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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

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

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CONTENTS

Page

Grower Summary

Headlines

- 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.
- 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.
- 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. This will be further investigated in experimental and commercial crops during 2005.
- An application has been submitted for a licence to release *P. maculipennis* in UK glasshouse crops.
- It is anticipated that one or both of these species will become a component in the whole IPM programme against mealybugs in tomato and pepper crops.
- A compatible second line of defence is under development. This will be used to redress the balance between pest and parasitoid should control with the primary control agent falter.

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.

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, investigated the increase in incidence of infestations of the *P. viburni* in UK tomato crops and began to formulate a control strategy. 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 chemical insecticides and is not consistent with the TGA's long term goal of pesticide-free crop production. Furthermore, it can not be applied to organic crops. There are still large gaps in the control strategy for organic crops. Sustainable control measures are also required to combat this pest on pepper crops.

To become more sustainable, the control strategy requires a biological component that will act continuously throughout the growing season. Preliminary investigations indicated that the parasitoids, *Leptomastix epona, Anagyrus pseudococci* and *Pseudaphycus maculipennis*, had potential to fulfil this role. *Leptomastix epona* and *Anagyrus pseudococci* can be released in UK crops but stocks must first be built up to match demand. *Pseudaphycus maculipennis* is not indigenous to the UK, but, as a specialist parasitoid of a glasshouse pest, it is anticipated that a licence to release in glasshouses could be obtained.

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

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

This project draws on complementary expertise from both research and industry backgrounds, including invertebrate biologists, specialists in parasitoid rearing, IPM practitioners, tomato crop agronomists and statisticians.

Summary of the work and main conclusions

Design of an IPM strategy

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

- Survival of mealy bugs on the glasshouse structure between crops - Hyvis 30 Emulsion (a polybutene based glue), Jet 5 (peroxyacetic acid) and undiluted vinegar have been shown to reduce the numbers of nymphs emerging from egg sacs on concrete by 50%, 67% and 81% respectively. Although this will reduce initial crop invasion, there may be up to 500 eggs in each egg sac and further control measures will be required.
- Initial crop invasion by overwintered survivors - A 2% dilution of Savona has been effective against first instar *P. viburni* as they colonise tomato plants, reducing numbers by up to 93%.
- Season-long suppression of mealybug population growth - Three parasitoids, *Leptomastix epona*, *Anagyrus pseudococci* and *Pseudaphycus maculipennis*, have potential for this role.
- A compatible second line of defence - This is 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 has also been shown to have a direct effect on other life cycle stages on the plant but the results have been variable; 2% and 4% dilutions giving 30-60% and 40-100% control respectively. Control will therefore be dependant on a series of applications. In addition, the effect of *Verticillium lecanii* (Mycotal WP) may 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*. These experiments are not yet complete but results to date indicate that all three parasitoids have a development time that is considerably shorter (*i.e.* approximately 50%) than the mealybug.
- 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 appear to be no fundamental reasons why these parasitoids will not become established in populations of *P. viburni* on tomato and pepper plants. However, there are questions over their ability to control *P. viburni* at the population level due to the huge numbers of offspring produced by the pest. *Anagyrus pseudococci* appears 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*, will be tested at the population level in larger glasshouse scale experiments in 2005 (subject to a licence being granted for *P. maculipennis*).

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.

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. Discussions within the project team will determine whether the combination of Mycotal and Savona should be pursued in commercial crops in 2005.

There is some evidence that parasitic nematodes will attack mealybugs and this is another option for a second line of defence treatment. However, this will require some preliminary research to determine the potential in commercial crops.

Financial benefits to growers

Surveys in 1998/99 confirmed that the incidence of *P. viburni* infestations was increasing on UK tomato crops, with approximately 7% of the national crop affected at that time. 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 the grower survey, 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 approximately £600 per hectare. The total cost of a mealybug infestation to a tomato business is therefore in the region of £3,800 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

The work is in the early stages so there are no new actions points at this stage.

Please refer to the report for HDC project PC 161 and HDC factsheet 25/00 'Mealybugs on protected tomato crops' for information on previous work.

Science Section

PART 1: GENERAL INTRODUCTION

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. There are three immature mealybug stages (first, second and third instar nymphs), which are similar in appearance to adult females. 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 the obscure mealybug (*Pseudococcus viburni*) on protected tomato crops in the UK.

HDC project, PC 161, which was completed in 2002, investigated the increase in incidence of infestations of the *P. viburni* in UK tomato crops and began to formulate a control strategy (Jacobson & Croft, 2002). The following notes summarise the key findings:

- The most effective and IPM compatible method of controlling mealybugs on tomato plants during the production season is the insect growth regulator, buprofezin (Applaud), but this may not be applied to organic crops. There is also a risk of resistance developing if control programmes become too heavily dependant on this chemical.
- Crop Oil proved to be very effective at removing wax from motile and egg stages of mealybugs, and this led to high levels of mortality. However, this is a paraffinic oil and may not be applied to organic crops.
- A 2% dilution of Savona was effective against first instar mealy bugs as they first colonised tomato plants, reducing numbers by 93%.
- Savona has also been shown to have a direct effect on other life cycle stages on the plant but the results have been variable; 2% and 4% dilutions giving 30-60% and 40-100% control respectively. Control would therefore be dependant on a series of applications.
- The effect of Mycotal WP on mealybugs may be enhanced by applying after Savona. The results of this sequence of treatments have been variable but 100% mortality has been achieved in some laboratory experiments. Once again, control would be dependant on a series of applications.
- Hyvis 30 Emulsion (a polybutene based glue), Jet 5 (peroxyacetic acid) and undiluted vinegar reduced the numbers of nymphs emerging from egg sacs on concrete by 50%, 67% and 81% respectively. However, there may be up to 500 eggs in each egg sac and these levels of control may be inadequate for commercial crops.

The measures listed above provide the basis of a control programme for conventional crops. However, the strategy is heavily dependant on chemical insecticides and is not consistent with the TGA's long term goal of pesticide-free crop production. Furthermore, the key product for conventional crops (buprofezin) may be withdrawn from the UK market (Hayman, pers. com.). There are still large gaps in the control strategy for organic crops. Sustainable control measures are now also required to combat this pest on pepper crops.

To become sustainable, the control strategy requires a biological component that will 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 indicate that the parasitoids, *Leptomastix epona* (EPPO, 2002) and *Pseudaphycus maculipennis* (Charles, 2001) have potential to fulfil this role. *Leptomastix epona* can be released in UK crops but stocks must first be built up to match demand. *Pseudaphycus maculipennis* is not indigenous to the UK but, as a specialist parasitoid of a glasshouse pest, it is anticipated that a licence to release in glasshouses could be obtained.

The development and successful application of biological control for mealybugs relies on an understanding of the biology and population dynamics of the pest and natural enemy (Gutierrez, 1993). Important factors to determine for the tomato and pepper crop environments are:

- Stage of mealybug life cycle attacked and rate of parasitism (Karamaouna & Copland, 2000a,b).
- Failure of parasitoids to survive within mealybugs due to encapsulation by the host (Blumberg, 1997; Blumberg & Van Driesche, 2001).
- The influence of plant architecture and morphology on the success of parasitism (Cloyd & Sadof, 2000). (The tomato plant environment is known to be hostile to some natural enemies [Jacobson & Croft, 2000]).

This project draws on the complementary expertise from both research and industry backgrounds, including invertebrate biologists, specialists in parasitoid rearing, IPM practitioners, tomato crop agronomists and statisticians.

Overall aim and objectives

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

The specific objectives are:

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

The project will progress in three sequential phases: i) collation of information and basic biological studies, ii) formulation of control strategies, and iii) implementation of strategies in commercial crops.

PART 2. DESIGN OF THE PROPOSED IPM PROGRAMME

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

1. Survival of mealy bugs on the glasshouse structure between crops.

Hyvis 30 Emulsion (a polybutene based glue), Jet 5 (peroxyacetic acid) and undiluted vinegar have been shown to reduce the numbers of nymphs emerging from egg sacs on concrete by 50%, 67% and 81% respectively (Jacobson & Croft, 2002). Although this will reduce crop invasion, there may be up to 500 eggs in each egg sac and further control measures will be required.

2. Initial crop invasion by overwintered survivors.

A 2% dilution of Savona was effective against first instar mealy bugs as they first colonised tomato plants, reducing numbers by 93% (Jacobson & Croft, 2002).

3. Season-long suppression of mealybug population growth by a primary biological control agent

To become more sustainable, a biological component is required that will act continuously throughout the growing season. Three parasitoids, *Leptomastix epona*, *Anagyrus pseudococci* and *Pseudaphycus maculipennis*, have potential to fulfil this role and will be evaluated in this project (Section 3).

4. A compatible second line of defence

This is 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 has also been shown to have a direct effect on other life cycle stages on the plant but the results have been variable; 2% and 4% dilutions giving 30-60% and 40-100% control respectively (Jacobson & Croft, 2002). Control will therefore be dependant on a series of applications.

The effect of *Verticillium lecanii* (Mycotal WP) on mealybugs may be enhanced by applying after Savona. The results of this sequence of treatments have been variable but 100% mortality has been achieved in some laboratory experiments (Jacobson & Croft, 2002). Once again, control would be dependant on a series of applications (Section 4).

There is some evidence that parasitic nematodes will attack mealybugs and this is another option for a second line of defence treatment. However, this will require some preliminary research to determine the potential in commercial crops.

PART 3: DEVELOPMENT OF A ROBUST BIOLOGICAL CONTROL MEASURE BASED ON PARASITOIDS

Background:

The parasitoids, *Anagyrus pseudococci* (Girault), *Leptomastix epona* (Walker) and *Pseudaphycus maculipennis* (Mercet) (Encyrtidae:Chalcidoidea), are all known to parasitise *P. viburni* and therefore have the potential for use in a control programme.

Although *A. pseudococci* and *L. epona* are known to attack other species of the *Pseudococcus* genus on a broad range of host plants, there is no relevant information about their performance on glasshouse pepper and tomato crops. It is particularly important to investigate their performance on tomato because these plants can present a fairly hostile environment to invertebrates (Jacobson and Croft, 2000) and impair the activities of beneficial species.

Pseudaphycus maculipennis is considered to be a specific parasitoid of *P. viburni* but there is little information available about its biology, behaviour and performance on different types of plants. It is therefore important to determine its potential performance on glasshouse tomato and pepper crops.

Anagyrus pseudococci and *L. epona* can be released in UK crops but *P. maculipennis* does not currently have official approval. However, as very specialised parasitoid of a non-indigenous pest that is restricted to semi-protected locations, the project team were reasonably confident that a licence to release in UK glasshouses could be obtained. Furthermore, there is a precedent because, following careful evaluation, it has been successfully introduced for the control *P. viburni* in New Zealand (John Charles, pers.comm.). As part of this project, the application for a licence to release *P. maculipennis* in the UK has been prepared and submitted to Defra.

The following series of experiments quantified key biological parameters of the three parasitoids to ascertain which of the species would provide the best level of control for *P. viburni* in glasshouse tomato and pepper crops.

3. 1. Development stages of *P. viburni* attacked by the parasitoids

Introduction:

Some species of parasitoid have a strong preference for specific instars or development stages of their insect host. For example, some species will not oviposit in the younger instars of their host because they are too small to allow their offspring to complete their development. This is important knowledge when designing a control programme because it helps to determine the correct time to release the parasitoids in relation to the pest population development. It also helps to predict the proportion of the pest population that will be attacked and the time that will be required to achieve control.

Psecdococcus viburni has three larval stages. The first instar is extremely small and is not attacked by *L. epona* (Karamaouna & Copland, 2000). However, there is little such information about the preferences of the other parasitoid species on the various mealybug life cycle stages.

The following experiment was designed to establish which development stages were attacked by *A. pseudococci*, *L. epona* and *P. maculipennis*.

Materials and methods:

Twenty mealybugs (*i.e.* five second instars, five third instars, five young adults and five egg-laying adults) were placed on parts of either tomato or pepper plants in a plastic ventilated box (25x11x12cm). The cut stems of the plants were held in damp cotton wool to keep them in good condition for the duration of the experiment.

For the first 24 hours, the three species of parasitoids were fed on diluted sugar solution (50%) and exposed to mealybugs on either tomato or pepper plants depending on their final destination. Single female parasitoids were then placed in each of the plastic boxes and kept in a controlled environment room (16L:8D, 21 ± 2^0C). The parasitoids were removed after 48 hours. The mealybugs were dissected after five days and the presence or absence of eggs was recorded.

There were 24 different combinations of parasitoids, pest life cycle stages and plants. Each was replicated four times.

Results and discussion:

The proportion of the various mealybug life cycle stages that were attacked by each species of parasitoid is shown in Table 1.

The results showed that all three species of parasitoids would lay eggs in all the *P. viburni* life cycle stages. The only failure was by *A. pseudococci* against second instar *P. viburni* on tomato. In general, the smallest instar (*i.e.* second) was the least preferred. The larger hosts are probably selected by the ovipositing females because they provide more food for the developing parasitoid larvae and therefore increase their chance of survival. The results suggest that the releases of all three species of parasitoids should be directed towards the larger life cycle stages.

The larger instars were used as hosts for the parasitoids in the subsequent experiments.

Table 1. The mean proportion (±sd) of mealybugs attacked at each development stage (five individuals per replicate, four replicates per development stage).

Plant species	Mealybug development stage	Species of parasitoid		
		L. epona	*P. maculipennis*	*A. pseudococci*
Pepper	2nd	0.25 (0.30)	0.30 (0.26)	0.25 (0.30)
	3rd	0.30 (0.20)	0.40 (0.33)	0.15 (0.30)
	Adult-young	0.45 (0.25)	0.45 (0.30)	0.25 (0.40)
	Adult-egg laying	0.40 (0.28)	0.50 (0.35)	0.15 (0.30)
Tomato	2nd	0.20 (0.16)	0.15 (0.10)	0.00 (0.00)
	3rd	0.40 (0.37)	0.30 (0.26)	0.10 (0.20)
	Adult-young	0.30 (0.20)	0.10 (0.12)	0.05 (0.10)
	Adult-egg laying	0.45 (0.34)	0.50 (0.12)	0.15 (0.19)

3. 2. Comparison of egg production by *A. pseudococci*, *L epona* and *P. maculipennis*

Introduction:

All three species of parasitoids are synovygenic; *i.e.* the females emerge with a relatively small number of mature eggs in their ovaries and they must feed to enable egg production to continue.
Preliminary studies by the authors and partners indicated that the females of these species did not feed directly on the mealybug hosts. Their most likely sources of nutrition would appear to be honey dew and nectar.

For a parasitoid to successfully parasitise a host, it must overcome the hosts' immune system. In most established parasitoid / host relationships, the parasitoid has successfully evolved a mechanism to achieve this. If a parasitoid oviposits in a non-suitable host, the host immune system will attack the parasitoids' egg or larvae through a process called encapsulation. This involves the parasitoid egg or larva becoming surrounded and killed by the hosts blood cells (haemocytes) and the pigment melanin. Although some parasitoids, such as *L. epona*, successfully attack some species of host, a percentage of the offspring are encapsulated and fail to complete their development. Blumberg & Driesche (2001) showed the encapsulation rates for *L. epona* on *P. viburni* to be 33.4% at a constant 23^0C, with temperature having an effect on these rates.

Fecundity in parasitoids can either be recorded as the potential fecundity (*i.e.* the number of eggs the parasitoid will produce) or the real fecundity (*i.e.* the actual number of offspring that complete their development and emerge as adults).

The following experiment compared the fecundity of *L. epona*, *P. maculipennis* and *A. pseudococci*. Three to four day old females were used, as this is generally the peak period for oviposition. Prior to the experiment, females were given a feed (50% sugar

solution) to optimise oviposition, and were exposed to *P. viburni* on peppers or tomatoes to provide some host experience. The latter can also influence oviposition. Encapsulation was also taken into account.

Materials and methods:

Thirty individual mealybugs (*i.e.* a mixture of third instars, young adults and pre-ovipositing adults) were placed on parts of tomato or pepper plants in a ventilated plastic box in a controlled environment room (16L:8D, 21 ± 2^0C) as described in section 3.1. One 2-3 day old female parasitoid (previously mated, fed and exposed to mealybugs on the relevant plants) was then placed in each box for 24 hours. The mealybugs were dissected after 7-10 days and the numbers of parasitoid larvae, and encapsulated eggs and larvae, were recorded. There were 16 replicates for each species of parasitoid with replication over time.

Analysis of the results was done using ANOVA and LSD on the square root transformed data.

Results and discussion:

The mean numbers of parasitoid offspring produced over 24 hours are shown in Table 2. These data include adult females that produced no offspring and encapsulated larvae.

Under the environmental conditions in the experiment, *P. maculipennis* produced the most offspring on both pepper and tomato plants (4.46 and 3.38 respectively). However, this was only significantly greater when compared to *A. pseudococci*. Although both *P. maculipennis* and *A. pseudococci* produced more offspring in *P. viburni* on pepper than tomato plants, these results were not significant at the 95% level ($p>0.05$).

Encapsulation of *L. epona* larvae (but not eggs) was observed, with 8% and 17% of offspring encapsulated in mealybugs on tomato and pepper respectively. However, some of these larvae were seen escaping from the encapsulation cocoon and so it is not known what proportion are prevented from completing their development. Furthermore, it is possible that temperature could affect both the rate and stage of encapsulation. More detailed studies would be required to quantify this effect under varying environmental conditions.

It is difficult to produce the optimum environment for oviposition in synovygenic species because feeding, temperature and pre-oviposition period requirements all have to be correct. It is therefore possible that the female parasitoids that failed to produce offspring in the experiment did so because there was something sub-optimal about the artificial environment within the experimental arena. To take this possibility into account, female parasitoids that produced no offspring were excluded from the statistical analysis of results shown in Table 3. These data show that *P. maculipennis* produced significantly more offspring ($p<0.05$) than *L. epona* and *A. pseudococci* on pepper, but only more than *A. pseudococci* on tomato.

Table 2. Mean number and square root transformed mean of offspring produced (over 24 hours) including adult females that produced no offspring and encapsulated larvae.

	Pepper		Tomato	
Species	Mean	Sqrt mean	Mean	Sqrt mean
L. epona	2.31	1.30	2.38	1.25
P. maculipennis	4.56	1.86	3.38	1.48
A. pseudococci	0.94	0.71	0.88	0.69
LSD (45df)		0.626		0.661

Table 3. Mean number and square root transformed mean of offspring produced, including encapsulated larvae (over 24 hours), but excluding adult females producing no offspring.

	Pepper		Tomato	
Species	Mean	Sqrt mean	Mean	Sqrt mean
L. epona	2.85	1.48[1]	3.45	1.81[1]
P. maculipennis	5.21	2.13[1,2]	4.50	1.97[1,2]
A. pseudococci	1.67	1.25[2]	1.56	1.22[2]
LSD[1]		0.591		0.493
LSD[2]		0.623		0.530

[1,2]For comparison of different means different LSD values were estimated

In conclusion, *P. maculipennis* was shown to be the most fecund species, but this was not always significantly greater than that of *L. epona*. This reflects the gregarious nature of *P. maculipennis*; *i.e.* up to seven offspring can develop within a single host. *Leptomastix epona* produces only one offspring per host but it is possible that she will attack more individuals per day. Further work would be required to establish which of these reproductive strategies would be the most successful against *P. viburni* in a tomato or pepper crop environment. *Anagyrus pseudococci* did not produce as many offspring as the other two species but it is possible that this tropical species would have greater fecundity at higher average temperatures.

3. 3. Development period of *A. pseudococci*, *L epona* and *P. maculipennis*

Introduction:

Development time is an important factor in predicting the potential of a parasitoid to provide control of the target pest. The shorter the development time of the parasitoid, the more generations that will occur within each pest generation. This will result in the production of more parasitoids relative to pests and improve the chances of keeping the pest population under control.

The following experiment compared the egg to adult development period for *L. epona*, *P. maculipennis* and *A. pseudococci* on *P. viburni* on tomato and pepper plants.

Materials and methods:

Ten mealybugs (*i.e.* five third and five young adult stages) were placed on parts of either tomato and pepper plants in a plastic box in a controlled environment room (16L:8D, 21±2^{0}C) as described in section 3.1. A single female parasitoid (previously fed and given host experience) was then placed in each box for 24 hours. The time taken for her offspring to develop to adult was recorded. There were 15 replicates per parasitoid species.

Results and discussion:

When this report was written, we were still waiting for the slower parasitoids to complete their development. As a consequence, the data are incomplete and have not yet been analysed. The results to date are summarised in Table 4. While they provide a slightly optimistic impression of development time, they are useful for drawing comparisons between the three species.

The mean development period from egg to adult is shorter for *P. maculipennis* than both *L. epona* and *A. pseudococci*. This will be an advantage when attempting to control *P. viburni*.

These results, and the low rates of fecundity that were recorded in section 3.2, suggest that *A. pseudococci* prefers a higher average temperature than is common in tomato and pepper crops. This observation is consistent with previous work, which showed that the optimum temperature for this parasitoid's development was 24.7^{0}C (Daane *et al*., 2004).

All three species have a more rapid generation time than *P. viburni*, which takes about 50 days to develop from egg to adult at 21 ± 2^{0}C (Heidari, 1989). This should aid control. However, *P. viburni* is highly fecund with egg sacs containing around 500 eggs. Further studies are required to determine whether the more rapid generation time of the parasitoids will compensate for the great number of eggs produced by the host.

Table 4. The mean development period (±sd) of *L. epona, P. maculipennis* and *A. pseudococci* in *P. viburni* on pepper or tomato plants.

Species of parasitoid	Sex	Mean development period of from egg to adults (no of days)	
		Pepper	Tomato
L. epona	Male		
	Female	25.5 (2.1)	
P. maculipennis	Male	18.0 (0.0)	18.0 (0.0)
	Female	18.0 (0.0)	18.2 (0.4)
A. pseudococci	Male	24.7 (0.6)	
	Female		

3. 4. The ability of *L. epona* and *P. maculipennis* to locate and oviposit in *P. viburni* on pepper and tomato plants.

Introduction:

Host finding and selection by parasitoids usually involves responses to a hierarchy of stimuli that ultimately bring the parasitoid into contact with its target. These steps to successful parasitisation, which can overlap and are not all obligatory, involve the following stages; i) host habitat location, ii) responses to indicators of host presence (e.g. honeydew), iii) structured searching patterns on host plants, iv) host location, v) host suitability, vi) host acceptance and vii) host regulation.

In experiment 3.2, the fecundity of the three parasitoids was compared over 24 hours in a laboratory environment. The method was designed to reduce the number of variable factors and allow the parasitoids to locate the insect host without first having to locate the host's habitat, as would be the situation in a commercial glasshouse.

The following experiments increased the number of variables by adding host habitat recognition and structured searching patterns on whole plants to the various stages associated with insect host location and acceptance. In other words, the parasitoids would have to recognise and accept tomato or pepper plants as a host habitat before finding and attacking the mealybugs. The fecundity of the two most promising parasitoid species, *L. epona* and *P. maculipennis*, was thus determined in a larger arena incorporating a more realistic range of behavioural variables.

Materials and methods:

Ten mealybugs (third instars and young adults) were placed on either the base of stems of tomato plants or the tops of pepper plants (0.5m tall). After a period of acclimatisation, the two species of plants were placed in separate screened experimental units. Approximately 20 female parasitoids (either *P. maculipennis* or *L. epona*) were released in each of the experimental units near to the position of the plants. After seven days the mealybugs were removed from the plants, dissected in the laboratory and the numbers of parasitoid larvae recorded. There were four treatments, comprising different combinations of plants and parasitoids, and five replicates per treatment.

Results and discussion:

The mean percentage of *P. viburni* parasitised by *P. maculipennis* and *L. epona* on whole pepper and tomato plants is shown in Table 5. Both *P. maculipennis* and *L. epona* were able to locate and oviposit in *P. viburni* on pepper and tomato plants. Both species located 18-26% of the mealybugs but there was a high variation around this mean.

The percentage of *P. viburni* attacked by both species of parasitoid was relatively low due to the short duration of the experiment. The large scale glasshouse trials designed for 2005 will provide a more realistic measurement of the parasioids' capabilities.

Table 5. The mean percentage (±sd) of mealybug (*P. viburni*) parasitised by *P. maculipennis* and *L epona* on pepper and tomato plants

Species of parasitoid	Pepper	Tomato
L. epona	26.0 (24.1)	22.0 (19.2)
P. maculipennis	24.0 (25.1)	18.0 (13.0)

The mean total number of larvae produced by the two species of parasitoid is given in Table 6. The numbers of larvae produced by *P. maculipennis* on pepper and tomato plants was 3.8 and 3.2 respectively, which was approximately 50% greater than the numbers produced by *L. epona* (2.6-pepper, 2.2-tomato). However, the large variations around the means did not allow any comparisons of statistical significance.

The combination of the results shown in Tables 5 and 6, demonstrate that the gregarious parasitoid, *P. maculipennis*, produces larger numbers of offspring from smaller numbers of attacked hosts than the solitary parasitoid, *L. epona.* This was consistent with observations in experiment 3.2.

Table 6. The mean numbers (±sd) of parasitoid larvae in *P. viburni* on tomato and pepper plants

Species of parasitoid	Pepper	Tomato
L. epona	2.6 (2.4)	2.2 (1.9)
P. maculipennis	3.8 (3.7)	3.2 (2.8)

These results clearly demonstrate that both *P. maculipennis* and *L. epona* are capable of locating *P. viburni* on whole tomato and pepper plants and that they should become established within populations of the pest. However, the level of control that could be achieved in a commercial crop has yet to be determined. The programme of work planned for 2005 will give a more realistic estimate of the parasitoids performance under glasshouse conditions.

Overall conclusions

This series of experiments has 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 develop in *P. viburni* on tomato and pepper plants:

- *Leptomastix epona*, *A. pseudococci* and *P. maculipennis* will 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 of the parasitoids in *P. viburni* on tomato or pepper plants over 24 hours. However, the fecundity of *P. maculipennis* and *L. epona* was higher than that of *A. pseudococci*.
- The development period of *P. maculipennis* was shorter than that of *L. epona* and *A. pseudococci*. Results to date indicate that all three 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 *P. viburni* on tomatoes and peppers, demonstrating that the habitat provided by those plants was acceptable to the parasitoids.

There appear to be no fundamental reasons why these parasitoids will not become established in populations of *P. viburni* on tomato and pepper plants. However, there are questions over their ability to control *P. viburni* at the population level due to the huge numbers of offspring produced by the pest. *Anagyrus pseudococci* appears to be the weakest candidate due to its poorer performance at the temperatures that are common in pepper and tomato crops in the early season. The stronger candidates, *L. epona* and *P. maculipennis*, will be tested at the population level in large glasshouse scale experiments in 2005 (subject to a licence being granted for *P. maculipennis*).

In the longer term, it is anticipated that one or both of these species will become a component in a larger IPM programme against mealybugs in tomato and pepper crops.

PART 4: FURTHER DEVELOPMENT AND EVALUATION OF SECOND LINE OF DEFENCE MEASURES IN COMMERCIAL CROPS

Background:

Some UK growers who used the fungal pathogen, *Verticillium lecanii* (Mycotal WP), against glasshouse whiteflies, reported incidental control of mealybugs but this was not confirmed in controlled experiments. Laboratory bioassays done under ideal conditions in the previous project (Jacobson & Croft, 2002) 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. In the present trial, the studies were scaled up to a commercial tomato crop within the Wight Salads Group.

Materials and methods:

The trial was established in ten rows (each 352 plants/heads) in glasshouse 11 at 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 three treatments; 4% Savona, 4% Savona plus Mycotal and untreated controls. Savona and Mycotal 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 Savona and Savona plus Mycotal treatments were each replicated in four complete rows. To reduce the risk to the rest of the site from untreated plants, the control was limited to a single row. However, to compensate, there were more sample points within that row.

A pre-treatment assessment of numbers of mealybugs on the horizontal tomato stem bundles was completed on 14 July 2004. This involved marking five 600mm lengths of stem bundles in each row and recording the number of healthy adults, nymphs and egg sacs present.

The first Savona and Mycotal treatments were applied on 15 and 16 July 2004 respectively. These were followed on 22 July 2004 by a post treatment assessment of the previously marked sections of stem.

At this point, a subsidiary experiment demonstrated that Savona and Mycotal could be mixed without any detrimental effect on the number of colony forming units (CFUs) produced by *V. lecanii*. Therefore, subsequent treatments of Savona and Mycotal were applied as tank mixes.

Savona and Savona plus Mycotal treatments were repeated twice; *i.e.* on 25 July and 5 August. The final post-treatment assessment was completed according to the above procedure on 17 August 2004.

Results and discussion:

As may be expected from a trial based on a naturally occurring infestation, the results were very variable and not suitable for statistical analysis. However, broad trends were observed:

Post-treatment assessments (22 July)

Most survivors were under the horizontal stems, particularly where these were pressed closely together in the bundles. This indicated a failure to achieve spray contact with the mealybugs. In summary, the results showed:

- Control - numbers of mealybugs increased by 132% overall
- Savona – numbers decreased by 52%
- Savona + Mycotal – numbers decreased by 61%

It was highly unlikely that the Savona and Savona plus Mycotal treatments were significantly different due to the inherent variation within treatments. This indicated that there had been no additional effect by the fungus. Furthermore, no fungal growth was evident on the dead mealybugs on the plants, nor did any develop when the cadavers were removed and incubated under ideal conditions in the laboratory.

There was no evidence of any natural mortality of mealybugs on the untreated control plants and their numbers increased between assessments. This unconstrained population growth was beginning to cause concern to the growers due to the risk of spread to the rest of their crops. The control had already provided an indication of the rate of population growth and was therefore terminated.

Post-treatment assessments (17 August)

Once again, most survivors were under horizontal stems suggesting inadequate spray coverage. In summary:

- Savona – numbers of mealybugs decreased overall by 57%
- Savona + Mycotal – numbers decreased overall by 7%

Although the overall reduction in numbers of mealybugs in the treatment of Savona with Mycotal was greater than with Savona alone, there was large inherent variation and some sample points still contained large numbers of healthy individuals. No fungal growth was recorded on the cadavers either in the glasshouse or in subsequent incubation tests.

Conclusion:

These results showed a small advantage in using Mycotal in addition to Savona over three applications at approximately seven day intervals. This was contrary to the previous small-scale studies at STC where the combined effect had been much greater. 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.

Discussions with the project team will determine whether the combination of Mycotal and Savona should be pursued in commercial crops in 2005.

There is some evidence that parasitic nematodes will attack mealybug-related insects (Verdun, pers. comm.). This will be investigated in the laboratory during 2005 with a view to instigating larger scale studies either within or in parallel to this project.

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