Project title:	Sweet pepper: Further development of IPM solutions for aphid infestations
Project number:	PC 295b
Project leader:	Dr R. Jacobson, RJC Ltd, 5 Milnthorpe Garth, Bramham, West Yorks, LS23 6TH.
Report:	Final report, December 2011
Previous report:	Not applicable
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Location of project:	RJC Ltd, West Yorkshire Imperial College, Silwood Valley Grown Nursery, Essex Cantelo Nurseries Ltd, Somerset
Industry Representative:	Mr G Taylor, Valley Grown Nursery Paynes Lane, Nazeing, Essex, EN9 2EX
Date project commenced:	1 February 2011
Date project completed:	31 December 2011

DISCLAIMER

[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

I declare that this work was done under my supervision according to the procedures described herein and that the report represents a true and accurate record of the results obtained.

Dr R Jacobson
Director
Rob Jacobson Consultancy Ltd

Signature *R J Jacobson* Date *December 2011*

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GROWER SUMMARY

Headline

Ten species of hyperparasitoids attacked primary parasitoids in pepper crops and *Asaphes* spp. are the main hyperparasite disrupting biological control programmes.

Background

British pepper growers lead the world with the implementation of integrated pest management (IPM) against a wide range of pests. These techniques are largely based on biological control with several different beneficial species being released against each pest species. The IPM programme would be very successful except for the breakdown in control of aphids which occurs every summer.

As IPM of aphids has become more refined in protected peppers, it has become clear that the performance of the primary parasitoids is being seriously impaired by hyperparasitism during the summer months. This is probably not unique to pepper crops. Aphids are serious pests of a wide range of protected and outdoor crops and continuous control with biological control agents has proved to be very difficult in most situations. It has become clear that the impact of hyperparaitoids must be reduced to optimise biological control of aphids within IPM programmes.

Hyperpasitoids are secondary insect parasitoids that attack biological control agents and thereby threaten the success of IPM programmes. Prior to 2010, there was very little information about levels of hyperparasitism in aphid populations in commercial pepper crops. Their presence was known as hyperparasitoid emergence holes had been observed in mummified aphids. However, there was no information on the extent of the problem or the species that were involved. Snap shot sampling in commercial pepper crops during 2010 revealed at least seven species of hyperparasitoids. The timing of emergence of the various hyperparasitoids suggested that they may be attacking each other and more thorough sequential sampling throughout the season was required to determine which species presented the greatest threat to the IPM of aphids. This was the first objective.

Open rearing units (ORUs) or banker plants have been used to boost numbers of parasitoids in protected cultivation for over 30 years. The objective is to sustain a reproducing population of the natural enemies and thereby provide season-long suppression of the pest species. ORUs were tested in commercial pepper crops during

2010 and appeared to be successful during the early season. However, by mid-May the units were infested with several species of hyperparasitoids. It was not know when the hyperparasitoids started to colonise the ORUs but it was clear that the control system had become compromised by mid-summer. Spanish research had indicated that ORUs could also be used to boost numbers of predatory syrphids (hoverflies). The researchers proposed that it may be possible to use ORUs based on parasitoids in the early season and then switch to ORUs based on syrphid flies in the summer. This was the second objective.

The researchers hypothesised that a thorough understanding of hyperparasitoid foraging behaviour could enable them to interrupt the process and thereby reduce the commercial impact of hyperparasitism. Ultimately, they envisaged identifying the chemical cues which hyperparasioids use to find their hosts and incorporate them into traps. Unfortunately, little information is available about the factors involved in host location. As a third objective, they proposed building a team of appropriate researchers who could address this issue generically across a wide range of agro-ecosystems and then deliver a project proposal to an appropriate funding organisation.

Summary

The work focused on *Myzus persicae* and *Aulacorthum solani* which had regularly caused economic damage in commercial pepper crops. Other aphid species such as *Macrosiphum euphorbiae* and *Aphis gossypii* occurred sporadically and locally but were usually controlled by the biological and / or chemical measures taken against the two main species.

The project built on previous work which had shown *Aphidius colemani*, *Aphidius ervi* and *Aphelinus abdominalis* to be the three most effective primary parasitoids against *M. persicae* and *A. solani*. An IPM programme based upon these natural enemies was established from the start of the season with weekly releases of *A. colemani*, *A. ervi* and *A. abdominalis* at 0.5, 0.25 and 0.5 / m^2 respectively. These rates were modified as necessary in response to aphid development and/or the use second line of defence treatments. On occasions, *Aphidius matricariae* were released instead of *A. colemani* or *A. ervi* due to supply issues. In addition, *Praon volucre* always invaded naturally and rapidly became established without the need to release purchased products

Objective 1. Study of hyperparasitism

The main body of this work was done in organic pepper crops in Somerset (Site 1). Samples of mummied aphids were collected in mid-March to obtain a base line for hyperparasitoid activity. The main sampling period was between mid-April and late-October. The crops were visited on 15 occasions during the 28 week period. On each occasion, the researchers attempted to collect 50-100 intact (*i.e.* non-emerged) mummies created by each of the *Aphidius*, *Aphelinus* and *Praon* parasitoids within colonies of both *M. persicae* and *A. solani*; *i.e.* up to six aphid / parasitoid combinations. In practice, it was not always possible to collect the full complement of all these combinations; intact mummies created by *Aphelinus* being particularly elusive. Each batch of each aphid / parasitoid mummies was incubated at room temperature and the emergent primary parasitoids and hyperparasitoids were identified. The establishment and development of the various species of parasitoids and hyperparasioids were plotted throughout the season.

The opportunity was also taken to collect batches of *M. persicae* based mummies from conventional pepper crops in Essex (Site 2), where we were particularly interested in the re-establishment of hyperparasitoids following mid-season treatments with Chess (pymetrozine). At this site, mummies were collected for emergence tests on eight occasions between mid-April and late September.

Ten species of hyperparasitoids were found during the study. The table below shows which of these were associated with each of the six different combinations of aphid and primary parasitoid (y = a positive record). Only *Asaphes suspensus* was found in every type of mummified aphid. This was also the most numerous species overall.

	Aulacorthum solani			*Myzus persicae*		*Macrosiphum euphorbiae*
	Aphidius	*Praon*	*Aphelinus*	*Aphidius*	*Praon*	*Aphelinus*
Alloxysta brevis	y			y		
Alloxysta brachyptera	y		y	y		
Alloxysta fulviceps				y		
Alloxysta victrix	y			y		
Asaphes suspensus	y	y	y	y	y	y
Asaphes vulgaris				y		
Pachyneuron aphidis			y	y		
Dendrocerus aphidum	y					
Dendrocerus laticeps	y	y				
Dendrocerus serricornis				y		

At Site 1, the proportion of primary parasitoids to hyperparasitoids fluctuated between 60:40 and 30:70 with an overall mean marginally in favour of the hyperparasitoids (44:56). This is clearly illustrated in Figure 1. A similar situation existed at Site 2 until the mid-season insecticidal treatment. This indicates that the hyperparasitoids coexist with their primary parasitoid hosts rather than eliminate them.

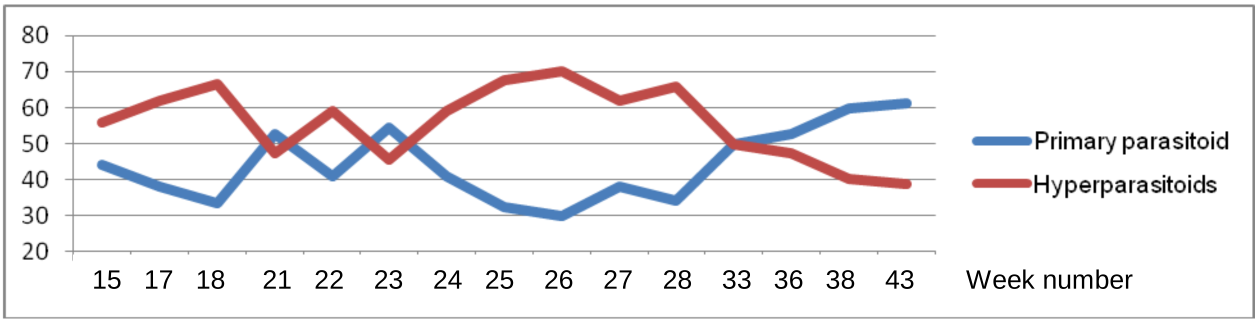

Figure 1. Proportion of primary parasitoids to hyperparasitoids at Site 1.

Overall, only 54% (range 20-80%) of mummies collected at Site 1 yielded an adult parasitoid (*i.e*. primary or hyper). The percentage was lower (26%) at Site 2 until the mid-season insecticidal treatment. We would normally expect 80-85% emergence of primary parasitoids from healthy cultures. Supplementary studies indicated that the difference between the normal expectation and the actual emergence could be attributed to the hyperparasitoids killing and feeding on the primary parasitoids without laying eggs. This is commonly known as 'host-feeding'.

At Site 2, numbers of the dominating hyperparasitoid, *Asaphes* spp., declined to below a detectable level following the mid-season insecticidal treatment. Aphids rapidly recolonised the crop as did the primary parasitoids which were still being released on a weekly basis. However, the hyperparasitoids were slower to recolonise and did not make a serious impact on the biological control programme for the rest of the season.

The population trends of the four genera of hyperparasioids at Site 2 are shown in Figure 2 and indicate that *Asaphes* spp. were suppressing members of the other three genera until the mid-season insecticidal treatment. This was also the case at Site 1.

An excellent model system was selected to study hyperparasitism on a generic level. This is based on the aphid, *Myzus persicae*, a primary parasitoid from the genus *Aphidius*, and a secondary parasitoid from the genus *Asaphes*.

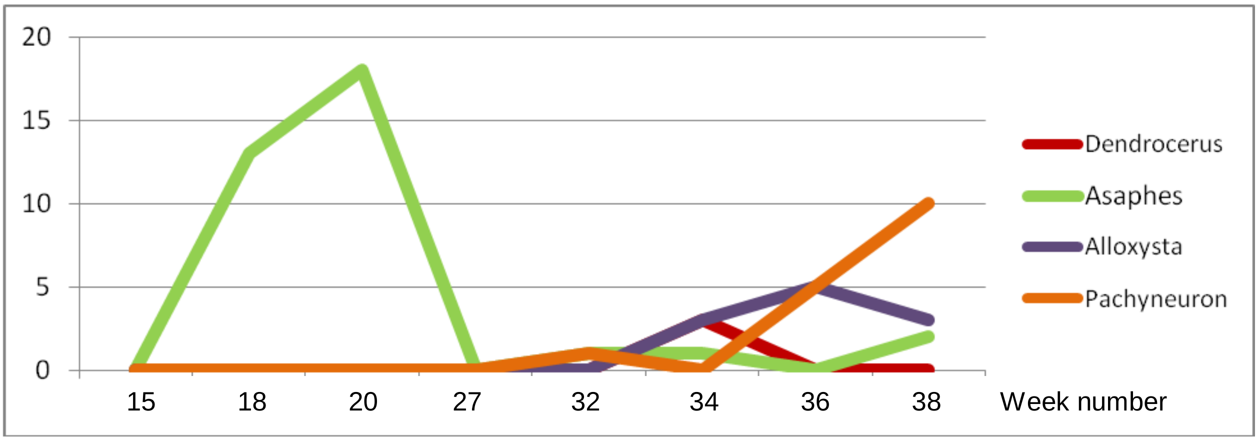

Figure 2. Percentage of mummified aphids which yielded each of the four genera of hyperparasitoids at Site 2.

Objective 2. Open rearing units

This trial was done in four blocks of conventional peppers at Site 2. All blocks received standard sequential release of *A. colemani*, *A. ervi* and *A. abdominalis* from the start of the season. In addition, Block 4 received ORUs for the production of parasitoids until mid-May and then switched to syrphid production. Blocks 1 and 2 received parasitoid-based ORUs until the end of April and then became a control for comparison with Block 4. Block 3 received no ORUs and thereby served as the overall control. The ORU placement programme built up to and then maintained eight active ORUs per hectare. Each ORU comprised a hanging basket containing wheat plants infested with the grain aphid (*Sitobion avenae*). The latter is a common host for the natural enemies without being a threat to the pepper crop. *Aphelinus abdominalis* were released onto the parasitoid-based units and *Episyrphus balteatus* were released onto the syrphid-based units.

The crops were monitored on a two week cycle until mid-July. On each occasion, all rows were walked and colonies of *M. persicae* and *A. solani* were recorded from individual units of three plants (nine heads). In addition, the percentage of aphids parasitised (i.e. showing as mummies) were recorded in two categories (0-50% or 50-100%). Samples of mummified aphids were collected from ORUs on five occasions and the emergent adult parasitoids identified. Numbers of immature *Episyrphus balteatus* present on the ORUs were recorded throughout June and July.

The ORUs produced numerous *A. abdominalis* until mid-April but thereafter became overrun by hyperparasitoids. The dominant hyperparasitoid species was *P. aphidis* with *A. brevis, A. brachyptera, A. vulgaris* and *D. laticeps* present in smaller numbers. This had been anticipated and was the principle reason for the switch to syrphid production in the ORUs from mid-May.

The cereal plants did not grow well during June / July and the cereal aphid populations did not thrive despite being ‘topped up’ from an on-site aphid production unit. The syrphids did not colonise the ORUs and very few were seen on the pepper plants during June. In July, populations of syrphids were numerous in outdoor habitats and it was impossible to distinguish between those released in the glasshouse and natural invaders. By then, the remnants of the ORUs had essentially become hyperparasitoid rearing units and they were removed from the glasshouse.

The aphids on the pepper plants were predominantly *A. solani* throughout this period. Primary parasitism was good until mid-May regardless of the presence of ORUs but declined rapidly thereafter in all treatment blocks. The decline coincided with a marked increase in hyperparasitoid activity. All blocks required supplementary treatments with Chess (pymetrozine) during early-mid June.

In summary, the combined use of primary parasitoid-based and syrphid-based ORUs may have assisted aphid control in the early season but did not obviate the need for mid-season treatments with an IPM compatible insecticide.

Objective 3. Pave the way for more generic studies

The results of a ‘spin-off’ project demonstrated the existence of a semiochemical released by mummified aphids which acted as an attractant to *A. suspensis*. Based on the work completed in this HDC project and the spin-off project, a team with complementary expertise prepared a Full Proposal for BBSRC funding via the ‘industry partner’ route. The proposal incorporates HDC and three private companies as industrial partners.

Financial Benefits

The cost of routine control measures applied against aphids in conventional pepper crops is about £5.8k per ha per season. Where difficulties occur with the control of aphids, the overall cost of additional biocontrols, sprays, labour to wash fruit and loss of marketable yield may exceed £100k per hectare per season. It is estimated that successful control measures developed by this project could ultimately save growers between £0.8k and £95k per hectare per season depending on the severity of the existing problems. In addition, the work paves the way for further studies aimed at providing more sustainable biologically-based solutions. This in turn will help growers to satisfy the standards sought by major food retailers and thus improve competitiveness of the UK industry

Action Points

- Open rearing systems can produce large numbers of primary parasitoids in the early season but are likely to become overrun by hyperparasitoids by mid-May. Where used, they should be removed from the glasshouse before the end of April.
- The results of this study do not support the use of a syrphid-based open rearing system in mid-season.
- In conventional crops, a single application of Chess (pymetrozine) in mid-season reduced numbers of the dominating hyperparasitoid to below detectable levels. Aphids and primary parasitoids rapidly recolonised the crop but hyperparasitoids did not make any further significant impact on the biological control programme.
- The results of this project have greatly improved our understanding of hyperparasitoids and have paved the way for more generic studies aimed at strengthening parasitoid-based solutions for aphid control across a wide range of commercial crops. Ultimately, this could save growers between £0.8k and £95k per hectare per season. In addition, it will help pepper growers to satisfy the standards sought by major food retailers and thus improve competitiveness of the UK industry.

SCIENCE SECTION

Introduction

Summary of work completed to date

HDC Project PC 295 devised a new IPM compatible strategy for aphid control in organic pepper crops (Jacobson, 2009). This consisted of primary biological control measures based on several species of parasitoids, supported by fatty acids (Savona) or natural pyrethrins (Pyrethrum 5EC) as a second line of defence (SLoD). A ‘proof of concept’ trial was very successful. However, some difficulties were encountered with the SLoD when implemented in commercial crops. Project PC 295a concentrated on improving the efficacy of the SLoD by: i) designing, fabricating and successfully testing a new spray boom configuration for wide bed organic crops and ii) developing a precise method of applying pymetrozine through the irrigation system in conventional crops (Jacobson, 2010).

As the IPM programme became more refined, it was clear that the performance of some biological control agents was being seriously impaired by intraguild predation (IGP) and hyperparasitism. Desk studies and preliminary work in commercial crops during 2010 provided an insight into both issues (Jacobson, 2010). Recommendations were made to avoid the use of the biological control agents that are most vulnerable to IGP but there was little more that could be done about that issue at that stage. However, hyperparasitoids presented a much greater problem and it became clear that their impact must be reduced if we were to optimise biological control of aphids within IPM programmes.

Hyperparasitism

Hyperpasitoids are secondary insect parasitoids that develop at the expense of biological control agents and thereby threaten the success of IPM programmes. Prior to 2010, we had very little information about levels of hyperparasitism in aphid populations in commercial pepper crops. We knew that they were present because we had seen typical emergence holes in mummified aphids. However, we had no information about the extent of the problem or the species that were involved. A desk study in 2010 compiled existing knowledge of hyperparasitism. Although information of direct relevance to pepper crops was very sparse, we were able to extrapolate from more general information about the biology and behaviour of hyperparasites in outdoor agro-ecosystems. In addition, snap shot sampling in commercial pepper crops during 2010 revealed at least seven species of hyperparasitoids. The timing of emergence of the various hyperparasitoids suggested that they may be attacking each other. However, more thorough sequential sampling throughout the season was required to determine which species presented the greatest threat to the IPM of our aphid pests (see Objective 1).

We hypothesised that a thorough understanding of hyperparasitoid foraging behaviour could enable us to interrupt the process and thereby reduce the commercial impact of hyperparasitism. Ultimately, we envisaged identifying the chemical cues which hyperparasioids use to find their hosts and incorporate them into traps. Unfortunately, little information was available about the factors involved in host location. We proposed building a team of appropriate researchers who could address this issue generically across a wide range of agro-ecosystems (see Objective 3).

Open **rearing units**

Open rearing units (ORUs) or banker plants have been used to boost numbers of parasitoids in protected cultivation for over 30 years (*eg* Jacobson *et al.*, 1998). The objective is to sustain a reproducing population of the natural enemies and thereby provide season-long suppression of the pest species. ORUs were tested in commercial pepper crops during 2010 and appeared to be successful during the early season (Taylor & Knight, pers. com.). However, by week 20 the units were infested with several species of hyperparasitoids (Jacobson, 2010). We do not know when the hyperparasites started to colonise the ORUs but it is clear that the control system had become compromised by mid-summer. Spanish research had indicated that ORUs could also be used to boost numbers of predatory syrphid flies during the summer (Pineda & Marcos-Garcia, 2008a/b). We proposed that it should be possible to use ORUs based on parasitoids in the early season and then switch to ORUs based on syrphid flies in the summer (see Objective 2).

General **Approach**

All practical work was done on commercial nurseries following the general approach that was successfully developed in HDC project PC 240 (Jacobson & Morley, 2007) and since adopted in HDC projects PC 251/251a (Jacobson, 2008) and PC 295/a (Jacobson, 2009 & 2010). This approach had immediately identified any important interactions with current agronomic practice and eliminated the need for additional exploitation phases to transfer the technology to the commercial situation. In all the examples provided above, the results of the research were implemented by growers during the projects. All trials were designed and data analysed with the assistance of Dr Fenlon (Warwick University).

The work focused on *Myzus persicae* and *Aulacorthum solani* which had regularly caused economic damage in commercial pepper crops. Other aphid species such as *Macrosiphum euphorbiae* and *Aphis gossypii* occurred sporadically and locally but were incidentally controlled by the biological and / or chemical measures taken against the two main species.

The project built on previous work which had shown *Aphidius colemani*, *Aphidius ervi* and *Aphelinus abdominalis* to be the three most effective primary parasitoids against *M. persicae* and *A. solani*. An IPM programme based upon these natural enemies was established from the start of the season under the guidance of Mr Robert Knight. This began with weekly releases of *A. colemani*, *A. ervi* and *A. abdominalis* at 0.5, 0.25 and 0.5 / m^2 respectively. These rates were modified as necessary in response to aphid development and/or the use second line of defence treatments. On occasions, *Aphidius matricariae* were released instead of *A. colemani* or *A. ervi* due to supply issues. In addition, *Praon volucre* always invaded naturally and rapidly became established without the need to release purchased products.

Materials and methods

Objective 1. Study of hyperparasitism

The main body of this work was done in organic pepper crops (Site 1 - Somerset) because there was less likelihood of the available SLoD treatments influencing the establishment of hyperparasitoids. Samples of mummied aphids were collected in week 11 2011 (mid-March) to obtain a base line for hyperparasitoid activity. The main sampling period was between week 15 2011 (mid-April) and week 38 2011 (late-September). The crops were visited on 14 occasions during that 23 week period. In addition, a final assessment was done in week 43 2011 to determine the level of hyperparasitoid activity as we approached the end of the season. On each occasion, we attempted to collect 50-100 intact (*i.e.* non-emerged) mummies created by each of the *Aphidius*, *Aphelinus* and *Praon* parasitoids within colonies of both *Myzus persicae* and *Aulacorthum solani*; *i.e.* up to six aphid / parasitoid combinations. In practice, it was not always possible to collect the full complement of all these combinations; intact mummies created by *Aphelinus* being particularly elusive. Each batch of each aphid / parasitoid mummies was subjected to emergence tests; *i.e.* the samples were incubated at room temperature ($20^{O}C$+/-$3^{O}C$) for three weeks and the emergence of parasitoids and hyperparasitoids was recorded daily. Adult parasitoids from each batch of mummies were sorted into genera by Dr Rob Jacobson or Ms Clare Tickle and sub-samples were sent to a specialist taxonomist (Dr Michael de Courcy Williams) for definitive identification. The establishment and development of the various species of parasitoids and hyperparasioids was thus plotted throughout the season.

The opportunity was also taken to collect batches of *M. persicae* based mummies from conventional pepper crops (Site 2 - Essex), where we were particularly interested in the re-establishment of hyperparasites following mid-season (week 24) treatments with Chess (pymetrozine). At this site, mummies were collected on eight occasions between weeks 15 and 38. Each batch was incubated and the emergent adult parasitoids identified as described above.

Objective 2. Open rearing units

The trial was done in four blocks of conventional peppers at Essex (Site 2). All blocks received standard sequential release of *Aphidius colemani*, *Aphidius ervi* and *Aphelinus abdominalis* from the start of the season. In addition, Block 4 received open rearing units (ORUs) for the production of parasitoids until mid-May and then changed to syrphid production. Blocks 1 and 2 received parasitoid-based ORUs until the end of April and then became a control for comparison with Block 4. Block 3 received no ORUs and thereby served as the overall control. The ORU introduction programme is shown in Table 1. Each ORU comprised a hanging basket containing wheat plants infested with the grain aphid (*Sitobion avenae*) (Figure 1). The latter is a common host for the natural enemies without being a threat to the pepper crop. *Aphelinus abdominalis* were released onto the parasitoid-based units and *Episyrphus balteatus* were released onto the syrphid-based units.

Table 1. Open rearing unit placement programme (NB: building up to and then maintaining active ORUs at the rate of eight per ha)

	Week 6	Week 8	Week 10	Week 12	Week 14	Week 16	Week 18	Week 20	
Block									TOTAL
1	5	4	4	4	4	4			25
2	3	2	2	2	2	2			13
4	13	10	10	10	10	10	10	10	83

The crops were monitored on an approximate two week cycle until week 27. On each occasion, all crop rows were walked and colonies of *Myzus persicae* and *Aulocorthum solani* were recorded in individual units of three plants (*i.e.* nine heads). Each monitoring unit was attributed to one of the following categories:

- Category 1: 1-10 aphids
- Category 2: 11-100 aphids
- Category 3: 101-1000 aphids
- Category 4: 1001-10,000 aphids
- Category 5: Over 10,000 aphids

In addition, the percentage of aphids parasitised and showing as mummies were recorded as 0-50% or 50-100% in each unit. A succinct summary of the data provided three statistics:

- the total number of 'positive' aphid records
- the mean category for those records
- the percentage of records that had high parasitism rates (*i.e*. 50 – 100%).

The three statistic summary was used to produce a record of infestation over time.

The condition of the ORUs was monitored on a regular basis. Samples of mummified aphids were collected on five occasions (*i.e.* in weeks 9, 15, 20, 26 and 27). Each batch was incubated and the emergent adult parasitoids identified as described above. Numbers of immature *Episyrphus balteatus* were recorded from the ORUs in weeks 22, 24, 26 and 27.

Figure 1. An individual ORU and four ORUs in position in Block 4 in late-February 2011

Objective 3. Pave the way for more generic studies

Our objective was to pave the way for more generic studies aimed at strengthening parasitoid-based solutions for aphid control across a wide range of commercial crops. This involved building a team of appropriate researchers and then preparing a proposal which could be submitted to appropriate funding organisations.

In a 'spin-off' project, Ms Clare Tickle (MSc student, Imperial College) working with Dr Jacobson and Dr Toby Bruce (chemical ecologist at Rothamsted Research), investigated the existence of semiochemicals released by parasitised aphids, which could act as attractants to hyperparasitoids. Volatile compounds were entrained from 100 *Aulacorthum* / *Aphidius* aphid mummies for a period of 96 hours. The mummies were placed into a glass vial and charcoal-filtered air was pumped over them and then drawn out through Porapak Q (0.05g) filters at 350mL/min. Diethyl ether was used to elute the volatile compounds from the Porapak Q filter. Behavioural bioassays were carried out in a perspex four-arm olfactometer (Petterson, 1970; Bruce *et al*, 2008), which was uniformly lit from above. The inlet port of one arm contained filter paper treated with 10μL of the volatile-ether solution and the filter paper in the inlet ports of the other three arms were treated with pure ether; thus forming blank controls. Air was drawn through the arms to the center at a rate of 350mL/min. Mated female *Asaphes suspensus* wasps were individually placed into the centre of the olfactometer, and the subsequent time spent and the number of entries made into each arm was recorded over a period of 16 minutes using OLFA (Udine, Italy) software. To avoid directional bias the apparatus was rotated 90 degrees every 2 min. The results of this preliminary study were incorporated into the funding proposal.

Results

Objective 1. Study of hyperparasitism

Site 1

In total, 3,306 intact mummies were collected for emergence tests from Site 1. The percentage of mummies yielding adult primary parasitoids and adult hyperparasitoids is shown in Figure 2. There were very few aphids (healthy or mummified) on the pepper plants at the first inspection in week 11 and no hyperparasitoids were detected at that stage. Thereafter, both primary parasitoids and hyperparasitoids were found at every assessment. Overall, 54% (range 20-80%) of mummies yielded an adult parasitoid (*i.e.* primary or hyper), while the remainder died within the mummy.

From wk 15 to wk 43, the proportion of primary parasitoids to hyperparasitoids fluctuated between 60:40 and 30:70 with an overall mean marginally in favour of the hyperparasitoids (44:56).

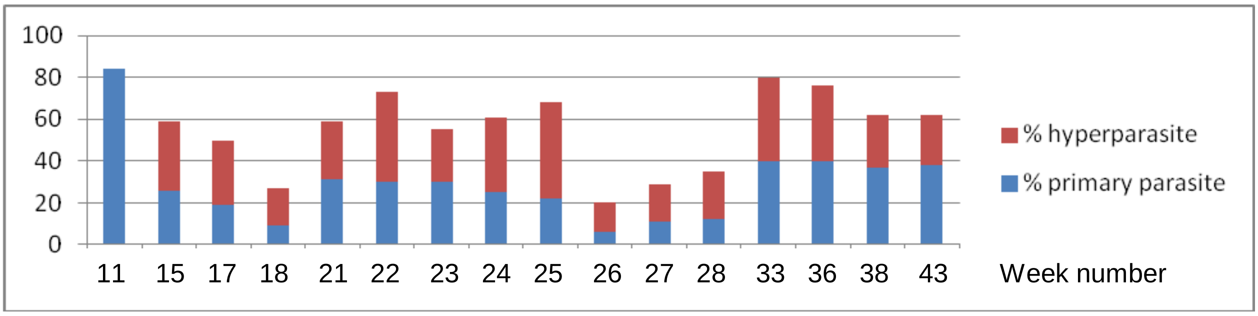

Figure 2. Percentage of adult primary parasitoids and hyperparasitoids emergent from all mummies collected at Site 1.

The results of formal identification of primary parasitoids were largely as expected for *Aphelinus* and *Praon*; *i.e.* all adults were *Aphelinus abdominalis* (Dalman, 1820) and *Praon volucre* (Haliday, 1833) respectively. In April, similar numbers of *Aphidius ervi* (Haliday, 1834) and *A. colemani* (Viereck, 1912) were identified from the emergence tests but no *A. colemani* were seen beyond May. *Aphidius matricariae* (Haliday, 1834) was found alongside *A. ervi* in late June and this species predominated by September.

The following seven species of hyperparasitoids were collected at Site 1 between weeks 11 and 43.

- CYNIPIDAE
  - Alloxystinae
    - *Alloxysta brevis* (Thompson, 1862)
    - *Alloxysta brachyptera* (Hartig, 1840)
    - *Alloxysta victrix* (Westwood, 1833)
- PTEROMALIDAE
  - Asaphinae
    - *Asaphes suspensus* (Nees, 1834)
  - Pteromalinae
    - *Pachyneuron aphidis* (Bouché, 1834)
- MEGASPILIDAE
  - Megaspilinae
    - *Dendrocerus aphidum* Rondani, 1877
    - *Dendrocerus laticeps* (Hedicke, 1929)

Figure 3 shows the percentage of mummies which yielded each of the four genera. *Asaphes suspensis* clearly dominated from week 15 onwards. The significance of the other six species in relation to their aphid / primary parasitoid host is described below.

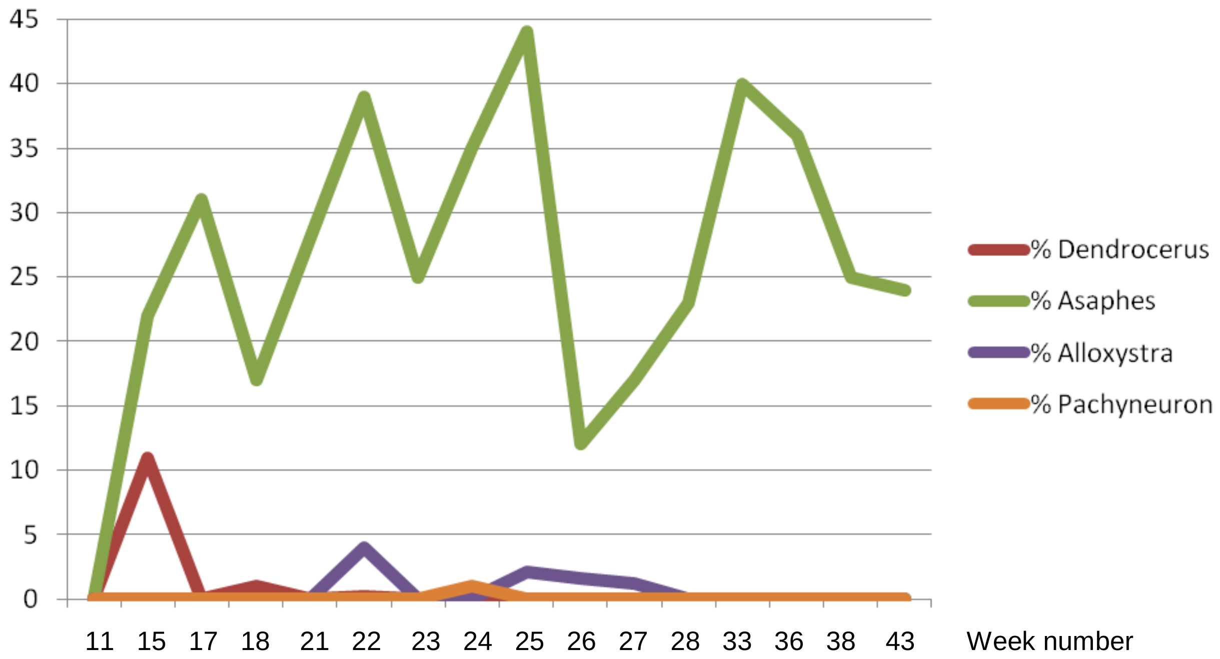

Figure 3. Percentage of mummified aphids which yielded each of the four genera of hyperparasitoids at Site 1 between weeks 11 and 43 2011.

Results from Aulacorthum-based samples at Site 1:

Aulacorthum solani was the most commonly found species of aphid from the beginning of the season until week 28 (mid July). Mummies created by both *Aphidius* and *Praon* were found throughout this period with about five times more *Aphidius* than *Praon*. Mummies created by *Aphelinus* were also present but in much smaller numbers and it was not always possible to collect an adequate sample.

The percentage of *Aulacorthum* / *Aphidius*-based mummies which yielded adult *Aphidius* or hyperparasitoids are shown in Figure 4. Overall, adult parasitoids (*i.e.* both primary and hyper) emerged from 51% (range 5%-73%) of the intact mummies collected. The remainder died within the mummy. The proportion of adult *Aphidius* to adult hyperparasitoids fluctuated between 61:39 and 20:80 with an overall mean clearly in favour of the hyperparasitoids (39:61). The following notes summarise the incidence of the various parasitoids:

- *Asaphes suspensus* were found from week 15 to 28, with a particularly large population around week 25. At its peak, this represented 44% of the mummies collected which was more than double the emergence of *Aphidius* spp. at that time.
- Two species of *Dendrocerus* (*D. laticeps* and *D. aphidium*) were found at the start of the monitoring period. This represented 17% of the mummies collected at week 15 but had

declined to less than 1% by week 18. None were found thereafter.

- Small numbers of *Alloxysta brevis* were recorded between weeks 22 and 27 but they never exceeded 3% of the mummies collected. Even smaller numbers of *A. brachyptera* and *A. victrix* were recorded between weeks 22 and 25 but they never exceeded 1% of the mummies collected.

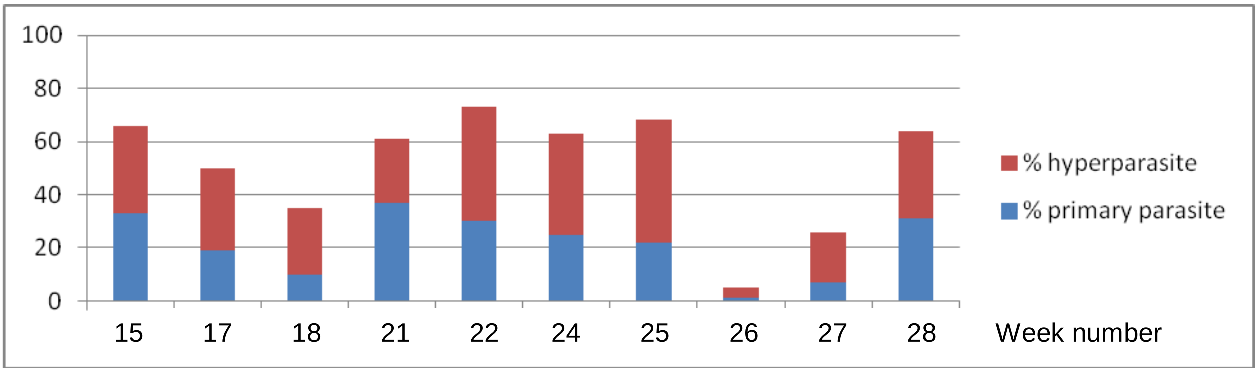

Figure 4. Percentage of *Aulacorthum* / *Aphidius-based* mummies which yielded adult *Aphidius* or hyperparasitoids at Site 1 between weeks 15 and 28.

The percentage of *Aulacorthum* / *Praon*-based mummies which yielded adult *P. volucre* or hyperparasitoids are shown in Figure 5. Overall, adult parasitoids emerged from 48% (range 13%-68%) of the intact mummies collected. This was similar to the overall emergence from *Aphidius*-based mummies*.* The proportion of adult *P. volucre* to adult hyperparasitoids fluctuated between 62:38 and 0:100 with an overall mean clearly in favour of the hyperparasitoids (35:65). Although the overall mean was similar to the *Aphidius*-based mummies, there was larger variation between samples. The result from week 26 was particularly notable because there was no adult *P. volucre* emergence at all. The following notes summarise the incidence of the various parasitoids:

- As with *Aphidius*-based mummies, *Asaphes suspensus* were the most numerous hyperparasitoid recorded throughout the monitored period.
- Small numbers of *Dendrocerus laticeps* were found between weeks 15 and 22. This represented 8% of the mummies collected at week 15 but only 1% in weeks 18 and 22. None were found thereafter.

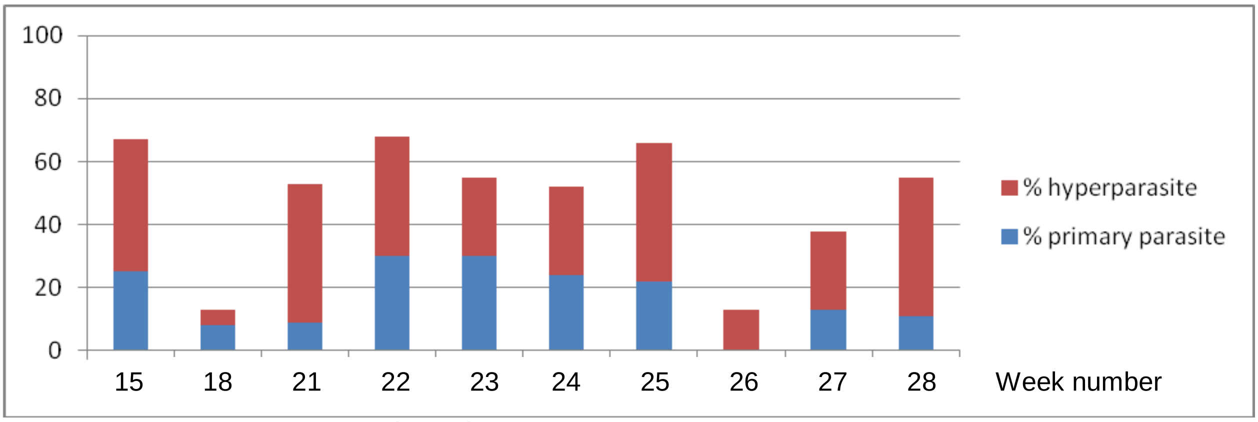

Figure 5. Percentage of *Aulacorthum* / *Praon*-based mummies which yielded adult *P. volucre* or hyperparasitoids at Site 1 between weeks 15 and 28.

Aulacorthum / *Aphelinus*-based mummies were found between weeks 15 and 27 but numbers were small and did not justify the production of a chart. Nonetheless, some useful information may be gleaned from the emergence tests. Overall, adult parasitoids emerged from 58% (range 0% - 76%) of the intact mummies collected. The proportion of adult *Aphelinus* to adult hyperparasitoids fluctuated between 100:0 and 50:50 with an overall mean in favour of the primary parasitoid 66:34. The following notes summarise the incidence of the various parasitoids:

- The main species of hyperparasitoid was once again *Asaphes suspensus*.
- Small numbers of *Alloxysta brachyptera* were recorded in week 26.
- Very small numbers of *Pachyneuron aphidius* were found in week 27.
- The conclusions which can be drawn from this data set are limited due to the relatively small sample size. However, they do show that *Aphelinus*-based *Aulacorthum* mummies are a suitable host for at least three species of hyperparasitoids.

Results from Myzus-based samples at Site 1:

Myzus persicae started to become more numerous around week 26 (late June) and was the most commonly found species of aphid from mid July until the end of the season. Mummies created by both *Aphidius* and *Praon* were found throughout this period with more than twice as many *Aphidius* as *Praon*. No mummies created by *Aphelinus* were collected from *Myzus* colonies.

The percentage of *Myzus* / *Aphidius*-based mummies which yielded adult *Aphidius* or hyperparasitoids are shown in Figure 6. Overall, adult parasitoids (*i.e.* both primary and hyper) emerged from 56% (range 24%-82%) of the intact mummies collected. This was broadly similar to the data collected from the *Aphidius*-based *Aulacorthum* mummies. The proportion of adult *Aphidius* to adult hyperparasitoids fluctuated between 59:41 and 25:75 with an overall mean marginally in favour of the hyperparasitoids (47:53). Once again, this was broadly similar to the *Aphidius*-based *Aulacorthum* mummies. The following notes summarise the incidence of the various parasitoids:

- All adult *Aphidius* emerging from these mummies between weeks 26 and 36 were identified as *A. matricariae.*
- *Asaphes suspensus* were found from week 26 to 43 and were the dominant hyperparasitoid.
- Small numbers of *Alloxystra brevis* were recorded in week 26 but this was only 2% of the mummies collected.

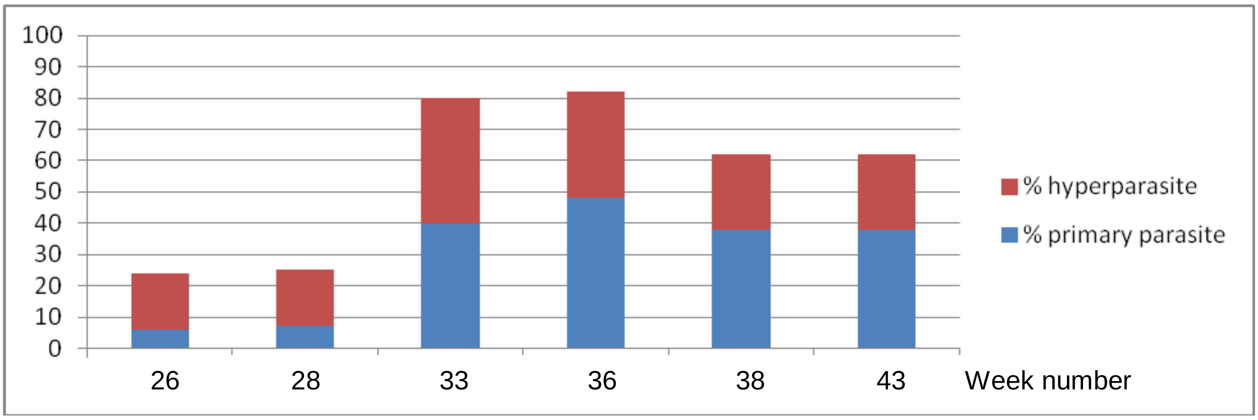

Figure 6. Percentage of *Myzus* / *Aphidius-based* mummies which yielded adult *Aphidius* or hyperparasitoids at Site 1 between weeks 26 and 43.

The percentage of *Myzus* / *Praon*-based mummies which yielded adult *P. volucre* or hyperparasitoids are shown in Figure 7. Overall, adult parasitoids emerged from 35% (range 3%-62%) of the intact mummies collected. This was the poorest overall emergence of all the various combinations of aphids and primary parasitoids. The proportion of adult *P. volucre* to adult hyperparasitoids fluctuated between 65:35 and 0:100 with an overall mean clearly in favour of the hyperparasitoids (36:64). This was comparable to the data from the *Praon*-based *Aulacorthum* mummies. The only hyperparasitoids collected from these mummies were *Asaphes suspensus.*

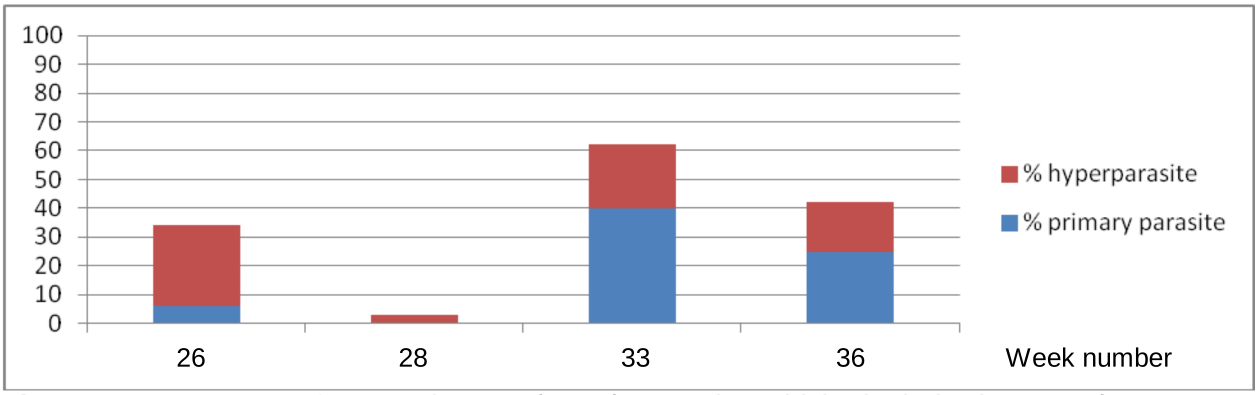

Figure 7. Percentage of *Myzus* / *Praon-based* mummies which yielded adult *P. volucre* or hyperparasitoids at Site 1 between weeks 26 and 36.

Site 2

In total, 576 intact mummies were collected for emergence tests from the crops at Site 2. The majority (94%) were *Myzus* / *Aphidius*–based mummies. There were also small numbers based on *Myzus / Praon* (2%) and *Myzus / Aphelinus* (2%) but neither type yielded any hyperparasitoids in emergence tests. In addition, samples were taken from a localised colony of *Macrosiphum euphorbiae* in week 18. The *Macrosiphum* / *Aphelinus*–based mummies were found to contain 80% *Asaphes suspensis* at a time when the *Myzus* / *Aphidius*–based mummies contained only 13% *A. suspensis.*

The percentage of mummies yielding adult primary parasitoids and adult hyperparasitoids is shown in Figure 8. Overall, adult parasitoids (*i.e.* both primary and hyper) emerged from 53% (range 18-84%) of the intact mummies collected. The mean emergence from samples collected in April / May was 26% while after the mid-season insecticidal treatments the mean increased to 77%. No hyperparasitoids were detected in the first emergence tests in April (week 15). The proportion of adult *Aphidius* to adult hyperparasitoids changed to 28:72 in May but returned to 100:0 after the mid season insecticidal treatments. The hyperparasioids then made a slow return in the late Summer / early Autumn, with proportions of *Aphidius* to adult hyperparasitoids changing from 99:1 in week 32 to 80:20 in week 38.

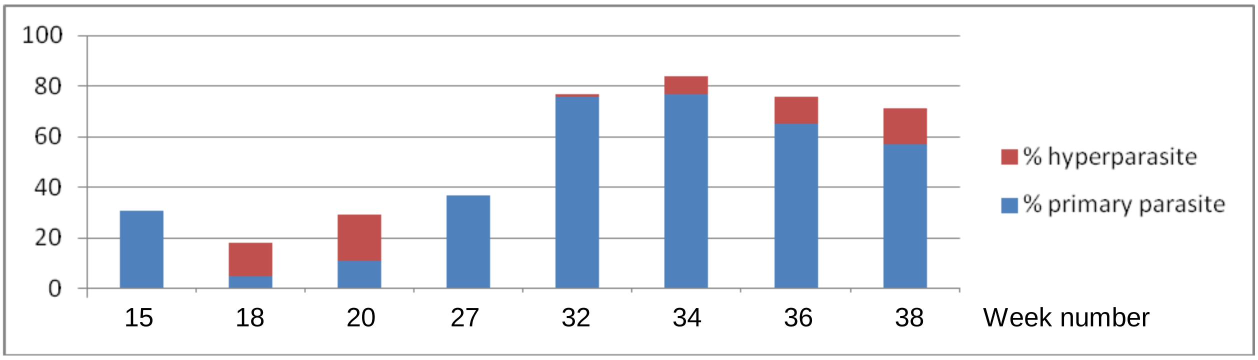

Figure 8. Percentage of adult primary parasitoids and hyperparasitoids emergent from all mummies collected at Site 2.

The results of formal identification of primary parasitoids were largely as expected. Both *Aphidius colemani and A. ervi* were identified throughout the season but no *A. matricariae* were found at this site. All the identified *Praon* were *P. volucre*.

The following eight species of hyperparasitoids were collected between weeks 15 and 38 2011.

- CYNIPIDAE
  - Alloxystinae
    - *Alloxysta brevis* (Thompson, 1862)
    - *Alloxysta brachyptera* (Hartig, 1840)
    - *Alloxysta fulviceps* (Curtis, 1838)
    - *Alloxysta victrix* (Westwood, 1833)
- PTEROMALIDAE
  - Asaphinae
    - *Asaphes suspensus* (Nees, 1834)
    - *Asaphes vulgaris* (Walker, 1834)
  - Pteromalinae
    - *Pachyneuron aphidis* (Bouché, 1834)
- MEGASPILIDAE
  - Megaspilinae
    - *Dendrocerus serricornis* (Boheman, 1832)

Figure 9 shows the percentage of mummies which yielded each of the four genera. *Asaphes* spp. dominated until the mid-season insecticidal treatments; all identified specimens being *Asaphes vulgaris.* However, there was a distinct change thereafter. The *Asaphes* population was at first undetectable and then very slow to return. In the absence of *Asaphes*, the other seven species of hyperparasitoids began to establish with *Pachyneuron aphidius* dominating by the end of season.

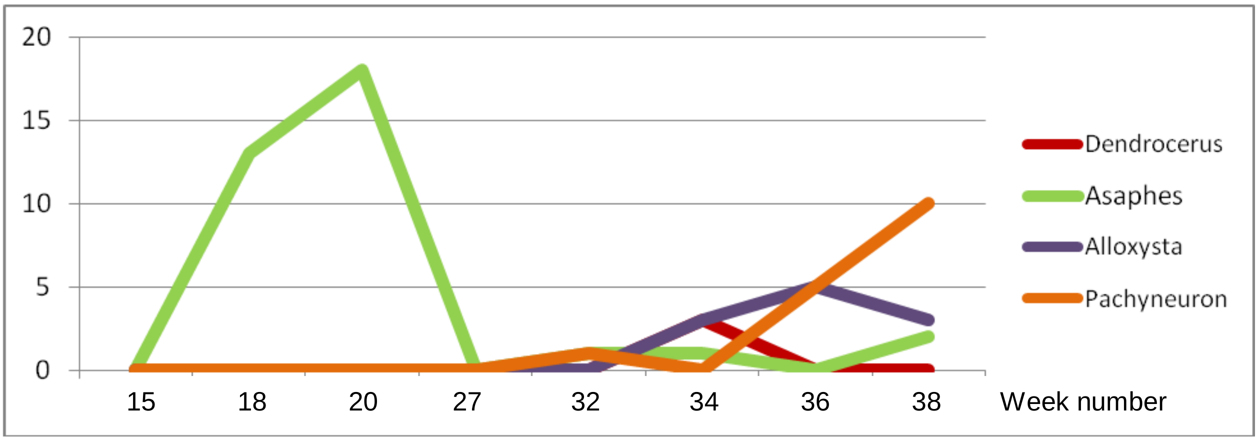

Figure 9. Percentage of mummified aphids which yielded each of the four genera of hyperparasitoids at Site 2 between weeks 15 and 38 2011.

Objective 2. Open rearing units

Aphid infestation over time

Figures 10 and 11 show the total number of 'positive' aphid records and the mean category score for those records for each block between weeks 14 and 27. Tabulated summaries of these data are included in Appendix 1. During that time the aphids were predominantly *A. solani*. The aphid damage was such that all blocks received supplementary treatments with Chess (pymetrozine) between weeks 23 and 25. Blocks 1 and 3 were sprayed in week 23, Block 4 in week 24 and Block 2 in week 25.

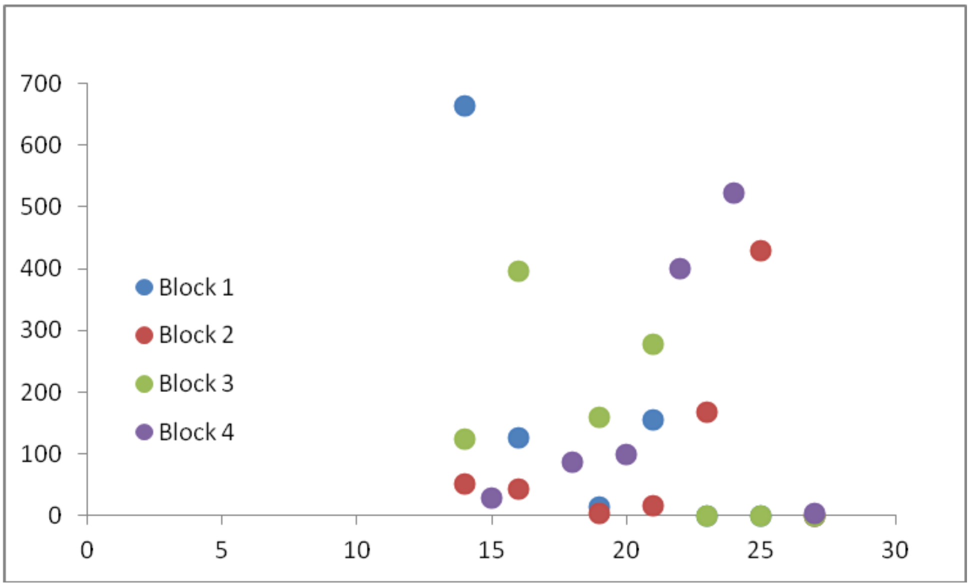

Figure 10. Number of 'positive' aphid records per sample week in each block

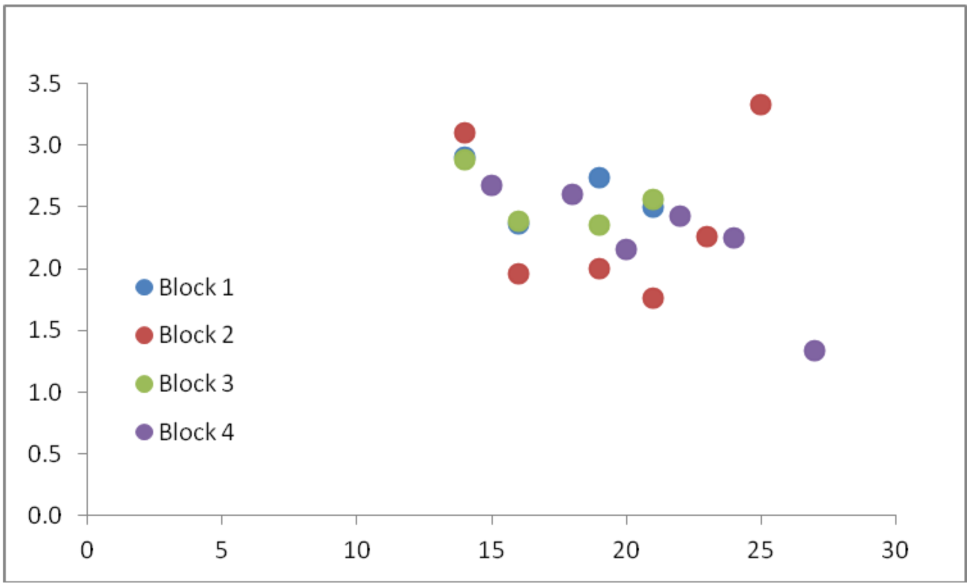

Figure 11. Mean category score of aphid colonies in each sample week in each block

Levels of parasitism

The proportion of aphid colonies within the higher (50-100%) parasitism category in each block between weeks 14 and 25 is shown in Table 2. Blocks 2, 3 and 4 had good levels of parasitism by *Aphidius* spp. and *Praon* spp. until week 19 (mid-May) but declined rapidly thereafter. Mummies formed by *Aphelinus* spp. were less numerous. Parasitism in Block 1 was never quite as good and it also declined after week 19.

Figure 12 shows a simple plot of the number of identified aphid colonies and the proportion of those colonies with parasitism in the higher category (*i.e.* 50 – 100%). There were 19 non-zero points across the four blocks. The correlation (r = –0.699 with 17d.f.) is significant at the 1% level demonstrating that as parasitism declines the number of aphid colonies increases.

Table 2. Proportion of aphid colonies within the higher (50-100%) parasitism category

Week Number 2011	Block 1	Block 2	Block 3	Block 4
14	7.2	96.1	99.2	67.9
16	69.3	86.4	70.8	
18				84.9
19	60.0	100.0	88.7	
20				54.5
21	30.1	58.8	64.7	
22				15.2
23	Sprayed	8.2	Sprayed	
24				8.6 (Sprayed)
25		1.6 (Sprayed)		

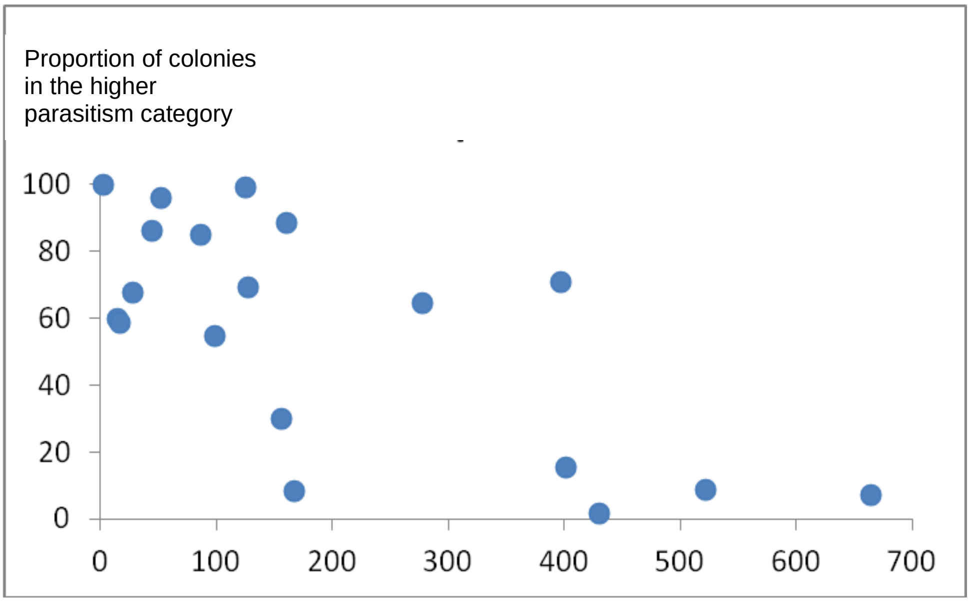

Figure 12. Correlation between the number of aphid colonies and the proportion of those colonies in the higher parasitism category.

Condition of open rearing units

The percentage of mummies collected from the ORUs which yielded adult primary parasitoids and adult hyperparasitoids is shown in Figure 13. In mid-April (week 15), 90% of the mummies yielded *Aphelinus abdominalis* and no hyperparasitoids were detected. By mid-May (week 20), the proportion of adult *A. abdominalis* to adult hyperparasitoids was 51:49 and by week 26 it was 13:87. *Pachyneuron aphidis* predominated throughout this period with *Alloxysta brevis, A. brachyptera, Asaphes vulgaris* and *Dendrocerus laticeps* present in smaller numbers.

In the early part of the season, each ORU remained productive for 10-12 weeks. A typical nine week old ORU in mid-April (week 15) is shown in Figure 14. Thereafter, the units deteriorated more rapidly and were producing few primary parasitoids by mid-May. The emphasis then switched to the production of syrphids. However, it proved very difficult to keep the ORUs in good condition during June and July. The cereal plants did not grow well in the warmer conditions and were vulnerable to disease (eg Figure 14). The cereal aphids did not thrive despite the populations being 'topped up' from an on-site aphid production unit. In addition, they were attacked by a wide range of natural enemies including primary parasitoids, predatory midges, Orius bugs and ladybirds. Very few syrphid larvae were recorded on the ORUs or on the pepper plants during June. The trial was terminated in mid-July.

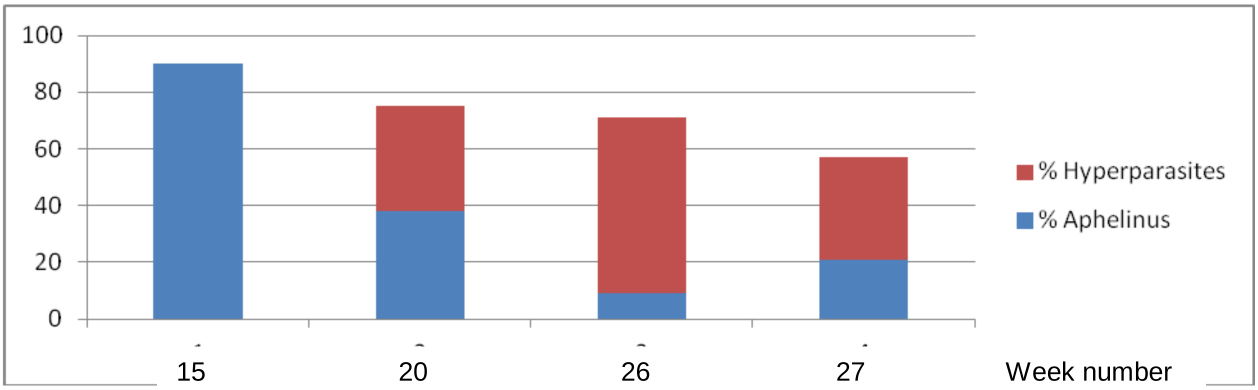

Figure 13. Percentage of adult primary parasitoids and hyperparasitoids emergent from open rearing units at Site 2 between weeks 15 and 27.

Figure 14. Condition of a typical nine week old ORU in mid-April (left) and a five week old ORU infected by mildew in early-June (right).

Objective 3. Pave the way for more generic studies

The results of the bioassay, testing the response of *A. suspensus* to volatiles collected from 100 mummified *A. solani*, reared on sweet pepper and parasitised by *Aphidius*, indicated an attraction to the treatment, with a mean time spent in the treatment arm of 4.71±0.40, compared with 2.80±0.21 minutes spent in the control (n= 5, $p<0.05$). In addition, *A. suspensus* made more entries into the treatment arm than into the control arms (treatment mean= 23±2.77, control mean= 17.60±2.21, n= 5, $p<0.05$) (Figure 15).

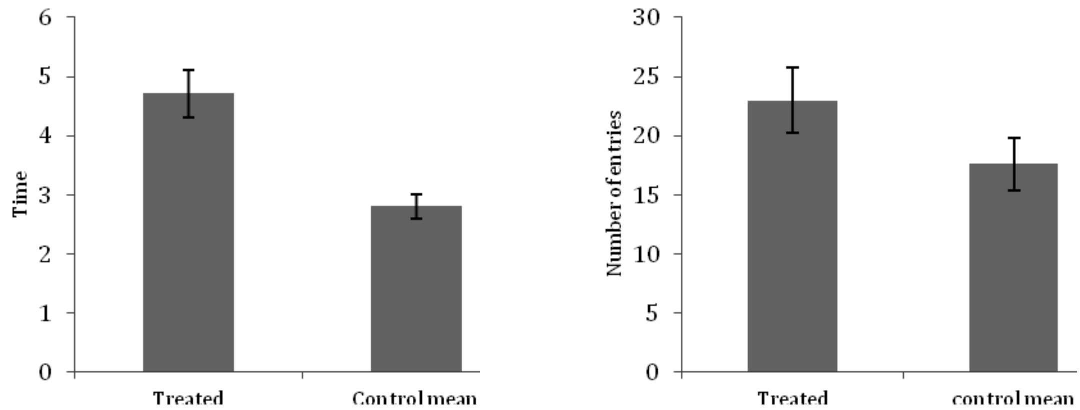

Figure 15. The response of the hyperparasitoid A. suspensus to headspace samples collected from mummified *A. solani*, parasitised by *A. ervi*. The chart on the left shows the time spent in the various arms of the olfactometer while the chart on the right shows the number of entries to each arm.

Based on the work completed in the present HDC project and the spin-off project, we have paved the way for more generic studies ultimately aimed at managing hyperparasitoids in a wide range of crops by incorporating the semiochemical in hyperparasitoid traps. A team of researchers with complementary expertise has been assembled at Rothamsted Research. Dr Toby Bruce has drafted a Full Proposal for BBSRC funding via the ‘industry partner’ route. This has already been approved for submission to BBSRC by the rigorous internal review procedure at Rothamsted Research. Valley Grown Nurseries, Cantelo Nurseries Ltd and Koppert UK have pledged their support to the proposal, thus building on their previous input to HDC projects PC 295-295b. Dr Bruce and Dr Jacobson have prepared a separate Proposal requesting that HDC become industry partners by contributing 10% of the project costs (submitted week 51 2011).

Discussion

Ten species of hyperparasitoids were found during the study. Table 3 shows which of these were associated with each of the six different combinations of aphid and primary parasitoid. Only *Asaphes suspensus* was found in every type of mummified aphid. This was also the most numerous species.

Table 3. Relationship between hyperparasitoids and six different combinations of aphids and primary parasitoids (y = recorded)

	Aulacorthum solani			*Myzus persicae*		*Macrosiphum euphorbiae*
	Aphidius	*Praon*	*Aphelinus*	*Aphidius*	*Praon*	*Aphelinus*
Alloxysta brevis	y			y		
Alloxysta brachyptera	y		y	y		
Alloxysta fulviceps				y		
Alloxysta victrix	y			y		
Asaphes suspensus	y	y	y	y	y	y
Asaphes vulgaris				y		
Pachyneuron aphidis			y	y		
Dendrocerus aphidum	y					
Dendrocerus laticeps	y	y				
Dendrocerus serricornis				y		

At Site 1, the proportion of primary parasitoids to hyperparasitoids fluctuated between 60:40 and 30:70 with an overall mean marginally in favour of the hyperparasitoids (44:56). This is clearly illustrated in Figure 16. A similar situation existed at Site 2 until the mid-season insecticidal treatment. This indicates that the hyperparasitoids coexist with their primary parasitoid hosts rather than eliminate them.

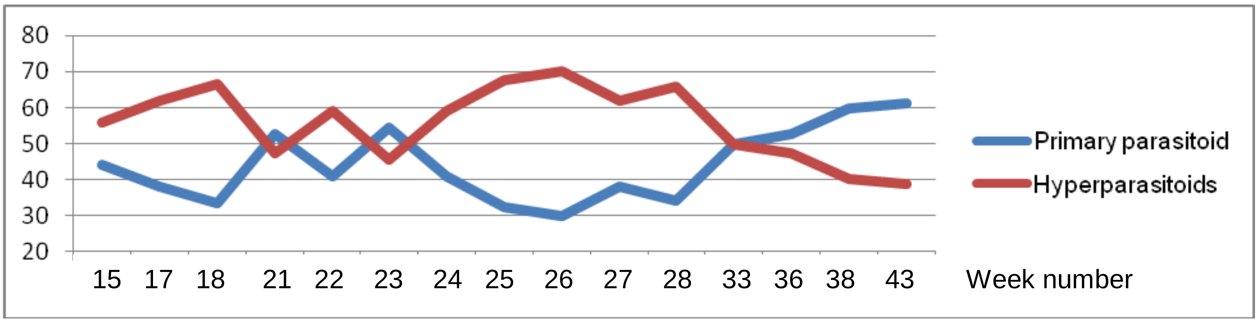

Figure 16. Proportion of primary parasitoids to hyperparasitoids throughout the season at Site 1.

Overall, 54% (range 20-80%) of mummies collected at Site 1 yielded an adult parasitoid (*i.e.* primary or hyper). The percentage was lower (26%) at Site 2 until the mid-season insecticidal treatment. We would normally expect 80-85% emergence of primary parasitoids from healthy cultures. Supplementary studies indicated that the difference between the normal expectation and the actual emergence could be attributed to host-feeding by adult hyperparasitoids. This was consistent with information gleaned from a German PhD thesis (Christiansen-Weniger, 1992), which states that female *Asaphes vulgaris* inject venom that kills the primary parasitoid pupae immediately before ovipositing or host feeding. The proteins gained from host feeding are important for the maturation of eggs. It would seem that host feeding by hyperparasitoids may be more detrimental to the primary parasitoid population than oviposition and development of offspring.

As a general trend, as the proportion of hyperparasitoids increased, the overall emergence from mummies decreased; *eg.* in week 26 there was a 70:30 balance in favour of hyperparasitoids and only 20% overall emergence. This trend is consistent with the assumption of host feeding by adult hyperparasitoids.

At Site 2, numbers of the dominating hyperparasitoid, *Asaphes vulgaris*, declined to below a detectable level following the mid-season insecticidal treatment. Aphids rapidly recolonised the crop as did the primary parasitoids which were still being released on a weekly basis. The hyperparasitoids were slower to recolonise and did not make a serious impact on the biological control programme for the rest of the season.

The population trends of the four genera of hyperparasioids shown in Figure 9 indicate that *Asaphes* spp. were suppressing members of the other three genera until the mid-season insecticidal treatment. This also seems to be the case beyond week 15 at Site 1 (Figure 3).

An excellent model system was selected to study hyperparasitism on a generic level. This is based on the aphid, *Myzus persicae*, a primary parasitoid from the genus *Aphidius*, and a secondary parasitoid from the genus *Asaphes*.

The ORUs produced numerous *Aphelinus abdominalis* until mid-April but thereafter became overrun by hyperparasitoids. The dominant secondary species was *Pachyneuron aphidis* with *Alloxysta brevis*, *Alloxysta brachyptera*, *Asaphes vulgaris* and *Dendrocerus laticeps* present in smaller numbers. This had been anticipated and was the principle reason for the switch to syrphid production in the ORUs from mid-May.

The cereal plants did not grow well during June / July and became infected by mildew. The cereal aphids were attacked by a wide range of natural enemies and the populations did not thrive despite being ‘topped up’ from an on-site aphid production unit. The syrphids did not colonise the ORUs and very few were seen on the pepper plants during June. In July, populations of syrphids were numerous in outdoor habitats and it was impossible to distinguish between those released in the glasshouse and natural invaders. By then, the remnants of the ORUs had essentially become hyperparasitoid rearing units and they were removed from the glasshouse.

The aphids on the pepper plants were predominantly *A. solani* throughout this period. Primary parasitism was good until mid-May regardless of the presence of ORUs but declined rapidly thereafter in all treatments. By cross reference to Figure 8, it can be seen that this decline coincided with a marked increase in hyperparasitoid activity. All blocks required supplementary treatments with Chess (pymetrozine) during early-mid June.

The results of the spin-off project demonstrated the existence of a semiochemical released by mummified aphids which acted as an attractant to *A. suspensus*. This was broadly consistent with the results of a previous study by Chritiansen-Wenger (1994) and helped to explain partially conflicting assumptions made by Buitenhuis *et al.* (2004). Based on the work completed in this HDC project and the spin-off project, a team with complementary expertise has prepared a Full Proposal for BBSRC funding via the 'industry partner' route. The proposal incorporates HDC and three private companies as industrial partners.

Conclusions

- Ten species of hyperparasitoids were found attacking primary parasitoids in UK pepper crops. The dominant species were members of the genus *Asaphes.*
- Hyperparasitoids coexist with their primary parasitoid hosts rather than eliminate them.
- Host feeding by hyperparasitoids appears to be more detrimental to the primary parasitoid population than oviposition and the subsequent development of offspring.
- Parasitoid-based ORUs produced large numbers of *A. abdominalis* in the early season but were overrun by hyperparasitoids by mid-May. It became difficult to maintain ORUs in good condition through June / July and the switch to a syrphid-based system was unsuccessful.
- The combined use of primary parasitoid-based and syrphid-based ORUs may have assisted aphid control in the early season but did not obviate the need for mid-season treatments with an IPM compatible insecticide.
- Numbers of the dominating hyperparasitoid, *Asaphes* spp., declined to below a detectable level following the mid-season insecticidal treatment of Chess (pymetrozine). Aphids and primary parasitoids rapidly recolonised the crop but hyperparasitoids did not make a serious impact on the biological control programme for the rest of the season.

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Appendix 1

Summary of crop monitoring data for Blocks 1 to 4 at Site 2 between weeks 14 and 27

Block 1

Week no.	total positive aphid samples	proportion in 50-100% parasitism category	mean aphid category
14	664	7.229	2.904
16	127	69.291	2.362
19	15	60.000	2.733
21	156	30.128	2.500
23	0		
25	0		
27	0		

Block 2

Week no.	total positive aphid samples	proportion in 50-100% parasitism category	mean aphid category
14	52	96.154	3.096
16	44	86.364	1.955
19	3	100.000	2.000
21	17	58.824	1.765
23	167	8.383	2.263
25	430	1.628	3.326
27	0		

Block 3

Week no.	total positive aphid samples	proportion in 50-100% parasitism category	mean aphid category
14	125	99.200	2.880
16	397	70.781	2.383
19	160	88.750	2.356
21	278	64.748	2.558
23	0		
25	0		
27	0		

Block 4

Week no.	total positive aphid samples	proportion in 50-100% parasitism category	Mean aphid category
15	28	67.857	2.679
18	86	84.884	2.605
20	99	54.545	2.162
22	401	15.212	2.424
24	522	8.621	2.255
27	3	0.000	1.333