Project Title	Protected herbs, ornamentals and celery: development of an on-nursery rearing system for *Atheta coriaria* for reduced cost biological control of sciarid and shore flies
Project number:	PC 239a
Project leader:	Jude Bennison, ADAS
Report:	First and final report, 10 August 2009
Previous report	First year report for project PC 239, January 2007 Second year report for project PC 239, September 2007 Third year report for project PC 239, March 2008
Key staff:	Jude Bennison, ADAS, Study Director Dr Pat Croft, STC, Site Manager for celery experiments Heather Maher and Kerry Maulden, ADAS, Site Managers for ADAS experiments
Location of project:	ADAS Boxworth, STC and commercial nurseries
Project coordinators:	Fay Richardson, Coletta & Tyson, 324, Hull Road, Woodmansey, Beverley, East Yorks, HU17 ORU Claire Donkin, Hill Farm, Sunderland Hill, Ravensden, Bedford, MK44 2SJ
Date project PC 239a commenced:	1 June 2008
Date completion due:	31 July 2009
Key words:	sciarid fly, *Bradysia difformis*, shore fly, *Scatella tenuicosta*, biological control, predatory beetles, *Atheta coriaria*, herbs, ornamentals, celery, lettuce.

Whilst reports issued under the auspices of the AHDB are prepared from the best available information, neither the authors nor the AHDB 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 a series of experiments 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

I declare that this work was done under my 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
Project Leader

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

Report authorised by:

Dr Tim O'Neill
Horticulture Research Manager
ADAS

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

TABLE OF CONTENTS

GROWER SUMMARY

Headline

- Growers could save money by on-nursery rearing of *Atheta* for control of sciarid and shore flies.

Background and expected deliverables

Sciarid and shore flies are common pests and contaminants respectively on many protected edible and ornamental crops. Sciarid fly larvae cause crop losses by damaging roots and shore flies can cause marketing problems in herbs, potted ornamentals and celery due to the presence of flies or droppings on the marketed plants or pots. Although entomopathogenic nematodes (e.g. *Steinernema feltiae*) and predatory mites (*Hypoaspis* spp.) are available for sciarid fly control, they are expensive and do not always give reliable control e.g. on protected herbs. Neither of these biological controls gives effective control of shore flies. An alternative is needed for reliable and cost-effective control of both pests within IPM programmes.

The predatory beetle, *Atheta coriaria* is known to feed on both sciarid and shore fly eggs and larvae, and has recently become commercially available. However, *Atheta* has given variable results in both research and grower experience on nurseries. An improved method for establishing high numbers of *Atheta* in susceptible crops, leading to more reliable and cost-effective control of target pests, is needed.

Following research from Canada on the use of an artificial diet (trout pellets) to rear *Atheta*, a UK nursery (WJ Findon & Son, now Bordon Hill Nurseries Ltd.) experimented with using *Atheta* 'breeding boxes' in crops of poinsettia and cyclamen in 2004. The system produced large numbers of the predators at low cost which gave good control of sciarid flies in both crops. Other growers of ornamentals tried the system with variable success. Fungal contamination in the boxes, variable *Atheta* production rates and potential negative interactions between *Atheta* and other biological control agents and naturally-occurring invertebrates need to be resolved. Further scientific development and testing of the system is needed.

The overall aim of project PC 239 was to develop a reliable and practical rearing system for *Atheta*, to enable growers to rear large numbers of the predator on their own nurseries, for improved, low-cost biological control of sciarid and shore flies.

Summary of project PC 239 and main conclusions

Year 1 (annual report, 2007)

Knowledge on *Atheta* biology, rearing methods and potential as a biological control agent within IPM programmes was reviewed and summarised. A simple, practical and inexpensive 'DIY' method using turkey-rearing food was developed for growers to rear and release large numbers of *Atheta.* A 'bait pot' system was developed for monitoring dispersal of *Atheta* in the glasshouse. In a commercial poinsettia crop, the system showed that *Atheta* dispersed from rearing-release boxes throughout the 2,000 m^2 glasshouse. Use of *Atheta* rearing-release boxes in a commercial 5-week crop of potted parsley reduced numbers of sciarid flies by 58%, when compared with a crop where *Steinernema feltiae* and *Hypoapis miles* were used for control.

Year 2 (annual report, January 2008)

Atheta adults added at 5 or 10 per parsley pot reduced mean numbers of sciarid flies 22 days later, from 11 per pot in untreated pots, to 3 and 1.7 per pot respectively (75% and 85% reductions respectively). These rates were equivalent to 500 or 1000 *Atheta* per m^2 respectively in potted herbs before spacing.

Atheta adults added at 25 per m^2 to soil samples collected from celery glasshouses reduced numbers of shore flies by up to 88%, when compared with numbers in samples of soil to which the grower had released *Atheta* at 5 per m^2.

These effective release rates in the pot herb and celery experiments would be commercially unacceptable if the grower used direct releases of *Atheta* bought from commercial suppliers, but might be possible if *Atheta* were reared on the nursery for direct release, or for use in a rearing-release system.

Numbers of *Atheta* leaving rearing-release boxes were manipulated by a feeding regime. Numbers leaving the boxes during week 1 in the glasshouse were increased from 6% to 47% by not feeding *Atheta* in the boxes the week before release.

In soil-grown crops e.g. celery, bait pots sunk into the ground were a better method for trapping and monitoring *Atheta* than those stood on the soil surface.

Atheta ate *Aphidoletes* larvae when offered as the only prey in Petri dishes. However, *Atheta* did not affect numbers of *Aphidoletes* larvae in the compost of aphid-infested parsley plants, when other prey e.g. sciarid flies were present.

Knowledge on the side-effects of pesticides on *Atheta* was summarised.

Year 3 (annual report, March 2008)

The self-watering rearing-release boxes allowed the release of similar numbers of *Atheta:* whether boxes were stood (i) on capillary matting and watered with sub-irrigation, or (ii) on woven ground-cover matting and watered with overhead irrigation. The methods are thus compatible with most glasshouse irrigation systems.

H. miles released at 300 per m^2 had no impact on numbers of *Atheta* in pots of parsley or in rearing-release boxes over a 4-week period. However, *Atheta* released from rearing-release boxes reduced numbers of *H. miles* in pots of parsley over a 4-week period, indicating that the use of both predators in IPM programmes would have a negative effect on *Hypoaspis* spp.

Expected deliverables in project PC 239a

A further project, PC 239a aimed to refine the *Atheta* rearing-release system before it could be tested further on commercial nurseries. Expected deliverables were to:

1. Quantify numbers of *Atheta* needed to control low densities of sciarid flies , and determine whether hungry *Atheta* give better control of sciarid flies than fully fed ones (ADAS).
2. Determine whether the feeding regime could be manipulated to achieve both quick and sustained release of *Atheta* (ADAS).
3. Determine whether *Atheta* leave rearing-release boxes and disperse in an early-season soil-grown lettuce crop (sub-contracted to STC).

Summary of project PC 239a and main conclusions

Quantifying numbers of Atheta needed to control low densities of sciarid flies and determining whether hungry Atheta give better control than fully fed ones (ADAS)

There were no differences in control of sciarid flies by fully fed or 'starved' *Atheta*.

Two, five or ten *Atheta* adults per pot significantly reduced numbers of sciarid fly adults emerging from pots of parsley infested with five sciarid fly eggs per pot. Five or ten *Atheta* adults per pot had similar effects on control of sciarid flies. Five adults per pot led to an 85% reduction in emerging flies when compared with the untreated control, i.e. to less than one fly per pot (see Figure 1). This release rate is equivalent to 500-660 *Atheta* per m^2 if released to small pots of herbs before spacing (depending on exact pot size and production system).

These effective release rates are much higher than those recommended by suppliers of *Atheta* (up to 10 per m^2) and would not be cost-effective. However, if *Atheta* were reared on the nursery, use at these rates should be cost-effective.

The results of experiments in PC 239 and PC 239a indicate that if effective *Atheta* rates were maintained in the glasshouse, the beetles should be able to reduce the sciarid fly population by 85% with each successive 5-week crop of parsley.

This strategy needs validating on a commercial herb nursery.

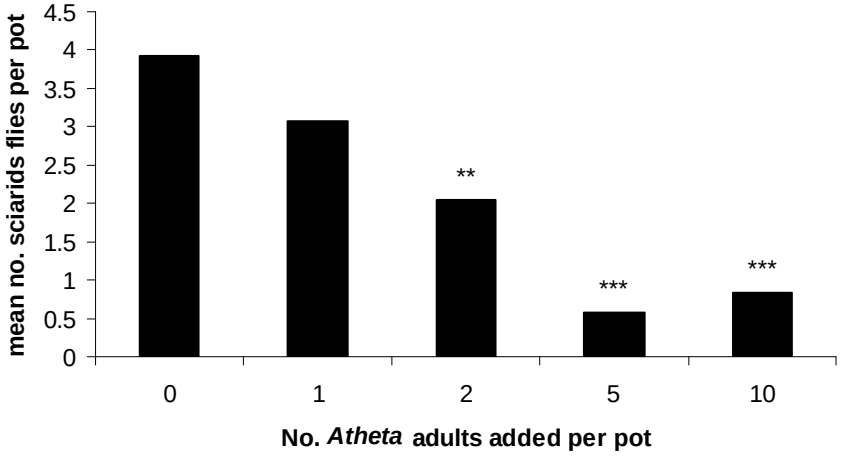

Figure 1. Mean numbers of sciarid fly adults emerged per pot of parsley, 28 days after adding *Atheta* adults at different rates per pot. ** significantly lower numbers than in untreated controls, $P<0.01$. *** $P<0.001$.

Determining whether the rearing-release box feeding regime could be manipulated to achieve both quick and sustained release of Atheta (ADAS).

An improved weekly feeding regime for rearing *Atheta* at 25ºC was 5g turkey crumbs per box in week 1; 10 or 15 g in week 2 and; 15 g in weeks 3 and 4. This regime produced over twice as many beetles after four weeks than the 'standard' feeding regime of 5 g per week. In two glasshouse experiments, both quick and sustained release of *Atheta* was given from rearing-release boxes over a six or seven week period.

Feeding the boxes every week or in alternate weeks led to similar total numbers (up to 2,800) of *Atheta* being released per box over the six or seven-week period.
Boxes fed every week contained more *Atheta* at the end of the experiments then boxes fed in alternate weeks and thus might have continued to release more *Atheta* for a longer period. Similarly, boxes fed with 15g turkey crumbs on each feeding occasion in the glasshouse contained more *Atheta* at the end of the experiments than boxes fed with 5g.

However, higher numbers of *Atheta* were released from boxes fed with 5g food than from boxes fed on 15g. This is likely to have been due to the higher glasshouse temperatures in the 5g experiment (August-October) than in the 15g experiment (November-December) leading to greater *Atheta* activity and flight.

Work in both PC 239 and PC 239a quantified numbers of *Atheta* needed to control sciarid flies at specific pest densities, using potted parsley as an example crop. It is likely that the most practical strategy for using *Atheta* would be to use high direct releases at the start to reduce the pest population in a crop with a high sciarid fly density. Such high release rates would only be cost-effective if the grower reared *Atheta* on the nursery. Once the sciarid fly density has been reduced, it may then be possible to maintain control by using lower direct releases of *Atheta,* or by using a rearing-release system. This strategy would need validating on commercial nurseries before firm recommendations can be given.

Determining whether Atheta leave rearing-release boxes and disperse in an early-season soil-grown lettuce crop (sub-contracted to STC)

Atheta adults left rearing-release boxes placed in a shore-fly infested lettuce crop shortly after planting in mid-May and within one week had dispersed and produced larvae in bait pots throughout a 2,000 m^2 glasshouse. 'Pitfall' bait pots sunk into the soil proved to be useful for detecting *Atheta* dispersal in the soil-grown lettuce crop. *Atheta* dispersal was not limited by mean soil temperatures of 16.5°C or by a temperature range of 11.2 to 27.9°C.

Financial benefits

The approximate retail price of *Atheta* is £25 for 500 (5 pence each) or £73 for 3,000 (2.4 pence each). With one commercial pack of 500 *Atheta*, a grower could set up eight rearing boxes, each with 60 'starter' adults. These should produce approximately 20,000 *Atheta* (2,400 per box) if kept for 28 days at 25°C. After investing £25 in one pack of *Atheta*, the grower could rear beetles worth £1,000 after four weeks (£125-worth per box). This represents a 4,000% return in investment.

The estimated grower cost for rearing one box of *Atheta* (substrate, turkey crumbs and labour) is 35 pence (0.015 p per beetle) over 28 days. Labour would account for over half this cost, but this could be reduced by using larger rearing containers. To control a heavy sciarid fly infestation on one 5-week crop of potted parsley, 500 *Atheta* per m^2 may be needed, costing approximately 7 pence per m^2. However, in subsequent parsley crops after the sciarid population had been reduced, numbers of *Atheta* needed and thus costs are likely to be much lower.

The average retail price of *Hypoaspis* sp. is 0.07 pence per predator, depending on how many are bought. Recommended release rates vary but for a heavy sciarid fly infestation, most growers use approximately 300 per m^2 as a preventive release, costing 20 pence per m^2. The average retail price of *S. feltiae* is £10-20 per 50 million, depending on how many are bought. The recommended preventive rate for sciarid fly control is 50,000 nematodes per m^2 (e.g. one pack of 50 million will treat 100 m^2), costing 10-20 pence per m^2.

Thus the estimated cost of growers rearing *Atheta* on the nursery would be less than buying commercial biological control agents for sciarid flies (not including application

costs). If *Atheta* gave adequate control of shore flies in addition to sciarid flies, this would add value to the grower using a 'DIY' system (*Hypoaspis* spp. and *S. feltiae* do not usually give adequate control of shore flies).

The full benefits of using a grower rearing system for *Atheta* rather than buying biocontrol agents would need to factor in heating costs for the rearing area if needed, labour needed for release or application of the different biocontrols, pest densities, crop area and value, and numbers of *Atheta* needed for effective control.

Strategies for using nursery-reared *Atheta* need validating on a commercial scale compared with other biological control options. However, some growers of protected herbs and ornamentals are already experimenting with their own *Atheta* rearing or rearing-release units and encouraging results are being reported.

Action points for growers

- Await the factsheet to be produced later in 2009, giving practical guidelines for rearing *Atheta* for direct release on the nursery.
- In the meantime, any growers interested in trying the system should contact Jude Bennison for details, tel. 01954 268225, email jude.bennison@adas.co.uk

SCIENCE SECTION

Introduction

Sciarid and shore flies are common pests and contaminants respectively on many protected edible and ornamental crops. Sciarid fly larvae cause crop losses by damaging roots and stems, and shore fly adults can cause crop rejections or marketing problems in herbs, potted ornamentals and celery due to the presence of flies or their droppings on the marketed plants or pots. Shore flies can also spread root diseases e.g. *Pythium*.

Although entomopathogenic nematodes and *Hypoaspis* spp. predatory mites are available for sciarid fly control, these are expensive and do not always give reliable control of sciarids on protected herbs. Neither of these biological control agents gives effective control of shore flies at economically viable application rates. An alternative control is needed as reliable and cost-effective biological control options are necessary for both pests for use within IPM programmes.

The predatory beetle *Atheta coriaria* is known to be an effective predator of both sciarid and shore fly eggs and larvae and has recently become commercially available. *Atheta* has been shown to reduce numbers of both sciarid and shore flies in ornamental crops when released as mixed life stages (R. GreatRex, personal communication). Recent grower-funded research by Stockbridge Technology Centre (STC) has indicated that *Atheta* can contribute to reducing shore flies and crop losses on celery, but further work is needed to confirm consistent control. A system to reduce the cost of the predators and to improve their performance on various protected crops is desirable.

To date, commercial releases of *Atheta* adults have given unreliable control of shore flies on susceptible pot herbs, where control needs to be very effective to meet the stringent standards set by the retailers. Releases of the predator as either adults or mixed life stages did not give successful control in a trial on a commercial herb nursery in HDC project PC 210 in 2004 (Bennison, 2005; Bennison *et al*, 2005). This was possibly due to poor beetle survival after their initial release due to variable availability of shore fly prey eggs before adult fly releases were made to the trial plots. An improved release method for successful predator establishment in the crop and thus more effective control is needed.

Following research in Canada on using an artificial diet (trout pellets) to rear *Atheta* (Carney *et al.*, 2002), a UK commercial nursery (W J Findon & Son, now Bordon Hill Nurseries Ltd.) experimented with using 'breeding boxes' for *Atheta* in crops of poinsettia and cyclamen during 2004. The system produced large numbers of the predators at very little cost and good control of sciarid flies was achieved in both crops. Other growers of ornamentals also tried the system with variable success.

Fungal contamination in the 'breeding boxes', potential problems with interactions between *Atheta*, other ground-dwelling biological control agents and other invertebrates attracted to the trout pellets, fungi or *Atheta* in the rearing boxes need to be resolved. With further scientific development and testing, a reliable on-nursery rearing system for maintaining a constant supply of large numbers of *Atheta* at very little cost has good potential for giving improved, low-cost control of both sciarid and shore flies.

The overall objective project PC 239 was to develop an effective, reliable and practical rearing system for *Atheta coriaria*, to enable growers to rear large numbers of the predators on their own nurseries, for improved, low-cost biological control of sciarid and shore flies on various protected crops. The results of project PC 239 are summarized in the Grower Summary in this report and are given in full in the three annual reports (Bennison 2007 and 2008).

The overall objective of project PC 239a was to further refine the rearing-release system. Detailed objectives were:

1.1. Determine how many *Atheta* are needed to control sciarid flies at a lower density than that used in PC 239 (ADAS).
1.2. Determine whether hungry *Atheta* give better control of sciarid flies than fully fed ones (ADAS).
1.3 Determine whether the feeding regime can be manipulated to achieve both quick and sustained release of *Atheta* (ADAS).
1.4 Determine whether *Atheta* leave rearing-release boxes and disperse in an early-season soil-grown lettuce crop (sub-contracted to STC).

1.1. and 1.2: Determine how many *Atheta* are needed to control sciarid flies at a lower density than that used in PC 239, and whether hungry *Atheta* give better control of sciarid flies than fully fed ones (ADAS).

Background

In year 2 of project PC 239, it was shown in an experiment done in June 2007 that 5-10 *Atheta* adults per parsley pot were needed to reduce high densities of sciarid flies (Bennison, 2008). This is a very high release rate for *Atheta* (500-1000 per m^2). Such high rates would be uneconomic if the grower bought the *Atheta* from a commercial supplier, but might be possible if the *Atheta* were reared by the grower on the nursery. Lower densities of sciarid flies than those used in this experiment can occur on herb nurseries in the early spring and could also occur later in the season if *Atheta* reduce the sciarid fly population with each successive crop.

In year 2 of project PC 239, it was shown in a glasshouse experiment done in August 2007 that the numbers of *Atheta* leaving rearing-release boxes might be manipulated by the feeding regime. Numbers of released predators during the first week were increased from 6% to 47% by not feeding the boxes with the artifical diet (turkey crumbs) for one week before allowing release (Bennison, 2008). This indicated that growers could starve the boxes for a week, then open the exit holes for a quick release of high numbers of (hungry) predators. Canadian laboratory research indicated that starved *Atheta* eat many more sciarid and shore fly prey than fully fed ones (Carney *et al*, 2002; Jandricic, 2005).

Experiment objectives

1.1. Test if lower numbers of *Atheta* can control a lower density of sciarid flies than that used in the PC 239 experiment in 2007. This could enable growers to start using the rearing-release system from early spring onwards, for sustained sciarid fly control throughout the season.

1.2. Compare fed and starved *Atheta* in the same experiment as in 1.1, to test if starving the *Atheta* before allowing them to leave the rearing-release boxes could allow lower numbers of the predator to control sciarid flies at a given density.

Materials and methods

The same method was used as in the experiment with a high density of sciarid flies in year 2 of PC 239 (which used *Atheta* from recently fed rearing boxes), but each pot was infested with equal numbers of sciarid fly eggs rather than using a natural infestation. On 14 May, 120 pots of newly emerged parsley plants supplied by a commercial pot herb nursery were infested with sciarid fly eggs. Five 1-day old sciarid fly (*Bradysia difformis)* eggs from the ADAS sciarid fly culture were added to the compost in each pot. This egg density was selected as in the PC 239 experiment which used a natural summer infestation of sciarid fly eggs, the pest was present at twice this density (a mean of 11 sciarid flies per untreated pot). Each pot (8 x 8 cm) was placed inside its own 'fly emergence pot' i.e. a larger (one litre) white plastic pot (12 cm diameter) with a disc of wet capillary matting in the base to maintain compost moisture. *Atheta* adults were added to 12 replicate pots at each of the following rates:

1. No *Atheta* per pot (untreated control).
2. Additional untreated control.
3. One starved *Atheta* per pot.
4. Two starved *Atheta* per pot.
5. Five starved *Atheta* per pot.
6. Ten starved *Atheta* per pot.
7. One fed *Atheta* per pot.
8. Two fed *Atheta* per pot.
9. Five fed *Atheta* per pot.
10. Ten fed *Atheta* per pot.

The 'starved' *Atheta* for treatments 3-6 were taken from an ADAS culture box that had not been fed with the artificial diet (turkey crumbs) for one week. The 'fed' *Atheta* for treatments 7-10 were taken from a culture box that had been fed with turkey crumbs the day before the experiment was set up.

A small yellow sticky trap (10x5 cm) was attached to the inside of each of the untreated control emergence pots, using a paper clip. Each pot was covered with a 'lid' of insect-proof mesh, secured with a rubber band. The pots were placed in a randomized block design on damp capillary matting in a research glasshouse. The matting was kept damp to maintain a high glasshouse humidity to favour sciarid fly survival. As the pots of parsley were inside sealed 'emergence' pots with no holes in

the base, the pots were watered as necessary through the mesh lid using a watering can with a fine rose, to keep the compost moist. The glasshouse temperature was set at a minimum of 17°C night, 19°C day, venting at 21°C, consistent with that used in the commercial herb glasshouse. A Tinytalk ® datalogger was placed in a pot of damp compost in an empty white plastic 'emergence pot' covered with insect-proof mesh. Daily mean, maximum and minimum temperatures were recorded in the pots throughout the experiment.

The sticky traps in the control pots were monitored regularly to determine when the first sciarid fly adult emergence occurred. Nineteen days after the experiment was set up, on 2 June, once the first sciarid flies were seen on the untreated control pot traps, sticky traps were added to the remainder of the pots (sticky traps were not added to the pots containing *Atheta* at the start of the experiment, to avoid trapping the predators). The pots were then left for a further nine days, to allow all the sciarid flies to emerge and get caught on the traps.

Assessments

Total numbers of sciarid fly adults per pot were recorded after counting numbers of sciarid flies on the trap, on the compost and on the inside of the emergence pot.

Statistical analysis

The mean numbers of sciarid flies per pot in the different treatments were compared using analysis of variance (ANOVA).

Results and discussion

Control of sciarid flies

In untreated control pots with no *Atheta* there was a mean of 3.9 sciarid adults per pot, 28 days after the pots had been infested with sciarid fly eggs (Table 1 and Figure 1). Thus a mean of 1.1 of the five sciarid fly eggs added to each untreated pot had not completed its development to a sciarid adult (20% natural mortality). This natural mortality in the sciarid fly life cycle is new information on the biology of *Bradysia difformis*.

There were no significant differences between numbers of sciarid fly adults per pot when treated with equal numbers of either fully fed *Atheta* or those which had not

been fed for one week. Laboratory studies in Canada where *Atheta* adults had been isolated and starved for six hours showed that starved beetles ate a mean of 154 sciarid fly eggs or 150 first instar sciarid fly larvae in Petri dishes (Carney *et al*, 2002). In a separate study, fully fed *Atheta* adults ate a mean of only three sciarid eggs or four first instar sciarid larvae (Jandricic, 2005). These results indicated that starved beetles could eat much higher numbers of sciarid prey than fully fed ones. However our HDC-funded experiment simulated more realistic commercial conditions than the Canadian laboratory bioassays in petri dishes. The 'starved' *Atheta* in our experiment were not isolated from the culture, but were kept in the rearing boxes and not fed with turkey crumbs for one week. This method was to simulate beetles leaving rearing-release boxes in the glasshouse and entering the pots of parsley. However, the *Atheta* in our 'starved' cultures could have eaten their own offspring in the absence of the artificial food source (turkey crumbs), which could explain the similar effects of fed and 'starved' *Atheta* on sciarid fly numbers.

As the fed and starved *Atheta* had similar effects on numbers of sciarid flies surviving to the adult stage, the data on the effects of fed and starved *Atheta* were combined in the analysis to determine reductions in sciarid flies by different numbers of *Atheta*. In pots treated with two, five or 10 *Atheta* adults per pot, there were significantly fewer (P<0.01) sciarid adults (means of 2.0, 0.6 and 0.8 respectively) than in untreated control pots (Table 1 and Figure 1). These reductions represented 48%, 85% and 79% control of sciarid flies by *Atheta* added at two, five or 10 per pot respectively (Table 1). Sciarid fly numbers were not significantly reduced in pots treated with one *Atheta* adult per pot.

Table 1. Mean numbers of sciarid fly adults emerged per pot of parsley, 28 days after adding *Atheta* adults at different rates per pot.

No. of *Atheta* adults added per pot	**Mean nos of sciarid flies per pot treated with starved and fed *Atheta***	**% control by starved and fed *Atheta* (compared with untreated)**
0	3.9	0
1	3.1	21%
2	2.0**	48%**
5	0.6***	85%***
10	0.8***	79%***

** significantly lower numbers than in untreated controls, P<0.01. *** P<0.001.

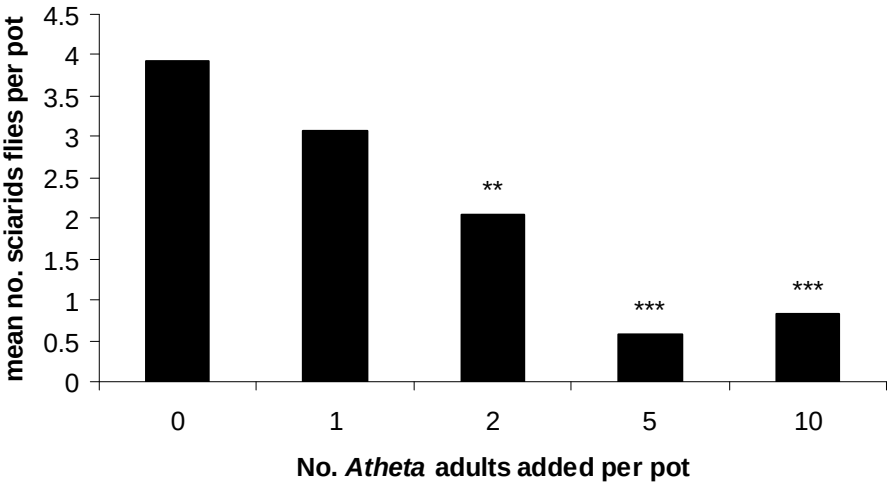

Figure 1. Mean numbers of sciarid fly adults emerged per pot of parsley, 28 days after adding *Atheta* adults at different rates per pot. ** significantly lower numbers than in untreated controls, $P<0.01$. *** $P<0.001$.

These results show that two *Atheta* adults per pot significantly reduced numbers of sciarid flies at a starting density of five eggs per pot, whereas in the experiment in year 2 of PC 239, a minimum of five *Atheta* adults were needed to significantly reduce a higher density of sciarid flies (11 adult sciarid flies per pot in untreated controls), Bennison 2008. In the experiment reported here, the effective rates of *Atheta* i.e. two, five and ten per pot, were equivalent to 200-264, 500-660 and 1,000-1,320 per m^2 respectively. This is based on commercial herb nurseries using 100 - 132 pots per m^2 before spacing, depending on exact pot size and production system. Such high rates of *Atheta* would be uneconomic if the grower bought them from a commercial supplier, but might be possible if the *Atheta* were reared on the grower's own nursery.

Furthermore, the combined results of the experiments in PC 239 and in this project indicate that if five or ten *Atheta* per pot could reduce a sciarid fly egg density of 11 per pot by 75-85% to 2-3 per pot in the first 5-week crop of parsley, then the same numbers of *Atheta* could reduce the remaining population of sciarid flies by a further 85%, to less than one fly per pot in the following 5-week crop. Thus if *Atheta* were maintained in the glasshouse, with every successive herb crop grown in the glasshouse, the beetles might reduce the sciarid fly population to very low numbers. Thereafter, only low numbers of *Atheta* might be needed to maintain the pest at

negligible levels. This potential strategy needs validating on successive crops of parsley or other sciarid-susceptible herbs on a commercial nursery.

Conclusions

- New information was given on the 20% natural mortality in the sciarid fly (*Bradysia difformis*) life cycle between the egg and adult stages.
- There were no differences in control of sciarid flies by fully fed or 'starved' *Atheta*.
- Two, five or ten *Atheta* adults per pot significantly reduced numbers of sciarid fly adults emerging from pots of parsley infested with five sciarid fly eggs per pot.
- Five or ten *Atheta* adults per pot had similar effects on control of sciarid flies. Five adults per pot led to an 85% reduction in emerging flies when compared with the untreated control, i.e. to less than one fly per pot. This release rate is equivalent to 500-660 *Atheta* per m^2 if released to small potted herbs before spacing (depending on exact pot size and production system).
- The effective release rates in this experiment are much higher than those recommended by commercial suppliers of *Atheta* (up to 10 per m^2) and would not be cost-effective for growers. However, if *Atheta* were reared on the grower's own nursery, cost-effective use of *Atheta* should be possible.
- The combined results of experiments in PC 239 and in the experiment reported here indicate that if effective *Atheta* rates were maintained in the glasshouse, the beetles should be able to reduce the sciarid fly population further with each successive 5-week crop of parsley.
- This strategy needs validating on a commercial herb nursery.

1.3. Determine whether the feeding regime can be manipulated to achieve both quick and sustained release of *Atheta* (ADAS).

Background

In year 2 of project PC 239, results showed that releases of *Atheta* from rearing-release boxes can be manipulated by the feeding regime (Bennison, 2008). Mean numbers of released *Atheta* during the first week in the glasshouse were increased from 6% of the total beetles in the boxes to 47%, by not feeding the boxes for a week before allowing escape of the predators. In addition to the stimulation of a quick release of *Atheta* when required, sustained release from the rearing-release boxes over time would also be necessary for effective control of sciarid or shore flies. The

first step in manipulating sustained release was to determine the optimum artificial food rate for maximum *Atheta* breeding.

In year 1 of project PC 239, a simple method for rearing *Atheta* on turkey crumbs was developed (Bennison, 2007). The method was adapted from a technique developed in Canada which used trout pellets as a food source (Carney *et al*, 2002). The work in PC 239 compared different animal feeds as an artificial food souce for *Atheta*. However, the work did not compare different amounts of food added to the *Atheta* cultures each week (2.5 or 5 g food per week was used, when starting each rearing box with 30 or 60 adult beetles respectively). ADAS *Atheta* cultures have been maintained since 2006 using this feeding regime, which typically leads to x 20 multiplication rates over a 4-week period when kept at 25ºC, i.e. boxes started with 60 beetles produce approximately 1,200 offspring after four weeks. Even higher numbers of beetles might be produced if the cultures are given more food. However, adding too much food can lead to problems with fungal contamination of leftover food in the rearing substrate.

Experiment objectives

Two experiments were designed with the following objectives:

1.3.1. Determine the optimum feeding regime to allow maximum breeding of *Atheta,* whilst avoiding fungal contamination problems with uneaten excess food.
1.3.2. Test feeding strategy to achieve both quick and sustained release of *Atheta*.

Experiment 1.3.1: Determine the optimum feeding regime to allow maximum breeding of Atheta, whilst avoiding fungal contamination problems with uneaten excess food.

Materials and methods

This experiment was done in October 2008.

Treatments
The following feeding regimes were tested:

1. 5g turkey crumbs per week i.e. on day 0, 7, 14, 21.

2. 5g turkey crumbs in week 1 (day 0), 10g in weeks 2, 3 and 4 i.e. on days 7, 14, 21.
3. 5g turkey crumbs in week 1 (day 0), 15g in weeks 2, 3 and 4 i.e. on days 7, 14, 21.
4. 5g turkey crumbs in week 1 (day 0), 10g in week 2 (day 7) and 15g in weeks 3 and 4 (days 14, 21).

There were eight replicate rearing boxes per treatment.

Setting up Atheta rearing boxes

32 boxes were set up, using the standard method developed in project PC 239 (Bennison, 2007). The rearing substrate (1.5 litres of a 1:1 mix of coir and vermiculite) was dampened with 150 ml water per litre of substrate and added to each plastic box (3 litre capacity). Sixty *Atheta* adults from the ADAS laboratory culture were added to each box. The appropriate number of grams of artificial food (turkey crumbs) were added to each of the eight replicate boxes per treatment, and incorporated into the substrate. Each box was sealed using a snap-on lid, fitted with two ventilation holes (2.5 cm diameter) covered with insect-proof mesh. The rearing boxes were held in a controlled-temperature laboratory at 25°C, 16:8 hour photoperiod for four weeks. Every week, the appropriate weight of turkey crumbs and water (if required) were added to each box and incorporated into the substrate.

Estimation of numbers of Atheta per box at end of experiment

Numbers of *Atheta* adults and larvae were estimated in each of the 32 boxes after 28 days in the controlled-temperature laboratory. Six 30 ml sub-samples of the substrate were taken, and passed through two sieves (gauge sizes 2 mm over 1 mm). *Atheta* adults and larvae collected on the finer sieve were turned onto a large white plastic tray and counted whilst being collected into a tube using a 'pooter'. All counted *Atheta* were returned to the respective box. Estimated numbers of *Atheta* adults and larvae per box were calculated.

Statistical analysis

Mean numbers of *Atheta* adults and larvae per rearing box in each treatment at the end of the experiment were analysed using analysis of variance (ANOVA).

Results and discussion

Mean numbers of Atheta per box at end of experiment

The 'standard' feeding regime of 5 g turkey crumbs per week produced a mean of 1116 *Atheta* adults and larvae per box after four weeks, which represents a multiplication rate of x19 from the starting numbers of 60 adult per box (Table 2 and Figure 2). This multiplication rate is typical of those produced using the standard ADAS *Atheta* production technique and feeding regime. Treatment 2 i.e. feeding the standard 5 g in the first week then increasing the feed to 10 g in the following three weeks, significantly (P<0.01) increased mean production to 1910 adults and larvae per box i.e. a mean multiplication rate of x32 (Table 2 and Figure 2). Treatments 3 and 4 produced the most beetles (P<0.001), with means of 2442 and 2432 adults and larvae per box at the end of the experiment i.e. x41 multiplication rates. No problems with fungal contamination of the feed occurred in any of the feeding regimes. All the feeding regimes used the standard rate of 5 g per week during the first week, when there were only the 'starter' 60 adults per box, as experience has shown that giving more feed at this stage is likely to lead fungal contamination of excess food. The mixing of the feed into the rearing substrate on every feeding occasion in the following weeks avoided any problems with fungal contamination.

Table 2. Mean numbers of *Atheta* adults and larvae per rearing box after four weeks in the controlled temperature laboratory held at 25ºC, using turkey crumbs in the different feeding regimes.

	Treatment 1 (5g in weeks 1, 2, 3 & 4)	**Treatment 2 (5g in week 1, 10g in weeks 2, 3 & 4)**	**Treatment 3 (5g in week 1, 15g in weeks 2, 3 & 4)**	**Treatment 4 (5g in week 1, 10g in week 2, 15g in weeks 3 & 4)**
Mean nos *Atheta* adults & larvae after 4 weeks	1116	1910**	2442***	2432***
Mean multiplication rate	X 19	X 32**	X 41***	X 41***

** significantly more produced than in the standard Treatment 1, P<0.01. *** significantly more produced than in the standard Treatment 1, P<0.001.

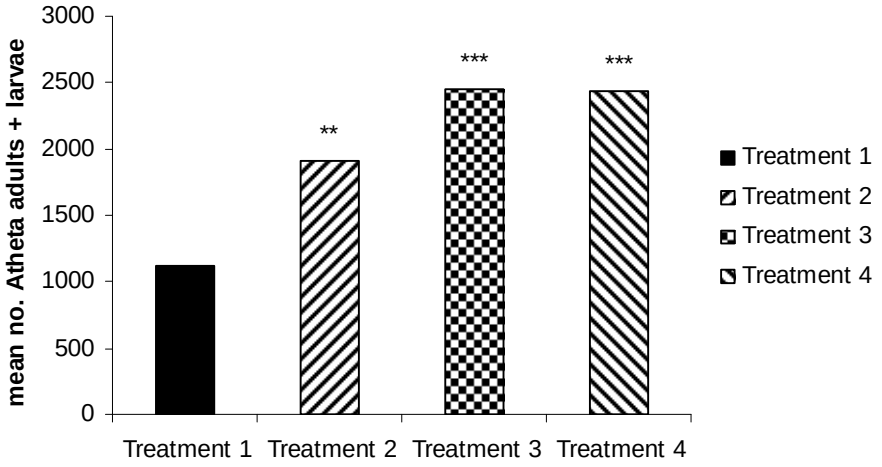

Figure 2. Mean numbers of *Atheta* adults and larvae per rearing box after four weeks in the controlled temperature laboratory held at 25ºC, using turkey crumbs in the different feeding regimes. ** significantly more produced than in the standard Treatment 1, $P<0.01$. *** significantly more produced than in the standard Treatment 1, $P<0.001$. See Table 2 for details of feeding regimes in Treatments 1-4.

Conclusion

The optimum feeding regime for rearing boxes starting with 60 adult *Atheta* was 5 g turkey crumbs per box in week 1, followed by 10 or 15 g in week 2, and 15 g in weeks 3 and 4. This regime produced over twice as many (x 41) beetles after four weeks than the standard feeding regime of 5 g per week (x 19).

Experiment 1.3.2: Test feeding strategy to achieve both quick and sustained release of Atheta

Materials and methods

Two experiments were done:

- Experiment A was done 22 August to 6 October 2008.
- Experiment B was done 29 October to 24 December 2008.

Experiment A was done before the experiment in 1.3.1 above was completed (optimum feeding for maximum breeding of *Atheta*), in order to do the experiment whilst ambient temperatures were still warm, i.e. when sciarid flies would be more of a problem than in cooler temperatures. Thus the feeding regime in experiment A

used 5g turkey crumbs on the feeding occasions, as the optimum weekly feed of 15g had not yet been determined in experiment 1.3.1.

Experiment B was done after the determination of the optimum weekly feed of 15g turkey crumbs, therefore the feeding regime used 15g of turkey crumbs on feeding occasions. This experiment was done during cooler glasshouse conditions than in experiment A, so that *Atheta* activity could be compared in the two experiment periods.

Preparation of Atheta boxes

A set of *Atheta* boxes in the ADAS *Atheta* rearing laboratory were not fed during the final week of their 4-week rearing time. This was to stimulate release of the *Atheta* from the boxes in the first week of the glasshouse experiment. The day before the glasshouse experiment was set up, eight boxes of *Atheta* with similar numbers of *Atheta* adults and larvae were selected for each of experiments A and B. Numbers of *Atheta* adults and larvae in six sub-samples per box were estimated using the same method as used at the end of the experiment in 1.3.1 above.

Setting up of glasshouse experiment

For each of experiments A and B, eight replicate insect-proof mesh cages (0.5 m x 0.5 m x 0.5 m) were placed on damp capillary matting on the floor of a research glasshouse. Four replicate cages were used for each of the following two treatments:

1. Boxes fed in alternate weeks starting one week after set-up i.e fed on days 7, 21 and 35.
2. Boxes fed every week starting one week after set-up i.e. days 7, 14, 21, 28 and 35.

In Experiments A and B respectively, the boxes were fed with 5 g or 15 g turkey crumbs respectively on each of the feeding occasions.

In each of experiments A and B, one *Atheta* box was placed in the middle of each cage. Before the boxes were placed in the cages, the insect-screening mesh covering the ventilation holes in the box lids was removed to allow the beetles to

leave of their own accord. The lids of the boxes were covered with aluminium foil to reflect direct sunlight and holes were made in the foil above the exit holes. The substrate in the boxes was kept damp using the self-watering system developed in project PC 239. This entailed using boxes with four holes (7 mm diameter) drilled in the bottom of each box. The holes were plugged with damp cotton wool, which acted as a wick from the damp capillary matting under the mesh floor of the cage. The capillary matting was kept damp using drip irrigation lines controlled by the glasshouse computer.

Glasshouse temperatures were set at minimum 19ºC night and day, venting at 21ºC. Natural daylength was extended to 16 hours in each experiment, using supplementary lighting. These conditions were consistent with those used in commercial pot herb production.

Blue sticky traps were placed on the floor of each cage around the lower edges of each box, to trap any *Atheta* adults and larvae crawling or jumping from the boxes. One yellow sticky trap was hung from the roof of each cage, to trap any flying *Atheta* adults.

Assessments

1. Temperatures in the *Atheta* substrate were recorded using a 'Tinytalk' datalogger, buried in the substrate of an additional box (with substrate only i.e. no beetles) placed alongside the cages on the matting. The datalogger was set up to record temperatures every hour, in order to calculate mean maximum, minimum and mean temperatures for each 24-hour period during the experiment.
2. The blue and yellow sticky traps in each cage were checked every week for six or seven weeks in Experiments A and B respectively, starting one week after experiment set-up (7, 14, 21, 28, 35 and 42 days after set-up). Records were made of numbers of *Atheta* adults and larvae on each trap, and of cumulative numbers of *Atheta* on all traps per cage.
3. At the end of the experiment, numbers of *Atheta* adults and larvae were estimated in six 30 ml sub-samples per box in each of the eight boxes, using the same method as used before the glasshouse experiment was set up.

Statistical analysis

In each of Experiments A and B, mean numbers of *Atheta* leaving the boxes in each of the two feeding regimes on each assessment date were compared using analysis of variance (ANOVA).

Results and Discussion

Experiment A

In Experiment A using 5g turkey crumbs per feeding occasion, mean numbers of *Atheta* adults and larvae per rearing-release box were similar (approximately 2,000 per box) for each feeding regime at the start of the glasshouse experiment (Table 3). As each box had been started with 60 *Atheta* adults per box, the mean multiplication rate per box during the box preparation period in the laboratory was x 36. Mean numbers of *Atheta* adults leaving each box in the glasshouse were statistically similar in both feeding regimes in weeks 1, 2, 4 and 5 (Figure 3). In week 1, around 25% of the 'starting' numbers of *Atheta* left the boxes in both feeding regimes (a mean of approximately 500 per box).

In week 3, significantly more *Atheta* (a mean of 304) left boxes fed in alternate weeks than those fed every week (a mean of 180), Figure 3. However, in week 6 at the end of the experiment, significantly more *Atheta* (a mean of 830) left boxes fed every week than those fed in alternate weeks (a mean of 441). Total numbers of *Atheta* leaving the boxes during the 6-week experiment were statisticially similar for each feeding regime i.e. means of 2152 and 2781 for boxes fed in alternate weeks and every week respectively, Table 3. Mean numbers of *Atheta* adults and larvae remaining in the rearing-release boxes at the end of the experiment were higher (1183) when fed every week than when fed in alternate weeks (744), Table 3.

These results indicate that over the 6-week experiment, the feeding regime did not affect total numbers of *Atheta* leaving boxes fed every week or those fed in alternate weeks. However, feeding the boxes every week maintained higher numbers of *Atheta* in the boxes at the end of the experiment. This result indicated that these boxes may have continued to release more *Atheta* than the boxes fed in alternate weeks, had the experiment continued for longer.

Table 3. Mean numbers of *Atheta* adults and larvae per box at the start and end of Experiment A and total numbers of *Atheta* leaving the boxes over the 6-week experiment.

	Boxes fed 5g turkey crumbs in alternate weeks	**Boxes fed 5g turkey crumbs every week**
Mean numbers of *Atheta* per box at start of experiment	2044	2236
Mean numbers of *Atheta* per box at end of experiment	744	1183
Mean numbers of *Atheta* leaving per box over the 6-week experiment	2152	2781

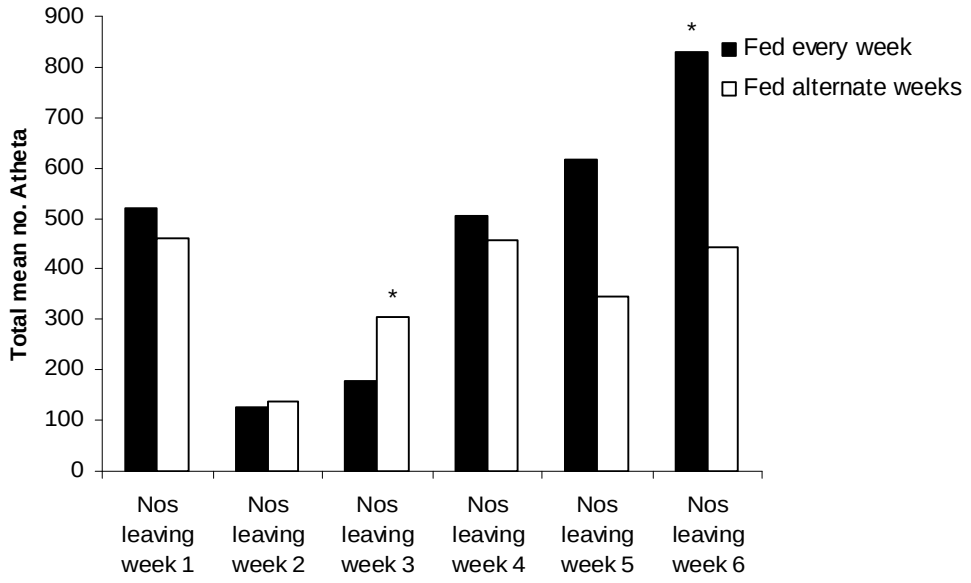

Figure 3. Mean numbers of *Atheta* adults and larvae leaving rearing-release boxes fed with 5 g turkey crumbs either every week or in alternate weeks between 29 August and 3 October 2008. * significantly more leaving than in the other feeding regime in any one week, $P<0.05$.

Daily mean, maximum and minimum temperatures in the rearing-release box substrate during Experiment A are shown in Figure 4. Temperatures remained within the known temperature range for *Atheta* development (15-32ºC, Miller & Williams, 1983) throughout the glasshouse experiment, except on 23 August, when the

maximum temperature reached 34 ºC. Mean temperatures during the 6-week experiment were 17-24ºC, minimum temperatures were 16-21ºC and maximum temperatures were 20-34ºC.

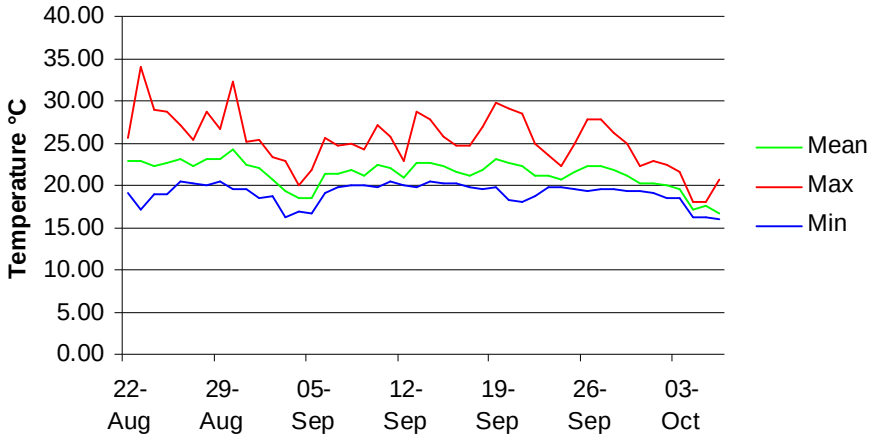

Figure 4. Daily mean, maximum and minimum temperatures during Experiment A.

Experiment B

In Experiment B using 15 g turkey crumbs per feeding occasion, as in Experiment A, mean numbers of *Atheta* adults and larvae per rearing-release box were similar (approximately 2,600 per box) for each feeding regime at the start of the glasshouse experiment (Table 4). As each box had been started with 60 *Atheta* adults per box, the mean multiplication rate per box during the preparation period in the laboratory was x 44. Mean numbers of *Atheta* adults leaving each box in the glasshouse were statistically similar in both feeding regimes in weeks 1, 2, 3, 4 and 6 (Figure 5). In week 1, around 12% of the 'starting' numbers of *Atheta* left the boxes in both feeding regimes (a mean of approximately 300 per box).

In week 5, significantly more *Atheta* (a mean of 142) left boxes fed in alternate weeks than those fed every week (a mean of 30), Figure 5. However, in week 7 at the end of the experiment, significantly more *Atheta* (a mean of 503) left boxes fed every week than those fed in alternate weeks (a mean of 284). As in Experiment A, total numbers of *Atheta* leaving the boxes during the 7-week experiment were statistically similar for each feeding regime i.e. means of 1272 and 1188 for boxes fed in

alternate weeks and every week respectively, Table 4. As in Experiment A, mean numbers of *Atheta* adults and larvae remaining in the rearing-release boxes at the end of the experiment were higher (2829) when fed every week than when fed in alternate weeks (1392), Table 4.

These results indicate that as in Experiment A, over the 7-week experiment, the difference in the feeding regime did not affect total numbers of *Atheta* leaving boxes fed every week or in alternate weeks. However, as in Experiment A, feeding the boxes every week maintained higher numbers of *Atheta* in the boxes at the end of the experiment and this is likely to explain why higher numbers of beetles left these boxes in week 7.

Table 4. Mean numbers of *Atheta* adults and larvae per box at the start and end of Experiment B and total numbers of *Atheta* leaving the boxes over the 7-week experiment.

	Boxes fed 15 g turkey crumbs in alternate weeks	**Boxes fed 15 g turkey crumbs every week**
Mean numbers of *Atheta* per box at start of experiment	2560	2712
Mean numbers of *Atheta* per box at end of experiment	1392	2829
Mean numbers of *Atheta* leaving per box over the 6-week experiment	1272	2829

Daily mean, maximum and minimum temperatures in the rearing-release box substrate during Experiment B are shown in Figure 6. Although glasshouse temperatures were set on the glasshouse computer as in Experiment A (minimum 19 ºC, venting at 21 ºC), ambient temperatures were lower than in Experiment A, which was done earlier in the year. Mean substrate temperatures in the rearing boxes were consequently lower in Experiment B than in Experiment A. Mean temperatures in Experiment B were 17-20 ºC, minimum temperatures were 16-19 ºC and maximum temperatures were 17 -22 ºC, Figure 6.

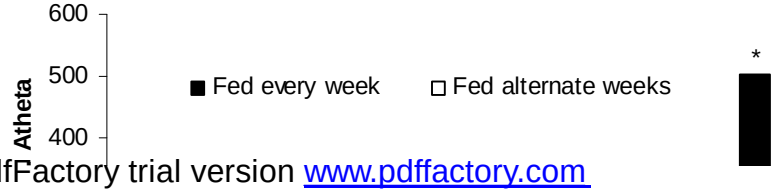

Figure 5. Mean numbers of *Atheta* adults and larvae leaving rearing-release boxes fed with 15g turkey crumbs either every week or in alternate weeks between 7 November and 18 December 2008. * significantly more leaving than in the other feeding regime in any one week, *P*< 0.05, *** P<0.001.

In Experiment B, although approximately twice the number of *Atheta* were left in the boxes at the end of the experiment than in Experiment A, the total numbers of *Atheta* leaving the boxes over the experiment period were lower than in Experiment A (Tables 3 and 4). This could have been due in part to the lower temperatures in Experiment B, as experience during PC 239 and PC 239a has shown that *Atheta* are much more active and the adults fly more readily at higher temperatures.

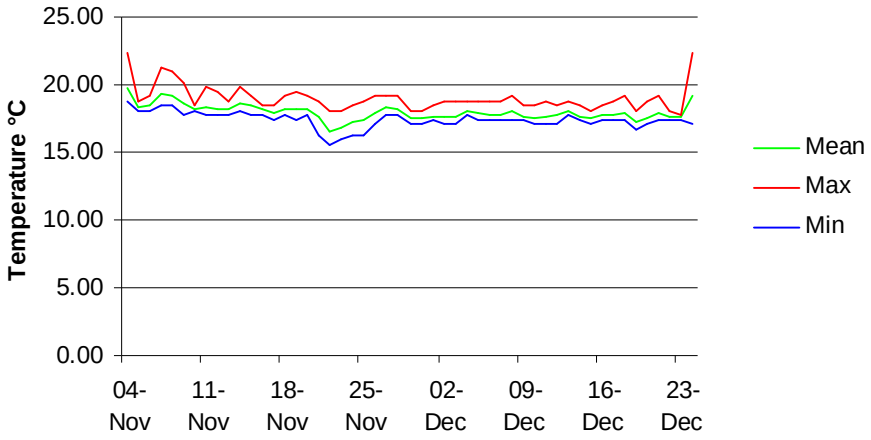

Figure 6. Daily mean, maximum and minimum temperatures during Experiment B.

Conclusions

- In both experiments A and B, both quick and sustained release of *Atheta* was given from the rearing-release boxes.
- Feeding the boxes every week or in alternate weeks did not affect total numbers of *Atheta* being released from the rearing-release boxes over a six or seven-week period.
- Feeding the boxes in alternate weeks led to significantly more *Atheta* being released in week 3 and 5 in Experiments A and B respectively, than from boxes fed every week.
- In both Experiments A and B, significantly more beetles were released from boxes fed every week in week 6 and 7 respectively, than from boxes fed in alternate weeks.
- In both Experiments A and B, feeding the boxes every week led to higher numbers of *Atheta* remaining in the boxes at the end of the experiment then feeding boxes in alternate weeks. Boxes fed every week might have continued to release more *Atheta* for a longer period, had the experiments continued for longer.
- Boxes fed with 15 g turkey crumbs on each feeding occasion led to higher numbers of *Atheta* remaining in the boxes than in boxes fed with 5 g of the food source. This result is consistent with the results of the optimum feeding experiment in 1.3.1.
- Higher numbers of *Atheta* were released from boxes in Experiment A (fed with 5 g food) than from boxes in Experiment B (fed on 15 g food). This is likely to have been due to higher glasshouse temperatures in Experiment A leading to greater *Atheta* activity and flight.
- The results of Experiments A and B would need to be validated on a commercial scale before recommendations could be given on the size and number of rearing-release boxes needed per unit area in commercial practice. The experiment in 1.1 in this report demonstrated that the number of *Atheta* needed to control sciarid flies on parsley depends on the pest density. It is likely that in a high sciarid fly density, the most practical strategy for using *Atheta* would be to use high direct releases to reduce the pest population. Such high release rates may not be cost-effective unless the grower rears *Atheta* on the nursery. Once the sciarid fly density has been reduced, it may then be possible to maintain control of the pest by using lower direct releases of *Atheta* or by using a rearing-release system. Practical strategies for using *Atheta* for low-cost control of sciarid and shore flies on a commercial scale on different protected crops at different times of year and

temperatures need validating on commercial nurseries before further recommendations can be given.

1.4. Determine whether *Atheta* leave rearing-release boxes and disperse in an early-season soil-grown lettuce crop (work in lettuce crop sub-contracted to STC)

Background

In year 1 of project PC 239, 'bait' pots containing damp compost and a small amount of turkey crumbs stood on capillary matting or on ebb and flood benches proved to be useful for monitoring *Atheta* dispersal in in protected crops of ornamental bedding crops and pot herbs (Bennison, 2007). However, *Atheta* were not detected in bait pots stood on the soil in a celery crop where rearing-release boxes were being tested (Bennison, 2007). Thus it was not possible to detect whether *Atheta* were leaving the boxes and dispersing in the celery crop. It was concluded that low temperatures (below 15°C) during April when the boxes were placed in the celery crop could have inhibited *Atheta* dispersal. At the time of the experiment, there was no information on *Atheta* biology below 15°C (the known temperature range of *Atheta* is 15-32°C, Miller & Williams, 1983). However, in year 2 of project PC 239, it was shown that bait pots sunk in the soil at a mean temperature of 19°C were more effective at trapping *Atheta* than those stood on the soil surface (Bennison, 2008). Thus, the bait pot technique used in the year 1 celery experiment could also have contributed to the absence of *Atheta* in the bait pots.

Experiment objective

1.4: Using the more effective 'pitfall' bait pot method tested in year 2, test if *Atheta* leave rearing-release boxes and disperse in an early-season soil-grown lettuce crop where shore flies are known to be a problem (practical work in lettuce crop sub-contracted to STC).

Materials and methods

An experiment was set up in a lettuce crop in autumn 2008 but the results were inconclusive as the grower applied cypermethrin (Toppel 100) for caterpillar control a few days after the experiment started. Very few *Atheta* were found in bait pots sunk

into the soil in the lettuce crop and this was probably due to adverse effects of Toppel 100 which is a pyrethroid insecticide, lethal to beetles. Thus a second experiment was set up in spring 2009.

Sites

- The practical work done by STC was sited in a commercial lettuce crop in North Yorkshire.
- The preparation of the *Atheta* rearing-release boxes for the experiment, estimates of numbers of *Atheta* in the boxes at the start and end of the experiment and statistical analysis of data were done at ADAS Boxworth.

Lettuce site details

There were two lettuce crops in the glasshouse, crops A and B, planted on 4 and 12 May 2009 respectively. The glasshouse was 66 m wide by 31 m long i.e. 2046 m^2. Each of crops A and B were 32 m wide and 31 m long, separated by a concrete path (2 m wide) running down the length of the glasshouse (see Appendix I). In crop A, there were five bays of lettuce, each 6.2 m by 32 m, with a soil path running down the middle of each bay. In crop B there were four bays of lettuce identical to those in crop A except that the fifth bay was occupied by a concrete path.

Experiment design (see Appendix I)

- On 15 May, one *Atheta* rearing-release box was placed at the concrete path end of each of the five soil paths in crop A and in each of the four soil paths in crop B (nine boxes in total).
- Five *Atheta* 'bait' pots were used in five replicate soil paths, in three paths in crop A and in two paths in crop B. The bait pots were set up at one, five, 10, 20 and 30 metres away from the rearing-release box at the concrete path end of the respective soil paths.
- Five yellow sticky traps were placed in four alternate replicate soil paths to those used for the bait pots, in two paths in each of crops A and B. The sticky traps were positioned at the same distances (one, five, 10, 20 and 30 m) away from the rearing-release boxes at the concrete path end of the respective soil paths.

Statistical analysis (ADAS)

Statistical analysis of numbers of *Atheta* per bait pot and numbers of shore flies per sticky trap on successive assessment dates was done by the ADAS statistician, using analysis of variance (ANOVA).

Setting up Atheta rearing-release boxes (ADAS)

Twenty rearing boxes of *Atheta* were set up four weeks before the glasshouse experiment was due to start, using the standard method developed in project PC 239 (Bennison, 2007). The rearing substrate (1.5 litres of a 1:1 mix of coir and vermiculite) was dampened with 150 ml water per litre of substrate and added to each plastic box (3 litre capacity). Sixty *Atheta* adults from the ADAS laboratory culture were added to each box. Fifteen grams of the artificial food source (turkey crumbs) were added to each box and incorporated into the substrate. This amount of food was used as results from the experiment in 1.3 in this report showed that significantly more *Atheta* were produced when fed on 15 g of turkey crumbs per week than on the 5 g per week used in project PC 239. Each box was sealed using a snap-on lid, fitted with two ventilation holes (2.5 cm diameter) covered with insect-proof mesh. The rearing boxes were held in a controlled-temperature laboratory at 25°C, 16:8 hour photoperiod for four weeks. Every week, 15 g of turkey crumbs and water (if required) were added to each box and incorporated into the substrate. No food was added to the boxes during the week before they were due to be set up in the experimental glasshouse, to ensure that the beetles were hungry at set-up (previous results in year 2 of project PC 239 showed that more *Atheta* leave the boxes when hungry, Bennison, 2008).

Estimation of numbers of Atheta per box (ADAS)

Nine of the 20 boxes were needed for the experiment. Numbers of *Atheta* adults and larvae were estimated in three of the nine boxes after the 4-week production period in the controlled-temperature laboratory. The estimates were done on 12 May, just before sending the boxes to STC for use in the glasshouse experiment. Six 30 ml sub-samples of the substrate were taken and passed through two sieves (gauge sizes 2 mm over 1 mm). *Atheta* adults and larvae collected on the finer sieve were turned onto a large white plastic tray and counted whilst being collected into a tube using a 'pooter'. All counted *Atheta* were returned to the respective box. Estimated numbers of *Atheta* adults and larvae per box were calculated.

Setting up Atheta boxes in the glasshouse (STC)

One *Atheta* box was placed at the end of each of the five soil paths in crop A and at the end of each of the four soil paths in crop B. The substrate in each box was kept damp using the self-watering system developed in project PC 239. This entailed removing the sticky tape over the four 7mm-diameter holes in the bottom of each box

and plugging the holes with damp cotton wool, which acted as wicks from the damp soil and capillary matting on which the boxes were stood. If the soil at the end of the paths was not already damp, the soil was dampened before positioning the boxes. Each box was placed onto a wetted piece of capillary matting slightly larger than the box. The soil under the matting was levelled as necessary, to ensure that the damp soil made good contact with the matting, and that the matting made good contact with the cotton wool plugs. The overhead watering used by the grower was expected to keep the matting and thus the substrate in the boxes damp.

The insect-proof mesh was removed from the holes in the box lids, thus allowing the *Atheta* to leave the boxes of their own accord. The lid of each box was covered with tinfoil, with holes inserted in the foil above each exit hole. The tinfoil was used to protect the *Atheta* from over-heating in strong sunlight and also to prevent algae from growing on the inside of the box lids.

Feeding the Atheta boxes in the glasshouse (STC)

Ready-ground turkey pellets were provided to STC by ADAS. Each box was fed with five grams of turkey crumbs, one and three weeks after setting up the experiment, on 22 May and 4 June. The turkey crumbs were sprinkled over the substrate and incorporated, together with water if required to keep the substrate damp, added using a hand-held mister. On each visit to the experiment, the capillary matting under the boxes was checked to ensure it remained wet.

Returning the Atheta boxes at the end of the experiment (STC)

When the experiment had finished on 18 June, all boxes were returned to ADAS for estimation of numbers of *Atheta* in the same three representative boxes used for estimates just before experiment set-up.

Setting up Atheta bait pots (STC)

Twenty-five plastic bait pots (15 in crop A and 10 in crop B) were set up in the glasshouse on the day the experiment was set up on 15 May, using the technique developed in year 2 of project PC 239. Five pots (8 cm diameter) were used in each of five replicate soil paths (three paths in crop A and two in crop B), at one, five, 10, 20 and 30 m away from the rearing-release box at the end of the respective paths. Each bait pot was filled with damp peat-based compost, with a ‘pinch’ of turkey crumbs incorporated in the compost before filling. The pots were sunk into the soil in the paths, so that the tops of the pots were level with the soil surface. The pots were

collected and replaced with fresh ones one, two, three and four weeks after experiment set-up. The pots set up after four weeks were collected immediately after the lettuce crop was harvested. On each collection date, each pot was placed into a sealed polythene bag and taken to the laboratory at STC for counts of *Atheta*.

Setting up sticky traps in glasshouse (STC)

Twenty half-sized yellow sticky traps (10 x 11 cm) were set up in the glasshouse at the start of the experiment on 15 May (10 traps in each of crops A and B). Five traps were used in each of four replicate soil paths (using alternate paths in between those used for the bait pots), at one, five, 10, 20 and 30 m away from the rearing-release box at the end of the respective paths. The traps were placed in a horizontal position on the soil in the paths, and secured with a clip to ensure they remained in place. The traps were collected and replaced with fresh ones after one, two and three weeks.

Temperature recording (STC)

Temperatures were recorded using two 'Tinytalk' dataloggers, buried in the soil half-way down two of the soil paths (one path in each of crop A and B), so that the sensors were just (3 mm) below soil level. The dataloggers were set up to record temperatures every hour, in order to calculate mean maximum, minimum and mean temperatures for each 24-hour period during the experiment.

Control of other pests

STC released aphid parasitoids to both crops on 22 and 29 May and on 4 June, as a precaution against aphid infestation. This was to reduce the risk of the grower needing to use an aphicide that would be harmful to *Atheta*. *Aphidius ervi* was released for control of the glasshouse and potato aphid, *Aulacorthum solani* and the potato aphid, *Macrosiphum euphorbiae*. *Aphidius colemani* was released for control of the peach-potato aphid *Myzus persicae*. If required, the grower agreed to apply pymetrozine (Chess) for aphid control, which should be safe to *Atheta* and currently has a Specific Off-label Approval (SOLA) for use on protected lettuce. However, use of Chess within two weeks of harvest would not be possible due to its 14-day harvest interval.

The grower agreed to apply *Bacillus thuringiensis* (Dipel DF) if necessary for caterpillar control. Dipel DF should be safe to *Atheta* and has a SOLA for use on protected lettuce.

Any pesticides used on the crops and dates of application during the experiment were recorded.

Assessments

Counting Atheta in bait pots (STC)

The bait pots were assessed for *Atheta* on the same dates they were collected from the glasshouse. The compost in each pot was turned out and gently spread onto a large white plastic tray. Numbers of *Atheta* adults, young (white) larvae and older (larger, yellow/brown) larvae were counted whilst collecting into a tube using a 'pooter'.

Counting shore flies on sticky traps (STC)

Numbers of shore fly adults and *Atheta* adults and larvae per trap were counted and recorded.

Results and discussion

Date of experiment set-up

Although it was intended to do the experiment in the early spring to allow the dispersal from rearing-release boxes to be evaluated at low temperatures, a suitable site was not found until mid-April. As the *Atheta* boxes for the experiment then took four weeks to prepare for the experiment, the start date was 15 May.

Numbers of Atheta in rearing-release boxes

Mean numbers of *Atheta* in the boxes estimated on 12 May at ADAS Boxworth before the experiment was set up by STC on 15 May were 1614 (1186 adults and 428 larvae). This indicated that the 60 adults that were used to start the rearing boxes four weeks earlier had multiplied by x27. This breeding rate was consistent with those given in the experiment in 1.3 above.

Mean numbers of *Atheta* adults and larvae in the boxes estimated on 22 June at ADAS Boxworth after the experiment was completed by STC on 18 June were 353 (22% of the starting numbers). These numbers were lower than those at the end of the ADAS experiment in 1.3.2 above, when 36% of the starting numbers were left in rearing-release boxes fed with 5g turkey crumbs in alternate weeks over a 6-week

period (Table 3). It was observed when the boxes were returned from the lettuce experiment to ADAS Boxworth for estimating numbers of *Atheta*, that the substrate in the boxes was very wet. This may have inhibited maximum production of *Atheta* in the rearing-release boxes during the lettuce experiment, as *Atheta* survival and breeding is favoured by damp but not over-wet substrate.

Numbers of Atheta in bait pots

Mean numbers of *Atheta* adults per bait pot on each assessment date at the different distances from the rearing-release boxes are given in Table 5 and Figure 6. Within one week of placing the boxes in the crop, *Atheta* adults had dispersed throughout the glasshouse to bait pots the furthest distance (30 m) away from the boxes. After one week, significantly more adults (a mean of 3.4 per pot) were found in the bait pots nearest to the boxes i.e. 1 m away (Table 5 and Figure 6). After two weeks, significantly similar numbers of *Atheta* adults were found in bait pots at all distances away from the boxes (overall mean of 2 per pot, Table 5 and Figure 6). After three weeks, no adults were found in bait pots 20 or 30 m away from the boxes and there was an overall mean of one adult per pot in bait pots one, five and 10 m away. After four weeks at the end of the experiment when the lettuce crop was harvested on 18 June, *Atheta* adults were only found in bait pots 20 m away from the boxes (a mean of 0.6 per pot).

These results demonstrated that *Atheta* adults left the rearing-release boxes within one week of placing the boxes in the lettuce crop and dispersed throughout the 2,000 m^2 glasshouse. Mean numbers of adults per bait pot reached two per pot within two weeks and then declined in the second two weeks of the experiment. The first two weeks of the lettuce production period are the most important for shore fly control, as shore fly adults are attracted to feed and lay eggs on the algae growing on the exposed soil between the young plants.

Table 5. Mean numbers of *Atheta* adults per bait pot between 22 May and 6 June at 1, 5, 10, 20 and 30 m from the rearing-release boxes

Date	1 m	5 m	10 m	20 m	30 m
22 May	3.4*	1.6	0.4	0	0.4
29 May	2.6	2.4	2	0.4	2.4
4 June	1.4	1.4	0.4	0	0
18 June	0	0	0	0.6	0

* significantly more in 1 m bait pots than those at other distances, $P<0.05$.

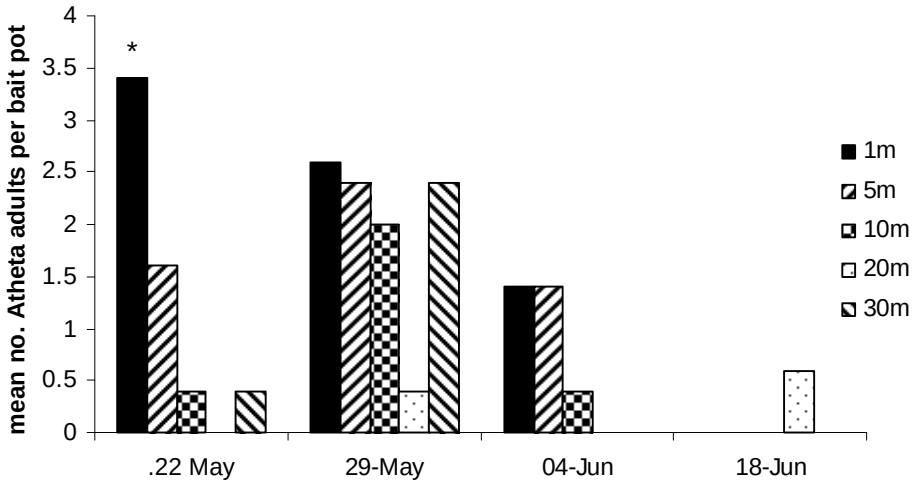

Figure 6. Mean numbers of *Atheta* adults per bait pot between 22 May and 6 June at 1, 5, 10, 20 and 30 m from the rearing-release boxes * significantly more in 1 m bait pots than those at other distances, $P<0.05$.

Mean numbers of *Atheta* larvae per bait pot are given in Table 6. *Atheta* larvae were found in the bait pots throughout the glasshouse on all assessment dates. There were no significant differences between numbers of larvae in pots at any distance away from the rearing-release boxes. As fresh boxes were set up every week during the experiment, the results demonstrate that the *Atheta* adults dispersing in the glasshouse were breeding in the pots within one week of colonising the pots.

Table 6. Mean numbers of *Atheta* larvae per bait pot between 22 May and 6 June at 1, 5, 10, 20 and 30 m from the rearing-release boxes (no significant differences in numbers at any distance on any one date).

Date	1 m	5 m	10 m	20 m	30 m
22 May	0	0.2	0.2	0	1
29 May	3	1	0.2	0	0.4
4 June	0.6	0.4	0	0	0.6
18 June	0.4	0.2	0.2	0.4	0

Numbers of shore flies on sticky traps

The shore fly density in the glasshouse was high when the experiment was set up on 15 May, indicated by the mean of 46 adults per trap at the end of the first week on 22 May (Table 7). Numbers of shore flies at all distances from the bait pots were statistically similar on each date, thus prey (shore fly eggs, larvae and pupae) were likely to be available for the *Atheta* throughout the glasshouse. Numbers of shore flies increased to overall means of 116 and 155 per trap on 29 May and 6 June respectively. However, none of the lettuce were rejected at harvest due to presence of shore flies. The aim of this experiment was to evaluate *Atheta* dispersal in a soil-grown lettuce crop where shore fly prey was not limiting, rather than to test *Atheta* control of shore flies in lettuce. It was unfortunate that the site was not identified earlier in the season, so that use of the *Atheta* rearing-release boxes could have been tested at lower temperatures and at lower shore fly densities.

Table 7. Mean numbers of shore flies per sticky trap at 1, 5, 10, 20 and 30 m away from the *Atheta* rearing-release boxes (no significant differences in numbers at any distance on any one date).

Date	1 m	5 m	10 m	20 m	30 m	Overall mean of all distances
22 May	34.5	43.0	51.0	37.2	64.2	46.0
29 May	112	116	154	122	79	116
6 June	148	156	139	157	175	155

Soil temperatures

Daily mean, maximum and mean temperatures during the experiment are shown in Figure 7. The published temperature range for *Atheta* development is 15-32°C (Miller & Williams, 1983). Since that data was published, research by ADAS in PSD-funded project PS 2120 showed that *Atheta* left rearing release boxes and flew to sticky traps in an unheated glasshouse in February, when mean, maximum and minimum temperatures were 11.2°C, 13.5°C and 9.5°C respectively (Bennison, 2008a). Research in the PSD project also showed that *Atheta* completed their life cycle at 15°C, whereas at 10°C adults survived for four weeks but no larvae developed (Bennison, 2009). In the lettuce experiment in this HDC project, mean daily soil temperatures were 16.5°C and fell to below 15°C on only one date, on 27 May (Figure 7). However, minimum daily soil temperatures were below 15°C on all dates except on 4 June and 13-16 June (Figure 7). Maximum soil temperatures reached 27.9°C on 29 May. The bait pot results demonstrate that *Atheta* were active in/on the soil between soil temperatures between 11.2°C and 27.9°C.

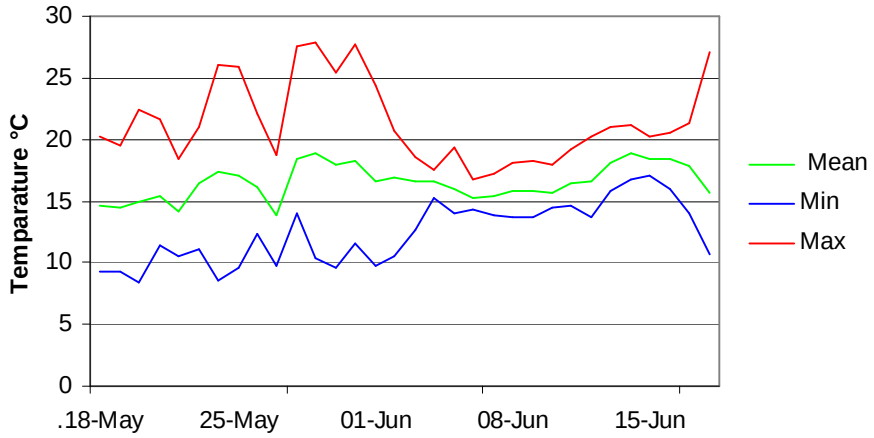

Figure 7. Daily mean, maximum and minimum soil temperatures in the glasshouse between 18 May and 18 June.

Control of other pests

The grower applied Chess on 17 May as a precaution against any aphids being in the crop. As Chess is an aphid antifeedant it should be safe to *Atheta*. No live or parasitized aphids were seen at any time during the experiment. Minor caterpillar damage was recorded but this did not warrant treatment. Thus no pesticides were used on the experimental crop that may have had adverse effects on *Atheta*.

Conclusions

- *Atheta* adults left rearing-release boxes placed in a shore-fly infested lettuce crop shortly after planting in mid-May and within one week had dispersed and produced larvae in bait pots throughout a 2,000 m^2 glasshouse.
- 'Pitfall' bait pots sunk into the soil proved to be useful for detecting *Atheta* dispersal in a soil-grown lettuce crop.
- *Atheta* dispersal was not limited by mean soil temperatures of 16.5°C or a temperature range of 11.2-27.9°C.

Technology transfer

- Jude Bennison sent details of the *Atheta* rearing system to individual HDC grower members on request, as agreed with HDC.
- Jude Bennison presented some of the results of both projects PC 239 and PC 239a at the UK vegetable industry conference, East of England Showground, Peterborough, 28 January 2009.

Acknowledgements

Thanks are due to the following for their help in project PC 239a:

- Richard GreatRex, Syngenta Bioline, for exchange of ideas and information and for supplying coir compost for the *Atheta* rearing substrate.
- Simon Budge, Humber VHB, for supplying parsley plants, free of charge.
- Norman Hinsley, Snaith Salads Ltd., North Yorkshire, for hosting the lettuce experiment.

References

Bennison, J. 2005. Protected herbs: best practice guidelines for integrated pest and disease management. *Defra (HH3118TPC) and HDC (PC 210) project annual report.*

Bennison, J., Green, K. and O'Neill, T. 2005. Best practice guide for integrated pest and disease management on UK protected herbs. *IOBC/wprs Bulletin* 28 (1): 15-18.

Bennison, J.A. (2007). Protected herbs, ornamentals and celery: Development of an on-nursery rearing system for *Atheta coriaria* for reduced cost biological control of sciarid and shore flies. *First Annual report for HDC project PC 239*.

Bennison, J.A. (2008). Protected herbs, ornamentals and celery: Development of an on-nursery rearing system for *Atheta coriaria* for reduced cost biological control of sciarid and shore flies. *Second Annual report for HDC project PC 239*.

Bennison, J.A. (2008a and 2009). Novel control of soil-dwelling horticultural pests using the staphylinid beetle *Atheta coriaria*. *First and second annual reports to PSD for project PS 2120*.

Carney, V.A., Diamond, J.C., Murphy, G.D. & Marshall, D. (2002). The potential of *Atheta coriaria* Kraatz (Coleoptera: Staphylinidae), as a biological control agent for use in greenhouse crops. *IOBC/wprs Bulletin* 25(1): 37-40.

Jandricic, S.E. (2005). The compatibility of *Atheta coriaria* (Kraatz) with greenhouse floriculture IPM programs for fungus gnat control. *MSc thesis, University of Guelph, Ontario, Canada.*

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

Appendix 1: Experimental plan *Atheta* dispersal in glasshouse