Know-how for Horticulture™

Improvements to biological control systems and development of biorational chemicals for integrated pest management of greenhouse vegetables

Dr Stephen Goodwin
NSW Agriculture

Project Number: VG00066

VG00066

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Project Title: Improvements to Biological Control Systems and Development of Biorational Chemicals for Integrated Pest Management in Greenhouse Vegetables

Authors: Dr Stephen Goodwin and Marilyn Steiner

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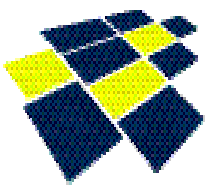

Horticulture Australia

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HAL Project Number: VG00066

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Purpose of Report:
This report describes the outcomes of ongoing research and development into improvements in biocontrol and the development of biorational chemicals for use in IPM programs by the greenhouse vegetable industry. Adequate access to effective biocontrol agents and safe, biorational chemicals is an essential requirement for any IPM program.

In Australia, greenhouse vegetable production lags behind other countries in its adoption of available sophisticated technology and in its use of IPM. For the latter part this is due to a lack of awareness of the commercial availability of biocontrol agents, the dearth of effective biocontrol agents for some key pests, the lack of availability of reduced risk chemicals that can be integrated with biocontrol agents to supplement their use and the lack of grower knowledge in correctly using these tools.

To a large extent the future of the Australian greenhouse vegetable industry rests as much with the development of sustainable crop protection systems as it does with the use of sophisticated production technology. This includes not only the adoption of existing IPM opportunities, but the development of new tools such as is occurring in our IPM research programs. This report details the outcomes of a comprehensive IPM research program conducted between 2000-2003.

The project reported on here also sought to provide greenhouse vegetable growers around Australia with support to familiarise them in the use of these tools through training programs and the provision of on-farm technical support.

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- Vegetable industry research and development levy

Date: 31 January 2004

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Media Summary

NSW Agriculture IPM researchers Stephen Goodwin and Marilyn Steiner continue to improve their track record in delivering new practical developments to the greenhouse vegetable industry.

Goodwin said " Aside from making sure the science in the work is right, we try to never lose sight of the need to provide practical solutions to greenhouse vegetable growers. Our recently completed HAL project on new biocontrol agents and reduced risk chemicals is a good example of this with some interesting developments coming out of it"

"A number of new biocontrol agents with potential for further investigation and commercial development were identified. These will add to the limited range of biocontrol agents presently available to Australian growers for aphid, spider mite, tomato russet mite and whitefly control" he said.

"One of the highlights of this part of our program was the effort that went into and continues to go into developing field use in greenhouse vegetable crops of the promising new biocontrol agent Montdorensis. We spent a lot of time testing the *How to Use Montdorensis* advice for growers in our greenhouses at the National Centre for Greenhouse Horticulture. In addition we have set up a mass rearing unit to supply interested growers with adequate numbers of the biocontrol agent, produced a fact sheet and provide on-farm technical support. This is an extra contribution to industry outside our funded research" Goodwin said.

Goodwin and Steiner also developed a new use for Montdorensis during the project. Tomato russet mite is a major damaging pest with no safe control solutions. New work has identified Montdorensis as an effective biocontrol agent against this pest as well as providing a back-up pesticide combination compatible with it.

Feeding trials on russet mite comparing a number of Australian and overseas predatory mites found Montdorensis was one of only a few species that ate large numbers of russet mite, but the only one that could establish and move around on tomatoes and could be mass reared. Greenhouse tomato growers using biocontrol can now use an IPM strategy involving Montdorensis for general russet mite control with a safe chemical combination for hot spots, without harming other biocontrol agents in the crop.

Steiner said "Our work done on testing the activity of reduced risk chemicals that have little effect on biocontrol agents will also greatly assist greenhouse vegetable growers with their biocontrol programs."

Another key area of discovery in the project was the progress made towards developing, for the first time in Australia, fungal biopesticide products for thrips, whitefly and aphids. Modern greenhouses can provide conditions to enable fungal biopesticides to work without creating conditions for plant disease to occur.

Goodwin said “We are collaborating with Somersby-based company BioCare Technology in this work. So far preliminary screening of isolates from Australia, the USA and Canada has identified several promising ones. The best of these will be developed into new commercial products in conjunction with BioCare in a new HAL project.

A major activity of the project was the continual testing of new IPM programs involving developments from their research. This was carried out in crops grown in modern computer-controlled greenhouses at NSW Agriculture’s National Centre for Greenhouse Horticulture at Gosford. In this way IPM programs for greenhouse cucumber, capsicum and tomato crops have continually been refined and improved.

“We are always happy to pass on this breaking news to growers. In 2003 we spoke to three groups in Victoria, two in SA and one in WA about some of these new developments for their herb and greenhouse vegetable crops.

“A key activity in this technology transfer was the development of an IPM training program *IPM Onfarm for Protected Cropping* for greenhouse vegetable producers by NSW Agriculture. This can be delivered in any State; already we have worked with Victorian and Western Australian growers.

“We were impressed by the interest shown by the growers in this information and in particular in their support for our continuing IPM research program” Goodwin concluded.

Technical Summary

Project VG00066, supported by the vegetable industry through Horticulture Australia Limited, undertook basic laboratory research and greenhouse crop developmental studies in IPM in 19 separate areas of work during 2000-03.

These included:

- Identified new biocontrol agents and developed rearing systems.

  Science: Involved collecting trips, establishing cultures at NCGH, in some instances developing mass rearing systems and conducting crop usage experiments and making some initial material available to cooperating growers.

  Future work: This work identified the following promising candidates for future investigation and development as commercially produced biocontrol agents. *Typhlodromips montdorensis* (Acari: Phytoseiidae) predatory mite for tomato russet mite, *Hippodamia variegata* (Coleoptera: Coccinellidae) and *Micromus tasmaniae* (Neuroptera: Hemerobiidae) predators of aphids and some other soft-bodied insects; *Feltiella* undescribed species (Diptera: Cecidomyidae) predator of spider mites, *Eretmocerus* spp (Hymenoptera: Aphelinidae) parasitoid for whitefly at high temperatures and *Aphidius colemani* (Hymenoptera: Braconidae) parasitoid of aphids.

  1. *Hippodamia* and *Micromus* are the subject of two postgraduate studies in a HAL application for 2004/05.
  2. Strong interest has been expressed by Biological Services, Loxton in the commercial development of *Aphidius* and *Eretmocerus* in the immediate future.
  3. Further work will be done on developing crop usage of *Feltiella* at NCGH.

- Evaluated new pesticides for side effects on the whitefly parasitoid *Encarsia formosa.*

  Science: Conducted replicated laboratory bioassay and greenhouse experiments with single dosages of 24 selected pesticides. Full data on work to date available. Partially completed.

  Future work: Complete laboratory program with nominated list of chemicals. Make results available to growers.

- Evaluated reduced risk chemicals against caterpillars, aphids, whitefly, thrips and mites in bioassays and crop trials in the laboratory and greenhouse.

  Science: Conducted preliminary replicated laboratory and greenhouse screening bioassays. Data analysed.

  Future work: Preliminary screening to be completed and greenhouse trials to develop crop usage protocols and data for registration conducted.

- Developed and improved IPM systems for greenhouse cucumber, capsicum and tomato crops with new biocontrol agents and reduced risk chemicals.

  Science: Tested crop usage releases of new biocontrol agents with particular emphasis on *Typhlodromips montdorensis*, and tested new reduced risk chemical treatments at NCGH. Crop monitoring data available.

  Future work: Continue to refine IPM programs in greenhouse vegetable crops at NCGH as new biocontrol agents and reduced risk chemicals become available. Provide data for permit/registration applications.

- Evaluated the potential for developing fungal biopesticides against thrips, whiteflies and aphids.

  Science: Conducted replicated laboratory bioassays to screen fungal isolates from three countries. Data analysed. Promising isolates identified.

  Future work: Undertake commercial development of new products for western flower thrips, greenhouse whitefly and aphids. Includes bioassays into dose-response and life-stage specificity, plus preliminary commercial production to identify cost-effective isolates, development of spore formulation technology, and conduct greenhouse bioassays and small crop greenhouse trials.

- Compared molecular relationships between local and some overseas phytoseiid mite species.

  Science: Collaborated with Macquarie University, Sydney in undertaking DNA sequencing of closely related phytoseiid mite species to clarify taxonomic relationships. Species important to biocontrol were investigated.

  Future work: Further DNA work is to be undertaken with strains of *Neoseiulus cucumeris,* an overseas phytoseiid species used commercially as a thrips biocontrol agent. The initial results showed some divergence in genetic profile within the species and more extensive strain comparison is to be conducted.

- Development and delivery of a new IPM training course for the greenhouse vegetable industry.

  Science: This was an extension activity. An accredited IPM training course *IPM Onfarm for Protected Cropping* was developed. Delivery was provided to two greenhouse vegetable groups at Shepparton, Victoria in late 2003. The course is available nationally. This industry now has available to it a set of practical IPM publications plus the above training course produced by NSW Agriculture at the NCGH.

  Future work: Trained growers should be supported by post-training on-farm technical support through HAL funded activities or by competent consultants.

- Technology transfer activities.

  Science: This was an extension activity. During the project overseas IPM specialists were brought to Australia. Dr Michael Brownbridge, University of Vermont, gave workshops on the development and use of fungal biopesticides to greenhouse vegetable groups in SA and Victoria. Ms Jude Bennison, ADAS, UK was funded by herb producers to deliver IPM workshops to herb producers in Victoria and to two groups in SA. A workshop for greenhouse vegetable growers in WA was conducted, followed by farm consultations.

  Future work: Attempts are being made to attract Dr Brownbridge to Australia to collaborate on the fungal biopesticide study. He would be available to network with growers and assist in the IPM adoption program.

The National Centre for Greenhouse Horticulture

The National Centre for Greenhouse Horticulture was established by NSW Agriculture at Gosford in 2001. It is a small complex of state-of-the-art greenhouses. In this report, NCGH refers to this Centre.

The NCGH was developed to provide quality facilities for the conduct of research and demonstration trials in crop production and crop protection. It was developed to a specification to demonstrate to industry a level of technology that they should be aiming for when considering investing in new structures and systems.

It comprises two larger structures of 500sqm and 600sqm and four smaller greenhouses each 54 sqm. In this report, the two larger greenhouses are referred to as the Square and Long Houses respectively and the smaller ones are referred to as the Propagators.

The Long House has twin-skinned inflated side-walls with misting to assist environmental management. Environmental and hydroponic management is provided by an ECOS controller.

The Square House is a twin span structure with side-wall ventilation and anti-virus mesh screening. It has thermal screening and a fogging system to assist with environmental management.

Both the Long House and Square House have double gull-wing ridge ventilation, 4m to the gutter, full hydroponic nutrition systems and hydronic heating. They have computer-controlled environmental and hydroponic systems. Crop trials, mainly on production research issues, are conducted in these two structures, with IPM investigations superimposed in the crop as well.

Unlike the larger greenhouses, the Propagators have no ridge ventilation, relying on a fan and pad system for ventilation and cooling. They are 3m to the gutter and have full hydroponic nutrition systems and hydronic heating. These four smaller structures are mainly used for IPM research. Environmental and hydroponic management for the Square House and the Propagators is provided by a PRIVA Maximiser controller.

All of the greenhouses, plus heating and hydroponic systems and the ECOS environmental and hydroponic controller for the Long House were provided by Harford Greenhouses. The greenhouse structures were erected by Harford Greenhouses. The PRIVA control system was provided and installed by AGCON Technology Ltd, New Zealand.

Introduction to Project

Despite the obvious advantage of climate to field production in Australia, greenhouse and hydroponic production has the potential to provide the market with a guaranteed supply of safe, quality produce in a range of vegetable and herb crops. The benefits of this production method are resulting in major new investment occurring throughout Australia. Currently the key crops are tomatoes, cucumbers and lettuce. Capsicum, while a major greenhouse crop overseas, is still mainly a field crop in Australia. The leaders in herb production are using greenhouse and hydroponic technology. There is also the potential for other crops to be produced using these methods.

Key to the claim of safe produce is the adoption of successful alternatives to synthetic pesticides that have formed the mainstay of all pest control programs in the past.

NSW Agriculture committed to the protected cropping industry by establishing the National Centre for Greenhouse Horticulture in 2001. One of the key activities at the Centre is the on-going program to develop and improve IPM systems. This includes the development of technical material, training courses, providing industry support through workshops, on-farm advice and encouraging and participating in the development of industry IPM groups. However most importance is attached to the on-going research program to develop new biocontrol agents and reduced risk or biorational chemicals that are safe to use with biocontrol agents. These were the targets of project VG00066.

Australia lags behind other countries in the availability and adoption of these tools. Amongst a number of Australian greenhouse vegetable producers there is strong interest in the development and adoption of IPM, but this cannot occur without industry support. To this end, aside from support from the vegetable levy, other industry bodies have also made voluntary contributions to this research effort to the amount of $2 for every $1 of levy contribution. As a result, although there is currently no compulsory tomato levy, outcomes from this project also targeted this crop.

The final report details the work conducted in a number of areas within these two general topics and good progress was achieved in the period 2000-2003. Research conducted in this project produced some completed outcomes such as the development of a new biorational insecticide, and tested IPM protocols for cucumbers and tomatoes with new biocontrol agents. In other instances the work conducted was part of on-going research. This included the identification of some new natural enemies with potential for further investigation and commercial development as biocontrol agents, plus some early developmental work. Also in another significant area some reduced risk chemicals were initially screened against key pests, while a comprehensive list of isolates of beneficial fungal pathogens were also screened against key pests. Each of these areas of work will be continued in subsequent R&D projects in an attempt to ultimately deliver additional commercial outcomes in integrated pest management to industry.

Description of Activities

Section A Development of an IPM program for greenhouse tomatoes

Background

Pests of greenhouse tomato crops in Australia are similar to those encountered worldwide. These include various thrips (western flower thrips, *Frankliniella occidentalis*, onion thrips, *Thrips tabaci*, and tomato thrips, *Frankliniella schultzei* (dark form)), mites (tomato russet mite, *Aculops lycopersici*, broad mite *Polyphagotarsonemus latus*, and two-spotted mite, *Tetranychus urticae*), whitefly (greenhouse whitefly, *Trialeurodes vaporariorum*), aphids (mostly green peach aphid, *Myzus persicae*), various caterpillars (cluster caterpillar*, Spodoptera litura*, Heliothis, *Helicoverpa* spp. and loopers, *Chrysodeixis* spp.), and fungus gnats (*Bradysia* spp). The three species of thrips that attack tomato rarely build up large numbers but they vector TSWV very efficiently so tolerance is very low.

In northern Europe, Canada, New Zealand and other developed countries, bumblebees perform pollination in the crop so tolerance to pesticides is very low and biocontrol of pests is widely practised. Tomato russet mite is still a problem, mostly in southern Mediterranean countries, as is 'carmine mite', an apparently toxic strain (or perhaps a different species) of two-spotted mite that *Phytoseiulus persimilis* is not able to control adequately because it has difficulty traversing the toxic glandular hairs of tomato. Control of greenhouse whitefly has been much improved by plant bugs such as *Macrolophus* in Europe and *Dicyphus* in Canada, and by a 'winter strain' of *Encarsia formosa* in Europe. There are likely no suitable types of plant bugs in Australia. *Nesidiocoris viridis* is a possibility but it has not shown much potential elsewhere and may damage the fruit. The carmine mite has not yet been recorded here. The predatory mite *Neoseiulus cucumeris* has been tried against thrips but does not perform well on tomato.

For Australian growers, the main pests are thrips, tomato russet mite and greenhouse whitefly, and research has concentrated on developing and improving a program for these pests.

<u>In this section the following trials were conducted:</u>

Biocontrol	Trial 1. Feeding capacity of phytoseiid predatory mites on tomato russet mite
	Trial 2. Evaluation of *Typhlodromips montdorensis* as a biocontrol agent for control of thrips and tomato russet mite
Other	Trial 3. Evaluation of the role of greenhouse whitefly in the distribution of tomato russet mite

Trial 1. Feeding capacity of phytoseiid predatory mites on tomato russet mite.

Background

Tomato russet mite has a world-wide distribution in temperate regions and is a serious pest of greenhouse tomato crops across Australia, particularly in SA, VIC and NSW. Its extremely small size makes it almost impossible to see individuals with the naked eye and therefore difficult to detect until symptoms of damage appear on the plant (purple-brown stems and rust-coloured, droopy leaves). The population density is very high wherever these symptoms are apparent, in the order of hundreds per square centimetre. The initial visible infestation is usually confined to a single plant or widely scattered plants, but by then mites have usually been carried on clothing, air currents or perhaps by insects to other plants, resulting in more extensive outbreaks a few weeks later. Sulfur, endosulfan, dicofol, and recently avermectin formulations are generally used to control the infestation. These products can interfere with biocontrol agents, either directly or by their negative effect on egg laying.

Biocontrol of tomato russet mite is difficult primarily because the tomato has glandular trichomes (hairs) which inhibit movement of predators. Phytoseiid mites appear to have most promise but the trichomes often gum up their legs and present an unfriendly environment for searching. An added difficulty is that preventative releases of predators are required to make sure russet mites are eaten before they have a chance to multiply to damaging numbers, and this requires a willingness by growers to spend money for a problem he/she is not sure exists at the time. If there is no russet mite, the predator has to find alternative food in order to survive, or regular releases are necessary. Predators that are also plant feeders are one possibility, but they should be able to feed on the plant without damaging it, and so far no such facultative predator has been found that will also feed on russet mite. Integration of phytoseiid mites with spot applications of relatively non-toxic pesticides still offers the best possibility for control within an IPM program at the present time.

Several issues surround the use of phytoseiid mites in tomato for TRM control. One issue relates to whether or not the mite will feed on TRM in sufficient numbers to eventually control the population. This may be temperature dependent. TRM tends to thrive in warm temperatures and low relative humidity. Its reproductive and survival capacity at temperatures >28^{o}C is probably more critical than those <25^{o}C, as most phytoseiids cannot tolerate high temperatures for long. Consumption rates for only a small number of phytoseiids have been reported in the literature.

Another issue is whether the phytoseiid is able to complete development on russet mite as its only food, at close to optimal levels for the species. If both these factors look promising, then the remaining important issue is whether it can survive and move on tomato. Even here, tomato types may affect the response, or the predator may adjust to this crop over time. The development of a tomato-adjusted *Phytoseiulus persimilis* for TSM control is a case in point, though there are few that believe the claims about this strain of predatory mite.

Other solanaceous plants may be attacked by TRM. In Australia, we have observed infestations on black nightshade, *Solanum nigrum*, and thornapple, *Datura* spp., in the wild, and on *Petunia* in home gardens. When the host plant dries up, or populations

become very numerous, TRM moves to leaf edges and 'stands' upright with anterior legs waving in the air. In this way it is probably more likely to be caught in wind currents or by passing animals, or on insects visiting the plant. It is suspected that flying pests such as thrips, whitefly and aphids, often observed on nightshade, are one vehicle for carrying TRM into greenhouses.

Black nightshade is relatively smooth-haired and readily cultivated, so we have used this plant to establish potential feeding capacity and fecundity of several phytoseiids on TRM. It also lends itself much more readily to seeing TRM and phytoseiid eggs on the surface than tomato. From there we plan to look at the wider issue of which species can be acclimatised to tomato.

Field surveys were conducted to find additional species of predators associated with TRM. The first was in June 2001, to Narrabri in north central NSW. We found large numbers of *Euseius victoriensis,* a native phytoseiid, at several sites east of Narrabri. James Altmann with Biological Services has been rearing the species for a year as it is a good predator of small mites in citrus and vineyards and he thought it would have some potential for broad mite in ornamentals. It has a high tolerance for hot dry conditions (the eggs hatch at about 25% RH). There were also *Feltiella* midge larvae present with TRM at this site and others.

The second and third surveys were conducted mid-late October 2001. The first area surveyed encompassed Gosford north to Coff's Harbour and inland through the Hunter Valley. The second was SW down to Albury, on the Victorian border, and back inland through Bathurst. Very few black nightshade plants were found and none of the predatory mites collected from a range of crops was of interest. After this period, the extended drought put an end to further surveys.

Materials and methods

The ability of several predatory mite species to survive and reproduce normally on TRM was evaluated first. We used black nightshade leaves on agar discs as it was much easier to see cast skins (an indication that the mite has moulted to the next stage) on these than on tomato. All species had some survivors, though the numbers were quite variable. From here we selected the best species and looked at survival on small tomato plants. We did not see many survivors, even though when we didn't introduce them we saw excellent establishment of *T. montdorensis*, *T. lailae* and *A. lentiginosus* on our TRM supply colony on tomato. Six phytoseiid mite species were finally selected for evaluation in feeding trials. These were *Typhlodromips montdorensis*, *Typhlodromalus lailae* (=*T. limonicus*), *Neoseiulus wearnei*, *Amblyseius lentiginosus*, *Euseius victoriensis* and *Neoseiulus cucumeris*. All except *N. cucumeris* were collected in Australia. For each species of phytoseiid, 50-100 eggs were collected from laboratory or greenhouse cultures, placed on a small piece of black filter paper, and the paper placed with a black nightshade shoot held in a small laboratory rearing unit. The unit consisted of two lidded drinking cups taped lid to lid with a hole between for stem insertion. The bottom cup held water, and the top unit had screened openings in top and side. A section of tomato leaf heavily infested with tomato russet mite was added to infest the nightshade. Units were held in a growth room at 25^{o}C. Phytoseiid eggs hatched in one to two days and all stages developed on TRM. After 11 days, when adult female phytoseiids were at their peak egg laying

period, they were extracted by washing through a screen, and placed individually on assay units.

The assay unit used was a 47 mm-diameter Millipore® petri dish, with a 30mm-diameter hole in the lid. This was screened with 105µm-sieve opening nylon mesh to allow air and water vapour exchange, but prevent escape of mites. About 4mL of a 1% agar solution was poured into each dish, and a 30mm diameter disc cut from a black nightshade leaf placed upper surface down on the surface just before setting point. For the first day, 100 late nymphal and adult TRM were transferred with a fine brush onto each leaf disk. They distributed themselves rapidly over the surface of the leaf. Between 10 and 25 units were set up for each phytoseiid species per run (two species per run), with an additional 10 units set up without predatory mites as a control. The time of predator addition was recorded for each unit. Units were placed screen-side down on a wire rack in plastic sandwich trays. A 60:40 glycerine:water mix in the bottom maintained relative humidity at approximately 75%. The trays were held in an incubator at 25^{o}C and 12hL:12hD period for a three-day assessment period. The first two days were regarded as a settling in period for the predator while it adjusted to a new environment and recovered from any previous food shortages, so they were kept on the same leaf disk.

Every 24h, dishes were examined under a microscope and the number of surviving TRM and the number of phytoseiid eggs were recorded. The eggs were removed. For the second and third day, unless all individuals had eaten less than 80 TRM, the number of TRM per unit was increased to 150 to allow for increased/decreased appetite after the settlement period. Occasionally an individual ate far more than the average, in which case the number was increased to 175, or rarely 200, for that individual alone. A surplus of TRM was maintained as far as possible throughout. Because it took time to reload each disk with TRM, the assessment time was recorded for each disk individually and the consumption rate and oviposition rate adjusted to a 24h period.

The oviposition and consumption rates are recorded as a daily mean for both the batch and survivors. Mortality was generally low and where present was not necessarily due to the unsuitability of TRM as food.

Results and discussion

It was hoped to do a minimum of three runs for each species but lack of russet mite delayed the trials and then other priorities intruded. All species completed their life cycle and were able to lay eggs (Table 1). Survival was high and where there were gaps it was mostly disappearances because they escaped or were overlooked. The order of maximum daily TRM consumption was *T. lailae*>*N. cucumeris*>*N. lentiginosus*>*T. montdorensis*>*N. wearnei*>*E. victoriensis.* Daily consumption declined over the three days for *E. victoriensis, N. wearnei* and *A. lentiginosus*. The result for *T. montdorensis* was variable and needs to be repeated. The order for egg production by survivors was *T. lailae*>*T. montdorensis*=*N. wearnei*>*A. lentiginosus*=*E. victoriensis*>*N. cucumeris*. Egg production increased for all species over the three days and appears to be in their normal range by the third day, indicating that TRM is a suitable food. The standout performer was *T. lailae*, for which unfortunately a mass rearing method has not yet been developed.

This research showed that TRM suitability as a food is not a limiting factor in the success of predators against TRM, but rather characteristics of the tomato plant, probably the glandular hairs. Finding a tomato-adjusted or tolerant predator was the next objective.

Table 1. Results of feeding trials with Australian phytoseiid mites and tomato russet mite (TRM) conducted at NSW Agriculture, National Centre for Greenhouse Horticulture, Gosford. Host plant is black nightshade.

Date tested	Phytoseiid species	N (batch)	Number of TRM eaten per day (batch average)			Number of TRM eaten per day (survivor average) (number of survivors)			Eggs laid per day (batch average)			Eggs laid per day (survivor average) (number of survivors)		
			Day 1	Day 2	Day 3	Day 1	Day 2	Day 3	Day 1	Day 2	Day 3	Day 1	Day 2	Day 3
26/7/02	*Euseius victoriensis*	22	59.6	42.7	35.1	69.0 (19)	59.1 (17)	51.4 (15)	1.2	1.5	1.7	1.4 (19)	2.0 (17)	2.6 (15)
26/7/02	*Neoseiulus wearnei*	23	82.8	60.2	49.1	90.7 (21)	72.9 (19)	59.4 (19)	1.7	2.4	2.4	1.9 (21)	3.0 (19)	3.0 (19)
20/3/02	*Typhlodromips montdorensis*	20	46.7	37.0	41.7	46.7	37.0	41.7	2.2	3.1	3.0	2.2	3.1	3.0
3/4/02		13	71.0	84.2	86.9	71.0	84.2	86.9	0.9	2.3	3.0	0.9	2.3	3.0
12/8/02		17	51.2	56.1	48.1	51.2	56.1	51.1 (16)	1.7	2.3	2.5	1.5	2.1	2.6 (16)
20/3/02	*Typhlodromalus lailae*	20	83.0	83.1	108.0	83.0	87.5	113.6	1.8	4.4	4.5	1.8	4.7	4.8
3/4/02		10	96.8	133.4	131.4	93.1	133.4	131.4	0.8	3.3	4.3	0.6	3.3	4.3
19/8/02		19	137.1	108.1	106.5	137.1	108.1	106.5	1.27	2.7	4.5	1.27	2.7	4.5
19/8/02		19	125.5	106.6	117.9	125.5	106.6	117.9	1.4	3.7	4.4	1.4	3.7	4.4
3/4/02	*Neoseiulus cucumeris* (ex UK originally)	20	77.0	94.7	90.9	79.5 (19)	105.9 (17)	101.6 (17)	1.2	1.3	2.2	1.2 (19)	1.4 (17)	2.4 (17)
19/8/02		14	141.7	102.8	83.6	141.7	102.8	83.6	1.1	1.8	2.4	1.1	1.8	2.4
12/8/02	*Amblyseius lentiginosus*	10	95.7	123.1	81.1	95.7	123.1	81.1	1.6	2.1	2.7	1.6	2.1	2.7

Trial 2. Evaluation of *Typhlodromips montdorensis* as a biocontrol agent for thrips and tomato russet mite.

Background

A crop of tomatoes was planted at NCGH in October 2002 to look at issues related to succession planting with cucumbers. This presented an opportunity to evaluate the predatory mite *Typhlodromips montdorensis* for control of thrips. This predator was known to feed on thrips larvae and had also been found in appreciable numbers on tomato feeding on tomato russet mite. Tomato crops offer a different challenge than cucumbers and capsicums to predatory mites because of the glandular trichomes, which inhibit movement and searching. There is also the potential lack of prey early in the crop to sustain them. A strategy of continually introducing *T. montdorensis* until there is enough prey was adopted, but this leads to practical considerations of whether a grower would be willing to pay for frequent introductions in advance of actually seeing a problem. The aim of the experiment was to determine whether *T. montdorensis* would establish on a commercial tomato crop and was able to keep thrips at a level where they inhibited or prevented TSWV transmission, and to put in abeyance practical ways of maintaining the population at reasonable cost.

Tomato russet mite invaded the crop several weeks after planting, and provided some additional opportunities to evaluate the effectiveness of *T. montdorensis* and also that of a species of *Feltiella* midge, the larvae of which were found feeding on TRM at several locations in NSW, including NCGH, Narrabri and the Warrumbungles. The species is undescribed and will be called *Feltiella* II here. Another undescribed species, *Feltiella* I, feeds only on two-spotted mite. A rearing method was developed for both that is described elsewhere in this report.

Materials and methods

A tomato crop cv Labell was planted 11-14 October 2002 in a 500 sq.m. area of the Long House, NCGH. A whole crop IPM program was set up before the crop was planted as a guideline for action (Table 2). Four yellow sticky traps (Seabright®) were set out in rows 2, 5, 7 and 8 and monitored weekly for flying pests. The crop was walked weekly and often biweekly, checking leaves randomly and looking for any signs of pests or diseases. Leaf washes were also conducted weekly by randomly collecting the terminal leaflet from a lower leaf of 10 plants in each of the 9 rows and passing the material through a series of screens. This was primarily to monitor for thrips and spider mites, which more commonly infest lower leaves. *Encarsia* were released weekly at 1/sqm from 11 October; this was increased to 2/sqm when whitefly increased in late December. *Stratiolaelaps* were released at the high rate of 100/sqm on 17 October; *Feltiella* I *and* II were released periodically as available from 12 November.

Table 2. IPM program set up for Long House tomato crop, October 2002 conducted at NSW Agriculture, National Centre for Greenhouse Horticulture, Gosford.

PEST	BIOCONTROL AGENT	SOURCE	RATE	TIMING OF INTRODUCTION	PESTICIDE BACK-UP
Spider mites	*Phytoseiulus persimilis* *Feltiella* sp.	NCGH	*P. persimilis*-2/sqm plus 20/infested leaf *Feltiella*-1/sqm/week, as available	When mites are first seen, then weekly in hot spots until established	Eco-Oil®, Torque®
Whiteflies	*Encarsia formosa*	Biological Services, SA	1/sqm/week if no whitefly seen, then 2/sqm when seen, then 0.75/sqm when 70% of scales are black	Two weeks after planting, then weekly	Eco-Oil®, Natrasoap®
Thrips	*Typhlodromips montdorensis*	NCGH	20/sqm, repeat in two weeks, then as necessary to maintain good predator distribution	If no thrips, apply 1-2 weeks after planting, repeat when thrips appear on traps or leaves or flowers. If thrips present, one week after planting, then two weeks later.	(Spinosad)
Fungus gnats	*Stratiolaelaps* (Hypoaspis) *scimitus* *Steinernema feltiae*	NCGH	100/sqm applied to growing medium at base of stem. Decrease to 20/sqm if rockwool or inorganic medium used	One week after planting out. Repeat if high trap count, poor *Stratiolaelaps* numbers	None necessary
Aphids	*Aphidius colemani*/lacewings (experimental)	NCGH	0.05-0.10/sqm	At first sign of aphids, biweekly until well established (mummies/lacewings present).	Pirimor®
Broad mite	*T. montdorensis*	NCGH	20/infested plant	When mites or damage first seen-apply to new growth weekly.	Vertimec®
Caterpill-ars	Bt formulation	Commercial product	Label rate	When damage first seen, seasonal. Hand pick caterpillars.	None necessary

Typhlodromips montdorensis was released at 20/sqm every two weeks as soon as thrips were noted in the crop, and thereafter to control TRM on an as-needed basis. Because tomato plants were small at first and the vermiculite with the predators was difficult to place on the leaf, various application methods were trialed with the first release, including placing vermiculite with mites at the stem/media interface, sprinkling over the plant, and misting the leaf to improve adherence. 10 terminal leaflets from each row were collected 6 and 13 November and washed to extract predator mites. The numbers recovered did not allow a proper analysis but predators established in similar numbers in all treatments except where they were sprinkled over the plant, where establishment was poor.

Several products were trialed against tomato russet mite after it became a problem after mid-December 2002.

Pulse® Penetrant, an agricultural adjuvant, was applied experimentally on 17 December at 60mL/100L on hot-spots of TRM, to evaluate the ability to control it, but it appeared only to kill *T. montdorensis*. Natrasoap® was also tried on 8 January, to no effect.

The miticide Calibre® with an oil as sticker/spreader was applied to nine plants on a trial basis on 10 and 14 January (Calibre 10%WP at 50g per 100L + 0.25% Eco-oil), and Vertimec® at a low rate of 30mL/100L to seven other plants. Both were very effective, though mortality of *T. montdorensis* was evident in the Vertimec® treatment. Predators had returned on Vertimec®-treated plants one week later, so residual effects were short.

The whole house was sprayed with Calibre® plus Eco-oil® on 22 January 2003 and again on 5 February. No further treatments for TRM were necessary until mid-March. As the current formulation of Calibre® is 10% EC, and the label rate is 25 mL/100L, a further trial spray was applied on 17 March 2003. Rows 1-5 were treated as a high volume spray with the low rate of 25mL Calibre®/100L, and rows 6-9 with the high rate of 50mL/100L. 0.25% Eco-oil® was added to both mixes, as laboratory trials indicated no activity of either product when applied separately. Leaves from eight heavily infested plants were flagged in each treatment, along with some with high numbers of predators. These were examined three days later under a microscope. Results are reported under the following section.

Formal monitoring concluded early April, once the pest situation stabilised, though the crop was walked weekly after this. The cropping situation became complex as cucumbers replaced tomatoes in most of the house.

Results and discussion

Thrips were first noted on 23 October 2002 during crop monitoring and on traps. *Typhlodromips montdorensis* was released at 20/sqm on three occasions at two-week intervals (28 October, 13 November and 1 December). Temperatures >35^{o}C were recorded in the last two weeks of October.

Several species of thrips were caught on yellow sticky traps (Fig. 1), including onion thrips, WFT and tomato thrips, all vectors of TSWV. Onion thrips were by far the

most numerous but populations declined after mid-December and did not recur in any significant numbers. Three plants confirmed infected with TSWV were removed on 13 November, 18 December 2002 and 22 January 2003. An additional yellow trap was placed where the plant was removed to trap possibly infected thrips. No further plants became infected. The only other pests to occur in any numbers on traps were fungus gnats and whitefly. Large populations of *Stratiolaelaps* were present in most bags and the level of fungus gnats was not considered a problem. Whiteflies were introduced inadvertently on a second tomato crop that went into the remaining 100sqm in early November but which was removed shortly. The peak in mid December is a result of this introduction. Most whiteflies were caught on a single trap. They were rarely observed in the main crop until much later in the year after new crops were interplanted with the old, and the weekly introduction rate had been reduced in error from two to one per square metre.

Only onion thrips were collected in leaf washes (Fig. 2). Average numbers were low which further emphasises the low tolerance for thrips when the species is a vector of TSWV. *Typhlodromips montdorensis* was not introduced after 14 January but was able to maintain itself in the crop and keep thrips at very low levels during summer, despite greenhouse temperatures frequently in excess of 30^{o}C. The numbers of predators never reached the levels that occurred in the cucumber crops, though high numbers were found in tomato russet mite infestations, including large numbers of eggs (Fig. 3). Russet mite was probably the means by which *T. montdorensis* maintained its presence in the crop. Leaflets were only taken from lower leaves as thrips were the main pest of interest so leaf wash counts may not reflect whole plant populations.

Tomato russet mite became the dominant pest in this crop. It is known to be a problem in the summer months during hot dry weather. It was first noted on 11 December on four plants. General releases of *Typhlodromips montdorensis* were suspended in favour of concentrated releases on TRM hot spots, at 500-1000 per infested plant. Approximately 10,000 predators were released on 18, 27 and 31 December, 3, 10 and 14 January 2003. No further releases were made. Despite predators cleaning up the existing infestation in about three days, new outbreaks kept occurring in an unpredictable fashion, so that it eventually became impractical to continue the hot-spot release strategy. The TRM specialist midge *Feltiella* II was not often seen. It was possibly unable to recycle on the plastic flooring, but on one occasion *T. montdorensis* was observed feeding on the midge larval stage.

Excellent control of TRM was achieved with Calibre®10% EC plus 0.25% Eco-oil at Calibre® rates of both 25 and 50g/100L (Table 3). No mortality of predators was observed.

Two-spotted mite was found in leaf washes in small numbers but not in crop inspections, and did not cause any noticeable damage. They may have been controlled by *T. montdorensis,* which is known to feed on them, or by *Feltiella* I.

Sprays and whole house fogs were necessary for persistent cluster caterpillar. Avatar® (experimental only) was spot-sprayed 21 December and 5 May. Delfin® strain of *Bacillus thuringiensis* applied on 21 December was not effective against

cluster caterpillar, so the Xentari strain was applied instead. It was very effective applied through a cold fogger (31 Jan, 12 Feb, 15 and 20 March, 15 and 28 April).

New crops of cucumbers and tomatoes interspersed among older plantings after April created problems for pest monitoring and control. The status quo changed as pests such as whitefly and thrips moved to preferred new foliage on young plants. Cotton aphid became a problem that was in the end partially but never totally controlled by *Aphidius colemani*. Whitefly built up on new plants as *Encarsia* releases had been inadvertently halved during the winter and furthermore not concentrated on new plants. A fungal pathogen, believed to be *Verticillium lecanii*, eventually attacked the whitefly, and with increased *Encarsia* releases provided excellent control. Citrus mealybug also appeared on a few plants and will present a challenge to succeeding crops. One application of Success® was made to a small section of new cucumber plants but thereafter *Typhlodromips montdorensis* provided excellent control of thrips until crop removal in October 2003. Succession planting and mixed cropping, from a pest management viewpoint, can create pest and disease problems and is not to be recommended.

Table 3. Three days post-treatment effect of Calibre® 10% EC at two rates plus Eco-oil® on tomato russet mite (TRM) and *T. montdorensis* on tomato.

Treatment	**Pre-treatment rating***		**Post-treatment rating***	
	TRM	***T. montdorensis***	**TRM**	***T. montdorensis***
25mL Calibre®+ 250mL Eco-oil®/100L	3.1 (1-4)	2.1 (0-3)	<1 (0-<1)	2.3 (0-4)
50mL Calibre®+ 250mL Eco-oil®/100L	2.5 (1-3)	0.75 (0-3)	0.2 (0-1)	1.1 (0-3)

* Severity of infestation rating scale of flagged leaves of 1-5, where 1=low, 5=high. The range is given in brackets.

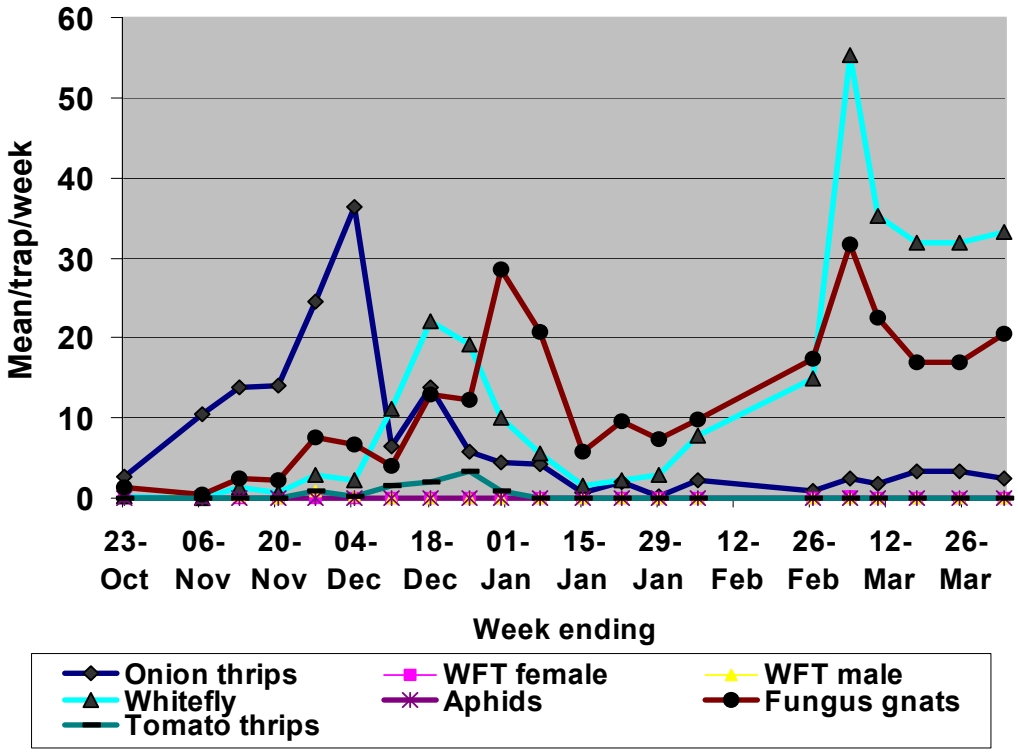


Fig. 1. Mean weekly yellow sticky trap catches of pests in a greenhouse tomato crop, NCGH Long House, Oct 2002-Mar 2003.

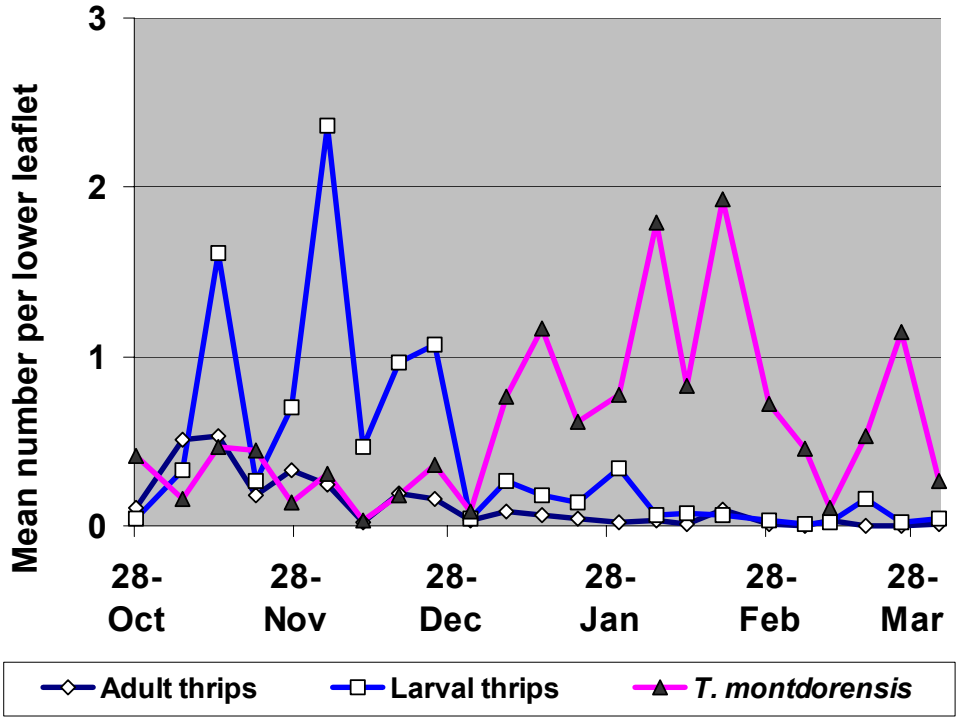


Fig. 2. Leaf wash data for onion thrips, *Thrips tabaci*, and *Typhlodromips montdorensis* on terminal leaflets of lower leaves of tomato, NCGH Long House, 2002-2003.

Fig. 3. *Typhlodromips montdorensis*, eggs and motile forms, on tomato feeding on tomato russet mite, conducted at NSW Agriculture, National Centre for Greenhouse Horticulture, Gosford, Long House, 2003.

Trial 3. Evaluation of the role of greenhouse whitefly in the distribution of tomato russet mite.

Background

Tomato russet mite (TRM) commonly infests tomato crops in the field and in greenhouses, with devastating effects on the tomato plant. Other host plants include nightshade, particularly black nightshade, petunia and potato.

The mite is too small to be seen with the naked eye so by the time infestations are apparent in the crop as rusty leaves or purple-brown stems (Fig. 4), the population of rust mites can be in the hundreds of thousands on one leaf. The pattern of infestation in the crop often appears to be random, with infested plants widely separated. The mode of entry into the greenhouse has not been studied, but is probably by wind. The legs of russet mite are all at one end of the body near the head, with an elongated abdomen behind. When russet mites become crowded on their host, they can be observed clustering at the edges of leaves or at high points, waving their legs in the air. This behaviour would lend itself to being carried by wind currents but also to being picked up by passing insects or vertebrates.

While the mode of entry is likely by wind, spread within the house is subject to other perhaps more likely means of dissemination, including being brushed off and carried on human clothing, or transport on an insect. During crop monitoring in the Long House tomato crop (2002/2003), tomato russet mite built up high populations but inspections of yellow sticky traps in the vicinity of infested plants only caught two TRMs, on separate traps, both very closely associated with a greenhouse whitefly adult.

A trial was set up to remove, as far as possible, wind and human vectoring, and to examine the possible role of another common tomato pest, the greenhouse whitefly, in transporting TRM into and within the greenhouse.

Materials and methods

The trial was carried out in the four 50 sq.m. Propagator greenhouses at NCGH (Houses 2, 3, 4, and 5). A crop of tomatoes cv Vulcan was seeded 29 January 2003 into rockwool blocks in one bay of a separate greenhouse. An additional 16 plants from the same batch were infested with TRM in a separate bay. These plants were in individual plastic nursery bags with potting mix, placed in a tray for ease of watering and to isolate them.

Except for the 16 additional plants, plants in rockwool blocks were set out in the Propagator houses on cocopeat bags on 27 February 2003. There were 5 rows and 70 plants per house (Fig. 5).

On 13 March, the additional tomato ‘banker’ plants, infested with TRM one to two weeks previously, were placed in the greenhouses, four per greenhouse. Their placement was at the end of the row away from the pad cooling system, which served as the sole cooling and venting system, so that air movement did not carry TRM into the crop. The doorway was entered through an enclosed vestibule, the outside door of

which was closed before opening the door to the greenhouse. A fine mesh cage, held up by a cylindrical wire frame, was placed over each infested plant. On 14 March, late instar greenhouse whitefly*, Trialeurodes vaporariorum,* (from Biological Services, Loxton, SA) were placed in each cage in Houses 2 and 3 only.

On 21 March, the tie at the top of the mesh in each cage was opened to allow emerged adult whitefly to exit. By this time, adult whiteflies and eggs were apparent in each infested cage and TRM infection was well established on at least one leaf of each plant. The mesh cages were removed entirely on 7 April. At this time TRM was not seen on the main crop in any of the four houses, but was well established on the four banker plants. Greenhouse whitefly was noted on the banker plants in Houses 2 and 3, and on a few plants in those houses, but not in 4 and 5. Broad mite was severely affecting some plants in rows 4 and 5 in House 2. Severe nutritional deficiencies were apparent in House 5 and later in House 4.

The crop in all four houses was examined twice weekly for any sign of TRM on the plants. Where TRM was noted, flagging tape was dated and placed over the plant so that the course of the infestation over time could be followed.

Staff working the crop was advised to avoid entry after visiting the infested Long House crop, to work Houses 4 and 5 first, to have minimal plant contact, and to wear rubber gloves and wash hands in Pyroneg® detergent between plants if pruning.

Yellow sticky traps (two per house) were placed in the centre of rows 2 and 4 (on the cocopeat bags so as not to remove whitefly) from 21-31 April to detect aerially dispersing TRM.

Results and discussion

The first plants with TRM were recorded on 15 April (Day 25). There was no obvious spread of TRM by whitefly, although the whitefly appeared reluctant to leave their banker plant host. Monitoring for TRM spread ceased 31 April, both because there was no obvious effect of the whiteflies, spread of TRM was already good, and the cable supporting the crop in House 4 snapped, putting plants in contact with each other.

The distribution of TRM through the crop was not even. As anticipated, most plants infested early were in the rows closest to the point source of the TRM (Figs. 6, 7). However, except in House 4, a few plants at the far end of the houses were also infested early. Although there may have been movement along a row to a neighbouring plant, TRM were apparently able to ‘jump’ plants. The spread across rows was also not even. The two lengthwise rows closest to the doorway were infested earlier and in greater numbers than those in the remaining three rows. The fan drawing air through the house via the cooling pad was located above row 5 (Fig. 5), so there appears to be a stronger influence from air movement entering through the doorway, or through people-transfer. After 41 days, 81.4%, 61.4%, 60%, and 65.7% of plants in Houses 2, 3, 4 and 5 respectively were infested with TRM.

Two houses (4 and 5) had nutritional problems caused by malfunctioning injectors. It was noted that these crops appeared much more prone to severe damage symptoms

than the faster growing crops receiving normal nutrition in the other two houses. House 2 was about 5°C warmer early in the trial, which may be the reason that it had the highest infestation rate. Broad mite became severe on a third of the plants in this house.

Although it appears that infestations did not develop until a month after the plants were potentially exposed to TRM, by the time symptoms were seen many thousands of mites were present so the first few could have arrived on the plant much earlier. The rapidity with which the rest of the crop was visibly infested suggests this to be the case and also that this mite is extremely mobile, even without obvious air movement.

The main stem, about 30cm off the ground, was the first to show symptoms of attack, in the form of a purplish-brown discolouration. The site of discolouration was initially on one side of the stem (often the opposite side to the point source), before spreading up the stem and to adjacent leaves, causing russeting, yellowing and drooping of those leaves.

The traps caught no TRM. There were a few whiteflies (1-8/trap) on traps from Houses 2 and 3, a few onion thrips (0-6/trap) from all houses, and large numbers of fungus gnats from all houses except House 5. The last mentioned are not obviously implicated in transfer. Traps from the Long House also did not find any TRM associated with fungus gnats over several weeks of trapping.

The ability of TRM to invade and spread in a crop remains somewhat of a mystery, but is probably a mixture of wind dispersal and physical transfer. Weeds such as black nightshade should not be allowed to remain in the vicinity of the crop, nor other plant hosts such as potato and petunia. Workers need to be trained to be on the lookout for damage symptoms on stems and leaves, and to presume that once symptoms are noticed, the infestation is very unlikely to be confined to one plant. The earlier symptoms are picked up, the more likely it is that spot-treatments with pesticide will be successful. Vigilance and the combination of a predator and compatible chemical(s) is the best solution for management.

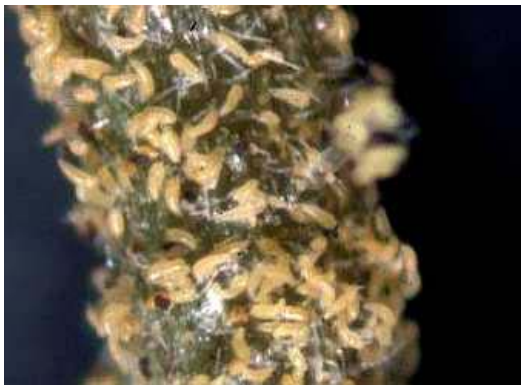


Fig. 4. Russeting of leaves caused by TRM (left) and TRM on stem (above).

Pad

X	X	X	X	X	14
X	X	X	X	X	13
X	X	X	X	X	12
X	X	X	X	X	11
X	X	X	X	X	10
X	X	X	X	X	9
X	X	X	X	X	8
X	X	X	X	X	7
X	X	X	X	X	6
X	X	X	X	X	5
X	X	X	X	X	4
X	X	X	X	X	3
X	X	X	X	X	2
X **R5**	X **R4**	X **R3**	X **R2**	X **R1**	1
TRM	**TRM**	**TRM**	**TRM**		

Fan Door

Fig. 5. Layout of greenhouses for tomato russet mite (TRM) trial conducted at NSW Agriculture, National Centre for Greenhouse Horticulture, Gosford. There were five rows of tomato plants, with four TRM point sources at the front of the house.

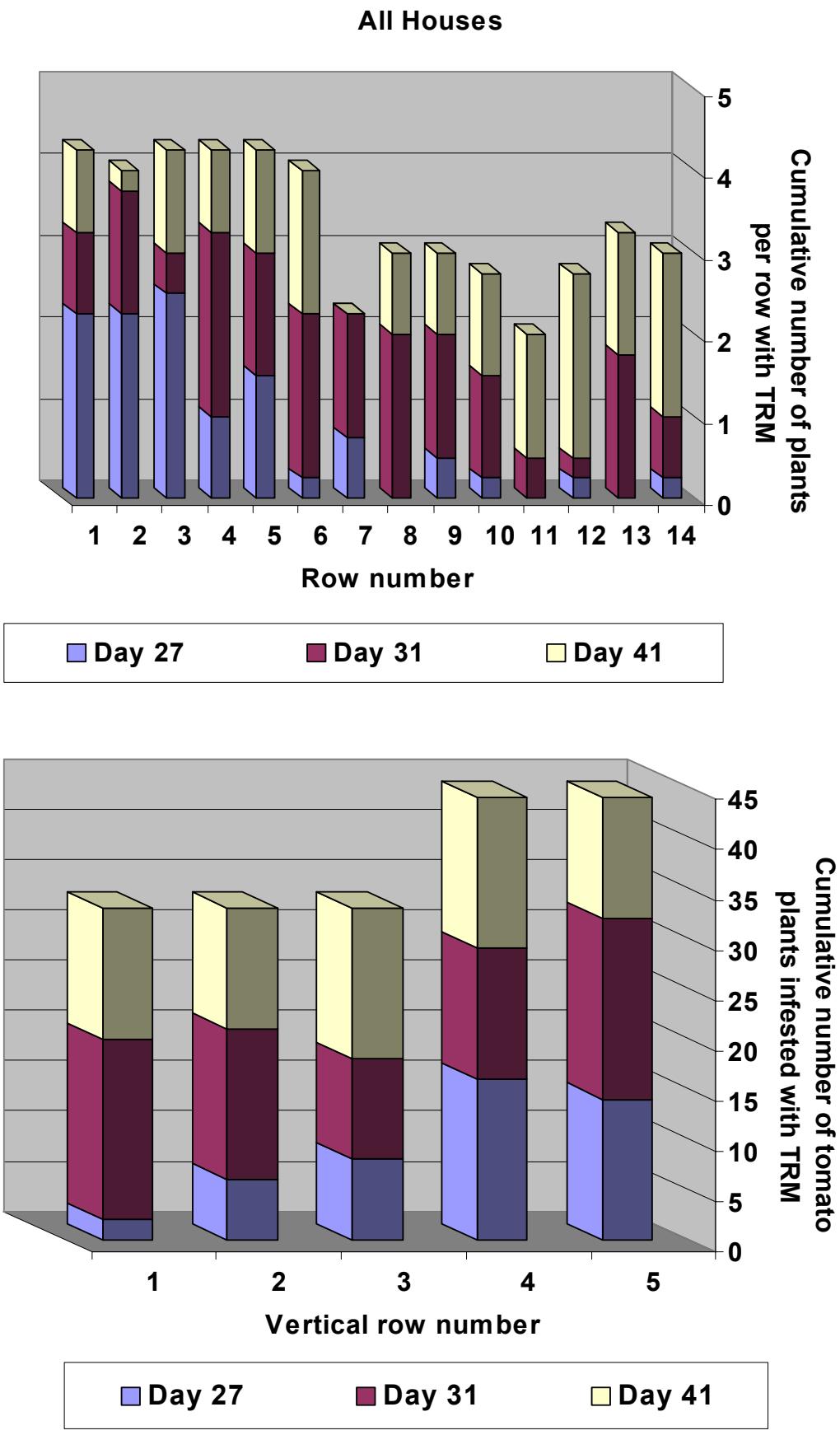


Fig. 6. Distribution of TRM infested plants within a greenhouse from four point sources at the beginning of horizontal row 1. There were five long (vertical) rows of 14 plants. The data is a mean of four houses.

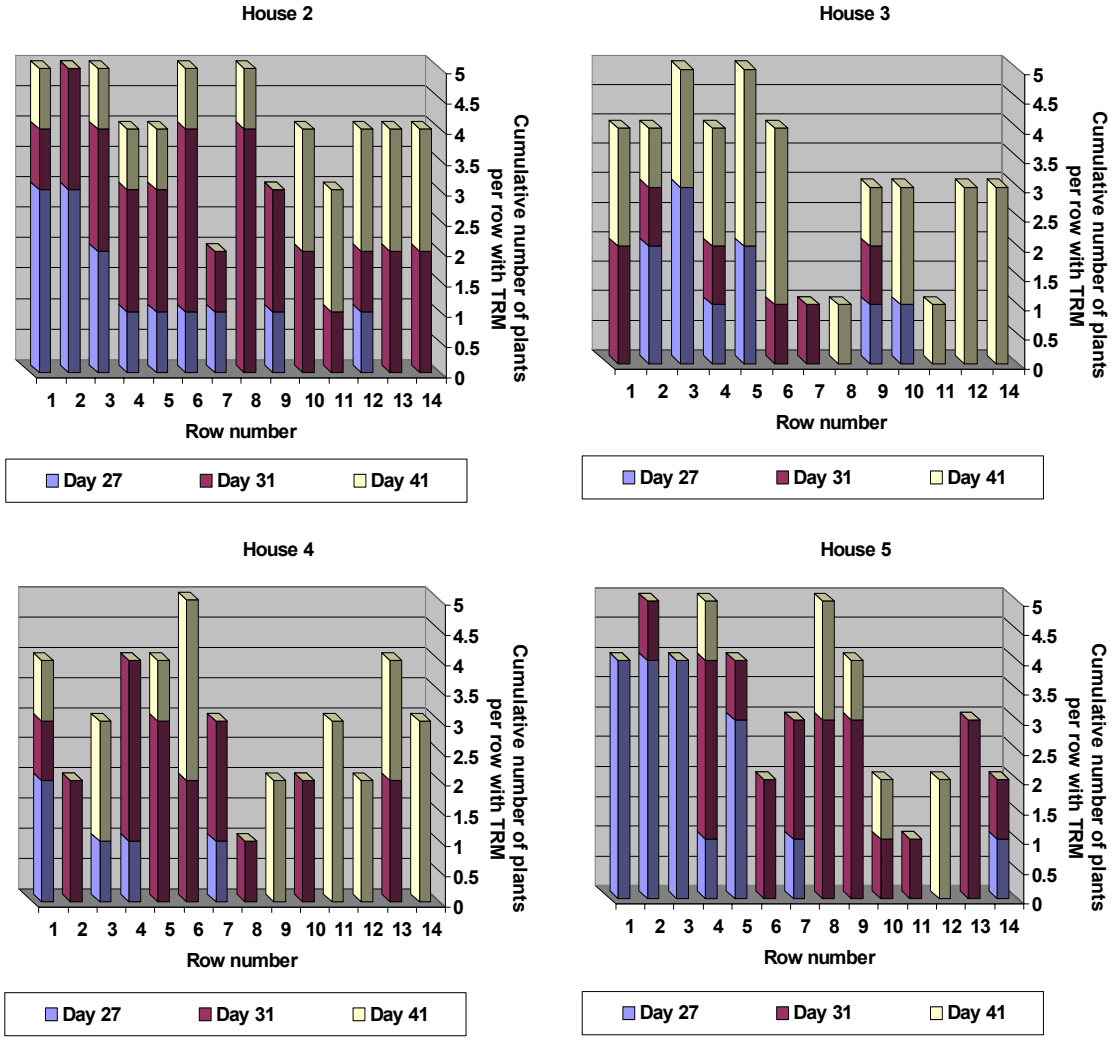


Fig. 7. Distribution of TRM infested plants within a greenhouse from four point sources at the beginning of horizontal row 1. Greenhouse whitefly was introduced into Houses 3 and 4 to examine their potential to spread TRM.

Summary of IPM research in greenhouse tomato crops at the National Centre for Greenhouse Horticulture 2000-2003

Research in tomato crops at the NCGH concentrated primarily on thrips and tomato russet mite management, while also managing other pests such as greenhouse whitefly, caterpillars, aphids and fungus gnats through the use of biocontrol agents or reduced risk pesticides.

No problems were encountered with whitefly when a weekly release schedule of *Encarsia formosa* was followed, nor with fungus gnats with a single release of the soil-inhabiting predatory mite *Stratiolaelaps.* Commercial growers using *Encarsia* frequently report whitefly problems in the winter. *Encarsia* must have a mean daily temperature over 18^{o}C to keep ahead of whitefly development, so too cool a temperature, along with low light, is the most likely explanation for failures. Once growers take corrective action with pesticides, the problems are often exacerbated. *Encarsia* rates are best increased during the winter to compensate for reduced activity. A short period of higher temperature during the day may also boost activity. Work is continuing on finding better reduced-risk pesticides to correct imbalances. Horticultural oils presently offer the best solution as they are both repellents and toxicants, though good coverage is critical. De-leafing and removing black parasitised scales from the house before the *Encarsia* have time to emerge is another cause of failures.

Cluster caterpillar control requires the Xentari strain of *Bacillus thuringiensis* and was very effective as a fog application. A permit is needed for any commercial application and it is recommended that this be sought. Similarly for any commercial use of Avatar®, which is very effective and also compatible with biocontrol agents, but which currently has no greenhouse registrations.

Thrips were managed very well by the predatory mite *Typhlodromips montdorensis,* but in the crop where it was trialed, populations were boosted by a continuous supply of tomato russet mite that they were feeding on. Without the TRM, it might be necessary to put in the predators on a fairly regular basis to make sure they are well distributed in the crop to control incoming thrips. A banker plant system that would supply pollen or an alternative food might be the solution.

Typhlodromips montdorensis was shown in laboratory and greenhouse trials to feed on tomato russet mite and to be able to complete its normal life cycle on this food. It was also able to establish very well on tomato, which is rare for a predatory mite. In a large greenhouse trial it provided a partial solution to TRM control but it needed supplementing with a compatible pesticide. Tomato russet mite is a very efficient disperser and can be present in very large numbers before damage symptoms appear on the plant. While TRM remains a problem, a solution looks promising. A combination of the miticide Calibre® and Eco-oil® provided very good control in a 500sqm crop, though laboratory and greenhouse bioassays, currently underway, are needed to confirm this. One other product in this trial, a general miticide, looks promising.

The midge *Feltiella* II specialises on Eriophyid mites and looked promising, but it did not perform well in a greenhouse with plastic flooring and *T. montdorensis* present. A

rearing system was developed for this midge but it was not able to produce sufficient numbers to make mass rearing feasible.

Section B Development of an IPM program for greenhouse cucumbers

Background

In this section the following trials were conducted:

IPM:	Trial 4. Developing and refining an IPM program in greenhouse cucumbers Trial 5. Positioning of yellow sticky traps in a greenhouse cucumber crop for better pest detection
Biocontrol	Trial 6. Evaluation of *Typhlodromips montdorensis* against western flower thrips in greenhouse cucumbers (see also Trial 4)
Biorationals	Trial 7. Evaluation of a ground treatment to control onion thrips in greenhouse cucumbers
Other	Trial 8. Influence of growing media on fungus gnat populations in a hydroponic system

Trial 4. Developing and refining an IPM program in greenhouse cucumbers

Background

Producers of greenhouse cucumbers in Australia rely heavily on pesticides to control insects, mites and diseases in their crops. In many countries, including New Zealand, Canada, and most European countries, pests are controlled primarily with biological control agents. The development of IPM programs relevant to Australian conditions is needed to provide a safe working environment and a sustainable pest management system. This program must seek to incorporate a range of control measures with the overall aim of reducing or eliminating the pesticide component, or at least substituting 'reduced risk' pesticides where immediate intervention is needed. 'Reduced risk' pesticides can be defined as products that are virtually non-toxic to people and have very short term or no impact on natural enemies, while providing good control of target pests.

A succession of cucumber crops were grown hydroponically for research and demonstration purposes in the Square and Long Houses at the National Centre for Greenhouse Research (See p7 for a description of the National Centre for Greenhouse Horticulture). These two houses were also used to evaluate and refine the IPM program, particularly the use of the new thrips predator *Typhlodromips montdorensis*.

The primary aims of the IPM program were to

(i) evaluate commercially available biocontrol agents and refine introduction rates
(ii) determine ability of some new biocontrol agents to establish and control the target pest, and refine rates of use
(iii) evaluate any promising 'reduced risk' pesticides for efficacy against pests and integration with biocontrol agents
(iv) establish sampling protocols for monitoring purposes.

Materials and methods

A list of cucumber cropping periods 2000-2003 is outlined in Table 4. A plan of each house was prepared, and a chart drawn up of anticipated pests and diseases (Table 5). Biocontrol agents, where available, were designated for each pest and disease as a first line of defence. The IPM program drawn up for the crop incorporated both biological and chemical elements (Table 6). A list of relatively safe pesticides was compiled that could be used as a backup if needed (Table 7). Pesticides can vary in their toxicity to different biocontrol agents (Table 8), allowing spot sprays or targeted treatments to be used in some cases where it might appear that their use would be incompatible.

Temperature and relative humidity were monitored regularly as part of general crop management, and mostly fell between 17^{o}C and 30^{o}C, considered suitable for good establishment of biocontrol agents. The main pests anticipated were two-spotted mite, *Tetranychus urticae*, greenhouse whitefly, *Trialeurodes vaporariorum*, western flower thrips, *Frankliniella occidentalis*, cotton aphid, *Aphis gossypii*, and fungus gnats, *Bradysia* spp.

At the time of the trials, commercially available biocontrol agents were *Encarsia formosa*, a parasitoid for greenhouse whitefly, *Phytoseiulus persimilis*, a predatory mite for two-spotted mite, and for fungus gnat control the predatory mite *Stratiolaelaps scimitus* (Hypoaspis) and the nematode *Steinernema feltiae*. The IPM program was reviewed prior to each experiment, with some rates adjusted as the need arose.

Table 4. Cropping periods for greenhouse cucumbers, NSW Agriculture, National Centre for Greenhouse Horticulture, Gosford .

House	Crop	Cropping period	Crop type
Square House	1	Jul-Sep 2001	Cucumber
Long House	2	Jul Sep 2001	Cucumber
Square House	3	Oct-Dec 2001	Cucumber
Square House	4	March-July 2002	Cucumber cv Mascott
Long House	5	May-Sep 2002	Cucumber cv Mascott
Square House	6	Oct-January 2003	Cucumber cvs Austin, Cobra and CU703
Long House	7	Oct 2002-June 2003	Tomato cv Labell
Long House	8	May-September 2003	Tomato + Cucumber cv Deena

Table 5. Pests and diseases anticipated in the cucumber crops, NSW Agriculture, National Centre for Greenhouse Horticulture, Gosford.

Pests anticipated		Diseases anticipated	
Major	Minor	Major	Minor
Two-spotted mite	Fungus gnats	Powdery mildew	Downy mildew
Bean spider mite	Caterpillars	Pythium root rot	Angular leaf spot
Whitefly	Broad mite	Gummy stem blight	Fusarium foot rot
Western flower thrips			Black root rot
Cotton aphid			Botrytis rot

In order to track pest populations, monitoring was conducted regularly and results recorded and graphed.

Monitoring methods Three monitoring methods were employed:

i) Trap catches. Yellow sticky cards (Seabright®) were hung at crop canopy level at a rate of ~1 per 200 sq.m. (four per house). One card was placed near the doorway and the others through the house. Cards were numbered and dated and checked weekly for flying pests (thrips, whitefly, fungus gnats and aphids). Numbers per card were recorded weekly and traps changed unless no pests were caught.

ii) Crop inspections. The crop was checked weekly (sometimes more often) by walking each row and looking for any signs of abnormality (scarring, stippling, distortion, honeydew, cast skins of aphids, ants active). Flagging tape was used to mark and date any problems as they were found. Different

coloured flagging tape was used to indicate different pests or different dates. About 10% of plants were also checked in a random fashion by examining undersides of leaves in different strata. Any plants with disease symptoms were noted. Pests and diseases were recorded on a monitoring sheet and need for action indicated. Biocontrol agent activity was also noted.

iii) Leaf washes. Leaf samples were collected randomly from upper, middle and lower leaves, bagged according to strata, and washed off through a screen, collecting the insects and mites for identification. 78 leaves were taken weekly, so weekly about 10% of plants had a leaf removed.

Leaf washes give useful information on relative numbers of certain pests and predators, and an average per leaf or flower. If this is graphed then it is easy to follow trends in population growth. The figure is only an average, and does not indicate whether the pests and predators are evenly distributed through the crop, or in different parts of the greenhouse, or in one corner of the greenhouse. Leaf washes alone do not substitute for walking the crop and checking for damage, or doing random crop inspections. Nor do they substitute for yellow sticky traps. They will, however, make it easier to find small insects and mites that can hide well, and can give an early warning of certain pests, such as spider mite, that can be followed up by closer inspection and releases of natural enemies.

Different pests and predators often inhabit different strata within the crop, so taking separate leaf samples in the upper, middle and lower canopy can tell where they are and how they are interacting.

Leaf washing is good for detecting thrips, predatory mites, spider mites, adult whitefly and aphids. It will not give any indication of immature whitefly or fungus gnats.

Recording methods. Monitoring data from leaf washes and traps were transferred to Microsoft Excel® for graphing and following trends. Action taken against pests and diseases was also recorded.

The pest management program relied heavily on non-chemical control. This required pro-active treatment and no delay in action taken. Where possible, spot treatments were recommended rather than a broad area spray. Compatible pesticides were on hand for prompt response.

Examples of record sheets that were maintained for plant inspections, sticky trap catches and leaf wash counts are in Appendices 1-3.

Sanitation The following procedures regarding sanitation were recommended to avoid bringing in unwanted pests and diseases:

- Keep doors closed and try to work infested areas last
- Restrict access to approved people only
- Use footbaths religiously and encourage others to do so. Keep them fresh
- Remove all weeds (especially along edges of greenhouse). They carry in pests and pathogens
- Avoid wearing white, yellow or bright blue clothing, as these colours attract thrips, whiteflies and aphids (red, grey, green, dark blue are usually acceptable).

- Pools of algal growth attract shore flies that carry soil pathogens. Fix the drainage problem.

Chemical issues There are a limited number of pesticides registered for cucumbers, and most of the insecticides and some of the fungicides are very harmful to biocontrol agents. Information sheets for registered products, and side effects of a range of products on biocontrol agents were distributed to staff involved in control decisions.

Crop 1. Square House cucumbers July-September 2001
Crop 2. Long House cucumbers July-September 2001

These crops were the first opportunity to trial establishment of the predatory mite *Typhlodromips montdorensis* on a commercial scale. Introduction rates were experimental and based on results in small greenhouse trials; initially 2 predators/plant (4/sqm) were introduced from the NCGH culture on 23 July, followed by three or four further introductions of 10-20/sqm to respond to an onion thrips, *Thrips tabaci,* infestation.

One introduction of *Stratiolaelaps* for fungus gnats, weekly or biweekly introductions of *Encarsia* for whitefly, and limited applications of *Phytoseiulus persimilis* and *Aphidius colemani* for spider mite and aphids respectively were made.

Crop 3. Square House cucumbers October-December 2001

This crop was designed to compare a run to waste system with NFT, and to look at alternative crop pruning methods. Because summer is usually a time when pest populations multiply rapidly and are difficult to control, weekly introductions of *T. montdorensis* were made for thrips and *Encarsia formosa* for whitefly. A single preventive introduction of *Stratiolaelaps* for fungus gnats and *Aphidius* for aphids was made, and two of *Phytoseiulus* when spider mites were first observed. Leaf washes were carried out on a weekly basis as a measure of relative thrips and predator populations. For this and subsequent crops, leaf washes and trap counts were adopted as the primary measure of pest monitoring, although the crop was walked every week by row and leaves randomly inspected to look at pest and BCA distribution in the crop. Plants with infestations of spider mite or aphids were flagged and dated with coloured tape and a section also tied to the nearby heating rail to make it easy to find for further checking.

Crop 4. Square House cucumbers March-July 2002

In this crop, various growing media were being compared with respect to yield in a hydroponic run-to-waste system. Cocopeat in long, mostly closed bags and NFT had been used previously. Present mixes were sawdust, cocopeat, rockwool, perlite or a composted bark potting mix in open bags.

During the previous crop, growers from the Sydney Basin attended a field day at NCGH in December 2001 on work in progress, and were asked for their opinion on costs of the biocontrol agents, as was the greenhouse project steering committee in February 2002. The general consensus was that this was too high and exceeded current costs of their chemical program. For Crop 4, introduction rates of some of the

biocontrol agents, specifically *T. montdorensis, E. formosa* and *Stratiolaelaps*, were reduced to cut costs. The crop was going into an autumn/winter production that should reduce the need for BCAs in any case. The revised program was to reduce *Encarsia* to 0.75/sqm/week, *T. montdorensis* to two introductions of 10/sqm and then on as-needed basis determined from monitoring, and *Stratiolaelaps* at 20/sqm.

Crop 5. Long House cucumbers May-September 2002

This was a crop planting density trial. The IPM program was modified on results from Crop 4, bearing in mind that this was a winter crop so rates of *Encarsia* needed to be raised to offset reduced light levels and activity. In this crop, introduction rates for *T. montdorensis* were two introductions, one two weeks after planting and the other two weeks later at 10/sqm to every plant, weekly releases of *E. formosa* at 1/sqm, two releases of *Stratiolaelaps* at 20/sqm (cocopeat bags), weekly releases of *Aphidius* at 0.2/sqm, and *Phytoseiulus persimilis* and *Feltiella* at first sign of spider mites. Pirimor® plus Ecocarb® were applied pre-planting as aphids and powdery mildew were noted on seedlings.

Crop 6. Square House cucumbers October-January 2002

This crop was designed to assess the impact of three different electrical conductivities on production from three cultivars. Planned introduction rates of biocontrol agents were similar to those used in the previous trial, but with a single high *Stratiolaelaps* release. These were *Encarsia* weekly at 1/sqm, 2 releases of Montdorensis at 10/sqm early in the crop, *Stratiolaelaps* at 100/sqm once, and *P. persimilis*, *Feltiella* and *Aphidius* at first sign of their respective pests.

Results and discussion

Crop 1. Square House cucumbers July- September 2001
Crop 2. Long House cucumbers July-September 2001

Biocontrol releases are detailed in Table 9. The crops were of short duration and assessment opportunities were limited; however, only one application of a pesticide was considered necessary-Eco-oil as a spot spray for bean spider mite on 31 August. All pest incidences were limited to a few plants except in late August, when onion thrips became well established in two rows on the north east (hotter) side of the Long House and at 47% incidence on plants. Trap catches were only 1.5/trap average at this time. By 9 September, incidence of thrips in the Long House was 46.7% of plants infested, but *T. montdorensis* was on 51.1 % of plants and present wherever there were larval thrips.

At the conclusion of the crop, 25 leaves were taken randomly from (i) upper (ii) middle and (iii) lower strata and washed through screens to extract thrips and predators. Table 10 lists means per leaf. On 13 September, the population of onion thrips was higher in the Square House where fewer *T. montdorensis* had been released early in the crop. On 27 September, there were high densities of *T. montdorensis* on leaves from the Long House in all strata (average of 12-20/leaf), and larval thrips had declined except on lower leaves (average 4.2/leaf), where they were now well outnumbered by predators.

This first trial was encouraging as it showed both good establishment and a fairly rapid response of *T. montdorensis* to increasing thrips density.

Crop 3. Square House cucumbers October-December 2001

Biocontrol releases are detailed in Table 11. Both western flower thrips and onion thrips were detected in the crop (Fig. 8); however, western flower thrips were mostly male, typical of very low populations in cucumbers, and were only found on sticky traps, from 9-23 November. Onion thrips population density was much higher, peaking the week ending 16 November at a mean of 40 adults per trap (four per lower leaf), and from there declining rapidly. *Typhlodromips montdorensis* numbers increased rapidly and maintained high populations for the duration of the crop (Fig. 9). Greatest numbers were on upper leaves (mean high 12 per leaf), while onion thrips were mostly found on lower leaves (Fig. 10). The ability of *T. montdorensis* to maintain populations greater than their primary prey was probably due to feeding on spider mites as well as thrips larvae. The only other controlling agent noted for thrips was a low incidence of the entomopathogenic fungus *Entomophthora*. Whitefly, aphid and fungus gnat population densities, as indicated by trap catches (Fig. 8) and crop inspections, were all very low. No sprays were applied for pests nor were necessary. Onion thrips is a leaf feeder and does not damage fruit so a higher tolerance than for WFT is allowed. The crop was of short duration but the biocontrol program put in place was very successful.

Some measure of costs is necessary to compare the costs of a biocontrol or integrated program with the chemical program that growers are used to applying. An approximation of costs for this program is detailed in Table 12. They were approximately 0.75-0.80 cents per plant or $1-$1.25 per square metre. In a longer-term crop, costs might be expected to continue for whitefly (weekly) and spider mites (sporadically), but should not be necessary for thrips and fungus gnats as the biocontrol agents present will persist and continue to perform.

Crop 4. Square House cucumbers March-July 2002

Releases of biocontrol agents into the crop are detailed in Table 13. The results were mixed.

Thrips. Excellent control of thrips was achieved with a single release of *T. montdorensis* as thrips were very late in establishing, and the predatory mites established well with the single introduction (Fig. 11). Western flower thrips was recorded as present very late and only in very low numbers on traps (Fig. 12). Onion thrips built up towards the end of the period to reach relatively high numbers (average 11/leaf) on lower leaves in mid June (Fig. 13). Boiler breakdown late April and early May, coupled with generally lower daytime temperatures, probably account for the plateau in predator numbers, which allowed onion thrips to increase rapidly towards the end of May. Once the temperature increased, predator populations multiplied rapidly to reach an average high of 18/leaf and brought the thrips under control again. Thrips were most numerous on lower leaves at the ends of the rows closest to the door and were unlikely to have had any impact on yield. Numbers on the leaves are not

reflected in high trap counts, which may be related to traps being located away from hot spots or lower leaves.

Whitefly. Excellent control of whitefly was achieved by weekly releases of *Encarsia* whether or not whiteflies were seen. In this crop, a low rate of 0.75/sqm was used instead of the previous rate of 2/plant. A few parasitised whitefly pupae were washed off leaves late in the crop, indicating that whiteflies were present and had been located by the parasite. They were otherwise not detected.

Spider mites. *Phytoseiulus persimilis* provided good control of both two-spotted mite and bean spider mite, but a quick response with the predators as soon as a patch of mites was detected was critical to containing them. Applications of Eco-oil + Ecocarb for powdery mildew 10 May, 31 May and 5 June may have assisted in control, but there are now suspicions about the negative impact of this combination on *P. persimilis.* The midge *Feltiella*, whose larva is a spider mite predator, was released on three occasions and was washed off leaves in small numbers, but it is not clear how large a role they played or whether it also may have been affected by the fungicide treatment.

Fungus gnats. A low introduction rate of *Stratiolaelaps*, 20/sqm instead of the previous 100/sqm on 19 March, a week after planting out, resulted in slow initial establishment. Some of the media types being trialed were clearly much favoured by fungus gnats, the 'cucumber mix' of composted bark in particular. A second release at 20/sqm was made three weeks after the first on 12 April. The entomopathogenic nematode *Steinernema feltiae* was applied through the drippers in May to try to speed control (three applications two weeks apart). This option added substantially to the cost of the program but brought about a rapid reduction in fungus gnat populations. It isn't clear whether nematodes or predatory mites were responsible for bringing the fungus gnats under control over the next few weeks. By the end of May, *Stratiolaelaps* had built up to very high numbers in open bag systems, particularly in cucumber mix, and few fungus gnats were in evidence (Fig. 12). Future recommendations on release rates should be media dependent, with two releases at 20/sqm two weeks apart probably the minimum for good early establishment and control.

Aphids. Cotton aphid, *Aphis gossypii*, was the major pest of concern in this crop. In the previous crop, a few *Aphidius colemani* and brown lacewing introduced at first sign of aphids (green peach aphid) totally controlled those aphids. In the present crop, an error was made in putting in too few *Aphidius* too late, and not spot spraying with Pirimor®. Cotton aphid can colonise cucumber very rapidly, with the result that a whole-crop spray was necessary 14 June to contain them. Regular weekly releases of *Aphidius* should have been made. Cotton aphid is not attracted to yellow sticky traps, so it is difficult to detect early enough to take corrective action. A regular preventative release strategy with *Aphidius* is probably advisable.

Diseases. Powdery mildew was present early in this crop and was more prevalent than in previous crops. Acquisition of a good greenhouse sprayer eventually brought it under control. Ecocarb+Eco-oil was applied 19 March, 17, 30 April, 3, 10, 27, and 31 May, and 14, 24 June. Amistar was used 3 May and 14 June, primarily for gummy stem blight. Difficulty in diagnosing nutritional deficiencies resulted in fairly severe

leaf damage. This made monitoring for pests and diseases rather difficult, as it tended to obscure any damage symptoms caused by them.

Some savings were made in the cost of the biocontrol program by reducing rates, particularly for *Encarsia* and *T. montdorensis* (Table 14), but this was compromised by too low initial rates of *Stratiolaelaps*, which necessitated a curative rather than a preventative control action, and too low rates of *Aphidius*, which necessitated a whole crop spray of Pirimor for aphid control. The costs of the pest control program (excluding fungicides) would have been substantially reduced without the additional cost of nematodes required to bring fungus gnats under control. The low rates of *Stratiolaelaps* might have been achievable in media such as rockwool or perlite, but not in media with high organic material. The moral is that biocontrol programs are not set in stone, but need to be adjusted depending on factors that might favour pests over beneficials. These include media, crop host, temperature, humidity, light levels, greenhouse structure etc., and need to be custom designed to some extent and modified by experience and by observations from regular monitoring. Also, even though limited spraying occurred, the side effects may have been substantial and upset the balance of pest and beneficial. Early intervention is preferable, where possible, to redress imbalances.

Crop 5. Long House cucumbers May-September 2002

Two-spotted mite was found to be widespread on young plants two weeks after planting out, necessitating early release of *P. persimilis*. Cotton aphid was also noted a week after planting out. Introductions are recorded in Table 15.

Thrips populations were very low on traps and in upper and middle leaf washes until mid July (Fig. 14), with both onion thrips and occasional WFT recorded. WFT were probably introduced inadvertently with *P. persimilis* on leaves from the NCGH culture. Plant inspections and leaf washes in early July indicated that onion thrips were increasing rapidly on lower leaves (Figs. 15, 16). Thrips predators were still low by late July and apparently not responding to increasing thrips densities. It is suspected that they had become sparse and poorly distributed because of the previous lack of prey. An additional release of 10/sqm was made 29 July, but only to 10% of plants. Despite a substantial increase in predator numbers within a week, it was soon apparent that this was too few, too late, to catch up with the onion thrips in time to prevent major leaf damage. The crop, except for the centre row of the nine rows, was sprayed with Success® at half rate (43mL/100L) on 30 August. This rate is usually relatively safe to *T. montdorensis*, but Agral® was added inadvertently as a wetting agent, and the mixture resulted in almost complete kill of both thrips and *T. montdorensis*. As the residual effect of Success® on predatory mites is short, a further application of *T. montdorensis* at 10/sqm was made to treated rows on 10 September. Meanwhile, *T. montdorensis* in the unsprayed centre row increased dramatically (Fig. 16) (the leaf wash count on 5 September is an average for the whole house). Onion thrips larval populations were very low by 12 September and adult populations by 26 September (Fig. 14).

It is likely that *T. montdorensis* could have controlled the thrips, which were almost all onion thrips, without intervention with Success®. The rapid rate of increase of *T. montdorensis* was astounding. Two lessons emerged from this trial. One is that *T.*

montdorensis needs to be reintroduced periodically if no food is available to them, to ensure that they are well distributed through the crop should there be a thrips incursion. The second is that traps above the crop will adequately detect WFT, but are incorrectly placed for onion thrips, which tends to favour lower leaves. At the end of June, traps were paired and one was placed in the top canopy and one near the base of the plant. Results of this placement on catches of the two thrips species and also whitefly and fungus gnats are detailed elsewhere in this report (Trial 5).

Other pests (whitefly, fungus gnats, spider mites) were adequately controlled by their appropriate biocontrol agents. *Feltiella* established but did not appear to recycle or contribute greatly to control. Because it is a ground pupating species, it is likely that the plastic floors are not suitable, or that they were preyed upon by *P. persi*milis or *T. montdorensis.* Very few applications of pesticides were necessary. The pre-plant application of Pirimor® and single application of Success® were the only applications made for pests. Powdery mildew required treatment early on and approximately fortnightly applications were made thereafter as a preventive measure (0.2% Ecocarb + 0.5% Eco-oil on 13, 17, 27 and 31 May, 14 , 21 June and 28 June). Bravo® was applied as a spot treatment for gummy stem blight on 14 June and 21 June, and copper oxide for the same disease on 28 June.

Crop 6. Square House cucumbers October 2002-January 2003.

Typhlodromips montdorensis were released at 10/sqm on 14 October and 28 October, but onion thrips built up very rapidly early in the crop (Fig. 17), and were particularly numerous on lower leaves by early November (Fig. 19). It is possible that they had been blown in from outside through the vents, as the screening was torn and in need of replacement. It appeared that *T. montdorensis* was well distributed and present in good numbers, so on the evidence from the previous crop that they might catch up, no sprays were applied. Instead, one additional release at 20/sqm was made on 13 November to plants on the ends and sides of the greenhouse where thrips were most numerous. By the third week in November, *T. montdorensis* populations exceeded thrips larval populations and by the end of November the thrips were no longer a problem (Fig. 18). Predator populations remained high to the end of the crop and as they far outnumbered thrips, were presumably feeding on spider mites. As with previous crops, western flower thrips was detected from early in the crop but in negligible numbers. A predatory thrips, *Desmothrips* sp., was detected on traps in low numbers but its response to thrips was either not density dependent or *T. montdorensis* failed to distinguish the larvae from those of onion thrips and ate them too.

Greenhouse whitefly was detected in all crops but only in very low numbers.

The main issue of concern was bean spider mite, and to a lesser extent two-spotted mite. Both were first noted in leaf washes from 31 October but not in crop inspections until 27 November. Releases of *Feltiella* were made on 12 and 28 November, 9, 13, and 19 December, and 15 and 22 January. The adult midges can fly and should be able to search the crop for prey. *Phytoseiulus persimilis* were released on 27 November, 3 and 19 December and 3 January into hot spots but were unusually slow to establish and spread. *Feltiella* were found in good numbers but did not appear to fly far from release points, nor to recycle. Of most concern was that many dead *P. persimilis* were found in leaf wash samples a few days after treatment with Eco-oil +

Ecocarb on 3 January 2003. Eggs appeared not to be affected as larval stages were present that must have hatched post-treatment. Populations of *T. montdorensis* appeared unaffected but *P. persimilis* numbers declined from a mean of 12.7 on 8 January to 1.5 a week later. Spider mites also appeared to decline so the mix may have had an impact on these also.

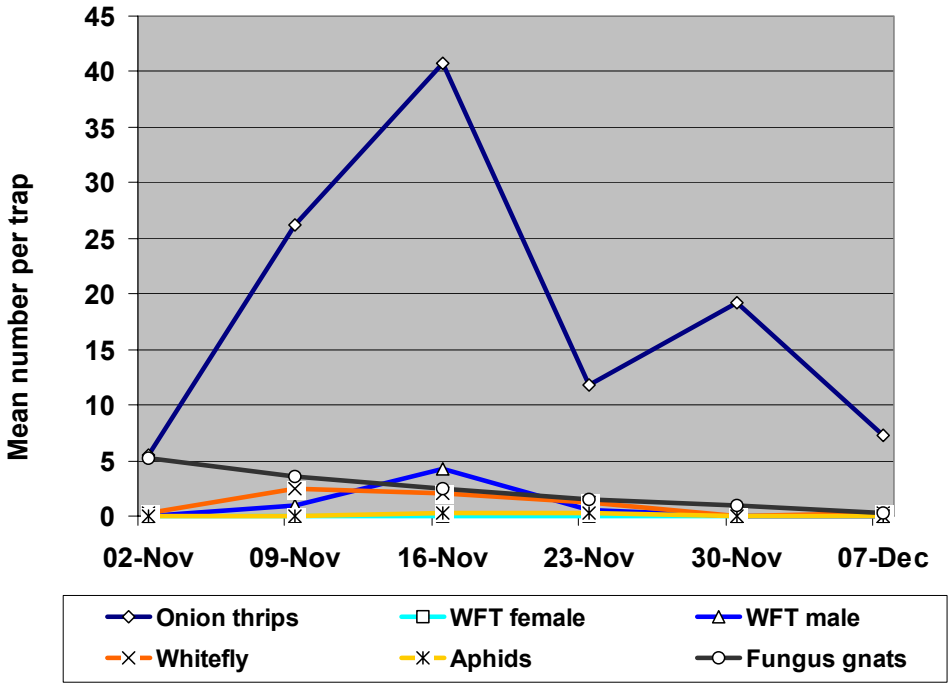


Fig. 8. Yellow sticky trap catches of pests in greenhouse cucumber, Square House, NCGH, Crop 3, 2001.

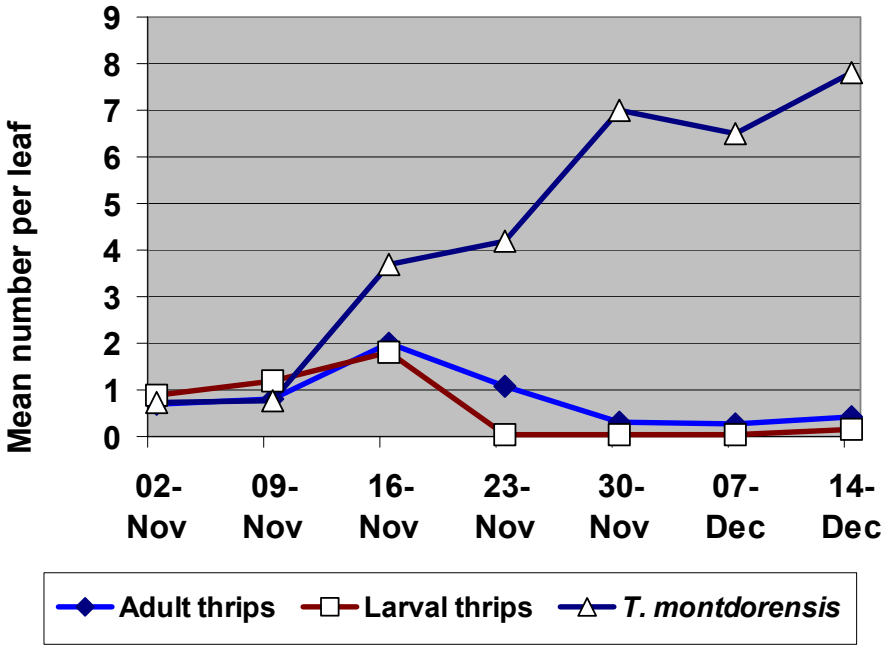


Fig. 9. Mean number of thrips (primarily onion thrips) and *T. montdorensis* on leaves of greenhouse cucumber, Square House, NCGH, Crop 3, 2001.

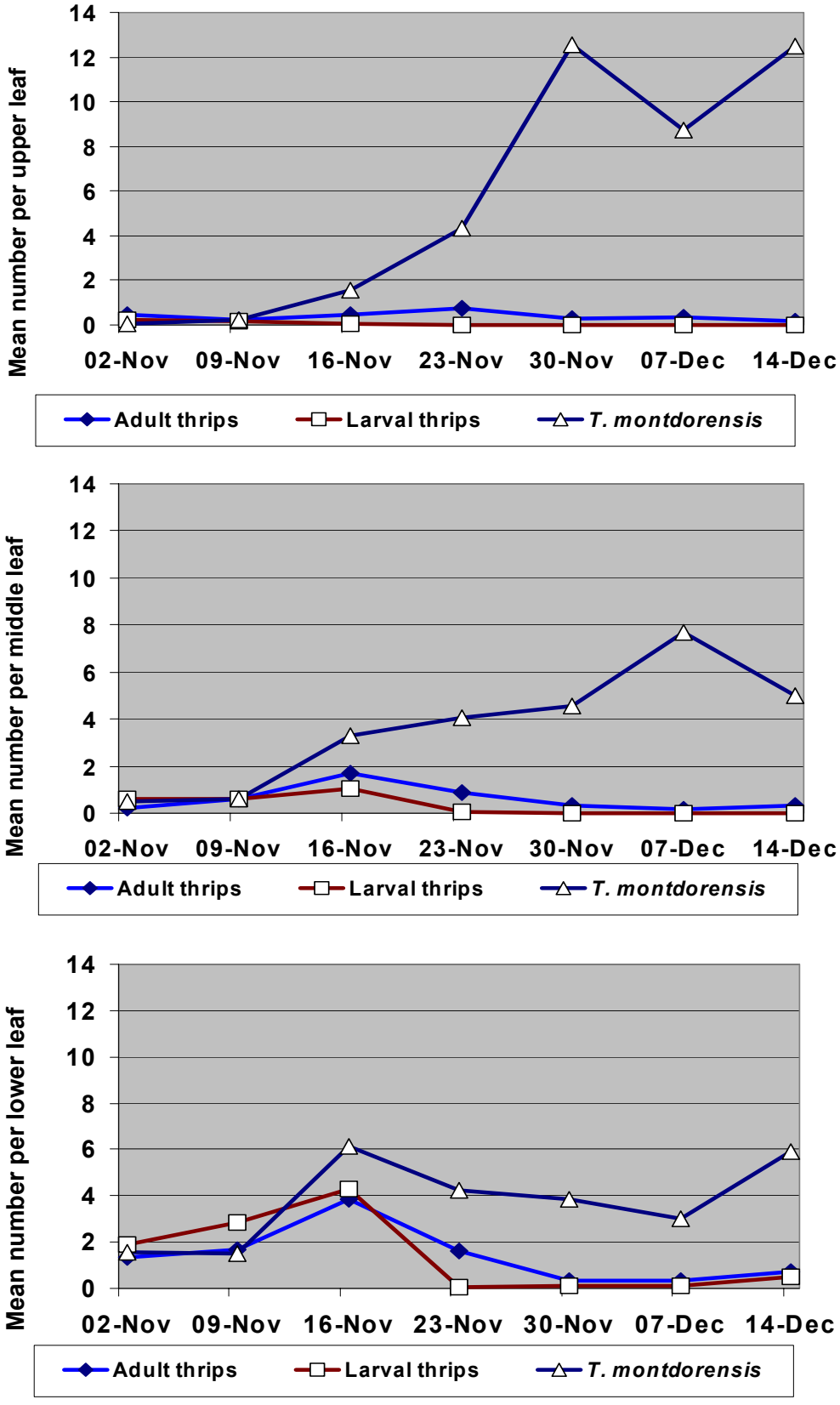


Fig. 10. Mean number of thrips and *T. montdorensis* on upper, middle and lower leaves of greenhouse cucumber, NSW Agriculture, National Centre for Greenhouse Horticulture, Gosford, Square House, Crop 3, 2001.

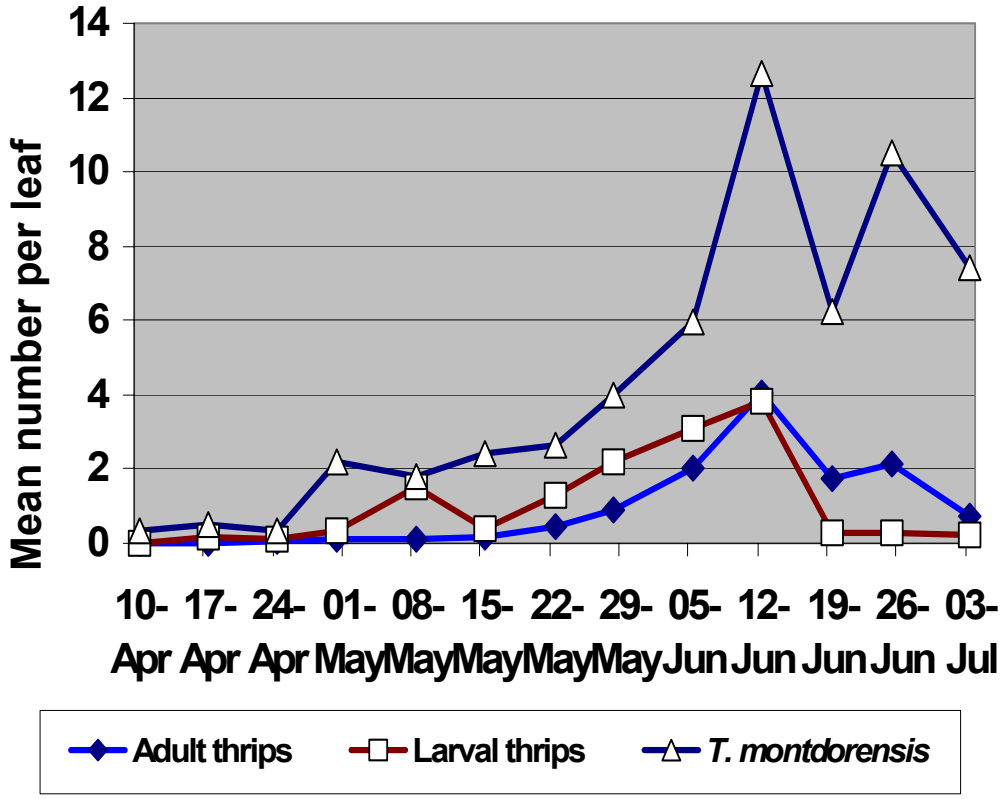


Fig. 11. Mean number of thrips (onion thrips) and *T. montdorensis* on leaves of greenhouse cucumber, Square House, NCGH, Crop 4, 2002.

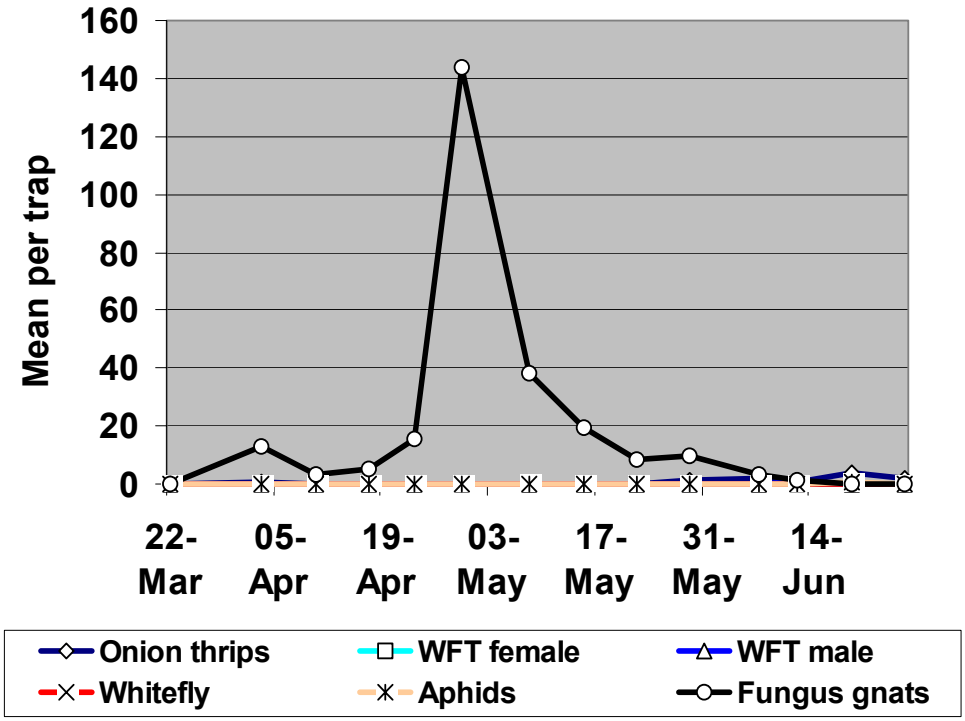


Fig. 12. Yellow sticky trap catches of pests in greenhouse cucumbers, Square House, NCGH, Crop 4, 2002.

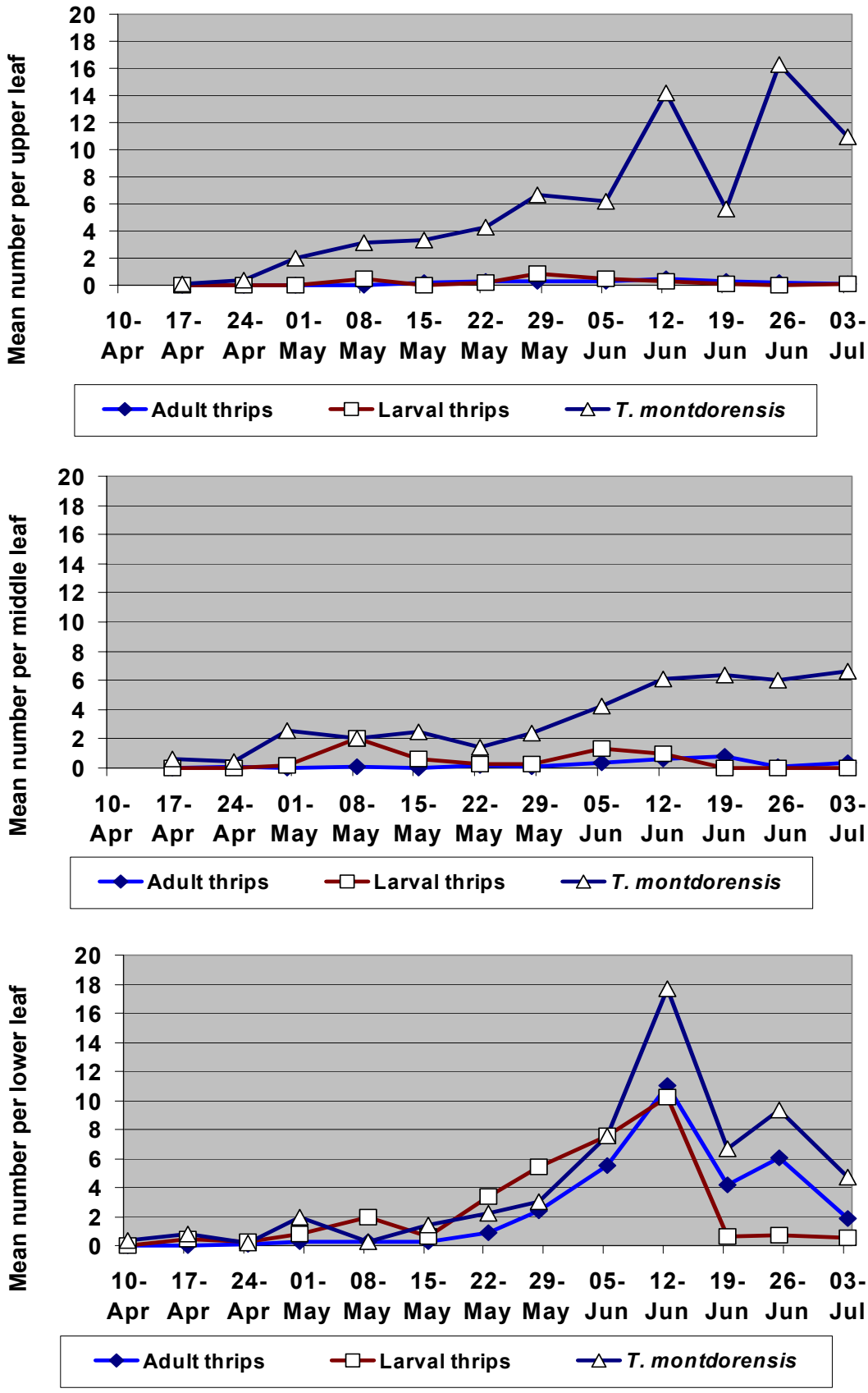


Fig. 13. Mean number of thrips and *T. montdorensis* on upper, middle and lower leaves of greenhouse cucumber, Square House, Crop 4, NCGH, 2002.

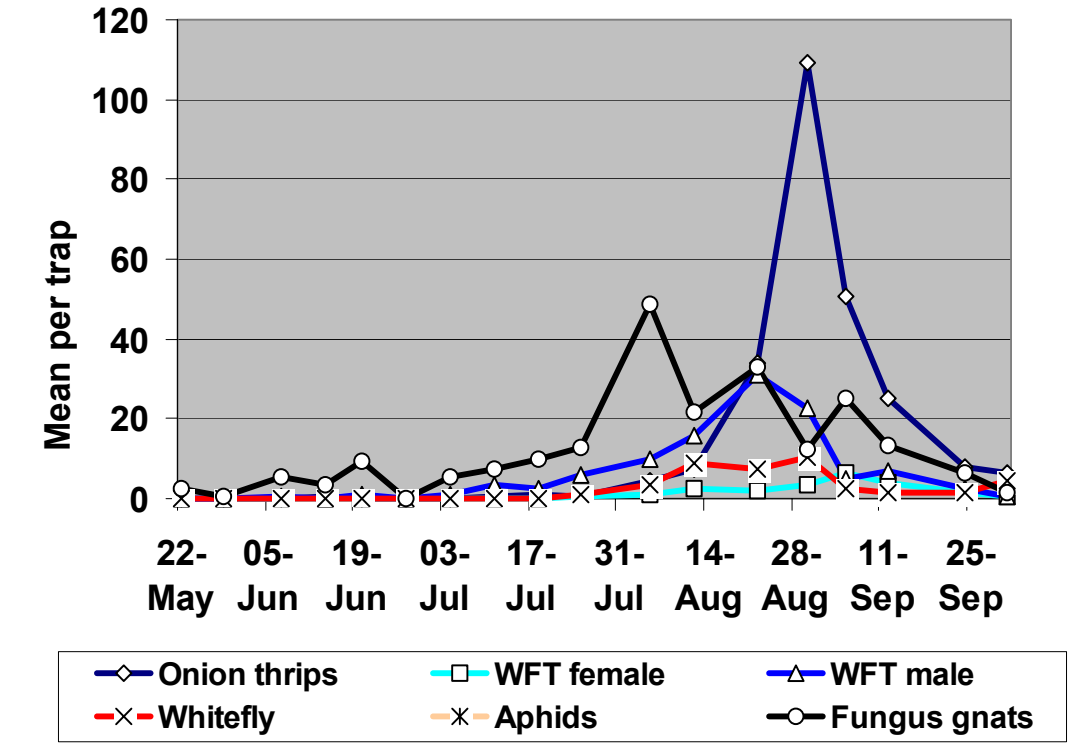


Fig. 14. Yellow sticky trap catches of pests in greenhouse cucumbers, Long House, NCGH, Crop 5, 2002.

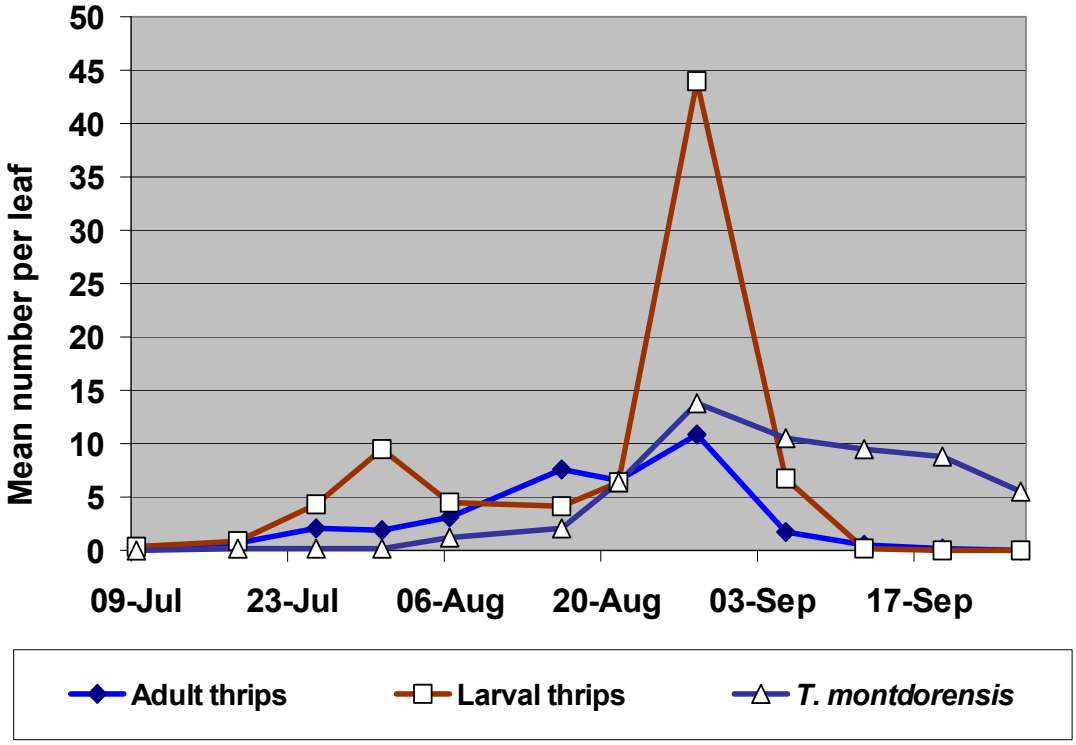


Fig. 15. Mean number of thrips (mostly onion thrips) and *T. montdorensis* on leaves of greenhouse cucumber, Long House, NCGH, Crop 5, 2002.

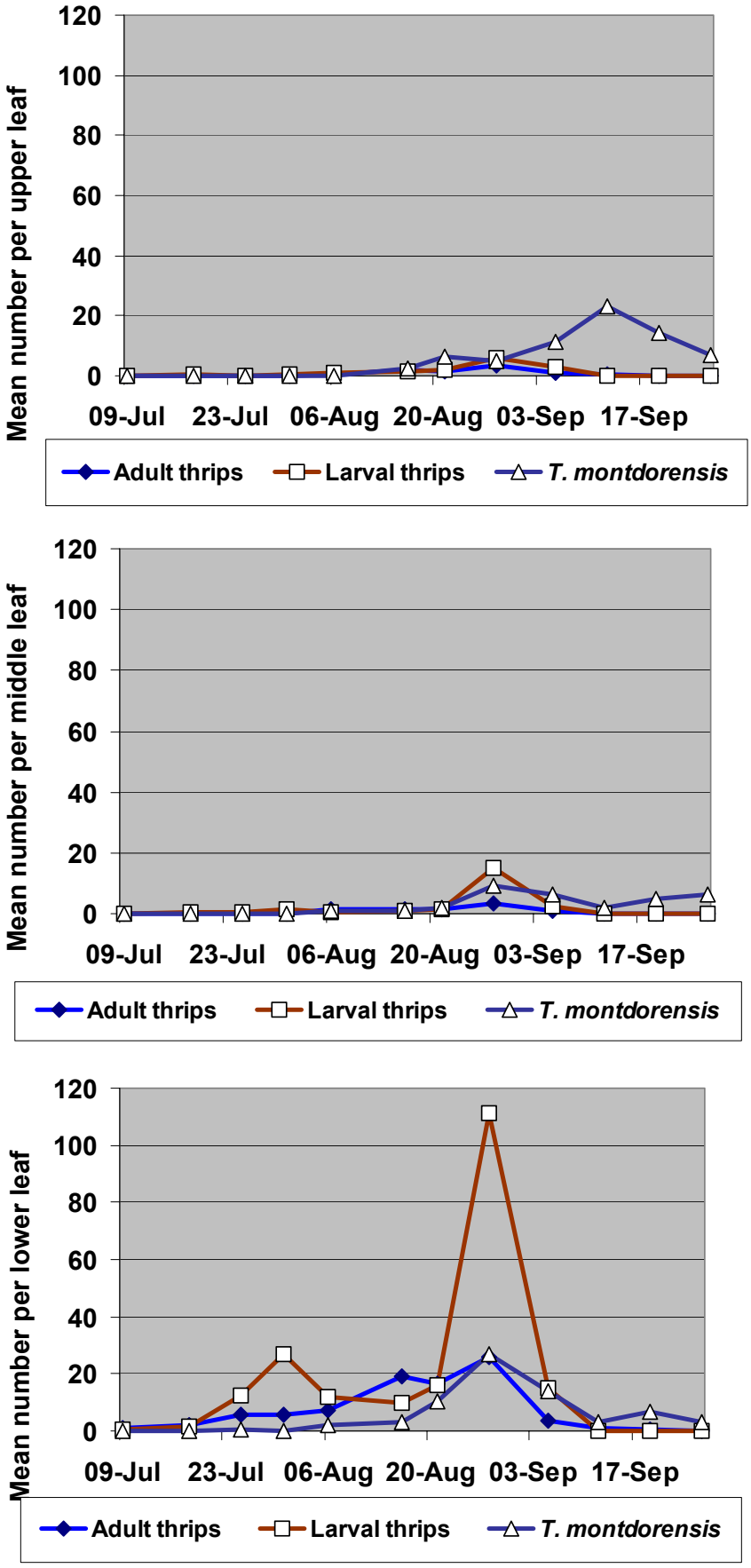


Fig. 16. Mean number of thrips (mostly onion thrips) and *T. montdorensis* on upper, middle and lower leaves of greenhouse cucumber, Long House, NCGH, Crop 5, 2002.

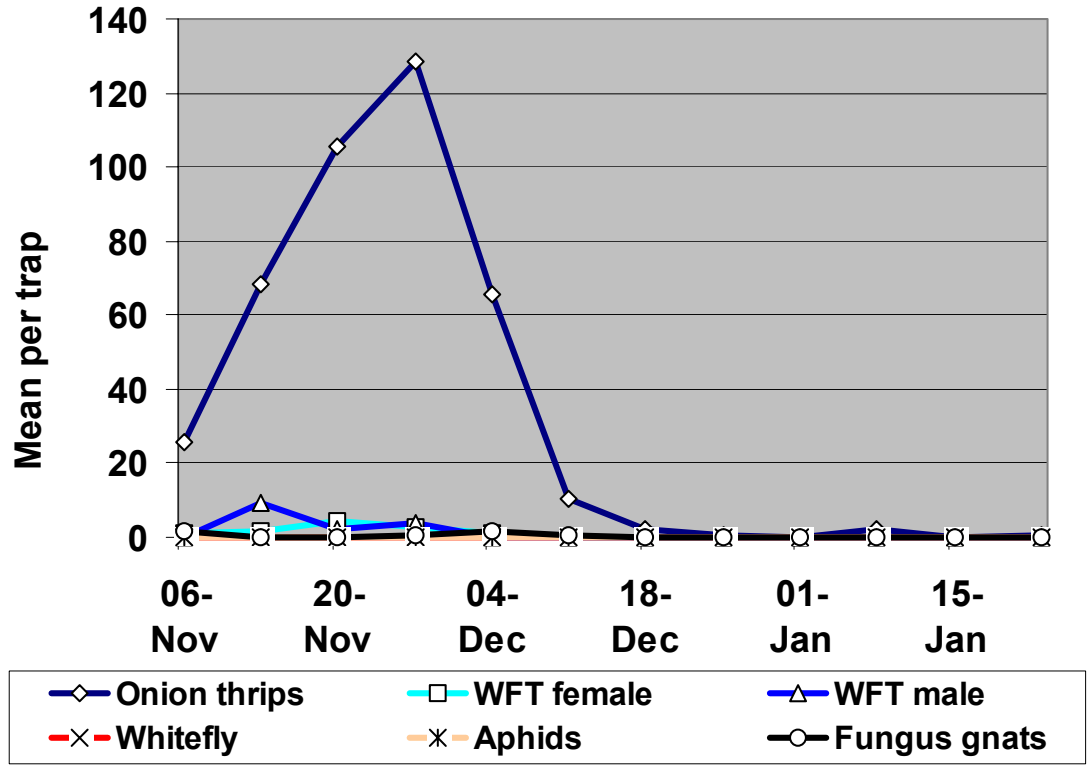


Fig. 17. Yellow sticky trap catches of pests in greenhouse cucumbers, Square House, NCGH, Crop 6, 2002.

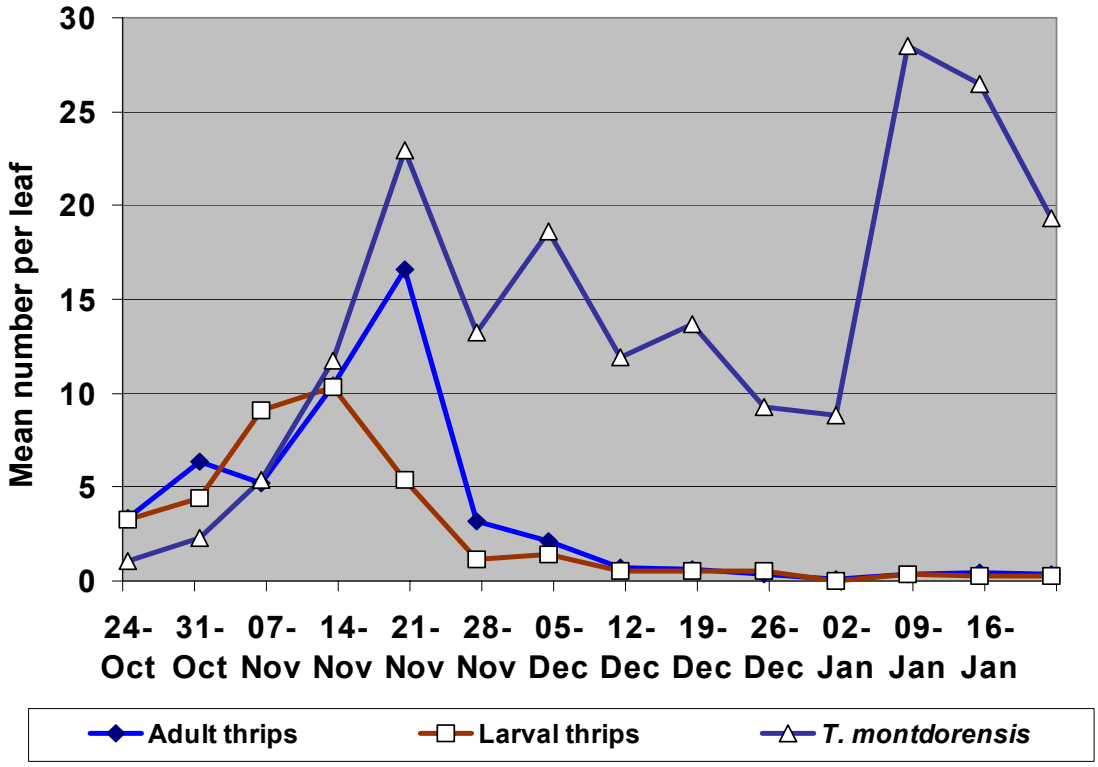


Fig. 18. Mean number of thrips (WFT) and *T. montdorensis* on leaves of greenhouse cucumber, Square House, NCGH, Crop 6, 2002.

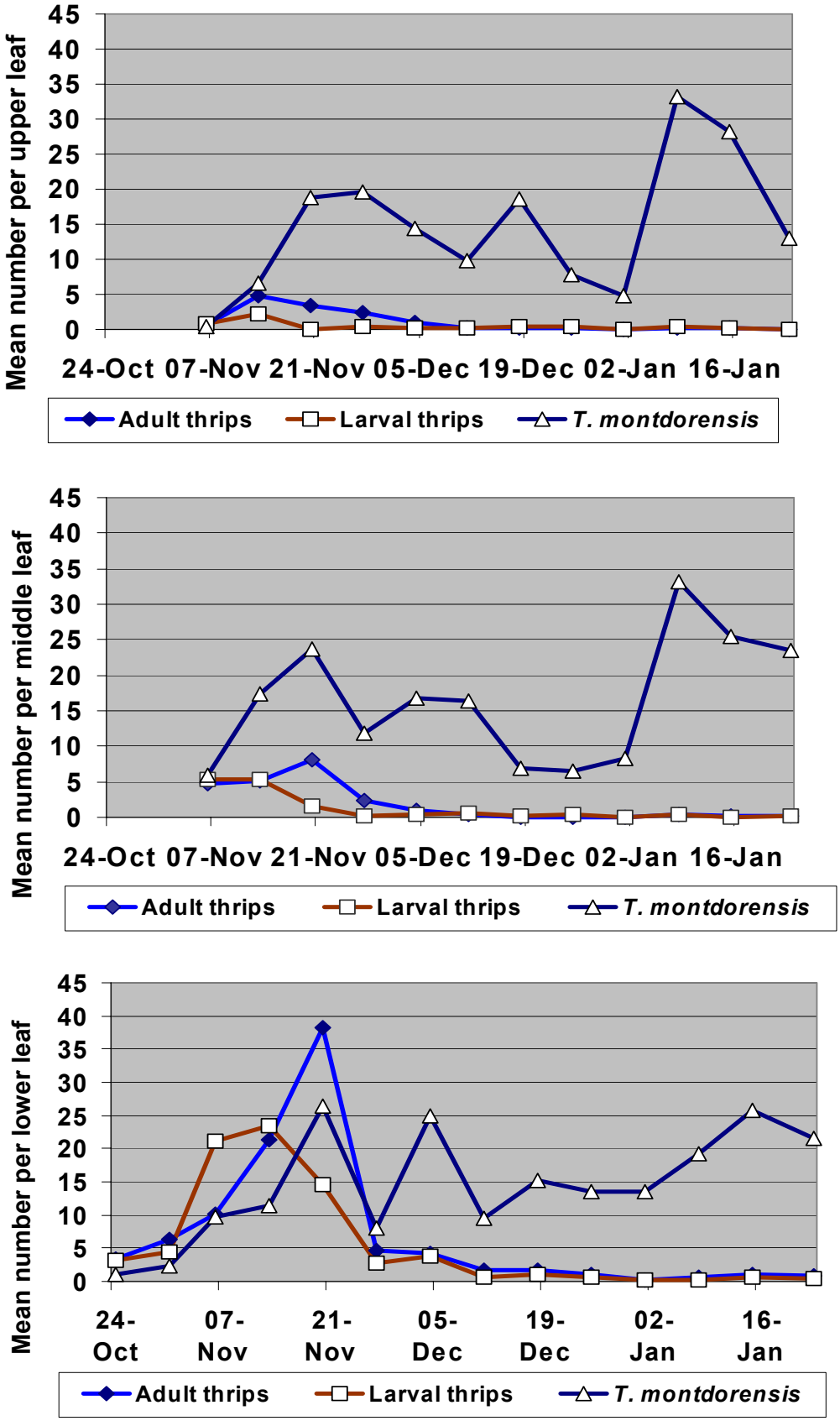


Fig. 19. Mean number of thrips and *T. montdorensis* on upper, middle and lower leaves of greenhouse cucumber, Square House, NCGH, Crop 6 2002.

Table 6. General integrated pest management program for pests in cucumbers at NCGH.

Pest	Biocontrol agent	Source	Rate	Timing of introduction	Pesticide back-up
Spider mites	*Phytoseiulus persimilis* *Feltiella* sp.	See IPM Manual NCGH	Pp-2/sqm plus 20/infested leaf *Feltiella*-1/sqm/week, as available	When mites are first seen, then weekly in hot spots until established	Eco-oil, Torque
Whiteflies	*Encarsia formosa*	Biological Services, SA	0.75/sqm/week if no whitefly seen, then 2/sqm when seen, then 0.75/sqm when 70% of scales are black	Two weeks after planting, then weekly	Eco-oil, Natrasoap
Thrips	*Typhlodromips montdorensis*	NCGH	10/sqm, repeat in two weeks, then as necessary to maintain good predator distribution	If no thrips, apply 1-2 weeks after planting, repeat when thrips appear on traps or leaves or flowers. If thrips present, one week after planting, then two weeks later.	(Spinosad)
Fungus gnats	*Stratiolaelaps (Hypoaspis) scimitus* *Steinernema feltiae*	NCGH Ecogrow	20/sqm applied to growing medium at base of stem. Increase to 50/sqm if cocopeat, sawdust or composted mix used. Minimum rate if numbers too high	One week after planting out. Except for rockwool, perlite, repeat in 2 weeks and if trap count>50/trap for two weeks running High trap count, poor Strats numbers	None necessary
Aphids	*Aphidius colemani* *Hippodamia variegata*	NCGH	0.05-0.10/sqm Hot-spot at 2-5/infested plant	At first sign of aphids, biweekly until well established (mummies present). As necessary	Pirimor
Broad mite	*T. montdorensis*	NCGH	20/infested plant	When mites or damage first seen-apply to new growth weekly.	Vertimec
Caterpillars	Bt formulation	Commercial product	Label rate	When damage first seen, seasonal. Hand pick caterpillars.	None necessary

Table 7. Pesticides registered for use on greenhouse cucumbers and their safety to biocontrol agents.

Insecticides			Fungicides		
Safety	**Active ingredient**	**Trade names**	**Safety**	**Active ingredient**	**Trade names**
NOT SAFE	dimethoate	Dimethoate, Romethoate, Rogor, Dimethomax	NOT SAFE	benomyl	Benlate
				carbendazim	Bavistin
	endosulfan	Endosulfan, Endosan		mancozeb	Dithane
	maldison	Maldison, Hy-mal		oxythioquinox	Morestan
	diazinon	Diazinon		pyrazophos	Afugan
	bifenthrin	Talstar	SAFE	azoxystrobin	Amistar
	imidacloprid	Confidor		chlorothalonil	Bravo
	oxythioquinox	Morestan		copper hydroxide/oxide	
	methamidophos	methamidophos		copper oxychloride	
	pyrazophos	pyrazophos		metiram	Polyram
	chlorpyriphos	Chlorpyriphos, Lorsban etc.		procymidone	Sumisclex
				triadimefon	Triadimefon
SAFE	pirimicarb (drench)	Pirimor		zineb	Zineb
	petroleum oil	Sunspray Ultrafine			
	botanical oil	Eco-oil			
	fatty acid soaps	Natrasoap			
	Bt	Delfin, Xentari			

Table 8. Side-effects chart for pesticides and biocontrol agents

Active ingredient	Pests controlled	Side-effect on biocontrol agents: S=safe; SH=slightly harmful; MH=moderately harmful; H=harmful. Figures indicate weeks persistent if known.*=no specific registration						
		Persimilis	Mont-dorensis	Encarsia	Stratio-laelaps	Lace-wings	Aphidius	Nema-todes
botanical oil (low rate)	aphids, whitefly, spider mites	S	S	S	S	S	S	S
petroleum oil (low rate)	aphids, whitefly, spider mites	S	S	S	S	S	S	S
insecticidal soap*	whitefly, spider mites	SH 0	SH 0	H 0	S	H 0	H 0	S
pirimicarb (foliar spray)	aphids	SH	S	MH 1	S	MH 1	MH	S
pirimicarb (soil drench)	aphids	S	S	S	?	S	S	
Bacillus thuringiensis*	caterpillars	S	S	S	S	S	S	S
maldison	aphids, leafhoppers	H	H	H	H	H	H	S
dimethoate	aphids, leafhoppers, mites, thrips	H	H	H	H	H	H	SH
endosulfan	aphids, leafhoppers, thrips	H	H	H	H	H	H	S
diazinon	caterpillars, thrips	H	H	H	H	H	H	SH
bifenthrin	Heliothis, whitefly, thrips, spider mite	H	H	H	H	H	H	S
imidacloprid (fs)	aphids, whitefly	H 0	H	H 3	MH 0	H 4	H	S
imidacloprid (sd)	aphids, whitefly	S	S	S	H 2-4	S	S	S
oxythioquinox	spider mite							
methamidophos	WFT	H	H	H	H	H	H	H
pyrazophos	WFT	H	H	H	H	H	H	S
chlorpyriphos	WFT, whitefly	H	H	H	H	H	H	MH

Table 9. Biocontrol agents released in NCGH cucumber, Crops 1 and 2, July-September 2001.

House	BCA applied	Pest	Date	Rate
Square House	*Typhlodromips montdorensis*	Thrips	23 July	4/sqm
			10 August	10/sqm
			24 August	10/sqm
			4 September	20/sqm
			12 September	10/sqm
	Encarsia formosa	Greenhouse whitefly	25 July	2/sqm
			3 August	4/sqm
			10 August	4/sqm
			24 August	4/sqm
			5 September	4/sqm
	Stratiolaelaps scimitus	Fungus gnats	24 July	20/plant
	Aphidius colemani	Cotton aphid	24 August	100/house
	Phytoseiulus persimilis	Two-spotted mite	4 September	5/sqm
Long House	*T.montdorensis*	Thrips	23 July	4/sqm
			10 August	10/sqm
			24 August	20/sqm
			4 September	20/sqm
	Encarsia formosa	Greenhouse whitefly	25 July	2/sqm
			3 August	4/sqm
			10 August	4/sqm
			24 August	4/sqm
			5 September	4/sqm
	Stratiolaelaps	Fungus gnats	24 July	20/plant
	Aphidius colemani	Cotton aphid	26 July	20/house
			24 August	100/house
	Phytoseiulus persimilis	Two-spotted mite	4 September	2/sqm

Table 10. Populations of onion thrips and *T. montdorensis* on cucumber crops from leaf washes in three strata, NCGH, Crops 1 and 2, September 2001.

Date sampled 2001	Pest/predator	Mean per leaf					
		Long House			Square House		
		upper	middle	lower	upper	middle	lower
13 Sept	Adult thrips	1.08	4.16	0.44	1.36	0.32	5.24
	Larval thrips	2.28	2.56	0.48	3.92	1.76	8.0
	T. montdorensis	3.72	3.00	2.20	0.76	0.92	0
27 Sept	Adult thrips	3.48	1.80	8.08	-	-	-
	Larval thrips	0.08	0.20	4.20	-	-	-
	T. montdorensis	20.40	12.00	16.40	-	-	-

Table 11. Biocontrol agents released in greenhouse cucumber crop, NCGH Square House, Crop 3, October-November 2001.

BCA applied	Pest	Date	Rate	Total
Typhlodromips montdorensis	Thrips	10 October	3/sqm	28,000
		15 October	3/sqm	
		24 October	10/sqm	
		31 October	10/sqm	
		5 November	10/sqm	
		14 November	10/sqm	
		21 November	10/sqm	
Encarsia formosa	Greenhouse whitefly	2 November to 30 November, weekly	3/sqm	7,500
Stratiolaelaps scimitus	Fungus gnats	10 October	100/sqm	50,000
Aphidius colemani	Cotton aphid	22 November	30	30
Phytoseiulus persimilis	Bean spider mite, two-spotted mite	23 November	4/sqm	7,000
		28 November	10/sqm	

Table 12. Estimated costs of biocontrol agents listed in Table 8. Costs decline substantially with increasing volume and frequency of order. These quantities were for approximately 700 plants in a 500sqm greenhouse, and were preventative introductions. Costs were about $1-$1.25 per square metre or 75-80 cents per plant.

Biocontrol agent	Quantity used	Cost/unit	Total (estimate)
Typhlodromips montdorensis	28,000	$60/10,000	$168
Encarsia formosa	7,500	$20/1000	$150
Phytoseiulus persimilis	7,000	$45/2000 $70/5000	$115
Stratiolaelaps scimitus	50,000	$30/15,000	$100
Aphidius colemani	30	Experimental, no current charge	-

Table 13. Biocontrol agents released in greenhouse cucumber crop, NCGH Square House, Crop 4, March-July 2002.

BCA applied	Pest	Date	Rate	Total
Typhlodromips montdorensis	Thrips	19 March	10/sqm	5,000
Encarsia formosa	Greenhouse whitefly	19 March to 12 June, weekly for 13 weeks	0.75/sqm	5,200
Stratiolaelaps scimitus	Fungus gnats	19 March, 12 April	20/sqm	20,000
Steinernema feltiae		30 April, 14 and 28 May	125 million	375 million
Aphidius colemani	Cotton aphid	28 May	0.2/sqm	550
		30 May	0.3/sqm	
		7 June	0.8/sqm	
Phytoseiulus persimilis	Bean spider mite, two-spotted mite	1, 7, 10, 27 May, 6, 15 June	Hot spots	9,000
Feltiella sp.		24 April, 1, 9, 20 May	1/sqm (hot spots)	2000

Table 14. Estimated costs of biocontrol agents listed in Table 13. Costs decline substantially with increasing volume and frequency of order. These quantities were for approximately 700 plants in a 500sqm greenhouse, and were preventative introductions. Costs were about $0.70 per square metre or 50 cents per plant without the nematodes, and $1.60 per square metre and $1.15/plant including nematodes.

Biocontrol agent	Quantity used	Cost/unit	Total (estimate)
Typhlodromus montdorensis	5,000	$60/10,000	$30
Encarsia formosa	5,200	$20/1000	$104
Phytoseiulus persimilis	10,000	$45/2,000 $70/5,000	$182.50
Steinernema feltiae	375million	$60/50million	$450
Stratiolaelaps scimitus	20,000	$30/15,000	$40
Feltiella sp.	2,000	Experimental, no current charge	?
Aphidius colemani	550		?

Table 15. Biocontrol agents released in greenhouse cucumber crop, NCGH Long House, Crop 5, 7 May-19 September 2002.

BCA applied	Pest	Date	Rate	Total
Typhlodromips montdorensis	Thrips	21 May, 4 June, 29 July, 10 September	10/sqm	24,000
Encarsia formosa	Greenhouse whitefly	15 May to end of crop, weekly for 19 weeks.	1/sqm	11,400
Stratiolaelaps scimitus	Fungus gnats	21 May and 6 June	20/sqm	24,000
Aphidius colemani	Cotton aphid	17 May, 24 May, 29 May, 7 June	0.15-0.5/sqm	650
Phytoseiulus persimilis	Bean spider mite, two-spotted mite	16 May, 6, 15 June, 4, 6 July, 28 July	Hot spot	2000+
Feltiella sp.		21 June weekly to mid September	Variable	5,000

Trial 5. Positioning of yellow sticky traps in a greenhouse cucumber crop for better pest detection

Background

Yellow sticky traps are useful tools for detecting and sometimes predicting pest populations in greenhouse crops. The general recommendation is to keep the trap at crop canopy height to optimise catches of primary pests such as whiteflies and thrips. In several cucumber crops at NCGH, traps failed to adequately warn of damaging levels of onion thrips. Leaf washes in the upper, middle and lower canopy of the crops found that the greatest onion thrips density was on lower leaves (Figs. 13, 16 and 19). It was decided to look at the relative usefulness of traps at two levels in order to provide better predictive advice for whiteflies, onion thrips, western flower thrips and fungus gnats.

Materials and methods

Traps were set out late June until 5 September 2002 in a mature cucumber crop in the Long House, NCGH (Crop 5). They were placed in pairs facing the same direction and oriented the same way, one at ~2m and the other at 0.5m. There were four replicates of each pair except in the final two weeks, when there were three. They were collected and changed weekly for nine weeks. The numbers of each pest were recorded.

Results and discussion

Low-placed traps caught more onion thrips (86.2% of total) and more fungus gnats (81.2% of total) than high-placed traps (Fig. 20), whereas the latter caught more WFT (74.6% of total) and whiteflies (96.3%, not shown). The trend was the same in each of the 11 weeks and on a per trap basis. Far more onion thrips were trapped (4479) than WFT (631). These results show that placement of traps in the lower canopy is strongly advised in mature cucumber crops for early detection of potentially damaging levels of onion thrips and fungus gnats. Traps should continue to be placed in the upper canopy for WFT and whiteflies.

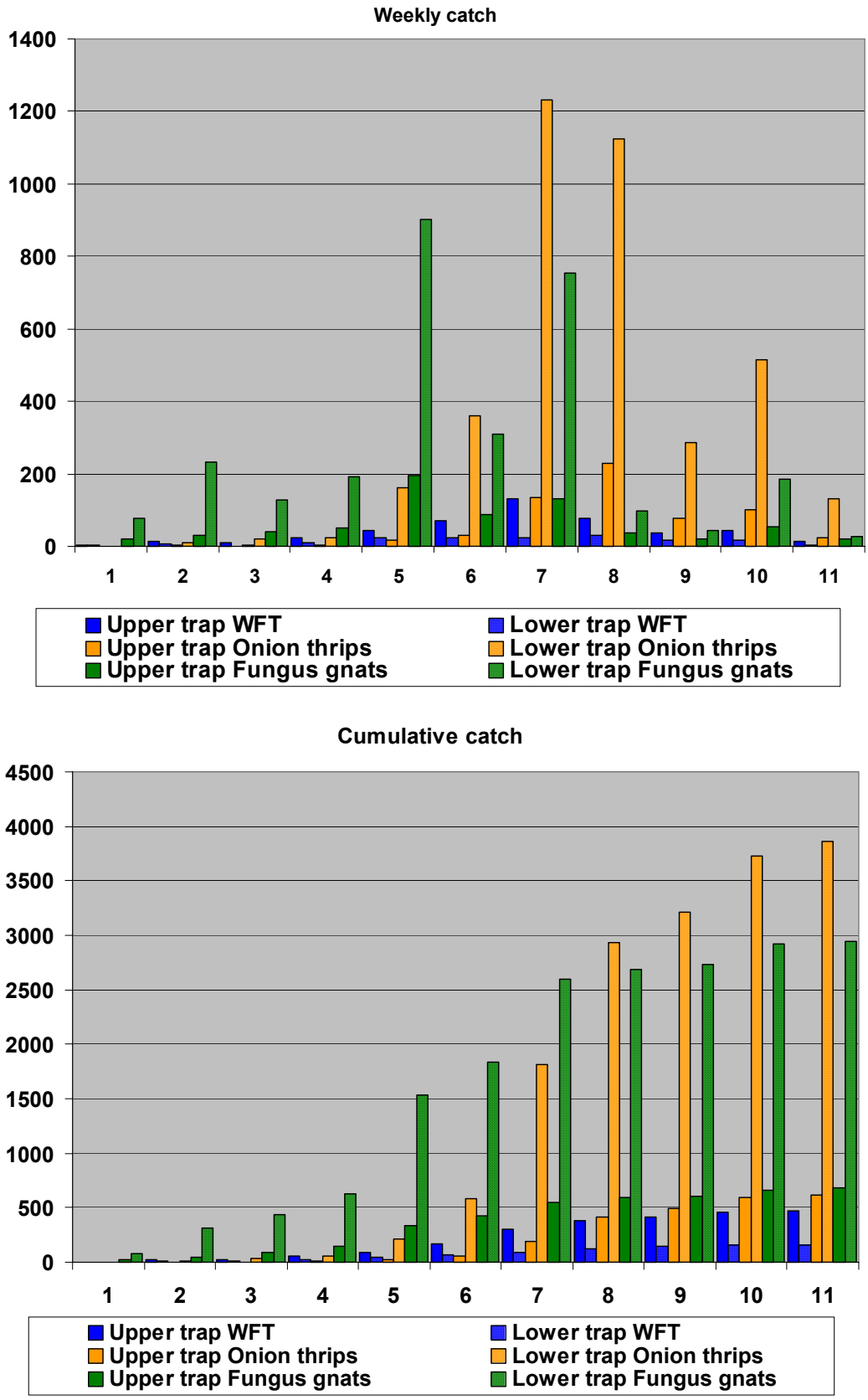


Fig. 20. Weekly (top) and cumulative catch (bottom) of pests on yellow sticky traps in a greenhouse cucumber crop, where traps were placed either high or low in the canopy.

Trial 6. Evaluation of *Typhlodromips montdorensis* against western flower thrips in greenhouse cucumbers (see also Trial 4)

Background

Typhlodromips montdorensis is known to feed on western flower thrips larvae. In a normal greenhouse situation, western flower thrips are usually invading from outside the greenhouse. A trial was set up in a 50sqm polyhouse at NCGH to assess the ability of *T. montdorensis* to control WFT in cucumber at low introduction rates when thrips are invading constantly. In the Long House and Square House crops, WFT were present but remained at very low levels. Was this a result of predation by *T. montdorensis* or an establishment problem for WFT? Onion thrips populations usually overwhelmed those of WFT.

Materials and methods

Cucumber cv Tandoora were planted out in five rows at the first-leaf stage on 19 April 2002 (89 plants). Adult female WFT were introduced twice-weekly at 250 per house per introduction from 23 April 2002 until 25 June. Predators were introduced twice only at 10/sqm on 26 April and 3 May to the crop. The crop was monitored weekly with yellow sticky traps (two/house), plant inspections and leaf washes. For leaf washes, two upper, two middle and two lower leaves per row were removed randomly and washed through screens. At the conclusion of the trial, the greenhouse was cleaned out and a new crop planted. The second crop ran from 1 July to 6 September 2002. WFT were released as before but no predators were introduced. The crop was maintained at 20-30^{o}C.

Results and discussion

Although this trial was unreplicated, the very large differences between thrips populations in the two crops confirms observations made in the Long House and Square House that *T. montdorensis* is an effective control agent against western flower thrips. Onion thrips were only detected in small numbers.

Trap catches reached a peak of 96 WFT per trap (20% females) in week 7 where predators were present, and 958 per trap (49% female) in week 6 where predators were absent (Fig. 21). Trap catches were predominantly male throughout the first crop and until 9 August in the second. Males are usually predominant at low population densities.

In leaf washes, the maximum mean number of thrips per leaf was 2.7 with *T. montdorensis* present (mostly adult), and 119 (mostly larvae), where predators were absent (Fig. 22). The entomopathogenic fungus *Entomophthora* was noted infecting thrips adults and larvae in the second crop on 16 August (week 7), becoming increasingly more prevalent and probably responsible for the decline in thrips numbers after this date.

The high trap catches in the first crop would normally have raised an alarm, but the leaf washes showed that few thrips were surviving predation. Predator numbers were

also very low, not exceeding 1.2/leaf, but this may have reflected a lack of alternative food such as spider mites.

Typhlodromips montdorensis at 10/sqm applied twice early in the crop was able to control a WFT adult 'incursion' of 10/sqm weekly for the nine week life of the crop.

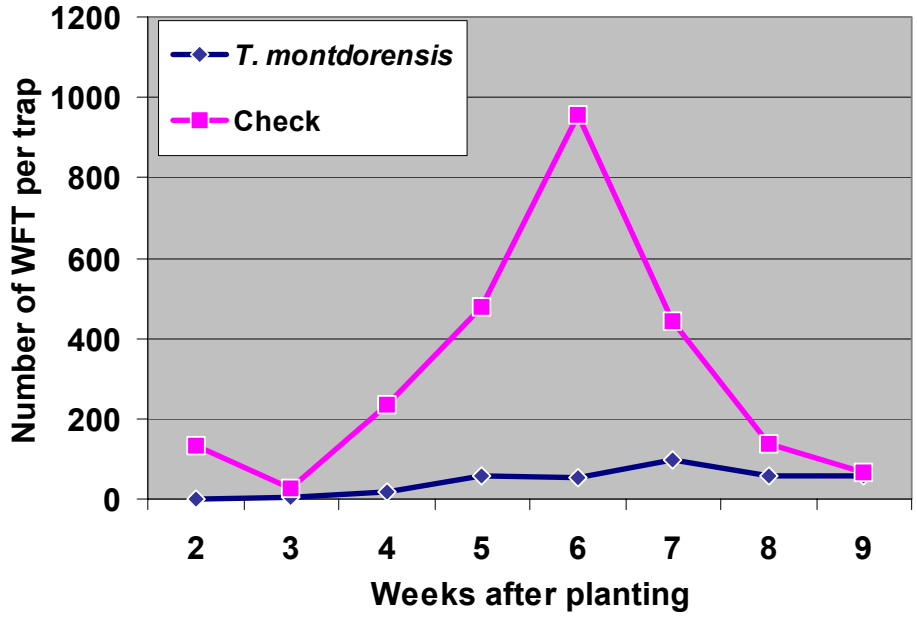


Fig. 21. Yellow sticky trap counts of western flower thrips in two successive crops of greenhouse cucumber, one where *T. montdorensis* were released at 10/sqm on 26 April and 3 May 2002, and the other where no predators were released.

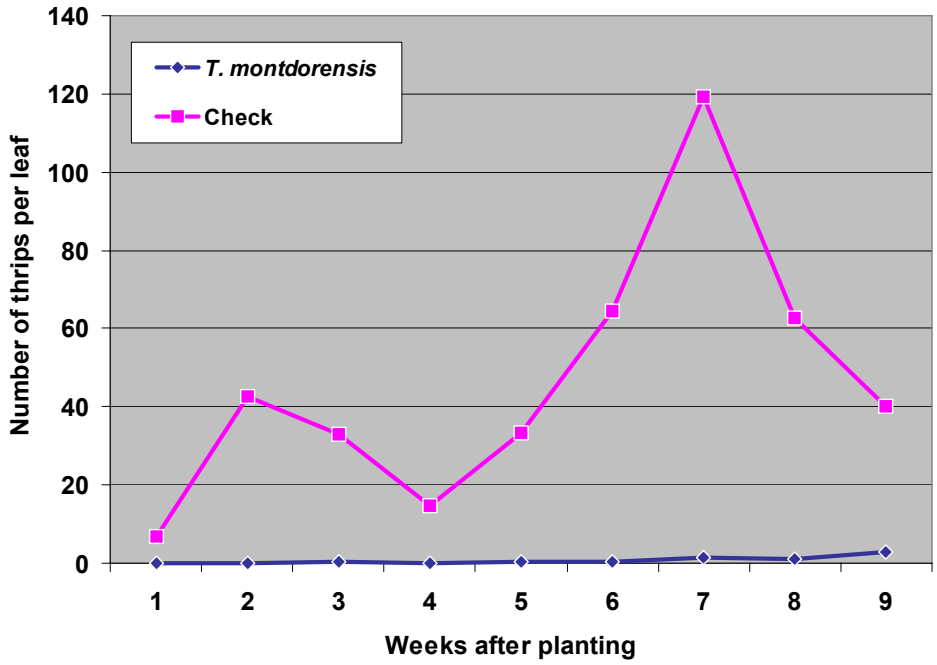


Fig. 22. Leaf wash counts on cucumber crops with and without *T. montdorensis*. Counts include adults and larvae but are mostly larvae where predators were absent.

Trial 7. Evaluation of a ground treatment to control onion thrips in greenhouse cucumber

Background

Onion thrips, *Thrips tabaci*, was a persistent pest in several greenhouse cucumber crops at the NCGH during 2000-2003, far outranking western flower thrips, the traditional major pest, which caused very little damage and was rarely observed. The situation would probably be reversed if pesticides were used, because onion thrips is more susceptible to chemicals. Onion thrips are all female, and thus can establish populations very rapidly. Western flower thrips are predominantly male at low population densities, so where predators are operating efficiently, they can keep that population low and prevent females from predominating. *Typhlodromips montdorensis* tends to distribute itself all over the plant and not in any particular strata. Western flower thrips are more commonly found on middle leaves but can be all over the plant. Onion thrips predominantly attack lower leaves so may build up high populations here while predators are building up elsewhere on the plant, delaying control of the population as a whole.

Both species of thrips drop to the ground as mature larvae and find shelter to pupate. There are currently no predators that are working effectively at this level. There is thus a need to control thrips here to break the cycle. A floor treatment that will restore the balance without upsetting predator and parasitoid populations is desirable. Spinosad (Success®) is very active against thrips as a foliar spray and is relatively harmless to most biocontrol agents except microhymenoptera. A floor spray effective against thrips would conceivably cause little disruption to foliar-inhabiting biocontrol agents.

Materials and methods

The crop was a mature cucumber crop (13 rows) grown in various hydroponic media in the 500 sq.m. Square House. The flooring was white plastic weed matting. A pair of metal heating pipes ran the length of the row in the isle. The crop was divided into four evenly sized blocks, excluding one outside row. There were three treatments, repeated randomly in each block (four replicates). These were as follows: T1, water only; T2, Success® (spinosad) at 41 mL/100L (5g ai /100L) to floor and bags only; and T3, Success® at 82 mL/100L to floor and bags only. Agral® was added to all treatments at 0.2ml/15L (0.001%) as a spreader. Each treatment area consisted of two rows of cucumbers. Treatments were applied as a coarse spray at steady walking speed to wet the plastic and media under the crop canopy (except the 0.8m width between the rails). None was applied to the plants. A total of 15L of solution was used for each treatment, applied from both sides of the centre line. The treatments were applied twice (5 and 12 July 2002).

To monitor adult thrips populations before and after treatment, two yellow Seabright™ sticky traps were placed landscape–wise within each treatment block, one per row. Placement was vertically oriented, bottom edge 10 cm from the medium, oriented north/south. Traps were set out six days before treatment, and one day after each treatment application to avoid any irritant effect of the chemical. They were left for ~6 days (28 June-4 July, 6 July-11 July, 13 July-19 July) and then removed and

checked for thrips. A media sample (20 mL, six replicates/treatment from all blocks) was taken from one of the media types, Australian Native Landscape mix, where *Stratiolaelaps* could be readily observed. Sample dates were 4 July and again on 12 and 19 July from the same bag, to assessed any impact of the treatments on *Stratiolaelaps* numbers. To bring mites to the surface, the bag was first tapped sharply and the surface layer mixed to distribute mites evenly before sampling.

Results and discussion

None of the treatments had any significant impact on either thrips numbers caught on traps or *Stratiolaelaps* populations in the media (Table 16). *Stratiolaelaps* populations remained relatively stable over the three-week period, whereas thrips numbers declined in all treatments, reflecting a general decline due to *T. montdorensis* predation on thrips larvae on the leaves.

Table 16. Populations of onion thrips on yellow traps and *Stratiolaelaps* in media pre- and post-treatment with Success® to the floor beneath the leaf canopy in a cucumber crop.

Date assessed	**Mean numbers of onion thrips per yellow sticky trap (range)**		
	Control	**41 mL Success®/100L**	**82 mL Success®/100L**
4 July	18.50 (2-43)	33.13 (15-59)	22.63 (8-60)
11 July	7.88 (4-20)	9.25 (5-20)	9.13 (2-19)
19 July	5.75 (2-13)	5.38 (2-12)	5.25 (1-12)
Date assessed	**Mean numbers of *Stratiolaelaps* per 20mL sample (range)**		
	Control	**41 mL Success®/100L**	**82 mL Success®/100L**
4 July	10.83 (8-13)	7.50 (4-11)	12.33 (4-25)
11 July	10.17 (7-15)	15.50 (10-23)	7.67 (3-13)
19 July	9.33 (6-15)	13.33 (7-18)	7.00 (4-10)

Trial 8. Influence of growing media on fungus gnat populations in a hydroponic system

Background

Fungus gnats are common in growing media and are difficult to control with pesticides. A research study looking at hydroponic growing media at the National Centre for Greenhouse Horticulture provided an opportunity to look at the media with respect to their attractiveness to fungus gnats. The ground dwelling predatory mite *Stratiolaelaps scimitus*, available commercially as Hypoaspis, has provided excellent control previously in Growool and soil-based media on the Station, including previous cucumber crops. Suggested introduction rates are two applications two weeks apart early in the crop at 100 mites per square metre.

For the media trial, five mixes were used. These were sawdust and a composted bark mix (ANL cucumber mix) in double white plastic grocery bags, Cocopeat in black growbags, Perlite in elongated closed shallow bags, and Growool in elongated blocks.

Materials and methods

In a bid to cut costs, the application rate in the autumn/winter cucumber crop in the square house was reduced from 100 to 20 mites per square metre, applied 19 March 2002 (a week after planting the crop out). A second application at the same rate was applied 12 April, as the mites could not be seen in the media. By late April, adult fungus gnats were visibly numerous, particularly hovering over bags with ANL cucumber mix. This media mix appeared very wet and perhaps not conducive to predatory mite establishment. To prevent damage to the cucumber roots, entomopathogenic nematodes (*Steinernema feltiae*) were ordered and applied on three occasions two weeks apart. Dates of application were 30 April, 14 and 28 May. They were applied through the drippers because of ease of application, though a whole-media soil drench might have been more effective. The rate was 125 million in 200L of water on the first dates and 100 million on the third.

To detect changes in fungus gnat adult populations coming off the five different media, a yellow sticky trap (Seabright®) was attached to a short stake and placed vertically just above the mix (Fig. 23). There were three replicates for each media type, one of each type in rows 3, 4 and 5. The traps were checked and changed weekly from 2 May (Week 1) to 27 June (Week 9), and adult fungus gnat numbers recorded. Unfortunately the traps were not put out in time to record the pre-treatment count, though nematodes do not affect adult gnats, nor pupae in the medium at time of first treatment, so the count on Week 1 is probably indicative of pre-treatment numbers. General trap catches from those placed at canopy level record a peak of 144 per trap for the week ending 29 April (Fig. 12 Trial 4). Traps in the upper canopy catch far fewer fungus gnats than those placed at ground level, but catches over 50/trap/week are usually considered an indication of a problem population.

Results and discussion

Media which were based on organic materials (ANL mix, cocopeat and sawdust) generated far more fungus gnats than inorganic ones (Growool and Perlite).

Populations as recorded on the sticky traps are presented in Fig. 24. Composted bark mix>cocopeat>sawdust>Perlite>Growool. Fungus gnat adult catches declined substantially in all media over the six-week period, to reach acceptable levels. It was noted in early June that *Stratiolaelaps* was now extremely numerous in ANL mix, so whether it was the nematodes or the predatory mites (or both) that could be credited with the decline has not been established. Further trials are planned to examine the relative importance of the two treatments. As *Stratiolaelaps* are known to feed on nematodes (but not such a degree as to interfere with their effectiveness as biocontrol agents), a possible strategy is to apply a low dose of both nematodes and *Stratiolaelaps* early in the crop to establish the latter in advance of fungus gnats.

Figure 23. Yellow sticky trap placement to detect adult fungus gnats emerging from media.

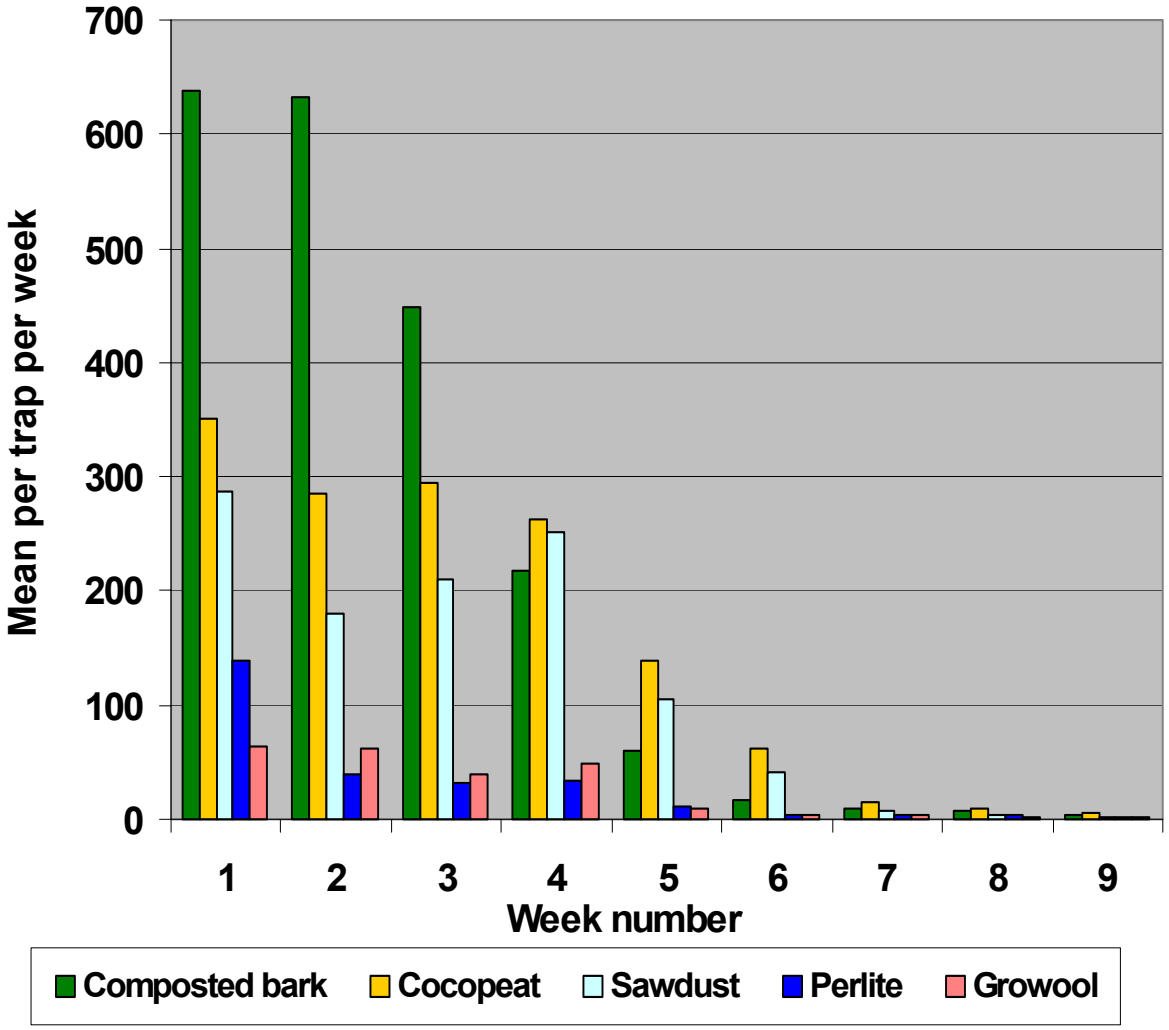


Fig. 24. Yellow sticky trap catches of adult fungus gnats over various media, Square House cucumber crop 4, 2 May-27 June 2002.

Summary of IPM research in cucumber crops at the National Centre for Greenhouse Horticulture 2000-2003

Greenhouse cucumber pests such as thrips, greenhouse whitefly, spider mites, aphids and fungus gnats were successfully managed in six crops through the use of biocontrol agents applied as preventive controlling agents instead of the traditional approach of using pesticides as curative agents. One pesticide spray was applied against thrips, one whole plant spray and occasional spot-sprays against cotton aphid, and one spot-spray against spider mite during this period.

The newly commercialised predatory mite *Typhlodromips montdorensis*, developed at NCGH, was very successful in controlling western flower thrips to below damaging levels in six successive crops when applied at low introduction rates early in the crop. Onion thrips were more of a problem in some crops but damaged leaves rather than fruit. The ratio of thrips to predators was critical, with control following rapidly when predator numbers equalled or exceeded thrips larval numbers. Early introduction to every plant gave the best control, with the number of introductions varying (Table 17) but ranging from 1-7. Two introductions at 10/sqm two weeks apart is probably adequate where thrips are present but in very low numbers, with repeat applications sometimes necessary to maintain good distribution of predators in the crop or where major incursions of adult thrips upset the balance between predators and thrips. The predators are capable of increasing very rapidly in warm, humid conditions where food is available and where there are no negative pesticide residues. A ground treatment with Success® at two rates to control larvae dropping off leaves to pupate failed to provide any reduction of thrips populations, but this strategy needs to be pursued with other products.

Yellow sticky traps placed in the upper canopy to detect thrips were found to provide adequate warning of western flower thrips but not of onion thrips as the latter reside mostly in the lower canopy. Traps at two levels are advisable.

Whitefly control was excellent in all crops following a strategy of weekly releases of the parasitoid *Encarsia formosa* at rates of 0.75-2/sqm. The low rate is probably adequate while whitefly are barely detectable.

Fungus gnat control with the predatory mite *Stratiolaelaps scimitus* (Hypoaspis) was excellent at an early introduction rate to media of 100/sqm. The type of media used greatly influenced fungus gnat numbers, with organic media very conducive to outbreaks and requiring higher rates of Hypoaspis to keep them under control. The rate could be reduced to 25/sqm in non-organic growing media. The entomopathogenic nematode *Steinernema feltiae* was used very successfully in one crop as a supplemental control to bring about a rapid decline in fungus gnat populations. A neem-based product, AzaMax, applied as a soil drench, provided some control of fungus gnat larvae.

Aphid control remains somewhat of a problem, with the parasitoid *Aphidius colemani* sometimes providing good control and other times not. The rate and timing of introduction is probably critical. While a rate of 1/sqm weekly at first sign of aphids is probably necessary rather than the lower rate used, there is as yet no commercial supplier in Australia and so not enough were available. Careful management with

parasitoids and spot treatment with Pirimor is currently the best option. Delays in treatment can lead to problems quite rapidly as cucumber is a very good host of cotton aphid. Small numbers of brown lacewing and the ladybug *Diomus notescens* were introduced but did not establish well on this occasion.

Spider mite was only a problem in one crop, with bean spider mite more prevalent than two-spotted mite. The predatory mite *Phytoseiulus persimilis* was introduced as a hot-spot treatment in this crop but it failed to establish well or give adequate control. A pesticide applied for powdery mildew control may be partially responsible. The spider mites supported very high populations of *T. montdorensis*, which did not control them but may have interfered with *P. persimilis* in some way. A predatory midge was tried as a supplemental control in three crops but despite occasionally being found in high numbers it did not appear to recycle well. Failure may have been partly due to interference by *T. montdorensis* or *P. persimilis*, by the inability to pupate on the plastic flooring, or by application of fungicides.

The biocontrol program (beneficials only) was costed for two (short) crops and ranged from 0.70 to $1.25/sqm (nematode costs excluded).

A necessary component of a successful biocontrol program such as this is lack of pressure from pests outside the greenhouse through installation of screening on vents, a double doorway with positive pressure fans, and weed management. The design of the greenhouse structure is also important. Good height to gutter and computer controls allows environmental management that favours both optimum crop growth and avoids the extremes that inhibit biocontrol agents and which favour pests and diseases.

Table 17. Releases of the predatory mite *T. montdorensis* in cucumber crops at NCGH, 2000-2003.

House	Crop	Cropping period	Number of releases
Square House	1	Jul-Sep 2001	5
Long House	2	Jul Sep 2001	5
Square House	3	Oct-Dec 2001	7
Square House	4	March-July 2002	1
Long House	5	May-Sep 2002	4
Square House	6	Oct-January 2003	3

Section C Development of an IPM program for greenhouse capsicums

Background

Although capsicums, or sweet peppers as they are known in some other countries, are a major greenhouse crop overseas, in Australia the great majority of production occurs in the field. This is reflected in variable product quality and price as supply can be interrupted by climatic events. In New Zealand capsicum is a key greenhouse crop and New Zealand produce is being exported to Australia. In Australia the Northern Adelaide Plains is the only area where capsicums are produced in any significant quantity, albeit in antiquated technology. There are also a number of producers in very old glasshouse structures in the Dareton region that have produced capsicum, although their technology is poor. There are occasional greenhouse producers of capsicums in Victoria, NSW and Queensland. It is a crop that will see greater production under cover developing over time in Australia, as has happened with tomatoes.

Capsicum is a crop that is well suited to successful biocontrol and IPM through its ample pollen and nectar production. It is also a crop that can be devastated by tomato spotted wilt virus. This R&D program has attempted to address some of these issues.

In this section the following trials were conducted:

Biocontrol	Trial 9. Management of western flower thrips and tomato spotted wilt virus using *Typhlodromips montdorensis* and development of an IPM program for greenhouse capsicums
Biorationals	Trial 10. Management of western flower thrips and tomato spotted wilt virus in greenhouse capsicums using virus transfer inhibitors Trial 11. Control of aphids on greenhouse capsicums

Trial 9. Management of western flower thrips and tomato spotted wilt virus using *Typhlodromips montdorensis*, and development of an IPM program for greenhouse capsicum.

Background

The tolerance for thrips vectors of tomato spotted wilt virus is very low in capsicums, particularly when they are young plants. In a previous trial at NCGH ending in January 2002, anti-transpirants and oils were applied to control TSWV but infection did not occur until the end of the trial and none of the products tested would have been suitable in any case.

After this experiment, the plants were left in place and TSWV infection established and spread. It was decided to use these plants as sources of infection in two subsequent trials.

Success has been achieved in controlling thrips overseas with predatory mites. We wanted to see if the native predatory mite *Typhlodromips montdorensis* would establish well in capsicum, in adequate numbers to control thrips below damaging levels to fruit, but also below levels that would spread TSWV. Predatory mites only eat larval thrips, but it is only larval thrips that can acquire virus, develop to adults and then transmit it. By introducing predators in advance of thrips, it was hoped to prevent transfer of virus from infested plants by their removing all larvae before they could complete development.

Because management of thrips with predators requires minimal pesticide use for other pests, a program was put in place to manage all pests biologically where possible.

Two successive capsicum crops were grown. The first ran from 15 March to 28 June, and the second from 9 August to 30 October 2002. The predatory mite *T. montdorensis* was released in the first crop only.

Materials and methods

Trial I. *Typhlodromips montdorensis* released. The trial was conducted in a 50 sq.m. polyhouse at NCGH (House 5) (Fig. 25). There were five rows of capsicum. The two outside rows (14 plants in each) were retained from the previous trial, and were all infected with TSWV. After cutting back, they were sprayed with Success® (spinosad) on 22 February to remove existing thrips, and as an added precaution, all buds, flowers and fruit were removed at the start of the experiment. The remaining three rows (28 plants in each), consisted of young plants (cv Zirconio) seeded 30 January 2002 and set out 15 March as they were just starting to branch. There were thus 112 plants in total, 25% with TSWV infection, at the start of the experiment.

The greenhouse had no vents but was cooled by a pad and fan system. A double positive pressure entry system reduced incursions of pests from outside. Temperature was maintained between 18^{o}C and 35^{o}C.

The IPM program planned is set out in Table 18. Plants were pruned weekly or biweekly to maintain two leaders. One fruit was allowed to set on each main stem node after the fourth node from the fork. Three to five leaves were allowed to remain on side shoots, pinching out beyond this. Mature red fruit was harvested twice weekly. Hands were dipped in milk between each plant to minimise physical transfer of virus.

The crop was monitored weekly for thrips and predators by examining the upper eight main stem leaves on 10 randomly selected plants in each of the three centre rows, and 10 flowers per row. Flowers were selected that had pollen just starting to spill.

Four yellow sticky traps were set out from 15 March and traps changed weekly, counting thrips, whitefly, aphids, fungus gnats, and shoreflies.

Plants with symptoms of TSWV (Fig. 26, 27) were flagged and confirmed positive with Agdia® test kits (Fig. 28).

Fig. 25. TSWV trial in greenhouse capsicum, NCGH. Plants in outside rows were pre-infected with TSWV.

Fig. 26. Capsicum leaves infected with TSWV.

Fig. 27. Capsicum fruit infected with TSWV.

Fig. 28. Agdia® test kit for TSWV. The second horizontal violet line indicates a positive test for virus.

Table 18. IPM program established for greenhouse capsicum, NCGH, March 2002.

Pest	Biocontrol agent	Source	Rate	Timing of introduction
Spider mites	*Feltiella* sp. *Phytoseiulus persimilis*	NCGH See IPM manual	Feltiella-1/sqm, as available Pp-2/sqm plus 20/ infested leaf	When mites are first seen, then weekly until established. Use Persimilis only if TSM are getting out of hand.
White-flies	*Encarsia formosa*	Biological Services, SA	0.75/sqm/week if no whitefly seen, then 2/sqm when seen, then 0.75/sqm when 70% of scales black	Two weeks after planting, then weekly
Thrips	*Typhlodromips montdorensis*	NCGH	10/sqm	One week after planting, then when WFT is first found in flowers, or any thrips on leaves, until established on all plants with thrips in similar numbers
Fungus gnats	*Stratiolaelaps* (Hypoaspis) *scimitus*	NCGH	20/sqm applied to growing medium at base of stem	At planting out. Repeat in 2 weeks and if trap count>50/trap for two weeks running
Aphids	*Aphidius colemani* /lacewings (experimental)	NCGH	0.05-0.10/sqm	At first sign of aphids, biweekly until well established (mummies/lacewings present)
Broad mite	*T. montdorensis*	NCGH	20/infested plant	When mites or damage first seen-apply to new growth weekly
Cater-pillars	Bt formulation	Commercial product	Label rate	When damage first seen, seasonal. Hand pick caterpillars

Typhlodromips montdorensis was introduced in a vermiculite carrier at 10/sqm in advance of thrips, on 19 and 25 March, a few on every plant, and allowed to establish for two weeks. The bran mite *Tyrophagus putrescentiae* (Tp) was released at 1Tm: 100 Tp with the first batch only because of concerns that no food would be available to the predators until the thrips were released or flowers were available with pollen. The capsicum leaves were small and it was difficult to place the vermiculite without it falling off. A non-scheduled third release was made on 9 April because of this

potential loss of predators. For the final release, the vermiculite with mites was placed at the base of the main stem. Field releases in capsicum in QLD and in greenhouse tomatoes at NCGH showed that predators were able to climb onto the plant from here.

500 adult WFT were introduced on 5 April (flowers present). Further releases of thrips were not considered necessary because they could be found readily in flowers after release.

Stratiolaelaps and *Encarsia* were released from 19 March according to the planned schedule, and five brown lacewing adults released at the same because cotton aphids were present. 100 *Feltiella* were released 3 April for spider mite and 10 *Diomus* on 4 April for aphids.

Trial II. No *Typhlodromips montdorensis* released. The trial was set up again in the same way, but no predators were introduced. Young TSWV-infected plants from Trial 1 were treated with Pirimor® for aphids on 5 August and pyrethrum on 5 and 7 August to kill *T. montdorensis*, and used to replace older infected plants in the outside rows. Capsicum cv Zirconio was seeded 14 June 2002 and planted out 9 August in the three centre rows. 500 WFT adults were introduced on 20 August, prior to flowers being present. This first introduction didn't establish well, possibly because of infection with the fungus *Entomophthora*, which was prevalent at the time, or possibly the absence of flowers. A second batch was reluctantly released at the same rate a month later on 17 September (Week 6), which did establish.

At the conclusion of this trial, the novel insecticide Mospilan® 20% SL (acetamiprid) was applied 9 October 2002 as a drench against green peach aphid on plants in one outside row. The rate was 0.5mL product/L at 100mL/rockwool cube. There were 4 cubes per cocopeat bag and the crop was mature. This product had previously given good control of green peach aphid and potato aphid as a foliar spray at the same rate.

The newly introduced ladybeetle *Hippodamia variegata* was also released in small numbers late in this crop for aphid control.

Results and discussion

In Trial I, the first plant showing TSWV symptoms (small yellow leaves, discoloured fruit) was recorded on 2 May (Week 7). By 28 June (Week 15), the percentage of infected plants in the centre three rows had levelled out at 28.6% (Fig. 29). As symptoms can take weeks to develop, most of the infection clearly took place early in the crop, carried by adult thrips that developed from larvae that had managed to escape being eaten. Trap catches showed thrips at very low numbers (Fig. 30), with adult WFT not exceeding 13/trap/week, and being mostly males. *Typhlodromips montdorensis* established very well and reached high densities (Fig. 31). The maximum mean was 10.6/leaf on 9 May, Week 8. Pollen probably assisted in keeping the population high as there were very few thrips to feed on. The thrips population was maintained at a very low level, with mean larval density on leaves never exceeding 0.04/leaf (Fig. 31). The number of thrips recorded in flowers reached a maximum of 3.8 adults in Week 6 and then declined. No damage to fruit or leaves was observed.

In Trial II, the percentage of plants visibly infected with TSWV increased dramatically 3-4 weeks after WFT populations increased, and 100% of plants were infected by Week 13 (Fig. 29). No fruit was harvested from this second trial because of TSWV infection, whereas several weeks of picking were achieved in the first. Fruit damage caused directly by thrips feeding (silvering) was very prevalent. The number of thrips on traps (Fig. 30), and in flowers (Fig. 31) was still increasing at the end of the trial. The maximum mean on traps was 308 in Week 11, and in flowers 6.3 adults and 8 larvae, which seems somewhat of a disparity. Until Week 9, most thrips on traps were male. The switch to mostly females in Week 10 led to a rapid increase in population in the final two weeks.

While 25% infection rate of TSWV in the first trial may seem high, the 'ask' was enormous. Even one thrips larva acquiring TSWV and surviving to adulthood can potentially transmit to several plants. The trials indicate (i) the extremely low tolerance level for thrips when TSWV is present, (ii) that *T. montdorensis* establishes very well on capsicum, even at low pest levels, and (iii) that *T. montdorensis* is capable of controlling WFT well below levels that can cause direct damage to leaves and fruit.

Symptoms of TSWV show up some weeks after the infection is present, so removing plants with TSWV as soon as symptoms are seen is necessary but not the whole answer. A resistant cultivar should be the first line of defence to increase tolerance for thrips. The crop is most susceptible to infection when young, so a compatible pesticide applied in the first few weeks is another option, though none of the existing products is safe to all biocontrol agents necessary to control the range of pests likely to be encountered in capsicum. We obtained excellent control of whitefly, fungus gnats and spider mites solely with commercially available biocontrol agents. Cotton aphid was a concern early in the crop and then green peach aphid, but they were controlled by *Aphidius colemani*, with some help from the lady beetle *Diomus notescens* and the brown lacewing *Micromus tasmanii.* A hyperparasite of *Aphidius colemani*, *Alloxysta victrix*, began to interfere with control later in the crop. This parasite is known to interfere with efficacy overseas.

Mospilan® applied for aphid control in one row as a drench appeared at first to give poor control, but a week later almost no aphids were visible on the plant. There were live WFT and *Phytoseiulus persimilis* present. Apart from the excellent control, a drench treatment may have an advantage in avoiding contact with leaf-inhabiting natural enemies. The fact that aphids dropped off the plant is also an advantage as there are no dead bodies.

The ladybeetle *Hippodamia variegata* established and bred very well on the capsicum and shows promise for supplemental aphid control.

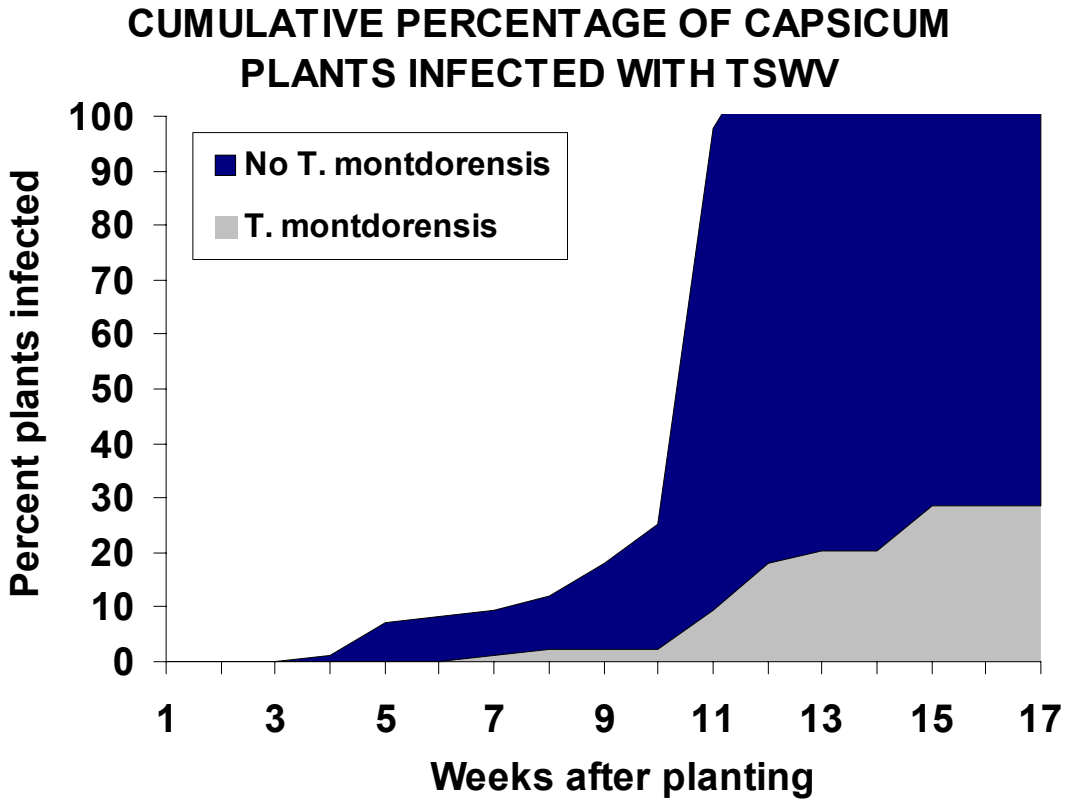


Fig. 29. Cumulative percentage of capsicum plants infected with TSWV in two successive crops, in only one of which *T. montdorensis* was released. Adult WFT (500) were released early in the crop in each.

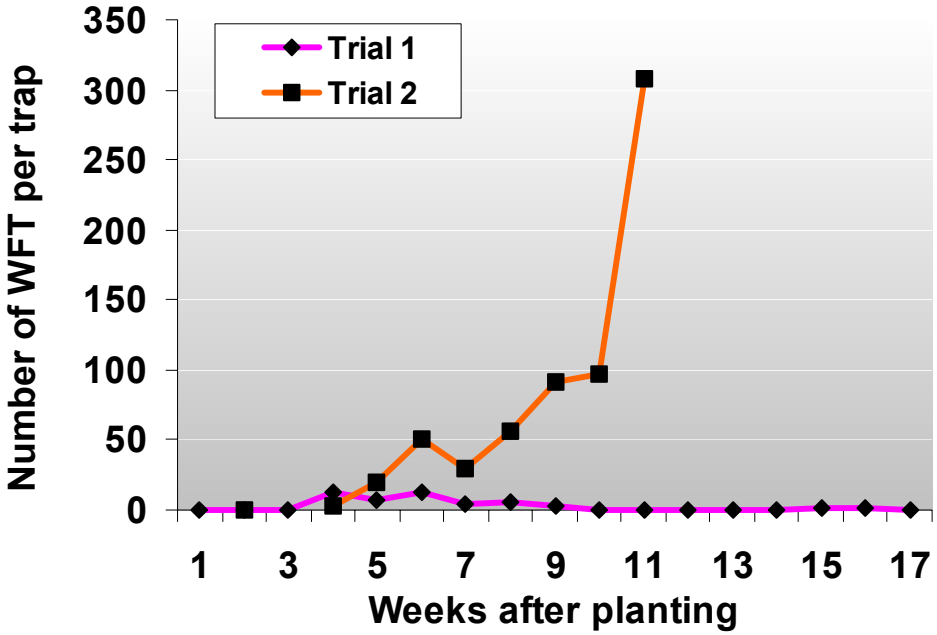


Fig. 30. Yellow sticky trap catches of WFT in two capsicum crops at NCGH. In Trial 1, *T. montdorensis* was introduced early in the crop to control the thrips, whereas no predators were introduced in Trial 2.

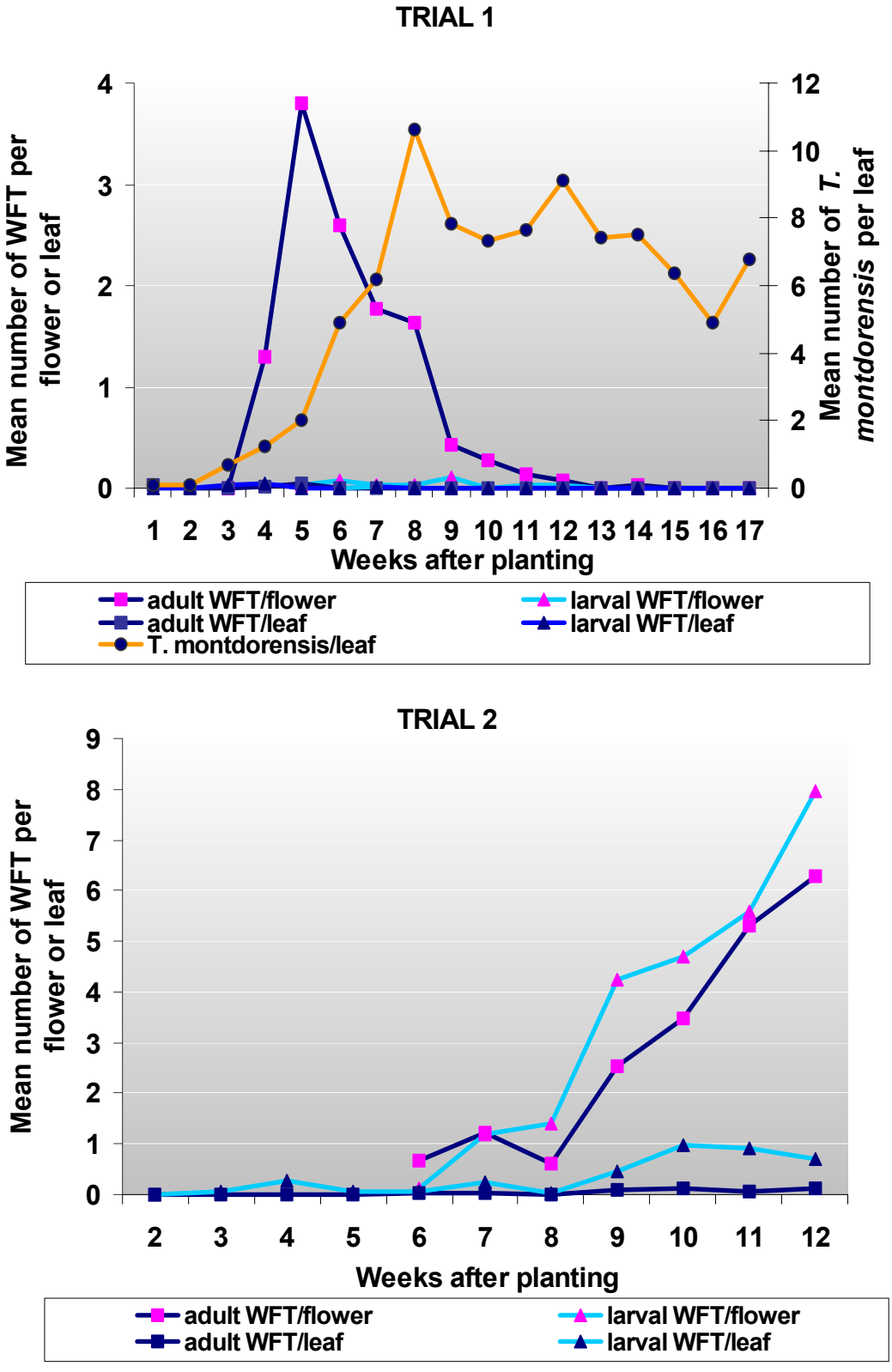


Fig. 31. Population density of WFT on leaves and in flowers of capsicum in two trials at NCGH. In Trial 1, *T. montdorensis* were released early in the crop, whereas no predators were released in Trial 2.

Trial 10. Management of tomato spotted wilt virus in greenhouse capsicums using virus transfer inhibitors

Background

Tomato spotted wilt virus is a common disease of capsicums in both field and greenhouse capsicum. It is vectored by thrips species, particularly western flower thrips, *Frankliniella occidentalis.* Other known vectors in Australia are onion thrips, *Thrips tabaci*, and tomato thrips, *Frankliniella schultzei*. Vector thrips pick up the virus by feeding on infected plants as larvae. The virus then replicates within the thrips larva and is transmitted to new plants by the more mobile adult stage. Thrips larvae can acquire the virus in only 15min of feeding. The tolerance for vector thrips is therefore very low and growers typically spray routinely against them. Many common weed species, including mallow, sowthistle and chickweed, can be infected with TSWV, and also are hosts to vector thrips. Some weed species, and even crops such as cucumber, can be infected but do not show symptoms. It is therefore crucial to control weeds both in and around the greenhouse and be aware of the dangers in crop rotation or mixed plantings. Screening of vents is also important to restrict entry of thrips. Symptoms of TSWV may not show up for several weeks after the original infection, so while it is important to remove infected plants as soon as symptoms are noted, it is likely that once one plant is noticed others will follow. However, young plants are much more prone to infection than older plants, so early protection is most critical.

Thrips can cause damage to capsicum in their own right. Feeding on fruit causes silvering, and is particularly prevalent where fruit is in contact with leaves or other fruit. While the tolerance for thrips increases if no TSWV is present, thrips populations should not be allowed to multiply unchecked or fruit will be downgraded.

There are few chemicals that are effective against western flower thrips, and few of these are registered in capsicum, Biological control agents are widely used overseas. These include the predatory mites *Neoseiulus cucumeris* and *Stratiolaelaps miles*, and various pirate bugs, *Orius* spp. The native predatory mite *Typhlodromips montdorensi*s is being evaluated at NCGH, and can build up good populations on capsicum. If this can be combined with a treatment that will inhibit or prevent transfer of TSWV in the early crop, then biocontrol becomes a viable option to reduce heavy use of chemicals. A 1993 Canadian study reported that Sunspray® petroleum oil and Wilt-Pruf® (di-1-p-menthene), an anti-transpirant, reduced transmission of TSWV by 57% and 73% respectively in Petunia. Wilt-Pruf® and products with similar active ingredients were not available in Australia, so we substituted two anti-transpirants readily available, of different chemical composition.

Materials and methods

Capsicums cv Spirit were planted out into cocopeat bags on 3 August 2001 in two 50sqm polyhouses (Houses 3 and 5). There were five rows in each house. There were 14 plants in the outer rows (treated as buffer rows) and 28 in each inner row. Each of the inner rows was treated as a block and each treatment was replicated randomly once in each block, with four plants per treatment. Two plants at the end of each row were left as buffers. Three replicates were in one house and three in the other.

The six treatments were as follows:
T1=Check
T2=Sunspray® Ultrafine oil (petroleum oil, Sunoco) at 1%
T3=Eco-oil® (vegetable oils, Organic Crop Protectants) at 1%
T4=Envy® at 50mL/L (carboxylated hydrophilic polymer, Agro Best Australia Pty Ltd)
T5=Antistress 2000® at 50mL/L (44% acrylic polymers, Friendly-Ag Products Pty Ltd))
T6=Resistant cultivar.

The crop was pruned to two leaders in late September. The soil-inhabiting predatory mite *Stratiolaelaps scimitus* was released once at 20/plant on the bags for fungus gnat control on 5 October, and *Encarsia formosa* for whitefly control weekly at 1/sqm from 15 October. Yellow sticky traps (Seabright®) (2/house initially then 4/house from 30 November) were hung at crop canopy level and changed weekly. Thrips and other pests were recorded. Western flower thrips were released weekly once flowers were present. Mancozeb Plus® was applied 15 November to control *Typhlodromips montdorensis* to allow thrips to establish. *Phytoseiulus persimilis* was released 16 November to control two-spotted mite.

The first spray application was made on 21 November 2001, with subsequent sprays applied weekly until 9 January 2002 (8 applications). Antistress® was not applied after 12 December because of delays in delivery.

TSWV was applied by physical inoculation on three occasions. The first was obtained from infected *Lisianthus*, and was applied to one upper leaf of three plants in each side row on 23 November. The second was from infected capsicum leaves and was applied to the same plants and to one buffer plant at the end of treatment rows (12 plants/house) on 29 November. In the absence of symptoms, a third application from infected *Amaranthus* was made 17 December on additional buffer plants. WFT were also reared on infected capsicums and released into the house periodically from 29 November. The delay in first application of TSWV and sprays was a result of the difficulties in obtaining infected thrips and infected plants, as the thrips must complete a life cycle to transmit and the plants may take weeks to show symptoms. Assessments of thrips populations was made weekly from 26 November, by counting adult and juveniles thrips in one flower and under the calyx of one ripe fruit in each plot. All plants were examined for symptoms of TSWV. A final assessment was made on 9 January 2002. At this time only one plant from the Envy® treatment in House 3 was showing symptoms of TSWV infection. Samples of leaves were bulked for each treatment in each house and tested with an Agdia® TSWV kit. Only the Envy® treatment in House 3 showed positive.

A count of set fruit on nodes 1-6 in each plot was made on 9 January 2002.

Results and discussion

Only one plant showed infection with WFT at the conclusion of the experiment. The lack of TSWV infection may have been a result of the age of the plants making them less susceptible to infection.

Most of the thrips caught on traps and on the plant were plague thrips, a non-vector of TSWV. WFT trapped were predominantly male throughout, despite the relatively high populations. WFT increased in population density towards the conclusion of the trial and plague thrips declined (Fig. 32).

Damage to fruit from thrips was evident throughout the experiment and was caused by all species of thrips, not just WFT. There were no obvious differences in thrips populations between treatments (Table 19). Populations in flowers tended to be more variable than on fruit, probably reflecting differences relating to age of flower. Overall, adult thrips were significantly more numerous in flowers than on fruit, whereas larvae were only slightly more numerous in flowers (Fig. 33).

It was noted that there appeared to be a reduction in fruit set with some treatments, confirmed in a count on 9 January 2002. The petroleum oil Sunspray Ultrafine® at 1% appeared to reduce fruit set significantly, with Eco-oil® also showing an effect Table 20). Both treatments left fruit feeling oily. Fruit set on the resistant cultivar was also reduced. The two anti-transpirants left a visible deposit on the fruit surface, which was unacceptable. They were also too expensive to apply regularly. None of the treatments therefore shows promise for use on capsicums, even if it had been shown to prevent TSWV transmission. The active ingredient in Wilt-Pruf®, di-1-p-menthene, is now available in Australia under a different name and is worthy of trial.

Stratiolaelaps, normally a soil dwelling mite, was found under the calyx of some fruit on each date, particularly 19 December, and *T. montdorensis* was also found in small numbers from 2 January. Both probably reduced thrips larval populations to some extent.

Table 19. Mean number of thrips (all species) on a ripe capsicum fruit or in flowers from 29 November 2001to 9 January 2002 under various weekly treatments (n=36).

Treatment	Thrips on fruit		Thrips in flowers	
	adults	larvae	adults	larvae
Check	1.9	5.7	8.2	8.1
Sunspray Ultrafine® oil	1.4	4.7	11.3	6.6
Eco-oil®	2.2	8	8.5	8.5
Envy®	2.3	5.8	8	5.9
Resistant cultivar	3.3	7.6	9.3	9
Means	2.4	6.6	8.9	7.5

Table 20. Number of set capsicum fruit on nodes 1-6, 9 January 2002.

Treatment	Mean fruit per stem (range)
Check	4.71 (3.2-5.5)
Sunspray Ultrafine® oil	2.06 (1.63-2.75)
Eco-oil®	3.33 (3-3.63)
Envy®	4.53 (3.63-5)
Antistress®	4.31 (3.75-5.38)
Resistant cultivar	2.91 (2.25-4.14)

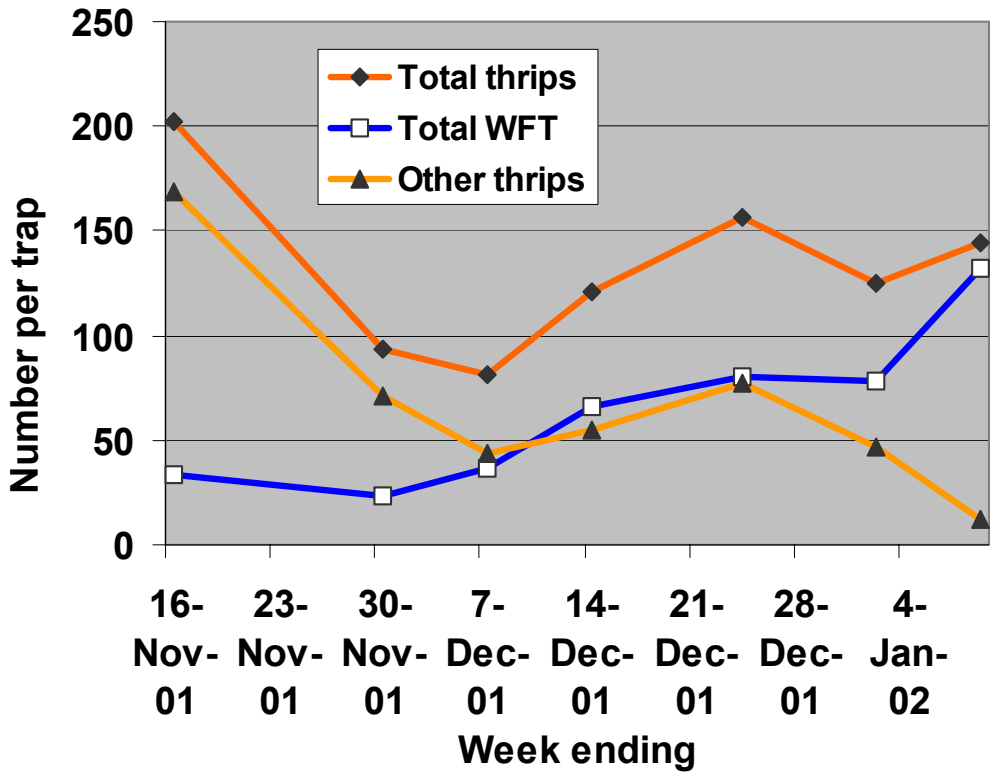


Fig. 32. Mean number of WFT and other thrips (mostly plague thrips) on yellow sticky traps in a capsicum crop in two greenhouses at NCGH.

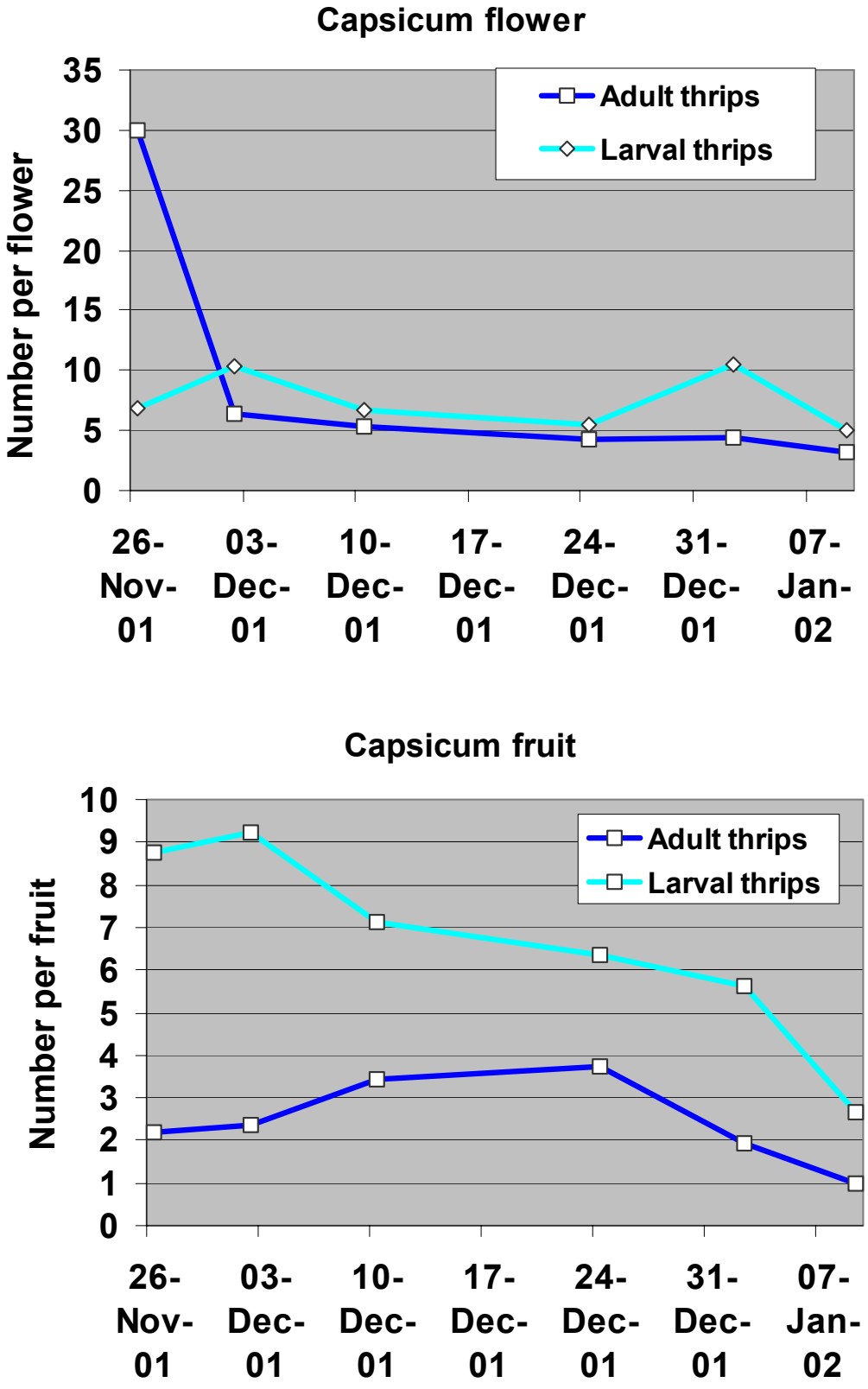


Fig. 33. Number of adult and larval thrips on fruit and in flowers of greenhouse capsicum, NCGH, November 2001-January 2002.

Trial 11. Control of aphids on greenhouse capsicums
Trial 11a Cotton aphid and cowpea aphid

Background

Capsicums are prone to attack by several aphid species, primarily green peach aphid, *Myzus persicae*, cotton aphid, *Aphis gossypii*, and occasionally cowpea aphid, *Aphis craccivora* (Fig. 34). Pirimor® (pirimicarb) is generally applied for control, either as a foliar spray or as a root drench. For a crop grown under minimal pesticide use, particularly where biocontrol agents are used, alternative 'soft' pesticides are needed that can be recommended to growers.

The opportunity to evaluate some reduced risk pesticides was presented when cotton aphid became established in a 50 sq.m. capsicum crop (House 5) at the end of another experiment. The crop was planted out 3 August 2001, and the aphids were first noted 20 December, at which time 10 brown lacewing (*Micromus tasmaniae*) adults (Fig. 35) were released. By 2 January 2002 the aphids had spread throughout the house and lacewings were still rarely seen. On 18 January low numbers of cowpea aphid were noted in another capsicum planting in House 3. A small black and red native lady beetle, *Diomus notescens* (Fig. 35), was noted in both Houses.

Fig. 34. Cotton aphid (left) and cowpea aphid (right) on greenhouse capsicum. Note bicolour legs in cowpea aphid.

Fig. 35. Brown lacewing (left) and *Diomus* (right), predators of aphids.

Materials and methods

The first trial was set out in House 5 on 24 January 2002 by counting aphids on previously flagged leaves. A pre-treatment and one-day post treatment count were completed but aphid populations dropped substantially on check leaves as well as treated leaves on Day 1, so the experiment was repeated. It was suspected that Agral applied as a wetting agent had caused mortality. The Agral was omitted from other treatments in the subsequent trial but was included as a treatment in its own right. Additionally, the same treatments were applied in House 3, against cowpea aphid.

Individual leaves within 0.5m of the top of the plant were checked to ensure a substantial aphid population, flagged, and randomly assigned to a treatment. In House 5 there were four replicates per treatment, and in House 3, with far fewer plants infested, there were three. Aphid numbers were reduced to <300/leaf where necessary to make counting manageable. Treatments were applied 30 January 02 with a hand sprayer to wet undersides of flagged leaves. Counts were carried out one, three and seven days post-treatment. Treatments were as follows, with no wetter added.

T1=Check (water)
T2=Azamax® at 3mL/L
T3=Eco-oil® at 0.5%
T4=Chess® at 0.2g/L
T5=Natrasoap® at 1%
T6=Pirimor® at 0.5g/L
T7=Agral® at 2mL/15L

Results and discussion

The delay in treatment coincided with a sudden increase in predator populations that had a serious impact on aphid counts, particularly three and seven days post-treatment in House 5. In House 3, the main impact of the predators was on Day 7 in Check, Azamax® and Eco-oil® treatments. The main predator in House 5 was brown lacewing and in House 3 was *Diomus notescens*. The results are presented in Fig. 36, but counts after Day 1, particularly in House 5, should be regarded as suspect because of the influence of the predators.

The most effective treatment for both aphid species was Pirimor®. Azamax® and Eco-oil® were effective against cowpea aphid, but less so against cotton aphid. Aphid numbers appeared to increase by the third day, particularly for Eco-Oil®. Natrasoap® was only moderately effective, though results from the aborted first trial suggest that addition of Agral® may markedly improve control. Chess® was more effective against cowpea aphid than cotton aphid. It is known as a slow acting material. Here it needed seven days for the aphids to be clearly dead, which is not a disadvantage if they are no longer feeding. Agral® did not appear to have any impact against aphids as a stand-alone treatment, but should be re-evaluated in combination with the pesticides tested. 'Safe' pesticides also need re-testing for safety to natural enemies when Agral® is added.

Short residual products such as Azamax®, Eco-oil® and Natrasoap® have an important place in IPM programs as products that can be used to knock back aphid

populations but which have minimal effect on natural enemies. Chess® (pymetrozine) is known to be safe for most natural enemies but to have a deleterious effect on aphid parasitoids and green lacewings for a week after application.

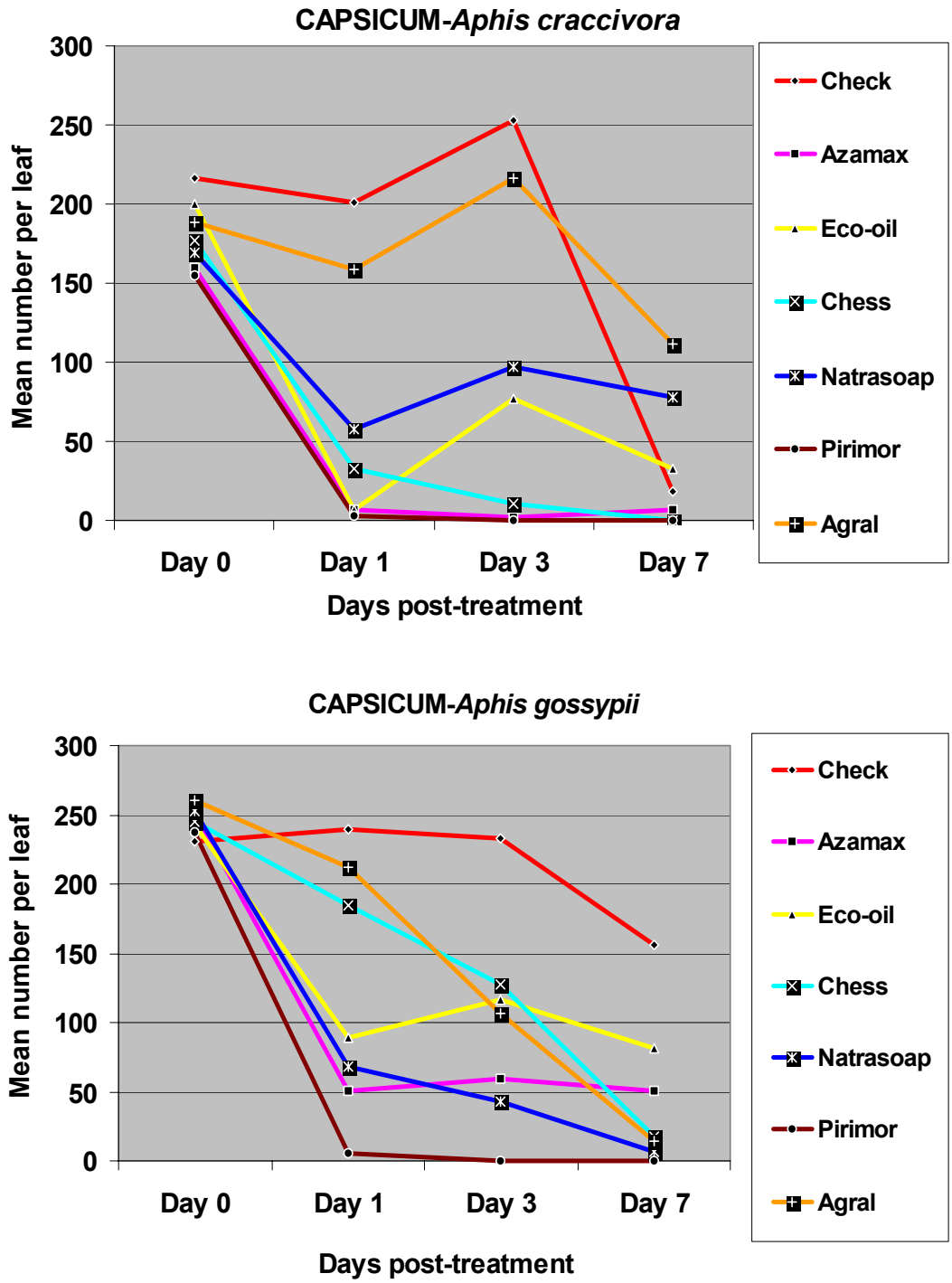


Figure 36. Mean leaf populations of cowpea aphid (upper) and cotton aphid (lower) pre-treatment and one, three and seven days post-treatment. Populations after Day 1 were impacted by brown lacewing, *Micromus tasmaniae*, in House 5 (cotton aphid) and *Diomus notescens* in House 3 (cowpea aphid).

Trial 11b Green peach aphid and potato aphid.

Background

A 50 sq.m. capsicum crop grown in propagation House 5, NCGH for a tomato spotted wilt virus trial became infested with two aphid species late in the trial. The two species were green peach aphid, *Myzus persicae*, which commonly attacks capsicum, and potato aphid, *Macrosiphum euphorbiae*, a large green aphid (Fig. 37). The opportunity arose to evaluate the same pesticides that had been tried in an earlier crop against cotton aphid, *Aphis gossypii*, and cowpea aphid, *Aphis craccivora*. The crop (cv Zirconia) had been planted out in mid March 2002, with three central rows of 28 plants each, and two side rows of 14 plants. Potato aphid was first noted in the third week of June in one row, but had spread to other rows at the time of the trial. Green peach aphid was in the majority in the rows assessed. Potato aphid was excitable as a wingless adult and dropped off leaves when they were disturbed, making accurate assessment difficult without spraying large areas. The target pest was therefore green peach aphid, though variable numbers of immature potato aphids were also present. Two consecutive trials were carried out. In the first trial, no wetting agent was applied, whereas Agral® was added to all treatments in the second trial to see if control could be improved.

Materials and methods

The first trial was set out on 24 July 2002 and the second on 30 July. One leaf within 0.5m of the top of a plant was checked to ensure a substantial aphid population, flagged, and randomly assigned to a treatment. The rows were divided into six blocks with each treatment replicated within each block. Adult potato aphids were removed and the remaining aphids on the leaf underside counted and recorded. Treatments were applied with a hand sprayer immediately after counting, to wet undersides of flagged leaves. Counts were carried out one, three and six days post-treatment, on the same leaves. The second trial was set out and assessed in the same way, using different leaves. In this case Agral® was added to all treatments, including the control, at 2mL/15L. Adult potato aphid numbers were recorded separately, as they tended to be migratory and moved onto treated leaves. The treatments were as follows (product rates).

T1=Check (water)
T2=Azamax® (azidirachtin) at 3mL/L
T3=Eco-oil® (vegetable oils) at 0.5%
T4=Chess® (pymetrozine) at 0.2g/L
T5=Natrasoap® (potassium salts of fatty acids) at 1%
T6=Pirimor® (pirimicarb) at 0.5g/L
T7=Mospilan® (acetamiprid) at 0.5mL/L

Results and discussion

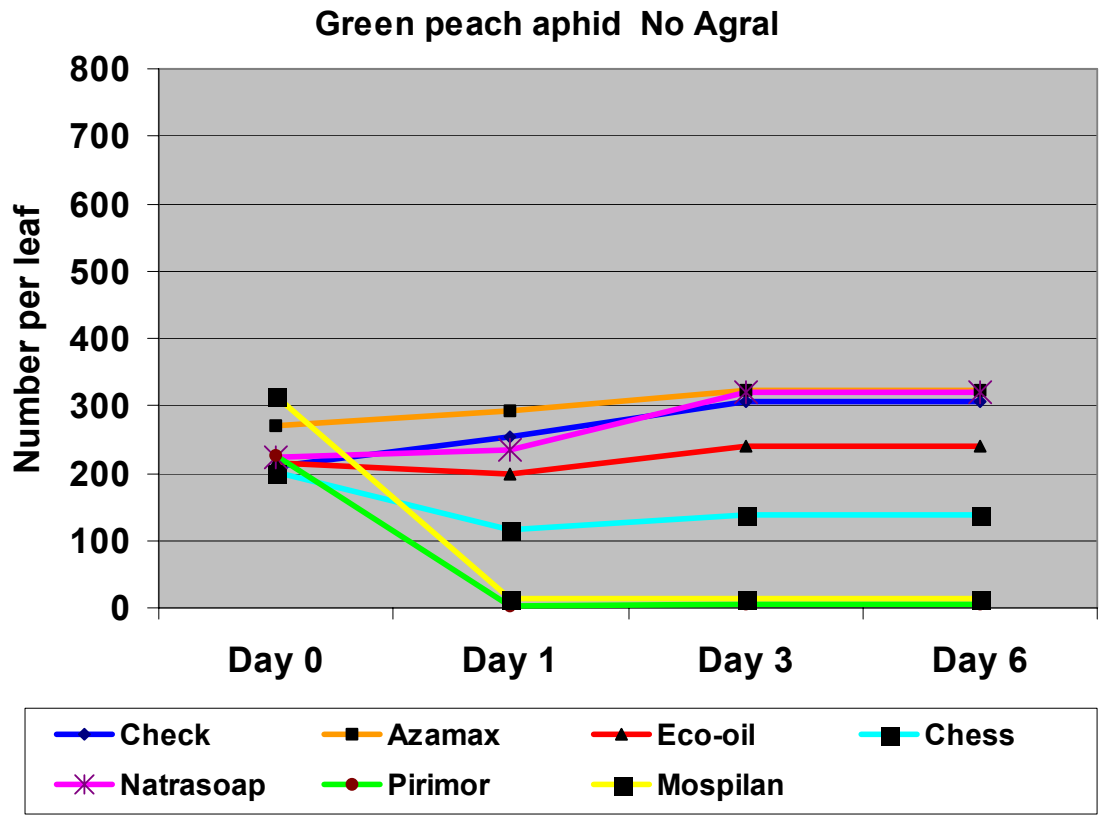
The most effective treatment for green peach aphid was the old standard, Pirimor®. Mospilan® was equally effective. Both gave rapid control with good residual control for at least 6 days, with or without Agral® (Fig. 38). Chess® is known to provide slow control of aphids. Without Agral® it gave poor control of green peach aphid

over six days, but moderate control when Agral® was added. Azamax®, Eco-oil® and Natrasoap without Agral® had very little effect, even on the first day after treatment. Addition of Agral® improved the efficacy of Azamax® slightly on the first day, but not that of Natrasoap® and Eco-Oil®. If there was a repellent effect for Azamax®, it was not clear and was not persistent for green peach aphid.

Adult potato aphids were apparently not affected by Eco-Oil®, Azamax®, Chess® or Natrasoap® either with or without Agral® (Fig. 39). Pirimor® and Mospilan® provided good control in both trials. Because adults are migratory they may have continued to arrive after treatment and confounded results over the longer period.

Acetamiprid (Mospilan®) is reported to be toxic to *Orius* and *Encarsia*, but safe to *Phytoseiulus*. It is moderately persistent. Pirimicarb (Pirimor®) is toxic to *Encarsia, Trichogramma* and predatory bugs and slightly to moderately harmful to predatory mites. Persistence is short. Pymetrozine (Chess®) is harmful to *Aphidius* but short-lived and relatively safe to other biocontrol agents. All are systemic so could be applied as a soil treatment, though there may be toxicity to *Stratiolaelaps.*

Fig. 37. Green peach aphid (left) and potato aphid (right) on capsicum



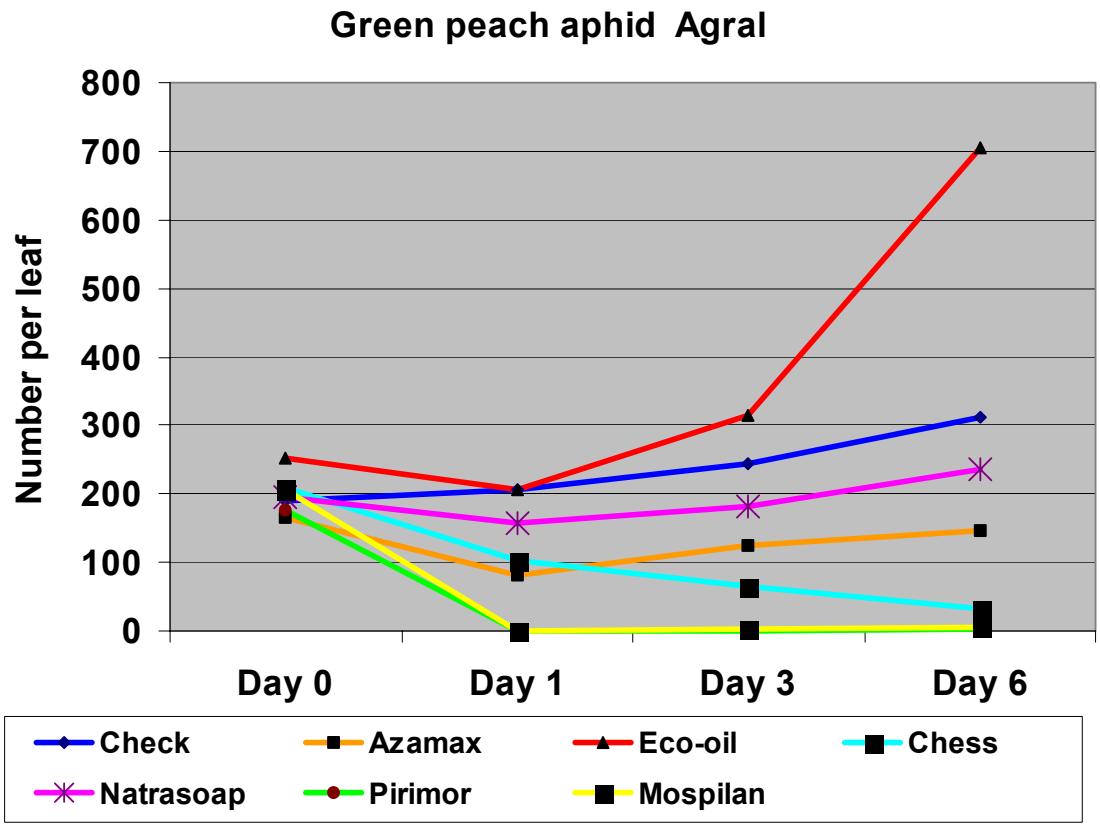
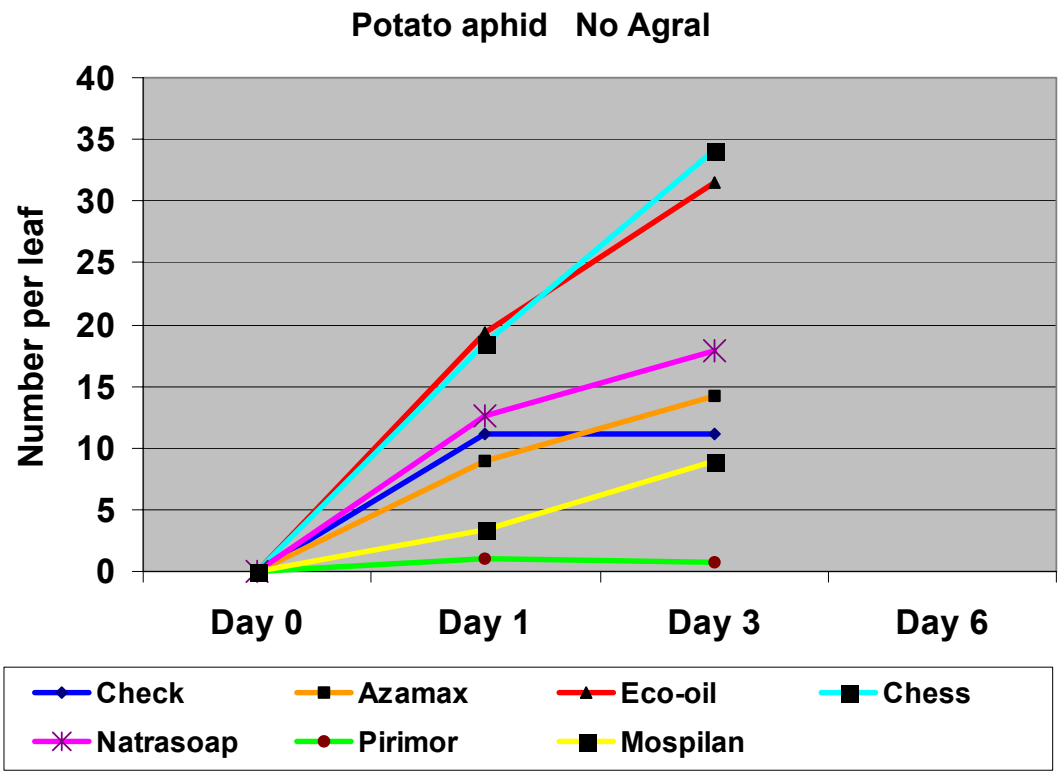


Fig. 38. Green peach aphid populations on leaves of capsicum pre-treatment, and one, three and six days post-treatment. Agral® was added to treatments in one trial only.



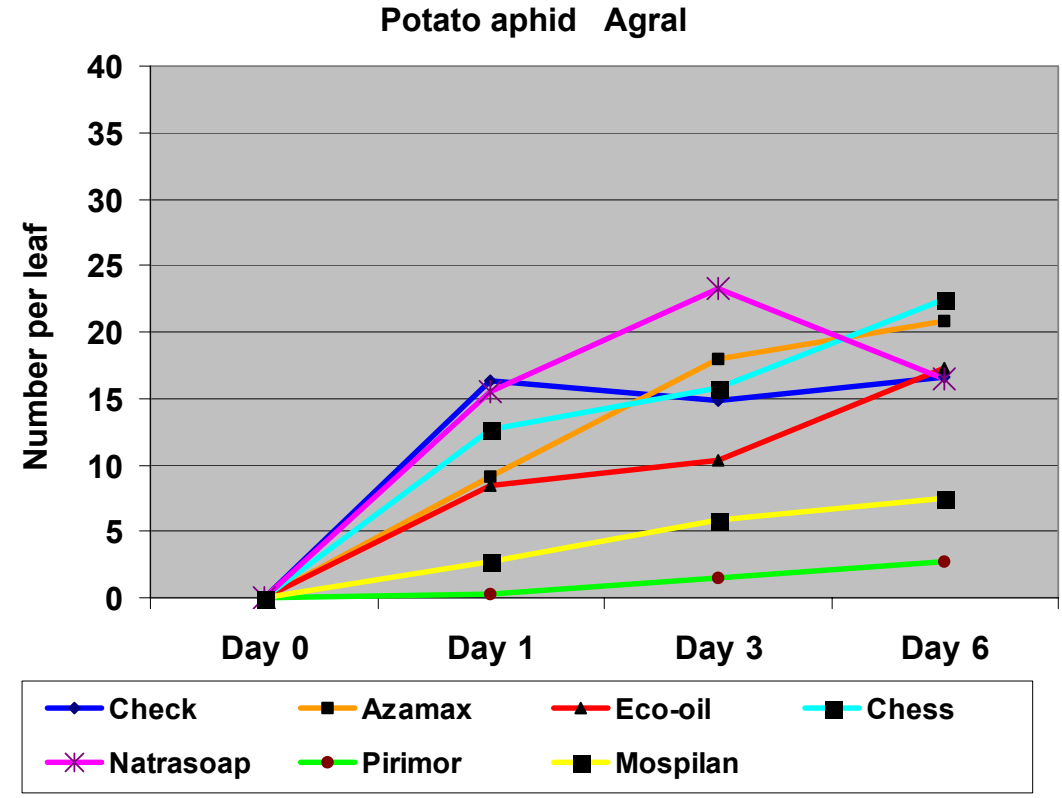


Fig. 39. Potato aphid populations on leaves of capsicum pre-treatment, and one, three and six days post-treatment. Agral® was added to treatments in one trial only. Potato aphid numbers were not recorded on Day 6 in one trial.

Summary of IPM research in capsicum crops at the National Centre for Greenhouse Horticulture 2000-2003

Trials were conducted in three successive capsicum crops at NCGH in 2001-2002, primarily to find non-chemical alternatives for pest management, in particular western flower thrips. WFT is a major vector of TSWV. There are few effective chemical treatments, and these may sometimes irritate thrips and cause increased spread of virus. A primary aim was therefore to manage WFT to a level where TSWV spread is also minimised, and a multi-pronged approach was adopted.

The use of protectant crop films that potentially inhibit virus transfer was evaluated but none of those tested (oils and acrylic polymers) was suitable. This does not mean that other materials may not be suitable, so this line of research should be pursued.

The recently developed thrips predator *Typhlodromips montdorensis* was evaluated and found to be highly effective in keeping thrips at levels low enough to protect fruit from damage. The introduction rate was three releases at 10/sqm, a relatively low rate. Despite a source of TSWV in the greenhouse (25% of plants with TSWV), virus spread to the new crop was greatly reduced (28% infected) compared with a predator-free crop (100% infected). When used with good crop husbandry practices, weed management, screened houses and environmental controls, these predators offer a viable alternative to pesticides in managing both thrips and virus. Resistant cultivars would also be of benefit and in conjunction with predators might prolong the life of these cultivars by taking the virus pressure off them. Crop yield needs to be comparable or better than existing cultivars.

Capsicums are very susceptible to attack by aphids. At least five species are known to attack them, and four of these showed up in the first two crops (cotton aphid, cowpea aphid, green peach aphid and potato aphid). The missing aphid is foxglove aphid, *Aulacorthum solani*. The opportunity was taken to evaluate several reduced–risk pesticides. None was better than the standard, Pirimor®, but resistance to the latter product is common overseas and alternatives are needed. Chess® and Mospilan® were the two most effective alternative products but they are not safe with all biocontrol agents and may not be equally effective against all species of aphids. In one trial, the braconid wasp *Aphidius colemani* successfully controlled green peach aphid, but not before numbers had built up to a level where honeydew was evident. A hyperparasite has the potential to interfere with control. Brown lacewing and *Diomus* established very well in two crops where they entered of their own accord, but not did not establish well in two later winter crops where they were introduced. This may be a seasonal issue that needs to be explored further.

Whitefly, two-spotted mite and fungus gnats were successfully controlled with releases of *Encarsia formosa*, a natural invasion of *P. persimilis,* and a single release of *Stratiolaelaps* respectively. The elements of a biocontrol program for capsicum are therefore already in place except for aphid control, where compatible chemicals can be employed as spot treatments within the IPM program so long as action is prompt.

Section D Non crop-specific IPM developments

Background

A range of experiments and trials relevant to all greenhouse crops are reported on in this section.

In this section the following trials were conducted:

Biocontrol
- Trial 12. Identification and development of new biocontrol agents for protected cropping
- Trial 13. Evaluation of the side effects of pesticides against the whitefly biocontrol agent *Encarsia formosa*
- Trial 14. Evaluation of the toxicity of Eco-Oil and Ecocarb to the phytoseiid predatory mite *Phytoseiulus persimilis*, a biocontrol agent of two-spotted mite
- Trial 15. DNA identification of some phytoseiid predatory mites to determine conspecific relationships

Biorationals
- Trial 16. Evaluation of the effectiveness of beneficial fungal pathogens against western flower thrips, greenhouse whitefly and green peach aphid
- Trial 17. Evaluation of a neem insecticide against western flower thrips, two-spotted mite, greenhouse whitefly, green peach aphid and tomato russet mite
- Trial 18. Evaluation of a neem insecticide as a soil drench against fungus gnats
- Trial 19. Evaluation of insect growth regulator insecticides against caterpillars.

Trial 12. Identification and development of new biocontrol agents for protected cropping

Background

There are currently seven producers of biocontrol agents in Australia (Table 21). The range of biocontrol agents is narrow compared with North America and Europe, where >35 are available. Development of native beneficials is considered a more viable option than attempting to introduce non-indigenous species from overseas with uncertain risk to native flora and fauna. In some cases, those BCAs used overseas are present here but no insectary has taken on board the mass rearing. This may be because the rearing method is a closely guarded secret, or that money required for research and development is not considered a high priority until there is market demand.

Table 21. Commercial producers of biocontrol agents in Australia

Producer	Address	Biocontrol agent(s) produced
Bugs for Bugs	Bowen Street, Mundubbera , QLD 4626	*Aphytis lingnanensis, Chilocorus circumdatus* and *C. baileyi, Cryptolaemus montrouzieri, Leptomastix dactylopii, Mallada signatus, Trichogramma pretiosum* and *T. carverae*, *Eusieus victoriensis*
Horticultural Crop Monitoring	PO Box 3025, Caloundra DC, QLD 4551	*Phytoseiulus persimilis, Neoseiulus wearnei*
Beneficial Bug Company	PO Box 436, Richmond NSW 2753	*Phytoseiulus persimilis*
Ecogrow	Unit 12, 5-11 Hollywood Avenue, Bondi Junction NSW 2022	*Heterorhabditis bacteriophora, H. zealandica, Steinernema carpocapsae, S. feltiae*
Bio-Protection Pty Ltd	140 McDougals Road, PO Box 384, Kilmore, VIC 3764	*Phytoseiulus persimilis*
Biological Services	PO Box 501, LOXTON, SA 5333	*Aphytis melinus, Encarsia formosa, Stratiolaelaps scimitus, Typhlodromus occidentalis, Typhlodromips montdorensis, Euseius victoriensis*
Bioworks	PO Box 203, Nambucca Heads, NSW 2448	*Phytoseiulus persimilis*

Interest in biocontrol of greenhouse pests in Australia has increased markedly in the last five years. The industry itself is also becoming much more sophisticated and experiencing a rapid growth phase. The NCGH has taken on the role of undertaking some of this basic research and development to be able to provide insectaries with a near market system for critical agents that they can exploit rapidly when there is market demand. Gaps identified are primarily for control of thrips, aphids, and tomato russet mite. Grower experience is also that *Encarsia* and *Phytoseiulus* are not effective in winter and early spring. We have concentrated our search on finding new biocontrol agents for pests where there are gaps and at looking for agents more effective under cool or extreme conditions. These are discussed below in more detail.

Thrips. The biocontrol agent that has been worked on most intensively is the predatory mite *Typhlodromips montdorensis*, primarily for thrips control, but also for tomato russet mite and broad mite. A commercial scale rearing system has been developed, though commercial agreements preclude detailed description. These predators have been in commercial use for three or four years now, and while reports have been mixed, notable success has been achieved in cucumber, herb and gerbera crops. In the last two years, 5 million predators have been shipped to over 35 growers. Uptake by commercial insectaries has been slower than was hoped, but refinements to rearing and harvesting systems in the last part of the project have resulted in considerable improvements in predictability of supply and time management. Until recently, demand has outstripped supply, which is only healthy if a rapid response is made to fill the void.

Tomato russet mite. This pest is quite common in summer months in NSW, SA and VIC on tomatoes. We have identified the weed black nightshade, *Solanum nigrum*, as a major carrier of russet mite in the wild, and in field surveys have concentrated on finding predatory mites associated with it. We have made three surveys during the project. The first was in June 2001, to Narrabri in north central NSW. As well as predators for tomato russet mite, we were also looking for other *Typhlodromips* species on *Echium* (Paterson's curse) that Dr Jenny Beard had described as being associated with thrips. We did not find *Typhlodromips* but we did find one phytoseiid in large numbers clearly feeding on tomato russet mite. This species was *Euseius victoriensis,* a native phytoseiid common in inland areas on citrus and grapevines. James Altmann with Biological Services has been rearing it for two years as it is a good predator of small mites and he believes it has some potential for broad mite in ornamentals. It has high tolerance for hot dry conditions (the eggs hatch at about 25% RH). There were also midge larvae (*Feltiella* II) present with TRM in Narrabri. The species was included in feeding trials described elsewhere in this report, and a rearing method was developed for it that is described below.

The second and third surveys were conducted mid-late October 2001. The first area surveyed was north from Gosford as far as Coffs Harbour, and back inland through the Hunter Valley; the second was SW down to Albury, on the Victorian border, and back inland through Bathurst. Very few black nightshade plants were found and none of the predatory mites collected from a range of crops was of interest. The main points of interest were that the entomopathogenic fungus *Entomophthora* is very prevalent at that time of year in the thrips population, and that brown lacewing, *Micromus tasmaniae*, was everywhere. *Phytoseius* spp. were fairly common where there were thrips but rearing attempts have not been successful.

A request for black nightshade samples was made to colleagues in SA, and both Narrabri and Yanco, but little was received. The drought precluded other surveys.

Aphids. The parasitoid *Aphidius colemani* was introduced into Australia several years ago and is well established in NSW and elsewhere. This species is used extensively for control of several aphid species overseas. Small quantities have been reared at NCGH to provide material for trials in the NCGH greenhouses, and have been provided for a few growers, with generally good results. Two different hosts are used; green peach aphid is reared on capsicum and cotton aphid on cucumber. Rearing is carried out in screened cages and only on a small scale at this stage. There are several other species of *Aphidius* in Australia that we have attempted to have identified, however the expert on the group in Canberra (Dr Mary Carver) is retired.

We have had a major interest in the brown lacewing, *Micromus tasmaniae*. There is a fairly extensive literature base on it and a recent book on lacewings provides general information. It is very widespread here and occurs in a variety of habitats at all times of the year, so it presumably does not diapause. It also establishes breeding populations in greenhouses, which is of particular interest. Both adults and larvae feed on aphids. A recent trial with biorational chemicals in capsicum against two aphid species (*Aphis craccivora* and *Aphis gossypii*) came to an early conclusion because of a population explosion of brown lacewing in one house and a small coccinellid beetle, *Diomus notescens*, in the other. Only 10 lacewings had been introduced, and no *Diomus*. Brown lacewings commonly enter greenhouses, even those apparently well sealed. We did some earlier work trying various diets for brown lacewing but found they accepted only aphids. They commonly lay eggs near spider mite colonies and are reported to eat Heliothis in cotton, so further research may prove beneficial.

Diomus notescens is a small native ladybeetle. It is quite common in NSW and QLD in lucerne and other crops, but no information has been published on its lifecycle or feeding preferences. We established a colony for a short time but were not able to rear large numbers with the facilities available. They do not clean off leaves as well as brown lacewing but appear to be more catholic in their tastes so may lend themselves to mass rearing.

Spider mites. In work on thrips management in hydroponic strawberries in NSW, *P. persimilis* was less effective than a *Feltiella* species that appears early in the season and stays through hot weather. It is present before spider mites have built up large populations. The taxonomic expert on the group, Dr Ray Gagne, identified it as *F. pinae*, a North American species, but he did not have the benefit of larvae and we have ruled this out. It appears to be an undescribed species. It pupates off-leaf so needs rearing differently than *F. acarisuga*. A rearing method was successfully developed and limited greenhouse trials conducted. An ARC grant application was submitted to enable the life-cycle and taxonomy to be studied, but was not successful. The rearing method is described below.

Another phytoseiid predatory mite, *Neoseiulus wearnei*, is commonly found in the field and in strawberries. It appears to like hot, dry conditions. It is very close to *N.*

fallacis, an introduced species. Small scale rearing was attempted but it did not build up large numbers.

Whitefly. We have not found any promising predators for whitefly here. There is a native *Eretmocerus*, *E. warrae*, inland in NSW that Dr. Paul DeBarro (CSIRO) feels may have potential as a parasitoid of greenhouse whitefly. We hoped to collect this from Narrabri, but again, the drought precluded collections. It is not uncommon in greenhouses that are unsprayed.

The only predator of whitefly that we have encountered is a plant bug, *Nesidiocorus viridis.* This is fairly widespread and has been found in our whitefly cultures, where it did not appear to have a substantial impact on populations. It has been reported elsewhere as a potential plant pest.

Rearing methods developed for mass production of natural enemies

A description of the rearing procedures developed for the *Feltiella* species is given, with comments on rearing of *Aphidius* and *T. montdorensis*. Both *Feltiella* species were collected in NSW but probably occur elsewhere. They are undescribed. *Feltiella* I attacks spider mites, whereas *Feltiella* II is associated with tomato russet mite on black nightshade. Because the adult is winged, it can potentially find target hosts faster than a predatory mite. Overseas, *Feltiella acarisuga* is sold for supplementary two-spotted mite control in crops such as cucumbers and roses. It pupates on the plant so is difficult to extract. The Australian species was found to pupate on the ground so rearing on a commercial scale is potentially easier.

Feltiella I. This species was reared on a two-week cycle at 25^{o}C in two small greenhouses. Lima beans were grown in soil media in four planter boxes and used after the first leaves were fully expanded. Any new growth was pinched out to keep them compact. The boxes were placed in a screened cage on Monday. The foliage of green beans heavily infested with spider mites was cut and placed on the Lima bean foliage. They were left there for a day to dry out and the mites transferred to the Lima beans. Green beans have hairs that trap midge larvae so cannot be used in rearing them directly. The following day, the dried green bean foliage was removed and the Lima bean leaves sprayed with a 15% sugar solution as food for adult midges. One to three day-old adult midges (~2400) were released into the cages and the screen closed. On the following Monday, the boxes were removed and taken to an adjoining greenhouse where they were placed over a V-shaped water collection unit. A pump continuously recycled water down the sides of the shute, which was sloped to one end. The water exiting the shute was directed through a large funnel into two sieves, one course and one fine. Midge larvae when mature throw themselves off the foliage at night. They were washed down the shute into the fine sieve. They were collected daily and further processed into small vials, where they pupated on fine damp sand, emerging a week later to start the cycle again.

Feltiella II. This species was reared in a similar way but tomatoes were used as host for tomato russet mite. The stock culture was maintained on large plants in a separate greenhouse and used to infest small tomato plants in planter boxes. The same water shute system as for *Feltiella* I was used to collect the larvae. These larvae did not all drop at night but otherwise they behaved in a similar manner. The life cycle was

approximately a day longer than for *Feltiella* I, which was not a problem as there was some flexibility in the collection period, and the temperature could be adjusted to shorten the cycle if necessary.

Rearing of *Feltiella* I was relatively simple once the logistics were established, and produced >10,000/week from four planter boxes. Ants and algal growth in the shute were the main problems, and occasionally invasion of *Phytoseiulus persimilis* and *T. montdorensis. Feltiella* II was more difficult to rear, primarily because of difficulties in getting an even infestation of tomato russet mite (and keeping *T. montdorensis* out of the stock tomato russet mite cultures).

While pupation off-leaf is very useful for insectaries, it creates a potential problem in modern hydroponic crops because plastic flooring presents an unfavourable environment for pupation. Greenhouses with dirt floors or crops such as gerberas with more debris at ground level have much more potential to use these *Feltiella*.

Aphidius colemani The greatest difficulty is in keeping *Aphidius* out of the aphid stock cultures, and in finding host plants that do not collapse under high aphid pressure. A hyperparasite of *Aphidius, Alloxysta victrix*, has also decimated colonies at NCGH on two occasions. Scaling up of rearing is dependent on additional greenhouse space becoming available as plant raising, aphid rearing and parasitoid rearing must be carried out in secure greenhouses in separate areas.

Typhlodromips montdorensis production has been affected by lack of adequate production facilities, but is currently returning >10,000/sqm/week (6 week cycle) and has the potential to meet industry needs. Predators are shipped weekly in 2.2L buckets, each holding 10,000 mites in fine vermiculite, so there are no concerns about plant material carrying other pests or diseases, a particular concern in WA. Predators are sprinkled on all plants to distribute them evenly in the crop.

Trial 13. Evaluation of the side-effects of pesticides against the whitefly biocontrol agent *Encarsia formosa*

Industry collaborator: Biological Services, Loxton, SA

Background

Integrated pest management in greenhouse vegetable crops utilising biocontrol agents (BCAs) is a proven approach to managing key pests in these crops. Success though does not always rely solely on the BCAs. There can be occasions when remedial chemical treatment is required. It is important therefore to be able to provide growers with information on the risk of using some chemical treatments on BCAs. The whitefly parasitoid *Encarsia formosa* is an important BCA for greenhouse vegetable crops. The International Organisation for Biological Control (IOBC) has undertaken extensive side effects testing of chemicals against this organism. Oomen (1985) described three laboratory tests and a single field test for this purpose. These methods measured mortality effects on pupae and adults, and side-effects on the parasitic performance of wasps surviving an earlier residue test on adults. They were adopted by the IOBC/WPRS working group on testing the side-effects of pesticides on beneficial insects and mites and by the European and Mediterranean Plant Protection Organisation (EPPO) in a 'Sequential Decision-Making Scheme' (SDMS), that provides the basis for classification of harmful impact of pesticides for *E. formosa* (OEPP/EPPO 1989). These methods were used to produce the data published in tables of chemical compatibilities for this biocontrol agent, made available by overseas biocontrol companies, and included in Handy Guide 6 in the NSW Agriculture manual '*Integrated Pest Management in Greenhouse Vegetables: Information Guide'*.

However, this has not kept pace with the development of new chemicals, particularly biorationals. As part of this project the IOBC established methodology has formed the basis for testing a range of new chemicals against *E. formosa* at the National Centre for Greenhouse Horticulture, Gosford.

The methods used are:

1. laboratory test on wasp mortality using fresh chemical residue
2. laboratory parasitism test using the surviving wasps from the first test
3. laboratory/greenhouse residue test using residues applied to French bean leaves with adults, and
4. contact mortality test on parasitised whitefly pupae.

Details are provided on progress to date. Testing will continue beyond the timeframe of this project until all of the procedures have been used with each of the chemicals.

Materials and methods

A list of chemicals tested, product concentration and rate applied is given in Table 22. Each chemical test is replicated three times. The methods used to date are briefly described.

(a) Laboratory bioassay test to evaluate the residual toxicity of pesticides against adult *Encarsia formosa*

In the first test method, a modification of the IOBC test method was adopted. The IOBC test method utilises a Munger Cell constructed of re-useable brass ring and glass plates. We have developed a modified method using a disposable 55mm diameter x 18mm deep plastic Petri dish for greater convenience, without sacrificing accuracy. Fresh test units are used each time and chemical is applied to all internal plastic surfaces of the test unit by Potter Precision Spraying Tower to deliver a deposit of approximately 2mg/sq.cm. The test unit is shown in Figures 40 and 41. A modified micropipette tip with mesh over the narrow end is used, in which a small droplet of 1:1 honey:water has been inserted to provide nourishment to the wasps during the assessment period (Figure 42). A series of Petri dishes each with a different chemical treatment is connected by soft aquarium tubing to a Welch Wobble Plate vacuum pump WE 2510C-02 (230V, 50Hz, 292 mbar vacuum pressure), to enable a gentle air flow to be drawn through each test unit without air from one unit entering another test unit (Figure 43). Prior to reaching the pump, air containing pesticide vapour passes through a 500mL plastic aspiration jar to prevent particulate matter from entering the pump. Approximately 30 up to 24 hour old *Encarsia* wasps are introduced into each test unit at 0 and 4 days after treatment and residual mortality is recorded after 1 and 4 days, and 1 and 3 days, respectively. Each treatment is replicated three times. Where obvious anomalies occur in the results, testing will be repeated until three consistent data sets are produced.

(b) Laboratory-greenhouse bioassay test to evaluate the residual toxicity of pesticides applied to French bean leaves against adult *Encarsia formosa*

In the second test method reported on here, chemical is applied to a French bean plant by hand sprayer to incipient run-off and allowed to dry. Sprayed plants are held in a glasshouse. At weekly intervals a treated bean leaf is removed and set up as shown in Figure 44. Petri dish arenas for a batch of chemicals are held in a plastic tray covered with Gladwrap to prevent leaf tissue from drying out (Figure 45). Approximately 30 0-24hour old *Encarsia* wasps are introduced into each test unit at 0 days after treatment and mortality recorded after 1 day and 4 days.

Results and discussion

Data are presented in Tables 23 and 24. Testing with the above methods is incomplete and further work will be required to produce three acceptable data sets for each chemical. At the commencement of the testing program, the laboratory non-ionic surfactant, Triton X-100® was used. This was replaced with Agral Spray Activator Spreading and Wetting Agent®, a non-ionic surfactant commonly used as a wetting agent for pesticides in horticulture. Some chemicals were tested with and without Agral to identify any mortality effect that might have been caused by the wetter.

Table 22. List of chemicals tested for side effects against the whitefly biocontrol agent *Encarsia formosa*

Chemical	**Trade Name**	**Product Concentration**	**Rate tested per litre**
abamectin	Vertimec	18g/L	0.5mL
acetamiprid	Mospilan	200g.L	0.5mL
azadirachtin	Azamax	12mL/L	2mL
botanical oil	Eco-Oil	20mL/L	5mL
chlorothalonil	Bravo	720g/L	2g
dichlofluanid	Euparen	500g/kg	2g
emamectin	Proclaim	50g/kg	3g
endosulfan	Thiodan	350g/L	1.9mL
imidacloprid	Confidor	200g/L	0.25mL
myclobutanil	Systhane	400g/kg	0.12g
phosphonic acid	Fungi-fos	200g/L	0.16mL
pirimicarb	Pirimor	500g/kg	0.5g
potassium bicarbonate	Ecocarb	856g/L	2g
potassium phosphate	Agri-50	10g/kg	1.33mL
potassium salts	Natrasoap	oleate 216g/L, linoleate 196 g/L, palmitate 11g/L, stearate 7g/L	10mL
propargite	Omite	300g/kg	1g
pymetrozine	Chess	500g/kg	0.2g
pyrethrin	Pyrethrum	4g/L	12.5ml
pyrimethanil	Scala	400g/L	2mL
spinosad	Success	120g/L	0.42mL
triadimenol	Bayfidan	250g/L	0.4mL
triforine	Saprol	20g/L	1.3mL
wettable sulfur	Sulphur Spray	800g/kg	3g
zineb	Zineb	800g/kg	1.5g

The following preliminary conclusions are provided. On the basis of results obtained from the first test method (Table 23), the fungicides Bravo®, Ecocarb®, Saprol®, Systhane® and Zineb® could be classified as harmless. Wettable sulfur produced a variable response. Of the 13 insecticide/miticides, AzaMax®, Eco-Oil®, Natrasoap® and Chess® could be classified as harmless, however, Vertimec® would appear to be toxic for a minimum of 4 days after treatment, and possibly longer; Thiodan® toxic for a minimum of 7 days, although there was some variability experienced between 4 and 7 days; Confidor® toxic for a minimum of 7 days, Malathion® toxic for a minimum of 5 days, Omite® toxic for a minimum of 4 days, but possibly harmless soon after, Pirimor® toxic for a minimum of 4 days, Proclaim® toxic for a minimum of 7 days, Pyrethrum® toxic for a minimum of 4 days and Success® toxic for a minimum of 5 days.

In a comparison of these results with those obtained from the second test method where residues were allowed to degrade on plant surfaces in a glasshouse (Table 24), the fungicides Bravo®, Ecocarb®, Systhane® and Saprol® and the insecticide Eco-

Oil® were all confirmed as harmless. Of those insecticides classified as harmful in test method 1, Proclaim® was confirmed as harmful with high mortality recorded for a minimum of 5 weeks after treatment. The apparent rapid decline in the toxicity of Omite® after 4 days to be harmless at 7 days was reinforced in the leaf residue trial where residues were harmless at 7 days. While Thiodan® was recorded as toxic for a minimum of 7 days in the previous test method, this contrasted with results obtained from the leaf residue test, where it demonstrated harmlessness at 7 days.

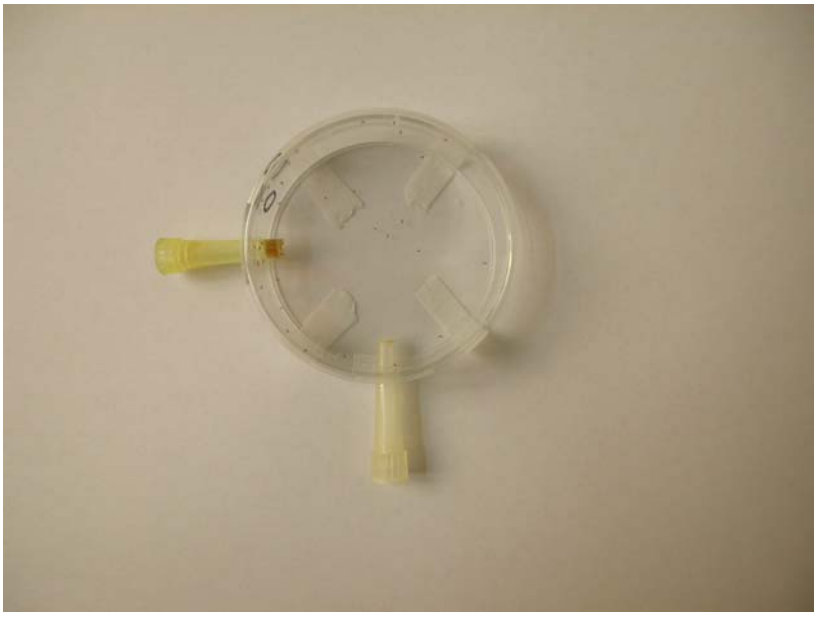


Fig. 40. Plastic Petri dish test arena with honeyed tip and ventilation tip inserted

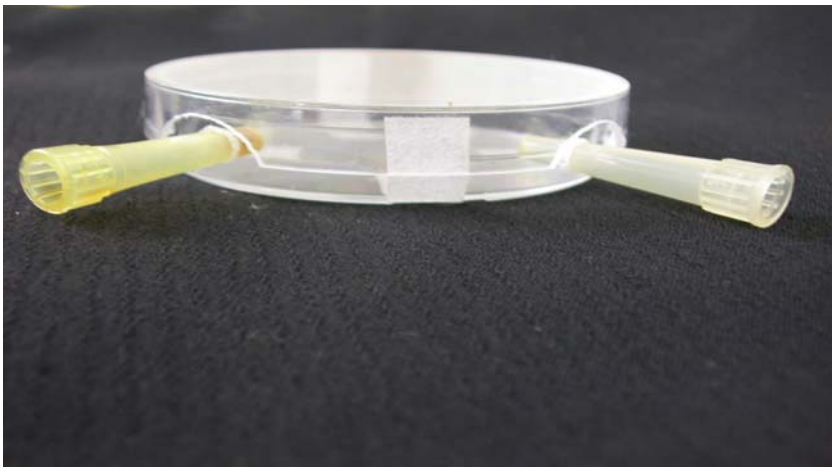


Fig. 41. Side elevation of Petri dish test arena.

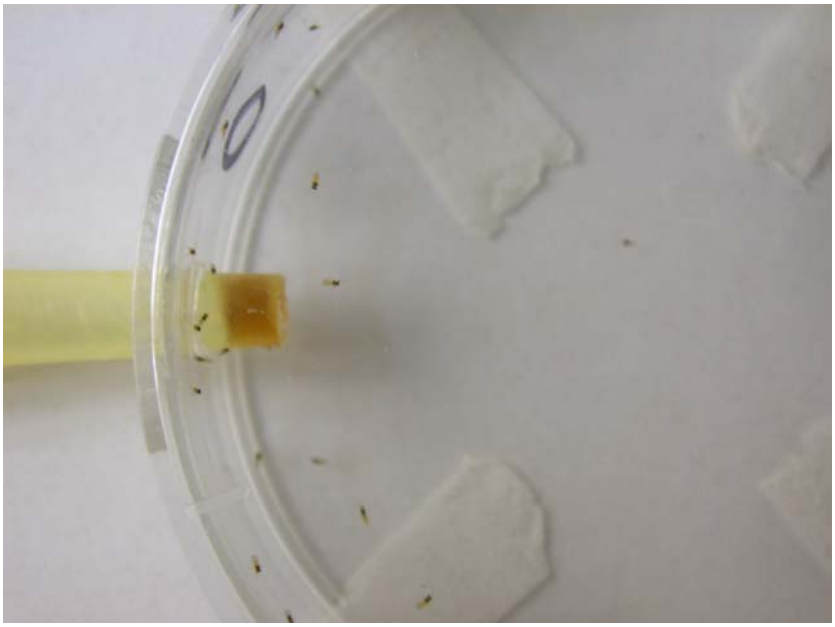


Fig. 42. Close-up of honeyed tip used to provide sustenance to wasps during assessment period.

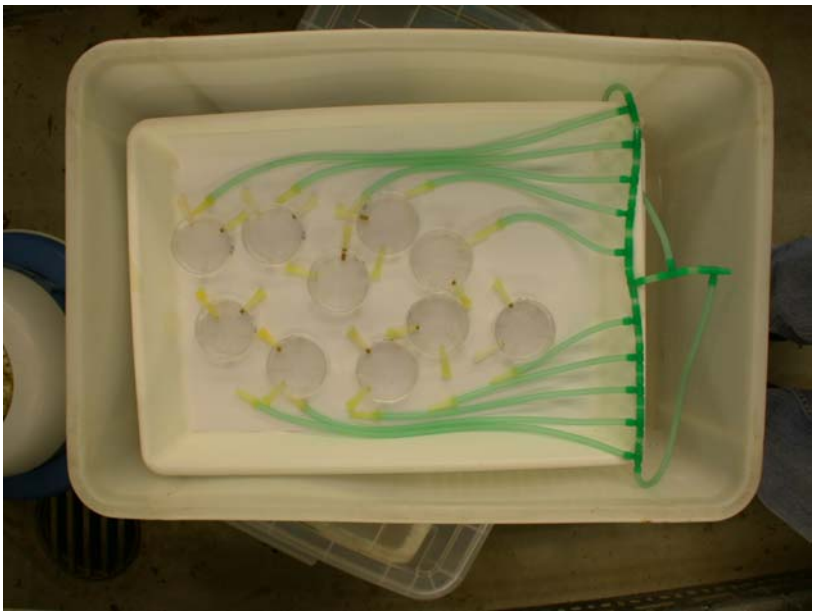


Fig. 43. Series of Petri dish test arenas each with a different chemical treatment connected to a vacuum pump for ventilation during assessment.

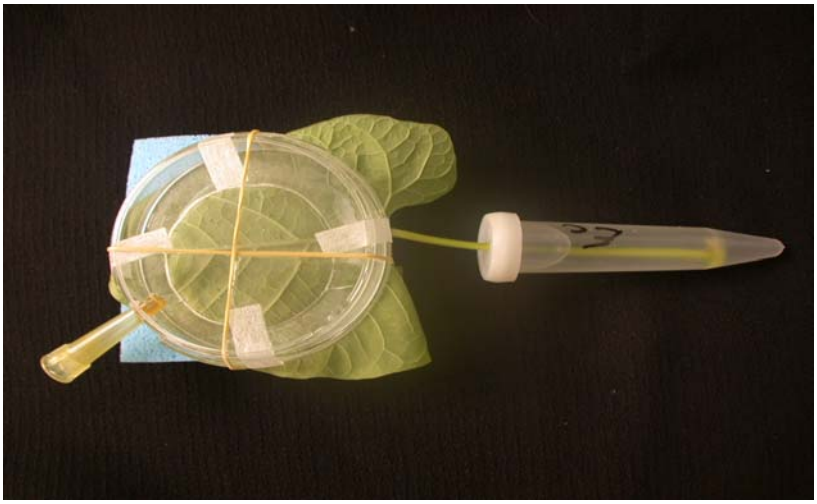


Fig. 44. Test arena for residual toxicity of chemical residue on French bean leaf assessed every 7 days after treatment.

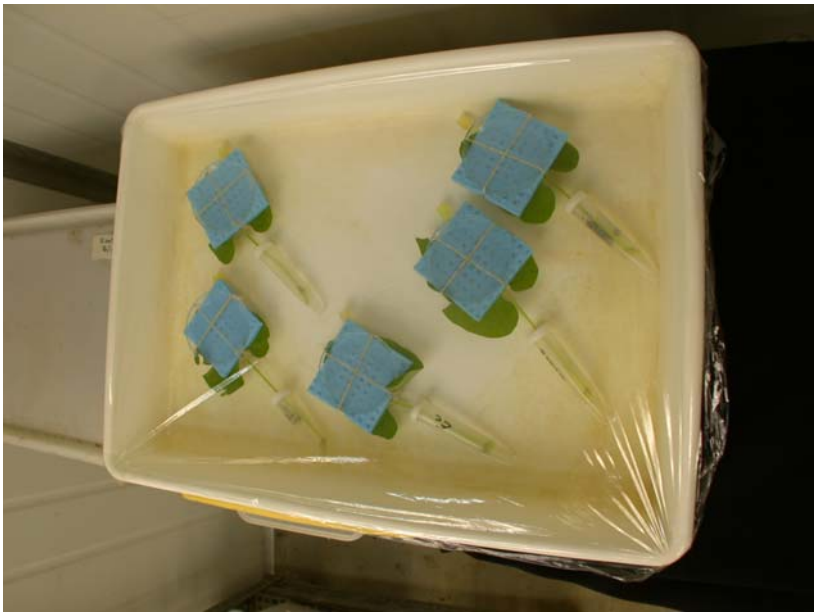


Fig. 45. Series of leaf test arenas each representing a different chemical.

Table 23 Residual toxicity of pesticides applied to a plastic Petri dish against adult *Encarsia formosa* using a standardised laboratory test method

Test	Chemical Trade Name	Chemical Name	Age of residue (days)	Days post-exposure	% mortality (adjusted by Abbot's formula)				
PT	Bravo + Agral	Chlorothalonil	0	1	0	0.1	11.8	14.7	
			0	4	0	0	8.8	14.9	
			4	1	0.1				
			4	3	0				
	Ecocarb + Agral	Potassium bicarbonate	0	1	0	0	3.2	0	
			0	4	2.7	2.6	0	0	
			4	1	8				
			4	3	2.5				
	Saprol + Agral	Triforine	0	1	2.6	10.5	0	3.1	
			0	4	0	10.5	0	0	
			4	1	0				
			4	3	0				
	Systhane + Agral	Myclobutanil	0	1	5.3	2.8	0	3.2	
			0	4	2.8	0	0		
			4	1	2.6				
			4	3	2.5				
	Sulfur + Agral	Wettable sulfur	0	1	0	0	0	4.8	
			0	4	76.7	5.7	43.5	38.1	
	Zineb + Agral	Zineb	0	1	13.9	11.8	6.1	6.1	
			0	4	11	11.8	0	31.1	
			4	1	5.3				
			4	3	5.3				

PT = Potter Precision Spraying Tower used in test method 1.
Agral = non-ionic wetting agent commercially used in horticultural spraying

Table 23. Continued

Test	Chemical Trade Name	Chemical Name	Age of residue (days)	Days post-exposure	% mortality (adjusted by Abbot's formula)				
PT	Vertimec + Agral	Abamectin	0	1	0	0	0	0	
			0	4	100	91.3	100	100	
	Vertimec + Triton	Abamectin	0	1	10.53	13.33			
			0	4	84.2	90			
			4	1	5.9	7.7			
			4	3	8.8	92.3			
	AzaMax + Agral	Azadirachtin	0	1	0	0	0.4	1.9	
			0	4	0	0	0	0.6	1.9
	AzaMax + Triton	Azadirachtin	0	1	3.3				
			0	4	3.3				
			4	1	0	3.5			
			4	3	10.3	3.5			
	AzaMax	Azadirachtin	0	1	0	0	0.4		
			0	4	0	29.3	0.9		
	Eco-oil + Agral	Botanical oil	0	1	0	0	3.2	0	
			0	4	2.7	2.6	0	0	
			4	1	8				
			4	3	2.5				
	Eco-oil + Triton	Botanical oil	0	1	3.8	0	0		
			0	4	22.4	0			
			4	1	9.1	8.5			
			4	3	12.1	8.5			
	Eco-oil + Triton	Botanical oil	0	1	13.1	0	3.5		
			0	4	100	0			
			4	1	6.7	8.5			
			4	3	13.3	12.5			

Triton = non-ionic wetting agent used in the laboratory

Table 23. Continued

	Chemical Trade Name	Chemical Name	Age of residue (days)	Days post-exposure	% mortality (adjusted by Abbot's formula)				
	Thiodan + Triton	Endosulfan	0	1	78.8	100	100		
			0	4	100	100			
			4	1	0	72.4			
			4	3	3.2				
	Thiodan + Agral	Endosulfan	0	1	100	100	100	100	
			0	4	100	100	100	100	
			4	1	5.3	3.5			
			4	3	100	100			
	Confidor + Agral	Imidacloprid	0	1	69.8	96.3	0	24	
			0	4	100	100	100	100	
	Confidor + Triton	Imidacloprid	0	1	69.7	91.9	71.2		
			0	4	100	100			
			4	1	71	100			
			4	3	100	100			
	Malathion + Triton	Maldison	0	1	97	100	93.4		
			0	4	100	100			
			4	1	100	100			
	Natrasoap + Agral	Potassium salts	0	1	9.3	0	23.5	6.5	
			0	4	5.3	2.7	53.5	6.5	

Table 23. Continued

Test	Chemical Trade Name	Chemical Name	Age of residue (days)	Days post-exposure	% mortality (adjusted by Abbot's formula)				
	Natrasoap + Triton	Potassium salts	0	1	0				
			0	4	8.8				
			4	1	0	0			
			4	3	0	0			
	Omite + Agral	Propargite	0	1	15.4	12.9	0	3	
			0	4	17.9	100	96.7	90.6	
			4	1	0	0.3			
			4	3	0	45.3			
	Pirimor	Pirimicarb	0	1	100	68.6			
			0	4	100	72.6			
	Pirimor + Agral	Pirimicarb	0	1	100	92	100		
			0	4	100	100	100		
	Poclaim + Agral	Emamectin	0	1	100	100	100	100	
			0	4	100	100	100	100	
			4	1	17.1	100			
			4	3	100	100			
	Chess	Pymetrozine	0	1	0	0			
			0	4	0	0			
	Chess + Agral	Pymetrozine	0	1	2.6	3.3	0		
			0	4	1.8	6.8	0		
	Pyrethrum + Agral	Pyrethrin	0	1	100	100	100		
	Pyrethrum + Triton	Pyrethrin	0	1	100	100			
			4	1	100	100			

Table 23. Continued

Test	Chemical Trade Name	Chemical Name	Age of residue (days)	Days post-exposure	% mortality (adjusted by Abbot's formula)				
	Success	Spinosad	0	1	0	100			
			0	4	100	100			
	Success + Agral	Spinosad	0	1	100	100	100		
	Success + Triton	Spinosad	0	1	100	90			
			0	4	100	100			
			4	1	100	100			

Table 24 Residual toxicity of pesticides applied to French bean leaves on adult *Encarsia formosa* using a standardised laboratory test method

Test	Chemical Trade Name	Chemical Name	Age of residue (days)	Days post-exposure	% mortality (adjusted by Abbot's formula)				
Plant	Bravo + Agral	Chlorothalonil	7	1	0	0			
			7	4	0	2.5			
	Ecocarb + Agral	Potassium bicarbonate	7	1	0	0			
			7	4	0	0			
	Systhane + Agral	Myclobutanil	7	1	0	0			
			7	4	0	0			
	Saprol + Agral	Triforine	7	1	0.1	0			
			7	4	11.8	0			
	Eco-oil + Agral	Botanical oil	7	1	0	0			
			7	4	0	7.8			
	Omite + Agral	Propargite	7	1	0	0			
			7	4	0	0			
	Proclaim + Agral	Emamectin	7	1	45.5	25			
			7	4	100	100	100		
			14	1	2.6	67.7	33.2		
			14	3	100	100	100		
			21	1	0				
			21	4	100	100			
			28	1	71.9				
			28	3	100				
			35	1	0				
			35	3	100				
	Thiodan + Agral	Endosulfan	7	1	0	0			
			7	4	0	0			
	Zineb + Agral	Zineb	7	1	0	0			
			7	4	0	0			

Trial 14. Evaluation of the toxicity of Eco-Oil™ and Ecocarb™ to the phytoseiid predatory mite *Phytoseiulus persimilis*, a biocontrol agent of two-spotted mite

Background

In a few instances a conclusion on the side effects of a pesticide based on laboratory bioassay data has differed with subsequent field experiences. An example of this was the fungicide mancozeb, initially considered to be safe on the basis of laboratory results, but which was subsequently found to be harmful against *P. persimilis* in the field. On the basis of testing conducted in the laboratory at Gosford it was concluded that Eco-Oil™ was safe to use with *P. persimilis*, however some field reports and direct observations in a research greenhouse at the NCGH have suggested that this may not be the case. Consequently, a replicated experiment was conducted in the greenhouse at Gosford to examine the influence of Eco-Oil™ and Ecocarb™ applied individually and in combination against the predatory mite.

Materials and methods

The experiment was conducted on young cucumber plants grown in 10 cm pots and previously treated with Cultar™ dwarfing agent to provide a test plant with limited growth. The experiment was conducted in a temperature-controlled glasshouse maintained at 25±2 ^{o}C. Prior to the experiment each plant was infested with two-spotted mite, followed by a release of *P. persimilis* after one day (10-20 adult females/plant).

A pre-treatment count of *P. persimilis* motile stages was made after a further two days by directly counting adults and nymphs on each plant in the laboratory using a stereoscopic microscope. The experiment compared the side-effects of Eco-Oil™ and Ecocarb™ on *P. persimilis* applied separately, and in combination, when applied once and also three times at 7 day intervals, with each treatment replicated four times. Chemical treatments were applied to incipient run-off on plants using a 1L hand-held sprayer and allowed to air dry. Treated plants were then arranged in randomised blocks in an experimental design produced by the Neil Coombes Digger program and held in the greenhouse. Plants were staked in an upright position to keep them apart and each plant was held in 2.5 cm water in a 4.5L ice cream container to prevent transfer of *P. persimilis* between plants. Despite the chemical treatments *P. persimilis* numbers increased and two-spotted mite as food was replenished every second day to maintain the surviving predator population.

Assessment of the single-application treatment schedule was made three days after the application, while the three-application schedule was assessed three days after the final application. Plants were assessed by removing the leaves and washing off the mites through fine sieves and preserving them in AGA for counting at a later date. At the time of the final assessment there were about 7 leaves per plant. Predatory mites alive at the time of washing were identified in the AGA preservative by the outstretched appearance of their legs as opposed to mites that were identified as dead at the time of washing and preservation by the curled appearance of their legs against the body.

The four chemical treatments were:

Eco-Oil	0.25%
Eco-Oil	0.5%
Ecocarb	0.3%
Eco-Oil + Ecocarb	0.25% + 0.3%
Eco-Oil + Ecocarb	0.5% + 0.3%
Control	

Data were analysed by fitting a Generalised Linear Mixed Model with Poisson errors and logarithmic link function to the surviving mites.

Results and discussion

Results are given in Table 25. After a single application all treatments except Eco-Oil at 0.25% produced a significantly lower predator population than the control. However, after three applications, only the high rate of Eco-Oil™ plus Ecocarb™ produced a significantly lower *P. persimilis* population than the control.

While there was an obvious negative effect after a single application, with most treatments reducing *P. persimilis* to about a third of the untreated population, the population recovered despite a further two applications, with only the high rate of the combination treatment having a negative impact over the long term.

Table 25. Mean counts of total *P. persimilis* mites after treatment with Eco-Oil™ and Ecocarb™.

Treatments	**Mean counts of total mites after treatment**	
	Single application	**Three applications**
Control	137.14 a	343.09 a
Ecocarb 0.3%	60.89 b	265.87 a
Eco-Oil 0.25%	110.39 b	259.56 a
Eco-Oil 0.5%	49.95 b	364.31 a
Eco-Oil 0.25% + Ecocarb 0.3%	45.56 b	475.33 a
Eco-Oil 0.5% + Ecocarb 0.3%	44.35 b	115.12 b

Means followed by different letters are significantly different from each other (p=0.05).

Trial 15. DNA identification of some phytoseiid mite species to establish conspecific relationships

Background

The predatory mite family Phytoseiidae is an important group of beneficials that has been extensively used as biocontrol agents against a wide range of phytophagous pest species in a range of different crops around the world. Taxonomic relationships in the Phytoseiidae to date have been established exclusively on morphological characters that, while providing separation of genera and species, has also been subject to frequent revisions. Taxonomists can be categorised as 'lumpers' or 'splitters'. The genus *Amblyseius* has been widely used for a number of species, however more recent revisions using external morphological characters have resulted in the placement of several of these species into alternative genera. Despite this there still exists some concern about the relationships between so called separate species based on this approach, and DNA sequencing is being used to provide clarification of these relationships.

Associate Professor Michael Gillings, Department of Biological Sciences, Macquarie University, Sydney was approached to undertake DNA separation of three pairs of phytoseiid mite species of interest to us as biocontrol agents where each pair was morphologically very similar. In addition he also included a number of other Australian species that also had potential as biocontrol agents and were of interest.

Materials and methods

Specimens were obtained from mite cultures established and maintained by Ms M. Steiner and Dr S. Goodwin of the National Centre for Greenhouse Horticulture, Gosford. Mites were transported in 90% ethanol. Experimental populations were received under ethanol and originated in the laboratories of Dr N. Martin (New Zealand) and Dr P. Ramakers (Netherlands). Reference species of Phytoseiidae were identified by Steiner and Goodwin with the key of Schicha (1987), and confirmed by Dr D. Walter previously of the University of Queensland, Department of Zoology. Details of the origins of mite lines are given in Table 26.

Mites were examined microscopically to determine that no extraneous material or other invertebrates were present, and were then washed in 70% ethanol several times. Between 20 and 50 individual mites were selected for analysis. Samples were subjected to the DNA procedures and PCR products were purified and sequenced.

Results and discussion

The DNA sequence data were aligned and used to construct phylogenetic trees (unpublished data).
From it the following conclusions were reached. *Neoseiulus barkeri* and *A. masiaka* are the same species, as are *A. limonicus* and *A. lailae*. The last pair are particularly important because they have been investigated as potential biocontrol agents in different countries in the belief that they were separate species. In both cases mass-rearing difficulties were experienced that have prevented them from being commercially available to date. There were some unexpected differences between

strains of *N. cucumeris*, an important biocontrol agent of thrips overseas. Further DNA work is planned for this species.

Table 26: List of mite species and strains, with information on their origin.

Species	Strain No.	Location
Amblyseius herbicolus	712	Green Pt., NSW
	816	Narara, NSW
Amblyseius lailae	127b	Narara, NSW
	480v	W. Australia
Amblyseius largoensis	739	Pt. Douglas, Qld
	787	Brisbane, Qld
Amblyseius lentiginosus	654	Narara, NSW
Amblyseius limonicus	940	New Zealand
	941	Netherlands
Amblyseius masiaka	535	Narara, NSW
	550	Narara, NSW
	602	Qld
	710	Narara, NSW
	531	Not known
	780	Narara, NSW
Amblyseius montdorensis	323	Adelaide, SA
	748	Cairns, Qld
	752	Atherton, Qld
	753	Tolga, Qld
	759	Titchum Ck, NT
Amblyseius peltatus	737	Daintree, Qld
Amblyseius sullivani	604	Bilouela, Qld
	732	Cooktown, Qld
	755	Fitcham Ck, NT
	780	Katherine, NT
	720	Narara, NSW
	529	Narara, NSW
	721	Narara, NSW
	771	Stapelton Ck, NT
	766	NT
	770	NT
	799	Qld
	800	NT
Neoseiulus barkeri	942a	Netherlands
	942b	Netherlands
	4.3.97	Netherlands
Neoseiulus fallacis	1005	Canada
Neoseiulus cucumeris	660	United Kingdom
	814	New Zealand
	944	Netherlands
	996	Victoria
	658	United Kingdom
	725	unknown
Neoseiulus wearni	884	Queensland
	885	NSW

Trial 16. Evaluation of the effectiveness of beneficial fungal pathogens against western flower thrips, greenhouse whitefly and green peach aphid

Industry collaborator: BioCare Technology Ltd, Somersby.

Background

Modern computer controlled greenhouse technology can control the greenhouse environment for the benefit of both crop production and crop protection programs. This includes temperature and humidity management. Humidity management means that pest control programs utilising beneficial fungal pathogens can be developed for greenhouse crops. Currently in Australia there are no fungal biopesticides registered for use in greenhouse crops.

This project was established to commence a program of work to identify promising isolates of fungal pathogens and to commercially develop products for registration and use by growers in an IPM program. The aim of this initial work was to conduct laboratory screening trials to compare the efficacy of 54 isolates of a number of entomopathogenic fungi (*Beauveria bassiana*, *Metarrhizium anisopliae* and *Verticillium lecanii*), against western flower thrips, greenhouse whitefly and green peach aphids. The trials utilised standardised laboratory bioassay techniques.

This area of research is a collaboration involving researchers from Australia, USA and Canada, with isolates provided by each country for testing. While the collaboration has been restricted to a comparison of the isolates on strains of western flower thrips in each country, in Australia the research program has also included evaluation of these isolates against aphid and whitefly species.

The research program commenced with the visit of Dr Michael Brownbridge, University of Vermont, one of the overseas collaborators and a specialist in the field of microbial insecticides, during October and November 2001. During his stay the following research and extension activities were undertaken:

- In the limited time available Dr Brownbridge and NSW Agriculture researchers tested laboratory screening procedures for western flower thrips and aphid species. The focus of the collaboration was in the former pest. Dr Brownbridge conducted tutorials in microbial procedures including culturing fungal pathogens, spore viability testing and spore counting. In the development of bioassay procedures, experiments were conducted to investigate the mortality effects of wetting agents against the target organisms and on spore viability and to compare some alternative bioassay methods against aphids and western flower thrips.

- Grower meetings were conducted in SA and Victoria to present information on the emerging use of microbial products internationally and the value of this project to Australian producers.

Materials and methods

All experiments were conducted in controlled temperature and humidity facilities and insect test material was provided from cultures maintained at the NCGH, Gosford. In the experimental procedures each week a group of 10 isolates was selected for testing. Each isolate was cultured to produce adequate spore material, which was harvested

after a two week incubation, counted and tested for viability. Spore solutions were then corrected to produce a spore concentration of 1 x 10^7 used for testing. Testing procedures comprised the following:

Western flower thrips. Four small disposable plastic Petri dishes 35mm diameter were used per isolate. A disc of French bean was cut and placed over a filter paper disc sat in the bottom of the dish and moistened with 30µL distilled water to maintain freshness. The four dishes were sprayed with 2mL of spore suspension using a Potter precision spray tower and allowed to air dry for 8 minutes. A Petri dish lid was placed on each unit and sealed together around the middle with Parafilm™. Ten adult female WFT were introduced into the unit through a small hole located in the centre of the lid and a square of Parafilm™ was then stretched tight over the lid to prevent the thrips from escaping. The dishes were held at 22°C in a controlled environment room and mortality and infectivity were assessed after 6 days. A water only control was used with each weekly batch of isolates tested and each treatment was replicated three times.

Greenhouse whitefly. A single stem of French bean containing two cotyledon leaves was placed through a small hole in the snap-top lid of a 75mm orchid vial containing water and 2-3 slow-release fertiliser granules for nutrition. The vial with bean stem was placed into a hole in a polystyrene slab to hold them in place and covered with a 10cm deep clear plastic cup with a screened hole on the side for ventilation. A hole in the top of the cup for the introduction of 25-30 adult whitefly was covered with the top half of a second cup to prevent the whitefly from escaping. The whitefly adults were allowed to oviposit for 24 hours before they were removed. The units were then held for 10 days at 22°C to allow development of first and second instars. Each pair of bean leaves was sprayed with 2mL of spore suspension using a Potter precision spray tower, removed and covered with the clear plastic cup with screened holes on the top and side for ventilation. Four units were tested per isolate. A water only control was used with each weekly batch of isolates tested and each treatment was replicated three times on separate occasions. Whitefly scales were assessed for mortality and infectivity after six days.

Green peach aphid. A capsicum leaf disc was set in agar with the underside uppermost in a 35mm Petri dish base. Ten adult aphids were introduced onto the disc and allowed to lay young for 24 hours, providing about 15 live young for testing. A creamer cup with a screened hole for ventilation in the top was placed over the Petri dish base as a cover. The adults were removed after the laying period and the young aphids allowed to develop to the second instar. Each unit was sprayed with 2mL of spore solution using a Potter precision spray tower and allowed to air dry before the units were placed in a controlled environment room at 22°. Four units were sprayed per isolate, a water only control was included with each test batch and each treatment was replicated three times. Aphids were assessed for mortality and infectivity after 6 days.

Data analysis. Each species was analysed separately. All data were corrected for control mortality using Abbott's formula prior to analysis and any data set with greater than 15% mortality was rejected. A Generalised Linear Mixed Model (GLMM) described below was fitted to the data with binomial errors and logit link function:

Logit(insects dead) ~ mean + isolate + *isolate.time*,

where the italicised terms are included in the model as random effects. ASReml was used to fit the model. Mean proportions were then predicted and confidence intervals calculated.

Results and discussion

From the analysis the resultant data gave the predicted efficacy (proportion of insects killed) of the isolates with 95% confidence intervals listed in descending order. The complete data set is not presented here. Isolates were compared for performance against the three target insects and on this basis the all-round best 17 fungal isolates given in Table 27 were selected for further study.

Table 27. Summary of best performed fungal isolates from the preliminary screening bioassays against western flower thrips (WFT), greenhouse whitefly (GWF) and green peach aphid (GPA).

Group	Isolate	Origin	Organism#	Bioassay data – Mean % mortality					
				WFT	Rating*	GWF	Rating	GPA	Rating
1. Strong performance against 3 pest species.	A1	BioCare	Bb	83.27	10 – 4	82.52	3 – 4	68.99	6 – 3
	A2	USA	Bb	87.62	3 – 2	84.54	1 – 6	64.03	12 – 15
	A3	Canada	Bb	90.50	1 – 1	83.87	2 – 1	74.88	2 – 2
	A4	Canada	Bb	85.45	8 – 10	75.98	5 – 3	70.59	5 – 6
	A5	USA	Bb	80.75	13 – 14	68.94	12 – 21	68.51	7 – 11
	A6	USA	Bb	83.92	9 – 13	68.67	13 – 14	64.70	10 – 10
	A7	CSIRO	Bb	86.73	7 – 5	73.38	6 – 5	66.82	8 – 8
	A8	Canada	Bb	87.52	4 – 11	79.69	4 – 2	71.51	4 – 5
2. Strong performance against both WFT & GWF, or WFT on own.	B1 both spp.	USA	Ma	79.07	16 – 15	69.59	10 – 16	53.94	31 – 27
	B2 both spp.	USA	Bb	82.00	12 – 12	67.73	14 – 13	54.42	27 – 35
	B3 both spp.	CSIRO	Bb	86.78	6 – 3	72.29	7 – 7	58.74	22 – 28
	B4 WFT only	CSIRO	Bb	87.08	5 – 7	65.04	23 – 14	71.98	3 – 4
	B5 WFT only	CSIRO	Bb	89.61	2 – 6	62.89	26 – 23	59.90	20 – 9
	B6 WFT only	CSIRO	Ma	83.18	11 – 8	65.17	21 – 15	64.24	11 – 7
3. Miscellaneous	C1	NSW Ag-aphid	?	68.06	28 – 26	53.23	37 – 36	85.91	1 – 1
	C2	CSIRO	Bb	77.27	19 – 17	69.98	9 – 8	61.29	15 – 19
	C3	Canada	Bb	77.09	20 - 22	69.33	11 - 9	62.52	14 - 21

- Numbers separated by a hyphen are the isolate's ratings according to whether the mean was calculated arithmetically or by biometrical analysis.

Bb = *Beauveria bassiana;* Ma = *Metarrhizium anisopliae*

Trial 17. Evaluation of a neem insecticide against western flower thrips, two-spotted mite, greenhouse whitefly, green peach aphid and tomato russet mite

Industry collaborators: Organic Crop Protectants Pty Ltd, Sydney and EID Parry (India).

Background

While there are a number of neem-based insecticidal products 'on the market' in Australia, none is registered. The fundamental problem with most of them is that they were produced from crushed neem seed extract and batches of product contain inconsistent amounts of the active ingredient azadirachtin. Recently a new neem-based product was introduced into Australia. Overseas it is known as NeemAzal T/S, although in Australia it is called AzaMax™. This product differs from all the others in that it contains azadirachtin extracted from neem seed and reconstituted in sesame oil at 1.2%. This product is manufactured by EID Parry (India) and is being developed in cooperation with Organic Crop Protectants, Sydney for the Australian protected cropping industry.

The aim of this research was to develop efficacy and phytotoxicity data to support an application for the registration of this product in greenhouse vegetable and ornamental (nursery and cutflower) crops. The work was supported by a substantial industry contribution that justified the inclusion of ornamental and tomato crops.

Greenhouse and container trials were conducted under controlled conditions against a range of vegetable and ornamental insect and mite pests.

A summary of the trial outcomes is presented in Table 28. Results of the trials are confidential to the sponsoring companies and HAL.

Table 28. Trials conducted to evaluate the efficacy and phytotoxicity of AzaMax® in greenhouse crops

Crop	Trial Subject	Comments
Cucumber	Western flower thrips	Recommended for registration
Gerbera	Western flower thrips	Recommended for registration
Cucumber	Two-spotted mite	Recommended for registration
Rose	Two-spotted mite	Recommended for registration
Strawberry	Two-spotted mite	Twice failed to establish trial
Tomato	Greenhouse whitefly	Recommended for registration
Gerbera	Greenhouse whitefly	Recommended for registration
Cucumber	Cotton aphid	Recommended for registration
Capsicum	Green peach aphid	Recommended for registration
Tomato	Tomato russet mite	Not recommended for registration
Tomato	Tomato russet mite	Not recommended for registration
Tomato	Tomato russet mite	Not recommended for registration
Ornamentals	Phytotoxicity	Safe
Vegetables (tomato, cucumber, capsicum, lettuce, basil, bean)	Phytotoxicity	Safe
Strawberry	Phytotoxicity	Safe

Trial 18. Application of a neem insecticide as a soil drench against fungus gnats

Background

The neem-based insecticide Azamax™ is an insect growth regulator that also has systemic properties. Reports from overseas and anecdotal reports from Australian growers suggest that it could be applied as a drench as well as a foliar spray. It is known to have activity against a range of pests.

Materials and methods

Lima beans were seeded into 7L black plastic bags in a compost mix and allowed to grow to the full size true leaf stage. There were eight bags with several plants per bag. The greenhouse had a long history of fungus gnats attacking lima beans so a natural infestation was allowed to develop, with no vermiculite added to the media surface to deter oviposition.

The bean plants were placed in another greenhouse on 21 March 2002 and maintained on separate trays. Four bags were treated with AzaMax™ as a soil drench on 25 March (day 1), 1 and 8 April, and four were left untreated (water only). The media was drenched with either (i) 1 L water or (ii) 3mL 1.2% AzaMax™ in 1L water. Water was applied equally to all bags as necessary between treatments to maintain soil moisture enough to prevent wilting. Application dates were 29 March (1L/bag) and 4, 11, 16, 17 and 20 April (500mL/bag).

Individual plants were caged after 7 days (1 April) by placing cylindrical fine mesh bags over a wire frame that was inserted at soil level (Fig. 46). Small sticky traps were used to assess emergence of adults. Traps were 7.4 cm x 2.4 cm cut-outs from a Seabright® trap, attached to a stake and placed vertically within the canopy, with the bottom narrow edge just above soil level. They were placed in the cages for 24h on days 2, 3, 9, 10, 15, 16, 22 and 23. The number of adult fungus gnats were counted and recorded after 24h in each case and traps removed. Traps 9, 15 and 22 recorded emergence over the previous 6 days and traps 3, 10, 16 and 23 catch over a 24h period. Because AzaMax™ is a growth regulator that affects larval development, trapping continued for two weeks after the last treatment to allow the larvae to become adults.

Results and discussion

There was a marked decline in fungus gnat emergence from AzaMax™-treated soil (Fig. 47). Though control was not absolute, the results are encouraging. Following the trial there was some question about the shelf-life of AzaMax™ and the batch tested, so a repeat is planned with new product and variable rates.

Fig. 46. Lima bean plants enclosed in mesh cages to assess fungus gnat emergence from soil treated with AzaMax as a drench. A small strip of yellow sticky trap was placed within the cage.

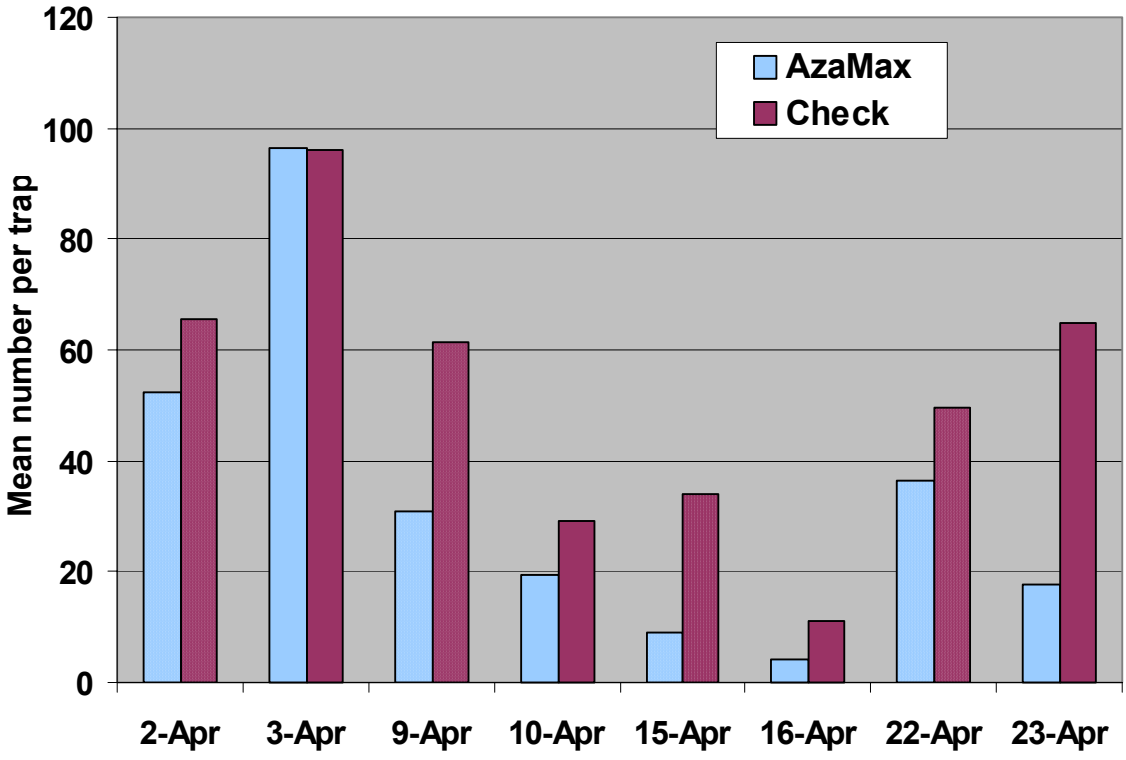


Fig. 47. Catches of fungus gnats over a 24h period from treated and untreated cages. AzaMax was applied as a drench at 0.3% on 25 March, 1 and 8 April 2003.

Trial 19. Evaluation of insect growth regulator insecticides against caterpillars.

Background

In terms of frequency of infestation, caterpillar species would not be classified as a key pest group in most greenhouse crops, yet when they are present severe economic damage can occur if not promptly controlled.

Key species for this industry are cluster caterpillar, *Spodoptera litura,* corn earworm, *Heliothis* spp., looper, *Chysodeixis* spp. and light brown apple moth, *Epiphyas postvittana.* To date control action has relied on spraying toxic chemicals and this has led to resistance problems particularly in *Heliothis* spp. Alternative approaches such as pheromone traps to identify timed spraying opportunities, biocontrol with the egg parasitoid *Trichogramma* spp. and the use of reduced risk chemicals such as the new insect growth regulators are starting to be tested by growers.

This trial aimed to assess the effect of four reduced risk insect growth regulator chemicals applied to gerberas infested with light brown apple moth.

Materials and methods

The trial conducted in gerberas grown hydroponically in environmentally controlled greenhouses at the National Centre for Greenhouse Horticulture was repeated on two separate occasions on 27.6.2001 and 22.8.2001. The target caterpillar species was light brown apple moth, *Epiphyas postvittana.* In each experiment two identical greenhouses were used, each with three double rows of flowering gerbera plants. There were 52 plants per double row and a single row of plants on either side acted as buffer rows. Treatments were applied by knapsack sprayer to the point of run-off and each treatment was replicated three times in each experiment. A pre-treatment and single post-treatment assessment was conducted by visually counting larvae on leaves and in flowers on 10 plants per treatment plot. The treatments are given in Table 29.

Table 29. Chemical treatments used against light brown apple moth infesting gerbera.

Chemical	Trade name	Product concentration	Application rate per litre
azadirachtin	Azamax	12g/L	2mL
indoxacarb	Avatar	400g/kg	0.25g
novaluron	Rimon	75g/kg	0.65g
tebufenozide	Mimic	700g/L	0.86g
control – water + wetter			

Post-treatment counts were analysed using a generalised linear mixed model with Poisson errors and log link function. Pre-treatment counts were included as an offset. The following model was fitted using ASReml:

$$\text{Log (count)} = \text{offset} + \text{treat} + \textit{house} + \textit{house:block} + \textit{error,}$$

where offset = log (pre-treatment counts), and the italicised terms were assumed to be random. The pre-treatment counts were adjusted for natural reduction in the untreated

plots using Abbott's formula. The efficacy value and its 95% confidence interval were then calculated by {1-EXP(mean)} and {1-EXP(mean ± 1.98 x SE)}.

Results and discussion

Results are given in Table 30. Novaluron (78%), indoxacarb (87%) and tebufenozide (91%) all provided effective control. They were not different from each other, while providing significantly better control than azadirachtin (46%), which was considered ineffective. Any of the first three IGRs would be suitable for inclusion in an IPM program. They provide effective caterpillar control and are safe to use with biocontrol agents.

Table 30. Effectiveness of reduced risk chemicals against light brown apple moth larvae

Chemical	Efficacy
novaluron	0.7799 a
indoxacarb	0.8861 a
tebufenozide	0.9071 a
azadirachtin	0.4642 b

Means followed by different letters are significantly different from each other (p=0.05).

Technology Transfer

The project was guided by a steering committee that ensured industry was kept informed.

Throughout the project, presentations on progress were made at industry Conferences for example Hydroponic Farmers Federation 2002, Australian Hydroponic & Greenhouse Association 2001, 2003.

A visiting scientist, Dr Michael Brownbridge brought to Australia from the USA to participate in the development of fungal biopesticides, travelled to SA and Vic to participate at industry workshops organised by greenhouse vegetable growers, and to visit some growers on their farms.

Accredited IPM training was delivered to two greenhouse vegetable groups in Shepparton and an IPM workshop to a greenhouse vegetable group in Perth in October 2003. New developments from the project were passed on to the growers on these occasions.

Articles were written for Practical Hydroponics & Greenhouses, Good Fruit & Vegetables and in the Western Flower Thrips Newsletter on IPM developments arising from the project. Media articles were also published in print and carried on television.

These were:
Steiner, M.Y. and Goodwin, S. (2000) IPM Notebook Friendly bugs for the new millennium. Australian Horticulture 98(2): 39-40.

Steiner, M.Y. and Goodwin, S. (2000). Biological control. Western Flower Thrips Newsletter No. 20. December 2000: 24.

Steiner, M.Y. and Goodwin, S. (2000). Biological control. Western Flower Thrips Newsletter No. 19. March 2000: 12.

Goodwin, S. (2001) New national centre for greenhouse horticulture Good fruit and Vegetables 11(9): 35-37.

Steiner, M.Y. and Goodwin, S. (2001). WFT management in strawberries. Western Flower Thrips Newsletter No. 24 December 2001: 17.

Steiner, M.Y. and Goodwin, S. (2001). Update on programs at the National centre for Greenhouse Horticulture, Horticultural Research and Advisory Station. Western Flower Thrips Newsletter No. 24 December 2001: 29.

Goodwin, S. and Steiner, M.Y. (2001). Development of beneficial fungal pathogens against western flower thrips. Western Flower Thrips Newsletter No. 24 December 2001: 30.

Steiner, M.Y. (2001). WFT management in strawberries. Western Flower Thrips Newsletter No. 23. September 2001: 30.

Steiner, M.Y. (2001). Biocontrols for WFT. Western Flower Thrips Newsletter No. 23. September 2001: 30.

Steiner, M.Y. (2001). Relationship between WFT, environmental factors and fruit/flower damage in strawberries. Western Flower Thrips Newsletter No. 21 March 2001: 12.

Steiner, M.Y. (2001). Promising pesticides for sustainable management of WFT in strawberries. Western Flower Thrips Newsletter No. 21 March 2001: 13-16.

Steiner, M.Y. (2001). Monitoring of WFT populations and damage in commercial strawberry crops. Western Flower Thrips Newsletter No. 21 March 2001: 17-18.

Goodwin, S. and Steiner, M. (2001) Practical integrated pest management. Proc. Australian Hydroponic & Greenhouse Conference 2001: 83-85.

Steiner, M.Y. (2002). New biological control agents for thrips. Practical Hydroponics and Greenhouses Issue 63, March/April: 36-42.

Steiner, M.Y. (2002). Ditching pesticides. Are we ready? Practical Hydroponics and Greenhouses Issue 65, July/August : 30-37.

Steiner, M.Y. (2002). Those pesky flies. Practical Hydroponics and Greenhouses Issue 66, September/October : 58-62.

Goodwin, S. and Steiner, M.Y. (2002). Pesticides to biocontrol…a bridge worth crossing. Practical Hydroponics and Greenhouses Issue 66, September/October: 68-75.

Steiner, M.Y. and Goodwin, S. (2002). Western flower thrips research at the National centre for Greenhouse Horticulture, HRAS, Gosford, NSW. Western Flower Thrips Newsletter No. 28 December 2002: 16-18.

Goodwin, S. and Steiner, M.Y. (2002). Significance of WFT/TSWV in NSW. Western Flower Thrips Newsletter No. 28 December 2002: 18-19.

Hardy, S.M. and Steiner, M.Y. (2002). Western flower thrips damages coastal stone fruit this season. Western Flower Thrips Newsletter No. 28 December 2002: 33-36.

Steiner, M.Y. and Goodwin, S. (2002). TSWV and western flower thrips management in greenhouse capsicums. Western Flower Thrips Newsletter No. 26 September 2002: 21-22.

Steiner, M.Y. and Goodwin, S. (2002). Thrips management in greenhouse cucumbers. Western Flower Thrips Newsletter No. 26 September 2002: 22-23

Steiner, M.Y. and Goodwin, S. (2002). WFT management in strawberries. Western Flower Thrips Newsletter No. 25. March 2002: 15-16.

Steiner, M.Y. and Goodwin, S. (2002). Use of anti-transpirants to protect capsicums against TSWV. Western Flower Thrips Newsletter No. 25 March 2002: 16.

Steiner, M.Y. and Goodwin, S. (2002). Progress with Montdorensis. Western Flower Thrips Newsletter No. 25. March 2002: 17.

Goodwin, S. and Steiner, M. (2002) New educational and training package produced for greenhouse vegetable producers by NSW Agriculture. Proc. Hydroponic Farmers Federation Conference July 2002: 52-55.

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Steiner, M.Y. (2003). Managing tomato spotted wilt virus in greenhouse capsicum. Good Fruit and Vegetables 13(9): 18-19.

Goodwin, S. and Steiner, M.Y. (2003). Thrips and TSWV hit NSW in 2002/03. Western Flower Thrips Newsletter No. 29 March 2003.

Goodwin, S. (2003). Friendly fungi for greenhouse. NSW Agriculture Today November 27, 2003. Also video story run on nine regional TV stations.

Steiner, MY (2003). Looking out for the good guys, NSW Agriculture Today November 27, 2003. Also video story run on seven regional TV stations.

Adoption of the new predatory mite *Typhlodromips montdorensis* was assisted through the establishment of a commercial rearing unit at the National Centre for Greenhouse Horticulture and the provision of direct support to growers using it. The aim was to make available to growers commercial quantities of the predator and to encourage its use; also to develop a market for the biocontrol agent to encourage uptake by a commercial biocontrol producer.

Recommendations

1. That the preliminary screening of fungal isolates be continued to commercial development of new fungal biopesticide products for the greenhouse vegetable industry.

Action: Subject of new HAL project VG03012 2003-06

2. That promising new biocontrol agents *Hippodamia variegata* and *Micromus tasmaniae* be further studied to provide information on their predatory performance, biological limitations and to develop usage strategies. That postgraduate study be the most appropriate way to obtain this information.

Action: Subject of a new HAL proposal 2004-07. Collaboration involving Dr Stephen Goodwin, Marilyn Steiner, Dr Sandra McDougall and Gus Campbell, NSW Agriculture and Sydney University and La Trobe University.

3. That promising new biocontrol agents *Aphidius colemani* and *Eretmocerus* sp. be put into commercial production.

Action: Biological Services, Loxton has expressed interest in commercially producing both biocontrol agents.

4. That promising new biocontrol agent *Feltiella* sp. be further evaluated before commercialising.

Action: Development of mass rearing system to be continued at NCGH by Goodwin & Steiner.

5. That promising reduced risk chemicals identified in VG00066 be further developed

Action: Further screening and small greenhouse crop trials be conducted at NCGH by Goodwin & Steiner

6. That issues of chemical toxicity to biocontrol agents identified in screening of chemicals against whitefly parasitoid *Encarsia formosa*, be further studied and overcome.

Action: Subject of new HAL project VG03008 2003-06.

7. That market development of Montdorensis biocontrol agent be continued by developing a client base and encouraging commercial uptake.

Action: Mass rearing unit established at NCGH, Gosford. Subject of a new HAL proposal 2004-05.

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NSW Agriculture is thanked for providing infrastructural and logistical support without which the work could not have been undertaken.

Horticulture Australia, formerly Horticultural Research and Development Corporation, and the Vegetable Industry Advisory Committee are thanked for recognising the Australian Protected Cropping Industry and for providing financial support to the newly formed National Centre for Greenhouse Horticulture established to meet the R&D needs of this industry.

Members of the Greenhouse Project Steering Committee; Dr Alison Anderson, NSW Vegetable Industry Development Officer (Chair), Jonathan Eccles, Senior Program Manager, Horticulture Australia Limited, Anne Wilson, greenhouse grower Victoria and Vice President, Hydroponic Farmers Federation, Anthony Brandsema, greenhouse grower Tasmania, Joe ElBustani, greenhouse grower NSW and President Greenhouse Vegetables NSW and Craig Feutrill, SA Vegetable Industry Officer are thanked for their contributions to project management.

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APPENDICES
Appendix I. SAMPLE RECORD SHEET: CROP INSPECTION DATA-PESTS

LOCATION: **CROP:**
Person monitoring: **Date:**

***Indicate species if known**

Row	Plant #	Tick for presence, circle if actionable*											*Action recommended*
		White-fly	Thrips	WFT u m l	Spider mites	Aphids	Cater-pillars	Pp	Ef	Tm	Ac	Lw	

Appendix II. SAMPLE RECORD SHEET: STICKY TRAP CATCH DATA-PESTS

LOCATION: **CROP:**
Person monitoring: **Date:**

Row	**Trap #**	**Record number per trap, circle if actionable**										***Action recommended***
		White -fly	**Thrips**	**WFT**	**Aphids**	**Fungus gnats**	**Shore-flies**					

Appendix III. SAMPLE FORM: Leaf Wash Data

Greenhouse: **Number of leaves/sample:** **Crop:**

Date	Mean/leaf																		
	Upper leaf					Middle leaf					Lower leaf					Other pests			Comments
	T. tabaci		WFT		Montdor ensis	T. tabaci		WFT		Montdor ensis	T. tabaci		WFT		Montdor ensis	Upper leaf	Middle leaf	Lower leaf	
	A	L	A	L		A	L	A	L		A	L	A	L					