REPORT
Supplementary work

To:
Horticultural Development Council
Bradbourne House
Stable Block
East Malling
Kent, ME19 6DZ

Mushrooms: integrated pest management of sciarid flies

M 42 supplementary work

Jane Smith, David Chandler

January 2005

Commercial - In Confidence

Project title:	Mushrooms: integrated pest management of sciarid flies
Project number:	M 42
Project leaders:	Jane Smith Warwick HRI University of Warwick Wellesbourne, Warwick CV35 9EF
	Dr David Chandler Warwick HRI University of Warwick Wellesbourne, Warwick CV35 9EF
Report :	Supplementary Work report, 31st September 2005
	First annual report, 31st March 2005
Previous reports:	Interim report, 31st January 2005
Location:	Warwick HRI
Project co-ordinator:	Peter Davies Shackleford Mushrooms Godalming Surrey GU8 6AE
Date commenced:	1st April 2004
Date completion due:	31st march 2006
Key words:	sciarids, mushroom, insect pest, Gnatrol, Thiacloprid, Nemasys.

The results and conclusions in this report are based on a single investigation. 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.

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 *L. 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 one chemical has approval for use against mushroom sciarid larvae, Dimilin (diflubenzuron). 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 March 2005).
4. To transfer knowledge to the industry (target date June 2006).

All experiments are being done with *L. ingenua* as a representative sciarid species.

In Objective 1, four products were identified which gave good control of *L. ingenua* populations in mushroom compost and casing in laboratory bioassays. These were Dimilin, Nemasys M, Calypso and Gnatrol. In Objective 2, these control agents were evaluated by applying them to either compost or casing substrates on a crop scale. In order to make the

treatments economic, the same amount of control agent was applied to compost and casing, even though the compost layer has larger volume than the casing. Adult sciarid flies were used to infest the compost, and the amount of control was measured by counting the numbers of adults flies that emerged from the first filial generation (approximately 21 days after application of the parent flies) and subsequent generations (which emerged at approximately 42 days). None of the agents gave good control of the first generation of flies. However, good control of the second generation was obtained with control agents applied to casing. In addition, Nemasys M applied to compost gave good control of the second generation of flies, suggesting that it was able to persist and multiply and migrate into the casing. Poor control of the first generation may have been caused by the fact that the concentration of control agent in the compost was low relative to that applied to the casing. It was therefore agreed to investigate first generation sciarid control using a range of dose rates for Nemasys M and Gnatrol. The effect on sciarids of Calypso, applied as a drench to compost, was also investigated. A dose response with Calypso was to be carried out separately, funded by Bayer.

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. 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 an aspirator. The filial generation of adult *L. ingenua* emerged in approximately 21 days.

Mushroom unit trial

In this experiment, mushroom compost was infested with gravid female sciarids on two occasions to simulate natural infestation conditions. HRI Phase II mushroom compost was placed into large polythene bags (25 kg per bag, 1 bag per treatment) and sealed with a cotton wool plug. Fifteen females were introduced into each bag and the bags were left on the floor of a growing chamber for three days. The compost was then spawned with *Agaricus bisporus* (Sinden A15) spawn at 0.5% and placed into pots (plastic plant pots 25 cm x 30 cm diameter, 2.5 kg compost per pot). There were eight replicate pots for each treatment, including the

untreated control. All treatments were applied to the compost immediately after spawning as a drench using a spray bar attached to a Zipette (Jencons Scientific, Bedforshire, UK. 50 ml). Nemasys M and Gnatrol were applied at different rates (0.75N, N, 1.5N, 2N and 4N) where N represents the manufacturer's recommended rate (Nemasys M, N = 3 x $10^6/m^2$, Gnatrol, N = 14.6 g/m^2). In addition, one treatment of Calypso was included at the manufacturer's recommended rate (150 ppm w/w product substrate).

The pots were enclosed in polythene bags to contain the sciarids used to infest the compost and to avoid cross infestation of the filial generation of sciarid flies between treatments. The bags were sealed with a rubber band around a 5 cm wide plug of non-absorbent cotton wool to allow gaseous exchange. The pots were arranged in a random order on shelves in the growing chamber and, after two days, 5 gravid female sciarids were introduced into each bag. The cropping chamber was operated according to Warwick HRI standard procedure (HRIW027). After 18 days the pots were cased and an inverted sticky trap (Oecos, Ltd, Kimpton, Hertfordshire UK, 10 cm x 20 cm) was placed on the casing of each to trap the emerging flies.

During the course of the experiment, carbon dioxide levels within the bags rose above the normal cropping level (ca. 1000 ppm). Consequently, holes were cut in the top and bottom of the bags, and one day after this the bags were opened. The high CO_2 delayed mushroom development and there is a risk that it may have affected the activity of the control agents. It was decided, therefore, to continue the experiment to record the second generation of sciarids.

A regression analysis was done on the relationship between the numbers of adult sciarid flies counted on sticky traps (transformed to the $\log_{10}$ scale) and the dose rate of the control agents, which was expressed as multiples of N. A test for curvature on the log transformed data was carried out but proved insignificant. Estimates were made from the regression model of the dose rates required to give 50%, 75% and 95% control of sciarid populations.

Results and Discussion

Data on the effect of the control agents on the numbers of adult sciarid flies trapped from mushroom pots are shown in Table 1. The estimated dose rates needed for 50%, 75% and 95% control calculated from the regression analysis are shown in Table 2.

Nemasys M

There was a significant effect (p=< 0.01) of dose rate on control of sciarid emergence for both 1st and 2nd generations. The estimated dose rates needed for 50%, 75% and 95% control of the 1st generation were 1.3 N, 2.4 N and 5.2 N respectively, where N = 3 x 10^6 m^{-2}. For control of the second generation, rates of 1.1 N (50% control), 2.8 N (75%) and 7.0 N (95%) were estimated.

Gnatrol

There was a significant effect of dose rate on the emergence of the 1st generation of sciarids (p=<0.01) but not for the 2nd generation. The estimated dose rates needed for 50%, 75% and 95% control of the 1st generation were 0.2 N, 2.8 N and 7.5 N respectively, where N = 14.6 g m^{-2}. None of the dose rates gave good control of the second generation. It would appear that Gnatrol does not give persistent control in mushroom compost and therefore a supplementary treatment to the casing would be required. In previous work funded by Valent Biosciences, Gnatrol (2N) gave >70% control of sciarid populations, and hence the levels of control in the present experiment were unexpectedly low.

Calypso gave good control of both 1st and 2nd generation sciarids (82% and 76% respectively).

The numbers of sciarids from the control pots were lower than expected and this may have artificially reduced apparent percentage control given by treatments. In the previous HDC funded crop scale trial, Nemasys M (N) mixed into the compost gave better sciarid control of the 2nd generation (72%). In this experiment, the regression analysis shows that the recommended rate (N) gave 49% control of sciarids from the 2nd generation. It is also possible that the nematodes are more effective when mixed into the compost than when applied as a drench.

The high levels of CO_2 created by containment of the pots in polythene bags may have influenced the results of this experiment. However, the bags were opened soon after emergence of the first generation of sciarids and CO_2 levels quickly returned to normal.

Conclusions

Where infestations of sciarids start early in the crop, and when sciarid numbers are high, it is important to get good control of the first generation. Sciarids can infest the compost

immediately after pasteurisation, at spawning and through the early stages of crop growth, and where infestation is occurring at these stages, it would be beneficial to use control agents in the compost.

Calypso gave good control of first and second generation sciarids when used as a compost drench.

Overall, Nemasys M also gave encouraging levels of control. Our results indicate that 2.4 and 2.8 times the recommended dose rate would be required to give 75% control of first and second generation sciarids respectively. We would consider this to be a reasonable level of sciarid control. It is noteworthy that the nematodes applied to compost continued to control the second generation of sciarid larvae, which result from eggs laid into the casing layer

Gnatrol gave good control of the first generation of sciarids. Our analysis showed that for 75% control of the first generation, the dose rate would need to be 2.2N. However, Gnatrol did not persist to control the second generation even at higher dose rates. In a crop therefore, any sciarids surviving from the first generation, or sciarids infesting after casing, would not be controlled. From the previous work, Gnatrol was shown to be more useful for overall control as a casing treatment. If used as a compost treatment to control first generation sciarids, it would need to be supplemented with a casing treatment which may not be economically feasible.

The extra cost involved in using Nemasys M and Gnatrol at the higher rates could be offset by the higher yields these treatments are known to produce. In this trial, mushroom yield was not assessed but the previous HDC trial showed that compost treated with Nemasys M (N) and Gnatrol (N & 1.5N) yielded 11.3%, 13.8% and 24.6% more mushrooms respectively than the untreated compost.

Table 1. Mean numbers of sciarids caught on sticky traps placed over mushroom compost treated with control agents at different rates

Control agent	Sciarid generation	Dose rate					
		0	0.75 N	N	1.5N	2N	4N
Nemasys M	1st	-	41	52	38	20	7
Nemasys M	2nd	-	208	163	179	151	79
Gnatrol	1st	-	29	20	13	32	16
Gnatrol	2nd	-	254	273	323	332	326
Calypso	1st	-	-	10	-	-	-
Calypso	2nd	-	-	89	-	-	-
Untreated	1st	54	-	-	-	-	-
Untreated	2nd	378	-	-	-	-	-

Manufacturer's recommended rate (N) as follows:

Nemasys N = 3 x 10^6 /m^2;

Gnatrol N = 14.6 g/m^2;

Calypso N = 115 ppm w/w product/substrate

Table 2. Dose rate of Nemasys M and Gnatrol needed to give 50%, 75% and 95% control of 1st and 2nd generation sciarids.

	Dose rate		
	50% control	75% control	95% control
Nemasys M			
1st gen	1.3N	2.4N	5.2N
2nd gen	1.1N	2.8N	7.0N
Gnatrol			
1st gen	0.2N	2.2N	7.5N
2nd gen	-	-	-

Manufacturer's recommended rate (N) as follows:

Nemasys N = 3 x 10^6 /m^2;

Gnatrol N = 14.6 g/m^2;

Fig 1a. Mean emergence of 1st and 2nd generation sciarids with different dose rates of Nemasys.

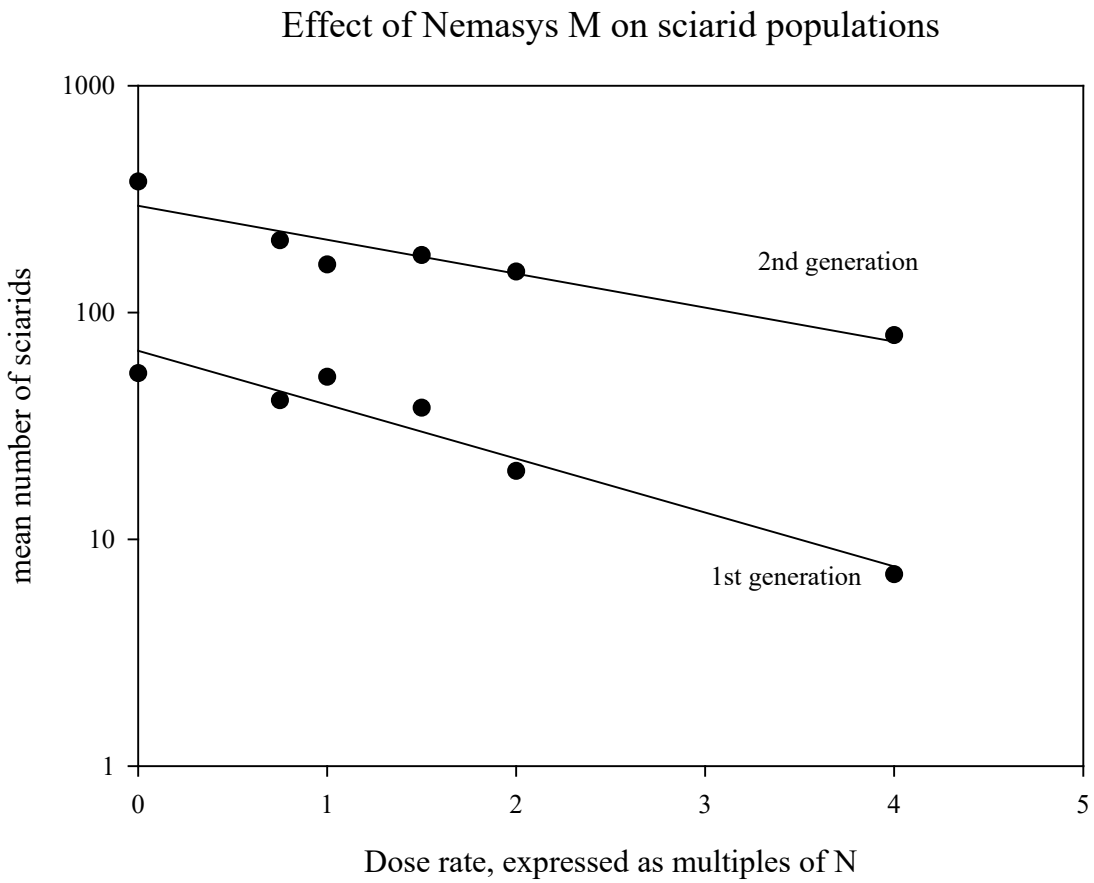

Fig 1b. Mean emergence of 1st and 2nd generation sciarids with different dose rates of Gnatrol.