FINAL REPORT

To:
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Sweet pepper: Short term solutions for leafhopper and aphid infestations

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Specialists in Integrated Pest Management, Research Facilitation and Horticultural Project Management

29 November 2009

Commercial – In Confidence

Project title	Sweet pepper: Short term IPM solutions for leafhopper and aphid infestations
Project number:	PC 295
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Report:	Final report [December 2009]
Previous reports	Not applicable
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Date project commenced:	1 April 2009
Date project completed (or expected completion date):	31 December 2009
Key words:	sweet pepper, *Empoasca decipiens*, *Myzus persicae*, *Aphidius colemani*, *Aphelinus* spp., *Praon* spp., indoxacarb, natural pyrethrins, fatty acid

The results and conclusions in this report are based on investigations conducted over a two-year period. The conditions under which the experiments were carried out and the results have been reported in detail and with accuracy. However, because of the biological nature of the work it must be borne in mind that different circumstances and conditions could produce different results. Therefore, care must be taken with interpretation of the results, especially if they are used as the basis for commercial product recommendations.

AUTHENTICATION

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

Dr R Jacobson
Director
Rob Jacobson Consultancy Ltd

R J Jacobson *29 November 2009*

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

CONTENTS

HEADLINES

- Indoxacarb (Steward) provides a short term solution to leafhopper infestations within existing IPM programmes in conventional crops.
- IPM of *Myzus persicae* can be based on a combination of parasitoids and Pyrethrum 5EC or Savona.

BACKGROUND AND EXPECTED DELIVERABLES

IPM in sweet peppers is well understood and has been extremely successful in northern Europe over the last two decades. However, if a broad spectrum pesticide is applied against any one of the pest species that attack the crop it can cause a complete breakdown of the IPM programme and result in total dependence on pesticides against all pests for the remainder of the season. In recent years, poor control of leafhoppers and aphids has led to such a scenario; *eg*. the use of thiacloprid (Calpypso) against leafhoppers in mid-season killed biological control agents resulting in secondary problems with western flower thrips (WFT). This led to populations of WFT being carried over to the following season causing direct damage from planting and, in some cases, transmission of tomato spotted wilt virus.

This study focused on finding IPM compatible control measures for leafhoppers and aphids in pepper crops with emphasis on solutions that could be immediately implemented in commercial crops.

Leafhoppers

Leafhoppers have been a pest in some UK tomato and pepper crops since the early 1990s. Trials in tomato in 1994, demonstrated that a carefully timed application of the IPM compatible product, buprofezin (Applaud), in the early season could provide control of *Hauptidia maroccana* (glasshouse leafhopper) for over three months. This became a standard commercial control measure for many years. However, buprofezin was removed from the market in 2008.

The insecticide, indoxacarb (Steward), was approved for control of caterpillars on protected peppers just before this project began. A preliminary literature search revealed that it had been used successfully against three species of leafhoppers on

other crops in other countries; *i.e.* potato leafhopper (*Empoasca fabae*), western grape leafhopper (*Erythroneura eleantula*), white apple leafhopper (*Typhlocyba pomeria*). A second literature search indicated that indoxacarb should be compatible with many biocontrol agents used in the pepper IPM programme. It appeared to have the potential to replace buprofezin within the IPM programme.

Indoxacarb could not be used in organic production and an alternative product was sought for these crops. Natural pyrethrins (extracts of African chrysanthemum) were approved for organic crops and were known to kill insects related to leafhoppers. Although natural pyrethrins are non specific and potentially harmful to biocontrols, studies on organic tomatoes in HDC Project PC 240 had shown how they could be successfully integrated into an IPM programme by separating them from the biocontrols in either time or space.

This project investigated the efficacy of indoxacarb and natural pyrethrins against *Empoasca decipens* (green leafhopper) in conventional and organic sweet pepper crops respectively.

Aphids

The primary biological control measures used against aphids on organic peppers are parasitic wasps (eg *Aphidius* spp.). They are usually released weekly from early in the season so that they are already present in the crop when the aphids arrive. This strategy is usually successful until mid-summer when the aphid population growth often outstrips that of the parasitoids.

We hypothesised that either natural pyrethrins (as Pyrethrum 5EC) or fatty acids (Savona) could be used as a secondary control measure to redress the balance between the pest and beneficial populations when the pest damage approached the economic damage threshold. An opportunity arose to test this hypothesis against a large aphid population in a commercial crop of organic peppers in October 2008. A second trial was organised in 2009 to consolidate the “proof of concept” findings and to test the strategy within the whole IPM system.

Prior to each trial, the aphid population was tested for resistance to natural pyrethrins. In addition, the impact of the second line of defence treatments on other biological control agents used in the overall IPM programme was noted.

SUMMARY OF THE PROJECT AND MAIN CONCLUSIONS

Indoxacarb against *Empoasca decipens* (green leafhopper)

Four separate trials evaluated the efficacy of indoxacarb when applied as a high volume (HV) spray, an ultra low volume (ULV) application and a high volume spray in a tank mix with pymetrozine (Chess).

The HV sprays of indoxacarb alone reduced leafhopper numbers by over 90% within two days and by 98-99% after 12-18 days. The leafhopper population did not recover within 34 days of the spray application. Results were broadly comparable when the product was applied with pymetrozine. The ULV application was less effective giving approximately 60% control at 5 days and this had not improved by 12 days.

After 24 hours, the indoxacarb residue on fruit was 0.04 mg/kg. The UK and Codex MRL is 0.3 mg/kg and the limit of detection is 0.02 mg/kg.

The predators, *Orius* spp. and *Amblyseius* spp., were found alive on leaves post-treatment. Over 70% of adult *Aphidius* spp. hatched from mummified aphids that were on the sprayed leaves. Based on these observations, a literature search and the biocontrol suppliers' recommendations, indoxacarb should be compatible with the predatory insects and mites used in the IPM programme in pepper crops. However, further clarification is required about the possible impact of the chemical on oviposition by *Orius* spp. The situation is less clear with parasitic wasps. It would appear that direct contact with the spray is harmful to adult wasps but larvae are reasonably well protected within the mummified aphids.

Natural pyrethrins against *Empoasca decipens* (green leafhopper)

There was a clear and rapid effect from the Pyrethrum 5EC treatment with leafhopper numbers dropping by over 99% during the first 24 hours post-treatment.

Natural pyrethrins are known to have very short persistence and it was considered unlikely that there would be any residual effect against nymphs which hatched from any eggs that survived the treatment. However, very few nymphs were found on the plants 12 days post-treatment. The potential impact of Pyrethrum 5EC on biological control agents is discussed below.

Aphids: Proof of concept trial

The “proof of concept” study focused on natural pyrethrins (as Pyrethrum 5EC) as the secondary control measure. Prior to treatment, there were an average of 110 healthy aphids per leaf with associated honey dew and sooty mould on both leaves and fruit. The Pyrethrum 5EC spray was applied high volume to the top 0.6m stratum of the plants. The day after treatment, the level of kill of aphids was quite variable. There was over 95% reduction in numbers on the sprayed foliage adjacent to the path. However, numbers had only been reduced by 40% on leaves within the middle of the dense wide-bed canopy and by even less at the very top of the central heads. This pattern of survival suggested poor spray contact rather than resistance to the product. This was consistent with the results of the formal resistance tests which showed the population to be susceptible to natural pyrethrins.

Pyrethrum 5EC was harmful to adult parasitoids but relatively safe to immature parasitoids within the mummified aphids. Approximately 80% of *Aphidius* spp. adults and 70% of *Praon* spp. adults emerged from the mummified aphids collected from the plants after treatment. This was consistent with normal expectations.

At the time of treatment, 5% of the aphid population was parasitised by approximately equal numbers of *Aphidius* spp. and *Praon* spp. The assessment four weeks post-treatment showed a quite remarkable shift in the balance of aphids and parasitoids with 95-98% of individuals being mummified. At that time, there were very large numbers of adult parasitoids flying within the crop canopy, new growth was completely “clean” and newly developing fruit were no longer contaminated by honey dew and sooty mould.

The proof of concept trial clearly demonstrated that an extremely large and damaging population of *M. persicae* could be controlled quickly and effectively with a combination of parasitoids and Pyrethrum 5EC.

Control of aphids with a combination of parasitoids and a “soft” chemical

The objectives of this trial were to consolidate the proof of concept findings and to test the second line of defence strategy at a lower level of aphid infestation within the whole IPM system.

Aphidius colemani, *Aphidius ervi* and *Aphelinus abdominalis* parasitoids had been released in the crop from early season and were established within the population of *Myzus persicae.* In addition, small numbers of *Praon* spp. had become established from the natural population. The aphids were just beginning to cause sticky patches on leaves and fruit in localised areas in late June, which was an indication that the infestation was approaching the economic damage threshold. This was considered to be the optimum time to apply the second line of defence treatment of either Pyrethrum 5EC or Savona. The high volume sprays of Pyrethrum 5EC and Savona were applied to the upper half of the plant canopy in 0.1ha plots.

The weather during the week immediately following application of the treatments was hot and dry. Aphid numbers rose very rapidly in the untreated control plot and there was a marked increase in stickiness on leaves and fruit. The damage rapidly became unacceptable on these plants and a corrective spray had to be applied. This confirmed that the spray applications in the trial area had been accurately timed.

As in the proof of concept trial, spray coverage was less than ideal confirming that improved application techniques are required for wide bed organic crops. Both of the second line of defence treatments initially suppressed aphid population growth compared to the untreated control plot. The day after treatment, aphid numbers were reduced by 98% in the tops of the plants in the Pyrethrum 5EC plot and by 58% in the Savona plot.

Parasitoids appeared to be taking control in both plots by day 13. Thereafter, the Savona plot came under complete control by day 22, which was broadly similar to the result in the proof of concept trial. However, there was a set back in the Pyrethrum 5EC plot due to hyperparasitism impairing the performance of the *Aphidius* spp. Aphid numbers eventually crashed in this plot due to a naturally occurring infection by entomopathogenic fungi (predominantly Entomophthorales).

Numbers of *Orius* spp. declined following both treatments but recovered more rapidly on the plants treated with Savona. It had proved difficult to integrate Pyrethrum 5EC with this predator because it inhabited the same stratum of the crop as the target pest. As a consequence, plants treated with Pyrethrum 5EC could have been vulnerable to attack by thrips although this did not happen in this trial.

FINANCIAL BENEFITS

The project rapidly developed an effective IPM compatible control measure for use against leafhoppers in conventional sweet pepper crops. There had been an urgent need for this in the Lea Valley area where previous treatments had disrupted the whole IPM programme and led to secondary problems with WFT and TSWV. One grower estimated his additional costs and yield losses to have been equivalent to £41.5k per hectare in the 2007/08 season. An interim report and an HDC News article conveyed the results of the project to the affected growers during the project. The new techniques were immediately adopted and were successfully implemented in commercial crops in that area. No further difficulties with leafhoppers, or associated problems with WFT, were reported during 2009. We may therefore conclude that the total cost of this project was recovered from the savings made by a single grower in the 2008/09 season. All other financial benefits have been a bonus.

The project has prepared the foundation of a strategy for the control of aphids within an IPM programme for organic sweet pepper crops. It has also identified the limiting factors which require further fine tuning in order to make the programme more robust.

ACTION POINTS FOR GROWERS

- Indoxacarb (Steward) provides a short term solution to leafhopper infestations within existing IPM programmes for conventional crops. The product should be diluted at the label rate for caterpillars (*i.e.* 125g per 1,000 litres water) and applied high volume (HV) to the point of run-off ensuring that good cover is achieved on both sides of the leaves.
- Natural pyrethrins (Pyrethrum 5EC) will control leafhoppers within existing IPM programmes and this treatment is suitable for organic crops. The product should be diluted at the rate of 20ml per 5 litres of water and applied HV to the point of run off ensuring good cover to the undersides of the leaves throughout the canopy. This treatment may have an adverse effect on *Orius* spp. and therefore should be restricted to hot spots of leafhopper activity.
- Both Pyrethrum 5EC and Savona can be used against aphids as a secondary control measure to support the primary biological control agents. However, the following factors must be taken into account:

- Appropriate parasitoids should be released weekly from planting. The release programme must be tailored to suit each individual cropping situation. If in doubt, growers should seek guidance from their biocontrol supplier or an IPM specialist.
- The second line of defence treatment should be applied when the aphids are just beginning to cause sticky patches on leaves and fruit in localised areas of the crop.
- In trials, Pyrethrum 5EC was diluted at the rate of 20ml per 12 litres water and applied HV to the point of run off ensuring good cover to the undersides of the leaves in the upper half of the crop. The latter helps to minimize the impact on beneficial organisms in the crop. While harmful to adult parasitoids, Pyrethrum 5EC did not appear to harm immature parasitoids within the mummified aphids. However, numbers of *Orius* spp. declined following treatment and had not recovered within 34 days of the treatment.
- In trials, 2% Savona was applied HV to the point of run off ensuring good cover to the undersides of the leaves in the upper half of the crop.

- This project has provided the basis for an IPM strategy against aphids in organic pepper crops. However, the following components require further investigation:
  - spray coverage in wide-bed organic pepper crops
  - hyperparasitism of *Aphidius* spp.
  - interaction between *Orius* spp. and *Aphidoletes aphidimyza*
- In addition, the following topics should be drawn into the aphid research programme:
  - alternative second line of defence treatments, such as entomopathogenic fungi for organic crops and pymetrozine (Chess) applied through the irrigation system for conventional crops.
  - a review of existing knowledge of open rearing systems (also known as banker plants) to explore their potential as a breeding base for novel biological agents.

SECTION 1. BACKGROUND TO LEAFHOPPER STUDIES

IPM in sweet peppers is already well understood and has been extremely successful in northern Europe over the last two decades. For example, Ramakers (2004) stated "sweet pepper is now considered the best example of successful IPM in protected cultivation with respect to complexity and duration". However, if a broad spectrum pesticide is applied against any one of the pest species that attack the crop it can cause a complete breakdown of the IPM programme and result in total dependence on pesticides against all pests for the remainder of the season.

In recent years, poor control of leafhoppers and aphids has led to such a scenario; *eg*. the use of thiacloprid (Calpypso) against leafhoppers in mid-season has killed biological control agents resulting in secondary problems with western flower thrips (WFT) and aphids. This led to populations of WFT being carried over to the following season causing problems from planting. In some cases, this included transmission of tomato spotted wilt virus. This study focused on finding an IPM compatible control measure for leafhoppers with emphasis on a solution that could be immediately implemented in commercial crops.

Leafhoppers have been a pest in some UK tomato and pepper crops since the early 1990s. Trials in tomato in 1994, demonstrated that a carefully timed application of buprofezin (Applaud) in the early season could provide control of *Hauptidia maroccana* (glasshouse leafhopper) for over three months (Jacobson & Chambers, 1996). This became a standard commercial control measure in tomatoes for many years (Jacobson, 2004). However, the success of buprofezin was at least partially dependent on its vapour action and it proved less effective in mid-season when glasshouse ventilators could be open for much of the day and part of the night. Buprofezin was removed from the market in 2008 and is no longer available to growers.

The insecticide, indoxacarb (Steward), had been approved for control of caterpillars on protected peppers just before the start of this project. A preliminary literature search revealed that it had also been used successfully against three species of leafhoppers on other crops in other countries; *i.e.* potato leafhopper (*Empoasca*

fabae), western grape leafhopper (*Erythroneura eleantula*), white apple leafhopper (*Typhlocyba pomeria*) (Dupont, 1999; McKinley *et al*., 2002).

Indoxacarb is an insecticide in a new class of chemistry (the oxadiazines) with a new mode of action. It affects insects from direct exposure to spray droplets and through ingestion of treated plant material. Once absorbed it kills by binding to a site on the sodium channel and blocking the flow of sodium ions into nerve channels. The result is impaired nerve function, feeding cessation, paralysis and finally death. Once indoxacarb is ingested, the insect stops feeding immediately but may take several days to die. Indoxacarb has no vapour action and is not systemic but does have translaminar movement into the mesophyll. While water solubility is very low, it is lipophilic which facilitates transport into the waxy leaf surface where it is protected from weathering. It has good photostability and rainfastness. (DuPont, 1999; McKinley *et al*, 2002; Sherrod, 1999).

A second literature search indicated that indoxacarb should be compatible with many biocontrol agents used in the pepper IPM programme (Dupont, 1999; Sherrod, 2000; Dinter & Wiles, 2000; McKinley *et al*, 2002). The findings relating to compatibility with biocontrols have been summarised in Section 4.

Indoxacarb could not be used in organic production and an alternative product was sought for these crops. Natural pyrethrins (extracts of African chrysanthemum) were approved for organic crops and were known to kill insects related to leafhoppers. Although natural pyrethrins are non specific and potentially harmful to biocontrols, studies on organic tomatoes in HDC Project PC 240 (Jacobson & Morley, 2007) have shown how they can be successfully integrated into an IPM programme by separating them from the biocontrols in either time or space. This project investigated the efficacy of natural pyrethrins against leafhoppers organic sweet pepper crops.

SECTION 2. PRELIMINARY PRACTICAL STUDIES WITH LEAFHOPPERS IN CONVENTIONAL CROPS

Key sites that had experienced difficulty controlling leafhoppers in recent seasons were monitored during May and June 2008 with the objective of detecting an early infestation that would provide a suitable site for this study. The first opportunity occurred at Abbey View Produce Ltd (Waltham Abbey) in a discrete area of one glasshouse.

The pests were identified by the author as *Empoasca decipiens* (green leafhopper) and this was confirmed by the Insect Identification Service of the Central Science Laboratory (now Fera). Further samples collected from other pepper nurseries in the Lea Valley area were subsequently found to be the same species. All trials reported below made use of naturally occurring populations in commercial greenhouses and there were no attempts to manipulate pest numbers prior to application of treatments.

A preliminary study showed that the leafhoppers were positioned at all levels in the crop with the exception of the lowest 0.3-0.4m. An assessment procedure was developed which involved examining 15-20 leaves evenly distributed between this position and the top of the plant. Each leaf was examined in situ and the numbers of leafhoppers were recorded. *Empoasca decipens* has seven life cycle stages; *i.e.* adult, egg and five nymphal stages (Jervis & Kidd, 1993). As it is difficult to accurately differentiate between nymphal stages in situ, this assessment procedure simply recorded them in three categories; small, medium and large. This was sufficient to enable us to distinguish between nymphs which had survived treatments and those which had hatched from eggs since treatment.

SECTION 3. LEAFHOPPER TRIALS IN CONVENTIONAL CROPS

INTRODUCTION

Four separate trials investigated the control of leafhoppers with indoxacarb (Steward) in commercial crops of sweet peppers. In the first trial, a high volume (HV) spray treatment was limited to eight crop rows and an adjacent area was used as an untreated control. The whole area was monitored over 35 days.

The first trial was highly successful and prompted a second study to evaluate a less labour intensive method of application via an Enbar ultra low volume (ULV) applicator. This was found to be less effective than the HV spray application and the trial was not repeated.

Two further HV spray trials were done to confirm the results of the first study. By this time, leafhopper populations had substantially increased and the pests were beginning to cause damage to both foliage and fruit. In both trials, whole crops were sprayed and the effects of the treatments were determined by comparing the size of the leafhopper populations before and after treatment.

Additional information was collected at every opportunity during the four trials. For example, fruit was tested for residues of indoxacarb (Trial 1) and the presence of live biological control agents was recorded after all treatments.

TRIAL 1

Materials and methods

Indoxacarb (Steward) was applied to eight rows of sweet peppers (cv Kelly) in Block B with a total area of 518m^2 on 17 July 2008. An adjacent untreated area was used as a control. The treatments were applied from a tank and pump situated on the central roadway using a retractable hose and hand lance fitted with a multi-nozzle head. The product was diluted at the label rate for caterpillars (*i.e.* 125g per 1,000 litres water) and applied HV to the point of run-off ensuring that good cover was

achieved on both sides of the leaves. The volume applied was equivalent to 1,930 litres per hectare. After an interval of seven days (*i.e.* on 24 July), a second treatment was applied by exactly the same method to half of the trial area.

There were eight sample points in each treated plant row and a further eight points in the untreated control; *i.e.* 72 sample points in total. At each sample point, 15 leaves were selected which were evenly distributed from the top down to 0.4m above the base of the plant. Each leaf was examined in situ and the number of leafhopper adults, small nymphs, medium nymphs and large nymphs were recorded separately. An initial assessment was done 24 hours prior to application of the first treatment. Further assessments were done 6 days after the first treatment, and 12 and 28 days after the second treatment.

The effect of indoxacarb was determined by comparing the size of the leafhopper population before and after each treatment. In addition, the size of the leafhopper populations in the treated areas were compared to the untreated control at the end of the trial.

Samples of fruit were taken from indoxacarb treated plants 24 hours after the application of the first treatment and tested for residues by the Eurofins laboratory.

Results and discussion

The mean numbers of leafhoppers recorded in each treatment on each assessment date, together with the percentage change over time, are shown in Table 1. There was a four fold increase in the numbers of leafhoppers recorded in the untreated area of the crop during the course of the trial. In contrast, where a single treatment of indoxacarb was applied, numbers had declined by 92%, 98% and 99% after 6, 18 and 28 days respectively. No additional benefit was seen from the second application of indoxacarb. The differences between treatments were so clear that no further analysis of the data was deemed necessary. The failure of the leafhopper population to recover over 34 days, or to reinvade from surrounding areas, indicated that the effect of indoxacarb was quite persistent.

Table 1. The mean numbers of leafhoppers (all life cycle stages) recorded on each assessment date in each treatment in Trial 1.

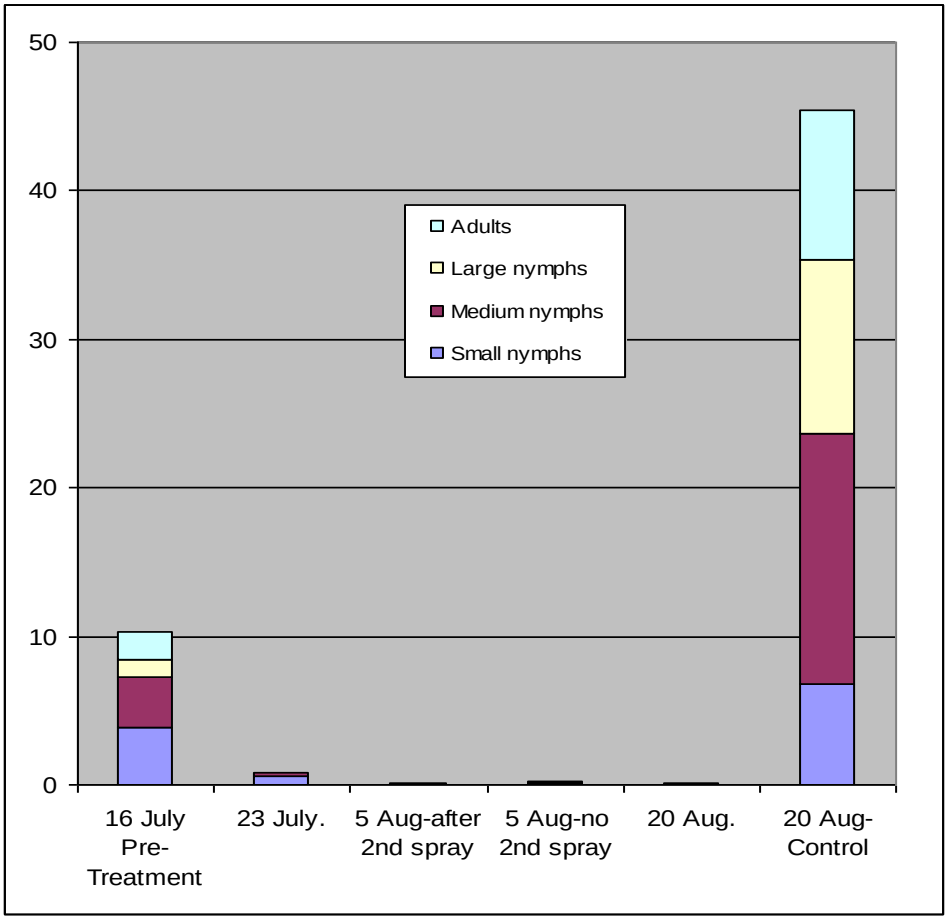
<table>
<tr><td rowspan="2">Treatment</td><td colspan="4">Mean numbers of leafhoppers (all life cycle stages combined) per sample point on the following assessment dates:
[percentage relative to original assessment]</td></tr>
<tr><td>16 July</td><td>23 July</td><td>5 August</td><td>20 August</td></tr>
<tr><td>Control</td><td rowspan="3">10.3
[100%]</td><td>-</td><td>-</td><td>45.38
[440%]</td></tr>
<tr><td>Single HV Spray</td><td rowspan="2">0.8
[8%]</td><td>0.2
[2%]</td><td rowspan="2">0.1
[1%]</td></tr>
<tr><td>Two HV Sprays</td><td>0.2
[2%]</td></tr>
</table>

The mean numbers of leafhoppers per sample point, broken down to provide an indication of the life cycle stages present, are shown for each treatment in Figure 1. Six days after the first treatment, there was a predominance of small nymphs on the leaves and it was assumed that they had hatched from eggs after the plants had been sprayed. A second spray of indoxacarb was therefore applied to half of the trial area to determine whether this would be necessary to kill the hatching nymphs. The next assessment (12 days after the second application) showed that there was no difference between the leafhopper population in the areas which had and had not received the second spray. This indicated that the first indoxacarb treatment was sufficiently persistent to kill the hatching nymphs.

After 24 hours, the indoxacarb residue on fruit was 0.04 mg/kg. The UK and Codex MRL is 0.3 mg/kg and the limit of detection is 0.02 mg/kg.

The population of *Orius* spp. was quite small prior to application. Nonetheless, all motile life cycle stages could be found alive on sprayed leaves on all post-treatment assessment dates. Similarly, live *Amblyseius* spp. were found on sprayed leaves on all post-treatment assessment dates. Mummified aphids were collected after both sprays and incubated in Petri-dishes in the laboratory. Adult *Aphidius* spp. successfully hatched from over 70% of the mummies and subsequently lived for at least 48 hours.

Figure 1. Mean numbers of leafhoppers per sample point, broken down to provide an indication of the life cycle stages present, for each treatment on the first three assessment dates in Trial 1



TRIAL 2

Materials and methods

Indoxacarb (Steward) was applied to the whole crop of peppers (cv Fiesta) in Block D (total area of 5,400m^2) on 8 August 2008 using an Enbar ULV applicator. The quantity of product applied was equivalent to 133gm in 11.1 litres water per hectare.

There were eight sample points in each of three plant rows; *i.e.* 24 sample points in total. At each sample point, 20 leaves were selected which were evenly distributed from the top down to 0.4m above the base of the plant. Each leaf was examined in

situ and the number of leafhopper adults, small nymphs, medium nymphs and large nymphs were recorded separately. An initial assessment was done prior to application of the treatment. Further assessments were done 5 and 12 days post-treatment.

The effect of indoxacarb was determined by comparing the size of the leafhopper population before and after each treatment.

Results and discussion

The mean numbers of leafhoppers recorded on each assessment date, together with the percentage change over time, are shown in Table 2. Five days after the single application of indoxacarb by Enbar, numbers of leafhoppers were reduced by 58%. The numbers were similar after a further 7 days.

Table 2. The mean numbers of leafhoppers (all life cycle stages) recorded on each assessment date in Trial 2

Treatment	Mean numbers of leafhoppers (all life cycle stages combined) per sample point on the following assessment dates: [percentage relative to original assessment]		
	2 August	13 August	20 August
Indoxacarb by Enbar	31.8 [100%]	13.3 [42%]	13.6 [43%]

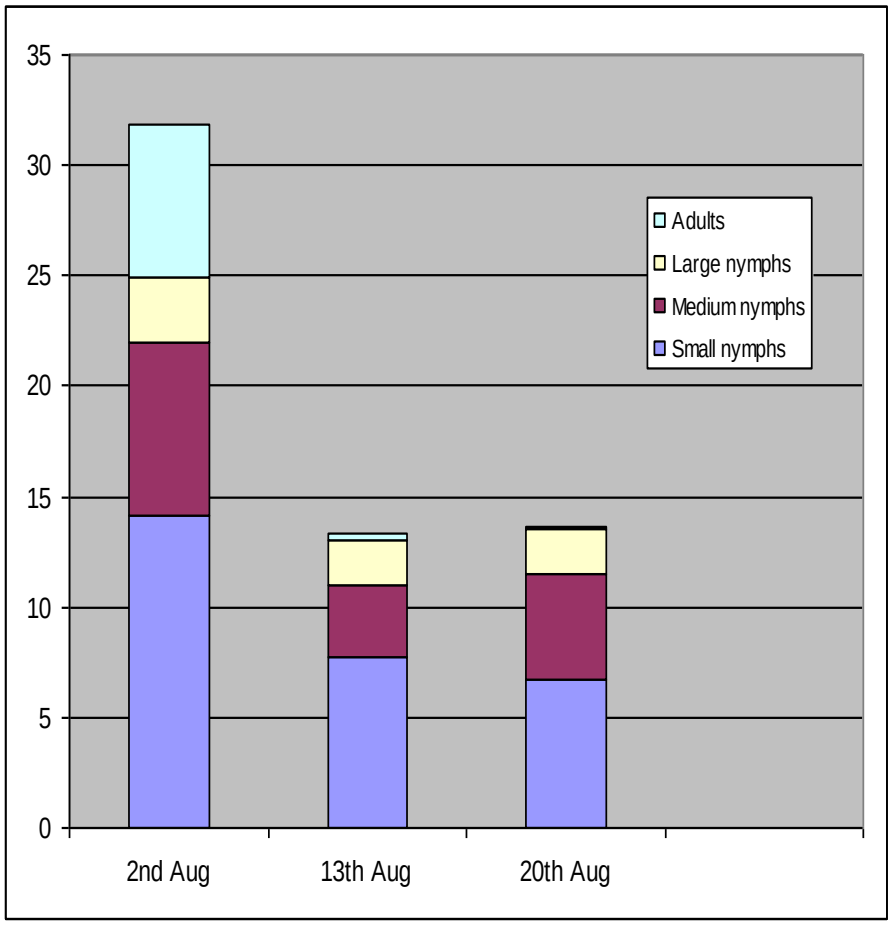
The mean numbers of leafhoppers per sample point, broken down to provide an indication of the life cycle stages present, are shown for each assessment date in Figure 2. By comparing the first post-treatment assessment with the pre-treatment assessment, it can be seen that the Enbar treatment achieved 95% reduction in numbers of adult leafhoppers. This was broadly consistent with the results achieved by the HV spray application in Trial 1. However, over the same period, there was only a 50% reduction in the numbers of leafhopper nymphs, which could only be partly explained by post-treatment egg hatch. It has been hypothesised that the adults,

which are very active and often in flight, are more likely to pick up a lethal dose of the ULV pesticide than the nymphs, which remain on the undersides of leaves.

Given the poorer results achieved by the Enbar ULV applicator and the possibility of encouraging resistance by exposing a proportion of the leafhopper population to sub-lethal doses, it was decided not to pursue this method of application.

Orius spp. were numerous on the plants post-treatment. Interestingly, one *Orius* spp. nymph was observed attacking and feeding upon a small leafhopper nymph.

Figure 2. Mean numbers of leafhoppers per sample point, broken down to provide an indication of the life cycle stages present, on each assessment date in Trial 2



TRIAL 3

Materials and methods

Indoxacarb (Steward) was applied to the whole crop of peppers (cv Kelly) in Block B (total area of 5,000m^2) on 28 August 2008 in a tank mix with pymetrozine (Chess). The latter was incorporated to control an infestation of aphids in the same crop. The single spray was applied as described for Trial 1 and the total volume was equivalent to 2,600 litres per hectare.

Sampling was done as described for Trial 2. An initial assessment was done prior to application of the treatment. A further assessment was done 9 days post-treatment.

The effect of indoxacarb was determined by comparing the size of the leafhopper population before and after each treatment.

Results and discussion

The mean numbers of leafhoppers recorded on each assessment date, together with the percentage change over time, are shown in Table 3. Nine days after the single HV application of indoxacarb, numbers of leafhoppers had been reduced by 97%. The differences between treatments were so clear that no further analysis of the data was deemed necessary.

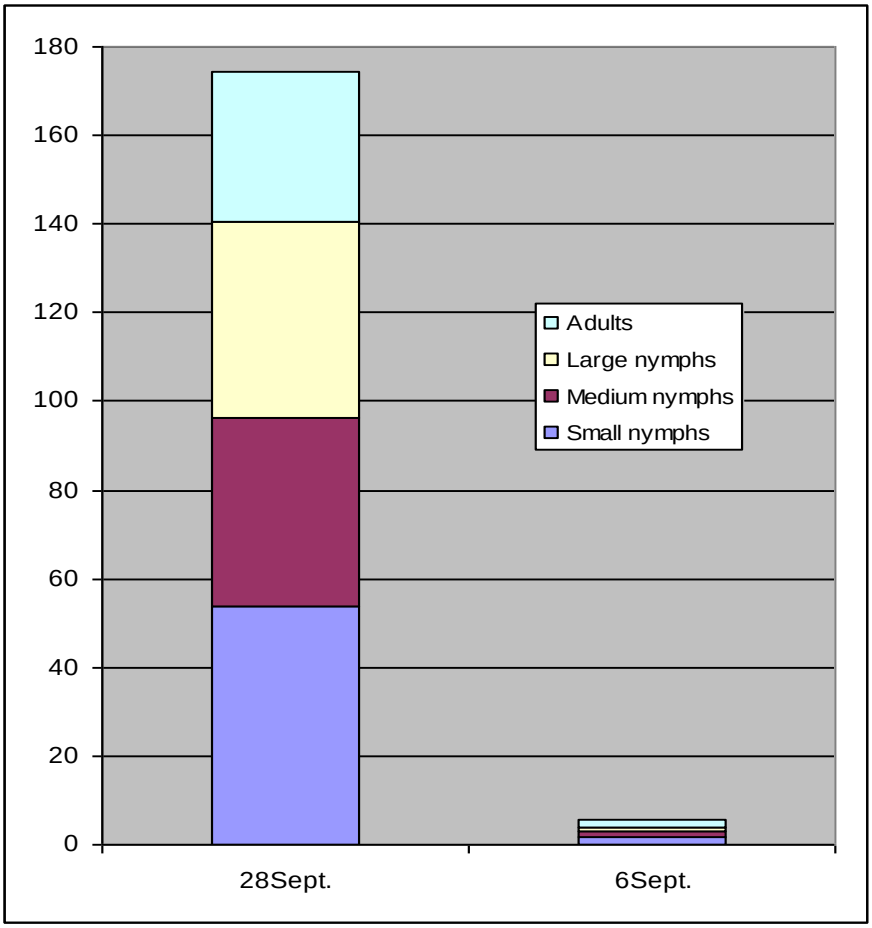
Table 3. The mean numbers of leafhoppers (all life cycle stages) recorded on each assessment date in Trial 3

Treatment	Mean numbers of leafhoppers (all life cycle stages combined) per sample point on the following assessment dates: [percentage relative to original assessment]	
	28 August	6 September
Indoxacarb with pymetrozine as HV spray	174.3 [100%]	5.5 [3%]

The mean numbers of leafhoppers per sample point, broken down to provide an indication of the life cycle stages present, are shown for each assessment date in Figure 3. In this case, the populations before and after treatment were comprised of similar proportions of individuals of each life cycle stage.

Amblyseius spp. mites were numerous in parts of this crop at the post-treatment assessment.

Figure 3. Mean numbers of leafhoppers per sample point, broken down to provide an indication of the life cycle stages present, on each assessment date in Trial 3



TRIAL 4

Materials and methods

Indoxacarb (Steward) was applied to the whole crop of peppers (cv Ferrari) in "New" Block (total area of 16,000m^2) on 4 September 2008. The single spray was applied as described for Trial 1 and the total volume was equivalent to 2,500 litres per hectare.

Sampling was done as described for Trial 2. An initial assessment was done prior to application of the treatment. Further assessments were done 2 and 12 days post-treatment. The effect of indoxacarb was determined by comparing the size of the leafhopper population before and after each treatment.

Results and discussion

The mean numbers of leafhoppers recorded on each assessment date, together with the percentage change over time, are shown in Table 4. Two days after the single HV application of indoxacarb, numbers of leafhoppers had been reduced by 93%. This was the shortest interval between the spray application and the post-treatment assessment in any of the four trials and shows that the product acts quickly. Twelve days after application of indoxacarb, numbers of leafhoppers had been reduced by 99%, which was broadly comparable with results in Trials 1 and 3. As with previous trials, the differences between treatments were so clear that no further analysis of the data was deemed necessary.

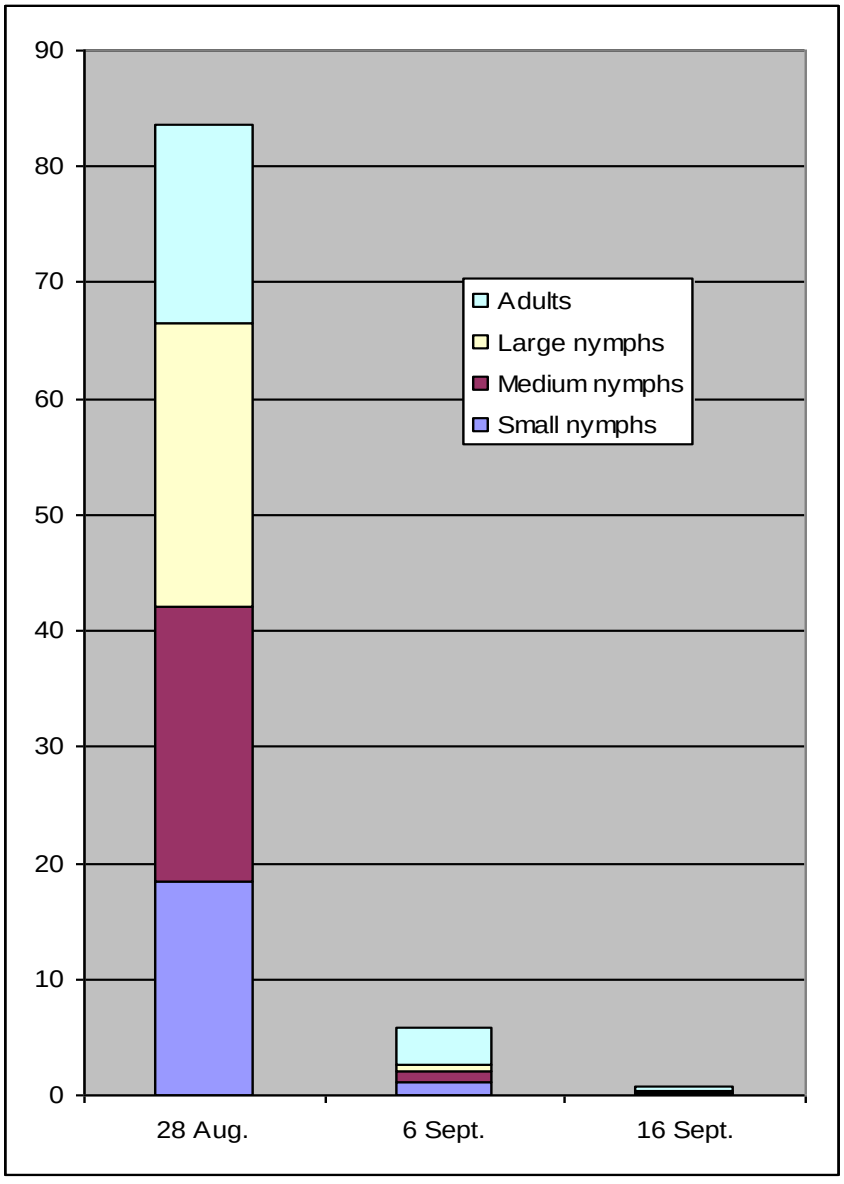
Table 4. The mean numbers of leafhoppers (all life cycle stages) recorded on each assessment date in Trial 4

Treatment	Mean numbers of leafhoppers (all life cycle stages combined) per sample point on the following assessment dates: [percentage relative to original assessment]		
	28 August	6 September	16 September
Indoxacarb HV spray	83.7 [100%]	5.8 [7%]	0.7 [1%]

The mean numbers of leafhoppers per sample point, broken down to provide an indication of the life cycle stages present, are shown for each assessment date in Figure 4.

Both *Orius* spp. and *Amblyseius* spp. were readily found at the post-treatment assessment.

Figure 4. Mean numbers of leafhoppers per sample point, broken down to provide an indication of the life cycle stages present, on each assessment date in Trial 4



SECTION 4. COMPATIBILITY OF INDOXACARB WITH BIOCONTROL AGENTS

Indoxacarb has a broad spectrum of activity against insects but is said to be selective due to its mode of action. McKinley *et al* (2002) stated that dried residues of indoxacarb (as Avaunt) did not significantly affect a wide range of beneficial arthropods and they cited as examples several species of predatory bugs (including *Orius* spp.), lacewing larvae, spiders, predaceous mites and parasitic wasps. They claimed this was a consequence of very limited ingestion due to the feeding habits of these insects and due to the lack of uptake via tarsal (*i.e.* 'feet') exposure.

Dinter and Wiles (2000) state that "indoxacarb preserves in-field predatory mite and beneficial insect populations which makes it an ideal choice in IPM programmes". They provide evidence to show that indoxacarb is safe to many predatory insects, including *Orius laevigatus*, and to predatory mites, including *Typhlodromus pyri*. However, inspection of that package of data suggests that aphid parasitoid populations may be partially affected for a short time following application of the insecticide. Dinter and Wiles specifically state that *Aphidius colemani* was found to be sensitive to indoxacarb under worst case scenarios (*i.e.* as a direct spray).

In a presentation posted on his web site, Sherrod (1999) provided a simple summary of the impact of indoxacarb on a wide range of beneficials. Of particular interest to UK pepper growers are:

Beneficial arthropods:		Impact of indoxacarb:
Parasites	*Aphidius colemani*	Moderate
	Aphidius rhapalosiphi	Slight
	Aphelinus mali	Slight
Predators	Predaceous mites	Slight
	Predaceous Diptera	Slight
	Orius spp.	Slight

There is some conflicting information provided from the biocontrol suppliers. The Biobest website (2008) states that indoxacarb is "non-toxic" to *Amblyseius cucumeris* nymphs and adults, *Phytoseiulus persimilis* nymphs and adults, *Orius laevigatus*

nymphs and adults, *Orius insidiosus* nymphs and adults, and *Aphidius* spp. adults and larvae.

In contrast, Koppert rank the effects of indoxacarb as very harmful to *Amblyseius cucumeris* adults and *Aphidius colemani* adults, and moderately harmful to *Orius laevigatus* adults and *Amblyseius swirskii* adults. Apparently, the ranking for *Orius laevigatus* was upgraded in 2008 because Koppert discovered that indoxacarb could stop ovipositon and nymphal development (R. Knight, Koppert, pers.com., 2008). Koppert rank indoxacarb as harmless to *Phytoseiulus persimilis* adults, *Feltiella acarisuga* adults / larvae, and *Episyrhus balteatus* larvae.

The author's personal experience following applications of indoxacarb (as Steward) in commercial pepper crops which were already using IPM techniques was that predaceous mites and *Orius* spp. could still be found on the sprayed plants after treatment. However, it was not known what proportion of the pre-treatment population this represented. In addition, adult *Aphidius* spp. were successfully hatched from over 70% of the mummified aphids collected from crops after treatment.

On balance, it would seem that the use of indoxacarb should be compatible with the predatory insects and mites used in the IPM programme in pepper crops. However, further clarification is required about the impact of the chemical on populations of *Orius* spp. following the information released by Koppert in 2008. The situation is less clear with parasitic wasps. It would appear that direct contact with the spray is harmful to adult wasps but larvae are reasonably well protected within the mummified aphids.

It is interesting to note that UK pepper growers who have used indoxacarb against caterpillars and leafhoppers during 2008 and 2009 have not reported any subsequent surge in non-target pest populations as had previously been observed following application of other broad spectrum insecticides such as thiacloprid (Calypso).

SECTION 5. EFFICACY OF NATURAL PYRETHRINS AGAINST LEAFHOPPERS

Materials and methods

The components and properties of natural pyrethrins are discussed in section 6.

Natural pyrethrins (Pyrethrum 5EC) were applied to approximately 400m^2 of sweet pepper (cv Boogie) at Barton Grange Nursery on 16 September 2009. The treatments were applied from a tank and pump situated on the central roadway using a retractable hose and hand lance fitted with a multi-nozzle head. The product was diluted at the rate of 20ml per 5 litres of water and applied high volume to the point of run off ensuring good cover to the undersides of the leaves throughout the canopy. The volume applied was equivalent to approximately 2,800 litres per hectare. An adjacent crop area of similar size was left unsprayed as an untreated control.

Assessments were done in the central three rows in both the untreated and treated plots. There were eight sample points per row (ie 24 per plot). At each sample point, twenty leaves were selected randomly from the middle stratum of the canopy. Each leaf was examined in situ and the numbers of leafhopper adults, small nymphs, medium nymphs and large nymphs were recoded separately. The first assessment was done pre-treatment on 10 September. The second and third assessments were done 1 day and 12 days respectively after application of Pyrethrum 5EC.

The effect of the Pyrethrum 5EC treatment was determined by comparing the size of the leafhopper population before and after the spray application, and by comparison to the untreated control.

Results and discussion

The mean numbers of leafhoppers recorded per sample point in each treatment on each assessment date are shown in Table 5 and Figure 5. Overall numbers were similar in the treated and untreated plots prior to application of Pyrethrum 5EC. In the Pyrethrum 5 EC plot, numbers dropped by over 99% during the first day after the spray application. Meanwhile, numbers in the untreated control rose slightly.

There was a clear and rapid effect from the Pyrethrum 5EC treatment. No nymphs were recorded at the first post-treatment assessment. The few adults that were found on that day were around the periphery of the plot and had probably moved in from the adjacent unsprayed plants.

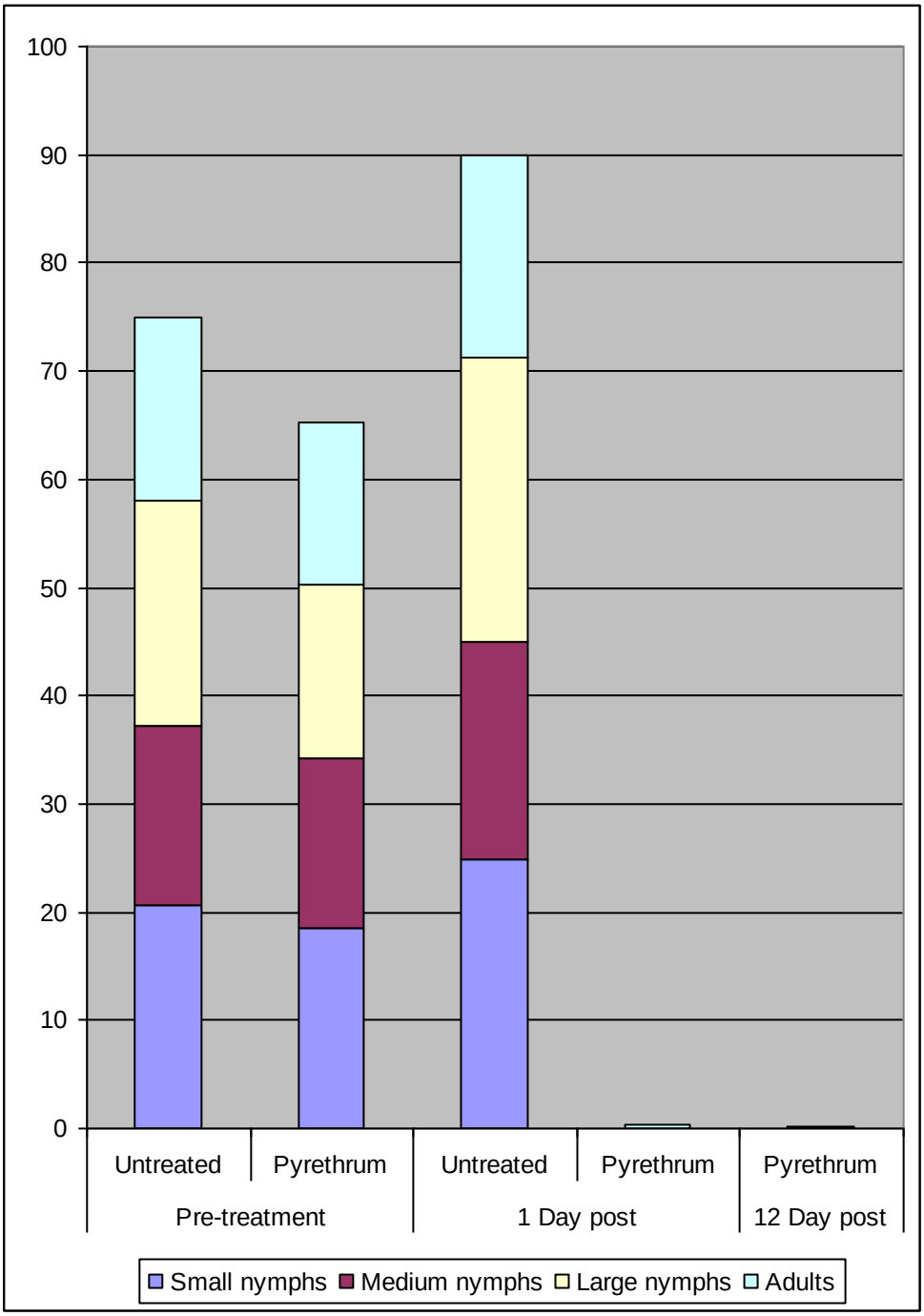
Natural pyrethrins are known to have very short persistence and it was considered unlikely that there would be any residual effect against nymphs which hatched from any eggs that survived the treatment. The second post-treatment assessment was done to determine how quickly the population might recover from the effect of the spray application. That assessment focussed on nymphs and was timed so that the first to hatch would not yet have become adults. On that basis, any adults that were recorded could be considered to have reinvaded the plants rather than survived the treatment. It may be seen from the results that very few nymphs were found 12 days post-treatment.

The impact of natural pyrethrins on biological control agents is discussed in Section 8 of this report.

Table 5. Mean numbers of leafhoppers recorded per sample point in each treatment on each assessment date

Assessment	**Treatment**	**Number of leafhoppers per sample point**			
		Small nymphs	**Medium nymphs**	**Large nymphs**	**Adults**
Pre-treatment	Control	20.6	16.7	20.8	16.9
	Pyrethrum	18.5	15.8	16.0	14.9
Post-treatment 1 day	Control	24.8	20.2	26.2	18.7
	Pyrethrum	0.0		0.0	0.3
Post-treatment 12 days	Control	No assessment			
	Pyrethrum	0.06	0.03	0.0	0.1

Figure 5. The mean numbers of leafhoppers recorded per sample point, broken down to provide an indication of the life cycle stages present, on each assessment date



SECTION 6. BACKGROUND TO APHID STUDIES

One of the specific objectives of this project was to investigate methods of integrating the use of natural pyrethrins with natural enemies to combat aphids on organic pepper crops. Pyrethrins are natural insecticides, which are extracted from the dried flowers of African chrysanthemum (*Chrysanthemum cinerariaefolium*). The extracts, which are collectively known as pyrethrum, consist of a mixture of several active ingredients; *i.e.* three esters of chrysanthemic acid (Pyrethrins I) and three corresponding esters of pyrethrin acid (Pyrethrins II). Pyrethrins II cause rapid knockdown of insects and this is followed by death associated with Pyrethrin I.

The commercial product, Pyrethrum 5EC, contains natural pyrethrins and the synergist piperonyl butoxide (PBO). The latter is also of natural origin, being prepared from oil of sassafras. PBO inhibits a class of enzymes known as mixed function oxidases, which contribute to the process by which insects detoxify pesticides, and it thus enhances the activity of the natural pyrethrins. The risk of insects developing resistance to Pyrethrum 5EC is low because the product contains more than one active ingredient and because PBO inhibits one of the principle mechanisms by which insects detoxify insecticides. Nonetheless, there had been a recent report of Pyrethrum 5EC failing to control a population of *Myzus persicae* in a crop of organic peppers in the south west of England and this had to be investigated before this work could begin.

Pyrethrum acts as both a contact and stomach poison. There is no vapour action, systemic activity or leaf penetration, so kill is largely dependent on direct contact. It has very short persistence and breaks down quickly under natural conditions. Previous tests have failed to detect any residues in samples of tomatoes collected 16 hours after application (Morley pers. com., 2006). Pyrethrum has a broad range of insecticidal activity and appears to be particularly effective against aphids, caterpillars, plant bugs and certain beetles. Previous HDC funded trials have also shown Pyrethrum 5EC to be effective against *Macrolophus caliginosus*, some life cycle stages of mealybugs and spider mites although this does not appear on the product label (Jacobson & Morley, 2006 & 2007).

There is little doubt that the broad spectrum activity of pyrethrum makes it potentially harmful to natural enemies. However, the short persistence of the product is a

tremendous advantage because this allows us to separate the control measure from natural enemies and biocontrol agents in time and / or space. For example, adult parasitic wasps (*eg Diglyphus, Dacnusa, Encarsia, Aphidius*) are vulnerable but the immature stages should be protected within the plant or their hosts. This has been demonstrated in previous HDC funded work in organic tomato crops (Jacobson & Morley, 2007).

We hypothesised that pyrethrum could be used as a secondary control measure to support the primary biological control agents. This strategy would require appropriate species of parasitic wasps to be released into the crop as early as possible and then pyrethrum used as a second line of defence to redress the balance between the pest and beneficial populations if the pest damage approached the economic damage threshold.

An opportunity arose to test this hypothesis in a large commercial crop of organic peppers in October 2008. Parasitoids had been released in the earlier stages of the crop and had become well established within the resident population of peach potato aphid, *Myzus persicae*. However, there had been a late season increase in aphid numbers and the pest was now causing significant damage to foliage and fruit (*eg.* Figure 6).

Figure 6. Example of aphid damage to plants in trial area on 14 October 2008

SECTION 7. PROOF OF CONCEPT TRIAL

The Objectives

To evaluate:

- the efficacy of Pyrethrum 5EC against *Myzus persicae*
- the compatibility of Pyrethrum 5EC with aphid parasitoids
- the combined effect of Pyrethrum 5EC and parasitoids in controlling a serious aphid infestation

Materials and methods

The pests and resistance tests

The infestation comprised both red and green forms of *M. persicae*, which were usually found co-existing on the same leaves (Figure 7). Given the recent report of control failure with Pyrethrum 5EC, samples of both colour forms were collected from the crop and sent to Rothamsted Research for formal resistance tests. In addition, the method of applying the pesticide to the crop was critically analysed.

Figure 7. Red and green forms of *Myzus persicae* co-existing on a pepper leaf

Although aphid specialists at Rothamsted Research consider the two colour forms to be the same species, they were cultured and tested separately using the following two techniques:

1. Conventional topical application bioassays using technical pyrethrins from Botanical Resources, Australia.
2. Total esterase assays using 1-naphthyl acetate and electrophoresis followed by esterase staining.

The parasitoids

Aphidius ervi and *A. colemani* had been released under the guidance of Mr R Knight (Koppert UK) throughout the season. 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 Pyrethrum 5EC. In addition to *Aphidius* spp., a natural population of *Praon* spp. had become established within the *M. persicae* population.

Treatments

Pyrethrum 5EC was applied at the rate of 1 litre per 600 litres water on 14 October 2008 using a hand held lance attached by a retractable hose to a tank and pump on the pathway at end of the crop rows. The intention was to apply the spray to the point of run off to the top 0.6m of the plants. There was no untreated control area because the damage was already unacceptable and the plants would have been destroyed unless the aphids were brought under control.

Assessments

In the absence of an untreated control, the effect of the treatment was assessed by comparing numbers of pests and parasitoids before and after treatment. A pre-treatment assessment was completed on 14 October 2008. The average number of healthy *M. persicae* per leaf was determined by counting the number of aphids on 100 leaves taken at random from the upper and middle strata of the crop. In addition, numbers of mummified aphids were recorded on the same leaves. No attempt was made to assess parasitised aphids that were not yet showing as mummies as this would have required dissection.

A post-treatment count of live aphids on 100 leaves taken at random from the upper and middle strata of the crop was done on 16 October 2008 to determine the efficacy of the product against the pest.

Samples of both *Aphidius* spp. and *Praon* spp. (as mummified aphids) were collected from sprayed leaves on 16 October 2008. They were placed in ventilated Petri dishes and incubated at 21-23^{o}C (Figure 8) to determine the proportion that had survived the Pyrethrum 5EC treatment.

Figure 8. Example of dishes used in parasite survival / emergence test

A further post-treatment assessment was completed on the 14 November 2008. On this occasion, numbers of healthy and mummified aphids were counted on 100 leaves taken at random from the upper and middle strata of the crop. This was done to determine the change in the proportion of aphids that were parasitised before and after the application of Pyrethrum 5EC.

Results and discussion

Resistance tests

The full results as supplied by Dr Graham Moores of Rothamsted Research are provided in Appendix 1. In summary:

Conventional topical application bioassays using technical pyrethrins from Botanical Resources Australia produced dose-response curves with a resultant LD_{50} of 77 ppm for the green population and 58 ppm for the red population. Both of these figures are typical of a susceptible population.

Total esterase assays using 1-naphthyl acetate and electrophoresis followed by esterase staining reinforced the finding that both populations were insecticide susceptible.

Parasitoid survival

There were 117 mummies of *Aphidius* spp and 97 mummies of *Praon* spp. The dishes were examined daily and the final count was done on 2 November (ie 17 days after collection). Adult wasps were seen emerging from day 2. The final count was 93 live *Aphidius* spp. adults (ie 79.5% emergence) and 66 live *Praon* spp. adults (ie 71.0% emergence).

Aphid and parasitoid numbers in the crop

As may be expected with a natural infestation, the numbers of aphids pre-treatment were variable but they averaged 110 healthy individuals per leaf. There were an average of 6.5 mummified aphids per leaf with approximately equal numbers of *Aphidius* spp. and *Praon* spp. This represented about 5% of the aphid population. Adult parasitoids were obvious on the wing within the crop canopy but it was impossible to quantify the numbers present. Given the level of damage already occurring to the crop, it is highly unlikely that this population of parasitoids would have prevented the crop from being destroyed within a few weeks.

Following treatment, the level of kill of aphids was quite variable. There was over 95% reduction in numbers on the sprayed foliage adjacent to the path (*i.e.* 5-10 per

leaf remaining). However, numbers had only been reduced by 40% on leaves within the middle of the canopy (*i.e.* an average of about 50 per leaf remaining) and by even less at the very top of the central heads. This pattern of survival suggested poor spray contact rather than resistance to the product and this was consistent with the results of the formal resistance tests. By comparison to pre-treatment, there were very few adult parasitoids on the wing which indicated that they had been killed by the spray. The latter had been expected.

The assessment on the 14 November showed a quite remarkable shift in the balance of aphids and parasitoids. On average, there were now less than one live aphid per leaf. 95-98% of individuals on leaves were now mummified with approximately 60% of those being *Aphidius* spp. and the remainder being *Praon* spp. (Figure 8). Furthermore, there were very large numbers of adult parasitoids flying within the crop canopy. New growth was completely "clean" (Figure 9) and newly developing fruit were no longer being contaminated by honey dew and sooty mould.

Figure 8. Level of parasitism on 14 November 2008

Figure 9. Plants growing away from damage

Overall conclusions

- The proof of concept trial clearly demonstrated that Pyrethrum 5EC could be successfully used as a secondary control measure to support the primary biological control agents.
- Both red and green forms of this *M. persicae* population were susceptible to Pyrethrum 5EC. However, the overall effect of the treatment was limited by poor spray penetration into the dense crop canopy of the wide organic beds.
- While harmful to adult parasitoids, Pyrethrum 5EC did not appear to harm immature parasitoids within the mummified aphids.
- Further information is required about the impact on other biological control agents of Pyrethrum 5EC when applied to part of the crop.
- This trial showed that an extremely large and damaging population of *M. persicae* could be controlled effectively and quickly with a combination of parasitoids and Pyrethrum 5EC. However, further work is required to determine the optimum time to apply the spray treatments so that crop losses are avoided.

SECTION 8. CONTROL OF MYZUS PERSICAE WITH A COMBINATION OF PARASITOIDS AND A 'SOFT' CHEMICAL

Objectives

- To consolidate findings from 2008, which had shown that a 'soft' chemical could be integrated with parasitoids to control an advanced aphid infestation.
- To time the application of such a treatment so that aphids could be controlled before causing economic damage.

Introduction

Aphidius colemani, *Aphidius ervi* and *Aphelinus abdominalis* parasitoids had been released in the crop from early season under the guidance of Mr R Knight (Koppert UK) and were established within the population of *Myzus persicae.* In addition, small numbers of *Praon* spp. had become established from the natural population. It was estimated that the parasitoids were present in the following proportions; 90% *Aphidius*, 2% *Aphelinus* and 8% *Praon*. *Aphidoletes aphidimyza* had also been released in the crop and small numbers were recorded in the preliminary assessment.

The aphids were just beginning to cause sticky patches on leaves and fruit in localised areas (Figure 10), which was an indication that the infestation was approaching the economic damage threshold. This was considered to be the optimum time to apply a second line of defence treatment.

Orius spp were well established throughout the trial area and were probably helping to suppress the aphid population growth. They were included in the assessments in order to determine any negative side effects from the second line of defence treatments. *Orius* spp. were occasionally seen feeding on *A. aphidimyza* larvae indicating that these two biocontrols are not compatible.

There was a trace of thrips damage on the pepper leaves and very small numbers of adult thrips were seen during the preliminary assessments. A major concern was that the second line of defence treatments would affect the control of thrips and lead to damaging infestations of this pest later in the season. To cover this possibility,

Savona (fatty acids) was included in the trial because it was expected to be less harmful to *Orius* spp. than pyrethrum.

Figure 10. Approaching economic damage in week 26, 2009. Note early signs of stickiness on this fruit and some cast aphid 'skins'

Materials and methods

The trial was done in three 0.1ha bays of a 3ha organic pepper crop at Bradon Farm. Each bay consisted of four rows of 130m length.

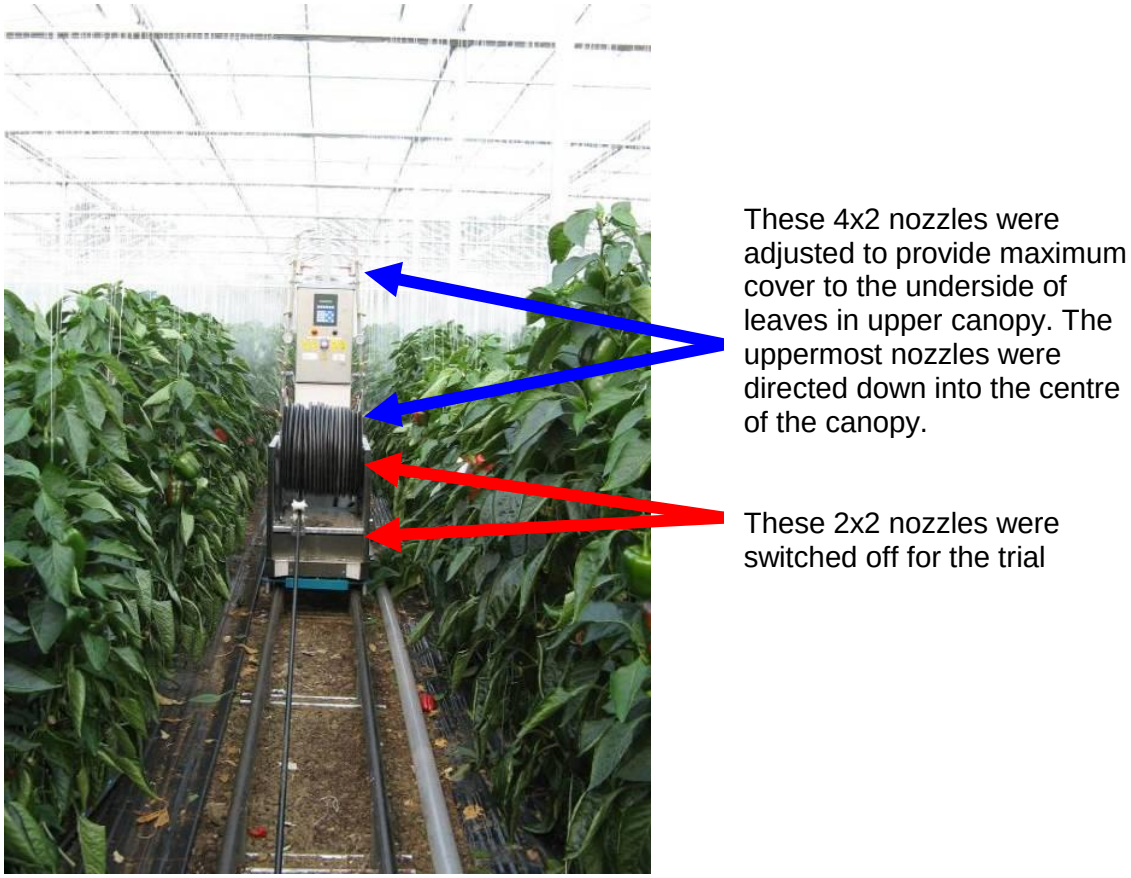
There were three treatments, each applied to all the plants in one bay:

1. Bay 8 – Pyrethrum 5EC (diluted 266ml per 100 litres or 1.6 litres per ha) applied at 80 litres of diluted spray per row (equivalent to 3,200 litres per ha).
2. Bay 9 – 2% Savona applied at the same volume.
3. Bay 10 – No treatment

The treatments were applied with a robotic sprayer (Figure 11). After preliminary trials, it was decided to run the machine at 25% of maximum speed with the lower four nozzles switched off. The upper eight nozzles were adjusted to give maximum cover to the underside of the leaves in the top half of the plant. This was difficult to achieve in the dense canopy and the underside of some leaves remained untreated

while spray ran off the upper surfaces of many others. The sprays were applied between 17.00 and 20.00 hrs on 24 June 2009.

Figure 11. Sprayer in action – note density of crop canopy which caused difficulty in achieving good cover to underside of leaves



The assessments were concentrated in one of the central rows of each bay. There were 15 assessment points distributed evenly along the row. As the intention was to spray only the top half of the plants, separate assessments were done in the upper and lower strata of the crop canopy. For each assessment point at each level, the total numbers of apparently healthy aphids, mummified aphids, *Orius* spp. and *A. aphidimyza* were counted on three randomly chosen leaves. The first assessment was done just before the sprays were applied on the 24 June and the first post-treatment assessments were done the following morning (*i.e.* 16-18 hours post-treatment). The subsequent post-treatment assessments were done 7, 12 and 22 days after the spray applications.

Aphids were collected from the crop and sent to Rothamsted Research to be tested for resistance to natural pyrethrins (as described in Section 7).

Statistical methods

Although there is no true replication in this trial, the analysis is based on the sampling variation within the three trial areas. Thus the major tool of analysis is that of analysis of variance in which the sampling variation within each treatment area is combined and used as a test for differences between means of the Pyrethrum 5EC, Savona and control samples. A simple t-test was done for each species × position count on the four post-treatment assessments as well as an analysis of the three 'treatments' at the pre-treatment stage. An extra analysis was also done of the Pyrethrum 5EC versus Savona profile over time, treating time as a factor.

Results and discussion

The mean numbers of aphids, mummified aphids and *Orius* spp. per sample point in the upper and lower crop strata of each treatment on each assessment date are shown in Table 6. The table also includes the percentage change from pre-treatment on each assessment date. To aid interpretation of the aphid results, the data in Table 6 are presented graphically for the upper and lower strata of the crop canopy in Figures 13 and 14 respectively. Significant differences between treatments are marked on these charts.

The weather during the week immediately following application of the treatments was hot and dry. Aphid numbers rose very rapidly in the untreated control plot and there was a marked increase in stickiness on leaves and fruit. This is clearly illustrated by comparing the images in Figure 10, taken at the threshold for application of treatments, and Figure 12, which was taken one week later. The damage had already become unacceptable in the untreated control plot and a corrective spray of Pyrethrum 5EC was applied to the upper half of the crop canopy. This confirmed that the applications in the trial area had been timed accurately. It also showed that both treatments had successfully suppressed the development of the aphid damage during that week. Thereafter, comparisons were drawn between the efficacy of the Savona and Pyrethrum 5EC treatments.

Figure 12. Example of fruit in the untreated plot 7 days into the trial illustrating why supplementary action was required

Table 6. Mean numbers of aphids, mummified aphids and *Orius* spp. per sample point and percentage change on each assessment date

Assessment days post-treatment	Mean number (% change from original count)						
	Upper plant				Lower plant		
	Aphid	Mummy	Orius (leaf)	Orius (flower)	Aphid	Mummy	Orius (leaf)
Pyrethrum 5EC Pre-treatment	26.7	1.6	1.8	1.6	16.7	1.9	2.4
Pyrethrum 5EC 1d post-treatment	0.4 (-98%)	1.3	0 (-100%)	0 (-100%)	12.9 (-23%)	1.1	1.3 (-46%)
Pyrethrum 5EC 7d post-treatment	2.9 (-91%)	2.2	0 (-100%)	0.1 (-94%)	15.2 (-9%)	2.5	0.7 (-70%)
Pyrethrum 5EC 12d post-treatment	22.6 (-15%)	1.9	0 (-100%)	0.2 (-88%)	24.3 (+45%)	3.1	0.6 (-75%)
Pyrethrum 5EC 22d post-treatment	42.8 (+60%)	8.1	0.1 (-96%)	0.2 (-88%)	7.2 (-57%)	4.8	0.4 (-84%)
Savona Pre-treatment	28.3	1.2	0.3	1.7	28.0	1.3	1.1
Savona 1d post-treatment	12.0 (-58%)	1.0	0.1 (-67%)	0.5 (-71%)	30.0 (+7%)	1.0	0.9 (-18%)
Savona 7d post-treatment	15.6 (-45%)	2.5	0.2 (-33%)	0.7 (-59%)	21.5 (-23%)	3.0	0.5 (-55%)
Savona 12d post-treatment	8.1 (-71%)	1.8	0.1 (-67%)	0.9 (-47%)	12.4 (-55%)	3.1	0.7 (-36%)
Savona 22d post-treatment	1.7 (-94%)	3.2	0.7 (+230%)	0.6 (-65%)	1.7 (-94%)	3.9	0.7 (-36%)
Untreated Pre-treatment	15.0	0.8	0.4	1.1	18.3	1.0	1.1

Figure 13. Mean numbers of aphids per sample point in the upper crop stratum of each treatment on each assessment date

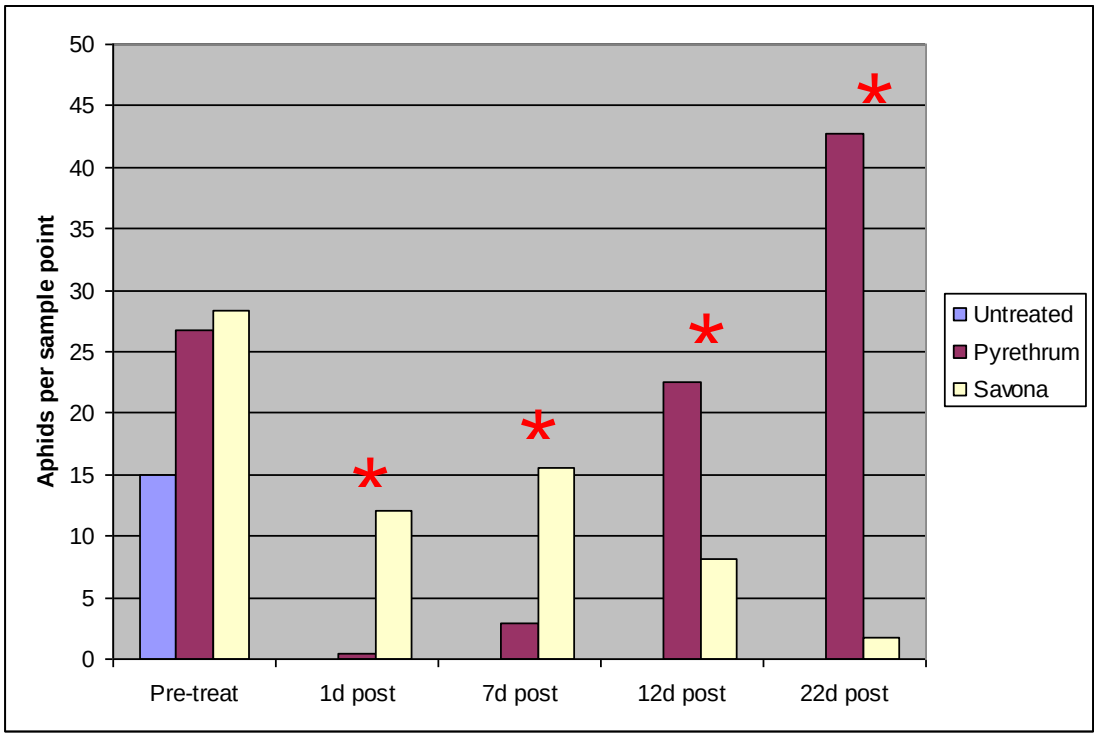
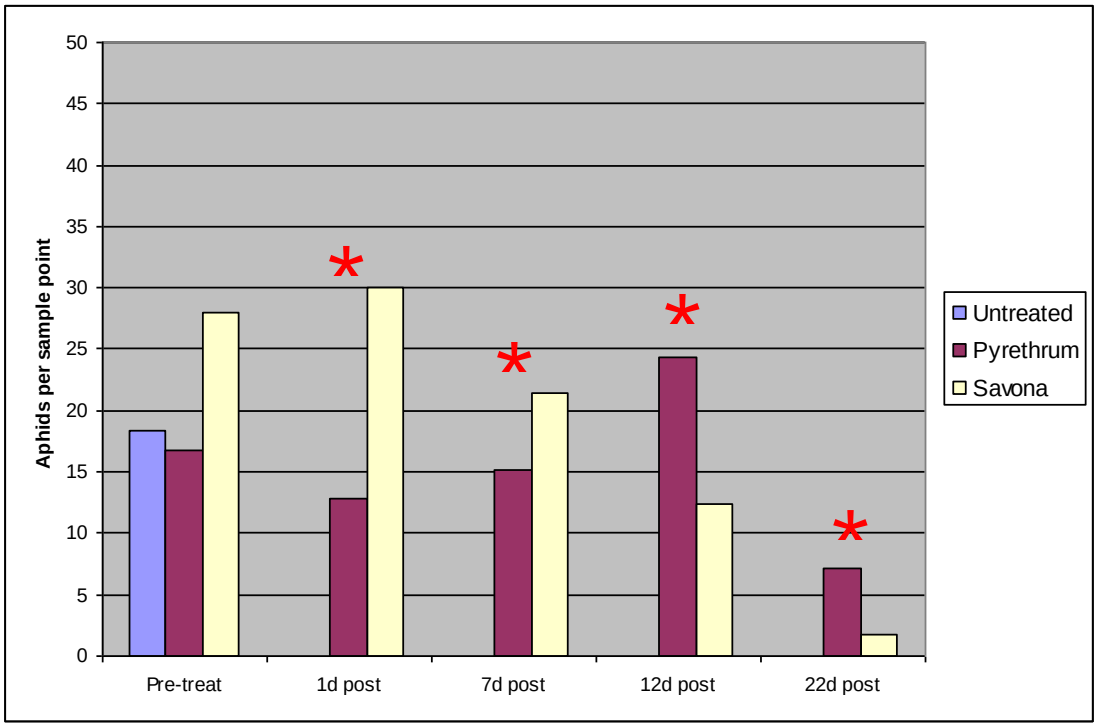


Figure 14. Mean numbers of aphids per sample point in the lower crop stratum of each treatment on each assessment date



* Indicates significant difference (*P<0.001*) between treatments on that date

Pyrethrum 5EC

The full resistance test results as supplied by Dr Graham Moores of Rothamsted Research are provided in Appendix 2. In summary: Conventional topical application bioassays using technical pyrethrins from Botanical Resources Australia produced a dose-response curve with a resultant LD_{50} of 77 ppm for this population. This is typical of a susceptible population. Furthermore, total esterase assays using 1-naphthyl acetate reinforced this finding. Total esterase levels were slightly higher than a laboratory susceptible clone, but lower than the R2 standard clone. The slight increase in esterase was probably due to the esterase activity of parasitoid contaminants, but even if this were not the case the levels were not high enough to confer resistance.

The day after treatment, aphid numbers were reduced by 98% in the top of the plant and by 23% in the unsprayed lower canopy. The reduction on the lower leaves was probably due to partial run-off from upper leaves. The numbers remained similar throughout the crop canopy during the following week but thereafter began to increase. At 22 days post-treatment, aphid numbers and associated damage in the top of the plant had become unacceptable and further sprays were applied.

Immediately before the original treatment was applied, about 8% of the aphid population was parasitised throughout the whole crop canopy. This had improved to 18% by 7 days post-treatment. At that point, the plants were beginning to grow away from the aphid infestation and we anticipated further improvement comparable to that observed in the proof of concept trial. However, the situation regressed and close examination revealed that the parasitoid population growth was being suppressed by hyperparasitoids in part of the plot. Externally, the aphid mummy gives no indication of the presence of a hyperparasitoid and so the scale of the problem can be underestimated. It is only when the hyperparasitoid emerges that the distinction can be made because the exit hole is quite different to that of an *Aphidius* spp. (Figure 15). At the end of this trial, mummies were collected for emergence tests which showed over 20% to be attacked by hyperparaitoids. The species was believed to be *Dendrocerus* spp., although this is still to be confirmed.

Very few *A. aphidimyza* were detected during the assessments. Numbers of *Orius* spp. declined markedly following the original treatment with Pyrethrum 5EC and numerous dead bodies were found in the tops of the plants at the first post-treatment

assessment. The *Orius* spp.population did not recover during the trial. Although small numbers of thrips were present throughout the crop, the population did not reach damaging levels.

Figure 15. Hyperparasitoid emergence hole

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

Savona

The initial aphid kill was poorer than in the Pyrethrum 5EC treatment with 58% reduction on the upper leaves and no reduction on the lower leaves. The aphid damage became worse during the following week but then aphid numbers decreased and very few remained at the final assessment. Figure 16 shows the plants growing away from the aphid damage at the end of the trial.

Immediately before the original treatment was applied, about 4% of the aphid population was parasitised throughout the whole crop canopy. By 12 days post-treatment, this had increased to 24% parasitism and by the end of the trial there were more mummies than healthy aphids in this plot. This had followed the pattern observed in the proof of concept trial. Hyperparasites were detected in this plot but less than 2% of the aphid population were affected.

In common with the Pyrethrum 5EC plot, very few *A. aphidimyza* were detected during the assessments. Overall, numbers of *Orius* spp. declined by about 50% following treatment but they were recovering towards the end of the trial.

Figure 16. Typical condition of plants in Savona plot 22 days post-treatment

Additional observations 34 days post-treatment

There was a general crash in aphid numbers between 22 and 34 days post-treatment due to a naturally occurring infection by entomopathogenic fungi (predominantly Entomophthorales). This was no doubt assisted by warm humid weather conditions in the intervening period. Other natural enemies, in particular syrphids, were now present and contributing to the control of the pest. It was also interesting to note that the proportion of parasitised aphids that had been attacked by *Praon* spp. had increased from 8% at the start of the trial to about 50% at day 34. This was no doubt due to the impact of hyperparasitoids on *Aphidius* spp.

Overall conclusions

- Spray coverage was less than ideal in the wide bed organic crop.
- An appropriate time to apply the second line of defence treatment was when the aphids were just beginning to cause sticky patches on leaves and fruit in localised areas of the crop.
- Both second line of defence treatments initially suppressed aphid population growth compared to the untreated control plot.
- Parasitoids appeared to be taking control in both plots by day 13. Thereafter, the Savona plot came under complete control by day 22. However, there was a set back in the Pyrethrum 5EC plot due to hyperparasitism which impaired the performance of *Aphidius* spp.
- Numbers of *Orius* spp. declined following both treatments but recovered more rapidly on the plants treated with Savona. As a consequence, plants treated with Pyrethrum 5EC could have been vulnerable to attack by thrips although this did not happen in this trial.
- Following the experience of this season, the potential role of entomopathogenic fungi within the IPM programme should be revisited.

<u>Recommendations for further work:</u>

This project has provided the basis for an IPM programme against aphids in organic pepper crops. However, the following components require further investigation:

- spray coverage in wide-bed organic pepper crops
- hyperparasitism of *Aphidius* spp.
- interaction between *Orius* spp. and *Aphidoletes aphidimyza*

In addition, the following topics should be brought into the research programme:

- alternatve second line of defence treatments:
  - entomopathogenic fungi in organic crops
  - pymetrozine (Chess) through the irrigation system in conventional crops
- review existing knowledge of open rearing systems [also known as banker plants] and explore their potential as a breeding base for novel biological agents

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.

ACKNOWLEDGEMENTS

My thanks go to all who have participated in these studies, in particular the members of the Pepper Technology Group who went to great lengths to make funding available; Gary Taylor and Gill Wardell who were very helpful in finding suitable sites for leafhopper trials (Gill also organised the indoxacarb residue tests); and both Bob Knight and Dr Caroline Reid for sharing crop monitoring information. Last but by no means least, Alan Turner and Alan Richardson for hosting trials in their crops and to their staff who applied the sprays.

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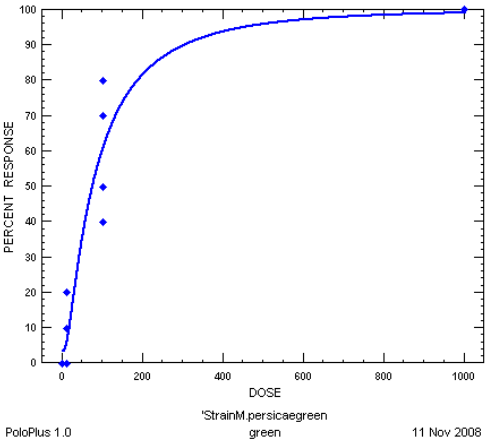
APPENDIX 1

Resistance to pyrethrin:
Results of tests on two *Myzus persicae* clones (green and red)

Autumn 2008

Green population

Bioassay (topical application)



Polo results
'StrainM.persicaegreen
green subjects 180 controls 29
slope=2.125+-0.444 nat.resp.=0.018+-0.018
heterogeneity=0.84

LD10=19.121	95% limits:	5.488 to 34.029
LD50=76.651	**95% limits:**	**47.503 to 110.588**
LD90=307.272	95% limits:	195.260 to 756.836
LD95=455.476	95% limits:	265.895 to 1431.296
LD99=953.072	95% limits:	463.011 to 4847.145

'StrainM.persicaegreen

	parameter	standard error	t ratio
green	-4.005	0.907	-4.416
NATURAL	0.018	0.018	0.995
SLOPE	2.125	0.444	4.785

Variance-Covariance matrix

	green	NATURAL	SLOPE
green	0.822737	-0.856571E-02	-0.395616
NATURAL	-0.856571E-02	0.311360E-03	0.388143E-02
SLOPE	-0.395616	0.388143E-02	0.197308

Chi-squared goodness of fit test

prep	green	dose	n	r	expected	residual	probab	std resid
		0.000	10.	0.	0.18	-0.176	0.018	-0.423
		0.000	10.	0.	0.18	-0.176	0.018	-0.423
		0.000	10.	0.	0.18	-0.176	0.018	-0.423
		0.000	10.	0.	0.18	-0.176	0.018	-0.423
		10.000	10.	0.	0.47	-0.471	0.047	-0.703
		10.000	10.	0.	0.47	-0.471	0.047	-0.703
		10.000	10.	0.	0.47	-0.471	0.047	-0.703
		10.000	10.	1.	0.47	0.529	0.047	0.790
		10.000	10.	2.	0.47	1.529	0.047	2.282
		100.000	10.	4.	6.04	-2.040	0.604	-1.319
		100.000	10.	5.	6.04	-1.040	0.604	-0.673
		100.000	10.	8.	6.04	1.960	0.604	1.267
		100.000	10.	7.	6.04	0.960	0.604	0.621
		100.000	10.	5.	6.04	-1.040	0.604	-0.673
		1000.000	10.	10.	9.91	0.087	0.991	0.297
		1000.000	10.	10.	9.91	0.087	0.991	0.297
		1000.000	10.	10.	9.91	0.087	0.991	0.297
		1000.000	10.	10.	9.91	0.087	0.991	0.297
NATURAL		29.	1.	0.51	0.491	0.018	0.694	

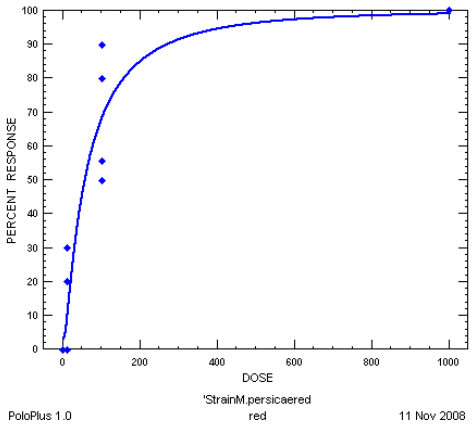
chi-square: 13.501 degrees of freedom: 16 heterogeneity: 0.844

Effective Doses

		dose	limits	0.90	0.95	0.99
LD10	green	19.121	lower	7.395	5.488	2.425
			upper	31.634	34.029	38.707
LD50	green	76.651	lower	52.385	47.503	37.320
			upper	104.117	110.588	125.520
LD90	green	307.272	lower	207.384	195.260	175.015
			upper	613.190	756.836	1335.890
LD95	green	455.476	lower	284.848	265.895	235.300
			upper	1090.096	1431.296	3010.497
LD99	green	953.07	lower	506.29	463.01	396.30
			upper	3273.0	4847.1	14299.

Red population

Bioassay (topical application)



Polo results
'StrainM.persicaered
red subjects 177 controls 30
slope=1.898+-0.293 nat.resp.=0.016+-0.016
heterogeneity=1.12

LD10=12.224	95% limits:	4.331 to 21.775
LD50=57.884	**95% limits:**	**35.794 to 90.472**
LD90=274.093	95% limits:	160.853 to 691.326
LD95=425.938	95% limits:	230.429 to 1315.072
LD99=973.804	95% limits:	441.560 to 4499.735

'StrainM.persicaered

	parameter	standard error	t ratio
red	-3.345	0.556	-6.019
NATURAL	0.016	0.016	1.008
SLOPE	1.898	0.293	6.472

Variance-Covariance matrix

	red	NATURAL	SLOPE
red	0.308805	-0.286283E-02	-0.156154
NATURAL	-0.286283E-02	0.247395E-03	0.123421E-02
SLOPE	-0.156154	0.123421E-02	0.859618E-01

Chi-squared goodness of fit test

Prep red	dose	n	r	expected	residual	probab	std resid
	0.000	9.	0.	0.15	-0.146	0.016	-0.386
	0.000	10.	0.	0.16	-0.163	0.016	-0.407
	0.000	10.	0.	0.16	-0.163	0.016	-0.407
	0.000	10.	0.	0.16	-0.163	0.016	-0.407
	10.000	10.	0.	0.89	-0.886	0.089	-0.986
	10.000	10.	0.	0.89	-0.886	0.089	-0.986
	10.000	9.	0.	0.80	-0.798	0.089	-0.935
	10.000	10.	3.	0.89	2.114	0.089	2.352
	10.000	10.	2.	0.89	1.114	0.089	1.239
	100.000	10.	5.	6.79	-1.790	0.679	-1.213
	100.000	10.	8.	6.79	1.210	0.679	0.819
	100.000	9.	5.	6.11	-1.111	0.679	-0.793
	100.000	10.	5.	6.79	-1.790	0.679	-1.213
	100.000	10.	9.	6.79	2.210	0.679	1.497
	1000.000	10.	10.	9.91	0.093	0.991	0.306
	1000.000	10.	10.	9.91	0.093	0.991	0.306
	1000.000	10.	10.	9.91	0.093	0.991	0.306
	1000.000	10.	10.	9.91	0.093	0.991	0.306
NATURAL		30.	1.	0.48	0.524	0.016	0.766

chi-square: 17.977 degrees of freedom: 16 heterogeneity: 1.1236

Effective Doses

	dose	limits	0.90	0.95	0.99
LD10 red	12.224	lower	5.542	4.331	2.143
		upper	19.993	21.775	25.744
LD50 red	57.884	lower	39.401	35.794	28.187
		upper	83.177	90.472	110.415
LD90 red	274.093	lower	174.291	160.853	137.138
		upper	556.110	691.326	1280.396
LD95 red	425.938	lower	252.087	230.429	193.461
		upper	1004.322	1315.072	2847.145
LD99 red	973.80	lower	494.09	441.56	356.33
		upper	3103.3	4499.7	13198.

Total esterase assay using standard *M. persicae* clones (esterase protocol)

Slope — Plate#1

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.017	118.29	141.21	154.29	136.29	108.20	135.21	164.23	0.917	0.762	0.000	0.000
B	0.000	76.879	67.464	79.720	78.105	59.444	56.550	70.121	0.743	0.762	0.000	0.000
C	0.000	6.965	6.220	8.240	6.921	6.842	6.469	4.635	0.714	0.000	0.058	0.031
D	0.000	4.672	4.285	5.971	4.551	4.333	4.040	4.875	0.786	0.733	0.022	0.116
E	0.030	7.766	9.024	11.777	6.844	10.379	8.664	9.873	0.755	0.757	-0.056	0.019
F	-0.027	6.075	9.573	6.397	7.177	8.352	8.289	8.988	0.847	0.646	0.048	0.015

Kinetic
Time: 5:00
Interval: 0:10
Reads: 31

Lm1 450

Automix: Off
Calibrate: On

Lag Time: 0:00
End Time: 5:00
OD Min: 0
OD Max: 0.15

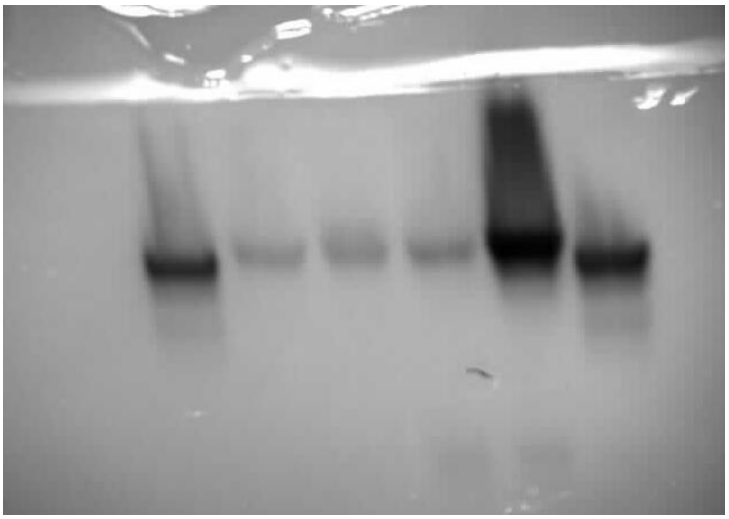
Plate Last Read:
11:18 23/10/2008

Plate:
A2-A8: R3 esterase level
B2-B8: R2 level
C2-C8: USIL susceptible
D2-D8: 4106A susceptible
E2-E8: M. Pesicae (Rob Jacobson) (green)
F2-F8: M. persicae (Rob Jacobson) (red)

Electrophoresis

1 R2, 2 Rob (green), 3 Rob (green), 4 Rob (red), 5 R3, 6 FE4

Aphids from Rob Jacobson are typical susceptible appearance



APPENDIX 2

Resistance to pyrethrin:
Results of tests on *Myzus persicae*

Summer 2009

Bioassay (topical application).

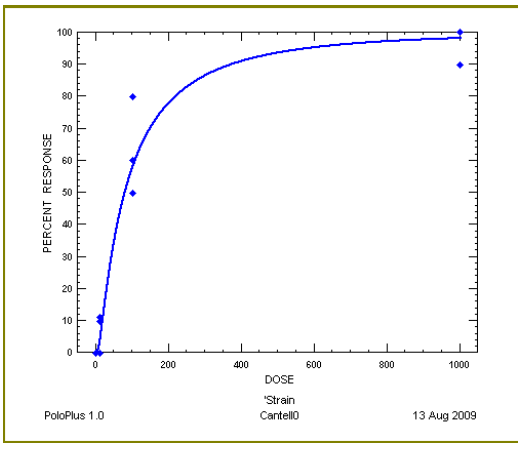
Chi-squared goodness of fit test

prep	dose	n	r	expected	residual	probab	std resid
	0.000	10.	0.	0.00	-0.002	0.000	-0.043
	0.000	10.	0.	0.00	-0.002	0.000	-0.043
	0.000	10.	0.	0.00	-0.002	0.000	-0.043
	0.000	9.	0.	0.00	-0.002	0.000	-0.040
	0.000	9.	0.	0.00	-0.002	0.000	-0.040
	10.000	10.	0.	0.47	-0.467	0.047	-0.700
	10.000	10.	0.	0.47	-0.467	0.047	-0.700
	10.000	10.	1.	0.47	0.533	0.047	0.799
	10.000	10.	0.	0.47	-0.467	0.047	-0.700
	10.000	9.	1.	0.42	0.580	0.047	0.916
	100.000	10.	5.	5.83	-0.830	0.583	-0.532
	100.000	10.	5.	5.83	-0.830	0.583	-0.532
	100.000	10.	8.	5.83	2.170	0.583	1.392
	100.000	10.	6.	5.83	0.170	0.583	0.109
	100.000	10.	6.	5.83	0.170	0.583	0.109
	1000.000	10.	10.	9.82	0.180	0.982	0.428
	1000.000	10.	9.	9.82	-0.820	0.982	-1.949
	1000.000	10.	10.	9.82	0.180	0.982	0.428
	1000.000	10.	10.	9.82	0.180	0.982	0.428

chi-square: 9.833 degrees of freedom: 17 heterogeneity: 0.578

Effective Doses

	dose	limits	0.90	0.95	0.99
LD50 Cantello	77.443	lower	57.318	53.908	47.488
		upper	105.052	111.900	127.642
LD90 Cantello	369.893	lower	247.558	231.948	205.821
		upper	658.092	760.192	1058.205
LD95 Cantello	576.222	lower	362.080	336.347	294.230
		upper	1146.005	1364.804	2043.287
LD99 Cantello1	323.469	lower	729.058	664.617	562.910
		upper	3287.521	4156.959	7173.620



Total esterase assay using std *M. persicae* clones and sample

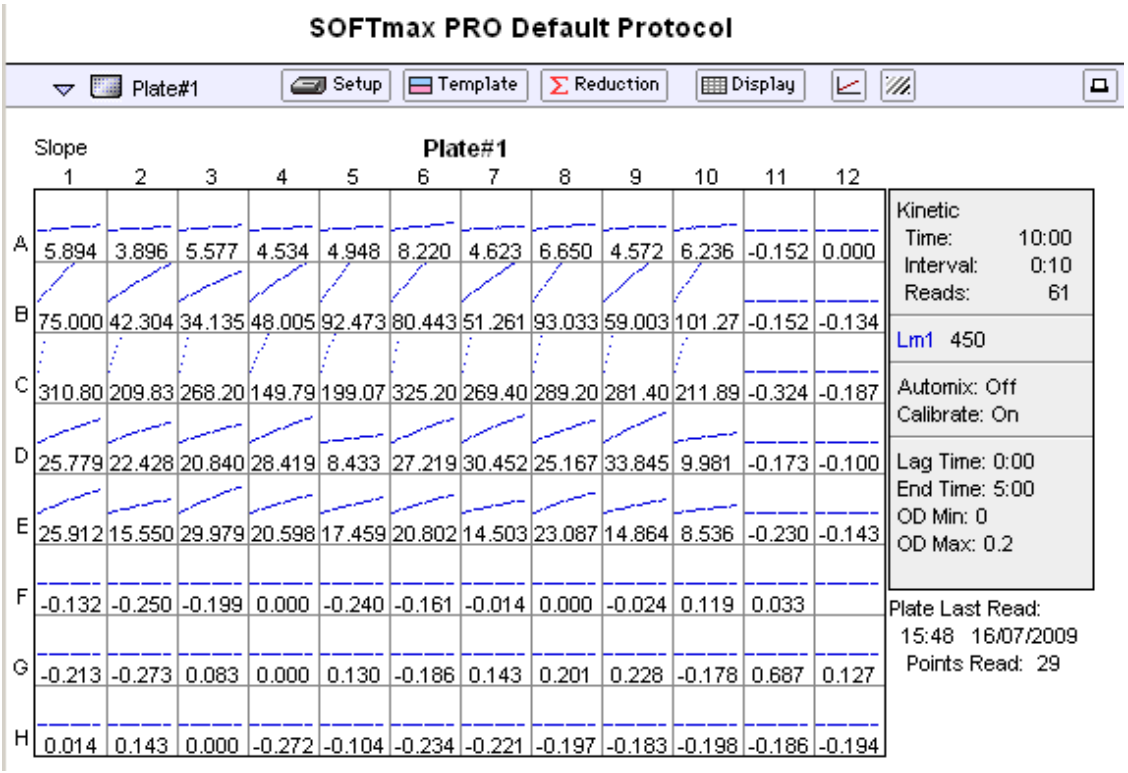


Plate:

A1-A10: S esterase level
B1-B10: R2 esterase level
C1-C10: R3 esterase level
D1-D10: Cantello sample
E1-E10: Cantello sample

