INNOVATE AG

SERO-X®

PESTICIDE

Technical Manual

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Contents

Introduction

Sero-X is an outcome of a collaborative research project between Growth Agriculture of Wee Waa NSW and the Cotton Catchment Communities Co-operative Research Centre. This project was instigated and led by Dr Robert Mensah PSM.

Sero-X is a world first natural chemical pesticide. It has a revolutionary set of peptides as the primary active compounds. The active constituent of Sero-X is *Clitoria ternatea* Extract which is the first approved active constituent to contain the bio-active peptides known as cyclotides. Ultra stable peptides like cyclotides meet the challenge of providing environmentally positive and socially acceptable food security to the world's growing population.

Sero-X's active constituent is an extract of *Clitoria ternatea,* commonly known as Butterfly Pea, a plant that has shown to protect itself from a range of pests including arthropods and diseases. The product minimises damage caused by a range of arthropod pests and diseases in a wide range of target food, fibre and foliage crops.

Sero-X and its bio-active compounds are the centre of much global research but brought to you in a product for the very first time, right here in Australia by an Australian regional company. Never before has Australian agricultural research and development had a new active constituent and formulated product pass the regulatory approval requirements and given Australian growers first access to such an exciting new product.

- Safe for Bees and other pollinators – with no observed behavioural changes.
- Leading the field in natural crop protection.
- Regional Australian owned and developed.
- Non toxic to mammals and predatorial arthropods.
- APVMA approved and registered product.
- Controls a broad spectrum of pests with no withholding periods and no residues.
- An organic, non-synthetic extract with high levels of efficacy against phytophagous arthropods.
- Cutting edge research in agricultural applications of bio-active peptides.
- Important tool in resistance management.

SERO-X® PESTICIDE

Allowed Input
Cert. No. 20021

AUSTRALIAN MADE

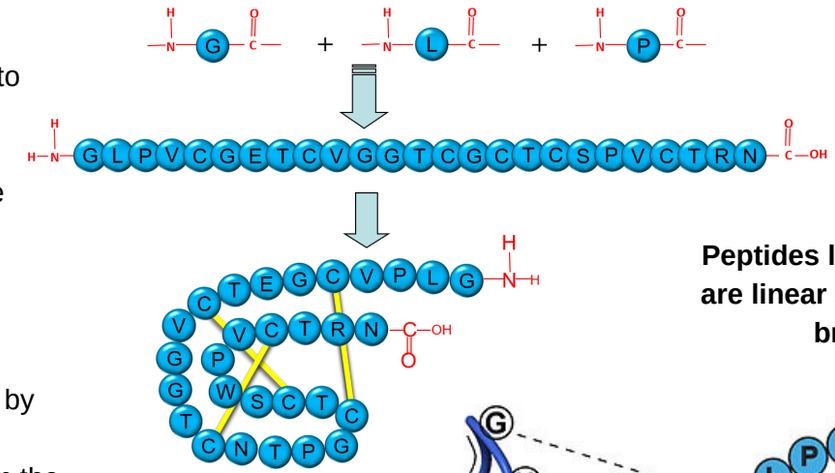
The Actives - Cyclotides

Significant research and investment has gone into investigating the active constituents of Sero-X. The answer lies in a unique group of compounds called cyclotides. The discovery of these compounds and their application to agriculture is set to revolutionise the approach to pest control.

Professor David Craik Director of the Australian Research Council Centre of Excellence for Innovations in Peptide and Protein Science and Fellow of the Royal Society says *"The really great thing about cyclotides is that they are eco-friendly insecticides that are non-toxic to beneficial pollinators such as bees, it's so rewarding to see that peptide-based products are having a real impact in protecting Australia's food and fibre crops"*

Peptides

- Peptides are a small chain of amino acids that aren't quite long enough to be considered a full protein (less than 50 units).
- They are, in essence, the building blocks that create a protein.
- Peptides are commonly used in human health as therapeutics for a wide range of issues.



Peptides look like this - they are linear and susceptible to breakdown.

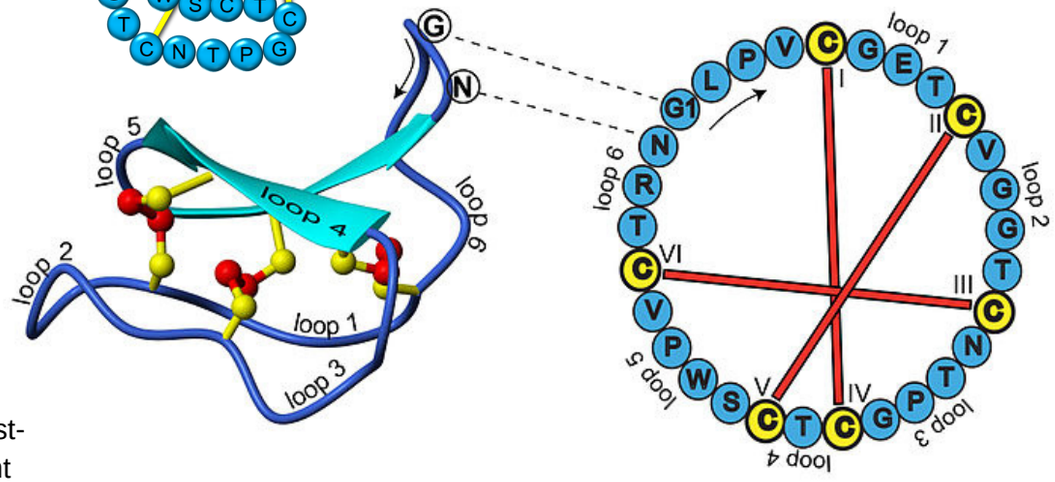
Cyclotides

- Cyclotides are small peptides isolated from plants.
- Cyclotides are exceptionally stable and are resistant to being denatured by thermal, chemical or enzymatic treatments.
- They continue to perform their function after manipulation/extraction from the plant.
- They are defensive peptides that protect against pests.

Biological Significance

Cyclotides have been proven to have a wide range of biological activities, including anti-HIV, insecticidal, anti-tumour, antifouling, anti-microbial, hemolytic, neurotensin antagonism, trypsin inhibition and uterotonic activities.

The potent pesticidal activity of cyclotides indicate that cyclotides act as plant host-defence agents. The observations that dozens or more cyclotides may be present in a single plant and the cyclotide architecture displayed suggest that cyclotides may be able to target many pests/pathogens simultaneously.



Cyclotides look like this - the cyclic structure is comparatively robust and resistant to breakdown.

How Sero-X is Made

The production process of Sero-X is a complex procedure that involves husbanding the *Clitoria ternatea* crop to maximise the expression of the natural pest defence compounds. It is the ultra stable peptides that provide the bioactivity of Sero-X and after the crop has been harvested a specific procedure is undertaken to extract these compounds and formulate the product into an emulsified concentrate.

Being a plant extract there are natural variations from season to season in the amount of cyclotide yielded, however, with our validated methodology for assessing the weight of cyclotides in each batch of *Clitoria ternatea* Extract made – we can insure that each batch is within specification to maintain our quality assurance.

Crop Production

At various locations in regional Australia *Clitoria ternatea* is grown under contract by primary producers.

Harvest and transport

The crop is harvested, baled and transported to the production facility in Goondiwindi QLD.

Extraction/Active Constituent

Chaffed plant material is put through an extraction process that targets the bio-active compounds. These compounds are dissolved into solution creating an emulsified concentrate.

Formulated Product

After pressing and filtering out the inert plant material, the active constituent *Clitoria ternatea* Extract is formed. *Clitoria ternatea* Extract is then formulated into the final product - Sero-X.

Pest Control & Mode of Action

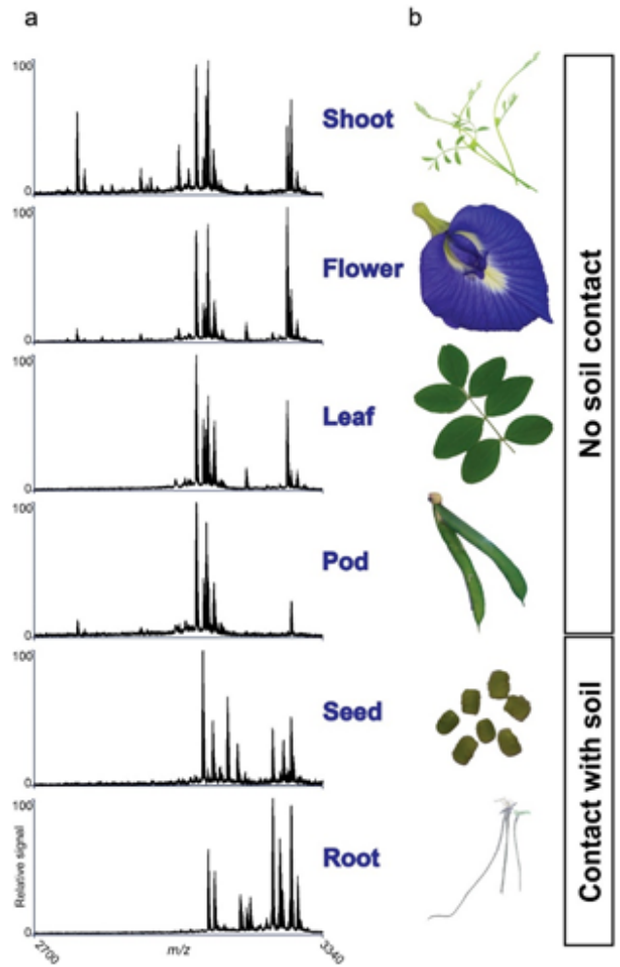
The active constituent in Sero-X is an extract of *Clitoria ternatea* (Butterfly Pea). The bioactive compounds in Butterfly Pea are a group of ultra stable cyclic peptides that have distinct biological activities and perform different roles.

Each of the more than 50 active cyclotides in Butterfly Pea have various roles to play in pest control and are active against different types of pests. They are chemically diverse depending on which part of the plant they come from e.g. leaves, stems, roots or seed.

Clitoria ternatea Extract includes many different biologically active compounds which in combination reduce the economic damage caused by target pests through insecticidal and behaviour modification activity such as egg laying disruption and anti feeding.

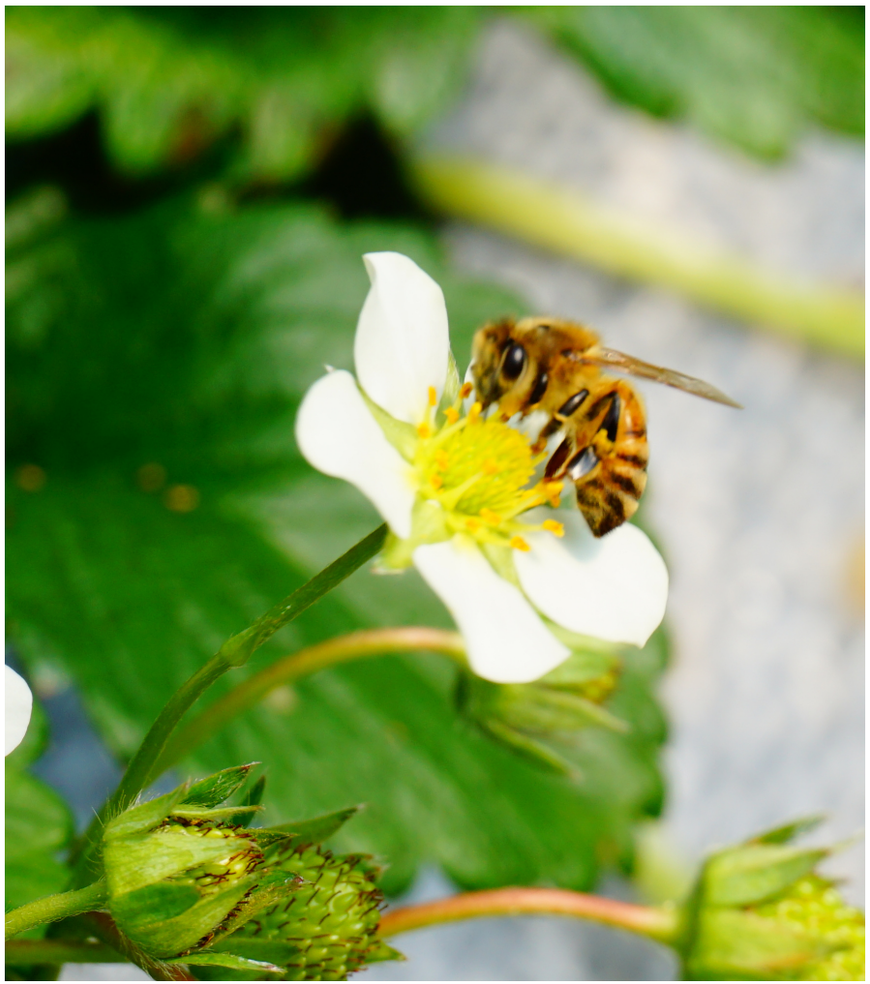
When we test extracts from different plant organs:

1. Those from aerial parts interact with insect-like membranes to disrupt them and inhibit microsclerotia development of *verticillium dahliae*.
2. Those from parts that contact soil are effective against early-stage nematodes.
3. Work continues to identify the specific cyclotides responsible for the pesticidal activity.



These compounds are only active against phytophagous species, that is, it only works against arthropods that feed on plant material and have no toxicity to predatory arthropods and pollinators.

Pest Control & Mode of Action



Sero-X provides control and suppression of pest through a number of distinct modes of action:

1. *Direct Toxicity:* soft bodied-small larvae and nymphs may be killed directly when contacted by the product.

2. *Anti-feedant/Repellence:* the presence of the cyclotides from Sero-X on treated plants deter pest feeding. Pests choose to starve rather than eat a crop treated with Sero-X.

3. *Ovi-position Deterrent:* the presence of the residues of Sero-X on treated plants can deter pest egg lay. Pests may avoid landing or laying eggs on areas treated with Sero-X.

4. *Microsclerotia inhibition:* the presence of Sero-X inhibits the growth and development of the microsclerotia of *verticillium dahliae* – reducing the infection of soils from tissue.

With these multiple modes of action the likelihood of resistance developing amongst target pests is highly unlikely.

In regard to arthropod pest control it can be used as a protective treatment when applied at regular intervals or as a knockdown treatment to control existing pests. This product is suitable for use in a Integrated Pest Management (IPM) program.

Current Label

The label claims for Sero-X are being continually updated. The following label claims are current May 2021 but please scan QR code on page 10 for the latest APVMA approved Label.

KEEP OUT OF REACH OF CHILDREN
READ SAFETY DIRECTIONS BEFORE OPENING OR USING

SERO-X®

INSECTICIDE

Active Constituent:
400 g/L *CLITORIA TERNATEA* EXTRACT

For the control or suppression of green mirids, silver leaf white fly (biotype b) and heliothis in cotton, and suppression of diamondback moth in brassicas as specified by the Directions for Use table.

CONTENTS: 20 L

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CROP	PEST	STATE	RATE	CRITICAL COMMENTS
Cotton	Cotton bollworm (*Helicoverpa armigera*) Native budworm (*Helicoverpa punctigera*) Silverleaf whitefly (biotype b) (*Bemisia tabaci*) Green mirid (*Creontiades dilutus*)	Qld, NSW and WA only	2 L / ha	Apply as indicated by field checks and pest presence thresholds to a maximum of 5 applications per growing season. Ensure good coverage. Note treatment effects may not be seen for 3 or more days. Suppression of pest numbers rather than control may occur if sufficient exposure is not possible. Best results are obtained when Sero-X Insecticide is applied before pest populations build up to damaging levels. Budworm and Bollworm: Applications should be timed to coincide with egg hatch and when small larvae up to 5 mm are present. A second application at 7 days may be required if conditions favour pest development. Silver leaf white fly, Green mirid: Apply at recommended threshold levels as indicated by field checks. A repeat application may be required at 14-20 days if conditions favour pest development.
Cotton	*Verticillium dahliae* microsclerotia	Qld, NSW and WA only	2 L / ha	Applications in the current season will reduce levels of *Verticillium dahliae* microsclerotia in soil following harvest. Mix with water and apply in 50L spray mix per hectare. Three applications are required at the following timing: 1. When majority of plants are between first square and first flower 2. When majority of plants are between mid to peak flowering 3. With the first defoliation
Brassicas	Diamondback moth (*Plutella xylostella*)	All states	2 L / ha	Apply as soon as diamondback moth appears and approach threshold levels. Ensure good coverage with a maximum 7 day retreatment interval to ensure that there is a constant exposure of new larvae to Sero-X.

Water & Dilution Rates

Many years of trials and several years of commercial applications have shown that Sero-X is most effective at 2lt per ha in 100lt.

This equates to a 2% v/v (volume by volume) dilution rate and current research indicates that the dilution/concentration rate is key to optimising the results of Sero-X.

In crops where high water rates are used it is important to maintain a % v/v rate of at least 0.8% (800ml of Sero-X in 100lt of water) however, a target of 2% depending on pest pressure and conditions is preferable where water rates allow.

Example

In 500lt of water 2% v/v = 10lt of Sero-X per ha has shown more consistent efficacy than in 1000lt of water 1%v/v= 10lt Sero-X per ha.

Users are encouraged to consider lowering total spray volume whilst ensuring optimal coverage.

Application

Horticulture

Water rates and Application

The main active compounds in Sero-X are not systemic or translaminar - so thorough coverage is essential.

An application volume of 2lt per 200-300 L/ha for vegetables early stage is recommended. For crops at a later stage of development: for example after head formation, apply Sero-X at 1-2L per 100L of water **(equivalent to 1-2% v/v)**, particularly if pest pressure is high and conditions are favourable for pest development.

Broadacre

Ground Rig

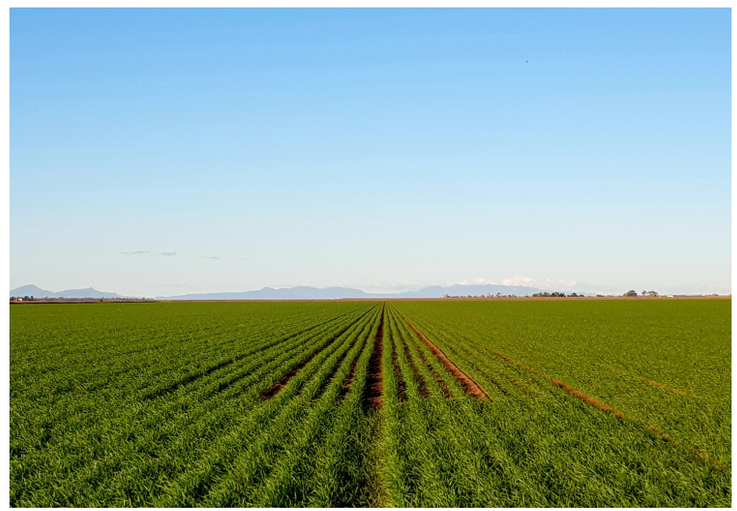
Thorough coverage is essential to ensure adequate control.

Applications should be made using nozzles, pressures and other spray conditions to produce a fine/medium spray. An application volume of 50-200 L/ha is recommended.

Aerial Application

Apply in a minimum of 30 L/ha water. Use appropriate water volume to achieve thorough coverage.

A strategy to minimise spray drift should be employed at all times when aerially applying sprays near sensitive areas. Such a strategy is illustrated by the cotton industry's Best Management Practice Manual.



Withholding period and MRL

The extremely low mammalian and non target species toxicity of Sero-X means there is:

- No maximum residue limit.
- No withholding periods.
- Re-entry permissible as soon as product has dried.

Impact on Non-Target Species

The actives in Sero-X are understood to only have an impact on plant eating arthropods and inhibit the growth of microsclerotia in certain Fungi.

There has been no impact observed on pollinators and either no or extremely low impact observed on predators.

Predatory Beetles	Red & Blue Beetle	VL
	Minute 2-spotted ladybird beetle	M
	Other ladybird beetles	L
	Total	VL
Predatory Bugs	Damsel bugs	VL
	Big eyed bugs	VL
	Other Predatory bugs	-
	Apple Dimpling	L
	Total	VL
Lacewing Adults		VL
Spiders		VL
Hymenoptera	Eretmocerus	-
	Trichogramma	L
	Diadegma	L**
	Total (wasps)	L
	Ants	VH
Toxicity to Bees	*(Established from APVMA Public Release Summary)*	VL
Overall Rating		L

The table is an extract of TABLE 3: Impact of insecticides and miticides on predators, parasitoids and bees in cotton from the Cotton Pest Management Guide 2020-21

This can be viewed at the Cotton Info site here

***Impact rating (% reduction in beneficials following application, based on scores for the major beneficial groups)**

**** Not Established in Table 3 of CPMG**

VL (very low) Less than 10%

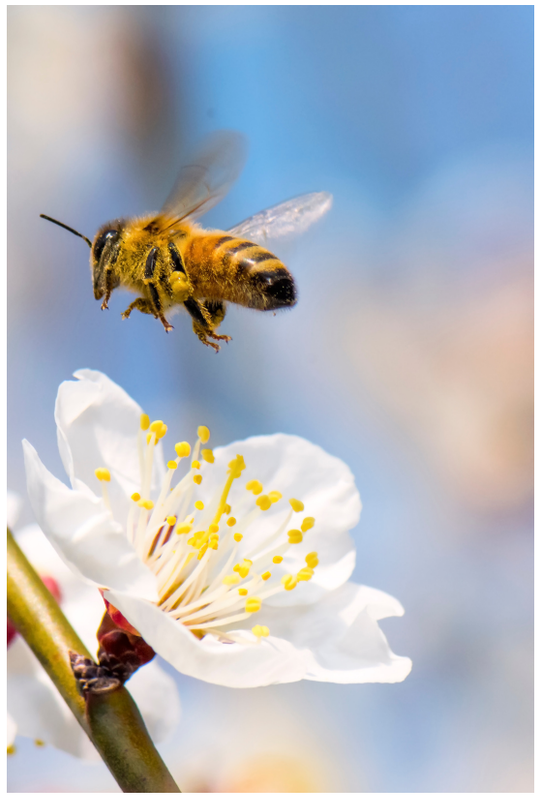
L (low) 10-20%;

M (moderate) 20-40%

H (high) 40-60%

VH (very high) >60%

A '-' indicates no data available for specific local species.



The fact that over a relatively short period of time (7-21 days) - peptides denature into amino acids and do not persist in the environment means chronic impact on pollinators and predators is also not observed.

Current Label & Permits

There are a number of Permits in place for use of Sero-X in crops outside the currently approved label claims.

Please scan the following QR code for links to current label and current permits.

Current Labels

Current Permits

Timing of applications - Arthropods

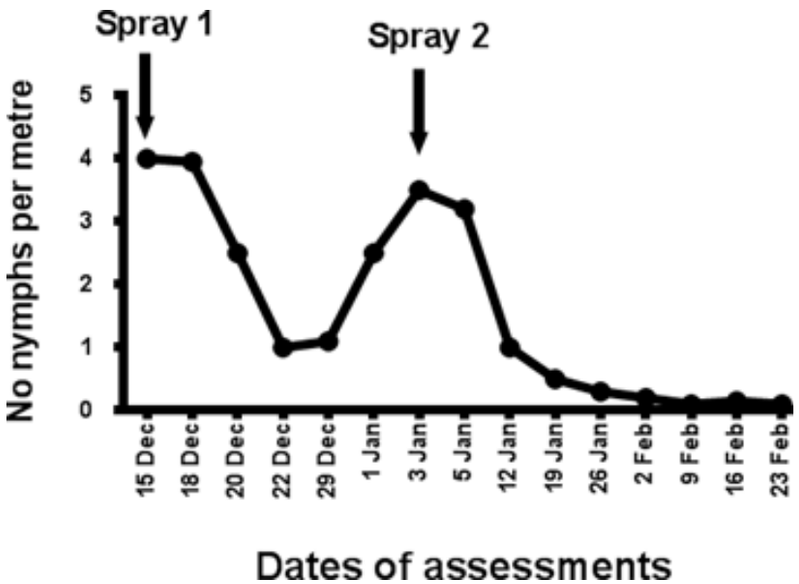
The timing of Sero-X applications is crucial to achieve consistent levels of insect control. The behaviour modification modes of action (repellency/ anti-feeding/ mating disruption) work better before high insect populations develop.

Using Sero-X as a prevention strategy in a consistent spray program is an excellent way to achieve high levels of control.

When used as a remedial in response to insect pressure the presence of adults means eggs are already laid on the crop.

In this situation two consecutive sprays 7-10 days apart may be required, because the eggs may have hatched to nymphs behind spray.

To effectively break the life-cycle a follow-up spray may be required.



In a spray programme

- Consider using as a preventative.
- 10-14 day spray intervals for maximum control.
- Heavy rain may require shortening time frames.

Remedial in response to pest populations

- Apply before high populations of adults develop.
- A second spray may be required after eggs hatch.

What will it do - Arthropods

Summary of efficacy work in Australia – note that extensive testing on these and many other species have been conducted with our European Partners Bi-PA.

INNOVATE AG

- *Helicoverpa spp.*
- Green Mirids
- Silver Leaf Whitefly
- Diamondback Moth

→ Label Claims

bi-pa
Biological Products for Agriculture

- Flower Caterpillar, *Lacebug*
- Thrips (various spp.)
- Mites (2spm and others)
- Aphids (Peach and others)

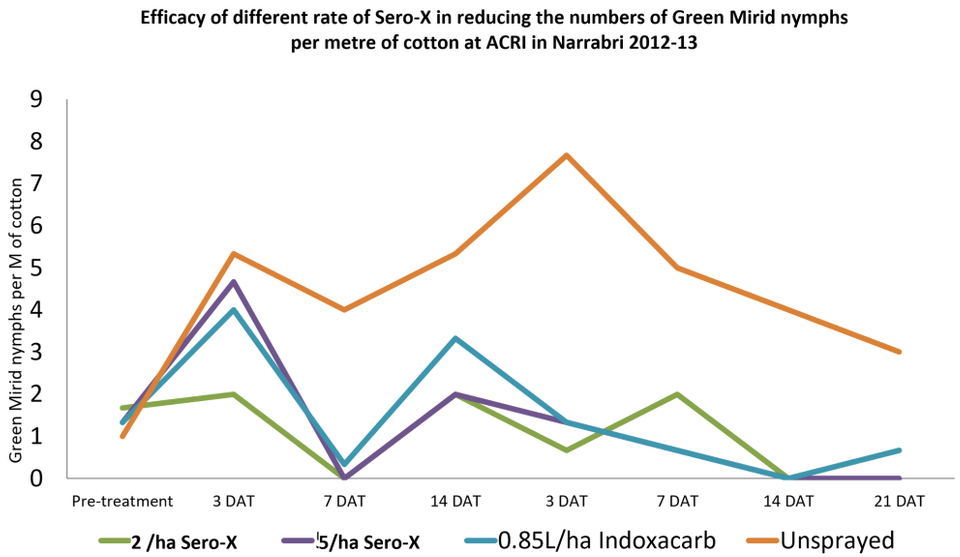
→ Established data. Confident of claim

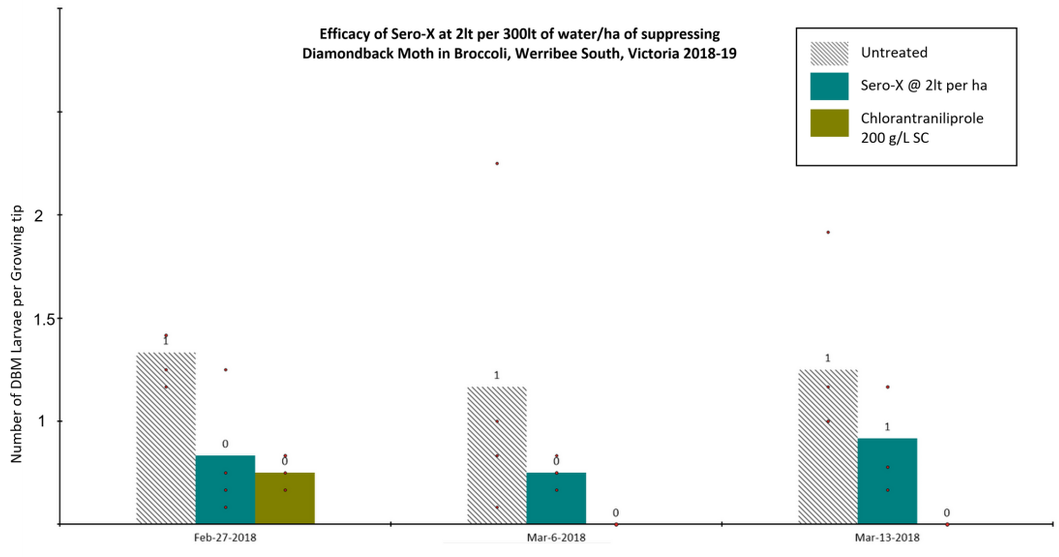
- Fruit Spotting bug
- Fall army worm & Cabbage cluster
- Rutherglen bug
- Seed treatment - soil pests

→ Indications of efficacy – rate & timing work required

Efficacy Example

The following are examples of the types of results that can be achieved using Sero-X. If you have questions about specific trial work on other pests or for more information not presented here please contact us at your convenience.





Green Mirids: With both anti-feedant and direct mortality Sero-X at 2lt per ha in 100lt of water can perform as well as commercial chemical standards.

Apply at recommended threshold levels as indicated by field checks. A repeat application may be required at 14 -20 days if conditions favour pest development.

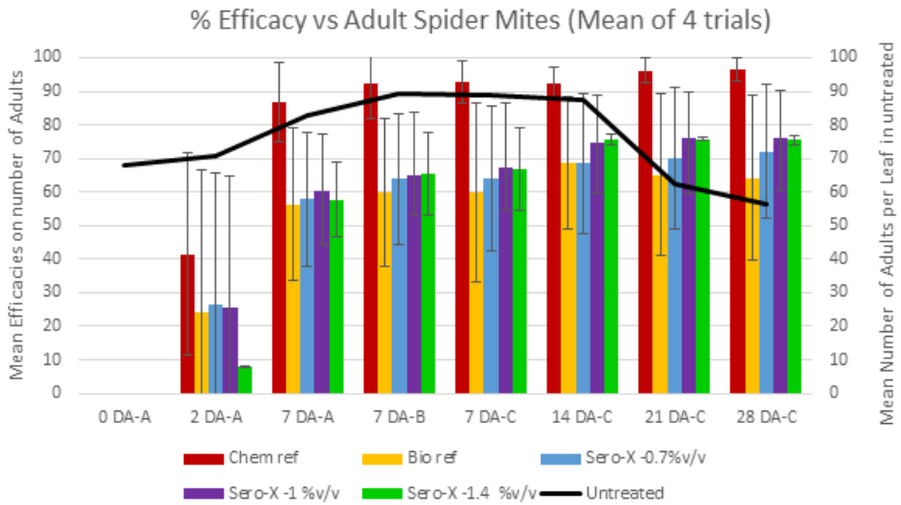
Diamond Back Moth: In trials across QLD and Victoria Sero-X provided effective suppression of DBM evident as reduced larvae and pupae numbers in brassicas.

In some trials Sero-X was also generally equivalent to Chlorantraniliprole 200 SC (200 g/L) at 100ml per/ha for the control of DBM.

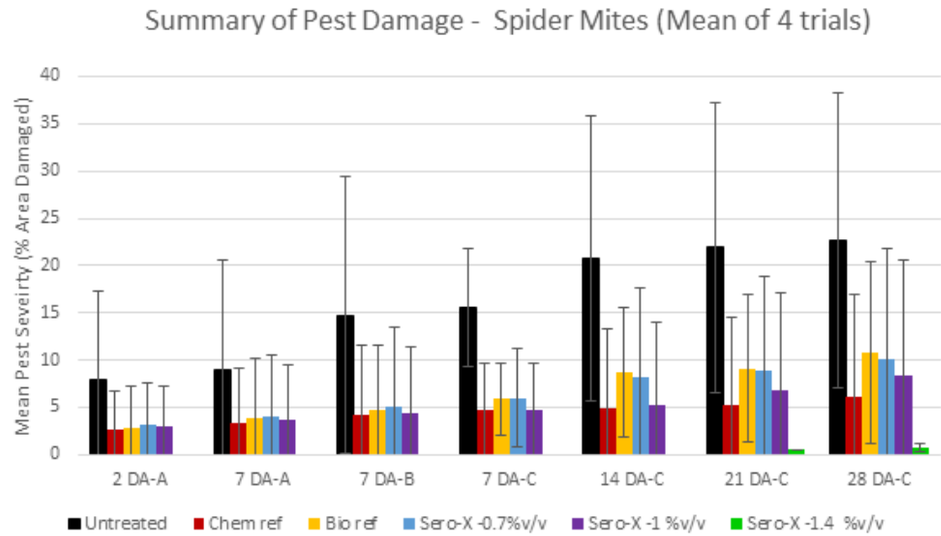
Efficacy Example - Direct toxicity vs pest damage

The following graphs demonstrate an example where direct toxicity against a target pest and the economic damage done by the target pest may not correlate when comparing a chemical standard vs Sero-X with its anti feeding properties.

These are an average of 4 trials with 3 applications 7 days apart of Sero-X and the reference products, conducted in various countries by our international partner Bi-PA



- From 7DA-A all Sero-X rates from 0.7% to 1.4% v/v Sero-X had between 55% and 75% direct toxicity.
- Sero-X was marginally higher than the biological reference, *Beauveria bassiana*.
- Sero-X efficacy was lower than the chemical reference miticide (abermectin or spirodiclofen).

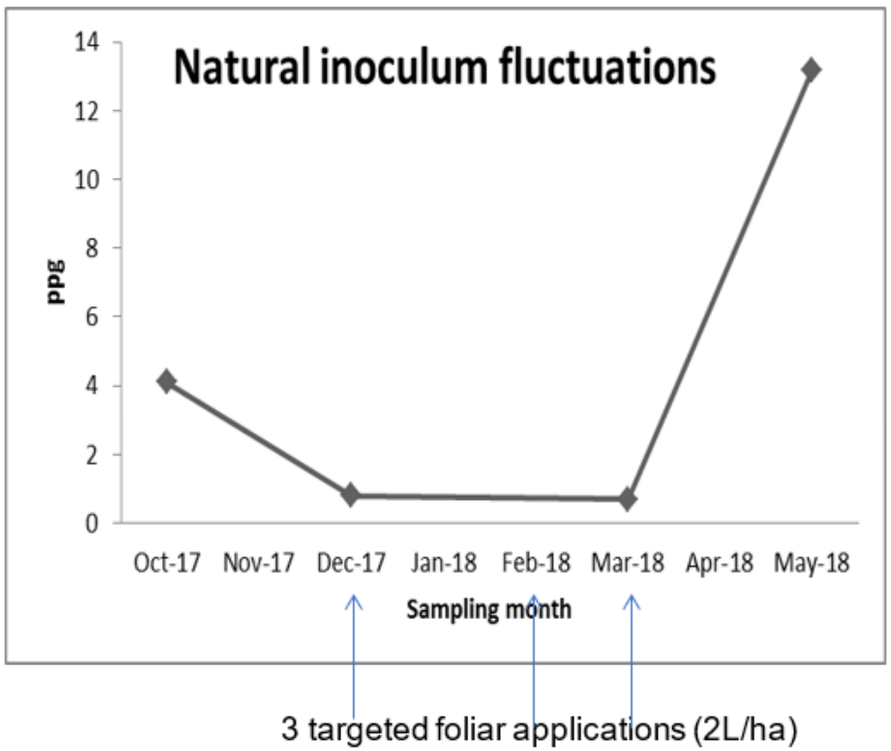


- From 2D-A till 7DA-C two lower Sero-X rates had similar levels of damage to both chemical and biological references.
- 1.4% v/v rate of Sero-X eliminated pest damage until 21 DA-C and was lower damage than the chemical reference.
- **In Sero-X treatments, the pest numbers may not be as low however the economic damage done by the pest may still be reduced further than the chemical reference.**

Timing of Application - Fungicide

To investigate the Verticillium wilt management strategy, foliar sprays were applied at intervals by a ground rig or by aircraft to foliage.

1. December or when majority of plants are between first square and first flower.
2. February or when majority of plants are between mid to late flowering.
3. March or with first defoliation.

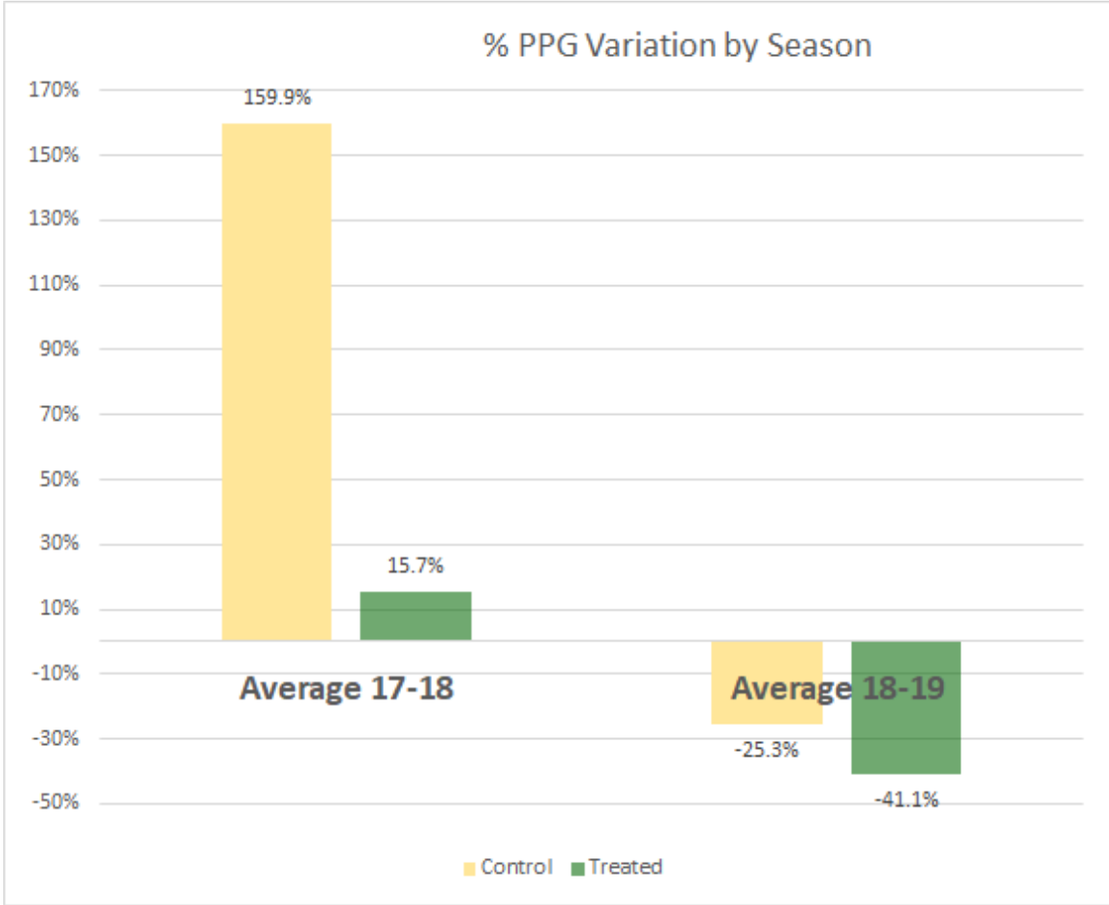


Measurements of inoculum levels (propagules per gram/ppg) were taken pre-plant and again during the growing season and post-harvest.

What will it do - Fungus

Sero-X helps to manage Verticillium wilt by minimising the amount of inoculum that returns to the soil at the end of the season by inhibiting the growth of the microsclerotia that forms on the defoliating plant tissue. No claim has been established for the treatment of the symptoms of Verticillium wilt in-season but it will target the cause of the disease.

In seasons where PPG significantly increases, Sero-X will limit the increase and in seasons where there would be a natural reduction, Sero-X can speed up the reduction by limiting the amount of infected tissue returning to the soil.



Trial Conclusions

Field and laboratory trial data confirm that Sero-X Pesticide containing 400g/L *Clitora ternatea* extract, provides effective suppression of microsclerotia of *verticillium dahliae* in cotton and would assist in management of Verticillium wilt as an alternative to crop rotation.

Cotton Field Research

- Our intent is to monitor more fields across multiple seasons across multiple valleys to further understand the impact of microsclerotial reduction.
- Monitoring of in season reduction of disease incidence and yield impact.
- Innovate Ag are keen to work with industry to develop a long term strategy to routinely monitor PPG levels in the soil - to treat the cause rather than the symptoms of this devastating disease.

General Instructions

Monitoring

Detailed checks of pest numbers as per best practice pest management requirements, are recommended to ensure application can be made at the earliest suitable time to achieve the best result.

Mixing

Shake or agitate the container prior to mixing with water. Add the required quantity of Sero-X Pesticide to clean water in a half filled spray tank with agitator or by-pass in operation.

Maintain agitation while filling tank with remainder of water. Agitation must also be maintained throughout the spray operation.

Storage and handling

Store in the closed, original container in a cool, well-ventilated area. DO NOT store for prolonged periods in direct sunlight.

Compatibility

Sero-X Pesticide is an emulsifiable concentrate and is likely compatible with commonly used organic liquid fertilisers. Always check the physical compatibility with other products using a jar test in the correct proportions.

Whilst physical compatibility is likely with commonly used fungicides and insecticides, chemical compatibility has not been tested.

Surfactants

Sero-X Pesticide contains a surfactant. Additional surfactant such as esterified vegetable oils may only be necessary on hard to wet plants. Use as per surfactant label instructions.

Collaborative Research & Future Work

Collaborative Research Projects

Peptides and particularly stable cyclic ones are a new frontier. Innovate Ag in conjunction with The University of Queensland Institute of Molecular Bioscience are at the forefront of this research and we will continue to be involved in this ground breaking and world leading research.

ARC Linkage Grant 1 – 2013-16

The University of Queensland – Institute for Molecular Bioscience & Innovate Ag

- Development of eco-friendly alternatives for crop pest management
- Assisted in achieving AC approval
- Gained understanding of Cyclotide Activity

ARC Linkage Grant 2 – 2021-2024

Eco-friendly pesticides for crop protection - The University of Queensland – Institute for Molecular Bioscience & Innovate Ag

- Continued cyclotide focus including mode of action and fungicidal activity

The ARC Centre of Excellence for Innovations in Peptide and Protein Science (CIPPS)

focuses on:

- Discovering new proteins and peptides,
- Decoding their biological functions, and developing new proteins and peptides to address challenges in health, agriculture and industry.

Innovate Ag is a partner organisation:

- Access to intellectual property in ag space
- Only ag focused partner organisation
- The future of crop protection could be uncovered in this project.

Bi-PA

- International development continues with our European partner Bi-PA