Young Plant Research Center

# Drenches of Entomopathogenic Nematodes for Control of Fungus Gnats in Poinsettia Liners



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Photo: Viable nematodes in solution before application.
http://www.mediashark.com/Pages/Projects/BioControl.html

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### Executive Summary

Fungus gnats (*Bradysia spp*.) are a common problem in greenhouse production. This study explored the efficacy of five entomopathogenic nematode applications during summer poinsettia propagation compared with Safari and an untreated control. The entomopathogenic nematode products we tested involved three species: *Steinernema carpocapsae* (SC Max); *Steinernema feltiae* (Nemasys, SF Max and Gnat Not) and *Heterorhabditis indica* (Symbion South).

There were an average of 3.4 emerging fungus gnats per strip of Oasis media compared with an average 12.9 fungus gnats for the peat/perlite (Sun Gro LA4) medium. No fungus gnat adults emerged on any strip for those plants treated with Safari.

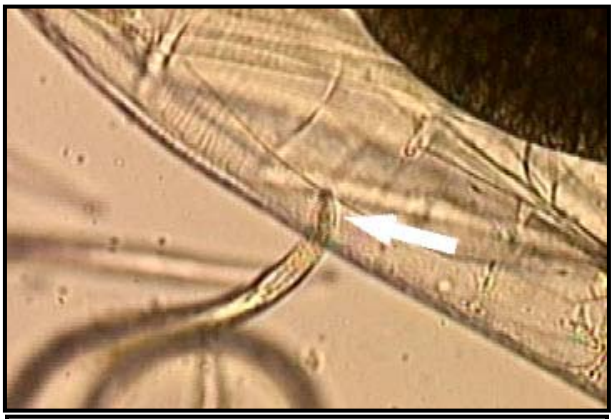
In the peat-perlite medium, only Symbion and Safari had significantly fewer entomopathogenic nematodes than the untreated control. The level of control provided by Symbion was surprising based on the low survival rate of the entomopathogenic nematodes in the viability test. Nemasys provided the next best level of control. For a fair product comparison, more evaluations would be needed given that batches of biological controls may vary in their viability.

## Introduction

The purpose of this study was to evaluate the effectiveness of drench applications of different entomopathogenic nematodes for controlling fungus gnats, compared with the insecticide dinotefuran (Safari™) and a water control.

Fungus gnats (*Bradysia spp*.) are a common problem in greenhouse substrates and are attracted to moist, growing medium, which is characteristic of a propagation environment. 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 disrupting the plant's ability to withdraw nutrients and potentially introducing plant diseases such as Pythium, Verticillium, Sclerotinia and Thielaviopsis (Drees, 1994).

Entomopathogenic nematodes (EPN) are a biological control agent that is increasing in popularity. Certain strains of EPN specifically target fungus gnat larvae. These EPN are able to detect larvae via the release of carbon dioxide and fungus gnat larvae excretion products. Infective juveniles (IJs) enter the hosts through natural openings, such as the mouth, anus or breathing pores (spiracles). Upon entering the host, the nematode releases a bacterium into the host, 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 host remains, completing two to three generations inside the host.



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

After consuming the host, large numbers of IJs leave the host and search for new hosts. At room temperature, it takes steinernematid nematodes up to 10 days from infection to the emergence of new IJs. Heterorhabditid nematodes take up to 15 days to emerge from a host.

Based on previous studies, dinotefuran (Safari™) was selected as the insecticide for comparison. There was also a water control (no treatment). The EPN treatments are shown in Table 1.

### Table 1: The entomopathogenic nematode products and active ingredients (species)

| Product | Active Ingredient |
|---|---|
| Gnat Not | *Steinernema feltiae* |
| Nemasys | *Steinernema feltiae* |
| SC Max (J-3Max Sc) | *Steinernema carpocapsae* |
| SF Max (J-3Max Sf ) | *Steinernema feltiae* |
| Symbion South | *Heterorhabditis indica* |

'Orion Red' poinsettia unrooted cuttings were received from Fischer USA and immediately stuck into growing medium on June 21, 2006 . Two growing media were used: Oasis foam or Sun Gro LA4 peat/perlite growing medium. Both types of growing medium were in Oasis wedge trays cut into strips to fit 5 cuttings per strip, with 70 strips (350 cuttings total) per growing medium type.

**Cuttings after being positioned in oasis strips.**

The greenhouse temperatures averaged 80.8°F day and 73.7°F night. The soil temperature averaged 82.5°F day and 73.2°F night. The cuttings were placed under mist that was scheduled to operate at a frequency of every 20 minutes for 6 seconds during the day and every 30 minutes for 6 seconds at night for the first two days. The mist frequency was reduced to 30 minutes during the day and 60 minutes at night for the following 8 days. The relative humidity was 80% for the duration of the study using a computer-controlled fogging system, and shade cloth was used to help maintain the relative humidity and cutting turgidity.

The cuttings were sprayed with Capsil immediately after sticking at a rate of 3 mL/1 gallon. On June 23 (day 2) and also on June 29 (day 8) the cuttings were sprayed with fenhexamid (Decree$^{TM}$) fungicide to prevent outbreaks of *Botrytis.*

## Research Protocol

The cuttings remained under mist for a total of 10 days after sticking to ensure sufficient rooting (plug was "pullable") before fungus gnats were released into the greenhouse compartment. Adult fungus gnats were collected from research compartments at the University of New Hampshire Greenhouse complex using insect aspirators and collecting the fungus gnats in plastic vials. Approximately 50 adult fungus gnats (mixture females and males) were released every second day, for a total of 350, into the compartment from June 30 through July 12, by gently emptying the plastic vial containing the fungus gnats in random locations on the benches between the poinsettia strips.

Two weeks after the cuttings were stuck the treatments were applied to the growing medium (July 5). The rates for the study were calculated using a 2 step dilution procedure to assure the same concentration of EPN were being applied per treatment (Table 1). The generalized protocol was as follows:

<u>Entomopathogenic nematode mixing and application method (photos at right)</u>

1. Expiration date was checked on each EPN package. All materials were delivered to UNH one week before application, and were stored in a refrigerator at 39$^{o}$F before application.
2. A container was filled with 1 to 2.5 liters of deionized water.
3. The EPN sponge was added to the water.
4. The sponge was agitated for 3 minutes to dislodge EPN.
5. A portion (from Table 2) of the solution in container 1 (maintaining agitation) was removed with a pipette and placed into container 2 (which held at least 1 liter of solution).
6. Container 2 solution was topped up to 1 liter with deionized water.
7. Viability was checked after mixing and before application to the substrate.

8. 30 mL from container 2 was applied to each strip (20 strips per treatment) in that group.
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 (2.5 liters) | Container 2 volume (1 liter) | | |
| **Treatment** | **Concentration (nematodes/L)** | **mL from container 1 added per liter** | **mL from container 2 added per strip** | **Theoretical # nematodes per strip** |
| Gnat Not | 2000000 | 63.1 | 30 | 3,788 |
| Nemasys | 20000000 | 6.3 | 30 | 3,788 |
| SC Max (J-3Max Sc) | 400000 | 315.7 | 30 | 3,788 |
| SF Max (J-3Max Sf) | 400000 | 315.7 | 30 | 3,788 |
| Symbion South | 2000000 | 63.1 | 30 | 3,788 |

**Nematode viability**: A viability test of the EPN was performed by taking 7.9 mL from container 2 and adding 2.1 mL of deionized water to attain a 1,000 EPN solutions. From the 10mL, 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 testing. 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 nematodes.

The number of "inactive" and "active" nematodes, indicated by whether nematodes were moving, was counted in each 40 microliter sample. A needle was placed into the sample to mechanically stimulate the EPN to check whether they were moved before concluding they were "inactive".

**Product efficacy for fungus gnat control:**

Two weeks after applying the treatments the poinsettia cuttings were cut level with the growing medium surface.

The strips filled with growing medium and plant roots were then placed into individual paper bags in Dillen 12-inch oval saucers filled with 200 mL of water to maintain a consistent moisture content. A yellow sticky card was placed on 2 clothes pins on top of the strips of growing medium to collect the emerging adult fungus gnats. The bags were then stored in a cooler between 60°F and 64°F for 4 weeks.

At the end of the 4 weeks the bags were opened, the sticky cards were removed and the fungus gnats were counted on each card and recorded.

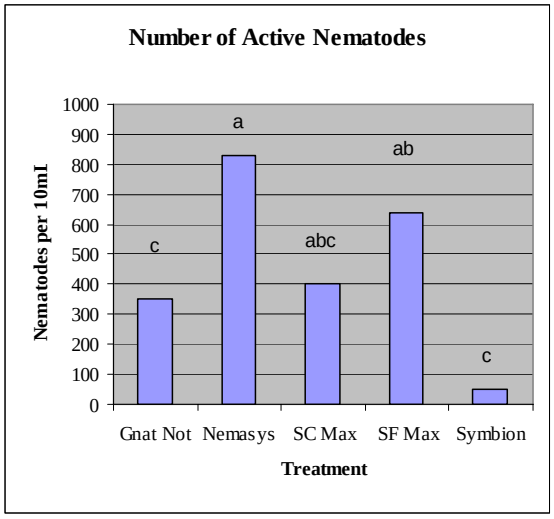
## Results and Discussion

Figure 1 and Table 3 show results from the nematode viability test. There were significantly more active nematodes in Nemasys and SF Max compared with the Gnat Not and Symbion.

Symbion had significantly more inactive nematodes than the other nematode treatments.

All products had fewer that the expected number (1000) of active organisms in the 10 mL sample, and only Nemasys had 1000 or more nematodes (including inactive organisms) in the sample.

**Figure 1. Number of active (left) and inactive (right) nematodes in 10 mL samples of five nematode products. The expected total number of nematodes (including active and inactive) in each sample was 1000. Each bar is based on the average of 25 replicate samples of 40 µL, scaled to 10 mL. Letters represent mean comparisons using Tukey's HSD at the p=0.05 level.**



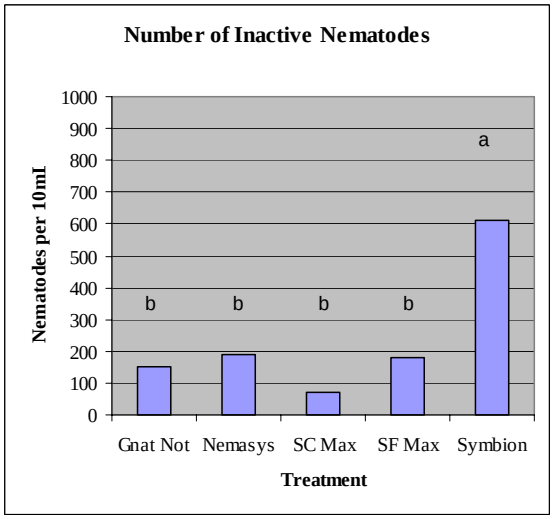
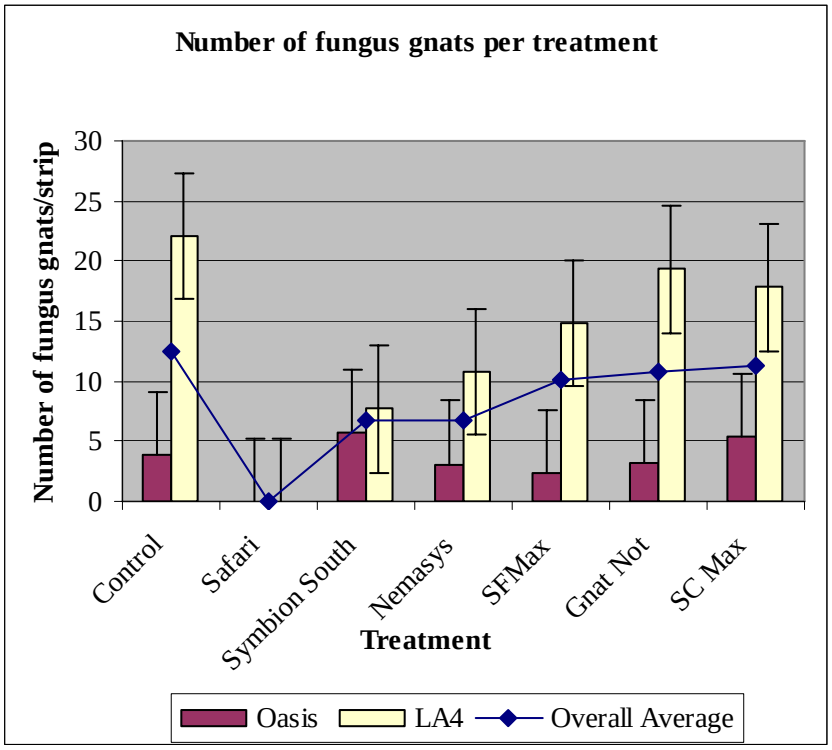




**Table 3. Number of active and inactive nematodes counted in the viability test. The expected number based on the product labels was 1000 per 10 mL sample and 3788 per strip. Actual counts and number of active individuals applied per strip are shown.**

| Trade Name | Nematode species | Total (active and inactive) nematodes per 10 mL | # Active per 10 mL | # Inactive per 10 mL | % Inactive | # Active nematodes applied per strip |
|---|---|---|---|---|---|---|
| **Gnat Not** | *Steinernema feltiae* | 500 | 350 | 150 | 30.0 | 1329 |
| **Nemasys** | *Steinernema feltiae* | 1020 | 830 | 190 | 18.6 | 3152 |
| **SC Max** | *Steinernema carpocapsae* | 470 | 400 | 70 | 14.9 | 1519 |
| **SF Max** | *Steinernema feltiae* | 820 | 640 | 180 | 22.0 | 2430 |
| **Symbion South** | *Heterorhabditis indica* | 660 | 50 | 610 | 92.4 | 190 |

**Figure 2. Product efficacy test for fungus gnat control. Error bars represent 95% confidence intervals.**




There was an average of 3.4 fungus gnats per strip for Oasis compared with 12.9 fungus gnats for the peat/perlite (LA4) medium. There were an average 4 or 22 fungus gnats in the untreated control strips for Oasis and peat/perlite, respectively.

No live fungus gnats emerged from any strip treated with Safari.

In the peat-perlite medium, only Symbion and Safari had significantly fewer nematodes than the untreated control. The high level of control from Symbion was surprising given the low number of active individuals for this product shown in the viability test (Figure 1). Therefore, the small number of active Symbion organisms must have been highly effective, or the viability test was not accurate. Nemasys was the second-best-performing nematode product.

For a fair product comparison, more evaluations would be needed given that batches of biological controls may vary in their viability. In discussing these trial results with BioControl Systems, Inc., which produces Gnat Not and Symbion, they indicated that no more than 10% of their organisms are normally inactive.

**Literature cited**

Drees, B.M. 1994. Fungus Gnat Management. The Texas Agricultural Extension Service, Texas A&M University System, Bulletin UC-028. http://insects.tamu.edu/extension/bulletins/uc/uc-028.html