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The results and conclusions in this report are based on a series of grower surveys and experiments conducted over a 4 year period. 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.

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GROWER SUMMARY

Headlines

- The most effective and IPM compatible method of controlling the obscure mealybug on tomato plants is the insect growth regulator, buprofezin (Applaud).
- A 2% dilution of Savona is effective against first instar mealybugs as they first colonise tomato plants, and hence offers a valuable alternative to Applaud for control at the start of the season. Savona is approved for use on organic tomato crops.
- The efficacy of Mycotal WP for the control on mealybugs on tomato plants will be enhanced if applied after Savona. Results can be variable but this sequential treatment ought to help to reduce mealybug numbers particularly in organic crops where the use of Applaud is prohibited.
- Hyvis 30 Emulsion (a polybutene based glue), Jet 5 (peroxyacetic acid) and undiluted vinegar will reduce the numbers of nymphs emerging from egg sacs on concrete dollies.

Background and expected deliverables

World-wide, mealybugs are one of the most significant pest groups, with over 3,000 species known to feed on a wide range of plant families in habitats varying from the soil to tree tops. This project was prompted by an apparent increase in the incidence of mealybugs on protected tomato crops in the UK.

The overall objectives were to determine the current pest status of mealybugs on protected tomato crops throughout the UK, to provide growers with information on the efficacy of current control techniques and advice on improving that control.

The expected deliverables from the work included:

- A clear indication of the scale of the threat posed by mealybugs to the UK tomato industry.
- An improved understanding of the biology and behaviour of mealybugs in tomato crops, with particular reference to their ability to survive in the empty glasshouse between crops.
- Guidance on the most effective IPM compatible control measures currently available.
- An evaluation of new biological and novel pesticidal control measures for both conventional and organic tomato crops.

Summary of the project and main conclusions

1. Survey of he pest status of mealybugs and available control measures

In the first year of the project (1998/1999), a grower survey confirmed that the incidence of obscure mealybug (*Pseudococcus viburni*) infestations was increasing on UK tomato crops, with approximately 7% of the national crop affected at that time. Reported yield losses were valued as high as £15,000 on one nursery with costs of control estimated around £3,000 per ha per season. The infestations most commonly resulted in damaged plant stems, contamination with sticky honeydew and secondary infections of *Botrytis*. Mealybugs were usually transported on to uninfested nurseries on infested plants (typically ornamental 'house plants') or on equipment. Spread within infested nurseries occurred when irrigation lines or packing boxes were moved from mealybug infested areas to new areas without first being cleaned and sterilised. Crop monitoring confirmed that individuals survived between crops as eggs, most commonly located on the concrete bases (dollies) of roof supports, on dwarf walls and on irrigation drippers.

The survey also compiled information about a wide variety of control methods that had been used in commercial crops. The most effective insecticides against the motile stages of mealybugs were Applaud, Decis and Malathion. Of these, Applaud was least damaging to the biological control agents used against other pests and was recommended as the first choice control measure for growers of conventional crops.

Physical control methods included hand rubbing, burning with propane burners and spraying with oils / detergents. Although these methods were less effective than chemical insecticides, they did suppress the pest population and prevented economic damage. The numbers of nymphs emerging onto the new crop were reduced by painting the concrete dollies in the affected areas with a thick paint or glue, covering them in polythene and sealing all joints with glue. Although effective, this method was labour intensive and expensive.

Details of the pest status of the obscure mealybug, and an assessment of control measures for tomato crops are provided in an HDC Fact Sheet (Reference 25/00).

2. Evaluation of new measures to control mealybug egg sacs

Growers require a cost-effective method of controlling mealybug eggs that survive between crops on concrete dollies and similar structures.

Four products (Hortichem Spraying Oil, Hyvis 30 Emulsion, Horticide and Malathion) were evaluated in a small-scale bioassay. Only Hyvis 30 Emulsion performed significantly better than controls sprayed with water only. This polybutene based "glue" reduced the number of surviving nymphs by 50%. However, there may be up to 500 eggs in each egg sac and this level of control may still be inadequate for commercial crops. The results were consistent with observations on commercial nurseries.

In laboratory bioassays, sprays of undiluted vinegar and Jet 5 reduced hatching and survival of first instar nymphs on concrete surfaces by 81% and 67%, respectively. Both treatments were more effective than the best product previously tested, *i.e.* Hyvis 30 Emulsion. However, with so many eggs in each egg sac even 81% control may be inadequate to protect a commercial crop. Further studies are required to determine whether repeated and well-targeted applications of vinegar or Jet 5 would provide more satisfactory control of egg sacs on concrete structures in the glasshouse.

3. Evaluation of new measures to control mealybugs at the start of the growing season

At the beginning of the tomato growing season, *P. viburni* first instar nymphs hatch from eggs on the fabric of the building and migrate onto the tomato plants. Previous studies within this project have demonstrated that these nymphs can be controlled with foliar applications of the insect growth regulator, buprofezin (Applaud). However, this product cannot be used on crops grown to organic standards.

Savona, vinegar and Mycotal (with and without the oil-based adjuvant, Addit) were evaluated against first instar mealybugs as they first colonised tomato plants. A 2% dilution of Savona was very effective against this life cycle stage, reducing numbers by 93%. The percentage of survivors in the Mycotal (+/- Addit), Addit and vinegar treatments were not significantly different to water applied alone; the overall reduction being 38%.

Therefore Savona at 2% provides a reasonably good method of controlling mealybug nymphs that invade tomato plants at the start of the season.

4. Evaluation of new measures to control mealybugs during the growing season

<u>Lacewing predators</u>
In laboratory bioassays, the predatory lacewing, *Chrysoperla carnea*, was shown to attack all mealybug life cycle stages, although first instar nymphs were their preferred prey (10 consumed per day). The results indicated that *C. carnea* had potential for use as a biological control agent against the obscure mealybug. However, further studies are required to determine whether the predators will remain on tomato plants, feed on the pests in that environment and form breeding colonies within the glasshouse.

<u>Hypoaspis mites</u>
Two species of predatory mites, *Hypoaspis miles* and *H. aculeifer*, were found to be unsuitable for the control of obscure mealybug on tomato crops despite being used by several growers.

<u>Chess (pymetrozine) – an antifeedant pesticide</u>
Chess (pymetrozine), an antifeedant chemical that is specific to some insect relatives of mealybugs (*e.g.* aphids and whiteflies), was evaluated against the obscure mealybug on tomato plants in a crop-scale experiment. The results showed that two applications, at rates of up to 80g product per 100 litre of water, provided some control but this was inadequate for commercial crops.

Pheromone traps

The female citrus mealybug, *Plannococcus citri*, produces a sex pheromone that has been synthesised and used to trap males of that species in commercial crops. Although sex pheromones are usually specific to individual species of insects, some will attract closely related species. The citrus mealybug pheromone was therefore incorporated into lures on sticky traps and tested in tomato crops that were heavily infested with the obscure mealy bug. The numbers of males caught were very small and it was concluded that the pheromone was not attractive to this species.

Fungal pathogens

Two commercial products of the fungal pathogen, *Beauveria bassiana* (Naturalis L and Botanigard WP), were evaluated against the obscure mealy bug in laboratory bioassays but neither product gave significant control of the pest.

Laboratory bioassays showed that another fungal pathogen, *Verticillium lecanii* (Mycotal), reduced the numbers of first instar nymphs by approximately 8%. It was proposed that this effect could be enhanced by applying *V. lecanii* in combination with dewaxing agents that could break down the mealybugs' waxy protection and allow more fungal spores to come into contact with the insect's body.

Fungal pathogens plus de-waxing agents

Savona, vinegar and Mycotal (with and without the oil-based adjuvant, Addit) were evaluated against older nymphs and adult mealy bugs, thus simulating control on plants during the main crop production season. Vinegar and Mycotal had little effect. The results with Savona were variable; 2% and 4% dilutions giving 30-60% and 40-100% control respectively. The level of control achieved with this soft soap is always likely to be variable because the results depend on a secondary effect; *i.e.* the primary effect being wax destruction and the secondary effect being dehydration.

A series of laboratory experiments were designed to investigate the possibility of enhancing the effect of Mycotal by applying it in combination with oils or wetters that could break down the waxy deposits and allow more spores to come into contact with the insect's body.

The first experiment examined the physical effects of a wide range of oils / wetters on the waxy deposits. In preliminary studies, Crop Oil had given good results in this respect and although it was not suitable for organic production it was included as a positive control. Overall, Crop Oil did prove to be the most effective product, almost completely dewaxing adults and egg sacs within 24 hours and causing high levels of mortality [see Plates A & B]. A 2% solution of Savona was also reasonably effective, removing wax evenly across the body and leaving just a light and incomplete sprinkling of white deposits [see Plates A & C]. A marginal improvement was obtained by increasing the concentration of Savona to 4%.

Plate A below: Typical untreated adult (left) and egg sac (right)

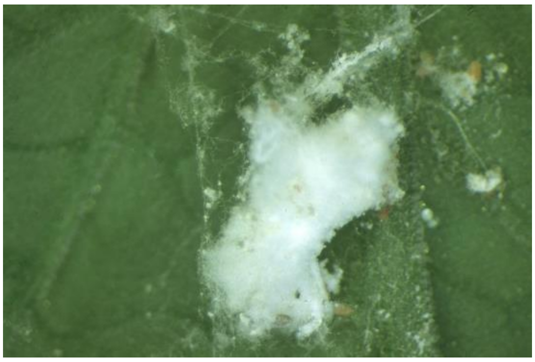
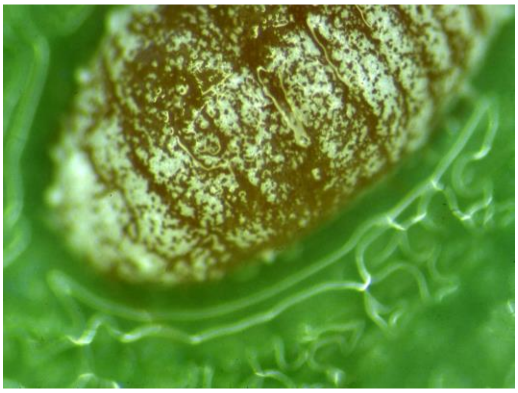
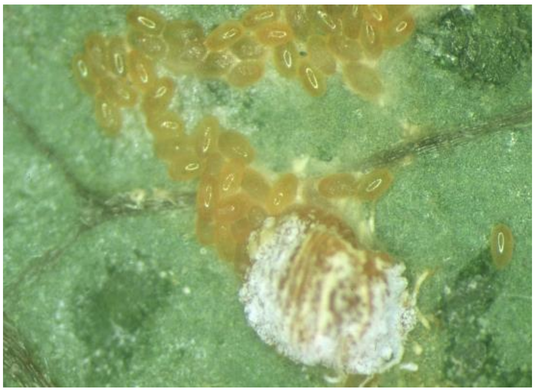


Plate B below: Adults (left) and egg sac following treatment with Crop Oil

Plate C below: Adults (left) and egg sac following treatment with 2% Savona





Sequential applications of a dewaxing agent followed by Mycotal were first evaluated against mealybug adults. Crop Oil and 4% Savona caused 80-100% and 40% mortality, respectively within 24 hours of application. This was too soon to have been due to the entomopathogenic fungus and therefore must have been a direct physical effect of the dewaxing agents.

The results with Savona and Mycotal were varied but at best the combined treatment provided 100% control and all dead mealybugs from these treatments were infected with *V. lecanii*. The results indicated that the strategy of using a dewaxing agent prior to the application of *V. lecanii* could enhance the efficacy of the entomopathogen against adult mealy bugs.

The work continued, focusing on the same sequence of Savona and Mycotal treatments, but now directed against all life cycle stages. There was considerable variation within these results, which was no doubt due to the fact that we were combining two control measures that were each inherently variable. However, we did again see an enhanced effect from using the combined Savona and Mycotal treatments against adults and nymphs (40-100% control) and, to a lesser extent, eggs. This control strategy clearly has potential but requires further refinement in commercial crops.

Potential of the parasitoid, Leptomastix epona

Published information on the biology of the mealybug parasitoid *Leptomastix epona*, was collated and compared to other parasitoids of the obscure mealybug, *P. viburni*. There is insufficient published data to characterise the most efficient parasitoid for the control of obscure mealybug, particularly in relation to tomato production. Both *L. epona* and *P. flavidulus* prefer young adult mealybugs, which are the most suitable host stage and give rise to higher numbers of females in the next generation. Although there is some indication that *P. flavidulus* will accept young host stages more than *L. epona* it is possible that if released together both parasitoid species would compete for the same host stage rather than be complementary.

Another species, *P. maculipennis*, has been introduced recently into New Zealand for the control of obscure mealybug on outdoor crops. However, this species is not native to the UK and hence presents difficulties with licensing agreements for use in the UK.

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 the grower survey in 1999, which averaged £3,100 per hectare. Despite the adoption of intensive control measures, the growers have 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 mealybug infestations to a tomato business is therefore in the region of £3,700 per hectare (£3,100 for control & £600 for crop damage/losses).

The project has identified some methods of improving mealybug control and has shown other measures that were being employed by growers to be ineffective. Both of these factors will help growers to reduce the overall financial impact of this pest. However, further evaluations of the techniques on commercial nurseries are required to determine the actual cost/benefit of these new approaches when applied on a large scale.

Action points for growers

- Do not bring ornamental plants on to a tomato nursery as they are a potential and likely source of mealybug insect pests.
- On an infected nursery, implement strict quarantine measures to prevent spread.

In addition to the control measures outlined in HDC Factsheet 25/00 'Mealybugs on protected tomato crops' the following points should be noted.

- A 2% dilution of Savona is effective against first instar mealybugs as they first colonise tomato plants, and hence offers a valuable alternative to Applaud for control at the start of the season. Savona is approved for use on organic tomato crops.
- The efficacy of Mycotal WP for the control on mealybugs on tomato plants will be enhanced if applied after Savona. Results can be variable but this sequential treatment ought to help to reduce mealybug numbers particularly in organic crops where the use of Applaud is prohibited. Control will be dependent on a series of applications.
- Hyvis 30 Emulsion (a polybutene based glue), Jet 5 (peroxyacetic acid) and undiluted vinegar will reduce the numbers of nymphs emerging from egg sacs on concrete dollies and hence should be used as part of the control strategy.
- Crop Oil proved to be very effective at removing wax from motile and egg stages of mealybugs, and its use should be considered at the end of the cropping season and during the clean up between crops. However, being a paraffinic oil, this product cannot be used in organic crops.

- Growers should note the following control measures that were shown to be ineffective for the control of obscure mealybugs on tomato crops:

    - The predatory mites, *Hypoaspis miles* and *H. aculeifer*.
    - The entomopathogenic fungus, *Beauveria bassiana* (Naturalis L and Botanigard WP).
    - Hortichem Spraying Oil, Horticide and Malathion were shown to be ineffective against mealybug eggs on concrete surfaces.
    - The anti-feedant chemical, pymetrozine (Chess), applied twice at rates up to 80g product per 100 litres water, provided inadequate control on plants.
    - The pheromone used for trapping *Plannococcus citri* (citrus mealy bug) did not appear to be attractive to male obscure mealybugs.

SCIENCE SECTION

PART 1. INTRODUCTION

Background

Mealybugs belong to the insect family, Homoptera, which also includes aphids, whiteflies and scale insects. They are soft-bodied insects with sucking mouthparts. Female mealybugs are wingless and covered in white waxy filaments, which gives them a 'mealy' appearance and provides protection against adverse conditions and insecticidal sprays. The males are small delicate winged insects with no mouthparts. They live only for a few days during which time they seek a female to mate. Eggs are laid in batches of 100-500 in cotton-like pouches made of wax. There are three immature mealybug stages (nymphs), which are similar in appearance to adult females. The smallest nymphs are pink but they become increasingly white as they produce more waxy filaments.

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. Mealybugs thrive in warm, humid, tropical conditions. In the UK, they are most commonly found on plants in heated glasshouses.

This project was prompted by an apparent increase in the incidence of mealybugs on protected tomato crops in the UK. Similar increases had also been reported on glasshouse crops in the Netherlands and France (Schoen and Martin, 1999). Further information was required to explain why the pest was becoming more common in the UK and to identify a control strategy.

Commercial objective

The commercial objective of this project was to determine the pest status of mealybugs on protected tomato crops in the UK, to provide growers with information on the efficacy of current control techniques and advice on improving that control.

Summary of work completed in the first phase of the project (to February 2000) (Sampson, 2000b)

The specific objectives for the first phase of the project were to:
1. Determine the pest status of mealybug on protected tomato crops in the UK.
2. Review the efficacy of current control methods available to growers.
3. Identify the location of egg masses between crops.
4. Produce a fact sheet based on the results.

Pest status of mealybug on UK protected tomato crops: In the1998 season, mealybug infestations were known to occur on 13 tomato nurseries throughout England. The total area affected was 20 ha, which is approximately 7% of the total UK tomato hectarage. Specimens collected from these crops were all identified as the obscure mealybug, *Pseudococcus viburni*. The grower survey confirmed that mealybug incidence had increased on UK tomato crops in recent years, with 70% of infestations occurring for the first time during the last decade. *Pseudococcus viburni* infestations most commonly resulted in damaged plant stems, contamination with sticky honeydew and secondary infections of *Botrytis cinerea*. Half the infested nurseries reported plant death and yield loss, with the most seriously affected losing nearly 50,000 plants in July and August. Another nursery estimated yield losses of £15,000. The cost of controlling *P. viburni* averaged £3,100 / ha / season, of which 75% were labour costs.

The spread of *P. viburni* between and within glasshouses: Mealybugs were usually transported on to uninfested nurseries on infested plants (typically ornamental 'house plants') or on equipment. Spread within infested nurseries occurred when irrigation lines or packing boxes were moved from mealybug infested areas to new areas without first being cleaned and sterilised. The waxy filaments make egg masses and mealybugs sticky and they are moved down rows attached to crop workers or birds (*e.g.* wagtails).

Survival between crops: Crop monitoring confirmed that most individuals survived between crops as eggs, most commonly located on the concrete bases (dollies) of roof supports, on dwarf walls and on irrigation drippers. They were also found on (or in) rockwool slabs, packing crates, strings, dried up plant debris, cracks in the soil, hollow metal posts and the edges of concrete roadways. At the start of the season, *P. viburni* eggs hatched in response to raised temperatures and the young nymphs moved on to the new plants, where they bred continuously throughout the season.

Control methods used against *P. viburni*: A wide variety of control methods were identified. Integrated control programmes using combinations of different methods were generally most successful:

- <u>Hygiene and quarantine</u> were shown to be important methods of limiting the spread of *P. viburni* within or between glasshouses. Growers were advised not to bring ornamental 'house' plants onto tomato nurseries and to restrict the movement of plant material or equipment from infested areas. It was also recommended that crop workers visit infested areas at the end of the day and wear overalls in those areas.
- <u>Insecticides</u> – Mealybugs are very difficult to control with insecticides. Their cryptic habits make it difficult to contact them with sprays and their waxy covering tends to repel water-based products. The insecticides that were most effective against the motile stages of *P. viburni* were Applaud, Decis and Malathion. Of these, Applaud has been recommended because it is least damaging to the biological control agents used against other pests. None of the chemicals were effective against eggs, so two to three applications were required at 14-day intervals to control hatching nymphs.
- <u>Timing of treatments on plants</u> - To minimise the survival of *P. viburni* eggs between crops, growers should attempt to kill all motile stages of the pest on plants at least two weeks before crops are pulled out. If *P. viburni* appears on the new crop, two treatments of Applaud are recommended against the first

generation of nymphs. Repeated use of Applaud through the season is to be discouraged as it could lead to the development of resistance to the pesticide.

- Physical control methods – Physical methods used by growers included hand rubbing, burning with propane burners and spraying with oils / detergents. Although these methods were less effective than chemical insecticides, they suppressed the pest population and prevented economic damage. Physical methods were used throughout the summer months with minimal disruption to the biological control of other pests. However, these methods were labour intensive and therefore expensive.
- Preventing survival between crops - None of the available insecticidal treatments were particularly effective against eggs on the structure of the glasshouse or on equipment. However, the numbers of nymphs emerging onto the new crop was reduced by painting the concrete dollies in the affected areas with a thick paint or glue, covering them in polythene and sealing all joints with glue. Although effective, this method was labour intensive and expensive.
- Biological control of *P. viburni* has not yet been successful on tomato crops in the UK, probably because the natural enemies that have been used develop too slowly at these temperatures.

Eradication of *P. viburni* from nurseries: Four growers have successfully eradicated *P. viburni* from their nurseries. This was achieved by the combined use of chemical treatments (Applaud or Decis) at the end of the season, together with a strict hygiene programme during the clean-up period between crops and the use of glues and traps to prevent emergence of nymphs the following season. Eradication may not be possible on nurseries that have a continuous invasion pressure from other sites, on nurseries that have a pesticide free policy or on nurseries growing to organic standards. Further research is required to identify integrated pest control strategies in such situations.

Fact Sheet: An HDC Fact Sheet (Reference 25/00) entitled "Mealybugs on Protected Tomato Crops" was produced and circulated to HDC levy payers (Sampson, 2000a).

Summary of work completed in the second phase of the project (to July 2001) (Jacobson, 2001)

The specific objectives for the second phase of the project were to:

1. Evaluate the following natural enemies as potential control agents of *P. viburni* in protected tomato crops:
   - *Hypoaspis* spp.
   - *Chrysoperla carnea*
   - *Beauveria bassiana*
   - *Verticillium lecanii*
2. Identify a product that could be used to control *P. viburni* eggs in concrete dollies, irrigation lines and elsewhere.
3. Screen pymetrozine as a possible chemical control of the active stages.
4. Evaluate a pheromone trap for catching male mealybugs.

Evaluation of potential biological control methods: In the second phase of the project, four methods of biological control were evaluated against *P. viburni*:

- *Hypoaspis* spp. – In the first experiment, *Hypoaspis miles and H. aculeifer*, were separately confined with all life cycle stages of *P. viburni* except adult males. The two species consumed similar numbers of prey; *ie* one egg or first instar nymph per day, or one second instar nymph per four days. The predators did not kill third instar nymphs or adult females, presumably because they were too large and/or too densely protected by wax. In a second experiment, batches of one hundred female predators were released at the base of tomato plants that had been artificially infested with first instar *P. viburni* nymphs. The predators did not reduce the number of *P. viburni* nymphs over 14 days and there was no evidence that they had climbed the plants. These experiments indicated that *H. miles* and *H. aculeifer* were not suitable biological control agents for *P. viburni* on tomato crops.
- <u>*Chrysoperla carnea*</u> – The lacewing, *C. carnea*, had been reported to attack some species of mealybugs but there was no information regarding its potential to control *P. viburni*. A small-scale study was therefore designed to determine the predation rate of *C. carnea* nymphs against all life cycle stages of *P. viburni*. Although the predators attacked all the mealybug life cycle stages, first instar nymphs were their preferred prey (10 consumed per day). The numbers of other life cycle stages consumed per day were roughly proportional to the size of the prey; *i.e.* 5 eggs, 3-4 second or third instar nymphs, and 1 adult female. The results demonstrated that *C. carnea* had potential for use as a biological control agent against *P. viburni*. However, further studies are required to determine whether the predators will remain on tomato plants, feed on the pests in that environment and form breeding colonies within the glasshouse.
- <u>*Beauveria bassiana*</u> – This is a fungal pathogen that has been reported to infect some species of mealybugs. Two commercial products (Naturalis L and Botanigard WP) were evaluated against *P. viburni* in laboratory bioassays but neither product gave significant control of the pest.
- <u>*Verticillium lecanii*</u> – Some UK growers who had used the fungal pathogen, *V. lecanii* (Mycotal WP), against glasshouse whiteflies, reported incidental control of mealybugs but this had not been confirmed. Laboratory bioassays done in this project showed that the pathogen reduced the numbers of first instar *P. viburni* nymphs by approximately 8%. It is possible that this effect could be enhanced by applying *V. lecanii* in an oil-based formulation because this would help to break down the mealybugs' waxy protection and allow more fungal spores to come into contact with the insect's body.

Control of *P. viburni* eggs/nymphs on the glasshouse structure: Growers require a cost-effective method of controlling *P. viburni* eggs that survive between crops on concrete dollies and similar structures. Four products (Hortichem Spraying Oil, Hyvis 30 Emulsion, Horticide and Malathion) were evaluated in a small-scale bioassay. Only Hyvis 30 Emulsion performed significantly better than untreated controls. This polybutene based "glue" reduced the number of surviving nymphs by 50%. However, there may be up to 500 eggs in each egg sac and this level of control may still be inadequate for commercial crops. The results were consistent with observations on commercial nurseries.

Evaluation of pymetrozine against *P. viburni*: Although Applaud has been identified as an IPM compatible control measure, repeated use throughout the season has been discouraged because it may lead to the development of resistance among pests. An alternative IPM compatible product is therefore required. Pymetrozine (Chess), an antifeedant chemical that is specific to some insect relatives of mealybugs (*e.g.* aphids and whiteflies), was evaluated against *P. viburni* on tomato plants in a crop-scale experiment. The results showed that two applications, at rates of up to 80g product per 100 litre of water, provided some control but this was inadequate for commercial crops.

Evaluation of sex pheromones as aid to trapping *P. viburni*: The female citrus mealybug, *Plannococcus citri*, produces a sex pheromone that has been synthesised and used to trap males of that species in commercial crops. Although sex pheromones are usually specific to individual species of insects, some will attract closely related species. The citrus mealybug pheromone was therefore incorporated into lures on sticky traps and tested in tomato crops that were heavily infested with the obscure mealy bug. The numbers of males caught were very small and it was concluded that the pheromone was not attractive to this species.

Future work

At the Project Review Meeting on 10 May 2001, it was decided that future work should focus on methods of controlling mealybugs on organic crops. The Review Panel suggested the following topics for further investigation:

- Evaluation of efficacy of peroxyacetic acid (Jet 5) and acetic acid (vinegar) against *P. viburni* egg sacs on the structure of the glasshouse.
- Evaluation of efficacy of Savona, vinegar and Mycotal WP +/- oil adjuvants against motile stages of *P. viburni.*
- Collation of information about the parasitoid, *Leptomastix epona*, the life cycle stages of *P. viburni* attacked and the parasitoids' potential in tomato crops.

Scientific targets of the third phase of the project (to October 2002)

1. Evaluate the efficacy of acetic acid and per acetic acid (as Jet 5) for the control of mealybug eggs on concrete dollies and other structural elements of the glasshouse.

2. Evaluate a range of control measures that are suitable for the control of mealybugs at the start of the growing season, in particular in organic tomato crops.

3. Evaluate a range of control measures that are suitable for the control of mealybugs during the growing season, in particular in organic tomato crops.

4. Evaluate the potential of specific parasitoids for the control of mealybugs in commercial tomato crops.

5. Monitor the efficacy of control measures for mealybug on commercial tomato nurseries.

PART 2. CONTROL OF *PSEUDOCOCCUS VIBURNI* EGGS ON CONCRETE DOLLIES AND OTHER STRUCTURAL ELEMENTS OF THE GLASSHOUSE.

Objective:

To evaluate the efficacy of acetic acid and peroxyacetic acid against *P. viburni* eggs and first instar nymphs on the concrete structure of the glasshouse**.**

Introduction:

Many *P. viburni* survive between crops as eggs attached to the concrete bases (dollies) of roof supports and other parts of the glasshouse structure. Up to 500 eggs may be encased within each thick waxy egg sac, which provides protection against adverse conditions and insecticidal treatments. At the start of the new season, with raised temperatures and increasing day length, first instar nymphs hatch from the egg sac and crawl onto the new tomato plants, rapidly establishing large infestations.

Three chemicals (Applaud, Decis and Malathion) have been effective against motile stages of *P. viburni* on tomato plants but none have been particularly successful against the eggs. In the second phase of this project, four products (Hortichem Spraying Oil, Hyvis 30 Emulsion, Horticide and Malathion) were evaluated in small-scale bioassays. Only Hyvis 30 Emulsion performed significantly better than untreated controls. This polybutene based “glue” reduced the number of surviving nymphs by 50%. However, with so many eggs in each egg sac, this level of control was considered inadequate for commercial crops. The results were consistent with observations on commercial nurseries.

At the Project Review Meeting on 10 May 2001, the review panel requested that the bioassay developed in 2001 be used to evaluate applications of acetic acid (applied as a proprietary vinegar product) and peroxyacetic acid (as Jet 5) against *P. viburni* eggs / nymphs on concrete surfaces.

Materials and method:

<u>Insect populations</u>

Pseudococcus viburni were reared on tomato plants (cv Spectra) at Stockbridge Technology Centre Ltd.

<u>Treatments</u>

1. Water (control)
2. 100% Sarson’s vinegar (6% acetic acid)
3. 50% Sarson’s vinegar (6% acetic acid) diluted with water.
4. Peroxyacetic acid (Jet 5) at 8ml product per litre water.

<u>Bioassay procedure</u>

The Treatments were applied to small (90 x 50 x 40 mm) concrete blocks that had been made for the experiment. A single egg sac was placed on the surface of each block and the approximate number of eggs within each sac was recorded. The sprays were applied to the point of run off with a fully calibrated aerosol sprayer. Following treatment, each concrete block was placed on a yellow sticky trap (150 x 200 mm) to capture hatching first instar *P. viburni* as they crawled from the block. The blocks/traps were then stored in a controlled environment room at 21 ± 2°C and 16:8 L:D. Preliminary studies had shown that the nymphs left the blocks soon after emerging from eggs and all the eggs hatched within three weeks. The numbers of nymphs captured on the sticky traps were therefore recorded after four weeks.

Each concrete block formed a replicate and there were ten replicates per Treatment. Data was analysed using analysis of deviance (analogous to ANOVA) from a Binomial model.

Results and discussion:

Analysis of deviance of the results in Table 1 showed there was a highly significant treatment affect ($p=0.005$).

Table 1. The mean (± se) number of surviving *P. viburni* first instar nymphs after the application of four different treatments (n = 10)

Treatments	Numbers of first instars	
	Pre-treatment count	Post treatment count
Untreated (water)	54.0 (10.8)	27.3 (10.4)
100% vinegar	100.0 (24.2)	22.3 (10.5)
50% vinegar	65.0 (11.9)	41.3 (8.8)
Jet 5	147.0 (24.6)	51.0 (11.6)

Analysis of the 'predicted' means showed that undiluted vinegar and Jet 5 reduced *P. viburni* hatching compared to diluted vinegar and the water control. Survival of first instar nymphs following application of undiluted vinegar and Jet 5 was reduced by 81% and 67% respectively. However, the inherent variability within the results means that there was no significant difference between these two Treatments.

Both Treatments were more effective than the best product tested in the bioassays completed in 2001 (see Introduction section above). However, with so many eggs in each egg sac even 81% control may be inadequate to protect a commercial crop. Further studies are required to determine whether repeated and well-targeted applications of vinegar or Jet 5 would provide satisfactory control of the egg sacs on the concrete structures.

The results in Table 1 show that application of water alone reduced the proportion of surviving first instar nymphs by 52%, which was broadly consistent with results obtained in bioassays in 2001. The physical action of spraying could either have partially removed the wax protection from the egg sac and thus rendered the eggs more vulnerable to unfavourable environmental conditions, or have caused direct physical damage to the eggs. Results in other experiments (see Part 3 of this report) suggest that first instar *P. viburni* nymphs are also vulnerable to the direct physical effect of spraying.

PART 3. CONTROL OF *PSEUDOCOCCUS VIBURNI* AT THE START OF THE GROWING SEASON.

Objective:

To evaluate a range of control measures that are suitable for the control of *Pseudococcus viburni* nymphs on tomato plants at the start of the growing season.

Introduction:

At the beginning of the tomato growing season, *P. viburni* first instar nymphs hatch from eggs on the fabric of the building and migrate onto the tomato plants. Previous studies within this project have demonstrated that these nymphs can be controlled with foliar applications of the insect growth regulator, buprofezin (Applaud). However, this product cannot be used on crops grown to organic standards.

At the Project Review Meeting on 10 May 2001, the review panel requested that a range of products that are allowed under organic crop production standards be evaluated in a small-scale glasshouse experiment. The chosen products were vinegar, the soft soap, Savona, and *Verticillium lecanii* (Mycotal). The latter was to be evaluated with and without the oil-based adjuvant, Addit.

Materials and Method:

Insect populations

Pseudococcus viburni were reared on tomato plants (cv Spectra) at Stockbridge Technology Centre Ltd.

Treatments

1. Untreated control
2. Water
3. 100% Sarson's vinegar (6% acetic acid)
4. 50% Sarson's vinegar (6% acetic acid) diluted with water.
5. 0.1% Mycotal (1g per litre water)
6. 0.1% Mycotal + 0.25% Addit (2.5ml per litre of water).
7. 0.25% Addit
8. 2% Savona (20ml per litre water)

Experimental procedure

Four rows of 12 tomato plants (cv. Espero) were grown hydroponically in rockwool blocks following normal commercial crop production practices. Three weeks after planting a single *P. viburni* egg sac was placed at the base of each plant. The numbers of first instar nymphs that subsequently emerged from the eggs sacs and moved onto the plants were recorded in a pre-treatment assessment. The eight Treatments were applied using a fully calibrated hand held 3 litre Hozelock Sprayer fitted with the standard spray nozzle. The numbers of surviving *P. viburni* nymphs were recorded seven days after application of treatments.

Each plant formed a replicate. There were six replicates per Treatment arranged in randomised blocks. The proportions of first instar *P. viburni* nymphs that survived the treatments were analysed using Binomial analysis using a general linear model.

Results and Discussion:

The proportion of first instar *P. viburni* nymphs surviving after application of the eight different insecticidal treatments are shown in Table2. Analysis of deviance of these results showed a highly significant ($p<0.001$) effect of treatment on the survival of first instar *P. viburni* nymphs.

Analysis showed that 97% of the nymphs survived in the untreated controls. Where water was applied as an additional control, only 62% of the nymphs survived the treatment. This indicates that first instar nymphs, like eggs, are vulnerable to the direct physical effect of spraying.

The numbers of survivors in the Mycotal (+/- Addit), Addit and two vinegar treatments were not significantly different to water applied alone; the overall mean for the six treatments being 59%. These results suggest that it was the physical action of spraying rather than any insecticidal property of the products that was the cause of the mortality.

Only 7% of the nymphs survived the application of Savona, which was significantly ($p<0.001$) better than any of the other treatments. This treatment therefore provides a reasonably good method of controlling of *P. viburni* nymphs that invade the plants at the start of the tomato season.

The application of undiluted vinegar to plants resulted in leaves going flaccid and then shrivelling. None of the other treatments caused obvious phytotoxic symptoms.

Table 2. The proportion of first instar *P. viburni* nymphs surviving after application of eight different insecticidal treatments (n=6).

Treatment	**Mean number of *P.viburni* nymphs**		**Proportion surviving (se)**
	Pre-treatment count	**Post-treatment count**	
Untreated	28.2	27.3	0.97 (0.029)
Water	21.5	14.7	0.62 (0.067)
100% Vinegar	46.7	30.8	0.65 (0.044)
50% Vinegar	32.5	21.2	0.58 (0.063)
Mycotal	26.0	13.2	0.47 (0.059)
Mycotal+Addit	28.8	19.8	0.65 (0.065)
Addit	17.0	10.3	0.57 (0.070)
Savona	20.5	1.0	0.07 (0.055)
s.e.d. (35 d.f.)	8.26	6.38	

PART 4. EVALUATION OF A RANGE OF CONTROL MEASURES THAT ARE SUITABLE FOR THE CONTROL OF *PSEUDOCOCCUS VIBURNI* DURING THE GROWING SEASON, IN PARTICULAR IN ORGANIC TOMATO CROPS.

Introduction:

In phase two of the project, the entomopathogenic fungus, *Verticillium lecanii* (Mycotal WP), was shown to infect *P. viburni* nymphs but only reduced the population by approximately 8% compared to untreated controls. It was proposed that this relatively poor infection rate was due to the protection afforded by the dense waxy deposits on the surface of the insects.

At the Project Review Meeting on 10 May 2001, the review panel requested that the research team investigate the possibility of enhancing the effect of *V. lecanii* by applying it in combination with oils or wetters that could break down the waxy deposits and allow more spores to come into contact with the insect's body.

A series of three laboratory experiments were designed to investigate the individual components of the proposed control strategy. The first experiment examined the physical effects of a wide range of oils / wetters on the waxy deposits and selected those with greatest potential for further investigation. The second experiment quantified infection rates by *V. lecanii* following application of the short-listed "dewaxing" agents and selected the most effective combinations. The third experiment compared the efficacy of the combined dewaxing agent / fungal pathogen treatment with Savona and vinegar.

EXPERIMENT 1

Objective:

To examine the efficiency of different oils and wetters as dewaxing agents to break down waxy deposits from the bodies of *P. viburni*.

Materials and method:

Insect populations

Pseudococcus viburni were reared on tomato plants (cv Spectra) at Stockbridge Technology Centre Ltd.

Treatments

1. Untreated control
2. Water
3. 100% Sarson's vinegar (6% acetic acid)
4. 50% Sarson's vinegar (6% acetic acid) diluted with water.
5. 0.5% Eradicoat Oil (5ml per litre water)
6. 0.05% Codacide – emulsified vegetable oil and polyethoxylate esters (0.5ml per litre of water)

7. 0.1% Codacide (1ml per litre of water)
8. 0.25% Addit (2.5ml per litre of water)
9. 2% Savona (20ml per litre water)
10. 4% Savona (40ml per litre water)
11. 1% Hortichem Spraying Oil (10ml per litre water)
12. 0.01% Experimental wetter "S" (0.1 ml per litre water)
13. 0.1% Experimental wetter "S" (1 ml per litre water)
14. 0.5% Experimental wetter "S" (5 ml per litre water)
15. 2.0% Experimental wetter "S" (20 ml per litre water)
16. 0.01% Experimental wetter "G" (0.1 ml per litre water)
17. 0.1% Experimental wetter "G" (1 ml per litre water)
18. 0.5% Experimental wetter "G" (5 ml per litre water)
19. 2.0% Experimental wetter "G" (20 ml per litre water)
20. 3% Crop Oil – a paraffinic oil (30ml per litre of water)
21. 1% Crop Oil Gold – methylated rapeseed oil (10ml per litre water)

Experimental procedure

Each treatment was tested against *P. viburni* adults and egg sacs. The insects were placed on an excised tomato leaf on a damp filter paper in a Petri dish and sprayed to the point equivalent to run off with the test product using a 500ml Hozelock hand held sprayer with standard nozzle. Each test was replicated three times. The results were assessed 24 hours after the application of the treatments. Due to the difficulty in quantifying the effect of the dewaxing agents, assessments were done by microscopic inspection and direct comparison with untreated controls and other treatments. To assist this procedure, each test insect / egg sac was photographed and the visual records were retained for further comparisons and reference.

Results and discussion:

Photographic records of the adult females and egg sacs from the untreated controls and the most effective treatments are shown in Figures 1-8. Overall, Crop Oil proved to be the most effective product, almost completely dewaxing the *P. viburni* adults and egg sacs within 24 hours (Figures 3 and 4). Savona (2%) removed most of the wax from the egg sacs (Figure 6) and over 90% (estimated) of the wax from adult females (Figure 5). With this treatment, the wax tended to be removed evenly across the whole body of the adults leaving just a light and incomplete sprinkling of white deposits (Figure 5). A marginal improvement was obtained by increasing the concentration of Savona to 4%. The effects of the two experimental wetters, S and G, were similar, with the 0.1% dilutions providing the most acceptable results. After application of these rates, an estimated 15-25% of eggs became exposed (Figure 8). The effect of these products on the adults was quite different to Savona; *i.e.* the wax was removed from a portion of the insect but left intact elsewhere (Figure 7) suggesting that the sprays were not spreading as effectively as the soft soap. At dilutions greater than 0.1%, there was evidence of the experimental wetters causing damage to the leaves, so those treatments were discounted. None of the other treatments were noticeably different to water sprays and were therefore discounted.

EXPERIMENT 2

Objective:

To quantify the infection rates of *P. viburni* adult females by *V. lecanii* (Mycotal) following application of four dewaxing agents.

Materials and method:

Insect populations

Pseudococcus viburni were reared on tomato plants (cv Spectra) at Stockbridge Technology Centre Ltd.

Treatments

1. Untreated control
2. 0.1% Experimental wetter “S” (1 ml per litre water) followed by 0.1% Mycotal (1g per litre water)
3. 0.1% Experimental wetter “S” followed by 0.1% Mycotal + 0.25% Addit (2.5ml per litre of water).
4. 0.1% Experimental wetter “G” (1 ml per litre water) followed by 0.1% Mycotal
5. 0.1% Experimental wetter “G” followed by 0.1% Mycotal + 0.25% Addit
6. 3% Crop Oil (30ml per litre of water) followed by 0.1% Mycotal
7. 3% Crop Oil (30ml per litre of water) followed by 0.1% Mycotal + 0.25% Addit
8. 4% Savona followed by 0.1% Mycotal
9. 4% Savona followed by 0.1% Mycotal + 0.25% Addit

Experimental procedure

Each *P. viburni* adult was placed on an excised tomato leaf on a damp filter paper in a Petri dish and sprayed to the point equivalent to run off with the test products using a 500ml Hozelock hand held sprayer with standard nozzle. The dewaxing agents (*i.e.* experimental wetters S and G, Crop Oil or Savona) were applied first, and the leaves then transferred to clean filter paper and allowed to dry before Mycotal (+/- Addit) was applied. The Petri dishes were stored in a controlled environment room (16L:8D, 21 ± 2^{o}C) and monitored daily. The numbers of live and dead *P. viburni* were recorded 1 and 5 days after application of the treatments.

Experimental design and analysis

Each Petri dish formed a replicate and there were five replicates per Treatment. In this trial five individuals were sprayed in a 4 x 2 factorial experiment (four wax-removal treatments together with Mycotal with or without the adjuvant Addit) together with an untreated control. Mortality at one day was analysed together with ‘further’ mortality after 5 days, *i.e*. the numbers of additional deaths. As in Parts 2 and 3 of this report, analysis was done using a simple binomial model.

Results and Discussion:

The proportion of adult female *P. viburni* that were dead 1 and 5 days after the application of the eight different combinations of dewaxing agents, *Verticillium lecanii* and Addit are shown in Table 3.

The results show that all the insects in the untreated controls survived and these were discounted from the analysis. The results for day 1 show highly significant (*p=0.001*) effects among the Treatments. The Treatments including experimental wetters S and G, Crop Oil and Savona all caused some mortality within 24 hours of application. This was too soon to have been due to the entomopathogenic fungus and therefore must have been a direct physical effect of the dewaxing agents. There was no additional impact from treatments that included Addit. Therefore, the overall mortality caused by the experimental wetters S and G, Crop Oil and Savona, was 10%, 20%, 90% and 40% respectively.

The column in Table 3 headed “Proportion of adults dead: 5 days” refers to the additional mortality that occurred from day 1 to day 5 post-treatment. The total effect of the combined dewaxing and entomopathogen treatments are shown in the column headed “Overall proportion dead”. The mortality recorded for the experimental product G and Savona had increased markedly by day five and all dead mealybugs from these treatments were infected with *V. lecanii*. This demonstrated that the strategy of using a dewaxing agent prior to the application of *V. lecanii* did enhance the efficacy of the entomopathogen. The same effect was noted, but less pronounced, with the experimental product S. The direct effect of Crop Oil on *P. viburni* at day 1 made it impossible to measure any additional control by *V. lecanii* at day 5. Analysis of data from day 5 did not show any additional impact of Addit in any treatments.

Table 3. The proportion of adult female *P. viburni* that were dead 1 and 5 days after the application of eight different combinations of dewaxing agents, *Verticillium lecanii* and Addit. (n=5)

Treatments	Proportion of adults dead:		Overall proportion dead
	1day	5 days	
Untreated	0.0	0.0	0.0
'S' (0.1%) + Mycotal (0.1%)	0.0	0.2	0.2
'S' (0.1%) + Mycotal (0.1%) / Addit (0.25%)	0.2	0.5	0.6
'G' (0.1%) + Mycotal (0.1%)	0.2	0.5	0.6
'G' (0.1%) + Mycotal (0.1%) / Addit (0.25%)	0.2	1.0	1.0
Crop oil (3%) + Mycotal (0.1%)	0.8	1.0	1.0
Crop oil (3%) + Mycotal (0.1%) / Addit (0.25%)	1.0	-	1.0
Savona (4%) + Mycotal (0.1%)	0.4	1.0	1.0
Savona (4%) + Mycotal(0.1%) / Addit (0.25%)	0.4	0.3	0.6

EXPERIMENT 3

Objective:

To compare the efficacy of the most effective combined dewaxing agent / entomopathogen control strategies with Savona and vinegar.

Materials and method:

Insect populations

Pseudococcus viburni were reared on tomato plants (cv Spectra) at Stockbridge Technology Centre Ltd.

Treatments

1. Untreated control
2. 100% Sarson's vinegar (6% acetic acid)
3. 0.1% Experimental wetter "G" (1 ml per litre water)
4. 0.1% Experimental wetter "G" followed by 0.1% Mycotal (1g per litre water)
5. 2% Savona (20ml per litre water)
6. 2% Savona followed by 0.1% Mycotal
7. 4% Savona (40ml per litre water)
8. 4% Savona followed by 0.1% Mycotal
9. Savona (4%) + Mycotal (0.1%)
10. 0.1% Mycotal
11. 0.1% Mycotal + 0.25% Addit (2.5ml per litre of water).

Experimental procedure

A single *P. viburni* adult, second instar nymph or egg sac was placed on an excised tomato leaf on a damp filter paper in a Petri dish and sprayed to the point equivalent to run off with the test products using a fully calibrated hand held aerosol sprayer. The dewaxing agents (*i.e.* experimental wetter G or Savona) were applied first and the leaves then transferred to clean filter paper and allowed to dry. Mycotal (+/- Addit) was then applied to the relevant Treatments. The Petri dishes were stored in a controlled environment room (16L:8D, 21 ± 2ºC) and monitored daily. The numbers of live *P. viburni* adults or nymphs were recorded 1 and 6 days after application of the sprays. The efficacy of the applications against egg sacs was measured by recording the numbers of eggs that successfully hatched or failed to hatch.

Experimental design and analysis

Each Petri dish formed a replicate and there were ten replicates per Treatment. Treatments were compared using analysis of deviance for a binomial model.

Results and Discussion:

Adults and nymphs

The proportion of *P. viburni* adult females and second instar nymphs that were dead 1 and 6 days after the application of the eleven different combinations of dewaxing agents, *Verticillium lecanii* and Addit are shown in Tables 4 and 5 respectively. There was only one mortality recorded for the control and vinegar treatments; *i.e.* a nymph in the vinegar treatment on day 1. These two Treatments were excluded from the analysis because they merely demonstrated that nearly all mortality recorded in the experiment was associated with the application of the other Treatments. Mortality on day 1, mortality from day 1 to day 6 (conditional on survivors at the end of day 1) and the total mortality over the 6 days were analysed. This latter is, of course, not independent of the other two.

The following key points may be drawn from the formal statistical analysis of adult survival data (*i.e.* binomial analysis of dead adult *P. viburni* as a proportion of live *P. viburni* at the start of the period):

- There was a highly significant ($p<0.001$) impact of main treatments at day 1, with 4% Savona causing greater mortality than other treatments.
- There were no subsidiary effects of Mycotal at this stage, which is not surprising because it would have been too soon for the entomopathogenic fungus to kill the insects.
- Mycotal applied alone or just with Addit (*i.e.* without a preliminary application of a dewaxing agent) had very little effect on adult *P. viburni*.
- The experimental wetter "G" appeared to enhance the effect of *V. lecanii* but the overall control achieved from this combination of treatments was not adequate for adoption in commercial crops.
- 2% Savona enhanced the effect of *V. lecanii* and the resulting combination of treatments provided total control of adult *P. viburni* under the conditions of this experiment.
- 4% Savona alone had a much greater impact on adult *P. viburni* and this masked any subsequent contribution by *V. lecanii*.

Formal analysis of nymph survival data provided results and conclusions that were broadly consistent with the key points listed for adults above.

Eggs

The proportion of *P. viburni* eggs / first instar nymphs that died after the application of the eleven different combinations of dewaxing agents, *Verticillium lecanii* and Addit are shown in Table 6. There was considerable inherent variability in the results, which made it difficult to detect real differences between the treatments.

Formally there was no significant difference between treatments, although some effects are worthy of comment. The Mycotal treatment alone seemed to be ineffective, which is consistent with results obtained against other life cycle stages. What was interesting, however, was the impact of applying Mycotal after both the experimental wetter G and 4% Savona. In these treatments, mortality was increased and *V. lecanii* growth was recorded on the newly hatched first instar *P. viburni*. While not totally conclusive, this result again indicates that the preliminary use of a dewaxing agent can enhance the subsequent effect of an entomopathogenic fungus.

Table 4. Proportion of *P. viburni* adult females that were dead 1 and 6 days after the application of the eleven different combinations of dewaxing agents, *V. lecanii* and Addit.

Treatments	**No. of dead mealybugs**		**Proportion dead**	**No. of dead mealybugs**		**Proportion dead**	**Overall Proportion dead**
	day 0	day 1		day 1	day 6		
Untreated	0	0	0.0	0	0	0.00	0.0
Vinegar	0	0	0.0	0	0	0.00	0.0
"G"	0	2	0.2	2	1	0.13	0.3
"G" + Mycotal	0	0	0.0	0	3	0.30	0.3
2% Savona	0	2	0.2	2	4	0.50	0.6
2% Savona + Mycotal	0	4	0.4	4	6	1.00	1.0
4% Savona	0	7	0.7	7	3	1.00	1.0
4% Savona + Mycotal	0	6	0.6	6	3	0.75	0.9
Mycotal	0	0	0.0	0	0	0.00	0.0
Mycotal + Addit	0	1	0.1	1	2	0.22	0.3

Table 5. Proportion of *P. viburni* second instar nymphs that were dead 1 and 6 days after the application of the eleven different combinations of dewaxing agents, *V. lecanii* and Addit.

Treatments	No. of dead mealybugs		Proportion dead	No. of dead mealybugs		Proportion dead	Overall Proportion dead
	day 0	day 1		day 1	day 6		
Untreated	0	0	0.0	0	0	0.00	0.0
Vinegar	0	1	0.1	1	0	0.00	0.1
"G"	0	2	0.2	2	3	0.38	0.5
"G" + Mycotal	0	1	0.1	1	3	0.33	0.4
2% Savona	0	3	0.3	3	0	0.00	0.3
2% Savona + Mycotal	0	0	0.0	0	5	0.50	0.5
4% Savona	0	6	0.6	6	4	1.00	1.0
4% Savona + Mycotal	0	5	0.5	5	4	0.80	0.9
Mycotal	0	0	0.0	0	1	0.10	0.1
Mycotal + Addit	0	0	0.0	0	1	0.10	0.1

Table 6. The proportion of *P. viburni* eggs / first instar nymphs that died after the application of the eleven different combinations of dewaxing agents, *Verticillium lecanii* and Addit.

Treatments	Mean number (se)		Proportion dead (se)
	No. of eggs	No. dead	
Untreated	57.1(12.6)	0.0 (0.0)	0.00(0.00)
Vinegar	32.5 (6.4)	8.3 (5.7)	0.25 (0.12)
"G"	36.9 (7.2)	6.5 (3.0)	0.18 (0.10)
"G" + Mycotal	18.6 (3.5)	8.7 (2.9)	0.47 (0.20)
2% Savona	26.0 (2.6)	9.4 (2.8)	0.36 (0.15)
2% Savona + Mycotal	25.4 (4.6)	8.5 (2.5)	0.34 (0.15)
4% Savona	40.3 (6.1)	15.8 (7.2)	0.39 (0.12)
4% Savona + Mycotal	24.9 (5.5)	13.9 (3.3)	0.56 (0.16)
Mycotal	48.9 (10.9)	3.4 (1.3)	0.07 (0.06)
Mycotal + Addit	52.6 (7.6)	5.8 (3.8)	0.11 (0.07)

PART 5. EVALUATE THE POTENTIAL OF SPECIFIC PARASITOIDS FOR THE CONTROL OF *PSEUDOCOCCUS VIBURNI* IN COMMERCIAL TOMATO CROPS.

Background

The most effective natural enemies of mealybugs are usually considered to be small parasitic wasps principally from the family Encyrtidae, but also include the families Aphelinidae and Pteromalidae. The main species that have been studied in relation to their use for the control of obscure mealybug are shown in Table 7. It is evident from the list that for most of the parasitoid species, including those in commercial production, there is a preference for host species other than the obscure mealybug. Therefore it is likely that successful biocontrol of obscure mealybug will rely ultimately on the use of a restricted range of natural enemies, which is in line with established practice in mealybug control (Moore, 1988).

Biological control of mealybugs

A range of natural enemies is known to attack and feed on mealybugs and when the use of disruptive pesticides is limited these can give successful biological control, most notably in outdoor situations. However, natural enemies for biological control of mealybugs have been investigated primarily for the control of the citrus mealybug, *Planococcus citri* Risso, both where it occurs naturally (Australia) and where it has been introduced, such as in North America, southern Europe and the Mediterranean Basin. Natural enemies have been reported as successful primary control agents of citrus mealybug in organic production and they now play an increasing role, along with the use of selective pesticides, in integrated fruit production in subtropical outdoor fruit production. Biological control has also been achieved, similarly in an outdoor environment, for the longtailed mealybug, *Pseudococcus longispinus* (Targioni-Tozzeti), on avocado in California and Israel (Bartlett, 1978; Swirski *et al.* 1980).

The longtailed mealybug belongs to a group of closely related species, which includes both the obscure mealybug, *Pseudococcus viburni* (Signoret) and the grape mealybug *P. maritimus* (Ehrhorn), collectively known as the *Pseudococcus maritmus-malacearum* complex (Wilkey & McKenzie, 1961; Miller *et al.* 1984; Gimpel & Miller, 1996). Intensive field work on what was considered to be “grape mealybugs” in North America during the 1960s lead to the discovery of this mealybug species complex and revealed that all three species have overlapping ranges of host plant species and natural enemy complexes. Furthermore, the work in America highlighted that there was a lack of effective natural enemies for the obscure mealybug in particular. Considerable research effort has been directed at using natural enemies that were found attacking one species in the complex for the biological control of other species in the group. This approach has been most successful in outdoor environments using strategies that augment the existing spectrum of natural enemies, usually where the pest has been introduced and naturally occurring predators and parasitoids are absent. The most recent example was in February 2001 when the parasitoid, *Pseudaphycus maculipennis* (Mercet), was introduced into New Zealand for the control of obscure mealybug on outdoor apple and pear (pipfruit) trees (Charles, 2001). However, control based on regular, inundative releases of natural enemies in protected

Table 7. Parasitoids of Obsure mealybug showing commercial availability within the European and Mediterranean Plant Protection Organisation (EPPO) region.

Parasitoid	Commercial use* / Host (start date)		Biogeography: Origin	Biogeography: Introduction
ENCYRTIDAE				
Leptomastix epona (Walker) [1] =*L. algirica* Trijiapitzn	✓ (1992)	Longtailed, Obscure, Citrus, & Citriculus =*P. cryptus* Hempel	Europe, Israel	USA, Chile
Pseudaphycus maculipennis (Mercet, 1923) [2]	✓ (1980)	Longtailed, Obscure	Europe	Georgia, 1976; Australia, 1997; New Zealand 2001
Pseudaphycus flavidulus (Brèthes) [1]	-	Grape, Obscure	Argentina & Chile	USA
Anagyrus fusciventris (Girault, 1915) [3]	✓ (1990)	Longtailed Obscure	Australia	Hawaii, USA West Indies, Israel, Europe
Anagyrus pseudococci (Girault, 1915) [3]	✓ (1995)	Citrus Longtailed? Obscure	Europe	Australia
Tetracnemoidea peregrina (Compére, 1939) [4]	-	Longtailed, (Obscure?)	S.America	USA, Europe, New Zealand
PTEROMALIDAE				
Ophelosia spp. [4 & 5] (also predator in egg sacs). *O. charlesii* Berry *O. keatsi* Girault	-	Citrophilus, =*P.calceolariae* (Maskel), Longtailed, Obscure	Australia	New Zealand
APHELINIDAE				
Coccophagus gurnei Compére [4]	-	Citrophilus, Longtailed, Obscure	Australia	New Zealand 1933

* EPPO 2002. [1] (Daane, 1999, Karamaona & Copland, 2000a, b); [2] (Charles, 2001; Panis & Brown, 1971); [3] (Waterhouse & Sands, 2001); [4] (Charles, 1993); [5] (Malipatil *et al.*, 2001).

or enclosed environments has proved to be more difficult (Blumberg & Van Driesche, 2001).

Parasitoids in mealybug control

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 *et al.*, 1993). Host selection by mealybug parasitoids is an important driver of these processes as hosts that are not vulnerable to attack can act as refuges for the development and spread of mealybug populations. When multiple natural enemies that share the same host are used, host selection and host suitability are important in determining whether they can coexist and complement each other or will simply compete for the same host (Godfray & Waage, 1991). However, difficulties for the biological control of obscure mealybug are indicated in the few studies (albeit based primarily on initial laboratory investigations) that are available for the priority natural enemy species (Blumberg & Van Driesche, 2001; Karamaouna & Copland, 2000a, b).

With respect to their effect on obscure mealybug, basic published biological information is available for only a few of the species listed in Table 7. One important biological characteristic, the rate of parasitism, has been reported (Blumberg & Van Driesche, 2001) for just two species (Table 8). Although *L. epona* shows the highest rate of parasitism (on both obscure and citrus mealybug) it is comparable to that of *A. fusciventris* (62% and 54%, respectively). However, with obscure mealybug as a host, both parasitoid species are susceptible to high rates of host defence by egg encapsulation (33% and 70%, respectively; see Table 9). Encapsulated eggs result when they are enclosed by host blood cells to form a capsule-like envelope, which can cause death of the parasitoid by physical prevention of its further development. The frequency of encapsulation can vary with host species, life stage and condition as well as with host plant and environmental conditions, particularly temperature (Blumberg, 1997). Escape from egg encapsulation in obscure mealybug by both *L. epona* and *A. fusciventris* is very low (4%, see Table 10) and indicates that the parasitoids are not well suited to this host. From the data presented in Tables 9 & 10 it is evident that for both parasitoid species the longtailed mealybug is a more suitable host than the obscure mealybug. It appears that much of the emphasis on the parasitoids *L. epona, A. fusciventris* and *P. flavidulus* has derived from commercial mass production criteria as the obscure mealybug is easier to maintain in culture than the longtailed mealybug (Blumberg & Van Driesche, 2001; Daane, 1999). In addition the target species for control has usually been the longtailed mealybug, which is the preferred host and has previously had a greater pest status, notably on grapevine.

The successful development and emergence of adult parasitoids is dependent on host suitability, which varies with host age and size. It is evident that although *L. epona* will accept a wide range of host stages of obscure mealybug for oviposition not all stages are equally suited to the parasitoid (Table 11). Increasingly higher proportions of male offspring are produced when younger host stages are used for oviposition. It is clear that under more natural conditions, when the parasitoid can choose between different host stages, older hosts are preferred and more female parasitoid offspring are produced.

Table 8. Rate of parasitism (percent hosts parasitised) by three encyrtid parasitoid species on different mealybug hosts at 23°C (from Blumberg & Van Driesche. 2001)

Parasitoid species	Mealybug host species		
	Obscure (*P. viburni*)	Longtailed (*P. longispinus*)	Citrus (*P. citri*)
Leptomastix epona	62%	58%	66%
Leptomastix dactylopii	54%	46%	59%
Anagyrus fusciventris	54%	52%	16%

Table 9. Rate of parasitoid egg encapsulation[1] by different mealybug species at 23°C (from Blumberg & Van Driesche. 2001).

Parasitoid species	Mealybug host species		
	Obscure (*P. viburni*)	Longtailed (*P. longispinus*)	Citrus (*P. citri*)
Leptomastix epona	33%	11%	100%
Leptomastix dactylopii	100%	54%	0
Anagyrus fusciventris	70%	3%	86%
Anagyrus pseudococci	-	-	40%

[1] Percent of parasitised mealybugs that encapsulated all eggs

Table 10. Percent escape from encapsulation by hatching larvae of three encyrtid parasitoid species in different mealybug species at 23°C (from Blumberg & Van Driesche. 2001).

Parasitoid species	Mealybug host species		
	Obscure (*P. viburni*)	Longtailed (*P. longispinus*)	Citrus (*P. citri*)
Leptomastix epona	4%	19%	-
Leptomastix dactylopii	-	2%	-
Anagyrus fusciventris	4%	30%	0%

A similar pattern is shown by the parasitoid *P. flavidulus* (Table 12). In a choice situation older hosts are preferred because more eggs are laid per host and a higher number of female offspring are produced. However it is possible that a wider range of host stages is acceptable for *P. flavidulus* than for *L. epona* because there is a less marked effect on the sex ratio of the next generation.

Table 11. Probability of host stage use by the solitary parasitoid *Leptomastix epona* showing offspring survival (emergence from pupal mummy formed) and sex ratio (male : female) at 26°C (from Karamaouna & Copland, 2000a).

Host		Egg laying probability	No.eggs per host	Mummified host		Survival (ex pupa)	Sex ratio
Stage	Size			No Choice	Choice		
1st instar	0.3-0.5	-		-	-	-	
2nd instar	0.5-0.9	0.11		14%	-	60%	49:1
3rd instar	1.0-1.7	0.30		42%	31%	64%	4:1
Young adult	1.8-2.3	0.23		60%	78%	72%	1:1.5
Preovipositing adult	2.3-3.3	0.36	(1.2)[1]	61%	83%	55%	1:3.2

[1] Number of eggs laid at 23°C (Blumberg & Van Drische 2001)

Table 12. Probability of host stage use by the gregarious parasitoid *Pseudaphycus flavidulus* showing the number of eggs laid per host, offspring survival (emergence from pupal mummy formed) and sex ratio (male : female) at 26°C (from Karamaouna & Copland, 2000a).

Host		Egg laying probability	No.eggs per host	Mummified host		Survival (ex pupa)	Sex ratio
Stage	Size			No Choice	Choice		
1st instar	0.3-0.5	0.02	-	-	-	-	
2nd instar	0.5-0.9	0.16	1.0	18%	5%	87%	?
3rd instar	1.0-1.7	0.23	1.4	46%	18%	76%	1:3
Young adult	1.8-2.3	0.28	2.9	22%	23%	92%	1:4
Preovipositing adult	2.3-3.3	0.31	4.7	15%	38%	85%	1:6

Plant architecture

There have been very few systematic studies to evaluate the influence of plant architecture on attack rates of mealybug parasitoids. One study with a parasitoid, *L. dactylopii*, of citrus mealybug showed that all architectural characteristics of plant size used, including height, leaf number, leaf surface area and number of branches, influenced the level of control achieved (Cloyd & Sadof, 2000). This study indicates that to improve their effectiveness any convenient measure of plant size (e.g. height) should be used to modify parasitoid release rates, which are usually based on units of surface area.

Summary

There is insufficient published data to characterise the most efficient parasitoid for the control of obscure mealybug, particularly in relation to tomato production. A feature of most mealybug parasitoids is that young adults are the most suitable hosts. A consequence of this is an increased chance that larval stages may escape from attack and act as refuges for the development and spread of the pest outbreak. Both *L. epona* and *P. flavidulus* prefer young adult mealybugs, which are the most suitable host stage and give rise to higher numbers of females in the next generation. Although there is some indication that *P. flavidulus* will accept young host stages more than *L. epona* (see Tables 11 & 12) it is possible that if released together both parasitoid species would compete for the same host stage rather than be complementary.

A third species, *P. maculipennis*, which is not native to the UK and has been introduced recently into New Zealand for the control of obscure mealybug on outdoor crops (Charles, 2001).

PART 6. MONITOR THE EFFICACY OF CONTROL MEASURES FOR *PSEUDOCOCCUS VIBURNI* ON COMMERCIAL TOMATO NURSERIES.

The managers of two large commercial nurseries that grow both conventional and organic tomato crops have provided information throughout the project regarding the incidence of mealy bugs in their glasshouses and the success of their control measures. Information for the 2002 tomato season is summarised below:

Grower A

This grower now controls *P. viburni* successfully in conventional crops by careful monitoring and thorough applications of buprofezin (Applaud). Thiacloprid (Calypso) has also been used experimentally with equivalent success.

The situation is quite different in organic crops, where *P. viburni* is now considered to be the most important pest on the nursery. The principle control measure is Savona, which is applied at high pressure to the lower stems of infested plants twice per week. To increase the speed of spraying and thus reduce labour input and cost, alternate sprays are directed at the upper and under sides of stems. Although this approach is moderately successful against the mealy bugs, the damaged areas of stems often become infected with *Botyris cinerea*, which leads to additional losses and further sprays. The grower has reported that the intensive spray programme causes the pest to become more active, leading to more rapid dispersal throughout the crop.

Grower A estimated the cost of the control programme to be approximately £2000 per hectare (£1500 for labour and £400 for materials). Despite the control programme, he also estimated mealybug damage to the value of approximately £600 per hectare.

Grower B

This grower also controls *P. viburni* successfully in conventional crops by monitoring to enable prompt action with thorough applications of buprofezin.

In organic crops, *P. viburni* is now considered to be one of the three most difficult pests to control (*i.e.* with *Macrolophus caliginosus* and woodlice). Two control measures have been adopted. The first is Savona applied weekly in infested areas at 4% dilution. The approach to these treatments is quite different to Grower A, in that more time is taken to obtain good spray coverage. While the degree of control achieved is considered to be reasonable, it is inevitably limited by the difficulty in penetrating the horizontal bundles of haulm at the base of the crop. The second control measure is Eradicoat, which is applied at 2.5% dilution in a similar manner to Savona. The degree of control achieved is again considered reasonable. However, if the pest progresses up the stems, Eradicoat can result in unacceptable sticky deposits on the fruit.

All control measures are considered to be unacceptably time consuming and too expensive in terms of both materials and labour. The estimated cost of the control programme was approximately £4,500 per hectare (£1,200 for labour and £3,300 for materials). There was also some financial loss due to mealy bug damage but this was not quantified.

CONCLUSIONS

The first year's studies (Sampson, 2000b) drew conclusions regarding:

- Pest status of *P. viburni* on UK tomato crops.
- Spread of *P. viburni* between and within glasshouses.
- Timing and location of infestations.
- Control measures based on quarantine, hygiene, chemical insecticides, physical methods and biological methods.
- Eradication of the pest from nurseries.

The information was summarised in HDC Fact Sheet (Reference 25/00) which is appended to this report (Appendix 2).

Conclusions at the end of the second year's studies were:

- The most effective and IPM compatible method of controlling *P. viburni* on tomato plants that has been identified to date is the insect growth regulator, buprofezin (Applaud). However, this product cannot be used on organic crops.
- *Hypoaspis miles* and *H. aculeifer* are not suitable biological control agents for release against *P. viburni* on tomato crops.
- *Chrysoperla carnea* has potential for use as a biological control agent against *P. viburni*. However, further studies are required to determine whether the predators will remain on tomato plants, feed on *P. viburni* in that environment and form breeding colonies within the glasshouse.
- The entomopathogenic fungi, *Beauveria bassiana* (Naturalis L and Botanigard WP), did not give significant control of *P. viburni*.
- The entomopathogenic fungi, *Verticillium lecanii* (Mycotal WP), infected *P. viburni* nymphs and reduced the population by approximately 8% compared to untreated controls. The effect of Mycotal WP could possibly be enhanced by applying it in an oil-based formulation. This should help to break down the mealybugs waxy protection and may allow more spores to come into contact with the insect's body.
- Of four products (Hortichem Spraying Oil, Hyvis 30 Emulsion, Horticide and Malathion) that were evaluated against *P. viburni* egg sacs on concrete surfaces, only Hyvis 30 Emulsion performed better than untreated controls. This polybutene based "glue" reduced the number of surviving nymphs by 50%. However, there may be up to 500 eggs in each sac and so this level of control was considered to be inadequate for commercial crops.
- The anti-feedant chemical, pymetrozine (Chess), applied twice at rates up to 80g product per 100 litres water, provided some control of *P. viburni* but this was considered to be inadequate for commercial crops.
- The pheromone used for trapping *Plannococcus citri* (citrus mealy bug) did not appear to be suitable for use against *P. viburni*.

The work in the final phase of the project focused on the control of mealy bugs on organic tomato crops as directed by the Project Review Panel on 10 May 2001. The principle conclusions were:

- Crop Oil proved to be very effective at removing wax from motile and egg stages of mealy bugs, and this led to high levels of mortality. Being a paraffinic oil, this product can not be used in organic crops but was included in the experiments as a positive control.
- A 2% dilution of Savona was effective against first instar mealy bugs as they first colonised tomato plants, reducing numbers by 93%. This should be the preferred

course of action at that crop stage because it will help to avoid overuse of Applaud and thus reduce resistance selection pressure to that chemical.

- 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 can be enhanced by applying after Savona. It has been hypothesised that Savona removes wax and allows more spores to come into contact with the insect's body. The results of this sequence of treatments have been variable but 100% mortality has been achieved in some circumstances.
- Jet 5 (peroxyacetic acid) and undiluted vinegar reduced the numbers of nymphs emerging from egg sacs on concrete by 67% and 81% respectively. Although this was an improvement on previous control measures tested, the levels of control may still be inadequate for commercial crops.

An appraisal of the published information on the biology of the mealybug parasitoid *Leptomastix epona* in relation to other parasitoids of the obscure mealybug, *P. viburni*, concluded that:

- There is insufficient published data to characterise the most efficient parasitoid for the control of obscure mealybug, particularly in relation to tomato production.
- Both *L. epona* and *P. flavidulus* prefer young adult mealybugs, which are the most suitable host stage and give rise to higher numbers of females in the next generation. Although there is some indication that *P. flavidulus* will accept young host stages more than *L. epona* it is possible that if released together both parasitoid species would compete for the same host stage rather than be complementary.
- An other species, *P. maculipennis*, which is not native to the UK, has been introduced recently into New Zealand for the control of obscure mealybug on outdoor crops.

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APPENDIX 1

Examples of the effects of various "dewaxing agents" on *Pseudococcus viburni* adults and egg sacs.

Figures 1 and 2: Typical untreated adult (left) and egg sac (right)

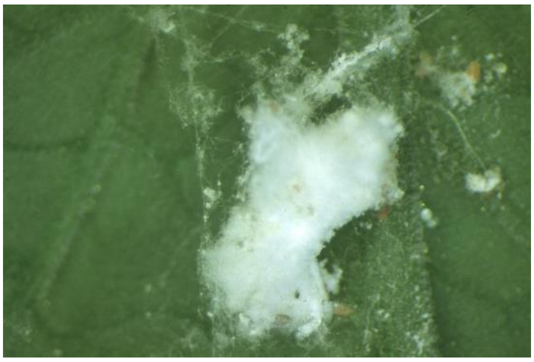

Figure 3 and 4: Adults (left) and egg sac following treatment with Crop Oil

Figure 5 and 6: Adults (left) and egg sac following treatment with 2% Savona

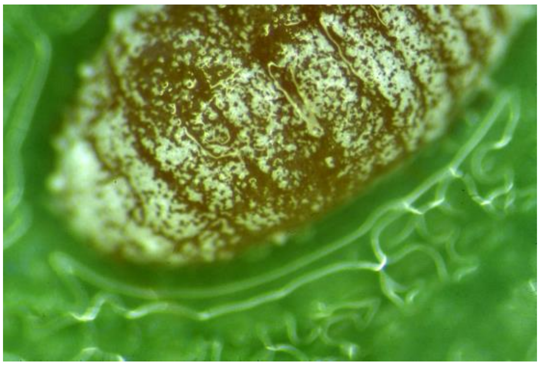

Figure 7 and 8: Adults (left) and egg sac following treatment with 0.1% Experimental Wetter % G.

APPENDIX 2

HDC FACT SHEET 25/00