Hort Innovation

Final Report

Understanding and managing the role of honey bees in CGMMV epidemiology

Project leader:

Dr Mary Finlay-Doney

Delivery partner:

Northern Territory Department of Primary Industries and Resources

Project code:

VM18008

Project:

Understanding and managing the role of honey bees in CGMMV epidemiology (VM18008)

Disclaimer:

Funding statement:

This project has been funded by Hort Innovation, using the melon 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 978-0-7341-4764-6

Published and distributed by: Hort Innovation

Level 7
141 Walker Street
North Sydney NSW 2060

Telephone: (02) 8295 2300

www.horticulture.com.au

Content

Summary

This project determined the mechanism(s) by which honey bees introduce *Cucumber green mottle mosaic virus* (CGMMV) into healthy cucurbit plants and developed recommendations to manage the transmission of CGMMV by honey bees.

Exposure to CGMMV is a national concern for melon producers and apiarists. CGMMV was first detected in Australia in 2014, and has now been found in the majority of states and territories. CGMMV is a plant virus (Tobamovirus) that infects cucurbits and can cause substantial crop losses. CGMMV is most likely introduced into a crop through infected planting material (seed or seedlings). The virus is mechanically transmitted e.g. using secateurs to prune plants or by driving a tractor through a crop.

Cucurbit crops are almost 100% pollinator dependent, requiring insect pollination for successful fruit set and production. In Australia, honey bees are regularly used to provide managed pollination services. *Cucumber green mottle mosaic virus* is not known to affect bee health. The main concern is that if honey bees are exposed to CGMMV and then moved significant distances within or between states, they may move this highly destructive plant virus to new locations.

We used lab and field trials to investigate CGMMV transmission by pollinating honey bees. We demonstrated that when CGMMV is already present in a crop, honey bees visiting flowers within the crop can transmit CGMMV between infected and healthy plants. This does not represent a significantly greater risk than any other mechanical transfer of the virus. We also demonstrated that if honey bees visit the flowers of CGMMV positive plants and their hive is then moved to a new location, bees foraging at the new location are able to cause CGMMV infection in cucurbit plants. This is the first time this transmission pathway has been confirmed.

CGMMV accumulates in honey bee hives when bees collect nectar and pollen from CGMMV positive plants. Standard hive management practices (spinning off honey, changing frames) reduced the detectability of CGMMV inside bee hives. CGMMV on bees from within the hive was detectable up to one month after the hive had been exposed to CGMMV positive plants. CGMMV was detectable in honey from within the same hives for over 12 months, but the virus was not viable.

Pollen samples from honey bee hives were analysed. Although 151 plant species were identified, we were unable to infer the plant source of the CGMMV detected. We produced an industry a factsheet on the ability of honey bees to transmit plant pathogens. Little is known outside of a limited number of specific pathogens but tobamoviruses and pathogens associated with pollen were identified as potential future concerns.

This project supports the informed management of CGMMV risk associated with the engagement and delivery of pollination services for cucurbit crops. Recommendations are:

For growers:

- Manage your farm biosecurity. Contact your industry representative or local state agency for advice.
- Discuss the CGMMV status of your crop with your apiarist.
- Discuss the CGMMV status of any bee hives that you bring onto your property.
- Ensure hives used on your property have not been exposed to CGMMV within the past month
- Ensure that hive materials (wax frames, honey) are not discarded in your cucurbit production areas.

For apiarists:

- Manage your apiary's exposure to CGMMV by knowing the status of the cucurbit crops you service
- If a hive has been exposed to CGMMV within the past 2 weeks the bees may be able to transfer CGMMV to other cucurbit plants when visiting flowers
- keep concise and accurate records on hives and loads, to enable trace back to determine the source of the disease
- physically separate of loads based on the sites they have worked
- remove CGMMV positive material from hives

- store equipment and consumables on the apiary in such a fashion that bees cannot access it
- honey supers should be separated at the extraction plant and not interchangeable between loads
- hive equipment should be cleaned between loads to ensure all wax and honey debris is removed.

Feedback from industry and government indicates that the recommendations of this project are adequate to practically manage the biosecurity risks around CGMMV. There remain several details we do not know and these may provide avenues for future research and development if desired:

- What is the likelihood of transmission of CGMMV by pollinating honey bees? Experiments in this project identified a 30% chance of transmission within a 24 hour window. Larger scale field trials may allow us to put more statistical confidence around these numbers.
- Can plant variety affect transmissibility of the virus? The field transmission trials in this project were conducted with a commercially available seeded variety. Commercially available cucurbit varieties may differ in their susceptibility to transmission from visiting honey bees.
- Why do bees stop being able to transmit CGMMV from CGMMV positive hive? We do not know the mechanism behind the identified 24hr window for disease transmission by pollinating honey bees.
- When does CGMMV inside the hive stop being viable (<12 months, >6 months)?

Keywords

biosecurity; *Cucumber green mottle mosaic virus*; cucurbits; honey bees; mechanical transmission; pollination; Tobamovirus; viability

Acronyms used in this report

AHBIC	Australian Honey Bee Industry Council
AMA	Australian Melon Association
CGMMV	*Cucumber green mottle mosaic virus*
COVID-19	Coronavirus disease 2019, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)
NGS	Next generation sequencing
PRG	Project reference group
PRSV	Papaya ringspot virus (potyvirus)
VG15013	Improved Management options for *Cucumber Green Mottle Mosaic Virus* (CGMMV) (vegetable levy funded Hort Innovation project, completed. Project Leader Dr Lucy Tran-Nguyen, NT DITT)
VG16086	Area wide management of vegetable diseases: viruses and bacteria (vegetable levy funded Hort Innovation project, running concurrently to VM18008. Project Leader Dr Cherie Gambley, QDAF)
ZYMV	Zucchini yellow mosaic virus (potyvirus)

Introduction

Exposure to *Cucumber green mottle mosaic virus* (CGMMV) is a national concern for melon producers and apiarists. CGMMV was first detected in Australia in 2014 (Tesorio *et al* 2016), and has now been detected in the majority of states and territories. This plant virus is highly destructive and contagious. In Israel it has reduced cucurbit production in affected properties by up to 90% (Darzi et al 2018).

Cucurbit crops are almost 100% pollinator dependent, they require insect pollination for successful fruit set and production. In Australia, honey bees are regularly used to provide managed pollination services to broad acre watermelon cropping. *Cucumber green mottle mosaic virus* is not known to affect bee health. The main concern is that if honey bees are exposed to CGMMV and then moved significant distances within or between states, they may move this highly destructive plant virus to new locations.

Based on previous research conducted in Australia and elsewhere we know that honey bees are able to move CGMMV around in the environment and that the virus can persist in bee hives for at least six months. Two honey bee trials conducted in the Northern Territory under VG15013 found CGMMV in flowers suggesting an introduction by pollinators. As part of VG15013, bee hive products from the Northern Territory and Queensland were tested for the presence of CGMMV both as fragments and as viable virus. All hive products (adult bees and brood, pollen, empty cells and propolis) contained CGMMV. However only pollen, honey and adults were shown to contain viable virus (capable of causing infection in plants). From these results we developed a sampling protocol for the detection of CGMMV in bee hives and recommendations for managing apiary exposure to CGMMV.

Although the CGMMV present in honey bee hives has been shown to remain viable for at least six months post-exposure (VG15013) it is unknown if honey bees are able to transmit the viable virus from inside their hives into clean melon crops. The possibility that hives could carry a highly destructive plant virus with them and transmit it into new areas (for at least six months post virus exposure) is a significant concern, both to melon producers (2019/20 industry value: $152 million) and the apiary industry (2018/19 industry value: $147 million).

The project presented here investigated the epidemiology of CGMMV transmission by honey bees. It has contributed to Objective 2 of the Australian Melon Industry's Strategic Investment Plan 2016-2021 – Protect the viability of the Australian melon industry through efficient pest management systems and biosecurity management. This project also aligns with the vision statement of the Australian Honey Bee Industry Council Strategic Plan 2018-2023 which is to be "a sustainable and profitable Australian honey bee industry which provides food security and market opportunities", and their goal of maintaining strong biosecurity and a healthy bee population.

Methodology

The specific activities we addressed in this project are outlined below.

CGMMV is a serious and readily transmissible plant virus. We upheld stringent biosecurity measures during our research. Full personal protective equipment was worn during field sampling. Sampling equipment was disposable or sterilised after a single use and on-farm biosecurity measures were adhered to. Equipment, surfaces and vehicles were decontaminated.

All transmission trials were conducted at NT government research facilities in the Northern Territory.

1. Field surveillance of current CGMMV status of apiaries in areas known to be affected by CGMMV

Apiaries from known CGMMV affected areas were sampled across three years. Adult bees, brood, pollen, capped and uncapped honey were collected from three hives per apiary (Figure 1). Samples were stored at 4°C until tested for the presence of CGMMV using a range of RT-PCRs and RT-qPCR using methods and protocols developed in VG15013 (see **Appendix 1** for detailed methods).

Figure 1: Honey bee sample collection in the field

2. Can honey bees transmit CGMMV from bee hives to cucurbit plants?

Laboratory experiments

Two experiments investigated the potential for honey bees to transfer CGMMV to cucurbit flowers (Figure 2). Experimental bees were individually collected from the landing board of a honey bee hive into 60mL vials and starved for 2hrs. The vial was then placed on a series of petri dishes which held excised cucurbit flowers. Caution was taken to avoid any physical contact between flowers and the vial.

In the first trial the bee was CGMMV free and there were three flowers – a CGMMV positive flower, a clean experimental flower and a clean control flower. The bee was allowed to feed and walk freely on the three flowers in turn.

In the second trial the bee came from a CGMMV positive hive and there were two flowers - a clean experimental flower and a clean control flower. At the end of both experiments bees, bee visited healthy flowers and the control flowers were tested for the presence of CGMMV.

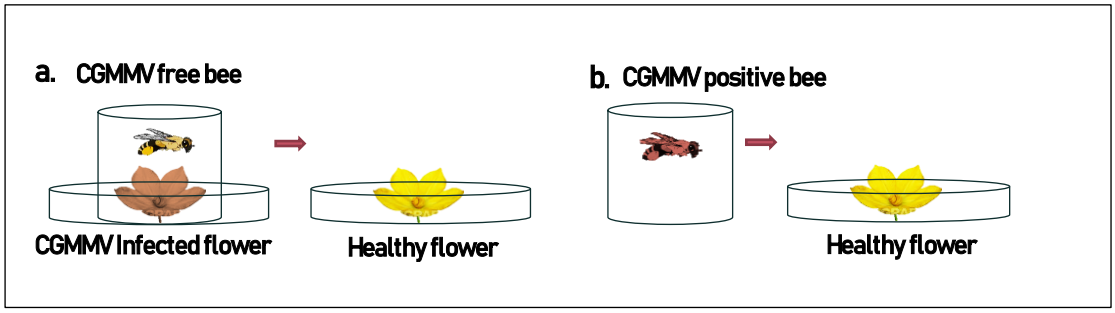


Figure 2: Laboratory experiment to test the potential for CGMMV transmission from (a) a flower infected with CGMMV flower (n = 27) or (b) a honey bee from a hive that has recently been exposed to CGMMV (n = 34), to a healthy flower. Control flowers not illustrated.

Field experiments

Two separate field experiments investigated the potential for honey bees to transfer CGMMV to cucurbit flowers and cause a systemic infection. These experiments followed on conceptually from the lab experiments detailed above. All plants used for the field experiments were grown in 2L plastic pots and trained around bamboo sticks. Following their field exposure to foraging honey bees they were transferred to a biosecure (insect free) glasshouse and grown for 8-10 weeks. During this time they were monitored and tested for the presence of CGMMV.

a) Virus transmission from infected plants to healthy plants

Forty CGMMV infected plants were placed in an open grass area. Five experimental plants (healthy with flowers) and three control plants (healthy without flowers) were placed among the infected plants (Figure 3) and exposed to CGMMV free bee hives. Experimental plants were placed in the field for five consecutive days between 08:00-12:00 and monitored for bee visitation. Outside of these times experimental and control plants were contained in separate mesh cages to avoid further bee visits. The experiment was conducted twice resulting in a total of ten experimental and six control plants.

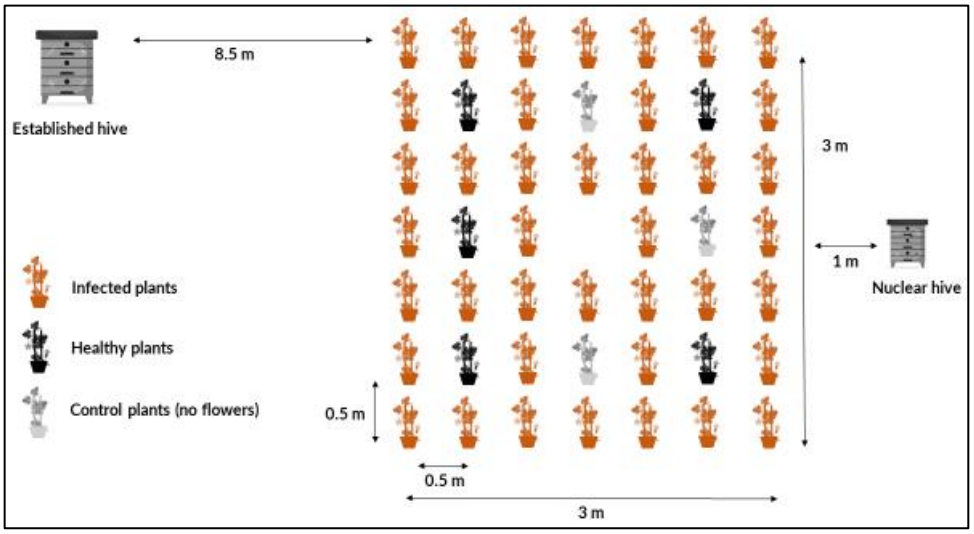


Figure 3: Schematic of field experiment to test the virus transmission from bees foraging between infected plants to healthy plants

b) Virus transmission from positive hive to healthy plants

Ten experimental plants (healthy, flowering) and four control plants (healthy, not flowering) were placed on a horticulture bench in an open grass area. A honey bee hive that had been recently exposed to CGMMV positive plants was placed near the plants (Figures 4 and 5). A total of 40 experimental and 16 control plants were exposed to the bees from 08:00-12:00 noon in four different plant treatments (10 experimental and 4 control plants per treatment). Outside of these times experimental and control plants were stored individually in mesh cages. The treatments were:

1) one day exposure, within 12 hours of hive exposure to CGMMV
2) five day exposure, after 36 hours of hive exposure to CGMMV
3) one day exposure, two weeks after hive exposure to CGMMV
4) five day exposure, two weeks after hive exposure to CGMMV

Experimental plants were observed. The number of flowers open on each day and the number of bee visits received were counted.

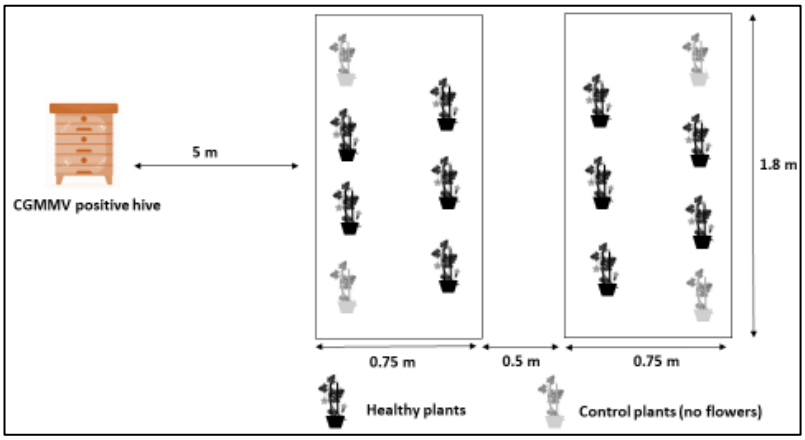


Figure 4: Schematic of field experiment to test the virus transmission from bees from a CGMMV positive hive to healthy plants

Figure 5: Experimental and control plants in the field and growing in the bio secure glass house

3. How long can CGMMV remain viable inside honey bee hives?

Individual portions of honey comb (9x14 cm) from CGMMV positive bee hives were placed in separate containers (Figure 6) and stored in a temperature controlled cabinet (35°C). Honey samples from four cells of each comb were collected monthly for 14 months and stored at 4°C until tested for CGMMV. The viability of CGMMV detected (12, 13 and 14 month) was tested (see **Appendix 2** for plant assay methods, Figure 7).

Figure 6: Portions of CGMMV positive honey comb stored in containers in the temperature controlled cabinet. Uncapped cells show where monthly samples of honey have been collected

Figure 7: Plant viability studies - cucumber plant inoculation and trial set up inside the biosecure glasshouse

4. What role alternative hosts play as a source of CGMMV inoculum in honey bee vectoring of the virus?

Pollen samples (n = 100) were collected from honey bee hives that provided pollination services to cucurbits in CGMMV affected areas in the Northern Territory and Queensland (2017-2021) and analysed using Next Generation Sequencing (NGS) at AgriBio Victoria, Melbourne. Species of plant pollen was identified and correlated with CGMMV presence to infer the role of alternative hosts in providing CGMMV inoculum. This work was conducted in collaboration with research being done in *VG16086 Area wide management of vegetable diseases: viruses and bacteria* (see **Appendix 3** for NGS methods).

5. Do honey (and pollen) extraction practices remove viable virus and, if so, to what extent does this remove the risk of virus transmission from a hive?

Honey and pollen were removed from a hive containing viable CGMMV. The hive was transferred to a resting site where there was no known source of CGMMV and provided with protein and sugar supplements until it established food sources from the natural environment. In the first week adult bees, brood and a swab of the wax comb surface were collected daily. Further samples were collected weekly for a month, monthly for five months and once at 14 months. Honey and pollen samples were collected whenever available. All samples were tested for the presence of CGMMV.

6. Can other mosaic viruses affecting cucurbits (e.g. *Zucchini yellow mosaic virus*, *Papaya ringspot virus type W*) potentially be transmitted by honey bees?

A proof of concept study targeting common cucurbit pathogens that were not of biosecurity concern was undertaken. Samples were collected from 18 apiaries that delivered managed pollination to cucurbits in NT and NSW (2019 - 2021). Three bees were collected from each of three hives per apiary. Extracted bee RNA was tested for the presence of these two potyviruses (see **Appendix 1**).

7. Project management, governance and communication

A start up project meeting was held via teleconference. The project reference group (PRG) was established with members from QDAF, Hort Innovation, Plant and Food Research NZ, NT Farmers Association and melon and apiary industry representatives. PRG members met via videoconference every six months to advise on project activities (see **Appendix 4** for a full list of members).

The management recommendations that were developed from the findings of this project were reviewed by industry bodies, industry representatives and the PRG.

The monitoring and evaluation plan for this project was developed and submitted with MS102. Reporting in line with this plan can be found in the Monitoring and Evaluation section of this report.

8. Project extension

Project updates and extension materials were produced regularly with new information being provided six monthly throughout the life of the project. These materials were primarily distributed through the Australian Melon Association however, where relevant and possible, material was provided to AUSVEG, the Australian Honey Bee Industry Council, bee biosecurity officers and other stakeholders.

This research project would not be possible without the ongoing support that we had from growers and apiarist who allowed us to collect and repeatedly sample from their production systems. These sampling activities provided us with material but also gave us opportunities to discuss the project progress and results with the people who could most benefit from the information. For reasons of privacy we cannot list their names but we are grateful to them all.

Project members participated in multiple industry and scientific meetings. This was most often done virtually due to the impacts of COVID-19. In the NT we were able to attend a number of face to face industry meetings to discuss and share the outputs of our work.

See the Outputs section on the following page for a full list of the extension activities we completed.

Outputs

In accordance with the agreed upon deliverables for this project, the following outputs were prepared and are included in the Appendices.

Fact sheets

1. Bees and *Cucumber green mottle mosaic virus* (**Appendix 5**)
2. *Cucumber green mottle mosaic virus* (CGMMV) and honey bees (**Appendix 6**)
3. The role of honey bees in *Cucumber green mottle mosaic virus* (CGMMV) epidemiology (**Appendix 7**)
4. Pollinating honey bees: do they spread plant diseases? (**Appendix 8**)

Industry newsletters

1. Volunteer apiarists need for research project, *Monthly news*: June/July 2019, Australian Honey Bee Industry Council (**Appendix 9**)
2. Bees and CGMMV, *Melon Enews*: August 2019, Australian Melon Association (**Appendix 10**)
3. CGMMV and Bees, *Melon Enews*: September 2019, Australian Melon Association (**Appendix 11**)
4. Understanding the role of honey bees in CGMMV, *Melon News*: June 2020, Australian Melon Association (**Appendix 12**)
5. CGMMV and Bees webinar, *Melon Enews*: June 2020, Australian Melon Association (**Appendix 13**)
6. CGMMV and Bees (VM 18008) update, *Melon Enews*: February 2021, Australian Melon Association (**Appendix 14**)
7. Managed pollination and the transmission of a plant – what's going on and should we be worried? *Monthly news*: March 2021, Australian Honey Bee Industry Council (**Appendix 15**)
8. Managed pollination and the transmission of a plant virus – what's going on and should we be worried? Territory Rural review: April 2021, Department of Industry, Tourism and Trade, NT Government (**Appendix 16**)
9. *Cucumber green mottle mosaic virus* (CGMMV) and bees project – August 2021 update. Melons Australia website https://www.melonsaustralia.org.au/cucumber-green-mottle-mosaic-virus-cgmmvand-bees-project-august-2021-update/ (**Appendix 17**)
10. CGMMV and Bees Project update, *Melon News*: December 2021, Australian Melon Association (**Appendix 18**)
11. VM18008: CGMMV and bees *AusVeg weekly update* (scheduled December 2021, factsheets and project outputs, not yet published)
12. Special edition: CGMMV and bees *AHBIC Newsletter* (scheduled, factsheets and project outputs, not yet published). Australian Honey Bee Industry Council
13. Article for AusVeg winter 2022 edition (scheduled July 2022). AUSVEG

Stakeholder meetings

1. Project reference group (PRG) meetings: April 2020, October 2020, March 2021, October 2021 (minutes have been provided to Hort Innovation in previous project reports)
2. Dr Mary Finlay-Doney & Dr Darsh Rathnayake had face to face meetings and teleconference conversations

with NT apiarist and melon farmers during the project

3. Dr Mary Finlay-Doney prepared slides for NSW apiarists meeting. Information delivered by Ashley Zamek, Hort Innovation - 2021 04 09 VM18008 NSW apiarists.pptx. April, 2021 (**Appendix 19**)
4. Dr Mary Finlay-Doney attended NTG Honey bee stakeholder meetings in Darwin. January 2021, June 2021
5. Dr Mary Finlay-Doney, Dr Brian Thistleton and Dr Darsh Rathnayake attended the Darwin stakeholder meeting which was held at Berrimah Farm Science Precinct on 22nd November 2021. The meeting was attended by ten Darwin based melon growers and apiarist.
6. NT Farmers/VegNet Katherine meeting scheduled for 30th November 2021. Dr Mary Finlay-Doney and Dr Maxine Piggott to attend. Postponed until Feb 2022 due to COVID-19 lockdowns and lockouts. Ten melon growers & apiarists individually contacted with results.

Webinars

1. Dr Darsh Rathnayake given an CGMMV-bee project update via Ag Webinars 2020: *Cucumber green mottle mosaic* virus bee research on 19th June 2020 (Ag Webinars 2020: Cucumber green mottle mosaic virus bee research - YouTube)
2. Dr Mary Finlay-Doney and Dr Darsh Rathnayake presented the final CGMMV-bee project results via Australian Melon Association (AMA) webinar on 15th November 2021 (Understanding and managing the role of honey bees in CGMMV epidemiology - Webinar - YouTube)

Scientific conferences

1. Dr Darsh Rathnayake presented a talk “Be(e) aware: Can honey bees carry viruses between flowers?” at the joint NT AES/APPS/NAQS seminar day. 24th September 2020 at Darwin Convention Centre
2. Shreya Patel presented a talk “Investigating the role of honey bees in CGMMV epidemiology” at the Australian Plant Pathology Society Conference. 24th November 2021
3. Dr Darsh Rathnayake presented a poster ”Be(e) aware: Pollinating Honey bees can be a vector for CGMMV in watermelons” at the Australian Plant Pathology Society Conference. 25th November 2021
4. Dr Darsh Rathnayake presented a talk “Honey bee assisted transmission of *Cucumber green mottle mosaic virus* (CGMMV) in watermelon” at the Australian Entomological Society Conference. 8th December 2021
5. Dr Mary Finlay-Doney presented a talk “Investigating the role of honey bees in CGMMV epidemiology” at the Australian Entomological Society Conference. 8th December 2021

Information sessions for growers and general public

1. CGMMV and honey bee information table was displayed and attended by Dr Mary Finlay-Doney during Katherine Show from 16th to 17th July 2021. The stall was attended by Katherine based growers, apiarists and general public over the three days.

Figure 8: CGMMV and honey bee information boards set up for the Katherine Show

2. CGMMV and honey bee information table was displayed and attended by Dr Darsh Rathnayake for year 11 Taminmin College students at Berrimah Farm Science Precinct. 24th May 2021.
3. CGMMV and honey bee information table was displayed and attended by Dr Darsh Rathnayake and Shreya Patel at the Department of Industry, Tourism and Trade open day at the Berrimah Farm Science Precinct. 18th May 2021.

Project factsheets and extension material distributed

1. Australian Melon Association – Industry Biosecurity Officer Joanna Embry (hardcopy)
2. Australian Honey Bee Industry Council – AHBIC Chair Trevor Weatherhead
3. NT Farmers – Vegetable Industry Development officer Amélie Corriveau (hardcopy)
4. AusVeg - National Communications officer Shaun Lindhe
5. Hort Innovation extension officers (hardcopy)
    a. Olive Hood - Toowoomba
    b. Sarah Strutt - Townsville
6. NTG biosecurity officers in Darwin and Katherine (hardcopy)
7. National bee biosecurity officers in every State and Territory (https://www.planthealthaustralia.com.au/national-programs/national-bee-biosecurity-program/bee-biosecurity-officers/)

Outcomes

This project addresses farmer productivity, resource use and management within both the current and future Melon Strategic Investment Plans (2016-2021 and 2022-2026). Specifically, the innovative research presented here provides data to show how CGMMV can be spread by honey bees and investigates the potential for managed honey bees to spread other plant diseases. These data underpin:

- best practice management of pollination services for cucurbits, and
- improved industry preparedness and resilience to biosecurity threats

In addition, this project also aligns with the vision statement of the Australian Honey Bee Industry Council Strategic Plan 2018-2023 which is to be "a sustainable and profitable Australian honey bee industry which provides food security and market opportunities", and their goal of maintaining strong biosecurity and a healthy bee population.

The project had the following intermediate and end of project outcomes:

Intermediate project outcomes

1. An understanding of the mechanism(s) by which honey bees move CGMMV into melon plants
2. Better informed business management and government policy for the management of apiaries, melon production and managed pollination

There was an additional outcome identified in the original project outline which was to develop a greater understanding of the potential for other mosaic viruses of melons to be transmitted by honey bees. This included a case study to test bees for the presence of specified potyviruses and a small literature review. This outcome was revised during the course of the project to encompass a much broader literature review. We will discuss this further below.

End of project outcomes

1. A solid understanding of the risk of CGMMV transmission by honey bees during the provision of managed pollination services

Intermediate project outcomes

Mechanism(s) by which honey bees move CGMMV into melon plants

Foraging honey bees are able to cause CGMMV infection in clean plants. The honey bees introduce the virus into healthy flowers, either directly from their bodies, or indirectly through the deposition of CGMMV positive pollen. Laboratory and field experiments confirmed that honey bees are capable of transferring the virus when flying between infected flowers and from CGMMV positive bee hives. CGMMV transmission on to healthy flowers was much lower when bees travelled from a CGMMV positive bee hive compared to when they travelled directly from a CGMMV positive flower (Figures 7 & 9). We conducted field trials to reflect actual commercial growing practices in Australia and to confirm that the virus that we were detecting in the flowers was actually capable of causing disease in living plants.

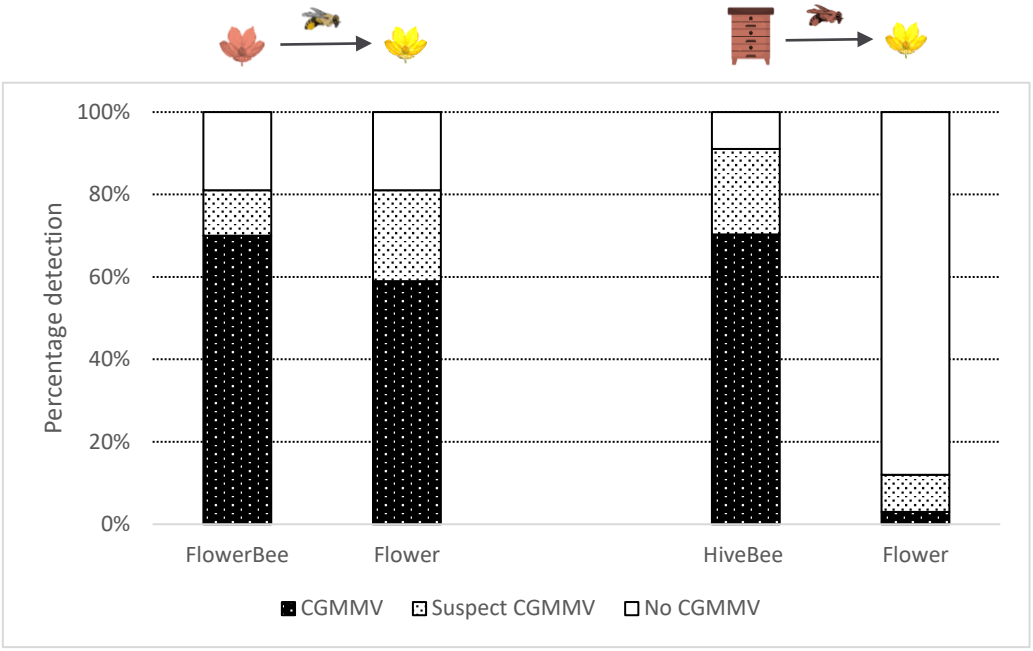


Figure 7: Laboratory trial: transmission of CGMMV by foraging honey bees onto excised healthy flowers. a) from CGMMV positive flowers (left hand side) and, b) CGMMV positive bee hives (right hand side)

In the field when honey bees visited healthy plants directly after foraging on CGMMV infected plants developed visible virus symptoms (Figure 8) within 4 weeks.

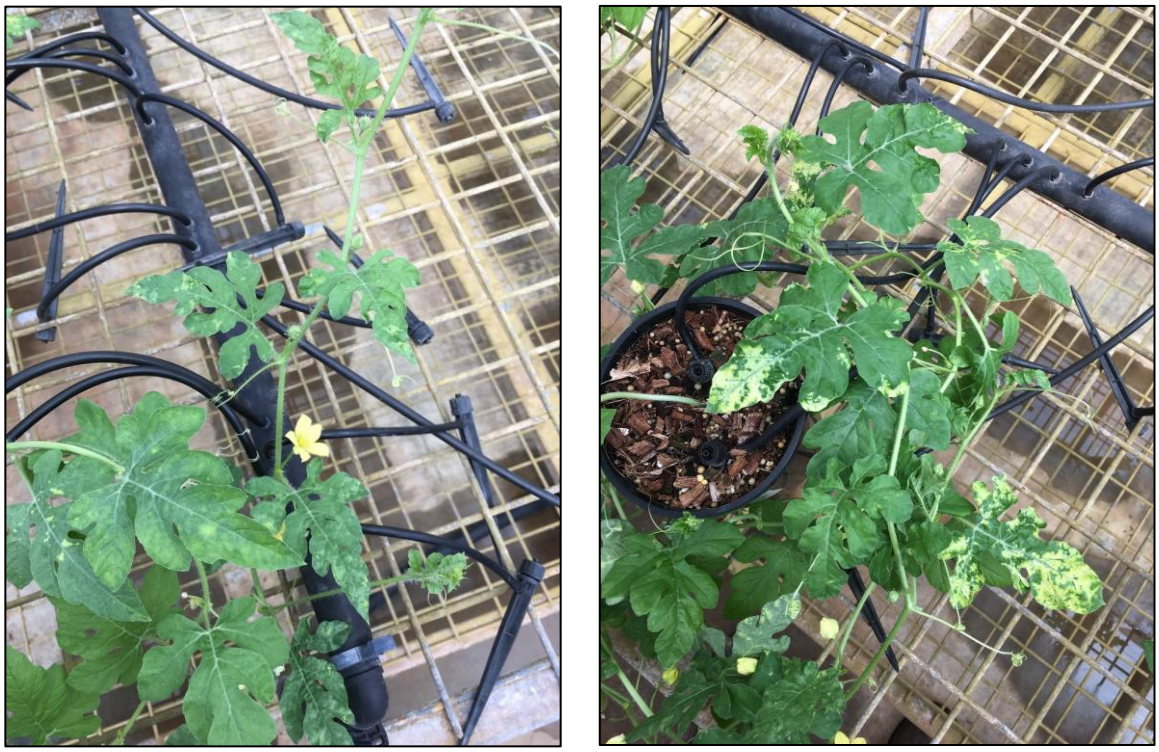


Figure 8: CGMMV symptoms on watermelon plants

When honey bees from a CGMMV positive hive introduced the virus into healthy plants the onset of infection was slower than when the bees introduced the virus directly from a CGMMV positive plant (Figure 9). Virus transferred by CGMMV positive bees was detected in plant leaves, and some growing fruit but no visible viral symptoms were observed over a 10 week period. The single fruit that tested CGMMV positive in this trial grew on a CGMMV negative parent plant, and was aborted before it set seed. Our results demonstrate that CGMMV transmission and plant infection is possible by bees from a CGMMV positive hive but that nature of the transmission is complex.

Figure 9: Developing fruit from a bee visited plant and experimental plants growing in the biosecure glasshouse

One very important finding of this field trial is that the period of time when it is possible for honey bees from a CGMMV positive bee hive to transmit the virus is limited. In our experiments, foraging bees only transmitted CGMMV within the 24 hours of the hives exposure to CGMMV positive plants. Virus transmission (by honey bees) was not observed after this 24 hour window (up to a period of 3 weeks; Figure 8). In these field experiments CGMMV infection was shown to enter healthy plants via both male and female flowers. No relationship was observed between CGMMV infection and the number of bee visits or the number of male or female flowers.

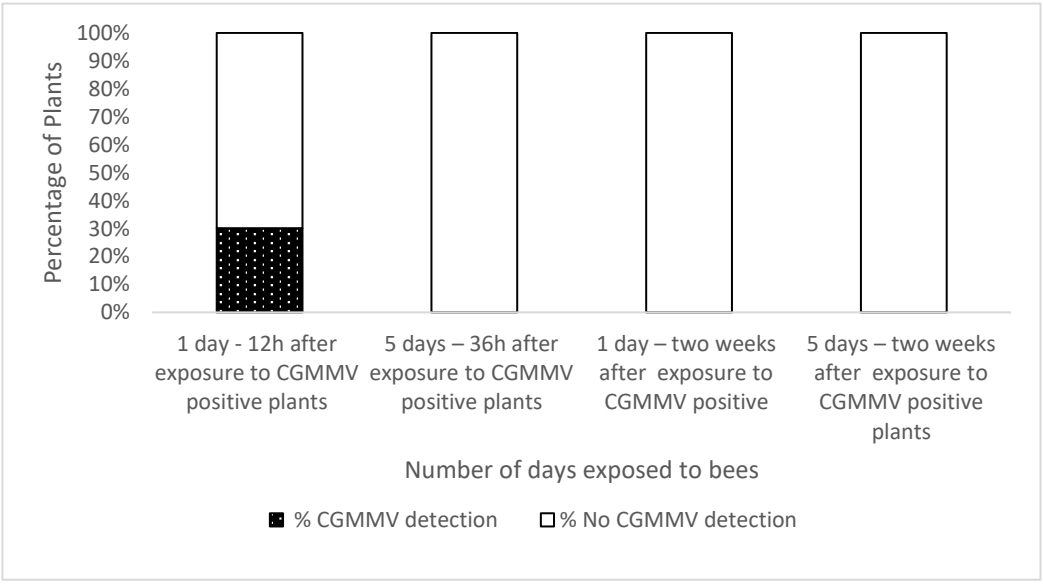


Figure 10: Field transmission trial: percentage of CGMMV infections in plants visited by honey bees from a CGMMV positive hive (n = 10 for each column).

Standard apiary practices involve changing frames, spinning off honey and, sometimes, the removal of pollen from hives at least once a year. Our experiment showed that resting a CGMMV positive bee hive after changing the frames and removing excess honey and pollen is a good practice to reduce CGMMV load. However, we observed that this method does not completely remove detectable CGMMV from within the hives. CGMMV was detectable in honey even after 14 months. We will discuss the management implications of this result further below. This experiment also showed that CGMMV was no longer detectable in the bees from the resting hive after one month (Figure 11). We conclude that there is no way that bees could transmit CGMMV from a CGMMV positive hive after one month of resting.

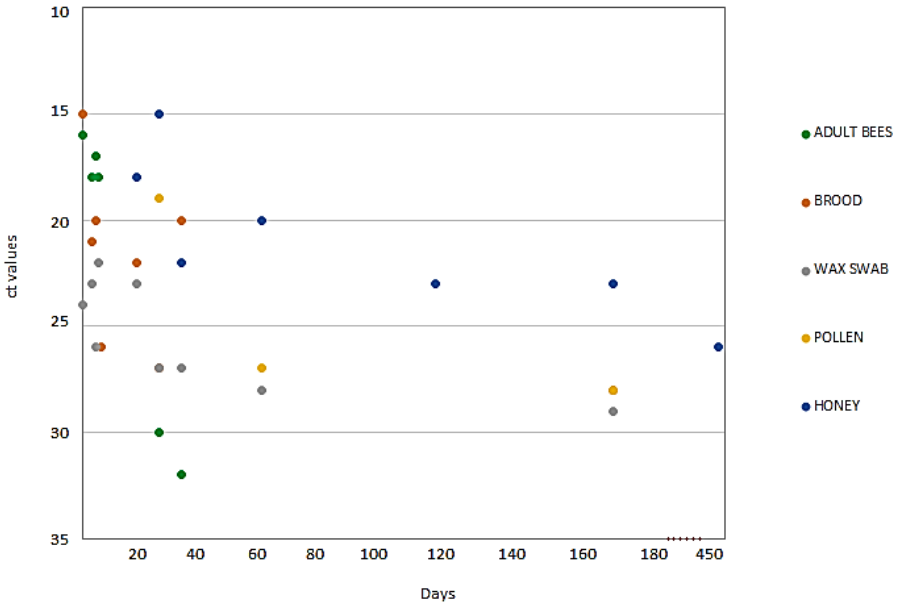


Figure 11: Detection of CGMMV in a bee hive at a resting site (as indicated by ct values from qPCR diagnostics), after exchange of frames and removal of honey and excess pollen

Although CGMMV is detectable in honey within a bee hive on a resting site up to 14 months after exposure to CGMMV positive plants (Figure 9) this virus is not viable. Our temperature controlled cabinet studies mimicked the temperature within a bee hive whilst allowing us to say with certainty that no new CGMMV material had been introduced. We stored honey frames for 14 months and tested samples from 12, 13 and 14 months post exposure to CGMMV positive plants. Molecular results indicated that the honey frames still had high levels of CGMMV in them, but when we inoculated plants with the CGMMV material detected in the honey it did not cause infection. It is clear that the CGMMV detected was not viable. In the previous CGMMV project (VG15013) it was found that CGMMV will remain viable inside a bee hive, for up to six months. Therefore, we conclude that CGMMV inside bee hives can survive between 6-12 months, but the specific point where the virus ceases to be viable is unknown. We know that apiary materials (capped honey, wax frames) can contain viable CGMMV for at least 6 months. Foraging bees themselves do not harbor any detectable CGMMV after 4 weeks and it is safe to use hives that have previously been exposed to CGMMV positive plants for pollination after this time. However, it is important to manage the specific biosecurity risks associated with CGMMV remaining inside the bee hive – it is not advised to work bee hives or spin off honey on a melon production property until 12 months post CGMMV exposure.

Managing the CGMMV risks associated with honey bees includes understanding when and where honey bees are exposed to the virus. VG15013 identified a range of common weeds (**Appendix 20**) that were potential CGMMV hosts. It was unclear if honey bees were encountering CGMMV in commercial cucurbit production or from weeds species, either around commercial production areas or elsewhere. To explore this question we established a collaboration with VG16086 *Area wide management of vegetable diseases: viruses and bacteria exposure* led by Dr Cherie Gambley (Queensland Department of Agriculture and Forestry). Joanne Mackie is a PhD student based at AgriBio Victoria in Dr Fiona Constable's lab and working within VG16086. Joanne conducted metagenomic analysis of the plant species present in bee hives, in the form of pollen. One hundred samples which were collected from honey bee hives as part of VG15013 and VM18008 were analysed. Plant species within these pollen samples were identified. Plant species and CGMMV presence was compared across samples to see if CGMMV presence could be correlated with any particular plant species or group. We particularly looked for plants in the family Cucurbitaceae, or any of those alternative weedy host identified in VG15013 (**Appendix 20**).

A brief summary of the results is provided below (Table 1). A table of plant species identified in CGMMV pollen samples can be found in **Appendix 21**. Fifty four samples were analysed in detail – 26 were CGMMV positive and 28 were CGMMV negative. There were 366 plant species from 72 families identified from this subset of 54 samples. There was massive variability in the plant species identified from each hive and even from pollen cells within the same hive. The number of plant species detected in a single pollen sample ranged from 1 to 38. There were no common species detected across all pollen samples. Only one pollen sample contained a known alternative weed host (caltrop, *Tribulus terrestris*). No cucurbits were identified in this same sample although it was CGMMV positive. The analysis did not find any impact of sample age (2017-2021) on number of plant species identified in a sample. In-depth analysis of these data is ongoing and will be published in collaboration with VG16086.

Our conclusion is that this method is not suitable to identify the source of CGMMV found in honey bees. We cannot be certain that the CGMMV detected in these pollen samples is even associated with any of the plant species identified in these same samples. Honey bee hive sampling in VG15013 has shown that CGMMV is detectable on the surface of the wax comb inside hives. In addition, anecdotal evidence (sampling bee hives) has shown that CGMMV is detected in bee hives that have been on managed pollination of cucurbits and is rarely detected in bee hives that are on native honey flows or resting sites away from commercial cucurbit production areas.

Table 1. Summary of the pollen analysis results from a subset of 54 pollen samples collected from honey bee hives that had provided pollination services to cucurbit crops.

	CGMMV status of pollen sample	
Percentage of samples	**Present (n = 26)**	**Absent (n = 28)**
Contained Cucurbitaceae	58%	75%
Did not contain Cucurbitaceae	41.06%	25%
Contained known alternative weed hosts	0.04 %	na

Understanding of the potential for other mosaic viruses of melons to be transmitted by honey bees

Zucchini yellow mosaic virus (ZYMV) and *Papaya ringspot virus type W* (PRSV-W) were chosen as the target mosaic viruses to address this question. These two viruses are established around Australia and are not of biosecurity concern. Therefore their detection on honey bees that have been providing pollination services for cucurbits does not have any biosecurity implications. The targeted viruses were not detected in the 18 apiary samples that we tested. These samples were collected over 3 years and each sample represented 9 bees. Joanne Mackie also tested for these potyviruses in the 100 pollen samples (collected over 5 years) that she analysed using metagenomics. PRSV-W was detected in 2 samples, ZYMV was not detected at all. There is one published paper in which these two viruses were detected in adult bee samples (from the NT) using high throughput sequencing (Roberts *et al*., 2018). The samples tested in Roberts *et al*., 2018 were collected during a single month in a single year and represent 1400 individuals. Potyviruses are known to be vectored by aphids and there is no evidence that these viruses can be transmitted by foraging honey bees.

A literature review on "Managed pollinators as plant disease vectors: what risk do they pose" is in preparation. This review is a significant increase in scope and complexity from what was originally proposed in the project submission documents. The original topic was a review of the current knowledge of potyvirus distribution in Australia and their known vectors. The new topic, as supported and developed by the PRG was to:

- review plant pathogens known to be transmitted by honey bees
- assess the survival of these pathogens in bees and bee hives, based on currently available information
- outline mechanisms of transmission, both known and hypotheticals
- specifically review high or medium risk pathogens of cucurbits from current industry biosecurity plans and assess the likelihood of their transmission by honey bees

The review will include all pathogens that are known to be spread via honey bees, mechanisms of transmission and the ability of pathogens to survive in bee hives (**Appendix 22**). This review is needed to synthesise a dispersed and fragmented interdisciplinary body of research but it will not provide information that is easily accessible to Australian cucurbit growers. To directly address the needs of Australian cucurbit growers and the apiarists who provide pollination services to them we have summarized the salient information generated in preparing the literature review and prepared a factsheet directly related to cucurbit pests of industry biosecurity concern in Australia (**Appendix 8**).

Better informed business management and government policy for the management of apiaries, melon production and managed pollination

The project outcomes and its implications were discussed with the field experts in government and private businesses during and at the end of the project. Project outcomes and derived management recommendations were accepted by policy makers, scientists and farmers. The results from this research do not necessitate a significant change in practice for melon growers or apiarists in most jurisdictions (see Monitoring and evaluation for further discussion). These results emphasise the importance of good biosecurity management, within the farm or apiary, and provide demonstrated evidence to underpin business decisions that producers affected by CGMMV are already making.

"I think you guys have done such a good job with this project. It is really interesting the results that you are getting" VIROLOGIST, QDAF

"This research informs interstate movement… [it has] really put some rigor behind a question that hadn't been answered before. Congratulations a great job. Well done." INDUSTY DEVELOPOMENT OFFICER, NT

"if they are only transmitting within 12-24hrs of being [exposed to CGMMV positive plants]. I would just take them away to a property, and then just to be extra safe, far away from any melons. For a week to be safe." APIARIST, NT

"when I do it [pollinate watermelons] we would basically only service 1 farm take them away and wouldn't bring them back for a couple of months" APIARIST, NT

"we mark/colour code our hives and have dedicated loads for each farm that we service" APIARIST, NT

Round table discussions) with melon farmers and apiarists during stakeholder meetings (Figure 12) were used to explore issues associated with managed pollination and the practical application of the project recommendations. These discussions were a useful platform for finalising the specific management recommendations and policies for both melon farmers and apiarists. Further, 100% of the meeting attendees acknowledged that after these meetings they had:

a) a better understanding of how honey bees move CGMMV

b) a better understanding of the risk of CGMMV transmission in different circumstances

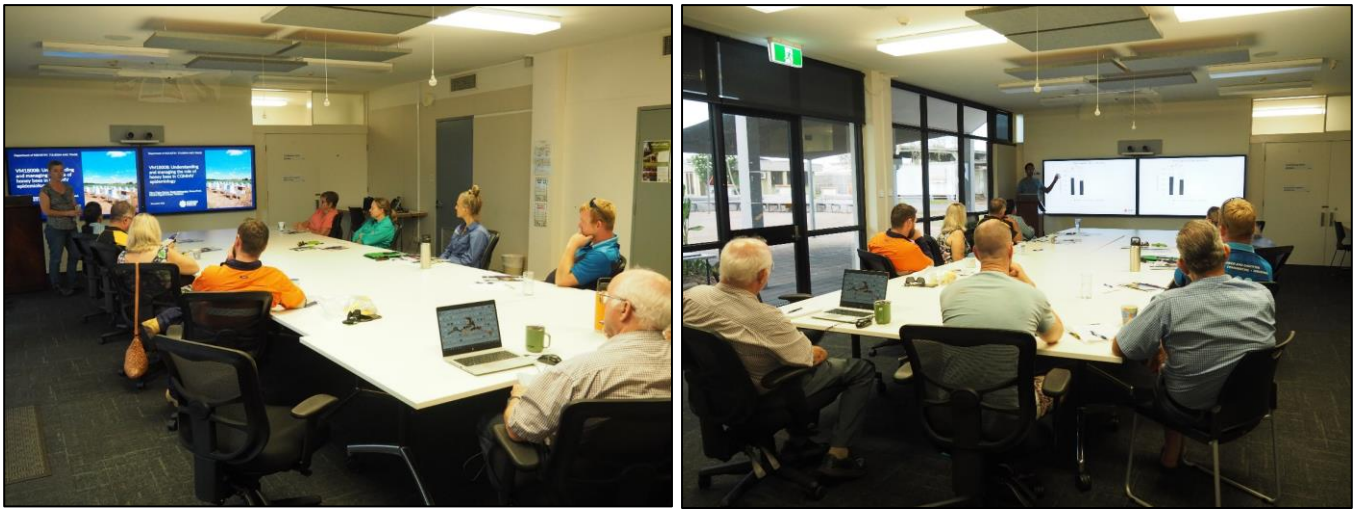


Figure 12: Darwin stakeholder (melon growers and apiarists) meeting at Berrimah Farm Science Precinct

Figure 13: Engagement with apiarists in the field while collecting hive samples

End of project outcomes

A solid understanding of the risk of CGMMV transmission by honey bees during the provision of managed pollination services

Our results indicate that honey bees can introduce CGMMV when pollinating infected and healthy crops at the same time. However, intensity of this transmission is dependent on the availability of the infected plants in the property. The risk of transmission is high if CGMMV infected plants are already present. In this way, honey bees are no different from any other mechanical vector that can spread CGMMV within a site. Early detection and proper removal of CGMMV infected plants remains the most important biosecurity measure to manage CGMMV.

The evidence from this project is that the risk of foraging honey bees introducing CGMMV into new areas when their hives are moved is low. Our field transmission trials showed that the window for transmission of CGMMV by foraging bees from a hive that has recently been exposed to CGMMV positive plants is less than 24 hours. This short window of possibility for transmission has been reported for other honey bee transmitted pathogens, although the length of the time for each specific pathogen varies (Alexandrova *et al*, 2002; Bristow & Martin, 1999; Mink, 1983). Our resting site hive trials showed that CGMMV was no longer detectable on honey bee bodies after one month. Therefore, we recommend at least one month resting of CGMMV positive bee hives at a CGMMV free location to stop CGMMV transmission by honey bees from CGMMV positive hives.

Research in this project and VG15013 has shown that CGMMV can survive in honey inside a bee hive for at least 6 months. We recommend avoiding bee hive management practices (such as hive splitting and honey extraction) while hives are present at a site of cucurbit production. This is because it could be possible to physically infect living cucurbits with viable CGMMV still contained in bee hive materials (such as wax frames or honey) when they are removed from the hive.

Therefore, the recommended management strategies to reduce CGMMV transmission via foraging bees and positive bee hives are:

- proper and early removal of infected plant material from the property
- at least one month break for CGMMV positive hives before introducing to another cucurbit crop, and
- avoid conducting hive management practices for suspected CGMMV positive hives on cucurbit producing properties.

Managed pollinators and their ability to transmit CGMMV are part of broader understanding of managing farm and apiary biosecurity. Honey bees that have been exposed to CGMMV are not the most significant source of CGMMV. It is a plant virus that is most frequently introduced in seed/infected planting material and can be moved in soil, plant material or on contaminated equipment. Apiary equipment (boxes, hive stands) and vehicles could carry CGMMV infected soil/plant material and also need to be managed. These management recommendations should be incorporated into on-farm biosecurity plans, apiary biosecurity plans and communicated to farm staff and visitors. Open communication between cucurbit producers and apiarists is necessary for these management strategies to be effective. Please read Recommendations section of the report to read more specific management practices to avoid CGMMV transmission via bees and positive bee hives.

Monitoring and evaluation

Key Evaluation Questions (Table 2) were developed to guide the project M&E Plan. The M&E Plan was submitted with Milestone Report MS102 in November, 2019. Outputs and outcomes that addressed each KEQ are discussed below.

Table 2: key evaluation questions for VM18008

Key evaluation questions	Project specific questions
Effectiveness	
To what extent has the project achieved its expected outcomes?	To what extent has the project increased the understanding of the mechanism(s) by which honey bees move CGMMV into melon plants? To what extent are business management and government policy better informed for the management of apiaries, melon production and managed pollination?
Relevance	
How relevant was the project to the needs of intended beneficiaries?	To what extent has the project met the needs of industry levy payers? To what extent has the project delivered new information for the melon and honey bee industries?
Process appropriateness	
How well have intended beneficiaries been engaged in the project?	To what extent were the target engagement levels of industry levy payers achieved? Have regular project updates been provided through linkage with the industries?
To what extent were engagement processes appropriate to the target audience/s of the project?	Did the project engage with industry levy payers through their preferred learning style? How accessible were extension events to industry levy payers?
Efficiency	
What efforts did the project make to improve efficiency?	How often were extension activities combined with survey trips?

To what extent has the project achieved its expected outcomes?

The project has successfully identified the specific risks around the spread and introduction of CGMMV and the use of managed pollinators. Honey bees are as capable as any other mechanical vector of moving CGMMV around in an environment where it is already present in live plants. Plant systems where European honey bees have been demonstrated to vector of plant pathogens are very limited. This research provides extensive data on this novel pathway.

The information generated from this project is positive for both the melon and apiary industries. We have demonstrated that the window for CGMMV transmission by pollinating honey bees is short (24hrs). For the majority of Australian cucurbit growing systems the risk of honey bees introducing CGMMV into new areas is relatively easy to manage and can be achieved using current business practices. The outputs of this project allow growers and apiarist to make business decisions based on sound evidence. The impact of this research has been acknowledged by industry.

"It was hell in 2014 and now we know so much more" MELON GROWER, NT

"[this research has] really put some rigor behind a question that hadn't been answered before" INDUSTRY DEVELOPMENT OFFICER, NT

"Your results make sense, which is good…If the [management recommendations] weren't working we would have expected to see [more CGMMV transmission]…We are not seeing in other states the kind of results that suggest that [moving bee hives] is a problem" GOVERNMENT POLICY ADVISOR, QLD

These experimental results were combined and analysed to provide evidence based management options for melon growers and apiarists (see **Appendices 6 and 7**). Electronic and hardcopy versions of these factsheets and extension documents were distributed to the three target industries (melon, vegetable and apiary) and through department channels. Project members attended several industry stakeholder meetings and information sessions (see Outputs for more details) to inform farmers, apiarists and the general public about the level of risk associated with managed pollination and to advise precautions to avoid the risk. The evidence from this project is that the risk of foraging honey bees introducing CGMMV into new areas when their hives are moved is low. This is a beneficial result for industry which is discussed further in the End of project outcomes.

How relevant was the project to the needs of intended beneficiaries?

Prior to this project business and policy decisions about managing the CGMMV biosecurity risk of cucurbit production and the provision of pollination services were being made in an information vacuum. The project outcomes allow private business and government agencies to make informed decisions to manage the CGMMV risk associated with honey bees and the movement of honey bee hives. Our research has shown that pollinating honey bees themselves pose a limited, and relatively easily managed, risk of introducing CGMMV into new environments. The project findings do not require a dramatic change in how melon growers and apiarists manage the delivery of pollination services. Instead, these data provide evidence to support current business practices.

Our work in this and previous projects (VG15013) has shown that CGMMV can remain viable within honey bee hives for at least six months, but that it is no longer viable at 12 months. Therefore bee hive material (frames and honey and pollen contained in them) may pose a risk to introducing CGMMV into new environments for significantly longer than the pollinating bees. This finding highlights the importance of continued on farm biosecurity measures to manage the biosecurity of CGMMV across all farm activities.

AHBIC is preparing a special edition of their newsletter with the results of this project for distribution to apiarists nationally. Trevor Weatherhead, AHBIC chair said the results were reassuring for the managed pollination industry. He did however highlight that there may be some challenges in the Burdekin region (north Queensland, south of Townsville) where apiarists provide pollination services to melons and then move their hives directly on to pumpkins, both of which are cucurbit species. This region has been identified as an important area for further education and extension of these project results in the future by the Australian Melon Association's Biosecurity Officer Joanna Embry.

The project has produced three factsheets containing industry recommendations, all of which are available on the Hort Innovation project page https://www.horticulture.com.au/growers/help-your-business-grow/research-reports-publications-fact-sheets-and-more/vm18008/. There are currently three manuscripts under preparation which will be submitted for peer review in 2022.

"Some very interesting results but also reassuring that the transmission risk for CGMMV is less than what was feared." GOVERNMENT BIOSECURITY EPIDEMIOLOGIST, NSW

How well have intended beneficiaries been engaged in the project?

Stakeholder communications were delivered through industry bodies – Australian Melon Association, Australian Honey Bee Industry Council and AusVeg. The AMA biosecurity officer, Joanna Embry was an essential contact for facilitating the dissemination information to the national melon growing community. Regular national communications were sent through the AMA channels. Locally in the NT we maintained regular informal connections with commercial melon growers and apiarists throughout the life of the project.

The project reference group (PRG) met every six months as scheduled to discuss project progress and outcomes. Industry feedback was useful in targeting effort and tailoring messaging. A specific example was that the scope of

the literature review that had originally been proposed for the project (a review of the current potyvirus distribution in Australia and their known vectors) was revised based on feedback from Dr Cherie Gambley who identified that it had some overlap with work being done in VG16086. Discussions within the PRG identified a much broader area of interest (honey bee transmission of plant viruses generally) and the project team the helped hone that question to focus on high and medium priority biosecurity threats to the Australian cucurbit industry.

For additional details regarding stakeholder engagement please see the response below and the Outputs section in this report.

To what extent were engagement processes appropriate to the target audience/s of the project?

Information about the project was provided in email updates and online webinars. Whilst these modes are flexible and allow people to access the information in their own time we actually had the greatest success in face to face meetings, Project Reference Group discussions and one on one interactions during field work or phone calls. In these direct, personal interactions industry partners and researchers were able to ask questions and provide immediate feedback. These conversations were valuable in shaping the direction of the research and how the researchers communicated their findings.

The target engagement levels were met across all aspects of the project. COVID-19 meant that some interactions were virtual, or via telephone, rather than face to face, but all planned engagement activities went ahead. Field sampling and surveillance was conducted in 2019 (7 sites), 2020 (5 sites) and 2021 (2 sites). Each surveillance event involved two members of the project team meeting with individual apiarists and melon growers in the NT to collect specific samples discuss project progress and current research outcomes. These face-to-face interactions were a valuable way for project members to share and gather information.

We committed to delivering half yearly project updates to the Australian Melon Association and the Australian Honey Bee Association. A list of the updates published in industry newsletters is provided in Outputs.

Project members also attended industry annual meetings and scientific conferences to disseminate the work. Many of these meeting were virtual due to the impacts of COVID-19 (see Outputs p XX for full details). Three end of project stakeholder meetings were organized

- national, Australian Melon Association
- regional, Darwin NT
- regional, Katherine NT

The national stakeholder meeting was delivered as a webinar and organized by the AMA. This meeting was scheduled after business hours in an effort to make it more accessible to growers and promoted nationally through AMA mail outs and phone calls. Although there were 10 registered participants, on the day only 2 people attended. After the webinar we reviewed the situation with Joanna Embry (AMA Biosecurity Officer). We considered what factors may have contributed to low turn out: perceived lack of relevance (growers not thinking it was relevant or as important as other production issues, growers not having a culture of following levy funded research because the fund is young) or lack of time (November is a busy time in the melon growing season nationally) but could not identify any definite reasons. Research on CGMMV transmission has had strong interest in annual Melon Association Conferences. In collaboration with AMA, we have made a concerted effort to communicate project results through other industry channels and in concert with other industry projects (e.g. VG16086). We would recommend carefully considering the suitability of delivering information to the national melon industry via live webinars in the future

The Darwin & Katherine stakeholder meetings were organized by the project team and NT Farmers and were promoted through emails, newsletters and industry webpages. In Darwin, the meeting was well attended. All commercial apiarists and 50% of commercial watermelon growers were present (10 people). Following the presentation and group discussion one hundred percent of attendees said that they had:

a) a better understanding of how honey bees move CGMMV

b) a better understanding of the risk of CGMMV transmission in different circumstances

The meeting was well received and discussions highlighted the importance of a broader understanding of managing farm and apiary biosecurity. Honey bees that have been exposed to CGMMV are not the most significant source of CGMMV. It is a plant virus that is most frequently introduced in seed/infected planting

material and can be moved in soil, plant material or on contaminated equipment. Additional information following on from this discussion was distributed to attendees and incorporated into other stakeholder engagement activities.

“It is great that you have followed up on the discussions from the meeting.” STAKEHOLDER, NT

The Katherine stakeholder meeting was cancelled due to the ongoing impacts of COVID-19 in the Katherine region. It is planned to be rescheduled in early 2022. In the interim project outputs have been emailed or posted to all stakeholders who had been invited to attend. Project leader, Dr Mary Finlay-Doney, phoned ten growers and apiarists directly to discuss the project outcomes and answer any questions that arose.

Direction and feedback from industry representatives was essential for ensuring that project outcomes were delivered clearly and to the relevant people.

“Thank you for asking for my contribution – it is great to synchronise our messaging” INDUSTRY DEVELOPMENT OFFICER, NT

In addition to these stakeholder meetings we also prepared a number of special project summary reports for the Australian Melon Industry, AUSVEG and the Australian Honey Bee Industry Council. These reports will be published nationally in industry magazines, and special editions of newsletters in early 2022.

What efforts did the project make to improve efficiency?

This project was developed based on obstacles identified by industry. The field surveillance was conducted on melon grower’s properties and in close association with apiarists. Throughout the life of the project we maintained regular personal contact with professional apiarist and melon growers within the NT and reached out to stakeholders interstate through the AMA biosecurity officer, VG16086 project members and State and Territory apiary and bee biosecurity officers. Project findings and progress were regularly discussed in informal conversations before being officially communicated. This allowed us to develop and hone our messages for greatest impact/reach.

Recommendations

Management practices for CGMMV require continuous implementation of biosecurity measures

For growers:

- Manage your farm biosecurity. Contact your industry representative or local state agency for advice.
- Discuss the CGMMV status of your crop with your apiarist.
- Discuss the CGMMV status of any bee hives that you bring onto your property.
- Ensure hives used on your property have not been exposed to CGMMV within the past month.
- Ensure that hive materials (wax frames, honey) are not discarded in your cucurbit production areas.

Successful apiary management practices minimise the introduction and possible spread of CGMMV within a

beekeeping enterprise. Management practices aim to:

- prevent or control the introduction of CGMMV into bee hives
- increase the likelihood of being able to trace detections back to the source
- reduce the likelihood of hives becoming a source of CGMMV infection.

The principles of apiary management are:

- using appropriate biosecurity practices to minimise the likelihood of CGMMV introduction, such as not working crops known to be CGMMV-positive and resting hives at least 5km away from known CGMMV-positive sites
- keeping concise and accurate records on hives and loads, to enable trace back to determine the source of the disease
- physically separating loads based on the sites they have worked
- removing CGMMV-positive material from hives, for example, changing frames and spinning off honey immediately after a known exposure to plants infected with CGMMV
- storing equipment and consumables on the apiary so that bees cannot access it
- ensuring hive components are only interchangeable within a load
- making sure honey supers are separated at the extraction plant and not interchangeable between loads
- cleaning hive equipment between loads to ensure all wax and honey debris is removed, typically by using hot water or steam cleaning. Note that although these techniques will remove honey and wax, which may be carrying CGMMV, they have not been demonstrated to decontaminate CGMMV. If possible, have separate equipment for different loads.

Management practices are context-specific and can be developed to suit commercial or individual needs.

- CGMMV is not known to affect bee health or hive strength.
- Ensure you understand how bees and hives are exposed to CGMMV.
- Manage your apiary's exposure to CGMMV by knowing the status of the cucurbit crops you service.
- Make sure you have a clear understanding of how your management systems operate.
- Ensure clear permanent marking and identification of hives (individually or in loads) and their components.
- Keep accurate and concise records for all apiary activities.
- If a hive has been exposed to CGMMV within the past month, the bees may be able to transfer CGMMV to other cucurbit plants when visiting flowers.
- If a hive has been exposed to CGMMV do not conduct hive maintenance on that hive in a cucurbit production area.
- Keep physical separation between loads of hives that possibly contain CGMMV and those that don't contain CGMMV.
- Attend to hives that contain CGMMV last in the workflow, and use separate hive tools and bee-keeping gear for these hives.
- Restrict movement of people, vehicles and animals to any hives you suspect contain CGMMV.

Refereed scientific publications

Journal articles in preparation

Rathnayake, D. N, Patel, S., Piggott, M., Thistleton, B.M, Finlay-Doney, M. (in prep) Honey bee assisted transmission of *Cucumber green mottle mosaic virus* (CGMMV) in watermelon.

Patel, S., Rathnayake, D. N, Finlay-Doney, M., Piggott, M., Thistleton, B.M (in prep) Investigating the role of honey bees in CGMMV epidemiology.

Finlay-Doney, M., Rathnayake, D. N. (in prep) Managed pollinators as plant disease vectors: what risk do they pose?

Mackie, J., Rathnayake, D.N., Finlay-Doney, M., Tran-Nguyen, L., Campbell, P.R., Rodoni, B.R., and Constable, F.E. (in prep) Metatranscriptomic detection of plant and virus species present in hive-stored pollen. *Scientific Reports*.

References

ALEXANDROVA, M., PORRINI, C., BAZZI, C., CARPANA, E., BIGLIARDI, M. & SABATINI, A. 2002. *Erwinia amylovora* longevity in beehives, beehive products and honeybees. *IX International Workshop on Fire Blight* 590, 201-205.

BRISTOW, P. & MARTIN, R. 1999. Transmission and the role of honeybees in field spread of *blueberry shock ilarvirus,* a pollen-borne virus of highbush blueberry. *Phytopathology,* 89**,** 124-130.

DARZI, E., SMITH, E., SHARGIL, D., LACHMAN, O., GANOT, L. & DOMBROVSKY, A. 2018. The honeybee *Apis mellifera* contributes to *Cucumber green mottle mosaic virus* spread via pollination. *Plant Pathology,* 67**,** 244-251.

MINK, G. 1983. The possible role of honeybees in long-distance spread of *Prunus necrotic ringspot virus* from California into Washington sweet cherry orchards.

ROBERTS, J. M., IRELAND, K. B., TAY, W. T. & PAINI, D. 2018. Honey bee-assisted surveillance for early plant virus detection. *Annals of Applied Biology,* 173**,** 285-293.

TESORIERO, L., CHAMBERS, G., SRIVASTAVA, M., SMITH, S., CONDE, B. & TRAN-NGUYEN, L. 2016. First report of *cucumber green mottle mosaic virus* in Australia. *Australasian Plant Disease Notes,* 11**,** 1-3.

Intellectual property, commercialisation and confidentiality

No project IP, project outputs, commercialisation or confidentiality issues to report.

Acknowledgements

This work would not have been possible without the support and cooperation of many people.

We would like to sincerely thank all the growers and apiarists who supported this project, particularly those who allowed us to sample from their apiaries and properties. This research would not have been possible without your ongoing commitment.

The Project Reference Group members provided valuable oversight and suggestions throughout the life of the project.

Joanna Embry, the Industry Biosecurity Office for the Australian Melon Association was a great source of support and open and honest feedback. We also thank Dianne Fullelove who provided industry advice and encouragement (previously with AMA).

Ashley Zamek (R & D Manager, Hort Innovation) provided support. We were grateful for her speedy feedback throughout the project.

NT Farmers provided support for industry engagement.

Lucy Tran Nguyen (NT DITT), Fiona Constable (DJPR) and Cherie Gambley (QDAF, project leader VG16086) gave willingly of their expertise and knowledge. Their technical advice and feedback was greatly appreciated.

Jo Mackie (DJPR) did the seemingly impossible and got species identifications from bee hive pollen samples. Thanks for the really interesting data and discussions. David Lovelock (DJPR) conducted seed testing on our bee pollinated, glasshouse grown watermelons.

Thanks to NT DITT staff: Michael Neal, Peter Bergin, Mark Traynor, Sharl Mintoff, Merran Neilsen and Mook Crothers

Vicki Simlesa was integral to starting this whole area of research way back in 2014. Vicki facilitated industry communication and provided apiary advice, assistance and expertise throughout the life of the project.

Thank you,

Mary Finlay-Doney, Darshana Rathnayake, Shreya Patel, Brian Thistleton and Maxine Piggott

Appendices

Appendix 1: Molecular diagnostic methods for plant and bee hive materials

CGMMV diagnostics

Testing was based on molecular assays using RT-PCR and RT-qPCR targeting CGMMV genes including the coat protein (CP; Reingold et al 2013), movement protein (MP; Ling et al 2014) and the RNA helicase subunit (Aviv; Shargil et al 2019). Positive PCR products were then sequenced and bioinformatics conducted for identification using the Geneious software. RT-qPCR was also conducted based upon the assays described by Berendsen et al 2015 and Hongyun et al 2008. Cycle thresholds above 35 are regarded as negative and Ct values of 30-34 are regarded as suspect CGMMV and Ct values below 30 was CGMMV positive.

Table A1 details the sample types tested.

Table A1: Samples and quantity used for RNA extraction for CGMMV testing

Test	Sample type	Quantity used for RNA extraction
Hive testing	Adult bees	3 individuals
	Pollen	0.5 – 1.0g
	honey	200µL
Bee testing	Adult bee	Whole bee
Flower testing	Flower	Half of the flower
Plant testing	Leaves	Two top leaves (about 4 cm in size)
	Fruits	1cm long piece of peduncle and $1cm^3$ piece of fruit rind
	Inoculation node	All nodes of the bee visited flower regions

Potyvirus testing

Conventional PCR was performed using MyTaq™ One-Step RT-PCR Kit (Meridian Bioscience, USA) following the manufacturer's instructions (see Table A2 for primers and cycling conditions).

References:

1. Berendsen, S. and J. Oosterhof (2015). TaqMan Assays Designed on the Coding Sequence of the Movement Protein of *Cucumber green mottle mosaic virus* for its Detection in Cucurbit Seeds. Phytopathology, 105 (11): 14-15
2. Ha, C., et al. (2008). "Design and application of two novel degenerate primer pairs for the detection and complete genomic characterization of potyviruses." Arch Virol 153(1): 25-36.
3. Ling, K. S., et al. (2014). "First report of *Cucumber green mottle mosaic virus* infecting greenhouse cucumber in Canada." Plant Disease 98 (5): 701-701.
4. Reingold, V., et al. (2013). "First report of *Cucumber green mottle mosaic virus* (CGMMV) symptoms in watermelon used for the discrimination of non-marketable fruits in Israeli commercial fields." New Disease Reports 28 (11).
5. Shargil, D., Zemach, H., Belausov, E., Lachman, O., Luria, N., Molad, O., Dombrovsky, A. (2019). Insights into the maternal pathway for *Cucumber green mottle mosaic virus* infection of cucurbit seeds. Protoplasma, 256: 1109-1118.

Appendix 2: Plant viability assay methods

Inoculum was prepared by adding 1:5 (v/v) ratio of CGMMV positive honey and 0.01M phosphate buffer. The concentrated inoculum was then diluted 1:1 with phosphate buffer (pH 7.0) and 100 mg of silica carbide was added as an abrasive. Two leaves of each plant were inoculated by adding a drop of inoculum on each leaf and rubbing it in a circular motion using a gloved finger. Positive control plants were inoculated with positive plant inoculum. Experimental plants were inoculated with CGMMV positive honey inoculum. Inoculated plants were grown in a biosecure glasshouse. Plants within each treatment were placed in separate benches and spaced to avoid contact with each other. Plants were checked daily and symptoms recorded as soon as first signs of mottling occurred.

Inoculated plants were tested at 4, 8 and 10 weeks post inoculation.

Appendix 3: Metagenomic sequencing of hive-collected pollen samples

One hundred pollen samples were collected during bee hive surveillance. Total RNA was extracted and sequencing libraries were prepared. A custom blast database was constructed using full length CGMMV, PRSV and ZYMV genomes and was used to identify virus-specific reads from the trimmed read pool. A database of plant protein coding regions was constructed using GenBank accessions for plant species belonging to the class of flowering plants. The database contains 93576 taxa. Contigs were then compared to the plant protein coding region database to identify possible plant species present in the pollen samples. Contigs with matching percentage identity of greater than 98.5% were included in the final list of host species.

References:

1. Altschul, S.F. *et al.* (1990) 'Basic local alignment search tool', *Journal of Molecular Biology*, 215(3), pp. 403–410. doi:10.1016/S0022-2836(05)80360-2.

2. Bankevich, A. *et al.* (2012) 'SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing', *Journal of computational biology : a journal of computational molecular cell biology*, 19(5), pp. 455–477. doi:10.1089/cmb.2012.0021.

3. Haas, B. *et al.* (2013) 'De novo transcript sequence reconstruction from RNA-Seq using the Trinity platform for reference generation and analysis', *Nature protocols*, 8, pp. 1494–1512. doi:10.1038/nprot.2013.084.

4. Kim, D. *et al.* (2019) 'Graph-based genome alignment and genotyping with HISAT2 and HISAT-genotype', *Nature Biotechnology*, 37(8), pp. 907–915. doi:10.1038/s41587-019-0201-4.

5. Krueger, F. (2019) *Trim Galore!*, *Babraham Bioinformatics Trim Galore*. Available at: https://www.bioinformatics.babraham.ac.uk/projects/trim_galore/ (Accessed: 31 March 2021).

Appendix 4: Full list of VM18008 Project Reference Group members

Name	Role
Dr Mary Finlay-Doney	VM18008 project leader
Dr Brian Thistleton	VM18008 alternate project leader, project member
Dr Darsh Rathnayake	VM18008 project member
Shreya Patel	VM18008 project member
Dr Maxine Piggott	VM18008 project member
Ashley Zamek	Hort Innovation R & D Manager
Dr Lisa Evans	Scientist, Fruit Crops Physiology, Sustainable Production, Plant and Food Research Australia
Greg Owens	NT Farmers, AusVeg representative
Dr Cherie Gambley	QDAF, Principal plant pathologist, project leader of VG16068
Teagan Alexander	Red Dirt Melons, Katherine, agronomist, to replace Dianna Renfree
Joanna Embry	Biosecurity Officer for the Australian melon industry
Sam Curtis	Kings Bees, Katherine, apiarist and melon grower
Stephen Pace	Pace Farming, melon grower
Partial participants (changed roles, or seconding)	
Vicki Simlesa	Technical Officer Apiary and Crocodiles, DPIR, VM18008 project member
Dianna Renfree	Red Dirt Melons, Katherine, agronomist
Dr Paul Campbell	QDAF, plant pathologist (virology)

Appendix 5: Bees and *Cucumber green mottle mosaic virus*

Background

Cucumber green mottle mosaic virus (CGMMV) is a plant virus that infects cucurbit crops including watermelon, cucumber, melons, zucchini and pumpkin.

CGMMV can cause substantial crop losses. CGMMV is most likely introduced into a crop through infected planting material (seed or seedlings) and can be mechanically transmitted, by something as small as using secateurs to prune plants, or something as large as driving a tractor through a crop.

Cucurbit crops are almost 100 per cent pollinator-dependent. They require insect pollination for successful fruit set and production. In Australia, honey bees are regularly used to provide managed pollination services to broadacre watermelon cropping.

This raises the question: Are honey bees able to transmit CGMMV?

The short answer is yes. However, there are gaps in our knowledge. The Northern Territory-based project team is trying to determine exactly how this may impact Australian apiarists and melon producers.

Current situation

CGMMV is not known to affect bee health.

However, a key concern is that if honey bees are exposed to CGMMV and then moved within or between states, they may move this plant virus to new locations.

In the past year, the research team has made several major discoveries.

- When CGMMV is already present in a crop, honey bees travelling from flowers on CGMMV-positive plants to flowers on clean plants cause infection in the clean plant.
- CGMMV can be detected in capped honey for up to 12 months after initial exposure.
- For a bee hive that contains CGMMV, removing the honey frames and placing the hive on a resting site (with no cultivated cucurbits nearby) can reduce the levels of CGMMV detected in the hive after 12 months.

Hort Innovation Strategic levy investment | MELON FUND

This project has been funded by Hort Innovation using the melon research and development levy and funds from the Australian Government.

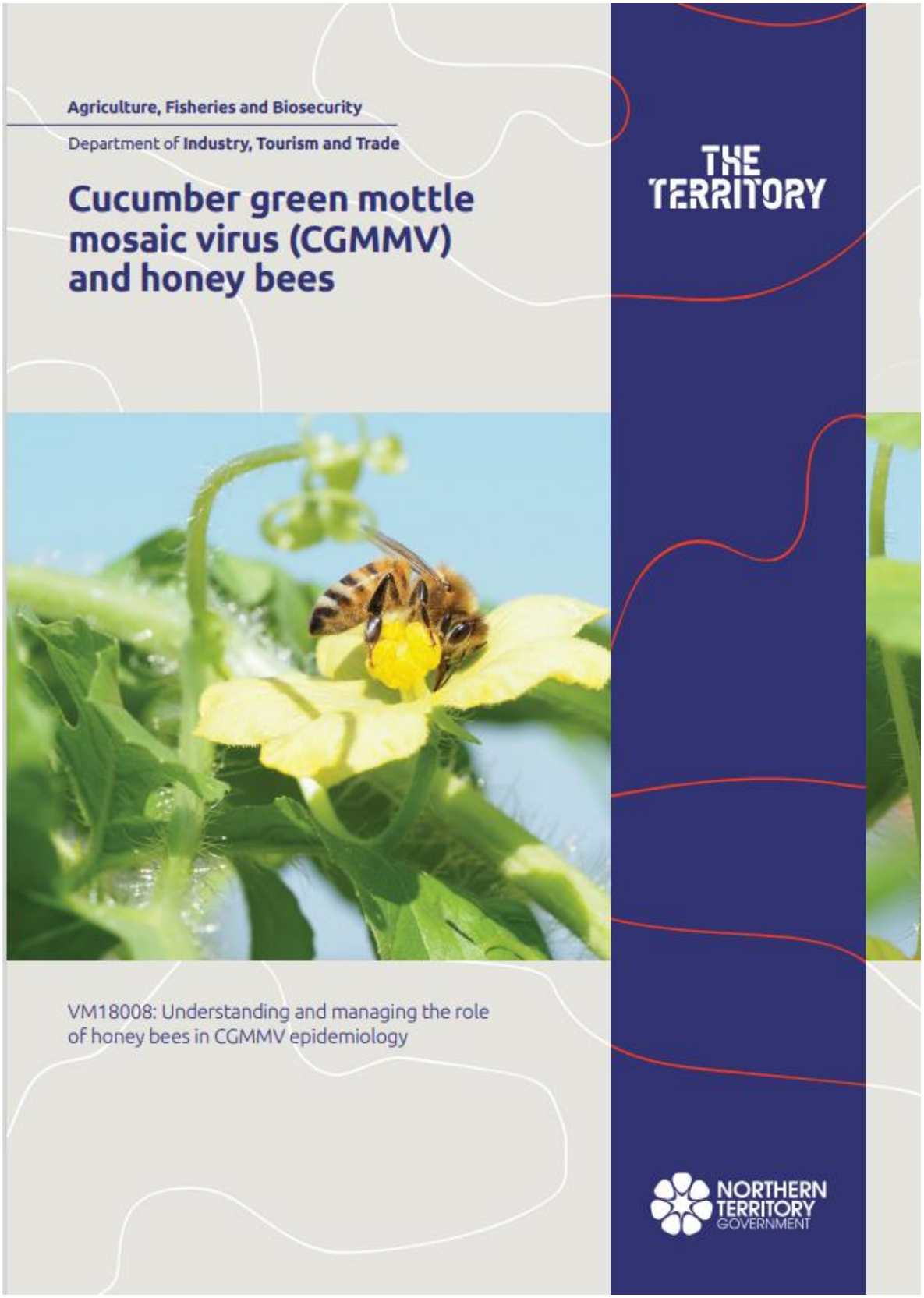
For more information on the fund and strategic levy investment visit horticulture.com.au

For more information, go to industry.nt.gov.au

Department of Industry, Tourism and Trade
T: 08 8999 2006

NORTHERN TERRITORY GOVERNMENT | THE TERRITORY

Appendix 6: *Cucumber green mottle mosaic virus* (CGMMV) and honey bees



In Australia, honey bees are regularly used to provide managed pollination services to broadacre watermelon cropping.

Epidemiology and management

Cucumber green mottle mosaic virus (CGMMV) is a plant disease of cucurbits (such as watermelon, cucumber and pumpkin). CGMMV can cause substantial crop losses. As a trade sensitive pest, there is a national plan for managing CGMMV in Australia to prevent spread and reduce impacts on currently affected regions to mitigate trade impacts.

CGMMV is most likely to be introduced into a crop through infected planting material (soil, seed or seedlings) and can be mechanically transmitted - by something as small as using secateurs to prune plants, or something as large as driving a tractor through a crop.

Cucurbit crops are almost 100% pollinator-dependent, requiring insect pollination for successful fruit set and production. Honey bees come into contact with CGMMV when collecting pollen and nectar through their regular foraging activities. Although live CGMMV has been identified in bee hives, there is no evidence that CGMMV affects the health of bee hives.

How do honey bees transmit CGMMV?

Honey bees are a vector for CGMMV when it is present in plants.

- Bees vector CGMMV in the field when pollinating both infected and healthy plants at the same time.
- Early detection and removal of CGMMV-positive plants is crucial to control or stop further spread of the virus via mechanical transmission, including by honey bees.
- The infection rate (percentage of healthy plants infected by pollinating bees) is likely to depend on the number of infected plants present at the site.

Honey bees recently exposed to CGMMV are capable of transmitting the virus to healthy plants if their hive is moved to a new site.

- CGMMV was detected in cucumbers and watermelons that had been visited by bees from CGMMV-positive hives. These hives were recently exposed to CGMMV-positive plants.

Cucumber green mottle mosaic virus (CGMMV) is a Tobamovirus.

This group of viruses is not commonly known to be insect transmitted.

- First detected in Australia in 2014.
- Now considered established in the Northern Territory and Western Australia, and under management in Queensland, South Australia and New South Wales.
- Causes leaf mottling and mosaic, yellowing and distortion.
- Affected fruit is unmarketable.

Honey bees are able to transmit CGMMV during pollination activities and cause infection in healthy plants.

When the hive had been away from CGMMV-positive plants for more than 24 hours, we did not detect CGMMV transmission to cucurbit plants. However, we were still able to detect CGMMV on bees from within the hive up to one month after the hive had been exposed to CGMMV-positive plants.

CGMMV in honey is detectable, but not viable, 12 months after the original exposure to CGMMV positive plants.

- We do not know exactly when CGMMV in bee hives becomes non-viable. At 6 months after exposure to CGMMV-positive plants, viable CGMMV can still be detected in honey. Removing this honey from the hive and physically rubbing it onto a cucurbit plant could infect the plant.
- To avoid human-assisted CGMMV transmission, do not conduct any bee hive maintenance, such as bee hive cleaning, splitting, honey and pollen extraction on cucurbit production sites.

CGMMV load in positive bee hives can be reduced by removing most of honey and pollen from the hives and placing them in an area without any CGMMV host plants.

- This can be an agricultural production site as long as the plants being grown are not CGMMV hosts. CGMMV non-hosts include sweetcorn, snake bean, okra, capsicum and peanuts.

How do I know if my hives have been exposed to CGMMV?

The most likely place for bee hives to come in contact with CGMMV is from bees visiting flowers of CGMMV-positive plants. In Australia, as well as being found in melons, zucchinis and other plants, it has also been found in several weedy cucurbit species and in several non-cucurbit species, including amaranth (*Amaranthus viridis*) and black nightshade (*Solanum nigrum*). Their importance in the disease cycle has not been fully determined.

If honey bees have been visiting CGMMV-positive plants it is very likely they will have picked up the virus and it will be detectable in the honey and pollen in the hives as well as on the bees themselves.

How do I know if hives contain CGMMV?

There will be no symptoms or indications that a bee hive contains CGMMV. CGMMV is a plant virus. In plants, it can cause leaf mottling and deformation, and soft fruit with rotten flesh. The only way to confirm if honey bee hives contain CGMMV is to test them in the laboratory using a series of molecular tests.

Management recommendations

Management practices for CGMMV require continuous implementation of biosecurity measures.

For growers

- Manage your farm biosecurity.
- Discuss the CGMMV status of your crop with your apiarist.
- Discuss the CGMMV status of any bee hives that you bring onto your property.
- Ensure hives used on your property have not been exposed to CGMMV within the past month.
- Ensure that hive materials (wax frames, honey) are not discarded in your cucurbit production areas.

For apiarists

- CGMMV is not known to affect bee health or hive strength.
- Manage your apiary's exposure to CGMMV by knowing the status of the cucurbit crops you service.
- If a hive has been exposed to CGMMV within the past month, the bees may be able to transfer CGMMV to other cucurbit plants when visiting flowers.
- If a hive has been exposed to CGMMV do not conduct hive maintenance on that hive in a cucurbit production area.

For more information, go to horticulture.com.au:

- Factsheet: Virus diseases of cucurbits in Australia (VG16086)
- VG15013 factsheets

Project leader

Mary Finlay-Doney: mary.finlay-doney@nt.gov.au

Authors

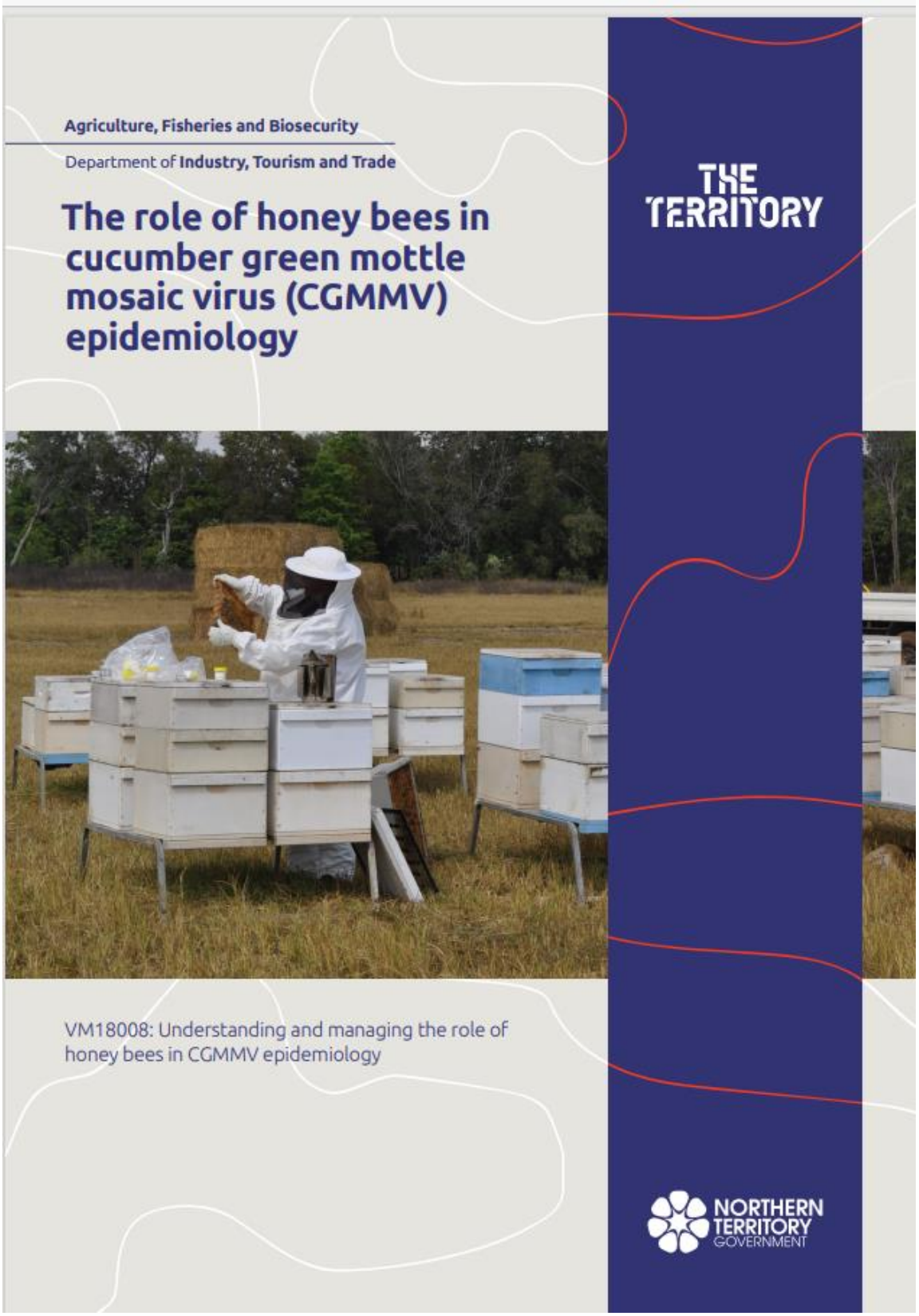
Mary Finlay-Doney and Darsh Rathnayake: darshana.rathnayake@nt.gov.au

This project has been funded by Hort Innovation using the melon and vegetable research and development levy and funds from the Australian Government.

For more information on the fund and strategic levy investment visit horticulture.com.au

For more information, go to industry.nt.gov.au
Department of Industry, Tourism and Trade
T: 08 8999 2006

Appendix 7: The role of honey bees in *Cucumber green mottle mosaic virus* (CGMMV) epidemiology



In Australia, honey bees are regularly used to provide managed pollination services to cucurbit crops.

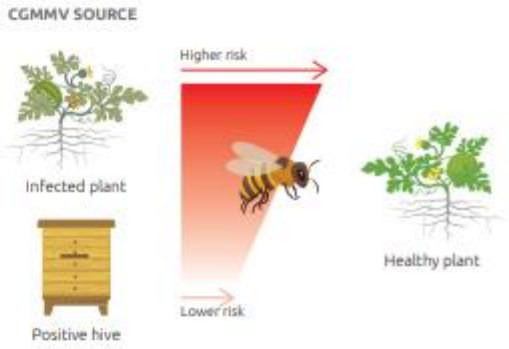
Honey bees are a mechanical vector of cucumber green mottle mosaic virus (CGMMV). CGMMV is a plant virus and is not known to replicate in honey bees or in their hives. If honey bees visit a flower on a plant infected with CGMMV and then visit a flower on a clean cucurbit plant, they are likely to transmit the virus. CGMMV accumulates in honey bee hives when bees collect nectar and pollen from plants infected with CGMMV.

Once inside a honey bee hive, CGMMV can remain viable in honey and pollen for at least 6 months. This could be of concern if you are working your hives and place CGMMV-positive hive material on the ground.

However, the CGMMV on forager bees is not able to be transmitted for the same period. If a bee hive is moved away from a source of CGMMV infected plants, after some time the honey bees from that hive are unlikely to introduce CGMMV into clean plants.

The risk of pollinating honey bees transferring CGMMV by moving hives between cucurbit crops is low if you have a resting period of one month between sites.

Figure 1: Honey bee CGMMV transmission pathways



Biosecurity management of hive materials and the external surfaces of hives (ensuring they are clear of soil, debris and plant material) is still necessary to manage the risk of transporting CGMMV on equipment.

Chlorine treatment for hive tools and external surfaces

Sterilization of vehicles, equipment, plant trays, tools and footwear with potassium peroxymonosulfate or freshly prepared 1% sodium hypochlorite (NaOCl) bleach can help limit the spread of CGMMV.

Repeated use of chlorine solution will damage hive tools, bee suits and boxes. It is recommended that you rinse these items with fresh water after the completion of treatment.

Honey bees are able to transmit CGMMV during pollination activities and cause infection in healthy plants.

Principles of CGMMV management in apiaries

Successful apiary management practices minimise the introduction and possible spread of CGMMV within a beekeeping enterprise.

Management practices aim to:

- prevent or control the introduction of CGMMV into bee hives
- increase the likelihood of being able to trace detections back to the source
- reduce the likelihood of hives becoming a source of CGMMV infection.

The principles of apiary management are:

- using appropriate biosecurity practices to minimise the likelihood of CGMMV introduction, such as not working crops known to be CGMMV-positive and resting hives at least 5km away from known CGMMV-positive sites
- keeping concise and accurate records on hives and loads, to enable trace back to determine the source of the disease
- physically separating loads based on the sites they have worked
- removing CGMMV-positive material from hives, for example, changing frames and spinning off honey immediately after a known exposure to plants infected with CGMMV
- storing equipment and consumables on the apiary so that bees cannot access it
- ensuring hive components are only interchangeable within a load
- making sure honey supers are separated at the extraction plant and not interchangeable between loads
- cleaning hive equipment between loads to ensure all wax and honey debris is removed, typically by using hot water or steam cleaning.

Note that although these techniques will remove honey and wax, which may be carrying CGMMV, they have not been demonstrated to decontaminate CGMMV. If possible, have separate equipment for different loads.

Management practices

Management practices are context-specific and can be developed to suit commercial or individual needs.

- Ensure you understand how bees and hives are exposed to CGMMV.
- Make sure you have a clear understanding of how your management systems operate.
- Ensure clear permanent marking and identification of hives (individually or in loads) and their components.
- Keep accurate and concise records for all apiary activities.
- Keep physical separation between loads of hives that possibly contain CGMMV and those that don't contain CGMMV.
- Attend to hives that contain CGMMV last in the workflow, and use separate hive tools and bee-keeping gear for these hives.
- Restrict movement of people, vehicles and animals to any hives you suspect contain CGMMV.

For more information, go to horticulture.com.au:

- Factsheet: Cucumber green mottle mosaic virus and honey bees (VM18008)

Project leader

Mary Finlay-Doney: mary.finlay-doney@nt.gov.au

Authors

Mary Finlay-Doney and Darsh Rathnayake: darshana.rathnayake@nt.gov.au

This project has been funded by Hort Innovation using the melon and vegetable research and development levy and funds from the Australian Government.

For more information on the fund and strategic levy investment visit horticulture.com.au

For more information, go to industry.nt.gov.au
Department of Industry, Tourism and Trade
T: 08 8999 2006

Appendix 8: Pollinating honey bees: do they spread plant diseases?

Managed pollination

In Australia, 65% of horticultural and agricultural production is pollinator dependent. Managed pollination services are predominantly provided by *Apis mellifera* (Hymenoptera: Apidae), the European honey bee. The provision of pollination services can improve both the quality and the quantity of production. What is less well understood is how managed pollinators can negatively impact production by spreading plant pathogens.

Pollinators as vectors of plant disease

There are relatively limited examples of demonstrated spread of plant pathogens by floral visitors e.g. thrips spreading *Prunus necrotic ringspot virus* (PNRSV, Bromovidae) in stone fruit and chrysomelid beetles spreading bacterial wilt (*Erwinia tracheiphila*) to cucurbits.

Bees spreading plant pathogens is even less reported.

Buzz pollinators, like bumble bees, can introduce plant pathogens when they damage flowers and surrounding plant tissue with their buzzing. Buzz pollinators are used in solanaceous crops.

There are few examples of demonstrated plant pathogen spread by European honey bees (*Apis mellifera*). All of the examples below involve plant pathogen entry via flowers:

* *Cucumber green mottle mosaic virus* (CGMMV, Tobamovirus) in melons and watermelons
* *Prunus necrotic ringspot virus* (PNRSV, Bromoviridae) in cherries
* *Blueberry shock ilarvirus* (BlShV, Ilarvirus) in highbush blueberry

Where honey bees have been demonstrated to spread plant pathogens the bees are likely to act as mechanical, rather than propagative/circulative, vectors. This means that honey bees physically carry the plant pathogen to new plants during floral visits. This may either be in pollen or by plant pathogens adhering to their bodies. Some fungi that are expressed in flowers have been demonstrated to be carried by honey bees. The fungi in Table 1 below all affect plant roots and are soil borne.

There are very limited examples of plant pathogens replicating inside honey bee bodies.

When bees are capable of spreading a plant pathogen they do not generally represent the most important source or carrier of the pathogen, although the long distance movement of bee hives can present a particular risk of introducing plant pathogens into new environments.

Honey bees and plant pathogens of biosecurity concern to Australian cucurbit production

There are 13 plant pathogens of cucurbits listed as high or medium risk to the Australian vegetable and melon industry (Industry Biosecurity Plans, 2020; Table 1). They are predominantly viruses and fungi but include one bacteria and one nematode. None of these pathogens have been demonstrated to be transmitted by honey bees.

It is difficult to generalise about potential spread of plant pathogens by pollinating honey bees. In Table 1 we have assessed the likelihood of these plant pathogens being spread by honey bees. We have used the following criteria:

- is the pathogen expressed in the pollen;
- are there other known insect vectors;
- are there other pathogens in that group that are spread by insects?

Based on these criteria we have assigned a rating of HIGH to:

- the tobamoviruses: because two other tobamoviruses (CGMMV and *Tomato brown rugose fruit virus* (ToBRFV)) have been shown to be transmitted by bees
- Cucurbit bacterial wilt (*Erwinia tracheiphila*): because a closely related bacteria, *Erwinia amylovora* is very likely to be transmitted by honey bees.

Table 1. High and medium priority pests of cucurbits identified in the Australian Melon and Vegetable Industry Biosecuity Plans (IBP 2020)

Cucurbit plant pathogens				Risk of honey bee transmission		
Plant pathogen group	Name	IBP[a]	Overall risk[b]	Found in pollen[c]	Known insect vectors	Likelihood of honey bee transmission
Nematode	Root knot nematode (*Meloidogyne enterolobii* (Syn. *Meloidogyne mayaguensis*))	V	High	No	No. Moved in soil and plant material	Negligible
Virus Orthotospovirus (Tospoviridae)	*Groundnut bud necrosis virus* (GNNV)	V & M	High	Unknown	Thrips (Thysanoptera)	Low
	Watermelon bud necrosis virus (WBNV)	V & M				
	Melon yellow spot virus (MYSV), *Watermelon silver mottle virus* (WMSMOV) and serogroup IV, *Melon severe mosaic virus*	M				
Virus Tobamovirus (Virgaviridae)	Kyuri green mottle mosaic virus (KGMMV), Zucchini green mottle mosaic virus (ZGMMV), Watermelon green mottle mosaic virus (WGMMV), Cucumber fruit mottle mosaic virus	M	Medium	Yes	No	High
Virus Gammacarmovirus (Tombusviridae)	*Melon necrotic spot virus* (MNSV)	M	Medium	Unknown	Unlikely. Moved in soil and by oomycetes.	Medium
Bacteria	Cucurbit bacterial wilt (*Erwinia tracheiphila*)	M	Medium	Yes	Beetles (Coleoptera)	High
Fungi	Fusarium root and stem rot of melons (*Fusarium oxysporum f.sp. melonis* (exotic races), *Fusarium oxysporum f.sp. niveum* (exotic races), *Fusarium oxysporum f.sp. radiciscucumerinum*, *Fusarium oxysporum f.sp lagenariae*)	M	High	Unknown	No. Moved in soil and plant material	Negligible
	Monosporascus root rot (*Monosporascus cannonballus*)		Medium			
	Melon root rot (*Acremonium cucurbitacearum*)					
	Melon black rot (*Phomopsis cucurbitae*) (syn. *Diaporthe melonis*)					
	Texas root rot (*Phymatotrichopsis omnivora*)					
	Sudden collapse of melons (*Rhizopycnis vagum*)					

[a] V = vegetable, M = melon

[b] Overall risk as estimated in the IBP

[c] This information was not available for each individual species so is provided for the plant pathogen group as whole

What else influences pathogen transmission?

It is difficult to generalise about potential spread of plant pathogens by pollinating honey bees because there is little specific published information. In the absence of specific details there are some additional factors to consider:

- Pathogen transmission is likely to be time dependent. That is, the time that has lapsed since the honey bees visited a plant affected with a pathogen and the survivability of the pathogen inside a bee hive will affect honey bees' ability to transmit it. The window for transmission will be specific to each pathogen and ranges from several hours to several weeks in currently available published studies.
- Floral traits can influence the transmission of vectored plant pathogens. This includes the likelihood of pathogen establishment and the likelihood of pathogen transmission
- Pollen transmission of viruses is complex. Transmission may be horizontal but not vertical, that is, pollen from one plant may infect another plant, without the virus being carried to their resultant offspring. Additionally, viruses may be detectable on pollen surfaces but not be naturally transmitted to plants

For further reading

Industry biosecurity plans
https://www.planthealthaustralia.com.au/about-us/documents/

References used to prepare this document are available at https://www.horticulture.com.au/growers/help-your-business-grow/research-reports-publications-fact-sheets-and-more/vm18008/

Project leader
Mary Finlay-Doney

Authors
Mary Finlay-Doney and Darsh Rathnayake
darshana.rathnayake@nt.gov.au

This project has been funded by Hort Innovation using the melon research and development levy and funds from the Australian Government. For more information on the fund and strategic levy investment visit horticulture.com.au

Hort Innovation Strategic levy investment | MELON FUND

MELONS AUSTRALIA

For more information, go to industry.nt.gov.au
Department of Industry, Tourism and Trade
T: 08 8999 2006

NORTHERN TERRITORY GOVERNMENT | THE TERRITORY

Pollinating honey bees: are they plant disease vectors?

A summary of the potential risks to the Australian cucurbit industry
VM18008 Understanding and managing the role of honey bees in CGMMV epidemiology

THE TERRITORY

References

Alexandrova, M., *et al.* (2002). "The role of honeybees in spreading *Erwinia amylovora*." Acta Horticulturae **590**: 55-60.

Andersen, C. (2013). Factsheet: Texas root rot. Plant Health Australia. https://www.planthealthaustralia.com.au/wp-content/uploads/2013/03/Texas-root-rot-FS-Cotton.pdf. Accessed November 2021.

Antignus, Y., *et al.* (2007). "Spread of Tomato apical stunt viroid (TASVd) in greenhouse tomato crops is associated with seed transmission and bumble bee activity." Plant disease **91** (1): 47-50.

Armengol, J., *et al.* (2003). "Identification, occurrence and pathogenicity of *Rhizopycnis vagum* on muskmelon in Spain" Plant Pathology **52**: 68-73.

Blanco, R. & Aveling, T.A.S. (2018). Seed-borne Fusarium pathogens in agricultural crops. Acta Horticulturae. **1204**: 161-170.

Bristow, P. R. & R. R. Martin (1999). "Transmission and the role of honeybees in field spread of *Blueberry Shock Ilarvirus*, a pollen-borne virus of highbush blueberry." Phytopathology **89** (2): 124-130.

Card, S. D., *et al.* (2007). "Plant pathogens transmitted by pollen." Australasian Plant Pathology **36**(5): 455-461.

Clarke, M. & D. Le Feuvre (2020). Size and scope of the Australian honey bee and pollination industry – a snapshot. *Publication No. 20-136. Project No. PRJ-012405.* Wagga Wagga, AgriFutures Australia: 54.

Coudriet, D. L., *et al.* (1979). "Transmission of *Muskmelon Necrotic Spot Virus* in muskmelons by cucumber beetles." Journal of Economic Entomology **72** (4): 560-561.

Darzi, E., *et al.* (2018). "The honeybee *Apis mellifera* contributes to *Cucumber green mottle mosaic virus* spread via pollination." Plant Pathology **67** (1): 244-251.

EPPO (2001) Mini data sheet on *Acremonium cucurbitacearum, Monosporascus cannonballus* and *Rhizopycnis vagum* https://gd.eppo.int/download/doc/994_minids_ACRECU.pdf. Accessed November 2021.

Garibaldi, A *et al.* (2011). "First report of Black Rot caused by *Phomopsis cucurbitae* on cantaloupe (*Cucumis melo*) in the Piedmont region of northern Italy." Plant Disease **95** (10): 1317-1317.

Ghignone, S., *et al.* (2003). "Development of specific PCR primers for identification and detection of *Rhizopycnis vagum*." European Journal of Plant Pathology **109**: 861–870.

Goldberg, N. & P. Lujan (2020). Guide A-229: Phymatotrichum Root Rot. College of Agricultural, Consumer and Environmental Sciences, New Mexico State University. www.aces.nmsu.edu/pubs. 4pp. Accessed November 2021.

Li, J. L., *et al.* (2014). "Systemic spread and propagation of a plant-pathogenic virus in European honeybees, *Apis mellifera*." mBio **5** (1) e00898-13.

McArt, S. H., *et al.* (2014). "Arranging the bouquet of disease: floral traits and the transmission of plant and animal pathogens." Ecology Letters **17** (5): 624-636.

Martyn, R.D. (2002). Monosporascus root rot and vine decline of melons. Updated 2009. The Plant Health Instructor. https://www.apsnet.org/edcenter/disandpath/fungalasco/pdlessons/Pages/Monosporascus.aspx. Accessed November 2021.

Matsuura, S., *et al.* (2010). "Transmission of *Tomato chlorotic dwarf viroid* by bumblebees (*Bombus ignitus*) in tomato plants." European Journal of Plant Pathology **126** (1): 111.

For more information, go to industry.nt.gov.au

Department of Industry, Tourism and Trade
T: 08 8999 2006

NORTHERN TERRITORY GOVERNMENT

THE TERRITORY

Mink, G. I. (1983). The possible role of honeybees in long distance spread of *Prunus necrotic ringspot virus* from California into Washington sweet cherry orchards. Plant virus epidemiology. R. T. Plumb and J. M. Thresh. Oxford, United Kingdom, Blackwell Scientific Publications: 85-91.

Okada, K., *et al.* (2000). "Tobacco mosaic virus is transmissible from tomato to tomato by pollinating bumblebees." Journal of General Plant Pathology **66** (1): 71-74

Parish, J. B., *et al.* (2019). "Survival and probability of transmission of plant pathogenic fungi through the digestive tract of honey bee workers." Apidologie **50** (6): 871-880.

Pattemore, D. E., *et al.* (2018). Assessment of the risks of transmission of myrtle rust (*Austropuccinia psidii*) spores by honey bees (*Apis mellifera*). Prepared for Biosecurity New Zealand By New Zealand Plant & Food Research: 18.

Pattemore, D. E., *et al.* (2014). "Evidence of the role of honey bees (*Apis mellifera*) as vectors of the bacterial plant pathogen *Pseudomonas syringae*." Australasian Plant Pathology **43** (5): 571-575.

Plant Biosecurity and Product Integrity (2008). Primefact 1642: Fusarium root rot of melons. NSW Department of Primary Industries. https://www.dpi.nsw.gov.au/biosecurity/plant/insect-pests-and-plant-diseases/Fusarium-root-rot. Accessed November 2021.

Plant Health Australia (2018). Factsheet: Monosporascus root rot https://www.melonsaustralia.org.au/wp-content/uploads/2018/12/Monosparascus-root-rot-fact-sheet.pdf. Accessed November 2021.

Porta-Puglia, A., *et al.* (2001). "First report of *Rhizopycnis vagum* associated with tomato roots in Italy" Plant Disease **85** (11): 1210.

Punja, Z. K. & M. Parker (2000). Development of fusarium root and stem rot, a new disease on greenhouse cucumber in British Columbia, caused by *Fusarium oxysporum* f. sp. radicis-cucumerinum. Canadian Journal of Plant Pathology **22** (4): 349-363.

Shaw, D. E. (1999). "Bees and fungi, with special reference to certain plant pathogens." Australasian Plant Pathology **28** (4): 269-282.

Shipp, J., *et al.* (2008). "Vectoring of *Pepino mosaic virus* by bumble□bees in tomato greenhouses." Annals of Applied Biology **153** (2): 149-155.

Uppalapati, S.R., *et al.* (2010). Phymatotrichum (cotton) root rot caused by *Phymatotrichopsis omnivora*: retrospects and prospects. Molecular Plant Pathology **11**: 325-334.

This project has been funded by Hort Innovation using the melon research and development levy and funds from the Australian Government. For more information on the fund and strategic levy investment visit horticulture.com.au

Hort Innovation Strategic levy investment | MELON FUND

MELONS AUSTRALIA

For more information, go to industry.nt.gov.au

Department of Industry, Tourism and Trade
T: 08 8999 2006

NORTHERN TERRITORY GOVERNMENT

Appendix 9: Volunteer apiarists need for research project, *Monthly news*: June/July 2019, Australian Honey Bee Industry Council

AUSTRALIAN HONEY BEE INDUSTRY COUNCIL INC

ABN 63 939 614 424

Monthly NEWS

June/July 2019

To: **The Australian Honey Bee Industry**
www.honeybee.org.au

From: **Sarah Paradice, CEO**
ahbic@honeybee.org.au

The State conferences are done and dusted for 2019.
Congratulations to all State Associations!

AHBIC Resource Access Workshop, funded by Agrifutures, was held at the Hotel Grand Chancellor, Launceston, Tasmania on Sunday 30th June and Monday 1st July 2019.
Back row, left to right: Stephen Fewster (WA), Ian Cane (Vic), Neil Bingley (NSW), Danny Le Feuvre (SA), Steve Cunial (NSW), Lindsay Bourke (Tas), Leilani Leyland (WA), Doug Somerville (NSW, Chair Agrifutures Honey Bee and Pollination Advisory Panel), Peter Barnes (Qld), Clinton Ruge (Qld), Rod Pavey (WA), Rob McDonald (Vic), Tim Burfitt (facilitator), Michael Clarke (report writer)
Front row, left to right: Peter McDonald (Vic, AHBIC Chair), Sarah Paradice (AHBIC CEO), Ben Hooper (SA), David Leyland (WA)

June-July 2019 *Page 1 of 20*

FLASHBACK PHOTO!

Can you name these people? Email and let us know! Thanks to Shirley Stephens (Tasmania) for providing this fabulous photo of the first AHBIC meeting in 1998. And yes, it is Shirley who is outnumbered as the only female!

VOLUNTEER APIARISTS NEEDED FOR RESEARCH PROJECT

The NT DPIR has been successful in winning the Horticulture Innovation Australia tender VM18008: Understanding and managing the role of honey bees in CGMMV epidemiology. This 2 year research project is funded through the melon levy and will run until July 2021. Dr Mary Finlay-Doney is the project leader. Ms Vicki Simlesa is the apiary officer for the project. From the previous HortInnovation project (VG15013) we demonstrated that CGMMV present in honey bee hives can remain viable for at least six months post-exposure. It is unknown if honey bees are able to transmit the viable virus from inside their hives into clean melon crops. The objective of this new project is to determine the mechanism by which honey bees introduce CGMMV into clean melon plants and develop management practices for the melon and apiary industries to combat this.

The specific questions of the project are:

- Is the transmission of CGMMV by honey bees from plant to plant whilst foraging or are honey bees also able to transmit the virus from their hive to plants?
- Can CGMMV remain viable inside honey bee hives beyond 6 months?
- What role do alternative hosts (weeds) play as a source for CGMMV inoculum in honey bee vectoring of the virus?
- Do current honey (and pollen) extraction practices remove viable virus and, if so, to what extent does this remove the risk of virus transmission from a hive?
- What management of honey bee hives is required for both the cucurbit and apiary industries to be confident of clean honey bees for pollination?

We plan to conduct surveillance of bee hives that have potentially been exposed to CGMMV and conduct field trials on Berrimah Research Farm, NT.

We are seeking to establish a national Project Reference Group (PRG) (2-3 melon growers, **2+ apiarists**) to provide advice on the project and to be used as a pivot for greater industry engagement. The PRG will meet biannually via teleconference.

For further information about the project or to indicate your interest to participate in the PRG please contact Mary Finlay-Doney mary.finlay-doney@nt.gov.au , 0428 461 615.

Appendix 10: Bees and CGMMV, *Melon Enews*: August 2019, Australian Melon Association

Melon Enews August 2019

www.melonsaustralia.org.au Love Aussie Melons Loveaussiemelon Pinterest

In This Issue:

Register of Authorised Officers

The Department of Agriculture has established a public register of Australian plant export Authorised Officers (AOs) after consultation with the Horticultural Exporters Industry Consultative Committee (HEICC). The Melon Industry Development Manager is a member of the HEICC.
Contact details and accreditations for the AOs who have consented to having their details published are listed **here**.

Bees and CGMMV

A new melon levy project has begun in the Northern Territory to continue the research work on bees and Cucumber green mottle mosaic virus. The NT Department of Primary Industry & Resources will investigate whether honeybees play a part in the spread this virus.

Get the jump on nematodes

Growers are invited to participate in an online Hort360 workshop about managing nematodes on Wednesday 28 August 2019. *Get the Jump on Nematodes* will feature a presentation by Dr Jady Li from CQ University, drilling down into nematode biology, treatment options for nematodes, and how to prevent further spread of nematodes. Register **online** or contact Hort360 BMP Facilitator Michelle Haase on 0428 586 890 or at **mhaase@growcom.com.au**

Update on AVPMA spray drift policy

The APVMA has **released an update** to their spray drift policy. The key changes of the new policy include:

Appendix 11: CGMMV and Bees, *Melon Enews*: September 2019, Australian Melon Association

Melon Enews September 2019

www.melonsaustralia.org.au Love Aussie Melons Loveaussiemelon Pinterest

In This Issue:

The Australian Melon Association AGM will be held by teleconference on Wednesday 25th September, 2019 at 3pm AEST. All members are encouraged to attend. Further information wil lbe distributed closer to the date.

1

The levy-funded project, Food Safety Training, Extension and Capacity for the melon industry has released food safety guides specifically for rockmelon growers. The project leader, Dr SP Singh had been working closely with melon growers over the past twelve months to ensure that food safety practices are high. The new Guides will further cement that knowledge base. The Guides and a range of other useful and practical information for melon growers is available **here**.

CGMMV and bees

The levy funded project to better understand the interaction of bees and CGMMV has kicked off in the Northern Territory. Surveillance of all commercial apiaries in the NT is underway with sampling of additional hives interstate. The results will provide a stocktake of the current status of hives that were being monitored throughout the previous CGMMV project and will allow identification of sources of experimental material for the current project.

The next step is to set up two trials to test the longevity of CGMMV in bee hives. One trial will investigate how long CGMMV can remain viable in capped honey cells and the second will investigate if removing all honey and pollen from a working bee hive can effectively remove CGMMV in the hive. Trials will also occur at Berrimah Farm in early 2020 to determine how honey bees introduce CGMMV into clean cucurbit plants.

Horticulture Award – Overtime for casuals

Have your say on changes to the Horticulture Award in a National Survey now open. This survey is essential to capture impacts of changes to the Horticulture Award that came into effect in April, including overtime entitlements.

Complete the survey **here**.

Property Identification Code reforms

Reforms to the Property Identification system are currently underway. The reforms are aimed at developing nationally consistent arrangements for the allocation and use of property identifiers for the animal and plant production sectors. Property Identification Codes (PIC's) provide traceability to specific properties and have been present in the

Appendix 12: Understanding the role of honey bees in CGMMV, *Melon News*: June 2020, Australian Melon Association

Understanding the role of honey bees in CGMMV

The Northern Territory (NT) Department of Primary Industry and Resources is implementing a Hort Innovation funded project VM18008 'Understanding and managing the role of honey bees in CGMMV epidemiology' to investigate the role of honey bees in transmitting cucumber green mottle mosaic virus (CGMMV) in cucurbit plants.

Field surveillance commenced in 2019 with extensive field sampling of bee hives from commercial apiaries in Katherine, Mataranka and Darwin in the NT. Most of these apiaries were from areas affected by CGMMV, first detected in the Territory in 2014. All hive samples collected (adult bees, broods, honey and pollen) were laboratory tested for the virus and five apiaries in Katherine returned positive for CGMMV.

One infected working bee hive was tested to determine whether there was capacity to recover from the virus after removing all honey and pollen. After three months of honey and pollen removal from this hive, the virus was still detected from almost all samples of adult bees, broods, new honey and pollen taken.

A trial investigating how long CGMMV can remain viable in capped honey cells is under way, testing for the presence of the virus and its viability at different time intervals. The next step will be to conduct field trials to determine how honey bees introduce CGMMV into healthy cucurbit plants from infected plants and hives. The trials will be conducted at Berrimah Farm Science Precinct in Darwin later this year.

The study results will assist in management strategies for CGMMV, improving the health of Australian melons and the productivity of the industry. This project is funded by Hort Innovation, using the melon 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.

Contact:
Dr Brian Thistleton
Principal Entomologist
Northern Territory Department of Primary Industry and Resources
0448 687 921
brian.thistleton@nt.gov.au

The objectives of this project are to:

- ⇒ understand the mode(s) of transmission of CGMMV by honey bees, and their hives as source of the virus, and the epidemiological significance this has for managing the virus and using honey bees for pollination
- ⇒ determine whether other mosaic viruses affecting cucurbits (e.g. zucchini mosaic virus) can be transmitted by honey bees
- ⇒ work with the melon and apiary industries to develop an effective plan for managing the spread of CGMMV that is sustainable for both industries.

Appendix 13: CGMMV and Bees webinar, *Melon Enews*: June 2020, Australian Melon Association

Melon Enews June 2020

www.melonsaustralia.org.au Love Aussie Melons Loveaussiemelon Pinterest

In This Issue:

1

Variety trials go virtual

While Aussie melon growers are confined to their own paddocks this season, Melons Australia (AMA) is bringing the latest in research and development via a video series featuring new melon varieties.

Many new varieties were due to be showcased by seed companies at the 2020 Syngenta Australian Melon Conference and Field Day that was scheduled to be held in late March in Western Australia but cancelled due to pandemic restrictions.

Shot on location at Capogreco Farms at Hamel in Western Australia, the video series features five melon variety trial sites by seed companies Syngenta, Seminis, BASF Nunhems, HM Clause and Rijk Zwaan, as well as a highlight compilation video featuring all variety trials.

To view the videos visit the **Melons Australia website**.

CGMMV and bees webinar

Understanding and managing the role of honey bees in managing CGMMV is a melon levy funded project that is exploring whether honey bees are able to introduce CGMMV into melon crops from infested hives. The project will then develop management practices to combat this issue in the melon and apiary industries.

A webinar will be held on Friday 19 June at 3:00pm ACST with Dr Darsh Rathnayake, a research scientist with the Northern Territory DPIR. **Register online**.

Melon biosecurity information

Plant Health Australia has updated it's website to now include information on the Melon Biosecurity Program. The program is an initiative to improve the management of, and preparedness for, biosecurity risks in the Australian melon industry at the farm and industry levels and is funded through the melon PHA (biosecurity) levy. **Read more**.

Appendix 14: CGMMV and Bees (VM 18008) update, *Melon News*: February 2021, Australian Melon Association

CGMMV and bees (VM18008) Update

By Mary Finlay-Doney, Northern Territory Department of Industry, Tourism and Trade

Do bees transmit CGMMV? The answer is yes. The NT-based project team are trying to determine exactly how and when.

Beginning in July 2019, this Hort Innovation Melon Fund project is led by the Northern Territory (NT) Department of Primary Industry and Resources to explore how honeybees are able to introduce cucumber green mottle mosaic virus (CGMMV) into melon crops. It is also developing management practices to combat this transmission for both the melon and apiary industries.

Field trials in 2020 showed that honeybees can move CGMMV between melon plants by visiting flowers. Bees travelling from flowers on CGMMV-positive plants to flowers on clean plants caused infection in the clean plant.

We know that CGMMV can persist in capped honey for at least six months. We currently have two trials underway – we have just collected the 12-month samples from capped honey we have been monitoring in a temperature-controlled cabinet and samples from a bee hive that was exposed to CGMMV-infected melons 12 months ago and has been on a resting site ever since. Results will be available soon.

This field season we will be working on determining the viability of the CGMMV in various bee hive products (wax, honey etc) and running field trials to see if honeybees from a hive containing viable CGMMV (in the wax and honey) are able to transmit the virus to clean plants.

The project will also produce a literature review of the capacity for managed pollinators (honeybees) in Australia to transmit other cucurbit pathogens aside from CGMMV. This review will cover viruses, bacteria, fungi and nematodes and will allow industry to assess and identify future threats.

The project wraps up in December 2021.

Hort Innovation Strategic levy investment | MELON FUND

This project has been funded by Hort Innovation using the melon research and development levy and funds from the Australian Government. For more information on the fund and strategic levy investment visit horticulture.com.au

Appendix 15: Managed pollination and the transmission of a plant – what's going on and should we be worried? *Monthly news*: March 2021, Australian Honey Bee Industry Council

- Managed pollination and the transmission of a plant virus –what's going on and should we be worried?

Hort Innovation VM18008:

Understanding and managing the role of honey bees in CGMMV epidemiology

Cucumber green mottle mosaic virus (CGMMV) is a plant virus that infects cucurbit crops including watermelon, cucumber, melons, zucchini and pumpkin. CGMMV can cause substantial crop losses. CGMMV is most likely introduced into a crop through infected planting material (seed or seedlings) and can be mechanically transmitted (by something as small as using secateurs to prune plants, or something as large as driving a tractor through a crop).

Cucurbit crops are almost 100% pollinator dependent, they require insect pollination for successful fruit set and production. In Australia, honey bees are regularly used to provide managed pollination services to broad acre watermelon cropping.

This raises the question are honey bees able to transmit CGMMV?
The short answer is yes. However, there are gaps in our knowledge. The NT based project team are trying to determine exactly how this may impact on Australian apiarists and melon producers.

CGMMV is not known to affect bee health. The main concern is that if honey bees are exposed to CGMMV and then moved significant distances within or between states, they may be move this highly destructive plant virus to new locations.

In the past year the research team has shown:

- when CGMMV is already present in a crop, honey bees travelling from flowers on CGMMV positive plants to flowers on clean plants cause infection in the clean plant;
- CGMMV can be detected in capped honey for up to 12 months after initial exposure;
- for a bee hive that contains CGMMV, removing the honey frames and placing the hive on a resting site (with no cultivated cucurbits nearby) can reduce the levels of CGMMV detected in the hive after 12 months.

This field season we will be working on determining if the CGMMV we have detected in various bee hive products (wax, honey, etc.) is alive and capable of causing plant disease, and running field trials to see if honey bees from a hive containing living CGMMV are able to transmit the virus to clean plants.

March 2021 *Page 9 of 15*

The project will also produce a literature review what is currently known about the ability of honey bees to transmit other pathogens aside from CGMMV. This review will cover viruses, bacteria, fungi and nematodes and will allow the apiary and horticulture industries to assess and identify future threats.

The project is due to wrap up in December 2021.

For more information about CGMMV see the department of agriculture website in your state or territory.

- https://www.dpi.nsw.gov.au/biosecurity/plant/insect-pests-and-plant-diseases/cgmmv
- https://www.business.qld.gov.au/industries/farms-fishing-forestry/agriculture/crop-growing/priority-pest-disease/cucumber-green-mottle-mosaic-virus
- https://www.agric.wa.gov.au/cgmmv
- https://nt.gov.au/industry/agriculture/food-crops-plants-and-quarantine/cucumber-green-mottle-mosaic-virus/cucumber-green-mottle-mosaic-virus
- https://pir.sa.gov.au/biosecurity/plant_health/emergency_and_significant_plant_pests/cucumber_green_mottle_mosaic_virus

Appendix 16: Managed pollination and the transmission of a plant virus – what's going on and should we be worried? Territory Rural review: April 2021, Department of Industry, Tourism and Trade, NT Government

NORTHERN TERRITORY GOVERNMENT | Department of Industry, Tourism and Trade

What are you looking for?

Home > Publications > Primary industry > Newsletters > Regional newsletters > NT Rural Review - May 2021 > Managed pollination and the transmission of a plant virus - what's going on and should we be worried?

Managed pollination and the transmission of a plant virus - what's going on and should we be worried?

Region: Greater Darwin, Katherine Region | Topic: Horticulture

Jan 2020

Caption: Get the buzz on the role of honey bees in CGMMV transmission

HortInnovation VM18008: Understanding and managing the role of honey bees in CGMMV epidemiology

In June 2020, the Australian melon industry was valued at $152.1 million. The Northern Territory (NT) produces 25% of all watermelons grown in Australia. *Cucumber green mottle mosaic virus* (CGMMV) is a plant virus first detected in the NT in 2014. CGMMV is a virus of cucurbit crops, including watermelon, cucumber, melons, zucchini and pumpkin. CGMMV can cause substantial crop losses. As a trade-sensitive pest, there is a national plan for managing CGMMV in Australia to prevent spread, reduce impacts on currently affected regions and mitigate trade impacts.

CGMMV is most likely introduced into a crop through infected planting material (soil, seed or seedlings) and can be mechanically transmitted, by something as small as using secateurs to prune plants, or something as large as driving a tractor through a crop.

Cucurbit crops are almost 100% pollinator-dependent, requiring insect pollination for successful fruit set and production. In Australia, honey bees are regularly used to provide managed pollination services to broadacre watermelon cropping.

This raises a question. Are honey bees able to transmit CGMMV?

The short answer is yes. However, there are gaps in our knowledge. The NT-based project team is trying to determine exactly how this may impact Australian apiarists and melon producers.

CGMMV is not known to affect bee health. The main concern is that if honey bees are exposed to CGMMV and then moved significant distances within or between states, they may move this highly destructive plant virus to new locations.

In the past year, the research team has shown that:

- honey bees travelling from flowers on CGMMV-positive plants to flowers on clean plants cause infection in the clean (uninfected) plant
- CGMMV can be detected in capped honey for up to 12 months after initial exposure.

The research has also found that removing the honey frames in a bee hive that contains CGMMV and placing the hive on a resting site (with no cultivated cucurbits nearby) can reduce the levels of CGMMV detected in the hive after 12 months.

This field season we will be working to determine if the CGMMV detected in various bee hive products (such as wax and honey) is viable and capable of causing plant disease, and running field trials to see if honey bees from a hive containing living CGMMV are able to transmit the virus to clean plants.

Caption: This season the department will test products from inside the beehive

The project will also produce a literature review of what is currently known about the potential for honey bees to transmit other pathogens aside from CGMMV. The review will cover viruses, bacteria, fungi and nematodes and will allow the apiary and horticulture industries to assess and identify future threats.

The project is due to wrap up in December 2021.

More information

Appendix 17: *Cucumber green mottle mosaic virus* (CGMMV) and bees project – August 2021 update.

Melons Australia website https://www.melonsaustralia.org.au/cucumber-green-mottle-mosaic-virus-cgmmvand-bees-project-august-2021-update/

MELONS AUSTRALIA

HOME NEWS & EVENTS CONSUMER INDUSTRY RESOURCES CONTA

INDUSTRY NEWS

CUCUMBER GREEN MOTTLE MOSAIC VIRUS (CGMMV)AND BEES PROJECT – AUGUST 2021 UPDATE

POSTED ON AUGUST 31, 2021 BY MELONS AUSTRALIA

CUCUMBER GREEN MOTTLE MOSAIC VIRUS (CGMMV) AND BEES PROJECT - AUGUST 2021 UPDATE

By Darshana Rathnayake, Northern Territory Department Industry, Tourism and Trade (DITT)

In May, we reported that placing CGMMV exposed hives on a resting site can reduce the amount of virus detectable in the hive. Through intensive glasshouse trials they have now shown that CGMMV present in honey frames, 12 months after exposure to the plant virus, is no longer viable. That is, it is not capable of causing disease in cucurbit plants.

Over the dry season, and through the one week COVID lockdown, we tested whether CGMMV positive bees can transmit CGMMV during managed pollination. We exposed potted flowering watermelon plants to a bee hive containing viable CGMMV (Figure 1).

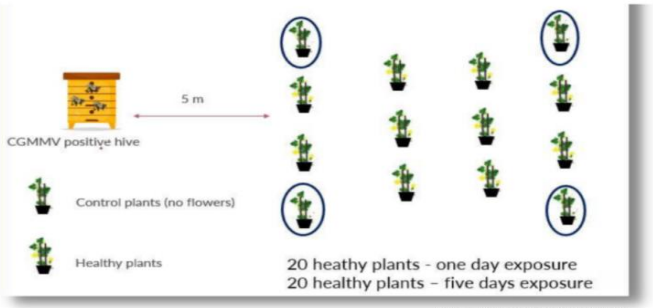


Figure 1 - Can bees move CGMMV around a field from positive hive to healthy plants?

It was observed bee visits to female and male watermelon flowers over five days (Figure 2). The trial was repeated four times and the plants that were visited by the bees are now being grown in a biosecure glasshouse. Experimental plants are tested and monitored for symptoms of CGMMV for eight weeks. Plant testing is at its final stage and the results will be available at the end of the year.

Figure 2 - observing bee visits to watermelon flowers

MELONS AUSTRALIA Hort Innovation MELON FUND

This project has been funded by Hort Innovation using the melon research and development levy and funds from the Australian Government. For more information on the fund and strategic levy investment visit horticulture.com.au

This project is funded by Hort Innovation, using the melon research and development levy and contributions from the Northern Territory Government. Project results will assist in management strategies for CGMMV, improving the health of Australian melons and the productivity of the industry.

Update August 2021

Appendix 18: CGMMV and Bees Project update, Melon News: December 2021, Australian Melon Association

NOT YET PUBLISHED

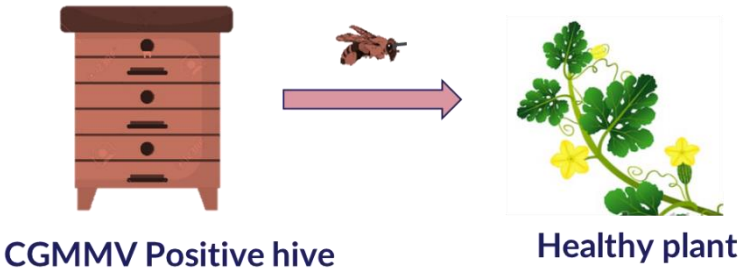
During the past two years the CGMMV-honey bee team in Darwin (Northern Territory (NT) Department of Industry, Tourism and Trade) has been busy conducting experiments for the project VM18008 – 'Understanding and managing the role of honey bees in CGMMV epidemiology'. This project is funded by Hort Innovation, through the melon research and development levy and with contribution from the NT Government. This project investigates the potential transmission of *Cucumber Green Mottle Mosaic Virus* (CGMMV) to cucurbit plants during pollination by honey bees.

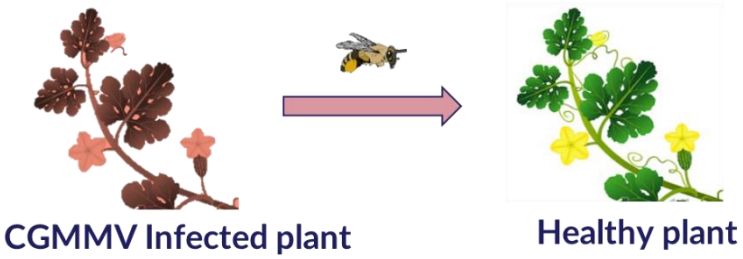
The team has carried out laboratory and field trials to answer the following questions: 1) Can honey bees transmit the virus from CGMMV positive hives or CGMMV infected plants to healthy plants? 2) Can commercial honey and pollen harvesting influence CGMMV presence in hives? 3) How long can CGMMV survive in a bee hive? 4) What pollen species are associated with CGMMV positive hives? and 5) Is there a potential for honey bees to carry other important cucurbit plant viruses?

After extensive field and laboratory trials the research team found that, during pollination activities, it is possible for honey bees to transmit CGMMV to healthy plants, resulting in disease. Transmission of CGMMV by honey bees is much more likely to happen when a bee travels between a plant already infected with CGMMV to a clean plant, compared with the situation in which a bee travels from a hive that contains CGMMV to a clean plant. The team also found that the CGMMV load in positive beehives can be reduced by removing honey and pollen from the hive (such as is done in the management of commercial apiaries) as well as by placing the hive in an area without any CGMMV host plants. Interestingly, whilst CGMMV can be detected from honey collected from a bee hive a year after original exposure to CGMMV positive plants, this CGMMV is not viable (that is, it is not capable of causing disease). The team also delivered an AMIA webinar in November and factsheets for the project are available on the Hort Innovation website (just search VM18008).

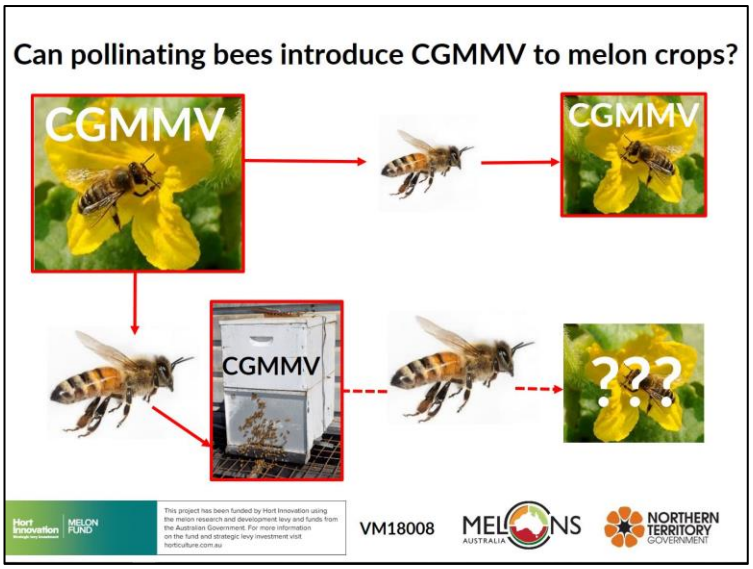
The conclusion of this research is that the most effective biosecurity measure is to keep your property CGMMV free. The most likely role of honey bees is to act as a mechanical vector of the virus in a location where it is already present. However, for those properties that have been infected, there are strategies that can be used to minimise the potential for honey bee hives to introduce CGMMV into new environments. The project findings provide a scientific basis for cucurbit producers and apiarists to reduce and manage the risk of CGMMV. The project factsheets provide guidelines on how to do this.

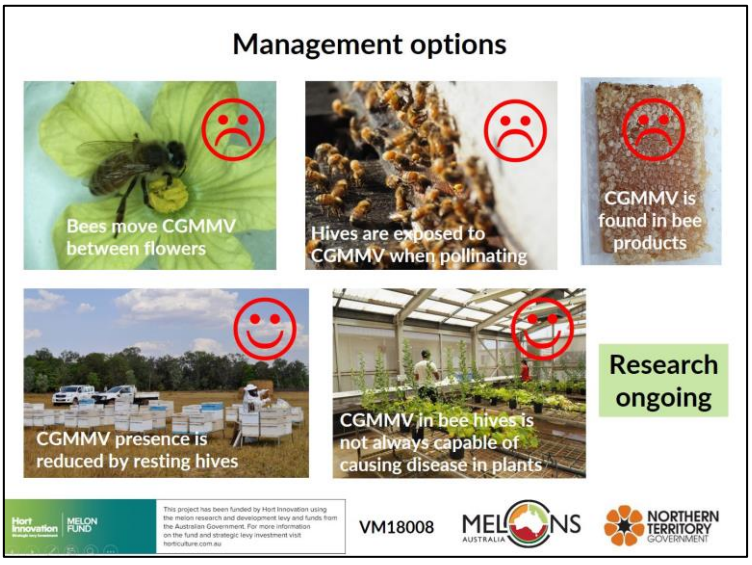
The project will be finalised at the end of 2021.


CGMMV Positive hive
Healthy plant


CGMMV Infected plant
Healthy plant

Appendix 19: Information for NSW apiarists delivered by Ashley Zamek, Hort Innovation - 2021 04 09





Appendix 20: Alternative hosts for *Cucumber green mottle mosaic virus*

- Black nightshade (*Solanum nigrum*)
- Amaranth (*Amaranthus viridis*)
- Pigweed (*Portulaca oleracea*)
- Sabi grass (*Urochloa mosambicensis*)
- Wild gooseberry (*Physalis angulata*)
- Crowfoot grass (*Eleusine indica*)
- Caltrop (*Tribulus terrestris*)

This information is from VG15013 Improved Management options for *Cucumber Green Mottle Mosaic Virus* (CGMMV)

Appendix 21: Pollen metagenomics results

Confidential, provided separately

Appendix 22: Draft literature review - Managed pollinators as plant disease vectors: what risk do they pose?

Confidential, provided separately