

Final Report

Project title:

Impact of Pesticides on Beneficial Arthropods of Importance in Australian Vegetable Production

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Delivery partner:

IPM Technologies

Project code:

VG 16067

Date:

27/04/2020

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No

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Funding statement:

This project has been funded by Hort Innovation, using the <insert industry> research and development levy and contributions from the Australian Government. Hort Innovation is the grower-owned, not-for-profit research and development corporation for Australian horticulture.

Publishing details:

ISBN <Hort Innovation to add>

Published and distributed by: Hort Innovation

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www.horticulture.com.au

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Summary

Pest management options can be described as falling into only 3 three categories – Biological control (predators, parasites and pathogens of the pests), Cultural controls (management practices) and Pesticides. Using as many of these three options in a compatible way is called Integrated Pest Management or IPM. Understanding the effects of pesticides on beneficial species is a key element of any successful IPM program. The reason for this is that to be able to utilize the benefits of biological control it is important to use pesticides in the least disruptive way possible. The aim of this project was to provide vegetable growers and advisors with information on the relative toxic effects of pesticides on beneficial species that are important in vegetable crops and to present this information as a series of crop-specific guides.

The reason for having crop-specific guides is that there are different products available in different crops and also often very different beneficial species of importance. The intended use of the guides is as support tools for growers and advisors wanting to use IPM or give IPM advice. They are not intended to describe any product as good or bad, or safe or not safe but to ensure that informed decisions can be made when choosing a pesticide.

The information is presented in a format where it is easy to see the relative impact of different products on the species relevant to each crop type. This makes it very much easier to use than the existing databases where it is necessary to search for each product on each species separately. It is also much more precise to see where exactly the toxicity fits on a continuum from 0 to 100% mortality instead of three or 4 broad categories.

For the guides to be successful they must be useable and contain information that is practical and relevant, for this reason much consideration was given to the presentation, content and target audience of the guides. The guides have been developed for seven crop types; Leafy vegetables and head lettuce, Cucurbits and Fruiting vegetables, Sweet corn, Legume vegetables, Stalk and stem, Root and Tuber and Brassicas. The information in the guides has been compiled from scientific literature, international data bases, field observations the results of testing conducted as part of this project.

The project was a collaboration between IPM Technologies, QDAF and SARDI.

The guides will be made available to download and print through Ausveg using the link below.

<https://ausveg.com.au/biosecurity-agrichemical/crop-protection/#IPM>

The guides will also will be available on the Hort Innovation website and are attached as appendices.

Keywords

Pesticides, beneficials species, IPM.

Introduction

The role of pesticides in Integrated Pest Management (IPM) has historically been difficult to reconcile. There are several reasons for this but the most relevant one is that while pesticides work, they are a much easier option than IPM which is often perceived to be difficult. In the last 15 or so years there has been a significant change in the types of pesticides available. There has been a shift by most of the major pesticide producing companies away from broad-spectrum products to selective or “soft” IPM compatible products. The availability of these newer products should have brought about wide scale adoption of IPM by making pesticides that are less disruptive to beneficial species available, however this has not been the case. Confusion about the benefits of selectivity, overstating the “safe” qualities of some IPM compatible products and the higher cost of new products compared to old have led to a change in spray programs but little increase in IPM adoption. What is sometimes difficult to understand is that one pesticide can be safe to some species and toxic to others while another pesticide can be safe or toxic to a different set of species. There is no way of predicting what any of these newer pesticides might do to any particular species, and so the only way to find out is to test each one. There is obviously an unending set of combinations of pesticide and beneficial species and so this project set out to test only the most relevant combinations for IPM users growing vegetables in Australia.

The most critical aspect of using IPM successfully is being able to make informed decisions on pesticide use and these decisions need to be made quickly and confidently. When using IPM the decision to apply a pesticide or not is based not only on the target pest but also life stage of the crop, beneficial species present, knowing what effect each product has on beneficial species and the potential for flaring other pests. Although some information on the toxicity of pesticides on beneficial species is already available it is not in a format that is readily accessible to the majority of vegetable growers. There are overseas data bases that provide some information but they are difficult to interpret without knowing the scientific names of the beneficial species and they include species that are not present in Australia. The Australian cotton industry has been proactive in providing toxicity information for cotton growers but again it is not relevant or usable for most vegetable growers.

This project aimed to address these problems by compiling a set of guides that can be used as IPM decision making support tools specifically for growers and advisors by providing information that is crop specific.

This type of information is needed by the vegetable industry for the successful implementation and adoption of IPM as it helps to integrate the use of pesticides on farms in the least disruptive way possible to biological control agents. This should result in improved use of pesticides and better pest management.

Methodology

The starting point for the project was to review the currently available information on the toxicity of pesticides on beneficial species. To narrow the search eleven beneficial species and 43 pesticides were selected. The species selection was based on key species that are important in a wide range of vegetable crops so as to be efficient and relevant. The species were also selected to be representative of particular groups (parasitic wasps, lacewings, predatory mites, predatory bugs and predatory beetles) and included some species that are native to Australia. In addition, availability either commercially or as laboratory colonies established from field collections was also taken into consideration as the testing phase of the project required large numbers of each species. The pesticides were selected firstly for having a registration or permit in vegetable crops and secondly for their potential to be used in IPM programs. Some products that are known to be highly toxic to beneficial species were included to provide a way of ranking products against each other. The review collated information on the selected species and pesticides from overseas data bases and scientific literature. From this review, gaps in knowledge were highlighted which were then filled, where possible, by bioassays conducted by IPM Technologies, QDAF and SARDI. As a result of the review 166 bioassays were conducted during this project.

Table 1. Species selected for testing in this project

Common name	Scientific name	Type of beneficial	Source	Statu
Green lacewing	<i>Mallada signatus</i>	Generalist predator	Commercially available	Native
Brown lacewing	<i>Micromus tasmaniae</i>	Aphid predator	Lab colony	Native
Damsel bug	<i>Nabis kinsbergii</i>	Caterpillar predator	Lab colony	Native
Orius	<i>Orius tantillus</i>	Thrips predator	Commercially available	Native
Ladybird	<i>Hippodamia vareigata</i>	Aphid predator	Lab colony	Naturalised
Californicus	<i>Neoseuilus californicus</i>	Predatory mite	Commercially available	Native
Persimilis	<i>Phytoseuilus persimilis</i>	Predatory mite	Commercially available	Naturalised
Diadegma	<i>Diadegma semiclausum</i>	Diamondback moth parasite	Commercially available	Naturalised
Aphidius	<i>Aphidius colemani</i>	Aphid parasite	Commercially available	Naturalised
Encarsia	<i>Encarsia formosa</i>	Whitefly parasite	Commercially available	Naturalised
Trichogramma	<i>Trichogramma pretiosum</i>	Moth egg parasite	Commercially available	Native

Table 2. Pesticides included in the review

Insecticides and miticides			
Active ingredient	Trade name	Active ingredient	Trade name
Abamectin	Vertimec	Flubendiamide	Belt
Afidopyrofen	Versys	Hexythiazox	Calibre
Bacillus thuringiensis	Dipel/Xentari	Imidacloprid	Confidor
Bifenazate	Acramite	Indoxacarb	Avatar
Bifenthrin	Talstar	Milbemectin	Milbeknock
Buprofezin	Applaud	NPV	Vivus
Clothianadin	Samurai	Permethrin	Ambush
Chlorpyrifos	Lorsban	Pirimicarb	Pirimor
Chlorantraniliprole	Coragen	Pymetrozine	Chess
Cyantraniliprole	Benevia	Pyroproxifen	Admiral
Emamectin Benzoate	Proclaim	Spinetoram	Success Neo
Etoxazole	ParaMite	Spirotetramat	Movento
Fipronil	Regent	Sulfoxaflor	Transform
Fonicamid	Mainman		
Fungicides			
Active ingredient	Trade name	Active ingredient	Trade name
Azoxystrobin	Amistar	Mancozeb	
Boscalid	Filan	Metalaxyl	Polyram
Boscalid, Kesoxim – Methyl	Colliss	Oxathiapiprolin	Zorvec
Chlorothalonil		Penthiopyrad	Fontelis
Cyazofamid	Ranman	Propamocarb Hydrochloride + Fluopicolide -	Infinito
Cyflufenamid	Flute	Triadimenol	
Cyprodinil+ Fludioxonil	Switch	Triadimefon	
Dimethomorph	Acrobat	Zineb	

The bioassay work was shared between the three collaborating organisations (IPM Technologies, QDAF and SARDI) and involved both acute and sub-lethal testing. Acute tests are designed to measure any toxicity effects of a product on the test species over a 24 or 48 – hour period. In these tests the beneficial species are directly exposed to the product as a dried deposit on a petri-dish. Protocols were developed at the beginning of the project and were based on IOBC (International Organisation for Biological and Integrated Control) guidelines. See Appendix 1.

An agreed protocol for conducting bioassays was developed and tested by the three organisations (IPM Technologies, QDAF and SARDI). In an initial trial to ensure standard procedures for testing, the same combination of insecticide, beneficial and bioassay was repeated until the results from each group were consistent. The insecticide used in these preliminary tests was Confidor (Imidacloprid) and the species used was *Aphidius* (aphid parasite). The type of test used was acute, treated surface, which means that the test species individuals were exposed to a dried deposit on a petri dish and mortality was assessed at 24, 48 and 72 hours. The protocol and testing cages were adapted from IOBC standards, and this included the application of the product. Following these preliminary tests, the protocol was refined, the application technique was improved, and it was agreed that modifications could be made to the testing cages to suit the species being tested. It was also agreed that the small parasitoids (*Encarsia* and *Trichogramma*) which are particularly sensitive would be assessed at 24 hrs to avoid high control mortality and the predators would be assessed at 48 hrs only which is the standard for this type of testing.

Initial tests for all products were “acute” treated surface. Both the top and bottom plates of petri dishes were sprayed with either a handheld atomizer, potter tower or track sprayer with the label rate and water volume appropriate for each product. The plates were then weighed to ensure that the correct amount of product had been applied. When dry the insects or mites were added and the petri dishes were assembled either side of a vented ring with a wick for moisture.



Figure 1: Testing chamber used in all bioassays.

For each bioassay there was a positive (Confidor) and a negative (water) control. The positive control has traditionally been a synthetic pyrethroid or other broad-spectrum insecticide, but it was decided that these products are too extreme in their toxicity and therefore not a good measure of the validity of each bioassay.

Another commonly used technique for acute bioassays is direct spray. In these bioassays the test species are sprayed directly with the product. It was the decision of the project leader not to use this technique as a routine part of this project. This was mainly for health and safety reasons as it requires more handling of the products and from experience (IPM Technologies testing) the results can be more inconsistent. However, two direct spray bioassays were completed to demonstrate that the results were equivalent to the treated surface results.

These types of bioassays are often referred to as “worst-case scenario” as they are likely to show higher mortality than may be observed in field situations. These bioassays are used because they have the advantage over field trials in that they are easier to replicate and control; but they only provide part of the answer. The results can be used to rank the toxicity of products and also to identify products that require further investigation. If a product is safe in the “worst- case scenario” tests, then it is considered safe under field conditions. If the product is not safe,

then further tests should be done to calibrate the results to be more indicative of field conditions. Tests were done by SARDI using a track sprayer on plant material and these results were compared to the sprayed petri dish results. The on-leaf track sprayer tests showed less toxic effects than the sprayed petri-dish. These results are in keeping with IOBC protocols and rating system.

Interpretation of data

The information presented in the guides on the toxicity of pesticides comes from various sources, these include the international data bases IOBC and Koppert, scientific literature and bioassays that have been conducted as part of VG16067. Information is presented differently by each of these sources. The data bases IOBC and Koppert use a toxicity rating for laboratory tests on artificial substrate which is divided into four categories and these are **0% to 30%** harmless, **31 -80%** slightly harmful and **81- 99%** harmful and **99 to 100%** very harmful. This is presented on the data bases as either a **1,2,3 or 4**. The project bioassay results are presented as the actual % mortality of the bioassays conducted and the scientific literature varies in how the results are presented and interpreted in each paper. In addition to the formal trial results, field observations must also be taken into account. Although observations are not quantifiable, they are still valid as an indicator of the accuracy of the laboratory testing. To add to the complexity are different testing protocols and different rates and application methods for testing the products. To convert the information from the different resources into a useable format it is first necessary to ask “who and what is this information for? The answer to this is that the information is for growers using IPM and for advisors giving IPM advise and it will be used alongside other information when choosing an IPM compatible pesticide. The list of pesticides, references and project results are attached as Appendix 2..

About the pesticides

The pesticides used in the guide were selected because they potentially have a place in IPM programs. However, this does not necessarily mean that they are ‘safe’ to all beneficial species and it is this that makes the role of pesticides in IPM confusing. Some older broad-spectrum products that are well known to be highly toxic to beneficials have also been included in the guides. Because of the varied sources of information and in some cases varied results of a single product on different species the most accurate and usable way of presenting the information is as a comparison of the toxicity between products. Products such as Chlorpyrifos are used to represent the extreme most highly toxic end of the scale and Dipel (a bacteria that only kills caterpillars) is at the other end which is very safe to all beneficial species. All other products fall somewhere in between. In the guides the results are presented as an arrow on a sliding scale, which is different from the traditional “traffic light” presentation of three categories of green as safe, yellow as slightly harmful and red as toxic. The arrow on the sliding scale provides a more accurate way of seeing the potential impact of each pesticide on a range of species. The positioning of the arrows has been determined after assessing all currently available information for each species and product. For some combinations this is very clear but for others it is less so. The potential for testing is ongoing and it is likely that some of results could change in the future as more information becomes available.

Development of the guides

The guides went through several drafts and were modified each time according to feedback from growers and agronomists who were invited to comment. General comments on the initial draft was that there was too much unnecessary information. It is important that the guides are as clear as possible and simple to understand.

Predators / Predatoren										Parasites / Parasieten										insecticide/acaricide				
Macrolophus caliginosus		Oriaus laevigatus + insidiosus + ruficornis		Poecilus fuscicornis		Aphidius colemani + ervi		Dacnusa sibirica Opus pallipes		Diglypha isaea		Encarsia formosa		Euclyptus variator		Trichogramma spp.		Hemiteles sp.		Stenomacrus		Vespa velutina		
type	adult	larvae	type	adult	larvae	type	adult	larvae	type	adult	larvae	type	adult	larvae	type	adult	larvae	type	adult	larvae	type	adult	larvae	
4			2	4	5		4	4	4			3	4	0-12		4	4	> 4	3		1	2	3	
			4	4			4	4	4			3												
1	1	0	1			1	1	0	1	0	1	0	1	1	0	1	1	0	1	1	0		1	0
3	1	0	1	1	0	1	1	0	1	0	1	0	1	1	0	1	1	0	1	1	0		1	0
4	4	0-12	4	4	0-12		4	4	4	0-12	4	4	4	0-12	4	4	4	0-12	2		2			
3	4	2	3	3	2		4	4	4	2	1	4	2	1	4	1	1	4	1	0	1	0	4	
4	4	0-12	4	4	0-12		4	4	4	0-12	4	4	4	0-12	4	4	4	0-12	1	0	1	0	1	0
3	3	1-2	1			1	1	0	1	0	1	1	0	1	1	0	1	1	0	1	0	1	0	0
			1			1	1	0	1	0	1	1	0	1	1	0	1	1	0	1	0	1	0	0
4	4	0-12	4	4	0-12		4	4	4	0-12	4	4	4	0-12	4	4	4	0-12	1	0	1	0	1	0
4	1		2	3		4	1	1	0	1	1	4	4	4	1	4								
			4			3	4				4	4			3	4								
3	1		2	3		4	4		4		2	4	4-5	3	4	4	2			2			4	
											2	4												
4	4	0-1	3	4	1	4	4	1	4	0	4	4	1	4	0-1	4	0-1	2			1	0	1	0

Figure.2 An example of how information is currently presented on existing data bases and charts.

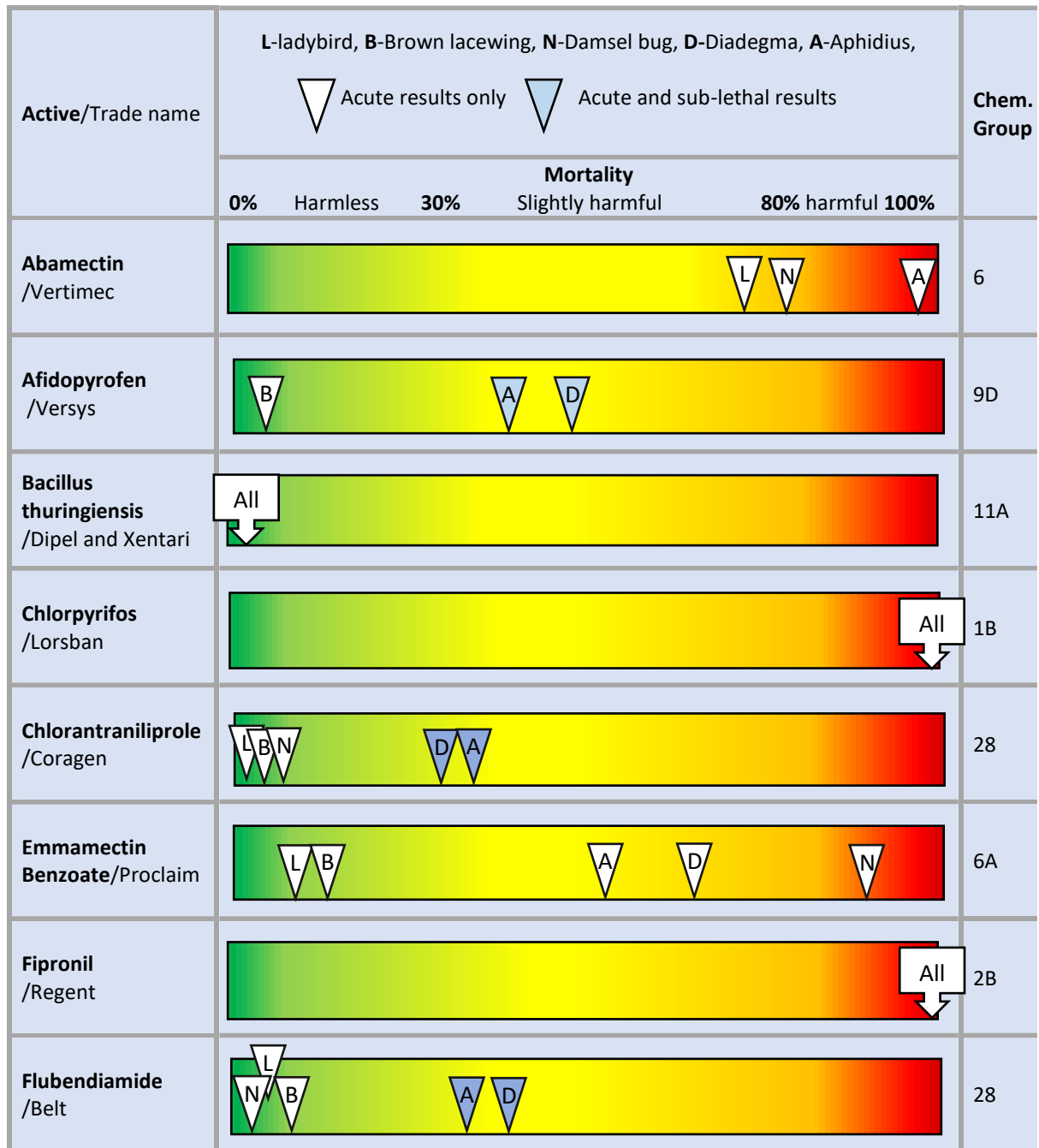


Figure. 3 An example of how the information is presented in the guides produced in this project.

Outputs

Monitoring and evaluation plan Appendix 3

List of pesticides registered and permitted in crops covered by the vegetable R&D levy (see guides)

Review of existing data on disruptive effects of pesticides on key beneficials relevant to the Australian vegetable industry, including a gap analysis. Appendix 4

Pesticide testing to fill gaps. The table below shows the testing which was completed as part of this project. The number of X's indicates how many tests were completed for each species. A total of 166 separate bioassays were completed as part of this project.

Species: **Orius** (*Orius tantillus*) **BLW** – brown lacewing (*Micromus tasmaniae*) **GLW** – green lacewing (*Mallada signatus*) **LB** – ladybird (*Hippodamia variegata*) **Tr**- Trichogramma (*Trichogramma pretiosum*) **Dia**-Diadegma (*Diadegma semiclausum*) **Aph**-Aphidius (*Aphidius colemani*) **Pp**- Persimilis (*Phytoseiulus persimilis*) **Ca**- Californicus (*Neoseiulus californicus*) **Hf**- hoverfly (*Syrphid spp.*)

Insert table heading

Product	Species											
	Orius	BLW	Nab	GLW	LB	En	Tr	Dia	Aph	Pp	Ca	Hf
Avatar		XX	X	XXX	XXXX	X	X	XX	X	X		
Belt			XXX	XX	XX	XX	X	X	XX	X		
Benevia	XX	XXXX	XXX	XX	XX	XX	XX	X	XX	X		
Confidor	X	X	X	X	X	XX	X	X	XXX	X		
Coragen			XX				X	X	XX	X		
Fontelis						X						
Infinito						X						
Mainman	X					XX	XX	XX	XX	X	X	
Movento	X	X	XXX	XX	XX	XX	XX	XX	XX	X		
Success Neo	XX	X	X	X	X	X	X	XX	XX	X		
Transform	XXX	X	X	X	X	X	X	XX	XX	X		X
Velifer	X								X	X		
Versys			XX					XXX	XXX		X	

One article per year for Vegetables Australia or Hortlink

<https://ausveg.com.au/articles/levy-funded-project-researching-impact-pesticides-beneficials/>

https://ausveg.com.au/app/uploads/publications/Vegetables-Australia_July-August-2018_Web.pdf

https://ausveg.com.au/app/uploads/publications/AUSVEG_VegetablesAustralia_2019_May-June_WEB-150DPI_F01v1.pdf

A set of user-friendly guides specific for each major vegetable crop type covered by the vegetable R&D levy (Appendix 5-11).

This is by far the most important output of the project. 7 guides to pesticide effects on beneficials have been developed as a result of this project. These will also be made available to AusVeg who will make them available via their website. They will be in a pdf format to be easily downloaded by growers or agronomists. Their availability will be advertised in a range of media including the AusVeg email newsletter, Vegetables Australia magazine and

direct communication with resellers. They will also be available on the IPM Technologies website.

Appendix 5: Brassicas – A guide to pesticide effects on beneficials

Appendix 6: Cucurbits and Fruiting Vegetables - A guide to pesticide effects on beneficials

Appendix 7: Leafy vegetables and Head lettuce - A guide to pesticide effects on beneficials

Appendix 8: Legume Vegetables - A guide to pesticide effects on beneficials

Appendix 9: - Root and Tuber A guide to pesticide effects on beneficials

Appendix 10: Stalk and Stem - A guide to pesticide effects on beneficials

Appendix 11: Sweet corn - A guide to pesticide effects on beneficials

Outcomes

Industry Outcome 4 in the Vegetable levy Strategic Investment Plan (SIP) highlights pest and disease management as an important area requiring ongoing levy investment. One specific area of concern in regards to pest management is around chemicals. Pesticide availability is becoming more limited due to resistance, withdrawal of products, MRL’s preventing access to export markets and an increase in consumer concerns about the health and environmental impacts of chemicals. These are all issues facing the vegetable industry. IPM is recognised as an important approach to improving pest and disease management which can also provide solutions to pesticide issues by shifting the industry to a more strategic use of ‘softer’ chemicals.

The end of project outcomes for this project align closely with those outlined in the SIP. The guides to the effects of pesticides on beneficial species will be useful tools for any vegetable grower, advisor or reseller wanting to make better informed decisions about the use of pesticides within an IPM program. A fundamental mistake that is often made with the promotion and extension of IPM is not acknowledging that pesticide decision making is, from a grower, agronomist and reseller point of view, the most important part of successfully using IPM. This project has taken information on the effects of pesticides on beneficial species that has largely been inaccessible by the vegetable industry and turned it into a useable set of crop-specific decision-making tools that will greatly support the uptake and the ongoing use of IPM by the vegetable industry.

The guides have been developed in consultation with growers, advisors, resellers, biological control producers, chemical companies and researchers. The comments on the final versions of the guides have all been positive. Key comments are that they are easy to use and the information is set out in a much clearer guide than is available anywhere else.

The guides will be received well by others outside the vegetable industry. Anyone wanting to use IPM successfully in any crop will find them of great value.

Monitoring and evaluation

The overall performance of the project was efficient with good communication and collaboration between the three organisations. Regular phone calls and emails were the primary methods of communication as well face to face meetings when possible.

Key evaluation questions	Relevant?	Project-specific questions
Effectiveness		
1. To what extent has the project achieved its expected outcomes?	Y	<i>To what extent has the project delivered to industry relevant information about the toxicity of pesticides on beneficials? The project has achieved its expected outcome of delivering to the vegetable industry information on the toxicity of pesticides on beneficial species.</i>

Key evaluation questions	Relevant?	Project-specific questions
Relevance		
2. How relevant was the project to the needs of intended beneficiaries?	Y	<i>To what extent has the information provided by this project met the needs of vegetable industry levy payers? This type of information is needed by the vegetable industry to support IPM decision making and to improve knowledge and understanding of the role of pesticides within IPM programs.</i>
Process appropriateness		
3. How well have intended beneficiaries been engaged in the project?	Y	<i>Were the guides given to the industry for feedback and was this feedback used in development of the final guides? The guides were developed with input from growers, agronomists, biological control producers and resellers to ensure that they met the needs of the intended audience.</i>
Efficiency		
5. What efforts did the project make to improve efficiency?	Y	<i>Were the bioassays completed in a cost-effective way? The bioassays were conducted in a cost-effective way.</i>
Other (if any)		
Include any project-specific question determined important for your project here	N	<i>To what extent has the project developed information that will have a lasting impact on the industry? The guides to the effects of pesticides on beneficials will have a significant and long-lasting impact on the vegetable industry by providing key information needed for IPM decision making.</i>

To ensure that the project would meet the intended outcome of providing the industry with a usable set of guides on the effects of pesticides on beneficial species feedback was sought from several people in the vegetable industry. Draft guides were sent to 10 vegetable farmers, 2 representatives from 2 chemical companies, 3 agronomists and a biological control producer. Feedback on the final version was 100% positive and some examples of comments are presented below.

“The guides are highly useful tools for growers, agronomists and resellers wanting to use IPM or give IPM advice.”

“The information is presented clearly and is easy to understand.”

“I really think this is great information, very easy to follow and understand.

I also showed it to my spray operator who also had no problem following it with little experience in the field”.

“Brilliant information, it will give resellers something to think about”

Recommendations

The value of the guides is in supporting growers and advisors using or wanting to use IPM. It is recommended that ongoing investment into the adoption of IPM in the vegetable industry is needed to address concerns relating to pesticide use.

Refereed scientific publications

N/A

Intellectual property, commercialisation and confidentiality


No project IP

Acknowledgements

The project leader would like to acknowledge the enormous amount of work and effort that the project team has put into achieving the aims of this project. Thank you to Janet, Lara, Zara, Paul, Maarten, Farrah and Kevin and also a very big thank you to Biological Services and Bugs for Bugs for supplying beneficial species for testing.

Appendices

Appendix 1. Protocols for bioassays

STANDARD OPERATING PROCEDURE		
 Queensland Government	Acute and sub-lethal bioassays on juvenile stages of predatory insects Market Access Team, H&FS, DAF Date: 30/11/2011 Version 1.0 Lara Senior, Zara Hall, Mona Moradi	
	<table border="1" style="width: 100%;"> <tr> <td style="width: 50%;">SOP No #35</td> <td style="width: 50%;">Version: 6</td> </tr> </table>	SOP No #35
SOP No #35	Version: 6	
HAZARDS	<ul style="list-style-type: none"> • A variety of pesticides may be used in the bioassays. Exposure to pesticides through spillage or accidental inhalation may be hazardous. • Manual handling issues associated with moving of the container of waste pesticide solution. 	
PERSONAL PROTECTIVE EQUIPMENT (PPE)	<ul style="list-style-type: none"> • Lab coat • Gloves • Goggles • Enclosed shoes 	
CRITICAL ADVICE	<ul style="list-style-type: none"> • A variety of pesticides may be used in the bioassays, with varying degrees of hazard to human health. 	
SAFE OPERATING PROCEDURES	<ul style="list-style-type: none"> • Before any staff use or come into contact with pesticides they must undergo training which includes reading the risk assessment, MAT standard operating procedure (SOP) and SDS for each pesticide. • All work with the pesticides (application, drying of pesticide residues, disposal of waste solution) must occur in a fume hood, or face masks should be worn. 	
EMERGENCY CONTACTS	Contact the Manager and first-aid officer if an incident has occurred.	
AUTHORISATION	All laboratory staff with adequate training are authorised to perform the bioassays	

STANDARD OPERATING PROCEDURE #35

Acute and sub-lethal bioassays on juvenile stages of predatory insects

Test endpoints

- A. Mortality of juvenile predatory insects during a 48-hour exposure period to dry residue.
- B. Survival to adult stage
- C. Mortality of adult predatory insects during a 48-hour exposure period to dry residue and subsequent reproductive performance over a 72-hour period.

Overview of bioassay

Assessments of treatment effects are to be made at 24 and 48 hours by recording mortality as moribund (unable to move away from a stimulus) and number alive (acute bioassay).

Assessments of survival to the adult stage are carried out only if the number of surviving insects is greater than 75% after 48 hours. Surviving juveniles are removed from the bioassay cages and placed in ventilated containers, provided with food and water, until they emerge as adults. Survival to pupal and adult stage is recorded. Reproductive assessments are carried out only if the number of surviving adults is greater than 50%. This stage of the bioassay is performed on fresh adults (NOT survivors from the juvenile bioassay). Mated, ovipositing adults are exposed to dry residues and assessments made at 24 and 48 hours by recording mortality as moribund (unable to move away from a stimulus) and alive. The number of eggs laid over a 72 hour period is recorded. The hatch rate of these eggs is assessed.

Description of Methods

A. Acute bioassay: mortality of juvenile predatory insects during a 48-hour exposure period to dry residue

1. Record bioassay details on results sheet, including source (commercial/field collected) and rearing details of test organism, and date bioassay set up.
2. Each bioassay will include a minimum of three treatments: 1. Test product, 2. Negative control (water), 3. Positive control (Confidor). More than one test product may be included in each bioassay.
3. Put on PPE: lab coat, gloves, goggles.
4. Label 55mm diameter disposable polystyrene petri dishes with the treatment and replicate using a permanent marker pen. Label the side of each lid and base.
5. Prepare sufficient petri dishes for 25 cages in total for each treatment (5 replicates of 5 cages). NOTE: if insufficient insects are available, a bioassay can be split by replicate over time (i.e. 3 replicates one week, the remaining 2 replicates at a later date).
6. Label 50 ml glass atomisers with the treatment name.
7. Working in a fume hood, measure out the required volumes of each pesticide and prepare the treatment solutions in 50 ml glass atomiser bottles. **Refer to the spray calculation (sample sheet attached).** Treatments should be applied at the highest label rate of product in a dilution of 500 L/ha.
8. Apply treatments to the petri dishes. The spray should be applied to the outer surface of the base and lid. Weigh petri dishes before and after spraying to demonstrate that the appropriate amount has been applied. Record the time that application of each treatment starts and finishes. **Record on the results sheet (Results sheet: Acute and sub-lethal bioassays on juvenile predatory insects) (attached).**
9. Leave sprayed petri dishes to dry in the fume hood for approximately 3 hours.
10. Following the 3 hour drying period, assemble the bioassay cages as per Figure 1 (minus the wick at this stage). Ensure the treated surfaces face inwards.
11. Dispose of waste pesticide solution according to ESP Chemical Waste Management SOP P0002.
12. FOR LACEWING BIOASSAYS ONLY: place a small quantity of moth eggs into each base, sufficient for the duration of the bioassay.



Figure 1. Bioassay cage

13. Place a minimum of 3 juveniles that are 2 days old in each bioassay cage, using a fine paintbrush. Select individuals of a similar size. Ensure separate brushes are used for each treatment and avoid touching treated surfaces. For each treatment, record the time that placement of the insects into the cages starts and finishes.
14. FOR LADYBIRD BIOASSAYS ONLY: Place 40-50 green peach aphids into the bioassay cage, using a fine paintbrush (excess food should be provided). Use separate brushes for each treatment.
15. Insert a damp wick into the hole used to introduce the adults into the cage. Ensure the hole is completely plugged.
16. Place the assembled bioassay cage into a container of water, such that one end of the wick is in the water.
17. Place all bioassay cages into a CER room at 24°C. Ensure a data logger is placed in the CER room to record temperature.
18. Carry out assessments of treatment effects at 24 and 48 hours after the insects are placed in the cages. Mortality should be recorded as number moribund (unable to move away from a stimulus) and number live. If mortality cannot be assessed visually, a probe may be inserted into the cage through the hole containing the wick, to gently prod the insects. If all insects are immobile the cage may be opened to assess mortality. **Record on the results sheet (Results sheet: Acute and sub-lethal bioassays on juvenile predatory insects) (attached).**
19. Also at 24 and 48 hours, place additional food into each cage. LADYBIRDS: canola seedlings infested with aphids are placed into the cages. The number of aphids will be based on observations of the number consumed, however, it is estimated that >40 will be required. LACEWINGS: ensure there are sufficient uneaten moth eggs remaining.
20. If the number of surviving insects at 48 hours is less than 75% (i.e. sub-lethal bioassay not required), carry out an additional assessment of mortality at 72 hours.

B. Sub-lethal bioassay: survival to adults.

21. If the number of surviving insects in a treatment is more than 75%, survival to adult will be assessed in a sub-lethal bioassay, compared with the control treatment and Confidor.
22. Record which treatments are continuing to sub-lethal bioassay **(Results sheet: Acute and sub-lethal bioassays on juvenile predatory insects) (attached).**
23. Place surviving juveniles from each replicate together in a container for a period of time until they reach the adult stage. Provide a water source and food source.
 - a. LADYBIRDS: estimated time from set-up of acute bioassay to first adult emergence is 12 to 14 days. The food source is cereal aphids on wheat or green peach aphid on canola seedlings.
 - b. LACEWINGS: estimated time from set-up of acute bioassay to first adult emergence is 17 to 18 days. The container consists of a 1000 ml takeaway container with ventilated lid (Figure 2). The food source is moth eggs.
24. Check regularly and record pupation, the number of adults which successfully emerge, and mortality. **Record totals on the results sheet (Results sheet: Acute and sub-lethal bioassays on juvenile predatory insects) (attached).** Insects should be checked at least every other day (excepting weekends), and any dead larvae removed and recorded.



Figure 2. Holding cage for juvenile predators and newly emerged adults

C. Sub-lethal bioassay: reproductive performance.

25. If the number of insects surviving to adult is more than 50%, effects on reproduction will be assessed in a further sub-lethal bioassay, compared with the control treatment and Confidor.
26. Record bioassay details on results sheet, including source, rearing details of test organism, date bioassay set up and treatments.
27. Label 1000 ml plastic takeaway containers and lids with the treatment and replicate using a permanent marker pen.
28. Cut out a 4 cm x 4 cm section from the lid and glue fine mesh in place. During spray application, tape the cut section loosely in place on the other side of the mesh, so that the mesh is sprayed. The section of plastic is removed after spraying.
29. Prepare 5 containers (= 5 replicates) per treatment.
30. Prepare pesticide solutions in 50 ml glass atomiser bottles as per the acute bioassay method.
31. Apply the treatments to the containers and lids. Weigh the containers and lids before and after application and record.
32. Leave to dry in the fume hood for approximately 3 hours.
33. Once dry, place 15 adult predators in each container. The adults should be mated and have begun oviposition. They will not be sexed at this stage but selected at random.
34. Supply each container with a water source and food source.
 - a. LADYBIRDS: food source is aphids, supplied on the host plant
 - b. LACEWINGS: food source is a 50:50 mix of honey and yeast autolysate mixed with water to form a thick paste.
35. Keep the adults in the treated container for 48 hours.
36. At the end of the 48 hour period, record mortality.
37. Transfer surviving adults to clean containers, one per container (figure 3). Containers should be labelled with the treatment, replicate and adult number (i.e. 1 to 15).
38. Each container is provided with food and water, and with paper towel placed beneath the lid as an oviposition substrate.
39. Record oviposition over a 72 hour period:
 - a. LADYBIRDS: count and remove eggs every 24 hours. If adults oviposit on the side of the container transfer the adult to a new container.
 - b. LACEWINGS: count and remove eggs at the end of the 72 hours.

40. Retain all eggs and check daily in order to record total viability for each batch, i.e. emergence of the first instar larva.
41. Kill adults and record the sex of each.
42. **Record total oviposition, viability and sex of adults on the results sheet (Results sheet: Acute and sub-lethal bioassays on juvenile predatory insects) (attached).**



Figure 3. Holding container for oviposition assessments

Protocol for Acute and Sub-lethal bioassays on parasitoid wasps.

Test endpoints:

Mortality of adult wasps during a 48-hour exposure period to dry residue.

Reproductive performance over a 24-hour period.

Evaluation:

Assessments of treatment effects are to be made at 24 and 48 hours by recording mortality as moribund (unable to move away from a stimulus) and number alive. Assessment should be made without opening test cages.

Reproductive performance assessments are carried out only if the number of surviving adults is greater than 75% (after either 24 or 48 hours). Surviving adults are to be caged with a suitable life stage of a host species for 24 hours and the subsequent level of parasitism is to be recorded as well as the number of progeny (newly emerged adults).

Description of Methods

Treated surface

Apply sprays to 6cm diameter disposable polystyrene petri-dishes, using either a hand-held atomiser or Potter tower with the highest label rate of product in a dilution of 500 L/ha. This is to be calibrated by weighing petri dishes before and after spraying to demonstrate that the appropriate amount of product has been applied. Sprayed petri dishes are left to dry for approximately 3 hours.

A minimum of 5 adults are placed in each testing cage, 25 cages in total for each treatment (5 replicates of 5 cages). Adults are fed honey and water. Three treatments are needed for each bioassay – 1. Product, 2. Negative control (water) 3. Positive control (Confidor).

Reproductive performance

Place surviving adults from each replicate together in a large cage with the appropriate life-stage of their host species for 24 hours. After 24 hours adult wasps are removed and hosts are kept until level of parasitism and

emergence of adults can be observed. This assessment is descriptive and is an indicator of sub-lethal effects only.

Protocol for Acute and Sub-lethal bioassays on the juvenile and adult stage of predatory insects

Test endpoints:

- (i) mortality of juveniles during a 72-hour exposure period to dry residue.
- (ii) Survival to adult and reproductive performance over a 72-hour period.

Evaluation:

Assessment of acute treatment effects is to be made at 48 and 72 hours by recording mortality as moribund (unable to move away from a stimulus) and number alive.

Sub-lethal assessments record the following:

- (i) number of juveniles that survive to become adults,
- (ii) mortality after 72 hours,
- (iii) the number of eggs laid during a 72-hour period and
- (iv) the viability of those eggs.

A second sub-lethal test involves spraying newly emerged adults and recording the number of eggs laid during a 72-hour period and the viability of those eggs.

Description of methods

Treated surface

Apply sprays to 6mm diameter disposable polystyrene petri-dishes, using either a hand-held atomiser or Potter tower with the label rate of product in a dilution of 500 L/ha. This is to be calibrated by weighing petri dishes before and after spraying to demonstrate that the appropriate amount has been applied. Sprayed petri dishes are left to dry for approximately 3 hours.

A minimum of 3 juveniles that are 2 days old are to be placed in each testing cage, 25 cages in total for each treatment (5 replicates of 5 cages). Juveniles are to be fed with an excess amount of food (which is added daily) and water. Three treatments are needed for each bioassay – 1. Product, 2. Negative control (water) 3. Positive control (Confidor).

Reproductive performance

Sub-lethal tests are carried out only if the number of surviving juveniles is greater than 75% after 72 hours. Surviving juveniles are to be removed from test cages and placed in large breeding boxes and provided with food and water and kept until they emerge as adults.

Record both the number of adults successfully emerging and mortality.

The newly emerged adults remain in boxes for approximately 5 days to allow for mating and for the preoviposition period. Females are then set up in individual containers for a 72-hour period and the number and viability of eggs is recorded and removed every 24 hours.

A second sub-lethal test is performed which is the same as the one described above except that the adults in this test are exposed to the dry deposit and not the juvenile stage.

For some products that are translaminar or systemic another sub-lethal test may be required for some species. In this test juveniles will be fed prey that has fed on treated plant material.

Abamectin - Vertimec					
Group	Species	Project Data (Percentage mortality)		Data from other sources (Ratings from 1 (safe); to 4 Highly Toxic)	
		Acute	Sub-lethal	Acute	Sub-lethal
Predators	<i>Hippodamia variegata</i>			3 IPM Technologies	
	<i>Orius sp</i>			4 Van de Veire & Tirry 2003	
	<i>Nabis kinbergii</i>			3 IPM Technologies	
	<i>Neoseiulus californicus</i>			3 Van de Veire et al., 2002	
	<i>Phytoseiulus persimilis</i>			3 Blummel&Hausendorf	
Parasitoids	<i>Aphidius colemani</i>			4 IOBC, 4 Koppert	
	<i>Trichogramma pretiosum</i>			3 IPM Technologies	
Afidopyrofen - Versys					
Group	Species	Project Data		Data from other sources	
		Acute	Sub-lethal	Acute	Sub-lethal
Predators	<i>Micromus tasmaniae</i>	10%			
	<i>Neoseiulus californicus</i>	66%			
	<i>Phytoseiulus persimilis</i>	23%			
Parasitoids	<i>Aphidius colemani</i>	55%			
Bacillus thuringiensis/Dipel/Xentari					
Bifenthrin/Talstar					
Group	Species	Project Data		Data from other sources	
		Acute	Sub-lethal	Acute	Sub-lethal
Predators	<i>Hippodamia variegata</i>			4 IOBC	
	<i>Orius sp</i>			4 IOBC	
	<i>Neoseiulus californicus</i>			4 Koppert	
	<i>Phytoseiulus persimilis</i>			4 Koppert	
Parasitoids	<i>Aphidius colemani</i>			4 Koppert	
Buprofezin/Aplaud					
Group	Species	Project Data		Data from other sources	
		Acute	Sub-lethal	Acute	Sub-lethal
Predators	<i>Mallada signatus</i> (related sp. <i>Chrysopa carnea</i>)			1 IOBC	
	<i>Orius spp</i>			2 IOBC	
	<i>Phytoseiulus persimilis</i>			1+2 IOBC	
Parasitoids	<i>Encarsia formosa</i>			1 IOBC	
Clothianadin/Samurai					
Group	Species	Project Data		Data from other sources	
Predators		Acute	Sub-lethal	Acute	Sub-lethal

	Coccinellidae			4 IOBC	
	<i>Neoseiulus californicus</i>			4 Koppert	
	<i>Phytoseiulus persimilis</i>			4 Koppert	
Parasitoids	<i>Aphidius colemani</i>			4 IOBC	
Chlorantraniliprole/Coragen					
Group	Species	Project Data		Data from other sources	
		Acute	Sub-lethal	Acute	Sub-lethal
Predators	<i>Micromus tasmaniae</i>			1 IPM Technologies	
	<i>Hippodamia variegata</i>	4%		1 IPM Technologies 1 E-Phy acute 2 Mills et al 2016	
	<i>Nabis kinbergii</i>			1 IPM Technologies	
	<i>Phytoseiulus persimilis</i>			1 Koppert	
Parasitoids	<i>Aphidius colemani</i>	3%	80% reduction	1 E-Phy, Brugger et al 2010,	Brugger et al 2010
	<i>Trichogramma pretiosum</i>			1E-Phy, Brugger et al 2010 Kahn et al 2017 acute and sublethal 2 IPM Technologies – acute adults.	
	<i>Diadegma semiclausum</i>	1%	79% reduction	1 IPM Technologies acute Brugger et al 2010 Brugger et al 2010 sublethal	
Chlorpyrifos/Lorsban					
Group	Species	Project Data		Data from other sources	
		Acute	Sub-lethal	Acute	Sub-lethal
Predators	<i>Micromus tasmaniae</i>			Cole et al 2010	
	<i>Mallada signatus</i>			4 IOBC	
	<i>Hippodamia variegata</i>			Cole et al 2010	
	<i>Nabis kinbergii</i>			Cole et al 2010	
	<i>Orius sp</i>			4 IOBC	
	<i>Neoseiulus californicus</i>			4 IOBC	
Parasitoids	<i>Phytoseiulus persimilis</i>			4 IOBC	
	<i>Aphidius colemani</i>			4 IOBC	
	<i>Encarsia formosa</i>			4 IOBC	
	<i>Trichogramma pretiosum</i>			4 IPM Technologies	
Cyantraniliprole/Benevia					
	Species	Project Data		Data from other sources	
		Acute	Sub-lethal	Acute	Sub-lethal
Predators	<i>Micromus tasmaniae</i>	22%			

	<i>Mallada signatus</i>	0% 99A%	17% P<0.05		
	<i>Hippodamia variegata</i>	3%	0 NS		
	<i>Nabis kinbergii</i>	12%	24%		
	<i>Orius tantillus</i>	31%			
	<i>Neoseiulus californicus</i>			1 Koppert	
Parasitoids	<i>Phytoseiulus persimilis</i>	17%	60%		
	<i>Aphidius colemani</i>	7%	31%NS		
	<i>Encarsia formosa</i>	43%	50%		
	<i>Trichogramma pretiosum</i>	31%	3%		
Emamectin Benzoate/Proclaim					
Group	Species	Project Data		Data from other sources	
		Acute	Sub-lethal	Acute	Sub-lethal
Predators	<i>Micromus tasmaniae</i>			1 IPM Technologies	
	<i>Hippodamia variegata</i>			1 IPM Technologies	
	<i>Nabis kinbergii</i>			3 IPM Technologies	
	<i>Orius sp</i>			4 Koppert	
	<i>Phytoseiulus persimilis</i>			4 Koppert	
Parasitoids	<i>Aphidius colemani</i>			1 E-Phy acute 1 IOBC 3 Koppert	2 Biondi et al 2013 sub-lethal 1 Bengochea 2012 sub-lethal
	<i>Diadegma semiclausum</i>			3. Haseeb et al 2000 Cordero et al 2007	
Etoxazole/ParaMite					
Group	Species	Project Data		Data from other sources	
		Acute	Sub-lethal	Acute	Sub-lethal
Predators	<i>Orius tantillus</i>			1 IOBC	
	<i>Neoseiulus californicus</i>			3 Koppert	
	<i>Phytoseiulus persimilis</i>			3 Koppert	
Parasitoids	<i>Aphidius colemani</i>			2 Koppert	
Fipronil/Regent					
Flonicamid/Mainman					
Group	Species	Project Data		Data from other sources	
		Acute	Sub-lethal	Acute	Sub-lethal
Predators	<i>Orius tantillus</i>	13%			
	<i>Neoseiulus californicus</i>			1 Koppert	
	<i>Phytoseiulus persimilis</i>	57%		1 Koppert	
Parasitoids	<i>Aphidius colemani</i>	0%	99%	2 Koppert Jansen et al 2011	Jansen et al 2011 sublethal
	<i>Encarsia formosa</i>	6%	31%NS	1 Koppert	
	<i>Trichogramma pretiosum</i>	96%		1.Kahn et al 2017	Kahn et al 2015 sub-lethal

Flubendiamide/Belt					
Group	Species	Project Data		Data from other sources	
		Acute	Sub-lethal	Acute	Sub-lethal
Predators	<i>Micromus tasmaniae</i>				
	<i>Mallada signatus</i>				
	<i>Hippodamia variegata</i>				
	<i>Nabis kinbergii</i>				
	<i>Orius tantillus</i>				
	<i>Neoseiulus californicus</i>			1 Koppert	
	<i>Phytoseiulus persimilis</i>	1%		1 Koppert	
Parasitoids	<i>Aphidius colemani</i>	4%	60%	1 Koppert	
	<i>Encarsia formosa</i>			1 Koppert	
	<i>Diadegma semiclausum</i>	3%	71%		
Hexythiazox/Calibre					
Group	Species	Project Data		Data from other sources	
		Acute	Sub-lethal	Acute	Sub-lethal
Predators	<i>Neoseiulus californicus</i>			1 Koppert	
	<i>Phytoseiulus persimilis</i>			1 Koppert	
Parasitoids	<i>Aphidius colemani</i>			1 Koppert	
	<i>Encarsia formosa</i>			1 Koppert	
	<i>Trichogramma pretiosum</i>			1 Koppert	
Imidacloprid/Confidor					
Group	Species	Project Data		Data from other sources	
		Acute	Sub-lethal	Acute	Sub-lethal
Predators	<i>Micromus tasmaniae</i>	100%		2 IPM Technologies acute larvae 2 IPM Technologies survival to adult	
	<i>Mallada signatus</i>	32% 72% Adults			
	<i>Hippodamia variegata</i>	98% 92%A		3 IPM Technologies acute larvae	
	<i>Nabis kinbergii</i>	98%		3 IPM Technologies acute nymphs	
	<i>Orius sp</i>	88%		3 Van de Veire & Tirry 2003 3 Angeli 2005	
	<i>Neoseiulus californicus</i>	58%		3 BioBest	
	<i>Phytoseiulus persimilis</i>	15%		2 Duso 2008 acute adults 3 Gentz 2010 3 Biobest	
Parasitoids	<i>Aphidius colemani</i>	10%	68%	3 Ketabi 2014 adults 3 Biobest	
	<i>Encarsia formosa</i>	73%		3 Biobest acute	

				3 Cotton management guide (Eretmoceras)	
	<i>Trichogramma pretiosum</i>	83%		3 Biobest	
	<i>Diadegma semiclausum</i>	53%	93%		
Indoxacarb/Avatar					
Group	Species	Project Data		Data from other sources	
		Acute	Sub-lethal	Acute	Sub-lethal
Predators	<i>Micromus tasmaniae</i>			1 IPM Technologies acute larvae	2 IPM Technologies
	<i>Mallada signatus</i>	14% 100%A	13%NS		
	<i>Hippodamia variegata</i>		5% 100%A	3 IPM Technologies acute larvae	
	<i>Nabis kinbergii</i>	16%			
	<i>Orius sp</i>			3 Van de Veire & Tirry 2003 2 Angeli 2005	
	<i>Neoseiulus californicus</i>			1 Biobest	
	<i>Phytoseiulus persimilis</i>			1 Biobest 1 Bostanian 2006	
Parasitoids	<i>Aphidius colemani</i>	57%		2 adult Koppert 3 Biobest 1 Stara et al 2011	
	<i>Encarsia formosa</i>	0%			
	<i>Trichogramma pretiosum</i>			1 IPM Technologies acute adult	
	<i>Diadegma semiclausum</i>	91%			
Milbemectin/Milbeknock 6					
Group	Species	Project Data		Data from other sources	
		Acute	Sub-lethal	Acute	Sub-lethal
Predators	<i>Orius sp</i>			2 Koppert	
	<i>Neoseiulus californicus</i>			4 Koppert	
	<i>Phytoseiulus persimilis</i>			4 Koppert	
Parasitoids	<i>Encarsia formosa</i>			1 Koppert	
NPV/Vivus					
Pirimicarb/Pirimor					
Group	Species	Project Data		Data from other sources	
		Acute	Sub-lethal	Acute	Sub-lethal
Predators	<i>Micromus tasmaniae</i>			1 IPM Technologies acute larvae	

				1 IPM Technologies survival to adult 2 Walker et al., 2007 – treated prey	
	<i>Hippodamia variegata</i>			1 IPM Technologies acute larvae	
	<i>Nabis kinbergii</i>			2 IPM Technologies acute nymphs 1 IPM Technologies survival to adult	
	<i>Orius sp</i>			2-3 IOBC Koppert	
	<i>Neoseiulus californicus</i>			2 Koppert	
	<i>Phytoseiulus persimilis</i>			2 Biobest 2 Koppert	
Parasitoids	<i>Aphidius colemani</i>			3 adult Koppert sp	
	<i>Encarsia formosa</i>			2 Koppert	
	<i>Trichogramma pretiosum</i>			2 IPM Technologies – acute adults.	
Pymetrozine/Chess					
Group	Species	Project Data		Data from other sources	
		Acute	Sub-lethal	Acute	Sub-lethal
Predators	<i>Micromus tasmaniae</i>			1 IPM Technologies acute larvae 1 IPM Technologies survival to adult	
	<i>Nabis kinbergii</i>			1 IPM Technologies acute nymphs 1 IPM Technologies survival to adult	
	<i>Orius sp</i>			2 Van de Veire & Tirry 2003 1 Koppert acute 1 Biobest acute	2 Moscardini et al 2013 sublethal
	<i>Neoseiulus californicus</i>			1 Koppert 2 Biobest	
	<i>Phytoseiulus persimilis</i>			1 Koppert 2 Biobest 2 Duso et al 2008	
Parasitoids	<i>Aphidius colemani</i>			1 Biobest 1 Jansen et al 2011	
	<i>Encarsia formosa</i>			2. Koppert acute adults 1. Biobest pupa	
	<i>Trichogramma pretiosum</i>			1 Biobest Jansen et al 2011 2 IPM Technologies acute adult	
	<i>Diadegma semiclausum</i>			1 IPM Technologies acute adult	
Pyrioxifen/Admiral Advance					
Group	Species	Project Data		Data from other sources	

		Acute	Sub-lethal	Acute	Sub-lethal
Predators	<i>Orius sp</i>			1 Koppert	
	<i>Neoseiulus californicus</i>			1 Koppert	
	<i>Phytoseiulus persimilis</i>			2 IOBC 2 Koppert	
Parasitoids	<i>Aphidius colemani</i>			2 IOBC 1 Koppert	
	<i>Encarsia formosa</i>			2 Koppert	
	<i>Trichogramma pretiosum</i>			2 IOBC 1 Koppert	
Spinetoram/Success Neo					
Group	Species	Project Data		Data from other sources	
		Acute	Sub-lethal	Acute	Sub-lethal
Predators	<i>Micromus tasmaniae</i>	96%			
	<i>Mallada signatus</i>	52%			
	<i>Hippodamia variegata</i>	14%			
	<i>Nabis kinbergii</i>	22%			
	<i>Orius sp</i>	100%		4 Koppert	
	<i>Neoseiulus californicus</i>			4 Koppert	
	<i>Phytoseiulus persimilis</i>			3 BioBest	
Parasitoids	<i>Aphidius colemani</i>	99%		4 Koppert	
	<i>Encarsia formosa</i>			4 Koppert	
	<i>Trichogramma pretiosum</i>	99%			
	<i>Diadegma semiclausum</i>	100%			
Spirotetramat/Movento +Hasten					
Group	Species	Project Data		Data from other sources	
		Acute	Sub-lethal	Acute	Sub-lethal
Predators	<i>Micromus tasmaniae</i>	22		1 IPM Technologies acute larvae	
	<i>Mallada signatus</i>	6 37A	34%		
	<i>Hippodamia variegata</i>	1%		1 IPM Technologies acute larvae	
	<i>Nabis kinbergii</i>			1 IPM Technologies acute nymphs	
	<i>Orius sp</i>	13%		2 Koppert	
	<i>Neoseiulus californicus</i>			3 Koppert	
	<i>Phytoseiulus persimilis</i>			3 Koppert	
Parasitoids	<i>Aphidius colemani</i>	0%		1 Koppert	
	<i>Encarsia formosa</i>	1%		2 Koppert	
	<i>Trichogramma pretiosum</i>			2 IPM Technologies – acute adults.	
	<i>Diadegma semiclausum</i>	1%			
Sulfoxaflor/Transform					
Group	Species	Project Data		Data from other sources	
		Acute	Sub-lethal	Acute	Sub-lethal

Predators	<i>Micromus tasmaniae</i>	100%			
	<i>Mallada signatus</i>	82%			
	<i>Hippodamia variegata</i>	93%			
	<i>Nabis kinbergii</i>	40%			
	<i>Orius tantillus</i>	100%			
	<i>Neoseiulus californicus</i>	38%		1 koppert	
	<i>Phytoseiulus persimilis</i>			1 koppert	
Parasitoids	<i>Aphidius colemani</i>	100%		4 Koppert	
	<i>Encarsia formosa</i>			4 Koppert	
	<i>Diadegma semiclausum</i>	97%			

Azoxystrobin – Amistar 11					
Group	Species	Project Data		Data from other sources	
		Acute	Sub-lethal	Acute	Sub-lethal
Predators	Ladybird larvae spp			1 IOBC	
	Orius			1 IOBC	
	Persimilis			1 IOBC	
Parasitoids	Aphidius			1 IOBC, 1 Koppert	
	Encarsia			1&2 Koppert	
	Trichogramma			1&2 IOBC	
Boscalid – Filan					
Boscalid, Kesoxim – Methyl – Colliss 7 11					
Group	Species	Project Data		Data from other sources	
		Acute	Sub-lethal	Acute	Sub-lethal
Predators	Californicus			1 Koppert	
Parasitoids	Aphidius			1 IOBC	
Bupirimate -Nimrod					
Group	Species	Project Data		Data from other sources	
		Acute	Sub-lethal	Acute	Sub-lethal
Predators	Orius			1&2 Koppert	
	Californicus			1 Koppert	
	Persimilis			1&2 Koppert	
Parasitoids	Aphidius			1 IOBC	
	Encarsia			4 IOBC, 2 Koppert	
	Trichogramma			1 Koppert	
Chlorothalonil M5					
Group	Species	Project Data		Data from other sources	
		Acute	Sub-lethal	Acute	Sub-lethal
Predators	Orius			1 Koppert	
	Persimilis			1 IOBC, 1 Koppert	
Parasitoids	Aphidius			2 IOBC, 1&2 Koppert	
	Encarsia			1 IOBC	
Cyazofamid - Ranman M5					
Group	Species	Project Data		Data from other sources	
		Acute	Sub-lethal	Acute	Sub-lethal
Predators	Ladybird larvae			1 IOBC	
Parasitoids	Aphidius			1 IOBC	
Cyflufenamid - Flute U6					
Group	Species	Project Data		Data from other sources	
		Acute	Sub-lethal	Acute	Sub-lethal
Predators	Green lacewing larvae			1 IPM Tech	
	Ladybird larvae			1 IPM Tech	
	Persimilis			1 IPM Tech	
Parasitoids	Aphidius			1 IOBC	
	Trichogramma			1 IPM Tech	
Cyprodinil+ Fludioxonil Switch 9 12					
Group	Species	Project Data		Data from other sources	
		Acute	Sub-lethal	Acute	Sub-lethal
Predators	Ladybird larvae			2 IOBC	
	Orius			2 IOBC	
	Persimilis			2 Koppert	
Parasitoids	Aphidius			1 Koppert +IOBC	
	Encarsia			1 Koppert +IOBC	
Dimethomorph - Acrobat 40					

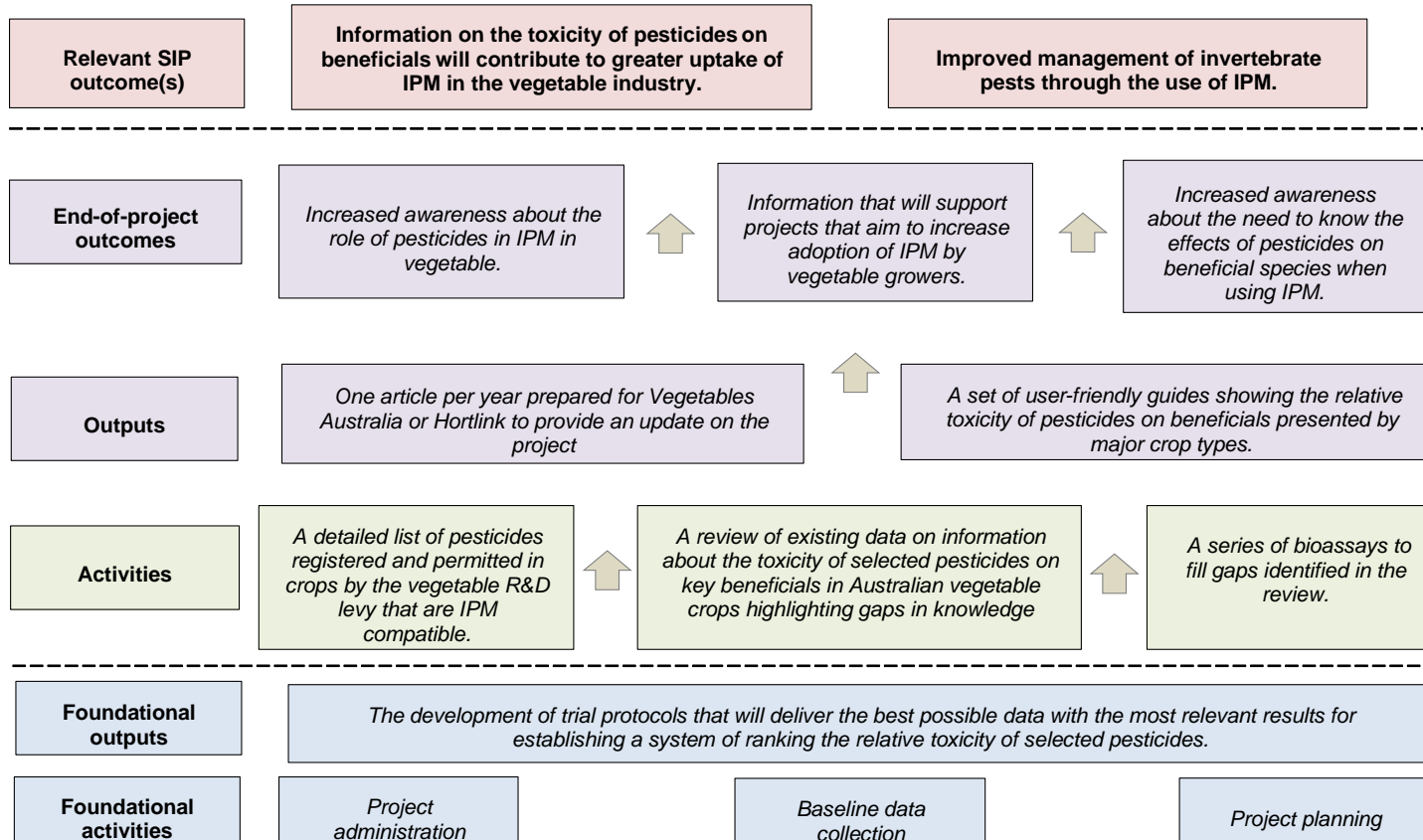
Group	Species	Project Data		Data from other sources	
		Acute	Sub-lethal	Acute	Sub-lethal
Predators	Orius			3 Koppert	
	Persimilis			1 IOBC, 1 Koppert	
Parasitoids	Trichogramma			1 IOBC	
Mancozeb - Y					
Group	Species	Project Data		Data from other sources	
		Acute	Sub-lethal	Acute	Sub-lethal
Predators	Orius			2&3 IOBC 1&2 Koppert	
	Californicus			2 Koppert	
	Persimilis			4 IOBC, 2 Koppert	2 Duso et al 2008
Parasitoids	Aphidius			2 IOBC, 1 Koppert	
	Encarsia			1 IOBC, 1&2 Koppert	
	Trichogramma			4 IOBC, 4 Koppert	
Mandipropamid- Revus					
Group	Species	Project Data		Data from other sources	
		Acute	Sub-lethal	Acute	Sub-lethal
Parasitoids	Aphidius			4 IOBC	
Metalaxyl - Y					
Group	Species	Project Data		Data from other sources	
		Acute	Sub-lethal	Acute	Sub-lethal
Predators	Orius			1 IOBC	
	Persimilis			3 Koppert	
Parasitoids	Aphidius			2 IOBC,	
	Encarsia			1 Koppert	
Metiram - Polyram					
Group	Species	Project Data		Data from other sources	
		Acute	Sub-lethal	Acute	Sub-lethal
Predators	Persimilis			1-2 IOBC 4 Koppert	
Parasitoids	Aphidius			1-2 IOBC 1 Koppert	
	Encarsia			1-4 Koppert	
	Trichogramma			1-4 Koppert	
	Diadegma				
Oxathiapiprolin - Zorvec					
Group	Species	Project Data		Data from other sources	
		Acute	Sub-lethal	Acute	Sub-lethal
Parasitoids	Aphidius			1 IOBC	
Penthiopyrad - Fontelis					
Group	Species	Project Data		Data from other sources	
		Acute	Sub-lethal	Acute	Sub-lethal
Parasitoids	Encarsia	0%			
Propamocarb Hydrochloride + Fluopicolide - Infinito					

Group	Species	Project Data		Data from other sources	
		Acute	Sub-lethal	Acute	Sub-lethal
Predators	Ladybird larvae			1 IOBC	
	Orius			1-2 Koppert	
	Californicus			1 Koppert	
	Persimilis			1 Koppert	
Parasitoids	Aphidius			1-2 IOBC, 1 Koppert	
	Encarsia	18%		1 Koppert	
	Trichogramma			4 IOBC, 1 Koppert	
Triadimenol-					
Group	Species	Project Data		Data from other sources	
		Acute	Sub-lethal	Acute	Sub-lethal
	Orius			1 Koppert	
	Californicus			1 Koppert	
	Persimilis			1 IOBC, 1 Koppert	
Parasitoids	Aphidius			1 IOBC, 1 Koppert	
	Encarsia			1 IOBC, 1 Koppert	
	Trichogramma			1 IOBC, 1 Koppert	
Triadimefon-					
Group	Species	Project Data		Data from other sources	
		Acute	Sub-lethal	Acute	Sub-lethal
	Ladybird larvae			1-2 IOBC	
	Orius			1-2 Koppert	
	Californicus			1 Koppert	
	Persimilis			1 Koppert	
	Encarsia			1 Koppert	
	Trichogramma			1 Koppert	
Zineb					
Group	Species	Project Data		Data from other sources	
		Acute	Sub-lethal	Acute	Sub-lethal
Predators	Orius			1 Koppert	
	Persimilis			1 Koppert	
Parasitoids	Aphidius			1 Koppert	
	Encarsia			1 Koppert	
	Trichogramma			1 Koppert	

Appendix 3. M&E plan

**Section A. Project Monitoring & Evaluation Planning –
TEMPLATE**

1. Program logic



1. Project M&E scope

a) Audience Table 1 M&E audience and their information needs

Audience	Information need
Primary	
Project team IPM Technologies, SARDI, QDAF	Bioassay protocols developed and trialled. Number of bioassays completed. Maintenance and establishment of insect colonies.
Hort Innovation	<i>Milestone reports that outline the progress of the project.</i> Review complete, number of bioassays, completion of guides and delivery of guides to the industry.

i. Key evaluation questions

Table 2 Project key evaluation questions

Key evaluation questions	Relevant?	Project-specific questions
Effectiveness		
1. To what extent has the project achieved its expected outcomes?	Y	<i>To what extent has the project delivered to industry relevant information about the toxicity of pesticides on beneficials?</i>
Relevance		
2. How relevant was the project to the needs of intended beneficiaries?	Y	<i>To what extent has the information provided by this project met the needs of vegetable industry levy payers?</i>
Process appropriateness		
3. How well have intended beneficiaries been engaged in the project?	Y	<i>Were the guides given to the industry for feedback and was this feedback used in development of the final guides?</i>
4. To what extent were engagement processes appropriate to the target audience/s of the project?	N	
Efficiency		
5. What efforts did the project make to improve efficiency?	Y	<i>Were the bioassays completed in a cost-effective way?</i>
Other (if any)		
Include any project-specific question determined important for your project here	N	<i>To what extent has the project developed information that will have a lasting impact on the industry?</i>

b. M&E budget

IPM Technologies: A total of 6 days @ \$1200 per day; 2 days to prepare M&E plan, plus 4 days to collect and synthesise data for mid-term review and final evaluation.

Performance expectations, data collection and analysis (refer to Section B - Guide; parts 3 and 4)

Table 3 Project monitoring plan

Logic level	What to monitor	Performance expectation (KPIs) and/or monitoring questions	Data collection – method (e.g. survey) and source (e.g. growers)	Timing of, and responsibility for, data collection
Foundational activities (list)	<i>Bioassay protocols Field collection of insects for Bioassays</i>	<i>- Initial testing of bioassay protocols - Establishment of insect colonies. - Number of bioassays completed- are we on target? Results of bioassays – are our trial protocols delivering consistent results?</i>	<i>Emails, meetings and phone conference calls between the three organisations.</i>	<i>Monthly or as needed (Project Leader)</i>
Activities and outputs (list)	<i>Annual article for Vegetables Australia and Hortlink.</i>	<i>Did we do it?</i>	<i>Email</i>	<i>Annually (Project Leader)</i>
Intermediate outcomes (list)	<i>Has the project gathered enough information for the successful completion of the guides?</i>	<i>Are the guides user-friendly? Do they provide appropriate information to their intended audience?</i>	<i>Draft guides given to selected members of the industry for feedback.</i>	<i>Final 6 months of the project.</i>
End-or-project outcomes (list)	<i>Completion of guides</i>	<i>Are the guides complete?</i>	<i>Observation Growers, agronomists, advisors, resellers.</i>	<i>As required.</i>

1. Evaluation

Table 4 Additional evaluation data requirements

KEQ	Data collection requirement	Source and method
	'NA'	

Table 5 Independent evaluation studies (as required by Hort Innovation)

Type of evaluation	When (start and finish)
<i>Mid-term evaluation</i>	<i>20/10/2018</i>
<i>Final evaluation</i>	<i>20/04/2020</i>

1. Reporting, learning and improvement (refer to Section B - Guide; part 6)

Table 6 Project progress reporting

Report type	To whom	Timing
<i>e.g. Milestone Reports</i>	<i>Hort Innovation</i>	<i>Six-monthly</i>
<i>e.g. Final Reports</i>	<i>Hort Innovation</i>	<i>At end of project</i>
<i>e.g. Articles</i>	<i>Industry magazine</i>	<i>Annually</i>

Table 7 Project continuous improvement activities

Continuous improvement process	Details	Timing
<i>Team meetings</i>	<i>Meeting between project team members to discuss project trials and their timing.</i>	<i>Quarterly or as needed</i>

Appendix 4. Review of existing data and gaps in knowledge

Table of results from the review highlighting knowledge gaps and potential bioassays.

Parasitoids 1 < 30 % harmless 2= 31% - 79% slightly harmful 3 > 81% harmful potential bioassays

Active	Trade name	<i>Diadegma semiclausum</i>	<i>Aphidius colemani</i>	<i>Trichogramma pretiosum</i>	<i>Encarsia formosa</i>
Permethrin	Ambush	3	3 Biobest	3	3 Biobest, 3 Hassan et al., 1988 acute
Imidacloprid	Confidor		3 Ketabi 2014 adults 3 Biobest	3 Biobest	3 Biobest acute 3 Cotton management guide (Eretmoceras)
Sulfoxaflor	Transform				
Flonicamid	Mainman		1 Biobest, Jansen et al 2011 Jansen et al 2011 sublethal	1.Kahn et al 2017 Kahn et al 2015 sub-lethal	1 Biobest 1 Koppert
Cyantraniliprole	Benevia		3 E-Phy acute		
Spinetoram	Success Neo				
Spirotetramat	Movento			2 IPM Technologies – acute adults.	
Chlorantraniliprole	Coragen	1 IPM Technologies acute Brugger et al 2010 Brugger et al 2010 sublethal	1 E-Phy, Brugger et al 2010, Brugger et al 2010 sublethal	1E-Phy, Brugger et al 2010 Kahn et al 2017 acute and sublethal 2 IPM Technologies – acute adults.	
Indoxacarb	Avatar		2 adult Koppert 3 Biobest 1 Stara et al 2011	1 IPM Technologies acute adult	

Emamectin	Proclaim	3. <i>Haseeb et al 2000</i> <i>Cordero et al 2007</i>	1 E-Phy acute 2 <i>Biondi et al 2013</i> sub-lethal 1 <i>Bengochea 2012</i> sub-lethal	3 IPM Technologies acute adult 2 E-Phy	3. E-Phy, <i>Sugiyama et al 2011</i> Acute 2 Koppert
Pyrethrum	Pyganic		3 Biobest	3 Biobest	
Pyroproxifen	Admiral		1 <i>Van Driesche et al</i>		
Milbemectin	Milbeknock		3 Biobest acute	3 Biobest acute	3. <i>Sugiyama et al 2011</i>
Bifenazate	Acramite		1 Biobest		
Abamectin	Vertimec		3 ?	3 IPM Technologies acute adult	
Pirimicarb	Pirimor		3 adult Koppert sp	2 IPM Technologies – acute adults.	
Pymetrozine	Chess	1 IPM Technologies acute adult	1 Biobest 1 <i>Jansen et al 2011</i>	1 Biobest <i>Jansen et al 2011</i> 2 IPM Technologies acute adult	2. Koppert acute adults 1. Biobest pupa
Fungicides					
Pyraclostrobin	Cabrio EC and WG		1 adults <i>Choi et al</i> 4 mummies <i>Choi et al</i>		
Carbendazim	Bavistin		1 acute and SL		
Mancozeb	Mancozeb		1 (Biobest acute, E-Phy)	2 Biobest acute 2 <i>Hafez 1999</i> sublethal	1. Koppert acute 1. Biobest acute

Predators

Active	Trade name	<i>Orius laevigatus</i>	<i>Nabis kinbergii</i>	<i>Micromus tasmaniae</i>	<i>Malada signatus</i>
Permethrin	Ambush	3 Biobest	3 IPM Technologies	3 IPM Technologies	3 IPM Technologies
Imidacloprid	Confidor	3 <i>Van de Veire & Tirry 2003</i> 3 <i>Angeli 2005</i>	3 IPM Technologies acute nymphs	2 IPM Technologies acute larvae 2 IPM Technologies survival to adult	
Sulfoxaflor	Transform				

Flonicamid	Mainman	2 E-Phy acute 1 Cole et al 2010 acute Cole et al 2010 sublethal			
Cyantraniliprole	Benevia	1 Koppert acute 3 Biobest acute 1 Fernandes et al 2016 sublethal 2 Gontijo et al 2015 sublethal			
Spinetoram	Success Neo				
Spirotetramat	Movento		1 IPM Technologies acute nymphs	1 IPM Technologies acute larvae	
Chlorantraniliprole	Coragen	1 Fernandez et al 2016 acute and sublethal 1.E-Phy acute 1 Broughten et al 2014 acute 3 Vasileiadis et al sublethal	1 IPM Technologies acute nymphs	1 IPM Technologies acute larvae	
Thiamethoxam	Actara	4 Van de Veire & Tirry 2003 2 Fernandes et al 2016 - lab & field - sig effect on fecundity		4 IPM Technologies- treated prey larvae	
Indoxacarb	Avatar	3 Van de Veire & Tirry 2003 2 Angeli 2005	3 IPM Technologies acute nymphs	1 IPM Technologies acute larvae 2 IPM Technologies survival to adult	
Emamectin	Proclaim	3. Koppert acute 3 Studebaker and King 2003 sublethal	3 Cole et al 2010, acute nymphs	1 IPM Technologies survival to adult	
Pyrethrum	Pyganic				
Pyroproxifen	Admiral	1 IOBC 1 Sterk et al adults and nymphs 1 Nagai 1990 - lab - sub-lethal 1 Moscardini et al sub-lethal 2 Simmons&Abd-Rabour - field trials			
Milbemectin	Milbeknock	2 Koppert acute			

Bifenazate	Acramite	1 Ahn et al acute			
Abamectin	Vertimec	4 Van de Veire & Tirry 2003	4 IPM Technologies acute nymphs		
Pirimicarb	Pirimor		2 IPM Technologies acute nymphs 1 IPM Technologies survival to adult	1 IPM Technologies acute larvae 1 IPM Technologies survival to adult 2 Walker et al., 2007 – treated prey	
Pymetrozine	Chess	2 Van de Veire & Tirry 2003 2 Moscardini et al 2013 sublethal 1 Koppert acute 1 Biobest acute	1 IPM Technologies acute nymphs 1 IPM Technologies survival to adult	1 IPM Technologies acute larvae 1 IPM Technologies survival to adult	
Fungicides					
Pyraclostrobin	Cabrio EC and WG	1 Choi et al – acute adult			
Carbendazim	Bavistin	1 Van de Veire et al., 2002			
Mancozeb	Mancozeb				

Predators

Active	Trade name	<i>Coccinella transversalis</i>	<i>Hippodamia variegata</i>	<i>Melangyna viridiceps</i>
Permethrin	Ambush	3	3	3
Imidacloprid	Confidor	3 IPM Technologies acute larvae	3 IPM Technologies acute larvae	
Sulfoxaflor	Transform			
Flonicamid	Mainman	2 E-Phy (related species)	2 E-Phy	1 Jansen et al acute (<i>E. balteatus</i>) 2 Moens et al 2011 sublethal
Cyantraniliprole	Benevia	2 E-Phy (related species)	2 E-Phy	
Spinetoram	Success Neo			
Spirotetramat	Movento	1 IPM Technologies acute larvae		
Chlorantraniliprole	Coragen	1 IPM Technologies acute larvae	1 E-Phy acute 2 Mills et al 2016	

Indoxacarb	Avatar	3 IPM Technologies acute larvae	3 IPM Technologies acute larvae	
Emamectin	Proclaim	1 IPM Technologies acute larvae 2 E-Phy (related species)	1 IPM Technologies acute larvae 3 Koppert acute	2 IPM Technologies acute larvae
Pyroproxifen	Admiral			
Milbemectin	Milbeknock			
Bifenazate	Acramite			
Abamectin	Vertimec	3 IPM Technologies acute larvae		
Pirimicarb	Pirimor	1 IPM Technologies acute larvae	1 IPM Technologies acute larvae	1 IPM Technologies acute larvae
Pymetrozine	Chess	1 IPM Technologies acute larvae	3 IPM Technologies acute larvae	1 IPM Technologies acute larvae
Pyraclostrobin	Cabrio EC and WG			
Carbendazim	Bavistin			
Mancozeb	Mancozeb	2 E-Phy (related species)	2 E-Phy	

Mites

Active	Trade name	<i>Phytoseiulus persimilis</i>	<i>Neoseiulus californicus</i>	<i>Neoseiulus cucumeris</i>
Permethrin	Ambush	3 Samsøe-Peterson 1985 3 BioBest	3	3
Imidacloprid	Confidor	2 Duso 2008 acute adults 3 Gentz 2010 3 Biobest	3 BioBest	3 Broughton reporting on lit.
Sulfoxaflor	Transform			
Flonicamid	Mainman	1 Koppert	1 Biobest	1 Koppert 1 Biobest 1 Yong in Kuk and San Soo kim 2017
Cyantraniliprole	Benevia	3 E-Phy 3 Koppert		3 E-Phy 3 Koppert
Spinetoram	Success Neo	3 BioBest		
Spirotetramat	Movento			
Chlorantraniliprole	Coragen	1 E-Phy	1 E-Phy	1 E-Phy

			1 Yong in Kuk and San Soo kim 2017	
Thiamethoxam	Actara	3 Biobest 3 Koppert 3 Sterk et al	2 Biobest 2 Koppert 1 Veire & Tirry	2 Koppert 3 1 Sang et al
Indoxacarb	Avatar	1 Biobest 1 Bostanian 2006	1 Biobest	1 Stara et al.,2009
Emamectin	Proclaim	3 IPM Technologies	1 Kaplan et al 2014	1 Yong et al 2016 2 IPM Technologies
Pyrethrum	Pyganic			
Pyroproxifen	Admiral	1,3,2 Repeat	2 Kurubal &Ay 2015	1
Milbemectin	Milbeknock	3 Koppert 3 Biobest	3 Koppert 3 Biobest	3 Koppert 3 Biobest
Bifenazate	Acramite	1 Steiner et al	1 Cloyd et al	1 Sang et al
Abamectin	Vertimec	3 IPM Technologies 3 Biobest 3 Blummel&Hausendorf	3 Van de Veire et al., 2002	
Pirimicarb	Pirimor	2 Biobest		
Pymetrozine	Chess	1 Koppert 2 Biobest 2 Duso et al 2008	1 Koppert 2 Biobest	1 Koppert 2 Biobest
Pyraclostrobin	Cabrio EC and WG			
Carbendazim	Bavistin	3 Biobest 3 Blummel&Hausendorf		
Mancozeb	Mancozeb	2 Duso et al 2008 sublethal	1 Castagnoli 2005 sublethal	1Cuthbertson et al 2012 sublethal