Project title:	Protected herbs: improved biological control of aphids (extension to PE 006)
Project number:	PE 006a
Project leader:	Jude Bennison, ADAS
Report:	Progress report, March 2013
Previous report:	-
Key staff:	Jude Bennison, Senior Entomologist Gemma Hough, Entomologist Chloe Whiteside, Research Technician Chris Dyer, Statistician, ADAS
Location of project:	ADAS Cambridge Boxworth
Industry Representative:	Claire Donkin, Lincolnshire herbs
Date project commenced:	1 April 2012
Date project completed (or expected completion date):	31 January 2014

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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 .....

Report authorised by:

Dr Tim O'Neill
Horticulture Research Manager
ADAS

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

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GROWER SUMMARY

Headlines

- *Ephedrus cerasicola*, *Praon volucre* and *Aphidius matricariae*, either as individual species or as a mix are effective against mint aphid
- *Aphidius matricariae* will be taken forward to the next experiment testing cost-effective release rates for mint aphid control.

Background

Until recently, biological control of aphids on protected crops relied mainly on three aphid parasitoid species:

- *Aphidius colemani* for control of the peach-potato aphid, *Myzus persicae* and the melon-cotton aphid, *Aphis gossypii*.
- *Aphidius ervi* and *Aphelinus abdominalis* for control of the potato aphid, *Macrosiphum euphorbiae* and the glasshouse-potato aphid, *Aulacorthum solani*.

On protected herbs, the peach-potato aphid is a common pest of basil and *A. colemani* usually gives effective control. However, grower experience indicated that two aphid species commonly occurring on all year round (AYR) protected herbs, the hawthorn-parsley aphid, *Dysaphis apiifolia* and the mint aphid, *Ovatus crataegarius*, do not seem to be parasitised by any of the above three parasitoid species.

Hawthorn-parsley aphid is a common and severe pest on AYR parsley, forming dense colonies at the base of the stems. Mint aphid is commonly found on mint and is often mistaken by growers as peach-potato aphid as it is similar in appearance. Commercial experience indicates that aphid predators (the predatory midge, *Aphidoletes aphidimyza* and the lacewing, *Chrysoperla carnea*) and the entomopathogenic fungus (‘Naturalis-L’) do not give effective control of hawthorn-parsley aphid and there has been little experience of using predators and fungi against mint aphid.

Chemical control on protected herbs is difficult due to the limited range of approved IPM-compatible aphicides and restrictions on frequency and timings of application. For example, pymetrozine (Chess WG) which has an Extension of Authorisation for minor use (EAMU, formerly known as a SOLA) for use on protected herbs, is effective against both target aphid species and is IPM-compatible, but must not be applied between 1 November and 1

March and has a 14-day harvest interval which is limiting on short-term herb AYR herb crops e.g. parsley which has a 5-week production time. In addition, growers are under increasing pressures to reduce the use of chemical pesticides and are keen to adopt more biological control strategies.

The new aphid parasitoid mix produced by Viridaxis in Belgium includes three newly available parasitoids in addition to the three species named above. The 'new' species are *Aphidius matricariae*, *Praon volucre* and *Ephedrus cerasicola*. The mix has given good control of a wide range of 'difficult' aphid species on strawberry, that were not been controlled by previously available parasitoids (Clare Sampson, personal communication). The mix has also given improved control of aphids on ornamental pot plants and HNS in BCP Certis trials (Clare Sampson, personal communication and subsequent grower use).

The aim of this project (PE 006a) was to develop a robust, cost-effective parasitoid release strategy for reliable control of hawthorn-parsley aphid and mint aphid on protected herbs using the effective parasitoids identified during PE 006.
The specific objectives were:

1. Demonstrate that *Aphidius colemani* will parasitise hawthorn-parsley aphid on pot-thick and spaced parsley plants in replicate cages in a commercial herb glasshouse
2. In small-scale research glasshouse experiments, develop an effective, robust parasitoid release strategy for control of hawthorn-parsley aphid and mint aphid.
3. In an experiment on a commercial herb nursery, validate the success and cost-effectiveness of the selected parasitoid release strategy for control of hawthorn-parsley aphid on parsley.

Summary

The results from this study so far indicate that parasitoids are more effective at parasitising hawthorn-parsley aphids in spaced pots of parsley than in those that are pot-thick. This indicates that the parasitoids might be inhibited from searching for this species of aphid (which infests the base of parsley plants) when closely spaced early in the production cycle. This might be one of the reasons why growers have not observed parasitized hawthorn-parsley aphids during the production cycle.

When comparing the effectiveness of individual and mixed species on the parasitism of mint aphid and hawthorn parsley aphid, the initial experiments had too much variation in the data from replicate cages within the treatments to make confident conclusions. Attempts were successfully made to reduce this variation and results from the second experiment on mint

aphid have indicated that this aphid is more effectively parasitized by a mix of parasitoid species (*E. cerasicola, A. matricariae* and *P. volucre)* or *E. cerasicola* alone, than by *A. matricariae* or *P. volucre* alone. When used in a species mix together with *A. matricariae* and *P. volucre*, *E. cerasicola* was responsible for 82% of the mummies. This result indicated that *E. cerasicola* is the superior parasitoid for mint aphid. Reasons for this could include enhanced host-searching ability and/or the mint aphid being more readily accepted as a suitable host by *E. cerasicola* compared with the other two parasitoid species.

When using mixed parasitoid species in a biological control programme there is the risk that competition between parasitoids for the host may occur and this could lead to reduced total parasitism and thus poorer aphid control. A recent study demonstrated that competition between larvae of *Aphidius ervi* and *Praon volucre* occurs within *M. euphorbiae*, with *P. volucre* being the superior competitor if both parasitoids lay eggs in the same host aphid. This could lead to the exclusion of *A. ervi* over time. It is possible that parasitoid larval competition could also play a role in mint aphid, with *E. cerasicola* larvae more successfully developing in aphids parasitized by multiple species.

During this study, it was also observed that while healthy aphid numbers were reduced significantly in treatments with each of the three parasitoids compared with the untreated controls, very few mummies were observed on the plants. Thus another factor in addition to parasitism may have contributed to aphid control. One possible factor could have been parasitoid host-killing via host feeding, as observed in PE 006 by *Aphidius ervi*, *Praon volucre*, *Ephedrus cerasicola* and *Aphelinus abdominalis* on mint aphid. Another factor could have been aphids dropping from the plant in response to the alarm pheromones produced by other aphids in the presence of parasitoid attack. Host killing and falling aphids are both factors which will enhance the impact of biological control by parasitoids. In the case of mint aphids, the reduction in numbers of healthy aphids by parasitoids, without the production of many mummies is an example of the ideal ‘overkill’ biological control strategy on a crop such as pot herbs, which are subject to retailer ‘zero tolerance’ of aphids or mummies.

The next stage of this study is to determine cost-effective release rates for the most effective individual or mix of parasitoids identified in the previous experiments. For mint aphid, the ideal candidate to take forward would be *E. cerasicola* but unfortunately this is not commercially available as a single species. Furthermore, the mix of the three species effective against mint aphid (*E. cerasicola, A. matricariae* and *P. volucre)* are only available as a mix of six parasitoids and it was shown in PE 006 that the three other parasitoids

(*Aphidius colemani*, *A. ervi* and *Aphelinus abdominalis*) do not parasitize mint aphid. Following consultation with the supplier of the parasitoid mix, Viridaxis in Belgium, it was confirmed that they do not currently plan to market a mix of parasitoids specifically for mint growers containing *E. cerasicola, A. matricariae* and *P. volucre* or make *E. cerasicola* available as a single species. Therefore *A. matricariae* was selected to take forward to the next step in the project to test release rates, as this is available as a single species from other suppliers e.g. Koppert. This experiment will commence during April 2013 for mint aphid. The initial experiment comparing single and mixed species for the control of hawthorn-parsley aphid will be repeated during April, using the amended protocol as used for the second mint aphid experiment, in order to select the parasitoid(s) to take forward to a release rate experiment.

Financial Benefits

None to date.

Action Points

None to date.

SCIENCE SECTION

Introduction

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Chemical control on protected herbs is difficult due to the limited range of approved IPM-compatible aphicides and restrictions on frequency and timings of application. For example, pymetrozine (Chess WG) which has an Extension of Authorisation for minor use (EAMU, formerly known as a SOLA) for use on protected herbs, is effective against both target aphid species and is IPM-compatible, but must not be applied between 1 November and 1 March and has a 14-day harvest interval which is limiting on short-term herb AYR herb crops e.g. parsley which has a 5-week production time. In addition, growers are under increasing pressures to reduce the use of chemical pesticides and are keen to adopt more biological control strategies.

The new aphid parasitoid mix produced by Viridaxis in Belgium includes three newly available parasitoids in addition to the three species named above. The 'new' species are *Aphidius matricariae*, *Praon volucre* and *Ephedrus cerasicola*. The mix has given good control of a wide range of 'difficult' aphid species on strawberry, that were not been controlled by previously available parasitoids (Clare Sampson, personal communication). The mix has also given improved control of aphids on ornamental pot plants and HNS in BCP Certis trials (Clare Sampson, personal communication and subsequent grower use).

Previous work from the current project PE 006 (1 April 2011-31 March 2012) have been very encouraging and have shown:

- *Aphidius colemani*, *Aphidius matricariae*, *Praon volucre*, *Ephedrus cerasicola* and *Aphelinus abdominalis* are able to successfully parasitise hawthorn-parsley aphids under laboratory conditions.
- A single introduction of *Aphidius colemani* significantly reduced hawthorn-parsley aphid populations under semi-field conditions. However, the single release rate was high, equivalent to 40/m^2. A single release equivalent to 16 *Aphidius colemani*/m^2 was less effective, indicating a need either for high release rates or regular introductions (the latter is the standard commercial strategy).
- Monitoring on commercial nurseries showed that hawthorn-parsley aphids were only found on older parsley plants (4-5 weeks after sowing), although grower observations suggest that this aphid species can sometimes be found earlier in the production line. If the aphids do not infest parsley plants until late in the production period, there will not be enough time for parasitized aphids to turn into visible mummies before the plants are sold.
- *Aphidius matricariae*, *Praon volucre* and *Ephedrus cerasicola* are able to successfully parasitise mint aphids under laboratory conditions.
- A single introduction of *Aphidius matricariae* significantly reduced mint aphid populations under semi-field conditions. However, the single release rate was high (equivalent to 40/m^2). As with *Aphidius colemani* and hawthorn-parsley aphid, the efficacy of a weekly release strategy needs testing.
- In the laboratory, there was evidence of host-killing behaviour by *Aphidius colemani*, *Ephedrus cerasicola* and *Aphelinus abdominalis* on hawthorn-parsley aphid and by *Aphidius ervi*, *Praon volucre*, *Ephedrus cerasicola* and *Aphelinus abdominalis* on mint aphid.

The aim of this project (PE 006a) was to develop a robust, cost-effective parasitoid release

strategy for reliable control of hawthorn-parsley aphid and mint aphid on protected herbs using the effective parasitoids identified during PE 006.

The specific objectives were:

1. Demonstrate that *Aphidius colemani* will parasitise hawthorn-parsley aphid on pot-thick and spaced parsley plants in replicate cages in a commercial herb glasshouse
2. In small-scale research glasshouse experiments, develop an effective, robust parasitoid release strategy for control of hawthorn-parsley aphid and mint aphid.
3. In an experiment on a commercial herb nursery, validate the success and cost-effectiveness of the selected parasitoid release strategy for control of hawthorn-parsley aphid on parsley.

Objective 1: Demonstrate that *Aphidius colemani* will parasitise hawthorn-parsley aphid on pot-thick and spaced parsley plants in replicate cages in a commercial herb glasshouse.

Materials and methods

Source of aphids

Hawthorn-parsley and mint aphids were collected from commercial nurseries during April and May in 2012. A culture of hawthorn-parsley aphids was set-up by placing infested curly parsley plants into gauze cages (50 x 50 x 50 cm). The cages were used to exclude any parasitoids and predators. These gauze cages were in turn placed in a computer-controlled glasshouse compartment on capillary matting, to allowing watering of the plants without needing to open the cages. The glasshouse compartment was set to maintain a temperature of approx. 20°C through the use of automatic heating, shading and ventilation. The culture was maintained by regularly replacing dead or dying plants with clean un-infested plants. By placing plants close together, aphids were able to easily move between plants and to infest newly introduced plants. The mint aphid culture was set up in exactly the same way, using mint plants in separate cages from the parsley plants.

Source of parasitoids

Aphid parasitoids produced by Viridaxis were supplied by BCP Certis for use in experiments completed in this project. For this objective, *A. colemani* was delivered as pupae within mummified aphids on 10 May 2012. The parasitoids of each species were transferred separately to a ventilated sandwich box which in turn was placed in a fridge to slow down development and emergence so wasps were ideally 48 hours old when used in the experiment. Boxes were moved to an ambient temperature on 14 May 2012. A piece of cotton-wool soaked in a honey solution (approx. 20% honey) was added to each box to

provide food for any emerging adult parasitoids. In addition, a piece of parsley was added so that emerging adult parasitoids were exposed to host plant cues associated with hawthorn-parsley aphid or mint aphid. The emerged parasitoids were left for two days in the boxes to allow them to mate.

Efficacy of A. colemani on pot-thick and spaced parsley plants

The experiment recorded the efficacy of *A. colemani* at parasitising hawthorn-parsley aphid on pot-thick and spaced parsley plants within cages placed in a commercial herb glasshouse. This was to determine whether parasitoid searching was inhibited by pot-thick plants which could explain why growers have not observed mummies. The experiment consisted of four treatments with four replicates of each (Table 1).

Table 1. Four treatments used in Objective 1

Treatment no.	No. plants per cage	No infested plants	No aphids per cage	No. female *Aphidius colemani*
1	2	2	50 (25/plant)	0 (untreated control)
2	16	2	50 (25/plant on 2 central plants)	0 (untreated control)
3	2	2	50 (25/plant)	5
4	16	2	50 (25/plant on 2 central plants)	5

On 14 May 2012, 25 mixed aged hawthorn-parsley aphids were transferred using a fine paintbrush onto 32 three-week old clean parsley plants. Two infested plants were placed into 16 insect proof cages (50x50x50) and an additional 14 clean parsley plants of the same age were added to eight of these cages (Figure 2).

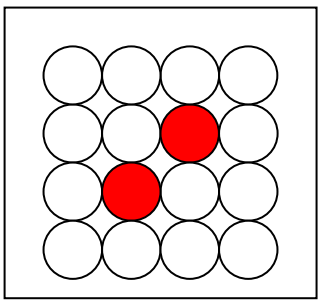

Figure 2. Arrangement of parsley plants in cages with 16 plants. Red circles indicate infested parsley plants and white circles indicate clean parsley plants

Cages were kept in a controlled glasshouse compartment at 20ºC 16L:8D to allow the aphids to settle before the cages were transported to Lincolnshire Herbs on 16 May 2012. A

pooter was used to transfer five adult mated female *A. colemani* (approximately 48 hours old) into eight specimen tubes which were then transported in a cool box. Prior to entering the nursery, five adult mated female *A. colemani* were released into eight of the cages (four cages with two plants and four cages with 16 plants) by placing an opened specimen tube in the centre of each cage. Cages where parasitoids were not released were the control treatments.

Cages were arranged in a randomised block design on a line in 3x6 rows and were treated in the same manner as the commercial parsley crops (20ºC, 12L:12D) and watered by capillary matting (Figure 3). Temperature data loggers were placed in two of the cages.

Figure 3. Arrangement of cages in the commercial glasshouse

After 10 days the cages were returned to ADAS Boxworth and the pots of parsley plants in each cage were destructively sampled by cutting the plants at their base, taking care not to dislodge the aphids. The number of healthy aphids and the number of aphid mummies within each cage was recorded, checking the plants, compost and the inside of the cage.

Once this assessment had been completed, portions of aphid-infested parsley plants from each cage were placed separately in ventilated sandwich boxes. Each sandwich box was placed in a controlled temperature room set to 20°C. Once the parasitoids began to emerge some were preserved in alcohol in order to confirm that they were *A. colemani.* Aphid-infested parsley plants were checked again on day 15 and 17, recording the number of additional mummified aphids. Percentage parasitism was calculated as followed: total number of mummies/ (total number of live aphids + total number of mummies x 100).

Statistical analysis

Data on the numbers of aphids and mummified aphids and percentage parasitism were analysed using an analysis of variance (ANOVA) in GenStat (12th Edition).

Results

Unparasitised aphids

The presence of *A. colemani* significantly affected the total number of healthy (unparasitised) hawthorn-parsley aphids recorded in each treatment (P<0.05). In the control treatments, numbers of aphids increased from 50 per cage to 488 – 753 per cage in 10 days (Figure 4). In both treatments where *A. colemani* were present, there were significantly lower numbers of unparasitised aphids per cage compared with the control treatments.

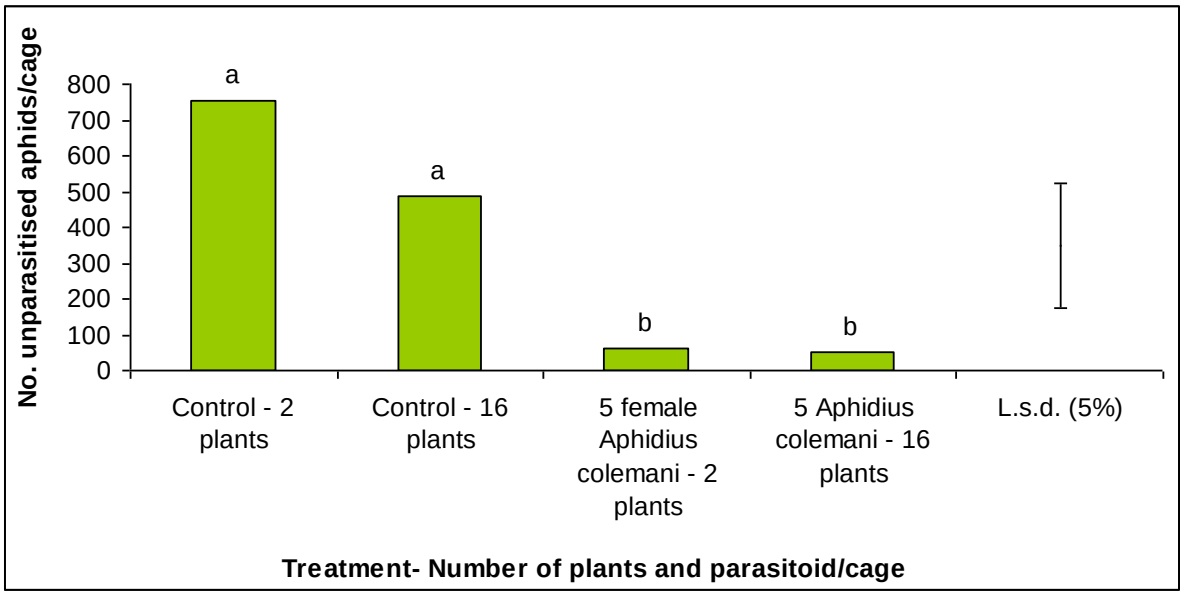

Figure 4. Mean number of unparasitised hawthorn-parsley aphids per cage (LSD 5%)

Mummified aphids

As expected mummies were only observed in cages where *A. colemani* were released which explains the significant effect of parasitoid treatment on the number of mummified aphids recorded (P<0.05). When looking at the subset data for treatments where parasitoids were released, a significant effect of the number of plants per cage was observed with more mummies being recorded in cages with two parsley plants compared to those with 16 parsley plants (P<0.05). This indicated that the parasitoids were more effective where the plants were spaced further apart than where the plants were unspaced (pot-thick) as occurs at the start of commercial production.

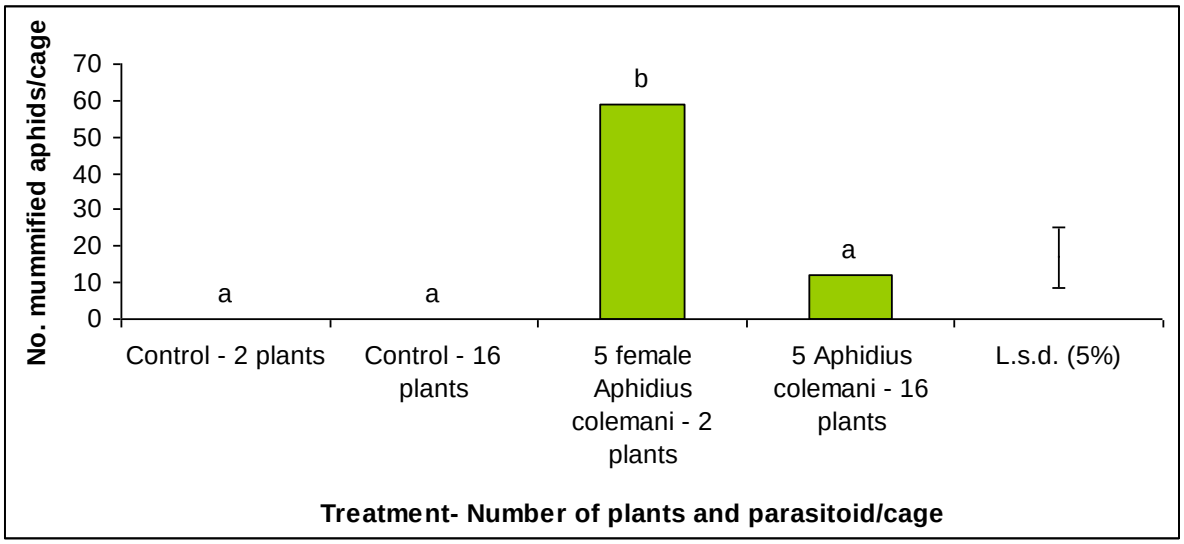

Figure 5. Mean number of mummified hawthorn-parsley aphids per cage (LSD 5%)

Percentage parasitized

No parasitism of hawthorn-parsley aphid occurred in cages where *A. colemani* was not released which again explains the significant effect of parasitoid treatment on the percentage of parasitised aphids (P=<0.05). When looking at the subset data for treatments where parasitoids were released, a significant effect of the number of plants per cage was observed on the percentage of parasitism recorded, with 43.8% more aphids being parasitized in cages with two plants (total of 64.3% parasitism) compared to 16 plants, (P<0.05). While percentage parasitism was lower in cages with 16 plants a single release of *A. colemani* resulted in 20.5% parasitism.

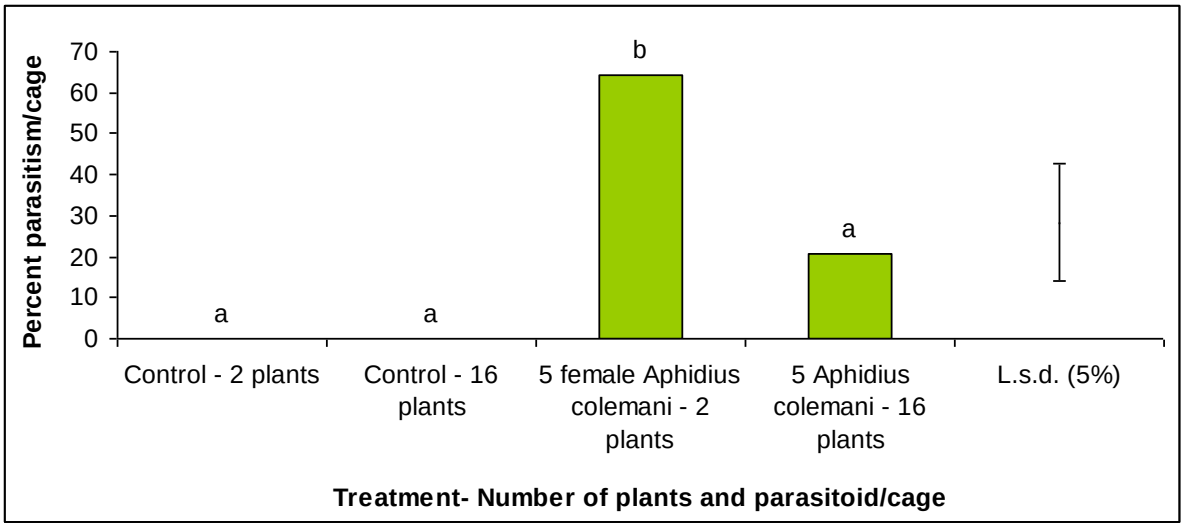

Figure 6. Mean percentage of parasitism per cage (LSD 5%)

Objective 2: In small-scale research glasshouse experiments, develop an effective, robust parasitoid release strategy for control of hawthorn-parsley aphid and mint aphid.

Three experiments on both aphid species are planned in this Objective:

The aim of Experiment 1: In small-scale research glasshouse experiments, compare the parasitism of hawthorn-parsley aphid and mint aphid by single parasitoid species and by mixed parasitoid species (using species shown to be effective against each aphid in PE 006).

Materials and methods

Sources of aphid and parasitoids

Aphids were sourced from the cultures maintained at ADAS Boxworth, Cambridgeshire.

Aphid parasitoids were produced by Viridaxis and supplied by BCP Certis.

Occasion 1: Efficacy of single and mixed parasitoid species for controlling mint aphid

This experiment recorded the efficacy of the single parasitoid species *A. matricariae, P. volucre* and *E. cerasicola* or a mix of these three species in parasitising mint aphid in cages in a commercial herb glasshouse. The experiment consisted of five treatments with four replicates of each (Table 7).

Table 7. Five treatments used in Objective 2, Experiment 1 Mint Aphid

Treatment no.	No. plants per cage	No infested plants	No aphids per cage	Female parasitoids released
1	16	2	50 (25 on each of 2 infested plants)	12x *Aphidius matricariae*
2	16	2	50 (25 on each of 2 infested plants)	12x *Praon volucre*
3	16	2	50 (25 on each of 2 infested plants)	12x *Ephedrus cerasicola*
4	16	2	50 (25 on each of 2 infested plants)	4x *Aphidius matricariae* 4x *Praon volucre* 4x *Ephedrus cerasicola*
5	16	2	50 (25 on each of 2 infested plants)	None (untreated control)

On 28 August 2012, 25 mixed aged mint aphids were transferred using a fine paintbrush

onto each of 40 clean mint plants. Before adding the aphids, the mint plants were trimmed to 10-15cm. Two infested plants were placed in the middle of 20 insect proof cages (50x50x50) and an additional 14 clean mint plants of the same age were added around the infested plants (Figure 8).

Figure 8. Insect proof cage containing 16 mint plants

Cages were kept in a controlled glasshouse compartment at ADAS Boxworth at 20ºC 16L:8D to allow the aphids to settle before the cages were transported to Lincolnshire Herbs on 29 August 2012. A pooter was used to transfer the adult mated female parasitoids (12 of each species or 12 mixed species) into separate specimen tubes which were then transported to the commercial nursery in a cool box. The mixed species were separated into three tubes and then combined on release. Prior to entering the nursery, the parasitoids were released into 16 of the cages as per the treatment list by placing the opened specimen tube between two plant pots.

Cages were arranged in a randomised block design on a line in 7x3 rows and were treated in the same manner as the commercial parsley crops (20ºC, 12L:12D) and watered by capillary matting (Figure 3). Two data loggers were placed in two cages.

On day 10 the cages were transferred back to ADAS Boxworth and kept at 21ºC 14L:10D. On day 15 the pots of mint plants were destructively sampled by cutting the plants at their base, taking care not to dislodge the aphids. The number of healthy aphids and the number of aphid mummies within each cage was recorded, checking both the plant and surrounding area. The mummies were recorded as *Aphidius, Praon* and *Ephedrus* which was determined by their colour. Mummies were kept and stored in petri dishes so that the adults could emerge and the species confirmed.

Once this assessment had been completed, live aphids on portions of mint plants were removed from the plants and kept in a ventilated sandwich boxes. On day 20 the aphids in the boxes were reassessed for swelling (as an indication of early parasitism) and mummies. Each sandwich box was placed in a controlled temperature room set to 20°C 14L:10D. Mummies were again collected. Once the parasitoids began to emerge some were preserved in alcohol in order to confirm the species. Percentage parasitism was calculated by using the total number of aphids mummified and the total number of healthy plus parasitised aphids.

Statistical analysis

Data on the numbers of healthy aphids and parasitised aphids were analysed using an ANOVA in GenStat (12th Edition).

Occasion 1: Efficacy of single and mixed parasitoid species for controlling hawthorn-parsley aphid

This experiment recorded the efficacy of the single parasitoid species *Aphidius colemani*, *Aphidius matricariae, Praon volucre*, *Ephedrus cerasicola* and *Aphidius abdominalis* or a mix of these species in parasitising hawthorn-parsley aphid in cages in a commercial herb glasshouse. The experiment consisted of seven treatments and three replicates but the remainder of the protocol was the same as that carried out for the mint aphid experiment (Table 9).

Table 9 Seven treatments used in Objective 2, Experiment 1 Hawthorn-parsley aphid

Treatment no.	No. plants per cage	No infested plants	No aphids per cage	Female parasitoids released
1	16	2	50 (25/plant)	10x *Aphidius colemani*
2	16	2	50 (25/plant)	10x *Aphidius matricariae*
3	16	2	50 (25/plant)	10x *Praon volucre*
4	16	2	50 (25/plant)	10x *Ephedrus cerasicola*
5	16	2	50 (25/plant)	10x *Aphelinus abdominalis*
6	16	2	50 (25/plant)	2x *Aphidius colemani* 2x *Aphidius abdominalis* 2x *Praon volucre* 2x*Ephedrus cerasicola* *2x Aphidius matricariae*
7	16	2	50 (25/plant)	None (untreated control)

On 13 August 2012, 25 mixed aged hawthorn-parsley aphids were transferred onto 42 clean curly parsley plants. Two infested plants were placed into the middle of each of 21 insect proof cages (50x50x50) and an additional 14 clean parsley plants of the same age were added around the infested plants. On 14 August 2012, the cages were transported to Lincolnshire Herbs and the parasitoids were released into the cages. On 23 August 2012 the cages were transferred back to Boxworth where they were destructively assessed on 24 August 2012. Another two assessments were then carried out on Day 16 and 22 on 29 August 2012 and 4 September 2012 respectively.

Results

Occasion 1: Efficacy of single and mixed parasitoid species for controlling mint aphid

Assessment

Control of mint aphid was similar regardless of whether a single parasitoid or a mixture of parasitoids was used. There were no significant differences observed between single or mixed parasitoids on the number of unparasitised mint aphids per cage, the number of mummies per cage and the percentage parasitism per cage (Figures 10, 11 and 12). This result was thought to be due to the large variation observed between the treatment replicates.

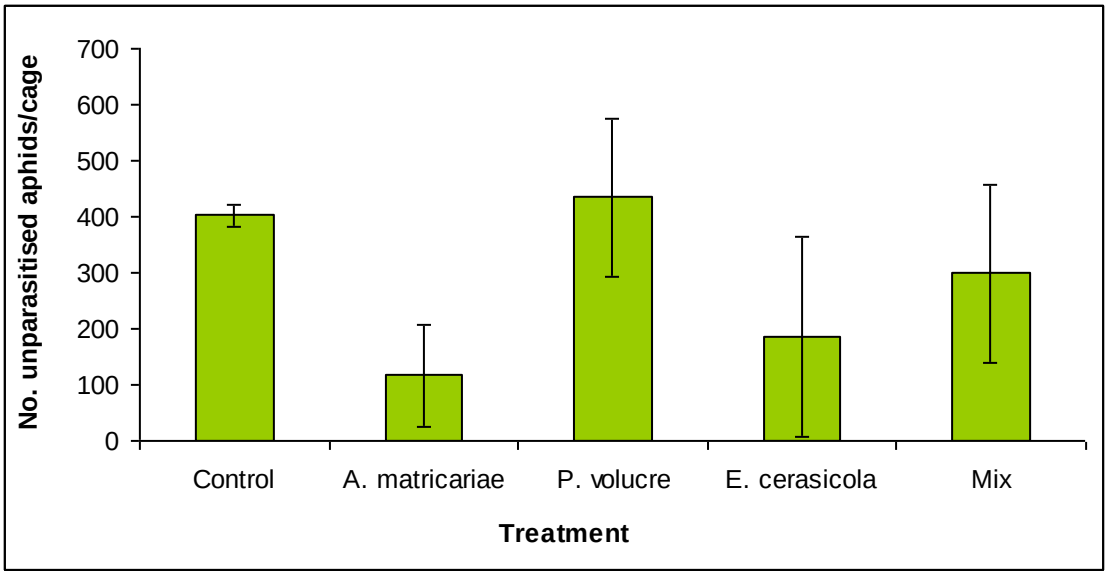

Figure 10. Mean number of unparasitised aphids per cage for the five treatments with the standard error of the mean (n=4 replicate cages)

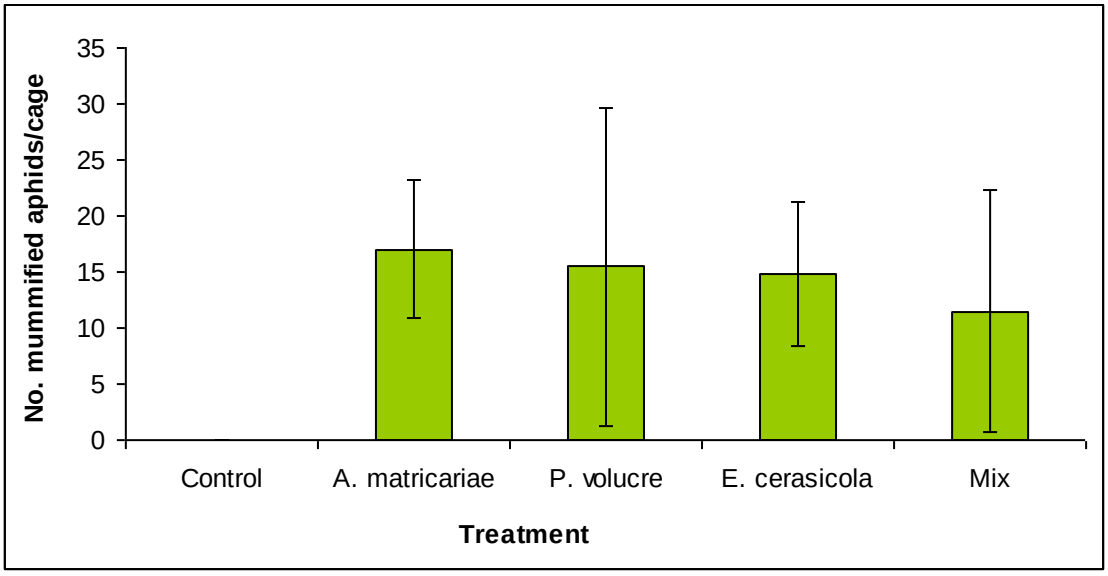

Figure 11. Mean number of mummified aphids per cage for the five treatments with the standard error of the mean (n=4 replicate cages)

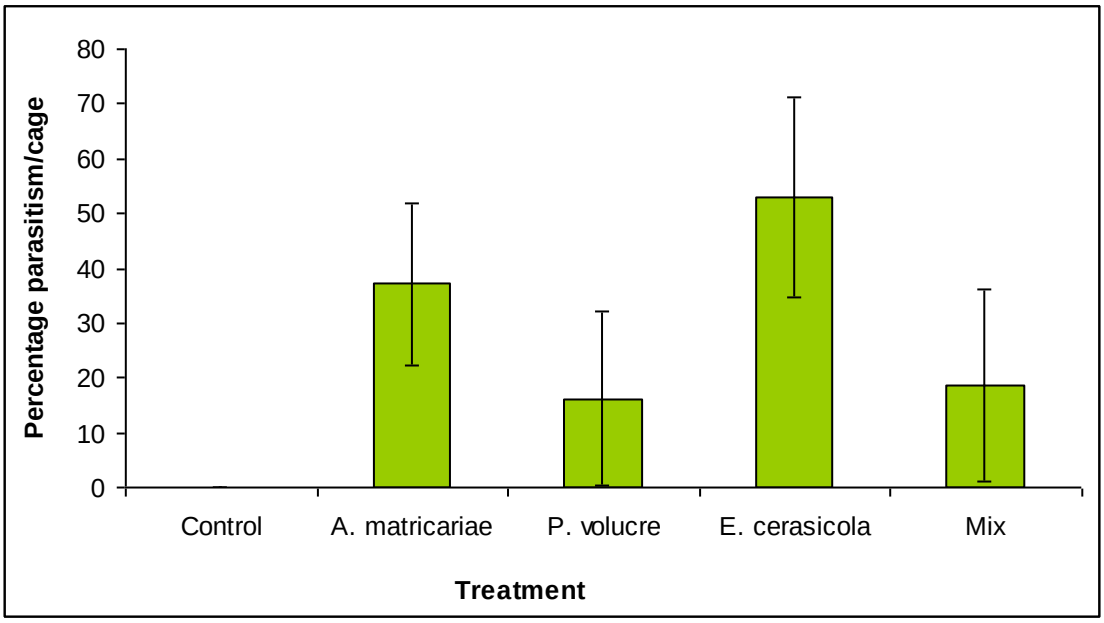

Figure 12. Mean percentage of parasitism per cage with the standard error of the mean (n=4 replicate cages)

Temperature

Figure 13 shows the mean average, maximum and minimum temperatures recorded in two cages throughout the experimental period. The mean temperature remained between 19-24°C which is higher than the 20ºC the glasshouse was set at. The warmer temperatures recorded could be due to the effect of the cage. A maximum temperature of 37°C was recorded on 28 August 2012 (prior to the cages being transported to the Lincolnshire Herbs) and a minimum temperature of 15°C on 12 September 2012.

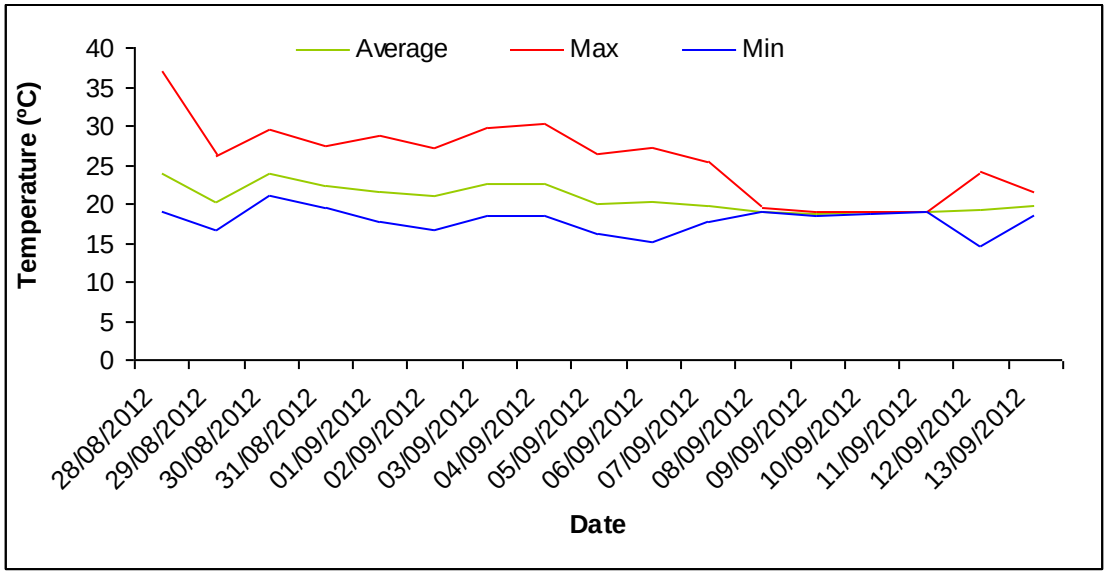

Figure 13. Mean average, maximum and minimum temperatures recorded throughout the experimental period

Occasion 1: Efficacy of single and mixed parasitoid species for controlling hawthorn-parsley aphid

Assessment

Control of hawthorn-parsley aphid was similar regardless of whether a single parasitoid or a mixture of parasitoids was used. There were no significant differences observed between single or mixed parasitoids on the number of unparasitised aphids per cage, the number of mummies per cage or the percentage parasitism per cage (Figure14, 15 and 16). Again this could have been due to the large variation observed between the treatment replicates.

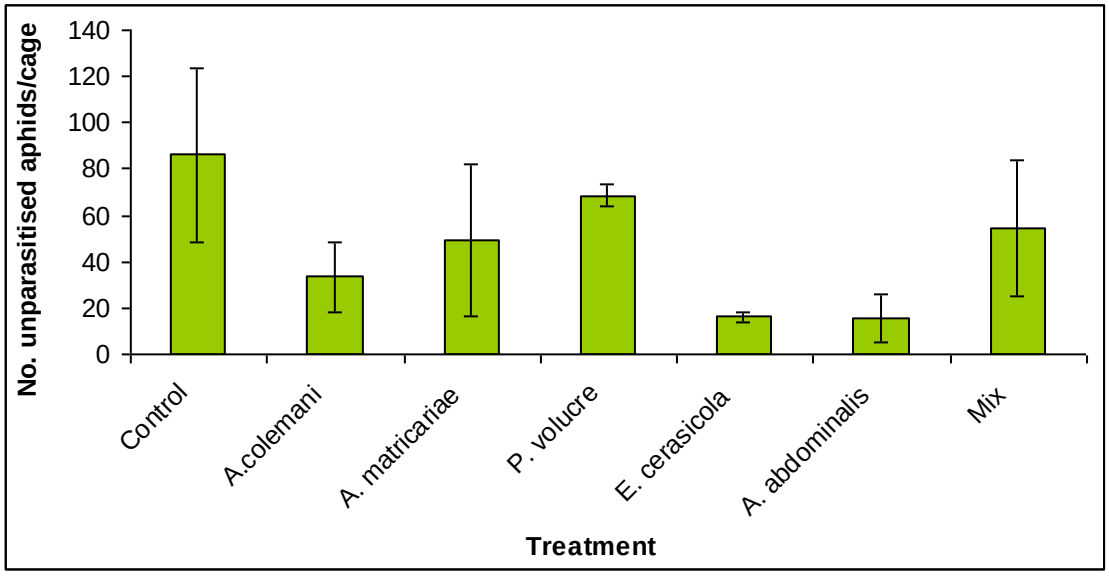

Figure 14. Mean number of unparasitised aphids per cage for the five treatments with standard error of the mean (n=3 replicate cages)

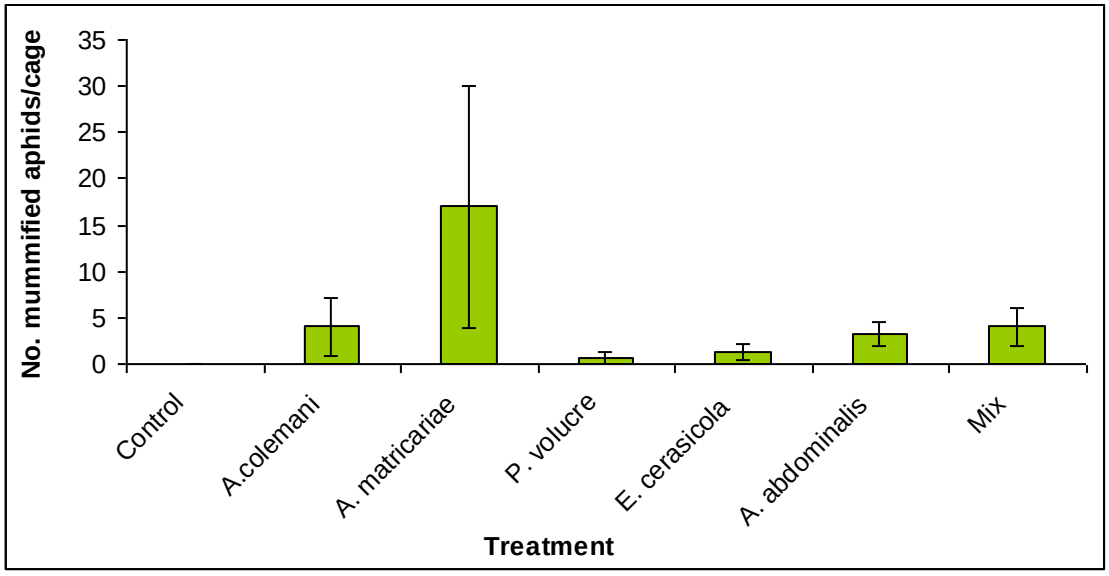

Figure 15. Mean number of mummified aphids per cage for the five treatments with standard error of the mean (n=3 replicate cages)

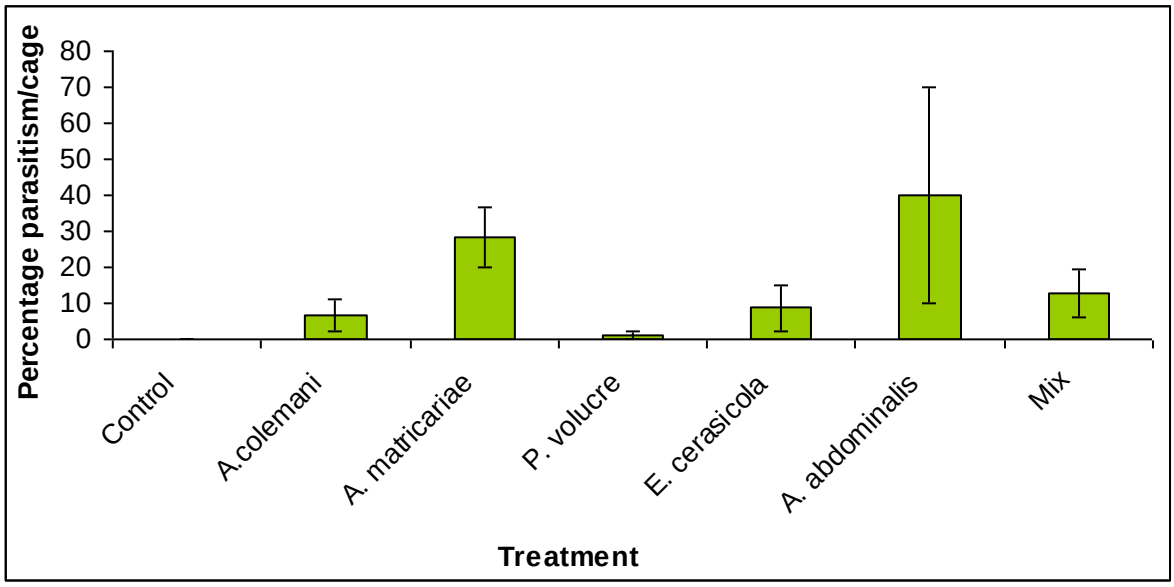

Figure 16. Mean percentage of parasitism per cage with standard error of the mean (n=3 replicate cages)

Temperature

Figure 17 shows the mean average, maximum and minimum temperatures recorded throughout the experimental period from a data logger placed in one of the treatment cages. The mean temperature remained between 20 and 25°C which is higher than the 20ºC the glasshouse was set at. The warmer temperatures recorded could be due to the effect of the cage. A maximum temperature of 32°C was recorded on 18 August 2012 and a minimum temperature of 16°C on 22/23 August 2012.

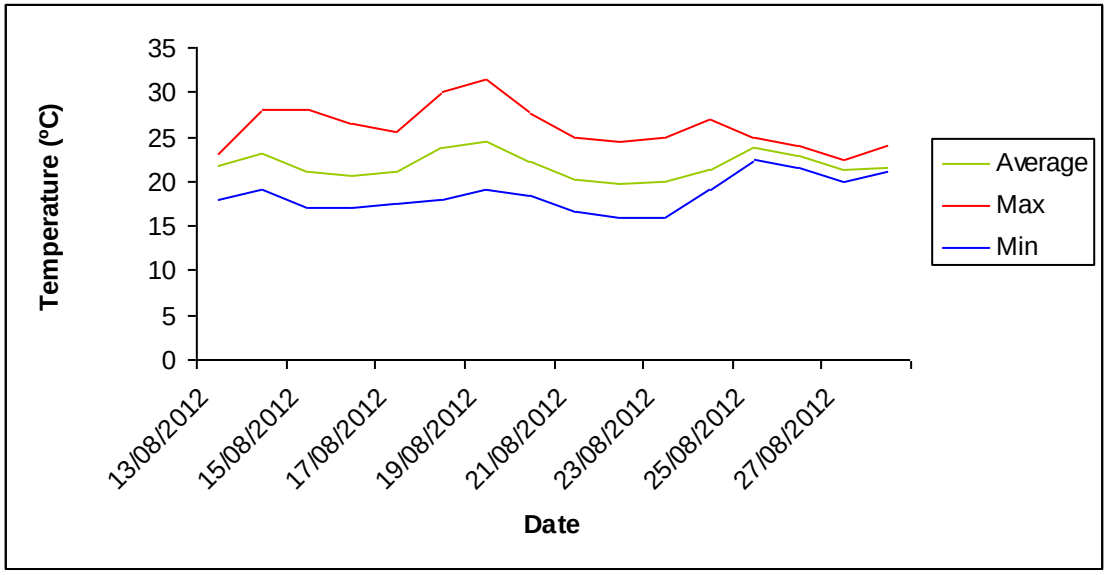

Figure 17. Mean average, maximum and minimum temperatures recorded throughout the experimental period

No differences were observed between the parasitism of individual species or a mix of species for both mint aphid and hawthorn-parsley aphid. As a parasitoid species or mix of species needed to be selected to develop a release strategy for each aphid species, it was decided to repeat Experiment 1 on both aphid species, with amendments to the methods in order to try to reduce the variation in results between replicate cages.

Materials and methods

Occasion 2: Efficacy of single and mixed parasitoid species for controlling mint aphid

The same five treatments were used as in Occasion 1, but instead of two of the 16 plants in each cage being infested with aphids (25 per infested plant), each of the plants was infested with smaller numbers of aphids. This was intended to test the parasitoid efficacy when a lower aphid density was present on all plants in the cages. On 18 February 2013, 180 mint plants were each infested with five mixed aged mint aphids which were transferred using a fine paintbrush. Mint plants were trimmed to 10-15cm before adding the aphids. Nine infested plants were placed into 20 insect proof cages (50x50x50). Nine plants were used on this occasion rather than 16 plants as in occasion 1, in order to reduce the time taken to complete the experiment. Thus on occasion 2, there were nine plants, each with five aphids, giving a total of 45 aphids per cage, whereas on occasion 1, there were 16 plants, with two of the plants having 25 aphids, giving a total of 50 aphids per cage.

Cages were kept in a controlled glasshouse compartment at ADAS Boxworth at 20ºC 16L:8D to allow the aphids to settle before the cages were transported to Lincolnshire Herbs on 19 February 2013. A pooter was used to transfer the adult female parasitoids (12 of each species and 12 mixed) into separate specimen tubes which were then transported to the commercial nursery in a cool box. To reduce possible damage to the parasitoids tissue was placed into the collection tube of the pooter before they were aspirated into the tubes. Prior to entering the nursery, the parasitoids were released into 16 of the cages as per the treatment list by placing the appropriate opened specimen tube between two plant pots.

Cages were arranged in a randomised block design on a line in 7x3 rows and were treated in the same manner as the commercial mint crops and watered by capillary matting. Two temperature data loggers were placed in two cages. The temperature in the commercial glasshouse was approximately 16°C at night and 20°C during the day.

On day 20 the cages were transferred back to ADAS Boxworth and kept in a controlled temperature laboratory at 21ºC 16L:8D. The following day the mint plants were destructively sampled by cutting the plants at their base, taking care not to dislodge the aphids. The number of healthy aphids and the number of aphid mummies within each cage was recorded, checking both the plant, the compost, the sides and bottom of the pots and the inside of the cages. The mummies were recorded as *Aphidius, Praon* and *Ephedrus* which was determined by their colour (pale brown, pale off-white and on a 'pedestal' and black respectively). Mummies were kept and stored in petri dishes. Once the parasitoids began to emerge some of each colour were preserved in alcohol in order to confirm the species. Percentage parasitism was calculated.

Statistical analysis

Data on the numbers of aphids and mummified aphids were analysed using an ANOVA in GenStat (12th Edition),

Results

Occasion 2: Efficacy of single and mixed parasitoid species for controlling mint aphid

Assessment

There was a significant effect of treatment on the number of healthy (unparasitised) aphids ($P<0.05$). All treatments containing parasitoids had significantly less healthy aphids (mean

of <59 aphids per cage) compared with the control which had a mean of 272 aphids per cage (Figure 18). Treatments containing *E. cerasicola* and the mix of three parasitoid species had the least number of healthy aphids with means of 11 and 5 per cage respectively. *Ephedrus cerasicola* and the mix were equally effective in reducing the number of healthy aphids. *Aphidius matricariae* and *P. volucre* were equally effective but were less effective than *E. cerasicola* and the species mix.

The highest number of mummies was recorded in cages with the parasitoid mix with a mean of 6 mummies per cage which is the equivalent to a mean of 1 mummies per plant (as each cage contained nine pots). However, none of the treatments were significantly different to the untreated treatment (Figure 19). No mummies were found in the control cages.

There was a significant effect of treatment on percentage parasitism ($P<0.05$). All treatments containing parasitoids had a significantly higher percentage parasitism compared with the control which had 0% parasitism (Figure 20). Treatments containing *E. cerasicola* and the mix of parasitoids had the highest percentage parasitism with 32.7% and 46.7% respectively. *Ephedrus cerasicola* and the mix were equally effective. *Aphidius matricariae* and *P. volucre* were also equally effective but gave significantly lower percentage parasitism compared with *E. cerasicola* and the mix.

Within the mixed treatments *E. cerasicola* was responsible for 81.8% of the mummies followed by *A. matricariae* (responsible for 13.6%) and *P. volucre* (responsible for 4.5%).

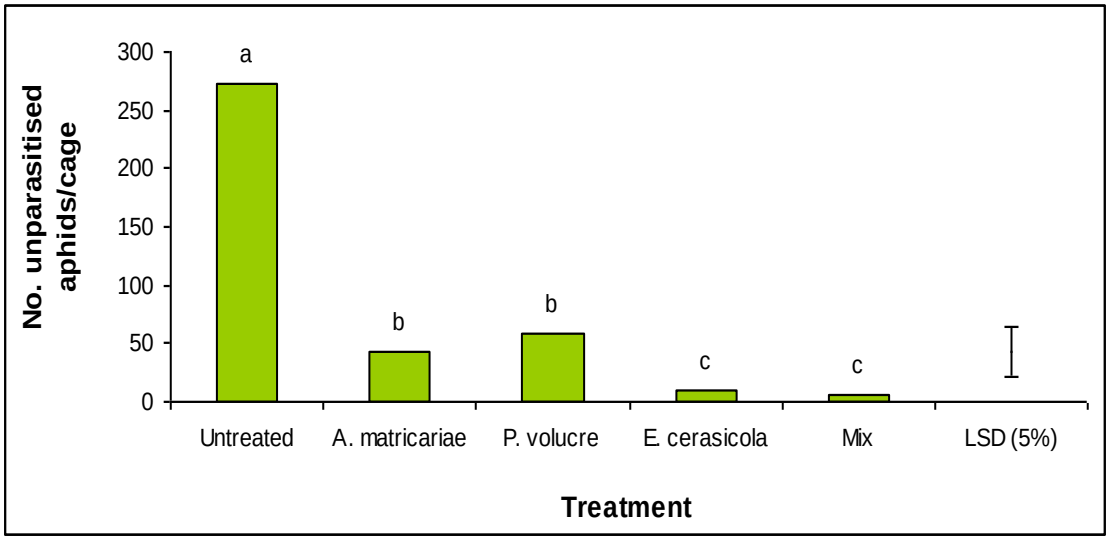

Figure 18. Mean number of healthy (unparasitised) aphids per cage for the five treatments (LSD 5%)

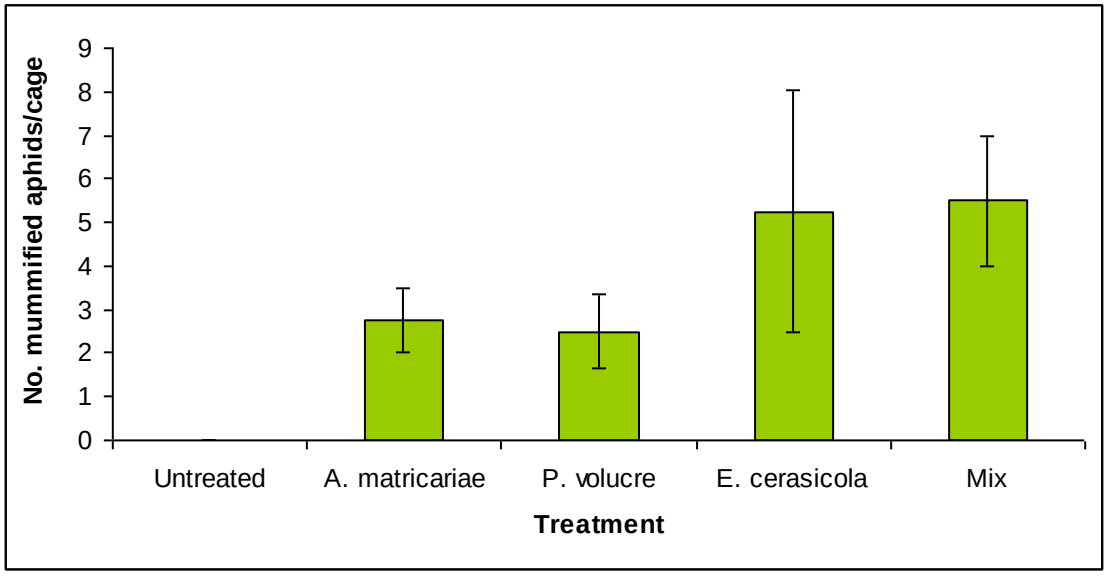

Figure 19. Mean number of mummified aphids per cage for the five treatments with standard error of the mean (n = 4 replicate cages)

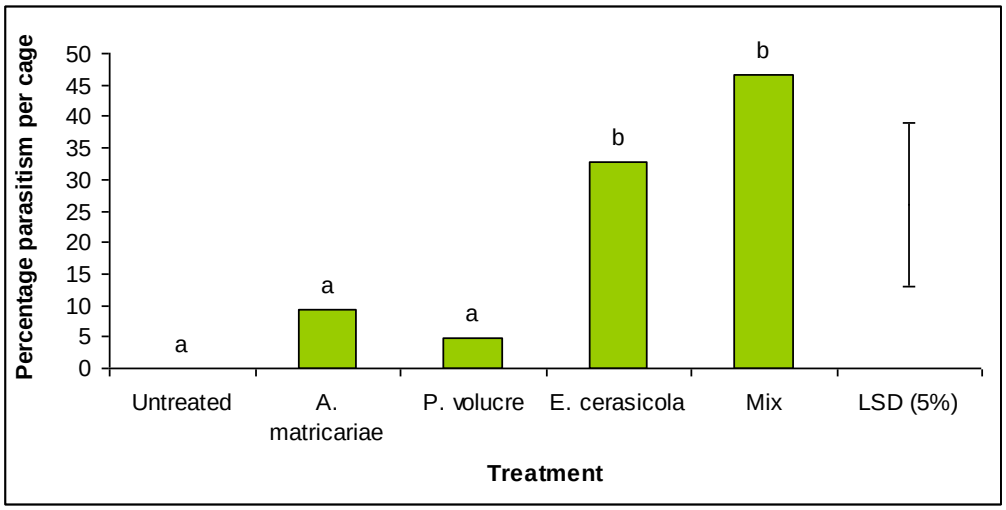

Figure 20. Mean percentage of parasitism per cage for the five treatments (LSD 5%)

Temperature

Figure 21 shows the mean daily average, maximum and minimum temperatures recorded throughout the experimental period from data loggers placed in two of the treatment cages. The mean temperature remained between 15 and 20°C, close to the 16°C night and 20°C day conditions the glasshouse was set at. A maximum temperature of 25°C was recorded on 4 March 2013. The two lowest minimum temperatures of 10.25 and 7.25°C were recorded on 19 February and 11 March 2013 respectively and these readings were taken during transportation of the cages between Boxworth and Lincolnshire Herbs. When the cages were in the glasshouse minimum temperatures were always above 12.5°C.

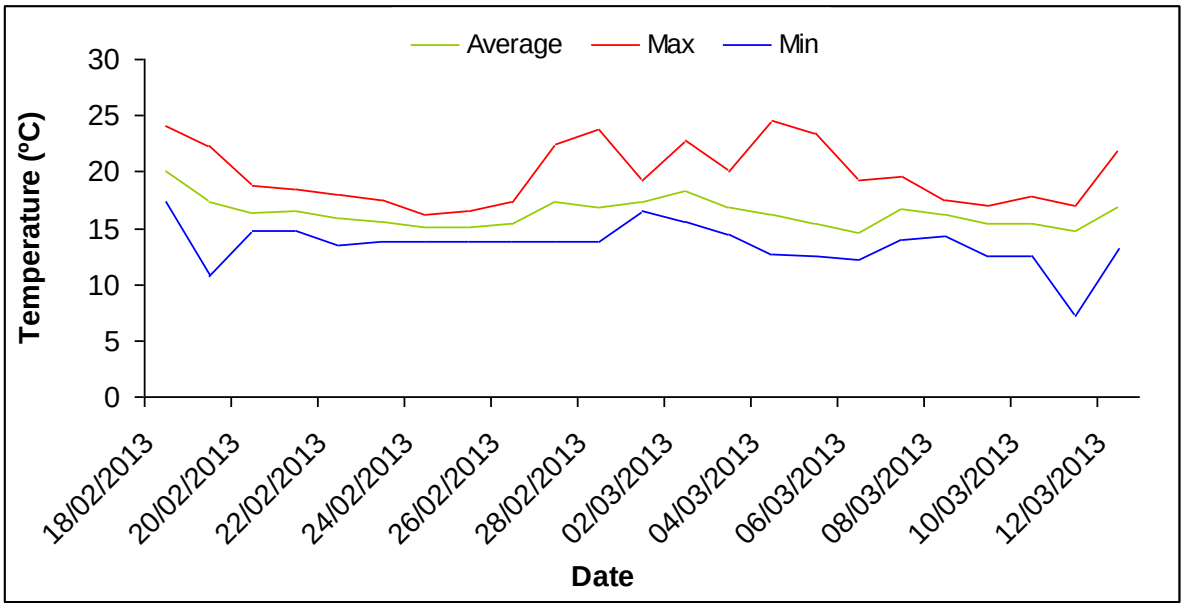

Figure 21. Mean average, maximum and minimum temperatures recorded throughout the experimental period

Discussion

The results from this study so far indicate that parasitoids are more effective at parasitising hawthorn-parsley aphids in spaced pots of parsley than in those that are pot-thick. This indicates that the parasitoids might be inhibited from searching for this species of aphid (which infests the base of parsley plants) when closely spaced early in the production cycle. This might be one of the reasons why growers have not observed parasitized hawthorn-parsley aphids during the production cycle.

When comparing the effectiveness of individual and mixed species on the parasitism of mint aphid and hawthorn parsley aphid, the initial experiments had too much variation in the data from replicate cages within the treatments to make confident conclusions. Attempts were successfully made to reduce this variation and results from the second experiment on mint aphid have indicated that this aphid is more effectively parasitized by a mix of parasitoid species (*E. cerasicola, A. matricariae* and *P. volucre)* or *E. cerasicola* alone, than by *A. matricariae* or *P. volucre* alone. When used in a species mix together with *A. matricariae* and *P. volucre*, *E. cerasicola* was responsible for 82% of the mummies. This result indicated that *E. cerasicola* is the superior parasitoid for mint aphid. Reasons for this could include enhanced host-searching ability and/or the mint aphid being more readily accepted as a suitable host by *E. cerasicola* compared with the other two parasitoid species.

When using mixed parasitoid species in a biological control programme there is the risk that competition between parasitoids for the host may occur and this could lead to reduced total parasitism and thus poorer aphid control. A recent study by Sidney *et al.* (2010) demonstrated that competition between larvae of *Aphidius ervi* and *Praon volucre* occurs within *M. euphorbiae*, with *P. volucre* being the superior competitor if both parasitoids lay eggs in the same host aphid. This could lead to the exclusion of *A. ervi* over time. It is possible that parasitoid larval competition could also play a role in mint aphid, with *E. cerasicola* larvae more successfully developing in aphids parasitized by multiple species.

During this study, it was also observed that while healthy aphid numbers were reduced significantly in treatments with each of the three parasitoids compared with the untreated controls, very few mummies were observed on the plants. Thus another factor in addition to parasitism may have contributed to aphid control. One possible factor could have been parasitoid host-killing via host feeding, as observed in PE 006 by *Aphidius ervi*, *Praon volucre*, *Ephedrus cerasicola* and *Aphelinus abdominalis* on mint aphid. Another factor could have been aphids dropping from the plant in response to the alarm pheromones produced by other aphids in the presence of parasitoid attack. Falling aphids have been observed by Growling and van Emden (1994), where 75% of rose-grain aphids, *Metopolophium dirhodum* fell or walked from cereal plants in the presence of parasitoids, with only 26% returning while the parasitoids were still present compared with 50% returning when the parasitoids were removed. Host killing and falling aphids are both factors which will enhance the impact of biological control by parasitoids. In the case of mint aphids, the reduction in numbers of healthy aphids by parasitoids, without the production of many mummies is an example of the ideal 'overkill' biological control strategy on a crop such as pot herbs, which are subject to retailer 'zero tolerance' of aphids or mummies.

The next stage of this study is to determine cost-effective release rates for the most effective individual or mix of parasitoids identified in the previous experiments. For mint aphid, the ideal candidate to take forward would be *E. cerasicola* but unfortunately this is not commercially available as a single species. Furthermore, the mix of the three species effective against mint aphid (*E. cerasicola, A. matricariae* and *P. volucre)* are only available as a mix of six parasitoids and it was shown in PE 006 that the three other parasitoids (*Aphidius colemani*, *A. ervi* and *Aphelinus abdominalis* do not parasitize mint aphid. Following consultation with the supplier of the parasitoid mix, Viridaxis in Belgium, it was confirmed that they do not currently plan to market a mix of parasitoids specifically for mint growers containing *E. cerasicola, A. matricariae* and *P. volucre* or make *E. cerasicola* available as a single species. Therefore *A. matricariae* was selected to take forward to the

next step in the project to test release rates, as this is available as a single species from other suppliers e.g. Koppert. This experiment will commence during April for mint aphid. The initial experiment comparing single and mixed species for the control of hawthorn-parsley aphid will be repeated during April, using the amended protocol as used for the second mint aphid experiment, in order to select the parasitoid(s) to take forward to a release rate experiment.

Conclusions so far

- Parasitoids are more effective at parasitizing hawthorn-parsley aphids on spaced pots of parsley than on pot thick pots.
- *Ephedrus cerasicola, Praon volucre* and *Aphidius matricariae*, either as individual species or as a mix are effective against mint aphid, with *E. cerasicola* and the species mix being the most effective. However, as *E. cerasicola* and the mix of the three species are not commercially available (only the mix of six species, three of which do not parasitise mint aphid), *Aphidius matricariae* will be taken forward to the next experiment testing cost-effective release rates for mint aphid control.

References

Growling, G.R. and van Emden, H.F. (1994). Falling aphids enhance impact of biological control by parasitoids on partially aphid-resistant plant varieties. *Annals of Applied Biology*, 125, 233-242.

Sidney, L.A., Bueno, V.H.P., Lins, J.C., Sampaio, M.V. and Silvia, D.B. (2010). Larval competition between *Aphidius ervi* and *Praon volcre* (Hymenoptera: Braconidae: Aphididae) in *Macrosiphum euphorbiae* (Hemiptera: Aphididae). *Environmental Entomology*, 39, 1550-1505.