Project title	Mushrooms: potential for biological control of sciarid and phorid flies using the predatory beetle *Atheta coriaria*
Project number:	M44
Project leaders:	Jude Bennison ADAS Boxworth, Boxworth, Cambridge, CB23 4NN Dr David Chandler Warwick HRI University of Warwick Wellesbourne Warwick CV35 9EF
Report:	Final report, 8 February 2008
Previous report	
Key staff:	Jude Bennison, ADAS, Study Director David Chandler, Warwick HRI, Principal Investigator Kerry Maulden, Senior Scientific Officer, ADAS, Site Manager for ADAS experiments Jane Smith, Senior Research Technician, Warwick HRI experiments
Location of project:	ADAS Boxworth, and Warwick HRI
Project coordinator:	
Date project commenced:	1 October 2006
Date project completed (or expected completion date):	30 September 2007
Key words:	Mushrooms, pest management, biological control, predator, sciarid fly, phorid fly, *Atheta coriaria*

Whilst reports issued under the auspices of the HDC are prepared from the best available information, neither the authors nor the HDC can accept any responsibility for inaccuracy or liability for loss, damage or injury from the application of any concept or procedure discussed.

The results and conclusions in this report are based on an investigation conducted over a one-year period. The conditions under which the experiments were carried out and the results have been reported in detail and with 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.

Jude Bennison
Senior Research Entomologist
ADAS

Signature ..... Date .....

David Chandler
Principal Investigator
Warwick HRI

Signature ..... Date .....

Report authorised by:

[Name]
[Position]
[Organisation]

Signature ..... Date .....

[Name]
[Position]
[Organisation]

Signature ..... Date .....

CONTENTS

	Page
Grower Summary	5
Headline	5
Background and expected deliverables	5
Summary of the project and main conclusions	6
Financial benefits	8
Action points for growers	8
Science section	9
Introduction	9
Objective 1: Control of mushroom fly pests by *Atheta*. Determine whether *Atheta* will feed on mushroom sciarid and phorid flies	10
Materials and Methods	10
Results and Discussion	14
Objective 1: Conclusions	17
Objective 2: Behaviour of *Atheta* in mushroom crops. Determine any potential problems with using *Atheta* in mushrooms.	17
Behavioural experiment: are *Atheta* potential contaminants on harvested mushrooms?	17
Materials and Methods	17
Results and Discussion	18
Experiment to quantify survival and reproduction of *Atheta* in spawned mushroom compost and casing	18
Materials and Methods	18
Results and Discussion	19
Objective 2: Conclusions	20
Overall conclusions of the project	20
Technology Transfer	21
Acknowledgements and References	21

Grower Summary

Headline

- In laboratory experiments, the predatory rove beetle *Atheta coriaria* successfully bred and dispersed in mushroom compost and casing, located and predated both eggs and larvae of sciarid and phorid flies.
- In an experiment with sciarid flies on mushroom compost, the beetles caused up to 70% reduction of sciarid fly populations.
- In a 'worst case scenario ' experiment to assess whether beetles could 'contaminate' a crop, a small number of beetles were observed on mushroom fruit bodies.

Background and expected deliverables

Mushroom sciarid and phorid flies are a constant threat to mushroom production, causing estimated annual losses of £8 million. Control of sciarid and phorid flies with chemical insecticides is becoming problematic due to pesticide withdrawals and to the development of fly resistance. Growers are under pressure from retailers to reduce or eliminate pesticide usage and to use non-chemical pest control in production for the organic market.

The insect-pathogenic nematode, *Steinernema feltiae* is being used increasingly for biological control of sciarid fly larvae, but is not effective against phorid larvae. *Atheta coriaria* is a small predatory beetle, which has recently been made commercially available and is used in glasshouse crops for control of sciarid and shore flies. HDC-funded project PC 239 is developing a low-cost 'DIY' rearing-release system for growers of protected crops. The system will enable growers to rear large numbers of the beetles on their own nurseries, and allow beetles to leave the rearing boxes of their own accord, to seek out prey within the glasshouse.

This pilot project aimed to evaluate the potential of using a grower 'rearing-release' system for *Atheta* in mushroom houses, for biological control of both sciarid and phorid flies.

The expected deliverables of the project were:

1. Determine whether *Atheta* will feed on mushroom sciarid and phorid flies.

2. Determine any potential problems with *Atheta* contamination of mushrooms at harvest.

3. Quantify survival and reproduction of *Atheta* in spawned mushroom compost and casing.

Summary of the project and main conclusions

1. Determine whether *Atheta* will feed on mushroom sciarid and phorid flies.

In laboratory bioassays done in Petri dishes, *Atheta* gave 100% control of both eggs and larvae of mushroom sciarid fly, and 52% control of mushroom phorid fly. In pots of mushroom compost and casing, *Atheta* gave up to 58% control of sciarid flies that were added as eggs to compost or casing.

In a more spatially complex experiment, where adult sciarid flies were introduced into containers of mushroom compost and allowed to lay eggs, *Atheta* gave 70% reduction of the subsequent sciarid population. The numbers of *Atheta* needed to control the target pests will be dependent on pest density, amongst other factors.

In a further experiment, where *Atheta* were introduced into a chamber containing pots of mushroom compost containing sciarid or phorid flies, the results were inconclusive, as unknown numbers of *Atheta* escaped from the 'treated' chambers and moved into untreated 'control' chambers.

2. **Determine any potential problems with Atheta contamination of mushrooms at *harvest.***

An experiment was done to test whether *Atheta* could be a potential contaminant on mushrooms at harvest. In a 'worst case scenario', a relatively large amount of *Atheta* rearing substrate with beetles was introduced on two occasions to a small enclosed space with spawned mushroom compost. At mushroom harvest, a small number of beetles were seen on fruit bodies (approximately one beetle on every 30 fruit bodies). It is not known if these beetles would have left the fruit bodies on disturbance during picking.

3. ***Quantify survival and reproduction of Atheta in spawned mushroom***

compost and casing

An experiment was done to test survival and reproduction of *Atheta* in replicated 5-litre pots, each with 1.5 litres of spawned mushroom compost. Thirty *Atheta* adults were added to each pot, in a small dish of the *Atheta* rearing substrate (mixture of coir compost and vermiculite). A food source (turkey grower crumbs) was added to the small dish every week. After 18 days in the dark at 25°C, there was a mean of 35 adults and 149 larvae per pot.

A replicate set of 5-litre pots of spawned mushroom compost, to which 30 *Atheta* adults had been added, were 'cased' with an equal weight of mushroom casing, after leaving the pots for 18 days in the dark at 25°C. The 'cased' pots were then left for 40 days in the dark at 20°C. At the end of the experiment, there was a mean of 17 adults and 13 larvae per pot in the casing, and a mean of 64 adults and 65 larvae per pot in the compost. The results demonstrate that *Atheta* can breed and disperse in mushroom compost and casing.

Conclusions

- This project has shown that *Atheta* will breed and disperse in both mushroom compost and casing, and will both locate and predate the eggs and larvae of both the mushroom sciarid fly, *Lycoriella ingenua* and the mushroom phorid fly, *Megaselia halterata*.
- There is potential for some movement of *Atheta* onto fruit bodies.
- Given that the beetles are inexpensive and simple to rear and maintain by growers, rather than having to be purchased regularly from biological control suppliers, and are already being used successfully for the control of sciarid flies on glasshouse crops, then these preliminary results are encouraging.
- Further work is warranted to investigate the control of sciarid and phorid flies on a crop scale, and to manipulate the numbers of beetles released, to ensure there are no potential contaminant problems at harvest. Control of sciarid flies by *Atheta* and by *Steinernema feltiae* should be compared, and the comparative cost benefits, including labour inputs and practicality, should be evaluated.
- Research in Canada has suggested that *Atheta* and *S. feltiae* are compatible. The compatibility of *Atheta* with selected pesticides used in mushroom production should be assessed, and the benefits of using a combination of the two biological control agents should be quantified in crop scale experiments.

Financial benefits

The annual value of UK mushrooms was estimated at £119 million and £107 million in 2003 and 2004, respectively (Defra Basic Horticultural Statistics, 2005). Fly pests are a constant threat to mushroom production, causing estimated annual losses of £8 million. Thus the

development of a practical, low-labour and low-cost strategy for effective, non-chemical control of both sciarid and phorid flies will give financial benefits to mushroom growers as part of an Integrated Pest Management approach.

Action points for growers

It is too early to give action points to growers at this stage, for commercial use of *Atheta* in mushroom production.

Science Section

Introduction

Fly pests are a constant threat to mushroom production, causing estimated losses of £8m p.a. (R, Gaze, personal communication). The most serious pests are mushroom sciarid flies, mainly *Lycoriella castanescens* and *L. ingenua*, and the mushroom phorid fly, *Megaselia halterata*. Sciarid larvae feed on mushroom compost and the growing edge of the mycelium. Phorid larvae feed only on fungal tissue and can cause considerable damage to the mycelium. Sciarid and phorid adults are also vectors of mushroom diseases, are a nuisance to pickers, and their presence on harvested mushrooms reduces crop value.

Control of sciarid and phorid flies with chemical insecticides is becoming problematic due to pesticide withdrawals and to the development of fly resistance. Diflubenzuron (Dimilin Flo) is the only pesticide currently available for sciarid fly control, and resistance to this compound is now developing. Growers are under pressure from retailers to reduce or eliminate pesticide usage and to use non-chemical pest control in production for the organic market.

Biological pest control within Integrated Pest Management (IPM) strategies reduces reliance on pesticides. IPM is used widely in glasshouse crops and is now having an impact in mushroom production, where the insect-pathogenic nematode, *Steinernema feltiae* (e.g. 'Nemasys') is being used increasingly for control of sciarid fly larvae. *S. feltiae* can be very effective against sciarid larvae but is not effective against phorid larvae. Additional or alternative biological control agents are needed for robust, flexible and sustainable control strategies for both sciarid and phorid flies.

Atheta coriaria is a small predatory staphylinid or 'rove' beetle, which has recently been made commercially available, and is used in glasshouse crops for control of sciarid and shore flies. Releases of the predators direct from suppliers have given promising results against both sciarid and shore flies in ornamental crops and celery, but control of shore flies on herbs (which need to meet more stringent retail standards) has been unreliable. ADAS and Stockbridge Technology Centre are currently doing HDC-funded research (PC 239) on developing an effective, reliable and practical 'DIY' on-nursery rearing system for *Atheta*, to enable growers to rear and maintain large numbers of the predator for improved, low-cost biological control of sciarid and shore flies.

The *Atheta* system being developed in project PC 239 could also have potential for control of mushroom flies. For this project, it was considered that *Atheta* could have advantages over *S. feltiae* in that they are excellent predators of fly eggs as well as larvae (Carney *et al.*

2002) and thus might control both sciarid and phorid flies at the egg stage, thus preventing damage by pest larvae. Unlike *S. feltiae*, *Atheta* are not time-consuming to apply as the adult beetles can fly to disperse, and both adults and larvae are very mobile and burrow into growing media, searching for prey. Recent research in Canada on the compatibility of *Atheta* with other biological control agents has shown that in laboratory studies, *S. feltiae* can kill *Atheta* larvae, but percentage kill was low (Jandricic *et al.* 2005a). *Atheta* might also feed on nematodes, including *S. feltiae*. Research by the same Canadian team on the compatibility of *Atheta* with pesticides showed that diflubenzuron was only slightly harmful to larvae (Jandricic *et al.* 2005b).

The overall aim of this project was to evaluate the potential of using a 'DIY' grower system for rearing *Atheta coriaria* in mushroom houses, for the biological control of both sciarid and phorid flies.

Objective 1: Control of mushroom fly pests by Atheta. Determine whether *Atheta* will feed on mushroom sciarid and phorid flies (Warwick HRI)

Materials and methods

Fungal culture

The A15 strain of *A. bisporus* (Sylvan, Peterborough, UK) was used in the study. This strain is grown commercially in the UK and elsewhere. A stock culture of the strain was stored in liquid nitrogen. Laboratory cultures were grown on 2% malt extract agar (Oxoid, Basingstoke, UK) at 25°C for 10 - 21 days. Mushroom spawn was prepared using rye grains as a substrate. The grains were stored at 4°C for up to 12 months before use. Composted substrate was prepared from wheat straw, poultry manure and gypsum according to standard commercial practice. Quantities were adjusted to give substrate N of 2.0 – 2.5%, pH 7 – 8. The mixed materials were stacked in windrows for 14 – 19 days for decomposition, and wetted approximately eight times to give a substrate moisture content of 75 – 76%. The composted material was pasteurised at 57°C for six hours in a controlled temperature chamber, then cooled to ambient temperature and aerated to reduce the ammonia content to below 0.06%. Casing comprised moist moss peat (35% moisture content). To produce composted substrate colonised by *A. bisporus* for bioassays, 500g of composted substrate was mixed with 1% mushroom spawn in polythene bags (ventilated with a cotton wool bung) and incubated at 25ºC in darkness for 14 – 21 days.

Fly pest cultures

Work on sciarid flies was done with *L. ingenua*, and work on phorid flies was done with *M. halterata*. Cultures of *L. ingenua* were reared on a mixture of 25 g soya flour (The Health

Store, Coventry, England) and 500 g autoclaved Irish moss peat (Vitax, Coalville, UK), pressed into the base of a plant propagator (25 x 18 x 20 cm, Stewart Plastics, Croydon, UK). The lid of each propagator was vented with two, 2 cm diameter holes plugged with cotton wool. New cultures were initiated by introducing 50 newly emerged adult *L .ingenua* (40 females, 10 males) into a propagator at 18-22°C for 5 days, after which time the adults were removed and the propagator was incubated at 25ºC in darkness. Adult flies of the next generation emerged in approximately 21 days.

Eggs of *L. ingenua* were obtained by incubating gravid adult females (180 - 220) for three days at 25ºC within an oviposition chamber. The chamber 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 Irish moss peat. 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 from the surface of the peat by flotation on water.

Cultures of *M. halterata* were reared on a mixture of 80 g composted substrate combined with 20 g composted substrate that had been colonised with *A. bisporus* spawn grains for 14 days as described previously, and pressed into polystyrene pots (11 x 8 cm). Groups of four pots were placed within plant propagators (described above). The base of each propagator was covered with moist absorbent paper, and sugar syrup (Lyle's Golden Syrup, Tate & Lyle, London UK) was smeared lightly on the inside surface of the propagator lid to provide a carbohydrate source for egg maturation. Cultures were initiated by placing 100 newly emerged adult *M. halterata* in a propagator at 18-22°C for 5 days, after which time the adults were removed and the propagator was incubated at 18-22°C in darkness. Adult flies of the next generation emerged in approximately 21 days.

<u>*Atheta* cultures</u>

Atheta coriaria were reared at ADAS Boxworth, using the method developed in HDC project PC 239 (Bennison, 2007). The beetles were reared in plastic boxes (3 litre capacity) sealed with a tight fitting snap-on lid, fitted with two ventilation holes (2.5 cm diameter) covered with insect-proof mesh. A coir and vermiculite (1:1 mix) substrate (1.5 litres) was added to each box, after dampening with 150 ml water per litre substrate. Sixty adult beetles, taken from the stock *Atheta* cultures at ADAS Boxworth, were added to each box. The artifical diet for the *Atheta* (turkey starter crumbs, Dodson & Horrell Ltd., UK) was sprinkled on top of the substrate and then incorporated by rotating the box through 360°. The rearing boxes were held in a controlled temperature room at 25°C, 16:8 photoperiod for 23 days, to produce the next generation of adults. Every week, 5-10ml of water (as required, to maintain the

dampness of the substrate) and 5g of turkey crumbs were incorporated into the substrate. Boxes of *Atheta* were supplied to Warwick HRI for the experiments in Objective 1.

Experiment 1: Petri dish bioassays of *Atheta* predation

Evaluation of *Atheta* predation of eggs and larval instars of *L. ingenua* and *M. halterata* was done in a Petri dish based assay. Adult *Atheta* were removed from the rearing substrate by sieving the substrate through 2 mm and 1.8 mm sieves into a holding pan, and were then collected using an aspirator.

L. ingenua: Petri dishes (9 cm diameter) were lined with filter paper (Whatman No. 1) moistened with water. Soya flour (Holland and Barrett, Leamington, Warks.) was placed in the centre of each dish (0.5 g per dish). Three treatments were then set up. Treatment 1 consisted of *L. ingenua* eggs only. The eggs were collected from a laboratory culture as described above. Batches of 50 eggs were placed adjacent to the soya flour in the Petri dishes (50 per dish). Treatment 2 consisted of *L. ingenua* eggs plus *Atheta* adults. Batches of 50 *L. ingenua* eggs were placed adjacent to the soya flour in Petri dishes as described above. Batches of five *Atheta* adults were then placed in each dish. Treatment 3 consisted of five *Atheta* adults per dish only (i.e. no *L. ingenua* eggs). There were three replicate Petri dishes for each treatment. The dishes were put into unsealed polythene bags to help to retain moisture in the dishes, but to allow gaseous exchange. The dishes were then placed into a controlled environment room at 25ºC without illumination. They were examined after five days and the number of sciarid fly larvae was counted.

M. halterata: Laboratory cultures of *A. bisporus* were grown on malt extract agar in 9cm Petri dishes as described above. Petri dishes were placed together in a chamber and the lids were removed. A pot of compost with newly emerging adult *M. halterata* was then placed in the chamber. The chamber was incubated at 25ºC in the dark in a controlled environment room. Two treatments were then set up. Treatment 1 consisted of *M. halterata* eggs only (three replicate dishes). Treatment 2 consisted of *M. halterata* eggs plus five *Atheta* adults per dish (six replicate dishes). The phorid fly eggs were laid in clumps underneath the fungal mycelium, hence it was not possible to accurately count the number of eggs per dish. The experiment was therefore conducted on the assumption that equal numbers of eggs were laid in each dish. Phorid fly larvae were washed out and counted from each dish after seven days.

Experiment 2: Evaluation of *Atheta* predation of *L. ingenua* in mushroom compost and casing (pot scale experiment)

Experiment 2 consisted of a bioassay testing *Atheta* predation of sciarid fly eggs placed onto mushroom compost or casing. This represented a more complex spatial and chemical

environment for prey location by *Atheta.* Polystyrene pots (11 x 8 cm) were filled with either 100g of mushroom compost or 50 g of mushroom casing, which were pressed by hand into the pots. The pots of mushroom casing were supplemented with 2.5 g soya flour placed in the centre of the surface of each pot. Batches of 50 sciarid fly eggs were collected as described previously, and placed onto the surface of each pot. Batches of *Atheta* adults (either two or five per pot) were then added. An additional treatment consisted of six adult sciarid flies (five females, one male) placed within a pot of compost. Either six or 12 *Atheta* adults were added to these pots. There were three replicates for each treatment. In total, 6 different treatments were set up plus 3 controls as follows:

1. Mushroom compost, 50 sciarid eggs, no *Atheta* (control).
2. Mushroom compost, 50 sciarid eggs plus five *Atheta* per pot.
3. Mushroom compost, 50 sciarid eggs plus two *Atheta* per pot.
4. Mushroom casing, 50 sciarid eggs, no *Atheta* (control).
5. Mushroom casing, 50 sciarid eggs plus five *Atheta* per pot.
6. Mushroom casing, 50 sciarid eggs plus two *Atheta* per pot.
7. Mushroom compost, six adult sciarid flies, no *Atheta* (control).
8. Mushroom compost, six adult sciarid flies plus six *Atheta* per pot.
9. Mushroom compost, six adult sciarid flies plus 12 *Atheta* per pot.

Each pot was covered by a vented lid and placed in a controlled environment room at 25ºC without illumination for 16 days, after which the numbers of emerged adult flies of the following generation were counted. This was done by placing a vented sticky trap (Oecos 10 cm^2 with a 1 cm hole in the centre, plugged with cotton wool) over each pot. The sticky traps were kept in place for seven days, after which time they were removed and the number of adult *L. ingenua* caught on each trap was recorded. The number of adult flies counted per trap was considered as a measure of the amount of pest control given by the *Atheta*.

Experiment 3: Evaluation of *Atheta* predation of *L. ingenua* and *M. halterata* in mushroom compost (chamber experiment)

Experiment 3 measured the ability of *Atheta* to predate the offspring of ovipositing adults of either sciarid flies or phorid flies, in replicated pots of composted substrate contained within a chamber. Unlike in the previous experiment, the pots were not covered with a lid to contain the *Atheta* or their prey. The bioassay against sciarid flies was done as follows: Groups of 10 polystyrene pots (A. W. Gregory & Co. Ltd., London, UK. No 16: 11 cm x 8 cm), each containing 100 g Phase II pasteurised mushroom compost were set out in two rows of five pots on moist capillary matting in a Perspex chamber (c. 3 l volume). Gravid female *L. ingenua* (30) and *Atheta* adults (30) were then introduced into the chamber. The control treatment consisted of a chamber of 10 pots of composted substrate into which 30 *L. ingenua* females were introduced, with no *Atheta*. There were three replicate chambers for both treatments. The bioassay against phorid flies was done as above, but in this case a pot containing mushroom spawn taken from a rearing culture of *M. halterata* (see above) was placed in each chamber, and hence acted a source of ovipositing adult phorid flies. The chambers were maintained in a controlled environment room at 25ºC without illumination. For the sciarid fly bioassay, the pots were removed after 19 days and the lids were covered with sticky traps (10 cm x 10 cm, Oecos, Hertfordshire, UK). The pots were maintained at 25ºC without illumination and the numbers of adult sciarid flies of the following generation were then recorded over 14 days. For the phorid fly assay, the pots were removed after 12 days and the lids were covered with sticky traps as above, prior to counting the numbers of adult phorid flies of the following generation.

Results and Discussion

Experiment 1: Petri dish bioassay of Atheta predation.

L. ingenua: In treatment 2 (sciarid fly eggs plus *Atheta* adults), all the sciarid fly eggs were consumed by the *Atheta* at day five. A mean of 34 sciarid fly larvae were recovered from the control treatment 1 (sciarid fly eggs and no *Atheta*). No records were kept of the survival of *Atheta*.

M. halterata: Addition of *Atheta* caused a significant reduction ($p < 0.05$) in the numbers of phorid fly larvae within the Petri dish arenas. In treatment 1 (untreated control, no *Atheta*), a mean of 27 (s.d. 4.0) phorid fly larvae were counted per dish at day 7. In treatment 2, a mean of 13 (s.d. 1.41) phorid fly larvae were counted per dish at day 7, i.e. a 48% survival compared to the control. No records were kept of the survival of *Atheta*. The phorid fly larvae buried into the mushroom mycelium after egg hatch, creating a more spatially complex environment for prey location than that found in the Petri dish bioassay used for *L.*

ingenua (above). This may account for the lower level of predation of *M. halterata* than of *L. ingenua* in this bioassay.

Experiment 2: Evaluation of Atheta predation of *L. ingenua* in mushroom compost and casing (pot scale experiment)

An accumulated analysis of deviance for treatments 1 – 6 indicated that addition of *Atheta* caused a significant reduction in the sciarid fly population ($p < 0.001$) and that there was a significant difference in the amount of control obtained with the addition of two or five *Atheta* ($p = 0.012$). Percentage survival of sciarid flies in the controls in treatments 1 and 4 was 86% and 80% respectively. In treatments 1 – 6, increasing the number of *Atheta* applied to the compost or casing caused an increase in the percentage mortality of the sciarid fly population (Table 1). Application of five Atheta per pot caused a reduction in sciarid fly populations of 56% and 58% in compost and casing treatments respectively. Application of *Atheta* to pots inoculated with adult sciarid flies (treatments 7 – 9) caused a reduction of c. 70% of the following generation compared to the control. Analysis of variance (based on a square root transformation of the data, and excluding one data point from the controls, which was an obvious outlier) indicated that this reduction in numbers of sciarid flies was significant ($p < 0.05$). However, there was no significant difference in the control achieved by *Atheta* when added at six or 12 per pot. These results were consistent with those in HDC project PC 239, which showed that in pots of parsley infested with sciarid fly (*Bradysia difformis*) eggs, *Atheta* introduced at five or 10 adults per pot significantly reduced numbers of sciarid fly adult developing in the pots, when compared with untreated controls, and with pots where lower numbers of *Atheta* were released (Bennison, 2007 b). The numbers of *Atheta* needed to control the target pest will be dependent on the pest density.

Table 1. Mean number of emerged sciarid flies, *L. ingenua*, on sticky traps in Experiment 2.

Treatment No.	**Substrate**	**Sciarid fly life stage added**	**No. *Atheta* added per pot**	**Mean No. emerged sciarid adults per pot (s.d.)**	**pest control (% reduction compared to control)**
1	compost	eggs	0	43 (4.0)	-
2			2	31 (4.2)	28%
3			5	19 (4.6)	56%
4	casing	eggs	0	40 (3.2)	-
5			2	23 (9.8)	43%
6			5	17 (2.0)	58%
7	compost	adults	0	226 (38.9)	-
8			6	64 (9.2)	72%
9			12	69 (34.6)	70%

Experiment 3: Evaluation of *Atheta* predation of *L. ingenua* and *M. halterata* in mushroom compost (chamber experiment)

For the bioassay testing *Atheta* against sciarid fly, a mean of 205 (s.d. 152) adults of the following generation were counted in pots treated with *Atheta*, and a mean of 196 (s.d. 140) adults were counted per pot in the controls (i.e. no significant difference between treatment and control). For the bioassay testing *Atheta* against phorid fly, a mean of 50.2 (s.d. 21.3) adults of the following generation were counted in pots treated with *Atheta*, and a mean of 34.0 (s.d. 32.6) adults were counted per pot in the controls. A t-test using square root transformed data indicated no significant difference between treatment and control in the phorid fly bioassays. The numbers of surviving *Atheta* were not counted in either the sciarid fly or the phorid fly bioassay. However, in both the sciarid fly and the phorid fly bioassays, *Atheta* adults were observed in the control chambers. Observations indicated that some *Atheta* moved under the capillary matting placed at the bottom of the chambers. The *Atheta* were thus able to escape from the chambers and move from the treated chambers to the controls. Unfortunately, because the numbers of *Atheta* observed within the chambers were not counted, it is not known how many moved out of the treated chambers and thus reduced the potential predation, or how many moved into the control chambers and thus predated sciarid or phorid fly eggs and larvae here.

Conclusions

- In laboratory bioassays done in Petri dishes, *Atheta* gave 100% control of both eggs and larvae of mushroom sciarid fly, and 52% control of mushroom phorid fly.
- In pots of mushroom compost and casing, *Atheta* gave up to 58% control of sciarid flies that were added as eggs to compost or casing.
- In a more spatially complex experiment, where adult sciarid flies were introduced into containers of mushroom compost and allowed to lay eggs, *Atheta* gave 70% reduction of the subsequent sciarid fly population. This result demonstrated the ability of *Atheta* to locate their prey within mushroom compost.
- The numbers of *Atheta* needed to control the target pests will be dependent on pest density, amongst other factors.
- In an experiment where *Atheta* were introduced into a chamber containing pots of mushroom compost with sciarid or phorid flies, representing an environment more similar to a mushroom crop, the results were inconclusive, as unknown numbers of *Atheta* escaped from the 'treated' chambers and moved into untreated 'control' chambers.

Objective 2: Behaviour of *Atheta* in mushroom crops. Determine any potential problems with using *Atheta* in mushrooms (Warwick HRI and ADAS)

Behavioural experiment: are *Atheta* potential contaminants on harvested mushrooms? (Warwick HRI)

Materials and methods

Fresh pasteurised mushroom compost was spawned with Sylvan A15 spawn at 1% w/w and pressed into pots (10 x 10 x 15 cm) - (350 g per pot, 16 pots). The pots were placed on moist capillary matting in a Perspex chamber (800 mm x 530 mm x 450 mm) and the chamber opening was covered with polythene to maintain humidity. A portion of *Atheta* rearing substrate (100 g) with beetles was placed in a container on the floor of the chamber. After 18 days, the compost was covered with a layer of casing material and then watered daily. The chamber was kept at 25°C until cased and then kept at 18°C, to mimic commercial crop growing temperatures. An unspecified number of additional *Atheta* were then introduced, and a piece of dry cat food was pressed into the casing of each pot to feed the beetles in the absence of fly eggs as prey. When the mushrooms were harvested, a count was made of the number of *Atheta* on mushroom bodies.

Results and Discussion

A total of 2505 g (148) mushrooms were harvested and a total of five *Atheta* adults were counted on the caps. However, it is not known whether these beetles were counted before or after the mushrooms were picked, nor whether the beetles left the mushrooms as a result of the disturbance of picking. This experiment was done as a worst case scenario for potential movement of *Atheta* onto mushroom fruit bodies, as the *Atheta* were confined in a small space with the crop and a large number of *Atheta* were used. The artificial food provided for the *Atheta* (dry cat food) may not have been as palatable as insect prey may have been, and this may have caused the *Atheta* to move onto the mushroom caps in search of more suitable food. Thus the results of the experiment should be treated with caution.

Experiment to quantify survival and reproduction of *Atheta* in spawned mushroom compost and casing (ADAS)

Materials and Methods

The survival and reproduction of *Atheta* was tested in spawned mushroom compost and in cased spawned mushroom compost. Twelve replicate white plastic 5-litre pots with snap-on lids were used for the experiment, six for the spawned mushroom compost, and six additional pots for cased compost. Ventilation and drainage holes were added to the lids and bases of the pots respectively, and these were screened with insect-proof mesh, to prevent *Atheta* escape. Mushroom spawn was mixed into the mushroom compost, at 0.5% of the compost weight. The spawned mushroom compost (1.5 litres) was added to each pot, and pressed down lightly.

Thirty *Atheta* adults were added to each pot, in a small dish of the *Atheta* rearing substrate (mixture of coir and vermiculite). A small amount of the food source for *Atheta* (turkey-rearing crumbs) was added to the small dish each week. The experimental set-up was designed to mimic placing an *Atheta* rearing-release box in a mushroom crop. The pots were stood on wet capillary matting and kept in a controlled temperature room in the dark, at 25°C for 18 days. After 18 days, *Atheta* adults and larvae in the mushroom compost in each of six replicate pots were extracted using Tullgren funnels, and then counted (Tullgren funnels use light bulbs as a source of heat to drive any invertebrates out of samples of substrate, into tubes of alcohol).

After the 18-day period, the mushroom compost in the remaining six replicate pots was ‘cased’. A layer of mushroom casing, equal in weight to the layer of compost, was placed on

top of the mushroom compost in each pot. A second introduction of 30 *Atheta* adults was made to each pot as before, in a small dish of the *Atheta* rearing substrate. The pots were placed on wet capillary matting in a controlled temperature room in the dark, at 20°C for 40 days. The *Atheta* were fed with a small amount of turkey crumbs each week and the casing was watered as required, to keep it damp. At the end of the experiment, the mushroom compost and casing in each pot was assessed separately for numbers of *Atheta* adults and larvae, using Tullgren funnel extraction.

Results and Discussion

After 18 days in the dark at 25°C, there was a mean of 35 *Atheta* adults and 149 larvae per pot of mushroom compost. This result demonstrated that the original 30 *Atheta* adults added to the small dish of rearing substrate in each pot, had dispersed into the mushroom compost and had produced larvae. After 18 days, the mean multiplication rate of *Atheta* in each pot was x6. Most of the new generation adults had not yet developed after 18 days, and this is consistent with results in HDC project PC 239, which showed that at 25°C, the time taken from *Atheta* adult to next generation adult is up to 23 days when fed on turkey crumbs (Bennison, 2007 a).

After casing the compost and leaving the pots for a further 40 days in the dark at 20°C, there was a mean of 17 *Atheta* adults and 13 larvae per pot in the casing, and a mean of 64 adults and 65 larvae per pot in the compost. These results demonstrate that *Atheta* can breed and disperse in mushroom compost and casing. The lower multiplication rate of *Atheta* at 20°C than at 25°C is consistent with published results on *Atheta* development rate at different temperatures, when fed on eggs of another beetle species (Miller & Williams. 1983). ADAS is currently determining the development rate of *Atheta* at various temperatures lower than 25°C, in PSD-funded project PS 2120, which is investigating the use of *Atheta* for the control of cabbage root fly and carrot fly in field vegetable crops.

Conclusions

- In a 'worst case scenario' experiment to test whether *Atheta* could be a potential contaminant on mushrooms at harvest, a small number of adults were seen on fruit bodies (approximately one per 30 mushrooms). However, a relatively large amount of *Atheta* rearing substrate with beetles had been introduced on two occasions to a small enclosed space with spawned mushroom compost. It is considered that the numbers of beetles observed on the mushrooms were lower than might have been expected, given the conditions of the experiment. It is possible that in a real mushroom crop, *Atheta* may have preferred to remain in the compost or casing, or may have left the fruit bodies on disturbance during picking. Further experimental work would be needed to assess the risk of *Atheta* being potential contaminants on harvested mushrooms.
- *Atheta* successfully bred and dispersed in both mushroom compost and casing, when fed on turkey crumbs and kept in conditions consistent with mushroom production, over an 18-day period of spawn run and a 40 day production period.

Overall conclusions of the project

In conclusion, this pilot project has shown that *Atheta* will breed and disperse in both mushroom compost and casing, and will both locate and predate the eggs and larvae of both the mushroom sciarid fly, *Lycoriella ingenua* and the mushroom phorid fly, *Megaselia halterata*. There is potential for some movement of *Atheta* onto fruit bodies, but the risk of the beetles representing a potential contaminant at harvest needs further evaluation in a real mushroom crop. Given that the beetles are inexpensive and simple to rear and maintain by growers, rather than having to be purchased regularly from biological control suppliers, and that they are already being used successfully for the control of sciarid flies in glasshouse crops, then these preliminary results on mushroom fly pests are encouraging. Further work is warranted to investigate the control of sciarid and phorid flies on a crop scale, and to manipulate the numbers of beetles released, to ensure there are no potential contaminant problems at harvest. Control of sciarid flies by *Atheta* and by *Steinernema feltiae* should be compared, and the comparative cost benefits, including labour inputs and practicality should be evaluated. Research in Canada has suggested that *Atheta* and *S. feltiae* are compatible. The compatibility of *Atheta* with selected pesticides used in mushroom production should be assessed, and the benefits of using a combination of the two biological control agents should be quantified in crop scale experiments.

Technology transfer

- Jane Smith presented the results of the project to date at the HDC mushroom panel meeting on 17 July 2007.
- David Chandler presented the results of the project at the HDC mushroom panel meeting on 6 November 2007.
- An article is scheduled for HDC News, in April 2008, on HDC-funded projects on *Atheta*. This article will include the key results from this project.

Acknowledgements

- Thanks to Martyn Dewhirst, for providing mushroom spawn for experimental work at ADAS Boxworth.

References

Bennison, J. 2007 a. Protected herbs, ornamentals and celery: development of an on-nursery rearing system for *Atheta coriaria* for reduced cost biological control of scarid and shore flies. *First year report to HDC for project PC 239.*

Bennison, J. 2007 b. Protected herbs, ornamentals and celery: development of an on-nursery rearing system for *Atheta coriaria* for reduced cost biological control of scarid and shore flies. *Second year report to HDC for project PC 239.*

Carney, V., Diamond, J., Murphy, G. and Marshall, D. 2002. The potential of *Atheta coriaria* Kraatz (Coleoptera: Staphylinidae), as a biological control agent for use in greenhouse crops. *IOBC/wprs Bulletin 28 (1):* 37-40.

Jandricic, S., Murphy, G., Broadbent, B., Scott-Dupree, C. and Harris, R. 2005a. Compatibility of *Atheta coriaria* with other biocontrol agents used in greenhouse production. *IOBC/wprs Bulletin 28 (1):* 135-138.

Jandricic, S., Scott-Dupree, C., Harris, R., Broadbent, B. and Murphy, G. 2005b. Toxicity of soil applied pesticides to *Atheta coriaria* Kraatz. *IOBC/wprs Bulletin 28 (1):* 139-142.

Miller, K.V. and Williams, R.N. 1983. Biology and host preference of *Atheta coriaria* (Coleoptera: Staphylinidae), an egg predator of Nitulidae and Muscidae. *Annals of the Entomological Society of America 76:* 158-161.