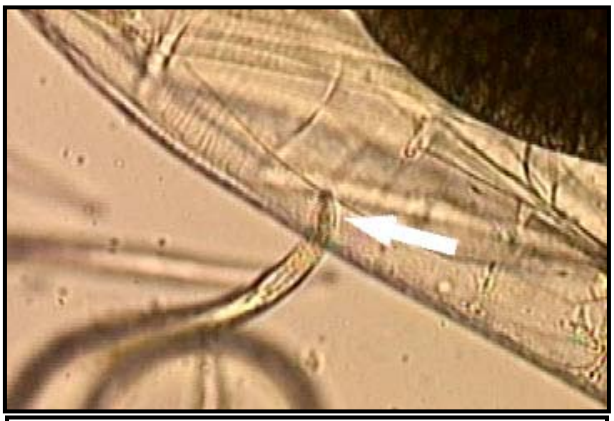
Viability of the EPN products varied both between products and between the two batches tested, ranging from 10% to 63% inactive EPN juveniles (presumed dead). However, more evaluations are needed given the variability between batches of EPN products. As a general commercial greenhouse practice, each batch of product received should be checked for viability.

Introduction

The purpose of this study was to evaluate the effectiveness of drench applications of different EPN products for control of fungus gnat larvae under warm greenhouse conditions. There was also a standard insecticide treatment, dinotefuran (Safari™) and a water control.

Fungus gnats (*Bradysia spp*.) are a common insect problem in greenhouses and are attracted to moist, growing medium, which is prevalent in propagation environments. Adult fungus gnats lay eggs in clusters just below the surface of the growing medium. The eggs hatch into larvae, which feed on plant roots thus inhibiting the plant's ability to withdraw water and nutrients. In addition, larvae may transmit plant diseases such as Pythium, Verticillium, Sclerotinia and Thielaviopsis.

Certain strains of entomopathogenic nematodes (EPN) specifically target fungus gnat larvae. These EPN are able to detect larvae via the release of carbon dioxide and products excreted by the larvae. Infective juveniles (IJs) enter larvae through natural openings, such as the mouth, anus or breathing pores (spiracles). Upon entering the host, the nematode releases a bacterium, which is actually responsible for killing the larvae. The bacteria kill the larvae, usually within 24 hours, by releasing protein destroying enzymes. The nematodes feed on the remains, and complete two to three generations inside the dead larvae. Eventually, large numbers of IJs leave the dead cadaver and search for new fungus gnat larvae.



EPN parasitizing a fungus gnat larva.
http://www.mediashark.com/Pages/Projects/BioControl.html

Research Protocol

Based on previous studies, dinotefuran (Safari™) was selected as the standard insecticide. There was also a water control. The EPN products used are presented in Table 1.

Table 1: Entomopathogenic nematode products and active ingredients (species)

Product	Active Ingredient (Species)
Nemasys	*Steinernema feltiae*
Scanmask	*Steinernema feltiae*
BioVector	*Steinernema riobrave*
Nemashield	*Steinernema feltiae*
J-3 Max Sf	*Steinernema feltiae*
Entonem	*Steinernema feltiae*
Nemasys-L	*Steinernema kraussei*

Unrooted cuttings of vegetatively propagated “Sutera Snowstorm” bacopa from Proven Winners were transplanted on May 23, 2007 into Fafard 2 Mix growing medium (55% peat, with the remainder perlite and vermiculite). Cuttings were inserted into 1206 trays, which were then excised into six-packs (6 cuttings per tray), with 480 trays (2880 cuttings) total.

Air and soil temperature were recorded once every 30 minutes throughout the experiment with a HOBO data logger. Greenhouse air temperature averaged 87°F during the day and 72°F at night, with a high of 100°F and a low of 69°F. The soil temperatures averaged 82°F during the day and 73°F at night. Cuttings were misted at a frequency of once every 20 to 30 minutes for 6 seconds during the day and every 30 minutes for 6 seconds at night for the first two weeks. Plants were then manually watered twice daily during weeks 3-4, and every other day after 5 weeks. Relative humidity averaged 73% (measured using a HortiMax humidity sensor) and daily light integral averaged 16.0 moles/m^2/day, measured as an average of two Apogee quantum sensors.

Adult fungus gnats were collected from the University of Florida greenhouses using insect aspirators and plastic vials. Approximately 75 mature (mixture of females and males) fungus gnats were released every other day, for a total of 450, into the compartment from June 1 through June 11, by placing the plastic vials randomly on the benches between the bacopa trays.

Two separate batches of each EPN products were received in 2-3 days prior to their application date on June 13 and June 20. Each batch was stored in a walk-in cooler at 40F until application. Viability was measured separately for each batch of EPN product immediately prior to drench application. Liner trays received either one application (the first June 13 batch), or two applications (the second June 20 batch) of each EPN product, with 20 replicate trays per application date and product. Safari was applied only once, on June 13. Control trays received a drench of deionized water.

An exception to other EPN products was Nemasys-L, which was applied 9 days after other treatments (June 22 and June 29) because of a lack of product availability. A separate group of control trays (different from the earlier control group) was sampled to compare with the Nemasys-L, because of the later EPN application date.

The rates used for the study were calculated based on a two-step dilution procedure to ensure the same concentration of EPN were being applied per treatment. This was appropriate since each control product did not have the same initial number of EPN. The generalized protocol was as follows:

Entomopathogenic nematode mixing and application method (photos at right)

1. Expiration date was checked on each EPN package. All materials were delivered to University of Florida within one week before application, and were stored in a refrigerator at 39°F.
2. A container was filled with 5 liters of deionized water.
3. The sponge containing the EPN was added to the water.
4. The sponge was agitated for 3 minutes to dislodge the EPN.
5. A portion (from Table 2) of the solution from container 1 (maintaining agitation) was removed with a pipette and placed into container 2 (which held 1 liter of solution).
6. The container 2 solution was filled to the top with 1 liter of deionized water.
7. Viability was determined after mixing and before application to the growing medium.
8. Approximately 30 mL from container 2 was applied to each tray (20 trays per treatment).
9. Steps 1-7 were repeated for each of the five EPN treatments.

Table 2: Dilution method for entomopathogenic nematode treatments

Two step dilution:	Step 1	Step 2			
	Container 1 volume (5 liters)	Container 2 volume (5 liter)			
Treatment	Concentration (nematodes/L)	mL from container 1 added per L	mL from container 2 added per tray	Estimated # nematodes per tray	Estimated # nematodes per square foot
Nemasys	10,000,000	23.7	100	4,740	39,500
Scanmask	5,000,000	47.4	100	4,740	39,500
BioVector	200,000,000	1.8	100	7,200	60,000
Nemashield Low	10,000,000	11.8	100	2,360	19,667
Nemashield Med	10,000,000	23.7	100	4,740	39,500
Nemashield High	10,000,000	32.2	100	6,440	53,667
J-3 Max Sf	200,000	237.1	100	4,740	39,500
Entonem	10,000,000	23.7	100	4,740	39,500
Nemasys-L	10,000,000	23.7	100	4,740	39,500

Nematode viability: Viability of the EPN was determined by taking a specified volume (see Table 3) of liquid from container 1 and diluting it with deionized water (see Table 3) to obtain solutions with approximately 1,000 EPN (except for J-3 Max Sf, which had a lower label concentration, at 200 EPN per mL). From this solution, 25 samples of 40 microliters were removed using an automatic pipette, and placed onto a petri dish (photo at right). A total of 1 mL was evaluated [40 microliters (µL) x 25 = 1,000 microliters (µL) = 1 mL].

Viability test. Photo shows the 40uL droplets being applied to the petri dish and then viewed through a dissecting scope to count the number of active and inactive EPN.

The number of "inactive" and "active" EPN juveniles, indicated by whether EPN were moving, was counted in each 40 microliter sample. A needle was inserted into the sample to mechanically stimulate the EPN in order to determine if they were active or not.

Table 3: Dilution method to assess entomopathogenic nematode viability

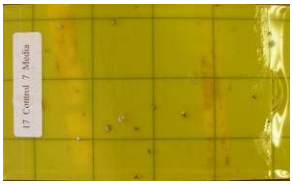
Treatment	mL taken from container 1 for container 3	mL deionized water added to container 3	Estimated nematode concentration EPN juveniles per mL
Nemasys	1	9	1,000
Scanmask	2	8	1,000
BioVector	1	599	1,000
Nemashield	1	9	1,000
J-3 Max Sf	10	0	200
Entonem	1	9	1000
Nemasys-L	1	9	1000

Product efficacy for fungus gnat control:
One week after applying the second EPN treatment (June 27 for all products except Nemasys-L, and July 6 for Nemasys-L) all bacopa cuttings were cut level with the growing medium surface.

The trays, filled with growing medium and plant roots, were then placed into individual paper bags in Dillen 12-inch oval saucers filled with 300 mL of water to maintain a consistent moisture content. A 3-in x 5-in yellow sticky card was positioned on 2 clothes pins on top of the trays to collect emerging adult fungus gnats. Bags were stapled closed and stored in a greenhouse for 3 weeks between 72°F and 100°F.

One week after placing pots in the bags, all the bags were opened and the water in saucers was topped off to ensure continued hydration of the growing medium. Because the growing medium may have dried, white plastic garbage bags were placed over each paper bag. Once a week for the next two weeks, ten randomly selected bags were opened to check moisture loss and then resealed. Media remained moist for the following 2 weeks after use of the plastic bags.

After 3 weeks, the bags were opened, sticky cards were removed, and the number of adult fungus gnats on each card was recorded.



Results and Discussion

Viability

Figure 1 and Table 4 present results from the EPN viability test. There were significantly more active EPN in Nemasys (861/mL) and Nemasys-L (742/mL) compared with the other EPN products.

However, J-3 Max Sf had a greater concentration than was indicated on its label. The label for J-3 Max Sf indicated that 200 EPN would be present per mL with our dilution rate. In contrast, we found 381 active, 89 inactive, and 469 total EPN per mL for J-3 Max Sf. Scanmask had a significantly higher proportion of inactive EPN (53%) than the other products.

All EPN products had fewer (<1000 EPN/mL) organisms than was expected based on the label rate, except for J-3 Max Sf (when corrected for the label dilution rate, as noted earlier), Nemasys-L (batch 1) and Nemasys (batch 2). Although we aimed for uniformity, sampling errors may still arise because of possible non-uniform mixing of the stock solution from which the viability samples were drawn, and potential clumping of nematodes.

In comparison, an evaluation by our research group during summer 2006 of a single batch of Nemasys, J-3 Max Sf, and three other EPN products not included in this trial, found that Nemasys had 1020 total nematodes/mL, of which 19% were inactive, and 820 total nematodes/mL for J-3 Max Sf, of which 22% were inactive (fairly similar to results from this trial).

Efficacy

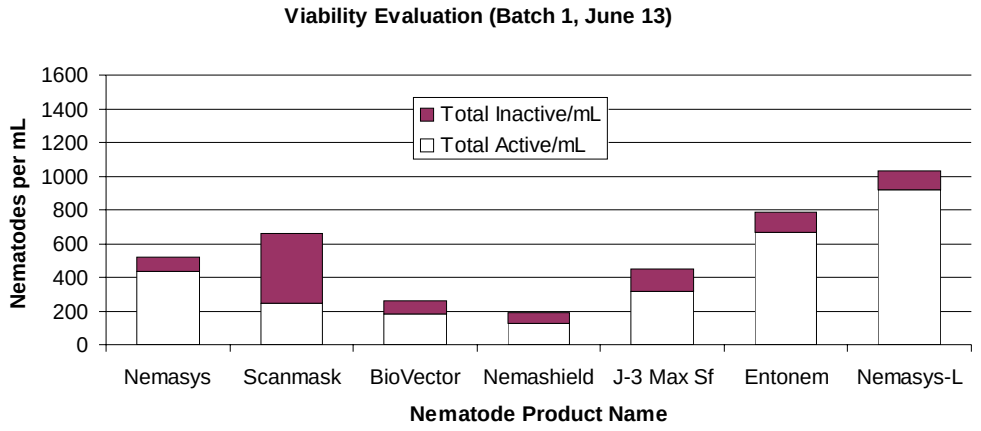
An average of 10 fungus gnat adults emerged from the control trays in the first sampling date (June 27) whereas a mean of 7.2 fungus gnat adults emerged from the second set of controls (used to compare against Nemasys-L on July 6, Table 5. A single drench application of dinotefuran (Safari) resulted in almost no emergence of fungus gnat adults.

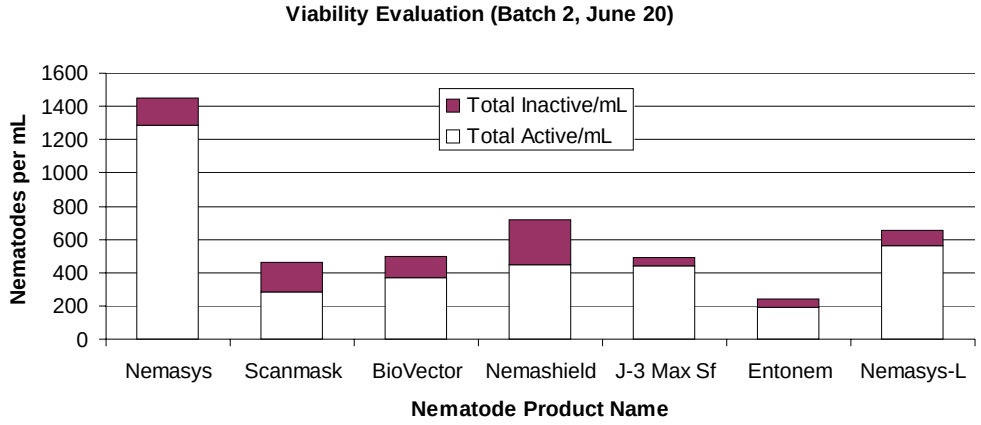
An increase in the number of EPN drench applications from one to two applications, one week apart, resulted in a marginal but statistically significant decrease (7.8 versus 8.9 emerging adults per tray). There was no interaction between products and number of treatments, and data were therefore pooled across one and two treatments in Table 5. Table 5 represents the average of emerging fungus gnat adults per tray across both one or two applications for each EPN product. Increasing the concentration (low to high) of Nemashield did not result in higher mortality of fungus gnat larvae.

When compared with the untreated control, only dinotefuran (Safari) and Entonem provided a significantly higher level of mortality of fungus gnat larvae compared to the other treatments. However, the standard insecticide treatment, dinotefuran (Safari) provided the highest level of fungus gnat larval mortality.

Efficacy of EPN was lower in this trial compared with our previous studies. For example, a trial in poinsettia crops in four locations (3 commercial locations, 1 university) in October 2005 found that the number of emerging fungus gnats per 6-in azalea pot following a single drench of Nemasys (*Steinernema feltiae*) was between 12 and 39% of control pots. In a summer trial with poinsettia liners at the University of New Hampshire in 2006, with very similar soil temperatures (83°F day/73°F night) to the Florida trial, the number of emerging fungus gnats per tray from a peat/perlite medium treated once with Nemasys was 49% of the control. A single application of Nemasys to phlox liners grown in peat/perlite during a Feb to Mar trial at the University of New Hampshire resulted in only 6% of the emerging adults per tray compared with untreated control trays.

Figure 1. Number of active and inactive entomopathogenic nematodes (EPN) per mL for ten EPN products with two separate evaluations (June 13 and 20). The expected total number of EPN (including active and inactive) in each sample was 1,000, except for J-3 Max Sf (expected 200 EPN/mL). Each bar is based on the average of 25 replicate samples of 40 µL. There were statistically significant differences between products and batches, which are detailed in Table 4.





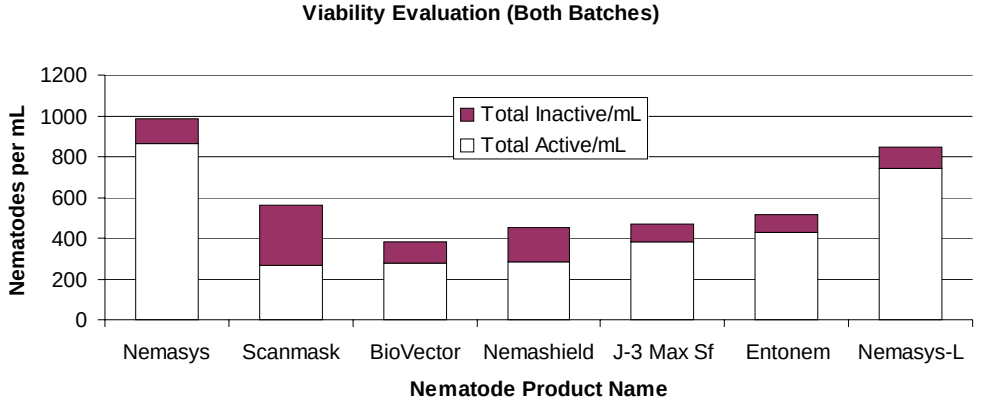


Table 4. Number of active and inactive entomopathogenic nematodes (EPN) in viability test 1 (top) and 2 (middle), and the mean from both batches (bottom). The expected number, based on the product labels, was 1,000 EPN per mL except for J-3 Max Sf (expected 200 EPN per mL). Actual counts of EPN are presented here and in Figure 1. Mean separation used Tukey's HSD at the p=0.05 level.

Batch 1 (June 13 application)

Product Name	Nematode Species	total active/mL	total inactive/mL	total/mL	% inactive
Nemasys	*Steinernema feltiae*	435c	87b	522cd	17
Scanmask	*S. feltiae*	245de	417[a]	662bc	63
BioVector	*S. riobrave*	184de	77b	261ef	30
Nemashield	*S. feltiae*	125e	65b	190f	34
J-3 Max Sf	*S. feltiae*	319cd	127b	446de	28
Entonem	*S. feltiae*	666b	118b	784b	15
Nemasys-L	*S. kraussei*	920a	114b	1,034a	11

Batch 2 (June 20 application)

Product Name	Nematode Species	total active/mL	total inactive/mL	total/mL	% inactive
Nemasys	*Steinernema feltiae*	1,288a	161b	1,449a	11
Scanmask	*S. feltiae*	287cd	174b	461c	38
BioVector	*S. riobrave*	372cd	128bc	500c	26
Nemashield	*S. feltiae*	447bc	270a	717b	38
J-3 Max Sf	*S. feltiae*	442bc	50d	492c	10
Entonem	*S. feltiae*	194d	49d	243d	20
Nemasys-L	*S. kraussei*	563b	93cd	656bc	14

Average Over Both Batches

Product Name	Nematode Species	total active/mL	total inactive/mL	total/mL	% inactive
Nemasys	*Steinernema feltiae*	861a	124c	986a	13
Scanmask	*S. feltiae*	266c	296a	562b	53
BioVector	*S. riobrave*	278c	103	381c	27
Nemashield	*S. feltiae*	286c	168b	454bc	37
J-3 Max Sf	*S. feltiae*	381bc	89c	469bc	19
Entonem	*S. feltiae*	430b	84c	523bc	18
Nemasys-L	*S. kraussei*	742a	104c	845a	12

Table 5. Entomopathogenic nematode (EPN) product efficacy test for fungus gnat control. Statistical significance is based on comparison against the control using Dunnett's Test at the 95% level for both one and two application treatments of the EPN products. Control 1 was used to calculate percent control and fungus gnat adult emergence for all products except Nemasys-L. A different set of control trays (Control 2) was used to determine percent control and reduction for Nemasys-L since Nemasys-L was applied later than other EPN products.

Product	Average Fungus Gnats per strip	Significantly different from control	% of control
Control 1	10.0		100%
Safari	0.1	Yes	1%
Entonem	6.7	Yes	67%
Scanmask	7.4		74%
Nemashield Low	7.6		76%
Nemasys	7.8		78%
Nemashield Med	8.0		80%
J-3 Max Sf	8.9		89%
Nemashield High	9.2		92%
BioVector	10.7		107%
Control 2	7.2		100%
Nemasys-L	7.7		107%

Acknowledgements

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