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### HAL R&D project number: VG10104

Project VG10104 is evaluating the use of integrated viral disease management systems for the prevention and/or delay of virus infection in vegetables.

- **Exploring a novel strategy to enhance efficacy of insect pathogens and disrupt cuticle hardening in insects.**

### HAL R&D project number: VG08053

Project VG08053 explored ways to enhance efficacy of insect pathogens and disrupt cuticle hardening in insects.

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# Management of virus diseases in vegetables (Milestone 3).

Capsicum plant severely affected by tomato spotted wilt virus.

**Facilitators:**

Milestone 3 of project VG10104 has recently been completed by Primary Investigator Denis Persley of the Department of Agriculture, Fisheries and Forestry (DAFF) Queensland.

## Introduction

Virus diseases are among the most challenging issues facing vegetable growers today, causing loss in a range of Australian vegetable crops including capsicum, vegetable cucurbits and lettuce.

Sap-sucking insects are the major vectors (carrier) of plant viruses, the most important of which are aphids, whiteflies, thrips and leafhoppers. Plant viruses can also be transmitted via other insects, mites, nematodes, fungi, infected or contaminated seeds, infected pollen, infected vegetative propagating material and/or contact between plants. The intricate relationship between the virus, host plants and vector often makes management systems problematic. As plants cannot be cured once infected, disease control aims to prevent or delay infection.

## About the project

Project VG10104 addressed several aspects of virus disease management in vegetables. Primary Investigator Denis Persley said because a single control method was not an effective strategy in reducing the disease, the project sought to facilitate the vegetable industry in adopting "…integrated viral disease management systems where all appropriate methods are used to reduce economic losses from disease."

The study developed findings attained in Project VG07128, a nationally focused project funded through the vegetable levy which identified the most damaging virus diseases in the Australian vegetable industry.

Intricate ringspots on capsicum leaf caused by tomato spotted wilt virus.

## Major project findings

Capsicum and cucurbit crops in the Gascoyne region of Western Australia have been severely affected by virus diseases over the last three years. The major pathogens were identified as the cucumber mosaic virus (CMV) in capsicums and zucchini yellow mosaic virus in cucurbits.

In capsicums, Brenda Coutts from the Department of Agriculture and Food Western Australia (DAFWA), conducted investigations into CMV – a major cause of crop losses at Carnarvon (Western Australia). When over 2,700 weed and crop samples were tested, the virus was found in weeds, particularly wild mustard, wild radish, sowthistle, wild lettuce, volunteer capsicum plants and discarded fruit.

"Comparison of the CMV strains found indicated that old capsicum crops, rather than weeds, were the most likely source of the virus. Unfortunately, none of the capsicum varieties tested in the field trial were resistant to CMV," Mr Persley said.

Mr Persley noted that disease control would therefore depend on reducing sources of CMV before planting new capsicum crops and managing the spread of aphid populations through farm hygiene.

Tomato spotted wilt virus (TSWV) is the primary cause of loss in greenhouse grown capsicum crops on the north Adelaide plain. The resistance of thrips to insecticides has resulted in frequent crop failures in recent years. According to Mr Persley, resistant varieties had quickly succumbed to the virus and the molecular and biological properties of the resistant-breaking strain are being investigated for alternative sources of resistance.

Vegetable cucurbits, aphid transmitted viruses, particularly papaya ringspot virus and zucchini yellow mosaic virus were found responsible for severe infection in plants.

"Surveys in the Gascoyne region of Western Australia found zucchini yellow mosaic virus in cucurbit crops on nine of 10 properties surveyed. These viruses are dispersed extremely quickly by aphids – the insect needs only to feed on an infected plant for less than one minute to obtain a charge of virus which it then transmits to another plant within the same feeding time," Mr Persley said.

"These viruses can also spread when infected plants contact healthy ones, for example, when damaged by machinery and equipment."

Tolerant varieties were found to be instrumental in reducing crop losses. In field trials at Gatton, Queensland, zucchini varieties tolerant to papaya ringspot virus produced up to 90 per cent of marketable fruit, where highly susceptible varieties had produced only 20 per cent of saleable fruit.

## Conclusion

Mr Persley said while no single method was likely to provide perfect control of the virus, he recommended a combination of management options to contain the disease.
"To minimise disease levels and yield reductions, growers should destroy harvested, infected crops prior to planting new crops," he said.
"New crops should be planted upwind from existing crops to reduce insect vector movement. Growers should also separate crops to maintain some distance and restrict insect movements between crops."
"Very encouraging results are currently being obtained in commercial capsicum crops in Virginia, South Australia, using biological control of thrips producing a major reduction in TSWV losses over the long cropping period," he said.
"This multi-discipline approach should provide a sustainable, profitable production system for growers."

### THE BOTTOM LINE: VG10104

- Plants cannot be cured once infected by a virus, but management methods can prevent or delay the infection.
- A key aspect of virus disease management is to accurately identify the virus causing the disease and then implement appropriate management strategies.
- Strategies for achieving virus management include the planting of resistant varieties.

## Acknowledgements

This project has been funded by HAL using voluntary contributions from The Department of Agriculture, Fisheries and Forestry, QLD and matched funds from the Australian Government.

# Exploring a novel strategy to enhance efficacy of insect pathogens and disrupt cuticle hardening in insects.

Crocidolomia parasitoid.

**Facilitators:**

Project VG08053 has been recently completed by Primary Investigator Sassan Asgari of the University of Queensland.

## Introduction

Resistance to chemical pesticides and issues with environmental contamination requires continued research into the use of non-chemical alternatives. Microorganisms employed as microbial control agents provide environmentally friendly alternatives with relatively low risk of resistance development in insect pests.
One of the major immune responses to microorganisms used for biological control of insect pests is the melanisation response, through which the formation of a dark-brown precipitate eliminates the pathogen. The melanin and the intermediate components produced during melanisation are toxic to both host and parasite and must be strictly regulated and localised so to not harm the insect.
Using natural components that can interfere with insect immune responses could be utilised to inhibit insect immune responses and therefore, increase the efficacy of microbial control agents.

## About the project

Project VG08053 studied the functional role of a venom protein isolated from a parasitoid wasp in inhibition of melanisation and its effect in a genetically modified insect and biopesticide.
Primary Investigator Dr Sassan Asgari explained that a particular parasitoid venom protein – Vn50 – has shown significant similarity to a host protein which activates a key enzyme in the melanisation process.
"By binding to this enzyme, instead of the host protein, Vn50 inhibits activation of the enzyme and therefore, no melanisation occurs," he said.
"With reference to the existing literature, we were aiming to increase our understanding of a key control point in melanin formation, by exploring basic insights into insect immunity. More specifically, we were also trying to test its potential value in applied pest control."

## Major project findings

"In this project, the investigators were interested in the melanisation response and the way the venom protein studied influences an enzymatic cascade in the host," Dr Asari said.
"The venom protein blocks one of the important immune responses of insects enabling pathogens to infect and reproduce more efficiently, ultimately causing higher mortality in insect pests."
During experimental procedures, the research team ubiquitously expressed the wasp venom protein (Vn50) in a model insect, which showed inhibition of melanisation in the host haemolymph (blood). The inhibition of activation of a key enzyme in the host haemolymph controls melanisation.
The following key findings were obtained:

- Several haemolymph components from the host bind to the venom protein upon exposure, especially those key enzymes involved in the melanisation cascade.
- The intact venom protein is required for its mode of action and none of the functional components of the protein on their own were able to inhibit melanisation.

- Production of venom protein in a genetically modified insect (vinegar fly) made it vulnerable to a fungal infection due to suppression of its immune system. However, the protein did not have a major effect on the fly's development or on its parasitoid success in parasitism.

In cases when the venom protein was produced by a genetically modified microbial control agent (baculovirus), it enhanced its efficacy, as significantly less of the virus was required to kill the host compared to the wild-type virus. Despite this, it was found that this enhancing effect would not occur in all host-virus interactions.

The results demonstrated the potential of the protein to increase the efficacy of microbial control agents so that fewer are needed to be sprayed in the field.

Dr Asgari said further research was needed into generating genetically modified viruses and testing them against a range of insect pests native to Australia.

"There is a strong possibility that genetically modified pathogens expressing Vn50 protein could be commercially produced and utilised in pest control," he said.

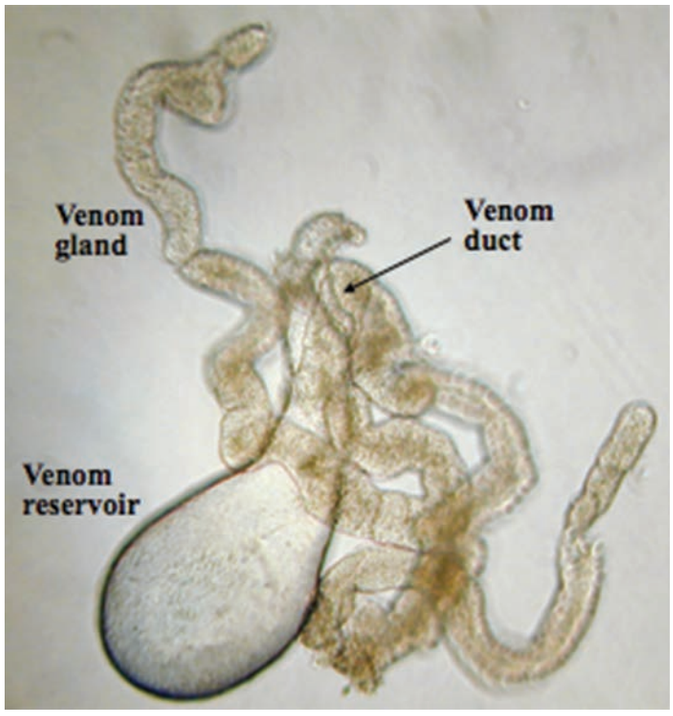
"Currently, many different strains of baculoviruses are commonly produced around the world, including Australia, and used in pest control."

## Conclusion

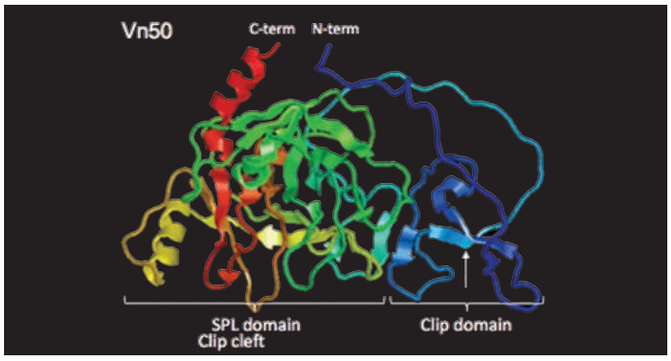
Dr Asgari said the research outcomes provided evidence that immune suppressors could potentially enhance the efficacy of microbial control agents.

"This could be achieved either by producing genetically modified insects that are more susceptible to microbial pathogens or genetically modified microbial control agents that have a superior efficacy over wild-type strains," he said.

Considering the effect of the venom protein in enhancing the efficacy of baculoviruses, Dr Asgari said it was worth constructing genetically modified baculoviruses based on the species present in Australia and testing them against a variety of insect pests.




Wasp Venom Apparatus.




A three-dimensional structure of the Vn50 venom protein.

### THE BOTTOM LINE: VG08053

- The venom protein Vn50 interferes with the immune system of insects, making them more vulnerable to infection by pathogens.
- Although the effect of Vn50 on other hosts, including plants and animals, has not been investigated, the absence of a similar melanisation process in plants and vertebrates suggests that Vn50 may not have any negative effects on these organisms.
- Recombinant viruses can be effectively used with crops expressing *Bacillus thuringiensis* toxins.

## Acknowledgements

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**Photo credits:**

VG10104 photos credit: Denis Persley.
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*Please contact Hugh Gurney at AUSVEG on 03 9822 0388 or email hugh.gurney@ausveg.com.au to submit topics for potential inclusion in future editions of* **vege**notes.

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PO Box 2042, Camberwell West, Vic, 3124
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