Project title:	Protected herbs: improved biological control of aphids (extension to PE 006)
Project number:	PE 006a
Project leader:	Jude Bennison, ADAS
Report:	Final report 2014
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Location of project:	ADAS Cambridge Boxworth Lincolnshire Herbs
Industry Representative:	Claire Donkin, Lincolnshire Herbs
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The results and conclusions in this report are based on an investigation conducted over a three-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.

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GROWER SUMMARY

Headline

The individual aphid parasitoids *Aphidius colemani* and *Aphidius matricariae* were as effective as a mix of parasitoid species in controlling hawthorn-parsley aphid and mint aphid respectively, giving 99% and 86% control respectively. However, the rates shown to be effective were high and would currently be uneconomic.

Background

Until recently, biological control of aphids on protected crops relied mainly on three aphid parasitoid species; *Aphidius colemani*, *Aphidius ervi* and *Aphelinus abdominalis*. An aphid parasitoid mix (BasilProtect) produced by Viridaxis includes these three parasitoid species as well as three 'new' species; *Aphidius matricariae*, *Praon volucre* and *Ephedrus cerasicola.* HDC-funded project PE 006 investigated the potential of each of the six parasitoid species against two 'problem' aphid species, hawthorn-parsley aphid and mint aphid, on all year round protected pot herbs. Under laboratory conditions, all of the parasitoids except for *A. ervi* parasitised hawthorn-parsley aphid and each of the three 'new' species (*A. matricariae*, *P. volucre* and *E. cerasicola)* parasitised 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 to:

- demonstrate that *A. colemani* would parasitise hawthorn-parsley aphid on unspaced and spaced parsley plants in replicate cages in a commercial herb glasshouse;
- in small-scale research glasshouse experiments, develop an effective, robust parasitoid release strategy for control of hawthorn-parsley aphid and mint aphid and;
- in an experiment on a commercial herb nursery, to validate the success and cost-effectiveness of the selected parasitoid release strategy in controlling hawthorn-parsley aphid on parsley.

Summary

Objective 1: Demonstrate that *Aphidius colemani* will parasitise hawthorn-parsley aphid on unspaced and spaced parsley plants

This experiment, set up in May 2012, compared the efficacy of *A. colemani* against hawthorn-parsley aphid on either unspaced or spaced parsley plants within insect-proof cages (0.5 x 0.5 x 0.5m i.e. 0.25m^2) in a commercial herb glasshouse. The aim of the experiment was to determine whether parasitoid searching is inhibited amongst unspaced plants, which might help to explain why growers have not observed parasitised hawthorn-parsley aphid 'mummies' after releasing *A. colemani* in parsley crops. There were four experimental treatments: two control treatments with either two or 16 parsley plants per cage but no aphid parasitoids and two parasitoid treatments with either two or 16 parsley plants per cage and five mated female *A. colemani* per cage (equivalent to 20 per m^2) Two central plants in each cage were infested with 25 hawthorn-parsley aphids before adult parasitoid release. The cages were placed onto capillary matting in the commercial glasshouse which allowed watering by sub-irrigation. After 17 days the numbers of healthy (unparasitised) aphids and parasitised aphid 'mummies' per cage were recorded and percentage parasitism calculated.

Numbers of healthy aphids: The presence of *A. colemani* significantly reduced the number of healthy (unparasitised) hawthorn-parsley aphids per cage. In the control cages without parasitoids, numbers of aphids had increased from 50 to 488 – 753 per cage in 17 days. In both treatments where *A. colemani* were present, there were significantly lower numbers of healthy aphids (64 and 52 per cage with two and 16 plants per cage respectively, equivalent to 92% and 89% control) compared with those in the control treatments.

Numbers of parasitised aphid "mummies': As expected, mummies were only observed in cages where *A. colemani* were released. Significantly more mummies and percentage parasitism were recorded in cages with two parsley plants (mean 59 mummies per cage and 64% parasitism) than in those with 16 parsley plants (mean 12 mummies per cage and 21% parasitism. This result indicated that more parasitism occurred where the plants were spaced than where the plants were unspaced as occurs at the start of commercial production. However, as the parasitoids led to 92% and 89% control of live aphids at both plant spacings, they must have reduced aphid numbers in other ways than parasitism, e.g. by host killing by host-feeding, as demonstrated in the laboratory in PE 006.

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

Six experiments were done in this Objective; three on each aphid species. The aim of Experiments 1, 2, 3 and 4 was to 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. The aim of Experiments 5 and 6 was to determine a cost-effective parasitoid release rate for each of the aphid species. As the first pair of experiments on each aphid species led to inconclusive results due to the large variability in aphid numbers building up in individual replicate treatments, only the results of the later experiments on each aphid species are presented in the Grower Summary. Full details of all experiments are given in the Science Section.

Experiment to compare single and mixed parasitoid species for controlling mint aphid

The experiment on mint aphid (in February 2013) compared the efficacy of the single parasitoid species *Aphidius matricariae, Praon volucre* and *Ephedrus cerasicola* or a mix of these three species in insect-proof cages in a commercial herb glasshouse. There were five experimental treatments with four replicates of each: an untreated control with no parasitoids, 12 mated female parasitoids per cage of either *A. matricariae, P. volucre* or *E. cerasicola* and a mix of the three species (four mated females of each species). As the cages were 0.5 x 0.5 x 0.5m (0.25m^2) the release rate was 40 per m^2. Nine mint plants were used in each cage and each plant was infested with five mint aphids before the adult parasitoids were released. The cages were placed onto capillary matting in a commercial herb glasshouse to allow watering by sub-irrigation. After 20 days the numbers of healthy aphids and parasitised aphid 'mummies' per cage were recorded at ADAS Boxworth and percentage parasitism calculated.

Numbers of healthy aphids: All the parasitoid treatments significantly reduced the numbers of healthy aphids compared with the untreated control which had a mean of 272 aphids per cage after 20 days. *Ephedrus cerasicola* and the mix of the three parasitoid species were equally the most effective treatments, reducing numbers of healthy aphids to 11 and five per cage respectively, equivalent to 96% and 98% control (Figure 1). *A. matricariae* and *P. volucre* were less effective, reducing numbers of healthy aphids to 43 and 59 per cage respectively, equivalent to 86% and 82% control respectively.

Numbers of parasitised aphid 'mummies': *Ephedrus cerasicola* and the mix of the three parasitoid species led to the highest percentage parasitism (33% and 48% respectively). *Aphidius matricariae* and *P. volucre* were equally effective but less effective than *E. cerasicola* and the mix, giving 9% and 5% parasitism respectively. As in the experiment in Objective 1, it is likely that in addition to parasitism, the parasitoids reduced the numbers of healthy aphids by host-feeding and possibly by stimulating the aphids to drop from the plants. Although *E. cerasicola* was the most effective single species at controlling mint aphid, it is not available as a single species. The mix of the three species is only available as a commercial mix of six species, three of which do not parasitise mint aphid. Therefore *A. matricariae* was selected as the species to test in the final experiment against mint aphid (determining effective release rate) as it is commercially available as a single species.

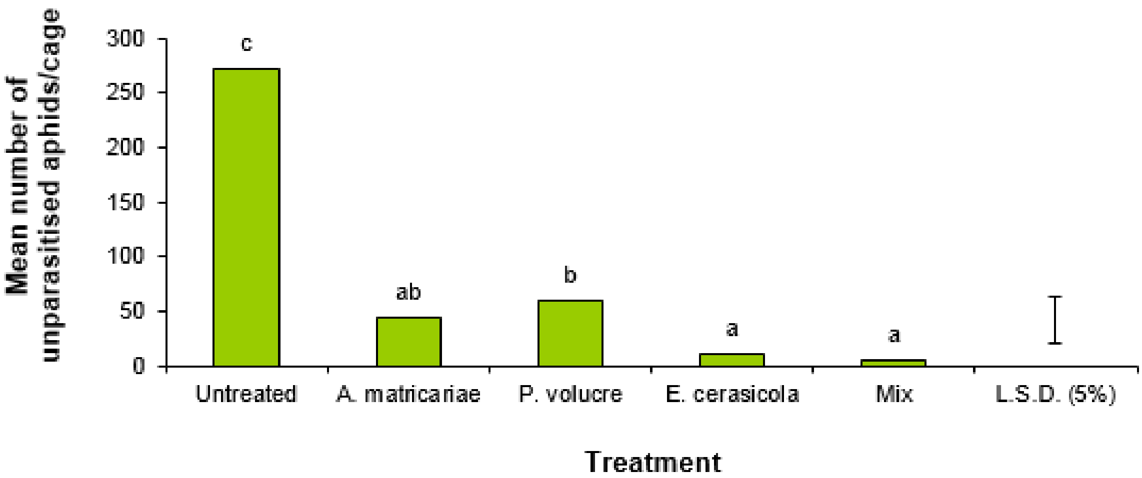

Figure 1. Mean number of healthy (unparasitised) mint aphids per cage. Bars with different letters are not significantly different from each other (P<0.05).

Experiment to compare single and mixed parasitoid species against hawthorn-parsley aphid

The experiment on hawthorn-parsley aphid (in May 2013) compared the efficacy of the single species *A. colemani*, *A. matricariae*, *P. volucre*, *E. cerasicola* and *Aphelinus abdominalis* or a mix of these five species against hawthorn-parsley aphid in insect-proof cages in a commercial herb glasshouse. There were seven experimental treatments including an untreated control, with three replicates of each treatment. Ten mated female parasitoids were added to each replicate cage (two of each of the five species in the parasitoid mix cages). Nine parsley plants were used per cage and each plant was infested with five hawthorn-parsley aphids. The experiment methods were the same as those carried out in the mint aphid experiments.

Numbers of healthy aphids: All the parasitoid treatments (used at 40 per m^2) significantly reduced numbers of healthy aphids (1-13 per cage) compared with the untreated controls (2,122 per cage, Figure 2). Each of the single species and the mix of species was equally effective, giving 95-99% control of hawthorn-parsley aphid over a 20-day period.

Numbers of parasitised aphid 'mummies': Ephedrus cerasicola and *Aphidius colemani* gave significantly higher numbers of 'mummies' (8 and 9 per cage respectively) but there were no significant differences between percentage parasitism (38-90% in cages treated with parasitoids). As in the mint aphid experiment, it is likely that in addition to parasitism, the parasitoids reduced the numbers of healthy aphids in other ways such as host-feeding. As all the parasitoids performed equally well, *A. colemani* was selected for the final experiment on hawthorn-parsely aphid to determine an effective release rate, as it is widely available as a single species and would be the most cost-effective.

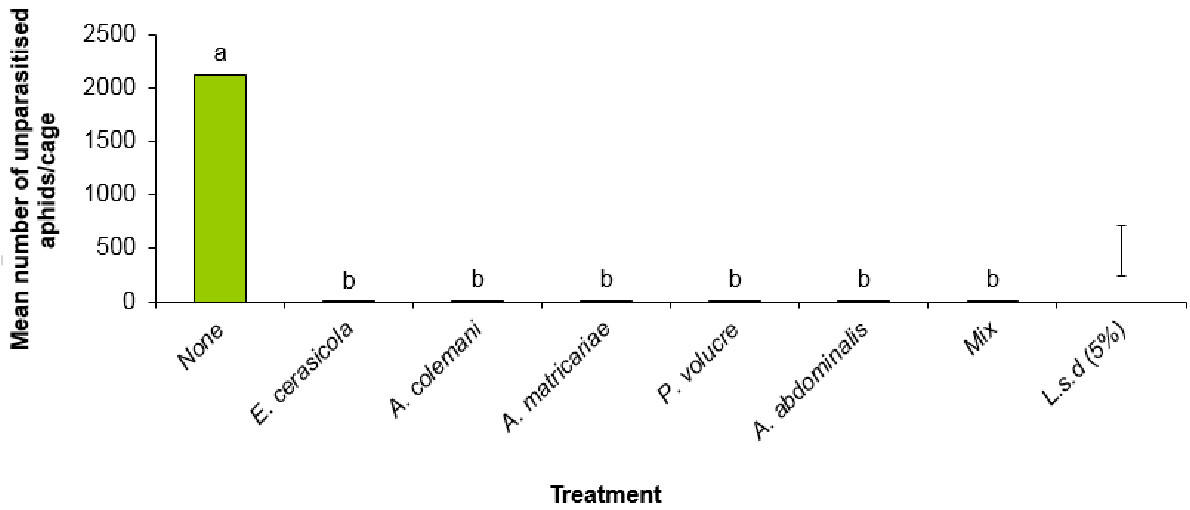

Figure 2. Mean number of healthy (unparasitised) hawthorn-parsley aphids per cage. Bars with different letters are significantly different from each other ($P<0.05$).

Experiment to determine effective parasitoid release rate for mint aphid

An experiment was set up in larger cages ($2.1m^2$) in April 2013 in order to test lower parasitoid release rates than in the previous experiments using $0.25m^2$ cages. There were four experiment treatments: an untreated control and *A. matricariae* at 1, 2 or 4 per m^2 and there were four replicate cages per treatment. In each cage there were 64 mint plants arranged to represent commercial spacing. Two plants in each cage were infested with two mint aphids. The cages were placed in a commercial herb nursery and the parasitoids were released on three weekly occasions, the first on the date of aphid infestation. Twenty days after set up, the plants were assessed for live and parasitised aphids. However, many of the plants had died due to the plants not having been watered adequately over a hot weekend just prior to the assessment date and no conclusive results could be given.

Experiment to determine effective parasitoid release rate for hawthorn-parsley aphid

A similar experiment to the one for mint aphid was set up on parsley in August 2013, but at ADAS Boxworth, so that watering could be carefully monitored during the continuing hot weather. The *A. colemani* release rates tested were the same as for *A. matricariae* in the mint aphid experiment. Twenty days after set up, although there were no significant effects on numbers of healthy hawthorn-parsley aphids or 'mummies' per cage due to the high variation between cages, a significantly higher percentage parasitism (12%) was recorded with the highest *A. colemani* release rate of 4 per m^2.

Objective 3: Experiment on a commercial herb nursery to validate the success and cost-effectiveness of the selected parasitoid release strategy for control of hawthorn-parsley aphid on parsley.

This experiment was due to be done during 2014 but hawthorn-parsley aphids did not infest parsley on the host nursery until late September. HDC and the BHTA were consulted and it was decided not to carry out this experiment.

Conclusions and financial implications

Mint aphid

- A single introduction of *A. matricariae* or a mix of *A. matricariae, P. volucre* and *E. cerasicola* at a high release rate (40/m^2) gave good control of mint aphid, reducing aphid numbers in cages by 86% and 98% respectively over a 3-week period. This release rate would not be currently cost-effective at an estimated £0.86 per m^2 *A. matricariae* and £2 per m^2 for the parasitoid mix, using representative prices from suppliers.

Hawthorn-parsley aphid

- A single introduction of *A. colemani* or a mix of *A. colemani*, *A. matricariae, E. cerasicola, P. volucre* and *A. abdominalis* at a high release rate (40/m^2) gave good control of hawthorn-parsley aphid, reducing numbers in cages by 99% over a 3-week period. This release rate would cost an estimated 82 pence per m^2 for *A. colemani* and £2 per m^2 for the parasitoid mix.
- A single release of *A. colemani* at 20/m^2 gave better control of hawthorn-parsley aphid (92% reduction) on spaced plants in cages than on unspaced plants (89% reduction). This release rate is higher than those routinely used by growers and would cost an estimated 41 pence per m^2 which is expensive. However, losses due to hawthorn-parsley aphids can exceed 20% and if aphicides are needed this incurs extra cost.
- Three weekly releases of *A. colemani* at 4/m^2 in cages did not reduce hawthorn-parsley aphid numbers compared with untreated controls. This release rate would cost an estimated 8 pence per m^2 per week but was ineffective.
- It is possible that a release rate for *A. colemani* in between 4/m^2 per week (ineffective) but lower than the 20/m^2 (effective but expensive) might be effective, depending on the density of aphids developing. However, it was not possible to test a cost-effective release strategy for *A. colemani* on a commercial parsley crop due

to the lack of hawthorn-parsley aphids on the host nursery until late in the 2014 season.

Action Points

- Ensure that aphids on parsley and mint are correctly identified. Use the HDC Crop Walkers Guide or the HDC Best Practice Guide www.hdc.org.uk/herbs/ for help with recognition
- On nurseries where hawthorn-parsley aphid occurs, consider preventive releases of *A. colemani* or the mix of six parasitoid species in 'BasilProtect' from various suppliers or 'Aphidsure mix' available from BCP Certis. These products include *Aphidius colemani* and four other parasitoid species which also attack this aphid. The lowest release rate shown to be effective in this project was $20/m^2$.
- On nurseries where mint aphid occurs, consider preventive releases of *Aphidius matricariae* or the mix of six species, three of which attack this aphid. The only release rate tested in this project was $40/m^2$ and this was effective.
- Do not release *Aphidius colemani* as a single species to mint as it will not control mint aphid.
- Parasitoids can kill aphids by 'host-feeding' where they act as a predator, in addition to parasitising aphids leading to 'mummies'. In this project host-feeding was thought to play a major role in controlling both aphid species. Therefore in addition to monitoring for mummies, judge aphid control by parasitoids by the number of healthy aphids.

SCIENCE SECTION

Introduction

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).

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 parasitised 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 unspaced 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 unspaced 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 2012. A culture of hawthorn-parsley aphids was set-up by placing infested curly parsley plants in mesh cages (50 x 50 x 50 m). The cages were used to exclude any other pests or aphid parasitoids and predators. These mesh cages were placed in a computer-controlled glasshouse compartment on capillary matting, to allow 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 unspaced and spaced parsley plants

The experiment recorded the efficacy of *A. colemani* at parasitising hawthorn-parsley aphid on unspaced and spaced parsley plants within cages placed in a commercial herb glasshouse. This was to determine whether parasitoid searching was inhibited by unspaced 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. *Aphidius colemani* females**
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 (50x50x50m) and an additional 14 clean parsley plants of the same age were added to eight of these cages (Figure 1).

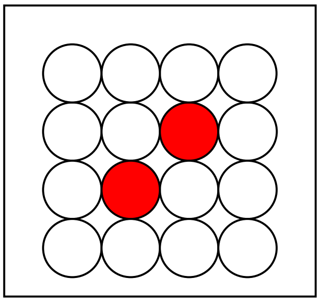

Figure 1. Arrangement of parsley plants in cages with 16 plants. Red circles indicate infested parsley plants and white circles indicate clean parsley plants

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' (aspirator) 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 2). Temperature data loggers were placed in two of the cages.

Figure 2. 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.* The portions of aphid-infested parsley plants were checked again on day 15 and 17, recording the number of additional mummified aphids. Percentage parasitism was calculated as follows: (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 (12^{th} 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 17 days (Figure 3). In both treatments where *A. colemani* were present, there were significantly lower numbers of unparasitised aphids (64 and 52 per cage with two and 16 plants per cage respectively, equivalent to 92% and 89% control) compared with those in the control treatments.

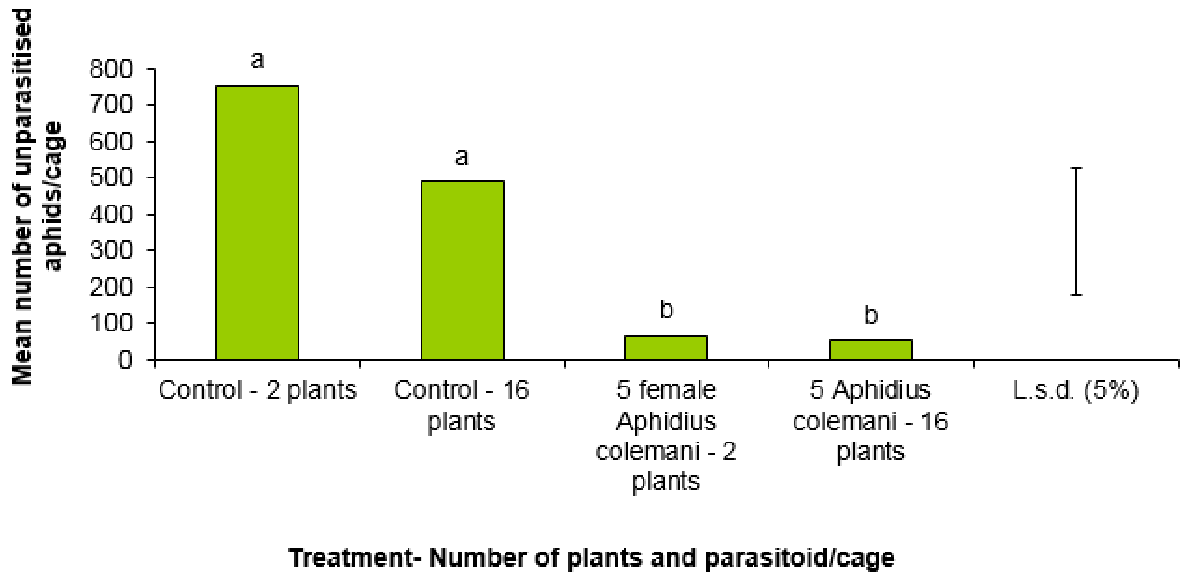

Figure 3. Mean number of unparasitised hawthorn-parsley aphids per cage (LSD 5%)

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) (Figure 4). When looking at the subset data for treatments where parasitoids were released, a significant effect (P<0.05) of the number of plants per cage was observed with more mummies being recorded in cages with two parsley plants (mean 59 mummies per cage) compared to those with 16 parsley plants (mean 12 mummies per cage). This indicated that the parasitoids were more effective where the plants were spaced further apart than where the plants were unspaced as occurs at the start of commercial production. However, as the parasitoids had led to 92% and 89% control of live aphids at both plant spacings, they must have reduced aphid numbers in other ways than parasitism, e.g. by host feeding as demonstrated in the laboratory in PE 006.

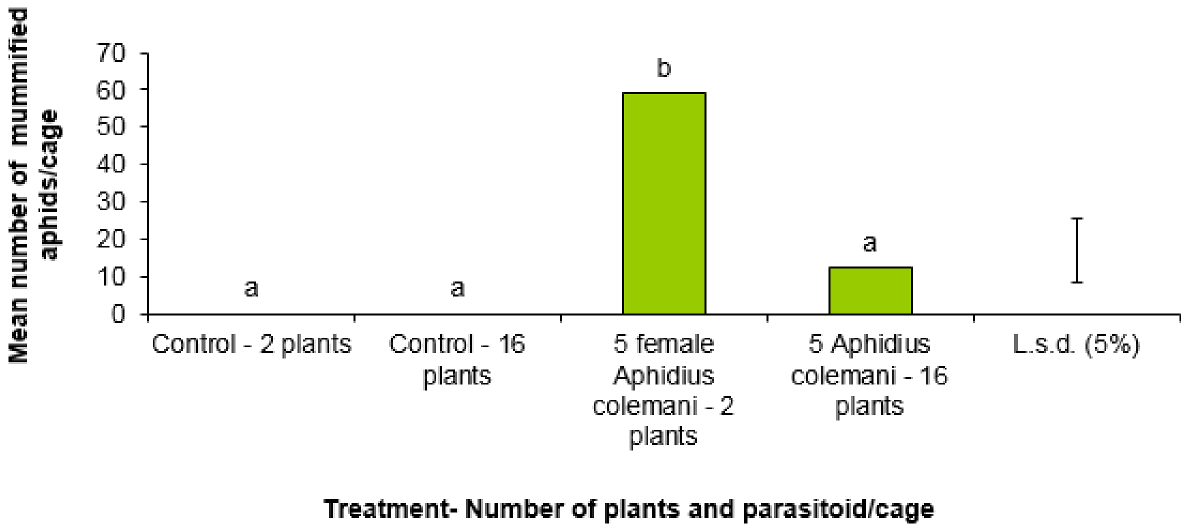

Figure 4. Mean number of mummified hawthorn-parsley aphids per cage (LSD 5%)

Percentage parasitism

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) (Figure 5). 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.

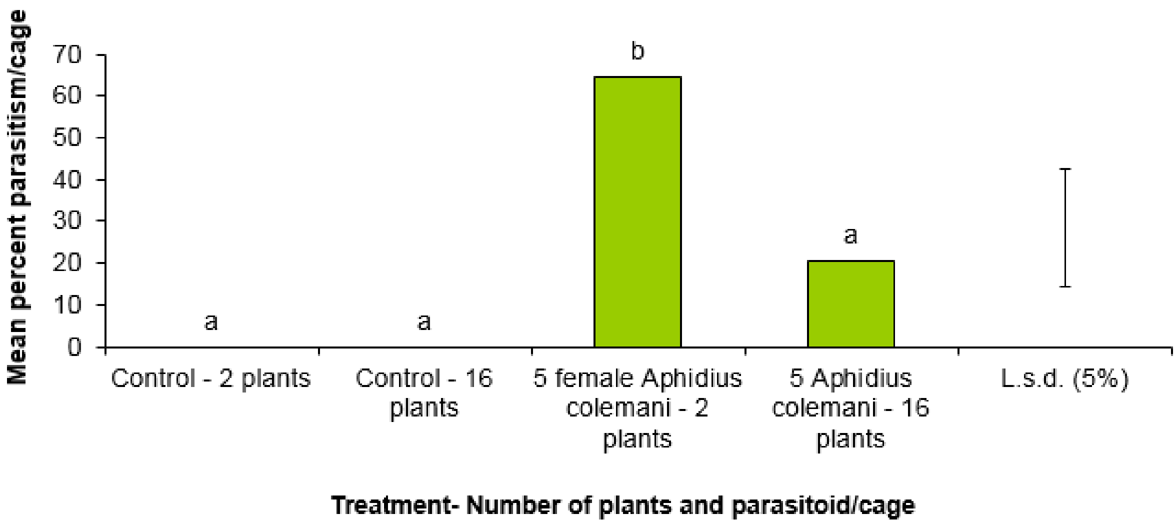

Figure 5. Mean percentage of parasitism per cage (LSD 5%)

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

Six experiments were done in this Objective, three on each aphid species.

Experiments 1-4: 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.

Experiment 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 2).

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

Treatment no.	No. plants per cage	No. infested plants	No. aphids per cage	No. and species of 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 (50x50x50m) and an additional 14 clean mint plants of the same age were added around the infested plants (Figure 6).

Figure 6. 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 the commercial herb nursery on 29 August 2012. A ‘pooter’ was used to transfer the adult mated female parasitoids (12 of each individual 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 two rows of seven cages and one row of six cages to fit in the available space on the line. The plants in the cages were kept under the same conditions as the commercial parsley crops (20ºC, 12L:12D) and watered by capillary matting (Figure 2). 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 plants and surrounding area. The mummies were recorded as *Aphidius, Praon* and *Ephedrus*, 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 any further 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).

<u>Experiment 2: Efficacy of single and mixed parasitoid species for controlling mint aphid</u>

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 with amendments to the methods in order to try to reduce the variation in results between replicate cages. The same five treatments were used as in Experiment 1, but instead of two of the 16 plants in each cage being infested with aphids (25 per infested plant), all plants were infested with smaller numbers of aphids. This experiment thus compared parasitoid efficacy when a lower aphid density was present on all plants.

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 (50x50x50m). Nine plants were used on this occasion rather than 16 plants as in Experiment 1, in order to reduce the time taken to complete the experiment. Thus in Experiment 2, there were nine plants, each with five aphids, giving a total of 45 aphids per cage, whereas in Experiment 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:8 D to allow the aphids to settle before the cages were transported to the commercial herb nursery 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 two rows of seven cages and one row of six cages to fit in the available space on the line. The plants in the cages were kept in the same conditions 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 from of each colour mummy 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

<u>Experiment 1: Efficacy of single and mixed parasitoid species for controlling mint aphid</u>

Numbers of healthy and parasitised aphids

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 the untreated cages and those with single or mixed parasitoids in the number of unparasitised mint aphids per cage, the number of mummies per cage and the percentage parasitism per cage (Figures 7, 8 and 9). This result was thought to be due to the large variation observed between the treatment replicates. Within the mixed treatments *E. cerasicola* was responsible for 87% of the mummies followed by *A. matricariae* (responsible for 13%).

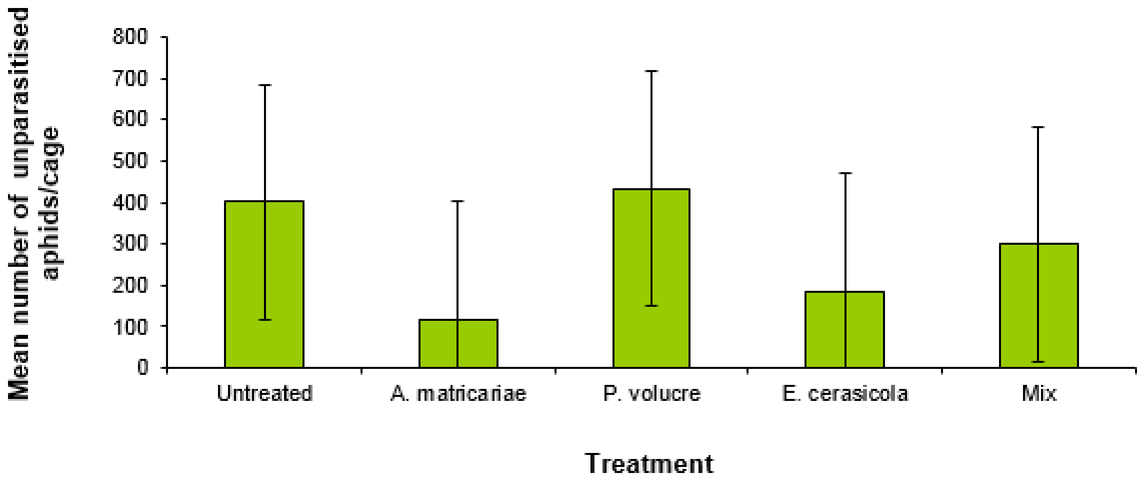

Figure 7. Mean number of unparasitised mint aphids per cage for the five treatments with 95% confidence limits (n=4 replicate cages)

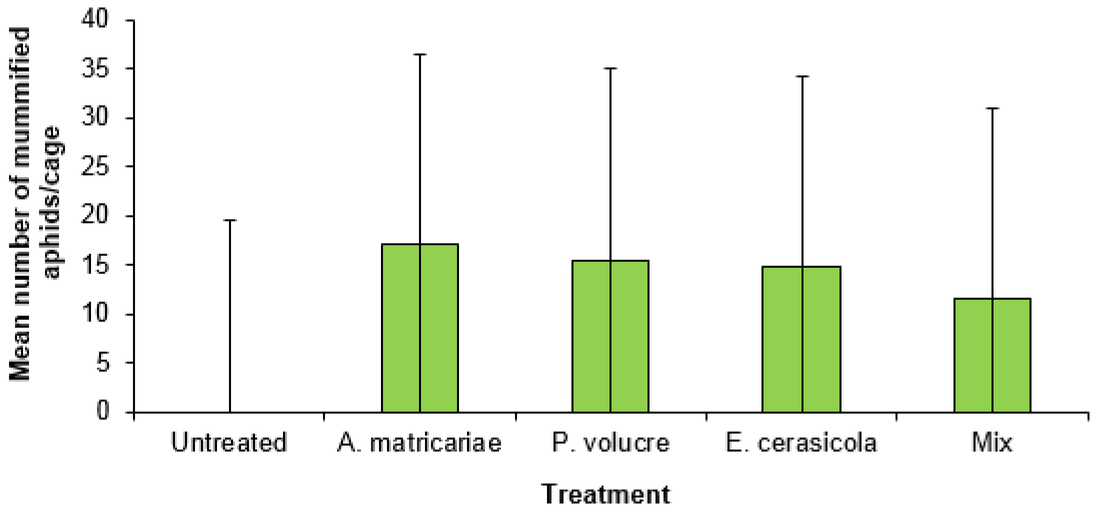

Figure 8. Mean number of parasitised mint aphids per cage for the five treatments with 95% confidence limits (n=4 replicate cages)

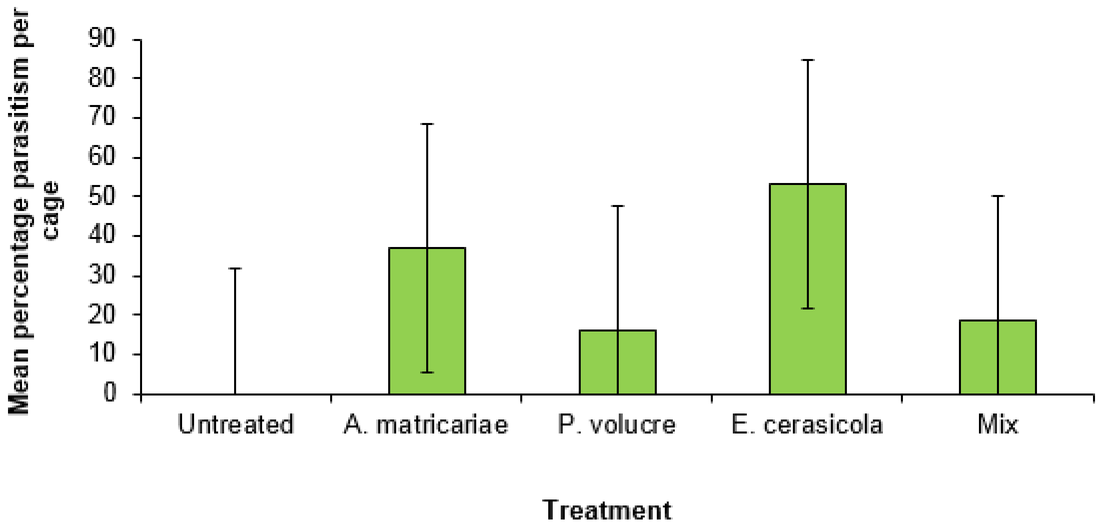

Figure 9. Mean percentage of mint aphid parasitism per cage with 95% confidence limits (n=4 replicate cages)

Temperature

Figure 10 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 commercial herb nursery) and a minimum temperature of 15°C on 12 September 2012. Temperatures were within the optimum range for aphid parasitoids (10-30°C) for most of the experiment period.

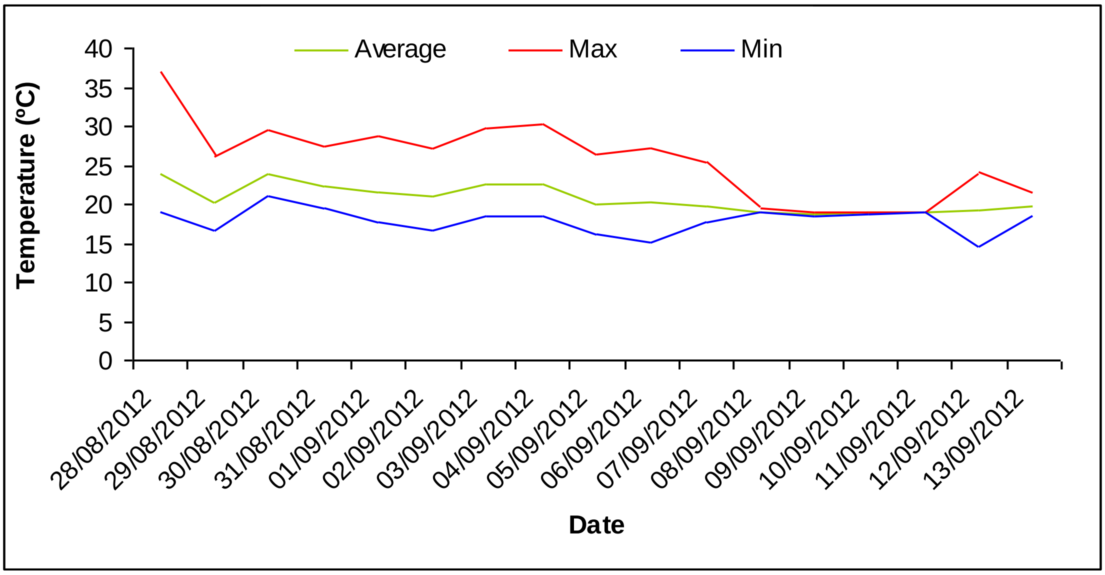

Figure 10. Mean average, maximum and minimum temperatures recorded throughout the experimental period

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

Numbers of healthy and parasitised aphids

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 11). 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, equivalent to 96% and 98% control. *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, reducing numbers of healthy aphids to 43 and 59 per cage respectively, equivalent to 86% and 82% control respectively.

The highest number of mummies was recorded in cages with the parasitoid mix with a mean of six mummies per cage which is the equivalent to a mean of one mummy per plant (as each cage contained nine pots). However, none of the treatments were significantly different to the untreated control (Figure 12). 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 led to a significantly higher percentage parasitism compared with the control which had 0% parasitism (Figure 13). Treatments containing *E. cerasicola* and the mix of parasitoids were equally effective and had the highest percentage parasitism with 32.7% and 46.7% respectively. *Aphidius matricariae* and *P. volucre* were also equally effective but gave significantly lower percentage parasitism (9% and 5% respectively) 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%).

As in the experiment in Objective 1, it is likely that in addition to parasitism, the parasitoids reduced the numbers of healthy aphids by host-feeding and possibly stimulating the aphids to drop from the plants.

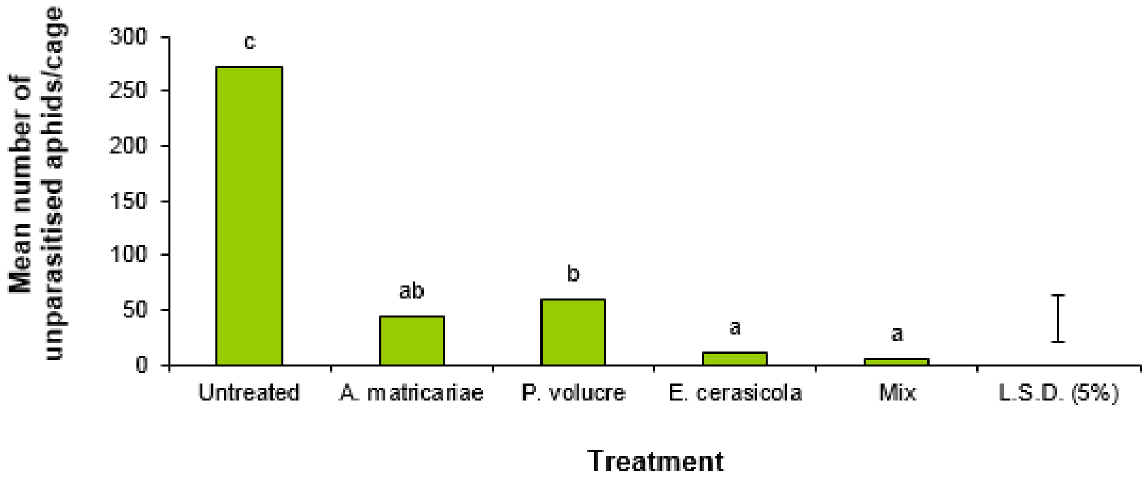

Figure 11. Mean number of healthy (unparasitised) mint aphids per cage for the five treatments (LSD 5%)

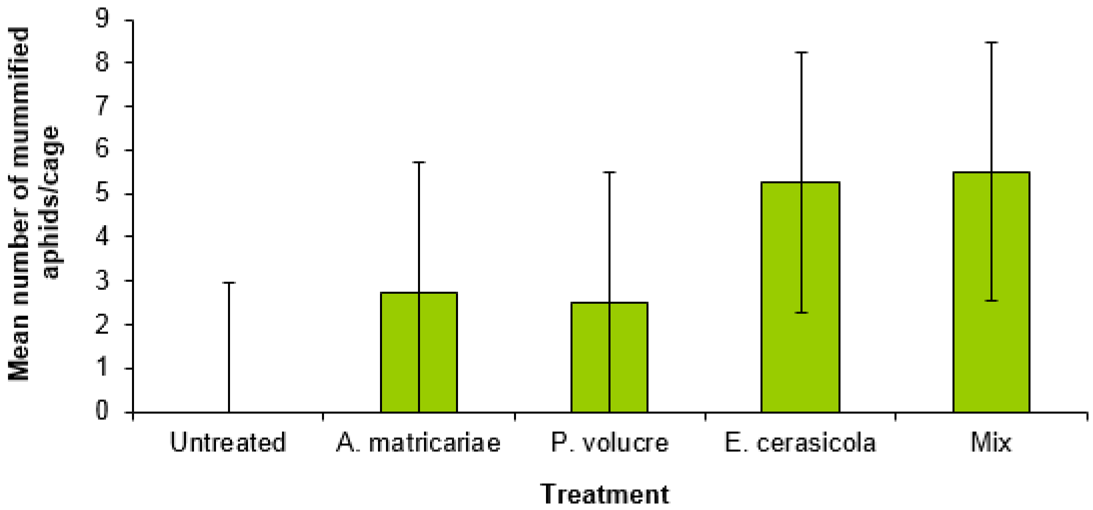

Figure 12. Mean number of parasitized mint aphids per cage for the five treatments with 95% confidence limits (n = 4 replicate cages)

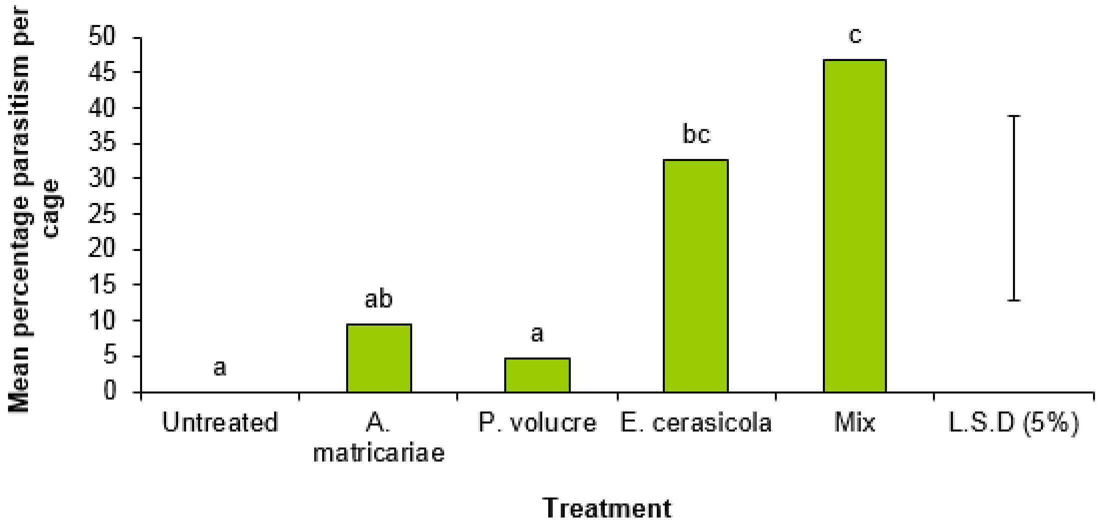

Figure 13. Mean percentage of mint aphid parasitism per cage for the five treatments (LSD 5%)

Temperature

Figure 14 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 the commercial herb nursery. When the cages were in the glasshouse minimum temperatures were always above 12.5°C. The temperatures remained within the optimum range for aphid parasitoids (10-30°C) for most of the experimental period.

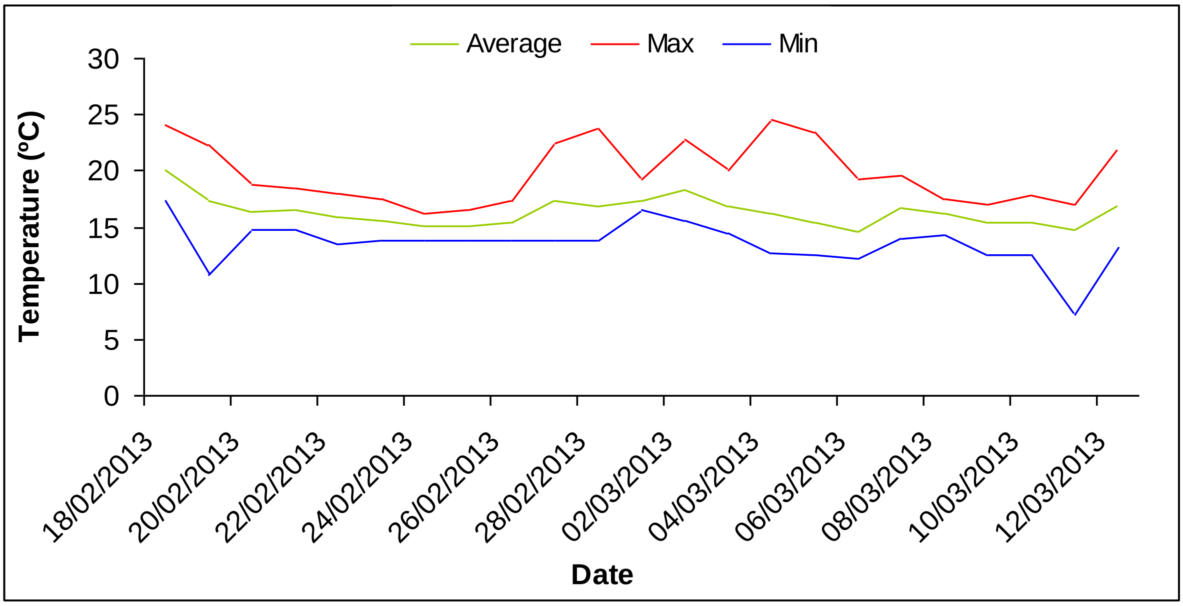

Figure 14. Mean average, maximum and minimum temperatures recorded throughout the experimental period

For mint aphid, the ideal candidate to take forward to Experiment 2 (determine effective release rates) would have been *E. cerasicola* but 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 parasitoid species and it was shown in PE 006 that the three other parasitoids (*Aphidius colemani*, *A. ervi* and *Aphelinus abdominalis*) do not parasitise 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 to 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.

Materials and methods

Experiment 3: 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 on parsley 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 in Experiment 1 (Table 3).

Table 3 Seven treatments used in Objective 2, Experiment 3 Hawthorn-parsley aphid

Treatment no.	No. plants per cage	No. infested plants	No. aphids per cage	No. and species of 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 (50x50x50m) 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 the commercial herb nursery 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 retained portions of aphid-infested plants on day 16 and 22 on 29 August 2012 and 4 September 2012 respectively.

Experiment 4: Efficacy of single and mixed parasitoid species for controlling hawthorn parsley aphid

As one 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 3 with amendments to the methods in order to try to reduce the variation in results between replicate cages.

The same seven treatments were used as in Experiment 3. The experimental method was similar to that used for mint aphid in Experiment 2. On 7 May 2013, 189 parsley plants were each infested with five mixed-aged hawthorn parsley aphids. Each cage contained nine plants which each had five aphids (45 aphids per cage) whereas in Experiment 3, 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 the commercial herb nursery on 8 May 2013. A ‘pooter’ was used to transfer the adult female parasitoids (10 of each species and 10 mixed) into separate specimen tubes which were then transported to the commercial nursery in a cool box. Prior to entering the nursery, the parasitoids were released into 21 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 parsley 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 16 the cages were transferred back to ADAS Boxworth and kept in a controlled temperature laboratory at 21ºC 16L:8D until day 20 when the assessment took place. Five plants in each cage were assessed in the same way as mint aphid in Experiment 2.

Statistical analysis

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

Results

Experiment 3: Efficacy of single and mixed parasitoid species for controlling hawthorn-parsley aphid

Numbers of healthy and parasitised aphids

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 (Figures 15, 16 and 17). Within the mixed treatments 75% were black mummies meaning either *E. cerasicola* or *A. abdominalis* was responsible. The remaining mummies had been parasitised by *P. volucre* (responsible for 25%).

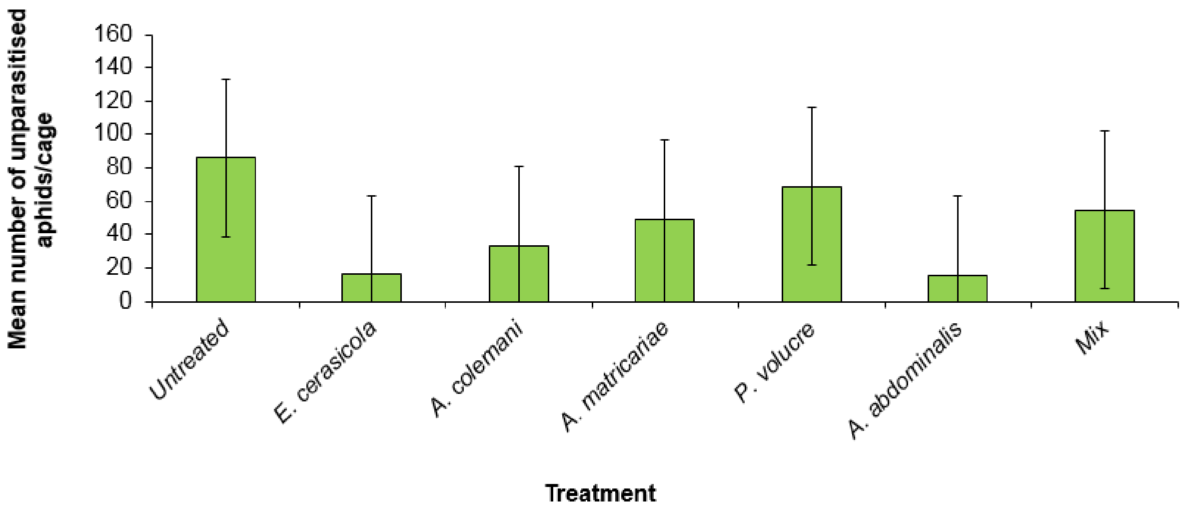

Figure 15. Mean number of unparasitised hawthorn-parsley aphids per cage for the seven treatments with 95% confidence limits (n=3 replicate cages)

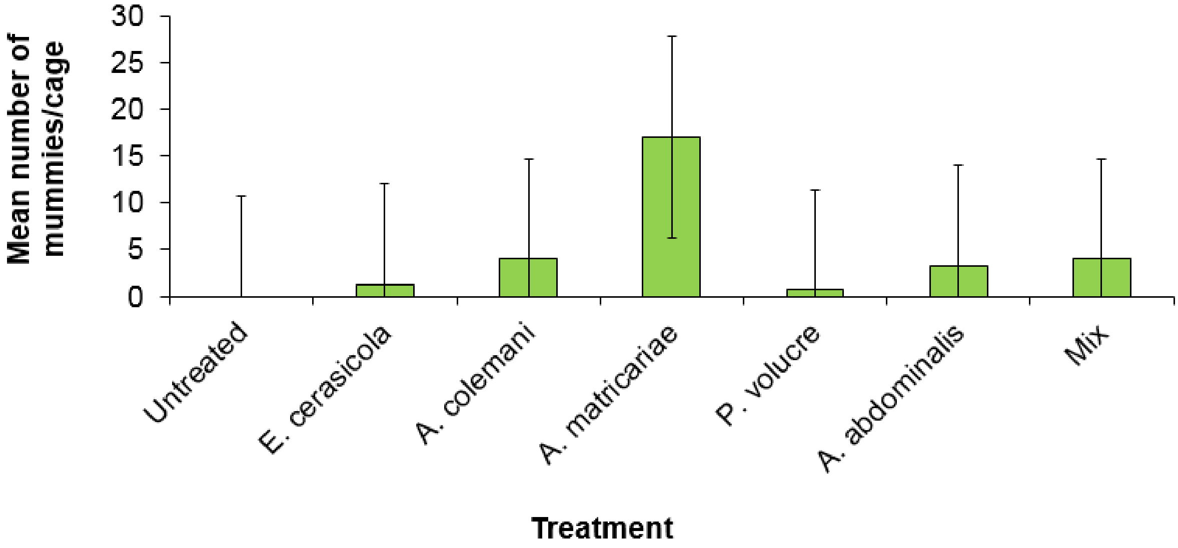

Figure 16. Mean number of parasitized hawthorn-parsley aphids per cage with 95% confidence limits (n=3 replicate cages)

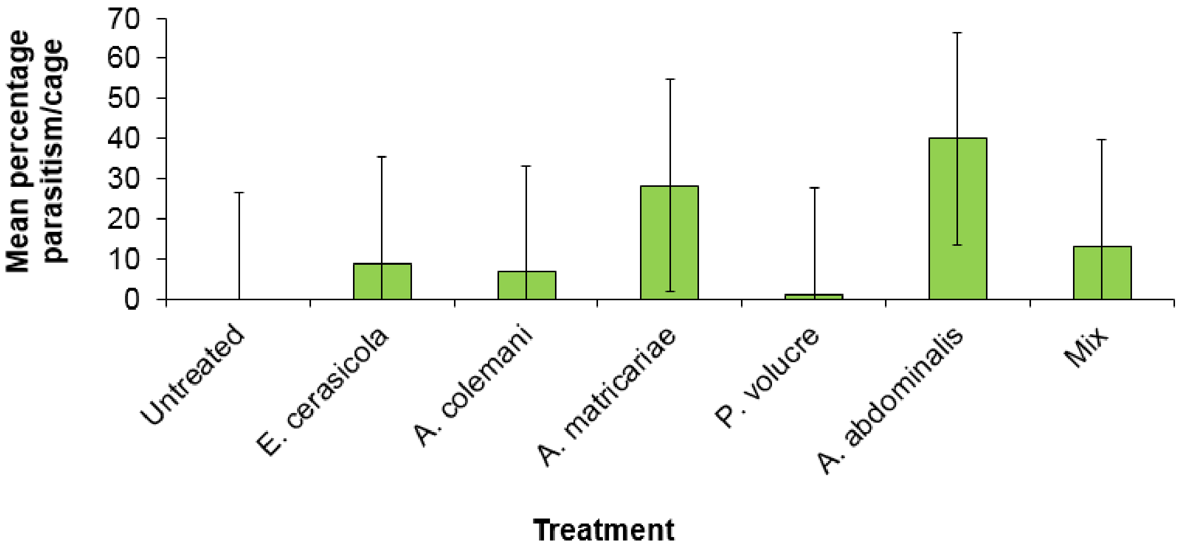

Figure 17. Mean percentage of hawthorn-parsley aphid parasitism per cage with 95% confidence limits (n=3 replicate cages)

Temperature

Figure 18 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. For most of the experimental period, temperatures were within the optimum range for aphid parasitoids (10-30°C).

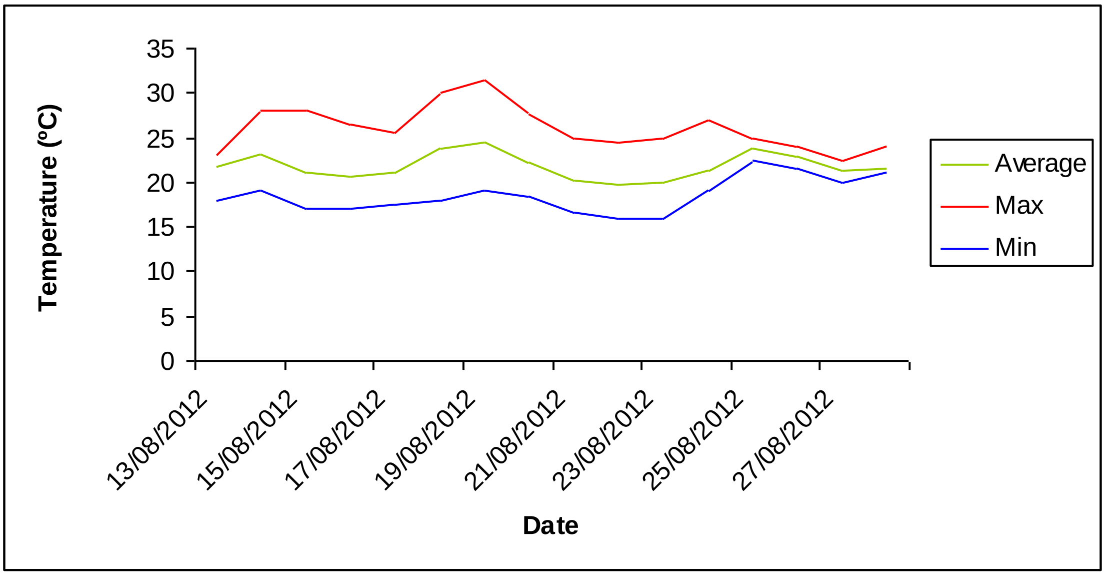

Figure 18. Mean average, maximum and minimum temperatures recorded throughout the experimental period

Experiment 4: Efficacy of single and mixed parasitoid species for controlling hawthorn parsley aphid

Numbers of healthy and parasitised aphids

Ephedrus cerasicola, Praon volucre, Aphidius matricariae, Aphelinus abdominalis and *Aphidius colemani* were all effective against hawthorn parsley aphid. Using any of the single species was just as effective as using a mix of the five species in reducing the number of live (unparasitised) aphids per plant to a mean of 1-13 per cage, compared with the untreated controls which had a mean of 2,122 per cage (Figure 19). Each of the single species and the mix of species gave 95-99% control of hawthorn-parsley aphid over a 20-day period. As all of the parasitoids performed equally well, *Aphidius colemani* was selected for the next experiment (determining effective release rates) against hawthorn parsley aphid as it is widely available as a single species and is the most cost effective.

Ephedrus cerasicola and *A. colemani* had a significantly higher mean number of mummies

(8 and 9 per cage respectively) than the untreated control (Figure 20). However, there were no significant differences in the percentage parasitism (38-90% in cages treated with parasitoids), (Figure 21). As in the mint aphid Experiment 2, it is likely that in addition to parasitism, the parasitoids reduced the numbers of healthy aphids in other ways such as host feeding. Within the mixed treatments *P. volucre* was responsible for 71.4% of the mummies followed by *A. abdominalis* (responsible for 14.3%) and *A. matricariae* (responsible for 14.3%)

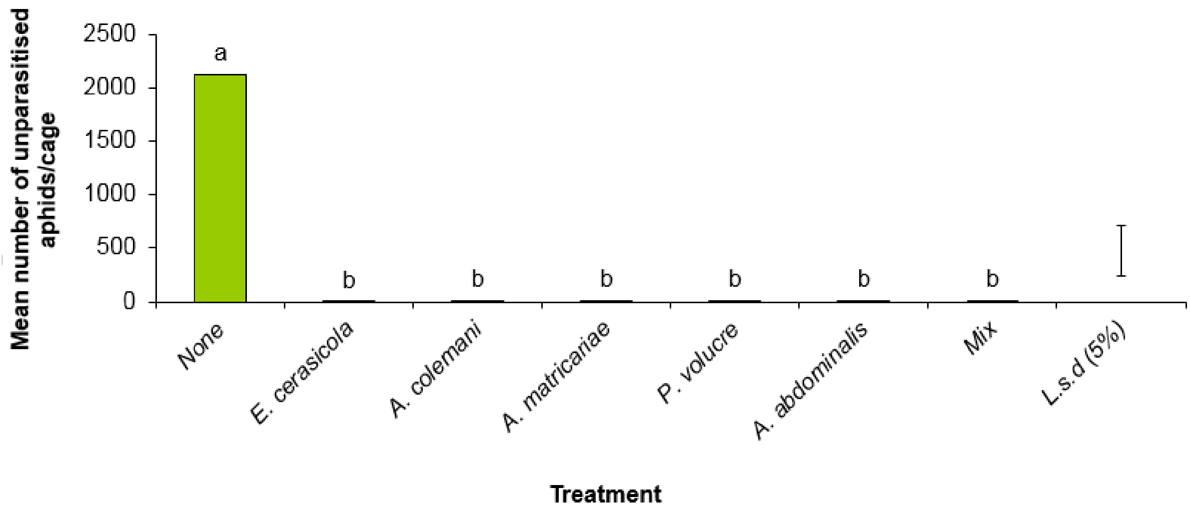

Figure 19 Mean number of unparasitised hawthorn-parsley aphids per cage for the seven treatments (LSD 5%).

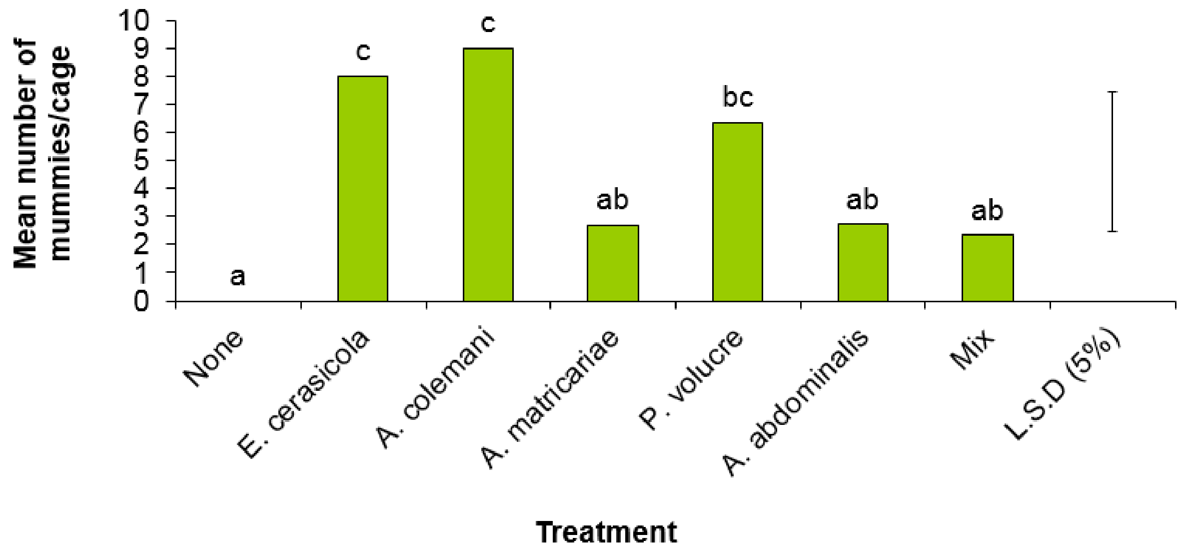

Figure 20 Mean number of parasitized hawthorn-parsley aphids per cage for the seven treatments (LSD 5%).

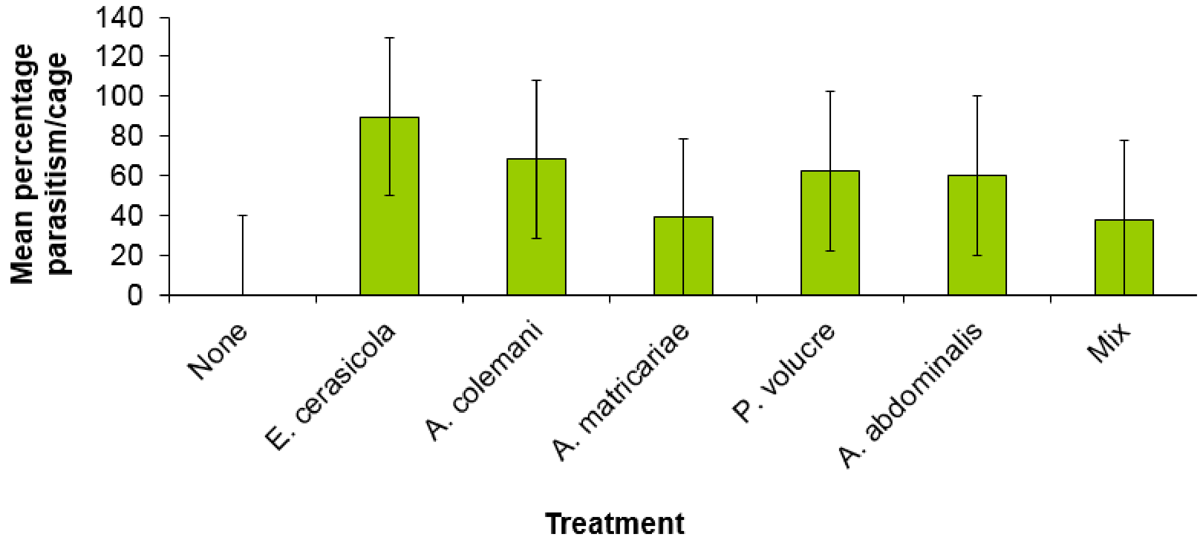

Figure 21 Mean percentage of hawthorn-parsley aphid parasitism per cage with 95% confidence limits (n=3 replicate cages)

Temperature

Figure 22 shows the mean average, maximum and minimum temperatures recorded throughout the experimental period from a data loggers placed in two cages. The mean temperature remained between 16.9 and 20.5°C. A maximum temperature of 25.5°C was recorded on 10 May and a minimum temperature of 14.5°C on 24 May. Temperatures remained within the optimum range for aphid parasitoids (10-30°C) during the experimental period.

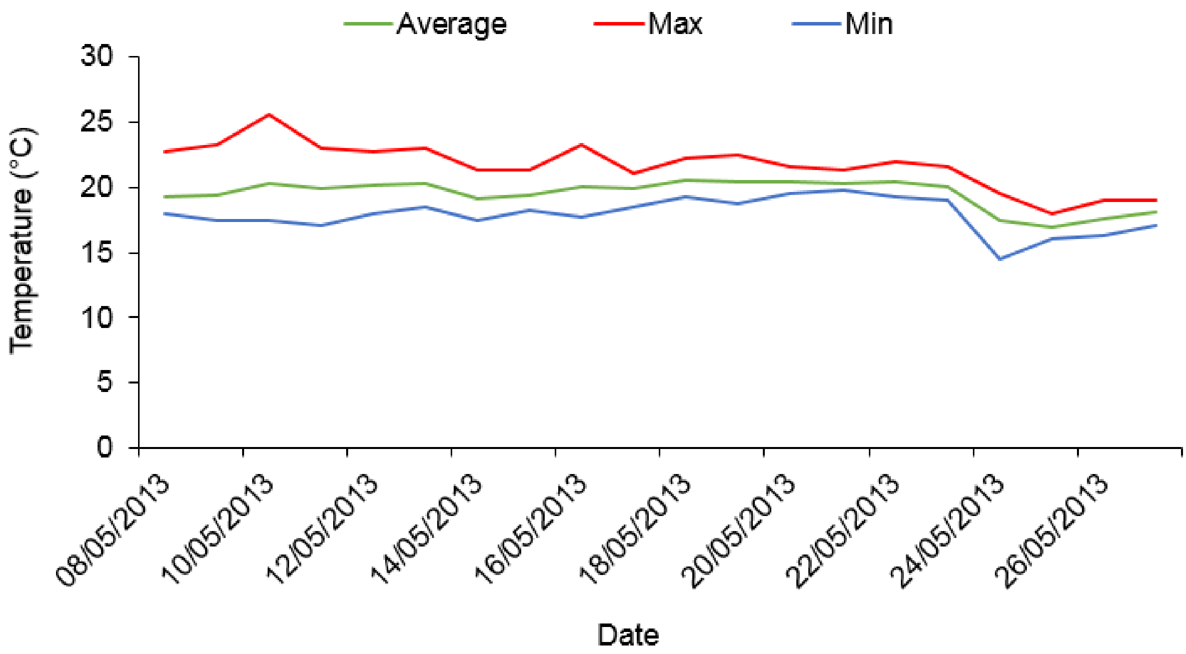

Figure 22. Mean average, maximum and minimum temperatures recorded throughout the experimental period

Experiments 5 and 6: **In small-scale experiments, determine an effective release rate of the selected single or mixed parasitoid species for control of hawthorn-parsley aphid and mint aphid.**

Materials and methods

Experiment 5: Determine a cost-effective release rate for mint aphid

This experiment aimed to determine the effective release rate of *Aphidius matricariae* for control of mint aphid. The experiment had four treatments (Table 4) and each treatment had four replicates. For the reasons previously discussed *A. matricariae* was selected for the development of a release strategy.

Table 4 *Aphidius matricariae* release rates and estimated cost per week

Treatment no.	Weekly release rate	Female *A. matricariae* parasitoids released per tent weekly (2.1m2)	Estimated cost
1	0	0	0
2	1 per m^2	2	£0.02/m^2 (£215/ha)
3	2 per m^2	4	£0.04/m^2 (£430/ha)
4	4 per m^2	8	£0.09/m^2 (£861/ha)

On 19 April 2013, 16 tent cages (145cm x 145 cm) were constructed and stood on the production lines on the commercial nursery (Figure 23). The floor of each cage was lined with capillary matting.

Figure 23 Tent cage mint aphid Experiment 5 on commercial nursery

Sixty-four mint plants were arranged in each cage to represent the commercial spacing of mint plants (Figure 24). Of these 64 plants, the same 18 plants (designated by black stars in Figure 24) in each tent were labelled to indicate that they were to be sampled.

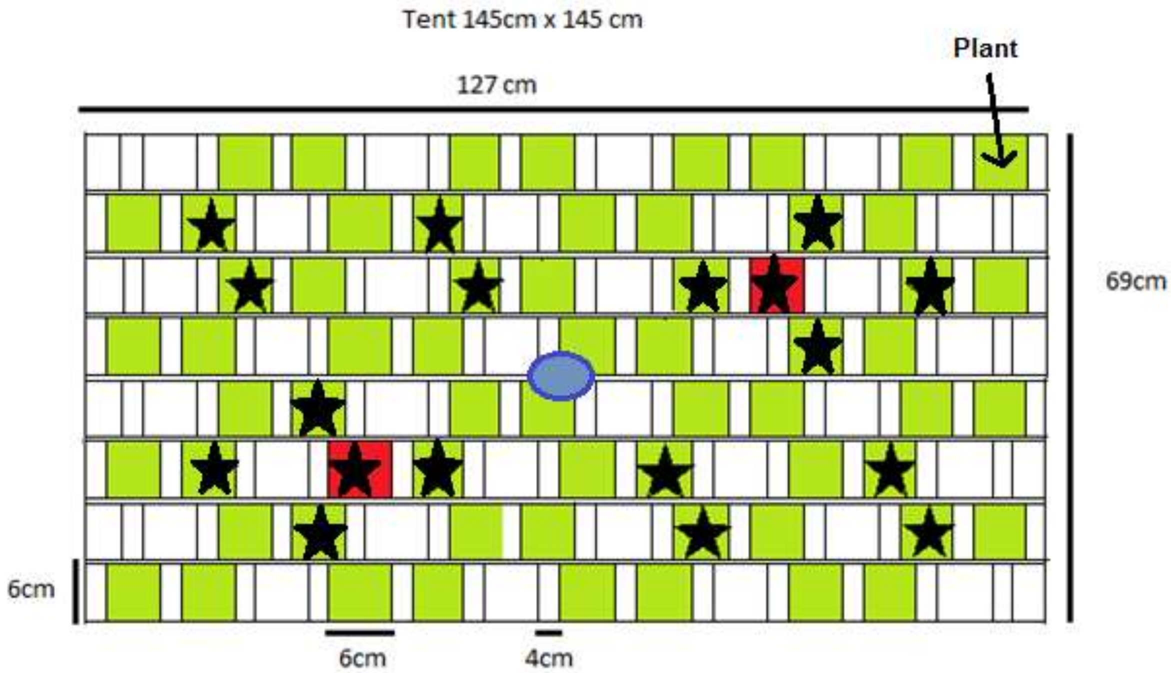

Figure 24 Arrangement of the 64 mint plants in each tent. Red plants are those which were inoculated with two mint aphids each. The 18 plants with black stars were sampled. The blue circle shows where the parasitoids were released from.

Mint aphids and parasitoids were brought from Boxworth for release into each cage. Sixteen petri dishes containing a sprig of mint and eight medium sized mint aphids were prepared. Two plants in each cage were infested with two aphids each using a fine paintbrush to transfer them (plants designated with a red square in Figure 24). Four tubes containing 2, 4 and 8 female *A. matricariae* were also prepared. The appropriate number of *A. matricariae* depending on the treatment were released into each tent by placing the opened tube on the base of the tent between two pots in the centre (as shown by the blue circle in Figure 24). A tube containing a 1:1 solution of honey and water covered with parafilm was also provided for the parasitoids to feed on. The plants were watered using the overhead irrigation.

Further weekly releases of *A. matricariae* were made on 26 April and 3 May. On 3 May it was observed that at the back of the line the plants in the centre of each cage had wilted and died due to drought.

On 8 May the plants were collected from the nursery. The protocol planned for the same 18 plants from each tent to have been collected into plastic boxes and brought back to ADAS, Boxworth for sampling. However, because some of the plants in each cage had died the nearest surviving plant had to be collected instead. All remaining plants were put into heavy duty bin bags and brought back to ADAS Boxworth for disposal.

At ADAS Boxworth, the number of healthy aphids and the number of aphid mummies on each assessment plant, compost and pot were counted. Plant material and live aphids sampled from each tent were placed in ventilated sandwich boxes and after approximately 14 days the number of additional mummies which developed were recorded.

Experiment 6: Determine a cost-effective release rate for hawthorn-parsley aphid

This experiment aimed to determine the effective release rate of *A. colemani* for control of hawthorn-parsley aphid. For reasons previously discussed, *A. colemani* was selected for the development of a release strategy. The release rates tested were the same as those tested for mint aphid; 0, 1, 2 and 4 parasitoids per m^2 (Table 5). The experimental method was also the same except that the trial took place in the glasshouse compartments at ADAS, Boxworth. It was decided to carry out the trial at ADAS, Boxworth, so that the watering regime could be carefully monitored due to the susceptibility of the small pots drying out over a short period of time.

Table 5 *Aphidius colemani* release rates and estimated costs

Treatment no.	**Weekly release rate**	**Female *A. colemani* parasitoids released per tent weekly (2.1m2)**	**Estimated cost**
1	0	0	0
2	1 per m2	2	£0.2/m2 (£205/ha)
3	2 per m2	4	£0.4/m2 (£410/ha)
4	4 per m2	8	£0.8/m2 (£819/ha)

Four glasshouse compartments were used and each contained a full set of treatments (Figure 25). The trial was set up on 13 August 2013 and the assessment took place on 2 & 3 September. Weekly releases of *A. colemani* were made on 20 August and 27 August. A temperature logger was placed in a tent cage in each of the four glasshouse compartments. The capillary matting in each tent cage was watered regularly.

Figure 25 Tent cage Experiment 6 at ADAS, Boxworth on hawthorn-parsley aphid.

Results

Experiment 5: Determine an effective release rate for mint aphid

Numbers of healthy and parasitised aphids

The overhead irrigation on the commercial nursery did not penetrate through the cages sufficiently and in combination with a very hot weekend many of the plants in each cage died from drought. While best efforts were made to assess the remaining plants no conclusive results could be drawn from the experiment.

Experiment 6: Determine an effective release rate for hawthorn parsley aphid

Numbers of healthy and parasitised aphids

No significant effects were observed for the mean number of live aphids per cage (Figure 26) or the mean number of mummies per cage (Figure 27) due to the high variation between cages. However, there was a trend for the highest release rate (4 per m^2) to have the lowest number of aphids and highest number of mummies. The mean percentage parasitism per cage was significantly higher at the highest release rate of 4 per m^2 compared with the other treatments (Figure 28)

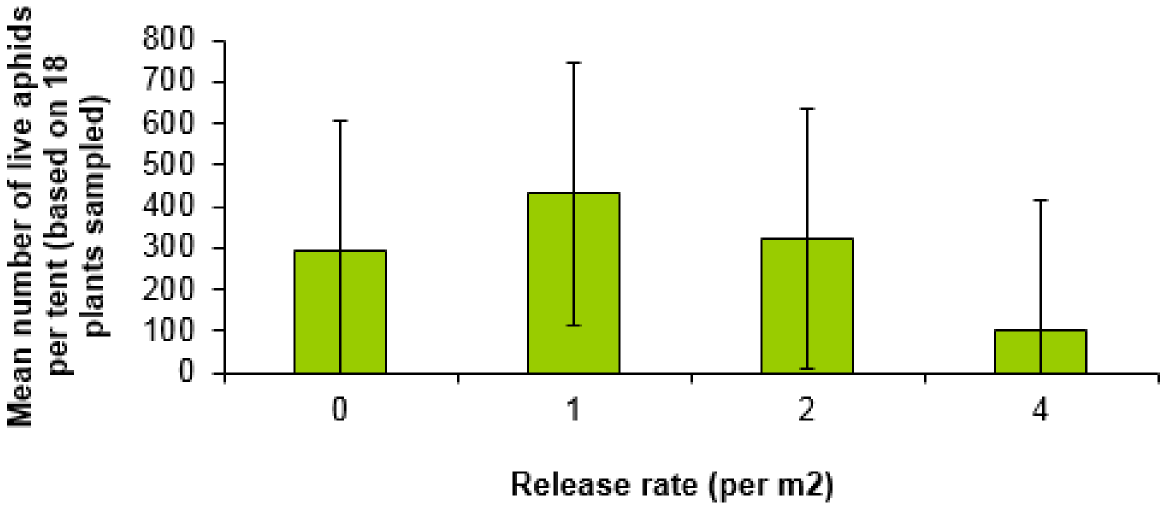

Figure 26. Mean number of unparasitised hawthorn-parsley aphids per cage for each release rate with 95% confidence limits (n=3 replicate cages)

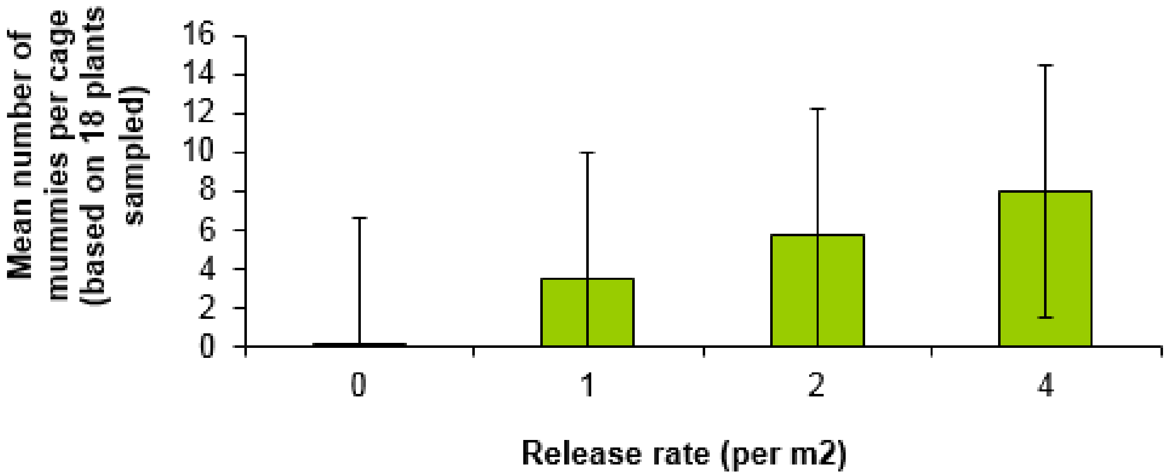

Figure 27. Mean number of parasitised hawthorn-parsley aphids per cage for each release rate with 95% confidence limits (n=3 replicate cages)

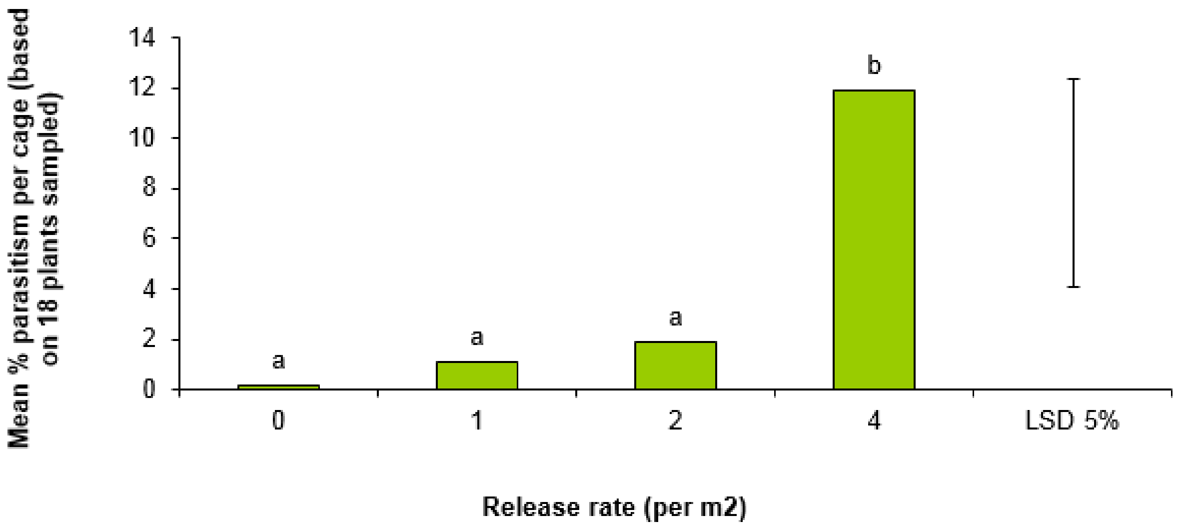

Figure 28. Mean percentage hawthorn-parsley aphid parasitism per cage for each release rate (LSD 5%)

Temperature

Figures 29, 30 and 31 show the mean average, maximum and minimum temperatures recorded throughout the experimental period respectively. The mean temperature remained between 19.4 and 24.3°C in all four compartments. A maximum temperature of 30.5 33, 31 and 28.5 °C was recorded on in glasshouse 1, 2, 3 and 4 respectively. A minimum temperature of 16, 15, 15 and 14°C was recorded on in glasshouse 1, 2, 3 and 4 respectively. Temperatures remained between the optimum range for aphid parasitoids (10-30°C) during the experimental period.

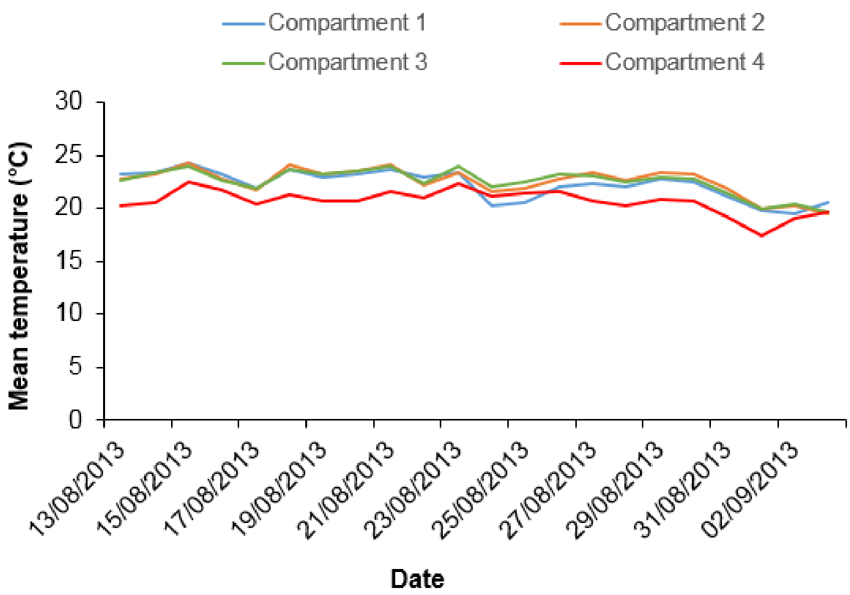

Figure 29 Mean temperature recorded throughout the trial period in the four glasshouse compartments

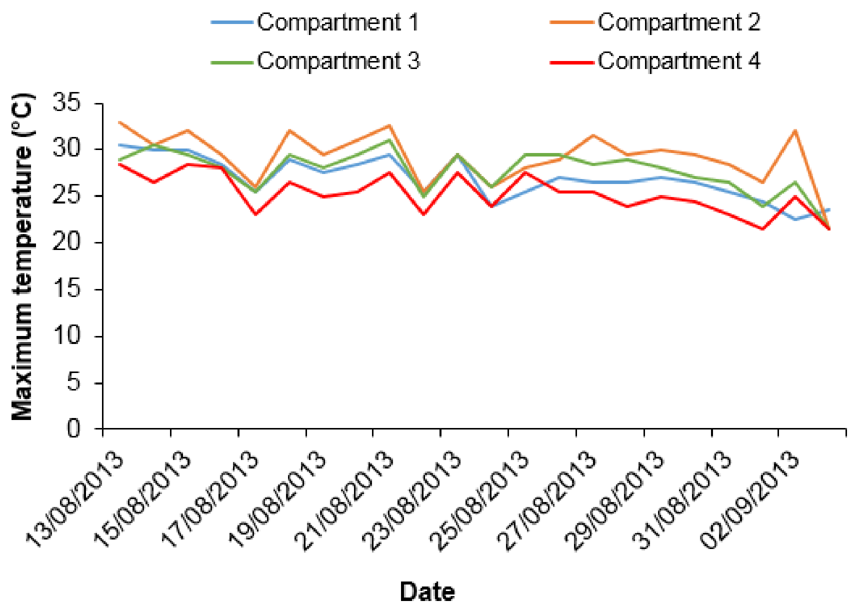

Figure 30 Maximum temperatures recorded throughout the trial period in the four glasshouse compartments

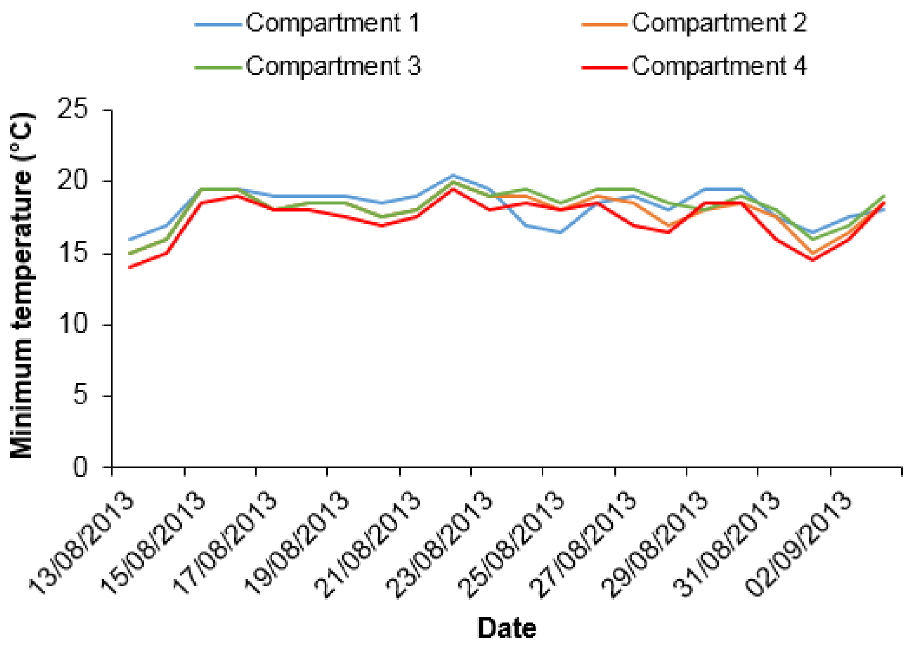

Figure 31 Minimum temperatures recorded throughout the trial period in the four glasshouse compartments

Objective 3: In small-scale research glasshouse experiments, test the robustness of the selected parasitoid release rate and frequency against a range of hawthorn-parsley aphid and mint aphid densities using repeated releases of parasitoids.

This experiment was due to be done during 2014 but hawthorn-parsley aphids did not infest parsley on the host nursery until late September. HDC and BHTA were consulted via the industry representative and it was decided not to carry out this experiment.

Discussion

Parasitoid searching in spaced and unspaced plants

The results from this study indicate that *A. colemani* was more effective at parasitising hawthorn-parsley aphids in spaced pots of parsley than in unspaced plants. This indicates that the parasitoids might be inhibited from searching for this species of aphid (which infests the base of parsley plants) when plants are closely spaced early in the production cycle. This might be one of the reasons why growers have not observed parasitised hawthorn-parsley aphids during the production cycle. However, as *A. colemani* led to 92% and 89% control of live aphids in spaced and unspaced plants respectively in this experiment, the parasitoids must have reduced aphid numbers in other ways in addition to parasitism (see below under heading 'Additional effects of parasitoids')

Parasitoid efficacy against mint aphid

When comparing the effectiveness of individual and mixed species on the parasitism of mint aphid, the initial experiment had too much variation in the data from replicate cages within the treatments to make confident conclusions. Steps were successfully made to reduce this variation and results from the second experiment indicated that mint aphid is more effectively parasitised 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 parasitised by multiple species.

However, *E. cerasicola* is not commercially available as a single species and is only available as a mix of six species, three of which (*Aphidius colemani, Aphidius ervi* and *Aphelinus abdominalis*) do not parasitise mint aphid. Therefore *A. matricariae* was selected to test in an experiment to determine a cost-effective release rate for control of mint aphid. However, this experiment on a commercial nursery gave inconclusive results due to many of the experimental plants in the cages dying from drought after a watering problem during a particularly hot weekend.

Parasitoid efficacy against hawthorn-parsley aphid

As with mint aphid, the first experiment to compare parasitoid efficacy gave inconclusive results due to large variation in aphid numbers between replicate treatments. In the second experiment using a different method, *Aphidius colemani*, *Ephedrus cerasicola, Praon volucre, Aphidius matricariae* and *Aphelinus abdominalis* were all effective against hawthorn- parsley aphid and using any of the single species was just as effective as using a mix of the five species. *A. colemani* was selected to test in an experiment in large cages to determine a cost-effective release strategy for hawthorn-parsley aphid, as it is widely available as a single species and would be more cost-effective. The highest weekly release rate (4 per m^2) was the most effective, giving significantly higher percentage aphid parasitism (12%). However, numbers of healthy aphids were not significantly reduced compared with those in untreated controls, thus it is likely that a higher release rate would be needed to control hawthorn-parsley aphid at the aphid densities used in this experiment. A trial on a commercial parsley crop was due to be done during 2014 to further test a release strategy, but hawthorn-parsley aphid did not infest parsley on the host nursery until late September and in consultation with the HDC and BHTA, it was decided not to carry out this experiment.

Additional effects of parasitoids

In experiments carried out on both aphid species in this project, numbers of healthy aphids were significantly reduced to very low numbers by the effective parasitoid species compared with in untreated controls, but percentage parasitism levels were lower than expected:

- A single introduction of *A. matricariae* and a mix of *A. matricariae, P. volucre* and *E. cerasicola* at a high release rate (40/m^2) reduced mint aphid populations in cages by 86% and 98% respectively over a 3-week period compared with those in untreated controls. However, percentage parasitism with the different species varied from only 5% to 48%.
- A single introduction of *A. colemani* and a mix of *A. colemani*, *A. matricariae, E. cerasicola, P. volucre* and *A. abdominalis* at a high release rate (40/m^2) reduced hawthorn-parsley aphid numbers in cages by 99% over a 3-week period. However, percentage parasitism with the different species varied from 38% to 90%.
- A single release of *A. colemani* at 20/m^2 reduced hawthorn-parsley aphid numbers in cages by 92% on spaced plants and by 89% on unspaced plants. However, percentage parasitism was only 21% in unspaced plants and 64% in spaced plants.

One possible additional mechanism for parasitoids to reduce numbers of healthy aphids could have been parasitoid host-killing via host feeding, where the parasitoid acts as a predator. This was observed in the laboratory 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. Another factor could have been aphids moving away from the plants when parasitised, possibly in an attempt to escape parasitism. This was observed with *E. cerasicola* as some black mummies were observed off the mint plants, for example on plant labels.

These additional effects of parasitoids could explain why very few mummies are seen on mint or parsley plants by growers after releasing parasitoids. The reduction in numbers of healthy aphids by parasitoids, without the production of many mummies on the plants 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.

Conclusions

- *Ephedrus cerasicola, Praon volucre* and *Aphidius matricariae*, either as individual species or as a mix were effective against mint aphid. *E. cerasicola* and the species mix were the most effective, with a single high release at 40 per m^2 reducing healthy aphid numbers by 86% and 98% respectively over a 3-week period. This release rate would not currently be cost-effective, at an estimated cost of 86 pence per m^2 for *A. matricariae* or £2 per m^2 for the parasitoid mix, using example costs from suppliers.
- *Ephedrus cerasicola, Praon volucre, Aphidius matricariae, Aphelinus abdominalis* and *Aphidius colemani* were all effective against hawthorn-parsley aphid. Using any of the single species was just as effective as using a mix of the five species. A single high release rate of either *A. colemani* or the mix of species at 40 per m^2 reduced healthy aphid numbers by 99% over a 3-week period. This release rate would not currently be cost-effective at an estimated cost of 82 pence per m^2 for *A. colemani* or £2 per m^2 for the parasitoid mix.

- *Aphidius colemani* was more effective at parasitising hawthorn-parsley aphids on spaced pots of parsley than on pot thick pots. However, a single release at 20 per m^2 reduced healthy aphid numbers by 92% on spaced plants and by 89% on unspaced plants. This release rate is currently expensive at an estimated cost of 41 pence per m^2, however losses due to hawthorn-parsley aphids can exceed 20% and if aphicides are needed this incurs extra cost.

- It is likely that parasitoids had additional effects to parasitism, such as host-feeding or causing aphids to drop off the plants. These factors could help to explain why growers who release parasitoids to mint or parsley do not see many parasitised mummies. If adequate control of live aphids is given, this 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.
- Using *A. colemani* at three weekly releases at a more cost-effective rate of four per m^2 (estimate cost eight pence per m^2 per week) did not give adequate control of hawthorn-parsley aphids on a 5-week crop of parsley in large cages. A further trial on a commercial crop planned to further test a cost-effective release strategy was not possible to complete due to hawthorn-parsley aphids infesting the parsley crop on the host nursery too late in the season.

Knowledge and Technology Transfer

Publications

- 'Aphidsure mix – Coming to the aid of protected herb growers'. *BCP Certis Technical Review*. March 2012.
- Bennison, J., Pope, T., Greetham, J., Evans, T. and Maher, H. (2012). Improved biological control of 'problem' aphids on protected herbs. *IOBC wprs Bulletin **80**, 155-158.*
- HDC News article July 2013

Presentations

- Update on project at BHTA Technical Meeting, Harper Adams University, October 2011.
- Presentation at Association of Applied Biologists (AAB) meeting 'Advances in Biological Control', Olde Barn Hotel, Marston, November 2011.
- Presentation at the IOBC working group meeting 'Integrated control in protected crops, Mediterranean climate, Sicily, September 2012.

- Presentation at the Royal Entomological Society Special Interest Group, Aphids, September 2013.
- Update to herb growers at the workshop on Integrated control of pests, diseases and weeds, Royal showground, Stoneleigh, February 2014.

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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.

