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The results and conclusions in this report are based on investigations conducted over a twelve-month 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
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Signature *RJ Jacobson* Date *December 2010*

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GROWER SUMMARY

Headline

- An IPM programme based on the systematic release of parasitoids and the application of 'soft' soap treatments through a modified vertical spray boom configuration controlled aphids in wide bed organic pepper crops.
- A precise method of applying pymetrozine through irrigation also controlled local infestations of aphids within conventional pepper crops.

Background and expected deliverables

The preceding HDC Project (PC 295) devised a new IPM compatible strategy for aphid control in organic pepper crops. This consisted of primary biological control measures 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 when implemented on a larger scale:

- Spray coverage of the SLoD products was inadequate in wide-bed organic crops.
- The performance of the parasitoids was impaired by hyperparasitism (*i.e.* naturally occurring parasitoids which attack the parasitoids being used as biological control agents).
- There appeared to be a negative interaction between some biological control agents utilised in the IPM programme.

The current work built on the findings of the first study by addressing the difficulties and introducing some additional options for aphid control.

Conventional growers have access to more effective SLoD products. One such product, pymetrozine (Chess WG), had recently received a Specific Off Label Approval (SOLA - Number 2024/2009) for application through the irrigation system. This project sought a means of using the irrigation system for localised rather than entire crop treatments.

The project also investigated the possibility of using open rearing units (ORUs) in pepper crops, with particular emphasis on their use as a breeding base for alternative parasitoids (*e.g. Aphelinus spp.*) and novel biological control agents (*e.g. syrphids*).

Summary of the project and main conclusions

Modification of vertical boom sprayer for wide bed organic crops

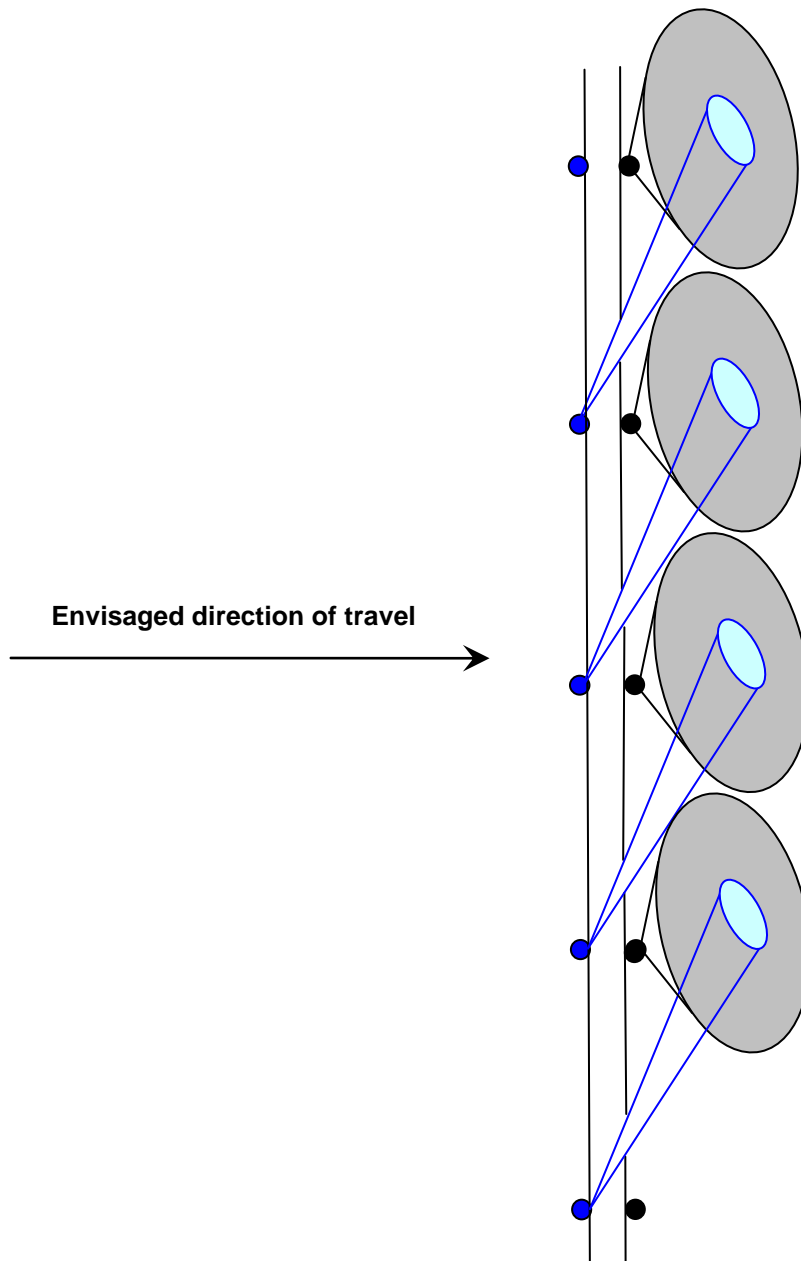
Some organic pepper crops are grown in double rows to facilitate incorporation of compost into the soil beds. This creates a wide canopy which is difficult to penetrate with sprays. Furthermore, many of the leaves on pepper plants hang at an angle of approximately 30° off vertical creating a virtual outer wall of foliage.

A critical assessment of the performance of the current robotic spray equipment was initially done. This was followed by the design and fabrication of a replacement unit. The important criterion was that the nozzles drove a spray underneath the near-vertical leaves, then lifted those leaves up momentarily to permit finer spray to penetrate and cover the lower leaf surfaces inside the canopy. Two types of nozzle were employed which were called 'lifters' and 'fillers' according to their purpose

A pair of replacement booms were built as a retro-fit to the robotic sprayers. The configuration incorporated ten lifters and ten fillers in separate rows on each boom. Each lifter was orientated upwards at 60° from the horizontal and fired into the spray cone of the filler nozzle on the tier above, close to the point where the spray met the outermost leaves of the crop. This had the effect of lifting the outer leaves and letting in the fine spray from the fillers. It also created maximum turbulence in a twisting motion so that no leaf stayed in one position for any length of time.

A pictorial illustration of the modified vertical boom sprayer as well as its image are given below (Figure i):

Figure i. Side of boom viewed from crop



(Note: Spray from the narrow-angle “lifter” nozzles is shown in blue, while spray from the finer “filler” nozzles is shown in grey.)

Figure ii. New booms fitted to the robotic sprayer:



(Note: At this stage the fans are still fitted to the base of the unit)

Biological evaluation of new spray booms

This part of the project served to evaluate the efficacy of the new spray booms and the efficacy of the SLoD strategy against a *Myzus persicae* (peach potato aphid) population. The parasitoids, *Aphidius ervi* and *A. colemani*, had been released systematically since the start of the season and, in addition, a natural population of *Praon* spp. had become established. Overall, approximately 8% of the aphid population was mummified by these parasitoids at the start of the trial.

Aphid numbers increased rapidly toward the end of May 2010 and some plants were starting to become sticky. This was the agreed signal to apply the SLoD treatment (1.5% Savona). The effect was compared to untreated controls. There were a total of 50 sample stations within clearly defined plots. Assessments were done immediately before the spray was applied and 1, 6 and 42 days post-treatment. On each occasion and at each sample station, numbers of aphids and mummified aphids were recorded

on one leaf in four positions within the crop canopy; *i.e.* upper inner, upper outer, lower inner and lower outer. In addition, intact aphid mummies were collected 1 and 42 days post-treatment and incubated in ventilated Petri-dishes to measure the proportion of parasitoids that successfully emerged from treated mummies. This provided a measure of the impact of the SLoD on the parasitoid population and a measure of the proportion of mummies that contained hyperparasites.

Following the application of Savona, there was an immediate reduction in aphid numbers and this was similar in all positions showing that the spray had successfully penetrated the whole canopy. Overall, the aphid numbers were reduced by approximately 90% at day 1 and by 97% at day 6. The aphid population recovered between day 6 and day 42 but numbers were still only one third of the pre-treatment count. No further spray interventions were required against aphids in that area. In contrast, in the untreated control, numbers of aphids increased nearly three fold during the first six days post-treatment leading to unacceptable quantities of sticky fruit and additional control measures were required.

Six days after application of the Savona treatment, mummies represented 66% and 11% of the remaining aphid population in the Savona and untreated plots respectively. Once again, there were no differences between sample points within the plant canopy. These results clearly illustrate how the SLoD treatment shifted the balance of the insect populations to the advantage of the parasitoids and thus helped to prevent any further fruit contamination. Hyper-parasitism by *Dendrocerus* increased from 5% to 15% during the trial but did not prevent season-long control of aphids in this case.

Pymetrozine applied via the irrigation system

The irrigation system can provide a useful vehicle for cost-effective application of systemic pesticides. When the product is applied at the central mixing point it is usually necessary to treat the whole area served by the system. However, aphid populations build up unevenly and SLoD treatments are only usually required in localised areas of crops. A more precise method of application was developed. This was based on a water powered Dosatron D20s applicator that accessed the system via individual irrigation manifolds thereby allowing the separate treatment of smaller areas served by each valve.

There was some debate over the interpretation of the information provided in the SOLA. The maximum individual dose for sweet pepper is 15 g product / per 1000 plants via the drip irrigation. The 'advisory information' suggests using this rate against whiteflies but a lower rate of 10 g product / per 1000 plants against aphids. The SOLA does not distinguish between 'plants' and 'heads', nor between young and mature plants, yet the quantity of foliage varies hugely at the extremes. All leaves are usually left on a pepper crop until the end of the season but, in this case, the lower leaves had been removed and it wasn't clear whether this would affect the uptake of the chemical or its distribution within the plant. In this first trial of the delivery system, the SOLA was interpreted literally in terms of 'plants' but the maximum individual dose rate was used due to the maturity of the plants.

There were two distinct phases to the study. In the first phase, a method of calibrating the equipment to determine the time required to take up and distribute the stock solution throughout the trial area was developed. The method is described in detail in the full report. Growers are advised to fully understand their own irrigation system before applying products through drippers.

In the second phase of the work, the efficacy of the technique against *Myzus persicae* on 1644m² of mature pepper crop was evaluated. There were approximately 4,000 plants which required 60g Chess WG applied from a 15 litre stock solution. The irrigation was turned off mid-afternoon to allow the plants to partially dry out the rockwool growing medium. The treatment was applied during cloudy conditions in the late afternoon. There were no further irrigation runs that day and drainage was reduced the following day to maximise the uptake and minimise flushing of the product through the growing medium.

Sample points to measure the size of the aphid population were established throughout the crop prior to treatment. Pymetrozine is known to have a slow effect and so the second assessment was delayed until 13 days post-treatment. On both occasions, the numbers of live and mummified aphids were recorded at every sample position. In addition, at the post-treatment assessment, mummified aphids were collected from the sample points and confined in dishes to determine whether parasitoids successfully emerged, thus indicating whether the treatment had been harmful to the immature wasps. This also provided a measure of hyperparasitism.

Chess WG was totally effective against *M. persicae* when applied by this technique. However, it may be necessary to repeat the trial in different situations; the most demanding being a fully mature crop with a full leaf canopy using the lower 'advisory' rate of 10g product per 1,000 plants. The impact of the treatment on parasitic wasps was unclear because the results were complicated by the presence of hyperparasites and this may require further investigation.

Hyperparasitism

Hyperparasitoids are secondary insect parasitoids that develop at the expense of biological control agents and thereby threaten the success of IPM programmes. Prior to this season, 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.

Large samples of mummified aphids were collected from commercial pepper crops and incubated at room temperature. The emerging wasps were sorted into genera and examples were identified by a specialist insect taxonomist. Five species of hyperparasites of the genera *Dendrocerus*, *Asaphes* and *Pachyneuron* were found associated with *Myzus persicae* / *Aphidius* mummies between June and September. Levels of hyperparasitism ranged from 8% to 63%; in some cases the population increased as the summer progressed but in others it decreased. The majority of *Aphidius* adults emerged within seven days of collection while hyperparasitoids typically emerged much later. Samples were also collected from ORUs based on barley plants infested with the cereal aphid, *Sitobion avenae*, and the primary parasitoid, *Aphelinus abdominalis*. 72% were found to be hyperparasitised, including three species from the genera *Dendrocerus* and *Alloxysta*. The timing of emergence of the various hyperparasitoids suggested that they may be attacking each other.

In addition to the practical surveys, a desk study sought to gain more background knowledge about hyperparasitism. Although information of direct relevance to protected peppers was sparse, general information about the biology and behavior of hyperparasites in other situations was compiled.

Dendrocerus, *Asaphes* and *Pachyneuron* are known as ectophagus hyperparasites. The female deposits her egg on the surface of the primary parasitic larva after the aphid is killed and mummified. The hyperparasitic larva then feeds externally on the primary host while both are still inside the mummy. We have detailed life-table data for *Dendrocerus carpenteri* on *Aphidius smithi* which may provide an approximate guide to the insect's development in UK pepper crops. Adult *D. carpenteri* emerge from the mummy approximately 16 days after oviposition. In the case of *Asaphes californicus*, the development from egg to adult takes about 21 days. Other species of the *Asaphes* genera are known to attack other hyperparasites.

Alloxysta are known as endophagus hyperparasites. The female deposits her egg inside the primary parasitoid larva while it is still developing inside the live aphid but before the aphid is mummified. The egg does not hatch until after the mummy is formed and then the hyperparasitic larva feeds on the primary larval host. In the case of *Alloxysta victrix*, the adult emerges 19 days after the original oviposition.

All the ectophagus species that we have identified in our crops are non-specific feeders; *i.e.* they attack a wide range of primary parasites irrespective of the aphid. Although the endophagus hyperparasitoids are usually host specific, at least one member of the *Alloxysta* genus is an exception to this rule.

A thorough understanding of hyperparasitoid foraging behaviour could enable us to interrupt the process and thereby reduce the commercial impact of hyperparasitism. Unfortunately, little information is available about the factors involved in host location. Generally, aphid honeydew provides information about the presence of aphids as the first step in locating the primary parasitoids and, thereafter, the female may be influenced by volatile chemicals released by the primary parasitoid.

Interactions between biocontrol agents

Conflicts between natural enemies are known as 'intraguild predation' (IGP). A few reviews have focused on IGP among the natural enemies associated with aphids. It is commonly believed that this constitutes one of the main forces influencing the dynamics of aphid feeding natural enemy populations and should be taken into account in all research studies. It is also widely acknowledged that the direct and indirect implications of IGP are complex and extremely difficult to study in the field.

Orius are generalist predators and are reported in the scientific literature to feed upon many beneficial species, including *Aphidoletes* eggs and larvae. This supports our more practical observations that they predate upon *Aphidoletes* larvae in pepper crops. Various species of *Orius* form a very important component of the overall IPM programme; for example their role in suppressing western flower thrips populations and transmission of tomato spotted wilt virus is indisputable. The available evidence strongly suggests that *Orius* should be retained in the pepper IPM programme in preference to *Aphidoletes*.

Researchers have investigated IGP between *Aphidoletes* and *Aphidius colemani*. They found that *Aphidoletes* larvae readily killed parasitised but not yet mummified *Aphis gossypii*. In practice, we have frequently seen *Aphidoletes* larvae feeding on *M. persicae* / *Aphidius* mummies. There are also records of predatory bugs feeding on mummified aphids; for example *Anthocoris nemorum* preyed readily on immature *A. colemani* contained within *M. persicae* mummies. Our own observations in pepper crops show that *Orius* larvae feed on *M. persicae* / *Aphidius* mummies but it is very difficult to determine the overall impact of this on the parasitoid population.

Open rearing units

Open rearing units (ORUs) or banker plants have been used to boost numbers of natural enemies in protected cultivation for over 30 years. The objective is to sustain a reproducing population of the natural enemies which provides season-long suppression of the pest species. Typical ORUs have been based on wheat, barley or maize infested with *Rhopalosiphum padi* (bird cherry aphid) or *Sitobion avenae* (cereal aphid). These species of aphids are a common host for parasitoids, such as *A. colemani*, *A. matricariae* or *A. ervi*, without being a direct threat to the crop.

A grower and a biological control specialist tested ORUs in commercial pepper crops during 2010. Eight ORUs per hectare were placed in the crops per week for three weeks in February. Each unit comprised a hanging basket of wheat plants, infested with *Sitobion avenae*, positioned above the crop and irrigated / fed by the irrigation system. The aphid populations were colonised with *Aphelinus abdominalis*, which was believed to be less susceptible to hyperparasitism than *Aphidius*. The units were refreshed periodically by adding presoaked wheat seed. The nursery's routine pest

monitoring procedures detected fewer colonies of aphids in glasshouses protected with ORUs than in glasshouses where the parasitoids had only been released systematically. Both the grower and the biocontrol specialist believed that the strategy had been successful up to week 20.

The units were examined in early July when many *Orius* and *Aphidoletes* were seen among the wheat plants. Although there was 93% adult wasp emergence from samples of intact mummified aphids, 72% were hyperparasitoids (*Dendrocerus* spp. and *Alloxysta* spp.). It was not clear when hyperparasites started to colonise the ORUs but it was clear that their value had become compromised by mid-summer.

Studies in Spanish pepper crops suggest that it may be possible to switch from ORUs based on parasitoids in the early season to ORUs based on syrphid flies in the summer. The aphid cultures in the ORUs provided an interim food source for larvae of released *Episyrphus balteatus* as well as increasing the ingress of other naturally occurring syrphid species. It has been shown that the presence of flowering plants (eg coriander or sweet alyssum) can enhance establishment of syrphids. However, analysis of the gut content of those predators has indicated that sweet pepper alone provides a suitable and adequate pollen source.

Financial Benefits

The cost of routine control measures applied against aphids in conventional pepper crops is about £5.8K per hectare 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 pepper growers to satisfy the standards sought by major food retailers and thus improve competitiveness of the UK industry.

Action Points for Growers

- Growers of wide bed organic crops should adopt the spray boom configuration developed in this project because it has been shown to greatly improve spray penetration and leaf coverage throughout the spray canopy.
- An IPM strategy based on *Aphidius colemani* and *Aphidius ervi* released systematically from planting and supported by a SLoD treatment of 1.5% Savona at first sight of sticky leaves / fruit provided effective aphid control except where the performance of the parasitoids was compromised by hyperparasites and / or intraguild predation.
- Growers of conventional pepper crops should consider using a SLoD treatment based on pymetrozine (Chess WG) applied via the irrigation system using a water powered Dosatron D20s applicator accessing the system via individual irrigation manifolds.
- The SOLA for Chess WG via the irrigation system requires clarification to take into account the difference between plants and heads, as well as the difference in the quantity of foliage on young and mature plants / heads. (*HDC note - It has been confirmed that the application rate for control of aphid is 10 g per 1000 plants (irrespective of head number or plant size)*)
- Further information is required about hyperparasitism of primary parasitic wasps with the aim of reducing the impact of this phenomenon on biological control of aphid pests in general.
- Growers should reconsider the use *Orius* and *Aphidoletes* within the same IPM programme due to intraguild predation. For sweet pepper crops, *Orius* should be retained in preference to *Aphidoletes*.
- Growers should consider boosting the numbers of biological control agents within their pepper crops by using parasitoid ORUs during the early season and syrphid ORUs during the summer. However, these techniques will require further refinement during the coming season.

SCIENCE SECTION

Section 1: Background to these studies

The preceding HDC funded Project (PC 295) sought short term IPM compatible solutions for leafhopper and aphid infestations in sweet peppers (Jacobson, 2009). It was successful in developing an IPM compatible solution to leafhopper infestations and the new control measure has since been adopted by all pepper growers who suffer infestations of that pest. In addition, a new IPM compatible strategy was devised for aphid control in organic pepper crops. This consisted of a primary control measure based on various parasitoids, supported by fatty acids (Savona) or natural pyrethrins (Pyrethrum 5EC) as a second line of defence (SLoD). A trial to establish “proof of concept” was very successful. However, the following difficulties were encountered when the strategy was implemented on a larger scale:

- Spray coverage of the SLoD products was inadequate in wide-bed organic crops.
- The performance of the parasitoids was impaired by hyperparasitism (*i.e.* naturally occurring parasitoids which attack the parasitoids being used as biological control agents).
- There appeared to be a negative interaction between additional biological control agents utilised in the programme (*i.e.* *Orius* spp. and *Aphidoletes aphidimyza*).

The present project (PC 295a) builds on the findings of that study by addressing the identified difficulties and by introducing some different options for aphid control.

Organic growers are somewhat limited in their options for SLoD treatments but conventional growers have access to other more effective products. One such product, pymetrozine (Chess), has recently received a SOLA for application through the irrigation system. This has the potential to greatly reduce the labour required for application and the quantity of product applied. The present project sought to optimise the use of pymetrozine through the irrigation system in conventional crops.

Previous work has demonstrated the benefits of using open rearing units (ORUs) to aid the establishment of primary biological control agents (*eg* Jacobson & Croft, 1998). However, they can also become a breeding site for hyperparasitoids, which is clearly counter productive. The present project revisited the possibility of using ORUs in crops, with particular emphasis on their use as a breeding base for alternative

parasitoids (eg *Aphelinus* spp.) and novel biological agents (eg syrphids). This involved reviewing existing knowledge and undertaking small scale studies with the biological control producer and Valley Grown Nursery.

Section 2: Modification of vertical boom sprayer for wide bed organic crops

2.1. Wide bed organic crops

Conventional pepper crops are grown in a single row of growing media slabs with plants / heads trained up vertical strings arranged in a V-formation (e.g. Figure 1a.). This provides a narrow crop canopy which is relatively easy to penetrate with crop protection sprays. In contrast, some organic crops are grown in double rows to facilitate incorporation of compost into the soil beds (Figure 1b). Plants / heads from each row are trained up vertical strings creating a wide canopy which is much more difficult to penetrate with sprays (Figure 1c). Furthermore, many of the leaves on pepper plants hang at an angle of approximately 30° off vertical creating a virtual wall of foliage which can be very difficult to penetrate (Figures 1 and 2).

Figure 1. Contrasting crop training systems in commercial pepper crops:

Figure 1a. Single row conventional crops



Figure 1b. Wide bed organic crops



Figure 1c. Breadth of wide bed organic crops



Trials in 2009, had shown that control of aphids with contact insecticides had varied from up to 95% on leaves at the outside of the wide bed canopy to only 20-40% within the canopy (Jacobson, 2009). It was clear that there should be an expert critical assessment of the performance of current robotic spray equipment with a view to optimising cover to the undersides of leaves throughout the crop canopy.

2.2. Assessment of original sprayer configuration

The original spraying equipment consisted of a Berg self-propelled, robotic unit, running up and down the pipe-tracks between each bed of peppers. The unit carried two vertical booms, one spraying to the left of the sprayer, the other to the right, partly treating two beds at a time (Figure 2). Complete treatment of a bed, on both sides, was achieved by further spray applied from adjacent tracks. The robotic unit could be programmed to spray at a given speed in a forwards direction, in reverse, or both. Booms could also be operated singly, for example when treating beds along the sides of the glasshouse. The robotic unit required a separate tender, which remained parked on the service road-way while spraying, comprising a mobile spray tank, pump and main pressure regulator (Figure 3). The spray-mix was sent to the robotic unit via a reel of pressure hose (approx. 150m long) carried on the robotic unit. The hose uncoiled as the unit ran up the track-way and recoiled on the return run. Fine adjustments to pressure could also be made on the robotic unit itself.

Each vertical 2m boom carried a single row of six nozzles at 35cm spacing, which treated the crop on each side across a vertical distance of approx. 2.5m. Spraying Systems 8002-VS (yellow) TeeJet flat-fan nozzles were fitted, probably operating at some 10 bars. Inspection of the crop after treatment by the above equipment suggested that appreciable numbers of leaves were being left untreated by the mutual shielding of one leaf against another, probably held against each other by the force of the spray applied. A high proportion of the spray from the fan nozzles was inevitably directed close to the horizontal, which was precisely the wrong trajectory because of the angle of the leaves. With a limited range of fan-angle adjustments, there was a risk that poor overlapping of spray-fans might also leave missed areas of the crop.

Figure 2. Original robotic sprayer showing configuration of nozzles (left) and in operation in the crop (right).



Figure 3. Mobile tender with tank and pump



Dense crops all over the world are renowned for the difficulties they create for insect control using potent insecticides, let alone the mild alternatives approved for use in organic pepper crops. Preliminary tests were conducted with a number of different

nozzles on the Berg booms and it was clear that the problem of mutual leaf shading would not be avoided without substantial changes to the nozzle configuration. A different approach was required which would include a high degree of tolerance.

The limited power available on the battery-operated sprayer units precluded any significant use of air to assist spray delivery. A single small fan had been fitted below each boom on one of the robotic sprayers prior to 2009 (Figure 4) but the effect was judged minimal. Adoption of a totally new, low-volume system based on rotary atomisers (with or without air) was vetoed for reasons of cost. This therefore meant that a solution must be sought using conventional hydraulic nozzles.

2.3. Replacement spray booms

Early in 2010, further tests were undertaken at length on a more comprehensive range of nozzles at pressures of up to 10 bar, seeking options that would achieve both penetration and coverage of the dense crop. An important criterion was that the nozzles selected must be able to drive spray underneath the near-vertical leaves of the crop canopy, to lift the leaves momentarily, so as to permit finer spray to penetrate and cover the under-leaf surfaces inside the canopy. The latter fine spray has no power to lift or penetrate as required but is needed to obtain the under-leaf cover. In contrast, a penetrating, lifting spray requires a narrow angled, coarse nozzle. It was therefore concluded that two types of nozzle had to be employed – here colloquially called ‘lifters’ and ‘fillers’ according to their purpose. In general terms, it was also considered that wide-angle nozzles would result in too much of the spray being wasted in useless trajectories, even where the fine ‘filler’ nozzles were concerned.

The narrow angled nozzles required as ‘lifters’ meant that the 35cm nozzle spacing on the Berg booms was excessive across a vertical distance of some 2.5m in crop height. A nozzle spacing of 20cm provided a better compromise, permitting a 2m boom to carry ten nozzles a side instead of six. However, in order to house an equivalent set of fine ‘filler’ nozzles, each boom also needed to include a second vertical row of 10 nozzles (*i.e.* 20 nozzles in total per boom). This number of nozzles also meant that low-output nozzles were required in order to avoid excessive volume rates of application. The only nozzles capable of meeting the above requirements with sufficient flexibility were the disc-core range of cone nozzles (*eg.* from Spraying

Systems, Hypro). To compare with the original 8002-VS nozzles employed on the Berg booms (which applied 1.02 l / min per nozzle at 5 bar), the following disc-core combination was considered to be most suitable:

Purpose	Disc size	Core type	Cone	Cone angle (5 bar)	Nominal flow (l/min/nozzle at 5bar)
'Lifter'	2	56	Solid	18°	1.20
'Filler'	1.5	45	Hollow	48°	0.81

The above combinations proved to be the smallest sizes of these nozzle types feasible for such work. Smaller disc sizes would be subject to much greater rates of wear and also showed appreciable loss of penetrative lifting power. This had consequences for the overall volume rates to be applied, especially in relation to the greater numbers of nozzles also required.

The 'lifter' nozzles must point upwards into the crop at some 60° off horizontal so as to lift the outer leaves of the crop effectively. The 'filler' nozzles also need to point upwards so as to permit the fine spray to penetrate. The adjustable Berg nozzle-holders did not offer such high angles and were also not adjustable except in the vertical plane. A decision was made to build a set of replacement booms from new as a retro-fit to the robotic sprayers. The new booms would incorporate an independent swivel at each nozzle position to permit complete flexibility in choice of trajectories in three dimensions. Not only would this permit selection of appropriate trajectories for each 'lifter' and 'filler' nozzle but would also permit selection of different options for the uppermost set of 'active' nozzles according to the height and stage of the crop to be treated.

Fabrication of the booms took place during April / May 2010 under contract by Spray-trac Systems Ltd (Marton-cum-Grafton, North Yorkshire). The new booms (Figure 4) were made from 25mm square-section stainless steel tube so that they would fit the same clamps on the robotic sprayer as used for the Berg booms. Each nozzle position incorporated an on / off valve and an anti-drip valve in addition to the swivels discussed above. Each boom was fitted with a 100-mesh, self-cleaning inlet filter, a wash-down or drain valve at the base, and a 10 bar pressure gauge. As on the Berg booms, a stop-valve and port was also incorporated at the top of the boom to permit use of boom extensions if required.

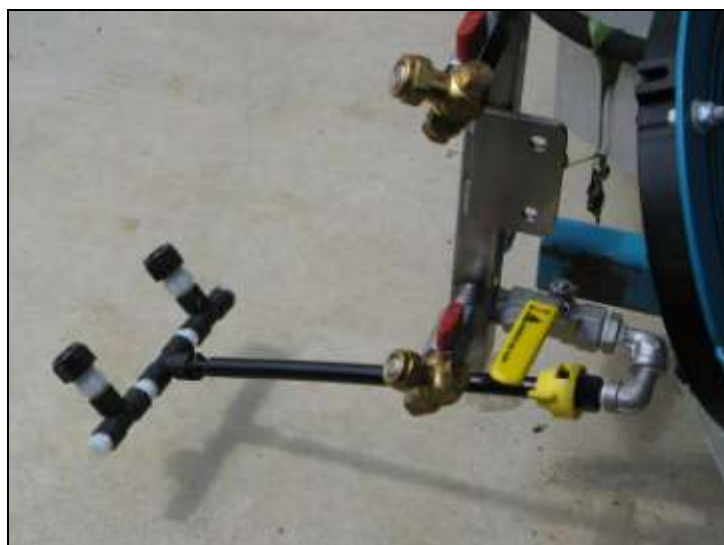
A further refinement was the addition of a 'Quick-Fit' basal extension piece and double-swivel to carry two extra nozzles that could direct spray upwards into the very base of the crop. These were fitted to the drain ports as an experimental measure. Figure 5 shows one of the flexible extensions in-place, as well as the plate welded to each boom to provide for a similar, more permanent attachment if required.

Figure 4. New booms fitted to the robotic sprayer



(Note: At this stage the fans are still fitted to the base of the unit)

Figure 5. Experimental, flexible nozzle extension at the base of the booms



The new booms were fitted to a robotic sprayer and tested at Bradon Nursery on 26 and 27 May 2010. There were subsequent visits during the season to discuss ongoing results, potential problems during use and to check and reset the boom configuration.

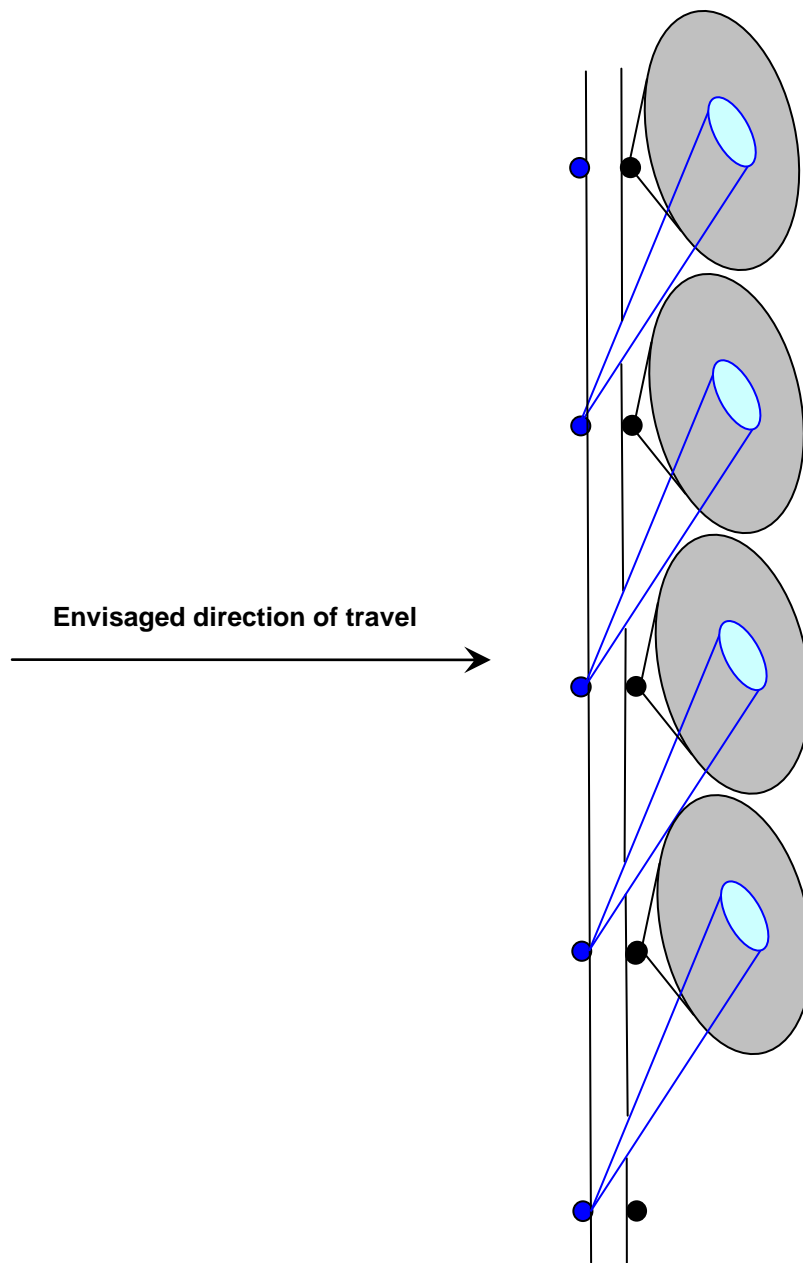
2.4. Overall performance of the new booms

A large number of possible permutations and combinations of the 'lifter' and 'filler' nozzles are possible on the two spray booms, including their trajectories. Just one combination appeared to have potentially greater merit than the rest and was adopted for the first tests of the booms at the nursery on 26 May 2010. This configuration involved fitting the 'lifters' and 'fillers' in separate rows on each boom. Each lifter was orientated upwards (some 60° from the horizontal) and fired into the spray cone of the 'filler' nozzle on the next tier above, close to the point where the spray meets the outermost leaves of the crop (Figure 6). This pattern was repeated the length of the boom for as long as nozzles needed to remain open as required by the height of the crop.

Because the 'lifter' jets are fired into the 'filler' cones with trajectories in different planes, this has the effect of lifting the outer leaves, letting in the fine spray from the 'fillers' but also creates maximum turbulence in a twisting motion so that no leaf stays in one position for any length of time. There has been no reason to change the configuration of nozzles as initially adopted. With the booms set-up throughout the season in this manner, it was reported on 7 September that no uncontrolled aphid problems had occurred in the wide bed crop. This was said to be the first time this had occurred in any year so far.

Not only did the new booms appear to give better crop cover during the preliminary tests in May, they also applied a higher volume rate than the original sprayer configuration. Compared with the 12-off 8002-VS nozzles previously used (applying some 12.25 l/min with all nozzles open at 5 bar), the 40 disc/core cone nozzles on the new booms would apply some 40.2 l/min with all nozzles open at 5 bar - an increase in volume of x 3.28. This ratio in applied volume rate may differ depending on the number of nozzle pairs operating at a given crop growth stage. Nevertheless, the increase in volume goes a long way to add extra insurance in respect of the intrinsic leaf coverage obtained, whatever the benefits of better spray targeting.

Figure 6. Side of boom viewed from crop



Notes:

- Spray from the narrow-angle “lifter” nozzles (D2/56) is shown in blue, while spray from the finer “filler” nozzles (D1.5/45) is shown in grey.
- Spray from the lifters travels faster and they were mounted on the rear side of the boom.
- Maximum turbulence is obtained by directing the lifting cones through the filler cones. Note that the cone from each “filler” intersects with the cone from a “lifter” in the tier below. The two sets of spray cones should meet more or less where they enter the crop.

Section 3. Biological evaluation of efficacy of new booms on robotic sprayer

Preliminary work

Aphid numbers increased rapidly during hot weather in weeks 21/22 2010 and some plants in an isolated area were just starting to become sticky. As this was the agreed signal to commence second line of defence (SLoD) sprays, the new booms on the robotic sprayer were put into action for the first time. Nursery staff sprayed the first seven sections of several bays with 1.5% Savona using the robotic sprayer which was programmed to spray in both directions. Observations post-treatment in week 23 indicated that this approach had been very successful against *Aulocorthum solani* (the species which had predominated in that area) and *Myzus persicae*. However, the quantity of spray delivered was very high (equivalent to about 8,000 litres per hectare).

In week 23, sticky plants were found in another area of the crop (Figure 7) and it was decided to take this opportunity for a more thorough evaluation of the modified sprayer. These infestations comprised almost entirely of the green form of *Myzus persicae* with an average of about 40 individuals per leaf (e.g. Figure 8).

Figure 7. Sticky leaves triggered second line of defence treatment



Figure 8. Typical aphid population at time of treatment



Preliminary runs with the sprayer indicated that coverage of the undersides of about 90-95% of the leaves could be obtained with a single pass. The effect was similar regardless of the direction; therefore it was decided to spray as the robot returned so that it was not passing through and disturbing wet crop. The lateral extensions at the bottom of the boom (Figure 5) were pushing the lower leaves inwards and thus partially blocking the spray. This was considered to be counter-productive and so the extensions were removed from the boom for these treatments. However, it was subsequently noted that where the lowest leaves were removed, the spray from the lateral extensions did penetrate the canopy from below and, in those circumstances, the modification appeared to be beneficial.

The effect of limiting the sprayer to one pass was to reduce the volume applied to the equivalent of 3,750 per hectare. Removing the lateral extensions further reduced the volume applied to the equivalent of 3,125 litres per hectare (still a high volume for a 1.6m high crop [Figure 9]). It was noted later in the spray operation that the uppermost 'lifter' nozzle was contributing little at this crop height and could have also been turned off. This could possibly have saved another 10% of spray reducing the volume to the equivalent of 2,812 litres per hectare.

Methods

The trial began on 11 June (week 23 2010) in a crop of organically grown sweet pepper (cv Ferrari) in block 15-17 at Cantelo Nurseries, Bradon Farm, Isle Abbots, Somerset.

Two areas of aphid infestation were chosen to i) receive a second line of defence treatment of 1.5% Savona and ii) to remain untreated. The former comprised 640m² in four rows of crop. The untreated area was smaller (approximately 80m²) to reduce any potential risk to the rest of the crop.

The sprayer was set up as follows:

- Lateral boom extensions removed
- Only lowest 5 pairs of nozzles on each boom were turned on
- Pressure 9-10 bar
- Spray only on reverse run
- Travel at 50% of maximum speed (considered to be the safest operating speed to reduce instability and the risk of catching plants/strings)
- Spray volume equivalent to 3,125 litres per hectare.

Figure 9. Height and breadth of crop at time of treatment



Aphidius ervi and *A. colemani* had been released under the guidance of a biocontrol specialist. The numbers and method of release were not relevant to this trial but rather the size of the population at the time of intervention with the SLoD. In addition

to *Aphidius* spp., a natural population of *Praon* spp. became established within the *M. persicae* population.

Assessments were done immediately before the spray was applied, 1 day post-treatment, 6 days post-treatment and 42 days post-treatment. On each occasion, five sample stations were selected at random within each of six 'plots' in the sprayed area (*i.e.* a total of 30 sample stations). In the unsprayed area, there were five sample stations in each of four plots. At each sample station, numbers of aphids, parasitised aphids (*i.e.* mummies), *Aphidoletes aphidimyza* and *Orius* spp. were recorded on one leaf in each of the following four positions in the crop canopy:

- within the upper 30cm of canopy adjacent to the path (designated upper/outer)
- within the upper 30cm in the middle of the canopy (designated upper/inner)
- within the lower 30cm of canopy adjacent to the path (designated lower/outer)
- within the lower 30cm in the middle of the canopy (designated lower/outer)

In addition, 1 day and 42 days post-treatment, intact aphid mummies were collected from the crop and incubated in ventilated Petri-dishes (Figure 10). There were two objectives to this exercise:

- to measure the proportion of parasites that successfully emerged from treated mummies and thus record any impact of the spray on the population
- to record the proportion of mummies that contained hyper-parasites

Figure 10. Ventilated incubation dish as used for parasitoid emergence tests



Statistical analysis

The primary method of analysis was analysis of variance. A preliminary plot of standard deviation of samples *versus* site means showed a very clear correlation between them suggesting that a square root or a logarithmic transformation of the data would give greater homogeneity of the data. The log transformation proved to be most effective and this was done for all data. The sampling configuration was spatially complex. A relatively simple model was used for the samples, *i.e.* to nest the replicate count analysis within the sampling sites but to extract the between row, between height and between position (inside and outside) effects together with the interaction between height and position. This was done for all sampling times. In addition, we examined the percentage reduction in numbers of aphid and *A. aphidimyza* at each canopy position from day 0 to day 1 and from day 0 to day 6.

Results and Discussion

Table 1 shows the mean numbers of live aphids at the four sample positions in the crop canopy for each Treatment on each assessment date. To aid interpretation of the results, the data for the sprayed area are also presented graphically in Figure 11.

Numbers increased nearly three fold in the untreated control during the first six days post-treatment leading to unacceptable numbers of sticky fruit (*e.g.* Figure 12). Additional control measures were applied to these plants on day 7. This confirmed that the Savona treatments had been timed accurately. The comparisons below focus on the effect of the Savona in the four positions within the crop canopy.

Following application of Savona, there was an immediate reduction in aphid numbers ($P < 0.05$) and this was similar in all sample positions indicating that the spray had successfully penetrated the whole canopy. Occasionally, a leaf was found which clearly had not received any spray, probably because it had been shielded by another. However, the incidence of this was much less than with the previous boom configuration. Overall, the aphid numbers were reduced by approximately 90% at day 1 and by 97% at day 6. No further sticky fruit were recorded which, when compared to the untreated control, clearly demonstrated that the SLoD treatment had been well timed and effective.

The aphid population recovered between day 6 and day 42 but overall numbers were still only 34% of the pre-treatment count. No further spray interventions were required against aphids in that area.

Table 1. Mean numbers of aphids in the four sampling positions in the plant canopy on each assessment date

Treatment	Sample position	Mean number of aphids per sample station			
		Pre-treatment	1 day post-treatment	6 days post-treatment	42 days post-treatment
Savona treatment	Upper outer	29.9	3.0	1.0	20.2
	Upper inner	37.0	3.6	0.9	7.5
	Lower outer	67.6	8.2	1.6	18.2
	Lower inner	21.4	3.2	1.6	7.5
	Overall mean	39.0	4.5	1.3	13.3
Untreated	Upper outer	11.1	13.9	35.8	-
	Upper inner	8.8	9.9	27.8	-
	Lower outer	20.4	27.5	79.5	-
	Lower inner	9.5	11.8	28.8	-
	Overall mean	12.5	15.5	43.0	-

Figure 11. Mean numbers of aphids per leaf at each position in the crop canopy before treatment with Savona and at three post-treatment assessment dates.

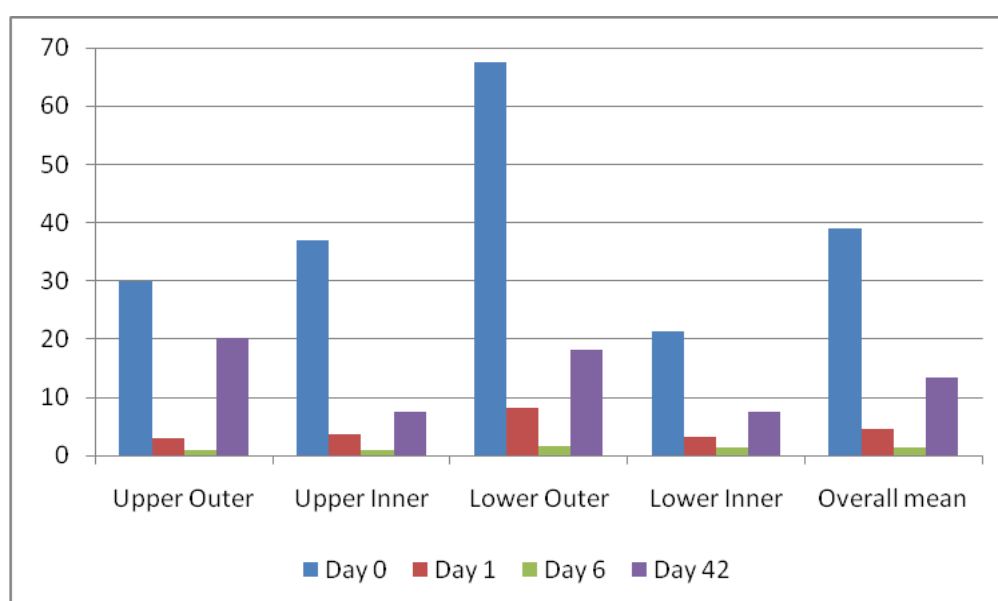


Figure 12. Example of unacceptable sticky leaves / fruit on untreated plants six days into the trial.



Table 2 shows the mean numbers of parasitised aphids showing as mummies in the four sample positions in the plant canopy on all assessment dates. Overall, approximately 8% of the aphid population was mummified at the start of the trial. Of course, this is an underestimate of total parasitism because it does not include those at an earlier (*i.e.* not yet visible) stage of development. There was no significant difference between numbers of mummies in the four canopy positions of the treated plots prior to treatment.

Table 2. Mean numbers of mummified aphids in the four sampling positions in the plant canopy on each assessment date

Treatment	Sample position	Mean number of mummies per sample station			
		Pre-treatment	1 day post-treatment	6 days post-treatment	42 days post-treatment
Savona treatment	Upper outer	2.8	2.8	2.0	0.9
	Upper inner	2.9	3.2	1.8	0.5
	Lower outer	3.2	3.6	4.1	0.9
	Lower inner	3.2	4.4	2.6	0.9
	Overall mean	3.0	3.5	2.6	0.8
Untreated	Upper outer	0.9	1.1	3.5	-
	Upper inner	1.2	1.7	9.4	-
	Lower outer	2.6	3.3	4.1	-
	Lower inner	1.8	2.2	4.7	-
	Overall mean	1.6	2.1	5.4	-

The day after treatment, mummies represented 44% and 12% of the remaining aphid population in the Savona and untreated plots respectively. Once again, there were no differences between sample points within the plant canopy. By day 6, the situation had changed and mummies represented 66% and 11% of the surviving aphid population respectively. These results clearly illustrate how the SLoD treatment shifted the balance of the insect populations to the advantage of the parasitoids and thus helped to prevent any further fruit contamination / damage.

Table 3 shows the mean numbers of *Aphidoletes aphidimyza* larvae. There was approximately one larvae per leaf at the start of the trial in the 'Savona plots' and this declined by about 75% ($P<0.05$) the day after treatment and by over 95% ($P<0.05$) by day 6. In contrast, numbers remained similar in the untreated plots up to six days post-treatment.

Table 3. Mean numbers of *Aphidoletes aphidimyza* in the four sampling positions in the plant canopy on each assessment date

Treatment	Sample position	Mean number of <i>Aphidoletes</i> per sample stations			
		Pre-treatment	1 day post-treatment	6 days post-treatment	42 days post-treatment
Savona treatment	Upper outer	1.0	0.1	<0.1	0.7
	Upper inner	0.6	0.1	0	0.1
	Lower outer	0.9	0.3	0	0.1
	Lower inner	0.8	0.1	0	0.1
	Overall mean	0.8	0.2	<0.1	0.2
Untreated	Upper outer	0.3	0.3	0.2	-
	Upper inner	0	0.1	0.1	-
	Lower outer	0.3	0	0.2	-
	Lower inner	0	0.1	0.1	-
	Overall mean	0.2	0.1	0.1	-

Table 4 shows the mean numbers of *Orius* spp. It was noted that there had been a considerable reduction in numbers of this predator throughout the whole crop since week 21. This was regardless of the spray history in the different areas. While we have no definitive explanation for this decline, it was suggested that it may have been due to a dearth of flowers for at least part of the intervening period. The numbers of *Orius* spp. recorded in these assessments were too small to draw any conclusions about the impact of the spray. However, the *Orius* spp. population had increased markedly by day 42 with an overall mean of 0.2 per leaf. Although not part of the

formal assessment, it was also noted that there was an overall mean of 1.5 *Orius* spp. per flower at day 42.

Table 4. Mean numbers of *Orius* spp in the four sampling positions in the plant canopy on each assessment date

Treatment	Sample position	Mean number of aphids per sample stations			
		Pre-treatment	1 day post-treatment	6 days post-treatment	42 days post-treatment
Savona treatment	Upper outer	0.1	0	0.1	0.9
	Upper inner	0.2	0.1	0.2	0.7
	Lower outer	<0.1	0.1	0	0.5
	Lower inner	0.1	<0.1	<0.1	0.4
	Overall mean	<0.1	<0.1	<0.1	0.6
Untreated	Upper outer	<0.1	<0.1	0	-
	Upper inner	0	0.1	0.1	-
	Lower outer	<0.1	0	0.1	-
	Lower inner	0	<0.1	0.1	-
	Overall mean	<0.1	<0.1	<0.1	-

Table 5 provides a summary of the size of the populations of *M. persicae* and the principal biological control agents; *i.e.* parasitised aphids (as mummies), *A. aphidimyza* and *Orius* spp. in the Savona treated plots on each assessment date. It can be seen that numbers of aphids increased between day 6 and day 42 but not to the pre-treatment level. The plants were slightly sticky in some small localised areas but it hadn't been necessary to resort to washing fruit. No further treatments had been deemed necessary during that time. Numbers of mummies caused by *Aphidius* spp. had declined which was contrary to expectations.

Table 5. Mean numbers of aphids, intact mummified aphids, *A. aphidimyza* and *Orius* spp. in the Savona treated plots on each of the four assessment dates.

	Overall mean number:			
	Pre-treatment	Days post-treatment		
		1	6	42
<i>Myzus persicae</i>	39.0	4.5	1.3	13.3
Mummified aphids	3.0	3.5	2.6	0.8
<i>Aphidoletes aphidimyza</i>	0.8	0.2	<0.1	0.2
<i>Orius</i> spp	<0.1	<0.1	<0.1	0.6

No evidence of hyperparasitism was seen on leaves in situ during the first assessment and only one hyperparasite emergence hole was noted in an empty mummy during the post-treatment assessments in the crop. Table 6 shows the percentage emergence of parasitoids and hyper-parasitoids from the mummies collected on days 1 and 42 post-treatment and subsequently incubated in ventilated Petri-dishes. We would normally expect between 70% and 85% emergence of *Aphidius* spp. from samples of mummies collected from the crop. Both of the sample batches from this trial were at the lower end of that range. Hyperparasitism by *Dendrocerus* spp. increased from 5% to 15% during the trial but that alone is unlikely to have caused the decline in numbers of parasitoids. Both *Aphidoletes* larvae and *Orius* spp. were observed feeding on aphid mummies (Figures 23 and 24) (see Section 6).

Table 6. Emergence of parasitoids and hyperparasitoids from mummies collected post-treatment

	Percentage emergence of:		
	<i>Aphidius</i> spp	Hyperparasites	Nothing
One day post-treatment (from 180 mummies)	68	5	27
42 days post-treatment (from 100 mummies)	69	15	16

In Summary:

- The SLoD treatment was appropriately timed at the first sight of sticky leaves and fruit in the area infested by aphids.
- The spray from the new boom configuration successfully penetrated the whole wide bed crop canopy.
- The SLoD treatment shifted the balance of the insect populations to the advantage of the parasitoids and thus helped to prevent any further fruit contamination / damage.
- Numbers of *A. aphidomyza* larvae declined by over 95% during the first six days post-treatment.
- Hyper-parasitism by *Dendrocerus* spp. increased from 5% to 15% during the trial but did not prevent season-long control of aphids in this case.
- Both *A. aphidomyza* larvae and *Orius* spp. were observed feeding on aphid mummies but it was not possible to quantify the overall impact on the parasitoid population.

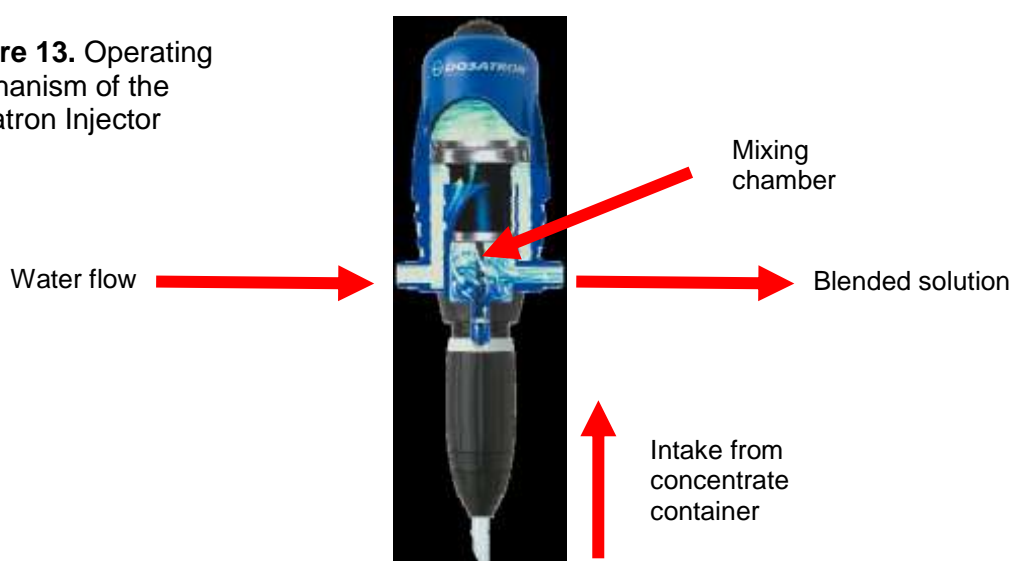
Section 4: Pymetrozine Via Irrigation

4.1. Introduction:

The irrigation system can provide a useful vehicle for cost-effective application of systemic pesticides. When the product is applied at the central mixing point it is usually necessary to treat the whole area served by the system. However, aphid populations build up unevenly and SLoD treatments are only usually required in localised areas of crops. Therefore, we aimed to develop a more precise method of application which would access the system via individual irrigation manifolds and thus allow separate treatment of the smaller areas served by each valve.

A preliminary trial attempted to use the nursery spray rig to pump pesticide directly into the irrigation manifold. This failed due the differences in volume and pressure between the two systems. We then focused on the use of purpose built equipment and opted for a water powered Dosatron unit which provides a reliable way to accurately inject chemicals into water lines. The Dosatron injector works using volumetric proportioning, ensuring that the chemical mixture remains the same regardless of variations in pressure and flow. When water enters the injector, it triggers the hydraulic motor, which begins moving up and down inside the body of the injector (Figure 13). On the up stroke, the Dosatron draws fluid up from the concentrate tank in an action similar to a hypodermic syringe. On the down stroke, the concentrate is displaced into the mixing chamber, where it is mixed with the water flowing through the unit. Then the water and chemical mixture is discharged into the water lines.

Figure 13. Operating mechanism of the Dosatron Injector



The anti-feedant, pymetrozine (Chess WG), had recently received a SOLA for application through the irrigation system (Notice of extension of use number 2024 of 2009). While this was expected to be an effective SLoD treatment against aphids, there was debate over the interpretation of the information provided in the SOLA:

- The maximum individual dose for sweet pepper is 15 g product / per 1000 plants via the drip irrigation. The 'advisory information' suggests using this rate against whitefly but a lower rate of 10 g product / per 1000 plants against aphids.
- The SOLA does not distinguish between 'plants' and 'heads'. Each plant is divided into three heads at a point approximately 0.4m above its base (e.g. Figure 14). Thereafter each head has the height and leaf canopy equivalent to a full plant. The implication of considering a 'head' to be a 'plant' would be a three fold increase in the overall dosage.
- The SOLA does not distinguish between young and old plants, yet the size of the plant and quantity of foliage will vary hugely at different times of the growing season. We were treating mature plants.
- All leaves are usually left on a pepper crop until the end of the season but, in this case, the lower leaves had been removed (Figure 14). We did not know whether this would affect the uptake of the chemical or its distribution in the plant.

In this first trial of the delivery system, we opted to take the SOLA literally in terms of 'plants' but used the maximum individual dose rate due to the maturity of those plants.

Figure 14. Examples of mature pepper crops showing the division of each plant into three heads (red arrow). The plants on the left have been subjected to lower leaf removal while those on the right have not.



4.2. Calibration of Dosatron:

Model:	Dosatron D20s
Flow rate:	1,000-2,000 litre / hr
Dosage rates:	0.2% - 2%
Pressure:	0.12 – 10 Bar
Set up:	See Figure 15
Site:	Block 4, Valley Grown Nursery
Area:	1644m ²
Plants:	2.38 / m ²
Heads:	3 per plant = 7.14 / m ²
Drippers:	2.5 / m ² (one per plant with extras at row ends) = 4,104 in total
Irrigation System:	
Pressure:	At manifold = 2.5 Bar
Flow rate:	15,245 litres / hr, equivalent to 254 litres / min

The Dosatron D20S was adjusted to provide 5.1 litres / min flow rate from the stock solution. Practice runs demonstrated that the equipment was working accurately and delivering the correct volume of stock solution.

Figure 15. Dosatron in situ showing stock tank (white container) and connections to manifold



4.3. Distribution of stock solution throughout the trial area:

The stock solution was coloured with iron chelate as LibFer (soluble powder formulation of ferric ethylenediamine bis – [2-hydroxyphenyl acetate]), which is usually used as a treatment of iron deficiency in crops. It was added at the rate of 100g of product per 20 litres water (Figure 16).

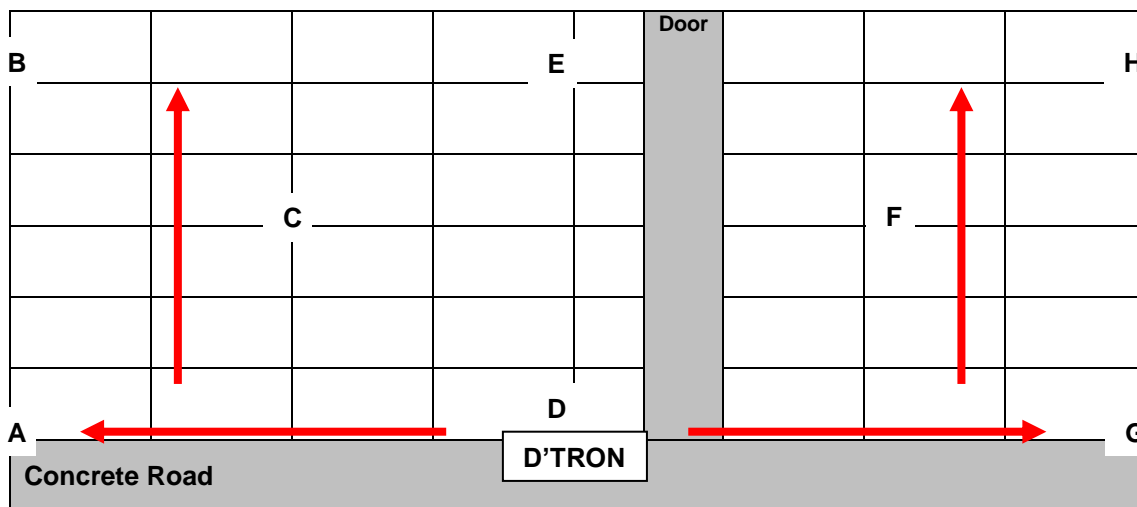
The irrigation system was initially run for 2min 30sec to remove 15 litres of coloured solution from the stock tank. The Dosatron was then bypassed and the irrigation was run in 14 short bursts of 30 seconds so that we could time how long it took to flush the coloured solution through to the plants. A single dripper was placed into a plastic cup in each of eight positions in the trial area ranging from adjacent to the Dosatron to the two most extreme points in the system (see Figure 17). The colour of the liquid in the cups was recorded at the end of each run.

Figure 16. Coloured solution in stock tank



Figure 17. Position of sample points during test of distribution of stock solution:

- Eight 8m bays across with 6 rows per bay.
- Rows are approx 27m in length, each consisting of six 4.5m sections.
- The eight sample points are labelled A to H.
- The sub-main delivery pipe prior to the Green Meteor irrigation lines runs along the edge of the concrete road adjacent to sample points A, D and G.
- The red arrows show the direction of irrigation flow from Dosatron and manifold.



The shaded areas in Figure 18 show i) the time of appearance of the coloured solution at each sample point, ii) the time for which it could be detected and iii) when it had been flushed out of the irrigation pipes. The solution emerged from the dripper adjacent to the Dosatron (*i.e.* sample point D) within 30 seconds of the start of the trial. It reached the central points of the trial area (*i.e.* sample points C and F) within 2.5 minutes and had been flushed clear within 3.5 minutes. The solution was first seen at the ends of the sub main delivery pipe within 3 minutes and was flushed clear of these positions in 4.5 minutes. However, it took considerably longer for the solution to reach the ends of the Green Meteor lines; *i.e.* 5, 6 and 6.5 minutes for points E, H and B respectively. It was noticeable that the solution also took longer to be flushed out of the system the further it was from the point of injection. At the most distant points, this was a total of 2-3 minutes. This is possibly because there is a certain amount of back flushing and mixing within the pipes when the irrigation was turned on and off during the test.

This work provided us with a good indication of how long the irrigation must be run to deliver the concentrate to all parts of the system and thus provided the methodology for the application of the SLoD treatment (Section 4.4.). It is recommended that other growers follow a similar procedure to fully understand their own irrigation system before applying products through the drippers.

Figure 18. Shaded cells show the time period that the coloured stock solution was emerging from the dripper in each of the eight sample points

	Time from start of trial (mins):													
	2.5	3	3.5	4	4.5	5	5.5	6	6.5	7	7.5	8	8.5	9
A														
B														
C														
D														
E														
F														
G														
H														

4.4. Efficacy of SLoD treatment delivered via irrigation

Methods

The treatment was applied in Block 4, Valley Grown Nursery, on the 7 September (week 36 2010). There were approximately 4,000 plants which required 60g Chess WG. This was applied from a 15 litre stock solution as described in sections 4.2 and 4.3 of this report. The irrigation was turned off mid-afternoon to allow the plants to partially dry out the rockwool growing medium and the treatment was applied during cloudy conditions in the late afternoon. The stock solution was taken up in 2.5 minutes and the irrigation was run for a further 6.5 minutes to flush the treated irrigation water through the furthest drippers. There were no further irrigation runs that day and drainage was reduced the following day to maximise uptake and minimise flushing of product through the growing medium.

Sample points to measure the size of the aphid population were established throughout the crop. Immediately prior to treatment, infested leaves were clearly marked at eight points in each of five evenly distributed rows. Pymetrozine is known to have a slow effect and so the second assessment was delayed until 20 September

(13 days post-treatment). On both occasions, the numbers of live aphids and mummified aphids were recorded at every position.

In addition, at the post-treatment assessment, mummified aphids were collected from the sample points and confined in dishes to determine whether parasitoids successfully emerged, thus indicating whether the treatment had been harmful to the immature wasps. This also provided a measure of hyperparasitism.

Results

The mean numbers of live *Myzus persicae* and mummified aphids per sample point in each crop row on each assessment date are shown in Table 7. Pymetrozine (as Chess WP) was totally effective against *M. persicae* when applied at 15gm per 1,000 plants via the irrigation system with the Dosatron D20s applicator. It may be necessary to repeat the trial in different situations; the most demanding being a fully mature crop with a full leaf canopy using the lower 'advisory' rate of 10g product per 1,000 plants.

Table 7. Mean numbers of live *Myzus persicae* and mummified aphids per crop row on each assessment date.

Row	Pre-treatment assessment		Post-treatment assessment	
	Aphids	Mummies	Aphids	Mummies
1	27.5	0.6	0	1.6
12	31.3	0.8	0	2.6
24	22.8	0.5	0	1.6
32	21.9	0.5	0	1.8
42	16.9	0.5	0	1.8
Overall mean	24.8	0.6	0	1.9

The types of parasitic wasps which emerged from the mummified aphids that had been collected post-treatment are shown in Table 8. Adult *Aphidius* spp emerged from only 6% of the collected mummies. Hyperparasitoids emerged from 21% of collected mummies with *Dendrocerus* spp. predominating. The reason for nothing emerging from the remaining 73% of collected mummies is not clear because the results are complicated by the large hyperparasitoid populations. As a consequence, the impact of pymetrozine on immature parasitoids developing within mummified aphids requires further investigation.

Table 8. Parasitoids and hyper-parasitoids which emerged from the mummified aphids collected post-treatment.

Type	Species (* to be identified)	Percentage of total mummies collected
Parasitoids	<i>Aphidius</i> spp	6
Hyperparasitoids	<i>Dendrocerus</i> spp	13
	<i>Asaphes</i> species A	4
	<i>Asaphes</i> species B	4
No parasitoid emergence	N/A	73

Section 5. Hyperparasitism

5.1. Background from scientific literature

Hyperparasitoids are secondary insect parasitoids that develop at the expense of a primary parasitoid, thereby representing a highly evolved trophic level. Only three insect orders are known to have evolved hyperparasitic behaviour; *i.e.* Hymenoptera, Diptera and Coleoptera. This brief summary will focus on the hymenoptera which attack parasitoids of aphids. The literature on this subject is quite limited and largely concerned with hyperparasitoids associated with aphids of outdoor crops. There is virtually no published information in the scientific literature which is directly related to protected peppers. Much of the more general information gleaned here has been sourced from three key review papers; *i.e.* Sullivan, 1987; Sullivan, 1988; Sullivan & Volkl, 1999.

To understand the behaviour of aphid hyperparasitoids, knowledge of the development of the primary parasitoids of aphids is essential. The latter are classified both taxonomically and behaviourally into only two families; *i.e.* Aphidiidae and Aphelinidae. Typically, the female wasp oviposits into the aphid and, over a period of about eight days, the parasitic larva gradually devours the aphid internally and kills it. The fourth instar larva spins a cocoon inside the dead aphid, whose exoskeleton becomes hard and changes colour. This is referred to as a 'mummy'. The larva then pupates and approximately four days later the new adult primary parasitoid cuts a circular emergence hole in the dorsum of the mummy (Figure 20) and pulls itself out.

Sullivan (1987) divides aphid hyperparasitoids into two categories based on adult ovipositional and larval feeding behaviours:

1. Endophagus: The female deposits her egg inside the primary parasitoid larva while it is still developing inside the live aphid but before the aphid is mummified. The egg does not hatch until after the mummy is formed and then the hyperparasitic larva feeds on the primary larval host. This category includes hyperparasitoid species of the genera *Alloxysta*, *Lytoxysta*, *Phaenoglyphis*, and *Tetrastichus*. In the case of *Alloxysta victrix*, the adult emerges from the mummy approximately 19 days after the original oviposition.
2. Ectophagous: The female deposits her egg on the surface of the primary parasitic larva after the aphid is killed and mummified. The hyperparasitic larva feeds externally on the primary host while both are still inside the mummy. This

category includes hyperparasitoid species of the genera *Asaphes*, *Dendrocerus*, *Pachyneuron* and *Coruna*. In the case of *Asaphes californicus*, the adult emerges from the mummy approximately 21 days after the original oviposition while development from egg to adult in *Dendrocerus carpenteri* takes about 16 days. In addition, *Aphidencyrthus* is a special case in which the larva is essentially endoparasitic but the adult can manifest either ovipositional behaviour.

At the next higher trophic level, aphid hyperparasitoids attack each other. Although difficult to prove in the field, it has been demonstrated in the laboratory that both intraspecific tertiary parasitism (autohyperparasitism) and interspecific tertiary hyperparasitism (allohyperparasitism) can occur (Bennett & Sullivan, 1981; Sullivan, 1972). Success in the competition between hyperparasitic larvae depends on the developmental stage of the hyperparasitoid larva already inside the mummy at the time of oviposition by the second hyperparasitoid. One of the best known examples of tertiary hyperparasitism is seen in a food chain in the alfalfa agroecosystem as illustrated in Figure 19 (van der Bosch *et al.*, 1982).

Figure 19. Food chain in alfalfa agroecosystem

<u>Primary producer</u>	<u>First trophic level</u>	<u>Second trophic level</u>	<u>Third trophic level</u>	<u>Fourth trophic level</u>
Crop	Herbivore	Primary parasite	Secondary parasite	Tertiary parasite
Alfalfa	<i>Acyrtosiphon pisum</i> (pea aphid)	<i>Aphidius smithi</i>	<i>Alloxystra victrix</i>	<i>Asaphes californicus</i>

Host specificity has received most attention at the level of primary parasitoids because it was thought that hyperparasitoids tended towards polyphagy (Vinson & Iwantsch, 1980). Contrary evidence from field and laboratory research, especially on the well-studied ecosystems in which aphids are insect pests, was reviewed by van der Bosch *et al.* (1982). He pointed out that feeding behaviour involves a continuum and that 'host-specificity' can range from monophagy to some level of oligophagy. It would seem that there is host specificity among the endoparasitoid aphid hyperparasitoids but much less, if any, in the ectoparasitoid genera.

In Europe, the genera *Dendrocerus* (6 species.), *Asaphes* (2 spp.), *Pachyneuron* (4 spp.), *Coruna* (1 spp.) and *Euneura* (2 spp.) comprise together 15 species of ectohyperparasitoids of aphids. Of these, 11 species have a very broad host range and attack various aphidiid genera and species, independent of the aphid host. Only *Pachyneuron gibbiscuta* and *Euneura laeviuscula* are believed to be host specific.

By contrast, endohyperparasitic species are much more numerous with more than 50 described species within the genera *Alloxystra*. There are only a few species attacking a broad range of unrelated aphid and primary parasitoid hosts, such as *Alloxystra victrix* and *Phaenaglyphis villosa*. The vast majority of the species, however, seem to be host specific, attacking either a single specific aphid host independent of the primary parasitoid or a single primary parasitoid genus independent of the aphid host.

This host specificity is also reflected by the composition of faunistic complexes. In native systems, host specific wasps are usually the most abundant hyperparasitoids, while generalist ectohyperparasitoids (especially *Asaphes* spp. and *Dendrocerus carpenteri*) are dominant in systems where the primary parasitoids have been artificially released.

A thorough understanding of hyperparasitoid foraging behavior could enable us to interrupt the process and thereby reduce the commercial impact of hyperparasitism. Unfortunately, there is little information available on the cues involved in host location. Generally, aphid honeydew may represent an unspecific cue providing information of the presence of aphids as first step in locating the primary parasitoids. There is evidence to suggest that the presence of honeydew increases residence times in females of *A. victrix* and *D. carpenteri* (Buitenhuis *et al.*, 2004). Attraction to host plant volatiles would provide the foraging female with additional reliable information (Sing & Srivastava, 1987). Thereafter, the female would have to acquire information about the presence of parasitised individuals and this would appear to be achieved by ovipositor contact (Sing & Srivastava, 1988). One species, *Asaphes vulgaris*, is known to use kairomones arising from the silky cocoon of aphidiid primary parasitoids for host finding (Christiansen-Weniger, 1994). Chow & Mackauer (1999) investigated host marking behaviour in *D. carpenteri* and concluded that the aphid mummy is marked with a contact pheromone after oviposition and this deters other females of the same species.

Walker & Cameron (1981) provide life-table data about *D. carpenteri* which provide an approximate guide to the insect's development in U.K. pepper crops. Interesting points include:

- *Aphidius smithi* was susceptible to oviposition by *D. carpenteri* at the age of 8.5 days but not at 8 days. The most significant event during that half day was that the aphid was killed by the primary parasitoid. At day 8, the *A. smithi* larva completely filled the body of the host aphid and at day 8.5 it had been secured to the substrate by the aphidiid larva (*i.e.* it was mummified).
- *Aphidius smithi* were available as hosts from day 8.5 to day 13. The primary parasitoid transformed into an adult on day 14, before emerging on days 15-16.
- At 19°C *D. carpenteri* adults lived for an average of 9.7 days. When presented with 30 mummies daily, each female produced an average of 75 progeny (maximum 120). Fecundity increased after day 1, reached a maximum of 10.2 eggs per day at day 5 and then declined. Over the life of 13 females studied, the average fecundity was 8.6 eggs per day.
- The maximum fecundity of *A. smithi* in the study was 300, significantly greater than the value of 120 recorded for *D. carpenteri*. A related species, *A. eadyi*, produced a maximum of 65 mummies per day (averaging 34 per day) compared with a daily maximum of 25 progeny for *D. carpenteri*.
- Mated female *D. carpenteri* produced an average of 69% female offspring and they generally emerged one day later than males.
- *D. carpenteri* developed slowly at 9.7°C. No larvae survived at 5.2°C. Survivorship at 9.7°C and 11.6°C was considerably lower than at 14.8°C and above.
- At lower temperatures it was noted that the hyperparasite required twice as long to develop into an adult than its host, whereas at 25°C rates of development were almost equal.
- At 10-15°C adult *D. carpenteri* lived for long periods (maximum 75 days at 11.6°C) but this was reduced at 5.6°C.
- The authors concluded that, at specific times, *Dendrocerus* spp. may slow the rate of increase the primary parasitoid sufficiently to reduce its impact on the primary host.

Field studies with hyperparasitoids raised the hypothesis that females of primary parasitoids tend to emigrate from an area populated hyperparasitoids. This has been supported by laboratory experiments with the specialised primary parasitoid,

Aphidius uzbekistanicus, and the hyperparasitoid, *Alloxystra victrix* (Hollier, *et al.* 1994). Boenisch (1995, unpublished thesis) showed that *D. carpenteri* also evokes an escape reaction in *A. uzbekistanicus*.

More recent studies have investigated how floral diversity impacts on parasitoid and hyperparasitoid development. Araj *et al.* (2006) demonstrated that the longevity of both parasitoids and their associated hyperparasitoids were enhanced by the presence of flowering plants. For example, buckwheat increased longevity of *Aphidius ervi* and *Dendrocerus aphidium* compared to controls. However, if the hyperparasitoids benefit more from the flowers than the primary parasitoids, then biocontrol of the aphid pest could be compromised. This could be the case in pepper crops.

5.2. Observations from commercial crops

Prior to this season, we had very little information about levels of hyperparasitism in aphid populations in commercial pepper crops. We knew that they were present because typical emergence holes had been seen in mummified aphids (Figure 20). However, we had no information about the species that were involved.

Figure 20. Typical emergence hole by *Aphidius* spp. (left) and a hyperparasite (right)



The hyperparasitoid emergence hole is typified by irregular edges, while *Aphidius* spp. emergence holes have a neater edge and usually retain a distinct 'lid'.

Between 91 and 180 mummified aphids were collected from commercial pepper crops on each of eight occasions during the 2010 growing season. On each

occasion, the mummies were placed in ventilated Petri dishes similar to those shown in Figure 10 and incubated at room temperature. The emerging wasps were sorted into genera and examples were identified by Dr Andrew Polaszek (Natural History Museum). The numbers of each species and approximate time of emergence were recorded.

Site 1. Organic pepper crop, Somerset

Samples of intact *Myzus persicae* / *Aphidius* spp. mummies were collected from three different cropping areas on 12 June and 22 July 2010. There was a large variation in the proportion of mummies from which wasps successfully emerged; *i.e.* 79%, 18% and 46% in areas 1, 2 and 3 respectively. It is possible that some of the failures may have been attacked by *Orius* spp. or *Aphidoletes aphidomyza* (see Section 6) or died of natural causes. There was no obvious explanation for the variance between the three areas.

Hyperparasitism was greater on 22 July than on 12 June in areas 1 and 3; *i.e.* increased from 8% to 17% and from 43% to 60% respectively. In contrast, it declined in area 2 from 63% to 24%. A total of five species were found:

- *Dendrocerus* spp. (Figure 21) including *D. carpenteri*, *D. aphidium* and *D. laticeps*.
- *Asaphes vulgaris*
- *Pachyneuron aphidis*

Figure 21. Example of a *Dendrocerus* spp. collected from Site 1.



The majority of *Aphidius* spp. emerged within seven days of collection while hyperparasitoids typically emerged during the period 4-10 days later. As a general rule, *Dendrocerus* spp. emerged before *Asaphes vulgaris*.

Site 2. Conventional pepper crop, Essex

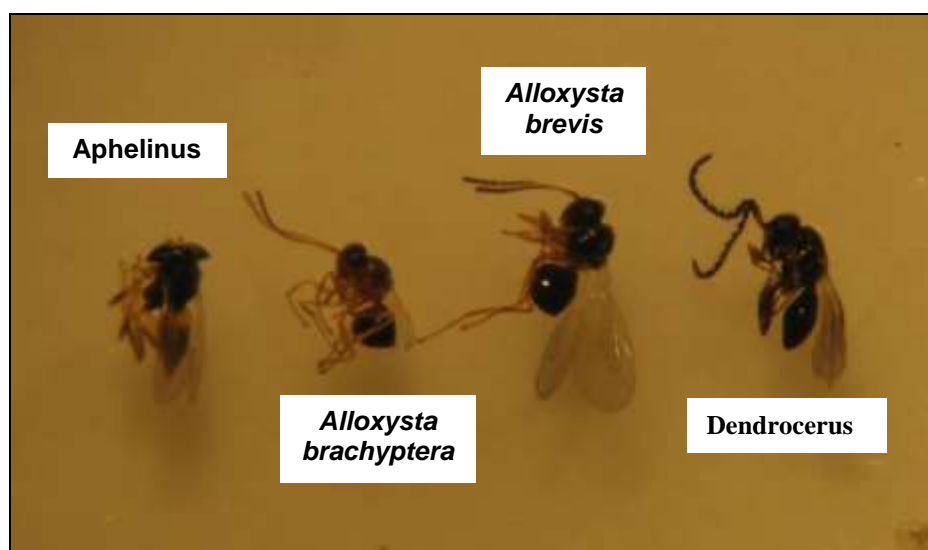
Samples from this site were collected from two very different situations. The first was a parasitoid open rearing unit (see Section 7) which consisted of *Aphelinus abdominalis* on *Sitobion avenae* on barley plants. The second was *Aphidius* spp. on *Myzus persicae* on pepper plants which had recently been treated with pymetrozine through the irrigation system (see Section 4).

The sample collected from the open rearing unit on 8 July had a very high percentage of successful adult wasp emergence (*i.e.* 93%). Of these, 28% were *A. abdominalis* and 72% were hyperparasitoids (Figure 22). The latter included:

- *Dendrocerus* spp. 10%
- *Alloxysta brevis* 36%
- *Alloxysta brachyptera* 26%

There was a less clear distinction between the time of emergence of the *A. abdominalis* and hyperparasitoids than had been seen between primary and secondary parasitoids at site 1.

Figure 22. Parasitic wasps from the open rearing unit at site 2.



Only 27% of the mummies collected from the conventional pepper crop yielded live adult wasps. Of these, 21% were *Aphidius* spp. and 79% were hyperparasitoids. The latter included two genera; *Dendrocerus* spp. (47%) and at least two species of *Asaphes* spp. (totaling 32%).

Section 6. Interactions between biocontrol agents

6.1. Introduction

Prior to this project a number of growers and IPM practitioners had questioned whether it was sensible to release the predators *Orius* spp. and *Aphidoletes aphidimyza* within the same IPM programme as the former may feed upon the latter.

If this was the case, then there were two questions to be answered:

- i) is the effect significant within the overall pepper IPM programme and
- ii) if so, which of the predators should be dropped from the programme?

In attempting to study the interaction between *Orius* spp. and *A. aphidimyza* in commercial pepper crops, it became clear that there were several other simultaneous interactions which could also have important consequences. These are discussed below.

6.2. Studying interactions

The conflicts between natural enemies have become known as 'intraguild predation' (IGP). Polis *et al.* (1989, 1992) defined a guild as including all species exploiting a similar resource, regardless of their nutrition mode, ecology or taxonomic position. Then they defined IGP as a predation event where a member of the guild preys upon another member of the same guild. The predator was defined as the IGP predator, the prey (or competing predator) as the IGP prey and their common resource as the extraguild prey.

A number of excellent papers have reviewed theoretical and empirical evidence and discussed the significance of IGP in its broadest context (e.g. Rosenheim, 1995; Arim & Marquet, 2004; Yano, 2006). However, they take the subject beyond the scope of this project. More relevant here are reviews that have focused on IGP among the natural enemies associated with aphids (e.g. Lucas, 2005). It is commonly believed that IGP constitutes one of the main forces influencing the structure and dynamics of aphidophagous guilds and must therefore be taken into account in all research studies. It is also recognised that understanding all the direct and indirect implications of IGP is complex and extremely difficult to study in the field.

As a consequence, it has become increasingly common to base IGP studies on a conceptual foundation, usually using mathematical models to help understand the population dynamics and to predict the outcome of any actions taken. Simulation models usually utilise empirical data from small scale laboratory experiments which eliminate many extraneous variables and inevitably lead to over simplification. While we may assume that placing IPM on a theoretical foundation will greatly reduce the risks of adverse effects from IGP, the models are rarely validated due to the complexity and cost of the field studies. This of course brings us full circle. When considering the value of conceptual studies, Barlow (1999) reminded researchers that “the goal is to understand, not the behaviour of models, but the behaviour of nature”.

6.3. IGP between *Orius* and *Aphidimyza*:

Where *A. aphidimyza* has been included in IGP studies, it has invariably been found to be the IGP prey. For example, Lucas *et al.* (1998) studied IGP among three species of aphid predators which commonly attack the potato aphid, *Macrosiphum euphorbiae*. Their studies included the specialist predator, *A. aphidimyza*, and two generalist predators, *Chrysoperla rufilabris* (a lacewing) and *Coleomegilla maculata lengi* (a beetle). The sessile and low mobility stages of *A. aphidimyza* were found to be extremely vulnerable to the two generalist predators.

Orius spp. are also generalist predators and where they have been included in IGP studies, they have been found to be the IGP predator. For example, in laboratory experiments against the predatory mites *Phytoseiulus persimilis* (Cloutier & Johnson, 1993), *Neoseiulus cucumeris* (Sanderson *et al.*, 2005; Gillespie & Quirling, 1992; Madadi, *et al.*, 2008) and *Iphiseius degenerans* (Brodsgaard & Enkegaard, 2005), and against the predatory bug, *Macrolophus caliginosus* (Jakobsen *et al.*, 2002), the *Orius* bugs were the aggressors.

The specific interaction between *Orius* spp. and *A. aphidimyza* was studied in the laboratory by Christensen *et al.* (2002). *Orius majuculus* consumed *A. aphidimyza* eggs in large numbers independent of the presence of aphid prey. Larvae of *A. aphidimyza* were also killed in substantial numbers when they were the only prey species but less so when aphids were offered as an alternative food source.

More recently, Hosseini *et al.* (2010) have studied the influence of plant quality on the interactions between *Aphis gossypii*, *A.aphidimyza* and *Orius laevigatus* in 25-day experiments on cucumber at various N fertilisation levels (90, 150, and 190 ppm) in microcosm set-ups under greenhouse conditions. The final aphid population size was significantly affected by an interactive effect of N fertilization and predator application. Regardless of the N fertilization levels, *O. laevigatus* alone was more effective in aphid suppression than *A. aphidimyza* alone. The *A. aphidimyza* population was suppressed by *O. laevigatus* in both the 90 and 150 ppm N treatments. However, there was no intraguild predation of *O. laevigatus* on *A. aphidimyza* at the 190 ppm N level.

In summary, the literature supports the more practical observations that *Orius* spp. predate upon eggs and larvae of *A. aphidimyza* in pepper crops. *Orius* spp. are a very important component of the overall IPM programme in peppers; their role in suppressing western flower thrips (*Frankliniella occidentalis*) development and subsequent transmission of tomato spotted wilt virus (O'Neill & Bennison, 2010) is indisputable. Furthermore, the work of Hosseini *et al* (2010) suggests that *O. laevigatus* may have a bigger impact on the aphid population than on *A. aphidimyza*. The available evidence suggests that *Orius* spp. should be retained in the pepper IPM programme in preference to *A. aphidimyza*.

6.4. Other interactions:

Enkegaard *et al.* (2005) investigated IGP between *A. aphidimyza* and *Aphidius colemani* in 24 laboratory experiments. They found that *A. aphidimyza* larvae readily killed parasitised but not yet mummified *Aphis gossypii* both when offered alone and when offered in combination with unparasitised aphids. The predator showed a slight preference for parasitised over unparasitised aphids. They did not observe predation on mummified *A. gossypii*, even when that was the only food available. The latter was in contrast to our own observations in pepper crops, where we frequently saw *A. aphidimyza* larvae feeding on *Myzus persicae* / *Aphidius* spp. mummies (eg. Figure 23).

There are also records of predatory bugs feeding on mummified aphids; for example Meyling (2002) reported that the predatory bug, *Anthocoris nemorum*, preyed readily on immature *A. colemani* contained within *M. persicae* mummies. Our own

observations in pepper crops show that *Orius* spp. larvae will feed on *Myzus persicae* / *Aphidius* spp. mummies (e.g. Figure 24).

Figure 23. *Aphidoletes aphidimyza* larva feeding on a *Myzus persicae* / *Aphidius* spp. mummy



Figure 24. *Orius* spp. larva feeding on a *Myzus persicae* / *Aphidius* spp. mummy



Section 7: Open Rearing Units

7.1. Background

Open rearing units (ORUs) or banker plants have been used to boost numbers of natural enemies 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 a pest species. Stacey (1977) published the first example in the scientific literature. His system was intended to suppress *Trialeurodes vaporariorum* (glasshouse whitefly) on tomato crops with the parasitic wasp, *Encarsia formosa*. Stacey grew tomato plants in a separate greenhouse, infested them with *T. vaporariorum* and allowed *E. formosa* to colonise the whiteflies. The plants were then moved into the tomato production unit. *Encarsia formosa* were sustained on the ORUs for over 8 weeks and were believed to contribute to the suppression of the pest during the early stages of the trials. However, the whiteflies eventually spread from the ORUs to the crop plants, which was clearly counter productive. The main lessons from this early pioneering work were that the plants used in ORUs should be botanically different to the crop and that the system should utilise herbivorous insects that do not attack the crop plant.

ORUs have received less attention from researchers than augmentative or conservation biological control techniques despite their obvious potential. Frank (2010) provided a useful review of the work done since 1977. He cited a total of 29 studies where ORUs had been implemented in crops and a further 30 studies which had addressed some aspect of natural enemy behaviour aimed at improving the efficiency of ORUs. Frank (2010) reported that aphids have become the main targets of ORUs. Typical ORUs have been based on cereals, such as wheat, barley, or maize infested with *Rhopalosiphum padi* (bird cherry aphid) or *Sitobion avenae* (cereal aphid). These species of aphids are a common host for parasitoids, such as *Aphidius colemani*, *A. matricariae* or *A. ervi*, without being a direct threat to the crop.

The system described by Jacobson and Croft (1998) to combat *Aphis gossypii* on cucumber provides a good example of what can be achieved against aphids using ORUs. They found that wheat, barley and oat plants were susceptible to plant pathogens in the greenhouse environment and this shortened the useful life of the ORUs. They subsequently worked with ORUs based on maize plants infested with *R. padi* and *A. colemani*, and compared their efficacy to multiple (or trickle) releases of

the same parasitoids. Effective application rates were determined in experimental crops for both systems during spring and summer. When labour to produce and maintain the ORUs were taken into account, the cost of the two systems was comparable. Under these circumstances, most UK cucumber growers opted for the trickle release because it was simpler to manage on a day to day basis.

It is interesting to note that hyperparasitism was not observed in the trials by Jacobson and Croft (1998). In fact, hyperparasitism is rarely mentioned in association with ORUs in the scientific literature despite it being a major threat to the adoption of this technique in commercial crops. This is possibly because most of the studies have been done in isolation and have not taken into account the difficulties caused by all the additional variables encountered when techniques are scaled up to full commercial production.

In the majority (92%) of published ORU studies, the aphids were targeted with parasitoids; particularly *A. colemani* (see Frank, 2010). However, predators have also been used alone or in conjunction with parasitoids. Several workers have attempted to develop ORUs to support populations of *Aphidoletes aphidimyza* (e.g. Hansen, 1983; Bennison & Corless, 1993; Bungler *et al.*, 1997; Kim & Kim, 2004). This approach would seem to have limited potential in commercial pepper crops due to intraguild predation by *Orius* spp. (see Section 6). The use of ORUs to support aphidophagous hoverfly populations may have more potential (see Section 7.3).

7.2. Practical experience with ORUs in pepper crops during 2010

In 2010, Taylor and Knight (unpublished data) tested ORUs in commercial pepper crops at Valley Grown Nurseries (Lower Nazeing, Herts). Eight ORUs per hectare were placed in the glasshouse per week for three weeks in February 2010. Each unit consisted of a hanging basket containing wheat plants infested with *Sitobion avenae* (Figure 25). The baskets were positioned above the crop, to reduce the problems with disease encountered by Jacobson and Croft (1998), and were irrigated / fed using the pepper irrigation system. The aphid populations were colonised with *Aphelinus abdominalis* (Figure 26), which was believed to be less susceptible to hyperparasitism than *Aphidius* spp. By 22 February (week 8), each of the first units contained very large numbers of aphids. Although no mummified aphids were

showing at that time, emergence tests similar to those described in Section 3 revealed a high level of parasitism.

The same ORUs remained in place for the next 20 weeks. Although the original plants deteriorated, the units were refreshed periodically simply by adding presoaked wheat seed. Inspection on the 12 May (week 19), revealed many mummified aphids (Figure 26). No attempt was made to quantify the actual number of parasitoids produced by the ORUs. However, the nursery's routine pest monitoring procedures detected fewer colonies of aphids in glasshouses protected with the ORUs than in glasshouses where the parasitoids had been released systematically. Both Taylor and Knight believed that the strategy had been successful up to that point.

Figure 25. ORUs in place above the pepper crop (left) and close up of a single unit (right) in week 8, 2010.



Figure 26. Condition of ORUs at Valley Grown Nursery in week 19; note the large numbers of mummified aphids (right).



The units were examined again on 8 July 2010 (week 27, 2010) when many *Orius* spp and *A. aphidimyza* were seen on the wheat plants. Samples of intact mummified aphids, collected for emergence tests in the laboratory, had a very high percentage of successful adult wasp emergence (*i.e.* 93%). However, only 28% of the emerging wasps were *A. abdominalis*; the remaining 72% being hyperparasitoids (Figure 22). The latter comprised 10% *Dendrocerus* spp., 36% *Alloxysta brevis* and 26% *Alloxysta brachyptera*. We do not know when hyperparasites started to colonise the units but it is clear that the value of the ORUs had become compromised by mid-summer.

7.3. Potential of ORUs for syrphids

Pineda and Marcos-Garcia (2008a) investigated the potential of aphid infested barley plants to enhance natural populations of syrphids in sweet pepper crops in Spain. They sowed 25cm diameter patches of barley in the soil at the rate of 3 patches per 100m², infested them with *Rhopalosiphum maidis* and then released marked *Episyrphus balteatus*. The aim of their work was to evaluate the potential of these aphid cultures to increase the residence time of the released syrphids and to enhance the natural populations of syrphids. Although the presence of the aphid cultures did not increase the residence time of the released syrphids compared to the controls, the provision of aphid reservoirs did significantly increase the ingress of four species of naturally occurring syrphids; *i.e.* *Sphaerophoria rueppellii* (81% of records), *S. scripta*, *Eupeodes corollae* and *E. balteatus*. Pineda and Marcos-Garcia found a high proportion of pepper pollen in the gut content of those predators indicating that sweet pepper provide a suitable pollen source.

The same authors investigated the benefits of using selected flowering plants (*i.e.* coriander and sweet alyssum) in greenhouses to enhance populations of syrphids (Pineda and Marcos-Garcia, 2008b). The flowering plants were reported to be “distributed in the greenhouses in several monospecific patches” but it is not absolutely clear how this was done or for what part of the season the plants were in flower. Nonetheless, the presence of these plants was said to significantly increase the numbers of both adult and immature life stages of naturally occurring syrphids, including *S. rueppellii*, *E. corollae* and *E. balteatus*. Analysis of gut contents showed that all three species fed on pollen from the flowering plants as well as on sweet pepper pollen.

Technology Transfer

- Jacobson, R.J. (2009). Taking control of leafhoppers. *HDC News*, March 2009. Vol 151. 22-23.
- Jacobson, R.J. (2009). Presentation to Pepper Technology Group. 5 March 2009. Stoneleigh, Warwickshire
- Jacobson, R.J. (2009). IPM: Plugging the gaps. Improving Production of Glasshouse Crops: The Pepper Experience. A Technical Seminar and Nursery Tour. Waltham Abbey Marriott Hotel and Valley Grown Nurseries. 29 September 2009.
- Jacobson, R.J. (2010). Spray support for front line agents. *HDC News*, April 2010. Vol 162. 20-21
- Jacobson, R.J. (2010). Developing IPM solutions for aphids. HDC / PTG Pepper Open Day. Chichester Park Hotel and Tangmere Airfield Nurseries, 21 September 2010.

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References

- Araj, S.E., Wratten, S., Lister, A. & Buckley, H. (2006). Floral diversity, parasitoids and hyperparasitoids – A laboratory approach. *Basic and Applied Ecology*. 9: 588-597.
- Arim, M. & Marquet, P.A. (2004). Intraguild predation: a widespread interaction related to species biology. *Ecology Letters* 7: 557–564
- Barlow, N.D. (1999). Models in biological control. In: *Theoretical Approaches to Biological Control*. Ed. Hawkins, B.A. & Cornell, H.V. Cambridge University Press, Cambridge, UK. 43-68pp.
- Bennett, A.W. & Sullivan, D.J. (1978). Defensive behavior against tertiary parasitism by the larvae of *Dendrocerus carpenteri*, an aphid hyperparasitoid. *Journal of New York Entomological Society* 86:1 53-60.
- Bennison, J.A & Corless, S.P. (1993). Biological control of aphids on cucumbers: further development of open rearing units or 'banker plants' to aid establishment of aphid natural enemies. *IOBC/WPRS Bulletin* 16, 5-8.
- Bunger, I., Leibig, H. & Zebitz, C.P.W. (1997). Erfahrungen mit der 'Offenen Zucht' von Blattlausantagonisten bei der Bekämpfung von *Aphis gossypii* Glover. *Mitteilungen der Deutschen Gesellschaft für Allgemeine und Angewandte Entomologie* 11, 327-330.
- Brodsgaard, H.F., and Enkegaard, A. (2005). Intraguild predation between *Orius majusculus*(Reuter) (Hemiptera: Anthocoridae) and *Iphiseius degeneras* Berlese (Acarina:Phytoseiidae). *IOBC/WPRS Bulletin* 28, 19-22.
- Buitenhuis, R., McNeill, J.N., Boivin, G. & Brodeur, J. (2004). The role of host searching of aphid hyperparasitoids. *Journal of Chemical Ecology*, 30:2 273-285.
- Christensen, R.K., Enkegaard, A. and Brodsgaard, H.F. (2002). Intraspecific interactions among the predators *Orius majusculus* and *Aphidoletes aphidimyza*. *IOBC/WPRS Bulletin* 25, 57-60.
- Christiansen-Weniger, P. (1994). Studies on semiochemicals affecting the host acceptance behavior of *Asaphes vulgaris* Wlk. *Norw. J. Agric. Sci.* 16:2 77-82.

- Cloutier, C., and Johnson, S.G. 1993. Predation by *Orius tristicolor* (White) (Hemiptera: Anthocoridae) on *Phytoseiulus persimilis* (Acarina: Phytoseiidae): testing for compatibility between biocontrol agents. *Environmental Entomology* 22, 477-482
- Chow, A. & Mackauer, M. (1999). Marking the package or its contents: Host discrimination and acceptance in the ectohyperparasitoid *Dendrocerus carpenteri* (Hymenoptera: Megaspilidae). *The Canadian Entomologist*. 131: 495-505.
- Enkegaard, A., Christensen, R.K. and Brodsgaard, H.F. (2005). Interspecific interactions among the aphid parasitoid *Aphidius colemani* and the aphidophagus gallmidge *Aphidoletes aphidimyza*. . *IOBC/WPRS Bulletin* 28(1) 83-86.
- Frank, S.D. (2010). Biological control of arthropod pests using banker plant systems: Past progress and future directions. *Biological Control*, 52, 8-16
- Gillespie, D. R., and Quiring, D. J. M. 1992. Competition between *Orius tristicolor* (White) (Hemiptera: Anthocoridae) and *Amblyseius cucumeris* (Oudemans) (Acari: Phytoseiidae) feeding on *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae). *Canadian Entomologist* **124**, 1123-1128.
- Hansen, L.S. (1983). Introduction of *Aphidoletes aphidimyza* (Rond.) (Diptera: Cecidiomyiidae) from an open rearing unit for the control of aphids in glasshouses. *IOBC/WPRS Bulletin* 6, 146-150.
- Hollier, C., Micha, S.G., Schulz S., Francke, W. & Pickett, J.A. (1994). Enemy-induced dispersal in a parasitic wasp. *Experimentia* 50; 182-185.
- Hosseini, M., Ashouri, A., Enkegaard, A., Weisser, W., Goldansaz, S.H., Mahalati, M.N., Sarraf M. & Hamid, R. (2010). Plant quality effects on intraguild predation between *Orius laevigatus* and *Aphidoletes aphidimyza*. *Entomologia Experimentalis et Applicata*, 135, 2, 208-216.
- Jacobson, R.J. (2009). Sweet pepper: Short term solutions for leafhopper and aphid infestations. *Report of contract work undertaken for HDC*, December 2009, 59pp
- Jacobson, R. J. & Croft, P. (1998). Strategies for the control of *Aphis gossypii* Glover

(Hom. Aphididae) with *Aphidius colemani* Viereck (Hym. Braconidae) in protected cucumbers. *Biocontrol Science and Technology*, 8 (3), 377-387.

Jakobsen, L., Enkegaard, A. and Brodsgaard, H.F. (2002). Interactions between the two polyphagous predators *Orius majusculus* and *Macrolophus caliginosus*. *IOBC/WPRS Bulletin* 25, 115-118.

Kim, Y. & Kim, J. (2004). Biological control of *Aphis gossypii* using barley banker plants in greenhouse grown oriental melon. In: Hoddle, M.S. (Ed), *California Conference on Biological Control IV, Berkeley, California, USA, 13-15 July 2004, Center for Biological Control, College of Natural Resources University of California, Berkeley, USA*.

Lucas, E. (2005). Intraguild predation among aphidophagous predators. *Eur. J. Entomol.* 102: 351–364.

Lucas, É., Coderre, D. & Brodeur, J. (1998). Intraguild predation among aphid predators: characterization and influence of extraguild prey density. *Ecology* 79:1084–1092.

Madadi, H., Enkegaard, A., Brødsgaard, H. F., Kharrazi-Pakdel, A.m Ashouri, A.& Mohaghegh-Neishabouri, J. (2008): *Orius albidipennis* (Heteroptera: Anthocoridae): Intraguild predation of and prey preference for *Neoseiulus cucumeris* (Acari: Phytoseiidae) on different host plants. *Entomol. Fennica* 19:

Meyling, N.V., Brodsgaard, H.F., and Enkegaard, A. (2002). Intraguild predation between the predatory flower bug, *Anthocoris nemorum*, and the parasitoid, *Aphidius colemani*. *IOBC/WPRS Bulletin* 25, 189-192.

O'Neill, T. & Bennison, J. (2010). Tomato spotted wilt virus in protected edible crops. HDC Factsheet 23/10.

Pineda, A. and Marcos-Garcia, A. (2008a). Introducing barley as aphid reservoir in sweet-pepper greenhouses: Effects on native and released hoverflies (Dipters: Syrphidae). *Eur. J. Entomol.* 105: 531-535.

Pineda, A. and Marcos-Garcia, A. (2008b). Use of selected flowering plants in greenhouses to enhance aphidophagous hoverfly populations (Dipters: Syrphidae). *Ann.soc. entomol.* 44 (4): 487-492.

- Polis G.A. & Holt R.D. (1992): Intraguild predation: The dynamics of complex trophic interactions. *Trends Ecol. Evol.* **7**: 151–154.
- Polis G.A., Myers C.A. & Holt R.D. (1989): The ecology and evolution of intraguild predation: Potential competitors that eat each other. *Annu. Rev. Ecol. Syst.* **20**: 297–330.
- Rosenheim, J.A., Kaya, H.K., Ehler, L.E., Marois, J.J., & Jaffee, B.A. (1995). Intraguild predation among biological-control agents: theory and evidence. *Biological Control* **5**, 303-335.
- Sanderson, J. P., Brodsgaard, H. F., and Enkegaard, A. 2005. Preference assessment of two *Orius* spp. for *Neoseiulus cucumeris* vs. *Frankliniella occidentalis*. *IOBC/WPRS Bulletin* **28**, 221-224.
- Sing, R & Srivastava, P.N. (1987). Potential host habitat location by *Alloxysta pleuralis* (Cameron). *Z. Ang. Zool.* 74:3 37-41
- Sing, R & Srivastava, P.N. (1988). Host acceptance behavior of *Alloxysta pleuralis*, a cynioid hyperparasitoid oa an aphid parasitoid *Trioxys indicus* on aphids. *Entomol. Exp. Appl.* 47: 89-94.
- Stacey, D.L. (1977). Banker plant production of *Encarsia formosa* Gahan and its use in control of glasshouse whitefly on tomatoes. *Plant Pathology* 26, 63-66.
- Sullivan, D.J. (1972). Comparative behavior and competition between two aphid hyperparasites: *Alloxysta victrix* and *Asaphes californicus*. *Environmental Entomology* 1:234-244.
- Sullivan, D.J. (1987). Insect hyperparasitism. *Annual Review of Entomology* 32:49-70.
- Sullivan, D.J. (1988). Aphid hyperparasitism. In *Aphids, Their Biology, Natural Enemies and Control*. Ed A.K. Minks & P. Harrewijn, 2B 189-203. Amsterdam: Elsevier. 382pp.
- Sullivan, D.J. & Volkl, W. (1999). Hyperparasitism; Multitrophic Ecology and Behavior. *Annual Review of Entomology* 44:291-315.
- van der Bosch, R. (1981). Specificity of hyperparasites. In *The role of hyperparasitism in biological control; A symposium* . Ed D. Rosen, Berkley California. Publication 4103, pp27-33.

- van der Bosch, R., Messenger, P.S. & Guitierrez, A.P. (1982). *An introduction to Biological Control*. New York: Plenum Press, 247pp.
- Vinson, S.B. & Iwantsch, G.F. (1980). Host selection by insect parasitoids. *Annual Review of Entomology* 21:109-133.
- Walker, G.P. & Cameron, P.J. (1981). The biology of *Dendrocerus carpenteri* (Hymenoptera: Ceraphronidae), a parasite of *Aphidius* species, and field observations of *Dendrocerus* species as hyperparasites of *Acyrtosiphon* species. *New Zealand Journal of Zoology*. 8 531-538.
- Yano, E. (2006). Effects of intraguild predation and interspecific competition among biological control agents in augmentative biological control in greenhouses. In: *Intraguild Predation and Interspecific Competition Among Biological Control Agents*. Second International Symposium on Biological Control of Arthropods. Davos, Switzerland - September 12-16, 2005. 8pp.