Extension to Greenhouse IPM Program

Stephen Goodwin
NSW Department of Primary Industries

Project Number: VG03109

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HAL Project Number: VG03109

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Purpose of the Report:

This report describes results of ongoing research and development on new tools for greenhouse vegetable IPM programs. These include investigations into new reduced-risk chemicals and new uses of some existing reduced-risk chemicals, plus the side effects of selected pesticides against some commercially-produced biocontrol agents. Additionally, although not a principal objective of this project, the release of three new biocontrol agents for commercial uptake is also reported on here.

The past three years have seen some encouraging signs of industry investment into greater areas of modern greenhouse production technology. With it, the potential of this industry as a supplier of quality, safe fresh produce and as a supplier of a greater range of fresh produce commodities is being realized.

However, as was reported in a previous final report (VG00066 'Improvements to Biological Control Systems and Development of Biorational Chemicals for Integrated Pest Management in Greenhouse Vegetables' 2000-03), 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 also the development of new tools such as is occurring in our IPM research programs.

Through projects such as VG03098 (WFT extension strategy for Sydney Basin vegetable growers), there is evidence of increasing interest in IPM and its adoption. However, IPM is only as successful as the available tools, and there is still some considerable way to go before Australian greenhouse vegetable growers have at their disposal the array of biocontrol agents and supporting reduced-risk pesticides available to producers in a number of developed countries overseas.

The project reported on here is a valuable contribution to the developmental effort and with the assistance of project VG05093 should see the development and release of a number of new reduced-risk pesticides, biocontrol agents, and information on pesticide use with biocontrol agents that will assist growers with appropriate greenhouse technology and training to implement IPM with greater surety in the future.

Acknowledgement of Funding Sources:

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- Vegetable industry research and development levy
- Becker Underwood (formerly BioCare Australia), Somersby, NSW
- Hydroponic Farmers Federation of Victoria
- Flavorite Tomatoes, Warragul, Victoria
- Duralite Horticultural Supplies, Monbulk, Victoria
- Freshzest Pty Ltd (Herbs), Leongatha South, Victoria
- Biological Services, Loxton, SA.

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MEDIA SUMMARY

Extension to Greenhouse IPM Program VG03109

This was a three-year project which greatly expanded information necessary to making informed choices in reducing toxic pesticide use in greenhouse vegetable crops. Principal researchers were Dr Stephen Goodwin and Ms Marilyn Steiner. The primary aim was to continue the development of effective, safe approaches to greenhouse pest management, and to provide the tools to do this with minimal risk of crop loss. With the rapid expansion and increasing environmental sophistication of the Australian greenhouse vegetable industry, pest management using biocontrol agents and reduced risk pesticides is now achievable as well as desirable.

The key objectives were

- Development of new reduced-risk pesticides and one or more fungal biopesticides to complement biocontrol agents in greenhouse vegetable IPM programs.
- Evaluation of the relative safety of residues of commonly used pesticides on key biocontrol agents to avoid improper use.
- Expansion of the range of natural enemies in commercial production.

The key outcomes were

- Identification of a local strain of the fungal pathogen *Beauveria bassiana* as an effective pesticide against both western flower thrips and greenhouse whitefly, and collaboration with an Australian partner, Becker Underwood, Somersby, NSW, in providing efficacy data necessary for commercialisation. This product is safe to both humans and biocontrol agents.
- Production of easy-to-read charts showing the short and long term effect of eight pesticides on five key biocontrol agents, not just on foliage, but also on plastics and in growing media. A synopsis chart of a broader range of contact and residual effects of greenhouse insecticides and miticides was also produced to allow growers to make informed choices of IPM-friendly pesticides and avoid costly mistakes.
- Identification of novel approaches to managing western flower thrips by targeting not just foliar but also ground dwelling stagess. Several new reduced-risk pesticides were identified for thrips, whiteflies, broad mite and tomato russet mite, which are both effective against pests and compatible with biocontrol agents. Targeted crops were cucumber, tomato, capsicum and lettuce.
- Assistance to Australian insectaries in collecting, rearing and providing them with several new natural enemies for commercialisation, including *Neoseiulus cucumeris* (thrips), *Dalotia coriaria* (fungus gnats, shoreflies), and *Aphidius colemani* (aphids). These are familiar to overseas producers but previously not found in Australia or not developed to commercialisation.

Recommendations are to continue this research in the project VG05093, by pursuing registration of the effective reduced-risk pesticides identified in this project, along with others that have been recently identified with good potential; evaluating their safety to biocontrol agents, and assessing some new indigenous natural enemies with promise for improved control of whitefly, thrips and soil pests. The need to provide an effective arsenal to combat pests in an environmentally safe manner is ongoing and a high priority.

TECHNICAL SUMMARY

Extension to Greenhouse IPM Program VG03109

This was a three-year project which greatly expanded information necessary to make informed choices in reducing toxic pesticide use in greenhouse vegetable crops. Pesticide residues which exceed the MRL are still a problem among segments of the industry. Providing growers with viable, safe alternatives demands a multi-pronged approach. Tools may include fungal biopesticides, none of which are currently registered in Australian greenhouses, a broader range of commercially available biocontrol agents suited to Australian conditions and pest types, information on the side-effects of pesticides used by the industry, and alternatives in reduced-risk pesticides which can safely be integrated with biocontrol agents where necessary. This is an ongoing need which will expand as the industry becomes more sophisticated and export oriented. Principal researchers were Dr Stephen Goodwin and Ms Marilyn Steiner.

The key objectives of this project were met. These were:

- Identification of a local strain of the fungal pathogen *Beauveria bassiana* as at least equivalent to the overseas commercial standard Botanigard™. It has great promise as an effective pesticide against both western flower thrips (WFT) and greenhouse whitefly. Collaboration to produce a commercially available product is continuing with the Australian industry partner, Becker Underwood, Somersby, NSW. Efficacy data for the final formulation is being collected to enable registration.
- Production of easy-to-read charts showing the short and long term effect of eight pesticides on local biotypes of five key biocontrol agents, not just on foliage, but also on plastics and in growing media. A synopsis chart of a broader range of contact and residual effects of greenhouse insecticides and miticides was also produced. The ability of some pesticides to be retained on plastic and differences in media absorption had not previously been reported. Detoxification attempts on plastics had only limited effect.
- Identification of novel approaches to managing WFT by targeting not just foliar stages but also ground dwelling stages. New biological information on WFT response to relative humidity was collected which will enable better targeting and manipulation of control measures. Several novel plastic floor treatments were devised which are inexpensive, provide >50% control, and can be safely used with natural enemies for pesticide-free control of WFT on foliage. Several new reduced-risk pesticides were identified for thrips, whiteflies, broad mite and tomato russet mite that provide safe alternatives for use alone or with natural enemies. Targeted crops were cucumber, tomato, capsicum and lettuce.
- Australian insectaries were provided with several natural enemies for commercialisation, including *Neoseiulus cucumeris* (thrips), *Dalotia coriaria* (fungus gnats, shoreflies), and *Aphidius colemani* (aphids). These are familiar to overseas producers but previously not found in Australia or not developed to commercialisation. Other indigenous natural enemies with potential for exploitation have been identified for a continuation of the project under Dr Leigh Pilkington.
- Attendance at an overseas workshop of the IOBC in Spain and post-workshop visit to Israel in May 2006 was very productive in terms of recommendations that could be made for greenhouse pest management in hot dry climates, and was reported on separately.

The strong recommendation is to continue this research in the project VG05093, by pursuing registration of the effective reduced-risk pesticides identified in this project, along with others that have been recently identified with good potential; evaluating their safety to biocontrol agents, and assessing some new indigenous natural enemies with promise for improved management of whitefly, thrips and soil pests. The need to provide an effective arsenal to combat pests in an environmentally safe manner is ongoing and a high priority for a technologically advancing industry.

1. EVALUATION OF REDUCED-RISK PESTICIDES FOR USE AGAINST GREENHOUSE PESTS

BACKGROUND

While natural enemies used against pests of protected cropping vary in their sensitivity to pesticides, as evidenced by results obtained during this project and by numerous other studies, most pesticides currently available have some negative side-effects. Because of the narrow range of available natural enemies in Australia, seasonally unsuitable temperatures for their use, and imbalances which may occur, growers must have access to pesticides in the category of reduced-risk. These products are either harmless to natural enemies or have short persistence so that they can be used as localised treatments to contain pest outbreaks in early intervention. Few such products are currently available. Those that are include viral and *Bacillus thuringiensis* (bacterial) formulations, soaps and oils. Many new chemistry products claim they are environmentally friendly, but in fact are not so, or only so to a narrow range of natural enemies. In this project we have extended previous work in identifying products safe or potentially safe to a broader range of natural enemies. The emphasis has been on evaluating efficacy of low-risk products against key pests. Work still remains on assessing their safety to a range of natural enemies.

Target pests include thrips, particularly western flower thrips, *Frankliniella occidentalis* (Pergande), two-spotted spider mite, *Tetranychus urticae* (Koch), greenhouse whitefly, *Trialeurodes vaporariorum* (Westwood), broad mite, *Polyphagotarsonemus latus* (Banks), and tomato russet mite, *Aculops lycopersici* (Massee). Trials were conducted between 2003 and 2006.

1.1 THRIPS

1.1.1 CONTROL OF WESTERN FLOWER THRIPS, *FRANKLINIELLA OCCIDENTALIS* (PERGANDE), IN GREENHOUSE CUCUMBERS USING GROUND TREATMENTS - GREENHOUSE TRIALS.

INTRODUCTION

Thrips at the late second larval stage are reported to drop down from plants to the ground to crawl to somewhere moist and sheltered in order to pupate. Several years ago, application of a product called Thripstick™ was advocated in Europe to kill larvae dropping to the ground. This was a sticky polybutene product mixed with deltamethrin for rapid kill. It was unpopular because it left plastic dirty and was unpleasant to walk on. It was also not particularly effective. Larval thrips are susceptible to dessication, so we explored the possibility that forcing them to cross a barrier which had dessicating properties might kill them and thus prevent successful pupation. If the treatments are successful, the adult population will decline over time. Conversely, the control population should increase over time. At 25^{o}C, the life cycle is completed in about 18 days, and the experiment needed to continue for at least two generations in the greenhouse. In the first trial, four dessicants were applied to polypropylene woven weed matting. In five subsequent trials, the strategy was altered. Weed matting was replaced by solid polyethylene Panda™ film, and treatments were chosen for deliquescent properties to maintain a liquid barrier at ground level.

Laboratory bioassays were conducted concurrently with these trials to select the most promising combinations of materials for treatments.

MATERIALS AND METHODS

Dessicant materials

Trial 1 The trial was set up in four adjacent but structurally separate 54sqm plastic-covered greenhouses. Cucumbers cv Kaspian RZ were seeded in 75mm Growool™ blocks. The seedlings were pre-infested with adult western flower thrips on 25 and 28 May 2004 while concentrated in the blocks. Vertical plastic sheeting barriers were installed in the greenhouses between rows to the height of the crop (~2m) to minimise the movement of thrips from one treatment row to another (Fig. 1). Cocopeat bags were laid out in the hydroponic channels in five rows. There were 5 treatments replicated 4 times, with greenhouses as replicates. They were applied 27/28 May to the ground cover in the greenhouses. Treatments were: Control; Surround™ (95% kaolin) at 60g/L; silica fume (Tasmanian Silica Pty Ltd) at 180g/L; Dryacide™ diatomaceous earth (900g/kg amorphous silica) at 120g/L (5L of slurry/100sqm); and Blue Circle™ Plaster Lime/hydrated lime (96% calcium hydroxide) at 180g/L. All products were applied as a slurry with an airgun to cover weed matting, tops of cocopeat bags, and sides of the plastic channels used to carry hydroponic nutrients and run-off, to cover as well as possible all thrips drop-off points from the crop canopy. Total amount of mix used per treatment was 12-14L (less where treatment used two outside rows). Once treatments had dried, plants at the second true leaf stage were set out 28 May in the cocopeat bags and tied to support strings. There were five rows in each house with 21 plants per row (3 plants/cocopeat bag). The treatment design was an incomplete (4 replicates) 5 x 5 Latin Square.

Adult and larval thrips were counted on a weekly basis, from 1 June to 14 July 2004. Leaf counts were made on one middle stratum leaf from each of ten plants in each row, starting with the sixth plant from the south end. Flower counts were also conducted once these were present, one flower from each of the ten plants. As flower age is important with regard to numbers of thrips present, on each plant the flower with most thrips was selected. This was generally the fully open one that was not faded. From weeks 7-11, yellow Seabright™ sticky traps were used instead of leaf and flower counts to monitor populations. The reasons for the change in methodology were a) thrips were able to fly over the top of the plastic dividers more readily as the crop had reached the horizontal support wire, so this was possibly confounding results, b) the population was high and was taking too long to count, c) flowers were at horizontal upper wire level so were potentially attracting thrips from neighbouring rows. Two traps were placed in each row. The traps were placed just above bag level to monitor new adult thrips emerging at ground level rather than leaf populations. This experiment was concluded 10 August 2004.

Statistical analysis

Trial 1

Adult and larval leaf counts The effect of different ground treatments on insect counts was modelled using a mixed linear regression approach (Searle 1971), which allowed for the separation of variance into fixed and random effects. Leaf counts from the 10 plants were totalled. Adult + 1 and larval + 1 counts were $\log_e$ transformed. Analysis was conducted in ASREML (Gilmour *et al.* 2000) and the fitted model is given by:

y = treatment + linear(time) + treatment.linear(time) + *spline(time)* + *factor(time)* + *house* + *row* + *house.row*, where y = transformed count and the italicised terms are included in the model as random effects.

Adult flower counts and trap counts This data was collected on four occasions only. The analysis was again conducted in ASREML on $\log_e$ transformed data, but each time was analysed separately. The fitted model is given by:

y = treatment + house + row, where y = transformed count and the italicised terms are included in the model as random effects.

Deliquescent materials

Trial 2 Table salt Only two of the 54 sqm greenhouses were available for this and subsequent trials, one of which was treated and the other left untreated in each case. Cucumbers cv Kaspian RZ were seeded into rockwool blocks and planted out on 11 July 2005 in three rows. The two side rows were left empty to avoid undue interference from proximity to side walls. There were 21 cucumbers per row, three plants per cocopeat bag, grown in a hydroponic system. White plastic (Panda film) was laid in each row to cover the floor (Fig. 2). It was taped up the side of the media bags to form a continuous surface to prevent thrips dropping to the ground to pupate from crawling under the bags. Plants were infested with WFT by introducing adult WFT from laboratory cultures twice a week from 22 July to 19 August. Approximately 2000 WFT were released in each house. The plants were pinched out at 2 metres on 15-19 August and the upper four side shoots were allowed to grow. Yellow sticky traps (Seabright™) were hung on 23 August at 2 m height between the rows, at two per inter-row (eight in total) per house. They were changed 29 August 2005 and weekly thereafter. Bottom leaves were removed to clear the ground on 29 August, prior to ground treatment. 10% table salt (sodium chloride) plus 0.1% Silwet-L77 as a spreader was applied as a ground spray in House 1 on 29 August (7 weeks post-planting) and 9 September. A total of 9L mix was used, at a cost of ~$4 per application. Silwet alone was applied in House 2. Thrips were monitored by weekly counts of adult thrips on traps. The trial was completed 27 September 2005.

Trial 3 Table salt + Eco-Oil Trial 2 was repeated but with 10% table salt plus 2% Eco-Oil™ as the treatment in House 1 and water only in House 2. Oil was added to try to keep the salt liquid as it was noted that at low humidity the salt crystallised and was of doubtful efficacy. Cucumbers were planted out 11 October 2005 and infested once with adult WFT on 28 October. Seabright yellow sticky traps were hung at canopy level on 21 October (8/house). Treatments were applied to the ground cover on 31 October and again on 7 November. Traps were changed 1 November 2005 and then weekly till 28 November when the trial was concluded. Thirty leaves from the middle stratum of each house were collected 28 November and washed through sieves to extract thrips.

Figure 1. Cucumbers with weed matting treated with silica fume. Rows/treatments were separated by plastic

Figure 2. Set up for treatment of Panda™ plastic with salts.

Trial 4 Pool salt + Eco-Oil. Trial 3 was repeated, using 10% pool salt (sodium chloride) instead of table salt, as pool salt has no additives that might affect the RH at which it liquefies. The crop was planted out 9 December 2005. A single application of 10% pool salt + 2% Eco-Oil was made a week later on 14 December 2005. Adult female WFT were released in both houses 13 and 16 December. Traps were set out from 15 December 2005 to 31 January 2006 and changed weekly (7 weeks). The trial was concluded 1 February 2006. Leaf washes of 30 leaves per house from lower stratum were conducted on 3 and 20 January 2006.

Trial 5 Calcium nitrate + Agri-Terra™. The failure of sodium chloride to liquefy at some greenhouse humidity levels prompted a search for salts that would do so at lower humidity. A series of laboratory bioassays was conducted to find a suitable alternative (see 1.1.5). Calcium nitrate had the requisite humidity response profile, but in laboratory bioassays was not toxic alone to WFT. One of the most promising combination treatments was calcium nitrate plus Agri-Terra. A cucumber crop was planted out in the two greenhouses 10 February 2006. A ground application of 10% calcium nitrate plus 0.5% Agri-Terra with 0.1% Silwet was made to Panda plastic on 16 February 2006 in one house, and again on 9 March as the material appeared to be either drying out or to be removed by foot traffic. The control greenhouse was treated with 0.1% Silwet only. A single release of WFT was made on 17 February. Traps were first placed on 21 February and replaced weekly. Six traps placed just above crop canopy within row were used in the first two weeks, and then eight traps inter-row, two per row at canopy height. Leaf washes of 30 lower canopy leaves per house were conducted on 8 and 24 March 2006. In addition, a thrips sample was taken from five areas of the plastic in the treated house under leaves close to the ground, where thrips were clearly visible as having been killed by the treatment. These were assessed for the percentage of individuals in each thrips stage.

Trial 6 Calcium nitrate + Agri-Terra + sodium chloride. Common salt has greater toxicity to WFT larvae than calcium nitrate, whereas the latter is liquid at a much lower RH. In laboratory bioassays, Agri-Terra improved toxicity of both materials. In this trial, greater longevity of the treatment was sought. The ground treatment applied, again to Panda plastic, was 10% pool salt + 10% calcium nitrate + 0.5% Agri-Terra with 0.1% Silwet as a spreader. The mix was liquid at 60% RH with a few crystals and some cloudiness when the two salts were mixed together. The control greenhouse was treated with 0.1% Silwet only. Cucumbers were planted out 24 May 2006. Approximately 3000 WFT adults/house were released 2 June and the floor treatment was applied 5 June and again 18 July. Traps were first set out 9 June in the upper canopy and changed weekly until 18 August (10 weeks). For the final three weeks, eight traps were also placed low in the canopy in each house. Leaf washes were conducted 14 July, 28 July, 10 August and 18 August, taking 30 leaves per house in the lower canopy. The rockwool blocks were also collected and washed through to look for ground dwelling predators.

Statistical analysis

Trials 1-6

Only two greenhouses were available so experiments were unreplicated. Population trends over time are shown for the treated and untreated greenhouses

RESULTS AND DISCUSSION

Dessicant materials

Trial 1 For adult and larval thrips on leaves (Figs. 3, 4), and adult thrips in flowers (Fig. 5), there was no statistically significant effect for any treatment (P = 0.05). For trap counts of adult thrips (Fig. 6), there was a significant treatment effect at all four times (P = <0.001) (Table 1). Only Dryacide appeared to provide substantial control of WFT, with >60% reduction in the population after 8 weeks. Despite the lack of significant differences in leaf and flower counts, a result of broad variation between counts on individual leaves and flowers, Dryacide stood out as the most effective treatment in plant counts also. There were edge effects due to the heating method (heating pipes along the sides) cooling method (fan and pad system with pad at the north end) and sunlight (greenhouses aligned north/south). Cooling was by fan and pad so cooler air was drawn across the greenhouse and may have affected ends of rows, although the experiment was conducted in winter months when the call for cooling was reduced. Relative humidity was variable between day and night and depending on outside weather conditions, so it was suspected that this may have affected the percentage of larvae dropping to the ground to pupate. While thrips numbers were not high on plants, larval thrips were increasing and control was considered inadequate as a sole treatment, though promising as a supplementary treatment to predators on leaves, particularly as only a single application was made.

Table 1. Effect of ground treatments to weed matting on yellow sticky trap catches of adult western flower thrips. Trap placement was near ground level.

Treatment	Predicted trap count			
	Week 8	Week 9	Week 10	Week 11
Control	223.73 a	268.19 a	449.62 ab	336.87 ab
Surround	206.30 a	214.95 a	356.74 b	234.51 bc
Silica fume	255.81 a	256.69 a	559.50 a	390.93 a
Hydrated lime	136.46 b	138.07 b	247.49 c	171.56 cd
Dryacide	74.85 c	93.72 c	155.26 d	157.25 d

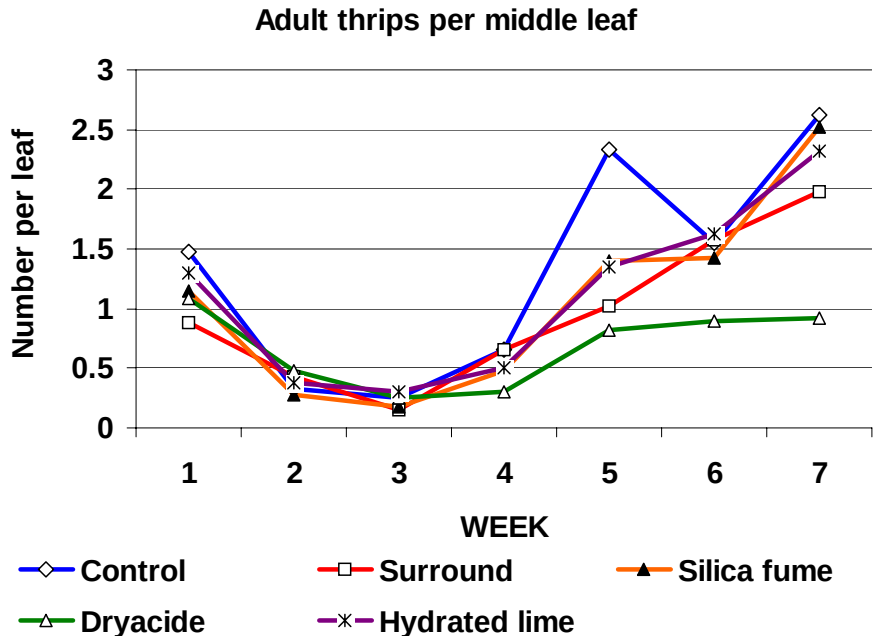

Figure 3. Mean number of adult WFT per middle stratum cucumber leaf, 1 June – 13 July 2004. Ground treatments were applied 27/28 May 2004.

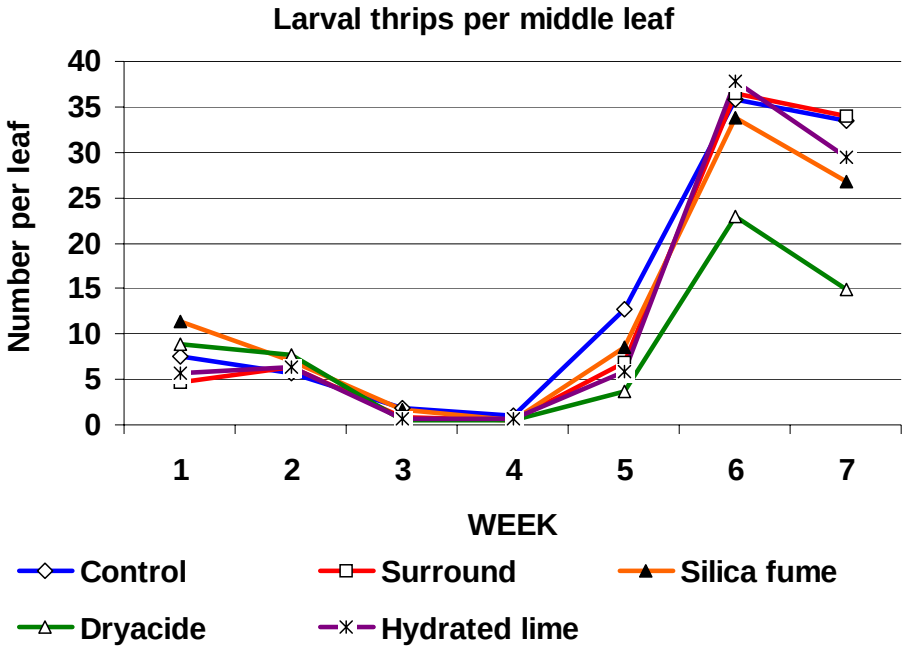

Figure 4. Mean number of larval WFT per middle stratum cucumber leaf, 1 June – 13 July 2004. Ground treatments were applied 27/28 May 2004.

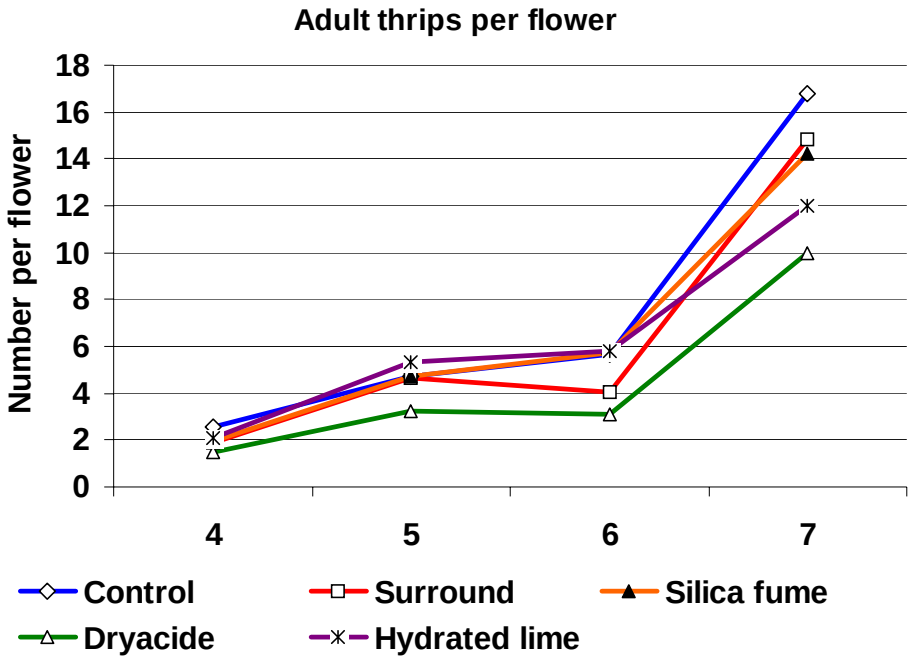

Figure 5. Mean number of adult WFT per cucumber flower, 22 June-13 July 2004. Ground treatments were applied 27/28 May 2004.

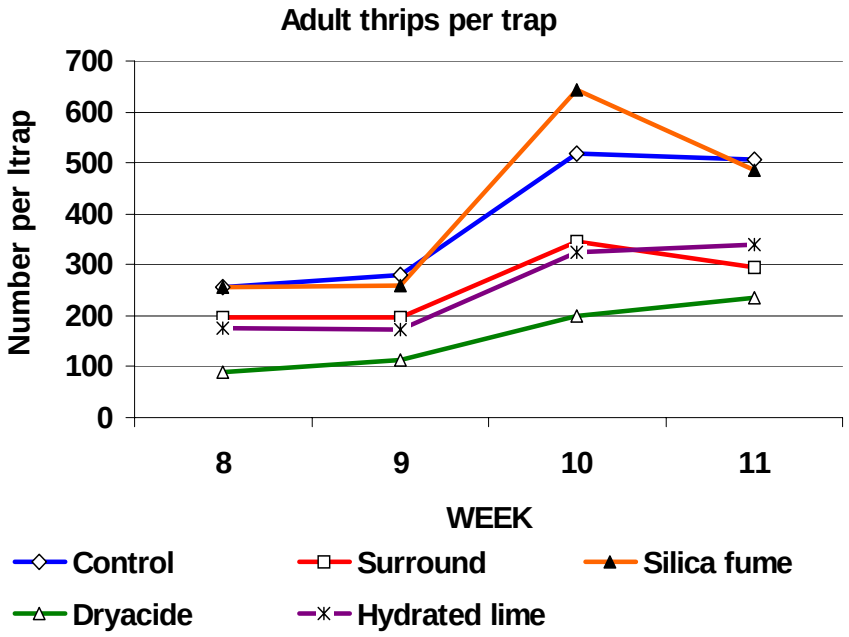

Figure 6. Mean number of adult WFT per yellow sticky trap, 14 July-10 August 2004. Ground treatments were applied 27/28 May 2004.

Deliquescent materials

Trial 2 Table salt Thrips populations at the time of first treatment were very high (Fig. 7), but almost double in the house that was treated with salt. In the first week post-treatment, thrips trap catches showed a 300% increase in the untreated house and then a rapid decline. In the treated house, there was only a 1% increase in the first week and catches remained relatively stable over the next three weeks. The results of this trial were confounded by an outbreak of the fungal pathogen *Beauveria* in the untreated house, first noted on 19 September. By trial end it had started to move into the other house. Its impact was to greatly reduce thrips numbers in the control (many dead adults and larvae were observed on the leaves). The predatory mite *Typhlodromips montdorensis* was also observed in the control house at trial end but was probably not yet in high enough numbers to have a serious impact on the thrips population.

The results with the salt were disappointing, but several factors contributed to this. When the weather was dry, overnight heating had the effect of reducing the relative humidity to a level where the salt was not deliquescing. During a rainy period, however, it was noted that the salt was liquid even during the day. Common salt should liquefy at ~ 75% RH. Failure to do so may reduce its activity against thrips, particularly during the early growth phase of the crop when canopy is sparse, and during winter when night-time heating dries out the atmosphere. Relative humidity was much lower during the day than at night. Daytime relative humidity ranged from 25-50%, whereas RH at night was 85-100%. The humidity generally started to increase late afternoon/early evening, a time when most thrips larvae preparing to pupate are reported to drop to the ground (pers. comm. William Kirk, 2006). Another factor contributing to poor results was that when the salt was liquid it ran into low spots leaving large areas of the plastic clear for walking thrips to circumvent it. With few exceptions, thrips populations were higher at the fan end, where temperatures were actually lower during the day. Late application of the treatment, when thrips populations were already high, may also have prejudiced results.

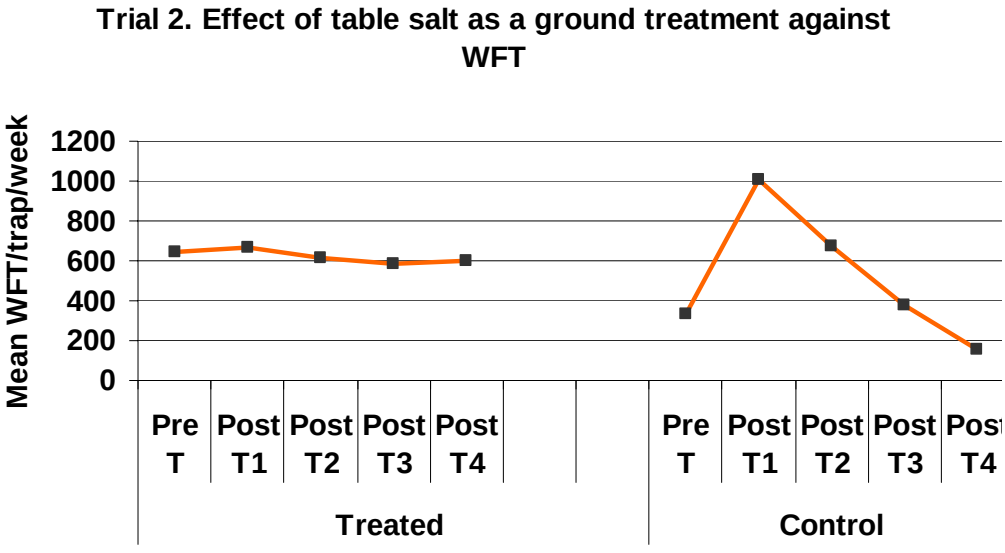

Figure 7. Weekly yellow sticky trap counts of WFT in greenhouse cucumber in treated (10% common salt on floor) and untreated greenhouses. Treatment was applied 29 August and 9 September 2005. An entomopathogen, *Beauveria* sp., substantially affected thrips in the control house.

Trial 3 Table salt plus Eco-Oil Results are presented in Table 2 and Fig. 8. Many dead larval and adult thrips were observed on the ground plastic in the treated house (Fig. 9), but not in the untreated house, so there is no doubt that the treatment killed substantial numbers of thrips.

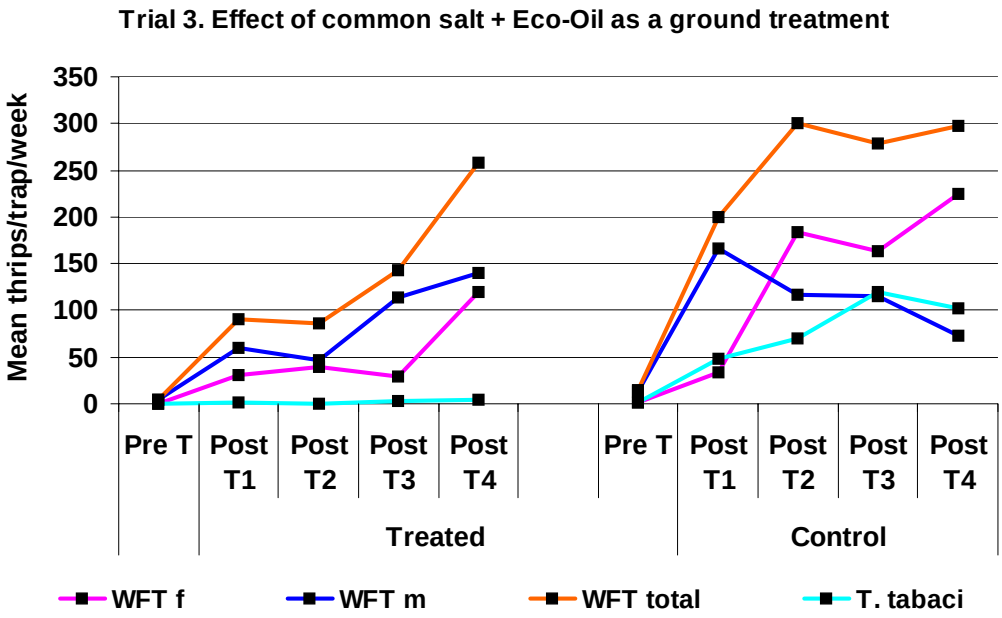

Figure 8. Effect of 10% common salt and 2% Eco-Oil as a ground treatment against thrips in greenhouse cucumbers. Two treatments were applied, on 31 October and 7 November 2005.

Figure 9. Dead thrips, mostly larvae, on treated plastic under cucumber leaf canopy.

Table 2. Thrips catches on yellow sticky traps following treatment with table salt plus Eco-Oil as a ground treatment for WFT control (T = treated, C = control).

Week	Mean/trap/7 days									
	WFT female		WFT male		WFT total		*T. tabaci*		*T. imaginis*	
	T	C	T	C	T	C	T	C	T	C
0	0.12	0.82	3.85	13.53	3.97	14.35	0.7	0.82	699.07	120.98
1	36.32	39.24	70.29	194.11	105.15	233.33	1.03	56.74	183.9	85.05
2	39.25	184	46.38	117	85.63	301	0.13	70.25	30.38	168.88
3	28.5	162.63	114.38	115.38	142.88	278.01	2.38	118.88	5.13	51.63
4	119.4	223.9	139.3	73.4	258.7	297.3	4	101.63	0.75	4.75

However, *Typhlodromips montdorensis* again invaded the crop in both houses, particularly in the control greenhouse, confounding results of the ground treatment. A leaf wash of 30 middle stratum leaves taken at the end of the trial produced a mean count of 4.27 thrips larvae and 7.40 predatory mites per leaf in the control greenhouse, and 30 thrips larvae and only 1.20 predatory mites per leaf in the treated house (Table 3). In previous experiments (Steiner & Goodwin 2004), rapid population declines of thrips have occurred when predator numbers equal or exceed thrips larval numbers, explaining the decline in thrips populations in the control house. The population of *Thrips tabaci* remained very low in the treated house, whereas it increased substantially in the untreated house. The reason why the effect of the treatment on WFT is less pronounced than on *T. tabaci* is possibly that large numbers of WFT pupated on the leaves instead of dropping to the ground. An unknown percentage of WFT therefore avoided the treatment, whereas it is suspected that onion thrips may not remain on the leaves to pupate. While thrips pupal numbers on the leaves were low in the leaf wash, taken late afternoon, it was observed that the number of pupae on leaves appeared far higher early the following morning. On traps, there was a male bias each week in the treated house, whereas the bias became female in week 2 in the control house, generally a prelude to a rapid increase in the WFT population.

Onion thrips populations on the middle stratum leaves were similar in both houses, but this may reflect the middle stratum sampled. Onion thrips much prefer lower leaves of cucumber (Steiner, pers. observ.). Plague thrips were numerous on traps at the start of the treatment and also declined more rapidly in the treated house. While they are believed to pupate at ground level, plague thrips do not generally feed or breed on cucumbers so the reason for the disparity in populations between treatments is not clear.

Table 3. Mean individual thrips and predatory mites per cucumber leaf (n = 30) from a middle stratum leaf wash on 28 November 2005.

	Mean per leaf				
	WFT adults	Thrips larvae	Thrips pupae	*Thrips tabaci*	*T. montdorensis*
Treated	1.00	30.0	0.60	1.10	1.20
Control	0.37	4.27	0.23	1.57	7.40

Trial 4 Pool salt plus Eco-Oil

Very few onion thrips were recorded. Western flower thrips populations on traps were lower in the treated house from week 2 after treatment (Figure 10). After 6 weeks, catches were approximately 50% lower in the treated house. Two leaf washes showed little disparity in thrips populations, but may have reflected reduced control following the single treatment. *Typhlodromips montdorensis* again invaded the crop, as shown by leaf wash samples (Table 4), reducing juvenile numbers, so the experiment was terminated after six weeks. Salt was evident as crystals during low humidity periods, but was liquid otherwise. Treatments should probably be repeated, possibly monthly.

Table 4. Mean individuals per cucumber leaf (n = 30) from a lower stratum leaf wash on 20 January 2006 (week 5).

Date collected		Mean per leaf				
		WFT adults	WFT larvae (late stage)	Thrips pupae	*Thrips tabaci*	*T. montdorensis*
20/1/06	Treated	2.73	2.5	5.83	0	0.17
	Control	5.13	1.63	3.87	0	1.3
30/1/06	Treated	8.77	4.17	13.77	0.2	6.0
	Control	7.16	4.77	12.07	0.23	2.97

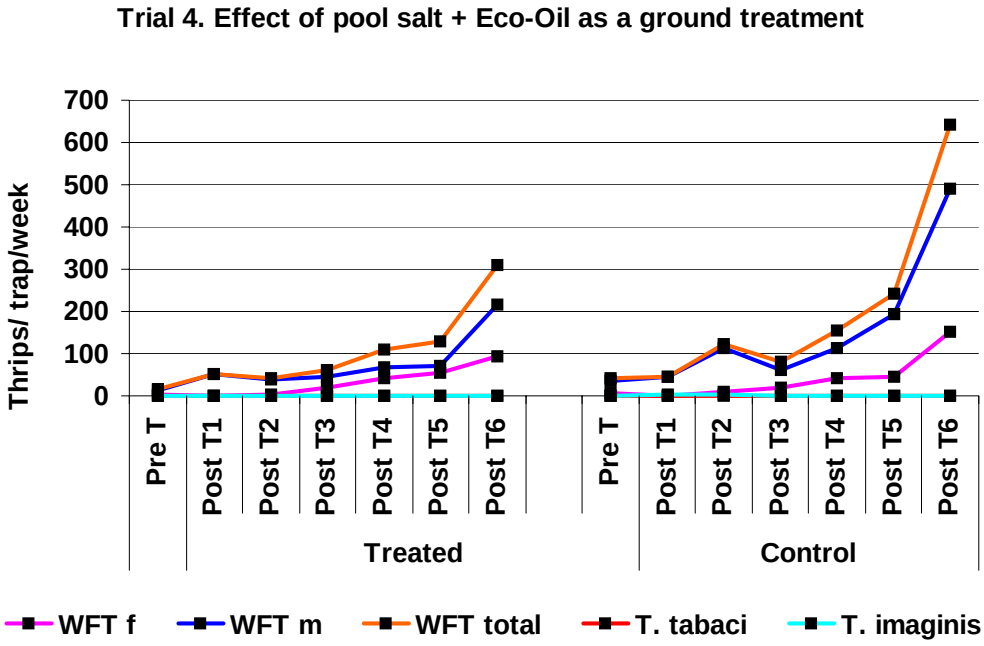

Figure 10. Effect of 10% pool salt + 2% Eco-Oil applied once on 14 December 2005 to a ground cover. Thrips were monitored with yellow sticky traps at canopy level from 15 December 2005-31 January 2006.

Trial 5 Calcium nitrate plus Agri-Terra.

The treatment gave good results against WFT as evidenced by trap counts (Fig. 11) and leaf washes (Table 5), though the material did not appear to have much longevity. The ratio of females to males was higher in the control population, predisposing it to a more rapid increase. Of the thrips collected from the plastic in the treated house, 6.1% were adult female, 8.8% adult male, 4.3% prepupae, 10.1% pupae, and 70.7% late stage larvae (n = 863). This confirms that late stage larvae are the predominant stage dropping to the ground, with a few pupae.

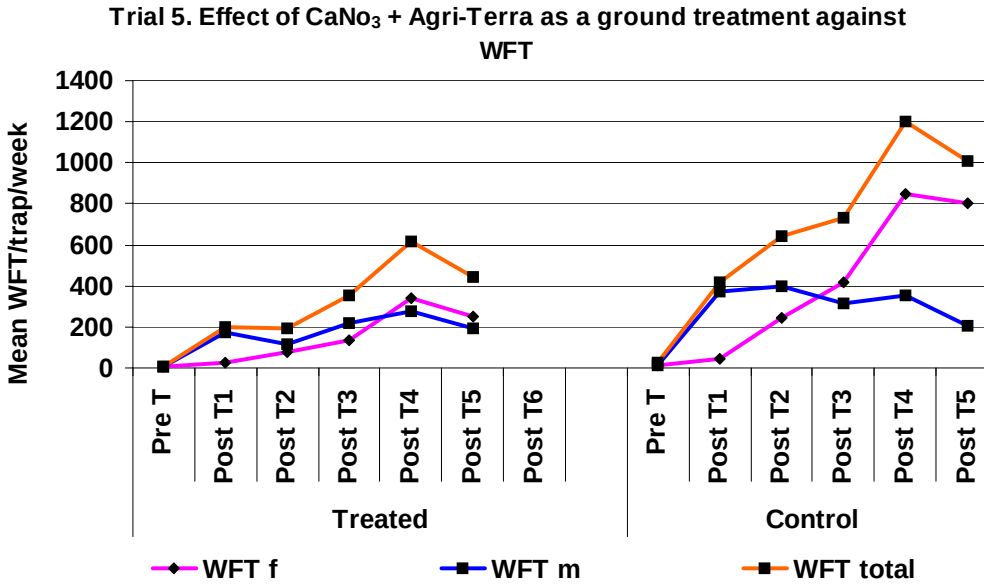

Figure 11. Effect of 10% calcium nitrate + 0.5% Agri-Terra applied 16 February and 9 March 2006 on western flower thrips in greenhouse cucumbers. Thrips populations were monitored with yellow sticky traps from 21 February to 5 April 2006.

Table 5. Mean individuals per cucumber leaf (n = 30) from a lower stratum leaf wash on 8 and 24 March 2006. (T = treated, C = control).

Date collected 2006	Mean per leaf							
	WFT adults		WFT larvae (late stage)		Thrips pupae		*Thrips tabaci*	
	T	C	T	C	T	C	T	C
8 March	2.93	5.90	2.97	2.63	4.17	5.90	0	0
24 March	11.46	25.74	6.93	18.2	16.66	32.0	0	0

Trial 6 Calcium nitrate + Agri-Terra + sodium chloride

Trap catches in the upper canopy showed low numbers of thrips in both treatments until the eighth week, with numbers higher in the treated house (Fig. 12). However, it was evident from visible leaf damage that in reality thrips were far more numerous in the untreated house. Leaf washes of lower stratum leaves confirmed this (Table 6). Traps hung in the lower canopy from week 8 to 10 indicated that WFT adults were distributed rather abnormally. In all previous experiments, upper traps captured more

WFT than lower ones, both for males and females. However, in this instance, females in the untreated greenhouse, but not in the treated one, were far more numerous on lower traps (Fig. 13). One possible explanation is that low traps were catching new females emerging at ground level rather than reflecting a general population, but this does not explain why a previous trial did not also detect this, or why males did not follow a similar pattern. Leaf washes of lower canopy leaves showed a female bias on each of the four dates in both houses.

Thrips tabaci and *T. montdorensis* were present in very low numbers. A small spider became numerous in both houses, but particularly in the untreated greenhouse. It was found on leaves, stem internodes and on the rockwool blocks and cocopeat bags, and was observed preying on thrips. Three species of laelapid mites were also found in washes of the rockwool blocks at the end of the experiment. They had been observed under the plastic sleeve of the rockwool cubes during the trial. Those in the control greenhouse were primarily *Stratiolaelaps scimitus*, whereas those in the treated greenhouse were *Hypoaspis* 'Gosford', an undescribed species already in culture at GHI. Fungus gnats were rare in both houses. Either predator may have contributed to thrips control at ground level.

Table 6. Mean individuals per cucumber leaf (n = 30) from a lower stratum leaf wash on four dates (T = treated, C = control).

Date collected 2006	Mean per leaf					
	WFT adults		WFT larvae		WFT pupae (% of immatures)	
	T	C	T	C	T	C
14 July	0.23	1.4	13.63	57.43	0.63 (4.42)	1.10 (1.88)
28 July	0.53	3.06	11.77	63.6	2.33 (16.52)	4.60 (6.74)
10 August	1.13	4.57	6.27	67.0	2.00 (24.18)	5.47 (7.55)
28 August	1.53	5.77	10.33	68.93	5.16 (33.31)	12.73 (15.59)

The respective ratios of larvae and pupae on leaves should give a rough measure of the percentage of thrips larvae dropping to the ground, as the ratio of time spent in each stage is constant at a fixed temperature. According to WFT life cycle data (Robb 1989), ~37% of immatures (pupae + larvae) should be pupae. In the literature, >90% of WFT late-stage larvae are reported to leave the plant to pupate in a range of crops, so that there should be very few pupae on the leaves. In Trial 6, the ratio as pupae on the leaves varied between the treated and untreated house (Table 6), but increased over time. Even accounting for some population increase, clearly an increasing percentage of larvae remained on the leaf to pupate rather than dropping to the ground, in the treated house perhaps almost all of them near the conclusion of the trial. This is most likely related to increasing humidity with greater crop canopy, but as there were no differences in RH and temperature between the two greenhouses, the disparity between them suggests another unknown factor is coming into play.

The application of a ground treatment was on the premise that most WFT larvae drop to the ground to pupate. This was clearly not the case in the greenhouses and crop under trial, and goes some way towards explaining why the results were not as good as expected for any of the treatments. Laboratory bioassays show few differences between most of the treatments trialled in the greenhouse, with efficacy >85% for all. Efficacy demonstrated in the greenhouse was closer to 50%, but it appears that this may be due to the failure of the thrips larvae to leave the plant under the conditions of the trial. More work is needed to determine whether relative

humidity is the determining factor for abandonment, and whether it can be manipulated to give better results. The advantage of any of these treatments is that they are relatively cost effective, not likely to result in any resistance problems, and can be integrated into an IPM program where predators such as *T. montdorensis* are used on the foliage, or even a laelapid mite in the media. The requirement is for full coverage of the floor with plastic, and debris to be kept to a minimum. Hydroponics systems using raised benches are ideal.

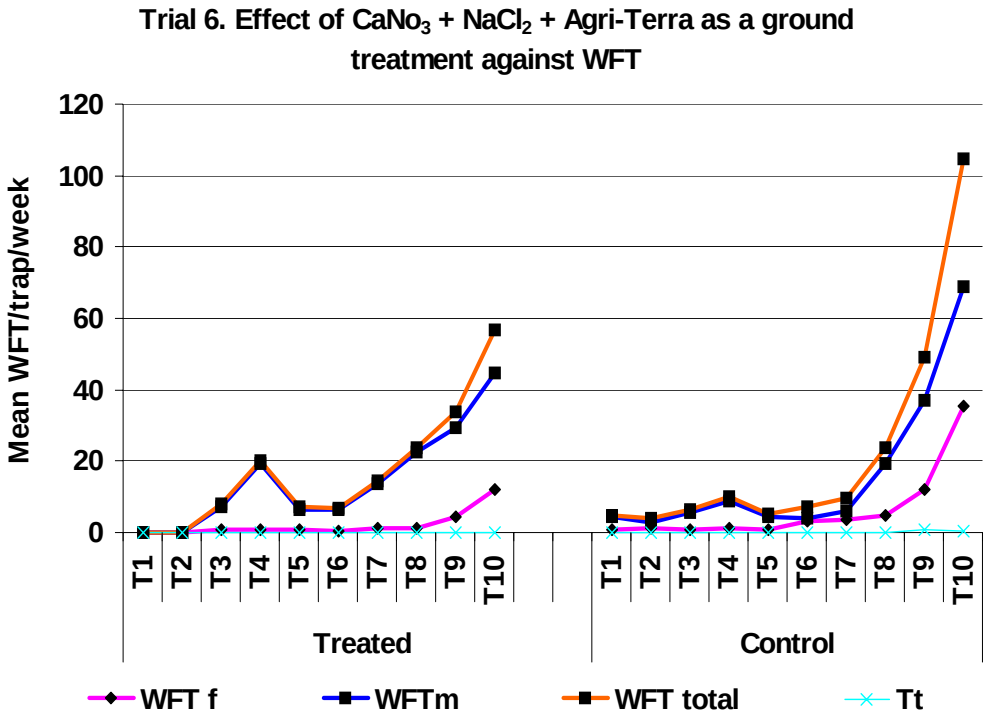

Figure 12. Effect of 10% sodium chloride + 10% calcium nitrate + 0.5% Agri-Terra applied 5 June and 18 July 2006 on WFT catches in greenhouse cucumbers. Thrips populations were monitored with yellow sticky traps placed in the upper leaf canopy from 9 June to 18 August 2006.

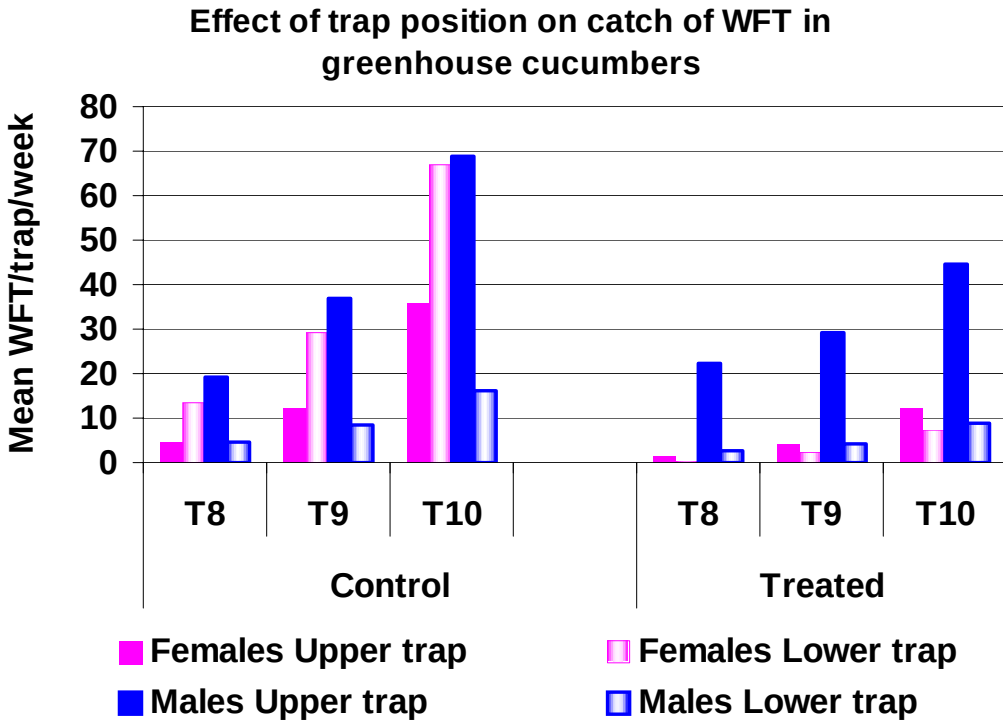

Figure 13. Catches of female and male WFT on yellow sticky traps in either the upper or lower canopy of a cucumber crop (n = 8). More WFT of both sexes are usually caught on upper canopy traps. The control house results are an anomaly.

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1.1.2 EVALUATION OF A KAOLIN FILM TO PROVIDE EARLY CROP PROTECTION AGAINST WESTERN FLOWER THRIPS, *FRANKLINIELLA OCCIDENTALIS* (PERGANDE) ON GREENHOUSE CUCUMBER AND CAPSICUM

INTRODUCTION

Western flower thrips (WFT) is a major pest of greenhouse vegetable crops, particularly cucumber and capsicum. Adults and larvae feed by rasping tissue and sucking sap from plant cells, causing direct damage in the form of leaf stippling and also scarring on cucumbers and stippling on capsicum fruit. Female thrips are attracted to nectar and pollen in flowers. In capsicum, plants are also very susceptible to infection by tomato spotted wilt virus, particularly when young. WFT is a very efficient vector of this virus.

Control of thrips is usually in the form of foliar sprays, which kill adult and larval thrips. There are few effective treatments, and rapid resistance to new pesticides such as spinosad has been documented (G. Herron, pers. comm. 2005). A predatory mite developed at GHI, *Typhlodromips montdorensis*, provides very good control by feeding on larvae, but it will not cope with large numbers of adult thrips. Tolerance of WFT is also very low early in a capsicum crop because of the virus issue. Most foliar sprays kill predators and parasitoids.

The aim of this experiment was to stop or deter thrips from laying eggs into foliage. This would reduce larval populations and thus possibilities for acquiring virus and carrying it as adults to new plants. A kaolin product (Surround™) has shown promise in protecting grapevines from leafhoppers and top fruit from various sucking pests. The clay coats the leaves and may deter oviposition and feeding. Because cucumber and capsicum are food crops and produce fruit early in their growth cycle, to avoid unsightly residues the product is only likely to be of use pre-fruiting. However, breaking the establishment cycle and reliance on toxic chemicals even for a 2-4 week period would be useful. Problems might be that some crops grow too fast to make this a practical treatment. The aim was to substantially reduce oviposition and feeding compared with untreated foliage.

MATERIALS AND METHODS

Cucumbers Two 54 sqm propagation greenhouses were used to assess treatments. In each house there were five rows of 28 plants, planted 3 October 2003 at the first true leaf stage. Planting density was four plants per cocopeat bag. The two outside rows were left untreated to build up thrips populations in the greenhouse. To start an infestation, adult WFT were taken from cultures and released in the outside rows on 3 and 7 October 2003. In each house the three treatment rows were each divided into three blocks of eight plants (Fig. 1). Of the eight plants in each block, four adjacent plants were randomly assigned to be treated and four were untreated. The crop was treated (i) immediately after planting out and (ii) as necessary to keep new leaves >4cm long covered with Surround on both surfaces. Control plants were water-treated on the same date. Surround was applied as a 6% W/V spray on 3, 7, 10, 13, 16, 20, 24, 28 October to new leaves only, and to whole plants on 30 October as the kaolin was flaking off older leaves and coverage had become poor.

Thrips were counted weekly for 5 weeks from 10 October. The underside of a lower leaf on each of the two central plants per plot (n = 18 in each house) was

checked. A middle stratum leaf was added from week 3. Larval thrips were counted as a measure of oviposition. Adult thrips were counted on the same leaves as a measure of settling. Feeding damage was difficult to see on kaolin-treated leaves so was not assessed. The trial was concluded when the first fruit was ~4cm long. There were 13-15 leaves at this time. Stem diameter and physiological responses to treatment were measured.

Capsicum The same two 54sqm greenhouses were replanted to capsicum on December 2003. Plants were set out in a similar fashion to the previous cucumber trial, with plants sprayed as necessary to cover new foliage. WFT were released 23, 30 December 2003, and 2, 9 January 2004. Surround was applied 2, 4, 9 and 16 January 2004 as a foliar spray. All leaves of the centre two plants in each plot were sampled weekly and numbers of adult and larval thrips were recorded. Predatory mites (*Typhlodromips montdorensis*) (~6000) were introduced into House 1 six days after final treatment (22 January) to assess their ability to establish on Surround-treated foliage. The number of thrips and predatory mites in 10 randomly selected, recently dehisced flowers in House 2, and 18 flowers in each treatment in House 1, were assessed 23 February 2004 by washing flowers and extracting with sieves of appropriate hole size.

Statistical analysis

The count data was $\log_e$ transformed prior to analysis to satisfy analysis assumptions. A split-plot-in-time ANOVA, which assumes that the correlation between insect counts on each treatment unit is the same between each assessment time, was used.

RESULTS AND DISCUSSION

Cucumbers There was a significant treatment effect, with fewer adult thrips on both middle and lower leaves of Surround-treated plants regardless of the assessment time. The treatment effect was consistent from week to week. For larvae, on lower leaves there were significantly fewer larvae on Surround-treated leaves only in weeks 2 and 5, with no significant difference at other times (Table 1). On middle leaves, larval counts were negligible for weeks 3 and 4 and no analysis was conducted. In week 5, Surround-treated leaves had significantly fewer larvae than the water control (Table 1). Data for middle and lower leaves and greenhouses were combined (Fig. 2). There were some onion thrips, *Thrips tabaci*, but most were WFT. There was a similar pattern of a larval peak in week 2 and an adult peak in week 3. Differences between treatments were greater for middle stratum leaves, and greater for larvae than for adults. No growth differences were apparent or measurable between treated and untreated plants. While some repellency may have occurred, inhibition of oviposition appears to be the main mechanism for reduced populations on treated foliage. Surround had limited stickability and flaked off lower leaves. With the greatly increased leaf growth following first treatment, this had a diluting effect on coverage. Whole plant treatment would help to mitigate this. Because of the rapid growth rate of cucumbers, application frequency was probably too often to make this an attractive control option, but would have a place if virus was an issue, there was a lack of alternative controls, or there were sequential plantings in the same greenhouse where the older crops were infested. The kaolin in Surround has a tendency to settle out rapidly and constant agitation was necessary in the spray tank, along with a coarse

nozzle to prevent clogging. At the conclusion of the trial, two fungal pathogens were observed affecting the thrips in both houses. One was subsequently captured and identified as *Beauveria bassiana*, and the other was tentatively identified as an *Entomophthora* sp.

Capsicum There were problems with the irrigation and fertiliser system in the second greenhouse so that only three of the nine blocks were useable. As with the cucumber crop, Surround application to foliage appeared to inhibit thrips egg laying, resulting in fewer larvae (Table 2). The general thrips infestation level was low, probably because adult thrips need a pollen source on capsicum to optimise egg laying, and the crop was not yet at flowering stage. Adult thrips were significantly lower on Surround-treated plants in the first week. Numbers were too low to analyse in weeks 2 and 3. Larval thrips were significantly lower on Surround-treated plants in weeks 2 and 3, and there was no significant difference in week 1. Predatory mites established in similar numbers on capsicum whether or not Surround had previously been applied (Table 3). Thrips populations were similarly controlled. Thrips populations in House 2, where predatory mites were not introduced, were considerably higher after 5 weeks than in House 1. Surround can thus be used early in a capsicum crop to protect plants against oviposition by thrips, prior to release of predators that might require pollen and the higher humidity of a full canopied crop. Capsicum is most susceptible to TSWV when young, so the protection afforded by Surround is an attractive and viable treatment. Plants do not grow as fast as cucumber so less frequent spraying is required to keep them fully covered. Surround had no discernable impact on growth.

Table 1. Populations of thrips (mostly western flower thrips) on kaolin-treated (K) and untreated (C) greenhouse cucumbers in two houses. Surround (kaolin) was applied to new foliage every 3-4 days over 5 weeks.

House	Week	Mean # adult thrips/leaf				Mean # larval thrips/leaf			
		Middle leaf		Lower leaf		Middle leaf		Lower leaf	
		K	C	K	C	K	C	K	C
1	1	-	-	1.72	3.11	-	-	0.28	2.89
	2	-	-	0.89	1.5	-	-	3.11	14.39
	3	12.67	28.38	14.61	19.83	0.06	1.33	3.28	1.22
	4	0.39	1.67	1.39	3.0	0	0.56	0.44	1.22
	5	0.22	1.06	0.72	2.11	0.17	3.72	2.28	9.89
2	1	-	-	2.33	6.83	-	-	0.06	2.0
	2	-	-	2.94	7.06	-	-	4.0	21.5
	3	35.5	43.5	36.5	41.72	0	2.33	3.39	5.0
	4	5.94	7.0	7.06	9.39	0.06	0.94	0.61	1.5
	5	3.83	6.83	5.39	6.61	0.78	10.33	14.22	27.33

Table 2. Populations of thrips on young capsicum plants treated or untreated with Surround (n = 24).

Week	Adult thrips/plant		Larval thrips/plant	
	Surround	Control	Surround	Control
1	0.21 ± 0.10	0.54 ± 0.19	0.33 ± 0.12	0.67 ± 0.22
2	0	0.29 ± 0.13	0.38 ± 0.22	3.08 ± 0.82
3	0.04 ± 0.04	0.08 ± 0.06	0	1.04 ± 0.31

Table 3. Populations of thrips and predatory mites (*T. montdorensis*) per capsicum flower on 23 February 2004, five weeks after final application of Surround. 6000 predatory mites were introduced on 22 January 2004 (~35/plant).

House	Mean # predatory mites/flower	Mean # thrips/flower		n
		Adult thrips	Larval thrips	
1 (Control)	32.1	1.46	0.17	18
1 (Surround)	42.9	2.08	0.11	18
2 (Control)	0	32.9	24.2	10

Figure 1. Greenhouse set-up with cucumber (left) and capsicum (below), with some plants treated with Surround film against thrips.

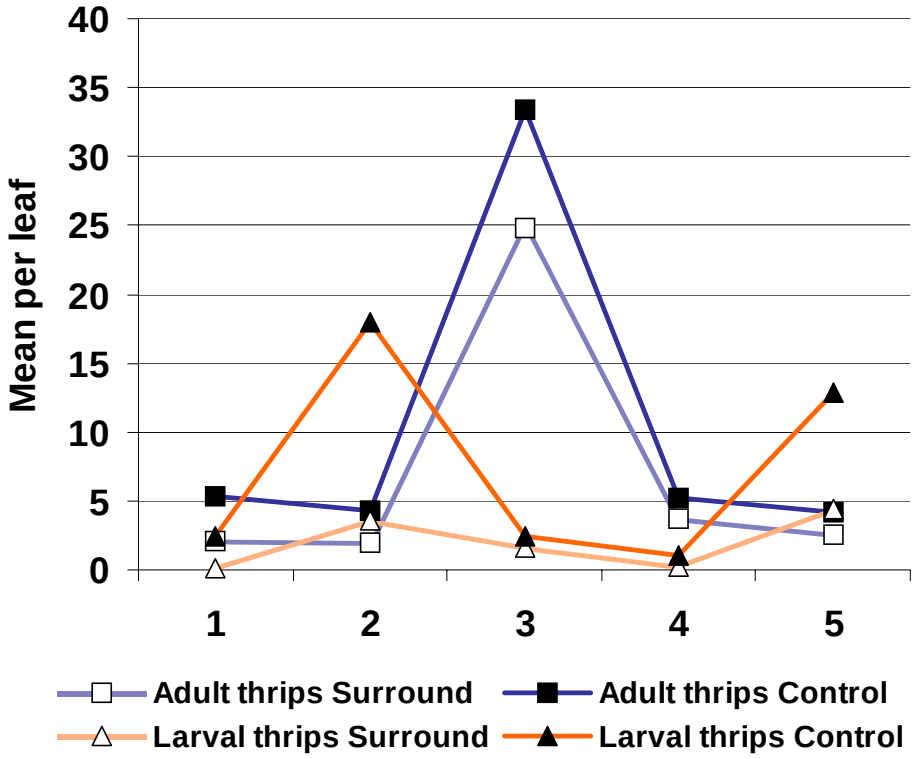


Figure 2. Populations of adult and larval thrips on middle and lower leaves of young greenhouse cucumber plants over 5 weeks. Plants treated with Surround (kaolin) were sprayed every 3-4 days to cover new foliage.

1.1.3. MANAGEMENT OF WESTERN FLOWER THRIPS, *FRANKLINIELLA OCCIDENTALIS* (PERGANDE), IN HYDROPONIC LETTUCE

1.1.3.1. EVALUATION OF ALUMINIUM FOIL AS A REPELLENT FOR WESTERN FLOWER THRIPS, *FRANKLINIELLA OCCIDENTALIS* (PERGANDE), IN HYDROPONIC LETTUCE

INTRODUCTION

Lettuce is a crop that is commonly infested with onion thrips, *Thrips tabaci* Lindeman, and western flower thrips (WFT) in New South Wales. Onion thrips is more commonly a field crop pest and western flower thrips occurs most frequently in hydroponic crops. Both thrips species vector tomato spotted wilt virus, and it is this rather than direct feeding damage that is the cause of most crop loss. Losses vary with the season and the year, but can be almost total. There are few effective toxicants for thrips management, especially in hydroponic crops where chemical residues are more persistent than in the field and present an additional problem. Overuse of the few pesticides registered has resulted in the development of resistance to these products.

Silver mulches have been used in field crops to repel virus vectors such as aphids and thrips. This experiment was designed to test whether aluminium foil applied over the irrigation channel might be an inexpensive option for partial thrips management in a hydroponic crop.

MATERIALS AND METHODS

Two $18m^2$ greenhouse units covered with reinforced plastic were set up for NFT hydroponic production. There were eight growing channels 12cm wide and 3m long in each unit, with 15 planting holes per channel, on a recirculated nutrient system. Lettuce seedlings of two cultivars, Green Oak Kristine and Red Oak Jamai, were set out on 9 March 2006. The foil-no foil treatments were assigned alternately to blocks two channels wide and half a channel long, with 7 plants in each half channel and the centre hole in each channel left empty. Within each block a split plot design was super-imposed so that one row was randomly assigned to the green cultivar and one to the red. This resulted in eight replicates per treatment in total. The foil was wrapped around the channel so that both upper and lower surfaces were covered. The foil was placed on 10 March and adult WFT were released from a laboratory culture on the same day by spreading vermiculite containing the thrips on the concrete floor below the benches holding the channels.

After 7 days, an assessment of thrips damage was made by grading leaf damage on each of the five centre plants per block on the scale 0 = no damage, 1 = very slight damage, 2 = 1-2 leaves damaged, 3 = >2 leaves damaged. These plants were then removed, minus roots, and bagged individually. Each plant was weighed and then the five plants per block were bulked and washed through to assess thrips numbers per plant. Counts were made separately of adult and larval thrips.

Statistical analysis

The experimental set-up was a split plot design with the main plot being the aluminium foil treatment and split for lettuce cultivar (2 x 2 factorial). Analysis of variance was used to test the effect of foil, cultivar and their interaction on thrips numbers, leaf damage and plant weight.

Adult and larval thrips counts
A log transformation on thrips numbers was undertaken to satisfy the assumptions of analysis of variance.

Leaf damage
This was assessed on an ordinal scale (0, 1, 2, 3), but since an average of five plants was analysed, methods appropriate for normally distributed data (ANOVA) were used.

RESULTS AND DISCUSSION

Thrips counts
Populations of both adult and larval WFT were significantly higher (P = 0.5) on the green lettuce cultivar (7 adults and 236.6 larvae per plant) than on the red cultivar (2.05 adults and 88.5 larvae per plant) (Fig. 1). Use of aluminium foil over the channels significantly reduced thrips numbers further in both cultivars (in green to 4.3 adults and 85.6 larvae, and in red to 1.4 adults and 47.6 larvae per plant).

There was also a significant linear relationship between the number of thrips larvae and adults per plant, which was the same for both cultivars. The rate of increase in thrips populations did not change when foil was used.

Plant weight
There was no significant effect of foil or lettuce cultivar on plant weight. The interaction of cultivar colour and foil was not significant, implying that these responses were consistent across treatments and cultivars.

Plant damage
Damage grading was significantly less in the lettuce from the treatments where foil was present (1.975 compared with 2.428). This response was consistent regardless of lettuce cultivar. The green cultivar had significantly higher leaf damage scores (2.712) than the red cultivar (1.7). This response was consistent regardless of foil presence or absence.

Silver colours reportedly repel adult thrips, hence the reduction in numbers. Once the plant grows over the silver foil, little impact could be expected. An advantage might be conferred in reducing initial thrips populations and thus the chances of thrips carrying TSWV to plants at their youngest and most susceptible stage. The short term effects would need to be justified by low cost of such a treatment.

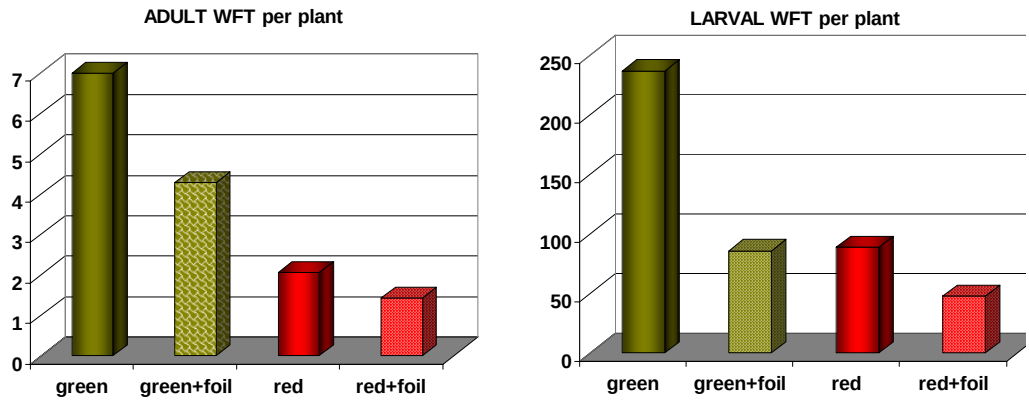


Figure 1. Populations of western flower thrips on green and red cultivars of hydroponically-grown lettuce with and without aluminium foil over the growing channels.

1.1.3.2 EVALUATION OF FOLIAR TREATMENTS AGAINST WESTERN FLOWER THRIPS, *FRANKLINIELLA OCCIDENTALIS* (PERGANDE), IN HYDROPONIC LETTUCE

INTRODUCTION

In a recent trial on a commercial hydroponic lettuce farm, it was noted that while treatments for tomato spotted wilt virus (TSWV) within the trial area were not effective, incidence of tomato spotted wilt virus in these beds was substantially lower than in adjacent grower beds. Two possible factors contributing to this reduced incidence were (i) in order to reduce TSWV spread, we removed plants once 25% of leaves showed symptoms of TSWV, and (ii) wet sprays, regardless of content, might have impacted on WFT establishment. Overhead irrigation is known to reduce thrips populations and fruit damage in strawberries. Water and other reduced-risk pesticides were trialled at Gosford on hydroponic lettuce to clarify whether water and also other reduced-risk pesticide treatments might have an impact on WFT populations.

MATERIALS AND METHODS

Trial 1 Two greenhouse units each 18m^2 covered with reinforced polypropylene plastic were set up with lettuce seedlings of the cultivar Green Oak Kristine on 29 June 2006 (Fig. 1). In each unit there were eight growing channels 12cm wide and 3m long, with 15 holes per channel, on a recirculated nutrient system. There were four treatments applied as foliar sprays to incipient run-off: 1. No treatment (control); 2. 0.1% Silwet™ L-77 as a surfactant applied twice weekly; 3. 0.5% Eco-Oil™ applied weekly; 4. DPI 9 (*Beauveria bassiana* Gosford-collected strain) at 22g spores plus 500mL oil and surfactant/100L, applied weekly. The four treatments were assigned randomly within blocks two channels wide and half a channel long, with six plants in each half channel, and two centre holes and one or two at each end left empty. This resulted in eight replicates per treatment in total. Adult WFT were released from a laboratory culture late afternoon on 4 July and again on 21 July by spreading vermiculite containing the thrips on the concrete floor below the benches holding the channels (~2000 thrips/unit on each occasion). Pesticide treatments were initiated the morning of 5 July. Approximately 300-400mL in total was applied for each material at each treatment date, increasing with greater leaf area.

After 4 weeks, on 31 July, an assessment of thrips damage was made by grading leaf damage on each of the four centre plants per plot on the scale 0 = no damage, 1 = very slight damage, 2 = 1-2 leaves damaged, 3 = >2 leaves damaged. These plants were then removed, minus roots, and bagged individually. Each plant was weighed and then washed through to assess thrips numbers. Counts were made separately of adult and immature (larval + pupal) thrips collected by washing through a screen (112µm hole size).

Trial 2 The two greenhouse units were set up in a similar way. The cultivar was Green Sun, a green type with somewhat more horizontal leaves. There were eight treatments with four replicates of seven plants (two replicates in each Bay) per treatment, arranged in a randomised block design. The plants were set out as seedlings 29 September 2006 and adult WFT released 3 October (~1500 per greenhouse unit) by sprinkling thrips in vermiculite on the floor beneath the channels. Treatments were applied 4 October as sprays to run-off. Treatments were: 1. No treatment; 2. water

only twice weekly; 3. 0.1% Silwet L-77 twice weekly; 4. 0.05% Silwet L-77 twice weekly; 5. 0.02% Silwet L-77 twice weekly; 6. 0.1% Agral twice weekly; 7. DPI 9 (*Beauveria bassiana* Gosford-collected strain) at 22g spores plus 500mL oil and surfactant/100L, applied weekly; 8. DPI 9 (*Beauveria bassiana* Gosford-collected strain) at 22g spores plus 500mL oil and surfactant/100L, applied twice weekly. Plants reached marketable size and were harvested on 27 October 2006. The five centre plants in each replicate were weighed individually and bulked for extraction of thrips as previously.

Statistical analysis

Analysis of variance of the plant weights and log transformed thrips counts was conducted.

RESULTS AND DISCUSSION

Trial 1 Leaf damage was very minor in all treatments so was not assessed beyond noting that there was no apparent leaf damage in T1 and occasional minor damage on outside leaves in the other three treatments. Leaf damage in the form of brown edges on new leaves was apparent in T2, and plants were visibly smaller. Plant weights and thrips numbers are shown in Figure 2.

The mean head weight of lettuce in T2 (water plus 0.1% Silwet) was significantly lower than in T1, T3 and T4, which were not significantly different from each other (Table 1). The most likely explanation for this is that Silwet was phytotoxic, borne out also by the brown tips to new leaves. Adult thrips populations were significantly lower in T2 than in all other treatments (Table 1), and T4 was significantly lower than T1, representing reductions of 97.2% (T2), 54.3% (T4), and 23.7% (T3). Juvenile thrips populations were significantly reduced by all treatments, representing reductions of 95.2% (T2), 59.1% (T4), and 33.9% (T3). Thrips populations were relatively low, which was partly due to a malfunction in temperature control providing low temperatures (12.8-23.7, mean 18.9^{o}C in Bay 1 and 8.0-23.7, mean 18.1^{o}C in Bay 2), and possibly due to a concrete floor which would have reduced survival of those larvae dropping to the ground to pupate because of the lack of shelter and low humidity (26.9-100%, mean 67% RH in Bay 1, and 23.1-98.8%, mean 64.8% RH in Bay 2). 50.6% of immature stages were pupae. The percentage of thrips in various life stages varied between treatments (Table 2). For T2, there is a marked reduction in the percentage of thrips in the pupal stage, and a corresponding increase in those in the adult stage. One hypothesis is that the treatment interferes with successful pupation of the larvae. It is apparent in the other treatments that a high percentage of thrips pupated within the lettuce plants rather than dropping to the ground to pupate. A few may have pupated under the plant or in the media and these were not counted. It is suspected that relative humidity may influence the decision to drop to the ground, and that in a more humid environment the percentage leaving the plant might increase.

The substantial reduction in WFT populations by application of water plus Silwet is very encouraging, but the influence of Silwet needs to be separated from water alone and the experiment repeated with lower rates of Silwet or an alternative wetter because of the phytotoxicity noted. No plant damage was noted from application of 0.5% Eco-Oil weekly, but the reduction in thrips populations was not

adequate. DPI 9 gave >50% control of WFT, which might be expected to improve with an increase in temperature and humidity.

Trial 2 Tip burn, possibly caused by temperature and humidity being too high and restricting calcium uptake, obscured any phytotoxicity due to treatment effect, except to note that plants treated with 0.1% Silwet were again visibly smaller. A subsequent trial conducted 13 November -11 December 2006 with cv Ember and the same rates of Silwet gave head weights of 58.93, 63.13, 56.58 and 47.29g for control, 0.02, 0.05 and 0.1% Silwet respectively. Head weights at 0.1% were significantly lighter than the other three rates (ANOVA, P = 0.05). Apart from visibly smaller plants, damage in the form of browning of a few leaves was evident at the 0.1% rate and rarely at the 0.05% rate.

Assessment of thrips populations was more complex because three additional species were present: plague thrips, *Thrips imaginis*, onion thrips, *Thrips tabaci*, and tomato thrips, *Frankliniella schultzei*. Onion thrips and tomato thrips are of interest because they may carry TSWV. Many of the plague thrips were shrunken and it is suspected that they do not survive on lettuce. Larval thrips could not be separated as to species. There were small numbers of orange larvae that were probably those of the darker *F. schultzei*.

Mean temperature over the four week period was ~21.0^{o}C (Table 3). Mean relative humidity was ~80-85% in the first two weeks and ~90% in the second period. Higher day temperatures equated to lower humidity. Because of less night heating and a rainy period the relative humidity was much higher and the temperature a little higher than in the previous trial.

An outbreak of lettuce aphid, *Nasonovia ribisnigri*, necessitated a foliar application of Pirimor™ to all plants mid-crop. Many dead thrips were noted after application, but as Pirimor is not known to affect onion thrips or WFT, it is suspected that these may have been *Thrips imaginis*, possibly affected by Pirimor, but more likely dying anyway from being on a non-host plant.

Differences in head weight were not significantly different statistically (Table 4). Thrips counts were analysed by comparing thrips immature stages and adults of the four species separately. For adult WFT, means for all treatments ranged from 4.25 to 14.4 per head, but there were no significant differences between treatments. For thrips larvae, means ranged from 6.35 to 33.05 per head with significant differences between some treatments (Table 4). Silwet at the two higher rates were the only treatments significantly different from the control. Clearly water alone at this application frequency did not reduce thrips populations and may have increased them. Major differences between replicates obscured treatment differences. For example, adult *Thrips tabaci* counts were higher in T2, T6 and T8 of Block 2, and T8 of Block 4, with higher larval counts here also, indicating that at least some of the larvae may have been those of *T. tabaci* rather than WFT. Adult counts of WFT were much higher in Block 4, T3, less so in T6 and T8, with notably higher larval numbers in T3 and T8, but not T6. Whether there was an uneven distribution of WFT or just a peculiarity of the greenhouse set-up is not known, but the high local adult population may have lowered the apparent efficacy of T3, T6 and T8 against WFT, and T2, T6 and T8 against *T. tabaci*. Mean numbers of *T. imaginis* per treatment varied between 17.57 and 34.14 per head, but because of the difficulty of discerning whether they were dead or alive when collected, statistical analysis of the data was not performed.

The percentage of thrips in the pupal stage relative to the larval stage is much lower in this trial than in the previous one. One possibility is that more thrips late-

stage larvae left the plant to pupate because of the more favourable relative humidity of the surrounding air, but this is contrary to experience in cucumbers, where more larvae remained on leaves at higher relative humidity. Differences in plant structure and thus localised humidity may also have played a part. The question of what influences thrips larvae to stay or leave the plant to pupate is an interesting one and has a bearing on the choice of control strategies in different crops.

The cultivar used in this trial was a green variety, but leaves were displayed horizontally making underleaf spray coverage poor, probably explaining the lower efficacy of 0.1% Silwet compared with the first trial. The increased adult WFT numbers in this treatment compared with the unsprayed control contrasted sharply with the very low numbers recorded in the first trial. Overall, Silwet still provided the best control of WFT, with the lower two rates preferable to minimize the possibility of phytotoxicity.

Figure 1. Set up for hydroponic lettuce trial.

Table 1. *Trial 1* Weight of hydroponic lettuce and western flower thrips populations after treatment with Silwet, Eco-Oil or DPI 9. (n = 32). Means followed by the same letter are not significantly different (P = 0.05).

	Head weight (g)	Adult thrips/head	Juvenile thrips/head
T1 (Control)	130.38 b	10.03 b	20.97 c
T2 (water + 0.1% Silwet)	98.27 a	0.28 a	1.00 a
T3 (0.5% Eco-Oil)	124.58 b	7.65 b	13.87 bc
T4 (0.022% DPI 9)	132.52 b	4.58 b	8.58 b

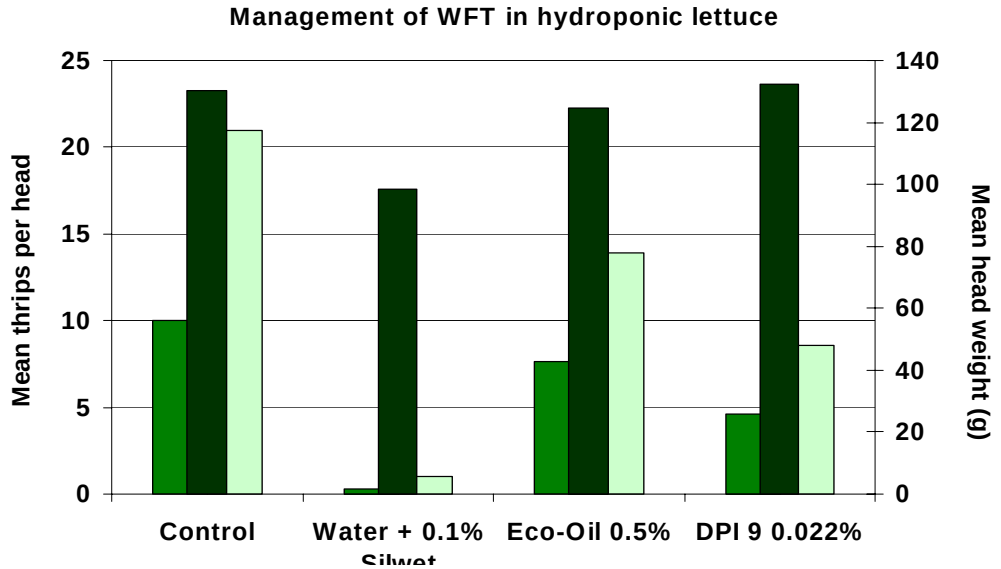


Figure 2. Western flower thrips populations and head weight of hydroponic lettuce after three weekly (Eco-Oil, DPI 9) and six biweekly (Silwet L-77) foliar applications.

Table 2. *Trial 1* Percentage of total WFT population in various life stages in four treatments.

	Adult female	Adult male	Larva	Prepupa	Pupa
T1 (Control)	7.32 ± 1.15 c	9.43 ± 1.55 c	43.42 ± 5.64 a	5.09 ± 1.19 a	34.74 ± 6.79 a
T2 (0.1% Silwet)	35.82 ± 7.64 a	16.42 ± 4.78 ab	34.33 ± 11.46 a	0.00 b	13.43 ± 5.25 b
T3 (0.5% Eco-Oil)	10.24 ± 1.47 b	11.15 ± 1.90 bc	35.28 ± 5.44 a	6.95 ± 1.76 a	36.38 ± 5.10 a
T4 (0.022% DPI 9)	9.02 ± 1.52 bc	18.31± 3.22 a	34.15 ± 5.17 a	7.38 ± 2.20 a	31.15 ± 6.95 a

Table 3. *Trials 1 and 2* Temperature and relative humidity means and ranges during trials evaluating reduced-risk pesticides on thrips in hydroponic lettuce.

Period (2006)	Temperature ^{o}C				Relative humidity %			
	Mean		Range		Mean		Range	
	Bay 1	Bay 2	Bay 1	Bay 2	Bay 1	Bay 2	Bay 1	Bay 2
29/6-1/8 (Trial 1)	18.9	18.1	12.8-3.7	8.0-23.7	67.0	64.8	26.9-100	23.1-98.8
28/9-19/10 (Trial 2)	21.0	20.6	15.4-9.4	12.0-8.3	79.8	84.9	36.4-100	35.4-100
19/10-27/10 (Trial 2)	21.0	21.0	15.4-7.6	15.4-7.2	90.6	92.1	55.0-100	54.31-100

Table 4. *Trial 2* Thrips populations on lettuce after treatment with Silwet, Agral and DPI 9 (*Beauveria bassiana*) (n = 32) and head weight. Means followed by the same letter are not significantly different (P = 0.05).

Treatment	Head weight (g)	Adult WFT/ head	Adult *T. tabaci*/ head	Adult *F. schultzei*/ head	Juvenile thrips/ head
Control	109.7 ns	11.25 ns	11.15 ns	1.1 ns	18.15 cd
Water 2x weekly	109.66	14.2	10.9	1.6	33.05 d
0.1% Silwet 2x weekly	104.33	13.3	5.8	1.15	6.35 a
0.05% Silwet 2x weekly	113.82	7.6	9.65	1.2	6.95 ab
0.02% Silwet 2x weekly	112.65	4.25	8.85	1.35	7.35 abc
0.1% Agral 2x weekly	111.33	9.8	13.65	1.05	12.5 abc
0.022% DPI-9 weekly	110.04	8.4	8.3	0.7	10.5 abc
0.022% DPI-9 2x weekly	113.6	14.4	12.3	1.75	14.8 bcd

Table 5. *Trial 2* Percentage of total WFT population in various life stages in four treatments.

	Adult female	Adult male	Larva	Pre pupa	Pupa
T1 (control)	22.5	11.03	52.31	1.79	12.37
T2 (water)	20.47	8.6	64.89	2.76	3.28
T3 (0.1% Silwet)	42.33	18.54	24.49	4.58	10.07
T4 (0.05% Silwet)	29.15	18.5	36.36	7.21	8.78
T5 (0.02% Silwet)	20.89	8.22	45.21	5.14	20.55
T6 (0.1% Agral)	25.96	15.74	49.57	3.62	5.11
T7 (0.022% DPI 9) weekly	25.06	12.2	45.23	1.33	16.19
T8 (0.022% DPI-9) 2x weekly	28.46	14.91	39.61	4.97	12.05

1.1.3.3 EVALUATION OF TWO ANTI-TRANSPIRANT FILMS FOR ABILITY TO REDUCE TOMATO SPOTTED WILT VIRUS TRANSMISSION BY WESTERN FLOWER THRIPS, *FRANKLINIELLA OCCIDENTALIS* (PERGANDE), IN LETTUCE.

INTRODUCTION

Tomato spotted wilt virus is a major problem in both field and greenhouse lettuce crops in the Sydney Basin. Onion thrips, *Thrips tabaci* Lindeman, is the primary vector in field crops, and western flower thrips (WFT) in crops under cover. Thrips can cause extensive feeding damage in their own right, leaving irregular spots on the foliage. Resistance to pesticides is common, primarily because both species are constantly invasive, the choice of effective pesticides is narrow, particularly for WFT, and multiple applications are common. If TSWV is also carried into the crop from surrounding infected weeds, then tolerance for thrips is much lower. Adult thrips carrying the virus may feed on several plants. If infected plants are not removed immediately, larvae developing on the infected plant can acquire the virus and once they are adult can increase its spread within the crop. 100% loss of crop in some cultivars is not uncommon.

Allen *et al.* (1993) tested the antidessicant Wilt-Pruf™ (1:6 β–pinene emulsion, a.i. di-1-p-menthene) and other film-forming products for their potential to inhibit virus transmission. Wilt-Pruf reduced transmission of TSWV by thrips on petunias by 73%. The product is slowly polymerised by sunlight into a persistent, clear, colourless and flexible film. Two recent formulations available in Australia are Stress-Ex™ (904 g/L di-1-p-menthene; Ekko Carrum Downs, VIC), which is sold as an anti-transpirant concentrate for fruit, vegetables and ornamentals, designed to last 60-90 days, and Flextend™ (859g/L di-1-p-menthene), a non-ionic extender-sticker-spreader designed to enhance the coverage and longevity of pesticides. Label rate for Stress-Ex for non-woody plants ranges from 0.5-2%, depending on use and crop type. The rate for Flextend is 300mL/ha (0.3% for field lettuce crops), except as a pesticide extender, when the rate is 1.2L/ha. The products are similar but the chain lengths of the molecules differ. Flextend has a shorter residual life.

An initial trial was conducted to assess plant safety of the two products. Two separate trials were then conducted on commercial lettuce grown hydroponically under cover to assess virus reduction. The first involved the application of Flextend and Stress-Ex and the second used only Stress-Ex. Both trials were conducted at a commercial site at Glenorie, NSW and were on hydroponic lettuce cv Red Coral.

MATERIALS AND METHODS

Phytotoxicity trial

Application of both Flextend and Stress-Ex to mature, hydroponically-grown lettuce in 18m^2 greenhouses at GHI was made on 21 December 2005 to cv Red Coral to cover all leaf surfaces. There were three replicates of 10 plants per treatment. Product rates were 0.5% and 1%, with a water control. An application was also made to green and red lettuce varieties under shade cloth on site. Plants were examined for phytotoxicity symptoms on 23 and 28 December.

TSWV inhibition trials
Trial 1 14 February-21 March 2006
The following treatments were applied:
1. Stress-Ex at 0.5% at transplanting, plus Flextend at 0.3% every two weeks
2. Stress-Ex at 1% at transplanting, plus Flextend at 0.3% every two weeks
3. Flextend at 0.3% at transplanting, and every two weeks
4. Flextend at 0.3% at transplanting, and weekly thereafter
5. Control (water)

Trial 2 28 March – 3 May 2006.
The following treatments were applied:
1. Stress-Ex at 5% at transplanting, and after two weeks then Stress-Ex 1% weekly thereafter
2. Stress-Ex at 5% at transplanting and after two weeks, then Stress-Ex 1% after two weeks
3. Stress-Ex at 1% at transplanting, plus Stress-Ex 1% every two weeks
4. Stress-Ex at 1% at transplanting, plus Stress-Ex 1% weekly thereafter
5. Control (water)

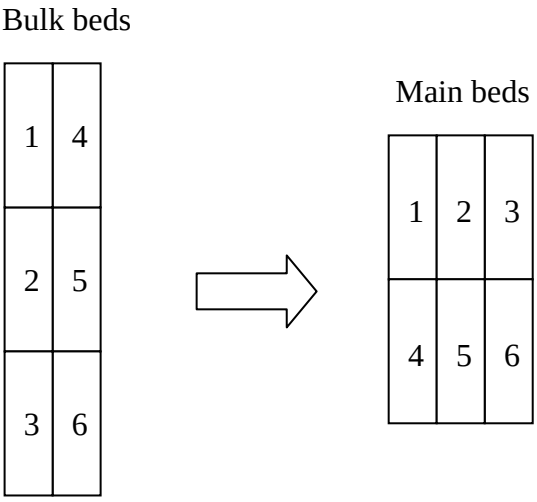
Stress-Ex was initially applied at 5% in T1 and T2 in Trial 2 to reflect the rate of Wilt-Pruf™ used in the Canadian trial (Allen *et al.* 1993), rather than the label field rate of 0.5 to 2%. It was lowered after the second week to avoid phytotoxicity or deposits at harvest.

The commercial lettuce crop was grown hydroponically in 8-row channels. As per cooperating grower practice, transplants were initially grown on for two weeks (16 days) in an 11-channel wide bulk bed where holes were more closely spaced and in two similar channels above the older crop. They were then moved to the main channel for a further two weeks (19 days) before harvesting. The transplants arrived from the propagators 13 February (Trial 1) and 27 March (Trial 2) and were moved to the initial channels on the same day. A block of these was assigned for the treatments. The set up for the five treatments was an extended Latin square with six replicates per treatment and 36 plants per plot. In the first channels, each treatment plot consisted of two adjacent rows of 18 plants. Guard plants were situated in one outside channel, five plants at the end of the channels and three plants between treatments. In the main channels, the 36 plants per plot were placed in three adjacent rows of 12 plants with two untreated guard channels between. Three 8-row sets of main channels were used, two replicates per set.

Treatments (Table 1) were applied with either a 2 litre hand-held pressurised sprayer or a 15 L knapsack sprayer depending on whether the bulk beds or main channels were being treated. Treatments not scheduled for a chemical spray were treated with water only. The first treatments were carried out early on the day following transplanting into the bulk beds, 14 February (Trial 1) and 28 March (Trial 2) respectively. Monitoring for virus symptoms was carried out twice weekly for Trial 1 and weekly for Trial 2. Plants with TSWV symptoms were classified into three categories as Trial 1 progressed ((Table 2). Plants showing >25% TSWV leaf symptoms (necrotic spots) were removed, bagged individually, and washed through screens to extract thrips. Thrips were separated into larval and adult thrips with adults separated to species and sex.

Agdia™ kits were used to confirm TSWV infection initially and where there was any doubt.

Three applications of treatments were undertaken on the bulk beds while the remaining two were carried out on the main channels. After the third treatment the plants were moved from the bulk beds to the main channels. Placement of the plants from the two-row arrangement of the 36 plants to the three-row arrangement followed a set pattern dependent on the position of the remaining plants in each plot:



Yellow sticky traps were placed 50cm over the bulk beds (four in Trial 1 and three in Trial 2) and six additional traps at each end of the main beds in both trials to monitor general population levels relative to seasonal levels previously recorded. The sticky traps were replaced weekly and thrips species and numbers recorded. A data logger was installed at the beginning of each trial, initially centrally placed over the bulk beds at a height of 50cm and then moved when the plants were transferred to the main channels to a position central between the three main channels.

Statistical analysis

TSWV inhibition

A generalised linear mixed model with logit link function and binomial errors was used to relate the proportion of lettuce plants exhibiting TSWV virus symptoms to treatments. The design elements, block and column, were included in the model as random effects. Treatment was included as a fixed effect. ASREML (Gilmour *et al*.) was used to conduct the analysis. Least significant differences at P = 5% were used to compare treatment effects on the logit scale. Virus symptoms were first scored 0 = no TWSV, or a, b, c representing increasing severity. For this analysis, scores of a, b, or c were classified as virus presence. The scoring classification was then changed to reflect the commercial reality-that the farmer would remove the virus-affected leaf of a plant with a score of ‘a’. Some replicates were lost in the second trial due to unauthorised removal of plants.

RESULTS AND DISCUSSION

Phytotoxicity trial

No phytotoxicity was noted. On close inspection, a deposit in the form of shiny spots was evident on lettuce sprayed with 1% Stress-Ex, but it was not considered a problem for marketing. No significant deposit was noted at rates up to 5% in the commercial operation.

Table 1. Treatments applied to two hydroponic lettuce crops to assess their ability to protect plants against TSWV.

Trial 1

Treatment	14th February	21st February	28th February	7th March	14th March	21st March
T1	Stress-Ex 0.5%	Water	Flextend 0.3%	Water	Flextend 0.3%	Final assessment
T2	Stress-Ex 1%	Water	Flextend 0.3%	Water	Flextend 0.3%	Final assessment
T3	Flextend 0.3%	Water	Flextend 0.3%	Water	Flextend 0.3%	Final assessment
T4	Flextend 0.3%	Flextend 0.3%	Flextend 0.3%	Flextend 0.3%	Flextend 0.3%	Final assessment
T5	Water	Water	Water	Water	Water	Final assessment

Trial 2

Treatment	28th March	5th April	12th April	19th April	26th April	3rd May
T1	Water	Water	Water	Water	Water	Final assessment
T2	Stress-Ex 1%	Stress-Ex 1%	Stress-Ex 1%	Stress-Ex 1%	Stress-Ex 1%	Final assessment
T3	Stress-Ex 5%	Water	Stress-Ex 5%	Stress-Ex 1%	Stress-Ex 1%	Final assessment
T4	Stress-Ex 1%	Water	Stress-Ex 1%	Water	Stress-Ex 1%	Final assessment
T5	Stress-Ex 5%	Water	Stress-Ex 5%	Water	Stress-Ex 1%	Final assessment

Table 2. Category definition for TSWV symptoms.

Category	Definition	Comment at harvesting
0	no TSWV	commercial
a	single leaf with TSWV	commercial if affected leaf removed
b	1-3 leaves with TSWV	maybe commercial if affected leaves removed
c	> 25% with TSWV	non-commercial

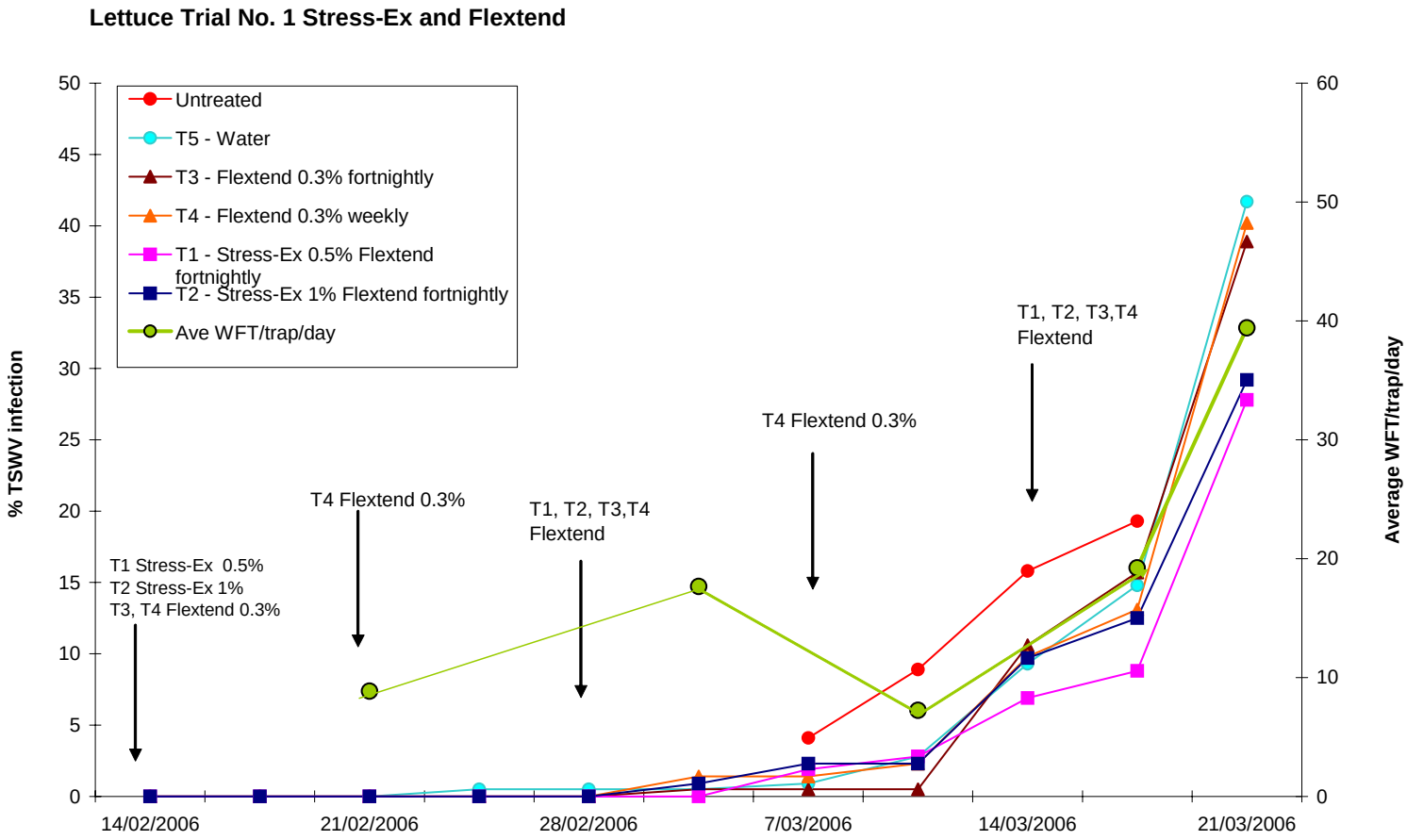


Figure 1. *Trial 1* Percentage of hydroponic lettuce with TSWV symptoms and yellow sticky trap counts of adult WFT following treatment with the anti-transpirants Flextend and Stress-Ex.

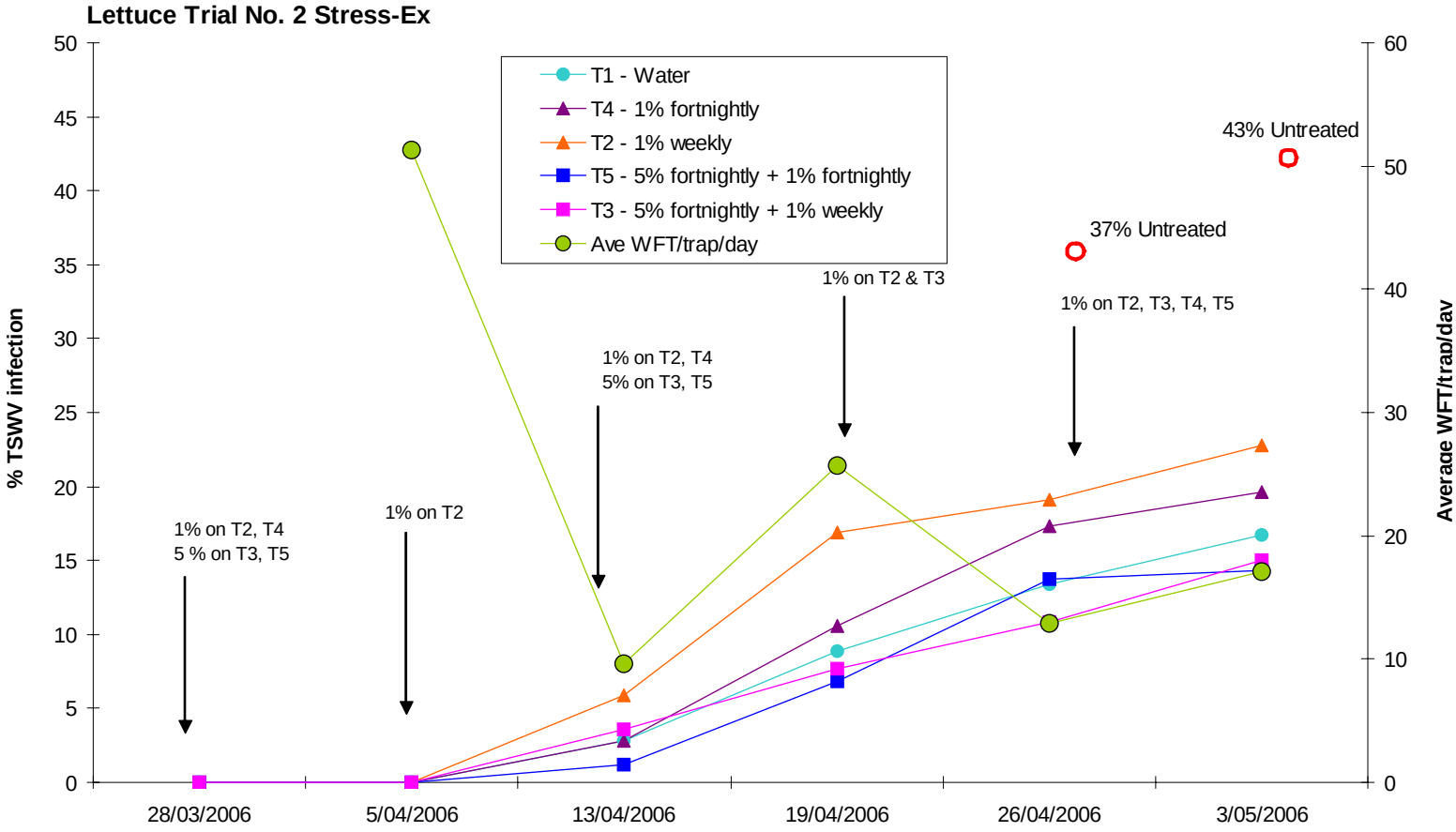


Figure 2. *Trial 2* Percentage of hydroponic lettuce with TSWV symptoms and yellow sticky trap counts of adult WFT following treatment with the anti-transpirant Stress-Ex.

TSWV inhibition trials

Trial 1 A significant treatment effect was observed using the first assessment method (Table 3). The proportion of lettuce plants with TSWV symptoms in the controls (42%) was significantly greater than the proportion with symptoms in either T1 (28%) or T2 (29%). The average proportion of lettuce plants with TSWV symptoms ranged from 26-33% at final assessment (Table 3 and Fig. 1). With the scoring change to rating only b and c as TSWV-positive, the treatment effect was not significant on any of the three assessment dates.

Table 3. Final assessment of TSWV incidence in Trial 1 (21 March 2006). Analysis was conducted on transformed means. Means (back transformed) in the same column followed by the same letter are not significantly different (P = 0.05%).

Treatment		Proportion with TSWV symptoms (a, b, or c)
1	0.5% Stress-Ex then 0.3% Flextend fortnightly	0.2834 a
2	1% Stress-Ex then 0.3% Flextend fortnightly	0.2925 ab
3	0.3% Flextend fortnightly	0.3639 abc
4	0.3% Flextend weekly	0.3777 bc
5	Control (water)	0.4155 c

Trial 2

Some plants were lost following their removal by farm staff from bulk beds just prior to moving to main beds. Two replicates were lost from T2. Regrettably, Blocks 5 and 6 were also removed by the grower from the trial beds on 2 May, the day before final assessment, to make up an order that was short.

Using the second 'commercial' scoring method, there was no significant difference between infection rates for any treatment on any assessment date. Average infection rates (a, b, c) on 3 May 2006 were 14-20% (Table 4). These rates were substantially lower than in Trial 1, possibly reflecting temperature differences as thrips populations were comparable. It was interesting that in the two adjacent beds outside the trial, infection rates were 37% and 43%. The higher infection rate outside the trial was also noted in the first trial (Fig. 1, untreated), and may be a consequence of removal of plants with 25% TSWV infection, or possible water application. The latter was not proven to be a factor in subsequent trials at GHI (Section 1.1.3.2), which suggests that early removal of plants with TSWV symptoms is very important in reducing the spread of infection.

Thrips populations on traps (mostly WFT) were high (Figs. 1, 2), averaging 8.9 (21 February) to 39.4 (21 March) thrips per day in Trial 1, and 9.6 (13 April) to 51.3 (5 April) in Trial 2.

The trial results were disappointing following the positive effects of Wilt-Pruf, with the same active ingredient, in the Canadian trial. In that case a single treatment was applied to an ornamental crop with >75% reduction in infection. In Trial 1, there was a positive treatment effect for 0.5 and 1.0% Stress-Ex with fortnightly application of Flextend if all plants with TSWV were considered. Because Flextend alone did not have an effect at the rates used, this product was not pursued in the second trial. Stress-Ex alone at the higher rate in Trial 2 had no apparent effect, although for a similar thrips population to Trial 1, TSWV infection rates in all treatments in Trial 2 were much lower.

Table 4. Final assessment of TSWV incidence in Trial 2 (3 May 2006). Analysis was conducted on transformed means. Means (back transformed) were not significantly different (P = 0.05%).

Treatment		Proportion with TSWV symptoms (a, b, or c)
1	5% Stress-Ex week 0 and 2, then 1% Stress-Ex weekly	0.1645 ns
2	5% Stress-Ex week 0 and 2, then 1% Stress-Ex fortnightly	0.1986
3	1% Stress-Ex fortnightly	0.1439
4	1% Stress-Ex weekly	0.1835
5	Control (water)	0.1388

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1.1.4 EFFECT OF RELATIVE HUMIDITY ON PUPATION SUCCESS OF WESTERN FLOWER THRI, *FRANKLINIELLA OCCIDENTALIS* (PERGANDE)

INTRODUCTION

Western flower thrips (WFT) are known to be sensitive to relative humidity at certain stages. Shipp & Gillespie (1993) studied the effect of temperature and vapour pressure deficit on various stages of WFT. Relative humidities (RH) tested at 25^{o}C were 12, 33, 53, 76 and 97%. At low RH, larval stages had the lowest survival rate off-leaf and pupae were mostly insensitive. However, the low humidity needed for an appreciable effect on larvae was below that experienced by greenhouses with a crop in situ, and thus has most relevance for between-crop sanitation. Most WFT larvae are reported to drop to the ground to pupate rather than remain on the plant. A limited number of workers have attempted to quantify this. It appears to be somewhat crop specific. Because of the sensitivity of larvae to low RH, it seems logical to presume that relative humidity influences this behaviour in some way. During greenhouse trials attempting to impact on those thrips larvae dropping to the ground to pupate (Section 1.1.1), it was apparent that even products highly effective in laboratory bioassays (Section 1.1.5) were no more than about 50% effective when applied in a greenhouse environment growing cucumbers. Here we examine the effect of a broader range of relative humidity on successful pupation of late stage WFT larvae in laboratory bioassays. Following results that showed survival of this stage was very much RH dependent, we questioned whether larvae were making a decision to pupate on or off-leaf based on the detected humidity. The mechanism by which thrips larvae leave the leaf may be by dropping off or by walking off. We examined the influence of various relative humidity levels on the decision whether or not to drop off leaves.

MATERIALS AND METHODS

Effect of RH on pupation success Known-age WFT larvae were obtained by allowing adult female WFT to oviposit on green bean, *Phaseolus vulgaris* L., pods for 24h. The thrips were then removed and the beans placed on two layers of Kimwipe® paper towel in screened boxes at 25^{o}C for approximately 7-8 days, until the first prepupae were observed. Cattail (*Typha* sp.) pollen and drops of honey were provided as additional food for larvae. Larvae were then removed and assigned randomly to an experimental unit, ten per unit. Each unit consisted of an inverted 55mm diameter by 14mm deep Petri® dish. A small plastic disc of polyethylene greenhouse ground cover (Panda™ film) was placed in the lower section to just fit. A 34mm diameter hole in the upper section was screened with 107µ hole size-nylon mesh to allow air and water vapour exchange, and the two dish sections sealed with Parafilm®. Thrips larvae were introduced via an aspirator through a small hole in the upper section, which was then sealed with Blu-tac™. Units with thrips were placed singly in 145-mm high by 67-mm wide plastic cylinders. The cylinders contained 250g of sulphuric acid solutions diluted with distilled water to give relative humidities ranging from 60% to 95%, in 5% increments (Solomon 1951). The Petri dish unit was attached to the lid of the cylinder with Velcro™ tape, with screen side-down. There were four replicates per treatment. The experiment was repeated twice for a narrower range of relative humidity around critical points (80-95%). The cylinders were kept in an incubator at constant 25±1^{o}C temperature and 12h photoperiod for 7-8 days to allow

time for completion of development to the adult stage. The number of thrips in each unit successfully completing development to the adult stage and the number of live pupae were recorded.

Influence of relative humidity on choice of pupation site
The experimental unit in this trial was a small Petri™ dish 35mm diameter, 10mm deep. A small amount of 1% agar was added to the dish and a green bean leaf disc of the same diameter placed upper surface down on top of the agar. Late stage thrips larvae six to seven days old were placed on the leaf surface, ~10 per unit, with a camel hair brush. A small drop of honey was placed on a leaf vein as supplementary food. Plastic lidded boxes 120mm x 85mm x 70mm deep (Prestige™) were filled with various concentrations of glycerol to a depth of 15mm. A recording temperature and relative humidity (Hastings Tinyview™) was attached to the lid of each box with fine wire and Velcro™, and beneath this the Petri dish containing the thrips larvae was also attached with Velcro, open surface down and about 5-7mm above the glycerol. The first two trials were conducted 13 and 17 October 2006 with RH ranges of 40 to 100% in 10% increments, the remainder on 31 October, 7 November, 15 November, 15 December 2006 and 8 January 2007 with RH ranges of 65-100% in 5 degree increments (vapour pressure deficits (VPD) 1.9-0 Kpa, Table 1). A more accurate RH measuring device, a probe (Vaisala HUMICAP® humidity and temperature meter HM 70) inserted into the lid of the box, was used to check RH at the conclusion of the trial because of discrepancies in TinyTag readings. All RH readings were within ± 0-2% of the set RH except 100% which showed 97% on the RH probe and was so recorded in the data. Measurements taken without the leaf disc unit in place indicated that the leaf disc unit disc raised the air temperature $<1^{o}C$ across the RH range. Trials were assessed after three days, except those on 15 November (5 days) and 8 January (4 days), which were left longer to allow completion of pupation. At the completion of the trial, the units were examined for the number and life-stage of thrips in the glycerol and on the leaf surface, and also within the box. The number of larvae found in the glycerol was expressed as a proportion of total thrips found.

Statistical analysis

Effect of RH on pupation success
Pupation success was defined as the presence of a live pupa or adult. A generalised linear model (GLM) with logit link function and binomial error distribution was used to relate the probability of an adult or pupa being alive to RH according to the following model:

$Log_e(p/(1-p)) = a + b.RH + error$

where p = probability of an adult or pupa being alive; a and b are regression coefficients.

Influence of relative humidity on choice of pupation site
The percentage of larvae in the glycerol compared to total thrips found (% drop) was calculated and used to represent choice of pupation site. The non-linear response of pupation behaviour to lower ranges of RH exhibited in the first trial suggested that a 'bent-stick' non-linear regression was appropriate. This method also allows the

estimation of the change-point between the two parts of the bent stick (the RH at which the % drop response changes).

RESULTS AND DISCUSSION

Effect of RH on pupation success All thrips died as larvae in the relative humidity range 60-75%. Survival at 80, 85, 90 and 95% RH was 2.91, 53.15, 97.78 and 94.22 % respectively (calculated probabilities are presented in Table 2). There was a slight delay in development at 90% compared with 95% RH. The % RH value corresponding to a particular probability of an adult or pupa being alive can be calculated by inverting the regression equation $\log_e(P/1-P) = -40.7 \pm 4.08 + 0.4713 \pm 0.0477$ RH, where P = probability of survival to pupa (Fig. 1).

Shipp & Gillespie (1993) reported that WFT pupae are not sensitive to relative humidity levels, in contrast to larvae. The authors examined a limited range of humidities and separated larvae only into 1st and 2nd instars and no particular age. Those tested here were at a stage when they were close to completing larval development and would normally seek shelter. It is likely that they would move to areas of higher humidity because of their observed sensitivity to relative humidity lower that 90%. The time to change from late second stage larva to prepupa once the pupation site is chosen is unknown, but probably of short duration, so there is only a small window of opportunity to negatively impact on pupation success by lowering the RH. Larvae that drop to the ground have a distinct preference for late afternoon (W. Kirk, pers. comm. 2006), suggesting that either daylight or relative humidity in the crop, possibly both, may be the trigger, as humidity rises during the night period.

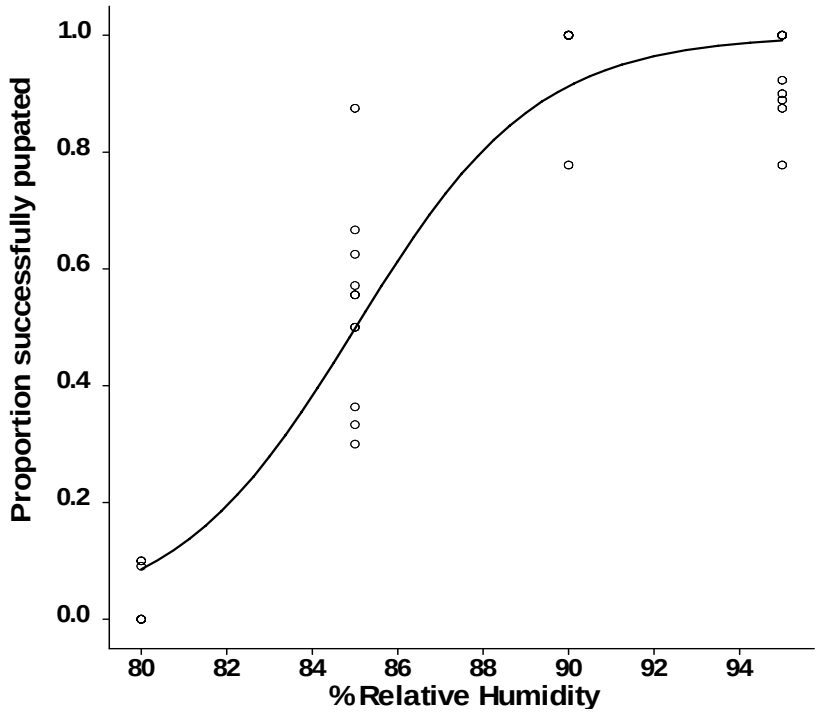

Figure 1. Predicted proportion of late stage WFT larvae that successfully pupate at varying relative humidities.

Table 1. Vapour pressure deficits (VPD) at various relative humidities (RH) at 25°C (saturated vapour pressure = 3.169).

RH	40	50	60	65	70	75	80	85	90	95	100
VPD (Kpa)	1.90	1.58	1.27	1.11	0.95	0.79	0.63	0.48	0.32	0.16	0

Table 2. Predicted proportion of WFT larvae surviving to pupal stage at four relative humidities. Means followed by the same letter are not significantly different (P = 0.05).

RH	Predicted proportion surviving
80	0.0390 a
85	0.5208 b
90	0.9792 c
95	0.9464 c

Influence of relative humidity on pupation choice The proportion of thrips larvae found in glycerol below a leaf surface decreased sharply at high RH values (Fig. 2). The break point at which the % drop response changed was 81.18% RH (~ s.e. 2.90 95% confidence intervals 77.00, 86.94). At this point 70.81% of larvae dropped into the glycerol. As RH declined to 40%, the proportion of larvae dropping increased gradually to a mean of about 0.8. At RH greater than the change point, the proportion found in the glycerol drooped rapidly to zero. A few pupae were found on the sides of the box and occasionally in the glycerol or on the data logger. The pupae in the glycerol were mostly live and floating on the surface. They may have fallen in inadvertently or dropped deliberately from the leaf surface, but it was not possible to determine this.

At 40-85% RH, the proportion of thrips of all stages found in the glycerol was variable and ranged from 0.67-0.82 (Fig. 3), whereas at 90-100% RH, that proportion was lower at 0.32-0.59. At 95% and 100% RH, 16-20% of thrips (mostly pupae) were off-leaf, but not in the glycerol, whereas at RH <75% they were either on the leaf disc or in the glycerol. Of thrips in the glycerol, in the lower range of RH, 91.1% were larvae and 8.9% pupae, compared with 40% larvae and 56.4% pupae in the higher range.

In the 15 December trial (excluded from the analysis), thrips larvae selected for placing on the leaf discs had already dropped to the ground litter in rearing boxes, and were 10 days old (25°C) instead of 6-7 days old. Relative humidities tested ranged from 65-100%. At 65-85% RH, 71.7% of thrips were subsequently found in the glycerol (n = 46), compared with 78.1% at 90-100% (n = 32), a minor difference. Of the thrips found in the glycerol, larvae, pupae and adults represented 27.8, 57.6 and 15.2% of the total respectively at the low RH range, and 12.0, 60.0 and 28% at the high range. RH thus had little or no apparent influence on dropping behaviour of pupae, only larvae, but it appears that some pupae also drop from the leaf, whether deliberately or inadvertently is not known. While there were differences in the percentage of these older larvae dropping in the two RH ranges, most appear to have remained on the leaf disc to complete pupation in the lower range, a difference in behaviour from slightly younger larvae.

We suggest two hypotheses for dropping behaviour. One, larvae choose to vacate leaves if the detected relative humidity is too low for survival, probably <85% on the strength of the survival data in the previous experiment. However, there are no reports to suggest that dropping is normal behaviour for feeding larvae. Two, larvae remain on the plant, seeking higher humidity locations, until they have ceased feeding in preparation for pupation. Moisture intake would then be reduced, and may trigger a change in behaviour to dropping to the ground in search of more favourable moisture conditions for survival to pupation. However, if the RH in the surrounding air is very high, they may elect to remain in or on the plant to pupate. It is clear that they are very sensitive to critical ranges of relative humidity. They may also drop at this stage for reasons not related to RH.

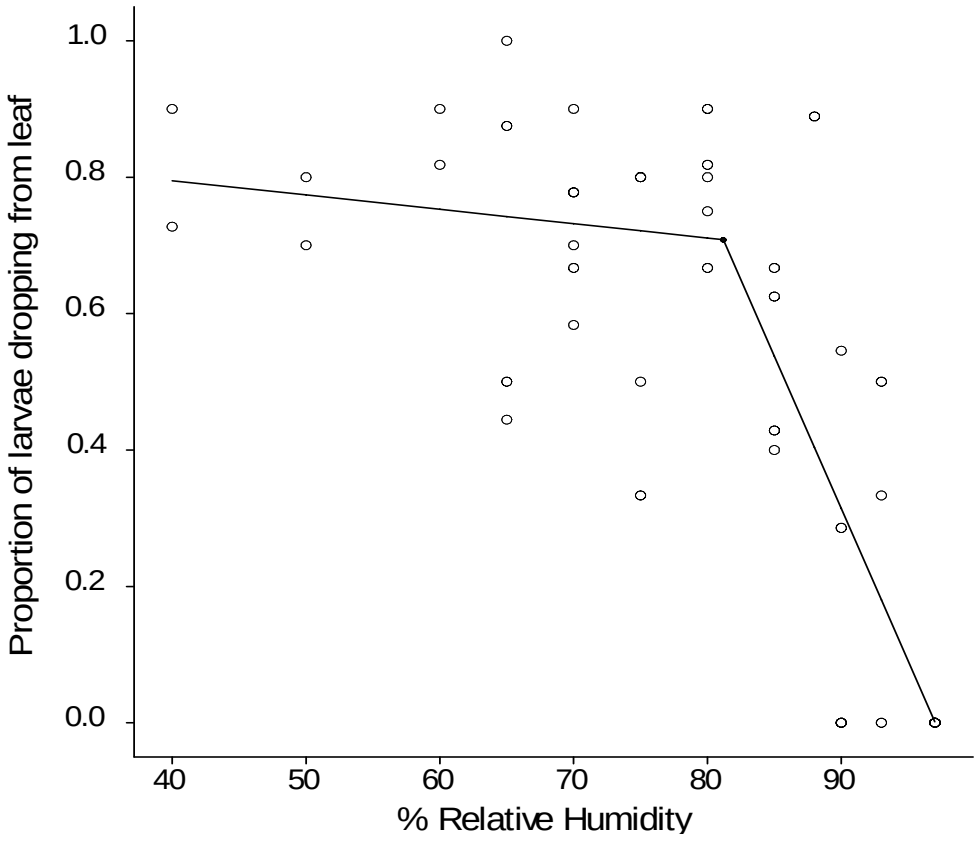

Figure 2. Proportion of WFT late-stage larvae dropping from a leaf surface to pupate at various relative humidities.

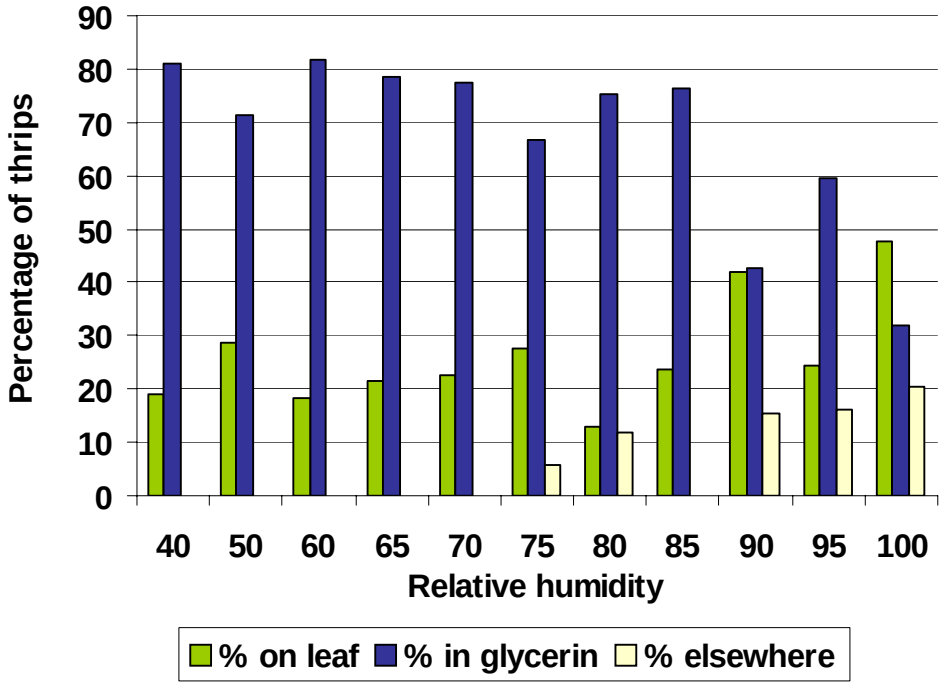

Figure 3. Disposition of WFT at various relative humidities 3-5 days after placing late-stage larvae on a leaf disc over glycerol.

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1.1.5 ASSESSMENT OF GROUND TREATMENTS TO REDUCE PUPATION SUCCESS OF WESTERN FLOWER THRIPS, *FRANKLINIELLA OCCIDENTALIS* (PERGANDE) - LABORATORY BIOASSAYS

INTRODUCTION

Management of western flower thrips (WFT) in any greenhouse crop is difficult and rarely successful if reliance is placed on pesticides alone. Most pesticides are applied as high volume foliar sprays, targeting either adult or larval stages. Few pesticides are effective for long, sometimes only for a season if chronically overused. Resistance has been reported to many older active ingredients, and also to new ones. The life cycle of thrips at warm greenhouse temperatures is short, as little as two weeks under optimal conditions, and the number of offspring numerous. Eggs are laid into plant tissue, so are protected from most sprays until they hatch. The two larval stages are mostly found on the undersides of leaves. Most late-stage larvae are reported to drop to the ground to pupate, and then to move to a sheltered site away from exposure to predators and adverse environmental conditions. Pupae do not feed and respire little. This is the second quite long period of several days when thrips are not contacted by pesticide foliar treatments. Allowing time for completion of development from pupa to adult is one reason for the regime of three consecutive sprays generally recommended in a resistance management strategy, with a decreasing interval between sprays with increasing temperature.

In a natural environment, the pupation site is a few millimetres below the soil surface. Shipp & Gillespie (1993) reported that larvae are very susceptible to mortality at low humidity, whereas pupae are not. We have shown that a relative humidity of 90% is needed to ensure 100% successful pupation of late-stage larvae, with almost none pupating at 80% RH (Section 1.1.4). In modern greenhouses with solid plastic or weed matting covering the soil, dropping larvae would need to move to shelter under pots and bags where humidity is higher. Treatments we evaluated were thus designed to kill the larvae before they could reach shelter, a time which we believe is relatively short.

There have been a few attempts to target the pupal stage of thrips in the soil, but few successes (Helyer *et al*. 1995; Pickford 1984; Steiner 1985). Steiner (1985) tested several products and found only bendiocarb to have an effect when applied under a cucumber crop. This product is one of the old carbamates and has no long-term future in the market place. Biocontrol agents such as *Stratiolaelaps scimitus* and *Steinernema feltiae* have some effect when applied to media, but thrips, when they drop to the ground, do not necessarily land in the media, particularly in modern, hydroponically grown crops where media may be in closed bags or otherwise covered. Laboratory experiments were therefore conducted to identify a product which would rapidly kill thrips larvae and/or pupae dropping on to plastic flooring. In the first greenhouse trial we conducted, several products were applied to plastic weed matting under a greenhouse cucumber crop (Section 1.1.1). An application of a drying agent to the plastic below the crop was envisaged to target the RH-susceptible larvae dropping to the ground. Following rather anomalous results in the greenhouse, laboratory bioassays were conducted to try to explain these results before returning to the greenhouse with the most promising products. In this series of laboratory bioassays, the emphasis shifted to evaluating deliquescent materials rather than dessicants, because of the probability of high relative humidity at night counteracting the effect of dessicants. The most promising of these materials were then applied as a

greenhouse ground cover to solid plastic sheeting (Panda™ film) (Section 1.1.1). The laboratory bioassays are described in this section.

MATERIALS AND METHODS

General methodology Known-age WFT larvae were obtained by allowing adult female WFT from a laboratory culture to oviposit in pods of green bean, *Phaseolous vulgaris* L., for 24h. The adult thrips were then removed and the beans with eggs placed in rearing boxes (Steiner & Goodwin 1998) at 25^{o}C for approximately 7-8 days. Cattail (*Typha* sp.) pollen and drops of honey were provided as additional food for larvae. When the first prepupal thrips were observed on the paper below the beans and larvae were observed coming to honey on the mesh, the largest late stage larvae were removed and assigned randomly to an experimental unit, 10-12 per unit. Each unit consisted of a 47.75mm diameter Millipore™ lidded dish. A 34mm diameter hole was cut in both upper and lower section and this was screened with 107µ hole size nylon mesh to allow air and water vapour exchange. A small disc of white plastic film (Panda™ film), used as a commercial floor covering in greenhouses, was the treatment surface. The solution to be tested was applied with a brush to cover both sides of the plastic, and allowed to dry each time. The treated disc was placed in the lower section of the Millipore dish to just fit. The two dish sections were joined and sealed with Parafilm™ along the join. Larvae were introduced via an aspirator tip through a small hole in the upper section of each unit, which was then sealed with Blu-tac™. There were four to six replicates per treatment. All units were held at high humidity while they were loaded (~30 min). Units were then placed in plastic lidded trays on a metal mesh stand over glycerol at 90-95% humidity (Fourney & Brandl 1992). The RH was high to ensure successful pupation was possible. The tray was kept in an incubator at constant 25±0.5^{o}C and 12hD:12hN photoperiod for 7-8 days to allow time for completion of development to the adult stage. The number of thrips in each unit successfully completing development to adult and the number of live pupae were then recorded, along with dead larval, pupal and adult stages. The percentage mortality from the treatment effect was then calculated, adjusting for control mortality.

Trial 1 Four concentrations (5, 10, 50 and 100g/L) of cooking salt (sodium chloride), were evaluated to establish optimum concentration for toxicity to late stage WFT larvae. Silwet L-77™® was added at 0.1% to all treatments, including a water control, to aid coverage. There were four replicates of each concentration. The experiment was set up 24 February 2005 and assessed 3 March 05.

Trial 2 Four materials previously evaluated in a greenhouse trial as ground treatments applied to weed matting (Section 1.1.1) were assessed, with the addition of cooking salt. Treatments were sodium chloride (cooking salt) at 100g/L, diatomaceous earth (Dryacide™) at 120g/L, kaolin (Surround™) at 60g/L, calcium chloride (hydrated lime) at 180g/L and silica fume at 120g/L. There were four replicates per treatment. The experiment was repeated four times, on 3 and 13 March and 10 and 17 June 2005.

Trial 3 A combination of 100g/L of cooking salt and Dryacide at 120g/L and the products separately were evaluated. The experiment was set up with six replicates per treatment and repeated four times on 24 March, 5, 13 and 27 May 2005.

Trial 4 In this trial, Eco-Oil™ was added to the salt component to improve spreadability and dispersion on plastic and to reduce the RH at which the salt deliquesced. Pool salt replaced cooking salt because the latter has an anti-caking material added to it (sodium ferrocyanide), which may have affected the relative humidity at which it deliquesced. Calcium nitrate was included as it liquefies at a much lower RH than sodium chloride and is less of a problem in potential run off or ground contamination. Trials were set up on 13 December 2005.

Trial 5 This trial was an observational one to better choose effective products under greenhouse conditions. Sodium chloride as cooking salt was shown to be effective against thrips larvae dropping to the ground to pupate. However, on its own it spread poorly, even with Silwet added to it, and tended to pool in low spots so that thrips may potentially avoid coming into contact with it. Eco-Oil added to the salt at 2% assisted in spread and in keeping the salt liquid, but it appeared to 'dry out' over time in the greenhouse, possibly reducing efficacy. A range of combinations of oil and the sticker di-1-p-menthene (Stress-Ex™) were evaluated to determine their ability to persist and to give good coverage. As per label instructions, Stress-Ex was sun-cured an hour to set it prior to exposure to thrips. Materials tested were: 1. 10% W/V calcium nitrate + 2% Eco-Oil; 2. 10% W/V pool salt (sodium chloride) + 2% Eco-Oil; 3. 2% Eco-Oil; 4. 10% calcium nitrate + 2% Sunspray Ultrafine™ horticultural oil; 5. 10% sodium chloride (pool salt) + 2% Sunspray oil; 6. 2% Sunspray oil; 7. 10% calcium nitrate + 1% Stress-Ex; 8. 10% sodium chloride (pool salt) + 1% Stress-Ex, with 0.1% Silwet added to each mix. There were five replicates per treatment. Materials were applied as dips to discs of plastic (Panda film) in Millipore dishes. Once dry, the disc was sandwiched between the upper and low dish section with an unscreened hole cut in the upper section (white surface of disc exposed to sunlight). The units were divided between two trays, treatments were randomised, and they were placed 21 December 2005 in two 54 sqm greenhouses on the sunny side at ground level for two weeks. The surface was then assessed visually under a microscope for oil/salt residue.

As a result of the longevity and coverage issues raised as a result of the previous experiment, a further series of experiments was conducted on mortality of various oil and chemical combinations.

Trial 6 The trial was set up on 6 January 2006 as previously for bioassays, with five replicates per treatment. Treatments were pool salt plus Stress-Ex or Sunspray oil, calcium nitrate plus Agri-50E™, Sunspray oil or Stress-Ex, and Agri-50E alone (Table 5).

Trial 7 Agri-Terra™ replaced Agri-50E in this trial, with Stress-Ex assessed alone (Table 6). The trial was conducted 13 January and repeated 20 January 2006. There were five replicates per treatment.

Trial 8 Some of the more promising treatments from previous trials were reassessed, with the addition of Abrade™, a silicon-based insecticide (Table 7). The trial was conducted 27 January 2006. There were five replicates per treatment.

Trial 9 Four of the calcium nitrate treatment combinations were repeated 3 February 2006, along with calcium nitrate plus Dryacide, and Abrade on its own (Table 8). The rate of Abrade was doubled, from 2.5g/L to 5g/L.

Statistical analysis

All trials were set up in a randomised complete block design**,** with a minimum of four replicates per treatment. Data were analysed using a generalised linear model (GLM) with binomial error distribution and logit link function. Replicates were fitted as a random effect in Trial 1 and experiment and experiment.rep in remaining trials. The number of dead (larvae + pupae) and total number of WFT (adults + larvae + pupae) were corrected for control mortality prior to analysis.

RESULTS AND DISCUSSION

Trial 1 Mortality of larvae and pupae when exposed to cooking salt at concentrations of 0.5-10% w/v varied between 62.9% and 98.5% with increasing concentration (corrected for control mortality) (Table 1, Fig. 1). There was no significant difference between 5% and 10%. A concentration of 10% (100g/L) was chosen for subsequent trials with salt.

Trial 2 The only products causing a significant reduction in thrips pupation success were Dryacide (55.6% reduction) and cooking salt (82% reduction) (Table 2, Fig. 2) Salt was significantly more effective than Dryacide. The results for Dryacide, Surround, hydrated lime and silica fume confirm rather indifferent efficacy results from the previous greenhouse trial.

Trial 3 All products provided significant control of thrips pupation (Table 3, Fig. 3); however, Dryacide alone was not very effective. Salt gave good control (>90%). Addition of Dryacide to salt did not provide significantly better control, but nor did it reduce the control effect. The combination may improve longevity, which has not been tested in these experiments.

Trial 4 Calcium nitrate plus Eco-Oil and sodium chloride plus Eco-Oil, which were equally effective, gave significantly better control than Eco-Oil or calcium nitrate alone (Table 4). Similarly, addition of Eco-Oil appeared to marginally improve the efficacy of salt. Control mortality was rather high (Fig. 4) due to a malfunction in the data logger and lower RH in the containers than necessary to ensure successful pupation.

Trial 5 All observations were taken at room temperature and RH (~21^{o}C and 60% RH).

1. Calcium nitrate plus Eco-Oil Disc surface appeared oily, variable size drops were still apparent; coverage was fairly even though a little variable between replicates.
2. Pool salt (sodium chloride) plus Eco-Oil Small crystals of salt were visible. Oily patches but irregular coverage.
3. Eco-Oil Appeared fairly dry, coverage of oil rather patchy where present.
4. Calcium nitrate plus Sunspray Oil Small drops of liquid, but generally good coverage of plastic disc.
5. Pool salt plus Sunspray Oil Salt crystals present of variable size. Fairly good coverage, better than 2.
6. Sunspray Oil Even coverage, very thin cover, much more even that Eco-Oil.
7. Calcium nitrate plus Stress-Ex Excellent coverage, oily appearance.
8. Pool salt plus Stress-Ex Crystals present, mostly small, not as good coverage as 7.

After two weeks, Sunspray petroleum oil applied to plastic discs showed more even coverage than the botanical oil formulation Eco-Oil. Pool salt, where used, crystallised out at room RH. Best coverage was obtained by a combination of calcium nitrate and Stress-Ex. Calcium nitrate plus Sunspray oil was also promising for good coverage.

Trial 6 All products caused significant mortality of WFT larvae (Table 5, Fig. 5). The most effective combinations were 10% pool salt plus 1% Stress-Ex (89.6% mortality), 10% calcium nitrate plus 0.3% Agri-50E (88.7% mortality), and either 10% pool salt or 10% calcium nitrate plus 2% Sunspray oil (84.7 and 86.9% mortality, respectively). Agri-50E alone was not very effective.

Trial 7 The most effective and consistent treatment was 10% calcium nitrate plus 0.5% Agri-Terra (94.2% mortality), with 10% calcium nitrate plus either 1% Stress-Ex or 2% Sunspray oil providing limited control but not significantly different from each other (Table 6, Fig. 6). The remaining treatments were not significantly different from the control. An infection of *Beauveria* was noted in the first assessment, which may have increased mortality.

Trial 8 All products provided excellent reduction of WFT pupation (Table 7, Fig. 7). Pool salt plus Agri-Terra provided the highest mortality, but this was not significantly different from other treatments.

Trial 9 The most effective treatments again were calcium nitrate with Agri-50E, Abrade, Agri-Terra or Stress-Ex (Table 8, Fig. 8). Calcium nitrate was not effective when used with Dryacide. Abrade was not effective on its own.

The mean mortality for each treatment combination has been ranked to allow an overall view of efficacy (Table 9). Several reduced-risk pesticides showed promise as potential ground treatments against WFT. Their activity is premised on thrips larvae dropping to the ground to pupate. A requirement for most of these bioassays was that the plastic surface will remain wet during the critical period after thrips drop. At 25^{o}C, sodium chloride deliquesces at approximately 75% RH and calcium nitrate at 60% RH. During periods of low RH, calcium nitrate would be the additive of choice, because sodium chloride would be crystallised. The advantage of sodium chloride is that it is a very good toxicant against WFT larvae alone, whereas calcium nitrate has low toxicity, and is cheap and readily attainable. On the other hand, sodium chloride might present an environmental risk if it were to run off outside the greenhouse, whereas calcium nitrate, as a common fertiliser, is of less concern for run-off, but likely to have restricted access. Several products enhanced the activity of calcium nitrate. These include Agri-Terra and Agri-50E, Abrade and Eco-Oil. None of these products was effective alone. Other products might be added to sodium chloride to improve its spreadability without detracting too much from efficacy. These include Eco-Oil and Sunspray oil. Agri-Terra, Agri-50E, Stress-Ex, Dryacide and Abrade added to salt were also quite effective, but how they would perform under greenhouse conditions at lower RH has not been assessed. The longevity of these treatments depends on foot traffic beneath the crop canopy and contamination with debris. Larvae remaining on the plant to pupate would not be affected. This proportion is reportedly minimal, but our experience in cucumber and lettuce crops suggests

otherwise. Recent research in the UK suggests that larvae dropping off the plant to pupate is not a random process but follows a diurnal pattern, with most dropping in late afternoon and a few in the early morning (J. Bennison, W. Kirk, pers. comm. 2006). They move to shelter or go down in the soil to pupate.

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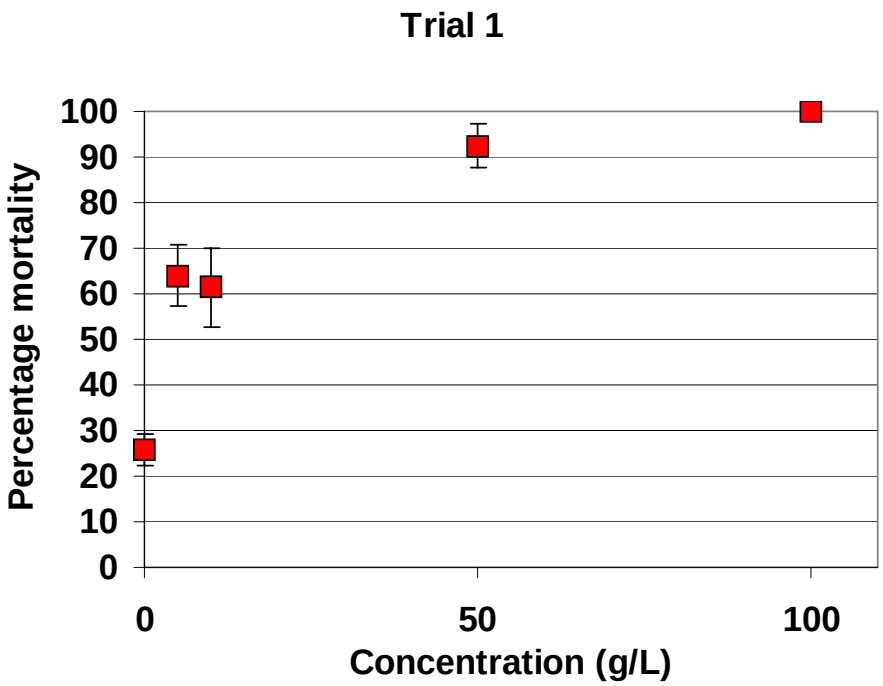

Figure 1. Effect of common salt at four concentrations on mortality of late-stage WFT larvae. Bars represent standard error of the mean (P = 0.05%).

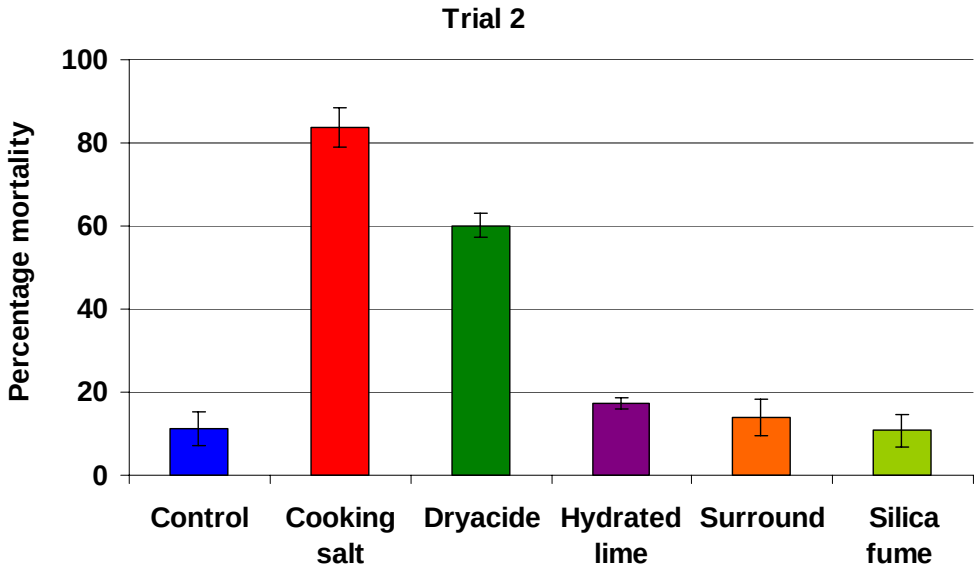

Figure 2. Mortality of late stage WFT larvae in contact with plastic treated with various chemicals. Bars represent standard errors of the mean (P = 0.05%).

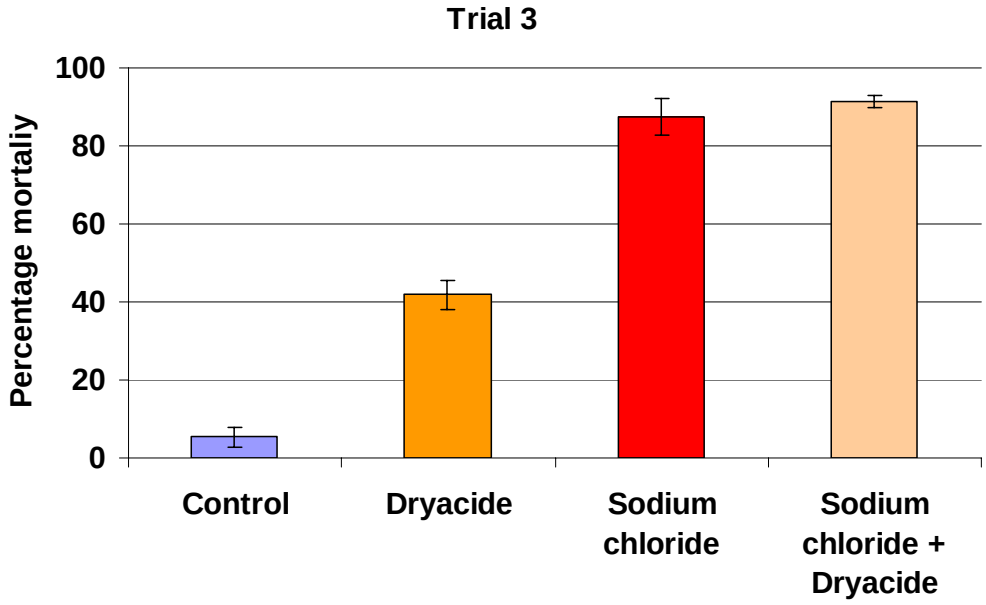

Figure 3. Mortality of late stage WFT larvae in contact with plastic treated with Dryacide or salt. Bars represent standard errors of the mean (P = 0.05%).

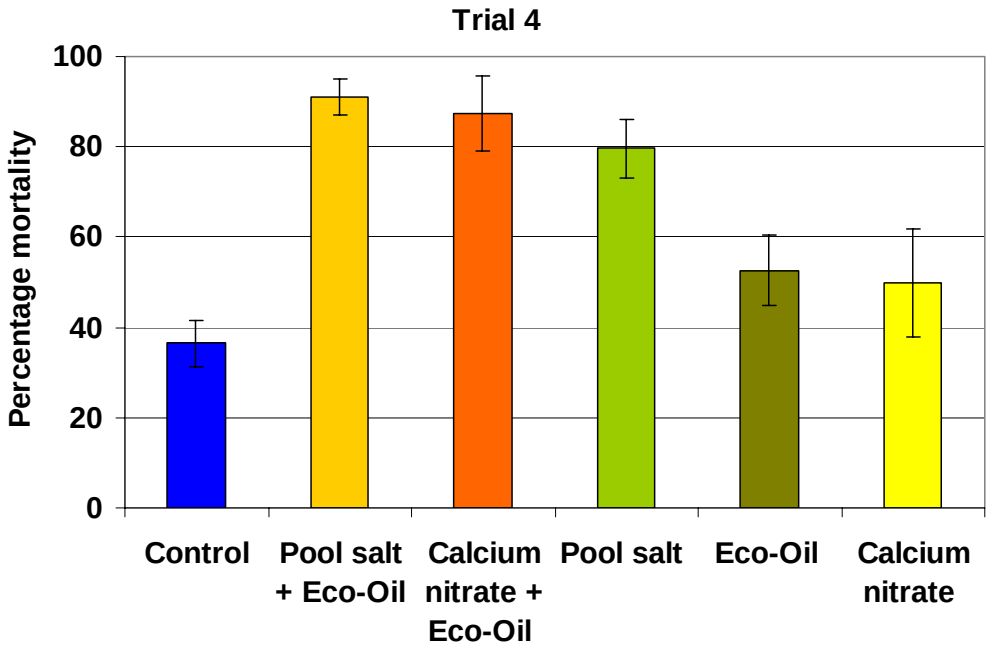

Figure 4. Mortality of late stage WFT larvae in contact with plastic treated with salts and Eco-Oil, 13 December 2005. Bars represent standard errors of the mean (P = 0.05%).

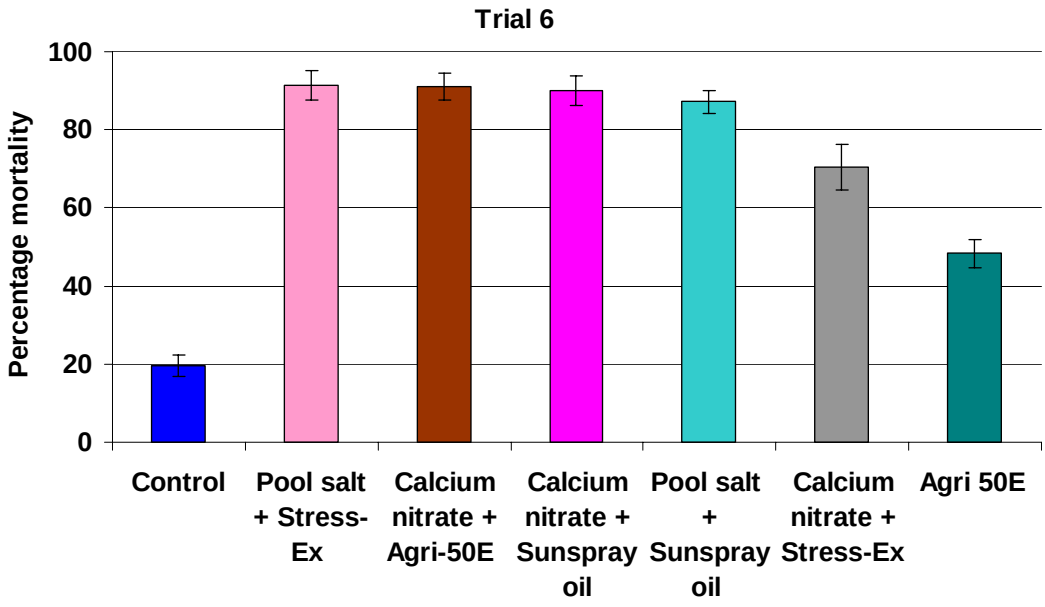

Figure 5. Mortality of late stage WFT larvae in contact with treated plastic, 6 January 2006. Bars represent standard errors of the mean (P = 0.05%).

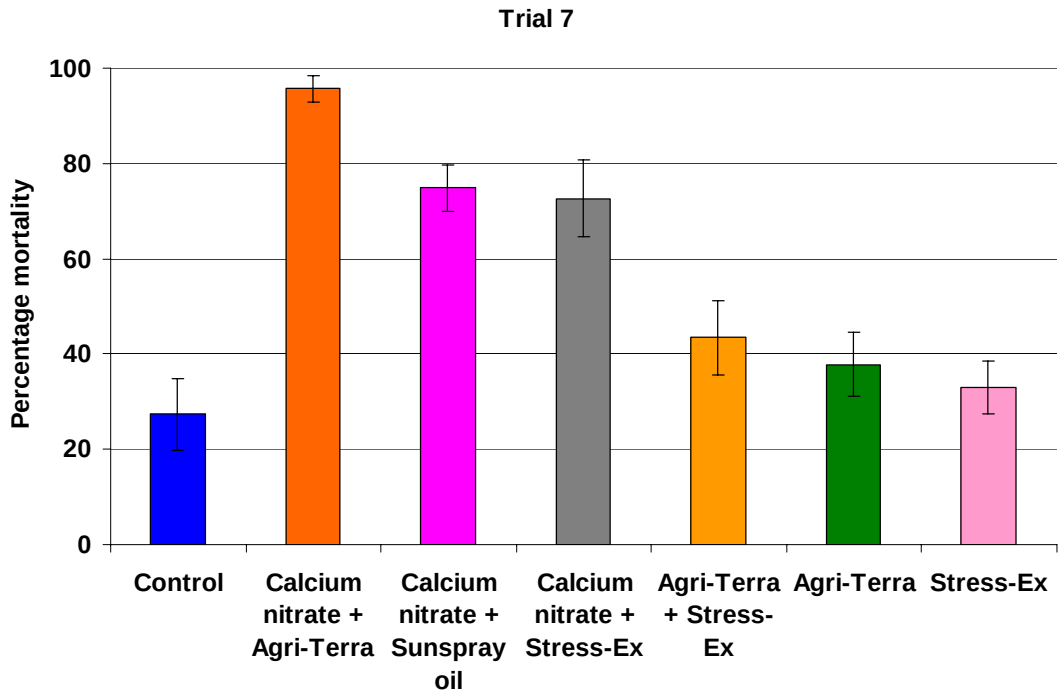

Figure 6. Mortality of late stage WFT larvae in contact with treated plastic 13 January and 20 January 2006. Bars represent standard errors of the mean (P = 0.05%).

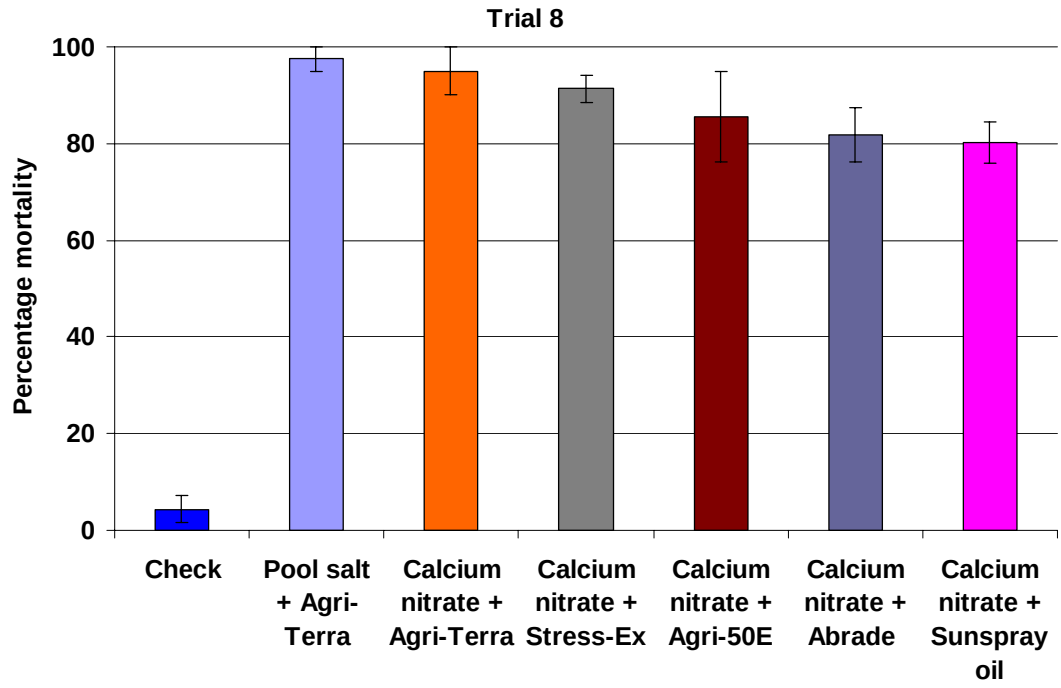

Figure 7. Mortality of late stage WFT larvae in contact with treated plastic on 27 January 2006. Bars represent standard errors of the means (P = 0.05).

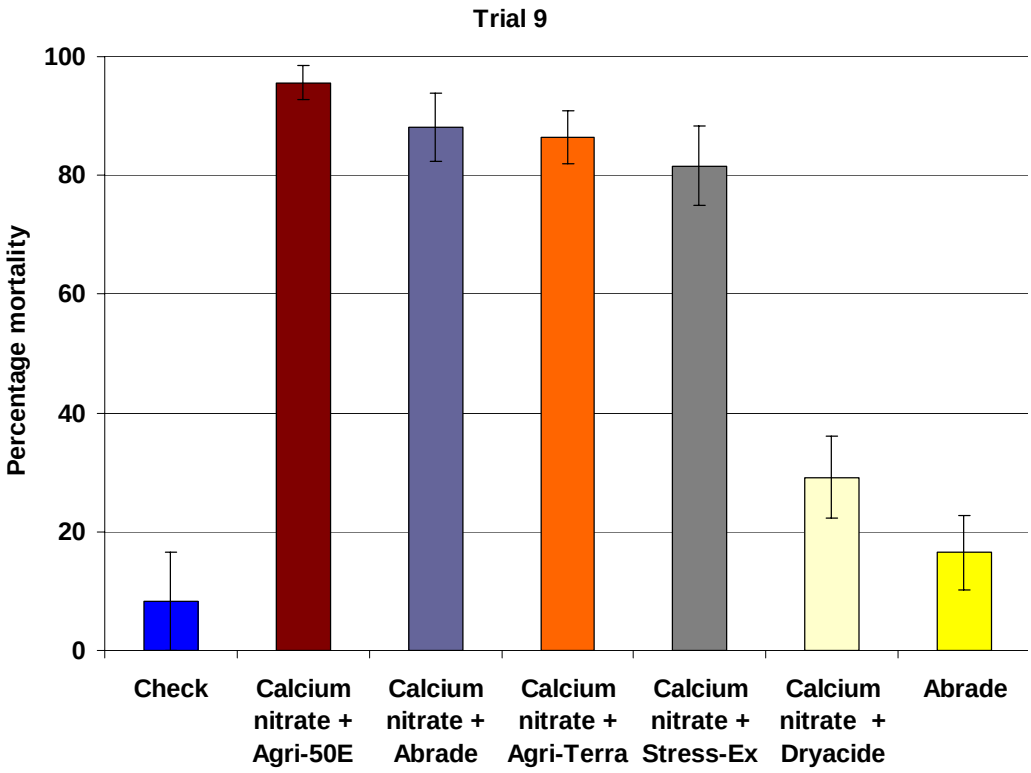

Figure 8. Mortality of late stage WFT larvae in contact with treated plastic on 3 February 2006. Bars represent standard errors of the mean (P = 0.05%).

Table 1. *Trial 1* Mortality of WFT immature stages when exposed to four cooking salt concentrations. Means followed by the same letter are not significantly different (P = 0.05).

Treatment*	Rate of product/L	Percent mortality
Sodium chloride (cooking salt)	100g	98.53 a
	50g	90.92 a
	10g	41.81 c
	1g	62.92 b

Table 1. *Trial 1* Mortality of WFT immature stages when exposed to four cooking salt concentrations. Means followed by the same letter are not significantly different (P = 0.05).

Treatment*	Rate of product/L	Percent mortality
Sodium chloride (cooking salt)	100g	98.53 a
	50g	90.92 a
	10g	41.81 c
	1g	62.92 b

Table 2. *Trial 2* Evaluation of materials applied to plastic to control WFT pupation. Means followed by the same letter are not significantly different (P = 0.05).

Treatment*	Rate of product/L	Percent mortality
Sodium chloride (cooking salt)	100g	81.97 a
Dryacide	120g	55.58 b
Hydrated lime	180g	11.03 c
Surround	60g	6.75 c
Silica fume	120g	4.17 c

*0.1% Silwet added to all treatments

Table 3. *Trial 3* Evaluation of cooking salt and Dryacide applied to plastic to control WFT pupation. Means followed by the same letter are not significantly different (P = 0.05).

Treatment*	Rate of product/L	Percent mortality
Sodium chloride (cooking salt)	100g	91.61 a
Sodium chloride + Dryacide	100g + 120g	90.16 a
Dryacide	120g	41.13 b

*0.1% Silwet added to all treatments

Table 4. *Trial 4* Evaluation of pool salt, calcium nitrate and Eco-Oil applied to plastic to control WFT pupation. Means followed by the same letter are not significantly different (P = 0.05).

Treatment*	Rate of product/L	Percent mortality
Sodium chloride + Eco-Oil	100g + 20mL	86.25 a
Calcium nitrate + Eco-Oil	100g + 20mL	85.17 a
Sodium chloride	100g	67.00 ab
Eco-Oil	20mL	29.73 b
Calcium nitrate	100g	25.14 b

*0.1% Silwet added to all treatments

Table 5. *Trial 6* Evaluation of various reduced-risk pesticides applied to plastic to control WFT pupation. Means followed by the same letter are not significantly different (P = 0.05).

Treatment*	Rate of product/L	Percent mortality
Pool salt + Stress-Ex	100g + 10mL	89.59 a
Calcium nitrate + Agri-50E	3mL + 100g	88.68 a
Calcium nitrate + Sunspray Oil	100g + 20mL	86.93 ab
Pool salt + Sunspray Oil	100g + 20mL	84.66 ab
Calcium nitrate + Stress-Ex	100g + 10mL	64.32 bc
Agri-50E	3mL	35.33 c

*Silwet at 0.1% was added to all treatments.

Table 6. *Trial 7* Evaluation of various reduced-risk pesticides applied to plastic to control WFT pupation. Means followed by the same letter are not significantly different (P = 0.05).

Treatment*	Rate of product/L	Percent mortality
Calcium nitrate + Agri-Terra	100g + 5mL	94.19 a
Calcium nitrate + Sunspray Oil	100g + 20mL	67.52 b
Calcium nitrate + Stress-Ex	100g + 10mL	62.96 b
Stress-Ex + Agri-Terra	10mL + 5mL	27.49 c
Agri-Terra	5mL	19.94 c
Stress-Ex	10mL	14.50 c

*Silwet at 0.1% was added to all treatments.

Table 7. *Trial 8* Evaluation of various reduced-risk pesticides applied to plastic to control WFT pupation. Means followed by the same letter are not significantly different (P = 0.05).

Treatment*	Rate of product/L	Percent mortality
Pool salt + Agri-Terra	100g + 5mL	98.16 a
Calcium nitrate + Agri-Terra	100g + 5mL	94.57 a
Calcium nitrate + Stress-Ex	100g + 10mL	90.74 a
Calcium nitrate + Agri-50E	100g + 3mL	84.79 a
Calcium nitrate + Abrade	100g +2.5mL	81.08 a
Calcium nitrate + Sunspray Oil	100g + 20mL	78.69 a

Table 8. *Trial 9* Evaluation of various reduced-risk pesticides applied to plastic to control WFT pupation. Means followed by the same letter are not significantly different (P = 0.05).

Treatment*	Rate of product/L	Percent mortality
Calcium nitrate + Agri-50E	100g + 3mL	94.99 a
Calcium nitrate + Abrade	100g +5mL	87.60 a
Calcium nitrate + Agri-Terra	100g + 5mL	85.21 a
Calcium nitrate + Stress-Ex	100g + 10mL	81.13 a
Calcium nitrate + Dryacide	100g + 120g	22.78 b
Abrade	5mL	12.36 b

Table 9. Summary of trials to assess efficacy of reduced-risk products and product mixes applied to plastic floor covering in preventing WFT pupation.

Treatment	% mortality	Trial
$CaNo_3$ 10% + Agri-Terra 0.5%	95.0	8
	94.2	7
	86.4	9
$CaNo_3$ 10% + Agri-50E 0.3%	91.0	6
	85.7	8
	95.6	9
$CaNo_3$ 10% + Sunspray Oil 2%	89.9	6
	71.1	7
	80.2	8
	79.6	7
$CaNo_3$ 10% + Stress-Ex 1%	91.3	8
	73.5	7
	70.4	6
	81.6	9
$CaNo_3$ 10% + Abrade 0.25%	81.8	8
$CaNo_3$ 10% + Abrade 0.5%	88.1	9
$CaNo_3$ 10% + Eco-Oil 2%	87.4	4
$CaNo_3$ 10% + Dryacide 12%	29.1	9
$CaNo_3$ 10%	49.8	4
$NaCl_2$ 10% + Agri-Terra 0.5%	95.0	8
$NaCl_2$ 10% + Sunspray Oil 2%	87.2	6
$NaCl_2$ 10% + Stress-Ex 1%	91.4	6
$NaCl_2$ 10%+ Eco-Oil 2%	90.9	4
$NaCl_2$ 10%+ Dryacide 12%	90.2	1
$NaCl_2$ 10% (cooking salt)	98.5	1
	82.0	2
	91.6	3
$NaCl_2$ 10% (pool salt)	79.6	4
Agri-50E 0.3%	48.3	6
Agri-Terra 0.5% + Stress-Ex 1%	46.0	7
Agri-Terra 0.5% + Stress-Ex 1%	41.3	7
Agri-Terra 0.5%	37.1	7
	38.3	7
Stress-Ex 1%	39.3	7
	28.0	7
Abrade 0.5%	16.5	9
Dryacide 12%	55.6	2
	41.1	3

1.1.6 EVALUATION OF FOLIAR TREATMENTS AGAINST WESTERN FLOWER THRIPS, *FRANKLINIELLA OCCIDENTALIS* (PERGANDE)

BACKGROUND

Since its introduction to Australia in 1993, western flower thrips (WFT), has established throughout Australia and is now regarded as a major horticultural pest problem. A combination of resistance to many pesticides and its aggressive vectoring of tomato spotted wilt virus has made WFT a key pest of the greenhouse vegetable industry. There is a dearth of effective pesticides legally available; however, modern greenhouses are capable of making effective use of biocontrol strategies, coupled with the assistance of reduced-risk pesticides in IPM programs, where they are available. In Australia, greenhouse vegetable growers are able to use the phytoseiid predatory mite biocontrol agents *Transeius* (*Typhlodromips*) *montdorensis* (Schicha) and *Neoseiulus cucumeris* (Oudemans) against this pest and other thrips pest species that breed in crops. However, there is still a demand for the development of effective reduced-risk pesticides to complement these two biocontrol agents in a biologically-based IPM program. This research investigated the efficacy of several products as foliar sprays against WFT to determine their ovipositional repellency and mortality effects.

Aside from trials against WFT reported on in this section, this study also included trials against greenhouse whitefly, *Trialeurodes vaporariorum* (Westwood), (GWF) and green peach aphid, *Myzus persicae* (Sulzer) (GPA). The list of pesticides used in this study is given in Table 1 and trials are reported in Sections 1.4 (GWF) and 1.5 (GPA).

Table 1. List of reduced-risk pesticides evaluated against WFT, GWF and GPA in pot trials conducted in a temperature-controlled small glasshouse.

Reduced-risk Pesticide	Type of product	Pest tested	Assessment*
Citrox	Cold pressed citrus product	WFT, GWF	OR, LM
Majestik	Starch-based	WFT, GWF	OR, LM
Agri-50E	Alginate	WFT, GWF	OR, LM
Eco-Oil	Botanical oil	WFT, GWF	OR, LM
Sunspray	Petroleum oil	WFT, GWF	OR, LM
Brella	Petroleum oil	WFT, GWF	OR, LM
Surround	Kaolin	WFT, GWF	OR, LM
Pulse	Silicone surfactant	WFT	LM
Silwet L-77	Silicone surfactant	WFT	LM
Freeway	Silicone surfactant	WFT	LM
NuFilm 17	Synthetic polymer	WFT, GWF	LM
Abrade	Amorphous silica	WFT, GPA	LM
Pest Off	Starch-based	WFT, GPA	LM
BioLink	Microbial mixture	WFT, GPA	LM

*OR = ovipositional repellence, LM = larval mortality

1.1.6.1 EVALUATION OF THE EFFICACY OF THE REDUCED-RISK PESTICIDES ABRADE™, PEST OFF™ AND BIOLINK™ AS FOLIAR SPRAYS AGAINST WESTERN FLOWER THRIPS, *FRANKLINIELLA OCCIDENTALIS* (PERGANDE)

INTRODUCTION

Three pesticides were brought to our attention as possible reduced-risk pesticides for greenhouse pest use in Australia. They were Abrade™, an amorphous silica-based product, registered in Australia as a stored grain protectant, Pest Off™, a starch-based product similar to Eradicoate™ and Majestik™, available in the UK and presently being targeted for registration in some other countries, plus Biolink™, an aqueous microbial mixture. An initial experiment was conducted to compare the efficacy of these three new reduced-risk chemicals against western flower thrips (WFT).

MATERIALS AND METHODS

Trial 1 The trial was conducted on potted plants in a small temperature-controlled glasshouse. Cucumber plants cv Kaspian RZ were propagated from seed in 30mm square Growool™ propagation blocks and transplanted into potting media in larger bags when they had reached single leaf stage. Seeds were sown on 13 May 2005 and seedlings transplanted on 25 May. All plants were drip irrigated hydroponically. Plants were infested on 7 and 8 June with 30 WFT larvae/plant. A pre-treatment assessment was made 8 June, just prior to treatment. Treatments were Abrade at 20mL and 40mL/L, Biolink at 50mL/L and Pest Off at 25mL/L, with a water only control. Treatments were applied once with a 1L hand-held applicator to incipient run-off. A treatment plot was a single plant and each treatment was replicated four times with treatments arranged randomly in each replicate block. Assessment was by direct counting total live thrips two days post-treatment.

Trial 2 Plants were germinated in 75mm GroWool™ blocks, transplanted into 15cm pots containing potting media for growing on, and placed on a bench in the glasshouse. The floor of the bench was covered with porous weed mat and the surface sprinkled with vermiculite to assist thrips pupation and successful adult emergence. The aim was to establish a breeding thrips population on the plants. Plants were infested with 30 WFT larvae/plant on two occasions: when the plants were at the true leaf stage and when plants had been topped to restrict growth after 5-6 true leaves had formed. No pre-treatment count was undertaken. There were two treatments, Abrade at 2.5ml/litre, and Pest Off at 25ml/litre, with a water only control, with all treatments replicated four times. The rate of Abrade was reduced from the previous trial to label rate. A surfactant (Silwet L77) was added to the Pest Off treatment, but not to the Abrade. Treatments were applied 1 and 5 July 2005 Assessments were made 3-4 days after each treatment application by counting numbers of live immature and adult thrips.

Statistical analysis

Data were log transformed for analysis by ANOVA.

RESULTS AND DISCUSSION

Trial 1 For the pre-treatment data, counts were not significantly different and uniformity was therefore assumed. For the post-treatment count, all treatments were significantly better than the control (P = 0.05), with Abrade at40mL/L different from Pest Off, Biolink and Abrade at 20mL/L. This was the first time any of these pesticides had been tested by us against WFT. Biolink will not be persevered with because its composition cannot be guaranteed from one batch to another. This would be unacceptable to the TGA and would be highly unlikely to gain clearance from them. Pest Off and Abrade, particularly the higher rate, would be worthy of further evaluation, particularly as the companies concerned have indicated their interest in registration with the APVMA should subsequent trials confirm their efficacy.

Trial 2 Data are not presented due to the lack of significant treatment differences for either adults or larvae at either of the assessments. The non-significant results were due to the degree of variability of the raw data and the lack of pre-treatment counts that prevented a test for uniformity from being carried out. Additionally, it was apparent that those treatment plants adjacent to unrestricted control plots heavily populated with WFT also became more heavily populated than might have been expected. Both products will be resubmitted for testing at a later date, although it is apparent from Trial 1 results that Abrade is unlikely to be effective at the label rate of 2.5mL/L.

Table 2. *Trial 1* Mortality effect of reduced-risk pesticides against WFT infesting cucumber plants. Means in the same column followed by the same letter are not significantly different (P = 0.05).

Treatment	Rate (mL/L)	% mortality (adjusted means).
Control	-	44.21 a
BioLink	20	28.16 b
Pest Off	20	26.79 b
Abrade	20	24.41 b
Abrade	40	9.59 c

1.1.6.2 EVALUATION OF THE OVIPOSITIONAL REPELLENCY AND MORTALITY EFFECTS OF REDUCED-RISK PESTICIDES AGAINST WESTERN FLOWER THRIPS, *FRANKLINIELLA OCCIDENTALIS* (PERGANDE)

INTRODUCTION

Separate trials to investigate the ovipositional repellency and mortality effects on WFT of candidate reduced-risk pesticides were conducted.

MATERIALS AND METHODS

Ovipositional repellence The trial compared seven pesticide treatments and a water only control, with each treatment replicated eight times (Table 1). French bean seedlings previously treated with Cultar™ dwarfing agent and grown in forestry tubes provided a test plant comprising a single pair of expanded cotyledons. Pesticides were applied 24 June 2004 to the pair of cotyledons to incipient run-off, using a 1L hand-held sprayer. Seedlings were air dried before being placed in a finely meshed ovipositional cage. Approximately 1000 adult female western flower thrips (WFT) were introduced for oviposition and disturbed three times over seven hours to ensure even distribution of egg laying throughout the block of seedlings. Oviposition was allowed to occur over 24 hours. Adult WFT were removed using an aspirator. The plants were then placed in randomized blocks in a separate greenhouse and held at approximately 25^{o}C. Assessment was undertaken in the laboratory by directly counting emerged WFT larvae four days after egg hatch (1 July).

Larval mortality The trial compared 11 pesticide treatments and a water only control with each treatment replicated four times (Table 2). Prior to treatment application, seedlings were placed in a finely meshed ovipositional cage, where approximately 1000 adult females WFT were introduced for oviposition 12 September 2003 and disturbed three times over seven hours to ensure even distribution of egg-laying throughout the block of seedlings. Oviposition was allowed to occur over 48 hours, following which the seedlings were taken from the cage, and WFT adults removed using an aspirator. Seedlings were held in a separate greenhouse to allow egg hatch to occur. A pre-treatment assessment was made by directly counting WFT first and second stage larvae on each plant on 17 September.

Pesticide treatments were applied 17 September to seedlings to incipient run-off, using a 1 L hand-held sprayer. After air drying they were placed in randomized blocks in a greenhouse and held at 25^{o}C. Post-treatment assessment was conducted in the laboratory two days after treatment by direct counting surviving thrips juveniles on each plant.

<u>Statistical analysis</u>

Trial 1 Data were log transformed and subjected to ANOVA.

Trial 2 Data were log transformed and subjected to ANOVA. Pre-treatment counts were not significantly different ($P<0.05$) and the populations were assumed to be uniformly distributed over the plants. Post treatment data were corrected for control mortality prior to analysis as follows:

Adjusted total = total *(1-control dead proportion)
Adjusted dead=dead - control dead proportion*total

Treatment effect was modelled using a mixed linear regression approach (Searle 1971), which allowed for the separation of variance into fixed and random effects.
A GLMM was fitted to the data with binomial errors and logit link function as follows:

Logit(insects dead) = mean + treat + *rep*,

where the italicised terms are included in the model as random effects. The analysis was conducted using ASREML. Treatment and time effects were examined for significance and means were compared using the least significance difference (LSD) technique at the 5% level and then back-transformed to the original scale.

RESULTS AND DISCUSSION

Trial 1
Ovipositional repellence Western flower thrips ovipositional repellency data are given in Table 3. Only two treatments, 6% Surround and 1% Sunspray oil, were significantly different ($P<0.05$) from the control, with Surround significantly better than Sunspray. None of the remaining treatments was different from the control, while Majestik and Eco-Oil were also not different from Sunspray.

Sunspray performed creditably as an oviposition repellent for WFT, as it did against greenhouse whitefly (Section 1.4). Unfortunately, for some unknown reason, the other petroleum oil product Brella was less well performed. Surround, the kaolin-based product was clearly the best oviposition repellent; however, the company concerned has decided not to proceed with developing it as a pesticide for this purpose. None of the remaining products in the ovipositional repellency trial demonstrated enough efficacy to warrant further investigation on their own, although combinations of products with others such as Citrox may hold promise.

Trial 2
Larval mortality WFT larval mortality data are given in Table 4. After correction for control mortality, the best performed treatment was the starch-based Majestik with 41.62% mortality. The remaining treatments were all significantly inferior to Majestik, performing quite poorly and offering little hope as potential IPM tools for WFT.

Overall, the larval mortality trial was disappointing. It may be worthwhile repeating this work to confirm or refute these findings.

Table 1. *Trial 1* Western flower thrips ovipositional repellency trial treatments.

Pesticide	Type of product
Citrox	Cold pressed citrus product
Majestik	Starch-based
Agri-50E	Alginate
Eco-Oil	Botanical oil
Sunspray	Petroleum oil
Brella	Petroleum oil
Surround	Kaolin

Table 2. *Trial 2* Western flower thrips larval treatments.

Pesticide	Type of product
Majestik	Starch based
Surround	Kaolin based
Pulse	Silicone surfactant
Silwet L77	Silicone surfactant
Eco-Oil	Botanical oil
Sunspray	Petroleum oil
Brella	Petroleum oil
Citrox	Cold pressed citrus
Freeway	Silicone surfactant
Agri-50E	Alginate based
NuFilm 17	Synthetic polymer

Table 3. *Trial 1*. Ovipositional repellence of reduced-risk pesticides against WFT. Means in the same column followed by the same letter are not significantly different (P = 0.05). Analysis was conducted on adjusted means.

Treatment	Rate applied (mL or g/L)	WFT larvae per plant (back transformed means)
Citrox	10	19.9 a
Control	-	18.7 a
Agri-50E	5	15.0 a
Brella	10	12.6 a
Majestik	50	10.6 ab
Eco-Oil	10	8.8 ab
Sunspray	10	4.5 b
Surround	60	0.4 c

Table 4. *Trial 2*. Efficacy of reduced-risk pesticides against WFT larvae. Means in the same column followed by the same letter are not significantly different (P = 0.05). Analysis was conducted on adjusted means.

Treatment	Rate applied (mL or g/L)	% mortality (back transformed means)
Majestik	50	41.62 a
Surround	60	18.23 b
Pulse	0.5	14.05 bc
Silwet L77	0.3	12.12 bc
Eco-Oil	10	10.66 bc
Sunspray	10	9.7 bcd
Brella	10	8.31 bcd
Citrox	10	8.13 cd
Freeway	0.5	6.12 cd
Agri-50E	5	6.03 cd
NuFilm 17	10	4.23 d

REFERENCE

Searle, S. R. 1971. Linear Models. John Wiley & Sons, New York.

1.2 EVALUATION OF PESTICIDES FOR EFFECTIVENESS AGAINST TOMATO RUSSET MITE, *ACULOPS LYCOPERSICI* (MASSEE), – LABORATORY BIOASSAYS

INTRODUCTION

Tomato russet mite (TRM) is a major problem in greenhouse tomato crops in the summer months in NSW, SA and VIC. The predatory mite *Transeius* (*Typhlodromips*) *montdorensis* (Schicha) will exert partial control but is not adequate on its own. Vertimec (abamectin) is very effective, but kills *T. montdorensis*, *Encarsia formosa* and other biocontrol agents. To avoid resistance problems, there are also restrictions on the permitted number of applications per season, from two to five. An alternative effective pesticide that is low risk to biocontrol agents is required. A combination of 0.25mL or g/L Calibre™ (10 EC or 10WP) (hexythiazox) and 0.25mL/L Eco-Oil™ (botanical oils) was very effective in a 500sqm tomato crop at GHI and in limited laboratory trials early in 2003, and had the required safety to natural enemies. More extensive trials of this and other products were conducted to provide information on candidates suitable for large scale greenhouse trials.

MATERIALS AND METHODS

A series of eleven trials were conducted between December 2003 and March 2006. Except for the last trial, which was conducted on cape gooseberry, *Physalis peruviana*, the host plant used was black nightshade, *Solanum nigra*, as tomato leaves are irregular and TRM difficult to see on this substrate. Both plants are solanaceous and hosts for TRM in the wild. Cape gooseberry has a very hirsute leaf and was difficult to transfer mites onto, but mite survival and leaf longevity were excellent. Leaf discs 30mm-diameter were cut from the plant and set in 1% agar, upper surface down, in screened Millipore™ dishes (one disc per unit). Tomato russet mites were taken from dense populations on tomato plants held in a small temperature-controlled greenhouse. Approximately 110 TRM were transferred to each disc using a camel hair brush late afternoon and allowed to settle overnight. There were 5-6 replicates per treatment. Pesticides were applied using a Potter's Spray Tower delivering 2 ± 0.5mg/cm^2. The control treatment was sprayed with water except in the final trial where 0.1% Silwet L-77, an organosilicone wetting agent, was added to all spray solutions. Treatments are summarised in Table 12 with trials in which they were assessed. Dishes were randomised and inverted with lower leaf surface down in a closed tray on a rack over a glycerol/water mix to give a relative humidity of approximately 75%. Relative humidity was measured by Hastings Tinyview™ data loggers. The relative humidity was varied for some experiments (65% and 80%), particularly for Calibre/Eco-Oil combinations, to see if this impacted on the results. After 3 days in an incubator at 25 ± 0.5 ^{o}C and 16:8 L: D photoperiod, live mites were counted while aspirating them off the leaf. Mites were counted as live if they were capable of movement when observed or touched.

Statistical analysis

All trials were analysed separately. The number of live mites after three days was subtracted from the initial number. The percentage mortality was corrected for control mortality prior to analysis.

Trial 1 Data were analysed using GLMM with binomial errors and logit link function fitted using ASREML; replicate was fitted as a random effect.
Trial 2 A t-test assuming unequal variance was used to compare the adjusted % mortalities of the two Eco-Oil treatments.
Trials 3 to 11 GLMM analysis was inappropriate for this data as the residual variance was too high. An ANOVA of the % adjusted mortality was conducted in GenStat.

RESULTS AND DISCUSSION

A summary of treatments and results is presented at the end of this section (Table 12).

Trial 1 (8-12 December 2003) Vertimec gave the best control, but Acramite and Calibre + Eco-Oil were also very effective (Table 1). Eco-Oil at 2.5mL/L was moderately effective. Mites were moribund in the Eco-Oil treatment. They were capable of leg movement, but not sustained walking; they were somewhat curled, but many were still plump. Eco-Oil was possibly repellent as 10-20% of mites were at the leaf margins. Most mites were mobile in the Calibre-alone treatment, whereas in the combination with Eco-Oil they were shrunken and the few survivors were at the leaf margins. Mites were still plump, but immobile in the Vertimec treatment, with some leg movement in survivors of the Acramite treatment, but otherwise immobile.

Table 1. Mortality of tomato russet mite on black nightshade leaves three days after a foliar spray application. Means in the same column followed by the same letter are not significantly different (P = 0.05%).

Treatment	Rate of product (mL/L)	% mortality (back transformed means)
Vertimec	0.75mL	100
Acramite	1.3mL	95.70 ± 1.84 a
Calibre 10EC	0.25mL	0.22 ± 0.17 c
Eco-Oil	2.5mL	74.84 ± 3.46 b
Calibre + Eco-Oil	0.25mL + 2.5mL	97.63 ± 1.48 a

Trial 2 (15-19 December 2003) Vertimec at all three rates, two of which were less than label rate of 0.6mL/L, provided total control of TRM (Table 2). Eco-Oil was also very effective at 5mL/L, but not at 2.5mL/L.

Table 2. Mortality of tomato russet mite on black nightshade leaves three days after a foliar spray application. Means in the same column followed by the same letter are not significantly different (P = 0.05%).

Treatment	Rate of product (mL or g/L)	% mortality (back transformed means)
Vertimec	0.75mL	100
Vertimec	0.50mL	100
Vertimec	0.25mL	100
Eco-Oil	2.5mL	42.63 a
Eco-Oil	5.0mL	92.69 b

Trial 3 (12-16 January 2004) Acramite at 1.3 and 1.5mL/L and Nufilm and Citrox with sugar provided excellent control of TRM. Silwet alone was not effective, unlike in previous trials with two-spotted mite, where it was very effective. For Acramite, TRM were plump, but there was no mobility. For Citrox + sugar, survivors were mobile, but slow. There was some leaf shininess, no eggs were seen. Survivors in the Nufilm + sugar treatment were more mobile than for Citrox; eggs were few in number. There were a few eggs in the Silwet treatment, and the leaf surface appeared slightly oily. Both Nufilm and Citrox with sugar added appeared promising for TRM control, but the result differed from that in Trial 6.

Table 3. Mortality of tomato russet mite on black nightshade leaves three days after a foliar spray application. Means in the same column followed by the same letter are not significantly different (P = 0.05%).

Treatment	Rate of product (mL or g/L)	% mortality (back transformed means)
Acramite	1.3mL	100
Acramite	1.5mL	100
Silwet-L77	0.1mL	21.68 a
Silwet-L77	0.2mL	48.77 a
Nufilm + sugar	10mL + 10g	90.34 b
Citrox + sugar	10mL + 20g	92.86 b

Trial 4 (19-23 January 2004) All oils except Eco-Oil at the lower rate of 2.5mL/L gave good to excellent control of TRM (Table 4). Survivors were mostly capable only of moving their legs and not walking. Humidity was high (80-87.5%).

Table 4. Mortality of tomato russet mite on black nightshade leaves three days after a foliar spray application. Means in the same column followed by the same letter are not significantly different (P = 0.05%).

Treatment	Rate of product (mL or g/L)	% mortality (back transformed means)
Eco-Oil	2.5mL	44.38 a
Eco-Oil	5mL	86.71 bc
Sunspray oil	5mL	96.11 bc
Sunspray oil	10mL	100
Brella oil	5mL	81.39 b
Brella oil	10mL	99.80 bc

Trial 5 (27-30 January 2004). Only Sunspray oil at 10mL/L provided good control (Table 5). The treatments were divided between two trays, one at 60% RH and the other at 80%. There were no consistent differences in mortality. Mites on discs treated with Citrox and Citrox + sugar tended to be at the edges of the disc, suggesting a repellent effect. Efficacy of both Citrox and Nufilm with sugar was much lower than in Trial 3, for unknown reasons, and not significantly different than the product without sugar. A similar result was obtained in Trial 6, though efficacy was better.

Table 5. Mortality of tomato russet mite on black nightshade leaves three days after a foliar spray application. Means in the same column followed by the same letter are not significantly different (P = 0.05%).

Treatment	Rate of product (mL or g/L)	% mortality (back transformed means)
Silwet	0.3mL	3.35 a
Calibre + Eco-Oil	0.25mL + 2.5mL	48.93 b
Citrox	10mL	44.64 b
Citrox + sugar	10mL + 20g	59.87 b
Nufilm	10mL	50.00 b
Nufilm + sugar	10mL + 10g	56.22 b
Sunspray oil	10mL	98.28 c

Trial 6 (3-6 February 2004). This was a repeat of Trial 5 as results were not consistent with previous results for Trial 3 for Nufilm and Citrox with sugar, or Calibre + Eco-Oil in Trial 1. Control was improved but the results show continued inconsistency and were not adequate for good control (Table 6). Lack of consistency may be partly due to borderline toxicity or delayed reaction. Sunspray at 10mL/L again gave excellent control.

Table 6. Mortality of tomato russet mite on black nightshade leaves three days after a foliar spray application. Means in the same column followed by the same letter are not significantly different (P = 0.05%).

Treatment	Rate of product (mL or g/L)	% mortality (back transformed means)
Silwet	0.3mL	7.30 a
Calibre + Eco-Oil	0.25mL + 2.5mL	71.85 b
Citrox	10mL	65.32 b
Citrox + sugar	10mL + 20g	78.38 b
Nufilm	10mL	58.33 b
Nufilm + sugar	10mL + 10g	62.84 b
Sunspray oil	10mL	100

Trial 7 (1 March-5 March 2004) Calibre + Eco-Oil treatments and control were distributed between two trays again, one at 50-55% RH, the other at 80-85% RH. The controls were not significantly different so data from both were combined. In this trial Calibre + Eco-Oil was more effective at low humidity than high (Table 7). Possibly low humidity speeds the process, or at least the mites appear more morbid. Control was still only moderate. Calcium chloride at 0.5% was added as a treatment as it is a humectant and may be useful to add where RH needs to be high. Sugar alone was not effective against TRM.

Table 7. Mortality of tomato russet mite on black nightshade leaves three days after a foliar spray application. Means in the same column followed by the same letter are not significantly different (P = 0.05%).

Treatment	Rate of product (mL or g/L)	% mortality (back transformed means)
Calibre + Eco-Oil high RH	0.25mL + 2.5mL	55.79 c
Calibre + Eco-Oil low RH	0.25mL + 2.5mL	72.34 d
Ca Cl_2, low RH	5g	36.09 b
Sugar, high RH	20g	1.65 a

Trial 8 (30 March – 2 April 2004) Results of this trial were similar to the previous trial (Table 8).

Table 8. Mortality of tomato russet mite on black nightshade leaves three days after a foliar spray application. Means in the same column followed by the same letter are not significantly different (P = 0.05%).

Treatment	Rate of product (mL or g/L)	% mortality (back transformed means)
Calibre + Eco-Oil high RH	0.25mL + 2.5mL	52.21 b
Calibre + Eco-Oil low RH	0.25mL + 2.5mL	72.05 c
Ca Cl_2, low RH	5g	37.92 b
Sugar, high RH	20g	17.23 a

Trial 9 (5 April–8 April 2004) This was a repeat of Trials 7 and 8, with poor control by Calibre + Eco-Oil at the rates used (Table 9). Control mites moved faster than treated ones, particularly in the sugar treatment, and were a little larger, so again, it is suspected that the variable control is related to borderline or delayed mortality.

Table 9. Mortality of tomato russet mite on black nightshade leaves three days after a foliar spray application. Means in the same column followed by the same letter are not significantly different (P = 0.05%).

Treatment	Rate of product (mL or g/L)	% mortality (back transformed means)
Calibre + Eco-Oil high RH	0.25mL + 2.5mL	33.32 a
Calibre + Eco-Oil low RH	0.25mL + 2.5mL	34.45 a
Ca Cl_2, low RH	5g	38.20 a
Sugar, high RH	20g	23.05 a

Trial 10 (20 April–23 April 2004) Calibre alone at 0.25 and 0.5mL/L had little effect on TRM (Table 10). For a Calibre + Eco-Oil combination, doubling either the Eco-Oil or Calibre rate in the mix gave excellent control of TRM, with the higher Eco-Oil rate giving the most consistent result. A mix of sugar and $Cacl_2$ was moderately effective and better than either product alone. Floramite, with the same active ingredient as Acramite, appeared to give much reduced control compared with Acramite at the

same rate (Trial 1 and 3), but there was considerable variation between replicates and this needs repeating.

Table 10. Mortality of tomato russet mite on black nightshade leaves three days after a foliar spray application. Means in the same column followed by the same letter are not significantly different (P = 0.05%).

Treatment	Rate of product (mL or g/L)	% mortality (back transformed means)
Sugar + $CaCl_2$	20g + 5g	70.34 b
Calibre + Eco-Oil	0.5mL + 2.5mL	93.23 ab
Calibre + Eco-Oil	0.25mL + 5.0mL	99.39 a
Floramite	1.3mL	54.34 b
Calibre	0.25mL	11.98 c
Calibre	0.5mL	14.59 c

Trial 11 (24 March-27 March 2006) Liquisulf and Vertimec provided excellent control of TRM, but Abrade, Applaud and Agri-50NF were not effective (Table 11). There were only occasional eggs in the sulphur treatment, a few in Agri-50NF and none in the Vertimec treatment.

Table 11. Mortality of tomato russet mite on cape gooseberry leaves three days after a foliar spray application. Means in the same column followed by the same letter are not significantly different (P = 0.05%).

Treatment	Rate of product (mL/L)	% mortality (back transformed means)
Liquisulf	3.5mL/L	98.86 c
Abrade	2.5mL/L	3.60 a
Agri-50NF	3mL/L	14.77 b
Applaud	0.6mL/L	7.58 ab
Vertimec	0.6mL/L	100

In summary, Calibre at 0.25mL/L plus Eco-Oil at 2.5mL/L as a mix gave inconsistent results when used at the rate which gave apparently excellent results in a $500m^2$ greenhouse. Calibre alone was not effective, whereas Eco-Oil gave moderate results at this rate and high mortality at 0.5mL/L. One replicate where either the Calibre rate was doubled to 0.5mL/L or Eco-Oil to 5mL/L in the combination gave high mortality; however, it is not possible to separate this from an effect of high Eco-Oil rate alone. It is possible that Calibre adds egg mortality to the mix, as there is little residual activity of the oil. Other products giving excellent control of TRM were Sunspray oil at 0.5 and 1%, Brella oil at 1%, Acramite at 1.3 and 1.5mL/L, Liquisulf at 3.5mL/L and Vertimec at 0.25-0.75mL/L. Floramite appeared to be less effective than Acramite, and Applaud, Agri-50NF and Abrade were ineffective. It is interesting to note the differences for these new products between their effect on TRM and broad mite. Acramite was not effective against broad mite, whereas Abrade and Agri-50NF were. Of the products tested against TRM, Calibre 0.5% + Eco-Oil 0.25%, Acramite, Liquisulf and Sunspray oil at 0.5% are of interest as likely to cause the least toxicity to natural enemies, while giving good to excellent control of TRM. While Vertimec at

the label rate is toxic and residual to natural enemies such as *Encarsia formosa* and predatory mites, it gave excellent control at 25mL/100L, well below label rate (60-90mL/100L), so could be used as a spot treatment or limited area treatment with minimal disruption.

Table 12. Summary of results of 11 bioassay trials assessing the effect of pesticides on tomato russet mite.

Treatment	Active ingredient	Rate (mL or g product /L)	Trial #	Adjusted % efficacy
Vertimec	18g/L abamectin	0.25	2	100
		0.5	2	100
		0.6	11	100
		0.75	1, 2	100, 100
Acramite	480g/L bifenazate	1.3	1, 3	95.7, 100
		1.5	3	100
Liquisulf 700 SC	700g/L elemental sulphur	3.5	11	98.9
Sunspray Ultrafine Spray Oil	988ml/L petroleum oil	5	4	96.11
		10	4, 5, 6	100, 98.3, 100
Brella	990mL/L petroleum oil	5	4	81.39
		10	4	99.80
Eco-Oil	850g/L botanical oils	2.5	1, 2, 4	74.8, 42.6, 44.4
		5	2, 4	92.7, 86.7
Calibre 10EC	100g/L hexythiazox	0.25	1, 10	0.2, 12.0
		0.5	10	14.6
Calibre + Eco-Oil	hexythiaxox + botanical oils	0.25 + 2.5	1, 5, 6, 7, 8, 9	97.6, 48.9, 71.9, 55.8, 72.3, 52.2, 72.1, 33.3, 34.5
Calibre + Eco-Oil	hexythiaxox + botanical oils	0.5 + 2.5	10	93.2
		0.25 + 5.0	10	99.4
Citrox 14W	10% citrus extract	10	5, 6	44.6, 65.3
Citrox + sugar		10 + 20	3, 5, 6	92.9, 59.9, 78.4
Nufilm-17-17	96% pinolene	10	5, 6	50, 58.3
Nufilm + sugar		10 + 10	3, 5, 6	90.3, 56.2, 62.8
Silwet L-77	80% polyalkylencoxide modified heptamethyltrisiloxane	0.1	3	21.7
		0.2	3	48.8
		0.3	5, 6	3.4, 7.3
Sugar	sucrose	20	7, 8, 9	1.7, 17.2, 23.1
Sugar + $CaCl_2$		20 + 5	10	70.3
Calcium chloride		5	7, 8, 9	36.1, 37.9, 38.2
Floramite	480g/L bifenazate	1.3	10	54.3
Abrade 50	450g/L amorphous silica	2.5	11	3.6
Agri-50NF	280g/L propylene glycol alginate	3	11	14.8
Applaud	440g/L buprofezin	0.6	11	7.6

REFERENCE

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1.3 EVALUATION OF PESTICIDES FOR CONTROL OF BROAD MITE, *POLYPHAGOTARSONEMUS LATUS* (BANKS), ON CAPSICUM

INTRODUCTION

Broad mite is a major problem in basil, capsicum and several other plants with soft foliage. It is often not observed until plant damage occurs. Broad mites prefer high humidity and warm temperatures, and are generally found in growing tips, on new leaves and fruit. Males disperse the population by carrying immature females to new leaves. Several species of phytoseiid mites feed on broad mite, but populations can build up rapidly and damage to growing tips often occurs before the cause is recognised. Immediate action is often required, but one that will leave residues not harmful to subsequent release of predatory mites such as *Transeius* (*Typhlodromips*) *montdorensis* and *Neoseiulus cucumeris*.

MATERIALS AND METHODS

Broad mites from basil, *Ocimum basilicum* (Leongatha, Freshzest Herbs, Victoria) were used to infest young capsicum plants at GHI. Three trials were conducted to evaluate pesticides with potential for integration in IPM programs using biocontrol agents. Treatments were arranged in a randomised block design in a small glasshouse. Plants were fertigated by drip irrigation. Temperature was 25 ± 5^{o}C, with variable humidity.

Trial 1 Plants were pruned to leave one upper leaf with reasonable numbers of broad mite and the growing tip. Pesticides were applied to run off with a hand sprayer, five replicates per treatment, on 17 October 2005, and assessed 20 October by placing leaves individually in ziplock bags and counting live mites under a microscope. No surfactant was added. Treatments were: Liquisulf 700 SC (Ekko, Vic) (700g/L sulphur) at 3.5mL/L, Abrade (450g/L amorphous silica) at 2.5mL/L, Eco-Oil (850g/L botanical oils) at 5mL/L, Acramite (480g/L bifenazate) at 0.65mL/L, and an untreated control.

Trial 2 Plants were pruned to leave two upper leaves with 20-70 broad mites plus the growing tip. Pesticides were applied to run off, five replicates per treatment on 3 March 2006, and assessed 6 March 2006 as previously. Treatments were Liquisulf at 3.5mL/L, Abrade at 2.5mL/L, Eco-Oil at 5mL/L, Agri-50NF (28% propylene glycol alginate) at 3mL/L, Applaud (440g/L buprofezin) at 0.6mL/L, Biocover Horticultural Oil (840g/L petroleum oil) at 10mL/L and an untreated control.

Trial 3 The previous trial was repeated on 28 August 2006 and assessed three days later. There were six replicates per treatment. The same pesticides were applied at the same rates, except Biocover oil, which was reduced to a rate of 5mL/L.

Statistical analysis

Analysis of variance was conducted on the ($\log_e$ + 1) transformed data. LSD of the means was used to separate treatment effects (P = 0.5%).

RESULTS AND DISCUSSION

Trial 1 After three days, mortality was very high with Abrade, Eco-Oil and sulphur (Table 1). There were a few very young mites on Eco-Oil-treated leaves so a second treatment to newer leaves would be recommended. Abrade and Liquisulf both left a visible deposit on the leaf surface, which may be an issue.

Table1. Assessment of a single foliar spray against broad mite (3 days post-treatment). Means in the same column followed by the same letter are not significantly different (P = 0.05).

Treatment	Rate (product/L)	Mean number of broad mites/leaf (back transformed means)
Control	-	105.7 a
Acramite	0.65mL	107.9 a
Abrade	2.5mL	7.7 b
Eco-Oil	5mL	1.9 b
Liquisulf	3.5mL	0

Trial 2 Both Liquisulf and Eco-Oil gave excellent control of broad mite (Table 2). Liquisulf again left a visible white deposit. Biocover oil gave excellent control except on one leaf, but left a visible oily deposit. Abrade also gave good control. It left a greyish deposit which was not as noticeable as with Liquisulf. Both Agri-50NF and Applaud killed some mites, but gave inconsistent results. The efficacy of Applaud, an insect growth regulator, may improve with time, but many eggs were noted.

Table 2. Assessment of a single foliar spray against broad mite (3 days PT) n = 5. Means in the same column followed by the same letter are not significantly different (P = 0.05).

Treatment	Rate (product/L)	Mean number of broad mites/leaf (back transformed means)
Control	-	21.4 a
Applaud	0.6mL	7.5 b
Agri-50NF	3mL	2.4 c
Abrade	2.5mL	1.4 cd
Biocover oil	10mL	0.3 d
Eco-Oil	5mL	0
Liquisulf	3.5mL	0

Trial 3 Liquisulf again gave excellent control of broad mite (Table 3). Biocover oil at 0.5%, half the previous rate, also provided excellent control and oily residue was not as noticeable. Eco-Oil and Agri-50NF were moderately effective, whereas Abrade and Applaud were not very effective.

Table 3. Assessment of a single foliar spray against broad mite (3 days PT) n = 6. Means in the same column followed by the same letter are not significantly different (P = 0.05).

Treatment	Rate (product/L)	Mean number of broad mites/leaf (back transformed means)
Control	-	42.3 a
Applaud	0.6mL	21.6 ab
Abrade	2.5mL	17.4 b
Agri-50NF	3mL	6.7 c
Eco-Oil	5mL	3.9 c
Biocover oil	5mL	0.4 d
Liquisulf	3.5mL	0.1 d

In summary, Liquisulf, Eco-Oil, and Biocover Horticultural Oil gave excellent control of broad mite on capsicum. Re-application of Eco-Oil may be necessary as some eggs survived. Abrade and Agri-50NF provided moderate control. Liquisulf and Abrade left a visible deposit on the leaf, which may present a problem in some crops. Biocover left an oily deposit at the high rate that was not noticeable at the lower rate. Applaud provided a low level of control, and Acramite was ineffective. Liquisulf is a so-called liquid sulphur, which is reportedly safer to natural enemies than the powder formulation; however, this should be evaluated. Sulphur and oils should not be used together or at close time intervals to avoid potential plant damage.

1.4 EVALUATION OF REDUCED-RISK PESTICIDES FOR USE AGAINST GREENHOUSE WHITEFLY, *TRIALEURODES VAPORARIORUM* (WESTWOOD)

INTRODUCTION

Despite the availability of the parasitoid biocontrol agent *Encarsia formosa*, greenhouse whitefly (GWF), continues to be a key pest of greenhouse vegetable crops. Under optimal temperature conditions, *Encarsia* can be an effective whitefly management tool; however, in Australia conditions often exceed this range, making it difficult for effective management to be achieved without the assistance of effective reduced-risk pesticides. A series of preliminary small-scale trials was conducted to evaluate the efficacy against GWF of some promising reduced-risk pesticides for ovipositional repellency and larval and pupal mortality.

MATERIALS AND METHODS

French bean seedlings were treated with Cultar™ dwarfing agent and grown in forestry tubes to provide a test plant comprising a pair of expanded cotyledons.

Ovipositional repellency trial

Trial 1 Seven pesticides (Table 1) were applied to plants to incipient run-off using a 1L hand-held sprayer. Each treatment was replicated four times and there was an untreated control. The seedlings were air dried before being placed in a finely meshed ovipositional cage and infested with adult whitefly. Approximately 1000 adult female GWF were introduced into the cage for oviposition and disturbed three times over seven hours to ensure even distribution of eggs throughout the block of seedlings. Oviposition was allowed to occur over 24 hours, following which the seedlings were taken from the cage, individually inspected for egg deposition, and whitefly adults removed using an aspirator. The plants were then placed in randomized blocks in a separate greenhouse and held for four days at approximately 25^{o}C. Assessment was undertaken in the laboratory by directly counting whitefly larvae four days after egg hatch.

Mortality trials

Trials 2, 3 and 4 Two trials against larvae and one against pupae were conducted. Seedlings were infested with whitefly as described in Trial 1 and held in a separate greenhouse to allow egg hatch to occur. For Trial 2, a pre-treatment assessment was made 24 September 2003 by directly counting whitefly II-III stage larvae on each plant in the laboratory.

Pesticide treatments (Tables 2 and 3) were applied 24 September, 28 November and 17 November in Trials 2, 3 and 4 respectively to incipient run-off, using a 1 L hand-held sprayer. They were allowed to air dry, then placed in randomized blocks in a greenhouse and held at 25^{o}C. All trials had an untreated control and each treatment was replicated four times. Post-treatment assessment was conducted in the laboratory 29 September, 2 December and 24 November for the respective trials, by directly counting surviving larvae or pupae on each plant.

Table 1. Pesticides evaluated in GWF ovipositional repellency trial. *Trial 1*

Pesticide	Type of product
Citrox	Cold pressed citrus product
Majestik	Starch-based
Agri-50E	Alginate
Eco-Oil	Botanical oil
Sunspray	Petroleum oil
Brella	Petrolem oil
Surround	Kaolin

Table 2. Pesticides evaluated against GWF larvae. *Trial 2*.

Pesticide	Type of product
Citrox	Cold pressed citrus product
Majestik	Starch-based
Agri-50E	Alginate
Eco-Oil	Botanical oil
Sunspray	Petroleum oil
Brella	Petroleum oil
Surround	Kaolin
NuFilm-17	Synthetic polymer

Table 3. Pesticides evaluated against GWF larvae *(Trial 3)* and pupae (*Trial 4*).

Pesticide	Type of product
Silwet	Silicone based surfactant
Citrox	Cold pressed citrus product
Citrox + Brella	Cold pressed citrus product + petroleum oil
Citrox + sugar	Cold pressed citrus product + sucrose
Eco-Oil	Botanical oil
Citrox + Eco-Oil	Cold pressed citrus product + botanical oil
Brella	Petroleum oil
Sunspray	Petroleum oil
NuFilm-17	Synthetic polymer
NuFilm + sugar	Synthetic polymer + sucrose

Statistical analysis

Trial 1 Ovipositional repellency trial
Data were log transformed and subjected to ANOVA.

Trials 2 and 3 Larval mortality trials
Data were log transformed and subjected to ANOVA. In Trial 2, counts were significantly different prior to the application of treatments. Non-uniformity was assumed and the pre-treatment counts were included as a covariate in the analysis of post-treatment data. In Trial 3, pre-treatment counts were not significantly different ($P<0.05$) and the populations were assumed to be uniformly distributed over plants.

Trial 4 Pupal mortality trial
Data were log transformed and subjected to ANOVA. Pre-treatment counts were not significantly different ($P<0.05$) and the populations were assumed to be uniformly distributed over plants.

RESULTS AND DISCUSSION

Ovipositional repellency
Trial 1 All treatments except Agri-50E™ resulted in significantly lower whitefly oviposition than the control (Table 4). The two most effective treatments were the petroleum oil treatments, Sunspray™ and Brella™, which were not significantly different from each other. Surround™, Eco-Oil™, Majestik™ and Citrox™ were the next best products, and not significantly different from each other. The rate of Eco-Oil 10mL/L is twice that recommended.

Larval mortality
Trial 2 All treatments were significantly different from the control ($P<0.05$) (Table 5). Excellent larval control was provided by Brella, Sunspray and Eco-Oil. Surround was the worst performed, although it was not different from Agri-50E and NuFilm-17. Its usefulness is thus as a repellent rather than a toxicant. Again, the rate of Eco-Oil 10mL/L is twice that recommended.

Trial 3 All treatments except Silwet™ were significantly different from the control (Table 6). Several products provided good to excellent control, particularly Citrox and those with Citrox added. Citrox enhanced the effectiveness of Eco-Oil and Brella, but was much more effective alone than in the previous trial. The two petroleum oils, Sunspray and Brella, were used at half the rate (5mL/L) in Trials 3 and 4 as in the previous two trials.

Pupal mortality
Trial 4. All treatments except Silwet and Sunspray were significantly different from the control (Table 7). Citrox and Brella were the most effective treatments, with Citrox in this case again appearing to enhance the activity of Eco-Oil and Brella, but not significantly. The remaining treatments performed similarly. Eco-Oil was significantly better than Sunspray oil in this trial at the same rate, a result that should be repeated.

The addition of sugar did not enhance the efficacy of pesticides against larvae or pupae in these trials. It acts as a phagostimulant and enhances efficacy of some products against thrips, but against sap suckers it may not work in the same way.

These preliminary trials produced some encouraging results. Clearly petroleum oil products can have an important role in preventing GWF populations from becoming established. Brella was a Caltex product under development for the horticultural market, but it has since been abandoned. In the larval mortality trials the two petroleum oil products were highly effective at 1%, but less so at 0.5%. Although the botanical oil product Eco-Oil was the next best treatment, it was obviously inferior to the petroleum oils. With the exception of Silwet, most of the other treatments produced encouraging results against GWF juvenile life stages. Agri-50E was

disappointing given that it was being developed specifically with whitefly in mind. This product has since been improved and is now being marketed as Agri-50NF.

Table 4. *Trial 1* Effect of reduced-risk pesticides as ovipositional repellents of GWF. Analysis was conducted on transformed means. Means in the same column followed by the same letter are not significantly different (P = 0.05).

Treatment	Rate of application (mL/L)	Mean GWF larvae per leaf (back transformed means)
Control	-	264.07 a
Agri-50E	5	153.47 ab
Citrox	10	82.93 bc
Majestik	25	42.38 c
Eco-Oil	10	32.45 c
Surround	60	31.79 c
Brella	10	8.49 d
Sunspray	10	3.85 d

Table 5. *Trial 2* Effect of reduced-risk pesticides on GWF larvae. Analysis was conducted on transformed means. Means in the same column followed by the same letter are not significantly different (P = 0.05).

Treatment	Rate of application (mL/L)	Mean GWF larvae per leaf (back transformed means)
Control	-	230.60 a
Surround	60	185.23 b
Agri-50E	10	158.81 bc
NuFilm-17	10	143.03 bc
Majestik	50	141.02 c
Citrox	10	138.91 c
Eco-Oil	10	32.41 d
Sunspray	10	5.95 e
Brella	10	0.72 f

Table 6. *Trial 3* Effect of reduced-risk pesticides on GWF larvae. Analysis was conducted on transformed means. Means in the same column followed by the same letter are not significantly different (P = 0.05).

Treatment	Rate of application (mL or g/L)	Mean GWF larvae or pupae per leaf (back transformed means)
Control	-	329.30 a
Silwet L77	0.2	169.72 ab
Eco-Oil	5	83.77 bc
NuFilm-17	10	82.93 bc
NuFilm + sugar	10 + 10	56.97 bc
Sunspray	5	53.05 c
Brella	5	28.96 cd
Citrox	10	11.93 de
Citrox + sugar	10 + 10	8.88 e
Citrox + Eco-Oil	10 + 5	5.89 e
Citrox + Brella	10 + 5	0.97 f

Table 7. *Trial 4* Effect of reduced-risk pesticides on GWF pupae. Analysis was conducted on transformed means. Means in the same column followed by the same letter are not significantly different (P = 0.05).

Treatment	Rate of application (mL or g/L)	Mean GWF pupae per leaf (back transformed means)
Silwet L77	0.2	387.22 a
Sunspray	5	339.34 a
Control	-	322.79 a
Eco-Oil	5	122.12 b
NuFilm-17 + sugar	10 + 10	111.50 b
Citrox + Eco-Oil	10 + 5	103.85 b
NuFilm-17	10	99.19 b
Citrox	10	85.03 bc
Brella	5	75.64 bc
Citrox + sugar	10 + 10	61.99 bc
Citrox + Brella	10 + 5	38.13 c

1.5. EVALUATION OF A REDUCED-RISK PESTICIDE FOR USE AGAINST GREEN PEACH APHID, *MYZUS PERSICAE* (SULZER)

INTRODUCTION

Three candidate reduced-risk pesticides were previously described in Trial 1.1.6.1 against western flower thrips. Of these, Pest Off was also evaluated for efficacy against green peach aphid (GPA) in this trial.

MATERIALS AND METHODS

The trial was conducted on potted plants in a small temperature-controlled glasshouse. Capsicum plants cv Santino were obtained from Leppington Speedy Seedlings and potted 4 June 2005 in 15cm pots. All plants were irrigated hydroponically using a cucumber formula with a single dripper per plant. Pest Off at 25mL/L was compared against a water only control. Treatments were applied by a 1L hand-held applicator to incipient run-off on three occasions seven days apart (8, 15 and 22 June 2005). A treatment plot was a single plant and each treatment was replicated four times with treatments arranged randomly in each replicate block.

Aphids from a GPA culture on capsicum were brush-transferred onto each plant in the trial. Assessment was by direct counting. There was no pre-treatment count. Post-treatment counts of GPA were made 14, 17 and 24 June by counting aphids on a single shoot six days after each treatment application. The shoot comprised three mature leaves, with a second pair of opened leaves that had not developed into the dark colour of a mature leaf, and any newly opened or opening leaves in the growing tip,. Selected shoots on each stem were marked with a spot of white correction fluid so that they could be identified at subsequent assessments.

Statistical analysis

Data were analysed separately at each assessment time with counts log transformed for analysis by ANOVA. Data were then back transformed.

RESULTS AND DISCUSSION

Pest Off treatment was significantly different from the control at both the second and third assessment. However, after three applications it was unable to prevent the aphid population from growing to an unacceptable size. This was not an encouraging outcome and Pest Off would be unlikely to be persevered with for this purpose.

Table 1. Mortality effect of a reduced-risk pesticide, Pest Off, against green peach aphid infesting capsicum. Applications were made three times, 7 days apart. Means in the same row with the same letter are not significantly different (P = 0.5). Analysis was conducted on transformed means. Means are back transformed.

Assessment	Mean number of aphids/plant	
	Control	Pest Off
1	5.78 a	4.90 a
2	972.6 a	146.9 b
3	4350.3 a	639.7 b

2. DEVELOPMENT OF FUNGAL BIOPESTICIDES AGAINST KEY GREENHOUSE PESTS

BACKGROUND

Concerns over the development of resistance to synthetic insecticides, and the potential deleterious effects of pesticides on the environment and human safety, have resulted in research being conducted into a number of alternative IPM tools for use in greenhouse crops. Modern greenhouse technology can provide good environmental control, affording opportunities for sophisticated integrated pest management (IPM) strategies that can negate the need for synthetic insecticides. Overseas, biocontrol agents (BCAs) are widely used and there is a wide range of commercially-produced BCAs regularly used by greenhouse vegetable producers. Reduced-risk pesticides are another integral part of an IPM strategy. In this context, the problems associated with pesticide use have provided impetus for the development of entomopathogenic fungi as an IPM tool, to complement biocontrol agents for use against key greenhouse pests.

Entomopathogenic fungi in the Class Hyphomycetes are most commonly targeted for this purpose; existing overseas commercial products include BotaniGard™, Naturalis O™ (*Beauveria bassiana*), Mycotal™, Vertalec™ (*Verticillium lecanii*) and PreFeRal™ WG (*Paecilomyces fumosoroseus*). In Australia, there are fewer commercially-produced BCAs and microbial reduced-risk products on the market. There are no fungal products registered for greenhouse pest control.

In this project, a research program has been conducted to identify fungal isolates with potential for development against thrips, whiteflies and aphids in greenhouse vegetable crops.

This project undertook to:

- Conduct a preliminary screen by laboratory bioassay of a range of fungal isolates obtained from sources in Australia, Canada, South America and the USA against thrips, whiteflies and aphids.
- Evaluate the spore yield obtained from a small-scale commercial system of fungal isolates identified as having promise.
- Conduct dose-response analysis by laboratory bioassay against thrips, whiteflies and aphids of promising fungal isolates with commercially-acceptable spore production potential.
- Select the most promising fungal isolate(s) for commercial development.
- Conduct small greenhouse pot and crop trials to investigate the efficacy of selected isolates against WFT and GWF.
- Conduct preliminary investigations on the influence of relative humidity on fungal isolate performance.

These objectives were met.

2.1 LABORATORY BIOASSAYS OF A RANGE OF ENTOMOPATHOGENIC FUNGAL ISOLATES AGAINST KEY GREENHOUSE PESTS

INTRODUCTION

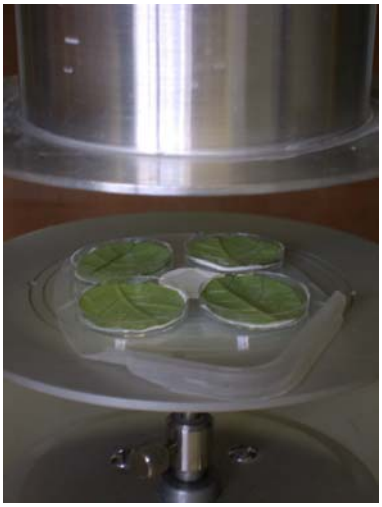
A range of fungal entomopathogenic isolates obtained from Canada, Australia, South America (Colombia) and the USA were compared for mortality and infectivity against western flower thrips (WFT), *Frankliniella occidentalis* (Pergande), greenhouse whitefly (GWF), *Trialeurodes vaporariorum* (Westwood) and green peach aphid (GPA), *Myzus persicae* (Sulzer). Laboratory bioassay experiments using a single spore concentration were conducted as a preliminary screen to identify promising isolates for further development.

MATERIALS AND METHODS

Insect rearing Cultures of WFT, GPA and GWF were maintained on green beans, Chinese cabbage and tobacco plants, respectively. Life stages of all species were produced for the bioassays to deliver 0-24 hour old specimens at the time of spraying.

Fungal isolates A preliminary bioassay involving the adult life stage and a single spore concentration (1×10^7 spores/g) was conducted on 54 fungal isolates, mainly *Beauveria bassiana*, with some *Metarhizium anisopliae* and fewer *Lecanicillium* (*Verticillium*) *lecanii* and *Paecilomyces fumosoroseus.* Isolates are listed in Table 1.

Laboratory bioassay methods
Thrips test method
The thrips laboratory bioassay method used a French bean leaf disc with the underside uppermost. This was set on a slightly moistened filter paper disc and placed in the shallow lid of a 35mm diameter plastic Petri dish as the test unit. Solutions of fungal spores were previously prepared by harvesting spores in 0.02% Silwet™ from agar culture grown on 0.25 SDAY media. Spores were counted and their viability checked. Spore concentration in aqueous solution was then adjusted to a predetermined concentration of 1×10^7 viable spores/g for the bioassay. Spore counts were made by including a glass cover slip on the spraying platform at the time of leaf disc spraying. Two ml of spore solution were applied by Potter Precision Spraying Tower at 11psi to the leaf discs and cover slip for each fungal isolate, to deliver a deposit of $2mg/cm^2$. The sprayed leaf discs were air dried in a fume cabinet for 10 minutes. Illustrations of the bioassay technique are given in Figs. 1 and 2. Ten adult thrips, previously counted into vials and covered with Parafilm™, were gassed for 5 sec with CO_2 and tapped out onto the surface of a dry, freshly-sprayed leaf disc, then quickly covered with the deeper section of the Petri dish as the lid. The unit was sealed with a strip of Parafilm™ stretched tight around the circumference and overlapping. Sealed units were held at 22^o C for 6 days prior to assessment. Each unit was assessed for mortality and infection by counting live and dead thrips under a binocular microscope. The thrips bioassay was conducted in two parts. Seven new isolates from California, plus one collected from an epizootic found infecting a thrips population in a NSW DPI research greenhouse at Gosford, were obtained and tested at a later date (Table 2). These were analysed differently to the first, larger batch of isolates. This work was undertaken between January and August 2003.



(a)

(b)

Figure 1. (a) Potter spray tower with four green bean leaf discs ready for fungal isolate deposition in WFT bioassay and (b) holder containing the leaf discs and a cover slip in the centre to collect spray droplets for spore counting.

(a)

(b)

Figure 2. (a) Bioassay units set out for fungal isolate application at different spore concentrations against WFT and (b) a sealed WFT bioassay unit after the fungal spray had dried and the WFT introduced.

(a) (b)

Figure 3. (a) Green bean shoot infested with GWF larvae, with petiole in water in a rooting tube set up on a clip as a holder during spraying in a Potter spraying tower and (b) sprayed GWF units being held in a controlled temperature room at 25^{o}C.

Figure 4. GWF larvae infected with DPI 9 fungal isolate on a green bean leaf after application through a Potter spraying tower in a laboratory bioassay.

Whitefly test method

The whitefly laboratory bioassay method used a freshly-cut single French bean leaf, with the stem placed through the hole in a lid into water in a germination tube as the test unit. Each tube containing a bean leaf shoot was placed in one of a series of holes in a polystyrene board for support, with a clear-plastic, screened, vented cup placed over the leaf as cover. Twenty to thirty 24 hour old adult whiteflies were aspirated into each cup and allowed to oviposit for 24 hours, after which they were removed. Leaves containing second instar nymphs (II instars) and a separate batch of leaves for adult residue tests were sprayed by holding the leaf, with underside uppermost, over the plate of a Potter Precision Spray Tower. Illustrations of the bioassay test unit and infected whitefly infesting a bean leaf are given in Figs. 3 and 4. Two ml of fungal isolate solution, previously prepared as described above, were applied to each unit and the leaves were allowed to air dry. The treated whiteflies were held at 23^{o}C for 6 days prior to assessment for mortality and infection.

Aphid test method

The aphid laboratory bioassay method used the bottom of a 35mm plastic Petri dish. A 35mm deep creamer cup, vented and screened at the top, was inverted and placed

over it as the test unit. The Petri dish base had a capsicum leaf disc set in agar in it. A leaf disc containing 12-20 second instar nymphs (II instars) was sprayed with 2ml of fungal solution using a Potter Precision Spray Tower as described for thrips and whitefly. For the first 24 hours after spraying, a non-vented cup was placed over the top of the vented cup to maintain a high humidity, after which it was removed and the vented cups held at 22^{o} C for 6 days prior to assessment for mortality and infection.

RESULTS AND DISCUSSION

Mean mortality data with 95% confidence intervals were compared for WFT (Tables 1 and 2), GWF (Table 3) and GPA (Table 4). Isolates that were not significantly different from the best performed isolate for each organism were then selected for further testing. On the basis of this, 27 isolates were selected from the original list for the next stage of the evaluation and development. Results of the analysis of the preliminary screening demonstrated a similarity between the order of the responses to the list of fungal isolates for WFT and GWF, while GPA performed differently. Follow-up studies have concentrated on the identification of the best isolate(s) for possible commercial development in the first instance, for WFT and GWF, followed by a separate one for GPA. Interestingly, two local isolates performed very well in each of the two groups, DPI 9 against thrips and whitefly, and DPI 1 against GPA.

Not all dead thrips were visibly infected by fungi. Tables 1 and 2 list both proportion dead and infected.

Table 1. Mortality effect of a single spore concentration of a range of entomopathogenic fungal isolates against WFT (CI = confidence intervals).

Isolate	Proportion dead	Lower CI	Upper CI	Isolate	Proportion infected	Lower CI	Upper CI
ARC5	0.9836	0.9187	0.9969	ARC5	0.8572	0.6932	0.941
UV CA2	0.9814	0.9279	0.9954	ARC8	0.8514	0.6837	0.9382
BC667	0.9748	0.8968	0.9942	CS1378	0.8102	0.6733	0.8984
CS827	0.9733	0.9102	0.9924	CS1409	0.7973	0.6363	0.8983
CS1378	0.9719	0.9186	0.9907	BC667	0.765	0.5635	0.8914
CS1409	0.9669	0.9	0.9896	CS827	0.7529	0.578	0.8714
CS1274	0.9659	0.9093	0.9877	UVCA2	0.7401	0.5629	0.863
CS1248	0.9593	0.8947	0.9849	ARC7	0.7287	0.5208	0.8691
ARC7	0.9548	0.8584	0.9866	UV726	0.7213	0.5606	0.84
UV726	0.9544	0.887	0.9824	CS1014	0.6812	0.4641	0.8406
UV0511B	0.9519	0.8705	0.9831	CS1274	0.6762	0.5096	0.8075
CS1013	0.9507	0.8662	0.9829	ARC4	0.6681	0.4483	0.8329
UV ER24	0.9503	0.8665	0.9825	CS1248	0.6534	0.4842	0.791
ARC8	0.9491	0.8471	0.9843	CS1013	0.644	0.4534	0.7978
UV1080	0.9347	0.8379	0.9754	UV0511B	0.5898	0.3992	0.7569
UV0500B	0.9205	0.7876	0.9731	ARC6	0.5556	0.3319	0.7588
CS1110	0.9061	0.7878	0.9617	UVER24	0.5387	0.3502	0.7168
BC601	0.8948	0.7414	0.9619	UV0500B	0.5233	0.3107	0.7278
CS1014	0.8891	0.7349	0.9586	CS1110	0.5098	0.3245	0.6924
UV3540	0.8747	0.7332	0.9466	BC613	0.5025	0.2899	0.7141
UVER20	0.8603	0.7146	0.9381	NSWA1	0.4944	0.3086	0.6819
BC668	0.8546	0.7038	0.9356	UV1080	0.4902	0.3078	0.6753
ARC4	0.8282	0.6386	0.9293	UV3540	0.4812	0.2995	0.6681
BB	0.7828	0.603	0.8953	BC668	0.479	0.2983	0.6655
ARC6	0.7657	0.547	0.8984	BC601	0.478	0.2729	0.6909
NSWA6	0.7637	0.5785	0.8838	BB	0.4778	0.2951	0.6667
NSWA1	0.7547	0.5679	0.878	NSWA6	0.4454	0.2674	0.6387

Table 1 (continued)

Isolate	Proportion dead	Lower CI	Upper CI	Isolate	Proportion infected	Lower CI	Upper CI
BC613	0.7449	0.5255	0.885	BC608	0.4302	0.2353	0.6494
NSWA5	0.7211	0.4945	0.8723	UVER20	0.4255	0.2544	0.6164
BC621	0.7084	0.5094	0.8504	BC618	0.4239	0.2271	0.6482
BC649	0.7064	0.48	0.8625	BC607	0.4049	0.2171	0.6253
BC640	0.7048	0.4764	0.8623	BC640	0.3647	0.19	0.5843
NSWA4	0.6863	0.4874	0.8343	NSWA5	0.3499	0.1793	0.5699
BC607	0.6774	0.4457	0.8458	BC649	0.327	0.1655	0.5435
BC608	0.6768	0.4458	0.8449	BC619	0.307	0.1523	0.5222
BC618	0.6752	0.4419	0.8452	BC614	0.3067	0.1687	0.491
BC614	0.6721	0.4719	0.8246	NSWA4	0.3006	0.1642	0.4847
BC619	0.6409	0.4079	0.8222	BC621	0.2985	0.1623	0.483
BC615	0.6239	0.4216	0.7905	BC615	0.2975	0.1623	0.4808
NSWA2	0.6176	0.4337	0.7731	BC636	0.2402	0.1103	0.4464
BC644	0.5872	0.3837	0.7647	BC617	0.2251	0.1028	0.4241
BC604	0.5705	0.3374	0.7759	NSWA7	0.2226	0.1002	0.4243
BNI	0.5412	0.269	0.7909	BC644	0.221	0.113	0.387
BC623	0.5256	0.3459	0.6989	NSWA2	0.2201	0.1202	0.3683
BC638	0.5158	0.3195	0.7073	BC635	0.2135	0.0956	0.4107
BC635	0.5083	0.2829	0.7305	BC604	0.189	0.0834	0.374
BC636	0.4922	0.2697	0.7177	BC623	0.1872	0.0999	0.3235
BC616	0.4917	0.2704	0.7162	BC639	0.1692	0.0822	0.3166
BC617	0.491	0.272	0.7135	BC616	0.145	0.0604	0.309
BC605	0.4711	0.2816	0.6693	BC638	0.1365	0.064	0.2677
PF	0.4535	0.2671	0.654	BNI	0.1287	0.0426	0.3292
BC639	0.4274	0.2465	0.6299	BC605	0.1252	0.0578	0.2503
NSWA7	0.3743	0.1869	0.6089	VT53	0.1237	0.0498	0.2755
VT53	0.3688	0.1815	0.6062	PF	0.1118	0.0505	0.2294

Table 2. Percentage mortality and visible infection of WFT at a single spore concentration of some supplementary entomopathogenic fungal isolates. The local isolate is DPI 9.

Isolate	% mortality	Isolate	% infected
DPI 9	95.42 a	**DPI 9**	86.69 a
UVCA155	89.29 b	UVCA155	86.56 a
UVCA1	87.19 b	UVCA1	86.2 a
UVCA171	73.65 c	UVCA171	60.89 b
UVCA721	63.73 cd	UVCA270	56.97 b
UVCA270	62.24 d	UVCA613	48.81 b
UVCA613	60.12 d	UVCA721	41.52 b
UVCA633	53.01 d	UVCA633	38.65 b

Table 3. Percentage mortality and standard deviation of GWF at a single spore concentration of a range of entomopathogenic fungal isolates.

Isolate	Mean	Std Dev	Isolate	Mean	Std Dev
CA-2	84.71	5.34	BB	60.65	16.82
ARC5(64)	83.87	2.68	BC613	60.31	8.66
BC667	82.52	2.99	BC649	60.08	17.84
ARC8(63)	79.69	2.79	BC640	59.58	15.60
ARC7(62)	75.98	8.96	BC614	57.07	13.01
FI-827	73.38	12.60	BC618	55.61	6.52
FI-1378	72.29	15.06	ARC6(66)	55.24	11.11
#3540	71.03	9.44	BC604	54.62	8.80
FI-1110	69.98	12.67	BC601	53.81	18.21
#1080	69.59	6.58	NSWA-1	53.23	11.64
ARC4(67)	69.33	2.17	BC607	51.28	16.68
#726	68.94	14.32	BC623	50.60	12.59
ER-24	68.67	16.56	NSWA-4	49.59	20.12
0511-B	67.73	9.09	BC605	49.25	4.28
FI-1013	66.98	19.59	BC644	48.18	14.85
NSWA-6	66.94	10.92	BC617	47.89	15.39
ER-20	66.29	8.95	NSWA-2	46.98	16.76
0500-B	65.84	4.85	BC608	45.67	13.09
BC668	65.54	13.77	BC639	45.58	10.35
BC635	65.52	8.13	BC638	45.38	12.38
FI-1248	65.17	16.65	BC615	44.61	12.51
FI-1014	65.10	11.15	BC616	42.76	8.66
FI-1274	65.00	19.38	BC619	40.99	15.89
NSWA-5	64.97	4.53	BNI	38.82	10.60
BC621	63.10	11.47	NSWA-7	36.10	4.70
FI-1409	62.89	15.85	VT53	34.16	16.01
BC636	61.96	9.17	PF	27.18	14.91

Table 4. Percentage mortality of GPA at a single spore concentration of a range of entomopathogenic fungal isolates.

Isolate	Mean % mortality	Std Dev	Isolate	Mean % mortality	Std Dev
DPI 1	85.91	10.18	BC644	54.30	27.01
ARC5(64)	74.88	17.06	BC618	54.11	27.68
FI-1274	71.98	15.10	BC623	54.09	30.04
ARC8(63)	71.51	17.10	#1080	53.94	24.47
ARC7(62)	70.59	17.37	BC636	53.34	25.61
BC667	68.99	17.28	ER-20	52.59	18.49
#726	68.51	16.90	BC640	51.77	29.11
FI-827	66.82	21.16	BC607	51.50	23.79
BC668	66.74	15.33	BC639	51.43	33.06
ER-24	64.70	22.11	BB	50.93	16.28
FI-1248	64.24	21.69	BC621	50.9	27.94
CA-2	64.03	17.64	0500-B	50.67	17.20
NSWA-6	62.60	17.90	BC614	49.11	26.03
ARC4(67)	62.52	20.25	NSWA-5	48.81	19.62
FI-1110	61.29	23.17	BC608	46.79	21.25
BC615	61.17	17.44	FI-1014	46.68	7.30
BC635	60.94	24.69	BC605	46.08	24.14
FI-1013	60.15	22.60	NSWA-4	43.48	24.17
ARC6(66)	60.02	24.67	BC616	43.48	25.30
FI-1409	59.90	30.62	BC649	39.95	24.81
BC617	59.44	19.15	#3540	34.46	8.82
FI-1378	58.74	18.83	NSWA-2	33.98	29.27
BC613	57.34	25.98	BC601	31.70	8.52
BC619	56.63	17.29	NSWA-7	30.178	21.81
BC638	56.44	23.88	PF	26.95	23.78
BC604	54.91	23.19	BNI	26.43	3.91
0511-B	54.42	21.23	VT53	25.28	23.01

2.2 EVALUATION OF SPORE YIELD OF SELECTED ENTOMOPATHOGENIC FUNGAL ISOLATES IN A SMALL-SCALE COMMERCIAL SYSTEM

INTRODUCTION

In conjunction with the preliminary laboratory bioassay of 65 fungal isolates and the more detailed dose response work, it was necessary to identify those isolates that had commercial promise by subjecting them to a small-scale commercial spore yield trial. Twenty seven fungal isolates selected from the original list as most effective against thrips and whitefly were included. This was conducted by the commercial partner, Becker Underwood Australia at Somersby, NSW. The methodology for this procedure is commercial-in-confidence and cannot be described here.

RESULTS AND DISCUSSION

Spore yield of the 27 selected isolates are given in Figure 1. Twelve isolates, while shown to be promising against thrips and whiteflies, failed to demonstrate commercial viability and were discarded. Ten new isolates, obtained from the USA and Australia subsequent to the preliminary work, were added to the testing program. Efficacy data for eight of these are given in Table 2 in Section 2.1, while the remaining two isolates, UVCA603 and ESC-1, are excluded. Of these, DPI 9 was a recent discovery in a virulent infection of WFT in the DPI research greenhouses at Gosford Horticultural Institute. Isolates that equalled or exceeded a nominal threshold for commercially-acceptable spore yield (4×10^9 spores/g) were retained and subjected to dose response bioassay evaluation. Four of the late-obtained isolates were added to the list.

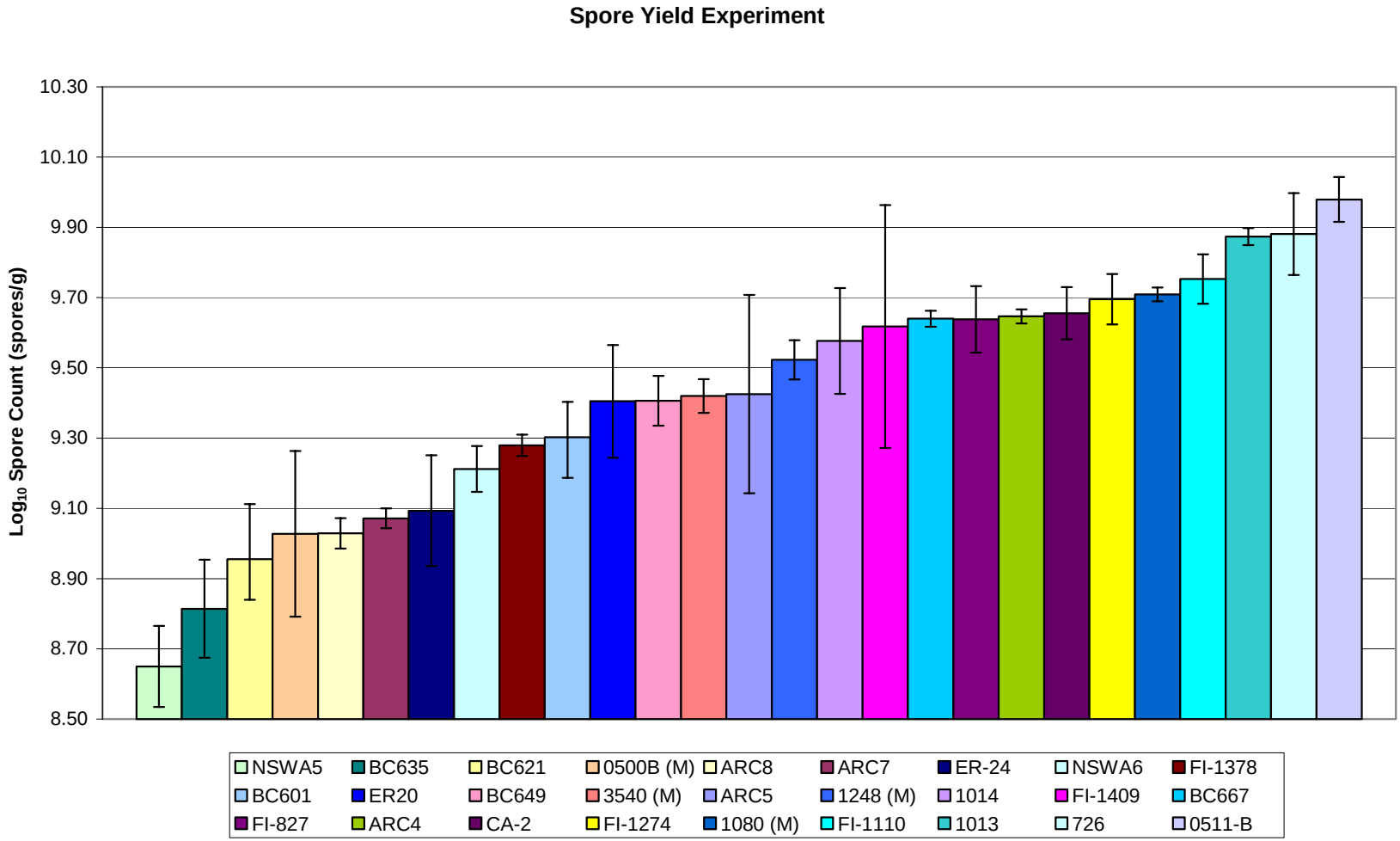


Figure 1. Spore yield of 27 selected entomopathogenic fungal isolates produced in a small-scale commercial production system.

2.3 DOSE-RESPONSE OF WESTERN FLOWER THRIPS, *FRANKLINIELLA OCCIDENTALIS* (PERGANDE), GREENHOUSE WHITEFLY, *TRIALEURODES VAPORARIORUM* (WESTWOOD) AND GREEN PEACH APHID, *MYZUS PERSICAE* (SULZER) TO SELECTED ENTOMOPATHOGENIC FUNGAL ISOLATES

INTRODUCTION

Following an initial laboratory bioassay of nearly 70 fungal isolates conducted against western flower thrips (WFT) and greenhouse whitefly (GWF) reported in Section 2.1, 27 were selected for further testing. Some of these were eliminated because they failed to meet a spore production threshold when subjected to a small-scale commercial production trial (Section 2.2). The surviving isolates were then subjected to intensive dose-response laboratory bioassays, where the mortality response of adult and juvenile life stages of WFT and GWF, and the juvenile stage of green peach aphid (GPA), was measured. Assessments were made of mortality and infection six days after treatment with each pest/life stage.

MATERIALS AND METHODS

WFT and GWF dose-response bioassays
Nineteen isolates surviving the single spore concentration preliminary screen and the spore yield trial were compared against the commercial isolate GHA (Botanigard™) by dose-response bioassay. Dose-response tests were conducted using the laboratory bioassay techniques previously described for WFT and GWF in Section 2.1 with five spore concentrations (1×10^6, 4×10^6, 1×10^7, 4×10^7 and 8×10^7 spores/g) and two life stages, adult and second stage larva. Each treatment was replicated three times on separate occasions using fresh spore solution from separate isolate colonies. Cultures were prepared by Becker Underwood Australia. Harvesting of spores, spore counting, spore viability tests and spore solution made up to a prescribed concentration was undertaken at the NSW DPI research laboratories prior to each bioassay. Assessments were made of mortality and infection six days after treatment with each pest/life stage.

GPA dose-response bioassays
Eight isolates surviving the single spore concentration preliminary screen and the spore yield trial were compared by dose-response bioassay. Dose-response tests were conducted using the laboratory bioassay technique previously described for GPA in Section 2.1 with five spore concentrations (1×10^6, 4×10^6, 1×10^7, 4×10^7 and 8×10^7 spores/g) and a single life stage, second instar nymphs. Each treatment was replicated three times on separate occasions using fresh spore solution from separate colonies of fungal isolate. Spore preparation and assessments are as described for WFT and GWF.

RESULTS AND DISCUSSION

Results of probit analysis for the top 10 isolates tested against GWF, WFT and GPA are given in Tables 1, 2 and 3 respectively. Lethal Dose (LD) for 50% and 99% mortality for all tested life stages of each pest are given.

A review of the data for both life stages of each insect target identified four isolates worthy of selection for commercial development. These were DPI 9 from

GHI, UVCA603 from University of California, BU667 from Becker Underwood and CS827 from CSIRO. These isolates did not necessarily occupy the top four rankings in each table, but were consistently well performed. There can be substantial differences between laboratory and greenhouse performances, hence four isolates were selected for potential development as a stand by. However, it has been decided that commercial development will focus on DPI 9 and possibly also UVCA603 in the first instance. UVCA603 has not been subjected to the small-scale commercial spore yield evaluation as yet, but this will be completed soon. Subject to this, it may be that it proves to be a very productive spore producer that demands further assessment in greenhouse trials. To date only DPI 9 has been subjected to greenhouse crop evaluation against WFT and GWF.

Table 1. LD_{50} and LD_{99} results for dose response bioassays of the ten best performed fungal isolates and late addition isolates against adult and larval GWF. CI = confidence interval. Those in bold were selected for further development.

Isolate	LD_{50} Adult GWF	Lower 95% CI	Upper 95% CI	Isolate	LD_{99} Adult GWF	Lower 95% CI	Upper 95% CI
DPI 9	1.41	0.02	7.34	**BU667**	4300.75	680.81	291060.3
CS1013	2.32	0.91	4.17	**CS827**	15957.3	3530.26	187374.2
ARC4	2.51	0.62	5.28	UV726	34310.62	6103.48	6.32E+05
UVCA2	2.6	1.12	4.51	CS1409	38933.37	2452.76	87425760
ARC5	2.84	0.47	6.63	CS1110	44958.91	6390.68	1527544
CS827	3.07	1.76	4.64	CS1013	48187.8	5435.91	3636621
UV726	3.09	1.69	4.78	CS1274	57993.6	9897.28	1090276
CS1110	3.26	1.65	5.29	ARC4	92111.83	5169.93	1.5E+08
BU667	3.44	1.27	6.45	UVCA2	102916.3	10753.28	756896.5
BUBB	4.14	0.47	11.41	ARC5	437693.9	10058.55	8.28E+10
+**UVCA603** 12th				+ **DPI 9** 16th +**UVCA603** 17th			

Isolate	LD_{50} Larval GWF	Lower 95% CI	Upper 95% CI	Isolate	LD_{99} Larval GWF	Lower 95% CI	Upper 95% CI
DPI 9	6.73	4.28	10.05	**BU667**	1718.92	486.63	1.81E+04
BU667	7.26	4.11	11.56	**UVCA603**	1740.01	542.49	12907.97
UVCA2	7.97	4.62	13.11	**CS827**	3137.96	732.72	49821.99
CS827	8.06	4.69	13.19	ARC5	3770.38	1415.54	1.59E+04
UVCA603	9.09	5.85	13.85	**DPI 9**	3967.21	1069.48	3.63E+04
CS1409	12.96	8.48	20.19	UVCA2	4849.08	1011.89	9.69E+04
ARC5	13.37	9.98	18.08	CS1013	5245.64	1228.59	8.03E+04
CS1110	14.57	10.53	20.21	CS1110	7142.86	2176.38	4.63E+04
CS1013	18.8	12.15	30.7	CS1409	7537.62	1791.44	89593.45
CS1274	26.24	16.81	47.04	UVCA1	9.62E+03	1858.71	2.35E+05

Table 2. Results of dose response bioassays of the ten best performed fungal isolates against adult and larval WFT. CI = confidence interval. Those in bold were selected for further development.

Isolate	LD_{50} Adult WFT	Lower 95% CI	Upper 95% CI	Isolate	LD_{99} Adult WFT	Lower 95% CI	Upper 95% CI
UVCA603	1.08	0.6	1.66	UVCA2	43.4	34.4	58
DPI 9	1.28	0.765	1.85	ARC5	51	35.5	84.4
CS1274	1.83	1.24	2.47	CS1013	67.7	47.5	108
CS1248	2.08	1.33	2.93	CS1409	72.7	54.3	106
CS827	2.19	1.56	2.87	**UVCA603**	72.8	49.6	123
UV726	2.22	0.67	4.25	**CS827**	74.7	54.8	111
UVCA2	2.22	1.63	2.83	**DPI 9**	81.6	56.8	135
UV0511B	2.46	1.81	3.16	CS1274	87.4	62	137
CS1409	2.59	1.92	3.29	**BU667**	119	63.8	329
ARC5	2.88	1.85	3.97	ARC4	154	104	257
BU667 12th							

Isolate	LD_{50} Larval WFT	Lower 95% CI	Upper 95% CI	Isolate	LD_{99} Larval WFT	Lower 95% CI	Upper 95% CI
UVCA2	2.96	1.29	5.05	CS1274	177	87	578
CS1248	3.87	2.87	5	**UVCA603**	413	244	832
UVCA603	4.57	3.48	5.76	**CS827**	451	280	832
CS1274	5.06	3.01	7.53	CS1013	579	236	2570
DPI 9	5.62	3.82	7.75	ARC5	626	280	2150
CS827	5.76	4.63	7.01	UVCA2	649	218	5060
UVCA155	6.18	4.96	7.54	**BU667**	896	424	2630
ARC4	9.33	7.58	11.4	CS1248	1130	587	2700
CS1013	9.45	6.35	13.8	ARC4	1140	624	2520
CS1409	9.65	6.39	14.4	UVCA155	1450	780	3240
BU667 13th				**DPI 9** 13th			

Table 3. Results of dose response bioassays of the eight best performed fungal isolates against juvenile green peach aphid (GPA). Those in bold were selected for further development.

Isolate	LD_{50} Nymphal GPA	Lower 95% CI	Upper 95% CI	LD_{99} Nymphal GPA	Lower 95% CI	Upper 95% CI
DPI 1	4.9×10^{6}	2.7×10^{6}	7.7×10^{6}	5.2×10^{8}	1.8×10^{8}	3.6×10^{9}
CS1274	1.2×10^{7}	8.8×10^{6}	1.8×10^{7}	9.3×10^{9}	2.6 x 109	7.2×10^{10}
DPI 6	9.3×10^{6}	3.4×10^{6}	2.2×10^{7}	1.5×10^{10}	1.2×10^{9}	5.3×10^{13}
BU668	2.4×10^{8}	1.0×10^{8}	1.9×10^{9}	7.3×10^{10}	5.8×10^{9}	5.0×10^{13}
ARC 5	2.2×10^{8}	1.2×10^{8}	6.0×10^{8}	8.8×10^{10}	1.4×10^{10}	2.4×10^{12}
BU667	4.6×10^{8}	1.9×10^{8}	2.4×10^{9}	7.5×10^{11}	5.2×10^{10}	1.5×10^{14}
CS827	2.4×10^{8}	5.3×10^{7}	5.2×10^{10}	5.2×10^{12}	1.8×10^{10}	4.1×10^{22}
DPI 9	2.9×10^{9}	5.4×10^{8}	3.7×10^{11}	1.4×10^{13}	1.7×10^{11}	6.5×10^{18}

2.4 VARIABILITY IN RESPONSES OF GENETICALLY-DISTINCT STRAINS OF WESTERN FLOWER THRIPS, *FRANKLINIELLA OCCIDENTALIS* (PERGANDE), TO ENTOMOPATHOGENIC FUNGAL ISOLATES

INTRODUCTION

Resistance to pesticides is identified in the laboratory by comparing the results of dose-response bioassays of a susceptible strain of the target organism, such as western flower thrips (WFT), with those of a field strain suspected of being resistant to the pesticide in question. A susceptible strain is one that, to the best of anyone's knowledge, has never been subjected to pesticides. They are hard to locate. In NSW DPI, Dr Grant Herron undertakes this work. However, to date there has not been a study done anywhere in the world on the responses to entomopathogenic fungal isolates of genetically-distinct strains of an insect pest. In this project, NSW DPI participated in an international collaboration with the Alberta Research Council, Vegreville, Alberta, Canada and the University of Vermont, USA, in a study to compare the dose-response of three strains of WFT sourced from Australia, Canada and the USA to eight entomopathogenic fungal isolates. Dose response refers to the mortality response of the target organism to a range of pesticide concentrations or in this case a range of entomopathogenic fungal spore concentrations. In the first instance, we collaborated with the laboratory of A/Prof Michael Gillings, Centre for Biodiversity, Macquarie University, to determine that the three strains of WFT cultured in the respective laboratories were genetically distinct.

MATERIALS AND METHODS

Rearing of WFT and fungal culturing techniques were the same as described in Section 2.1. Eight fungal isolates were compared in this study (Table 1). While ideally the methodology adopted in the assays should have been the same, there was some variation (Table 2).

The WFT strains from Australia, Canada and the USA were genetically compared using BOX and ERIC (enterobacterial repetitive intergenic consensus) families of short interspersed repetitive elements (Gillings and Holley 1997a, b). Thrips material for the PCR tests from the research colonies of the three collaborating laboratories was provided in 90% ethanol.

RESULTS AND DISCUSSION

DNA fingerprinting of WFT strains

The DNA sequences provided by both methods (Fig. 1) confirmed that the three western flower thrips strains were genetically distinct and could be used to compare the responses to fungal isolates.

Laboratory bioassays

A comparison of the bioassay methods used in each laboratory (Table 2) shows that while there were some minor differences in the equipment used, the basic approach was the same and would not be a factor in the response comparisons. Adult and larval responses to the fungal isolates varied between the three WFT strains.

Adult data (Table 3) showed a similarity in response range in all three WFT strains, with Australia (91.81 – 35.05%), Canada (95.57 – 5.44%) and USA (100 – 39.28%).

Larval data (Table 4) demonstrated a similar, but lower, range of mortality responses in the Australian (66.52 – 25.40%) and US strains (66.76 – 6.67%), but different from the Canadian strain (33.03 – 5.7%), which was lower. For the Australian and US strains of larval WFT, six of the eight fungal isolates had similar response rankings (UVCA603, CS1248, CS827, BU667, CS1013 and ARC5), while UVCA2 and CS1378 responded quite dissimilarly: UVCA2 ranked 4th at 58.60% in the Australian strain, and ranked 7th at 22.72% in the US strain, and CS1378 ranked 8th at 25.40% and 3rd at 48.48%, respectively.

In each WFT strain, for each life stage, the majority of the fungal isolates were not significantly different from each other. In all data sets, there were mostly 1-2 isolates at the upper response range that were different from the bottom 1-3 isolates. Between these, there were 5-6 isolates where the responses were not significantly different, except for US strain adult responses, where the top four isolates were different from the next two, which were different from the final isolate. In the US strain, three isolates CS1378, BU667 and CS827, produced 100% mortality against adults and were excluded from the analysis. It is likely that they were not different (P = 0.05) from UVCA2, but different from the rest.

Interestingly, when larval and adult mortality data were pooled for the three strains and ranked (Table 5), each isolate demonstrated a high degree of constancy in its rankings across strains and life stages, although most of these isolates had at least one abnormal ranking. Where WFT strain mortality data were pooled for each life stage (Table 6), adults were more susceptible to the fungal isolates than were larvae ($P<0.05$) with proportion difference in each isolate ranging from 1.6 to 3.7x greater susceptibility of adults over larvae. Percentage mortality ranged between 5-100% for adults against 5-66% for larvae.

In summary, there is a detectable difference in responses to the fungal isolates between genetically-distinct strains of WFT and between life stages. The former should be taken into consideration when preparing to use a new fungal biopesticide product in the field for the first time, until its performance can be clearly identified.

Table 1. Entomopathogenic fungal isolates compared against three strains of western flower thrips.

Isolate	Organism	Origin
UVCA-2	*Beauveria bassiana*	Uni. Vermont, USA
UVCA603	*Beauveria bassiana*	Uni. Vermont, USA
CS827	*Beauveria bassiana*	CSIRO, Australia
CS1013	*Beauveria bassiana*	CSIRO, Australia
CS1378	*Beauveria bassiana*	CSIRO, Australia
BU667	*Beauveria bassiana*	Becker Underwood, Australia
ARC5	*Beauveria bassiana*	Alberta Research Centre, Canada
CS1248	*Metarrhizium anisopliae*	CSIRO, Australia

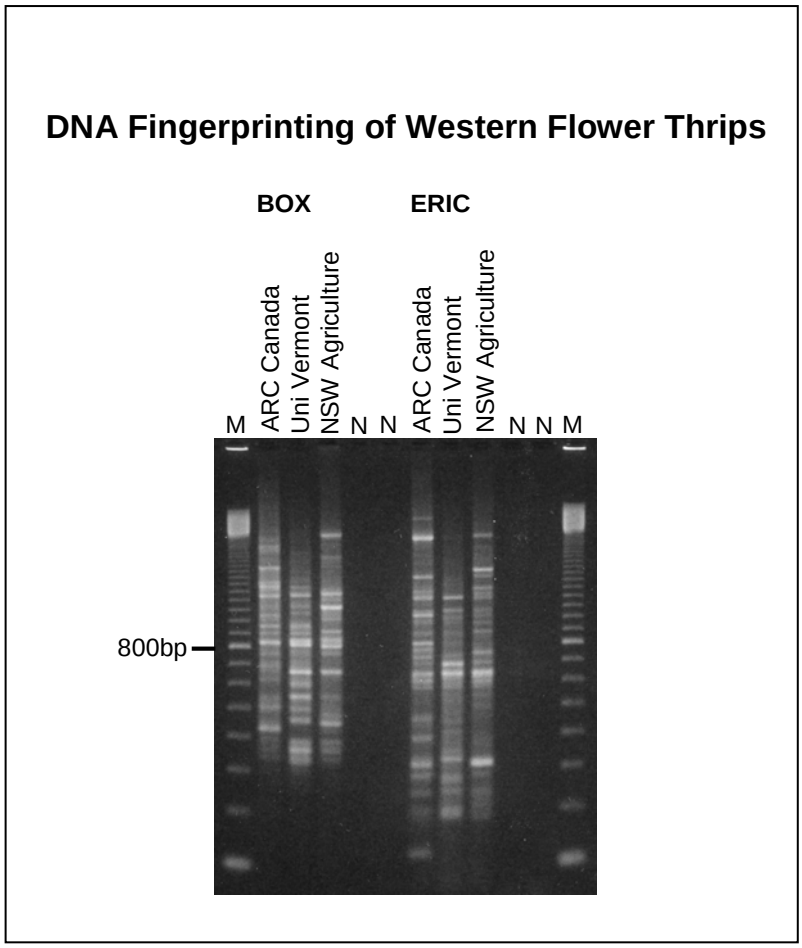


Figure 1. DNA sequences obtained using BOX and ERIC methods of three strains of western flower thrips from Australia, Canada and the USA.

Table 2. Bioassay methods adopted in the three laboratories.

Variable	Source of WFT strains		
	Australia	Canada	USA
Volume of water on paper disc	30µl	25µl	80µl
Spraying method	PT/12psi	Plexiglass/12psi	PT/12psi
Volume sprayed	2ml	1ml	3ml
Drying time	8 mins	10 mins	10 mins
Holding conditions	Light 23^{o}C/6d	Dark 25^{o}C/6d	Dark 25^{o}C/6d
Spore concentration	1 x 10^{7}	1 x 10^{7}	1 x 10^{7}
Spore deposit/mm^{2}	417	554	?
WFT stages tested	L2, adult	L2, adult	L2, adult

PT = Potter precision spraying tower
Plexiglass = material used to construct a cylindrical spraying tower containing an airbrush sprayer (Aztec™ 3000s airbrush, Testor Corp., Illinois).

Table 3. Adult responses of three genetically-distinct strains of WFT to eight entomopathogenic fungal isolates. Analysis was conducted on transformed means. Means followed by the same letter in the same column are not significantly different (P = 0.05).

Australia		Canada		USA	
Isolate	% mortality	Isolate	% mortality	Isolate	% mortality
UVCA603	91.81 a	CS1248	95.53 a	CS1378	100
UVCA2	90.00 ab	UVCA603	85.46 ab	BU667	100
ARC5	87.10 ab	UVCA2	80.55 bc	CS827	100
CS827	86.32 ab	CS827	79.62 bc	UVCA2	98.93 a
CS1013	76.27 ab	CS1013	74.31 bc	UVCA603	92.82 b
CS1248	72.89 bc	BU667	64.31 bc	CS1013	86.85 b
BU667	71.98 bc	CS1378	56.23 bc	CS1248	74.40 bc
CS1378	35.05 c	ARC5	5.44 c	ARC5	39.28 c

Table 4. Larval responses of three genetically-distinct strains of WFT to eight entomopathogenic fungal isolates. Analysis was conducted on transformed means. Means followed by the same letter in the same column are not significantly different (P = 0.05).

Australia		Canada		USA	
Isolate	% mortality	Isolate	% mortality	Isolate	% mortality
UVCA603	66.52 a	CS1248	33.03 a	CS1248	66.76 a
CS1248	62.94 a	CS1013	23.66 ab	UVCA603	62.63 a
UVCA2	58.60 ab	BU667	14.16 abc	CS1378	48.48 ab
CS827	57.96 ab	UVCA2	13.08 abc	CS827	46.94 ab
BU667	47.65 ab	CS827	11.29 abc	CS1013	42.27 ab
CS1013	45.95 ab	CS1378	9.53 bc	BU667	33.28 ab
ARC5	40.81 ab	UVCA603	8.06 bc	UVCA2	22.72 bc
CS1378	25.40 ab	ARC5	5.74 c	ARC5	6.67 c

Table 5. Ranked responses of adult and larval life stages of three genetically-distinct strains of WFT to eight fungal isolates.

Isolate	Australia		Canada		USA	
	Adult	Larva	Adult	Larva	Adult	Larva
UVCA603	1	1	2	7	5	2
CS1248	6	2	1	1	7	1
UVCA2	2	3	3	4	4	7
CS827	4	4	4	5	1	4
BU667	7	5	6	3	1	6
CS1013	5	6	5	2	6	5
ARC5	3	7	8	8	8	8
CS1378	8	8	7	6	1	3

Table 6. Comparison of adult and larval mortality data of three genetically-distinct strains of WFT to eight fungal isolates.

Isolate	Australia		Canada		USA	
	Adult	Larva	Adult	Larva	Adult	Larva
UVCA603	91.81	66.52	85.46	8.06	92.02	62.63
CS1248	72.89	62.94	95.53	33.03	74.40	66.76
UVCA2	90.00	58.60	80.55	13.08	98.93	22.72
CS827	86.32	57.96	79.62	11.29	100.00	46.94
BU667	71.98	47.65	64.31	14.16	100.00	33.28
CS1013	76.27	45.95	74.31	23.66	86.85	42.27
ARC5	87.10	40.81	5.44	5.74	39.28	6.67
CS1378	35.05	25.40	56.23	9.53	100.00	48.48

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2.5 INVESTIGATIONS INTO THE EFFECT OF RELATIVE HUMIDITY ON THE RESPONSE OF WESTERN FLOWER THRIPS, *FRANKLINIELLA OCCIDENTALIS* (PERGANDE), TO ENTOMOPATHOGENIC FUNGAL ISOLATES

INTRODUCTION

A key element in the successful application of beneficial entomopathogenic fungi as a pesticidal product for greenhouse crop use is tolerance to low humidity. Fungal spores require a humid environment to germinate. For growers to successfully use a fungal biopesticide they need to know the conditions under which these products will perform.

In an initial investigation, a laboratory experiment to compare the effect of three constant humidity levels, 50%, 70% and 90%, at a constant temperature (25^{o}C) on the response of western flower thrips (WFT) to a fungal isolate was conducted.

MATERIALS AND METHODS

Constant humidity was provided by solutions of glycerol and water in a plastic sandwich tray with lid, and the whole unit sealed inside a plastic bag and maintained at 25^{o}C. A wire mesh platform inside each sandwich tray held the test units above the humidity solution. Each test unit was comprised of a Millipore™ dish with a fine screen covering a hole in the lid to allow air exchange over the humidity solution. A fresh leaf disc cut from a French bean leaf was placed upside down in agar inside the dish base. Two ml of fungal solution prepared in 0.1% Silwet were applied at 8 x 10^{7} spores/g by Potter spraying tower onto each leaf disc and the discs were allowed to air dry for 10 minutes. Each unit was then infested with 15-20 adult thrips, anaesthetised with $CO_{2,}$ tapped out onto the leaf surface, sealed with the screened lid and placed upside down on the wire mesh over the constant humidity solution. There were four units per treatment plot/rep, a total of 60-80 thrips per treatment replicate. The control was 0.1% Silwet and each treatment was replicated four times. The sealed sandwich tray(s) were held in a controlled environment room at 25^{o}C and assessed after 6 days by counting dead and infected thrips.

Four isolates selected from the dose-response screening (DPI 9, CS827, BU667, UVCA155) were compared in this experiment.

Statistical analysis

Final adult mortality counts were corrected for controls prior to analysis (as described in previous analyses.) No correction factor was needed for the infection counts – all controls had zero infection. Larval counts were excluded from the analysis since this data was not replicated.

It was assumed that the experiment was a 4 treatments (+ Control) * 3 humidities factorial with 3 replications and no other blocking. Data were analysed using ASREML.

RESULTS AND DISCUSSION

There was no overall significant difference in treatment effect between the fungal isolates when humidity data were pooled, partly due to a high variance (Fig. 1); however, CA155 gave consistently lower mortalities at each RH than the other three isolates (Fig. 2). There was a significant effect between relative humidity and fungal isolates. When the isolate data for each RH were pooled, low relative humidity had a significant negative effect on their performance (Fig. 3). Mean mortalities of 88.71% at 90% RH, 58.59% at 70% RH and 20.45% at 50% RH were all different at $P<0.05$.

Clearly relative humidity is a concern for the successful performance of fungal isolates against WFT as it needs to be high for effective use. Relative humidity is generally not constant in a greenhouse crop. It can vary with outside weather, day or night (night is usually much higher except when heating pipes are on in winter), and crop type, density and age. Further experiments are planned to investigate the minimum period of optimal humidity that will ensure successful spore germination and host infection, and also the maximum period of exposure to sub-optimal humidities before physiological damage occurs to the spores, preventing their germination and growth. This is being undertaken in project VG05093.

Isolate	% mortality
DPI 9	71.4 ns
BU667	66.82
CS827	61.71
CA155	33.34

Figure 1. Effect of selected entomopathogenic fungal isolates on adult WFT at three relative humidities (humidity data were pooled). Differences were not significantly different (P = 0.05).

Isolate	% relative humidity	% mortality
BC667	90	93.4 a
DPI 9	90	92.46 a
CS827	90	88.6 ab
CA155	90	73.83 bc
DPI 99	70	69.97 bc
BC667	70	67.01 c
CS827	70	62.23 cd
DPI 9	50	35.25 de
CA155	70	33.95 de
CS827	50	24.63 e
BC667	50	22.13 e
CA155	50	7.94 f

Figure 2. Effect of relative humidity on individual fungal isolates. Analysis was conducted on transformed means. Means in the same column followed by the same letter are not significantly different (P = 0.05).

% RH	% mortality
50	20.45 c
70	58.59 b
90	88.71 a

Figure 3. Effect of relative humidity on pooled data of four selected entomopathogenic fungal isolates against adult WFT. Analysis was conducted on transformed means. Means in the same column followed by the same letter are not significantly different (P = 0.05).

2.6 GREENHOUSE TRIALS TO EVALUATE EFFICACY OF SELECTED ENTOMOPATHOGENIC FUNGAL ISOLATES AGAINST WESTERN FLOWER THRIPS, *FRANKLINIELLA OCCIDENTALIS* (PERGANDE), AND GREENHOUSE WHITEFLY, *TRIALEURODES VAPORARIORUM* (WESTWOOD)

2.6.1 EVALUATION OF THE MORTALITY EFFECT OF DIFFERENT SPORE CONCENTRATIONS OF A FUNGAL ISOLATE IN RATES OF OIL AGAINST WESTERN FLOWER THRIPS, *FRANKLINIELLA OCCIDENTALIS* (PERGANDE)

INTRODUCTION

The development of a commercial biopesticide requires the determination of minimum effective fungal isolate and carrier concentrations. In this research program, following the completion of the dose-response experiments in the laboratory, promising isolates were identified for commercial development against the three target pest species. However, the rate equivalent to that recommended for BotaniGard™ overseas, is not an economically viable proposition with the preferred isolate for western flower thrips (WFT) here in Australia. The commercial partner, Becker Underwood Australia, has requested that trials be undertaken to identify the minimum effective fungal isolate and carrier concentrations.

MATERIALS AND METHODS

An initial experiment was conducted to compare the efficacy of two spore concentrations of the fungal isolate DPI 9 against WFT, plus phytotoxicity of two rates of oil. Single cucumber plants of the Rijk Zwaan cv Kaspian were grown from seed in 75mm Growool blocks and transplanted into15cm pots. Plants were set up in a temperature-controlled glasshouse on a bench covered with black weed mat and with coarse vermiculite over the surface to act as a pupation refuge. WFT larvae were transferred onto the plants and allowed to establish and breed for seven days.

A pre-treatment assessment was undertaken by counting WFT adult and larval numbers on each plant. Treatments were applied on two occasions seven days apart, using a 1L hand-held sprayer to apply to both sides of the leaf to run-off. Plants were allowed to dry, arranged into randomised blocks in the glasshouse, and covered with muslin mesh suspended on a wire frame over each pot. The cages confined the thrips to the treated plant. Post-treatment assessments were conducted seven days after each treatment application by counting total thrips numbers on each plant.

The treatments were:
Water control
1% oil control
0.5% oil control
Full spore concentration + 1% oil
Full spore concentration + 0.5% oil
Half spore concentration + 1% oil
Half spore concentration + 0.5% oil

Statistical analysis

Adult, larval and total count data were normalised using a $\log_e$ transformation. A preliminary analysis of variance was conducted on each separate variate at each time. Means were separated using the LSD procedure at P = 0.05.

RESULTS AND DISCUSSION

Non-significant differences in all pre-treatment counts indicated a uniform WFT population (Table 1). Similarly, differences were not detected in the first post-treatment assessment. After two applications, the full spore concentration at both oil rates were not different from each other, but were significantly better than all the other treatments. Neither rate of oil was phytotoxic.

Table 1. Comparison of efficacy of fungal spore and oil concentrations against WFT. Means followed by the same letter in the same column are not significantly different (P = 0.05).

	Mean total WFT/plant		
Treatment	Pre-treatment	7 DAT 1	7 DAT 2
Water control	36.6 ns	47.5 ns	98.5 a
1% oil control	39.6	78.3	47.9 a
0.5% oil	32.5	121.5	78.0 a
Full spore + 1% oil	34.1	17.8	2.30 b
Full spore + 0.5% oil	71.5	18.7	5.60 b
Half spore + 1% oil	50.4	57.4	27.2 a
Half spore + 0.5% oil	35.5	32.8	25.8 a

Although this trial produced a statistical separation in the final assessment, overall, the trial was compromised by the variability in the thrips populations between replicates within treatments. In both of the first two assessments, there were some large mean variations between some of the treatments yet they were not significantly different. This trial will be repeated.

2.6.2 COMPARISON OF THE EFFICACY OF FOUR ENTOMOPATHOGENIC FUNGAL ISOLATES AGAINST WESTERN FLOWER THRIPS, *FRANKLINIELLA OCCIDENTALIS* (PERGANDE) AND GREENHOUSE WHITEFLY, *TRIALEURODES VAPORARIORUM* (WESTWOOD)

INTRODUCTION

There was no single fungal isolate that dominated all others against WFT and GWF in the dose-response bioassays. However, four isolates showed consistent promise. They were separately compared with each other against WFT and GWF infesting cucumber plants in a small research greenhouse, to determine whether the results obtained under laboratory conditions could be repeated in a greenhouse.

MATERIALS AND METHODS

An initial small greenhouse experiment was conducted to compare the efficacy of the four isolates DPI 9, CS827, BU667, UVCA155. Following the completion of this trial, it was discovered that UVCA155 in fact should have been UVCA603. The trials were conducted on potted cucumber plants in a small temperature-controlled glasshouse.

The treatments were:
Water control
0.5% oil control
DPI 9 + 0.5% oil
CS827 + 0.5% oil
BU667 + 0.5% oil
UVCA155 + 0.5% oil

Western flower thrips Single cucumber plants cv Kaspian RZ were grown from seed planted 11 August 2005 in 75mm Growool blocks and transplanted into 15cm pots on 26 August. Plants were set up in a temperature-controlled glasshouse on a bench covered with black weed mat and with coarse vermiculite over the surface as a pupation refuge. The trial area was subdivided by suspending clear plastic film down the centre of the benches and across the bench to isolate areas for each potted plant to minimise adult thrips migrating from one plant to another and confounding the trial outcomes. WFT larvae were transferred onto the plants 30 August and allowed to establish and breed for 7 days. An assessment prior to the first treatment application was undertaken 5 September by counting WFT adult and larval numbers on each plant. Treatments were applied 5, 12 and 19 September by 1L hand-held sprayers to both sides of the leaf to run-off. Plants were allowed to dry and then arranged in randomised blocks in the glasshouse. Post-treatments assessments of adult and larval thrips were conducted on 12, 19 and 26 September.

Greenhouse whitefly The trial was set up in the same way. Cucumbers were seeded 21 September and used 11 October 2005. Plants were set up in a temperature-controlled glasshouse on a bench. The trial area was subdivided by suspending clear plastic film down the centre of the benches and across the bench to isolate areas for each potted plant, to minimise subsequently emerging adult whitefly migrating from one plant to

another and confounding the trial outcomes. 2000 GWF adults were released onto the plants on 17 October and allowed to oviposit for two days, then removed. After egg hatch, the larval stages were allowed to develop for 13 days to second stage nymphs before commencing the trial.

The experimental unit was a single potted cucumber plant. Treatments were applied using a 1L hand-held sprayer to both sides of one leaf to run-off, with four replicates per treatment. Plants were allowed to dry and then arranged in a randomised pattern in the glasshouse. Pre-treatment counts of GWF juveniles were undertaken 1 and 7 November, and sprays were applied 2 November and 9 November. Post-treatment counts were undertaken 9 and 16 November.

Statistical analysis

Counts of larval and adult thrips and juvenile and pupal whitefly were log transformed and analysed using ANOVA in Genstat 9. Means were back transformed after analysis.

RESULTS AND DISCUSSION

Western flower thrips Pre-treatment populations were not significantly different and uniformity was assumed. Throughout the post-treatment assessments DPI 9 performed consistently well, as did BU667 (Table 1). CS827 was also promising. UVCA155 was consistently the worst performed isolate and not different from either or both of the controls on each occasion.

Greenhouse whitefly Pre-treatment counts were not significantly different, indicating a uniform population in all treatments. After treatment application, the local fungal isolate DPI 9 was numerically the best performed (Table 2), although statistically not significantly different from BU667 and CS827. DPI 9 was selected as a suitable candidate fungal isolate for commercial development for GWF, the major pest of greenhouse tomatoes and a significant pest in many other greenhouse crops.

Table 1. Predicted counts of adult and larval western flower thrips treated with four selected entomopathogenic fungal isolates and positive and negative control treatments. Means were transformed prior to analysis. Means in the same column followed by the same letter are not significantly different (P = 0.05).

Treatment	WFT predicted Counts											
	Pre-treatment			Post-treatment 1			Post-treatment 2			Post-treatment 3		
	Adults	Larvae	Total	Adults	Larvae	Total	Adults	Larvae	Total	Adults	Larvae	Total
Water control	3.63 ns	66.02 ns	69.41 ns	6.23 a	141.18 a	148.41a	3.18 ns	39.25 a	42.95 ab	2.03 ns	78.26 a	79.84 a
Oil control	4.38	72.98	77.48	4.94 a	151.41 a	157.59 a	1.08	5.64 b	7.92 cd	3.76	66.69 a	70.81 a
DPI 9	5.77	103.54	108.85	0.19 b	18.92 c	19.49 c	0.26	2.89 b	3.29 d	0.26	10.49 b	10.80 b
CS827	5.13	109.95	115.58	2.83 ab	79.84 ab	83.10 ab	1.72	15.96 ab	17.99 bc	1.61	31.19 ab	32.79 ab
BU667	4.72	102.51	107.77	3.22 a	43.38 bc	46.53 bc	0.58	6.05 b	6.62 cd	1.61	7.39 b	9.68 b
UVCA155	5.31	105.64	112.17	4.60 a	68.72 ab	72.97 ab	0.72 a	46.53 a	47.94 a	1.	107.77 a	109.95 a

Table 2. Comparison of four fungal isolates against greenhouse whitefly infesting cucumbers. Analysis was conducted on the transformed means. Means (back transformed) followed by the same letter in the same column are not significantly different (P = 0.05).

Isolate	Mean numbers of GWF nymphs pre-treatment	Mean numbers of GWF nymphs and pupae 7 days post-treatment
Water control	121.51 ns	73.0 a
0.5% oil control	114.43	27.8 ab
UVCA155 + 0.5% oil	159.17	20.8 bc
CS827 + 0.5% oil	95.58	6.8 cd
BU667 + 0.5% oil	141.17	3.5 de
DPI 9 + 0.5% oil	121.51	0.9 de

2.6.3 GREENHOUSE CROP EVALUATION OF THE ENTOMOPATHOGENIC FUNGAL ISOLATE DPI 9 AGAINST WESTERN FLOWER THRIPS, *FRANKLINIELLA OCCIDENTALIS* (PERGANDE), INFESTING CUCUMBER AND GREENHOUSE WHITEFLY, *TRIALEURODES VAPORARIORUM* (WESTWOOD) INFESTING TOMATOES

INTRODUCTION

Following the identification of promising fungal isolates from a comprehensive laboratory bioassay screening process, greenhouse crop trials were conducted to evaluate the efficacy of selected isolates against western flower thrips (WFT) and greenhouse whitefly (GWF). The Australian isolate DPI 9, an isolate of *Beauveria bassiana*, was discovered as an epizootic infecting WFT in a Gosford Horticultural Institute greenhouse. It has been selected for commercial development with Becker Underwood Australia. In the initial greenhouse crop trials, the label rate for the commercially-developed *Beauveria bassiana* product BotaniGard™ was adopted. In the first WFT trial, there was a water only control and not the formulation oil product used with the DPI 9 spores. In the interests of accuracy the trial was repeated in September 2005, this time with oil as the control treatment.

Greenhouse whitefly is a major pest problem in greenhouse tomatoes and cucumbers in most regions where they are grown. In Queensland, silverleaf whitefly, *Bemisia tabaci* type b, is the prevailing whitefly pest species owing to the warmer temperatures that favour it. An effective fungal biopesticide would be of great benefit to producers throughout Australia as it would be a useful addition to the meagre armoury of IPM tools currently available. Following the success of the first trial, a further trial was conducted to evaluate the efficacy of DPI 9 at a reduced spore concentration under the same conditions.

MATERIALS AND METHODS

Western flower thrips

Trial 1 Cucumber seeds of the Rijk Zwaan cultivar Kaspian RZ (F1-Hybrid *Cucumis sativus*) were sown in 75cm GroWool™ blocks and held in a temperature-controlled glasshouse at 23^{o}C.

Two 54m^2 greenhouses with 3m sidewalls, hydronic heating and fan and pad cooling, and no roof ventilation, were used for this unreplicated trial with the fungal treatment in one greenhouse and the control in the other. Seedlings were transplanted into cocopeat bags at 3 - 4 true leaf stage with three plants per bag and irrigated at 2.2EC, pH 5.3 – 6.2 thereafter. Humidity was maintained in an uncontrolled way through the moisture from the greenhouse cooling pads.

Each greenhouse was planted with three rows of approximately 20 plants per row (Fig. 1). Two-spotted mite was observed on the left hand side row in greenhouse 2 and *Phytoseiulus persimilis* was released for control. Western flower thrips was released into both greenhouses by evenly distributing larvae and adults on the plants in the greenhouses on 27 and 28 June and 5 July 2005. Side-shoots were removed and plants trained as needed, EC and pH were monitored and temperature and humidity recorded using a Hastings Tinyview™ data logger.

A pre-treatment assessment of WFT populations in both greenhouses was conducted 14 July, nine days after the final thrips release, by randomly selecting 10 plants per row,

excluding the two end plants in each row which acted as buffers. Adult and larval thrips were counted on a single leaf per plant. A single treatment application was applied using a knapsack sprayer the following day. Treatments were a formulated DPI 9 product containing 2.6 x 10^9 viable spores/L dilute spray and a water only control, applied thoroughly, but not to runoff. Post-treatment assessments at 7 and 14 days after treatment (22 and 29 July) were undertaken following the same procedure as described for pre-treatment counts. Leaf nodes sampled were: pre-treatment, the 4th node; post-treatment 1, the 5th node, and post-treatment 2 the 7th node. Adult thrips were also monitored on two sticky traps per row and traps changed every seven days of the trial.

Figure 1. Layout of greenhouse cucumber trial.

Trial 2 The experiment was set up in the same way as previously. There was a substantial carry-over of WFT in the greenhouses from the previous trial, derived from pupae that were secreted under the plastic irrigation channels. These had to be tapped off the plants into alcohol and removed from the two greenhouses to allow the young plants to grow. At an appropriate stage of growth a pre-treatment assessment was undertaken on 2 September 2005 of the remaining established thrips population, using the same assessment method as in Trial 1. Treatments were applied three times on 2 September, 9 September and 16 September 2005 using a knapsack sprayer. Treatments were a formulated DPI 9 product containing 2.6 x 10^9 viable spores/L dilute spray, and 0.5% Synertrol HortiOil™ (850g/L emulsifiable botanical oil, Organic Crop Protectants, Lilyfield) as the control, both applied thoroughly, but not to runoff. Post-treatment assessments were made 7, 14 and 21 days after the first spray and

immediately prior to subsequent applications. Adult thrips were also monitored on two yellow sticky traps per row, six per greenhouse. Traps were changed every seven days of the trial, with the first set of traps installed on 28 August and the fourth set of traps removed on the 30 September 2005.

Greenhouse whitefly

Trial 3 Tomato seeds of the Rijk Zwaan cultivar Tradiro were sown on 21 September 2005 in 75mm GroWool™ blocks and held in a temperature-controlled glasshouse at 23^{o}C. They were irrigated with tomato hydroponic nutrient formula. Seedlings (3-4 leaf stage) were transplanted on 20 October into cocopeat bags in two research greenhouses. In each greenhouse there were three rows with 21 plants per row. The greenhouses were 54m^2 with 3m sidewalls, hydronic heating, fan and pad cooling, and no roof ventilation. The treatments were unreplicated, with the fungal treatment in one greenhouse and the control in the other. Side shoots were removed and plants trained as needed, EC and pH were monitored and temperature and humidity recorded using a Hastings Tinyview™ data logger.

8000 adult GWF were released into each of the greenhouses on 31 October, with further releases of 1000 and 500 in each greenhouse again on 3 November and 4 November 2005, respectively, a total of 9500 adult GWF into each greenhouse. Following the establishment of the whitefly population, the populations in both greenhouses were assessed prior to treatment on 15 November 2005 by randomly selecting 10 plants per row, excluding the end two plants in each row, which acted as buffers. Whitefly nymphs and pupae were counted on each of two leaves per plant. Treatments were applied three times on 16 November, 23 November and 30 November 2005 using a knapsack sprayer. Treatments were a formulated DPI 9 product containing 2.6 x 10^9 viable spores/L dilute spray, and 0.5% Synertrol HortiOil (850g/L emulsifiable botanical oil, Organic Crop Protectants, Lilyfield) as the control, both applied thoroughly, but not to runoff. Post-treatment assessments were undertaken 7, 14, 21, 28 and 35 days after the first spray and immediately prior to subsequent spray applications. Adult whiteflies were also monitored on two sticky traps per row, six per greenhouse. Traps were changed every seven days from 9 November to 20 December 2005.

Trial 4 Tomato seeds of the Rijk Zwaan cultivar Tradiro were sown on 16 January 2006 in 75mm GroWool™ blocks and held in a temperature controlled glasshouse at 23^{o}C. They were irrigated with tomato hydroponic nutrient formula. Seedlings (3-4 leaf stage) were transplanted on 3 February into cocopeat bags in two research greenhouses. In each greenhouse there were three rows with 21 plants per row. The greenhouses were as previously described. The treatments were unreplicated, with the fungal treatment in one greenhouse and the control in the other. Side shoots were removed and plants trained as needed, EC and pH were monitored and temperature and humidity recorded using a Hastings Tinyview™ data logger.

3000 adult GWF were released into each of the greenhouses in two releases on the 17 and 22 February 2006. Following establishment of the whiteflies, the populations in both greenhouses were assessed prior to treatment on 3 March 2006 by randomly selecting 10 plants per row, excluding the end two plants in each row which acted as buffers, and counting whitefly nymphs and pupae on each of two leaves per plant. Treatments were applied three times on 3 March, 10 March and 17 March 2006 using a knapsack sprayer. Treatments were a formulated DPI 9 product containing one quarter of the recommended BotaniGard rate or 0.65 x 10^9 viable spores/L dilute spray, and 0.5% Synertrol HortiOil (850g/L emulsifiable

botanical oil, Organic Crop Protectants, Lilyfield) as the control, both applied thoroughly, but not to runoff. Post-treatment assessments were undertaken at 7, 14, 21, 28 and 35 days after the first spray and immediately prior to subsequent spray applications. Adult whiteflies were also monitored on two sticky traps per row, six per greenhouse. Traps were changed every seven days from 27 February to 26 May 2006.

RESULTS AND DISCUSSION

Western flower thrips

Trial 1 A comparison of the DPI 9 treated population with the control population of WFT (Fig. 2), clearly demonstrated the efficacy of the fungal treatment, even in the short space of time of the trial. Late commencement of sticky trapping of adult populations resulted in insufficient data to assess impact (Fig. 3). The trial commenced on 14 July and adult counts two weeks later were still reflecting fresh emergences from pupal populations at ground level that were inaccessible to the sprays. This would explain the apparent greater proportion in the treated to untreated plots on the traps compared with the crop counts.

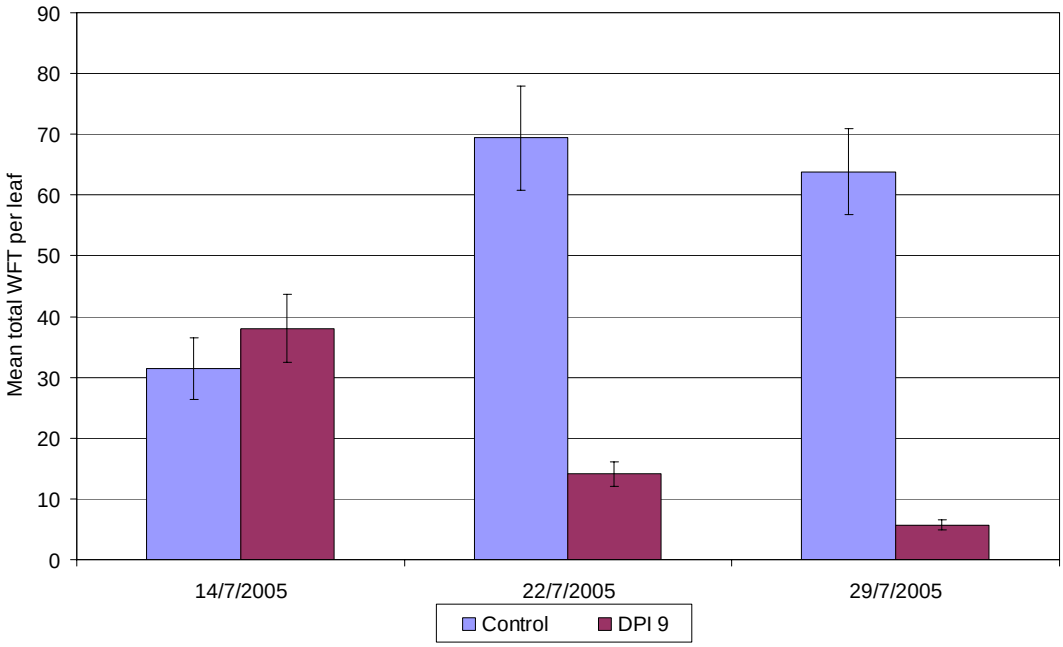

Figure 2. *Trial 1*. Effectiveness of DPI 9 against total life stages of WFT infesting greenhouse cucumbers, July 2005.

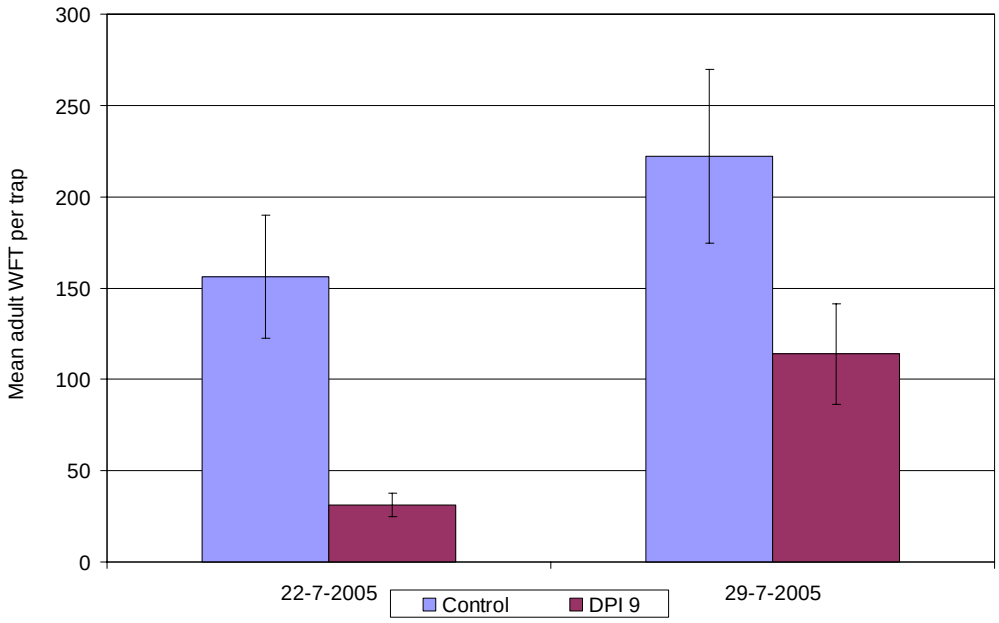

Figure 3. *Trial 1* Effect of DPI 9 on catches of adult WFT on yellow sticky traps.

Trial 2 Despite the initially higher population density of WFT, DPI 9 again gave outstanding control of WFT, as reflected in the comparison of treated and untreated crop (Fig. 4) and sticky trap populations (Fig. 5).

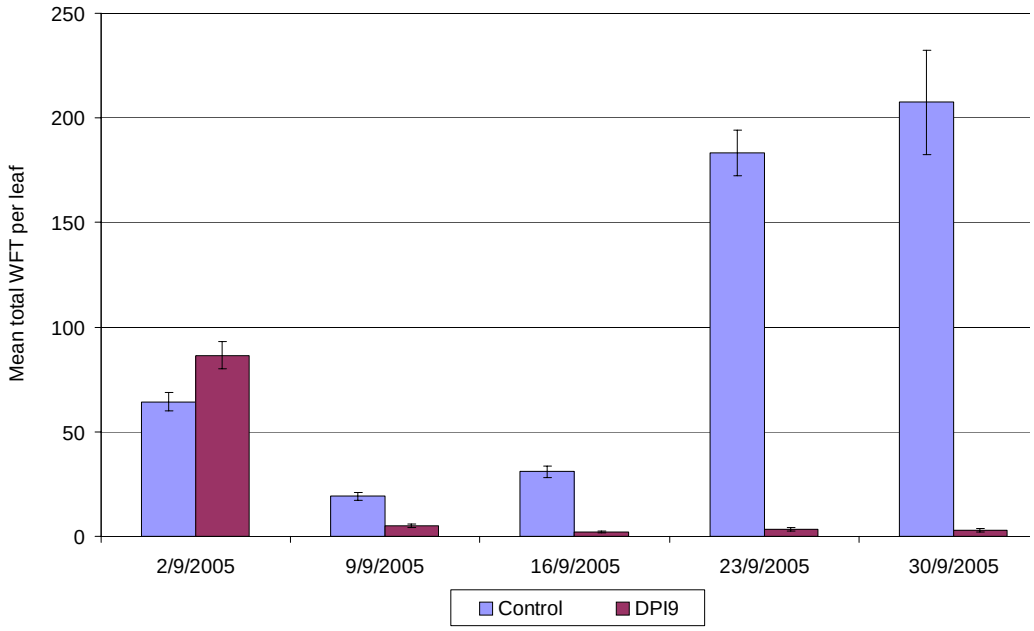

Figure 4. *Trial 2* Effectiveness of DPI 9 against WFT infesting greenhouse cucumbers, September 2005. Treatments were DPI 9 or oil.

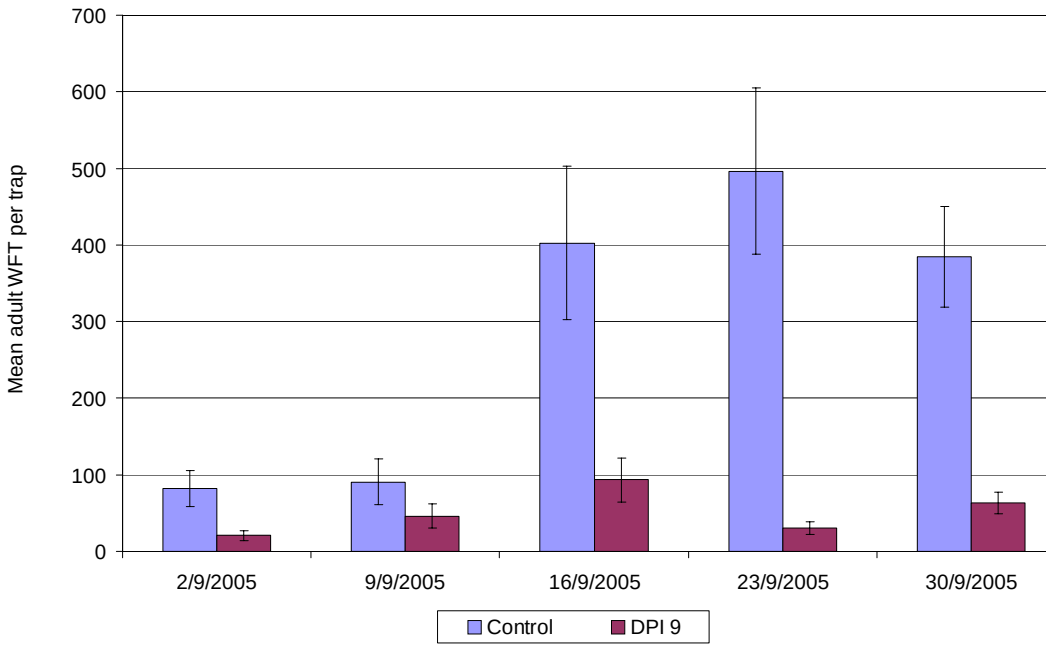

Figure 5. *Trial 2* Mean number of adult WFT on yellow sticky traps in greenhouse cucumber crops treated with DPI 9 or oil.

Greenhouse whitefly

Trial 3 Crop populations of nymphs, pupal and combined life stages of greenhouse whitefly in DPI 9 treated and the control greenhouses are compared in Figures 6, 7 and 8, respectively, and adult populations caught on sticky traps in the same greenhouses in Figure 9. DPI 9 demonstrated a clear ability to control this important crop pest in this six week trial, which is sufficient for a biocontrol program to be introduced or re-introduced after a period of whitefly activity. However, DPI 9 was used at the spore concentration recommended for the biopesticide BotaniGard™ registered for this purpose in the USA and elsewhere. It has been determined by the Australian business collaborator who would be responsible for the commercial development of DPI 9 that this is not commercially viable. It was recommended that the trial be repeated with a reduced spore concentration of DPI 9.

Trial 4 As in the previous trial, DPI 9 successfully controlled the GWF in the treated greenhouse, for a 10 week period (Fig. 10), although later in the crop, numbers per leaf increased. However, it was a good result at a quarter of the spore concentration applied in the previous trial. Numbers of adult whitefly on the sticky traps fluctuated over a 14 week period (Fig. 11). Still, DPI 9 demonstrated that it would be a useful tool against GWF in a biologically-based IPM strategy in greenhouse tomato crops. Although not specifically recorded, natural incursions of *Encarsia formosa*, a parasitoid of greenhouse whitefly, were observed in the DPI 9 treated crop and clearly survived treatment.

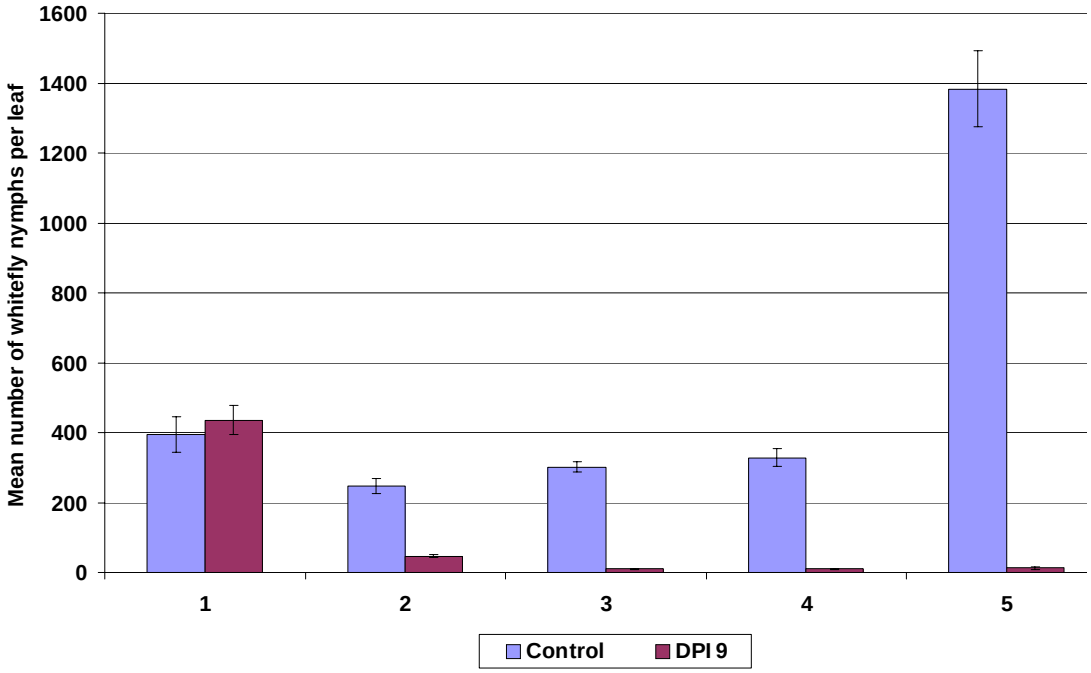

Figure 6. *Trial 3* Mean number of greenhouse whitefly nymphs/leaf in untreated and DPI treated greenhouse tomato crops.

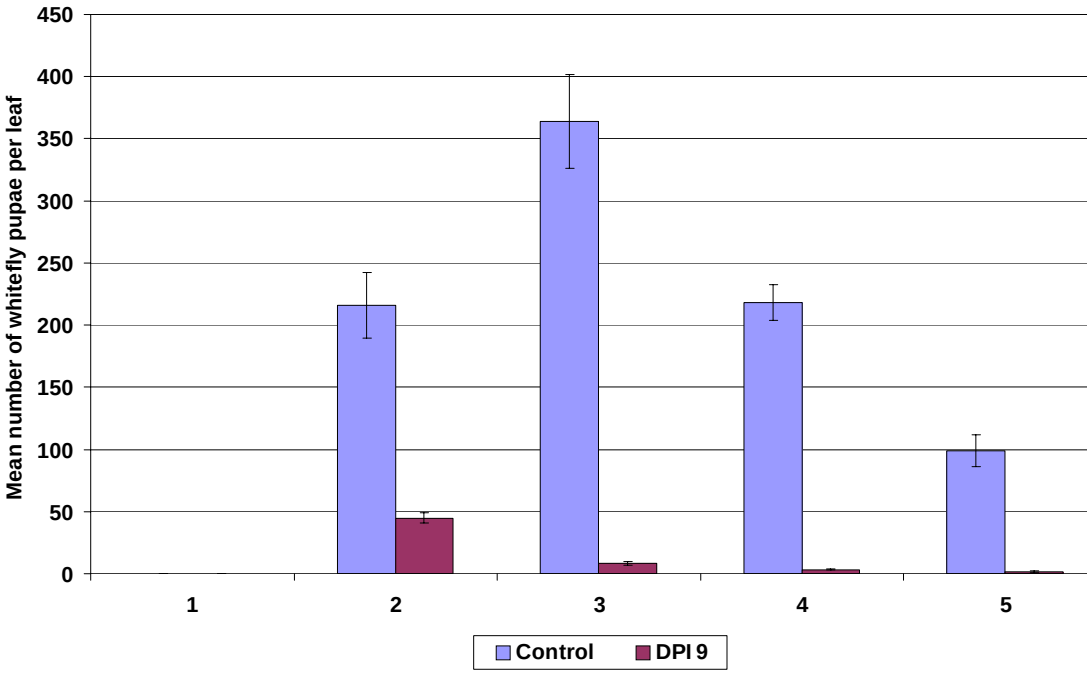

Figure 7. *Trial 3* Mean number of GWF pupae/leaf in untreated and DPI-treated greenhouse tomato crops.

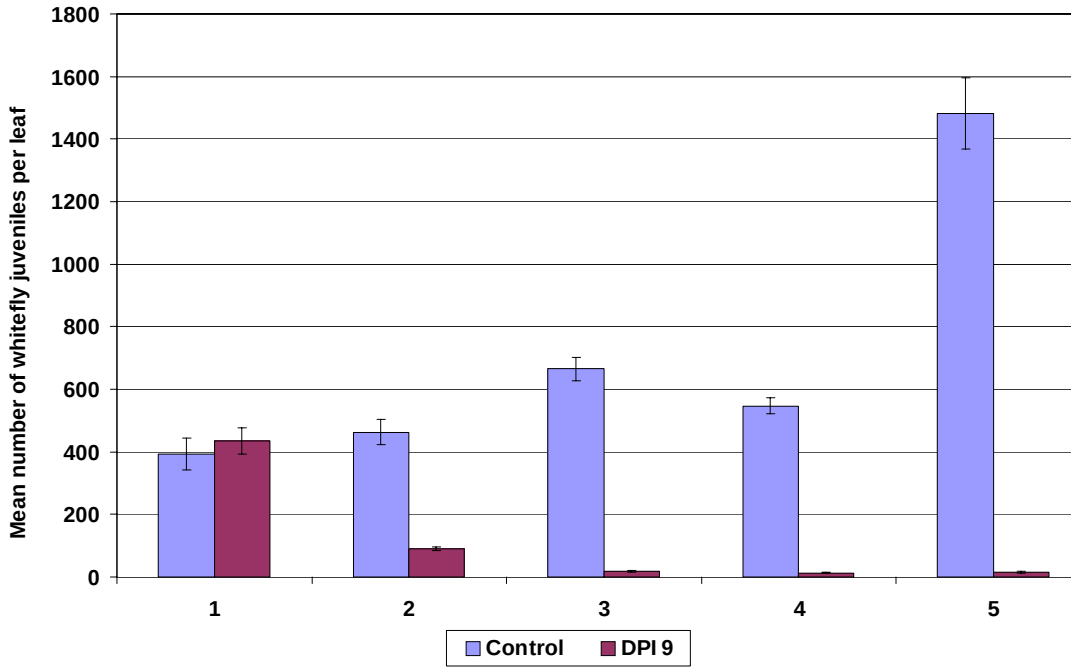

Figure 8. *Trial 3.* Comparison of mean number of greenhouse whitefly juveniles/leaf in untreated and DPI-treated greenhouse tomato crops.

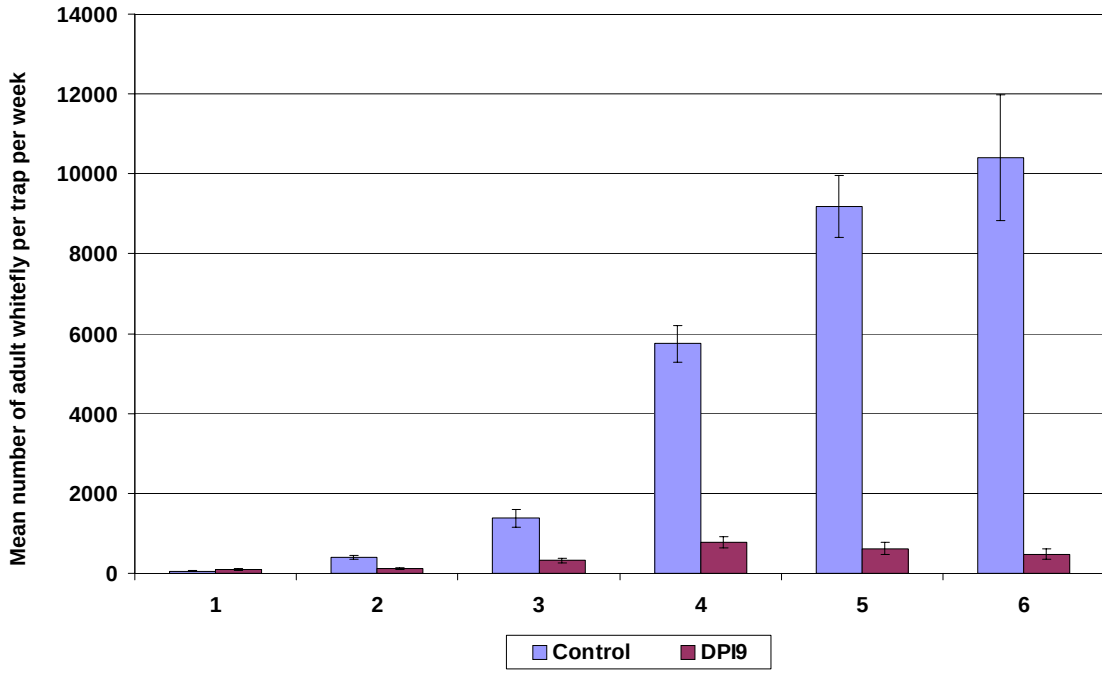

Figure 9. *Trial 3.* Comparison of mean number of adult GWF per yellow sticky trap in untreated and DPI 9-treated greenhouse tomato crops.

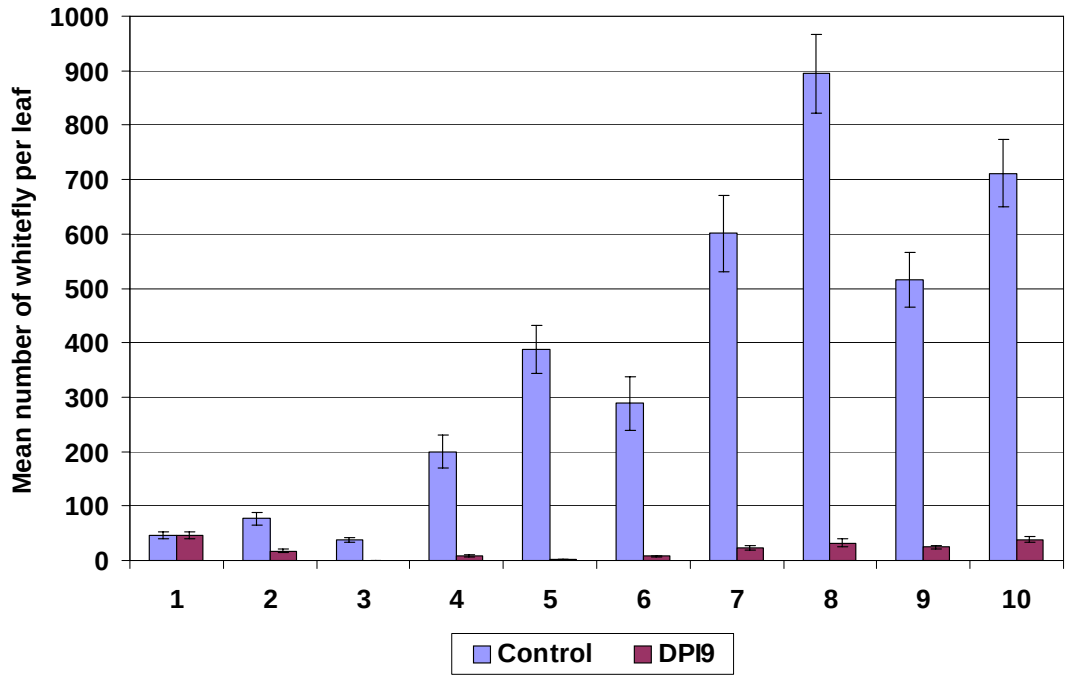

Figure 10. *Trial 4* Comparison of mean number of GWF juvenile stages/leaf in untreated and DPI 9- treated greenhouse tomato crops.

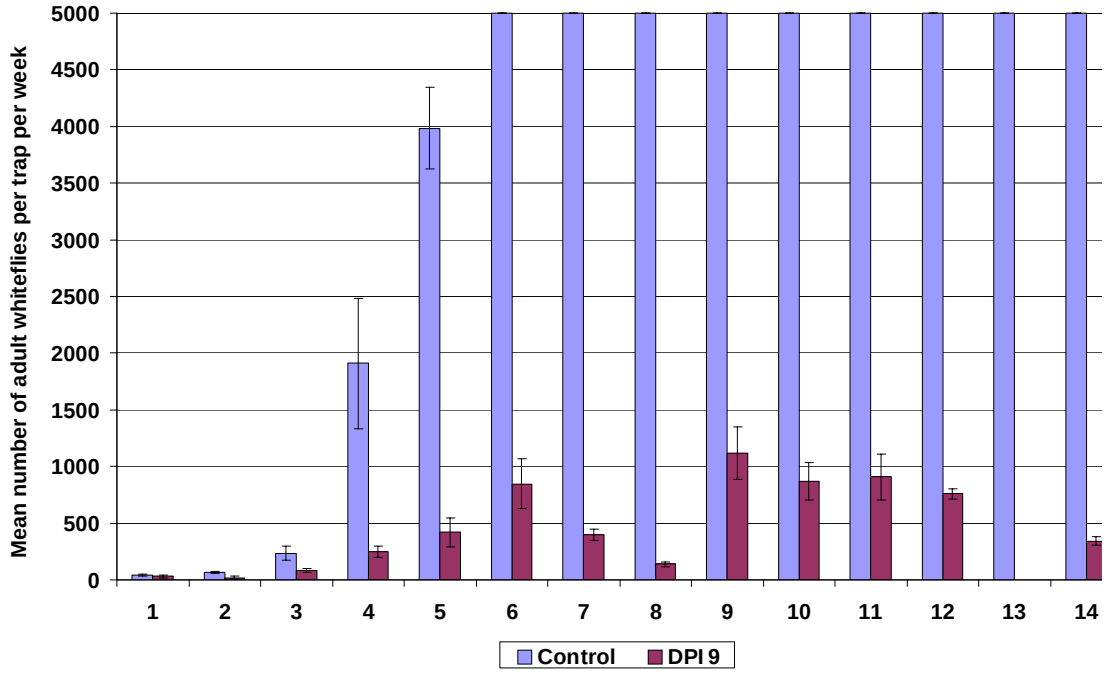

Figure 11. *Trial 4* Comparison of mean number of adult GWF per sticky trap in untreated and DPI 9-treated greenhouse tomato crops. Maximum values represent >5000 whitefly per trap.

3. IDENTIFYING AND RESOLVING PESTICIDE RISK FACTORS IN A GREENHOUSE IPM SYSTEM

3.1 IMPACT OF PESTICIDE RESIDUES IN A GREENHOUSE SYSTEM

INTRODUCTION

One of the factors presently hampering adoption of biocontrol for greenhouse pest management is a lack of so-called 'reduced-risk' pesticides that can be safely integrated with natural enemies. The definition of 'reduced-risk' in this context is that it is relatively harmless to natural enemies, rather than to humans, though the latter is generally the case also. The need for application of a pesticide may be to redress a situation where pest populations are too high to either start a biocontrol program or to continue one. Reasons for imbalance in pest/biocontrol agent populations may be many, from pests being favoured because the temperature is too low or too high, to the ratio of pests to natural enemies being too high initially, to a mistake being made on rates or choice of pesticide, or to massive periodic invasion of flying pests which overload the capacity of the natural enemy to cope, at least in the short term.

Pesticides act against natural enemies already present by direct contact with wet spray or drench, or indirectly through contact with residues. There is some information in the literature and on websites (e.g. http://www.koppert.ne and http://www.biobest.be) on these effects for the more commonly used natural enemies, although there are many gaps, particularly with regard to new chemistry products. Much information used to compile charts is anecdotal. Pesticides may have a negative (occasionally a positive) impact on natural enemies through post-application residues left on foliage, greenhouse structural components and in growing media. Less information is available on how long such residues might remain a problem for natural enemies. This information is needed to make recommendations on safe choice of pesticides, and when natural enemies can be released or re-released into the crop following a pesticide application. Biocontrol programs are preventive rather than curative and need to be started very early in the crop, thus propagators also need to know which pesticides they can use which will be compatible with natural enemies when the crop is grown on.

In this study, we examined the impact of residues of pesticides selected as being commonly used in Australian greenhouse crops (note Nitofol™ has no greenhouse registrations, but is still used by some growers), and test products that might remove them from the greenhouse environment. For some products, there were already strong anecdotal indications of toxicity, because of difficulty in starting biocontrol programs after their use; for others there was some suspicion that they may be having a negative impact. In any study of this type, there are many factors that may impinge on results, including the sensitivity of the natural enemy to a particular pesticide, the pesticide formulation, mode of application, rate of pesticide applied, growth rate of the crop, availability of refugia for natural enemies, mobility of natural enemies, crop type and structure, temperature and UV radiation, the degradation rate of the specific pesticide under varying conditions, and even an induced or innate resistance of a particular natural enemy population to the pesticide. The impact of the pesticides may be immediate and cause death of the individual, but it may also be chronic and affect long term survival, mating and egg laying capacity, searching ability, movement and a host of other capabilities. It is not possible to cover all these eventualities in the short term. In this study we have chosen only a few more commonly used pesticides and natural enemies,

and materials where pesticide residues might be retained. We have elected to follow only more obvious impacts for a period of up to two weeks post-application, with exposure to aged residues for three days. From this we can at least obtain a strong indication of which pesticides not to use, and an indication of where further study might be warranted on those of doubtful safety.

Among the natural enemies, we chose two foliar-inhabiting phytoseiid mites: the spider mite predator *Phytoseiulus persimilis* Athias-Henriot, which preys only on spider mites, and the more generalist predator *Transeius* (*Typhlodromips*) *montdorensis* (Schicha), which feeds primarily on thrips, mites and pollen. We selected one soil-dwelling predatory laelapid mite, *Stratiolaelaps* (*Hypoaspis*) *scimitus* (Womersley), which feeds on fungus gnat larvae and thrips pupae. We also chose two foliar-inhabiting parasitoids: the whitefly parasitoid *Encarsia formosa* Gahan, and the aphid parasitoid *Aphidius colemani* Viereck. We recorded survival and egg laying of the predatory mites, but only survival of adult parasitoids because egg laying requires hosts at the correct stage of development and the need to follow those through for at least a generation to see an effect, which was too time consuming and labour intensive for this project term. In describing the effect on natural enemies, we have followed the convention of the IOBC Working Group 'Pesticides and Beneficial Organisms' by assigning negative effects to four categories, where <25% reduction in control capacity is 'harmless', 25-50% is 'slightly harmful', 50-75% is 'moderately harmful', and >75% is 'very harmful'.

Residues of pesticides are generally assumed to have their maximum impact on natural enemies when applied to foliage (or media with respect to ground dwelling natural enemies). The contribution to mortality from pesticide residues on other surfaces that are contaminated is not known. These include plastic flooring, plastic roofing material and side wall cover, glass, plastic pots etc. Within the limited time period for this trial, we chose to study the impact of residues on three plastic materials, two used as ground covers (Panda™ film and weed matting) and a clear plastic commonly used for side walls.

Pesticides toxic to natural enemies may be applied either inappropriately, because the grower was unaware of the side-effects, or deliberately to rectify a pest imbalance when options are not considered available. In these circumstances the question is often asked whether there is any way to detoxify the chemical to enable a more rapid return to a biocontrol option. There are several products available for between-crop cleaning of plastic and removal of plant pathogens, but data relating to their usefulness as detoxicants of pesticide residues are scarce. Use of these products on plants and in growing media is unlikely because of phytotoxicity. Residues that might remain on plastics lend themselves more readily to removal, particularly as a wash down between crops. Four such products were selected from those cleaning agents commercially available, and tested on the three natural enemies that tested most sensitive to pesticide residues. The choice of pesticides was limited to those showing persistent high toxicity to these natural enemies.

3.1.1 RESIDUES ON FOLIAGE

MATERIALS AND METHODS

Three crop types, Lebanese cucumber cv Kaspian RZ, tomato cv Vulcan, and Basil were selected. Young plants were grown from seed and pinched out at six leaves (Fig. 1). These leaves were allowed to reach full size. Side shoots were removed in case of dilution of pesticide over time in new growth. Pesticides (Table 1) were applied at label rate to upper and lower foliage as a spray to incipient run-off, and allowed to dry. Treated plants (six replicates per treatment) were held in randomised blocks in a small glasshouse at 25 ± 5^{o}C under partial shade for two weeks. Aging residues were tested on the biocontrol agents *P. persimilis, T. montdorensis, E. formosa* and *A. colemani* at 0 (1-2 hours), 7, and 14 days post-application. To present the treated surface to each natural enemy, 30mm-diameter leaf discs (one per plant) were taken with a hole punch at the appropriate aging interval, and placed lower surface exposed on 1% agar in closed 47.8mm diameter x 7.6mm height Millipore dishes. The dishes were vented with a 30mm-diameter screen with 105µm sieve opening. They were held screen-side down in a tray over glycerol/water in an incubator at 25 ± 2^{o}C and ~80% RH for three days. Clip cages* in situ on the plants were initially trialled but were too difficult to handle and to examine for survivors. Natural enemies (5-7 individuals/unit) were introduced in cut-down pipette tips or with camel hair brushes. Parasitoids were fed with undiluted honey placed in small drops on the screening, *P. persimilis* with two spotted spider mite, *Tetranychus urticae* Koch, washed from green bean foliage and presented as a small clump, and *T. montdorensis* with cattail, *Typha* sp., pollen dusted on a small section of leaf. After three days, mortality was recorded, with those mites moribund or parasitoids unable to hop (*E. formosa*) or walk (*A. colemani*) counted as dead. Egg laying was recorded for *P. persimilis* and *T. montdorensis*. Counts of eggs and hatched larvae were combined and calculated as mean progeny/total adult mites at the start of the experiment.

* Clip cages were made similar to specifications published in a news release from the European Whitefly Studies Network 2001(http://www.whitefly.org/EWSN-NewDownLds-pdf/EWSN-ResPack-PA2-eq01.pdf.) and designed by John Innes Centre, Norwich Research Park, Colney Lane, Norwich NR4 7UH UK.

Table 1. Pesticides trialled as residues on foliage.

Trade name	Active ingredient	Chemical group	Application rate product/100L*
Talstar 80SC	bifenthrin	pyrethroid	50mL
Confidor 200SC	imidacloprid	neonicotinoid	25mL
Vertimec 18g ai/L	abamectin	macrocyclic lactone glycoside	50mL
Success 120g ai/L	spinosad	tetracyclic macrolide	80mL
Lannate L 225g ai/L	methomyl	carbamate	200mL
Nitofol 580g ai/L	methamidophos	organophosphate	50mL
Pirimor 50WP	pirimicarb	carbamate	50g
Dithane Rainshield 750g ai/kg	mancozeb	carbamate	200g

*0.01% Silwet was added to each chemical and to the control as a spreader.

Figure 1. Set-up for trials of residues on foliage. Leaf discs were removed 0, 7 and 14 days after pesticide application and biocontrol agents exposed to residues on the discs for three days.

The pesticides were divided into two batches of four products, each with a control, with *P. persimilis* and *E. formosa* as the first natural enemies evaluated, run concurrently, and *T. montdorensis* and *A. colemani* as the second, also run concurrently. Cucumbers, tomato and basil were run consecutively. *Encarsia formosa* and *A. colemani* were obtained from Biological Services, Loxton, SA, and *P. persimilis* and *T. montdorensis* from cultures maintained at GHI. None had a history of exposure to pesticides, as far as was known.

Statistical analysis

Effect on adults Final mortality counts were corrected for control mortality prior to analysis as follows:

% mortality = [Td (Tt.Cd/Ct)]/[Tt (1-Cd/Ct)] = Adjusted dead/Adjusted total x 100, where Td = treated dead, Cd = control dead, Tt = treated total, and Ct = control total.

Treatment effect was modelled using a mixed linear regression approach (Searle 1971) which allowed for the separation of variance into fixed and random effects. A GLMM was fitted to the data with binomial errors and logit link function as follows:

Logit (insects dead) = mean + treat + time + treat.time + *batch.rep*, where the italicised terms are included in the model as random effects. The analysis was conducted using ASREML (Gilmour *et al*. 2002).

Effect on production of progeny (mites only) Treatment means were used to calculate percentage reduction and these were adjusted for control mortality using the same formula as for adults. An analysis of variance could not be conducted on percentage reduction data because of negative values. Counts of progeny (eggs + larvae) per adult mite were log transformed and analysed using the same approach as for adults. The data were analysed using a mixed model approach, fitting spray, time and spray.time as fixed effects and rep and rep.spray as random effects. ASREML was used to fit the model.

RESULTS AND DISCUSSION

The data presented in Tables 2-17 show percentage mortality of the four natural enemies by crop and by time, with the addition of mean number of progeny (eggs plus larvae) of *P. persimilis* and *T. montdorensis* produced over three days after each degradation time period. The data are presented in pairs of tables with the same data, but analysed first by crop for each time period for all pesticides, and then by time period for each crop and individual pesticide. Statistical analysis was calculated on the basis of individual replicates and transformed for analysis as described. In some cases, there are discrepancies between results for crops and within a treatment, which was often due to considerable variation in oviposition rate or mortality within a treatment, and in some cases may be due to differences in crop physiology or structure, though there was no consistency in the latter trend. The main biological basis for variation in the mites is that female mites were of unknown age and thus not necessarily young or at peak egg laying stage. For the two parasitoids, adults were recently emerged from parasitised hosts and thus known to be young. Another source of variation was that some pesticides were of borderline toxicity. Thus mites may have qualified as ‘live’, but were too sick to function as effective predators or parasitoids.

Phytoseiulus persimilis
Mean residual toxicity of pesticides to adult female mites immediately after application was in the order Talstar > Nitofol > Lannate > Vertimec > Confidor > Success > Pirimor > Dithane (Table 2). Talstar, Nitofol and Lannate were very harmful immediately after application, with Vertimec slightly-moderately harmful. Other products were either not harmful or only slightly so. By Day 7, only Talstar was very harmful, and Nitofol and Confidor slightly so on some crops (Tables 2, 3). The effect on egg laying was more severe than on adult survival, indicating a sublethal effect on overall health (Tables 4-7). Where there was very high adult mortality (Talstar, Nitofol and Lannate), the number of eggs laid by the batch was naturally minimal. For Lannate, there was no residual effect after 7 days. For Vertimec, with moderate adult mortality only immediately post-spray, egg laying was severely reduced for at least 7 days and moderately reduced for 14 days. For Confidor, with low adult mortality, egg laying was severely to moderately reduced for at least 7 days. Results for remaining pesticides were not consistent between crops and weeks, but egg laying was less affected. Dithane has been known to reduce egg laying in predatory mites but this was not apparent in this trial, and previously may have been a formulation difference. Addition of wetting agents may greatly increase the toxicity of even ‘safe’ pesticides. It was

not possible to statistically test differences between the three crops, but there appeared to be either a similar trend, or no consistent pattern of differences.

Table 2. Percentage mortality by crop of adult *Phytoseiulus persimilis* exposed to 0, 7 and 14 day old residues of eight pesticides applied to foliage of three crops (Cuc = cucumber, Tom = tomato, Bas = basil). Means were adjusted for control mortality. Means followed by the same letter in the same column are not significantly different (P = 0.05) (analysis conducted on transformed data).

Phytoseiulus persimilis	% Adult mortality by crop								
Foliar residue	Day 0			Day 7			Day 14		
	Cuc	Tom	Bas	Cuc	Tom	Bas	Cuc	Tom	Bas
Talstar	100	100	100	80.30a	100	100	72.46a	87.45a	96.80a
Nitofol	96.48a	100	100	35.47ab	15.09ab	6.19b	46.90a	12.88b	19.83b
Lannate	49.67b	95.95a	84.71a	3.32bc	0	0	0	5.66b	9.80b
Vertimec	50.98b	70.06b	44.82ab	21.67bc	6.00b	21.27ab	3.09b	7.33b	5.48b
Confidor	10.46bc	41.59bc	0c	0bc	36.60a	50.00a	0	0	2.29b
Success	30.47bc	0.43d	1.07bc	2.03bc	24.95ab	15.09ab	0	17.81b	1.98b
Pirimor	2.52c	0d	17.70bc	0.11c	6.40b	2.91b	2.91b	6.49b	3.50b
Dithane	0	10.48cd	1.40c	2.73bc	0	3.09b	2.83b	0.20b	2.88b

Table 3. Percentage mortality over time of adult *Phytoseiulus persimilis* exposed to 0, 7 and 14 day old residues of eight pesticides applied to foliage of three crops. Means of the same pesticide and crop in the same horizontal row followed by the same letter are not significantly different (P = 0.05) (analysis conducted on transformed data).

Phytoseiulus persimilis	% Adult mortality over time								
Foliar residue	Cucumber			Tomato			Basil		
	Day 0	Day 7	Day 14	Day 0	Day 7	Day 14	Day 0	Day 7	Day 14
Talstar	100	80.30a	72.46a	100	100	87.45a	100	100	96.80a
Nitofol	96.48a	35.47b	46.90b	100	15.09b	12.88b	100	6.19a	19.83a
Lannate	49.67a	3.32b	0	95.95a	0	5.66b	84.71a	0	9.80b
Vertimec	50.98a	21.67a	3.09b	70.06a	6.00b	7.33b	44.82a	21.27ab	5.48c
Confidor	10.46a	0a	0	41.59a	36.60a	0	0a	50.00b	2.29a
Success	30.47a	2.03a	0	0.43a	24.95b	17.81ab	1.07a	15.09a	1.98a
Pirimor	2.52a	0.11a	2.91a	0a	6.40a	6.49a	17.70a	2.91a	3.50a
Dithane	0	2.73a	2.83a	10.48a	0	0.2a	1.40a	3.09a	2.88a

Table 4. Mean eggs and juveniles produced by *Phytoseiulus persimilis* after three days of exposure to residues of pesticides on three crops (Cuc = cucumber, Tom = tomato, Bas = basil), adjusted for control mortality. Residues were allowed to degrade over three time periods. Means in the same column are not significantly different (P = 0.05) (analysis conducted on transformed data).

Phytoseiulus persimilis	Progeny per female by crop								
Foliar residue	Day 0			Day 7			Day 14		
	Cuc	Tom	Bas	Cuc	Tom	Bas	Cuc	Tom	Bas
Control 1	6.17ab	5.14a	4.84a	7.08a	6.70a	5.27a	6.57ab	8.43a	4.97abc
Lannate	0.94d	0.26c	0.15d	7.43a	9.04a	4.94ab	5.76ab	7.75a	2.88cd
Dithane	4.97ab	6.47a	4.34ab	5.77a	7.51a	4.94ab	6.86a	8.80a	3.03bcd
Nitofol	1.00d	0	0.33c	3.13b	4.45b	3.78ab	4.44abc	6.79ab	3.50bcd
Pirimor	4.33bc	5.83a	2.76b	8.06a	6.56a	4.31ab	6.54ab	8.16a	3.58abcd
Control 2	6.68a	6.09a	6.38a	6.73a	8.06a	5.97a	2.97cd	6.77a	7.20a
Success	3.12c	6.99a	5.55a	6.5a	3.91b	4.09ab	4.04bc	6.44ab	6.08a
Confidor	1.39d	1.33b	4.46ab	1.55c	4.00b	3.10b	3.38cd	4.67b	5.66ab
Talstar	0	0	0	0.24d	0	0	0.35e	0.03c	0
Vertimec	0.94d	0c	0.53c	0.83cd	1.12c	1.06c	2.55d	3.33c	1.91d

Table 5. Mean eggs and juveniles produced over three days by adult *Phytoseiulus persimilis* exposed to 0, 7 and 14 day old residues of eight pesticides applied to foliage. Means of the same pesticide and crop in the same horizontal row followed by the same letter are not significantly different (P = 0.05) (analysis conducted on transformed data).

Phytoseiulus persimilis	Progeny per female over time								
Foliar residue	Cucumber			Tomato			Basil		
	Day 0	Day 7	Day 14	Day 0	Day 7	Day 14	Day 0	Day 7	Day 14
Control 1	6.17a	7.08a	6.57a	5.14a	6.70b	8.43b	4.84a	5.27a	4.97a
Lannate	0.94a	7.43b	5.76b	0.26a	9.04b	7.75b	0.15a	4.94b	2.88b
Dithane	4.97a	5.77ab	6.86b	6.47a	7.51a	8.80a	4.34a	4.94ab	3.03b
Nitofol	1.00a	3.13b	4.44b	0	4.45a	6.79b	0.33a	3.78b	3.50c
Pirimor	4.33a	8.06b	6.54b	5.83a	6.56a	8.16a	2.76a	4.31a	3.58a
Control 2	6.68a	6.73a	2.97b	6.09a	8.06a	6.77a	6.38a	5.97b	7.20a
Success	3.12a	6.5b	4.04ab	6.99a	3.91b	6.44a	5.55a	4.09a	6.08b
Confidor	1.39a	1.55a	3.38b	1.33a	4.00b	4.67b	4.46a	3.10a	5.66b
Talstar	0	0.24a	0.35a	0	0	0.03a	0	0a	0
Vertimec	0.94a	0.83a	2.55b	0a	1.12b	3.33c	0.53a	1.06a	1.91b

Table 6. Percentage reduction in egg laying of *Phytoseiulus persimilis* exposed to 0, 7 and 14 day old residues of eight pesticides applied to foliage (Cuc = cucumber, Tom = tomato, Bas = basil) (adjusted for control mortality).

Phytoseiulus persimilis	% Progeny reduction by crop								
Foliar residue	Day 0			Day 7			Day 14		
	Cuc	Tom	Bas	Cuc	Tom	Bas	Cuc	Tom	Bas
Talstar	100	100	100	96.41	100	100	88.35	99.53	100
Nitofol	83.80	100	92.11	55.88	33.46	28.18	32.45	19.46	29.59
Lannate	84.70	94.94	96.48	0	0	6.28	12.36	8.04	42.15
Vertimec	85.98	100	91.69	87.61	86.10	82.24	14.20	50.76	73.45
Confidor	79.19	78.10	30.12	77.01	50.36	48.06	0	31.05	21.38
Success	53.27	0	12.92	3.38	51.50	31.45	0	4.90	15.52
Pirimor	29.89	0	34.64	0	2.07	18.21	0.59	3.20	28.07
Dithane	19.49	0	0	18.52	0	6.28	0	0	39.07

Table 7. Percentage reduction in egg laying over time of *Phytoseiulus persimilis* exposed to 0, 7 and 14 day old residues of eight pesticides applied to foliage of three crops (adjusted for control mortality).

Phytoseiulus persimilis	% Progeny reduction over time								
Foliar residue	Cucumber			Tomato			Basil		
	Day 0	Day 7	Day 14	Day 0	Day 7	Day 14	Day 0	Day 7	Day 14
Talstar	100	96.41	88.35	100	100	99.53	100	100	100
Nitofol	83.80	55.88	32.45	100	33.46	19.46	92.11	28.18	29.59
Lannate	84.70	0	12.36	94.94	0	8.04	96.48	6.28	42.15
Vertimec	85.98	87.61	14.20	100	86.10	50.76	91.69	82.24	73.45
Confidor	79.19	77.01	0	78.10	50.36	31.05	30.12	48.06	21.38
Success	53.27	3.38	0	0	51.50	4.90	12.92	31.45	15.52
Pirimor	29.89	0	0.59	0	2.07	3.20	34.64	18.21	28.07
Dithane	19.49	18.52	0	0	0	0	0	6.28	39.07

Typhlodromips montdorensis

Mean residual toxicity of pesticides to adult female mites immediately after application was in the order Talstar > Nitofol > Lannate > Success (Tables 8, 9). Talstar, Nitofol and Lannate were very harmful immediately after application, with Success slightly-moderately harmful on tomato and basil. It did not cause mortality on cucumber, confirmed in a repeat experiment. Other pesticides were either not, or only slightly harmful. Effects of residues declined rapidly by Day 7 (Table 9), with only Talstar showing slight to moderate toxicity 7 and 14 days post-application, variable with crop. Adult *Typhlodromips montdorensis* were less sensitive to Talstar, Confidor and Vertimec than *P. persimilis*, and more sensitive to Success.

As with *P. persimilis*, the effect on egg laying was more severe than on adult survival and effects persisted for longer (Tables 10-13). Talstar was the only pesticide still very harmful after 14 days. Vertimec and Confidor were apparently not harmful to adult mites, but the effect on egg laying was severely and moderately harmful respectively immediately post-application, declining to slightly harmful for Confidor by 7 days, though the latter reduction was not significant, and relatively harmless for Vertimec. Sensitivity to the pesticides tested was similar to that of *P. persimilis*, except for an apparent greater tolerance of Vertimec.

Table 8. Percentage mortality of adult *Typhlodromips montdorensis* exposed to 0, 7 and 14 day old residues of eight pesticides applied to foliage of three crops (Cuc = cucumber, Tom = tomato, Bas = basil). Means were adjusted for control mortality. Means in the same column followed by the same letter are not significantly different (P = 0.05) (analysis conducted on transformed data).

Typhlodromips montdorensis	% Adult mortality by crop								
Foliar residue	Day 0			Day 7			Day 14		
	Cuc	Tom	Bas	Cuc	Tom	Bas	Cuc	Tom	Bas
Talstar	92.68a	100	100	71.46a	11.55ab	50.00a	22.22a	48.22a	61.52a
Nitofol	79.30a	100	67.88a	0	24.31a	18.61ab	0	0b	10.35b
Lannate	85.65a	88.45a	50.00a	0	2.83c	0	0	0	0
Vertimec	0	10.35d	0	0	0	0	4.00a	0	0
Confidor	0	3.78e	7.33b	0	0	10.71b	3.40a	0	3.51b
Success	0	50.00b	62.50a	0	0	0	3.64a	0	0
Pirimor	0	0	0	0	9.80b	0	3.40a	0b	0
Dithane	10.35b	21.20c	10.44b	3.51b	2.37c	0b	3.64a	0b	0

Table 9. Percentage mortality over time of adult *Typhlodromips montdorensis* exposed to 0, 7 and 14 day old residues of eight pesticides applied to foliage. Means of the same pesticide and crop in the same row followed by the same letter are not significantly different (P = 0.05) (analysis conducted on transformed data).

Typhlodromips montdorensis	% Adult mortality over time								
Foliar residue	Cucumber			Tomato			Basil		
	Day 0	Day 7	Day 14	Day 0	Day 7	Day 14	Day 0	Day 7	Day 14
Talstar	92.68a	71.46a	22.22b	100	11.55b	48.22a	100	50.00a	61.52a
Nitofol	79.30a	0	0	100	24.31a	0b	67.88a	18.61ab	10.35b
Lannate	85.65a	0	0	88.45a	2.83b	0	50.00a	0	0
Vertimec	0a	0	4.00a	10.35a	0	0	0	0	0
Confidor	0a	0	3.40a	3.78a	0	0	7.33a	10.71a	3.51a
Success	0a	0	3.64a	50.00a	0	0	62.50a	0	0
Pirimor	0	0	3.40b	0	9.80c	0b	0	0	0
Dithane	10.35a	3.51a	3.64a	21.20a	2.37b	0b	10.44a	0a	0

Table 10. Mean eggs and juveniles produced by *Typhlodromips montdorensis* after three days of exposure to residues of pesticides on three crops (Cuc = cucumber, Tom = tomato, Bas = basil). Residues were allowed to degrade over three time periods. Means in the same column are not significantly different (P = 0.05) (analysis conducted on transformed data).

Typhlodromips montdorensis	Progeny per female by crop								
Foliar residue	Day 0			Day 7			Day 14		
	Cuc	Tom	Bas	Cuc	Tom	Bas	Cuc	Tom	Bas
Control 1	4.23a	2.41a	2.83a	4.69a	5.12a	3.11a	4.60a	2.37c	3.29ab
Lannate	0.57c	0.149c	0.64b	4.62ab	2.81cd	3.00a	4.60a	4.15a	3.39a
Dithane	2.62b	1.24b	1.66a	4.75a	3.18bc	3.00a	2.53b	3.41abc	3.48a
Nitofol	0.28c	0	0	2.41c	1.73d	1.07b	3.59ab	2.13c	2.31ab
Pirimor	3.63ab	2.52a	3.38a	3.32abc	2.39cd	2.41a	3.13ab	2.47bc	2.04ab
Control 2	3.78*a	3.33a	1.73*a	2.90bc	4.93a	0.4bc	3.08ab	4.37a	2.21ab
Success	2.96ab	0.93b	0.44bc	4.75abc	4.28ab	0.37bc	3.60ab	4.45a	1.64ab
Confidor	2.29b	0.82b	0.27c	3.68abc	3.25abc	0.21c	3.60ab	3.48abc	2.07ab
Talstar	0	0	0	0	0.27e	0.07bc	0	0	0b
Vertimec	0.56c	1.07b	0.55bc	3.27bc	4.73ab	0.40b	3.68ab	3.90ab	2.17ab

*retransformed means used for this batch as some trials repeated

Table 11. Mean eggs and juveniles produced over three days by adult *Typhlodromips montdorensis* exposed to 0, 7 and 14 day old residues of eight pesticides applied to foliage of three crops. Means of the same pesticide and crop in the same row followed by the same letter are not significantly different (P = 0.05).

Typhlodromips montdorensis	Progeny per female over time								
Foliar residue	Cucumber			Tomato			Basil		
	Day 0	Day 7	Day 14	Day 0	Day 7	Day 14	Day 0	Day 7	Day 14
Control 1	4.23a	4.69a	4.60a	2.41a	5.12b	2.37a	2.83a	3.11a	3.29a
Lannate	0.57a	4.62b	4.60b	0.149a	2.81b	4.15c	0.64a	3.00b	3.39b
Dithane	2.62a	4.75b	2.53a	1.24a	3.18b	3.41b	1.66a	3.00ab	3.48b
Nitofol	0.28a	2.41b	3.59b	0	1.73a	2.13a	0	1.07a	2.31b
Pirimor	3.63a	3.32a	3.13a	2.52a	2.39a	2.47a	3.38a	2.41a	2.04a
Control 2	3.78*a	2.90a	3.08a	3.33a	4.93a	4.37a	1.73*a	0.4b	2.21a
Success	2.96a	4.75a	3.60a	0.93a	4.28b	4.45b	0.44a	0.37a	1.64b
Confidor	2.29a	3.68a	3.60a	0.82a	3.25b	3.48b	0.27a	0.21a	2.07b
Talstar	0	0	0	0	0.27a	0	0	0.07a	0
Vertimec	0.56a	3.27b	3.68b	1.07a	4.73b	3.90b	0.55a	0.40a	2.17b

Table 12. Percentage reduction in egg laying of *Typhlodromips montdorensis* exposed to 0, 7 and 14 day old residues of eight pesticides applied to foliage of three crops (Cuc = cucumber, Tom = tomato, Bas = basil). Means were adjusted for control mortality.

Typhlodromips montdorensis	% Progeny reduction by crop								
Foliar residue	Day 0			Day 7			Day 14		
	Cuc	Tom	Bas	Cuc	Tom	Bas	Cuc	Tom	Bas
Talstar	100	100	100	100	94.51	82.30	100	100	100
Nitofol	93.49	100	100	48.68	66.21	65.60	21.83	10.14	4.17
Lannate	86.49	93.81	77.25	1.50	45.17	3.40	0	0	0
Vertimec	81.42	67.88	79.32	0	4.03	0	0	10.68	1.82
Confidor	50.37	75.52	52.06	0	34.04	46.47	0	20.23	6.68
Success	12.88	72.05	33.19	0	13.18	8.50	0	0	26.02
Pirimor	14.28	0	0	29.24	53.36	22.36	31.86	0	15.51
Dithane	38.02	48.49	41.47	0	37.79	3.53	44.89	0	0

Table 13. Percentage reduction in egg laying over time of *Typhlodromips montdorensis* exposed to 0, 7 and 14 day old residues of eight pesticides applied to foliage. Means were adjusted for control mortality.

Typhlodromips montdorensis	% Progeny reduction over time								
Foliar residue	Cucumber			Tomato			Basil		
	Day 0	Day 7	Day 14	Day 0	Day 7	Day 14	Day 0	Day 7	Day 14
Talstar	100	100	100	100	94.51	100	100	82.30	100
Nitofol	93.49	48.68	21.83	100	66.21	21.83	100	65.60	4.17
Lannate	86.49	1.50	0	93.81	45.17	0	77.25	3.40	0
Vertimec	81.42	0	0	67.88	4.03	0	79.32	0	1.82
Confidor	50.37	0	0	75.52	34.04	0	52.06	46.47	6.68
Success	12.88	0	0	72.05	13.18	0	33.19	8.50	26.02
Pirimor	14.28	29.24	31.86	0	53.36	31.86	0	22.36	15.51
Dithane	38.02	0	44.89	48.49	37.79	44.89	41.47	3.53	0

Encarsia formosa

Only effect on mortality of adult wasps was assessed, so results reflect only gross impact on adults and not necessarily their ability to function as effective parasitoids. *Encarsia* was very sensitive to residues of all pesticides trialled except Pirimor and Dithane (Tables 14, 15). Talstar, Nitofol and Success were very harmful for at least 14 days, and Confidor and Vertimec for 7 days, with a decline in toxicity to moderately and slightly harmful respectively after 14 days. Lannate was very harmful immediately post-application, declining to moderately harmful after 7 days, though apparently safe on tomato.

Table 14. Percentage mortality of adult *Encarsia formosa* exposed to 0, 7 and 14 day old residues of eight pesticides applied to foliage (Cuc = cucumber, Tom = tomato, Bas = basil), adjusted for control mortality. Means in the same column followed by the same letter are not significantly different (P = 0.05) (analysis conducted on transformed data).

Encarsia formosa	% Adult mortality by crop								
Foliar residue	Day 0			Day 7			Day 14		
	Cuc	Tom	Bas	Cuc	Tom	Bas	Cuc	Tom	Bas
Talstar	100	100	100	100	100	100	100	100	100
Nitofol	100	100	100	100	83.74a	100	81.52a	24.31ab	100
Lannate	100	97.08a	93.63a	70.29a	10.79b	52.82b	2.40cd	0	2.80a
Vertimec	100	93.93a	95.05a	84.25a	92.10a	96.08a	26.51bc	62.48a	0a
Confidor	82.70a	90.80a	75.83a	95.49a	88.18a	92.38ab	50.91ab	64.53a	1.80a
Success	100	100	100	100	100	100	96.93a	100	100
Pirimor	0b	2.12b	1.28b	8.10b	6.46b	0	0d	2.49c	9.93a
Dithane	0	16.95b	28.39b	13.83b	27.72b	2.28c	27.82bc	4.95bc	5.75a

Table 15. Percentage mortality of adult *Encarsia formosa* exposed to 0, 7 and 14 day old residues of eight pesticides applied to foliage, adjusted for control mortality. Means of the same pesticide and crop in the same row followed by the same letter are not significantly different (P = 0.05) (analysis conducted on transformed data).

Encarsia formosa	% Adult mortality over time								
Foliar residue	Cucumber			Tomato			Basil		
	Day 0	Day 7	Day 14	Day 0	Day 7	Day 14	Day 0	Day 7	Day 14
Talstar	100	100	100	100	100	100	100	100	100
Nitofol	100	100	81.52a	100	83.74a	24.31b	100	100	100
Lannate	100	70.29a	2.40a	97.08a	10.79b	0	93.63a	52.82ab	2.80b
Vertimec	100	84.25a	26.51a	93.93a	92.10ab	62.48b	95.05a	96.08a	0b
Confidor	82.70ab	95.49a	50.91b	90.80a	88.18a	64.53a	75.83a	92.38a	1.80b
Success	100	100	96.93a	100	100	100	100	100	100
Pirimor	0a	8.10a	0a	2.12a	6.46a	2.49a	1.28a	0	9.93a
Dithane	0	13.83a	27.82b	16.95a	27.72a	4.95a	28.39a	2.28a	5.75a

Aphidius colemani

Success and Vertimec were very harmful to *A. colemani* for at least 14 days, Talstar for 7 days, and Nitofol only immediately post-application (Tables 16, 17). Confidor, Lannate, Pirimor and Dithane were not harmful to adult wasps. *Aphidius* was thus less sensitive than *E. formosa* to Talstar, Nitofol, Lannate, and Confidor, more sensitive to Vertimec, and equally sensitive to Success. Again, impact on overall health has not been determined, and thus it should not be presumed that those pesticides 'harmless' to adult wasps are necessarily safe to use. Further assessments should be made in this regard.

Table 16. Percentage mortality of adult *Aphidius colemani* exposed to 0, 7 and 14 day-old residues of eight pesticides applied to foliage of three crops, adjusted for control mortality. Means in the same column followed by the same letter are not significantly different (P = 0.05) (analysis conducted on transformed data).

Aphidius colemani	% Adult mortality by crop								
Foliar residue	Day 0			Day 7			Day 14		
	Cuc	Tom	Bas	Cuc	Tom	Bas	Cuc	Tom	Bas
Talstar	100	100	100	89.01a	82.22a	80.52ab	10.89b	46.72a	6.52b
Nitofol	100	100	100	3.50c	0.27b	23.50cd	8.25b	0	2.82b
Lannate	6.19b	22.28b	15.57b	2.74c	0	2.68d	2.25c	2.27b	0
Vertimec	100	96.95a	88.67a	100	97.13a	51.46bc	81.27a	77.00a	79.01a
Confidor	10.04b	27.29b	5.26b	24.12b	6.52b	0	0c	2.27b	9.72b
Success	100	100	97.39a	100	100	91.42a	100	88.31a	100
Pirimor	6.00b	0b	0	0	0.10b	2.62d	8.56b	0	0.35b
Dithane	22.81a	1.76b	0	0.19c	0	22.81cd	6.83b	0	0.08b

Table 17. Percentage mortality over time of adult *Aphidius colemani* exposed to 0, 7 and 14 day-old residues of eight pesticides applied to foliage of three crops, adjusted for control mortality. Means of the same pesticide and crop in the same row followed by the same letter are not significantly different (P = 0.05) (analysis conducted on transformed data).

Aphidius colemani	% Adult mortality over time								
Foliar residue	Cucumber			Tomato			Basil		
	Day 0	Day 7	Day 14	Day 0	Day 7	Day 14	Day 0	Day 7	Day 14
Talstar	100	89.01a	10.89b	100	82.22a	46.72a	100	80.52a	6.52b
Nitofol	100	3.50a	8.25a	100	0.27a	0	100	23.50a	2.82a
Lannate	6.19a	2.74a	2.25a	22.28a	0	2.27a	15.57a	2.68b	0
Vertimec	100	100	81.27a	96.95a	97.13a	77.00a	88.67a	51.46a	79.01a
Confidor	10.04ab	24.12a	0b	27.29a	6.52a	2.27a	5.26a	0	9.72a
Success	100	100	100	100	100	88.31a	97.39a	91.42a	100
Pirimor	6.00a	0	8.56a	0a	0.10a	0	0	2.62a	0.35a
Dithane	22.81a	0.19a	6.83a	1.76a	0	0	0	22.81a	0.08a

Sub-lethal effects on egg laying and other biological parameters need to be assessed before overall safety can be assumed. Prior exposure to these pesticides in the case of the parasitoids is also unknown, so it is not known whether lack of sensitivity indicates an innate or an induced resistance, perhaps even a strain difference. Differences in response to pesticides by two species of mites and two micro-hymenoptera suggest that generalisations should be made with caution. Parasitoids are generally more sensitive than predators, but there are exceptions and a wide range of responses may occur. Each species should be tested separately. Evaluation of strains with different exposure history to pesticides would give some indication of the possibilities for development of resistance. Because of the work involved, it would probably be better to err on the side of caution and provide advice based on strains not known to have exposure to pesticides. Some pesticides, such as Talstar, because of their broad spectrum toxicity and persistence, have no place in an IPM program. Success and Vertimec should not be used with *E. formosa* and *A. colemani*, and particularly for Vertimec, might be expected to impact negatively on a program relying on *P. persimilis* and/or *T. montdorensis* because of their effect on oviposition. Confidor is not suitable for use with *E. formosa*, *P. persimilis* or *T. montdorensis*, at least as a foliar application. Other pesticides with short-term toxicity may have a place in spot application or in applications timed to avoid periods when natural enemies are in the crop.

Variation between crops may occur because of crop canopy structure or leaf hairiness. Plants that were used in the experiment were pinched out to prevent further growth. In an actual greenhouse situation, new growth would be clean of pesticides, unless systemic and persistent, allowing colonisation of predators and parasitoids that inhabit this part of the crop canopy. Some variability in results is due to borderline toxicity, where individuals are sick but not yet dead. For *E. formosa* and *A. colemani*, the honey solution supplied as food may have dried out or been consumed to the point of being unavailable, causing death of otherwise healthy individuals. Lack of statistical separation of means for mite egg laying in particular is a result of varying age of females, so that some are at peak egg laying and others at the end of their reproductive life. Ideally, adult female mites, for example, should be 3-4 days into their oviposition period. It is possible to set up even-age females, but it takes considerably more time and within the scope of this project it was decided to take on a broader range of pesticides and species to gain an understanding of the general issues confronting choice of pesticides in IPM programs. Further work on specific pesticides of interest would be advantageous.

Compared with reports on side-effects on the Koppert and Biobest websites, there were some marked differences in effect, which may have been due to pesticide formulation, rate differences, or sensitivity of local strains of natural enemies. With respect to the two parasitoids, lesser sensitivity may be accounted for by not assessing effect on egg laying and other biological parameters in this trial. In regard to residual effects, our trials show Talstar as less toxic to *A. colemani*, Nitofol less toxic to *P. persimilis* and *A. colemani*, Lannate less toxic to *P. persimilis*, *A. colemani* and *E. formosa*, Confidor more toxic to *P. persimilis* and *A. colemani*, Vertimec more toxic to *P. persimilis*, *E. formosa* and *A. colemani*, Pirimor less toxic to *E. formosa*, and Dithane less toxic to *P. persimilis*.

Charts of the general effects of the eight pesticides tested as foliar sprays are appended (APPENDIX I A, B and C).

REFERENCE

Searle, S. R. 1971. Linear Models. John Wiley & Sons, New York.

3.1.2 RESIDUES IN MEDIA

MATERIALS AND METHODS

Media applications are most likely to affect soil-dwelling arthropods. A limited number of pesticides are applied directly to this zone. Confidor and Pirimor are two systemic pesticides that can also be applied as drenches. Talstar, a contact-acting pesticide known to have long-term negative effects on many natural enemies, was applied as a surface treatment to simulate potential run off as a result of foliar application use. The target biocontrol agent was *Stratiolaelaps scimitus*, widely used for control of fungus gnat larvae. The effect of media type on residuality of pesticides was unknown, thus several greenhouse media were included in the trial. These were a composted bark mix, vermiculite (#3 grade), Perlite, rockwool, pine sawdust, and cocopeat. 150mm-diameter plastic plant pots were filled with media to be tested, and media types divided into two batches of three types. Confidor and Pirimor were applied as drenches to saturation. The concentration used was that specified for foliar application, as, at the time, drench application was not a registered use pattern. A permit has since been issued to use Confidor as a drench against whitefly infesting greenhouse tomatoes. Because all growing media were initially at different moisture levels, the amount of liquid to drench was standardised by assessing the amount of water needed to wet each media type to point of run off. Amounts to saturate media for drenches of Confidor and Pirimor were as follows: vermiculite 350mL, compost 150mL, sawdust 150mL, cocopeat 250mL, perlite 300mL, and rockwool 200mL. The minimum was 150mL, so this amount was chosen as the drench volume of pesticide. The balance to drench for each media was pre-applied as water and mixed in well. For vermiculite, for example, 350mL/pot were needed to saturate it, therefore 200mL of water was added per pot and the vermiculite mixed to ensure uniform dampness. 150 ml of pesticide solution was then added as a top drench. For Talstar, 140 ml water was applied + 10 mL surface pesticide spray. The exception in media treatment was for rockwool, supplied as cubes. Each cube took 200mL to saturate, so this amount was applied as a drench for Confidor and Pirimor, and at 2.8mL surface spray/cube following a drench of 197.2mL water for Talstar. Three rockwool cubes were used instead of one 150mm-diameter pot (Fig. 2). Pesticide rates were as follows: 1. Confidor 200SC. 25mL/100L applied as a drench at 150mL/pot; 2. Pirimor 50WP, 50g/100L applied as a drench at 150mL/pot; 3. Talstar 80SC, 50mL/100L applied as a surface spray at 10mL/pot or 2.8mL/rockwool cube.

Plastic vials were used to confine *S. scimitus* to the media. The solid end was cut off a 120mL screw cap plastic vial, 45mm x 105mm, and a 34mm diameter hole was cut into the cap and screened with 105µm nylon mesh. The cut end was inserted into the media to a depth of ~80mm, close to the pot base, 1-2 hours after pesticide application. All three tubes were inserted at the same time to avoid disturbance later. With cap removed, 50 adult female *S. scimitus* were aspirated and introduced into one tube in each pot. A small vial cap containing 5mL of bran mite, *Tyrophagus putrescentiae,* from rolled oat sievings and a cube of soft dog food (food for the bran mites) was placed on the media surface as food. The screened cap was then screwed on the vial. After 7 days, the vial plus contents were removed and the media placed into a Ziplock bag. The empty vial minus cap was replaced in the pot to prevent collapse of the media into the hole. Mites were added to the remaining two vials 7 and 14 days post-treatment, and vial contents collected after 7 days exposure as previously. Treatments were randomised in a block, with four replicates. The pesticides were run in two batches. The pots were held on 150mm-diameter saucers in a screened glasshouse at 25±5^{o}C.

Water was added to the saucers in 50-100ml aliquots only as necessary to maintain moisture to within 1cm of the top of the media in the tube. The same amount was added to each media type, but because of absorption and evaporation differences between them some types received more water than others.

To extract mites and their progeny from the various media, variations on a basic technique were used. Prior to processing, the media from the vial was held in ziplock bags in a controlled environment room for up to two weeks at 17^{o}C. The low temperature was designed to arrest development, but possibly allowed some. Processing could take 30 minutes for one sample so it was not possible to do all on the same day. One replicate was processed before another was started. The material was washed through a series of three sieves-coarse, medium and fine (105µm). The medium sieve retained adults and some larvae, and the fine sieve eggs (rarely visible) and smaller larvae. The contents of the medium sieve were dried from below with paper tissue and approximately 50% by volume of #1-grade vermiculite added and mixed in. The vermiculite absorbed much of the water and allowed easier processing of the material. As mites had a tendency to go down into the media, bottom heat was used to drive them to the surface and increase their activity. This was provided by a polystyrene box in which a 40 Watt light bulb with metal guard had been inserted through the side. A loose-fitting plastic lid was placed over the top and a piece of Kimwipe™ placed on top of this to absorb moisture and allow manipulation of the media. Media was spread in a thin layer over the Kimwipe™ in 4-6 batches and active mites were aspirated and counted. The material was re-layered several times and the box tapped until no additional mites were found, being careful not to kill the mites by overheating. For perlite and rockwool, no vermiculite was added. For rockwool, which is naturally formed into layers, the plug was teased into thin layers under running water and mites collected in the sieves. The rockwool was retained, moisture squeezed out, and each layer held against a light to show up any residual mites trapped by the fibres. For each media type, material in the finest sieve was dried enough to remove excess water and examined under a microscope for larval stages. They were aspirated as seen. The sieve was also placed on the heat box briefly to encourage mites to come to the surface. The material was washed down and reprocessed once. Eggs were not counted as they were difficult to see and not mobile. Live food mite, *T. putrescentiae*, was present in good numbers in all samples so lack of food did not appear to be a factor in survival.

The first batch of media was set up 9 March 2005 and the second 27 April 2005. The Week 2 batch for compost, vermiculite and sawdust was lost when all mites died after storing at 10^{o}C to hold development instead of the normal 17^{o}C. This batch was repeated except for Pirimor in compost and for Confidor in the three media, as the previous trial had indicated no effect of treatment.

Statistical analysis

Adult count data

Final mortality counts were corrected for control mortality prior to analysis as follows:

% mortality = [Td (Tt.Cd/Ct)]/[Tt (1-Cd/Ct)] = Adjusted dead/Adjusted total x 100, where Td = treated dead, Cd = control dead, Tt = treated total, and Ct = control total.

50 insects were used to test each treatment. In rare cases where post-treatment counts were >50, the assumption was made that post-treatment count number = pre-treatment count number.

The effect of pesticide and media on mite survival was modelled using a mixed linear regression approach (Searle 1971) which allowed for the separation of variance into fixed and random effects. A GLMM was fitted to the data with binomial errors and logit link function as follows:

Logit(mites dead) = mean + pesticide + mean + pesticide.media + *rep* + *rep.pesticide*

where the italicised terms are included in the model as random effects. The analysis was conducted using ASREML (Gilmour *et al.* 2002).

Data were collected at three times and analysed separately for each occasion. Pesticide and media main effects and their interaction were examined for significance. Predicted means were provided for all interactions. The least significant difference (LSD) technique at the 5% level was used to separate means on the transformed scale.

Juvenile count data

Juvenile counts were also made. Three extra variates were derived from the adult and juvenile count data. These were

*number of juveniles/adult

*% reduction in juvenile numbers = (Control count-Treated count)/Control count*100

*% reduction in juvenile (j)/adult (a) numbers = (Control j/a count-Treated j/a count/Control j/a count*100.

These four variates were log transformed prior to analysis and then modelled in the same way as described for adult counts. Times were again analysed separately.

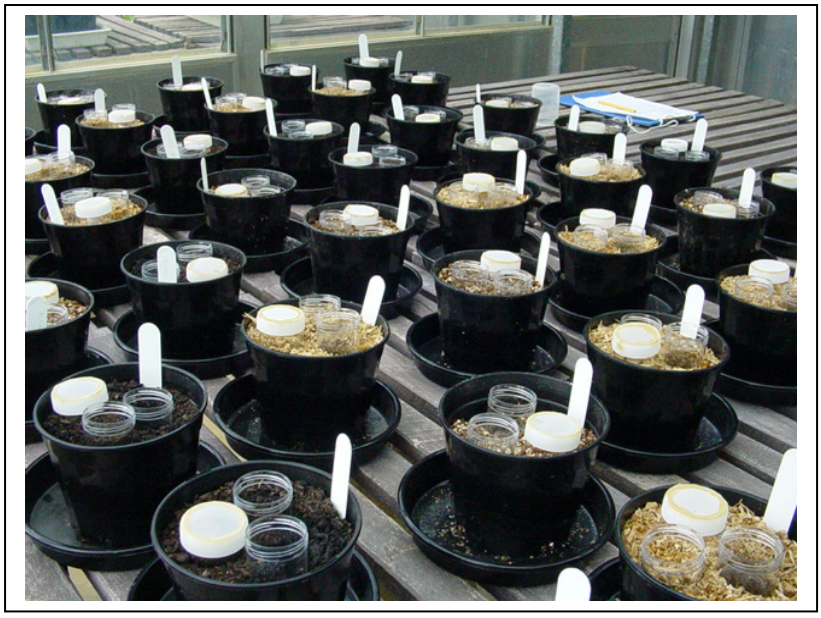

Figure 2. Bioassay set-up of pesticides applied to various media. Fifty *Stratiolaelaps scimitus* were added to one of the plastic vials in each of three successive weeks and collected for extraction after 7 days. Rockwool (above) was assessed similarly.

RESULTS AND DISCUSSION

There were significant differences between treatments in effect on *S. scimitus*, but media type also strongly influenced the effect (Tables 18, 19, 20). Talstar as a surface application was generally the most harmful pesticide, with residual effects of at least 14 days. Immediately after application, Talstar was moderately harmful to adult mites in compost, with survivors appearing very thin, and it was very harmful in all other media (Table 18). Egg laying in all media was minimal (Tables 19, 20). After seven days, the effect of Talstar on egg laying was still very harmful in all media, though of apparently low toxicity to adults in compost and moderate toxicity in sawdust. After 14 days, adult survival was still very low in cocopeat and perlite, but high in compost and sawdust, and moderate in rockwool. Egg laying was still severely affected in all media. Confidor was generally of low toxicity to adults and did not significantly affect egg laying. Pirimor was not harmful to adults in compost, cocopeat and sawdust, and did not affect egg laying, but was very harmful in rockwool, perlite and vermiculite for at least 14 days. This suggests that organic matter may tie up this pesticide, and perhaps others. In the case of Pirimor, this must have occurred rapidly and it was apparently not released later. An experiment conducted with drenches of Pirimor to a capsicum plant infested with green peach aphid grown in cocopeat or vermiculite confirmed that control was markedly reduced in a cocopeat medium. A spray application to foliage is reportedly safe to *S. scimitus* in media, according to the Koppert (http://www.koppert.nl) and Biobest (http://www.biobest.be) web-site side-effects databases, but no data is reported on drench application. Talstar is confirmed as not compatible in an IPM program using *S. scimitus*, because of the likelihood of pesticide contacting the surface of media from run-off applied to foliage.

The repeat experiment for Talstar and Pirimor in compost, sawdust and vermiculite returned much lower mortality of adult mites in both the first and second week, possibly a result of different environmental conditions or watering regime. A mean value is given in the Table. Mean temperature in the first run was 25.1^{o}C (range 20.8-28.3), mean relative humidity 82.6% (range 59.8-99.4), and in the second run 24.8^{o}C (21.1-27.9), relative humidity 53.4% (range 35.4-78.0). The drier conditions in the second run may have required more watering. 900-1050mL water were added to each unit, depending on the media, over the 3 week period in the first run, and 1150mL in the second, in each case added to the base of the media. Possibly the media was on the drier side in the second run.

Eggs could not be counted at assessment, but mean larval and nymphal progeny per female show preferences for high organic media (Table 19); however, eggs had been deposited in all media, showing possibilities for control of target pests in less favoured environments.

A chart of side-effects is appended (APPENDIX I D).

Side-effects on *Stratiolaelaps scimitus* listed on the 1. Biobest 2. Koppert website report that 1. Talstar as a spray is very harmful for >8 weeks 2. very harmful for 8-12 weeks, Confidor is 1. very harmful by spraying or as a drench, with no data on residual effect 2. moderately harmful but not persistent, and Pirimor 1. and 2. as a spray is safe. Data do not consider media type. Application rate of both Pirimor and Confidor that we used were those for a foliar application, and may have been lower than a drench rate. Both products gave control of aphids on small potted plants so this rate may have been adequate for this size pot.

Table 18. Percentage mortality of adult *Stratiolaelaps scimitus* exposed to residues of three pesticides in six media. Means are adjusted for control mortality. Means followed by the same letter within the same time period are not statistically different (P = 0.05) (analyses conducted on transformed data).

TREAT-MENT	Days Post-treat-ment	MEDIA TYPE					
		Compost	Cocopeat	Sawdust	Rockwool	Perlite	Vermic-ulite
Talstar	0	66.93bcde	87.91bc	78.88*bcd	100	100	98.87a
Confidor		16.73def	1.65f	42.06cdef	11.97def	26.03cdef	42.33cdef
Pirimor		0.80ef	2.20f	0.40*f	100	95.89ab	46.63*cdef
Talstar	7	19.54def	97.45ab	60.36*cde	97.37ab	97.40ab	97.79ab
Confidor		1.67ef	3.06ef	26.28cdef	25.66cdef	19.48cdef	21.54cdef
Pirimor		0ef	4.08*f	16.43*def	99.34a	88.31abc	77.02*bcd
Talstar	14	7.28cd	96.48a	15.05cd	55.35bc	98.63a	100
Confidor		-	0e	-	11.32cd	19.18cd	-
Pirimor		-	0	3.76de	95.60a	90.41ab	94.57a

* Repeat trial giving much lower mortality values (mean given)

Table 19. Mean number of juveniles per female mite in eight different media exposed to three pesticides, from eggs laid over 7 days (adjusted, transformed means). Means followed by the same letter within the same time period are not statistically different (P = 0.05).

TREAT-MENT	Days Post-treat-ment	MEDIA TYPE					
		Compost	Cocopeat	Sawdust	Rock wool	Perlite	Vermic-ulite
Control	0	0.49cde	3.68a	0.66cd	0.84c	0.16gh	0.14gh
Talstar		0.02h	0.05gh	0.01h	0	0.01h	0
Confidor		0.45def	2.79ab	0.58cde	0.73cd	0.30efg	0.16fgh
Pirimor		0.61cde	2.72b	0.51cde	0	0	0.01h
Control	7	1.05bc	1.60ab	1.19b	0.18f	0.1f	0.66cd
Talstar		0.22ef	0	0.23ef	0	0	0
Confidor		1.11bc	1.60ab	1.22ab	0.07f	0.03f	0.54de
Pirimor		1.17b	1.85ab	1.17b	0	0	0.02f
Control	14	1.27b	0.82c	2.08a	0.15ef	0.05f	0.35de
Talstar		0.16ef	0	0.43d	0.01f	0	0.01f
Confidor		-	1.08bc	-	0.09f	0.01f	-
Pirimor		-	0.92bc	2.29a	0	0	0.01f

Table 20. Percentage reduction in progeny of *Stratiolaelaps scimitus* exposed to residues of three pesticides in six media over three time periods. Data were corrected for control mortality. Figures in italics represent an increase in progeny numbers compared with the control

TREAT-MENT	Days Post-treat-ment	MEDIA TYPE					
		Com-post	Coco-peat	Sawdust	Rock wool	Perlite	Vermic-ulite
Talstar	0	95.25	98.63	98.66	100	97.14	100
Confidor		27.13	20.04	20.37	17.30	*88.57*	38.18
Pirimor		0	30.51	18.47*	100	100	100
Talstar	7	79.47*	100	82.81	100	100	100
Confidor		*14.36*	1.75	*54.97*	59.46	50	7.44
Pirimor		*23.76*	*10.82*	2.0	100	100	98.84
Talstar	14	88.01	100	78.88	96.67	100	98.68
Confidor		-	*39.52*	-	36.67	80	-
Pirimor		-	*22.75*	*7.64*	100	100	98.68

* Repeat trial giving much lower mortality values (mean given)

Table 21. Side-effects of pesticides applied as sprays, compilation from Koppert and Biobest websites (December 2006). T = harmfulness, R= length of residual effect in weeks (w) or days (d). 1 = harmless, 2 = slightly harmful, 3 = moderately harmful, 4 = very harmful.

TREAT-MENT	*P. persimilis*		*E. formosa*		*A. colemani*		*S. scimitus*	
	T	R	T	R	T	R	T	R
Talstar	**4**	8-12w	**4**	8-12 w	**4**	8-12w	**2 - 4**	0->8w
Nitofol	**4**	6-8w	**4**	4-8w	**4**	>4w	**2**	0
Lannate	**4**	4w	**4**	6-10w	**4**	8-12w	**2 - 4**	?
Vertimec	**2**	1-2w	**3**	5d	**4**	1w	**2**	5d
Confidor	**2 - 4**	0	**4**	>2	**4**	?	**3 - 4**	0
Success	**1 - 4**	1w	**2 - 4**	1-2w	**3 - 4**	1-2w	**1**	?
Pirimor	**2**	3-4d	**3**	3-4d	**1**	0	**1**	0
Dithane	**1 - 2**	0	**1**	0	**1**	0	**1**	0

REFERENCE

Searle, S. R. 1971. Linear Models. John Wiley & Sons, New York.

3.1.3 RESIDUES ON GREENHOUSE PLASTICS

MATERIALS AND METHODS

The previous experiments on crop foliage indicated that of the four commonly used natural enemies, the phytoseiid mite *P. persimilis* was more sensitive to selected pesticides than *T. montdorensis,* and the parasitoid wasp *E. formosa* more so than *A. colemani.* The pesticides most toxic to *P. persimilis* after one week were Talstar, Vertimec and Confidor, and to *E. formosa* were Talstar, Success, Nitofol, Confidor and Vertimec. For the soil-dwelling mite *S. scimitus,* Talstar and Pirimor were most toxic when applied to Perlite and vermiculite. These most toxic combinations were selected to examine residual effects on plastics.

Greenhouse material treatments were 1. clear covering plastic (CF), 2. Panda film (Propanda™, Redpath, Bendigo, VIC (PF), a solid polythene plastic used as a floor covering, white on one side and black on the reverse side, and 3. white, porous woven polypropylene ground cover (Reflectamat™, Redpath, Bendigo, VIC) (GC), also used as a floor covering. The materials were cut into 30cm x 45cm strips and first washed in Pyroneg™ to remove any existing surface residues, rinsed and dried. Pesticides were applied with a 2L hand sprayer to wet a 30cm x 45cm piece of the plastic (Table 22). Silwet was added as a wetting agent at 0.1mL/L to all treatments including the control. Panda film and ground cover were sprayed in a horizontal position and allowed to dry before use, whereas clear film was sprayed in a vertical position for *P. persimilis* and *E. formosa*, and in a horizontal position for *S. scimitus*. This was to reflect their normal and/or likely contact orientation in a greenhouse. After drying, materials were hung vertically from a wire on the eastern side of a plastic-covered greenhouse. The toxicity of dry residues was assessed by cutting pre-marked 50mm diameter discs from the treated materials at intervals of 0 (immediately after drying), 7 and 14 days. There were six such discs per treatment. These were placed individually in the lid of small plastic Petri dishes 55mm diameter x 14mm height, with treated surface up. A 34mm diameter hole in the base was screened with 105µm nylon mesh. A 1cm length of dental wick was sandwiched vertically between the screening and plastic disc and wetted to provide a water source. The appropriate biocontrol agent was aspirated (~8/dish) into a small pipette tip and introduced into the Petri dish with the tip. Food was provided as follows: *P. persimilis* - a dime-sized clump of *T. urticae* from a colony washed off leaves; *E. formosa* - 10% honey applied to the dental wick instead of water; *S. scimitus* - *T. putrescentiae* established on a small piece of dog food. The lid of the Petri dish was taped to the base with Parafilm to ensure a good seal. Petri dish units were placed in enclosed plastic trays over a glycerol and water mixture to give ~80% RH, and held in an incubator at 25 ± 1^{o}C for three days before assessing mortality. Egg laying was assessed for *P. persimilis* only, as it was the only biocontrol agent that would lay eggs under the trial conditions.

Statistical analysis

Adults The percent mortality of adult insects and mites was corrected for control mortality by the following procedure:

% mortality = [Td (Tt.Cd/Ct)]/[Tt (1-Cd/Ct)] = Adjusted dead/Adjusted total x 100, where Td = treated dead, Cd = control dead, Tt = treated total, and Ct = control total.

A GLMM with binomial errors and logit link function was fitted to the data - replicate was fitted as a random effect.
Eggs and juveniles Counts + 1 of (eggs + larvae) per adult were log transformed and the data analysed according to the model previously described. Mean percentage reductions of (juveniles + eggs) per adult mite were also calculated and corrected for control mortality as previously.

Table 22. Application rates of pesticides applied to greenhouse plastics and then exposed to selected biocontrol agents.

	Talstar	Confidor	Success	Pirimor	Nitofol	Vertimec
E. formosa	0.5mL/L	0.25mL/L	0.8mL/L	-	2mL/L	0.5mL/L
P. persimilis	0.5mL/L	0.25mL/L	-	-	-	0.5mL/L
S. scimitus	0.5mL/L	0.25mL/L	-	2.0g/L	-	-

RESULTS AND DISCUSSION

In general, pesticides were more toxic and for a longer period when applied to ground cover (polypropylene) than when applied to either Panda film or clear film (polyethylene).

Stratiolaelaps scimitus For the normally ground dwelling predatory mite *S. scimitus*, a surface application of Talstar was very harmful for at least 14 days when applied to ground cover, but not harmful when applied to clear film or Panda film (Table 23). This was a rather unexpected result. Pirimor was also harmless on clear film and Panda film, but slightly harmful on ground cover immediately after the application had dried. Toxicity did not persist. Confidor was tested only on ground cover, and was harmless.

Encarsia formosa For adult *E. formosa*, a normally foliar-inhabiting parasitoid, Talstar was very harmful and residual for at least 14 days when applied to ground cover, was moderately harmful on Panda film immediately after application, but only slightly harmful thereafter, and was harmless on clear film (Table 24), again, an unexpected result. Confidor was very harmful on all materials immediately after application, and retained toxicity except on clear film, with greatest persistence on ground cover. Success was very harmful on all materials for 14 days, except for moderate toxicity on clear film after 14 days. This was another unexpected result for residues to persist this long. Nitofol was very harmful on all materials immediately after application and on ground cover for 14 days. On Panda film, toxicity declined to moderate after 14 days, whereas on clear film it was not toxic at 7 or 14 days. For Vertimec, toxicity was high on ground cover and Panda film immediately after application, and moderately harmful on clear film. It was not harmful on clear film subsequently, but on Panda film was moderately harmful at both 7 and 14 days post-application. Contrary to results for other pesticides, Vertimec did not retain high toxicity on ground cover but was safe after 14 days.

Phytoseiulus persimilis For the normally foliar-inhabiting *P. persimilis*, mixed results were also obtained depending on the type of plastic and the post-treatment time (Tables 25-28).

For adult mites (Table 25), Talstar was very harmful for at least 14 days on ground cover, but was not toxic on Panda film. On clear film it was initially very harmful, but toxicity declined to low levels thereafter. Confidor was initially moderately harmful only on ground cover, with toxicity appearing to increase over time, though not significantly. Vertimec was safe on clear film, but very harmful on ground cover immediately post-application, declining to moderate after 7 days and slight at 14 days. On Panda film, it was moderately to very toxic for 7 days, but safe after 14 days. Effects on number of eggs laid (Tables 26 and 27) and percentage reduction in egg laying (Table 28) were relatively similar though inclined to be more severe.

The results should be interpreted cautiously because the degree of contact of a specific biocontrol agent with any particular type of plastic in a greenhouse situation has not been assessed. It is assumed that a soil-dwelling natural enemy such as *Stratiolaelaps* would be more likely to contact ground coverings, whereas a foliar-dwelling natural enemy would in all cases probably have little contact with side wall or ground materials, though this has not been looked at. There may be potential to contact various plastics in pot and media in bag materials. Support wires and poles are another potential source of contaminants that might be contacted, in particular by travelling predatory mites. The period of exposure is also critical. In the bioassays the natural enemy was obliged to cross a treated surface for three days to access water and food, but may have partially avoided contact if it stayed on the lid or was able to go under the plastic disc (they were very rarely found there). We have seen that assumptions based on looking at only one material of a common group such as plastics would lead to erroneous conclusions if applied to the whole. The bioassays indicate only the relative possibility of receiving a harmful dose, and some comparison between types of materials. Pesticides applied to polypropylene ground cover were more harmful than when applied to Panda film or clear film. In general, Success, Talstar and Nitofol were the most toxic and most residual pesticides; however, the low toxicity of Talstar to *Encarsia* when applied to clear film was a surprise, as difficulties in establishing *Encarsia* for months after Talstar use are a common grower complaint. Except for *S. scimitus*, application to clear film was made in a vertical position rather than horizontally as for the other two materials, thus probably reducing the deposition rate. Where possible growers should minimise run off onto ground covers.

A summary chart of side-effects of pesticides on plastics is appended (APPENDIX I E, F and G).

Table 23. Side-effects of pesticides applied to greenhouse plastics on adult *Stratiolaelaps scimitus.* CF = Clear plastic film (covering material); PF = Panda film (solid plastic floor covering); GC = Ground cover (white, porous woven polypropylene). Means were adjusted for control mortality and transformed for analysis. Means followed by the same letter in the same time period are not significantly different (P = 0.05).

Stratiolaelaps scimitus	% adult mortality after 3 days								
TREAT-MENT	Day 0			Day 7			Day 14		
	CF	PF	GC	CF	PF	GC	CF	PF	GC
Talstar	1.69b	5.86b	100	3.04a	2.38a	100	5.38b	-	92.01a
Pirimor	3.17b	6.10b	46.80a	1.75a	2.56a	10.20a	5.41b	-	0
Confidor	-	-	4.16b	-	-	1.87a	-	-	0

Table 24. Side-effects of pesticides applied to greenhouse plastics on adult *Encarsia formosa.* CF = Clear plastic film (covering material); PF = Panda film (solid plastic floor covering); GC = Ground cover (white, porous woven polypropylene). Means were adjusted for control mortality and transformed for analysis. Means followed by the same letter in the same time period are not significantly different (P = 0.05).

Encarsia formosa	% adult mortality after 3 days								
TREAT-MENT	Day 0			Day 7			Day 14		
	CF	PF	GC	CF	PF	GC	CF	PF	GC
Talstar	15.70c	62.4bc	100	9.86cd	10.30cd	100	1.79d	32.38bc	100
Confidor	90.19ab	100	98.24a	11.76cd	56.94bc	82.69ab	37.12bc	37.45bc	71.09ab
Success	97.34a	100	100	97.34a	97.68a	100	70.81ab	89.93a	100
Nitofol	100	100	100	7.60d	79.15ab	100	1.84d	69.19ab	100
Vertimec	68.89bc	92.98ab	94.23ab	6.27d	60.28bc	30.38bcd	12.37cd	69.19ab	1.00d

Table 25. Side-effects of pesticides applied to greenhouse plastics on *Phytoseiulus persimilis.* CF = Clear plastic film (covering material); PF = Panda film (solid plastic floor covering); GC = Ground cover (white, porous woven polypropylene). Means were adjusted for control mortality and transformed for analysis. Means followed by the same letter in the same time period are not significantly different (P = 0.05).

Phytoseiulus persimilis	% adult mortality after 3 days								
TREAT-MENT	Day 0			Day 7			Day 14		
	CF	PF	GC	CF	PF	GC	CF	PF	GC
Talstar	75.86ab	24.42cd	100	20.24d	22.76cd	100	28.89bc	1.48d	100
Confidor	13.67d	36.53bcd	70.57abc	6.48d	5.21d	77.93ab	2.04d	17.33bc	90.01a
Vertimec	18.91d	73.48abc	89.97a	3.37d	82.78a	72.41abc	8.66cd	0	46.72b

Table 26. Side-effects of pesticides applied to greenhouse plastics on *Phytoseiulus persimilis.* CF = Clear plastic film (covering material); PF = Panda film (solid plastic floor covering); GC = Ground cover (white, porous woven polypropylene). Means were adjusted for control mortality and transformed for analysis. Means followed by the same letter in the same time period are not significantly different (P = 0.05).

Phytoseiulus persimilis	Mean progeny per adult mite								
TREAT-MENT	Day 0			Day 7			Day 14		
	CF	PF	GC	CF	PF	GC	CF	PF	GC
Control	4.46ab	6.83a	4.03b	6.01abc	7.27a	4.28bc	9.27ab	9.89ab	6.96abcd
Talstar	0.30d	4.34ab	0	3.74c	5.01abc	0	4.43d	6.69bcd	0
Confidor	4.18b	1.19c	0.31d	5.44abc	7.00a	0.55d	10.67a	6.42bcd	0.43e
Vertimec	3.23b	0.31d	0.07d	6.29ab	0.48d	0.65d	8.44abc	5.83cd	1.53e

Table 27. Side-effects of pesticides applied to greenhouse plastics on *Phytoseiulus persimilis.* CF = Clear plastic film (covering material); PF = Panda film (solid plastic floor covering); GC = Ground cover (white, porous woven polypropylene). Means were adjusted for control mortality and transformed for analysis. Means of the same pesticide and material in the same row followed by the same letter are not significantly different (P = 0.05).

Phytoseiulus persimilis	Mean progeny per adult mite over time								
TREAT-MENT	Clear film			Panda film			Ground cover		
	0	7	14	0	7	14	0	7	14
Control	4.46a	6.01ab	9.27b	6.83a	7.27a	9.89a	4.03a	4.28a	6.96b
Talstar	0.30a	3.74b	4.43b	4.34a	5.01a	6.69a	0	0	0
Confidor	4.18a	5.44a	10.67b	1.19a	7.00b	6.42b	0.31a	0.55a	0.43a
Vertimec	3.23a	6.29b	8.44b	0.31a	0.48a	5.83b	0.07a	0.65b	1.53c

Table 28. Side-effects of pesticides applied to greenhouse plastics on *Phytoseiulus persimilis.* CF = Clear plastic film (covering material); PF = Panda film (solid plastic floor covering); GC = Ground cover (white, porous woven polypropylene). Mean percentage reduction in progeny numbers were adjusted for control mortality.

Phytoseiulus persimilis	% reduction in progeny after 3 days								
TREAT-MENT	Day 0			Day 7			Day 14		
	CF	PF	GC	CF	PF	GC	CF	PF	GC
Talstar	91.29	32.14	100	35.53	27.86	100	48.87	16.81	100
Confidor	0	79.98	91.70	9.12	2.17	83.62	0	31.72	91.30
Vertimec	27.55	93.45	97.94	27.94	93.29	83.92	9.35	27.60	76.59

3.1.4 DETOXIFICATION OF PESTICIDE RESIDUES

MATERIALS AND METHODS

After the previous series of experiments was completed, those pesticides causing >75% mortality to the most sensitive biocontrol agents were selected to test four potential decontaminants. Treatments were: 1. Control (water + 0.1% Silwet); 2. Virkon-S™ disinfectant (20.4% potassium peroxymonosulphate, Antec International) at 10g/L; 3. Sporekill Agricultural Disinfectant™ (120g/L didecyldimethyl ammonium chloride, Ekko, Carrum Downs, VIC) at 1mL/L; 4. All Clear™ spray tank cleaner and decontaminator (5-15% anionic surfactants, 5-15% 2-aminoethanol, 1-5% nonionic surfactants, 1-5% sodium 1-hydrxyethylidene-1,1-diphosphonate, AgNova Technologies Pty Ltd, Eltham, VIC) at 5mL/L; and 5. Duraclean™ greenhouse film exterior cleaner (<10% ionic surfactants, <5% sodium metasilicates, penta, <5% complex phosphates, <5% butoxyethanol, <5% quaternary ammonium chloride, Redpath Ideal Greenhouse Pty Ltd) at 40mL/L. Silwet at 0.1% was added to all pesticide treatments and to the control as a wetter, but was not added to the detoxicants as it was felt that these were already incorporated, nor to water washes following pesticide application. Only the Sporekill label indicated it might be safe to use on and around plants, and only All Clear claimed it could detoxify pesticides.

Trials were conducted from 28 July to 4 September 2006. Treatments were applied to two plastics: woven ground cover (polypropylene) and clear film plastic for side walls (polyethylene). Originally targeted biocontrol agents/pesticide combinations were *E. formosa*/Talstar (ground cover and cucumber) *E. formosa*/Success (clear plastic and cucumber), *P. persimilis*/Talstar and *P. persimilis*/Confidor (ground cover and cucumber (Sporekill only)), *P. persimilis*/Vertimec (cucumber, Sporekill only), and *S. scimitus*/Talstar (ground cover), *S. scimitus*/Talstar (Rockwool and Perlite, Sporekill only) and *S. scimitus*/Pirimor (Rockwool and Perlite, Sporekill only). Not all these combinations were pursued, for reasons explained below.

Treatments (Table 29) were applied to pre-cleaned strips of the plastic 30cm wide x 40cm long, using a small hand sprayer to completely wet the surface. Ground cover plastic was laid horizontally on wire mesh benches and clear plastic pegged vertically off bench edges for spraying. The first trial examined toxic effects of the detoxicants themselves to *E. formosa*. No toxic effects were noted for Virkon; however, other products indicated varying levels of low to moderate toxicity, thus only Virkon was pursued initially. Pesticides were applied first, and allowed to dry for one hour in a glasshouse. One batch was left without further treatment to evaluate pesticide effect alone, and selected detoxicants with water wash as a secondary control were applied to other batches to thoroughly wet. Once the plastic was again dry, after approximately one hour, pre-marked discs 34mm-diameter were cut out, and the same assessment procedure was followed as in the previous trial with plastics, with *E. formosa* as the first target natural enemy. Results from the trials with *E. formosa* demonstrated that none of the four cleaning products was effective in detoxifying Talstar, so planned combinations of detoxicants and Talstar with *P. persimilis* and *S. scimitus* were not pursued. There was also some doubt about the safety of Sporekill to natural enemies, so work on cucumbers and media with this product was also not pursued.

Because all remaining combinations of treatments could logistically not be conducted simultaneously, several trials were conducted with some treatments repeated (Table 30). There were eight trials for *E. formosa* and one for *P. persimilis*.

Statistical analysis

Adults Final adult mortality counts were corrected for control mortality prior to analysis (as described in previous analyses.) A GLMM was fitted to transformed data with binomial errors and logit link function as follows:

Logit(insects dead) = mean + treat +*time.rep,*

where the italicised terms were included in the model as random effects. The analysis was conducted using ASREML (Gilmour *et al*. 2002). All treatments were analysed simultaneously for each natural enemy.

Treatment and time effects were examined for significance and means were compared using the least significance difference (LSD) technique at the 5% level and then back-transformed to the original scale.

Eggs and juveniles Counts + 1 of (eggs + larvae) per adult were log transformed and the data analysed according to the model previously described. Mean percentage reductions of (juveniles + eggs) per adult mite were also calculated and corrected for control mortality as previously.

Table 29. Treatments applied to two types of greenhouse plastics to test the ability of four cleaning agents to detoxify selected pesticides. Treated plastics were exposed to *E. formosa* and *P. persimilis* for 3 days to evaluate residual toxicity.

TREATMENT		Clear film	Ground cover	Ground cover
		E. formosa	*E. formosa*	*P. persimilis*
1	Water	x	x	
2	Water + Silwet	xxxx	xxx	x
3	Virkon	xx	xx	x
4	Sporekill	xxx	xx	x
5	All Clear	xx	xx	x
6	Duraclean	xx	x	x
7	Virkon + water	x		
8	Talstar	x	xx	
9	Talstar + water	xx	x	
10	Talstar + Virkon	xx	x	
11	Talstar + Sporekill		x	
12	Talstar + All Clear	x	x	
13	Success	xxxx		
14	Success + water	xx		
15	Success + Virkon	xxxx		
16	Success + Sporekill	x		
17	Success + All Clear	x		
18	Success + Duraclean	x		
19	Confidor			x
20	Confidor + water			x
21	Confidor + Virkon			x
22	Confidor + Sporekill			x
23	Confidor + All Clear			x
24	Confidor + Duraclean			x

Table 30. Treatments applied in detoxicant evaluation trials. See Table 29 for explanation of treatment number. GC = polypropylene ground cover, CF = clear film polyethylene side wall cover.

Date of trial (2006)	Natural enemy	Plastic	Treatment #
28/7	*E. formosa*	GC	1, 3, 4, 5, 6
7/8		CF	2, 3, 4, 5, 6
7/8		GC	2, 3, 8, 10
11/8		CF	1, 2, 3, 4, 7, 8, 9, 10, 13, 15, 13, 14
18/8		CF	2, 9, 10, 13, 14, 15
18/8		GC	2, 4, 5, 8, 11, 12
25/8		CF	2, 4, 5, 6, 13, 15, 16, 17, 18
25/8		GC	2, 9
4/9	*P. persimilis*	GC	2, 3, 4, 5, 6, 19, 20, 21, 22, 23, 24

RESULTS AND DISCUSSION

There was no significant difference between water and Silwet controls so these treatments were combined in the analysis.

Encarsia formosa Of the prospective detoxicants, once dry, Virkon and Sporekill were safe to adult *E. formosa* on both ground cover and clear film; All Clear was safe on clear film and slightly toxic on ground cover, and Duraclean was slightly harmful on both plastics (Table 31). Of the pesticides, Talstar was very harmful on ground cover and slightly harmful on clear film. None of the cleaning agents reduced the toxicity of Talstar on ground cover, while on clear film results with either a Virkon or water wash were inconsistent and thus inconclusive. Success was very harmful to *E. formosa* on clear film, confirming results from the previous trial assessing residues on plastics. Virkon was the only cleaning agent that detoxified Success, rendering it slightly harmful.

Phytoseiulus persimilis All tests were conducted on ground cover plastic. All Clear, Sporekill and Duraclean were not harmful, whereas Virkon exhibited slight toxicity though not significantly different from All Clear and Duraclean (Table 31). Egg laying was not affected, except for a possible stimulatory effect for Duraclean and particularly All Clear that might be worth investigating further. Confidor was very harmful to adult mites (96.7% mortality) and to egg laying (91.7%). For adults this is higher than in the previous trial on ground cover plastic (70.6%), but very similar for egg laying. A water wash had no significant effect on reducing Confidor toxicity, whereas the four detoxicants were effective to varying degrees in improving adult survival and egg laying. Mortalities for adult mites ranged from 33-60% (slightly-moderately harmful), but were not significantly different from each other. Egg laying was normal for Sporekill, All Clear and Duraclean, and considering adult mortality levels, again suggests a stimulatory effect.

In summary, no single product tested detoxified all pesticides on all materials, and none completely. Variations should be expected for other pesticides, particularly as the contact period is relatively short. Virkon was most successful in partially detoxifying Success, with

some activity against Confidor, but none against Talstar. All Clear, Duraclean and Sporekill were not effective in detoxifying Talstar or Success, but were moderately effective in reducing the toxicity of Confidor to *P. persimilis* on ground cover. Because of the lack of materials identified to detoxify Talstar, this product should not be used in greenhouses where it is intended to use biocontrol agents, particularly those using polypropylene. Further testing may be warranted with a broader range of pesticides and cleaning agents. Cleaning agents should also be tested for direct toxicity against biocontrol agents if intending to use them where these are active.

Table 31. Percentage reduction in *E. formosa* and *P. persimilis* in contact with newly dried residues of three pesticides and following application of cleaning agents to clear film or ground cover. Means were adjusted for control mortality and transformed prior to analysis. Means in the same column followed by the same letter are not significantly different (P = 0.05). Figures in italics represent an increase in means.

TREATMENT	Clear film	Ground cover			
	E. formosa (adults)	*E. formosa* (adults)	*P. persimilis* (adults)	*P. persimilis* (progeny)	*P. persimilis* (progeny)
	% reduction				Mean per mite
Water	-	-	-	-	-
Water + Silwet	-	-	-	-	2.70bcd
Virkon	7.65ef	2.09f	34.88bc	18.08	3.01abcd
Sporekill	15.94ef	9.71ef	1.24d	*74.89*	4.99a
All Clear	9.16ef	26.94e	10.14cd	*16.46*	3.60abc
Duraclean	33.61de	37.42de	22.65c	*51.92*	4.17ab
Virkon + water	2.25f	-	-	-	-
Talstar	42.28cde	100	-	-	-
Talstar + water	12.28ef	100	-	-	-
Talstar + Virkon	38.76de	100	-	-	-
Talstar + Sporekill	-	100	-	-	-
Talstar + All Clear	-	100	-	-	-
Success	95.78ab	-	-	-	-
Success + water	89.77abc	-	-	-	-
Success + Virkon	32.93e	-	-	-	-
Success + Sporekill	85.91abcd	-	-	-	-
Success + All Clear	98.49ab	-	-	-	-
Success + Duraclean	98.54a	-	-	-	-
Confidor	-	-	96.83a	90.68	0.25e
Confidor + water	-	-	85.71ab	89.11	0.27e
Confidor + Virkon	-	-	57.95bc	24.92	1.76d
Confidor + Sporekill	-	-	60.49bc	11.75	2.34cd
Confidor + All Clear	-	-	33.06bc	9.81	2.58bcd
Confidor + Duraclean	-	-	40.13bc	27.27	3.10abcd

3.2 ASSESSMENT OF THE IMPACT OF BIFENAZATE ON THE SPIDER MITE PREDATOR *PHYTOSEIULUS PERSIMILIS* ATHIAS-HENRIOT

INTRODUCTION

Insecticides, miticides and fungicides can be harmful to many natural enemies. Over the years, a Working Group of the International Organisation for Biological Control Global has sponsored testing of pesticides against a range of predators and parasites in commercial production, results of which are reported in IOBC Bulletins. These tests are not exhaustive and results cannot always be extrapolated to species not tested, or even to the same species if it is subject to different pesticide pressures or there are other strain differences. Pesticide formulations may also vary in different countries, particularly with regard to carriers.

Questions about why a supposedly effective predator fails to establish in a commercial crop often lead back to harmful residues on the crop or structure. Knowing which pesticides can safely be integrated with predators is important in making recommendations in a crop where several pests and diseases may require treatment to keep them below damaging levels.

A pesticide may have both direct and indirect effects on the natural enemy. It may kill immediately or produce chronic effects. It may inhibit movement, stop or slow down egg laying, kill only one stage, but not others, have short term or long term effects, or be repellent. It may be harmful at some rates but not at others. All these combinations take a great deal of work and time to find answers to. Recently, there have been conflicting reports of the effect of the novel acaricide bifenazate on oviposition by the phytoseiid mite *Phytoseiulus persimilis* Athias-Henriot. Kim & Yoo (2002) evaluated bifenazate (Acramite® 20% SC) at a rate of 100mg ai/L, applied as a spray to bean leaf discs holding adult females or eggs of *P. persimilis*, and also fed them treated two-spotted mite, *Tetranychus urticae* Koch, as prey. Mortality of adult females and larvae was slightly higher than for the control mites, and egg production was not significantly different. Adult predators feeding on treated prey for 8 days produced about 10% fewer eggs but were otherwise unaffected. Van de Veire and Tirry (2003) found bifenazate harmless to the phytoseiid mite *Neoseiulus californicus* (McGregor) when eggs were placed on capsicum leaves treated with bifenazate at a rate of 150mg a.i./L. Kim & Seo (2001) evaluated bifenazate (23.5% SC) at a rate of 118mg a.i./L, applied as a spray to bean leaf discs holding adult females or eggs of the phytoseiid mite *Neoseiulus womersleyi* (Schicha), and also fed them treated two-spotted mite prey. There was only a slight reduction in oviposition and survival. James (2002) evaluated Acramite® 50WS on adult females of *Galendromus occidentalis* (Nesbitt), *Neoseiulus fallacis* (Garman) and *Amblyseius andersoni* (Chant) at rates of 225-900mg a.i./L (misreported as 25-100mg a.i./L), and reported mortalities of 57.3, 67.2 and 80.8% respectively at the high rate, and much lower mortality at the low rate.

We report here data on short term-testing of two bifenazate formulations on *P. persimilis*. Four basic tests were carried out. The first was a direct contact toxicity test, the second an indirect test designed to measure the short term effect of residues on a leaf surface, the third a combined direct contact plus indirect residual effect, and the fourth was designed to detect repellency of residues over the short term.

MATERIALS AND METHODS

Phytoseiulus persimilis were obtained from a culture maintained for several years on two-spotted mite on green bean, *Phaseolus vulgaris* L., plants at the Gosford Horticultural Institute site at Narara, NSW. Neither predator nor prey had been exposed to pesticides in recent years. Acramite™ SC miticide (480g/L) is registered in Australia (Crompton Specialties PTY, Regency Park SA 5010) on stone and pome fruit at 65mL product/100L (310mg a.i./L).

Residual effect Six leaf discs of 30mm-diameter were cut from green bean, leaves. Half the leaf discs were treated with water (control) and half with Floramite™ 2L SC (240g a.i./L bifenazate) at 1.3 mL/L (310mg ai/L) through a Potter Spray Tower depositing 2 ± 0.5mg/cm^2. The leaf discs were allowed to dry and set singly, upper surface down, on 1% agar in 47 mm-diameter Millipore™ dishes. The lid of each dish was screened with 105 µm hole size nylon mesh to allow air and water vapour exchange. Eggs of *Phytoseiulus persimilis* from the culture were then placed in batches of 50-60 on each of the treated and untreated leaf discs. Eggs and motiles of two-spotted mite were added as food. Dishes were placed screen-side down on a rack over a glycerol/water mix in closed plastic trays to give a relative humidity (RH) of 85 ± 0.5% (Fourney & Brandl 1992). Temperature and relative humidity were monitored in the trays using a Hastings Tinyview™ data logger. The trays were held in a controlled environment cabinet at a set temperature of 25 ± 0.5^{o}C with 12h photoperiod.

On Day 3, 30 fresh leaf discs were made up and treated as before with Floramite or water. Once the discs were dry, *P. persimilis* from the first set of discs were transferred to them, five per dish, and spider mite again added. Spider mite was added daily as required to provide surplus food, particularly to the treated discs as spider mites died from the treatment. The first adult predators and their eggs were noted on Day 5. Males from the relevant treatment were redistributed to ensure each disc had at least one so that females were mated. On Day 6, females were separated and transferred to newly-treated leaf discs, one only per dish. A male from the same treatment was added where available. Oviposition was recorded for four 24h periods, removing eggs as counted and bulking on a single leaf disc per treatment for each daily period. These discs had been similarly treated on Day 6. Each female had thus been exposed to residues of Floramite for 10 days, with fresh residues on Days 1, 4 and 6. The experiment was repeated with Acramite SC (480g a.i./L) at 0.65mL/L (310mg a.i./L).

Contact effect

To provide even-age immature predatory mites for testing, three Millipore dish units were set up as described previously with untreated green bean leaf discs. Eggs of *P. persimilis* <24h old were placed in batches of ~100 on each leaf disc, with two-spotted mite as food. They were maintained at 25 ± 0.5^{o}C and allowed to hatch and develop to larval and protonymph stages (Days 2 and 3). A modification of the method of Dennehy *et al.* (1993) was used to evaluate contact toxicity effects of Floramite on survival and subsequent egg laying. This method used two 1mL disposable micropipette tips. The distal tip, cut down to 22 mm in length, was placed over the narrow end of the proximal tip, cut to 60 mm in length. The latter was screened at the tip with 105µm nylon mesh. The unit was attached by a length of clear plastic tubing to a vacuum pump operating at minimum pressure. A solution of Floramite (1.3mL/L) was prepared and the non-ionic surfactant Teric X10™ (1% octyphenol

ethoxylate) was added to both treatment and water control solutions at a rate of 0.2mL/L. Ten to twelve mites at a time were gently drawn into the distal tip and held against the screen of the proximal tip, which prevented the mites being sucked up the vacuum line. The still-connected tips were removed and attached to a micropipette (GILSON P-1000 pipetman™), then 0.25mL of treatment solution was drawn into the tip to fill the distal and a short length of the proximal tip, keeping the mites immersed for 30 secs. The solution and mites were then gently expelled onto filter paper and each batch of treated mites transferred onto an untreated bean leaf disc using a fine brush. There were four batches of 10-12 mites for each treatment. These were checked daily until mating was noted (Days 4 and 5). Adult females (20/treatment) were removed and placed individually on untreated green bean leaf discs set on agar, as previously described, and fed daily with surplus two-spotted mite. They were checked at four 24h periods at which time eggs were counted and removed. Daily egg batches from each treatment were retained to check for successful hatch. The experiment was repeated with Acramite at 0.65mL/L.

Combined contact and residual effect Effect on both protonymphs and adult females was assessed. Approximately 200 eggs of *P. persimilis* were collected from the main culture 15 November 2004 and set up in five Millipore dishes with active stages of two-spotted mite as food (day 1) on green bean leaf discs. On day 2, when the mites had developed to protonymph stage, 100 were transferred with a fine brush to new leaf discs, two mites per disc (50 units). Half the units (leaf disc side only) were sprayed with water and half with Acramite at 0.65mL/L through a Potter Spray Tower depositing 2 ± 0.5mg/cm^2, checking each disc immediately before spraying to ensure the mites were on the leaf disc. Once the spray had dried, two-spotted mites were supplied as food. The latter were obtained by washing them off leaves and collecting all stages in a fine sieve. A small amount was collected with a brush and smeared along a section of leaf vein. Remaining predatory mites from day 2 were retained till they had reached the adult stage. Females were placed individually in units as described for protonymphs and similarly sprayed (day 8) with water (22 units) or Acramite (21 units). For both protonymphs and adults, the discs were checked for *P. persimilis* mortality and oviposition five days after treatment.

Repellent effect The experimental unit was a 9cm-diameter plastic Petri dish with a 30 mm-diameter hole in the lid screened with 105µm nylon mesh to provide ventilation. Eight 30mm-diameter green bean leaf discs were sprayed with water or 1.3mL/L Floramite®, using a Potter Spray Tower. A 1% agar solution was poured in the base of the Petri dishes. Just prior to the agar setting, the leaf discs were placed in a treated-untreated pair upper surface-down on the surface of the agar. The disc pairs were separated by 1-2cm, with four such dishes set up on three occasions. A batch of two-spotted mite eggs and motile stages washed off infested green bean leaves was placed alongside the central vein in each leaf disc as food. Batches of 20 adult female *P. persimilis* from a laboratory colony were aspirated into 200µL pipette tips. Each tip was then shortened and attached to the lid of the Petri dish with BluTac™, so that the mites were free to leave the tip and move to either of the leaf discs. A strip of Parafilm™ was used to seal the Petri dish unit. The units were inverted over a glycerol/water mix in closed plastic trays as previously. A Hastings Tinyview™ was used to record RH and temperature. The tray was held in a controlled environment cabinet at a set temperature of 25 ± 0.5^{o}C at 85 ± 0.5% RH with 12h photoperiod. Units were examined 24h and 48h post-treatment. The number of females on each leaf disc was recorded on each

occasion. The number of predator eggs was recorded only on the final observation. The experiment was repeated with Acramite at 0.65mL/L as the treatment.

A second experiment was carried out in the same way, except that two-spotted mites were pre-established in high numbers on all leaf discs 24h prior to treatment, and the assessment was conducted 3h post-treatment. In this way we wanted to ensure that TSM was evenly distributed on both treatments at the start of the experiment and initial distribution of predatory mites was not influenced by spider mite distribution.

Statistical analysis

Residual and contact effect A t-test, with unequal variance assumption, was conducted on the total number of eggs laid over the four days. A REML analysis (split-plot in time) of all data was conducted, assuming that the residuals are normally distributed and that the variances for each treatment are similar.

Combined residual and contact effect A generalised linear model with logit link function and bionomial error distribution was fitted to the mortality data in order to test the treatment effect on mortality. Similarly, a generalised linear regression model with log link function and Poisson error distribution was used to test treatment effect on number of progeny.

Repellent effect The differences between the number of mites on the control leaf disc and the Floramite/Acramite-treated leaf disc was calculated for each dish. For each day, a t-test was used to test whether the mean of these differences was equal to zero. If there was no repellent effect we would expect that there would be the same number of mites on each leaf disc, regardless of treatment.

RESULTS

Residual effect

There was no adult mortality. For Floramite, development of *P. persimilis* to maturity was slower on treated than on untreated leaf discs. Seven females failed to lay any eggs on day 1, and the variance was higher than in the control on subsequent days. Using the t-test, total oviposition per mite was significantly less for predators on Floramite-treated leaves (16.05) compared with the control (20.09) ($P = < 0.001$) (Table 1). When day 1 was omitted from the analysis, there were still significantly fewer eggs laid on Floramite-treated leaves ($P = 0.005$) for days 2, 3 and 4. Analysis by REML found significant effects of treatment, day and the interaction between treatment and day. Mites laid significantly fewer eggs than the control on days 1 and 2 ($P < 0.05$) but on days 3 and 4 there were no significant differences between treatments. Results were similar for Acramite (Table 1). Females laid significantly fewer eggs in total over four days ($P < 0.05$) than the control mites. REML analysis found significant treatment and day effect, confirming there were significantly fewer eggs laid on Acramite-treated leaves than in the control overall, and on day 1. Eggs laid on days 2, 3, and 4 were not significantly different from each other for either treatment. The interaction between day and treatment was not significant, implying that the treatment effect was consistent from one day to the next i.e. Acramite treatment always resulted in fewer eggs. While significant, the reduction in oviposition was <20%, and under IOBC side-effects testing-notation would be considered ‘safe’.

Because two-spotted mites were killed within a few hours by bifenazate, it is possible that food supply was more limited for these females and development and oviposition were affected negatively during the developmental period to mature adult. One female died in the Floramite and in the control treatment, and one in the Acramite treatment. Approximately one third of the females in the Floramite-treated units were found on the lid and not on the leaf disc each day, suggesting a repellent effect. It is also possible that predators were searching for live food, though they were observed feeding on dead adult spider mites. This repellent effect was not noted in the Acramite treatment. All eggs in both treatments were deposited on the leaf disc. Eggs collected from both treatments hatched by the second day and larvae appeared normal. Exposure to residues of Floramite and Acramite therefore had minimal effect on oviposition or mortality of *P. persimilis* during the treatment period.

Contact effect Overall, there was no significant difference between the mean numbers of eggs laid per day by predatory mites that had been immersed in Floramite or Acramite as nymphs compared with those in the control batch (Table 2). No differences in behaviour were noted. All eggs laid in both treatments hatched after 1-2 days and larvae appeared normal. Two control mites and one treated in the Acramite experiment became sick and laid fewer eggs.

Combined contact and residual effect There was no significant difference in mortality of Acramite-treated protonymphs. For adults, 18.2% of the control mites died and none of the Acramite-treated mites (Table 3). Predatory mites developed faster in untreated protonymph units. For protonymphs, where there were female/male pairs, an average of 9.6 eggs were laid over 5 days in the control protonymph batch and none in the Acramite-treated batch. However, of the pairs placed in the units, ~50% of the units ended up with males needed for mating and oviposition, whereas only 20% of Acramite units had males. Combined with more immature females in the latter, egg production was understandable lower. The delay in maturity may have been a result of less or lower quality food as Acramite killed the spider mite. For adult mites, the control mite batch laid on average 21.59 eggs and Acramite-treated mites 19.81 eggs over 5 days, not significantly different.

Repellent effect In the Floramite treatments, significantly fewer mites were found on treated discs than on untreated discs both 24h ($P = <0.001$) and 48h ($P = <0.001$) post-treatment (Table 4), suggesting Floramite had a repellent effect. For Acramite, the difference was not significantly different from zero at 24h, but was at 48h ($P = 0.001$). The proportion of mites on untreated discs increased over time, and may have been partly a result of the absence of live two-spotted mites on bifenazate-treated leaf surfaces. Eggs were laid on both treated and untreated discs, but far more were laid on untreated discs. Most eggs on the bifenazate-treated discs were laid under the patch of two-spotted mites where there were still some survivors, whereas on untreated discs, eggs were deposited mostly over the leaf surface. Floramite appeared to have more of a repellent effect on adult female *P. persimilis* than Acramite.

Where two-spotted mites were pre-established on all discs, and *P. persimilis* distribution assessed 3h post-treatment with Floramite, a mean of 10.25 ± 1.06 predatory mites were found on untreated discs and only 2.88 ± 0.61 on treated ones ($n = 8$). The repellent effect is therefore not affected by distribution or population density of the spider mite prey.

Neither formulation of bifenazate tested had an effect on oviposition rate of *P. persimilis* when applied as a contact treatment, and only a minor effect as a residue when no choice in treated or untreated foliage was presented. When presented with such a choice,

Floramite had a repellent effect on *P. persimilis* at 24h. After 48h, both products showed repellency. It was suspected that this may be a result of live prey no longer being available on the leaf surface in treated units. However, for adult females, substantial differences in occupancy between treated and untreated leaves were observed 3h after treatment where there was a pre-established spider mite infestation and still several live on treated leaves. This suggests repellency rather than simply a lack of live food. Insufficient live prey in the treated units is the most likely explanation for delayed maturity where protonymphs were treated by contact plus residual application.

In a crop situation, repellency may cause predators to move to untreated or new foliage, but this would happen anyway as prey is no longer available on treated foliage. The disruptive effect of bifenazate in a crop should therefore be minimal, particularly if applied as a spot-treatment. Other authors have not reported a repellent effect, but the testing method may not have lent itself to this observation, or not been evident at the lower rates tested. Rates as low as one tenth of that tested provided almost 100% control of two-spotted mite in small scale testing (Goodwin & Steiner 2003), suggesting a lower rate may be applicable where *P. persimilis* is also present.

Differences in susceptibility to a specific pesticide may occur between species of phytoseiid mites, so results showing safety to one species cannot necessarily be extrapolated to others. The rate used in this experiment (310mg ai/L) was higher than those reported as safe for phytoseiid mites (100-150mg a.i./L) (Kim & Seo 2001, Kim & Yoo 2002, van de Veire & Tiry 2003), and the methods differed, so a direct comparison is not possible. The high mortality of Acramite reported by James (2002) to *N. fallacis*, *G. occidentalis* and *A. andersoni* was a result of misreported rates (D. James, pers. com. 2004). The rates tested were actually, 225, 450 and 900mg ai/100L, and the low rate was safe.

It would appear that use of Acramite SC and Floramite SC against spider mites at a rate as high as 310mg a.i./L is compatible with *P. persimilis*, though best used as a spot treatment because of high mortality of its prey, two-spotted mite.

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Table 1. Oviposition by *Phytoseiulus persimilis* reared for a single generation on green bean leaf discs treated or not treated with Floramite or Acramite. Means in the same column with the same letter are not significantly different (P = 0.05).

	Phytoseiulus persimilis eggs laid /day ± SE (n)				
	Day 7	Day 8	Day 9	Day 10	Total
Floramite	4.00 ± 0.44 (13)	5.00 ± 0.20 (18)	5.06 ± 0.19 (19)	4.94 ± 0.17 (17)	16.05 ± 0.96a
Control	5.11 ± 0.13 (19)	5.32 ± 0.17 (19)	5.37 ± 0.18 (19)	5.11 ± 0.11 (19)	20.89 ± 0.25b
Acramite	2.39 ± 0.24 (18)	4.94 ± 0.21 (18)	4.61 ± 0.33 (18)	4.94 ± 0.34 (18)	16.89 ± 0.63a
Control	3.50 ± 0.47 (12)	5.08 ± 0.42 (12)	5.17 ± 0.17 (12)	5.25 ± 0.18 (12)	19.0 ± 0.70b

Table 2. Oviposition by *Phytoseiulus persimilis* exposed as nymphs to Floramite or Acramite by micro-immersion. Mature females were placed on untreated green bean leaf discs for oviposition. Means in the same column with the same letter are not significantly different (P = 0.05).

	Phytoseiulus persimilis eggs laid /day ± SE (n)				
	Day 7	Day 8	Day 9	Day 10	Total
Floramite	4.42 ± 0.22 (19)	5.30 ± 0.18 (20)	5.40 ± 0.15 (20)	4.85 ± 0.13 (20)	19.75 ± 0.37a
Control	4.56 ± 0.26 (18)	5.35 ± 0.18 (20)	5.50 ± 0.26 (20)	4.50 ± 0.31 (19)	19.45 ± 0.68a
Acramite	4.85 ± 0.27 (20)	5.60 ± 0.11 (20)	5.10 ± 0.10 (20)	4.45 ± 0.17 (20)	19.85 ± 0.68a
Control	5.20 ± 0.17 (20)	5.10 ± 0.14 (20)	4.55 ± 0.29 (20)	5.00 ± 0.41 (20)	20.0 ± 0.39a

Table 3. Mortality of *P. persimilis* five days after application of Acramite® to a bean leaf disc with either protonymph or adult female stage present. Progeny are also recorded. Means in the same row with the same letter are not significantly different (P = 0.05).

Stage treated	Percent mortality (n)		Mean eggs + immatures per female over 5 days (n)	
	Acramite	Control	Acramite	Control
Protonymph	12.82a (39)	16.28a (43)	0 (2*)	9.56 (9*)
Adult female	0 (21)	18.18 (22)	19.81a (21)	21.59a (22)

*adult females with males

Table 4. Mean number of adult female *Phytoseiulus persimilis* on untreated and bifenazate-treated leaf discs in a choice test 24 and 48h post-treatment, and total number of eggs laid at the final observation. There were 20 mites per dish. Means in the same column with the same letter are not significantly different (P = 0.05).

Treatment	Mean number of adult mites per leaf disc ± SE		Mean number of eggs laid
	24h	48h	48h
Floramite	2.33 ± 0.36 a	2.50 ± 0.31 a	22.00 ± 4.24 a
Control	12.33 ± 1.03 b	7.50 ± 0.79 b	88.75 ± 5.58 b

	Mean number of adult mites per leaf disc ± SE		Mean number of eggs laid
	24h	48h	48h
Acramite	3.58 ± 0.62 a	2.08 ± 0.43 a	27.42 ± 4.75 a
Control	5.42 ± 0.79 a	6.67 ± 0.70 b	48.33 ± 6.18 b

4. DEVELOPMENT OF NEW BIOCONTROL AGENTS FOR USE IN GREENHOUSE CROPS

BACKGROUND

While this area was not a formal topic of this project, we have continued to maintain an interest in the development of this field in Australia, and offer the following information for the interest of greenhouse vegetable producers and other interested stakeholders.

Natural enemies currently available from commercial insectaries are listed in Table 1 with their target pests, while work in progress on potential new agents is listed in Table 2. Further information can be found on the Australasian Biological Control Association (ABC) website http://www.goodbugs.org.au and in their book 'The Good Bug Book'. While the range of natural enemies has expanded considerably in the last few years, there are still gaps that need to be filled to provide a comprehensive response to all the major pests of greenhouse vegetables. Minor pests are unlikely to be a target for development of biocontrol agents, because the market is too small to make it economic. In some cases, minor pests are controlled by a biocontrol agent already on the market for a major pest, for example broad mite, which is adequately controlled by *T. montdorensis* or *N. cucumeris* (prime target is thrips). Reduced-risk pesticides are also a key component of an IPM program where minor pests are likely to occur, and which may build up as a result of broad spectrum, more toxic pesticides no longer being applied.

Because Australia has a hot, dry climate in most areas, some natural enemies not native to the country, for example *Encarsia formosa, Aphidius colemani* and *Phytoseiulus persimilis*, are not effective at extremes of the climate range. For this reason, and in the absence of permission to import new, more suitable biocontrol agents from overseas, it is important to search for and develop Australian natural enemies, which might perform under broader climate extremes. This is more difficult than it might appear. Not every effective predator or parasitoid lends itself to mass production and practical use, in fact, very few do. It is also important to realise that biocontrol agents cannot be tailor-made to suit all growing conditions, particularly those quite unsuited to growing crops. This is the situation with many of the low technology tunnel houses, particularly in the Sydney Basin, which struggle to grow cucumbers and tomatoes in temperature ranges of 0^{o}C to 55^{o}C. Once the crop growing conditions are optimised, the ability to use biocontrol agents and reduce the use of toxic pesticides will expand rapidly from its present doldrums. There is light at the end of the tunnel with recent major high technology developments, mostly for greenhouse tomatoes, but unreasonable demands for preventive application of pesticides for fruit fly threaten to railroad long-awaited adoption of biocontrols in pest management.

Key pests requiring additional biocontrol agents are whiteflies and thrips. For greenhouse whitefly, the predatory bug *Nesidiocoris tenuis* has shown potential in Mediterranean countries (Goodwin & Steiner 2006) and is fairly widespread in Australia. Two parasitoids are also of interest. The exotic *Eretmocerus hayati* was introduced two years ago into QLD by Dr Paul De Barro, CSIRO, after extensive (and expensive) research to find the best candidate for area-wide reduction programs for silverleaf whitefly. So far there is no commercial producer for greenhouse crops. *Eretmocerus warrae* is a native parasitoid species which occurs across Australia on greenhouse whitefly. It is widespread in eastern NSW in a range of extreme climates and not infrequently occurs in greenhouses. This species has been targeted for further research at GHI. Interest is also strong in phytoseiid mite predators of

whitefly eggs. *Amblyseius swirskii* has excited considerable interest in the rest of the world as an excellent whitefly egg predator, but is regrettably not permitted in Australia. One species that appeared promising in Dutch trials, *Euseius ovalis*, does occur here, and was collected by the authors in QLD. *Phytoseiulus persimilis* is also not very effective at high temperatures and low relative humidity, but *Neosiulus wearnei*, sold as *N. californicus* in Europe and North America, and a contaminant in QLD supplies of *P. persimilis*, is tolerant of these conditions and would be a useful addition to the armoury. Thrips management is still suffering from the lack of a good adult predator such as the pirate bug *Orius*. The only potentially effective Australian species, *O. armatus*, has not been collected in the last few years. *Typhlodromips montdorensis*, despite being an excellent thrips larval predator, has not been promoted strongly, because supplies are limited by current production methods. Nevertheless, 15 million have been sold in the last three years to ~ 40 customers.

The key to development of phytoseiid mites as commercially sustainable biocontrol agents is off-plant mass production systems. Koppert Biological Systems, a major insectary based in The Netherlands, developed a new system for *A. swirskii* that also has good potential for other phytoseiids such as *T. montdorensis*. Unfortunately this system is commercial-in-confidence. The dried fruit mite, *Carpoglyphus lactis*, is one of the food hosts named in the Koppert patent application for this system. After some searching, we found this species in dried figs imported from Turkey, and have been able to rear it in large quantities on a bran and dextrose-based medium. This is only a first step in developing a commercial rearing system. Artificial diets are also required for *Nesidiocoris tenuis*, lacewings and ladybugs.

Control of soil pests is now well served by a suite of predators and parasites. The laelapid mite *Stratiolaelaps scimitus*, also known by its old name *Hypoaspis miles*, is very effective against fungus gnats and assists with thrips control. It has become apparent though that there are severe limitations on its ability to survive temperatures below 11^{o}C and high temperatures above 30^{o}C. *Hypoaspis aculeifer* was recently discovered by Dr Irene Vaanenin in QLD and has now become commercially available. It is reportedly more effective against thrips and bulb mites. The beetle *Dalotia coriaria*, discovered at Gosford, is becoming popular in hydroponic systems as a mobile and persistent predator of fungus gnats, shoreflies and thrips. The nematode *Steinernema feltiae* is also popular among growers for rapid control of fungus gnat larvae. An undescribed species of predatory mite, *Hypoaspis* n sp., discovered by the authors in greenhouses in the Gosford area, has potential for fungus gnats, shore flies and thrips in wetter environments. Information is curiously lacking on the temperature tolerance of these species, which is urgently needed to clarify usage recommendations. Work is currently underway on comparative feeding trials for the three mite species and will be followed by trials on temperature tolerances and development rates.

DEVELOPMENT OF BIOCONTROL AGENTS

A soil-inhabiting predatory mite species was collected in January 2006 from Ramm Botanicals, an ornamental production nursery near Tuggerah, NSW, in plant pots where shoreflies had been prevalent. It was subsequently collected in our own greenhouses under the sleeves of rockwool blocks on cocopeat bags where thrips were the main pest. It has been observed feeding on thrips larvae and nematodes, and rears easily. Dr Bruce Halliday identified it as a *Hypoaspis* species, but was unable to find a record of it in Australia. Specimens were subsequently sent to Dr Evert Lindquist in Canada and to Dr Farid Faraji in The Netherlands. It appears to be an undescribed species. Dr Faraji offered to describe it and

we await the outcome. Our interest in it is that it appears to like wet habitats so has potential for shorefly control, along with fungus gnat and thrips. It may also be tolerant of a wider range of temperatures than *Stratiolaelaps scimitus*, while rearing easily in mass production systems.

There is renewed interest in the predatory bug *Nesidiocoris tenuis* after positive results against whitefly were reported by Mediterranean countries at an IOBC Integrated Control in Protected Crops workshop in Spain that we attended in May 2006 (Goodwin & Steiner 2006). Introduction rates are relatively low. Work has commenced on artificial diets at GHI to rear it in sufficient numbers to conduct trials.

The aphid parasitoid *Aphidius colemani*, the thrips predator *Neoseiulus cucumeris*, and the fungus gnat predator *Dalotia coriaria* were all found in Australia and turned over to Biological Services after an initial period of rearing up numbers. These natural enemies have been available from commercial insectaries overseas for several years. *Aphidius colemani* was an earlier introduction into Australia under a more liberal biosecurity climate for control of lucerne aphids, and is widespread, but not previously exploited for greenhouse use. *Dalotia coriaria*, which was developed as a biocontrol agent in Canada recently, and then made available in Europe, was reported from NSW under the name *Atheta australis* in Australia in the 1870's. We discovered it in 2004 in the *T. montdorensis* production system at GHI, feeding on fungus gnats, and so it is presumably widespread. Although *Neoseiulus cucumeris* was suspected as being present in Australia as *Amblyseius bellinus*, and is present in New Zealand, permission to release a strain we brought in from the UK in 1994 was refused by AQIS. It was fortunate that we found live specimens in several greenhouse ornamental crops in Victoria three years ago and were able to cooperate with Biological Services for its release onto the market. It is effective at a cooler temperature range than *T. montdorensis*. Although not as good a predator as *T. montdorensis* against thrips, an off-plant rearing system has made it more readily available and affordable. Some effort has gone into trying to develop an off-plant rearing method for *T. montdorensis*. Results so far have not been encouraging, but a cooperative venture with an Australian insectary and an overseas interest looks promising.

The native brown lacewing, *Micromus tasmaniae*, and the exotic ladybug *Hippodamia variagata*, are under study as part of two HAL-funded PhD projects that are supervised jointly through GHI and Charles Sturt University, Orange.

Evaluation of a locally collected strain of the entomopathogenic fungus *Beauveria bassiana* and its development to a commercial product against thrips and whitefly is reported elsewhere in this document.

REFERENCE

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Table 1. Biocontrol agents and suppliers for greenhouse vegetable pests in Australia, 2006.

Biocontrol agent	Predator or parasitoid	Target pest	Supplier
Phytoseiulus persimilis	Predatory mite	Spider mites	Various
Encarsia formosa	Parasitoid wasp	Greenhouse and silverleaf whiteflies	Biological Services, SA
**Typhlodromips* (*Transeius*) *montdorensis*	Predatory mite	Thrips, broad mite, tomato russet mite	DPI, NSW Beneficial Bug Company, NSW
**Neoseiulus cucumeris*	Predatory mite	Thrips, broad mite	Biological Services, SA
**Stratiolaelaps* (*Hypoaspis*) *scimitus*	Predatory mite	Fungus gnats	Biological Services, SA
**Dalotia* (*Atheta*) *coriaria*	Predatory beetle	Fungus gnats, shoreflies	Biological Services, SA
Steinernema feltiae	Parasitic nematode	Fungus gnats	Ecogrow, NSW
Trichogramma pretiosum, T. carverae	Parasitoid wasp	Caterpillars	Bugs for Bugs, QLD
**Aphidius colemani*	Parasitoid wasp	Aphids	Biological Services, SA
Mallada signata	Predatory lacewing	Aphids	Bugs for Bugs, QLD
Micromus tasmaniae	Predatory lacewing	Aphids	IPM Technologies, VIC
Cryptolaemus montrouzieri	Predatory beetle	Mealybugs	Bugs for Bugs, QLD
Euseius victoriensis	Predatory mite	Broad mite	Biological Services, SA
Hippodamia variegata	Predatory ladybeetle	Aphids, caterpillars	IPM Technologies, VIC

*Provided through GHI

Table 2. Natural enemies under development or with potential as biocontrol agents in Australia.

Natural enemy	Biocontrol action	Target pest	Developing agency
Hypoaspis n sp	Predatory mite	Fungus gnats, shoreflies	DPI, Gosford, NSW
Eretmocerus warrae	Parasitoid wasp	Greenhouse whitefly	DPI, Gosford, NSW
Eretmocerus hayati	Parasitoid wasp	Silverleaf whitefly	CSIRO, Indooroopilly, QLD
Beauveria bassiana	Fungal pathogen	Thrips, whiteflies, aphids	DPI, Gosford, NSW
Nesidiocoris tenuis	Predatory bug	Whiteflies	DPI, Gosford, NSW
Neoseiulus californicus (*wearnei*)	Predatory mite	Spider mites	DPI, Gosford, NSW
Orius armatus	Predatory bug	Thrips	DPI, Gosford, NSW
Euseius sp. *Neoseiulus* sp.	Predatory mites	Whiteflies	DPI, Gosford, NSW

Eretmocerus warrae, native parasitoid for greenhouse whitefly

Neoseiulus wearnei, indigenous predatory mite for spider mite

Nesidiocoris tenuis, indigenous predatory mirid bug for whiteflies

Orius armatus, native predator bug for thrips

Dalotia coriaria, indigenous beetle for soil pests

Hypoaspis n. sp., native predatory mite for soil pests

OVERSEAS TRAVEL

During the course of this project, two overseas trips were undertaken to attend greenhouse IPM related conferences and to meet with scientific colleagues to discuss research interests, to review research developments and to learn of new opportunities and methodology that might benefit the Gosford DPI greenhouse IPM research program. In addition, we both chaired Sessions at the workshop in Finland, at the request of the organizer, and made presentations.

They were:

1. Meeting of the International Organisation for Biological Control/Western Palaearctic Regional Section (IOBC/WPRS), Temperate Climate Working Group on Greenhouse IPM, held in Turku, Finland, April, 2005.

2. Meeting of the International Organisation for Biological Control/Western Palaearctic Regional Section (IOBC/WPRS), Mediterranean Climate Working Group on Greenhouse IPM, held in Murcia, Spain, May 2006.

Detailed reports on both overseas trips are submitted with this final report. Both trips brought substantial benefits to the NSW DPI IPM research program, both now and in the future, and have assisted in the identification of some new research IPM goals. Continued financial support from the vegetable industry will see these benefits flow on to the Australian greenhouse vegetable industry in the future.

TECHNOLOGY TRANSFER

The research findings of this project were communicated at industry conferences between 2003 and 2006: the Australian Hydroponic and Greenhouse Association national conferences in 2003 and 2005 and the Hydroponic Farmers Federation Conferences in 2004 and 2006. Presentations were made on each of the programs.

An important new initiative associated with these conferences was the sponsorship of a trade booth by the project. The booth made available the latest pest and disease research outputs, copies of the greenhouse vegetables IPM manual and field guide, posters alerting growers to new key disease issues such as tomato leaf curl virus, new pest and disease posters for cucumber, lettuce and tomato, the release of a new IPM educational tool, and the Pest Sense card game, to which we contributed. It also provided a site where growers could come up to discuss their problems with technical specialists from NSW DPI. We also had samples of biocontrol agents and a microscope available so that growers could familiarise themselves with what they looked like. In this regard we collaborated with Biological Services, Bugs for Bugs and Beneficial Bug Company at the booth to promote biocontrol.

Additionally, this project linked with VG03098 “Regional extension strategy for managing western flower thrips and tomato spotted wilt virus in the Sydney region” and was able to utilise extension activities organised on-farm and elsewhere, to pass on research findings from this project to Sydney Basin and North Coast greenhouse vegetable producers. This included talks on TSWV management and biocontrol options, and the use of screening to keep out pests.

Elsewhere, IPM workshops were conducted in Devonport and Hobart in Tasmania and Coffs Harbour in North Coast NSW for greenhouse vegetable growers in part to pass on current research findings and also to educate growers in IPM principles, pest diagnostics and commercial approaches to IPM adoption.

Research results from this project were presented at the 2006 Australian Entomological Society annual conference held in Adelaide and at the 2005 International Cucurbit Conference held in Townsville. We also attended the annual Australian Herb Industry Workshop at Hahndorf, VIC in September, and assisted in arranging the visit there and to GHI of Jude Bennison, ADAS, UK to cooperate in the production of an Australian IPM Guide for herb growers.

We also intend to publish articles in Practical Hydroponics & Greenhouses and Good, Fruit & Vegetables on research findings of the project. Already the first article for PH&G on biocontrol developments and new targets has been submitted.

PRESENTATIONS, PAPERS, ARTICLES AND WORKSHOPS

2003

Goodwin, S. 2003. Practical IPM: what you need to know to get started. Australian Hydroponic & Greenhouse Conference Proceedings, Melbourne, VIC 2003: 83 – 86. Paper and Powerpoint presentation.

Goodwin, S. & M.Y.Steiner. 2003. Greenhouse cucumber IPM. Workshop for WA greenhouse vegetable growers, Perth. Powerpoint presentation.

Steiner, M.Y. 2003. The IPM arsenal: what's hot, what's not. Australian Hydroponic & Greenhouse Conference Proceedings, Melbourne, VIC 2003: 97 – 98. Paper and Powerpoint presentation.

2004

Goodwin, S. & M.Y. Steiner. 2004. Outcomes of IPM research projects. Hydroponic Farmers Federation Conference Proceedings, Bendigo, VIC July 2004: 9pp. Paper and Powerpoint presentation.

Bennison, J. & M.Y.Steiner. 2004. Biological control of thrips in greenhouses: the European and Australian experience. International Congress of Entomology, Brisbane, QLD. August 2004. Powerpoint presentation.

Steiner, M.Y. 2004. Managing tomato russet mite, *Aculops lycopersici* (Massee), in greenhouse tomato crops. International Congress of Entomology, Brisbane, QLD. August 2004. Powerpoint presentation.

Steiner, M.Y., S. Goodwin & T. Wellham. 2004. *Typhlodromips montdorensis* (Schicha) (Acari: Phytoseiidae) - an effective predator for use against thrips in greenhouse vegetable crops. International Congress of Entomology, Brisbane, QLD. August 2004. Powerpoint presentation.

2005

Goodwin, S. 2005. Report on an overseas trip to Finland. April 2005. Workshop of the IOBC/WPRS Integrated Pest Management in Greenhouse Crops-temperate climate.

Goodwin, S. & M.Y. Steiner. 2005. Integrated pest management for the Australian Herb Industry. Annual Workshop, Australian Spice and Herb Industry Association. Brisbane, QLD. Powerpoint presentation.

Steiner, M. Y. and S. Goodwin. 2005. Managing tomato russet mite, *Aculops lycopersici* (Massee) (Acari: Eriophyiidae) in greenhouse tomato crops. Bull. IOBC/WPRS Vol. 28 (1), 245-248.

Steiner, M. Y. and S. Goodwin. 2005. Compatibility of two formulations of bifenazate with *Phytoseiulus persimilis* Athias-Henriot (Acari: Phytoseiidae). Bull. IOBC/WPRS Vol. 28 (1), 249-252.

Goodwin, S., M. Steiner and W. Liang. 2005. Development of new fungal biopesticides for the Australian greenhouse industry. Bull. IOBC/WPRS Vol. 28 (1), 107-110.

Goodwin, S. & M.Y. Steiner. 2005. Least toxic alternatives – developments in fungal biopesticides and reduced-risk chemicals. Australian Hydroponic & Greenhouse Conference Proceedings, Bundaberg, QLD 2005: 75 – 80. Paper and Powerpoint presentation.

Steiner, M.Y. & S. Goodwin. 2005. Challenges for the implementation of IPM in cucumber crops. International Cucurbit Conference, Townsville, QLD July 2005. Paper and Powerpoint presentation.

2006

Goodwin, S. & M.Y. Steiner. 2006. IPM update. Hydroponic Farmers Federation Conference, Geelong, VIC, July 2006: Powerpoint presentation.

Goodwin, S. & Steiner. 2006. Report on an overseas trip to Spain, the UK and Israel 3-29 May 2006.

Steiner, M.Y. & S. Goodwin. 2006. Side-effects of pesticide residues on biocontrol agents. Flowers Australia Conference, Gold Coast, QLD August 2006. Powerpoint presentation.

Steiner, M.Y. and S. Goodwin. 2006. Strategies to improve uptake of IPM in Australian greenhouses. Entomological Society of Australia annual conference, September, 2006. Adelaide, SA. Powerpoint presentation.

2007

Goodwin, S. & M.Y. Steiner. 2007. A new star is born: *Amblyseius swirskii*. Practical Hydroponics & Greenhouses Issue 93: 23 – 28.

RECOMMENDATIONS

There is a need for ongoing research into advancements in sustainable IPM programs for greenhouse vegetable crops in Australia. This is demonstrated by a comparison of the current limited numbers of commercially-produced biocontrol agents in Australia against the comprehensive biologically-based IPM systems available in Europe, the Mediterranean and North America. Also by the pressure on growers caused by failures with current biocontrol agents due to climatic extremes where climate-specific biocontrol agents were unavailable. We have made progress, but this is slow, due to the limited governmental resources available for this work and the demands of other areas of IPM research on time.

The two recent overseas trips conducted during this project period were very productive in identifying some new biocontrol research opportunities. These, plus some others previously known to us, are reflected in a new project involving Dr Leigh Pilkington, who has taken over the greenhouse IPM research responsibility in NSW DPI at Gosford Horticultural Institute. They can be found in project proposal VG05093.

In addition, aside from the urgent need for these new biocontrol developments, there is also a strong need for new, effective reduced-risk pesticides. To bring both of these fields together, information for growers on the side-effects of these and other new pesticides

coming onto the market on biocontrol agents is urgently required, to achieve confidence about the integration of both elements in biologically-based IPM strategies.

VG03109 has made progress in the development of side effects data by taking a new approach to this work that has real practical value to growers. Project VG05093 identifies new biocontrol agents, new reduced-risk chemicals and new information on side effects in its objectives.

There is a vast amount of R&D required in greenhouse IPM still to be undertaken that will require continuing funding. Note that overseas there are a large number of researchers and research organizations in many different countries that have contributed to the current situation over the past 30 years and continue to contribute to its development. In Australia, NSW DPI provides the only dedicated R&D opportunities into this field through Dr Pilkington, and in pathology, Mr Len Tesoriero. The authors will continue their involvement in this field for the greenhouse industry through the provision of IPM consultant services in retirement.

ACKNOWLEDGEMENTS

We would like to thank AusVeg and Horticulture Australia Limited for providing funding for this project. This final report marks not only the conclusion of a project, but also the end of our research careers in greenhouse IPM. To that end we would like to offer a special thanks to the vegetable producers who contribute to the national vegetable levy for approving the disbursement of their funds over an extended period of time into our research activities in this field. We hope we have contributed to the advancement in the development and adoption of IPM in this time.

Thanks are also due to industry cooperators Joe D'Anastasi, hydroponic lettuce producer, for making his farm available for trials and to John Vella, Leppington Speedy Seedlings, for providing vegetable seedlings for research trials.

We especially thank NSW DPI staff for all their hard work and support. They include Tony Wellham, technical officer (scientific) for 12 years of great support to this program, Fah Eagleton, technical assistant, for 10 years of doing all the jobs no-one else would do and in providing the underpinning support in the propagation of seedlings for trials, maintaining biocontrol and pest cultures for research programs, for taking over the commercial production of the Montdorensis culture and, in the face of this mountain of work, for maintaining her enduring cheerfulness every working day. More recently to Dr Debbie Kent and Dr Leigh Pilkington, for their involvement in the research program and finally, to our long suffering biometrical staff, Lorraine Spohr, biometrician and Anne Harris, biometrical technical officer, for their analysis of all the data.

APPENDIX I

Summary charts of results of testing the effects of pesticide residues at GHI 2003-2006.

A. Pesticide residues on foliage: Impact on adult *P. persimilis* and *T. montdorensis*
B. Pesticide residues on foliage: Impact on egg laying of *P. persimilis* and *T. montdorensis*
C. Pesticide residues on foliage: Impact on adults of *P. persimilis, T. montdorensis, E. formosa* and *A. colemani*.
D. Pesticide residues in media: impact on *Stratiolaelaps scimitus* (Hypoaspis)
E. Pesticide residues on greenhouse plastics: Impact on *Encarsia formosa*
F. Pesticide residues on greenhouse plastics: Impact on *Phytoseiulus persimilis*
G. Pesticide residues on greenhouse plastics: Impact on *Stratiolaelaps scimitus*

A

PESTICIDE RESIDUES ON FOLIAGE

IMPACT ON *P. PERSIMILIS* AND *T. MONTDORENSIS*

harmless 0-25% reduction	slightly harmful 25-50% reduction	moderately harmful 50-75% reduction	very harmful 75-100% reduction

First, second and third columns report impact on adult survival and number of progeny 0, 7 and 14 days post-application respectively. Data were collected on three crop types and averaged. Impact on egg laying is generally more severe than on adult mortality as adults may survive but be very sick.

	Phytoseiulus persimilis						*Typhlodromips montdorensis*					
	Adults			Progeny			Adults			Progeny		
	0	7	14	0	7	14	0	7	14	0	7	14
Talstar	🔴	🔴	🔴	🔴	🔴	🔴	🔴	🟠	🟡	🔴	🔴	🔴
Nitofol	🔴	🟡	🟢	🔴	🟡	🟡	🔴	🟢	🟢	🔴	🟠	🟢
Lannate	🔴	🟢	🟢	🔴	🟢	🟢	🔴	🟢	🟢	🔴	🟡	🟢
Confidor	🟡	🟡	🟢	🟠	🔴	🟢	🟢	🟢	🟢	🟠	🟡	🟢
Success	🟢	🟢	🟢	🟡	🟡	🟢	🟡	🟢	🟢	🟡	🟢	🟢
Vertimec	🟠	🟢	🟢	🔴	🔴	🟠	🟢	🟢	🟢	🔴	🟢	🟢
Pirimor	🟢	🟢	🟢	🟡	🟢	🟢	🟢	🟢	🟢	🟢	🟡	🟢
Dithane	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🟡	🟢	🟢

B

PESTICIDE RESIDUES ON FOLIAGE

IMPACT ON EGG LAYING OF *P. PERSIMILIS* AND *T. MONTDORENSIS*

harmless 0-25% reduction	slightly harmful 25-50% reduction	moderately harmful 50-75% reduction	very harmful 75-100% reduction

First, second and third columns report impact on egg laying 0, 7 and 14 days post-application respectively. Data were collected on three crops and averaged.

	Phytoseiulus persimilis			*Typhlodromips montdorensis*		
	0	7	14	0	7	14
Talstar	🔴	🔴	🔴	🔴	🔴	🔴
Nitofol	🔴	🟡	🟡	🔴	🟠	🟢
Lannate	🔴	🟢	🟢	🔴	🟡	🟢
Confidor	🟠	🔴	🟢	🟠	🟡	🟢
Success	🟡	🟡	🟢	🟡	🟢	🟢
Vertimec	🔴	🔴	🟠	🔴	🟢	🟢
Pirimor	🟡	🟢	🟢	🟢	🟡	🟢
Dithane	🟢	🟢	🟢	🟡	🟢	🟢

C

PESTICIDE RESIDUES ON FOLIAGE

IMPACT ON ADULT *P. PERSIMILIS*, *T. MONTDORENSIS*, *E. FORMOSA*, AND *A. COLEMANI*

harmless 0-25% reduction	slightly harmful 25-50% reduction	moderately harmful 50-75% reduction	very harmful 75-100% reduction

First, second and third columns report impact on survival of adults 0, 7 and 14 days post-application respectively. Data were collected on three crop types and averaged. Results should be checked against impact on egg laying as adults may survive but be very sick.

	Encarsia formosa			*Aphidius colemani*			*Phytoseiulus persimilis*			*Typhlodromips montdorensis*		
	0	7	14	0	7	14	0	7	14	0	7	14
Talstar	🔴	🔴	🔴	🔴	🔴	🟢	🔴	🔴	🔴	🔴	🟠	🟡
Nitofol	🔴	🔴	🔴	🔴	🟢	🟢	🔴	🟡	🟢	🔴	🟡	🟢
Lannate	🔴	🟠	🟢	🟢	🟢	🟢	🔴	🟢	🟢	🔴	🟢	🟢
Confidor	🔴	🔴	🟠	🟢	🟢	🟢	🟡	🟡	🟢	🟢	🟢	🟢
Success	🔴	🔴	🔴	🔴	🔴	🔴	🟢	🟢	🟢	🟡	🟢	🟢
Vertimec	🔴	🔴	🟡	🔴	🔴	🔴	🟠	🟢	🟢	🟢	🟢	🟢
Pirimor	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🟢
Dithane	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🟢

D

PESTICIDE RESIDUES IN MEDIA-IMPACT ON *STRATIOLAELAPS SCIMITUS* (HYPOASPIS)

harmless (0-25% reduction)	slightly harmful (25-50% reduction)	moderately harmful (50-75% reduction)	very harmful (75-100% reduction)

First, second and third columns report impact 0, 7 and 14 days post-application respectively. Designation of harmfulness is impact on adult survival and progeny produced over 7 days. Talstar was applied as a surface application, and Pirimor and Confidor as drenches.

	ADULT MITES									PROGENY								
	TALSTAR			CONFIDOR			PIRIMOR			TALSTAR			CONFIDOR			PIRIMOR		
	0	7	14	0	7	14	0	7	14	0	7	14	0	7	14	0	7	14
Compost	🟠	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🔴	🔴	🔴	🟡	🟢	🟢	🟡	🟢	🟢
Cocopeat	🔴	🔴	🔴	🟢	🟢	🟢	🟢	🟢	🟢	🔴	🔴	🔴	🟢	🟢	🟢	🟡	🟢	🟢
Sawdust	🔴	🟠	🟢	🟡	🟢	🟢	🟢	🟢	🟢	🔴	🔴	🔴	🟢	🟢	🟢	🟢	🟢	🟢
Vermiculite	🔴	🔴	🔴	🟡	🟢	🟢	🟠	🔴	🔴	🔴	🔴	🔴	🟡	🟢	🟢	🔴	🔴	🔴
Rockwool	🔴	🔴	🟠	🟢	🟡	🟢	🔴	🔴	🔴	🔴	🔴	🔴	🟢	🟠	🟡	🔴	🔴	🔴
Perlite	🔴	🔴	🔴	🟡	🟡	🟡	🔴	🔴	🔴	🔴	🔴	🔴	🟢	🟠	🔴	🔴	🔴	🔴

E

PESTICIDE RESIDUES ON GREENHOUSE PLASTICS-IMPACT ON *ENCARSIA FORMOSA*

harmless (0-25% reduction)	slightly harmful (25-50% reduction)	moderately harmful (50-75% reduction)	very harmful (75-100% reduction)

First, second and third columns report impact 0, 7 and 14 days post-application respectively. Designation of harmfulness is impact on adult survival. Panda film and weed matting were held vertically while spraying, and clear film horizontally.

	ADULTS									PROGENY (not assessed)								
	Clear film			Panda film			Ground cover			Clear film			Panda film			Ground cover		
	0	7	14	0	7	14	0	7	14	0	7	14	0	7	14	0	7	14
Talstar	🟢	🟢	🟢	🟠	🟢	🟡	🔴	🔴	🔴	–			–			–		
Confidor	🔴	🟢	🟡	🔴	🟠	🟡	🔴	🔴	🟠	–			–			–		
Vertimec	🟠	🟢	🟢	🔴	🟠	🟠	🔴	🟡	🟢	–			–			–		
Success	🔴	🔴	🟠	🔴	🔴	🔴	🔴	🔴	🔴	–			–			–		
Nitofol	🔴	🟢	🟢	🔴	🔴	🟠	🔴	🔴	🔴	–			–			–		

F

PESTICIDE RESIDUES ON GREENHOUSE PLASTICS-IMPACT ON *PHYTOSEIULUS PERSIMILIS*

harmless (0-25% reduction)	slightly harmful (25-50% reduction)	moderately harmful (50-75% reduction)	very harmful (75-100% reduction)

First, second and third columns report impact 0, 7 and 14 days post-application respectively. Designation of harmfulness is impact on adult survival and progeny produced over 3 days. Panda film and weed matting were held vertically while spraying, and clear film horizontally.

	ADULTS									PROGENY								
	0	7	14	0	7	14	0	7	14	0	7	14	0	7	14	0	7	14
	Clear film			Panda film			Ground cover			Clear film			Panda film			Ground cover		
Talstar	🔴	🟢	🟡	🟢	🟢	🟢	🔴	🔴	🔴	🔴	🟡	🟡	🟡	🟡	🟢	🔴	🔴	🔴
Confidor	🟢	🟢	🟢	🟡	🟢	🟢	🟠	🔴	🔴	🟢	🟢	🟢	🔴	🟢	🟡	🔴	🔴	🔴
Vertimec	🟢	🟢	🟢	🟠	🔴	🟢	🔴	🟠	🟡	🟡	🟡	🟢	🔴	🔴	🟡	🔴	🔴	🔴

PESTICIDE RESIDUES ON GREENHOUSE PLASTICS-IMPACT ON *STRATIOLAELAPS SCIMITUS*

harmless (0-25% reduction)	slightly harmful (25-50% reduction)	moderately harmful (50-75% reduction)	very harmful (75-100% reduction)

First, second and third columns report impact 0, 7 and 14 days post-application respectively. Designation of harmfulness is impact on adult survival and progeny produced over 3 days. Panda film and weed matting were held vertically while spraying, and clear film horizontally.

	ADULTS			PROGENY (not assessed)		
	Clear film	Panda film	Ground cover	Clear film	Panda film	Ground cover
Talstar	🟢 🟢 🟢	🟢 🟢 🟢	🔴 🔴 🔴	–	–	–
Confidor	–	–	🟢 🟢 🟢	–	–	–
Pirimor	🟢 🟢 🟢	🟢 🟢 🟢	🟡 🟢 🟢	–	–	–

APPENDIX 11

TABLE OF SIDE-EFFECTS OF PESTICIDES ON NATURAL ENEMIES

Pesticides targeting pests often impact negatively on natural enemies. The following table lists pesticides generally available to Australian growers* of cucumber, lettuce, capsicum and tomatoes, and their effects on some common biocontrol agents. It was sourced primarily from on-line information provided by the commercial insectaries Biobest and Koppert on their websites. Additional information has been added from our own testing (Goodwin & Steiner 2004, 2007), particularly for *Transeius* (*Typhlodromips*) *montdorensis*. Caution is needed in interpreting results. Only direct toxicity of products to natural enemies is described. Sublethal effects such as reduced fertility, altered searching behaviour, decreased parasitisation capacity or lifespan may not be included. Toxicity may vary greatly depending on pesticide formulation, species of natural enemy (even local strains of the same natural enemy), life-cycle stage contacted, climatic conditions, whether applied as full foliar sprays, fumigants or ground treatments, and many other factors. Where rating varies between sources a conservative toxicity rating has been selected. The chart should be used as a guide only. Note that addition of some spreaders and stickers may greatly increase toxicity of a 'safe' product. Even water may disrupt natural enemies if used at high volume. Readers will note that there are many gaps in the information (denoted by an empty circle), particularly with respect to residual activity and new pesticide actives. Old chemistry products are often broad spectrum and residual. New chemistry products are often touted as 'safe', but this is often from the human toxicity standpoint. There are few truly selective pesticides. Targeted treatments of non-residual, less-selective pesticides may be an acceptable option.

Koppert lists as its sources of information 'results of the IOBC Working Group 'Pesticides and Beneficial Organisms' and various research institutes. More than one hundred scientific publications were included in the comparative literature study. Much of the data was also derived from our own research and the experiences of Koppert B.V. employees. The data about the residual effects of the pesticides have been collected as much as possible from practical experience.'

Biobest states that 'the new side effects manual is the most comprehensive and accurate overview of the direct side effects of crop protection products on bumblebees and natural enemies used by the horticultural industry. Results were verified in trials under field conditions. Cooperation with several domestic and foreign research institutes, universities and the phyto-pharmaceutical industry as well as sources such as the 'Joint Testing Programs' of the IOBC workgroup 'Side Effects of Pesticides on Beneficial Organisms' was essential for the completion of this manual. The information presented is mainly based on species and strains of beneficial organisms as they are delivered by BIOBEST N.V. and its distributors all over the world. Natural enemies from other origins, even if they are the same species, may react differently to active ingredients mentioned in the manual'.

* Check current registrations and permits. Use must be approved by the APVMA and be specified on the label or permit.

INFORMATION SOURCED

Goodwin. S. & M. Y. Steiner. 2007. Extension to Greenhouse IPM Program. Final report to HAL/AUSVEG. Project # VG03109.

Goodwin. S. & M. Y. Steiner. 2004. Improvements to Biological Control Systems and development of Biorational Chemicals for Integrated Pest Management in Greenhouse Vegetables. Final report to HAL/AUSVEG. Project # VG00066.

On-line manual of side-effects of pesticides on natural enemies: http://www.biobest.bl

On-line database of side-effects of pesticides on natural enemies: http://www.koppert.nl

ACTIVE INGREDIENT	TRADE NAMES	PESTICIDE TYPE	TOXICITY TO GREENHOUSE BIOCONTROL AGENTS C = contact effect R = residual activity in days (D) or weeks (W)											
			Aphidius colemani		*Encarsia formosa*		*Neoseiulus cucumeris*		*Phytoseiulus persimilis*		*Steinernema feltiae*		*Stratioalaelaps scimitus*		*Transeius montdorensis*	
			harmless 0-25% reduction				slightly harmful 25-50% reduction				moderately harmful 50-75% reduction		very harmful 75-100% reduction			
			C	R	C	R	C	R	C	R	C	R	C	R	C	R
diazinon	Diazinon	organic phosphate			4-6W		3W		1W							
dichlorvos	DDVP, Dichlorvos	organic phosphate			1W		3D		1W							
dicofol	Kelthane	organic chlorine			>1W		4W		2W							
dimethoate	Cygon, Rogor	organic phosphate			>8W		>8W		8W							
emamectin benzoate	Proclaim*	macrocyclic lactone glycoside			>1W											
esfenvalerate	Sumi-alpha flex	synthetic pyrethroid		>8W		>8W		>8W		>8W				4W		
fenbutatin oxide	Torque	organic-tin							3D							
fluvalinate	Mavrik	synthetic pyrethroid														
gamma-cyhalothrin	Trojan	synthetic pyrethroid														
imidacloprid	Confidor	neonicotinoid		<1W		>2W		2W		1W				<1W		1W
lambda-cyhalothrin	Karate	synthetic pyrethroid		>8W		>8W		>8W		>8W				>8W		
maldison	Maldison, Malathion	organic phosphate		>8W		>8W		>8W		>1W						
methoxyfenozide	Prodigy	diacylhydrazine														

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			C	R	C	R	C	R	C	R	C	R	C	R	C	R
nuclear polyhedrosis virus	Vivus Gold, Gemstar	biological insecticide														
permethrin	Ambush, Pounce	synthetic pyrethroid		>8W		>8W		>8W		>8W				>8W		
petroleum oil	Biocover, Sunspray	petroleum oil														
pirimicarb	Pirimor	carbamate				3D		3D		3D						
potassium salts of fatty acids	Natrasoap, Neemtech	soap				0D		0D		0D						
propargite	Omite	organic complex				1W				3D		1W				
pymetrozine	Chess	azomethine		3D												
pyrethrins + pbo	Pyganic	synthetic pyrethroid				2W		1W		1W				1W		
spinosad	Success, Entrust	actinomycete fermentation product		>2W		>2W		2W		1W						<1W
sulphur	Liquisulf, Sulphur	element				1-4W				1W						
tau-fluvalinate	Mavrik	synthetic pyrethroid								6W						
thiodicarb	Larvin	oxime carbamate														
trichlorfon	Dipterex	organic phosphate				1W		2W		2W				0		

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			C	R	C	R	C	R	C	R	C	R	C	R	C	R
abamectin	Vertimec, Avermectin	macrocyclic lactone glycoside		>1W		3W		2W		>3W						<1W
acephate	Orthene, Lancer	organic phosphate				>8W		>8W		>3W		3D				
alpha-cypermethrin	Dominex	synthetic pyrethroid		>8W		>8W		>8W		>8W				>8W		
amorphous silica	Abrade*	diatomaceous earth														
B. thuringiensis israeliensis	Vectobac	biological insecticide														
B. thuringiensis var. *kurstaki*	Delfin	biological insecticide														
beta-cyfluthrin	Bulldock	synthetic pyrethroid														
bifenthrin	Talstar, Bifenthrin	synthetic pyrethroid		>8W		>8W		>8W		>8W				>8W		>2W
botanical oil	Eco-Oil	botanical oil														
buprofezin	Applaud	thiadiazine				4D										
chlorpyrifos	Lorsban, Chlorpyriphos	organic phosphate				>8W		6-8W		4D		2W				
cypermethrin	Cypermethrin	synthetic pyrethroid		>8W		>8W		>8W		>8W				>8W		
deltamethrin	Decis	synthetic pyrethroid		>8W		>8W		>8W		>8W				>8W		