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The results and conclusions in this report are based on an investigation conducted over one year. The conditions under which the experiment was carried out and the results obtained have been reported with detail and accuracy. However, because of the biological nature of the work it must be borne in mind that different circumstances and conditions could produce different results. Therefore, care must be taken with interpretation of the results especially if they are used as the basis for commercial product recommendations.

**AUTHENTICATION**

We declare that this work was done under our supervision according to the procedures described herein and that the report represents a true and accurate record of the results obtained.

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**GROWER SUMMARY**



**SCIENCE SECTION**

# Grower Summary

## Headline

In a laboratory experiment, mushroom sciarid flies were controlled by the novel insecticide Calypso (thiacloprid) and the biological control agents Nemasys (based on the insect pathogenic nematode *Steinernema feltiae*) and Gnatrol (based on the insect pathogenic bacterium *Bacillus thuringiensis*).

In a crop scale experiment good control was achieved by treating the casing with Dimilin (Dimilin susceptible population), Calypso or Nemasys, and by treating the compost with Nemasys and 1.5x rate Gnatrol.

## Background and expected deliverables

Mushroom sciarid flies are important pests of commercial mushroom production. The main species causing damage in the UK are *Lycoriella castanescens and Lycoriella ingenua*. The latter is of particular concern because it has started to develop resistance to diflubenzuron, the most widely used chemical insecticide for sciarid fly control. At present, only one other chemical insecticide is available, the pyrethroid deltamethrin. A biological control agent, the insect pathogenic nematode *Steinernema feltiae*, is also being sold, but it is reputed only to work when applied to mushroom casing.

In order to develop sustainable methods of controlling sciarid flies, new control agents need to be evaluated. More information is also required about the effectiveness of insect pathogenic nematodes. In addition, the potential to integrate different control agents needs to be assessed.

The aim of this project is to investigate an IPM strategy for controlling sciarid fly pests for the UK mushroom industry. The expected deliverables from this work include:

- An assessment of potential control agents and a control strategy based on current control methods.
- New information on control methods that could rapidly become available to the industry.

- Information made available to industry, via fact sheets, popular articles, training events and a project advisory group.
- Consideration of a strategy to minimise the development of insecticide resistance in sciarid flies.

## Summary of the project and main conclusions

### Laboratory evaluation of different control agents

Laboratory experiments were done to evaluate the effect of seven different control agents on *L. ingenua* populations in mushroom compost and casing. Control agents were applied as drenches and by mixing (= admix) into the substrate. *Lycoriella ingenua* were tested in two ways: (a) by applying eggs to the substrate three days before or after the application of the control agent; (b) allowing adult females to lay their eggs on substrate treated with the control agents. The control agents used are set out in the following table:

| Control agent | Type |
|---|---|
| Dimilin (Uniroyal) | Chemical insecticide (diflubenzuron) approved in UK for mushrooms |
| Decis (AgrEvo) | Chemical insecticide (deltamethrin) approved in UK for mushrooms |
| Nemasys (Becker Underwood) | Biological control agent, insect pathogenic nematode (*Steinernema feltiae*) available in UK |
| Calypso (Bayer) | Novel chemical insecticide (thiacloprid) approved in UK for other crops |
| Spinosad (Dow) | Novel chemical insecticide approved in UK as Conserve for other crops |
| Gnatrol (Valent Biosciences) | Biological control agent, insect pathogenic bacterium (*Bacillus thuringiensis*), sold for use on mushroom crops in USA |
| AGARIguard (ECOspray Ltd.) | Novel insecticide, based on food-grade garlic extract |

- <u>Dimilin</u> gave good control in casing and compost.
- <u>Decis</u> gave poor control in casing and compost.

- Nemasys gave good control in casing and compost.
- Calypso gave good control, except when mixed into compost against eggs.
- Spinosad gave poor control in casing, and mixed results in compost.
- Gnatrol gave good control in casing and compost.
- AGARIguard gave poor control in compost and casing. However, in previous work, it caused high levels of mortality when drenched directly onto *L. ingenua* eggs. It also gave better control when fly infestation occurred immediately after treatment. The poor control in this study may be due to poor persistence of AGARIguard in the substrate. It may have some ovipositional deterrent effect but this would also be detrimentally affected by poor persistence.

**Crop scale evaluation of selected control agents**

From the results of the laboratory experiments, Nemasys, Gnatrol, and Calypso were selected for evaluation on a crop scale. In accordance with commercial practice, the treatments were mixed into compost at spawning, or applied to casing as drenches. Dimilin was also included as a standard casing treatment, and the experiment included untreated controls.

- None of the replicated treatments gave good control of the first generation of sciarids (all gave less than 35% control compared to the untreated trays).
- Control of the second generation was improved in all treatments. Overall, good sciarid control was achieved by treating the casing with Dimilin, Calypso and Nemasys, and by treating the compost with Nemasys.
- Dimilin gave 80% control of the second generation.
- Control of the second generation with Calypso was less in the compost (46% control) than the casing (70% control).
- Nemasys performed equally well in compost and casing (70% control of second generation in both cases).
- Gnatrol gave about 50% control of the second generation in compost and casing, but it is possible that control could have been improved if a higher concentration had been used.
- Mushroom yield was increased with all treatments over untreated substrate. When compared to the Dimilin treated trays, higher yields were achieved where casing had been treated with Gnatrol and Calypso and where compost had been treated with Gnatrol and Nemasys.

## Financial benefits

The total cost of pest infestations to the UK mushroom industry is estimated at £11-12 million per annum, including direct loss from insect attack, transmission of mushroom pathogens, and costs of buying and applying insecticides. The financial benefits of this study cannot be ascertained at this stage, as they will depend upon a number of factors including the future availability of insecticides and the development and spread of insecticide resistance. However, a sustainable and effective method of sciarid fly management should be of financial benefit to growers. In the longer term, the development of methods that reduce the use of chemical insecticides, or enable insecticide-free production, may well give a marketing benefit.

## Action points for growers

- Laboratory studies suggest that garlic extract is unlikely to be effective against sciarid fly populations, because it persists for less than three days after application. There is a possibility that it may work if it contacts the pest very soon after application or as a deterrent to oviposition before it degrades.
- The nematode, *Steinernema feltiae*, is effective at controlling sciarid fly populations in mushroom casing and compost. At present, some growers use Nemasys in casing as a split dose at casing and then between mushroom flushes. However, it might be more effective to use it as a split dose in compost and casing.

# SCIENCE SECTION

## Introduction

Mushroom sciarid flies are important pests of the commercial mushroom, *Agaricus bisporus* (Lange) Imbach. The main species causing damage in the UK are *Lycoriella castanescens and Lycoriella ingenua*. The latter is of particular concern because it is extending its range and has started to develop resistance to diflubenzuron, the most widely used chemical for sciarid fly control. The total cost of pest infestations to the UK mushroom industry is £11-12 million per annum, including direct loss from insect attack, transmission of pathogens and insecticide costs. Sciarids can sometimes cause complete crop failures. Less quantifiable, but often just as important, are 'fly factors' such as nuisance to mushroom pickers, the presence of flies in mushroom pre-packs and flies getting into people's homes next to the farm.

The aim of this project is to investigate an IPM strategy for controlling sciarid fly pests for the UK mushroom industry. With the withdrawal of many insecticides for use in mushroom crops, the need for new methods of control is becoming urgent. Only two chemicals have approval for use against mushroom sciarid larvae, Dimilin (diflubenzuron) and Decis (deltamethrin). The most readily available biological control is the nematode *Steinernema feltiae* (Nemasys), which is applied to the casing layer, and is being used by growers with varying success.

The objectives of the project are as follows:

1. To evaluate the susceptibility of sciarid flies to a range of control agents in the laboratory, including novel insecticides and biological control agents, applied to both mushroom compost and casing (target date November 2004).
2. To assess selected control agents for their ability to control populations of sciarid flies in a crop scale experiment (target date May 2005).
3. To devise and evaluate an integrated pest management strategy which is intended to reduce the selection pressure for the development of resistance to chemical insecticides (target date December 2005).
4. To transfer knowledge to the industry (target date March 2006).

## Objective 1: Laboratory evaluation of control agents against *L. ingenua*

### Materials and methods

*Lycoriella ingenua cultures*

Cultures of *L. ingenua* originated from a grower's holding and were reared on a mixture of 25 g soya flour (The Health Store, Coventry, UK) and 500g Irish moss peat (35 % moisture content) (Vitax, Coalville, Leicestershire, UK) within a plant propagator (25 x 18 x 20 cm, Stewart Plastics, Croydon, UK) at 25ºC in darkness. The lid of each propagator was vented with two 2 cm diameter holes plugged with cotton wool. Cultures were initiated by introducing 50 gravid adult female *L. ingenua* into a propagator using a pooter. The filial generation of adult *L. ingenua* emerged in approximately 21 days.

A method for obtaining eggs of *L. ingenua* was adapted from Binns (1973). Gravid adult female *L. ingenua* (180 - 220) were incubated for 3 days at 25ºC within an oviposition chamber, which consisted of a cylindrical plastic frame (5 cm high x 6 cm diameter) covered in fine mesh nylon gauze, placed on the base of a Petri dish (7.5 cm diameter) which contained 25 g Vitax Irish moss peat (35% moisture content). The oviposition chamber was placed on moistened tissue paper in a plant propagator (23 cm high x 9 cm diameter, Stewart Plastics, Croydon, UK) to maintain a humid atmosphere. Eggs were extracted by flotation on water.

*Mushroom cultivation substrates*

Phase II mushroom compost was prepared at the Warwick HRI mushroom unit. Casing treatments comprised moist moss peat (35% moisture content) supplemented with 5% soya flour as a food source for sciarid larvae. Note that bioassays were not done using compost inoculated with *A. bisporus*, as high levels of mycelium are known to inhibit sciarid development (Binns, 1975), which could confound the results of experiments.

*The effect of different control agents on the survival of L. ingenua from egg to adulthood, and on the development of L. ingenua populations*

A bioassay was done to evaluate the effects of seven control agents (Table 1) on the survival to adulthood of *L. ingenua* eggs. Control agents were applied at the manufacturer's recommended rate (Table 1). The treatments were applied to both compost and casing substrates as drenches and by mixing (= admix). Controls were treated with distilled water. For drench treatment of compost, 100 g of compost was pressed into plastic pots (10 cm high x 8 cm diameter, with white polythene lids vented with a 1 cm hole plugged with cotton wool (A W Gregory & Co. Ltd., London). Treatments were then drenched onto the compost surface using a Gilson pipette at a rate of 9 ml per pot (equivalent to 1 L /$m^2$). For admix treatment of compost, treatments were mixed into compost at a rate of 9 ml per 100g compost. Compost was then pressed into plastic pots (as above) at a rate of 100 g per pot. For drench treatment of casing, 25 g casing substrate was pressed into plastic pots (8 cm high x 6 cm diameter, vented lids as above). Treatments were then drenched onto the compost surface using a Gilson pipette at a rate of 5 ml per pot (equivalent to 1 L/$m^2$). The casing was then pressed down using a second pot until 3 cm deep. For admix treatment of casing, treatments were mixed into casing at a rate of 5 ml per 25 g casing substrate. The substrate was then placed into plastic pots (as above), 25 g per pot, and pressed to a depth of 3 cm. *Lycoriella ingenua* eggs were pipetted onto the surface of cultivation substrates at a rate of 25 per pot for casing and 50 per pot for compost. For drench treatments, eggs were applied either three days before or three days after the drench was applied. This was done to prevent eggs becoming immersed in liquid suspensions of the control agents at the substrate surface, as previous work has indicated that this can give distorted results. For admix treatments, the eggs were applied three days after the application of the control agents. Each pot was covered with a vented sticky trap (Oecos 10 $cm^2$ with 1 cm hole in the centre plugged with cotton wool) and incubated at 25ºC in darkness in a controlled environment room for 17 days, after which the sticky traps were inspected daily for 14 days and the number of adult *L. ingenua* caught on each trap was recorded. There were three replicate pots of each treatment and three controls, and the experiment was repeated three times.

Following this, a second a laboratory bioassay was done which provided a compound measure of the oviposition of adult female *L. ingenua* and the subsequent development and survival of their offspring to adulthood. Control agents were admixed into cultivation substrates as described previously. For each treatment, six replicate pots were placed in a plant propagator (Stewart Plastics, Croydon, England, 25 x 18 x 20 cm). Thirty gravid female *L. ingenua* collected with a pooter from a culture 1 – 2 days after emergence were transferred to each propagator. After four days the pots were covered with a vented sticky trap and incubated at 25ºC in darkness in a controlled environment room for 17 days, after which the sticky traps were inspected daily for 14 days and the number of adult *L. ingenua* caught on each trap was recorded. The experiment was repeated on three separate occasions.

**Table 1:** Control agents evaluated in laboratory experiments against *L. ingenua*

| Treatment | Application rate |
|---|---|
| Dimilin (Uniroyal) | 30 ppm a.i. 40% |
| Decis (AgrEvo) | 3 ml product / 100 $m^2$ |
| Nemasys M (*Steinernema feltiae*) Becker Underwood) | 3,000,000 / $m^2$ |
| Calypso (Bayer) | 600 ppm product |
| Spinosad (Dow) | 30 ppm (a.i. 48 %) |
| Gnatrol (Valent Biosciences) | 3 lbs /1000 $ft^2$ (= 1465 g / 100 $m^2$) |
| AGARIguard (ECOspray Ltd.) | 1% substrate weight |

**Results**

Results of the bioassays are given in Tables 2 and 3.

Dimilin was effective against sciarid larvae in casing and compost but in commercial practice its use is limited to casing treatment as it is otherwise too expensive. In these bioassays a Dimilin susceptible culture of the mushroom sciarid *Lycoriella ingenua* was used. Where the sciarid population has become resistant, Dimilin will not be effective.

Gnatrol gave good control of *L. ingenua* in both casing and compost. Previous work on the Warwick HRI mushroom unit showed that Gnatrol only gave good control of *L. castanescens* when used at double the recommended dose rate (Valent Biosciences commercial report), which may indicate that this species exhibits less susceptibility than *L. ingenua*, although caution is required as the two experiments were done on very different spatial scales. Crop scale experiments in the USA have indicated that Gnatrol is effective against *L. ingenua* (Keil 2002) and hence further investigation would appear to be warranted.

Calypso gave good control, apart from the compost admix treatment with egg infestation.

Nemasys was effective as a control measure in both casing and compost. However, it has been suggested that compost colonised by mycelium might inhibit Nemasys by rendering the substrate too dry (Roma Gwyn, agricultural consultant, personal communication). As control of the first generation of sciarids is most important to prevent a large infestation in the crop, Nemasys might have a window of opportunity for control of this generation before the mycelium fully colonises the compost. In practice, growers often use Nemasys in casing as a split dose at casing and then again between mushroom flushes. It would be worthwhile to investigate sciarid control using the split dose at spawning and casing so that the compost is protected from the larvae from the start of a crop.

AGARIguard gave poor control in both compost and casing when eggs were introduced regardless of time of treatment. As previous experiments had shown the garlic treatment to be effective when applied on the same day as the egg infestation, this suggests that the garlic has poor persistence. As there was also poor control in these bioassays when the drench treatment was applied three days after egg infestation, it is possible that the larvae had migrated from the substrate surface before the treatment was applied. However, when the substrates were infested with flies, the control given by AGARIguard was higher (72.7% in casing and 84.5% in compost). This suggests that: (a) a large proportion of eggs were oviposited while AGARIguard was still active; and/or (b) it may have a negative effect on oviposition. The latter could be useful if used on compost immediately post pasteurisation, at spawning or on the casing.

Spinosad gave poor control in casing throughout. With compost it gave good control both when used as a drench treatment three days before egg infestation and when used as an admix before infestation with flies. However, it gave poor control when admixed into the compost and when used as a drench three days after egg infestation. Thus eggs laid in the compost a few days after an admix treatment or eggs already in the compost when a drench treatment was applied would not be controlled.

Decis gave poor control in casing and compost throughout.

**Table 2.** Susceptibility of L. ingenua to different control agents applied to compost and casing. The results are given as % control compared to untreated substrate.

| | % control | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Casing | | | | Compost | | | |
| Infestation type: | Egg | | | Adult | Egg | | | Adult |
| Introduced: | Day 0 | Day 3 | Day 3 | Day 0 | Day 0 | Day 3 | Day 3 | Day 0 |
| Treatment: | Drench | Drench | Admix | Admix | Drench | Drench | Admix | Admix |
| Applied at: | Day 3 | Day 0 | Day 0 | Day 0 | Day 3 | Day 0 | Day 0 | Day 0 |
| Dimilin | 79.8 | 94.2 | 94.2 | 97.7 | 68.6 | 85.9 | 83.8 | 89.1 |
| Gnatrol | 87.3 | 98.8 | 100 | 93.8 | 80.7 | 99.4 | 68.7 | 75.6 |
| Calypso | 88.5 | 95.9 | 94.9 | 95.9 | 68.8 | 87.8 | 37.1 | 79.4 |
| Nemasys | 95.9 | 96.5 | 96.5 | 95.6 | 96.7 | 91.9 | 94.8 | 90.7 |
| AGARIguard | 11.5 | 5.2 | 2.3 | 72.7 | 41.1 | 7.0 | 14.2 | 84.5 |
| Spinosad | 39.2 | 8.1 | 4.0 | -11.0 | 25.8 | 89.6 | 36.2 | 77.6 |
| Decis | 22.4 | 38.3 | 8.1 | 64.1 | 37.8 | 47.8 | 22.6 | 32.8 |

**Table 3**. Mean % control from all treatments

| | Dimilin | Gnatrol | Calypso | Nemasys | Agariguard | Spinosad | Decis |
|---|---|---|---|---|---|---|---|
| Casing | 91.5 | 95.0 | 91.3 | 96.1 | 22.9 | 10.1 | 33.2 |
| Compost | 81.9 | 81.1 | 68.3 | 93.5 | 36.7 | 57.3 | 35.3 |

## Objective 2: Crop scale evaluation of control agents against *L. ingenua*

### Materials and methods

From the results of Objective 1, Nemasys, Gnatrol, and Calypso were selected for evaluation on a crop scale. In accordance with commercial practice the treatments were mixed into compost at spawning or applied to casing as drenches. Dimilin_was also included as a standard casing treatment and the experiment included untreated controls. *L. ingenua* cultures were prepared as described previously. Trays of HRI mushroom compost (50 kg per tray) were spawned with *Agaricus bisporus* (Sinden A15) spawn at 0.5%. Eight trays were spawned for each treatment with a further eight for the untreated control. Compost admix treatments were added at spawning (Table 1). All the trays were covered with muslin held in place with staples and then sealed around the tray with 5 mm wide paper tape. The muslin was used to contain sciarid flies for the infestation and to ensure that equal numbers of flies were used to infest each tray. The trays were arranged in a random order in one growing chamber and 10 gravid female sciarids introduced into each corner under the muslin over each tray (40 flies per tray). The small holes made in the muslin to allow fly entry were plugged with cotton wool. The cropping chamber was operated according to Warwick HRI standard procedure (HRIW027). The trays were left covered until day 18 when the muslin was removed and the trays cased. The trays for each treatment were then separated into a different house to avoid emerging flies confounding results between treatments. Where necessary, casing treatments were applied by drench and then an inverted sticky trap (Oecos, Ltd, Kimpton, Hertfordshire UK, 10 cm x 20 cm) was placed on the casing of each tray to trap a sample of emerging flies. Fly numbers were recorded as well as yield and quality of mushrooms and the results were analysed by ANOVA. Control agents were applied at the manufacturers' recommended rate (See Table 1). However, in addition, one tray of Gnatrol was included at 1.5 x the manufacturers' recommended rate. The results of this treatment have been included, although the data need to be treated with caution because of the lack of replication.

### Results

Data for the effect of control agents on the numbers of adult sciarid flies trapped from mushroom trays are shown in Table 4. There was a significant difference ($P < 0.001$) in sciarid emergence between treatments. None of the replicated treatments gave good control of the first generation of sciarids (all less than 35% control compared to the untreated trays). Control of the second generation was improved in all treatments. Control of the second generation with Calypso was less in the compost than the casing, however Nemasys performed equally well in compost and casing. Although Gnatrol gave only about 50% control in compost and casing, the 1.5x dose applied to the compost gave the best overall control (care has to be taken with this result as it was not replicated) so it is possible that control when applied to the casing could have been improved if a higher concentration had been used.

**Table 4**. Sciarid emergence per tray and percent control compared with untreated compost.

| | | Number of sciarids per tray | | | % control | | |
|---|---|---|---|---|---|---|---|
| | | Generation | | | Generation | | |
| Treatment | Stage | 1st | 2nd | Total | 1st | 2nd | Overall |
| Control | | 632 | 2887 | 3519 | | | |
| Dimilin | casing | 991 | 569 | 1559 | -3.3 | 80.3 | 65.3 |
| Calypso | casing | 432 | 842 | 1274 | 31.7 | 70.8 | 63.8 |
| Gnatrol | casing | 618 | 1554 | 2172 | 2.3 | 46.2 | 38.3 |
| Nemasys | casing | 439 | 772 | 1211 | 30.6 | 73.2 | 65.6 |
| Calypso | compost | 480 | 1555 | 2036 | 24.0 | 46.1 | 42.2 |
| Gnatrol | compost | 423 | 1397 | 1820 | 33.0 | 51.6 | 48.3 |
| Nemasys | compost | 506 | 819 | 1325 | 20.0 | 71.6 | 62.3 |
| Gnatrol x 1.5 | compost | 133 | 602 | 735 | 79.0 | 77.1 | 77.5 |

Data for mushroom yields are shown in Table 5. There was a significant difference ($P < 0.001$) in yield between treatments. The untreated control had the lowest overall yield (10.815 kg/tray) and the highest percent of damaged mushrooms (19.3%). Compared with the yield from trays with the standard sciarid treatment Dimilin, Gnatrol in both compost and casing, Calypso in casing and Nemasys in compost increased the yield of mushrooms (4.8%, 9.8%, 9.8% and 2.5% respectively). There were significant differences ($P < 0.001$) in the percentage of damaged mushrooms between treatments, but there was no correlation between damage and sciarid control apart from the untreated control trays which had the highest percentage of fly damaged mushrooms and the largest emergence of sciarids. This may be because there was some sciarid control by all treatments and sciarid larvae tend to burrow into the stipes only when the infestation level is very high. (Hussey & Gurney 1968).

**Table 5.** Yield and quality of mushrooms per tray from all treatments.

| Treatment | Stage | Yield (kg) per tray | % damaged by sciarids | % buttons | % closed | % open |
|---|---|---|---|---|---|---|
| Control | | 10.815 | 19.3 | 7.5 | 55.5 | 17.7 |
| Dimilin | Casing | 11.745 | 9.8 | 8.6 | 66.5 | 15.2 |
| Calypso | Casing | 12.897 | 0.6 | 7.5 | 37.8 | 54.0 |
| Gnatrol | Casing | 12.903 | 3.4 | 8.1 | 48.8 | 39.7 |
| Nemasys | Casing | 11.170 | 5.4 | 7.7 | 54.4 | 32.5 |
| Calypso | Compost | 11.116 | 11.9 | 5.3 | 60.9 | 22.0 |
| Gnatrol | Compost | 12.307 | 5.7 | 3.3 | 54.5 | 36.6 |
| Nemasys | Compost | 12.042 | 7.4 | 5.3 | 54.5 | 32.8 |
| Gnatrol x 1.5 | Compost | 13.478 | 4.1 | 7.4 | 68.8 | 19.6 |

## Conclusions

Good sciarid control was achieved by treating the casing with Dimilin, Calypso and Nemasys, and by treating the compost with Nemasys. Apart from the unreplicated compost treatment with Gnatrol at 1.5x rate, none of the treatments gave good control of the first generation of sciarids.

Some facts have to be considered when evaluating these results.

- The sciarid infestation used in this experiment was manipulated to be much higher than that experienced on commercial crops. As we infested directly onto each treatment, there was maximum pressure possible for treatments to exert control. In a commercial practice the initial infestation would be far lower.
- The culture of *L. ingenua* used in this study was susceptible to Dimilin. Dimilin would not have such good control if the fly population were resistant.

- There were indications that the higher rate of Gnatrol in compost gave better results both in sciarid control and in higher yield but this was not a replicated treatment.
- The effect of Gnatrol may be species related. These experiments were carried out using *Lycoriella ingenua*. A previous crop scale experiment using *L. castanescens* showed Gnatrol at the normal dose rate to be less effective (23% control in casing; no control in compost).

Further investigation of Gnatrol at a higher concentration would be warranted, given that it may give good levels of control and, critically, be able to control the first generation of sciarid flies. Further investigation is also warranted of the effects of Nemasys in combination with other treatments or used as a split dose in compost and casing. A split dose used in casing has been shown to be more effective than a single dose.

## Technology Transfer

- HDC news article will be written for end May 2005.
- Scientific paper will be written at end of project.

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