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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.

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GROWER SUMMARY

Headline

New information has been provided on the potential of commercially-available parasitoids for biological control of hawthorn-parsley aphid and mint aphid.

Background and objective of project

Until recently, biological control of aphids on protected crops relied mainly on three aphid parasitoid species; *Aphidius colemani, Aphidius ervi* and *Aphelinus abdominalis*. A new aphid parasitoid mix produced by Viridaxis and available from BCP Certis includes these three parasitoid species as well as three 'new' species; *Aphidius matricariae, Praon volucre* and *Ephedrus cerasicola*. This mix of six parasitoid species has given good control of a wide range of 'difficult' aphid species on strawberry and ornamental pot plants.

This project investigated the potential of the six currently available parasitoid species (provided by Viridaxis through BCP Certis) against two 'problem' aphid species, hawthorn-parsley aphid and mint aphid, on all year round protected pot herbs.

Summary of the project and main conclusions

Objective 1: In laboratory studies, determine the parasitism and host-killing of hawthorn-parsley aphid and mint aphid by the six parasitoid species

Parasitism: the behaviour of Aphidius colemani, Aphidius ervi, Aphidius matricariae, Praon volucre, Ephedrus cerasicola and Aphelinus abdominalis was observed in Petri dishes when offered a mix of second and third instar aphids (the life stage preferred for parasitism). Either 20 hawthorn-parsley aphids or 20 mint aphids were offered on a piece of parsley or mint respectively. Each mated female parasitoid was observed using a binocular microscope for 10 minutes, recording the number of times that the parasitoid attacked an aphid with its ovipositor (egg-laying tube). At the end of the observation period the parasitoid was allowed to continue to parasitise the aphids for a further two hours. The parasitoid was then removed and the aphids transferred to clean potted parsley or mint plants, using a separate pot of plants for each dish of aphids. Each pot of plants was then covered with a perforated bread bag in order to prevent other parasitoids or predators from

attacking the aphids. Numbers of mummified (parasitised) aphids were recorded after 10 and 14 days.

Hawthorn-parsley aphid: all six parasitoid species attacked the aphids, although only one *Aphidius ervi* individual made a single attempt to parasitise a hawthorn-parsley aphid. *Aphidius colemani, Praon volucre* and *Ephedrus cerasicola* made the most attacks, although the number of attacks varied between individuals. All the parasitoid species except for *Aphidius ervi* successfully parasitised the aphids, confirmed by the presence of mummified aphids 14 days after the observations were completed. However, the number of mummified aphids was low and aphid mummies were not always recorded from dishes where attacks had occurred. This suggests that attacked aphids sometimes died before mummifying, that attacks did not necessarily result in an egg being laid in the aphid or that eggs were laid but that the parasitoid was not always able to develop within this host.

Mint aphid: five of the six parasitoid species (all except *Aphidius ervi*) were observed to attack the aphids. *Aphidius colemani, Aphidius matricariae* and *Ephedrus cerasicola* were recorded to make the most attacks, although the number of attacks varied between individuals. Mummified aphids were recorded 14 days after being attacked by *Aphidius matricariae, Ephedrus cerasicola* and *Praon volucre* but not by *Aphidius colemani.* The other parasitoid species did not successfully parasitise the mint aphids and did not lead to aphid mummies developing.

Host-killing – evidence of host-killing by Aphidius colemani, Aphidius ervi, Aphidius matricariae, Praon volucre, Ephedrus cerasicola and Aphelinus abdominalis was recorded by confining a mated female parasitoid in a Petri dish together with a mixture of second and third instar aphids for 24 hours. Ten aphids of either species were offered on a piece of parsley or mint respectively. After 24 hours the aphids in each dish were checked using a microscope, recording the numbers alive or dead.

Hawthorn-parsley aphid: the mean percentage of aphids dead after 24 hours ranged from 7.3% with *Aphidius matricariae* to 62.7% with *Aphidius colemani*. Aphid mortality when no parasitoid was confined with the aphids was 12.5%. Aphid mortality in dishes containing *Aphidius colemani*, *Ephedrus cerasicola* (46.7%) and *Aphelinus abdominalis* (48.3%) was significantly higher than in dishes not containing an aphid parasitoid. Aphid mortality in dishes containing *Aphidius colemani* was not significantly higher than in dishes containing *Ephedrus cerasicola* or *Aphelinus abdominalis*. The other parasitoid species did not significantly increase aphid mortality. Where aphid mortality was significantly increased by

the presence of an aphid parasitoid this may have been due to the parasitoids' host feeding on the aphids, or by repeated 'stings' during egg laying or by the parasitoids disturbing the aphids so that they left the plant material and starved.

Mint aphid: the mean percentage of aphids dead after 24 hours ranged from 9.8% with *Aphidius matricariae* to 54.3% with *Ephedrus cerasicola*. Aphid mortality when no parasitoid was confined with the aphids was 11.4%. Aphid mortality in dishes containing *Aphidius ervi* (32.5%), *Praon volucre* (42.5%), *Ephedrus cerasicola* and *Aphelinus abdominalis* (41.1%) was significantly higher than in dishes not containing an aphid parasitoid but did not differ significantly from each other. The other parasitoid species did not significantly increase aphid mortality. The reasons for the higher aphid mortality recorded are likely to have been as previously described.

Aphidius matricariae is reputed to be an aggressive parasitoid, with the females able to kill aphids by repeated 'stinging' with their ovipositors, in addition to acting as a parasitoid (de Menten, personal communication). However, results with hawthorn-parsley aphid and mint aphid found no evidence of this behaviour either in the number of attacks recorded or in aphid mortality

	Hawthorn-parsley aphid				Mint aphid			
Parasitoid species	Attacks recorded	Mummified aphids	Host- killing	Attacks recorded	Mummified aphids	Host- killing		
Aphidius colemani	✓	✓	✓	✓	-	-		
Aphidius ervi	√ *	-	-	-	-	✓		
Aphidius matricariae	\checkmark	\checkmark	-	\checkmark	\checkmark	-		
Praon volucre	✓	✓	-	\checkmark	\checkmark	\checkmark		
Ephedrus cerasicola	\checkmark	✓	✓	\checkmark	\checkmark	\checkmark		
Aphelinus abdominalis	\checkmark	✓	✓	✓	-	\checkmark		

 Table 1. Summary of results from laboratory experiments recording parasitism and hostkilling of six aphid parasitoid species.

✓ = attack recorded, *= only a single attack recorded, - = no attack recorded

Objective 2: In small-scale research glasshouse experiments, evaluate control of hawthorn-parsley aphid and mint aphid by individual or mixed parasitoid species.

Aphidius colemani and Aphidius matricariae were selected for these semi-field experiments to test control of hawthorn-parsley aphid and mint aphid on potted parsley and mint plants

respectively. The experiments were done using insect cages placed in a computercontrolled glasshouse compartment set to maintain a temperature of approximately 20°C.

To record parasitism of hawthorn-parsley aphids by *Aphidius colemani*, 12 pots of pest-free young parsley plants were each infested with 25 mixed-age hawthorn-parsley aphids. Two infested pots of plants were then placed into each of six insect cages. After 24 hours, five mated female *Aphidius colemani* were released into three of the cages (a rate equivalent to $40/m^2$). No parasitoids were released into the other three 'control' cages. After 10 days the numbers of live and mummified aphids were recorded and the numbers of additional mummies were recorded after a further seven days. To record parasitism of mint aphids by *Aphidius matricariae* a similar experiment was done but the numbers of live and mummified aphids were released and the final assessment of the numbers of mummified aphids done after a further nine days.

Hawthorn-parsley aphid: 10 days after releasing five mated female *Aphidius colemani* in cages containing 50 hawthorn-parsley aphids the mean number of aphids recorded remained largely unchanged at 56 per cage. However, of the aphids recorded, 57% had been parasitised. In comparison, in cages where no parasitoids had been released the number of hawthorn-parsley aphids had increased from 50 to a mean of 294 in 10 days.

Mint aphid: 10 days after releasing five mated female *Aphidius matricariae* in cages containing 50 mint aphids the number of aphids recorded had been reduced to a mean of 31 per cage. Most of these aphids were healthy but 9% had been parasitised. In comparison, in cages where no parasitoids had been released the number of mint aphids had increased from 50 to a mean of 142 per cage in 10 days.

There is published evidence that aphid parasitoids may attack aphids more readily if they have developed on an aphid of the same species or have already successfully attacked aphids of that species. To test this, an additional experiment using hawthorn-parsley aphids and *Aphidius colemani* was completed. Nine cages were prepared with aphid-infested parsley plants as previously described. Two mated female *Aphidius colemani* direct from BCP Certis were released into three of the cages (a rate equivalent to 16/m²). Two 'conditioned' mated female *Aphidius colemani* that had been reared on hawthorn-parsley aphids were released into three further cages. No parasitoids were released into the final three 'control' cages.

'Conditioned' *Aphidius colemani*: 10 days after releasing two mated female *Aphidius colemani* that had been reared on hawthorn-parsley aphids, in cages containing 50 hawthorn-parsley aphids the mean number of aphids recorded had increased to 149 per cage. Similarly, in cages where two mated female *Aphidius colemani* direct from BCP Certis were released, the mean number of hawthorn-parsley aphids increased from 50 to 115 per cage. In cages where no parasitoids had been released the mean number of hawthorn-parsley aphids had increased from 50 to 149. These results indicate that a single release of two *Aphidius colemani*, either 'conditioned' or direct from BCP Certis, did not effectively control hawthorn-parsley aphid when each plant was initially infested with 25 aphids.

Evidence to indicate that 'conditioned' parasitoids were more effective in controlling hawthorn-parsley aphid than parasitoids direct from BCP Certis was inconclusive. Parasitised aphids were only recorded in one of the three cages in which 'conditioned' parasitoids had been released, suggesting that in the other two cages the parasitoids died before parasitising any aphids or were unable to parasitise these aphids. Overall mean parasitism per cage was, therefore, low (14%) but in the cage where parasitism was recorded it was high (42%) and the number of healthy aphids low (46). In cages where parasitoids direct from BCP Certis were released, parasitism was recorded in each cage, with a mean of 22% (with a range of 15 to 30%) of aphids parasitised.

Objective 3: In an experiment on a commercial herb nursery, evaluate control of hawthorn-parsley aphid on parsley by selected individual/mixed parasitoid species.

The planned experiment could not be done as work in Objectives 1 and 2 took much longer than expected, by which time numbers of plants infested with hawthorn-parsley aphid on the host nursery were much lower than earlier in the season and this would have put the experiment at risk. Therefore, intensive monitoring of parsley crops on two commercial nurseries was done to establish the aphid infestation time during the 5-week production period and the percentage parasitism given by the growers' release strategies. On both crops, hawthorn-parsley aphids were found only on plants 4-5 weeks after they were sown. Parasitism was recorded at only one site and then at very low levels (2.3% of aphids parasitised). A tentative identification by the Natural History Museum indicated that the single parasitoid collected from this site was an *Aphidius matricariae* male. This parasitoid species must have been naturally-occurring as at this site, only *Aphidius colemani* had been released. At the second site no parasitism of hawthorn-parsley aphids was recorded even

though the mix of six parasitoid species had been released weekly. It is likely that the aphids infested the parsley too late in the production stage for any parasitised aphids to have developed into visible 'mummies' before the marketing stage.

Financial Benefits

Results from this project have shown that a single release of a high rate of *Aphidius colemani* and *Aphidius matricariae* can reduce populations of hawthorn-parsley aphid and mint aphid on parsley and mint respectively. Further work is needed to determine whether releases of multiple parasitoid species are more effective than those of a single species and to establish a cost- effective release strategy for successful biological control of both these aphid species. This work will be done in the extension project to PE 006 during 2012. Developing improved biological control strategies for these aphid species will contribute to more robust IPM programmes, reduced plant losses and reduced labour time needed for effective aphid control.

The new parasitoid mix includes three species able to successfully parasitise mint aphid, *Aphidius matricariae*, *Praon volucre* and *Ephedrus cerasicola*. Until recently these three species had not been available to growers. Results of this project will save growers wasting money on releasing single species of the previously available parasitoid species such as *Aphidius colemani* in mint crops.

Action Points

- Ensure that aphids on parsley and mint are correctly identified. Use the HDC Crop Walkers Guide for herbs or the HDC Herb Best Practice Guide <u>www.hdc.org.uk/herbs/</u> for help with recognition.
- On nurseries where hawthorn-parsley aphid occurs, consider releasing *Aphidius colemani* or the mix of six parasitoid species available from BCP Certis, which includes *A. colemani* and four other parasitoid species that also attack this aphid.
- Where mint aphid is confirmed on mint, consider releasing the mix of six parasitoid species available from BCP Certis. Three parasitoid species in this mix, *Aphidius matricariae*, *Praon volucre* and *Ephedrus cerasicola*, attack mint aphid.
- Do not release Aphidius colemani to mint as it will not control mint aphid.

SCIENCE SECTION

Introduction

Biological control of aphids on protected crops currently relies mainly on three aphid parasitoid species:

- Aphidius colemani for control of the peach-potato aphid, Myzus persicae and the meloncotton 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 *Aphidius colemani* usually gives effective control. However, 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 currently approved entomopathogenic fungi ('Vertalec' and '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 IPMcompatible aphicides and restrictions on frequency and timings of application. For example, pymetrozine (as Chess WG) 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 pesticides and are keen to adopt more biological control strategies. The new aphid parasitoid mix available from BCP Certis 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 have not been controlled by previously available parasitoids. The mix has also given improved control of aphids on ornamental pot plants and HNS in BCP Certis trials (Clare Sampson, personal communication). No work has yet been done on the potential of the mix against 'problem' aphid species on protected herbs. However, *Praon volucre* has a wide host range and has been recorded on mint aphid in Greece (Kavallieratos *et al.* 2005). *Aphidius matricariae* is reputed to be an aggressive parasitoid, with the females able to kill aphids by repeated 'stinging' with their ovipositors, in addition to acting as a parasitoid (de Menten, personal communication).

Each of the six aphid parasitoid species now available to growers is reared commercially by biological control companies under carefully controlled conditions on suitable aphid species. Growers then use these parasitoid species to control aphid species that can include different species to the one that the parasitoids have been reared on. Aphid parasitoids seem able to readily 'switch' between the aphid species on which they were reared to other aphid species as long as these aphids are within their host range. However, there is evidence that aphid parasitoids may show a preference to the aphid species and host plant on which they were reared (e.g. van Emden *et al.* 2002 & 2008). This preference is thought to be due to the chemical cues acquired as the parasitoid develops inside the aphid and then cuts its way out of the aphid mummy.

The aim of this project was to improve biological control of hawthorn-parsley aphid and mint aphid on protected herbs. Specific objectives were:

- 1. In laboratory studies, determine the parasitism and host-killing of hawthorn-parsley aphid and mint aphid by six commercially available aphid parasitoid species.
- 2. In small-scale research glasshouse experiments, evaluate the control of hawthornparsley aphid and mint aphid by selected individual or mixed parasitoid species.
- 3. In an experiment on a commercial herb nursery, evaluate the control of hawthornparsley aphid on parsley by selected individual or mixed parasitoid species.
- 4. Communicate the results to the industry.

Objective 1: In laboratory studies, determine the parasitism and host-killing of hawthorn-parsley aphid and mint aphid by the six parasitoid species

Materials and methods

Source of aphids

Hawthorn-parsley and mint aphids were collected from commercial nurseries during April and May in 2011. A culture of hawthorn-parsley aphids was set-up by placing infested curly parsley plants into gauze cages (50 x 50 x 50 cm). 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 heating, shading and ventilation. The culture was maintained by regularly replacing dead or dying plants with clean uninfested 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. Six species of parasitoid were delivered as pupae within mummified aphids (Table 2). The parasitoids of each species were transferred separately to a ventilated sandwich box, which in turn was placed in a controlled temperature room set to 20°C. A piece of cotton-wool soaked in a honey solution (approx. 20% honey) was added to each box to provide food for emerging adult parasitoids. In addition, a piece of parsley and/or mint was added so that emerging adult parasitoids were exposed to host plant cues associated with hawthorn-parsley aphid or mint aphid.

Table 2. Parasitoid species

Parasitoid species

Aphidius ervi
Aphidius colemani
Aphidius matricariae
Praon volucre
Ephedrus cerasicola
Aphelinus abdominalis

Parasitism of aphids

This experiment recorded whether each of the parasitoid species tested attempted to lay eggs in hawthorn-parsley aphids or mint aphids and whether these aphids subsequently mummified (indicating successful parasitism). To do this a piece of curly parsley was placed in a small glass Petri dish (55 mm diameter). Twenty 2nd and 3rd instar (aphid parasitoids readily attack aphids of this age, although younger and older aphids may also be attacked) hawthorn-parsley aphids were carefully transferred from the aphid culture to the parsley in the Petri dish using a fine paintbrush. Once the aphids had been transferred the top of the Petri was replaced and the aphids were allowed to settle on the parsley. Twenty-four Petri dishes containing hawthorn-parsley aphids were prepared in this way, four for each of the six parasitoid species. The same technique was used to infest a piece of mint with 20 mint aphids (Figure 1), again preparing 24 Petri dishes.



Figure 1. A piece of mint infested with mint aphids in a glass Petri dish

Adult female parasitoids were collected from the ventilated sandwich box in which they had emerged using a pooter. Female parasitoids used in this experiment were 48-72 hours old. As there were both female and male parasitoids in the sandwich box, it was assumed that females had mated after emergence.

Once collected each female parasitoid was transferred separately to a clean empty glass Petri dish and allowed to acclimatise to these conditions for 30-60 minutes. Four female parasitoids were prepared in this way for each of the six species tested (see Table 1). Once the aphids and parasitoids had acclimatised to the conditions within their respective Petri dishes a parasitoid was introduced to a dish of aphids by waiting for the parasitoid to walk onto the lid of the dish and then carefully swapping the lids. A Petri dish containing the aphids and a parasitoid was then placed under a low power stereomicroscope (Wild M8) using a cold (fibre optic) light source (Figure 2). The parasitoid was again allowed to acclimatise to this change in conditions for approximately five minutes. The behaviour of the parasitoid was then observed using the microscope for 10 minutes, recording the number of times the parasitoid attacked the aphids. An attack was recorded when the tip of the parasitoids abdomen touched an aphid in an attempt to lay an egg. For *Aphelinus abdominalis* an attack was recorded when the parasitoid's long ovipositor was seen to be inserted into an aphid.



Figure 2. Parasitoid behaviour observed using a low power stereomicroscope

After the 10 minutes observation period the Petri dish was placed to one side in the laboratory for a further two hours to allow the parasitoid to continue to parasitise the aphids. Then the parasitoid was removed from the Petri dish and the aphids were carefully transferred to a clean pot of curly parsley plants or a mint plant (depending on the aphid species) using a fine paintbrush. The pot of parsley plants or mint plant was then covered with a perforated bread-bag, which was secured around the plant pot using a rubber band, to prevent further parasitoids or predators attacking the aphids on the plant. The pot was then placed in a controlled temperature room set to 20°C. The plant was watered as required and after 10 days checked for evidence of mummified aphids. After 14 days the plant was destructively sampled and the number of mummified aphids recorded.

Evidence of host-killing by parasitoids

This experiment recorded whether there was any evidence of host feeding by each of the six parasitoid species in addition to any parasitism. Petri dishes containing a piece of parsley with 10 second or third instar hawthorn-parsley aphids were prepared as previously described. Similarly, a mated female parasitoid, 48-72 hours old, was collected and introduced into the Petri as previously described. Four Petri dishes were prepared in this way for each of the six parasitoid species. An additional eight 'control' Petri dishes were prepared in the same way except that a parasitoid was not released into the dish. Petri dishes containing mint and mint aphids were prepared in exactly the same way.

The prepared Petri dishes were placed on a tray, which in turn was placed in a controlled temperature room set to 20°C. After 24 hours each dish was carefully checked, recording the survival of the parasitoid and aphids.

Statistical analysis

Data on the number of attacks observed in each 10 minute period, numbers of mummified aphids and aphid survival in the parasitoid host-killing experiment were analysed using analysis of variance (ANOVA) in GenStat (12th Edition) for both the hawthorn-parsley aphid and mint aphid experiments. Individual comparisons between treatment means were completed using least significant difference (LSD) (P<0.05).

Results

Parasitism of aphids

Hawthorn-parsley aphid: all six species of parasitoid were recorded attacking (tip of abdomen touching an aphid in an attempt to lay an egg) hawthorn-parsley aphids when observed in the Petri dishes (Table 3). However, for *Aphidius ervi* only a single parasitoid was observed to make one attempt to attack a hawthorn-parsley aphid. For the other five species the number of attacks observed in each 10 minute period varied from 0 (for all species except *Praon volucre* where at least 2 attacks were recorded for each individual) to 20 (for one *Aphidius colemani* female). The large amount of variability in the number of attacks recorded meant that statistically there was no significant difference between each of the six parasitoid species.

For each of the aphid parasitoid species, with the exception of *Aphidius ervi*, mummified hawthorn-parsley aphids were recorded 14 days after the observation part of the experiment (Table 3). Numbers of mummified aphids were low and were not recorded in each replicate where attacks had been recorded. Again there was no significant difference in the number of mummified aphids recorded for each successful parasitoid species.

Table 3. Mean number of attacks on hawthorn-parsley aphids recorded in 10 minutes of observation and subsequent number of mummified aphids recorded for each parasitoid species

Parasitoid species	Mean number of attacks	Mean number of mummified
		aphids
Aphidius ervi	0.2	0.0
Aphidius colemani	6.5	0.8
Aphidius matricariae	3.5	0.5
Praon volucre	5.0	0.5
Ephedrus cerasicola	5.8	0.5
Aphelinus abdominalis	1.5	0.8
Significance (df = 5), $P =$	n.s.	n.s.
SED	3.74	0.54

Mint aphid: five of the six species of parasitoid were recorded attacking mint aphids when observed in the Petri dishes (Table 4). *Aphidius ervi* was the only species not observed to make any attacks. For the other five species the number of attacks observed in each 10 minute period varied from 0 (for all species except *Ephedrus cerasicola* where at least six attacks were recorded for each individual) to 12 (for a single *Aphidius colemani* and a single *Aphidius matricariae*). The large amount of variability in the number of attacks recorded meant that statistically there was no significant difference between each of the six parasitoid species.

Mummified mint aphids were recorded 14 days after the observation part of the experiment for only three of the parasitoid species; *Aphidius matricariae*, *Praon volucre* and *Ephedrus cerasicola* (Table 4). Numbers of mummified aphids were higher than in the hawthornparsley aphid experiment but again were not recorded in each replicate where attacks had been recorded. However, parasitoid species significantly affected the number of mummified aphids recorded.

Parasitoid species	Mean number of attacks	Mean number of mummified
		aphids
Aphidius ervi	0.0	0.0
Aphidius colemani	5.0	0.0
Aphidius matricariae	5.3	2.0
Praon volucre	3.0	0.8
Ephedrus cerasicola	8.0	1.8
Aphelinus abdominalis	1.0	0.0
Significance (df = 5), P =	n.s.	0.014
SED	2.81	0.66

Table 4. Mean number of attacks on mint aphids recorded in 10 minutes of observation and subsequent number of mummified aphids recorded for each parasitoid species

Figures in bold are significantly different from zero (P<0.05).

Evidence of host-killing by parasitoids

This experiment recorded the number of dead hawthorn-parsley aphids in a Petri dish 24 hours after a mated female parasitoid had been introduced. Before the start of this experiment, only *Aphelinus abdominalis* was seen to feed on hawthorn-parsley aphids in the previous experiment in which parasitoid behaviour was observed. No parasitoids were previously observed feeding on mint aphids.

Hawthorn-parsley aphid: mean aphid mortality in this experiment ranged from 7.3% for *Aphidius matricariae* to 62.7% for *Aphidius colemani* (Table 5). Overall parasitoid species significantly affected aphid mortality. Mean aphid mortality in dishes containing an *Aphidius colemani*, an *Ephedrus cerasicola* or an *Aphelinus abdominalis* female was significantly (LSD where P<0.05) higher than in control Petri dishes that did not contain a parasitoid. For the other parasitoid species mean aphid mortality did not differ significantly from the control.

Parasitoid species	Mean percent aphids dead after 24 hours
Aphidius ervi	18.3
Aphidius colemani	62.7
Aphidius matricariae	7.3
Praon volucre	35.0
Ephedrus cerasicola	46.7
Aphelinus abdominalis	48.3
Control	12.5
Significance (df = 6), $P =$	0.024
SED	15.74

Table 5. Mean percent of hawthorn-parsley aphids dead after 24 hours

Figures in bold are significantly different from the control (P<0.05).

Mint aphid: mean aphid mortality in this experiment ranged from 9.8% for *Aphidius matricariae* to 54.3% for *Ephedrus cerasicola* (Table 6). Again, overall parasitoid species significantly affected aphid mortality. Mean aphid mortality in dishes containing an *Aphidius ervi*, a *Praon volucre* or an *Ephedrus cerasicola* female was significantly (LSD where P<0.05) higher than in control Petri dishes that did not contain a parasitoid. For the other parasitoid species mean aphid mortality did not differ significantly from the control.

Parasitoid species	Mean percent aphids dead after 24 hours
Aphidius ervi	32.5
Aphidius colemani	24.7
Aphidius matricariae	9.8
Praon volucre	42.5
Ephedrus cerasicola	54.3
Aphelinus abdominalis	41.1
Control	11.4
Significance (df = 6), $P =$	0.024
SED	15.99

Figures in bold are significantly different from the control (P<0.05).

Objective 2: In small-scale research glasshouse experiments, evaluate the control of hawthorn-parsley aphid and mint aphid by selected individual or mixed parasitoid species.

Materials and methods

Immediately before these semi-field experiments were started the culture of hawthornparsley aphids became contaminated with an aphid parasitoid (Figure 3). Most of the aphids were parasitised and the culture was almost lost. This was a set-back to the project but presented an opportunity to identify the parasitoid species responsible. Specimens were sent to the Natural History Museum for identification and the parasitoid species was confirmed as *Aphidius colemani*. It is likely that one or more undetected female parasitoids of this species had been transferred to the cages on young parsley plants had been brought back to ADAS Boxworth to start the culture, as the grower had been releasing the parasitoid species mix to the commercial crop. *Aphidius colemani* had also been used extensively at ADAS Boxworth to control aphids on other unrelated experiments in adjacent glasshouses and it is possible that one or more female parasitoids were able to enter the cage containing the culture of hawthorn-parsley aphids. However, this is unlikely as the ADAS glasshouse compartments are insect-screened and the culture was in an insect-proof cage.



Figure 3. Parasitised hawthorn-parsley aphid

Efficacy of parasitoids under semi-field conditions

These experiments recorded the efficacy of *Aphidius colemani* in controlling hawthornparsley aphid on potted parsley plants and of *Aphidius matricariae* in controlling mint aphid on potted mint plants under semi-field conditions. *Aphidius colemani* was selected based on results from the laboratory studies as well as its ability to almost wipe out the hawthorn-parsley aphid culture. *Aphidius matricariae* was selected based on results from the laboratory studies with mint aphid. The experiments were completed using insect cages placed in a computer-controlled glasshouse compartment set to maintain a temperature of approximately 20°C (Figure 4).



Figure 4. Insect cages placed within glasshouse compartment

Hawthorn-parsley aphid: pots of clean young curly parsley plants were infested with mixed age hawthorn-parsley aphids. Each pot of plants was infested with 25 aphids, using aphids from the culture and carefully transferring each aphid using a fine paintbrush. Twelve pots of plants were prepared in this way. Two infested pots of plants were then placed into each of six insect cages.

The prepared insect cages were then left for 24 hours to allow the aphids to settle on the plants on which they had been placed. Next, 15 mated female *A. colemani*, each 48-72 hours old, were collected using a pooter, placing five parasitoids into each of three specimen tubes. Three cages were then selected at random and five parasitoids carefully introduced into the centre of each cage. After 10 days the pots of parsley plants were destructively sampled by cutting the plants at their base but taking care not to dislodge the aphids. For each cage the number of live and mummified aphids was recorded. Once this assessment had been completed, 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. Aphids were checked again after further seven days, recording the number of additional mummified aphids.

Mint aphid: this experiment was completed as described for hawthorn-parsley aphid but using *A. matricariae* in place of *A. colemani*. Also numbers of aphids and mummified aphids were recorded 12 days after the parasitoids were released and a final assessment of the numbers of mummified completed after a further nine days.

There is evidence that aphid parasitoids may attack aphids more readily if they have developed on an aphid of the same species or have already successfully attacked aphids of that species. To test this, an additional experiment using hawthorn-parsley aphids and *Aphidius colemani* was completed. Nine cages were prepared with aphid-infested parsley plants as previously described. Two mated female *Aphidius colemani* supplied direct from BCP Certis were released into three of the cages. Two mated female *Aphidius colemani* that had been 'conditioned', i.e. reared on hawthorn-parsley aphids (collected from the first semi-field experiment) were released into three further cages. No parasitoids were released into the final three 'control' cages. Numbers of live and mummified aphids were recorded 10 days after the parasitoids were released and a final assessment of the numbers of mummified aphids completed after a further four days.

Statistical analysis

Data on the numbers of aphids and mummified aphids were analysed using analysis of variance (ANOVA) in GenStat (12th Edition) for both the hawthorn-parsley aphid and mint aphid experiments.

Results

Recording efficacy of parasitoids under semi-field conditions

Aphidius colemani to control hawthorn-parsley aphid: the release of five mated female *Aphidius colemani* (at a rate equivalent to 40/m²) into cages containing 50 hawthorn-parsley aphids significantly reduced the number of healthy aphids to a mean of 20 after 10 days (Table 7). By comparison, in control cages in which no parasitoids were released there was an almost six-fold increase in the number of hawthorn-parsley aphids over the same period.

Releasing *Aphidius colemani* into cages also resulted in 57% parasitism of the aphids within 10 days.

	Mean numb	er of healthy	Mean number of		Mean % parasitism	
	apl	nids	parasitized aphids			
Treatment	Day 0	Day 10	Day 0	Day 10 & 17	Day 0	Day 10 & 17
Control	50	294	0	0	0	0
Aphidius	50	20	0	26	0	57
colemani						
Significance		0.003		0.013		<0.001
(df = 1), P =						
SED		42.60		6.06		2.53

Table 7. Mean numbers of healthy and parasitised hawthorn-parsley aphids per cage under semi-field conditions

Aphidius matricariae to control mint aphid: the release of five mated female *Aphidius matricariae* (a rate equivalent to 40/m²) into cages containing 50 mint aphids significantly reduced the number of healthy aphids to a mean of 28 after 10 days (Table 8). By comparison, in control cages in which no parasitoids were released there was an almost three-fold increase in the number of mint aphids over the same period. However, releasing *Aphidius matricariae* into cages resulted in lower levels of mint aphid parasitism (9%) than that given by *Aphidius colemani* with hawthorn-parsley aphid within 10 days.

	Mean number of healthy		Mean number of		Mean % parasitism	
	aphids		parasitized aphids			
	Day 0	Day 12	Day 0	Day 12 & 21	Day 0	Day 12 & 21
Control	50	142	0	0	0	0
Aphidius	50	28	0	3	0	9
matricariae						
Significance		0.048		0.016		0.003
(df = 1), P = SED		40.60		0.67		1.38

Table 8. Mean numbers of healthy and parasitised mint aphids per cage under semi-field conditions

'Conditioned' *Aphidius colemani* reared on hawthorn-parsley aphid to control hawthornparsley aphid: the release of two mated female *Aphidius colemani* reared on hawthornparsley aphid (a rate equivalent to 16/m²) into cages containing 50 hawthorn-parsley aphids did not significantly reduced the number of healthy aphids. Instead, in these cages there was an almost three-fold increase in the mean number of healthy aphids after 10 days (Table 9). Similarly, in cages where two mated *Aphidius colemani* supplied direct by BCP Certis were released there was an almost two-fold increase in the mean number of healthy aphids. In control cages, where no parasitoids were released, there was an almost threefold increase in the mean number of healthy aphids over the 10 day period.

Releasing *Aphidius colemani* supplied direct by BCP Certis into cages resulted in a mean of 23% parasitism (with a range of 15 to 30% parasitism recorded) of the aphids within 10 days. Although this level of parasitism was lower than when five parasitoids of this species were released into cages, the mean number of parasitised aphids was similar (23 in this experiment and 26 in the previous experiment with hawthorn-parsley aphids). In cages where 'conditioned' *Aphidius colemani* reared on hawthorn-parsley were released, percent parasitism was only 14%; however, this reflects the fact that parasitised aphids were only recorded in one of the three cages. Where parasitised aphids were recorded the rate of parasitism was 42%. Although no parasitised aphids should have been found in control cages where no parasitoids were released, a small number of aphid mummies (six) were recorded in one cage. This result suggests that one or more parasitoid(s) was able to get into the cage during the experiment and reflects the difficulty in excluding the parasitoids. The overall results from this experiment provide only inconclusive evidence to indicate that 'conditioned' parasitoids were more effective in controlling hawthorn-parsley aphid than parasitoids reared on another aphid species and supplied direct from BCP Certis.

	Mean number of		Mean number of		Mean % parasitism	
	healthy aphids		parasitised aphids			
	Day 0	Day 10	Day 0	Day 10 & 14	Day 0	Day 10 & 14
Control	50	147	0	2	0	2
Aphidius colemani	50	92	0	23	0	22
(supplied by BCP Certis)						
Aphidius colemani	50	138	0	11	0	14
(reared on hawthorn-						
parsley aphid)						
Significance (df = 2), P =		n.s.		n.s.		n.s.
SED		47.70		9.17		12.00

 Table 9. Mean numbers of healthy and parasitised hawthorn-parsley aphids per cage under semi-field conditions

Objective 3: In an experiment on a commercial herb nursery, evaluate control of hawthorn-parsley aphid on parsley by selected individual or mixed parasitoid species.

The planned experiment could not be done as work in Objectives 1 and 2 took much longer than expected, by which time numbers of plants infested with hawthorn-parsley aphid on the host nursery were much lower than earlier in the season and this would have put the experiment at risk. Therefore, intensive monitoring of parsley crops on two commercial nurseries was done to establish the aphid infestation time during the 5-week production period and the percentage parasitism given by the growers' release strategies.

Materials and methods

One crop of protected curly parsley was sampled on each of two commercial nurseries, recording numbers of healthy and parasitised aphids. Sampling was completed by carefully examining a minimum 10 pots per production week, recording the number of live and parasitised aphids (aphid mummies).

Site 1: one block of plants was sampled on 3 November 2011, assessing plants from each of the final three production weeks. Parasitised aphids were kept in the laboratory and successfully emerging parasitoids sent to the Natural History Museum for identification. Site 2: four blocks of plants were sampled on 25 November 2011, assessing plants from each of the five production weeks.

Results

During the monitoring of plants from the two commercial protected parsley crops, hawthornparsley aphids were only found on older plants (Table 10). No aphids were found on plants less than five weeks old at Site 1 and less than four weeks old at Site 2. In addition, the mean number of aphids per plant was higher on five week old plants than on four week old plants at Site 2. Relatively few plants were infested with aphids, 13% at Site 1 (70 plants sampled) and 2% at Site 2 (620 plants sampled). Parasitism was recorded only at Site 1 and only at a very low level (2.3% of the total aphids found). From the parasitised aphids collected, a single adult male was collected and sent to the Natural History Museum. As this was a male parasitoid species identification was difficult but the specimen was tentatively identified as *Aphidius matricariae*. This parasitoid species must have been naturallyoccurring as the grower at Site 1 had only released *Aphidius colemani*, at a rate of 0.24 per m² per week.

Approx. age of	Mean number of		Mean number of		Mean percent of	
plants (weeks)	aphids per plant		parasitised aphids		aphids parasitised	
		per plant				
	Site 1	Site 2	Site 1	Site 2	Site 1	Site 2
1	-	0.00	-	0.00	-	0.00
2	-	0.00	-	0.00	-	0.00
3	0.00	0.00	0.00	0.00	0.00	0.00
4	0.00	0.93	0.00	0.00	0.00	0.00
5	2.60	6.38	0.06	0.00	2.31	0.00

Table 10. Mean numbers of healthy and parasitised hawthorn-parsley aphids per plant on

 two commercial parsley crops

– no data available.

Despite the lack of recorded aphid parasitism at Site 2, the grower had released the parasitoid mix produced by Viridaxis at a minimum rate of 1.8 per m² per week (one tube per

 $130m^2$, with each tube releasing a minimum of 240 parasitoid adults). Of these 1.8 parasitoids per m², approximately 0.3 per m² are likely to have been *Aphidius colemani*, the remaining parasitoids being made up of the other five species in the mix. This rate of *A. colemani* was similar to that used (as a single species) at Site 1. In order to confirm that these parasitoids had successfully emerged, two tubes that had been released into the parsley crop several weeks earlier were assessed. This confirmed that tube one contained 483 aphid mummies and tube two 384 mummies. Of these mummies 82% in tube one and 92% in tube two had successfully emerged, i.e. 396 and 353 (a mean of 375) parasitoids had emerged from the two respective tubes. These numbers are, well above the supplier's minimum number per tube.

Discussion

There is limited published information on the parasitoid species recorded attacking hawthorn-parsley aphid and mint aphid. However, Kavallieratos *et al.* (2005) recorded *Praon volucre* recorded on mint aphid in Greece. More recently, during the course of the project Viridaxis produced information based on published literature and observations under commercial conditions in protected herb crops on the host ranges of each of the six parasitoid species tested here. This information is summarized below (Table 11):

Parasitoid species	Hawthorn-parsley aphid	Mint aphid
Aphidius ervi		
Aphidius colemani	++	
Aphidius matricariae	+	++
Praon volucre		+++
Ephedrus cerasicola	++	
Aphelinus abdominalis		

Table 11. Information on host-ranges of aphid parasitoid species – source Viridaxis.

NB: + = good efficacy; ++ = high efficacy; +++ = very high efficacy

Results from this study have increased our understanding of the host ranges of these six commercially available aphid parasitoid species (Table 12). In particular, there is now information that under laboratory conditions *Praon volucre* and *Aphelinus abdominalis* are able to successfully parasitise hawthorn-parsley aphid. There is also information that under laboratory conditions *Ephedrus cerasicola* is able to successfully parasitise mint aphid. Results for the other parasitoid species are in agreement with the Viridaxis information.

Parasitoid species	Hawthorn-parsley aphid	Mint aphid
Aphidius ervi		
Aphidius colemani	++	
Aphidius matricariae	+	++
Praon volucre	(+)	+++
Ephedrus cerasicola	++	(+)
Aphelinus abdominalis	(+)	

Table 12. Information on host-ranges of aphid parasitoid species – source Viridaxis and results from this project.

NB: (+) = parasitism confirmed under laboratory or semi-field conditions; + = good efficacy; ++ = high efficacy; +++ = very high efficacy

Growers have been unable to successfully control hawthorn-parsley aphid or mint aphid using releases of *Aphidius colemani*, *Aphidius ervi* and/or *Aphelinus abdominalis*. However, results from this project and the Viridaxis information indicate that *Aphidius colemani* may provide useful control of this aphid. This contradicts grower experience using this aphid parasitoid. There are several possible explanations as to why *Aphidius colemani* may be effective under laboratory and semi-field conditions but ineffective when currently used by growers:

- 1. Parsley plants are grown densely in pots and there is little space between pots, particularly when pot-thick before spacing. As a result, as the plants grow they form a thick canopy. Hawthorn-parsley aphids typically colonise the stems of the plants i.e. below the leaf canopy. It is possible that aphid parasitoids such as *Aphidius colemani* may find it difficult to locate and parasitise these aphids within this environment. In the semi-field experiment only two pots were placed in each cage meaning that the parasitoids would have been able to fly around each pot and may then have found it easier to locate and parasitise the aphids.
- 2. In this project, hawthorn-parsley aphids parasitised by *Aphidius colemani* took 10-14 days to mummify at 20°C. The time taken for adult parasitoids to emerge from these parasitised aphids would have been approximately 20 days. Given that the production time for potted parsley is approximately five weeks from seedling emergence to sale and that, based on evidence from commercial growers, hawthorn-parsley aphids are typically only found on older plants, there would not be enough time for parasitoids to complete their development before the plants are sold. As a result it is unlikely that parasitoids would be able to establish within this crop. Instead, many parasitised aphids

are likely to be discarded at the end of the production line, as they would be visually indistinguishable from healthy aphids.

- 3. *Aphidius colemani* is typically used at rates of 0.25 to 0.5 per m² for preventive or curative control respectively of susceptible aphid pests, with applications applied every week. In this project a single release equivalent to 40 per m² was effective against hawthorn-parsley under semi-field conditions while a release of 16 per m² was less effective. It is unclear whether lower rates would be effective in controlling this pest if they were applied weekly.
- 4. It is possible that the environmental conditions within commercial protected parsley crops are not suitable for *Aphidius colemani*. However, this explanation seems unlikely given that this parasitoid can provide effective control against peach-potato aphid on AYR basil crops grown in the same glasshouse as parsley.

Aphidius matricariae, Praon volucre and Ephedrus cerasicola have only recently been available for growers. All three species offer potential against hawthorn-parsley aphid, either on their own or as part of a mix of parasitoid species. However, the fact that no parasitised hawthorn-parsley aphids were recorded at a commercial nursery releasing these parasitoid species as part of the six species mix indicates that more work is required to develop an effective biological control strategy.

Results for *Aphidius colemani, Aphidius ervi* and *Aphelinus abdominalis* confirm grower experience in being unable to control mint aphid with these parasitoid species. However, all three of the newly available parasitoid species show promise against mint aphid. In particular *Aphidius matricariae* may provide useful control of this pest. However, as these results were under semi-field conditions using a single release of parasitoids, equivalent to 40 per m², many of the points raised above for *Aphidius colemani* against hawthorn-parsley aphid also apply to mint aphid control.

Although there is evidence that aphid parasitoids may show a preference to the aphid species and host plant on which it was reared (e.g. van Emden *et al.* 2002; 2008) results from this project were inconclusive. More work would be required to confirm whether 'conditioned' aphid parasitoids reared on hawthorn-parsley aphids or mint aphids are more effective biological control agents against these pests. However, as parasitoids supplied by Viridaxis through BCP Certis were able to successfully parasitise both species of aphid there is little apparent need to modify parasitoid production systems.

Conclusions

- 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/m². A single release equivalent to 16 *Aphidius colemani*/m² was less effective, indicating a need either for high release rates or regular introductions (the latter is the standard commercial strategy and will be further tested in the extension to this project during 2012).
- Evidence to support the hypothesis that 'conditioned' *Aphidius colemani* reared on hawthorn-parsley aphids provide more effective control of this pest is inconclusive.
- 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.
- 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/m²). As with *Aphidius colemani* and hawthorn-parsley aphid, the efficacy of a weekly release strategy needs testing.
- 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.

Potential further work (to be done in the extension to this project during 2012)

- Demonstrate that *Aphidius colemani* will parasitise hawthorn-parsley aphid on potthick and spaced parsley plants in replicate cages in a commercial herb glasshouse.
- Develop an effective, robust parasitoid release strategy for control of hawthornparsley aphid and mint aphid.
- Validate the success and cost-effectiveness of the selected parasitoid release strategy for control of hawthorn-parsley aphid on parsley on a commercial nursery.

Knowledge and technology transfer

Publications

Aphidsure mix – Coming to the aid of protected herb growers. *BCP Certis Technical Review*. March 2012.

Presentations

Protected herbs: Improved biological control of aphids. BHTA Technical Meeting, Harper Adams. October 2011.

Improving biological control of two 'problem' aphid species on protected herbs. *AAB* meeting 'Advances in Biological Control, Olde Barn Hotel, Marston. November 2011.

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References

Kavallieratos, N.G., Tomanovic, Z., Stary, P., Athanassiou, C.G., Fasseas, C., Petrovic, O., Stanisavljevic, L.Z. & Veroniki, M.A. (2005). *Praon* Haliday (Hymenoptera: Braconmidae: Aphidiinae) of Southeastern Europe: key, host range and phylogenetic relationships. *Zoologischer Anzeiger*. 243, 181-209.

van Emden, H. F., Eletherianos, I., Rose, J., Douloumpaka, S. & Pettersson, J. (2002) Aphid parasitoids detect that an alien plant was present nearby during their development. Physiological Entomology. 27: 199-205.

van Emden, H. F., Storeck, A. P., Douloumpaka, S., Eleftherianos, I., Poppy, G. M. & Powell, W. (2008) Plant chemistry and aphid parasitoids (Hymenoptera: Braconidae): Imprinting and memory. European Journal of Entomology. 105: 477-483.