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

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PRACTICAL SECTION FOR GROWERS

Commercial benefits of the project

The project has:

- Determined the scale of the threat that mealybugs pose to the UK tomato industry and identified measures that will help to prevent the pest becoming more widespread.
- Provided an improved understanding of the biology and behaviour of mealybugs in UK tomato crops, which has helped to more effectively target control measures on both plants and glasshouse structure.
- Reduced financial losses caused by the pest and the cost of control measures.
- Identified an effective and IPM compatible control measure (Applaud), which has reduced the dependence on broad spectrum insecticides and the disruption such products cause to biological control of other pests and biological crop pollination.
- Demonstrated that certain control measures previously used by growers are ineffective.

In the longer-term, the project aims to develop a mealybug control strategy that is not dependant on chemical insecticides and therefore of value to organic tomato growers.

Background and objectives

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. This project was prompted by an apparent increase in the incidence of the obscure mealybug (*Pseudococcus viburni*) on protected tomato crops in the UK.

The overall 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. The specific objectives for the second year 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.

Summary of results and conclusions

In the first year of the project, a survey confirmed that the incidence of mealybug infestations was increasing on UK tomato crops, with approximately 7% of the national crop affected at that time. 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. 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.

In the second year of the project, four methods of biological control were evaluated against mealybugs:

- *Hypoaspis* spp. – In the first experiment, *Hypoaspis miles and H. aculeifer,* were separately confined with all life cycle stages of mealybugs 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 nymphs. The predators did not reduce the number of mealybug 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 the obscure mealybug on tomato crops.
- *Chrysoperla carnea* – The lacewing, *C. carnea,* had been reported to attack some species of mealybugs but there was no information regarding its potential to control the obscure mealybug. A small-scale study was therefore designed to determine the predation rate of *C. carnea* nymphs against all life cycle stages of the obscure mealy bug. 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 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.

- *Beauveria bassiana* – 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 the obscure mealy bug in laboratory bioassays but neither product gave significant control of the pest.
- *Verticillium lecanii* – 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 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.

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 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.

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 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. Please note that pymetrozine (as Chess) is not approved in the UK for use on tomato crops.

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.

Action points for growers

- 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.
- Biological control measures that have been shown to have some potential against the obscure mealybug are:
    - *Chrysoperla carnea* - Further studies are required to determine whether these predators will be effective on tomato plants.
    - *Verticillium lecanii* (Mycotal WP) - The effect of this product may be enhanced by applying it in an oil-based formulation.
- The polybutene based glue, Hyvis 30 Emulsion, reduced the numbers of nymphs emerging from egg sacs on concrete by 50%. However, this level of control may be inadequate for commercial crops.
- Several control measures have been tested and shown to be unsuitable for use against obscure mealybugs on tomato crops, *i.e.*:
    - 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. Please note that pymetrozine (as Chess) is not approved in the UK for use on tomato crops.
    - The pheromone used for trapping *Plannococcus citri* (citrus mealy bug) did not appear to be attractive to male obscure mealybugs.

Practical and financial benefits from the study

The immediate benefits of the project will be:

- Improved control of mealybugs in protected tomato crops.
- Prevention of direct damage and financial losses caused by this pest.
- Reduced labour costs.

The ultimate benefits will be:

- Minimised chemical use following development of non-chemical methods.
- Reduced disruption to biological control agents and crop pollination due to chemical use.
- Satisfy demands of the UK's food retailers for reduced pesticide use and thereby improve competitiveness of UK tomato industry.
- Improved control of mealybugs in protected ornamental crops.

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 to develop effective control measures that are IPM compatible.

Summary of work completed in the first year of the project

The specific objectives for the first year 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:

- Hygiene and quarantine 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.
- Insecticides – 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.
- Timing of treatments on plants - 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).

Scientific targets of the second year of the project

The specific objectives for the second year 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.

PART 2. CONTROL OF *P. VIBURNI* WITH *HYPOASPIS* SPP.

Introduction:

Several growers have released *Hypoaspis* spp. on tomato crops in an attempt to control *P. viburni* but the results have been inconclusive (Sampson, 2000b). The experiments described here were designed to determine whether two species of *Hypoaspis* would feed on *P. viburni* and to evaluate their potential to control the pest on tomato plants. Adult female *Hypoaspis* spp. were used in the experiments. The predator populations were not synchronised because previous experiments with sciarid flies had shown that predation rates did not change with age (Wright and Chambers, 1994).

2.1. Laboratory Bioassays

Objective:

To compare the predation rates of *Hypoaspsis miles* and *H. aculeifer* against different life stages of *Pseudococcus viburni*.

Materials and Methods:

Treatments: *Hypoaspis miles* and *H. aculeifer* were evaluated separately against *P. viburni* adult females, eggs, first instar nymphs, second instar nymphs and third instar nymphs; *ie* 10 different combinations of predator and pest life cycle stages. In addition, there was an untreated control for each *P. viburni* life cycle stage, giving a total of total 15 Treatments.

Insect Populations: *Pseudococcus viburni* was reared on tomato plants (cv Spectra) at HRI, Stockbridge House. *Hypoaspis miles* and *H. aculeifer* were supplied by Biological Crop Protection Ltd. The latter were reared on *Tyrophagous* spp. mites.

Bioassay Procedure: The bioassays were done in 20 ml plastic tubes containing approximately 3 ml of sterile peat to provide a refuge for the mites and a 5 cm long piece of tomato stem (cv Spectra). Depending on the individual treatment, either three *P. viburni* adult females, 20 eggs, five first instar nymphs, five second instar nymphs or five third instar nymphs were placed on the tomato stem. The eggs were removed from their protective wax covering for this experiment. A single adult female *H. miles* or *H. aculeifer* was placed into each tube. In addition, similar numbers of each *P. viburni* life cycle stage were placed in tubes without predators as untreated controls. The tubes were closed with ventilated lids and stored upright in a controlled temperature room at 21 ± 2°C and 16:8 L:D.

Assessments: After 72 hours, the numbers of *P. viburni* that were live, dead or missing from each tube were recorded.

Experimental Design: Each tube comprised a replicate and there were 15 replicates per Treatment.

Statistical analysis: The numbers of *P. viburni* in each Treatment were compared by analysis of variance.

Results and Discussion:

There were no significant differences between the numbers of prey eaten by *H. miles* and *H. aculeifer*.

The numbers of *P. viburni* eggs, first instar nymphs and second instar nymphs were reduced ($P<0.01$) by the predators. The numbers eaten were dependent on the size of the prey. Both *H. miles* and *H. aculeifer* devoured one *P. viburni* egg or first instar nymph per day, or one second instar nymph per four days. Shereef *et al.* (1980) had previously reported that *H. miles* fed only on motile stages of prey but this experiment clearly demonstrated that they also fed on eggs.

The numbers of *P. viburni* adult females and third instar nymphs were not significantly reduced by either *H. miles* or *H. aculeifer*. The predators were probably unable to kill these two life cycle stages because they were too large and / or too densely protected by wax.

The limited predation rate of *H. miles* and *H. aculeifer*, suggested that large numbers of these predators would be required to control a population of *P. viburni* on tomato plants.

2.2. Plant scale experiment

Objective:

To evaluate the efficacy of *Hypoaspsis miles* and *H. aculeifer* against *Pseudococcus viburni* first instar nymphs on tomato plants.

Materials and Methods:

Location: Glasshouse F18, HRI Stockbridge House

Treatments: *Hypoaspis miles* and *H. aculeifer* were evaluated separately against *P. viburni* first instar nymphs at three positions on tomato plants; *ie* six

different combinations of predator and pest position. In addition, there was an untreated control for *P. viburni* at each position, giving a total of total 9 Treatments.

Insect Populations: *Pseudococcus viburni* was reared on tomato plants (cv Spectra) at HRI, Stockbridge House. *Hypoaspis miles* and *H. aculeifer* were supplied by Biological Crop Protection Ltd. The latter were reared on *Tyrophagous* spp. mites.

Procedure: The experiment was done in a 200 m² glasshouse on tomato plants (cv Spectra) grown on rockwool slabs under environmental conditions that were broadly consistent with normal commercial practice. The plants were spaced to minimise the risk of cross contamination by the insects and mites. On 13 September 2000, each plant was infested with 30 *P. viburni* first instar nymphs, which were divided equally between leaves in three positions; *ie* the lowest leaf, 0.5 m above ground and 1 m above ground. One hundred female *Hypoaspis* mites of the relevant species were then released on the rockwool block at the base of each plant. No mites were released on the untreated controls.

Assessments: Half the replicates were assessed 7 days after treatment and half were assessed 14 days after treatment. On each assessment date, the numbers of live *P. viburni* nymphs at each leaf position were counted and the presence or absence of *Hypoaspsis* spp. mites recorded.

Experimental Design: Each plant was an individual replicate and there were ten replicates per plot arranged in a completely randomised block design.

Statistical Analysis: The percentage of *P. viburni* nymphs that survived in each replicate was calculated. The data were subjected to angular transformation and Treatments compared by analysis of variance. Separate analyses of variance were done for each assessment date. The two data sets were then combined and analysed again.

Results and Discussion:

The percentages of *P. viburni* surviving at three positions on tomato plants in the presence and absence of *H. miles* or *H. aculeifer* are shown in Table 1. As there was no significant difference within Treatments at 7 and 14 days, the two data sets were combined.

Less than 50% of the released mealybugs were recovered at the end of the experiment. This was largely due to natural dispersal, with the greatest losses from the lower leaves.
There was no significant difference in the effects of *H. miles* and *H. aculeifer* on the numbers of *P. viburni* first instar nymphs that survived on the plants. When released at the

rate of 100 adult females per plant, the predators did not reduce the numbers of mealybugs on leaves up to 1 m above ground.

If the predators had climbed the plants and fed on the *P. viburni* nymphs at the predation rates recorded in the laboratory experiment (section 2.2.), it is estimated that all the prey would all have been consumed within two days. However, no *Hypoaspis* mites of either species were found on any leaves during the assessments. It is known that the mites had survived on the rockwool blocks because many were seen at the end of the experiment. Although *Hypoaspis* spp. are naturally soil dwelling creatures, they are known to climb to the aerial parts of plants because they have often been seen on leaves in cucumber crops. However, there is no evidence that they climb tomato plants. It is possible that they are deterred by the dense clothing of type VI trichomes on the stems and petioles of tomato plants.

These laboratory and plant scale experiments indicate that *H. miles* and *H. aculeifer* are not suitable biological control agents for release against *P. viburni* on tomato crops.

Table 1. The mean percentage (angular transformed mean) of *Pseudococcus viburni* surviving at three positions on tomato plants in the presence and absence of *H. miles* or *H. aculeifer*.

Leaf position	**Treatment**			**(LSD) 26 d.f.**
	Control	***H. miles***	***H. aculeifer***	
1m	58 (51.6)	51 (44.0)	43 (39.0)	(16.2)
0.5 m	47 (41.8)	45 (42.0)	46 (40.8)	(12.8)
Ground	29 (30.3)	23 (25.2)	20 (22.0)	(14.9)
All heights	45 (41.9)	40 (38.6)	36 (36.0)	(9.1)

PART 3. CONTROL OF *P. VIBURNI* WITH *CHRYSOPERLA CARNEA*

Introduction:

Chrysoperla carnea belong to the Neuroptera, a family of predatory insects commonly known as lacewings. The adult lays its eggs on the end of long hair-like filaments on leaves, thus providing some protection from other predators. The larva hatches after a few days and begins to feed on soft-bodied prey such as aphids. Both adults and larvae are voracious predators, injecting a paralysing venom into the prey before sucking out its body fluids. A larva may consume over 200 aphids in a week. Lacewings are also reported to attack cotton cushion scale insects, thrips, spider mites, small caterpillars and mealybugs (Krishnamoorthy & Mani, 1993). When such prey is in short supply, they may be cannibalistic. This small-scale study was designed to provide a preliminary evaluation of the potential of *C. carnea* to control *P. viburni*.

Objective:

To determine the predation rate of *C. carnea* second instar nymphs against all life cycle stages of *P. viburni*.

Materials and Methods:

Treatments: In five Treatments, *C. carnea* were evaluated against *P. viburni* adult females, eggs, first instar nymphs, second instar nymphs and third instar nymphs. There were untreated controls for each life cycle stage, giving a total of ten Treatments.

Insect populations: *Pseudococcus viburni* was reared on tomato plants (cv Spectra) at HRI, Stockbridge House. *Chrysoperla carnea* were supplied by Novartis BCM. The latter were reared on eggs of *Ephestia* spp. (meal moth).

Procedure: The experiment was done in 9 cm Petri-dishes with ventilated lids. A tomato leaf (cv Spectra) was placed on moist filter paper in each Petri-dish and infested with either 10 adult female *P. viburni*, 20 eggs, 20 first instar nymphs, 10 second instar nymphs or 10 third instar nymphs. One *C. carnea* second instar larva was then placed in each "treated" dish. All dishes were stored in a controlled temperature room at 21 ± 2°C and 16:8 L:D.

Assessments: After 24 hours, the numbers of live mealybugs in each Petri-dish were counted.

Experimental Design: Each Petri-dish comprised a replicate and there were 15 replicates per Treatment arranged in a completely randomised block design.

Statistical analysis: The numbers of each *P. viburni* life cycle stage that had been eaten by *C. carnea* were calculated. The data were subjected to square root transformation and Treatments compared by analysis of variance.

Results and Discussion:

Table 2 shows the natural mortality of *P. viburni* in the untreated controls and the numbers of each *P. viburni* life cycle stage that were eaten by *C. carnea*. There was little natural mortality of any of the *P. viburni* life cycle stages.

Chrysoperla carnea attacked and consumed all life cycle stages of *P. viburni* but the preferred prey were first instar nymphs. At 10 per day, there were twice as many of these nymphs killed as any other life cycle stage ($P<0.05$). The numbers of other *P. viburni* life cycle stages consumed per day were roughly in proportion to the size of the prey; *ie* 5 eggs, 3-4 second or third instar nymphs, and 1 adult female ($P<0.05$).

These results demonstrated that *Chrysoperla 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 *P. viburni* in that environment and form breeding colonies within the glasshouse.

Table 2. The mean numbers (square root transformed) of all life cycle stages of *P. viburni* that were eaten per day (from a total of N) by one *C. carnea* second instar larva compared to the number (square root transformed) that died of other causes in the untreated controls.

	N	Treated	Control	LSD
Eggs	20	5 (2.08)	0 (0.10)	(0.55) 17df
1st instars	20	10 (3.22)	0.9 (0.82)	(0.60) 18df
2nd instars	10	3 (1.52)	0.8 (0.61)	(0.69) 16df
3rd instars	10	4 (1.96)	0 (0)	(0.36) 17df
Adults	10	1 (0.71)	0 (0)	(0.48) 17df
(LSD) 85df			(0.52)	

PART 4. CONTROL OF *P. VIBURNI* WITH FUNGAL ENTOMOPATHOGENS

Objective:

To compare the efficacy of three fungal entomopathogen products against *Pseudococcus viburni*.

Introduction:

There is little published information on the efficacy of fungal entomopathogens against mealybugs. The possible use of *Hirsutella cryptosclerotium* against the mealybug, *Rastrococcus invadens*, has been discussed (Fernandez-Garcia *et al.*, 1990) but not investigated in field trials. *Beauveria bassiana* has been reported to infect the citrus mealybug (*Plannococcus citri*) and mango mealybug (*Drosicha mangiferae*) (Srivastave & Faish, 1988) but it has not been recorded on *P. viburni*. Where UK growers have applied *Verticillium lecanii* against glasshouse whiteflies, some incidental control of mealybugs has been reported though this has not been confirmed (Sampson, 2000b).

Three fungal entomopathogen products were selected for evaluation against *P. viburni* in this project; *ie* Naturalis L and BotaniGard WP, which both contain *Beauveria bassiana*, and Mycotal WP, which contains *Verticillium lecanii*. Naturalis L is supplied as an oil-based formulation, while the other two products are wettable powders. The two species of fungi were evaluated in separate experiments.

Materials and Methods - Experiment 1:

Treatments:
1. Naturalis L (4 ml product per 1 litre water)
2. BotaniGard WP (1.25 g product per 1 litre water)
3. Water only

Insect Population: *Pseudococcus viburni* were reared on tomato plants (cv 'Spectra') at HRI, Stockbridge House.

Bioassay procedure: The bioassay was done in 9 cm Petri-dishes with ventilated lids to reduce condensation. An excised tomato leaf (cv Spectra) was placed on a 2-3 mm layer of tap water agar in each dish. Ten *P. viburni* first instar nymphs were then released on the leaf. Each replicate dish was sprayed with 2 ml of diluted product in a Potter's Tower set at a pressure of 0.5 bar. The dishes were subsequently sealed with parafilm and placed in a high humidity chamber at 21±2°C and 16:8 L:D.

Assessments: Each dish was examined after 6 days and the numbers of live and dead *P. viburni* recorded. Dead *P. viburni* were collected and placed on damp filter paper in a Petri-dishes and incubated without light at 23°C. Fungal growth

(mycosis) on the dead insects was subsequently sub-cultured on growth media, incubated until sporulation occurred and identified.

Experimental Design: Each Petri-dish comprised a replicate and there were 10 replicates per Treatment arranged in a completely randomised block design.

Statistical analysis: Initially data were analysed using a simple analysis of variance on the angular transformation of the percentage of dead insects. However, analysis of the residuals showed the transformed data were not normally distributed. Because of this a Generalized Linear Model was used assuming the data (alive vs total) to be binomially distributed with a logit link function. Analysis of the residuals looked better in terms of the residual plots.

Materials and Methods - Experiment 2:

Treatments: 1. Mycotal WP (1.0 g product per 1 litre water)
2. Water only

The methodology was the same as experiment 1, except there were 15 replicates of each Treatment.

Results and Discussion:

The combined results of experiments 1 and 2 are shown in Table 3 and Figure 1. There was no significant difference in the mortality of the *P. viburni* first instar nymphs that had been sprayed with Naturalis L, Botanigard WP or water in experiment 1. However, in experiment 2, the application of Mycotal WP resulted in approximately 8% more ($P<0.05$) mortality than water only. Mycosis was observed on 75% of the dead insects collected from the Mycotal WP Treatment.

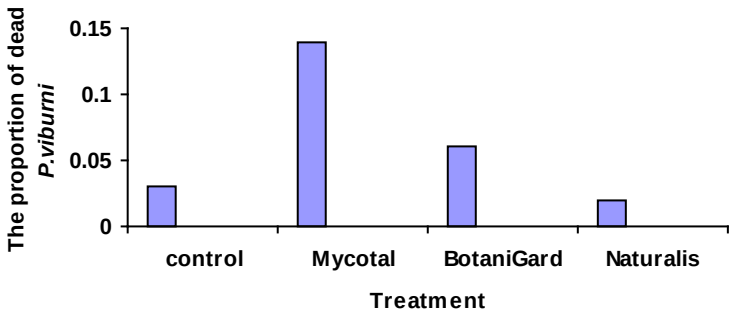
The effect of Mycotal WP may be enhanced by application in an oil-based formulation because this will help to break down the mealybugs waxy protection and allow more spores to come into contact with the insect's body.

Table 3. The mean proportion (standard error) of dead *P. viburni* six days post application of three entomopathogenic fungal products compared to controls treated with water only. The data is presented as a regression analysis on the predicted dead mealybugs.

Experiment	Treatments				d.f.
	Control	Naturalis L	BotaniGard WP	Mycotal WP	
1	0.03 (0.018)	0.02 (0.017)	0.06 (0.025)		27
2	0.07 (0.021)	-	-	0.14 (0.030)	28

There were significantly more ($P < 0.05$) dead *P. viburni* in the Mycotal Treatment than the respective control.

Figure 1. The proportion of dead *P. viburni* recorded six days post application of three entomopathogenic fungal products compared to controls treated with water only.



PART 5. CONTROL OF *PSEUDOCOCCUS VIBURNI* EGGS AND FIRST INSTAR NYMPHS ON THE CONCRETE STUCTURE OF THE GLASSHOUSE

Introduction:

Many *P. viburni* survive between crops as eggs on the concrete bases (dollies) of roof supports. There may be up to 500 individual eggs within a single egg pouch and they are densely protected by waxy filaments. At the start of the new season, *P. viburni* eggs hatch in response to raised temperature. The young nymphs move on to the new 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. This experiment was a small-scale bioassay designed to evaluate alternative products against egg masses on the dollies.

Objective:

To evaluate the potential of four treatments for the control of *Pseudococcus viburni* eggs and first instar nymphs on the concrete structure of the glasshouse.

Materials and methods:

Treatments:
1. Untreated control
2. Water
3. Hortichem Spraying Oil – 10 ml product per litre water
4. Hyvis 30 Emulsion (polybutene glue) – 10 ml product per litre water
5. Horticide - 20 ml product per litre water
6. Malathion – 1.9 ml product per litre water

Insect Populations: *Pseudococcus viburni* was reared on tomato plants (cv Spectra) at HRI, Stockbridge House.

Bioassay procedure: The Treatments were applied to small (90 x 50 x 40 mm) concrete blocks that had been made for the experiment. Four batches of approximately 100 *P. viburni* eggs were placed on the upper surface of each concrete block. The sprays were applied to the point of run off with a 3 litre Hozelock Sprayer fitted with the standard spray nozzle. Following treatment, each concrete block was placed on a yellow sticky trap (150 x 200 mm) and stored in a controlled environment room at 21 ± 2°C and 16:8 L:D until the final assessment had been completed.

Assessments: Preliminary studies had shown that the first instar nymphs left the blocks soon after emerging from eggs and became trapped on the sticky traps. In

those studies, all the eggs hatched within three weeks. In this experiment, the traps were changed after 3 weeks and the numbers of captured nymphs recorded. The traps were then changed at two week intervals until no further nymphs were found.

Experimental design: Each concrete block formed a replicate and there were eight replicates per Treatment (except the untreated control for which there were two) arranged in a randomised block design.

Statistical Analysis: The data were subjected to square root transformation and Treatments compared by analysis of variance.

Results and Discussion:

The mean numbers per Treatment of *P. viburni* first instar nymphs that survived the treatments and were caught on the sticky traps are shown in Table 4. There were no significant differences between the six treatments. This may be explained by the extended emergence time of the insects and the short persistence of the products under the alkaline conditions of the concrete surface. The polybutene glue (Hyvis 30 emulsion) dried rapidly on the absorbent surface and performed less well than might have been expected. As there had been some difficulty dissolving this product, it was re-tested in a second experiment. On this occasion, 50% fewer ($P<0.05$) nymphs survived the treatment than in the untreated control. However, this level of control was still not acceptable for a commercial treatment.

These results are consistent with the observations of growers on commercial nurseries.

Table 4. The mean numbers of *P. viburni* eggs / first instar nymphs that survived five different types of treatment on concrete blocks.

	N*	Mean numbers (square root transformed mean) of surviving mealybugs	
Horticide	8	127.75	(11.19)
Hyvis30	8	153.38	(12.11)
Malathion	8	106.63	(9.94)
Spraying oil	8	134.75	(11.29)
Water	8	130.75	(11.10)
Untreated	2	218.50	(14.91)
LSD (36 df)			(8.68) - to compare different N (5.49) – To compare identical N)

* Number of replicates

PART 6. CONTROL OF *P. VIBURNI* WITH PYMETROZINE

Introduction:

Pymetrozine (Chess) is an antifeedant chemical that is specific to some insect relatives of mealybugs; *e.g.* aphids and whiteflies. After it is imbibed by these sucking insects, it acts on the nervous system and prevents further feeding. The insects usually die of starvation between three and seven days after application of the product. Pymetrozine was evaluated against *P. viburni* on tomato plants in the present crop-scale experiment.

Objective:

To determine the efficacy of pymetrozine against active stages of *P. viburni* on protected tomato plants.

Methods:

Site: Glasshouse F18, HRI Stockbridge House

Treatments:
1. Two applications of pymetrozine (Chess) at 40g product / 100 l water, with a 10-day interval.
2. Two applications of pymetrozine (Chess) at 80g product / 100 l of water, with a 10 day interval.
3. Water only.

Application: All treatments were applied high volume to maximum leaf retention using a fully calibrated Oxford Precision sprayer. The first and second sprays were applied on 13 and 23 October 2000 respectively.

Growing conditions: Tomato plants (cv Favorita) were grown hydroponically in rockwool slabs with excess feed solution running to waste. The aerial environment was computer controlled with minimum day:night temperatures of 19^{O}C:16 ^{O}C and ventilators opening at 20-21 ^{O}C.

Insect Populations: *Pseudococcus viburni* was reared on tomato plants (cv Spectra) at HRI, Stockbridge House and released on the plants in the experimental crop in April 2000.

Assessments: Four plants were selected per plot and a 50 cm length of stem was marked on each. The numbers of each life cycle stage of *P. viburni* (live and dead) were recorded on each stem the day before the first application and seven days after the second application of pymetrozine.

Experimental design: There were twelve plants per plot and six plots per Treatment arranged in a randomised block design.

Statistical analysis: Due to the fact that there were statistical differences between numbers of *P. viburni* populations on pre-treatment plots, data were analysed using an analysis of covariance. The covariate used was the pre-treatment numbers of *P. viburni* per plot. All data were square root transformed prior to analysis.

Results and Discussion:

The effects of the two application rates of pymetrozine on mean numbers of *P. viburni* egg sacs, first instar nymphs, second instar nymphs, third instar nymphs and adult females are shown in Tables 5, 6, 7, 8 and 9 respectively.

After taking into account the pre-treatment differences in *P. viburni* population size, there was a slight trend towards lower numbers of all life cycle stages on the pymetrozine treated plants than on untreated controls at the end of the experiment. However, this was only significant in two situations; *i.e.* numbers of third instar nymphs were 28% lower ($P<0.05$) in the pymetrozine low rate treatment than in the controls, and numbers of egg sacs were 30% lower ($P<0.05$) in the pymetrozine low rate treatment than in the controls.

These results show that two pymetrozine applications at rates up to 80g product per 100 litre of water, do not provide adequate control of a *P. viburni* population on tomato plants.

Table 5. The effect of rate of application of pymetrozine on the mean numbers of *P. viburni* egg sacs per 50 cm length of tomato stem.

Treatment	Mean numbers of *P. viburni* egg sacs		
	Pre treatment	Post treatment	Means of square root transformed data adjusted for the covariates
Control	5.04	5.00	2.44
Low rate (40g/litre)	5.88	5.17	2.18
High rate (80g/litre)	7.42	4.79	1.71
LSD (5df)			0.46

Table 6. The effect of rate of application of pymetrozine on the mean numbers of *P. viburni* first instar nymphs per 50 cm length of tomato stem.

Treatment	Mean numbers of first instar nymphs		
	Pre-treatment	Post treatment	Means of square root transformed data adjusted for the covariates
Control	57.29	29.29	4.77
Low rate (40g/litre)	56.00	15.29	3.26
High rate (80g/litre)	50.96	16.33	4.13
LSD (5df)			2.23

Table 7. The effect of rate of application of pymetrozine on the mean numbers of *P. viburni* second instar nymphs per 50 cm length of tomato stem.

Treatment	Mean numbers of second instar nymphs		
	Pre-treatment	Post treatment	Means of square root transformed data adjusted for the covariates
Control	46.88	50.25	7.09
Low rate (40g/litre)	46.54	39.00	5.81
High rate (80g/litre)	37.38	34.75	5.81
LSD (5df)			1.69

Table 8. The effect of rate of application of pymetrozine on the mean numbers of *P. viburni* third instar nymphs per 50 cm length of tomato stem.

Treatment	Mean numbers of third instar mealybugs		
	Pre-treatment	Post treatment	Means of square root transformed data adjusted for the covariates
Control	8.71	33.04	5.80
Low rate (40g/litre)	7.38	21.08	4.19
High rate (80g/litre)	4.21	20.96	4.36
LSD (5df)			1.58

Table 9. The effect of rate of application of pymetrozine on the mean numbers of *P. viburni* third instar nymphs per 50 cm length of tomato stem.

Treatment	Mean numbers of adult mealybugs		
	Pre-treatment	Post treatment	Means of square root transformed data adjusted for the covariates
Control	0.63	8.28	2.81
Low rate (40g/litre)	1.29	4.71	2.12
High rate (80g/litre)	0.75	2.79	1.60
LSD (5df)			1.26

PART 7. MEALYBUG PHEROMONES

Introduction:

The female sex pheromone of the citrus mealybug, *Plannococcus citri*, has been identified (Bierl-Leonhardt *et al.*, 1981; Hwang & Chu, 1987), synthesised and is now used to trap males of this species in commercial crops. At the project review meeting in February 2000, participants expressed an interest in developing a pheromone trap for *P. viburni* but it was agreed that there were insufficient funds available in this project. Sex pheromones are usually specific to individual species of insects but some will attract closely related species. It was therefore agreed to determine whether the *P. citri* pheromone was attractive to male *P. viburni*. If a positive effect were demonstrated in the preliminary studies, more detailed experiments would be organised for 2001/02.

Materials and methods:

The observations were made during September and October 2000 in a 200 m² glasshouse containing a mature tomato crop (cv Favorita) that had been infested with *P. viburni* in April 2000. It was estimated that there were over 100,000 male mealybugs in the glasshouse when the traps were evaluated.

Each trap consisted of a rubber pheromone dispenser (1.5 x 1.0 cm) placed in the centre of a yellow sticky trap (25cm x 10cm). The dispensers of the baited traps were loaded with 200 µg of a solution of the *P. citri* pheromone (1 µg -isopropenyl –2-dimethylcyclobutanemethanol acetate per 1 µl acetone), which filled the dispenser. The dispenser was allowed to dry at room temperature before use. The unbaited control traps were loaded with acetone only.

On four separate occasions, two traps (one baited and one control) were placed in the crop. The traps were hung separately on wires, approximately 30 cm above the ground and 1 m from a mealybug infested plant. After seven days, the numbers of male mealybugs caught on each trap were counted.

Results and Discussion:

The numbers of male mealybugs caught per trap are shown in Table 10.

In total, four male mealybugs were caught on the pheromone traps, compared to one on the control traps. This was a very small catch considering the large number of *P. viburni* that were in the glasshouse at the time. Those that were caught were probably the result of incidental trapping. If the pheromone had any attraction for male *P. viburni*, it must have been masked by pheromones naturally produced by the females on the plants.

In summary, the pheromone used for trapping *P. citri* does not appear to be suitable for use against *P. viburni*.

Table 10. The numbers of male *P. viburni* caught on pheromone baited and control traps on four different occasions.

Date traps placed	Numbers of male mealybugs caught per trap, seven days after the trap was set up	
	Control	**Pheromone trap**
6 September	0	2
2 October	1	2
9 October	0	0
16 October	0	0

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.

Additional conclusions at the end of the second year's studies are:

- 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*.

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 Panel suggested the following topics for further investigation:

- Evaluation of efficacy of peracetic 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.

REFERENCES

Bierl-Leonhardt, B.A., Moreno, D.S., Schwarz, M., Fargerlund, J., and Plimmer, J.R. (1981). Isolation, identification and synthesis of the sex pheromone of the citrus mealybug, *Planococcus citri* (Risso). *Tetrahedron Lett.* 22: 389-392.

Fernandez-Garcia, E., Evans, H.C. and Samson, R.A. (1990). *Hirsutella cryptosclerotium* sp. Nov., an entomopathogen of the mealybug pest, *Rastococcus invadens*, in West Africa. *Mycological Research*. 90:8, 1111-1117.

Hwang, J.S. and Chu, Y.I. (1987). The development of sex pheromone traps for the citrus mealybug, *Planococcus citri* (Risso). *Plant Protection Bulletin, Taiwan*. 29:3, 297-305.

Krishnamoorthy, A. and Mani, M. (1989). Records of green lacewings preying on mealybugs in India. *Current Science*. 58;3, 155-156.

Sampson, C. (2000a). Mealybugs on Protected Tomato Crops. *HDC Fact Sheet*. 25/00, 4pp.

Sampson, C. (2000b). Protected tomatoes: Integrated control of mealybugs. Horticultural Development Council report for project PC 161. February 2000, 30pp.

Schoen, L and Martin, C., (1999). A "new" type of scale on tomatoes. *Pseudococcus viburni*, a potential greenhouse pest. *Phytoma*. 514, 39-40.

Shereef, G.M., Soliman, Z.R. and Afifi, A.M. (1980). Economic inportance of the mite *Hypoaspis miles* (Berlese) (Mesostigmata: Laelapidae) and its life history. *Bull. Zool. Soc. Egypt*, 30, 103-108.

Srivastava, RP and Fasih, M. (1988). Natural occurrence of *Beauvaria bassiana*, an entomogenous fungus on mango mealy bug, *Drosicha mangiferae*. *Indian Journal of Plant Pathology*. 6: 1, 8-10.

Wright, E.M. and Chambers, R.J. (1994). The biology of the predatory mite *Hypoaspis miles (Acari: Laelapidae)*, a potential biological control agent of *Bradysia paupera (Diptera: Sciaridae). Entomophaga.* 39 (2), 225-235.

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