Developing Strawberry IPM: testing OP-resistant predatory mites

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Statement about the purpose of the report:
This report details the research and extension delivery undertaken in Project BS09000 to develop an organophosphate-tolerant strain of predatory mite for commercial rearing to assist with the biological control of western flower thrips in Australian strawberry crops.

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2. MEDIA SUMMARY

The aim of this project was to develop a key component of an integrated pest management (IPM) system for strawberry production, namely the breeding of an organophosphate (OP)-tolerant predatory mite (*Gaeolaelaps aculeifer*) for inundative release to biologically control western flower thrips (*Frankliniella occidentalis*).

At the time this project was initiated the control of western flower thrips in commercial Australian strawberry crops was largely reliant upon insecticides, which was neither cost-effective nor sustainable because of the development of insecticide resistance. Some strawberry growers were using commercially-reared biocontrol agents such as *G. aculeifer* to manage western flower thrips infestation levels, and achieving considerable success. However this agent is vulnerable to broad-spectrum insecticides used for the control of other pests, most notably the OPs used for Rutherglen bug control.

In this project we have exposed a strain of commercially-reared *G. aculeifer* to repeated and progressively increasing doses of dimethoate and thereby created an OP-tolerant strain. This strain provides the Australian strawberry industry with a new means of limiting the disruption to the strawberry IPM system for western flower thrips during a Rutherglen bug invasion.

The OP-tolerant *G. aculeifer* strain developed in this project has been supplied to Biological Services (Loxton), the main Australian commercial producer of this agent. Interested growers can purchase this selected strain of *G. aculeifer* and obtain instructions for its field release and maintenance from Biological Services (Loxton) (see http://www.biologicalservices.com.au/ for contact details).

Further investment by Australia's horticultural industries, through Horticulture Australia Limited, in the development of more biological control agents to help manage the range of pests that attack horticultural crops across a broad range of crop environments would have genuine potential to reduce current dependence on pesticides.

3. TECHNICAL SUMMARY

3.1 The Problem

At the time this project was initiated the control of western flower thrips (WFT) in commercial Australian strawberry crops was largely reliant upon insecticides, which was neither cost-effective nor sustainable because of the development of insecticide resistance. Insecticide resistance evolves in a pest population through selection of resistance genes as a result of repeated exposure to an insecticide.

Some innovative strawberry growers were using commercially-reared biocontrol agents such as the predatory mite *Gaeolaelaps aculeifer* (Canestrini) (Mesostigmata: Laelapidae) to manage WFT infestation levels, and achieving considerable success.

However this predatory mite agent is vulnerable to broad-spectrum insecticides used for the control of other pests, most notably the organophosphates (OPs) used for Rutherglen bug control.

Hence in strawberry crops where an inundative release of biological control agents was being practiced the system commonly worked effectively early in the season, but was disrupted in summer if Rutherglen bug invaded and required insecticidal control.

The aim of this project was to develop a commercial strain of *G. aculeifer* with a level of OP-tolerance that would allow it to survive the periodic field application of dimethoate or related insecticides for the control of, transient pests such as Rutherglen bug, and hence to add a greater resilience to the biological control management system in strawberries.

3.2 The Project Science

In this project we selected a strain of commercially-reared *G. aculeifer* for OP-tolerance by exposing it fortnightly over a two-year period to a dose of dimethoate. The selecting rate that was used approximated to the LC_{50} (i.e. the dose that kills 50% of the test animals) and was periodically increased as the tolerance of the selected strain increased.

The tolerance level of the selected strain was quantified by conducting full dose-response bioassays using a Potter Tower to administer the doses, and analysing the mortality response by Probit analysis.

3.3 The Key Research Findings, Extension Highlights and Industry Outcomes

A moderate level of OP and carbamate resistance has been selected.

The selected resistance appears to be reasonably stable if moderate selection pressure is applied at intervals of 4-6 weeks.

The selected strain, along with guidelines for selection maintenance of the insecticidal tolerance, has been given to Biological Services (Loxton), the commercial producer of *G. aculeifer* in Australia. Interested growers can purchase this selected strain of *G. aculeifer* and obtain instructions for its field release and maintenance from Biological Services (Loxton) (see http://www.biologicalservices.com.au/ for contact details).

This project has provided the Australian strawberry industry with a more effective biological control agent for integration with insecticide controls, which should help limit the disruption to the western flower thrips IPM system during a Rutherglen bug invasion.

3.4 Recommendations

That Australia's horticultural industries, through Horticulture Australia Limited, invest in the development of more biological control agents to help manage the range of pests in the range of crop environments (eg. different host plants, temperature, humidity and insecticidal tolerances, etc) where biological control has genuine potential to reduce current over reliance on pesticides.

4. INTRODUCTION

Because of the development of high-level resistance to most pesticides available for western flower thrips (*Frankliniella occidentalis* (Pergande)) (WFT) and two spotted mite (Tetranychus urticae Koch) (TSM), the Australian strawberry industry in recent years has faced imminent failure of their insecticide management programs for the control of these pests. The slow entry rate of new chemistry precludes the likelihood of rehabilitating an insecticide-oriented control strategy. In turn, pesticide residues are an important risk factor for the industry. Encouragingly there are off-the-shelf commercially-available biocontrol agents for all the key strawberry pests except for Rutherglen bug (J. Altmann and L. Chilman, pers. comm.). The organophosphate (OP) and carbamate insecticides available for Rutherglen bug control are broad-spectrum and presently disruptive to the biocontrol of WFT and TSM.

Hence in this project, which was conceived in 2008, we proposed to develop a key component of a cost-effective IPM system for the strawberry industry, namely a commercial strain of the WFT predatory mite, *Gaeolaelaps aculeifer* (Canestrini) (Mesostigmata: Laelapidae), that had tolerance to field rates of the main OP's then used for Rutherglen bug control. The most commonly used insecticide for Rutherglen bug control at the time was dimethoate.

There is a well-established record of attempts to release and establish insecticide-tolerant strains of key natural enemies (Van Driesche and Bellows 1999), and of mites in particular (Hoy 1987; Roush and Hoy 1981). For many natural enemies, genetic variability exists that permits the development of insecticide-resistant populations under field or laboratory selection.

Preliminary SARDI screening of the *G. aculeifer* strain reared by Biological Services, Loxton indicated that this strain possessed low-level dimethoate tolerance. This provided encouragement that a deliberate selection program to breed an OP-tolerant *G. aculeifer,* as has been done in the pome-fruit industry with the main predatory mite of two spotted mite, was potentially feasible and could thereby provide the strawberry industry with a novel strategy for WFT management and a means of avoiding disruption to the IPM system during a Rutherglen bug invasion.

Whereas the intent of many of the programs to develop an insecticide-tolerant strain of a biological control agent is to permanently establish the tolerant form of the natural enemy in the field, the intent in this study was to develop the tolerant strain for annual inundative release into strawberry crops, with the strain's tolerance maintained by periodic selection in culture.

5. METHODS AND MATERIALS

***Gaeolaelaps aculeifer* cultures**

The *G. aculeifer* culture was sourced from Biological Services, Loxton, and a method to successfully rear *G. aculeifer* under small-scale laboratory conditions was developed.

Fig. 1. The insecticide treatment pots used for the selection study.

Full Dose-Response Bioassays

A full dose-response bioassay was conducted at the outset of the study to establish the 'base-line' susceptibility of the source population of *G. aculeifer* to dimethoate (Amgrow Chemspray Rogor® 100 Systemic Insecticide product containing 100 g ai L^{-1}), and after seven, 12, 31, 43 and 46 selections with dimethoate to determine the progressive change in the tolerance of the selected Strain 1.

These bioassays were conducted by transferring with a sable-hair brush approximately 20 adult *G. aculeifer* onto 90mm diameter cabbage leaf discs embedded in agar in 90mm diameter petri-dishes. Each petri dish was then placed in a Potter tower to administer a precise deposit (3.60±0.163mg cm^{-2}) of the test insecticide or control (RO water) using a 4 ml aliquot of the test solution. A series of six dimethoate concentrations were applied (4 replicates per dose). The Potter spray tower was triple rinsed with AR Acetone and RO water between each change in treatment. Once removed from the Potter spray tower the dishes were covered with plastic film that was secured with a rubber band. Fine holes were then punched into the plastic film using a micro needle to allow air exchange. The treated petri dishes were then held in an incubator at 20^0C until mortality assessment 48 hours post-treatment. The results were analysed by probit analysis (POLO-PLUS™, LeOra Software).

The Insecticide Tolerance Selection Procedure

For the insecticide selection six 150ml samples of the *G. aculeifer* culture were transferred from the culture containers to six 600ml cylindrical peat-based pots (Fig. 1). A 3ml sub-sample was then taken from each pot to estimate the density of *G. aculeifer*. The number of mites in these samples were counted on a black gridded Petri dish and recorded.

The chosen selecting dose of Rogor® (100 g dimethoate ai L^{-1}) was prepared in a 500ml garden spray bottle, and 50ml of this solution sprayed on to the surface of the culture in each pot. Following the application of the spray treatment 5ml of mite food culture mix was added to each pot, and the pots then incubated at 25°C. Forty-eight hours after treatment the density of surviving mites in each pot was estimated by assessing 3ml sub-samples.

Three clean plastic containers were then used to establish a new *G. aculeifer* culture with the survivors of the exposure to the selecting dose of dimethoate. These culture containers were then stored at 25°C in darkness for two weeks.

Two selected *G. aculeifer* strains were maintained. Strain 1 was treated fortnightly with a dimethoate dose that caused approximately 50% mortality (LC_{50}) of the treated mites. Strain 2 was treated fortnightly with a dimethoate dose that caused approximately 10% mortality (LC_{10}) of the treated mites (Nb. from the 32nd selection onwards Strain 2 was stopped). The specific selecting doses are presented in Table 2. A third strain which was left unsprayed was also maintained in case the selected strains experienced a serious population decline.

The fortnightly spray selection process is likely to have approximated the *G. aculeifer* generation time (Enkegaard *et al*. 1997).

Table 2: The selecting doses of dimethoate used during the selection study.

The number of selections from the start of the study	Dimethoate dose (g ai L^{-1})*	
	Strain 1	**Strain 2**
1-7	0.008**	0.002***
8-31	0.025**	0.008
32-43	0.05**	-
44-46	0.05	

*Note that the registered rate of dimethoate in strawberries was 0.3 g L^{-1}.

**These doses approximate the LC_{50} at the 1st, 7th and 31st selections respectively.

***This dose approximates the LC_{10} at the 1st selection.

Selections 1-43 occurred fortnightly, corresponding to a selection approximately each generation. Selections 44-46 occurred approximately monthly between January and March 2012. A nine week period without selection passed before the final bioassays were conducted on 15 and 22 May 2012.

Cross tolerance bioassays

A series of full dose-response bioassays were conducted (as described in the Full Dose-Response Bioassay methods above) with the unselected *G. aculeifer* strain and the alternative Rutherglen bug insecticide, the carbamate methomyl (Lannate® 225 g ai L^{-1}). The 'base-line' susceptibility of this unselected strain to this insecticide was then calculated by Probit analysis.

Following the 34th and 46th selection with dimethoate, full dose-response bioassays were conducted on the selected strain with methomyl to determine whether any cross-tolerance was conferred by the dimethoate selection to this alternative carbamate insecticide.

6. RESULTS

Once the initial difficulties with the mite rearing had been overcome, the dimethoate selection program commenced in January 2010.

The 'base-line' probit results for the unselected 'susceptible' (control) strain of *G. aculeifer* tested with dimethoate are presented in Table 2 (19/1/10 test date). The LC_{50} and LC_{99} were estimated to be 0.0078 and 0.0787 g ai L^{-1} respectively.

Despite encountering some difficulties due to the insecticide selections periodically reducing the mite population more than expected, the dimethoate selection program led to a steady (albeit relatively slow) increase in tolerance to dimethoate over time (See Table 3 and Fig. 4).

After the mite strain had been selected forty-three times (which approximated to a similar number of generations) it exhibited, relative to the unselected control strain, 2.8 and 21 fold tolerance to dimethoate at the LC_{50} and LC_{99} respectively. At this tolerance level it would be expected that at least a modest percentage (perhaps 10-20%) of this selected strain would survive a field rate dose of dimethoate. Given their high fecundity and short generation time this level of survivorship would be expected to result in a relatively quick recovery of the population if other sub-lethal effects of the insecticide treatment do not affect their reproduction and growth.

Following the January 2012 bioassay of the strain that had received the 43 selections, a further three selections at approximately monthly intervals were applied, and then a nine week period without selection occurred (15 March – 21 May 2012). The subsequent bioassay of 21 May 2012 indicated that the tolerance of the strain had not reverted with the lesser selection program, and that there had been a significant flattening of the dose response compared to the previous bioassays. As a result the resistance ratio at the LC_{50} and LC_{99} was estimated to be 3.7 and 1046 respectively. At this tolerance level 30-40% of the selected strain would potentially survive a field dose rate of dimethoate (0.3 g ai L^{-1}).

This 21 May 2012 bioassay result is most encouraging, as it suggests that the selected tolerance could be maintained in a culture with a selection frequency of around 4-6 weeks, and that the level of tolerance in this strain is now at levels that could provide a sufficient resilience for the strain to survive a periodic field spray application.

The bioassay results for the cross-tolerance study with methomyl are presented in Table 3 and Fig. 5. They reveal that a modest increase in tolerance to methomyl has been progressively conferred by the dimethoate selection program. There has been a significant flattening of the methomyl dose response from the start of the selection process to the most recent bioassay, which has resulted in the resistance ratio at the LC_{99} increasing to 134.7. At this tolerance level 20-25% of the selected strain would potentially survive a field dose rate of methomyl (0.3375 g ai L^{-1}).

Table 2. Full dose-response bioassay results of the dimethoate-selected *G. aculeifer* (Strain 1) tested against a commercial formulation of dimethoate. An unselected strain of *G. aculeifer* was used as the control.

Number of selections / Date (dd/mm/yy)	LC_{50} g ai L^{-1}	CL†	RR†	LC_{99} g ai L^{-1}	CL	RR	Slope+/- s.e.	N†
0 (19/1/10)	0.0078	(0.0049-0.0112)	1	0.0787	(0.0386-0.458)	1	2.320+/- 0.214	602
7 (3/5/10)	0.0095	(0.0051-0.0164)	1.2	0.304	(0.093-8.734)	3.9	1.544+/- 0.229	238
12 (14/7/10)	0.0255	(0.0124-0.0524)	3.3	0.620	(0.186-22.998)	7.9	1.678+/- 0.231	260
16 (20/9/10) ††	-	-	-	-	-	-	-	280
31 (17/5/11)	0.0416	(0.0327-0.0521)	5.3	1.03	(0.61-2.21)	13.1	1.670+/- 0.139	593
43 (17/1/12)	0.0218	(0.0103-0.0356)	2.8	1.649	(0.575-15.232)	21	1.238+/- 0.129	452
46 (21/5/12)	0.0291	(0.0180-0.0477)	3.7	82.32	(14.6-1569.5)	1046	0.674+/- 0.072	571

†CL=confidence limits of the LC_{50} and LC_{99} values respectively; RR=resistance ratio, i.e., the ratio of the LC value of the selected strain to the LC value of the unselected control strain; N=number of tested mites.

††High control mortality in this bioassay (conducted by a replacement technician) prevented probit analysis of the data-set.

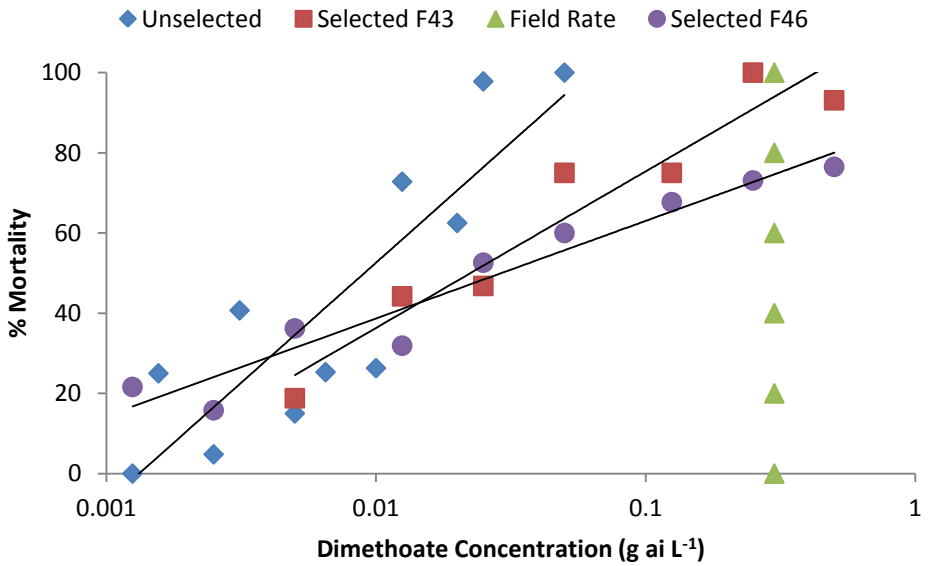

Fig. 4. The full-dose response data and probit regression lines of best fit for the *G. aculeifer* unselected strain, and for the selected strain after 43 (17 Jan 2012) and 46 (21 May 2012) selections with dimethoate.

Table 3. Full dose-response bioassay results of the dimethoate-selected *G. aculeifer* (Strain 1) tested against a commercial formulation of methomyl. An unselected strain of *G. aculeifer* was used as the control.

Number of selections / Date (dd/mm/yy)	LC_{50} g ai L^{-1}	CL†	RR†	LC_{99} g ai L^{-1}	CL	RR	Slope+/- s.e.	N†
0 (18/5/11)	0.0878	(0.0666-0.1136)	1	0.7461	(0.4354-2.0005)	1	2.502+/-0.218	393
34 (24/5/11)	0.108	(0.089-0.129)	1.2	1.57	(1.07-2.65)	2.1	2.002+/-0.172	489
46 (24/5/12)	0.0087	(0.0002-0.0236)	0.1	100.51	(5.65-3406.28)	134.7	0.573+/-0.138	399

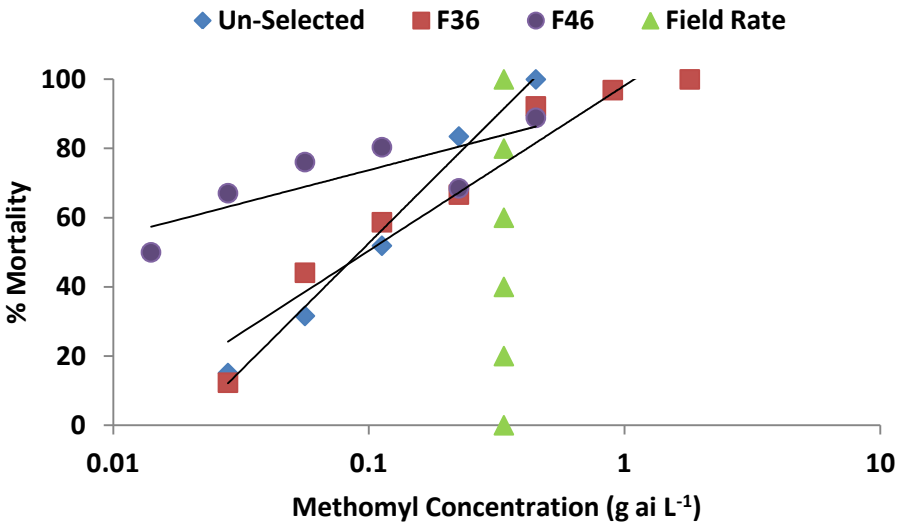

Fig. 5. The full-dose response data and probit regression lines of best fit for the *G. aculeifer* unselected strain, and for the dimethoate-selected strain after 43 (17 Jan 2012) and 46 (21 May 2012) selections when bioassay tested with methomyl.

7. DISCUSSION

On 6 October 2011 the Australian Pesticides and Veterinary Medicines Authority permanently suspended the use of dimethoate in a range of crops, including strawberries. Unfortunately this development undermined the specific intent of this project to select a dimethoate-tolerant mite strain.

However the modest level of cross-tolerance to methomyl that has resulted from the dimethoate selection is likely to permit a significant percentage of this selected strain to withstand a field spray with methomyl, particularly if applied at half-strength to minimize disruptive effects on beneficials.

Having completed this selection work, the South Australian Research and Development Institute has provided the insecticide-tolerant *G. aculeifer*, along with instructions to maintain the selected tolerance, to Biological Services (Loxton) for commercial production.

In summary, this project has developed an insecticide-tolerant biocontrol agent for western flower thrips (WFT) management that is likely to possess the field resilience to withstand the disruptive effects of a Rutherglen bug spray program. This will hopefully increase the overall success with WFT biological control in Australian strawberries, and help foster greater confidence by strawberry growers in the reliability of biological control.

8. TECHNOLOGY TRANSFER

The selected tolerant strain of *G. aculeifer* produced in this project has been given to Biological Services (Loxton), the commercial producer of this biological control agent in Australia, and hence is now available to assist strawberry growers that are endeavouring to implement a biological control based IPM system.

The contact details for Biological Services (Loxton) are available at:
http://www.biologicalservices.com.au/

9. RECOMMENDATIONS

That Australia's horticultural industries, through Horticulture Australia Limited, invest in the development of more biological control agents to help manage the range of pests in the range of crop environments (eg. different host plants, temperature, humidity and insecticidal tolerances, etc) where biological control has genuine potential to reduce current over reliance on pesticides.

That all strawberry growers use the services of an IPM consultant to assist them with their strawberry pest management and their use of biological control agents.

10. ACKNOWLEDGEMENTS

The strawberry industry is acknowledged for its forward thinking in supporting the uptake of biological control and a true integration of pest management practices.

We thank Mr James Altmann for the supply of the *Gaeolaelaps aculeifer* and *Carpoglyphus lactis* starter cultures, and initial advice on the rearing methods for these mites.

The project leader thanks the two SARDI Entomology staff, Ms Lakshmi Nacey and Mr Kevin Powis, who were responsible for the maintenance of the mite cultures and the performance of the bioassays and Probit analyses respectively, and Dr Peter Crisp for helping conceive the project.

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