ANNUAL REPORT

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Tomato: Further development of sustainable mealybug control strategies

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Commercial – In Confidence

Project Title:	Tomato: Further development of sustainable mealybug control strategies
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Report:	Second annual report
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The results and conclusions in this report are based on a series of carefully monitored applied studies in experimental facilities and large-scale commercial glasshouses. The conditions under which the studies were carried out and the results have been reported with detail and 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 the 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 this report represents a true and accurate record of the results obtained.

Signature……Date…….

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GROWER SUMMARY

Headline

- A sustainable IPM programme, consisting of a suite of compatible control measures, has been designed for use against obscure mealybug (*Pseudococcus viburni*) on organic tomato and pepper crops. It is being evaluated in the remainder of this project.

Summary of results

- Two species of parasitoids, *Leptomastix epona* and *Pseudaphycus maculipennis*, have been shown to be capable of locating, attacking and completing their development in most life cycle stages of *P. viburni* on both tomato and pepper plants. It is anticipated that one or both of these species will become a component in the whole IPM programme against mealybugs on tomato and pepper crops.
- *Pseudaphycus maculipennis* appears to be the stronger candidate and a licence has been obtained for trials in commercial crops in 2006.
- Questions remain over the ability of the parasitoids to control *P. viburni* at the population level due to the huge numbers of offspring produced by the pest.
- Both species will be further investigated in commercial crops during 2006 with the parasitoids released earlier in the season when mealybug numbers are still relatively low.
- Eradicoat T currently offers the best potential for a second line of defence treatment in organic tomato crops. However, the effectiveness will always depend on the contact of the product and the pest, which is difficult within the horizontal stem bundles. The insect growth regulator, buprofezin (Applaud), remains the most effective second line of defence for conventional crops.

Background and expected deliverables

HDC funded studies have been prompted by an increase in the incidence of the obscure mealybug (*Pseudococcus viburni*) on protected tomato crops in the UK. This species commonly occurs on a wide range of crops throughout the world. In addition to tomatoes, it has been recorded in the UK on other edible crops, including peppers, and on glasshouse-grown ornamental plants.

Female mealybugs are wingless, soft-bodied insects with sucking mouthparts. They are covered in white waxy filaments, which provide protection from adverse conditions and insecticidal sprays. The males are small delicate winged insects that only live for a few days. Eggs are laid in batches of 100-500 in cotton-like pouches made of wax. There are three immature mealybug stages (first, second and third instar nymphs), which are similar in appearance to adult females.

HDC project, PC 161, which was completed in 2002, formulated a control strategy for the obscure mealy bug on tomato. The most effective and IPM compatible method of controlling mealybugs on tomato plants during the production season was the insect growth regulator, buprofezin (Applaud). While this provides the basis of a control programme for conventional crops, the strategy is heavily dependant on the chemical insecticide and is not consistent with the TGA's long term goal of pesticide-free crop

production. Furthermore, it cannot be applied to organic crops. Sustainable control measures are now also required to combat this pest on pepper crops.

The overall aim of this project was to formulate sustainable strategies, based on a combination of new and existing control measures, for the control of *P. viburni* on UK tomato and pepper crops. The specific objectives were:

1. To develop a robust biological control measure based on parasitoids.
2. To construct sustainable mealybug control strategies tailored to the specific requirements of conventional and organic tomato and pepper crops.
3. To test and refine the control strategies in commercial crops.

Summary of work to date

It was considered highly unlikely that any one sustainable control measure would be successful against *P. viburni* on commercial crops. Therefore, a programme was designed consisting of a suite of compatible control measures that could be used to combat the pest at four distinct stages throughout the growing season:

- Survival on the glasshouse structure between crops (These control measures were developed in Project PC 161)**.**
- Initial crop invasion by overwintered survivors.
- Season-long suppression of mealybug population growth.
- A compatible second line of defence.

Initial crop invasion by overwintered survivors.

It has been shown that mealybug eggs on the structure of the glasshouse hatch within three weeks of the glasshouse being heated and they quickly migrate to the new plants. A 2% dilution of Savona has been effective against first instar *P. viburni* as they colonise tomato plants, reducing numbers by up to 93%. However, there is some concern over the effect of this product on young plants. Alternative products will be tested in the third year of the project.

Season-long suppression of mealybug population growth.

Much of the work in the first year of this project was aimed at developing a biological control measure for the obscure mealybug that would provide season-long suppression of the pests' population growth. A series of laboratory-based experiments provided important information about the key biological parameters that governed the ability of three species of parasitoids, *Leptomastix epona*, *Anagyrus pseudococci* and *Pseudaphycus maculipennis*, to successfully locate, attack and complete their development in the mealybugs on tomato and pepper plants. There appeared to be no fundamental reasons why these parasitoids would not become established in populations of *P. viburni* on both types of plants. However, there were questions over their ability to control *P. viburni* at the population level due to the huge numbers of offspring produced by the pest. *A. pseudococci* appeared to be the weakest candidate due to its poorer performance at the temperatures that are common in crops in the early season. The stronger candidates, *L. epona* and *P. maculipennis*, were tested at the population level in larger scale experiments in 2005.

Leptomastix epona could be released in UK crops but *P. maculipennis* was a non-native species and did not have official approval. As part of this project, an application for a licence to release *P. maculipennis* in the UK was successfully obtained from Defra. The studies in the second year of the project consisted of a detailed examination of the population growth of *L. epona* and *P. maculipennis*

against that of their host, *P. viburni*, within experimental glasshouse crops, and larger-scale observations of the establishment of *L. epona* in commercial tomato and pepper crops.

In contained glasshouse tomato trials at STC Research Foundation, results showed that 80-90% parasitism was achieved from a single release of either *P. maculipennis* or a combination of *P. maculipennis* / *L. epona*. A maximum level of 50% parasitism was achieved by *L. epona* alone. While both species could have the potential to become components in a mealybug control programme for tomato crops, *P. maculipennis* appeared to show the greatest promise. However, it was several weeks before they began to reduce the pest populations and there remain doubts as to whether these control measures will be successful in commercial crops.

The parasitoids did not perform so well in the contained glasshouse pepper trials at STC Research Foundation. In this case, a maximum of 40% parasitism was achieved by *P. maculipennis* but no parasitism was detected by *L. epona* throughout the trial. The differences in the results between tomatoes and peppers may be explained by the habitats occupied by *P. viburni* on these crops. On tomato plants, the mealybugs are situated primarily on the lower stems while on peppers they are usually found beneath the calyx of the fruits. It would appear that the parasitoids are more successful when attacking mealybugs situated on the open stems than when the pests are tightly encrypted beneath the calyx of the peppers. This may simply be because the parasitoids are unable to locate their hosts. The location of mealybugs on peppers may be an additional barrier to successful establishment of the parasitoids in commercial pepper crops because at least some of the developing wasps will be removed from the glasshouse with the harvested fruit.

The licence for release of *P. maculipennis* was not issued in time for the larger scale trials and so only *L. epona* could be evaluated in the commercial tomato and pepper crops. *L. epona* became established in the mealybug population on tomato plants following four weekly releases at the rate of 2 parasitoids per m^2 and achieved a level of 15% parasitism 70 days after the first release. Similar observations of *L. epona* within a commercial pepper crop recorded a level of 23% parasitism 77 days after the first release of the parasitoids. In both cases, the speed of establishment was inadequate to bring the pest population under control.

The generation time for *P. viburni* is slow, taking 50 days from egg to adult at 21±2ºC. This provides an advantage to the parasitoids, which complete their development in about the half the time. However, the pest has very high fecundity rates (adult female *P. viburni* can produce up to 500 eggs in its egg sac), which means the population could continue to grow rapidly while the parasitoids are becoming established. There is insufficient resource within this project to allow more detailed studies of the population dynamics within this biological system. However, to be successful, we believe that the parasitoids must be released in the early part of the growing season while pest numbers are still relatively small. The studies with parasitoids will continue in 2006.

A compatible second line of defence

This component was required for use mid-season to redress the balance between pest and parasitoid should control with the primary control agent falter. Applaud remains an effective second line of defence for conventional crops but an alternative is required for organic crops. The effect of Savona on first instar larvae is mentioned above. This product has also been shown to have a direct effect on other life cycle stages on the plant but the results had been variable; 2% and 4% dilutions giving 30-

60% and 40-100% control respectively. Control would therefore be dependant on a series of applications and there are now concerns about the direct effect that such a programme would have on the plants.

Some UK growers who used the fungal pathogen, *Verticillium lecanii*, against glasshouse whiteflies, reported incidental control of mealybugs but this had not been confirmed in controlled experiments. Laboratory bioassays done under ideal conditions showed that the pathogen reduced numbers of first instar nymphs but only by about 10%. It was presumed that the mealybugs were protected from infection by their waxy covering. However, it was hypothesised that the effect of the fungus could be enhanced by applying it after Savona because the soap would breakdown the waxy protection and allow more fungal spores to come into contact with the insect's body. Preliminary results were variable but 100% mortality of mealybugs was achieved in some laboratory experiments. The studies were scaled up to a commercial tomato crop in 2004. These results showed only a small advantage in using Mycotal in addition to Savona over three applications at approximately seven day intervals. This was contrary to the previous laboratory scale studies and was attributed to the difficulty in obtaining good spray cover among the horizontal spray bundles.

The studies were repeated in 2005 with Eradicoat T incorporated in the trial. This was a new formulation of a starch-based material with a physical mode of action. All three treatments (*i.e.* 4% Savona, 4% Savona plus 0.1% Mycotal, Eradicoat T) reduced numbers of mealybugs while the pest population continued to grow in the untreated controls. There was no apparent difference between the Savona and Savona plus Mycotal treatments (except in the number of viable egg sacs) thus indicating that there had been no additional effect by the fungus. This was supported by the fact that no fungal growth was evident on the dead mealybugs on the plants, nor did any develop when the cadavers were removed and incubated under more ideal conditions in the laboratory. Eradicoat T performed significantly better than the Savona on all development stages of the mealybug and currently offers the best potential for a second line of defence treatment in organic tomato crops. However, the effectiveness will always depend on the contact of the product and the pest.

Financial benefits to growers

The cost of control measures applied against patchy infestations of mealybugs on two monitored nurseries throughout 2002 varied from £2,000 to £4,500 per hectare. This was comparable to estimates received in response to a grower survey in the late 1990s, which averaged £3,100 per hectare. Despite these intensive control measures, the growers still suffered financial losses due to mealybug damage. These losses have been difficult to quantify but one grower estimated them to be over £1,000 per hectare in 2005. The total cost of a mealybug infestation to a tomato business is therefore likely to be in excess of £4,000 per hectare.

The most effective control measures identified to date are heavily dependant on chemical insecticides. The development of successful biological control against mealybugs will therefore provide another step towards the TGA's long-term goal of pesticide-free crop production. This will increase the desirability of TGA members' produce and strengthen their marketing position. Furthermore, the most effective existing control measures are not compatible with the standards that govern organic production. The development of successful biological control will therefore fill an important gap in organic growers' overall pest management armoury.

Action points for growers

- Inspect all plant material for mealybugs before introducing it into the greenhouse.
- Monitor plants regularly for presence of mealybugs concentrating on areas where it was found in the previous season and on the plants beside posts. If a few plants are heavily infested they should be removed and destroyed.
- Until both species of parasitoid described above are further investigated in commercial crops during 2006, Eradicoat T can be used on organic crops and Applaud on conventional crops as a second line of defence. Both products require good contact of the product and the pest.
- Carry out a thorough end of season clean up including the structure of glasshouses eg concrete dollies and irrigation tubes.

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SCIENCE SECTION

PART 1: GENERAL INTRODUCTION

Background

Mealybugs belong to the insect family, Homoptera, which also includes aphids, whiteflies and scale insects. Female mealybugs are wingless, soft-bodied insects with sucking mouthparts. They are covered in white waxy filaments, which provide protection from adverse conditions and insecticidal sprays. The males are small delicate winged insects that only live for a few days. Eggs are laid in batches of 100-500 in cotton-like pouches made of wax. World-wide, mealybugs are one of the most significant pest groups, with over 3000 species known to feed on a wide range of plant families in habitats varying from the soil to tree tops.

HDC funded studies have been prompted by an increase in the incidence of *Pseudococcus viburni* (Signoret) (the obscure mealybug) on protected tomato crops in the UK. This species is a polyphagous cosmopolitan pest (Ben-Dov, 1994). In addition to tomatoes, it has been recorded in the UK on other edible crops, including peppers, and on glasshouse-grown ornamental plants.

HDC project, PC 161, which was completed in 2002, investigated the increase in incidence of infestations of *P. viburni* in UK tomato crops and began to formulate a control strategy (Jacobson & Croft, 2002). The most effective and IPM compatible method of controlling mealybugs on tomato plants during the production season was shown to be the insect growth regulator, buprofezin (Applaud), but this may not be applied to organic crops. Furthermore, this dependence on a single chemical insecticide was not consistent with the TGA's long term goal of pesticide-free crop production.

To become sustainable, the control strategy required a biological component that would act continuously throughout the growing season. Project PC 161 evaluated several biological control agents (*eg Hypoaspis* spp., *Chrysoperla* spp., *Beauveria bassiana*) but none proved to be very promising. However, preliminary investigations indicated that the parasitoids, *Leptomastix epona* (EPPO, 2002) and *Pseudaphycus maculipennis* (Charles, 2001) had potential to fulfil this role. *L. epona* could be released in UK crops but *P. maculipennis* was not indigenous and therefore required a licence before it could be used in trials in glasshouses.

The overall aim of this project was to formulate sustainable strategies, based on a combination of new and existing control measures, for the control of *P. viburni* on UK tomato and pepper crops.

The specific objectives were:

1. To develop a robust biological control measure based on parasitoids.
2. To construct sustainable mealybug control strategies tailored to the specific requirements of conventional and organic tomato and pepper crops.
3. To test and refine the control strategies in commercial crops.

Summary of work completed in the first year (Jacobson & Croft, 2005):

Design of an IPM strategy

It was considered highly unlikely that any one sustainable control measure would be successful against *P. viburni* on commercial crops. Therefore, a programme was designed consisting of the following suite of compatible control measures:

- Survival of *P. viburni* on the glasshouse structure between crops**.**
  Hyvis 30 Emulsion (a polybutene based glue), Jet 5 (peroxyacetic acid) and undiluted vinegar had been shown to reduce the numbers of nymphs emerging from egg sacs on concrete by 50%, 67% and 81% respectively. Although this would reduce initial crop invasion, there may be up to 500 eggs in each egg sac and further control measures would be required.
- Initial crop invasion by overwintered survivors.
  A 2% dilution of Savona had been effective against first instar *P. viburni* as they colonised tomato plants, reducing numbers by up to 93%. However, there was some concern over the effect of this product on young plants.
- Season-long suppression of *P. viburni* population growth.
  Three parasitoids, *Leptomastix epona*, *Anagyrus pseudococci* and *Pseudaphycus maculipennis*, were considered to have potential for this role.
- A compatible second line of defence.
  This component was required for use mid-season to redress the balance between pest and parasitoid should control with the primary control agent falter. The effect of Savona on first instar larvae is mentioned above. This product had also been shown to have a direct effect on other life cycle stages on the plant but the results had been variable; 2% and 4% dilutions giving 30-60% and 40-100% control respectively. Control would therefore be dependant on a series of applications. In addition, it had been shown in the laboratory that the effect of *Verticillium lecanii* (Mycotal WP) could be enhanced by applying after Savona.

Season-long suppression of mealybug population growth with parasitoids

A series of experiments provided the following important information about the key biological parameters that govern the ability of *L. epona*, *A. pseudococci* and *P. maculipennis* to successfully locate, attack and complete their development in *P. viburni* on tomato and pepper plants:

- All three species of parasitoids will successfully parasitise most of the development stages of *P. viburni* (*i.e.* second and third instar larvae, and young and pre-ovipositing adults) on tomato and pepper plants.
- There was no significant difference in the fecundity (egg laying capacity) of the parasitoids in *P. viburni* on tomato or pepper plants. However, the fecundity of *P. maculipennis* and *L. epona* was higher than that of *A. pseudococci*.
- The development period (egg to adult) of *P. maculipennis* was shorter than that of *L. epona* and *A. pseudococci*. Results showed that all three parasitoids have a development time that is considerably shorter (*i.e.* approximately 50%) than *P. viburni.*
- In whole plant experiments, *L. epona* and *P. maculipennis* were able to locate and oviposit in the mealybug on whole tomato and pepper plants, demonstrating that the habitat provided by those plants was acceptable to the parasitoids.

There appeared to be no fundamental reasons why these parasitoids would not become established in populations of *P. viburni* on tomato and pepper plants. However, there were questions over their ability to control *P. viburni* at the population level due to the huge numbers of offspring produced by the pest. Following a review of the progress of the project, it was agreed that *L. epona* and *P. maculipennis* appeared the better parasitoids, and they would be tested at the population level in larger glasshouse scale experiments in 2005.

A compatible second line of defence

Some UK growers who used the fungal pathogen, *Verticillium lecanii*, against glasshouse whiteflies, reported incidental control of mealybugs but this was not confirmed in controlled experiments. Laboratory bioassays done under ideal conditions showed that the pathogen reduced numbers of first instar nymphs but only by about 10%. It was presumed that the mealybugs were protected from infection by their waxy covering. However, it was hypothesised that the effect of the fungus could be enhanced by applying it after Savona because the soap would breakdown the waxy protection and allow more fungal spores to come into contact with the insect's body. Preliminary results were variable but 100% mortality of mealybugs was achieved in some laboratory experiments (Jacobson & Croft, 2002).

The studies were scaled up to a commercial tomato crop within the Wight Salads Group. These results showed only a small advantage in using Mycotal in addition to Savona over three applications at approximately seven day intervals. This was contrary to the previous laboratory scale studies. However, it is not unusual to obtain poorer results with insect pathogens when the treatments are increased from experimental to commercial-scale. This is usually attributed to the difficulty in obtaining good spray cover and / or to the different environmental conditions.

PART 2: DEVELOPMENT OF ROBUST BIOLOGICAL CONTROL MEASURES BASED ON PARASITOIDS

2.1. Introduction

Of the three species of parasitoids studied in the first year of this project (Jacobson & Croft, 2005), *Pseudaphycus maculipennis* and *Leptomastix epona* appeared to have the greatest potential for incorporation in an IPM programme against *Pseudococcus viburni* on tomato and pepper crops. Both species were selected for further evaluation.

Leptomastix epona could be released in UK crops but *P. maculipennis* was a non-native species and did not have official approval. However, as a very specialised parasitoid of a non-indigenous pest restricted to semi-protected locations, the project team were reasonably confident that a licence to release in UK glasshouses could be obtained. Furthermore, there was a precedent because it had already been successfully introduced for the control *P. viburni* in New Zealand (John Charles, pers.comm.). As part of this project, an application for a licence to release *P. maculipennis* in the UK was prepared and submitted to Defra in 2004 and successfully obtained in 2005.

The studies included in this part of the project consisted of a detailed examination of the population growth of *L. epona* and *P. maculipennis* against that of their host, *P. viburni*, within experimental glasshouse crops, and larger-scale observations of the establishment of *L. epona* in commercial tomato and pepper crops.

2.2. Studies to evaluate the potential of *Leptomastix epona* and *Pseudaphycus maculipennis* to locate and parasitise *Pseudococcus viburni* in experimental tomato and pepper crops.

Materials and method

The experiment was done at STC Research Foundation in eight identical glasshouse sections (each measuring approximately 40m^2). Four of the sections each contained 50 tomato plants arranged in two equal rows. The other four sections contained a similar arrangement of pepper plants.

All plants were artificially infested with *P. viburni* taking care to establish populations as evenly as possible throughout each glasshouse. Seven plants with similar numbers of mealybugs were selected and tagged in each glasshouse section. The precise numbers of *P. viburni* were then recorded.

The following treatments were each applied to a glasshouse section containing tomato and pepper plants:

1. *P. maculipennis*
2. *P. maculipennis* and *L. epona*
3. *L. epona*
4. Untreated control (*i.e.* no parasitoids)

All parasitoids were released on one occasion at the rate of 2 individual adults per plant (1;1 ratio for the *L. epona/P. maculipennis* mix).
At 10 to 14 day intervals following the release of the parasitoids, one of the seven tagged plants was removed from each glasshouse for assessment in the laboratory.

On each occasion, the numbers of *P. viburni* were recorded before being dissected to determine whether they were parasitised. A mean count of *P. viburni* was done throughout the trial in the untreated control section.

Results and Discussion

Figure 4 shows the levels of parasitism of *P. viburni* achieved from single releases of *L. epona* and *P. maculipennis* in small experimental tomato crops. Seventy four days after the release of the parasitoids, parasitism of *P. viburni* was 97% and 89% with *P. maculipennis* and *P. maculipennis* / *L. epona* respectively. At the same assessment, *L. epona* alone had achieved 11% parasitism. The growth in the rates of parasitism within each glasshouse section suggested that *P. maculipennis* could achieve high levels of parasitism without *L. epona*. Figure 5 shows that, by the end of the experiment, there were fewer mealybugs in all the parasitoid treatments than in the untreated control on tomato plants.

The single release of the parasitoids within this trial should allow us to observe an increasing level of parasitism over time if parasitism is successful. This is because the mummies resulting from parasitism will accumulate on each of the replicates over time and we are recording the overall parasitism observed on each replicate over time. Generally there is an increase over time. However there are varying levels of parasitism as seen in Figure 4. For example the final percentage of *L. epona* (11%) was lower than a previous assessment (50%). This final lower figure and the peaks and troughs in Figure 4 show a variation that was independent of mealybug density on each of the plants and is possibly a reflection of the low replication and the inherent variation within biological systems.

Figure 6 shows the levels of parasitism of *P. viburni* achieved from single releases of *L. epona* and *P. maculipennis* over 52 days in small experimental pepper crops. A maximum of 40% parasitism was achieved by *P. maculipennis* but no parasitism was detected by *L. epona* throughout the trial (NB: 23% parasitism was achieved by *L. epona* following four weekly introductions in the commercial crop – see section 2.4).

The differences in the results between tomatoes and peppers may be explained by the habitats occupied by *P. viburni* on these crops. On tomato plants, the mealybugs are situated primarily on the lower stems while on peppers they are usually found beneath the calyx of the fruits. It would appear that the parasitoids are more successful when attacking mealybugs situated on the open stems than when the pests are tightly encrypted beneath the calyx of the peppers. This may simply be because the parasitoids are unable to locate their hosts. The location of mealybugs on peppers may be an additional barrier to successful establishment of the parasitoids in commercial pepper crops because at least some of the developing wasps will be removed from the glasshouse with the harvested fruit.

Figure 4. Percentage parasitism of *P. viburni* on glasshouse tomato plants after a single introduction of two species of parasitoid

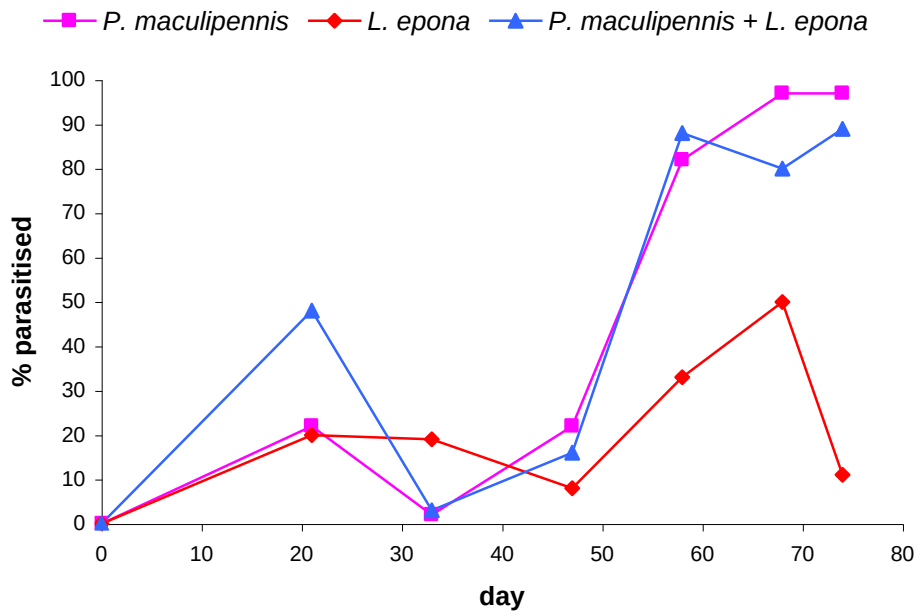


Figure 5. The numbers of live *P. viburni* on glasshouse tomato plants following a single release of two species of parasitoid.

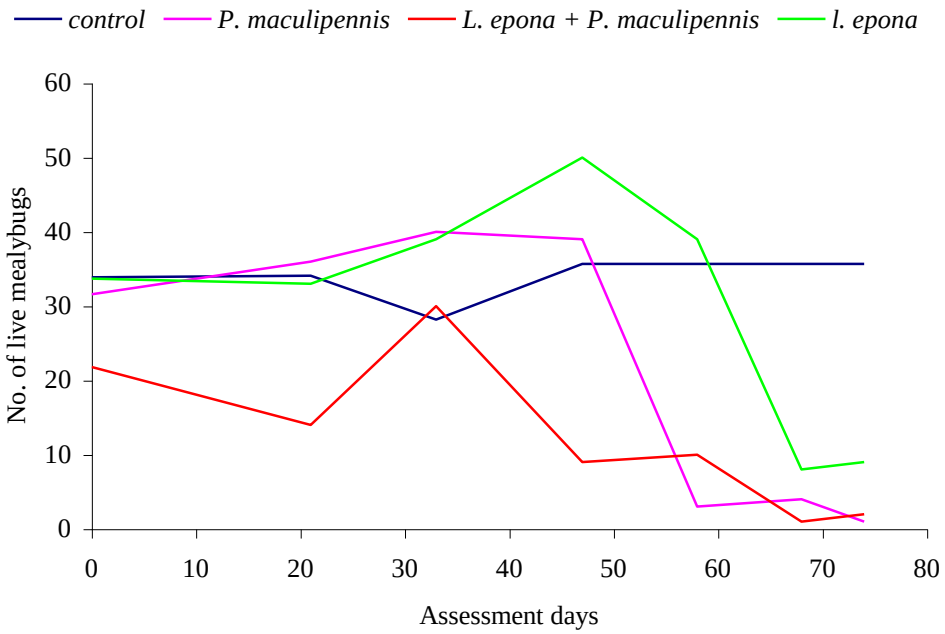
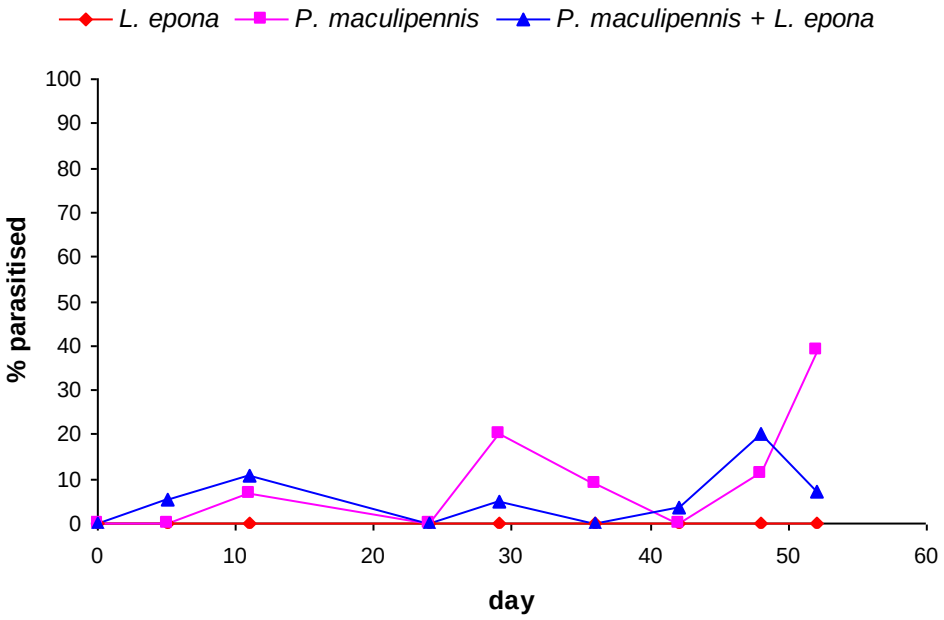


Figure 6. Percentage parasitism of *P. viburni* on glasshouse pepper plants after a single introduction of two species of parasitoid



Conclusions

While both species could have the potential to become components in a mealybug control programme for tomato crops, *P. maculipennis* appeared to show the greatest promise. However, it was several weeks before they began to reduce the pest populations and there remain doubts as to whether these control measures will be successful in commercial crops.

The generation time for *P. viburni* is slow, taking 50 days from egg to adult at 21±2°C (Heidari, 1989). This provides an advantage to the parasitoids, which complete their development in about the half the time. However, the pest has very high fecundity rates (adult female *P. viburni* can produce up to 500 eggs in its egg sac), which means the population could continue to grow rapidly while the parasitoids are becoming established. There is insufficient resource within this project to allow more detailed studies of the population dynamics within this biological system. However, to be successful, we believe that the parasitoids must be released in the early part of the growing season while pest numbers are still relatively small.

2.3. Monitoring the establishment of *Leptomastix epona* in a commercial tomato crop

Materials and methods

The trial was established in approximately 860m^2 of baby plum tomatoes (cv Conchita) in glasshouse 5 at WSG's Budbridge Manor Nursery. The plants had become infested at the beginning of the season by a population of *P. viburni* that was resident in the glasshouse. As is common with naturally occurring infestations, the individual colonies of pests were of variable size and unevenly distributed throughout the trial area.

The trial area was divided into two plots. *L. epona* were released in one plot at the rate of 2 (1:1 male:female ratio) per m^2 per week for four weeks from week 24 (15 June 2005). WSG's standard control programme, consisting of weekly high volume applications of 4% Savona, was applied to the stem bundles in the other plot during the same period.

Ten monitoring stations were established in each plot with the objective of comparing mealybug population growth trends and determining levels of parasitism. Each station comprised a 30cm length of the horizontal tomato stem bundle. The numbers of motile mealybugs and egg masses were recorded at each monitoring station at the beginning of the trial and at 21 day intervals thereafter (*i.e.* weeks 27, 30 and 33). On each occasion, 100 mealybugs (second and third instar larvae, and pre-ovipositing adults) were collected from stems adjacent to the sample stations and dissected to determine whether they were parasitised.

Monitoring results

At the pre-treatment assessment (seven days before release of the parasitoids), there were an average of 5 motile mealybugs and 5 egg masses at each monitoring station. However, there was potential for rapid population growth because the eggs were just beginning to hatch.

First post treatment assessment

By the first post treatment assessment (21 days after first parasitoid release), there were an average of 9.3 motile stages and 4.5 egg masses per monitoring station in the standard Savona treated plot. However, the intensive Savona applications were beginning to cause discolouration of the stems and it was feared that they would be weakened and become more vulnerable to fungal pathogens. As a consequence, the last application of Savona was applied seven days after first post treatment assessment. Numbers of mealybugs had risen more considerably in the *L. epona* plot, with an average of 25.1 motile stages and 11.1 egg masses per monitoring station. No parasitised mealybugs were recorded in the monitoring stations at that time, although a small number of parasitised adults were found during a larger inspection of the whole plot.

Second post treatment assessment

42 days after first parasitoid release, numbers of mealybugs had increased in the standard plot (12 motile stages and 6.7 egg masses per station) and in the *L. epona* plot (31 motile stages and 16.8 egg masses). At that time, 6% of second and third instar larvae, and pre-ovipositing adults were parasitised in the *L. epona* plot.

Third post treatment assessment

63 days after the first release of parasitoids, numbers of mealybugs were stabilising in both plots. There were now an average of 10.9 motile stages and 7.9 egg masses per monitoring station in the standard plot, and 26.4 motile stages and 12.2 egg masses in the *L. epona*. Parasitism had risen to 15% in the *L. epona* plot.

Conclusions

It is clear from these monitoring results that *L. epona* can become established in a mealybug population on tomato plants under commercial growing conditions, achieving a level of 15% parasitism 70 days after the first release. However, the speed of establishment was not adequate to bring the population under control. This was no doubt due to the large number of mealybug offspring emerging from the egg sacs in the crop.

2.4. Monitoring the establishment of *Leptomastix epona* in a commercial pepper crop.

Materials and methods

This trial was established in approximately 800m^2 of peppers (cv Ferrari) in a relatively small glasshouse at the Chilton Cantelo site of Cantelo Nurseries Ltd. As with the tomato crop in the previously described trial, the plants had become naturally infested by *P. viburni* at the beginning of the season and the individual colonies of pests were of variable size and unevenly distributed.

The trial area consisted of 13 rows of 190 plants. Each plant had been trained with three stems, giving a total of 7410 heads. Until the start of this trial on 2 June 2005, the fruits had been harvested green (*i.e* 28-35 days old) and were therefore picked before any mealybugs living under the calyx became mature and started to lay eggs. This had obviously restricted the pest's population growth. However, from 2 June 2005 onwards, the fruit were to be picked red and would therefore stay on the plant for approximately 60 days. From past experience, this change in agronomic practice was usually followed by a rapid increase in mealybug numbers.

L. epona were released in the trial area from 2 June 2005 at the rate of 0.8 (1:1 male:female ratio) per m^2 per week for eight weeks. The release rate was lower than in the tomato trial because the mealybug population was smaller. A mirror image of the trial area was untreated for comparison.

On the 1 June 2005, the crop was surveyed and all mealybug infestations were noted. Due to agronomic practice up to that date, the pest population growth had been restricted and relatively small numbers of mealybugs were found on medium to large fruit. Very few egg masses were seen. The pests were under the calyx of the fruits and

it was impossible to count them accurately without being destructive. Numbers were therefore estimated using the following categories:

VF	= <2 individuals	MN	= 6-15 individuals
F	= 2-5 individuals	LN	= > 15 individuals

Thirty monitoring stations, each consisting of three heads, were then selected in the “F” category and marked for future reference. The stations were examined seven, 28 and 56 days after the first release of the parasitoids. On each occasion, the size of the mealybug population was categorised and the presence of parasitised individuals were noted. In addition to these three on-site assessments, mature fruits harvested from these heads and were sent to STC for examination at weekly intervals.

Monitoring results

At the start of the trial, the mealybug colonies were all in the “F” category. The next on-site assessment was done 28 days after the first release of the parasitoids. The grower would normally have expected the mealybug numbers to increase markedly during this period but this did not happen and the population growth had remained at about the same level. However, there had been a shift in the structure of the population, with a noticeable reduction in numbers of large nymphs / adults and an increase in the number of egg masses which were beginning to release small nymphs. There was also evidence of parasitism among dead adult mealybugs. This was very difficult to detect due the position of the insects under the calyx. Those recorded as parasitised were obvious because they were darkly coloured due to the developing wasp inside. However, they were only seen when the wasp was almost ready to emerge and it is therefore likely that the in-crop assessments under-estimated the level of parasitism.

During the following month (to 56 days after first release of the parasitoids), counts on the marked plants showed a slight overall decrease in numbers of motile stages and many fewer egg masses. However, this was comparable to the untreated plants so we cannot assume that the parasitoids were suppressing the population.

The more detailed parasitism checks on harvested fruit, showed parasitism to be relatively low (maximum 7%) until 77 days after the first parasitoid release when it increased to 23%.

Conclusions

As with the tomato crop, these monitoring results show that *L. epona* can become established in a mealybug population on pepper plants under commercial growing conditions. However, the rate of establishment was again too slow to control the pest population.

Overall conclusion from commercial-scale studies

It is anticipated that *L. epona* could become a component in a larger IPM programme against this pest in tomato and pepper crops but the population dynamics will probably dictate that the parasitoids must be released before the first generation of mealybugs start to produce their egg sacs. However, it is not clear at this stage whether *L. epona* will be sufficiently active to locate the mealybugs at low densities under the shorter days and cooler conditions that prevail in the earlier part of the growing season.

PART 3: FURTHER DEVELOPMENT AND EVALUATION OF SECOND LINE OF DEFENCE MEASURES IN COMMERCIAL CROPS

Introduction

Following a review of the progress of the project in spring 2005, it was agreed that the commercial scale trial involving Savona and Mycotal (see Part 1 of this report) should be repeated paying more attention to application technique. In addition, Eradicoat T was incorporated in the trial. This was a new formulation of a starch-based material with a physical mode of action.

Materials and methods

The trial was established in 16 rows (each with 94 plants/heads) of baby plum tomato plants (cv Santa) in glasshouse 10 at WSG's Arreton Valley Nursery. The plants had become infested at the beginning of the season by a population of *P. viburni* that was resident in the glasshouse. As is common with naturally occurring infestations, the individual colonies of pests were of variable size and unevenly distributed throughout the trial area.

There were four treatments; 4% Savona, 4% Savona plus 0.1% Mycotal, Eradicoat T and untreated controls. All treatments were replicated in four complete rows. Savona, Mycotal and Eradicoat T were prepared and applied to the horizontal tomato stem bundles according to label recommendations by the nursery staff. This was a high volume spray applied manually using a Brinkman high volume glasshouse sprayer fitted with a multihead (*i.e.* x4) lance.

The whole trial area was surveyed prior to the first treatments being applied and twelve sample stations with similar numbers of mealybugs were selected in each plot. Each station consisted of a 600mm length of horizontal stem within the stem bundle. A pre-treatment assessment of numbers of mealybugs was completed on 29 July 2005. The numbers of healthy adults, nymphs and egg sacs were recorded separately.

Three treatment applications were made at a 10 and 11 day interval. Assessments were made eight days after the first and last application of treatments, on the previously marked sections of stem. The final assessment in the untreated controls was brought forward to one day after the final application of treatments.

Analysis was done on the numbers of *P. viburni* recorded on the final assessment (statistical analysis was also done on the pre-treatment counts to establish no significant difference between the numbers of *P. viburni* between the different treatments). Analysis was done using Anova on the square root transformed values and means were compared using Least Significant Difference.

Results and discussion

A summary of the numbers of mealybugs recorded on the three assessment dates is shown in Table 1. As may be expected from a trial based on a naturally occurring infestation, the numbers recorded in the individual sample stations were very variable. These results are more clearly illustrated in Figure 7, which shows mean numbers of motile stages (*i.e.* adults and nymphs) of mealybugs in the four treatments on all assessment dates.

The mealybug population grew rapidly in the untreated plots and by the end of the trial the numbers of motile stages and egg masses had increased by 88% and 354% respectively. At that stage, they were becoming a threat to the rest of the crop and the final assessment in the untreated plots was brought forward so that a control programme could begin.

By contrast, all three control treatments reduced the numbers of *P. viburni*. After the first application, numbers of motile stages had decreased by 39%, 35% and 52% in the Savona, Savona plus Mycotal and Eradicoat T treatments respectively. After the full sequence of three applications, numbers of motile stages had decreased by 57%, 50% and 67% in the Savona, Savona plus Mycotal and Eradicoat T treatments respectively. As in the previous trial (Jacobson & Croft, 2005), most survivors were under the horizontal stems, particularly where these were pressed closely together in the bundles. This once again illustrated the difficulty in achieving good spray coverage and contact with the mealybugs.

Table 1. Summary of numbers of *P. viburni* recorded on three occasions; *i.e.* before application of treatments, and eight days after the first and the final application.

Treatment	Life cycle stage	Pre-treatment	After first treatment		After final treatment	
		Mean / sample station	Mean / sample station	% change	Mean / sample station	% change
Untreated	Adults	5.4	6.5	+ 20	8.3	+ 28
	Nymphs	7.4	10.2	+ 38	15.8	+ 55
	Egg sacs	5.2	11.2	+ 115	13.2	+ 18
Savona	Adults	4.2	2.8	- 33	1.7	- 39
	Nymphs	7.3	4.2	- 42	3.2	- 24
	Egg sacs	3.7	4.2	- 13	2.7	- 36
Savona + Mycotal	Adults	3.4	1.7	- 26	1.3	- 71
	Nymphs	6.1	4.5	- 50	3.4	+ 50
	Egg sacs	7.0	3.5	- 50	1.2	- 66
Eradicoat	Adults	2.6	1.5	- 70	0.4	- 73
	Nymphs	5.1	2.2	- 15	2.1	- 4
	Egg sacs	6.2	2.9	- 53	0.7	- 76

Figure 7. Mean numbers of motile stages of *P. viburni* in the four treatments on three assessment dates

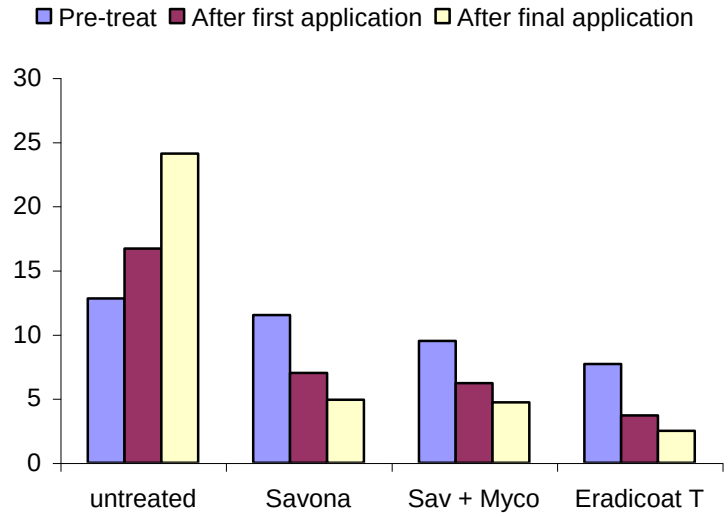


Table 2 shows the statistical comparison of the final count of *P. viburni* development stages. Eradicoat T significantly ($p<0.05$) reduced all of the three stages in comparison to Savona (4%) and Savona (4%) + Mycotal (0.1%).

There was no apparent difference between the Savona and Savona plus Mycotal treatments for the nymph and adult stages thus indicating that there had been no additional effect by the fungus. This was supported by the fact that no fungal growth was evident on the dead mealybugs on the plants, nor did any develop when the cadavers were removed and incubated under more ideal conditions in the laboratory. However, there was some improvement in the reduction of eggs sacs with the addition of Mycotal to Savona.

Table 2. A comparison of the mean (square root transformed mean) numbers of live *P. viburni* development stages following the application of three treatments

Development stage	Savona	Savona + Mycotal	Eradicoat T
Adults	1.67 (1.01)	1.33 (0.72)	0.42 (0.26)
Nymphs	3.17 (1.60)	3.42 (1.63)	2.08 (1.07)
Egg sacs	3.17 (1.59)	1.17 (0.80)	0.67 (0.45)
LSD (99df)	(0.68)		

Conclusions

Eradicoat T currently offers the best potential for a second line of defence treatment in organic tomato crops. However, the effectiveness will always depend on the contact of the product and the pest, which is difficult within the horizontal stem bundles.
The insect growth regulator, buprofezin (Applaud), remains the most effective second line of defence for conventional crops.

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