Final Report

Improved Management Options for Cucumber *green mottle mosaic virus*

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Northern Territory Department of Primary Industry and Resources

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VG15013

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Improved Management Options for Cucumber green mottle mosaic virus – VG15013

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Summary

Cucumber green mottle mosaic virus is an economically important *Tobamovirus* that infects cucurbits. In September 2014, it was detected in the Northern Territory. This was the first time it had been reported in Australia. This three year national project focused on the improved management options for *Cucumber green mottle mosaic virus*.

The three key research areas of this project were to:

1. Determine the importance of weeds, non-host plants and honeybees on CGMMV in disease epidemiology.
2. Examine the potential for in-field diagnostics to assist rapid detection of the virus on farms known/suspected to be infected with CGMMV.
3. Develop multilingual communication and extension materials to assist with management options to cucurbit growers including on-farm biosecurity protocols.

To address these research areas, five key activities were carried out. A summary of the findings from each activity are summarized below

Activity 1. CGMMV alternative host and non-hosts.

Activity 2. Understanding CGMMV biology in contaminated soil

Activity 3. Improving CGMMV diagnostics for plant and seed

Activity 4. Understanding the role of honey bees in CGMMV epidemiology

Activity 5. Extension and capacity building

Activity 1. CGMMV alternative host and non-hosts.

Six crops were investigated to determine whether the plants were hosts of CGMMV. These were mainly vegetable alternatives but a cover crop and legume were also identified after consultation with industry and research partners. These six crops, sweet corn, snake bean, capsicum, okra, sorghum and peanut were found to be non-hosts of CGMMV in both field and pot trials in the NT. Common weed species grown in Australian cucurbit production areas were surveyed and a common list was derived for pot trials in the NT and Western Australia. CGMMV positive weed species are *Citrullus lanatus* (wild melon), *Luffa acutangula* (wild luffa), *Amaranthus viridis* (Amaranth), *Portulaca oleracea* (pigweed), *Solanum nigrum* (black nightshade), *Chenopodium album* (fat hen), *Physalis angulata* (wild gooseberry) and *Urochloa mosambicensis* (sabi grass). Some positive test results for weed seeds also indicated this to be a pathway for CGMMV spread. However, it should be noted that the interaction between weed host and the virus is not clear cut and further work is required.

Activity 2. Understanding CGMMV biology in contaminated soil

Previous field trial work in the NT showed that CGMMV remained viable after at least 12 months without host plants. The immunocapture protocol to purify CGMMV particles was unreliable when using soil that was not heavily infested but worked with infected leaf material. Comparative experiments to investigate seedlings transplanted into potting mix contaminated with CGMMV resulted in 11/100 plants becoming infected. When seeds were directly sown into the potting mix with CGMMV sap, it was found that CGMMV only remained infectious in soil up to 36 weeks. Direct sowing of seeds in contaminated soil produced less infection numbers compared to transplants due to the damaged root systems allowing virus entry in the transplants.

Activity 3. Improving CGMMV diagnostics for plant and seed

Seed molecular testing used in this project was validated in a PBCRC project and was conducted in Victoria, the testing was extended to include Asian cucurbit seeds from seed extracted from the fruit of "Asian" cucurbit species including *Benecasa hispida* (two seed lots extracted from wax and hairy melon), *Lagenaria siceraria, Luffa acutangula, Luffa cylindrical, Momordica charantia* and *Trichosanthes cucumerina*. Seeds were divided into sub-samples of 100, 250, 500 and 1000 and spiked with the equivalent amount of one CGMMV contaminated seed (hybrid *Cucurbita maxima X Cucurbit moschata*)

for each seed type. CGMMV could be detected reliably in sub-samples of up to 500 seed of all species except *T. cucumerina,* for which sub-sample sizes of up to 250 seed were most reliable. Higher Ct values, indicating lower sensitivity, were observed in all seed sub-sample sizes of *M. charantia* and *T. cucumerina* compared to other seed types. These results show that the protocol for detection of CGMMV in seed is applicable to a broad range of cucurbit species and sub-sample sizes of up to 500 seed may be reliable for most cucurbit species.

In-field diagnostic testing a new version of the lateral flow dipstick on the market was able to detect the Australian strain of CGMMV in 10^3 dilution and was suitable for bulking up to 10 plants in a single assay. A new RT-RPA assay developed within this project in Queensland and preliminary results for the RT-RPA assay indicated that CGMMV could successfully be detected. The research pursued lateral flow detection and a multiplex RPA assay targeting the common cucurbit plant viruses (papaya ringspot virus, zucchini yellow mosaic virus, watermelon mosaic virus and CGMMV). A case study with PRSV and CGMMV commenced with the assay working well for CGMMV but not PRSV. But further refinement in readiness for validation showed cross binding issues that resulted in false positives and due to inconsistent, unreliable and non reproducible results, this test was not pursued.

Activity 4. Understanding the role of honey bees in CGMMV epidemiology

A variety of bee products were sampled since 2014 as part of the CGMMV incursion response and continued within this project until 2017. Over 150 pooled samples were tested for the presence of CGMMV, 89 tested for viability. Of these, CGMMV was present in bees, brood, pollen, honey, wax and propolis, but only viable CGMMV was found in adult bees, pollen and honey.

To compile a bee sampling protocol, 11 hives were sampled in each apiary, with apiaries varying in size from 29 – 124 hives. In each apiary one hive was sampled intensively (10 samples of both bees and pollen) and ten hives were used for extensive sampling (1 sample of both bees and pollen from each hive). A single sample of honey was taken from all hives, and this was included in the analysis as extensive sampling. It is recommend that extensive sampling of honey and either adult bees or pollen are undertaken. Testing of different bee products showed that CGMMV was present in bees, brood, pollen, propolis, wax and honey but only viable CGMMV was found in adult bees, pollen and honey. When specific hives were regularly sampled, over time, only the honey remained CGMMV infective.

Activity 5. Extension and capacity building

There have been extension activities by project members participating at growers meetings and scientific conferences. Research factsheets have been developed as research is updated or completed to inform industry stakeholders. The on-farm biosecurity manual is available in English, Vietnamese and Khmer.

The recommendations for the management of CGMMV are made on the basis of the work in VG15013:

- Plant only clean seeds that have been tested at the higher level of 9400 seed numbers per batch. Request documentary evidence of testing from the seed supplier
- Avoid sharing seeds and if you do, investigate the source and history of the material and obtain evidence that the seeds have been tested and is negative for CGMMV
- Do not save seed from any plant or crop suspected of being infected with CGMMV
- Adopt and maintain the on-farm biosecurity procedures, these include
    - 'Come clean, go clean'
    - Appropriate disinfection of tools, equipment, machinery and footwear
    - Exercise particular care with equipment and people if moving production to a new area from an area where CGMMV has occurred
- Plant crops in clean soil and grow non-hosts plants in infested CGMMV soils to reduce the virus load in the ground
- Learn to recognize CGMMV symptoms early and avoid disturbing the area once infection has been identified
- Rogue out symptomatic plants and add a buffer zone
- Know where the bee hives you use have previously worked

- Use the redeveloped field immunostrip available from Agdia (NB. We found this could cross react with PRSV) but also send samples into your state diagnostic laboratories for confirmatory testing.
- Seed testing of Asian cucurbits is reliable for subsamples up to 500 seeds for most species except *T. cucumerina* where the sample size should not exceed 250 seeds.

Future research needs

- Mode of transmission of CGMMV by honeybees and the epidemiological significance
- Use of disinfectants under commercial conditions to provide layer of protection for new seedlings
- Role of cover crops (non-hosts) to reduce CGMMV inoculum in soil, how long to grow and whether the reduction of the pathogen also leads to increase in beneficial microbes?
- Role of weed species and other alternative hosts in the epidemiology and survival of CGMMV
- Nature and value of cucurbit cultivars with resistance/ tolerance to CGMMV in disease management

Keywords

Cucumber green mottle mosaic virus; cucurbits; *Tobamovirus*; weeds; honeybees; non-hosts; seeds, on-farm biosecurity; extension; in-field diagnostics

Introduction

Cucumber green mottle mosaic virus (CGMMV) is a *Tobamovirus* that can infect cucurbit plants and is responsible for significant economic losses worldwide (CABI Crop Protection Compendium, January 2019; https://www.cabi.org/cpc/datasheet/16951). It was first reported in 1935 (Ainsworth, 1935), followed by a report by Hollings et al (1975) describing the first *Tobamovirus* infecting plants in Cucurbitaceae. The type strain is the most common strain in Europe and this strain does not produce fruit symptoms (Norwegian Scientific Committee for Food Safety). There are several strains of CGMMV worldwide and a source of spread is due to contaminated seed; this provides a route between countries and introduction into new uninfected cucurbit growing areas. International research has identified that current seed disinfection treatments do not significantly eliminate the infectivity capacity of CGMMV on cucurbit seeds (Reingold et al 2015). This highlights the need to screen seeds at a much higher stringency to allow detection and avoid entry of new CGMMV strains into Australia.

The Australian emergency measures required all seed of listed species proposed for import be tested for CGMMV using an International Seed Testing Association-accredited ELISA protocol on samples of 9400 seeds from 'large seed lots' or a sample of 20% of the seed lot in the case of 'small seed lots' (Constable et al 2018). Testing in two Australian laboratories showed that in large seed lots, one of 16 seed lots (6.3%) of cucumber seed tested positive for CGMMV and three of 19 seed lots (15.8%) of melon. Estimated prevalence of CGMMV in the four positive large seed lots ranged from 0.044 to 0.254%, the latter being recorded from a sample of melon seed of South American origin. In the case of small seed lots, seven of 86 seed lots (8.1%) of cucumber were found to be contaminated with CGMMV, as were 10 of 374 (2.7%) small seed lots of melon. CGMMV was also detected in one of 54 (1.9%) small seed lots of watermelon. Constable et al 2018 found that using a hypergeometric distribution, non-detection of CGMMV in a required sample of 9400 seeds from large seed lots indicated, with a probability of 0.99, that the prevalence of the virus is no higher than 0.0439%. The required sample of 20% of a small seed lot provided lower levels of sensitivity for prevalence compared to that for large seed lots, with statistical confidence varying with the number of seeds tested. As such, given equal prevalence, contaminated small seed lots have a lower probability of detection than larger seed lots.

CGMMV is a highly stable particle that can persist on plant debris, soil, water and seed, which can be the primary source of infection. Transmission in the ground occurs when seedlings come into contact with contaminated plant debris, contaminated soil, machinery or equipment, water, transplants and contaminated packing material. Infection of plants is via wounds allowing the virus to enter due to normal handling of plants especially when plants are pruned, staked or handled during planting. CGMMV infected plants display a range of symptoms from mosaic and mottling of leaves, bleaching or yellowing of leaves, fruit symptoms can include external mottling, yellowing, internal cavities and premature softening (Choi 2001, Varveri et al 2002, Boubourakas et al 2004, Shim et al 2005)

In September 2014, CGMMV was detected for the first time in the Northern Territory (NT) (Tesoriero et al 2014), Australia on mainly commercial watermelon farms. It has since been detected in a range of cucurbit vegetables such as pumpkin, cucumber, squash and Asian cucurbit vegetables. Since the initial detection in the NT, CGMMV is now found in isolated areas in Queensland (QLD) and South Australia (SA) and in cucurbit growing regions in Western Australia. The Australian CGMMV strain (Kehoe et al 2017) shares very high sequence similarity to the Indian bottle gourd strain and the Canadian CGMMV strain from cucumber. *Cucumber green mottle mosaic virus* is classed as endemic in the NT and WA, whilst under quarantine control in QLD and SA.

A critical part of CGMMV management is identifying the possible sources of CGMMV reservoirs that could potentially retain CGMMV levels and allow reinfection to occur. These may include susceptible weed hosts grown around and within cucurbit areas and the potential role that bees and apiaries play in the infection cycle. On the other hand it is also critical to identify non-hosts of CGMMV for potential cover crops to allow production on CGMMV infected land. Honeybee pollinators, which are used to improve yield and fruit quality in cucurbit crops, could represent a significant reservoir of CGMMV. Preliminary research by the NT DPIR has indicated that the virus remains detectable, and viable, within honeybee hives months after their last exposure to infected plants. The ability of the Australian strain of CGMMV to be transmitted from positive testing hives to uninfected cucurbits or weed species is unknown. Work is needed to clarify the

potential of bees to transmit the Australian strain of CGMMV in Australian agricultural settings, so that cucurbit growers are able to effectively manage the risk of further CGMMV spread through bee activity.

The three key research areas of this project were to:

1. Determine the importance of weeds, non-host plants and honeybees on CGMMV in disease epidemiology.
2. Examine the potential for in-field diagnostics to assist rapid detection of the virus on farms known/suspected to be infected with CGMMV.
3. Develop multilingual communication and extension materials to assist with management options to cucurbit growers including on-farm biosecurity protocols.

Methodology

Weed as alternative CGMMV hosts

Northern Territory

Throughout the project, weeds in the Katherine area were collected routinely to monitor the levels of CGMMV in and around previous infested properties. These included species listed in Table 1 and Crowsfoot grass (*Eleusine indica*) and caltrop (*Tribulus terrestris*).

Table 1. Weed species used in CGMMV trials in the Northern Territory and Western Australia.

Weeds species for CGMMV trials (NT)	Weeds species for CGMMV trials (WA)*
Pigweed (*Portulaca oleracea*)	Afghan melon (*Citrullus lanatus*)
Amaranth (*Amaranthus viridus*)	Paddy melon (*Cucumis myriocarpus*)
Native gooseberry (*Physalis angulata)*	Wild passiflora species (*Passiflora foetida*)
Black nightshade *(Solanum nigrum)*	Nightshade (*Solanum nigrum*)
Fat hen *(Chenopodium album*)	Wild luffa (*Luffa acutangula*?)
Sabi grass (*Urochloa mosambicensis*)	Wild pumpkin vine (*Operculina brownii*)
	Wild hibiscus (unknown species)

Four weed hosts; *Amaranthus viridis, Portulaca oleracea, Solanum nigrum, Physalis angulata*, one experimental weed host; *Chenopodium album* and one grass; *Urochloa mosambicensis*, were chosen to determine their CGMMV host status. The four weeds and grass were commonly found in or around cucurbit fields. To study this interaction further, 80 each of the weeds and grass were potted into individual pots, with clean soil and automatic watering. To inoculate the weeds, ~40µl of prepared inoculum was rubbed onto the surface of two leaves per weed with silicon carbide to create a small amount of damage on the surface and allow the virus to enter. The weeds were left to grow for a two-month period before they were sampled and tested for CGMMV.

Figure 1. *Physalis angulata* (Native gooseberry) flowering plant with seed pod (**A**) and its seedlings (**B**); and *Solanum nigrum* (Black nightshade) berries (**C**).

The ability of CGMMV to infect non-host plants

With the knowledge that CGMMV is viable in host free soils for a period for at least 1 – 2 years, alternative crops which are non-hosts of the virus were investigated. With industry consultation and project members from Australian jurisdictions, a list of crops was determined and tested to evaluate their CGMMV host status. The trials were conducted in the NT in both field (Figure 2) and pot trials. As there are two distinct seasons in the NT, dry (d) and wet (w), crops for each of the seasons are being investigated. These crops include; sweetcorn (d), snake bean (d), Okra (w), capsicum (d), peanuts (w), sorghum (w) and common grasses (w). Initially a field trial was to be conducted for both the dry and wet season trials. Due to poor infection rates within the positive plants and general health of the plants in the dry season trial, pot trials were conducted (Figure 3) for this particular trial.

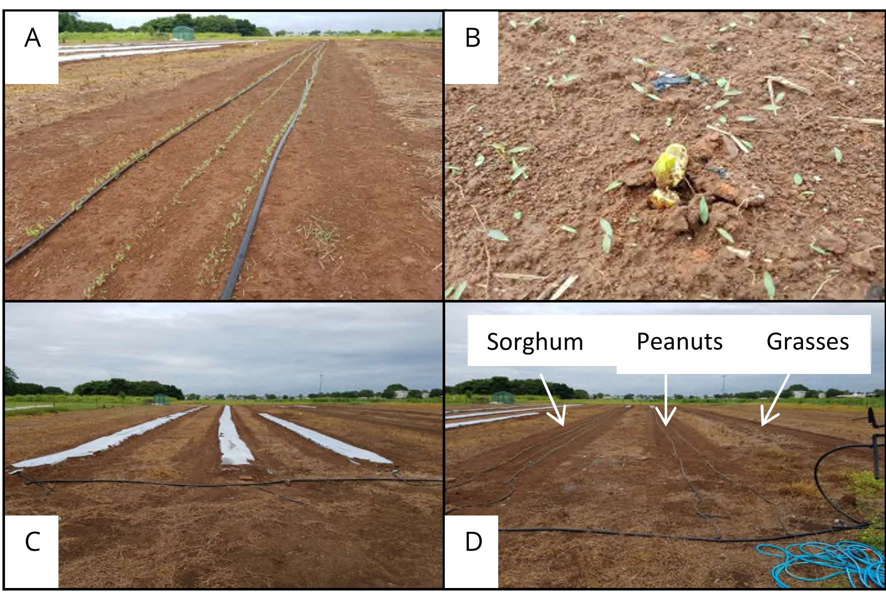

Figure 2. Germination of the non-host seeds **A)** sorghum and **B)** peanuts and the layout of the **C)** positive and **D)** non-host blocks for the wet season trial.

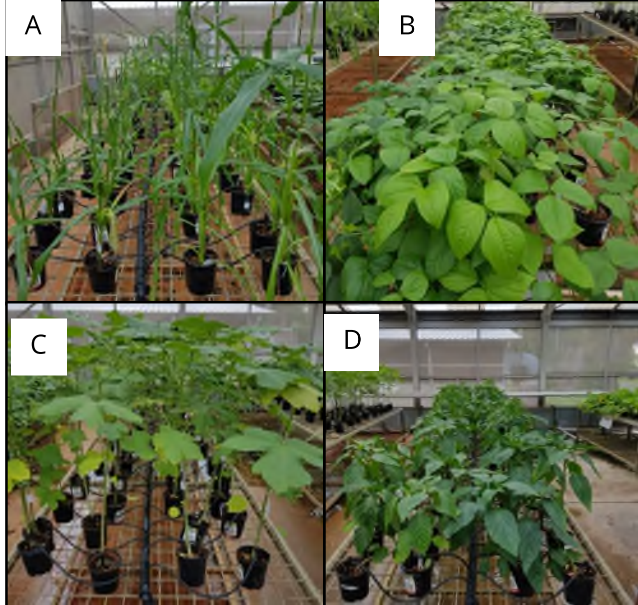

Figure 3. Non-host pot trial with **A)** Sweet corn, **B)** Snake bean, **C)** Okra and **D)** Capsicum at 5 weeks post inoculation with CGMMV.

Understanding CGMMV diagnostics for plant and seed material

Improving in-field diagnostics

Current technologies were evaluated for applicability of CGMMV diagnostics in a resource limited situation. The easily adapted and robust technologies for diagnosis of CGMMV from plant material are displayed in Table 2.

Table 2. Comparison of current technologies available for CGMMV diagnostics

Technology	Sensitivity	Specificity	Speed	Index ing	User Skill
Lateral Flow	Low	Med	Fast	Low	Low
LAMP	Med/High	High	Fast	Low	Med
RPA	Med/High	High	Fast	Low	Med
ELISA	Med	Med	Slow	Med	Med/High
Immuno Blotting	Low	Med	Slow	High	Med/High

As can be seen from the table, different technologies are more applicable depending on the testing situation; larger testing numbers will require techniques such as ELISA or Immunoblotting. The specificity and sensitivity of lateral flow, ELISA and Immunoblotting is related to the properties of the antibodies that used for the assays. Antibodies from different sources for CGMMV detection have been found to have differing sensitivities. The specificity of the nucleic acid based tests is both a positive and negative trait. Good design of primers means that there is little chance of false positives due to specific regions being targeted, but there is a possibility of false negatives if an isolate of CGMMV is varied enough from the isolate that the primers were designed to.

ELISA/Lateral Flow

In the early stages of the CGMMV incursion, the commercially available dipsticks were trialled for their reliability and effectiveness in the field. It was found that only 10% were effective compared to laboratory testing. In Queensland, testing of Agdia lateral flow strips with the Australian isolate of CGMMV yielded no detectable signal after 15 minutes, making it unsuitable for a field diagnostic. A newer version was released during the course of the project and was evaluated for sensitivity levels. Two different commercially available antisera were tested with the current isolate of CGMMV in DAS-ELISA.

Seed testing

VG15013 aligned with the PBCRC2148 “International acceptance of Australian solanaceous and cucurbit seed tests” project that was completed in June 2017. This PBCRC2148 project established that Australian developed molecular seed testing protocols could be used as an international standard for detection of CGMMV in seed. A proficiency test between three international seed testing laboratories was done to determine the most reliable test for detection of CGMMV. *Cucumber green mottle mosaic virus* contaminated melon, cucumber, watermelon and rootstock seed were diluted with uninfected seed at 1/100, 1/250 and 1/1000 and tested by ELISA and by RT-qPCR.

The rate of detection of CGMMV in individual seeds lots could be as low as one sub-sample of 100 cucurbit seeds in a 9,400 seed sample, highlighting the risk that internationally traded seed poses as a pathway for the introduction of seed-borne and transmitted pathogens into Australia. Statistical analysis in combination with the detection rates of CGMMV in seed lots supports the use of larger seed samples to increase the possibility of detecting contaminated seed. This project used bioinformatics analyses to evaluate and select conventional/end-point and real-time RT-PCR tests for detection of CGMMV. A molecular protocol for the

detection of CGMMV in cucurbit seed was developed. It was evaluated using several cucurbit species that are gown on a large commercial scale in Australia including cucumber (*Cucumis sativus*), rockmelon (*C. melo*), watermelon (*Citrullis lanatus*), squash (*Cucurbita pepo*), pumpkin (*C. maxima, C. moschata*, *C. maxima x C. moschata*) and rootstocks (*Lagenaria* sp.). The seed testing was then utilized in VG15013 to detect CGMMV in seed of other cucurbit species (Asian vegetables) including *Benecasa hispida, Cucumis anguria, Lagenaria siceraria, Luffa acutangula, Luffa cylindrical, Momordica charantia* and *Trichosanthes cucumerina*.

In addition, seeds from the NT weeds trial was also tested, 44 samples of seeds from Amaranthus (four samples), Black Nightshade (one sample), Chenopodium (16 samples), Gooseberry (five samples), Pigweed (16 samples), Sabi grass (one samples) and Watermelon (one samples). Up to 2000 seeds of each sample were used for testing and these were divided into sub-samples of up to 1000 seed, when more than 1000 seed were available to test. RNA was extracted from a total of 84 seed sub-samples and tested using the two endpoint RT-PCR tests (Ling et al, 2014; Reingold 2013) and the RZ real-time RT-PCR test (Berendsen and Oosterhof, 2015) for CGMMV, as described in the PBCRC protocol. They were also tested using the real-time RT-PCR described by Hongyun et al (2008).

Understanding the role of bees and the persistence of CGMMV in honey bee hives in CGMMV transmission

<u>CGMMV bee hive field trial</u>

Transmission trials were conducted at the NT DPIR Berrimah Research Farm. The trials sought to determine if honey bees could transmit CGMMV to clean plants and were set up to coincide with the CGMMV field trial at Berrimah Research Farm.

The bee trial was set up adjacent to the potentially infectious CGMMV trial. The first phase of the trial consisted of 80 potted cucurbits (40 squash and 40 watermelon plants) contained in large clear plastic tubs and placed within a bird free netted structure. Two active European honey bee hives were placed in the vicinity of the cucurbits which is adjacent to the inoculated CGMMV field site. The hives were sampled before the trial commenced, twice during the trial and will be sampled again at the end of the trial (8 weeks). The second trial phase had another 80 cucurbits (40 squash and 40 watermelon plants) replace the original plants.

Experimental plants were monitored three times a week at varying times of the day. The presence of flowers and insect activity, including but not limited to visiting bees, was noted.

Two European honey bee hives were located within 10 metres of the cucurbit enclosure. The orientation of the hives was changed fortnightly to encourage the bees to scout in close proximity to the experimental plants. At times the hives were placed inside the enclosure to protect them from predation from wildlife.

Extension and capacity building

<u>Industry engagement</u>

Where possible during the course of the project, biennial grower meetings were held in the NT in both Darwin and Katherine and facilitated by NT Farmers Association. For each meeting, a presurvey form was sent to growers to participate, updates on project progress, where available, factsheets were distributed.

In all jurisdictions, where possible, project members attended grower and industry meetings to provide updates. The agenda items included project introduction and the new CGMMV regulations and the on-farm biosecurity plan required to comply with the regulations in 2015/2016. In Katherine, 19 melon and vegetable growers and industry representatives met on 17 December 2015 and on the 14 March 2016 in Darwin which attracted 35 attendees. The scope and the focus of the VG15013 CGMMV R&D project was presented to the growers and to ensure that the proposed research would answer the questions posed by industry. The growers gave their input into what was needed to refine the draft on- farm biosecurity plan template that was to be used as the basis for the grower’s current market access and

disease management response.

Grower training on farm biosecurity plan and mock audit

Growers were then offered a training workshop to go through the on-farm biosecurity plan template and how to complete it for their farm and be compliant with interstate quarantine requirements. The training took place on farm in an industry leaders shed (Marrakai), and included a mock audit and site inspections of signage, footbaths, movement control and site for wash down facilities.

Outputs

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

Factsheets

Cucumber green mottle mosaic virus (CGMMV) (Appendix 2)

Symptoms of *Cucumber green mottle mosaic virus* (CGMMV) I (Appendix 3)

Symptoms of *Cucumber green mottle mosaic virus* (CGMMV) II (Appendix 4)

Cucumber green mottle mosaic virus (CGMMV) Symptoms and damage (Appendix 5)

Non-hosts of *Cucumber green mottle mosaic virus* (Appendix 6)

Weed hosts of *Cucumber green mottle mosaic virus* (Appendix 7)

VG15013 – Improved management options for *Cucumber green mottle mosaic virus* (CGMMV) (Appendix 8)

VG15013 – Improved management options for *Cucumber green mottle mosaic virus* – CGMMV and European Honey bees Research update (Appendix 9)

Management practices to minimise Cucumber green mottle mosaic virus in European honey bee hives" (Appendix 10)

CGMMV- Improved management options (Appendix 11)

Farm Biosecurity Plan – English (Appendix 12)

Farm Biosecurity Plan – Vietnamese (Appendix 13)

Farm Biosecurity Plan – Khmer (Appendix 14)

CGMMV Preliminary soil research industry summary

Australasian Plant Pathology Society Conference CGMMV poster

Report on CGMMV sampling from Charters Towers bee hives

Grower meetings

Denis Persley attended cucurbit growers meeting held in Virginia, South Australia and Ayr, Queensland (AMA) to discuss CGMMV management and provided images of the virus affecting fruit that were obtained from the research project.

Dr Mary Finlay-Doney had face to face meetings and telephone conversations with 87% NT apiarists in the last six months

CGMMV on-farm Biosecurity plan following Biosecurity WAs response to the CGMMV outbreaks in Perth, Geraldton, Carnarvon and Kununurra.

Dr Lucy Tran-Nguyen, Greg Owens and Denis Persley visited Bundaberg and presented to growers.

Two grower meetings each year in Darwin and Katherine.

Industry conferences

Most project members attended the 2016 Australian Melon Conference in Mildura March 2016 on CGMMV and Fusarium wilt of watermelon. The conference was attended by 230 people, 70 of which were growers. To coincide with the conference, a project meeting with all project members including

key stakeholders and Dr Aviv Dombrovsky was held to discuss and plan the project activities.

Dr Fiona Constable attended the Australian Melon Association Bayers Conference in Griffith, 2017 and presented the Plant Biosecurity seed testing and the VG15013 project updates.

Greg Owens and Denis Persley attend Hort Convention in 2017 where Dr Lucy Tran-Nguyen won the AusVeg Researcher of the year.

Scientific conferences

Dr Tran-Nguyen presented at the 13th International Plant Virus Epidemiology Symposium, Avignon on the “Cucumber Green Mottle Mosaic Virus in Australia – the story so far”.

Dr David Lovelock attend the American Phytopathological Society Conference, Texas in June 2017 and presented the CGMMV Australian situation and research updates.

Dr Lucy Tran-Nguyen, Dr David Lovelock and Dr Mary Finlay-Doney attended and presented at the 2017 Australasian Plant Pathology conference in Brisbane.

Dr Tran-Nguyen, Dr Fiona Constable and Denis Persley attended the International Congress on Plant Pathology in Boston, 2018 and co-facilitated the inaugural international research meeting with USDA on CGMMV with over 15 delegates from USA, Australia, Netherlands and Canada.

Dr Lucy Tran-Nguyen, Dr Fiona Constable, Dr Mary Finlay-Doney attended and presented at the melon conference in Townsville in September 2018.

Dr Lucy Tran-Nguyen will present all the CGMMV research findings at the Australasian Plant Pathology Conference in November 2019. https://www.apps2019.org/

International visits

The University of Davis part funded Dr Tran-Nguyen to visit, present an extended version of the IPVE presentation at the University and the California Department of Food and Agriculture to researchers, service providers, seed regulators and regulators. The discussions and linkages were beneficial to provide a clearer understanding on the CGMMV outbreak in the USA in 2013 and 2014.

Farm and/or rural shows

CGMMV soil poster for NT Department of Primary Industry Staff forum but will also be used for Research Farm days and Rural shows.

Information

Stakeholder engagement plan

Stakeholder engagement timeline

NT vegetable and melon preseason event report from NTFA

Article in Kimberly Echo Newspaper “Farmers get close took at virus-combating trials” pg3, 28th September, 2017

WA Grower article “Ord River Irrigation Area cucurbit virus disease research”, pg 28-31, Summer 2017 Issue.

Outcomes

Outcomes listed in the MERI plan that was submitted in MS103.

Vision	***Better understanding the role that weeds and bees play in the disease epidemiology of Cucumber green mottle mosaic virus and improved in field diagnostic tools for detection***
Strategic Objective	*Farm productivity, resource use and management (to enable growers to defend themselves from emerging pests and diseases) (AusVeg Industry Strategic Investment Plant 2012-2017)*
Project Outcomes **Longer Term**	• *Inform industry on disease epidemiology and roles that weeds and honey bees may have as disease reservoirs or vectors* • *Identify/recommend in-field diagnostics to allow rapid detection as a decision making tool* • *Disease management and preparedness for non-affected regions* • *National Diagnostic protocol validated*
Project Outcomes **(intermediate outcomes)**	• *Identify roles of weeds and honey bees in the disease epidemiology, identify non-host crops able to reduce and survive under disease pressure and reduce virus inoculum by year 3 of project* • *Understand virus persistence in different soils under different environmental conditions by year 2 of project* • *Develop a multilingual on-farm biosecurity manual by project completion*
Project Activities (immediate outcomes)	• *Current scientific literature on the virus reviewed* • *Engage key stakeholders to advise, update, extend and evaluate research outputs*
Foundational Activities	• Conduct and evaluate • Weed and non-hosts national surveys • Pot trials (contaminated soil and weeds) • In-field diagnostic technologies in the market • Seed testing (expand to include Asian cucurbit vegetable) • Grower engagement communications activities and meetings

Immediate project outcomes

Current scientific literature on the virus reviewed

A review paper on CGMMV was published in 2017 and co-authored by the project leader.

Dombrovsky, A, Tran-Nguyen, L.T.T., Jones, R.A.C. (2017). *Cucumber green mottle mosaic virus:* rapidly increasing global distribution, etiology, epidemiology and management. *Annual Review of Phytopathology*. https://doi.org/10.1146/annurev-phyto-080516-035349

Engage key stakeholders to advise, update, extend and evaluate research outputs
Refer to outputs section for details

- biannual grower meetings in the NT each year
- attendance and presentation at industry conferences
- attendance and presentation at scientific conferences
- grower meetings project members in WA, QLD and SA
- grower factsheets developed

The Darwin region melon and vegetable growers' preseason meeting was held on 23 March with 47 participants at Coastal Plains Research Station (Figure. 4). This was also the first field day for the

vegetable IPM demo site being developed in conjunction between NTDPIR, TNRM and NT Farmers. Lucy Tran-Nguyen (NT DPIR) provided an update on the research to growers and industry representatives and reinforced the procedures in place on-farm to protect farms as best as possible from any infection from this virus. Research entomologist, Mary Finlay-Doney presented her work on CGMMV and honey bees and the Plant Health NT provided an update on market access for cucurbits travelling interstate and CGMMV detections in WA and Qld.

Figure 4. Grower meeting at Coastal Plains Research Station, NT.

The information from this meeting was used to update the Information sheet which was disseminated through the stakeholder network, along some amendments to the CGMMV on-farm Biosecurity plan template that has been slightly modified by Biosecurity WA to use in their response to the CGMMV outbreaks in Perth, Geralton, Carnarvon and Kununurra.

The project leader, Lucy Tran-Nguyen (NT DPIR), Denis Persley (QDAF) and NT farmers IDM were invited by BFVG to address a group of cucurbit growers in Bundaberg following the detection of CGMMV in cucumber shade-houses in that area. The meeting was well attended by 25 participants and there was good interaction between the Bundaberg growers and the NT reps. The reaction was similar to when Dr Dombrovsky addressed the NT growers during the first phases of the outbreak in the NT. Growers being reassured that there was a program of on-farm biosecurity protocols that would protect cucurbit farms by people with actual experience of the disease and could relate real grower experience of the virus and the impacts of the quarantine. Bundaberg growers were provided with copies of the on-farm biosecurity plan templates, information sheets from the VG15013 project and QDAF information along with contacts and reference information.

VegNet activities were used to inform the wider vegetable and melon community about the disease and the on-farm protocols and resources for its management. NT Farmers was part of a combined VegNet presentation to Hort Connections in May 2017 that allowed NT Farmers to tell the story of CGMMV and how it impacted on growers. More particularly how Vegetable projects VG12113, VG15013 and VG15044 worked together to respond, research and manage this exotic virus incursion. Project member, Denis Persley (QDAF) also participated in the Hort Connections conference.

VegNet projects are producing videos as part of the extension process and the CGMMV incursion and response is the key focus of the biosecurity video just released for public viewing.
https://www.youtube.com/watch?v=xsuKyYQVRlU&feature=youtu.be

Biosecurity in the Top End was complicated by two more incursions in 2018. Citrus canker and Asian Honey bee were identified in the Darwin Region and the citrus canker impacted on many of the small Asian market gardeners that were also impacted by CGMMV. These incursions also impacted on NT Farmers ability to concentrate on CGMMV management and extension when these growers needed

assistance through the citrus canker response around interstate market access and owner reimbursement costs. A pleasing outcome was the way these growers could relate and use the general on-farm biosecurity principles and practices for CGMMV to protect themselves from citrus canker and the records necessary for early return to interstate trading.

NT Farmers surveyed the other VegNet officers around Australia to gauge the demand for translation of the on-farm biosecurity template for CGMMV into other languages besides the existing English and Vietnamese. This was done via emails and at the National VegNet meeting at Hort Connections. The only other language discussed was Khmer but that the demand was not strong from the VegNet partners.

NT Farmers in conjunction with the NT DPIR CGMMV team coordinated the vegetable and melon preseason meetings in Katherine at Katherine research station (Figure 5) on 11 April 2018 and for Darwin at Coastal plains research station (Figure 6) on 12 April. In total 38 farmers and industry representatives attended these meetings. NT Farmers have also conducted farm walks at the IPM Demonstration Plot at Coastal Plains Research Station where CGMMV was discussed and appropriate on farm biosecurity was demonstrated at these farm walks, and subsequent irrigation workshops. This leading by example helps to reinforce the practices required to minimise disease transmission. The IPM trial (Figure 7) also raises awareness of endemic insects and pathogens that can occur on vegetable crops but also allow monitoring for any exotics. Early detection is key to minimize the spread of disease and economic impact.

Figure 5. Vegetable preseason Katherine 2018

Figure 6. Preseason Darwin

Figure 7. Farm walk CPRF July 2018. NB Zucchinis included in the planting as a cucurbit requiring CGMMV awareness and management.

In March NT Farmers entered into an agreement with the Northern Australian Quarantine survey group (NAQS) to run a pilot project on-farm engagement with non-English speaking background growers to increase the number of surveys that occur on commercial farming properties. NT Farmers employed a casual second-generation Vietnamese grower to assist with this engagement. This proved beneficial when the NAQS botanist could correctly identify the weeds in local cucurbit farms that determined the probability of CGMMV remaining in the farm. This engagement is set to continue with an extension of this engagement role and help with reducing the impact of citrus canker on the smaller market gardeners.

Intermediate project outcomes

Non-hosts of CGMMV

A total of six species were identified as CGMMV non-hosts from pot and field trials in the NT. Of the 80 plants of each species, no CGMMV was detected (Table 3). It is acknowledged that it is difficult for growers to change to another crop with ease and it is also dependent on the region. However, this non-exhaustive list provides growers different options for alternate crops.

Table 3. Identification of CGMMV non-host species.

Crop	Season	Field Trial	Pot Trial
Sweetcorn	Dry	-	-
Snake bean	Dry	N/A	-
Capsicum	Dry	-	-
Okra	Wet	N/A	-
Sorghum	Wet	-	-
Peanut	Wet	-	-

Weeds as alternative hosts of CGMMV

The weed species that were identified as alternative hosts of CGMMV are listed in Table 4 below.

Table 4. Weed/Grass PCR and qPCR results two-months post inoculation with CGMMV.

Weed/Grass	Conventional PCR	qPCR
Amaranthus viridis	+	+
Portulaca oleracea	-	+
Solanum nigrum	-	+
Physalis angulata	-	-
Chenopodium album	-	+
Urochloa mosambicensis	-	+

These results may indicate that although CGMMV was detected through qPCR, the virus may be unable to replicate within the weed itself and the virus may be contained to a localised area.

Additional weed species identified near cucurbit production in WA and were inoculated with CGMMV to define CGMMV host range (Table 5). Only Afghan melon and wild luffa produced CGMMV symptoms (Figure 8).

Table 5. Weed species for CGMMV host range studies

Weeds species for CGMMV trials (WA)	No. plants CGMMV infected/total inoculated	Symptoms
Afghan melon (*Citrullus lanatus*)	8/8	Mild leaf mottle
Wild luffa (*Luffa acutangula*)	8/8	Mild leaf mottle
Nightshade (*Solanum nigrum*)	0/16 *	
Chenopodium quinoa	0/6	
Wild passiflora species (*Passiflora sp.*)	0/2	
Wild pumpkin vine (*Operculina brownii*)	Failed to germinate	
Paddy melon (*Cucumis myriocarpus*)	Failed to germinate	

Figure 8. Symptoms of CGMMV on hand pollinated afghan melon (left). Seedlings grown from seed of infected fruit (right) showed no symptoms of the virus and were negative for its presence by ELISA at 28 days after planting.

Honeybee sampling and trials

A variety of bee products were sampled, beginning in 2014 as part of the incursion response and continuing with the commencement of this project. Over 150 pooled samples were tested for the presence of CGMMV, 89 of these were tested for viability (Table 6).

Table 6. Bee products sampled and tested for CGMMV

	CGMMV	
Bee product	**Present % (n)**	**Viable % (n)**
TOTAL # SAMPLES	162*	89
Adult bees	37% (65)	15% (34)
Newly emerged bees	100% (3)	na
Brood	25% (16)	na
Pollen	77% (45)	18%
Honey	68% (28)	33% (21)
Wax	64% (12)	0% (6)
Propolis	100% (1)	na
Wax moth	uncertain (1)	na
Seeds	0% (360)	

In 2017 four apiaries were sampled, in Darwin and Katherine, to develop a preliminary sampling protocol for the detection of CGMMV in bee hives. This was three years post the original detection of CGMMV in the Northern Territory. Eleven hives were sampled in each apiary, with apiaries varying in size from 29 – 124 hives. In each apiary one hive was sampled intensively (10 samples of both bees and pollen) and ten hives were used for extensive sampling (1 sample of both bees and pollen from each hive). A single sample of honey was taken from all hives, and this was included in the analysis as extensive sampling. The

outcome of this study is to recommend extensive sampling of honey and either adult bees or pollen (Table 7).

Bee samples were collected quarterly from working and resting hives. When available, pollen and adults were always collected. Other bee products were collected opportunistically. Samples were pooled by apiary (three hives per apiary).

Table 7. The likelihood of detecting CGMMV in bee hives using extensive and intensive sampling of bee hive products. Data presented as proportions with confidence intervals (binomial analysis)

Hive product	Proportion of samples with CGMMV (s.d.)	Hive product
	Intensive	Extensive
Adults	0.2 (0.1)	0.1 (0.1)
Pollen	0	0.1 (0.1)
Honey	Na	0.9 (0.1)

Viable CGMMV was found in adult bees, pollen and honey. Field trials showed that CGMMV was detected in the flowers and not the plant, therefore, it was most likely that the virus was introduced by a pollinator. Honeybees are able to move CGMMV to healthy cucurbit plants but questions on transmission is still unclear. It is still uncertain how bees move CGMMV in the environment, how long the virus can survive in bee hives and is it being transported out to the hive. These questions require further investigation in future research.

European bees hive products were sampled eight times (Table 8). Plant samples were taken concurrently. Opportunistic samples from a native bee hive (*Tetragonula* sp.) were taken on three occasions, testing bees, pollen and brood. Both adult and brood *Tetragonula* tested positive on one occasion (data not presented here). CGMMV positive pollen sample underwent palynology studies at the Australian National University.

Table 8. Samples from European honey bee hives.

		Bee hive product		
Trial Phase	Sampling event	Trial Phase	Sampling event	Trial Phase
Pre Trial	1	-	-	-
Trial 1	2	+ (both hives)	+ (only one hive had pollen present)	-
	3	-	-	-
	4	-	-	-
	5	-	-	-
Trial 2	6	-	-	-
	7	-	-	-
	8	-	-	-

In both Trials 1 and 2 the presence of CGMMV in the bee hives increased over the course of the trial. In trial 3 CGMMV was never detected. In both Trials 1 and 2 flowers tested positive for the presence of CGMMV, but leaf and fruit material did not. We concluded that bees are able to move virus to uninfected plants. We

presume that under the field conditions tested here the bees introduce the virus to flowers during pollination activities. In both Trials 1 and 2 the level of CGMMV infection found in the experimental plants was very small. Three pollen samples (1 CGMMV positive, 2 CGMMV negative) collected during field trial 2 were sent to the Paleolab at the Australian National University for analysis (Fig. 9). These pollen samples were analysed for the presence of all currently known hosts of CGMMV and the plants being grown in the associated experiments. None of these plant species were definitively identified in any of the three pollen samples (Table 9).

Table 9. Plant species present in bee pollen samples. The plant species listed here are known hosts of CGMMV in the Northern Territory or were being grown in the field associated with Trial 2. The pollen samples were collected from the two bee hives being used in Trial 2.

Family	Genus	species	Common name	Comments about pollen sample
Amaranthaceae	*Amaranthus*	*retroflex*		Not present
Amaranthaceae	*Amaranthus*		weed	Not present
Amaranthaceae	*Chenopodium*	*album*		Not present
Amaryllidaceae	*Allium*	*fistulosum*	spring onion	Possibly
Cucurbitaceae	*Benincasa*	*hispida*		Possibly (image 4)
Cucurbitaceae	*Citrullus*	*lanatus*	Watermelon	Not present
Cucurbitaceae	*Cucumis*	*myriocarpus*	prickly paddy melon	Possibly (image 10)
Cucurbitaceae	*Cucumis*	*sativus*	cucumber	Possibly (image 10)
Cucurbitaceae	*Cucumis*	spp.		Possibly (image 10)
Cucurbitaceae	*Cucurbita*	*maxima*	pumpkin	Not present
Cucurbitaceae	*Cucurbita*	*moschata*	grammas	Not present
Cucurbitaceae	*Cucurbita*	*pepo*	zucchini, squash	Not present
Cucurbitaceae	*Luffa*	sp.		Not present
Cucurbitaceae	*Momordica*	*charantia*		Not present
Poaceae	*Urochloa*	*mosambicensis*	sabi grass	Not able to determine. Poaceae present
Poaceae	*Zea*	*mays*	sweet corn	Not able to determine. Poaceae present
Portulacaceae	*Portulaca*	*oleracea*	pig weed, purslane	Not present
Solanaceae	*Solanum*	*nigrum*	nightshade	Unlikely
Solanaceae	*Capsicum*	*annum*	capsicum	Possibly (image 8)

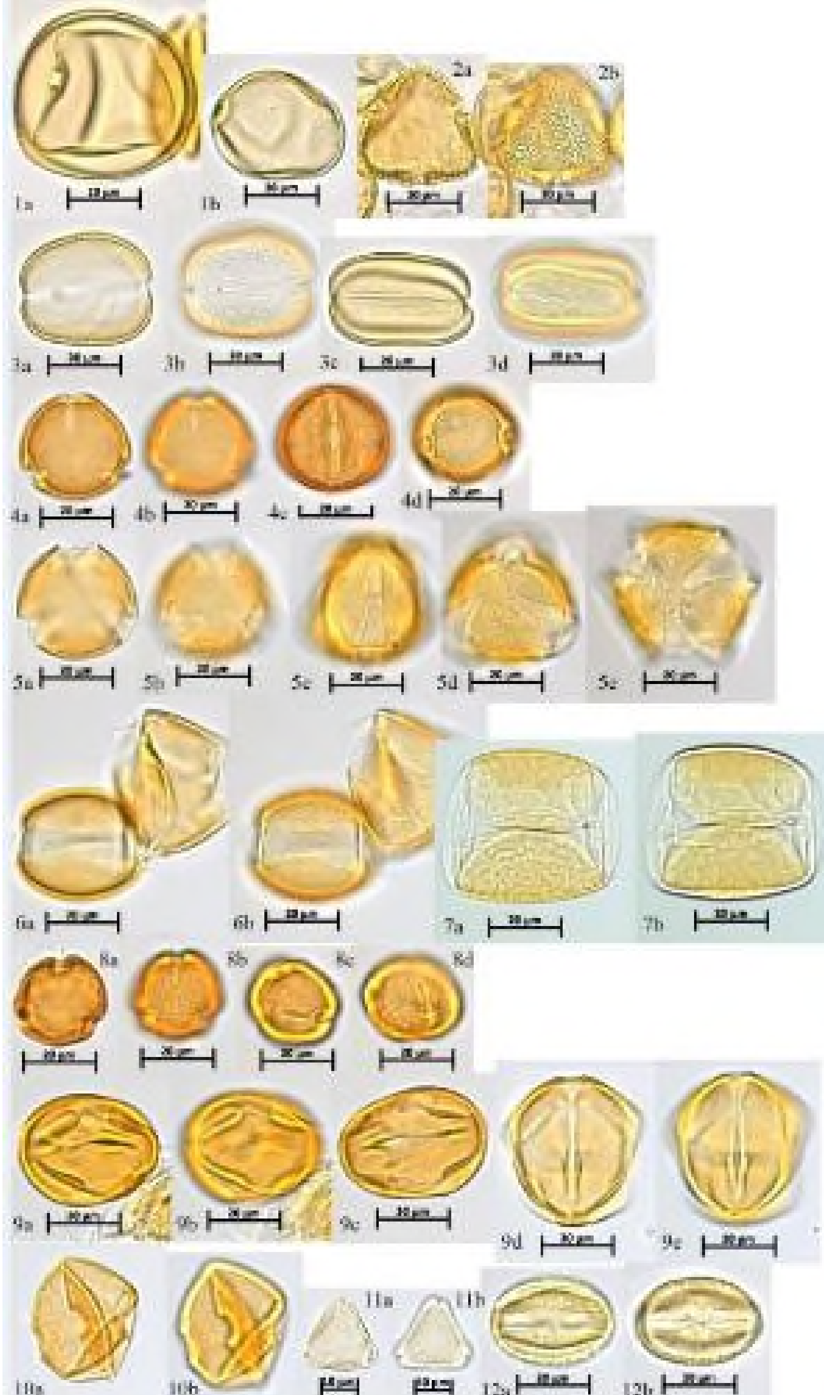

Figure 9. Pollen types collected from beehives.

The honey bee research in VG15013 is the first of its kind; when the research commenced there was very little published about the role of honey bees in the epidemiology of CGMMV. Angulatal trials in Israel and China had focused solely on virus expression in plants without directly testing bees and been conducted in artificial/enclosed environments not in the field. The behaviour of honey bees differs in enclosed structures compared to the open field. The two small scale field trials conducted in VG15013 showed that honey bees do have a role in the transmissibility but the mode of transmission still needs further work (i.e. bee to plant to bee or bee to hive to plant). VG15013 bee research has clearly shown that new funding and a pollinator-dedicated project to investigate the role of pollinators is critical to understand the insects’ role in plant virus epidemiology.

The results of Darzi et al 2017 are not immediately transferable to Australian conditions. Darzi et al 2017 conducted their trials in glasshouses with small nucleus hives. In Australia production is broad acre and managed pollinators are in full sized hives; honey bees will behave differently under these conditions. Honeybees are not used in glasshouses in commercial settings because they do not fly well and fail to thrive (over 50% of the bees in Darzi et al's experimental hives died out, Aviv Dombrovsky pers. comm. 13 Aug 2015). In addition there are still many questions about transmission that remain unanswered. We do not know how the bees move the virus around in the environment, how the virus is introduced to uninfected plants or how long the virus may persist in bee hives. These questions are important for the management of CGMMV both in Australia and globally.

Long term project outcomes

Identify/recommend in-field diagnostics to allow rapid detection as a decision-making tool

Seed molecular testing in VIC was extended to include Asian cucurbit seeds from seed extracted from the fruit of "Asian" cucurbit species including *Benecasa hispida* (two seed lots extracted from wax and hairy melon)*, Lagenaria siceraria, Luffa acutangula, Luffa cylindrical, Momordica charantia* and *Trichosanthes cucumerina*. Seeds were divided into sub-samples of 100, 250, 500 and 1000 and spiked with the equivalent amount of one CGMMV contaminated seed (hybrid *Cucurbita maxima X Cucurbit moschata*) for each seed type. CGMMV could be detected reliably in sub-samples of up to 500 seed of all species except *T. cucumerina,* for which sub-sample sizes of up to 250 seed were most reliable (Table 10). Higher Ct values, indicating lower sensitivity, were observed in all seed sub-sample sizes of *M. charantia* and *T. cucumerina* compared to other seed types. These results show that the protocol for detection of CGMMV in seed is applicable to a broad range of cucurbit species and sub-sample sizes of up to 500 seed may be reliable for most cucurbit species.

Table 10. The average and range of the RT-qPCR cycle threshold (Ct) values observed for the combined results of each seed sample size (100, 250, 500 or 1000) of the seven different cucurbit seed species including *B. hispida* (wax) *B. hispida* (hairy)*, L. siceraria, L. acutangula, L. cylindrical, M. charantia* and *T. cucumerina*

	100 seed	250 seed	500 seed	1000 seed
Number of positives (expected 14)	N = 13	N = 13	N = 12	N = 6
Ct range	26.4 to 32.3	27.4 to 32.1	28.9 to 32.8	30.4 to 32.2
Average Ct	28.900	29.800	30.800	31.000
Standard deviation (Ct)	1.99	1.51	1.28	0.064

CGMMV was not detected in the healthy controls. CGMMV could be detected reliably in sub-samples of up to 500 seed of all species except *T. cucumerina,* for which sub-sample sizes of up to 250 seed were most reliable. Higher Ct values, indicating lower sensitivity, were observed in all seed sub-sample sizes of *M. charantia* and *T. cucumerina* compared to other seed types. These results show that the protocol for detection of CGMMV in seed is applicable to a broad range of cucurbit species and sub-sample sizes of up to 500 seed may be reliable for most cucurbit species.

CGMMV was not detected using the endpoint RT-PCR tests. Suspect positive results, with cycle threshold values below 35, were obtained in 2 samples of pigweed using the Berendsen and Oosterhof (2015) and Hongyun et al 2008 real-time RT-PCR tests and in one additional pigweed sample and two chenopodium samples using the RZ RT-PCR only. These results suggest that some weed seeds are at risk of maintaining CGMMV inoculum in the environment. Further work is required to verify the detection of CGMMV in these weeds seeds and to determine transmissibility to seedlings but this is beyond the scope of this project. It is

anticipated these results will be further studied in the PhD project on viruses in weeds within the Hort Innovation funded project VG16086: Area wide management of vegetable diseases: viruses and bacteria, led by Queensland DAF.

The CGMMV testing has shown that RT-qPCR is more sensitive than ELISA. Positive results were obtained at a 1/1000 dilution of seed by RT-qPCR. A matrix effect is observed when rootstock and watermelon are spiked with the CGMMV contaminated rootstock, watermelon, melon and cucumber seed, indicating that they were inhibitory to ELISA and RT-qPCR tests. Low range positives or negative results were observed in ELISA for rootstock and watermelon seed, suggesting that this could lead to false negative results during routine ELISA testing. CGMMV was detected by RT-qPCR in the same seed samples but positive results occurred later with higher Ct values.

The adoption of RT-qPCR testing for CGMMV in seed is recommended. The results also suggested that 1000 seed samples could be used for detection of CGMMV by RT-qPCR.

However further validation of the RT-qPCR tests is required to determine an accurate cut-off for positive results and to determine a process to confirm suspect positive results occurring after cycle 35. The results of the proficiency test and some diagnostic testing indicate that positive results occurring beyond cycle 35 could be due to lack of specificity of the primers and probes. This observation was confirmed by next generation sequencing which could not detect any CGMMV sequence in these low level positive seed samples. Similar results have been observed by international colleagues.

Testing of the current CGMMV Lateral flow device by AGDIA

In-field diagnostics testing a new version of the lateral flow dipstick on the market was able to detect the Australian strain of CGMMV in 10^3 dilution and is suitable for bulking up to 10 plants in a single assay. A new RT-RPA assay is being developed within this project in QLD and preliminary results for the RT-RPA assay indicates that CGMMV can successfully be detected. However, more work is needed to convert this assay to lateral flow detection. In addition, a multiplex RPA assay will also target the common cucurbit plant viruses (*Papaya ring spot virus* (PRSV), *Zucchini yellow mosaic virus* (ZYMV), *Watermelon mosaic virus* (WMV) and CGMMV). A case study with PRSV and CGMMV has commenced with the assay working well for CGMMV but not PRSV.

Initial tests of a published LAMP assay did not work with our isolate. The newly designed assay worked, with a detectable signal in 25 minutes. Further work is needed in examining loop or stem primers to speed up amplification.

CGMMV positive control material (Vir-5311) and healthy cucumber material was diluted 1:10 in SEB buffer. The CGMMV was serial diluted in 300µL (each) of the healthy cucumber material at 10^0, 10^1, 10^2, 10^3, 10^4. Lateral flow dipsticks were inserted and left to develop for 15 minutes. The test was repeated three times.

The Australian strain of CGMMV was easily detected down to 1:100 dilution in healthy material (Figure 10). A faint band was visible in the 10^3 dilution and was only readily visible in 2 out of the three tests. It is recommended that the test is valid for bulking up to 10 plants in a single assay.

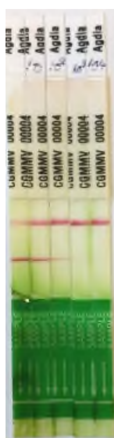

Figure 10. Detection level of AGDIA CGMMV LFD on the Australian CGMMV strain.

Disease management and preparedness for non-affected regions
During the life of the project, on-farm biosecurity protocols have been implemented as required by the national CGMMV management plan, that was developed in 2016 and reviewed in 2018. NT cucurbit growers have adopted the on-farm biosecurity protocols and most have been found to be compliant and similar CGMMV management strategies have been distributed and applied by other jurisdiction. At the end of the project, CGMMV was deemed established in the Northern Territory and Western Australia, under control in Queensland in Charters Towers and Bundaberg, under control in South Australia in Virginia. To date only Victoria, New South Wales, Australian Capital Territory and Tasmania remain CGMMV-free. Biosecurity awareness and weed management on and around cucurbit production areas remains ongoing. In the NT, apiarists are managing the biosecurity of their own apiaries. Many are choosing not to work on properties known to be previously infected with CGMMV.

Develop a multilingual on-farm biosecurity manual by project completion
The on-farm biosecurity manual was revised to include open fields (as recommended during the mid-project review) and is available in English, Vietnamese and Khmer (Appendix 12-14).

Monitoring and evaluation

Project activities and outcomes	Monitoring / evaluation activity
List of weeds as alternative hosts of CGMMV	• *Collate national list of weeds from Australian cucurbit production areas (Year 1)* • *Determine the top common weed species, collect seeds for pot trial bioassays (Year 1 and 2)*
List of non-hosts species of CGMMV	• *Collate national list of non-hosts species with consultation from industry (Year 1)* • *Conduct field and/or pot trials to identify CGMMV non-host status (Year 1 and 2)*
Evaluate honey bees role in CGMMV disease epidemiology	• *Evaluate data from field trials and test honey bee hive products for CGMMV and test for viability (Year 1)* • *Compile protocol to sample and test CGMMV in honey bee hives (Year 2)*
Evaluate current in-field diagnostic tools for rapid detection of CGMMV	• *Collate current tools available to rapidly detect CGMMV in field and identify and evaluate new technologies to the market (Year 2 and 3)*
Improving CGMMV diagnostics for plant and seeds	• *Finalise national diagnostic protocol for CGMMV testing for plants* • *Include Asian cucurbit vegetable seeds into the large subsample testing regime*
Understanding CGMMV biology	• *Virus purification from contaminated soils* • *Persistence in differing soils over time*
Extension and capacity building	• *Communications strategy and plans devised* • *Grower meetings*

National weed surveys around and on cucurbit production areas were conducted in Northern Territory, Western Australia, Queensland, New South Wales, South Australia and Victoria. Generally, weed species that were identified, by the CGMMV risk working group, was found in most jurisdictions. Mainly wild melons were opportunistic regrowth plants. Weed pot and field trials confirmed the weed species that are CGMMV hosts, however it was found that the virus distribution within the weed hosts differed compared to watermelon and cucurbit plants in general. This implies that the replication of the virus and systemic spread in the weed host is unclear and could be localized or the weed host plant plays a role in preventing virus particle movement in the host. Some seeds from the tested weed species were subjected to seed assays as per cucurbits and some were found to be positive for CGMMV. Hence there is a potential for CGMMV to spread via weed seeds as well. Further work is needed to investigate whether CGMMV in weed seeds are viable and can infection, however, it is out of the scope and timing of this current project and will be investigated in the following PhD study in VG16086. Weeds and the role they have in CGMMV epidemiology is not clear cut, consistent positive detections gives confidence behind the value of having on-farm biosecurity recommendation for weed management to control the spread of CGMMV.

The honey bee and pollen research has ascertained that honey bees do have a role in CGMMV transmission but the mode of transmission is unclear, whether it is bee – hive – plant and/or plant – bee – plant interactions. A two-year bee and bee product study identified that many bee products do contain CGMMV with viable CGMMV found in bees, pollen and honey. Over time, only the honey in the beehives remain infective. Studies of the pollen from hives to identify the origins of the plant hosts did

not, in most cases, include known CGMMV hosts (cucurbits and weeds). A gap of pollen identification tools to species level was identified during the research and potentially a pollen database would benefit the future understanding to build on CGMMV epidemiology. The introduction of CGMMV to flowers in two separate field trials by pollinators highlights the potential for bees to have a role in CGMMV transmission.

Field trials showed that CGMMV survival in soil is prolonged and can be over a 12-month period without the presence of host plants. While under a protected cropping system i.e. structure, where biosecurity measures of decontamination was sufficient to eradicate the virus from the shade house structure as pot trials conducted 12 months after decontamination was CGMMV negative. This was also evident in the many times, the biocontrol screen house at Berrimah Farm could be decontaminated between trials with no issue of cross contamination. Persistence of CGMMV in soils could differ between soil types, temperature, presence/absence of host debris. Pot trials conducted in both NT and WA showed that transplants in contaminated soils had higher infection rate compared to direct sowing. This is because transplants encounter more root damage and as such allows the virus to enter via the root system. Direct sowing provides time for plants to establish and grow before infection to occur, this allows time for growers to manage the disease should symptoms appear in early and young seedlings. Rouging out symptomatic plants and creating a buffer zone is a good management strategy to eliminate any source of infection and potential for mechanical transmission as plants get older.

In Israel, a management option was to add a disinfectant to the hole prior to planting to provide a layer of protection for cucurbit plants. In the NT, two common disinfectants are commonly used, are Virkon and bleach. A small pilot trial to test the efficacy of these two disinfectants at three contact times showed that they were proficient in killing the virus and stopping multiplication and spread. However, this still needs to be rolled out to the commercial field environment and whether it is cost beneficial to disinfect the irrigation tapes and planting holes prior to cucurbit plants are undertaken.

The soil assay to purify CGMMV particles was shown to be not a viable option due to too many technical difficulties encountered during the optimization and validation process. The protocol is based upon using magnetic beads and there was consistent non specific binding to the beads possibly due to metal ions within the soils. The soil assay appears to work well when used to test small scale leaf experiments and small volumes of water and heavily infested soils. However, too many false positives were encountered. When applied to soil with low levels of infection, the immunocapture technique was below detection level as observed with ELISA testing which showed the same soil sample contained CGMMV.

Improvements for in-field technologies were investigated during the course of the project. During the incursion, use of the current dipsticks available were not sensitive under the hot conditions of the NT with only 10% success rate and lack of specificity to the Australian strain compared to 100% in laboratory testing. The published CGMMV LAMP assay was also evaluated to determine its usefulness in field diagnostics. But it too failed to detect the Australian CGMMV isolate to satisfactory level, potentially due to nucleic acid mismatches in the assay primers. During the course of the project, a new dipstick was available and has been shown to be more reliable with reproducible results, however, it was prone to the occasional false positive with PRSV. Queensland project members developed a new RPA assay/lateral flow test strip which showed promising results early on, however, as substrates were produced in mass levels to enable validation in collaborating laboratories in the project, it was found to have technical issues in cross binding and produced false positives results. Several methods to wash the magnetic beads to prevent the cross binding failed and eventually did not produce a reliable, satisfactory and reproductive assay for in-field testing. This is an area that needs more funding and development time.

The implementation of the on-farm manual as part of the national management plan for CGMMV and the compliance was highly successful. When market access was re-established and the quarantine measures were lifted in February 2016, no infected fruit was intercepted at market for 1.5 years until July 2017. Thus it is necessary to maintain the on-farm management and be vigilant with weed management as the case of re-emergence of CGMMV in Charters Towers due to the presence of opportunistic wild melons regrowth. The manual is available in English, Vietnamese and Khmer.

The project encountered some setback in the first year due to equipment damage when soil probes were used at growers' properties to monitor soil temperature over time to determine whether

temperature would be enough to kill the virus in the NT soils. Technical issues arose for both immunocapture and in-field diagnostics that used magnetic beads. This may be due to high levels of metals in soils as the immunocapture protocol worked well using plant leaves. Cross binding prevented the RPA assay and immune RPA to be validated as the test was unreliable and could not be reproduced. In 2018, two new incursion outbreaks occurred in the NT that slowed the finalization of the weeds trial until late December 2018. These were citrus canker and Asian honey bee, which meant project team members had to respond to both outbreaks whilst trying to maintain research to ensure completion.

The national diagnostic protocol that was compiled at the start of the CGMMV incursion was adopted by project members. Non-specific binding of some weed species has raised some reservations in and further work is needed to refine the RT-qPCR and could most likely stem from Queensland work on developing the RPA primers based upon the movement protein gene after comparison of 56 whole CGMMV genomes. This will need further investigation.

Recommendations

The following recommendations for the management of CGMMV are made on the basis of the work in VG15013:

- Plant only clean seeds that have been tested at the higher level of 9400 seed numbers per batch. Request documentary evidence of testing from the seed supplier
- Avoid sharing seeds and if you do, investigate the source and history of the material and obtain evidence that the seeds have been tested and is negative for CGMMV
- Do not save seed from any plant or crop suspected of being infected with CGMMV
- Adopt and maintain the on-farm biosecurity procedures, these include
  - 'Come clean, go clean'
  - Appropriate disinfection of tools, equipment, machinery and footwear
  - Exercise particular care with equipment and people if moving production to a new area from an area where CGMMV has occurred
- Plant crops in clean soil and grow non-hosts plants in infested CGMMV soils to reduce the virus load in the ground
- Learn to recognize CGMMV symptoms early and avoid disturbing the area once infection has been identified
- Rogue out symptomatic plants and add a buffer zone
- Know where the bee hives you use have previously worked
- Use the redeveloped field immunostrip available from Agdia (NB. We found this could cross react with PRSV) but also send samples into your state diagnostic laboratories for confirmatory testing.
- Seed testing of Asian cucurbits is reliable for subsamples up to 500 seeds for most species except *T. cucumerina* where the sample size should not exceed 250 seeds.

Future research needs

- Mode of transmission of CGMMV by honeybees and the epidemiological significance
- Use of disinfectants under commercial conditions to provide layer of protection for new seedlings
- Role of cover crops (non-hosts) to reduce CGMMV inoculum in soil, how long to grow and whether the reduction of the pathogen also leads to increase in beneficial microbes?
- Role of weed species and other alternative hosts in the epidemiology and survival of CGMMV
- Nature and value of cucurbit cultivars with resistance/ tolerance to CGMMV in disease management

Refereed scientific publications

Constable, F., Daly, A., Terras, M.A, Penrose, L. and Dall, D (2018) Detection in Australia of *Cucumber green mottle mosaic virus* in seed lots of cucurbit crops. *Australasian Plant Disease Notes.* ***13****:18.*

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Finlay-Doney, M., Simlesa, V., Tran-Nguyen, L.T.T., Lovelock, D. A., Constable, F. E., Kelly, G., Kurz, N., Campbell, P. The presence of *Cucumber green mottle mosaic* virus in *Apis mellifera* hives: implications for managed pollination (in prep)

Draft manuscript in preparation on describing the CGMMV hosts and non-hosts (in prep).

Draft manuscript in preparation on describing the CGMMV detections and Australian strain based upon next generation sequencing of the NT and QLD isolates.

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Intellectual property, commercialisation and confidentiality

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Appendices

Appendix 1- Full methodology and experimental results

Appendix 2- *Cucumber green mottle mosaic virus* (CGMMV)

Appendix 3 - Symptoms of *Cucumber green mottle mosaic virus* (CGMMV) I

Appendix 4 - Symptoms of *Cucumber green mottle mosaic virus* (CGMMV) II

Appendix 5 - *Cucumber green mottle mosaic virus* (CGMMV) Symptoms and damage

Appendix 6 - Non-hosts of *Cucumber green mottle mosaic virus*

Appendix 7- Weed hosts of *Cucumber green mottle mosaic virus*

Appendix 8 - VG15013 – Improved management options for *Cucumber green mottle mosaic virus* (CGMMV)

Appendix 9 - VG15013 – Improved management options for *Cucumber green mottle mosaic virus* – CGMMV and European Honey bees Research update

Appendix 10 - Management practices to minimise Cucumber green mottle mosaic virus in European honey bee hives”

Appendix 11 - CGMMV- Improved management options

Appendix 12 - Farm Biosecurity Plan – English

Appendix 13 - Farm Biosecurity Plan – Vietnamese

Appendix 14 - Farm Biosecurity Plan – Khmer

Appendix 1. Full methodology and experimental results

Methods

Weed survey

Weed surveys around cucurbit production areas were conducted in all jurisdictions. The protocol that was used in the NT was distributed to all states to ensure consistency. To ensure the surveys were focused, the following natural weed hosts, identified by the CGMMV Risk Assessment Working Group, was targeted in each of the jurisdictions. The species included

- *Amaranthus blitoides* [Amaranthaceae]
- *Amaranthus retroflexus* [Amaranthaceae]
- *Amaranthus viridis* [Amaranthaceae]
- *Chenopodium album* [Chenopodiaceae]
- *Ecballium elaterium* [Cucurbitaceae]
- *Heliotropium europaeum* [Boraginaceae]
- *Portulacea oleracea* [Portulacaceae]
- *Solanum nigrum* [Solanaceae]

Trial preparations

In all instances, either for pot or field trials, cucurbit plants (watermelon, cucumber and/or pumpkin), tobacco (*Nicotiana benthamiana*), non-host plants (okra, snakebean, sweet corn, sorghum, peanut, capsicum) and weed species (pigweed, wild gooseberry, amaranthus, black nightshade, chenopodium, sabi grass) were sown from seed and used for trials after the first two true leaf stage. Before plant bioassay trials, all plants were initially tested for CGMMV. Each trial had both negative and positive controls. For consistency across all the trials, a biometrician was consulted for experimental design. In preliminary field experiments conducted in a small study prior to the commencement of VG15013, 80 plants total were used in each plot to determine CGMMV absence/present data. Biocontrol screenhouse trials were designed in a similar fashion where experimental benches with automated irrigation fitted 80 pots per bench. Equivalent numbers were used for the negative and positive control treatments. The negative control pots were hand watered twice daily to avoid cross contamination with treated plants. Following the end of each trial, 1% chlorine was twice flushed through the irrigation tubing and sprinkler heads twice before flush several times with tap water. All benches and floor was scrubbed with 1% chlorine and left for 24 hours between trials. All pot and field trials were left for 6-8 weeks before testing for CGMMV occurred. In all instances, the two controls were tested first then experimental plants. Bulk samples from each plant were taken (ie 1 leaf per plant, 1 bulk sample consisted of 10 leaves, each treatment had 8 bulk samples). In WA, CGMMV pot trials were conducted in a PC2 containment structure.

Inoculum preparations

CGMMV positive plants (watermelon, cucumber and/or tobacco) were used as a source of inoculum for plant bioassays. For each trial, 500 mg of dried CGMMV positive watermelon/cucumber was ground in 10 mL of 0.01 M potassium phosphate buffer (pH 7.0) or 5 mL with 250 mg of tobacco until a homogeneous solution was obtained. The concentrated inoculum was then diluted 1:1 with phosphate buffer (pH 7.0) and 100-200 mg of silica carbide was added as an abrasive. Two leaves closest to the base of the plant was chosen for inoculation, these were tagged and 30 uL of diluted inoculum solution was added carefully as a droplet and gently rubbed in a circular motion onto the leaf using gloved hands using the index finger. Plants were checked daily and symptoms recorded as soon as first signs of mottling occurred, generally two weeks post inoculation in cucurbit plants and 7 -10 days in tobacco plants.

Diagnostics

In the NT, testing was based on molecular assays using RT-PCR targeting CGMMV genes such as the coat protein (CP; Reingold et al 2013), movement protein (Ling et al 2014) and the RNA helicase (Dombrovsky, unpublished), any positive PCR products were then sequenced and bioinformatics conducted for identification using the Geneious software. RT-qPCR was also conducted for a rapid and

more sensitive test 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. ELISA testing were conducted in WA, QLD and Vic on routine basis and PCR testing when needed.

Amaranthus viridis *as a viable host for CGMMV*

During the initial sampling for CGMMV in the periods of 2015-2016, *A. viridis* was routinely sampled and often found to be suspect positive for CGMMV. For this reason, *A. viridis* was analysed further, with five pots or two leaves per plant taken to a total of 10 leaves and tested for the presence of CGMMV. *Amaranthus* continually tested positive and as such was selected for more intensive experiments to determine whether virus could replicate and multiple in the host, whether the infection was systemic in the plants and whether the infected *Amaranthus* plant could cross infect back into cucurbit host. Previously collected and stored *A. viridis* material was used as inoculum, with each inoculum transferred onto four watermelon plants. The inoculated watermelons were bulked together and tested for the presence of CGMMV.

Western Australia

Western Australia conducted CGMMV weed trials as part of the project "Resolving the critical disease threats to the Western Australian cucurbit industry from new and previous incursions of damaging cucurbit viruses" to complement the weed trials in the NT. The purpose being that more weed species could be evaluated for their susceptibility to CGMMV infection.

Pot trials using the species listed in Table 1 (see main report) and *Chenopodium quinoa* and *C. amaranticolor* was inoculated with CGMMV (six plants of each) on 3 to 4 lower leaves of each plant. At 28 days post inoculation, the inoculated leaves, mid leaves (non-inoculated), tip leaves and seed pods of plants were tested by ELISA.

Queensland

Queensland Department of Agriculture and Fisheries sampled and tested the following weed hosts for CGMMV using ELISA and/or PCR at the infested property at Charters Towers. The weeds sampled were *Abulilon oxycarpum*, *Amaranthus* sp., *Bidens pilosa*, *Cucumis anguria*, *Cucumis myriocarpus*, *Onopordum acanthium*, *Passiflora foetida*, *Portulaca oleracea*, *Portulaca* sp., *Solanum nigrum* and *Verbesina encelioides*.

CGMMV persistence in soil

The research project used a secure screen house for pot trials at Berrimah Farm and field trials on four previous infested properties (IP's) that had been CGMMV host-free for 8-12 months. The IPs were selected based upon geographic location, soil make-up, temperature and daylight period. Under permit to grow cucurbits on IPs with quarantine measures in place and approval by property owners to conduct CGMMV trials on their properties, the NTG funded CGMMV soil research commenced in August 2015. To evaluate the persistence of CGMMV in infected soil, it was proposed to collect soil samples from three different time points (0, 3 and 6 months). Time 0 month occurred in August 2015, when the project commenced. This involved sampling soil from the selected properties at the three time points, conduct pot trials at Berrimah Farm, and conduct field trials in conjunction with the 0 month soil sampling. In total three different methodologies would be evaluated to determine whether the CGMMV was present and infective from the IPs. This included growing cucurbits in field trials on IPs; growing cucurbits in contaminated soil collected from IPs and lastly, using an immunocapture technique to purify CGMMV particles from contaminated soil, inoculate susceptible plant hosts and determine whether the CGMMV was still viable and infective. All seedlings used in the research were tested for CGMMV prior to planting to ensure their CGMMV-free status. All cucurbit seeds used were previously tested for CGMMV and shown to be CGMMV-free.

For each property, 80 soil samples were collected from a 12m x 12m grid around a GPS coordinates where a known positive detection was determined at each site. Prior to soil samples being taken, farmer's ensured area was cleared and planting rows were set up. Field sites were set up using star pickets, wire and bee exclusion netting, following on from the soil sampling. These structures were

roughly 15m x 15m with an average height of 2m. Eighty susceptible watermelon/cucumber plants were planted at each site within 10-15cm of the soil samples, with the same number of plants per row as per soil samples. The plants were left for 5-6 weeks, after which point they were bulk sampled (1 leaf per plant, with a total of 10 plants per sample = 8 bulk samples). The pot trial was conducted within a secure screen house at Berrimah Farm. One susceptible plant (watermelon or cucumber) was then placed into each pot, with one person doing a single property. These plants were left for a period of 5 weeks before being bulk sampled (same as field trial) and tested for CGMMV presence. Soil from CGMMV positive pots were kept for future diagnostics.

In addition to investigating the persistence of CGMMV in soil from infested properties, the sole CGMMV infested property in the NT that grew cucurbit under protected cropping system was revisited. The property was placed under quarantine when squash seedlings tested positive for CGMMV in March 2015. Since this period, no cucurbits had been grown in the structures. A pot trial was conducted in June 2016 (15 months after the detection and no host plants). The trial consisted of 80 CGMMV-free cucurbit seedlings grown in the structure.

WA persistence in soil trials used soils remaining from previous CGMMV pot experiments, any stem and leaf material was removed and soil kept dry (with no watering) in the greenhouse under ambient conditions. Immediately following plant removal this soil was used to test if plants grown in CGMMV infested soil became infected. Seeds and seedling were introduced into the infested soil to determine transmission rate. The remaining pots of soil were kept for either 2, 4, 8, 12, 24, 36 weeks with soil at each time point tested by transplanting healthy cucumber 'Reko' into the soil. Plants were kept for 3 weeks post transplantation (to avoid any cross contamination) by which time symptoms started to appear – seedling were tested individually by ELISA.

NT - Effective contact times of two common disinfectants of CGMMV

To explore the efficacy of two common disinfectants, Virkon and Chlorine, were assessed for their ability to kill CGMMV particles using three contact times, 30 s, 1 min and 5 min. Solutions of the disinfectants are applied to the respective inoculum which in turn dilutes them to their working concentrations, Virkon 2% and Chlorine 1%. Five tobacco and five watermelon plants were then inoculated at each time-point with an inoculum that had either Virkon or Chlorine added. In addition five of each (watermelon and tobacco) were used for positive and negative controls. The plants were left for a period of 10 weeks before being tested for the presence or absence of CGMMV.

Soil assay using immunocapture magnetic beads to purify CGMMV particles

A protocol that allows CGMMV purification from soil using magnetic beads and CGMMV antibodies was provided by Dr Aviv Dombrovsky (pers comm) for validation in Australia. The protocol was initially tested using a small scale immunoprecipitation experiment with CGMMV antibodies from two different companies (Agdia and Prime Diagnostics), goat anti-rabbit IgG magnetic beads (New England Biolabs) and CGMMV positive tobacco leaves and water.

The protocol was based upon the use of magnetic beads, the primary antibodies (CGMMV-CO) from Prime Diagnostic was tested due to its higher level of sensitivity to all CGMMV strains, and the secondary antibody (Goat Anti-rabbit IgG magnetic beads) were from New England Biolabs. The sensitivity of the protocol was tested with and without soil to determine whether the soil was inhibitory and using different concentration of CGMMV inoculum (derived from dried infected cucumber leaves, Figure 1).

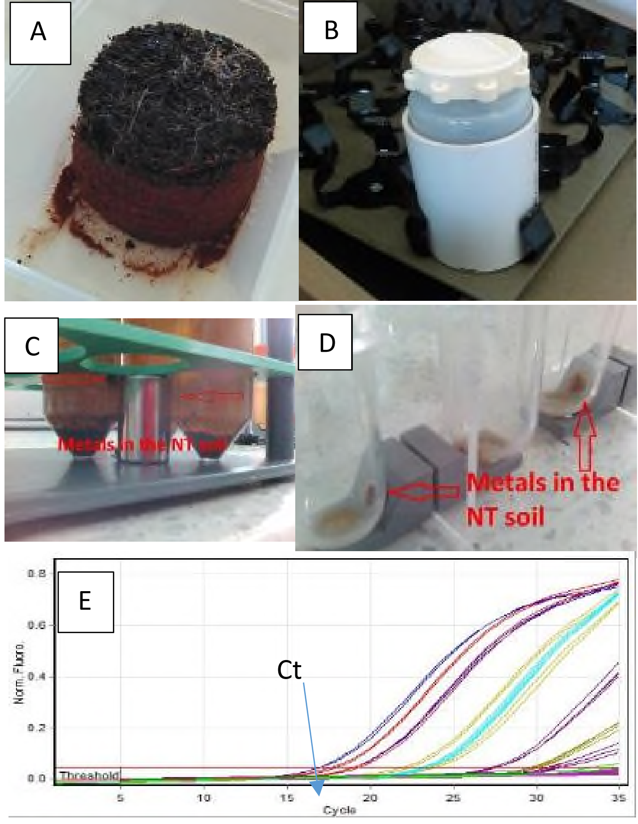


Figure 1. Immunocapture protocol to purify CGMMV particles from (A) contaminated soil, sedimentation of soil using low and high speed centrifugation (B), and magnetic beads (C, D) and reverse transcriptase qPCR showing the serial dilutions of CGMMV inoculum in samples (with and without soils), where Ct value is the point at which the threshold cycle number intercepts the sigmoidal curve (E).

The immunocapture protocol's sensitivity level was evaluated using 125 mg of infected leaf cucurbit material in 10 ml of phosphate buffer. This inoculum suspension was further diluted to 1:100, 1:1,000 and 1:10,000 fold. The immunocapture protocol was used to purify the virus from the supernatant (in absence and presence of soil), the final extract was split and one batch underwent RNA extraction and real time PCR assays and the second underwent plant bioassays.

Western Australia- Comparison of assays to detect CGMMV in soil containing infective roots

A soil/root mixture using roots of highly infected cucumber plants tested by several different methods

- Immunocapture protocol (Version 3, supplied by the NT) followed closely with 20g aliquot of soil tested, as well as plant leaf positive and negative controls.
- Second protocol developed in-house using 1g of same root/soil samples extracted in PBS-T buffer with ball bearings at 22 Hz and tested by traditional ELISA (Agdia antisera), RT-PCR (Qiagen RNEasy and Promega RT-PCR) or IC-RT-PCR.
- IC-RT-PCR involved binding CGMMV antibody to the side of PCR tubes adapted from a previously published protocol (Kamenova & Adkins, 2004, Plant Dis. 88:34-40) followed by standard RT-PCR (Figure 4).

Immunocapture protocol was validated in QLD

CGMMV Immunocapture was conducted on soils from the NT from infested properties, by the protocol optimized by the NT, with minor modifications. Following weighing of the soils, 50 mL of PBS was added, and shaken, centrifuged, strained, and decanted as per the protocol. The pH was checked following this and was found to be too low in a number of samples (Table 1). All soils had sufficient acid added to break the buffering of the PBS (pH 7.4). As the pH values was approaching the isoelectric point for CGMMV (pH 6), 3 ml of 1 M sodium phosphate buffer (pH7.2) was added to each sample to stabilise the pH. Soil samples were checked post addition and all were pH 7.2 indicating correct buffering.

Table 1. Soil samples pH post solubilisation

Soil	pH	Soil	pH
NT IP1 pots 61-70	6.3	NT IP1 pots 71-80	6.3
NT IP2 pots 41-50	7	NT IP3 pots 27-38	6.5
NT IP3 pots 31-40	6.4	NT IP3 pots 39-51	6.4

Addition of the anti-CGMMV antibody was also at a higher rate (3 µL of undiluted Prime antisera per sample), though still well below the binding capacity of the Goat anti-rabbit magnetic particles. The beads were also washed with PBS-Tween (not PBS). The cleaned beads were then used directly in the RT-PCR similarly to the field assay detection, without the losses of viral RNA during the RNA extraction.

Understanding CGMMV diagnostics for plant and seed material

Improving in-field diagnostics

Loop Mediated Isothermal Amplification (LAMP)/ Recombinase Polymerase Amplification (RPA)

Alignments of 56 full genomes of CGMMV from wide geographical areas, showed that the largest conserved regions was in the movement protein coding region. This area is also ideal for diagnostic design as the sub-genomic RNA of this region is produced in active infections. Presence of sub-genomic RNA means that there is the possibility of detecting the virus in crude extractions. The CGMMV particle is very stable, and disruption of this to expose the viral genome is difficult in quick extractions in resource limited scenarios. A new RT-PCR, LAMP and RT-RPA assays were designed to this conserved region.

The best option for in field diagnostics is the specificity of antibody detection with the sensitivity of DNA based amplification technologies. To this end a number of DNA fragments were developed for possible LAMP or RPA amplification. The DNA fragments were attached to antibodies and used in conjunction with a possible immunocapture of CGMMV for detection in crude extracts.

Results

Sixteen samples in total were tested for CGMMV, with 8 of 16 samples testing positive to conventional PCR tests and 12 of 16 testing positive to qPCR (Table 2).

Table 2. Testing of *A. viridis* for the presence of CGMMV.

Sample (Pots)	Conventional PCR	qPCR
1 (1-5)	-	-
2 (6-10)	-	+
3 (11-15)	+	+
4 (16-20)	-	-
5 (21-25)	+	+
6 (26-30)	+	+
7 (31-35)	+	+
8 (36-40)	+	+
9 (41-45)	+	+
10 (46-50)	-	-
11 (51-55)	-	+
12 (56-60)	+	+
13 (61-65)	-	-
14 (66-70)	-	+
15 (71-75)	+	+
16 (76-80)	-	+

To confirm that CGMMV was present, positive conventional PCR results were sent for sequencing, with only three samples returning confirmation of CGMMV. Individual pots were then selected for further analysis, with 15 pots sampled and tested individually for the presence of CGMMV. Of the 15 pots, half tested positive to CGMMV (Table 3), however sequencing was unable to confirm the presence of CGMMV.

Table 3. Individual *A. viridis* pots tested for the presence of CGMMV.

Sample (Pot)	Conventional PCR	qPCR
1 (36)	+	+
2 (37)	-	-
3 (38)	+	+
4 (39)	+	+
5 (40)	+	+
6 (41)	+	+
7 (42)	-	-
8 (43)	-	+
9 (44)	+	-
10 (45)	-	+
11 (71)	-	-
12 (72)	-	-
13 (73)	-	+
14 (74)	+	+
15 (75)	-	-

Three of the six watermelon bulk samples tested positive for CGMMV from bulk sample 7 (Pots 31 – 35) and the two individual *A. viridis* samples (Pots 36 and 39) (Table 4). Sequencing was able to confirm the presence of CGMMV from samples 5 (Pot 36) and 6 (Pot 39), indicating that *A. viridis* may be a host of CGMMV.

Table 4. Watermelon plants inoculated with *A. viridis* material positive for CGMMV.

Sample	Conventional PCR	qPCR
1 (Bulk sample 5)	-	-
2 (Bulk sample 6)	-	-
3 (Bulk sample 7)	+	+
4 (Bulk sample 8)	-	-
5 (Single pot 36)	+	+
6 (Single pot 39)	+	+

Weed sectioning to study the spread of CGMMV through weed hosts

At the completion of the weed trials, three weeds (four from *P. angulata*) from each species were chosen for sectioning, in which the roots, shoots and tips were separated and individually tested for the presence of CGMMV (Table 5). As a comparison, infected watermelon plants were also sectioned (Table 6).

Table 5. Weed sectioning and testing for the presence of CGMMV

Weed	Wild Gooseberry				Black Nightshade			Sabi Grass			Amaranth			Pigweed			Fat Hen		
#	1	2	3	4	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
Section 1 – Roots	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
Section 2 – Shoot	-	-	-	+	-	-	+	-	-	-	+	-	-	+	-	-	-	-	-
Section 3 – Shoot	+	-	+	+	-	+	-	+	-	-	+	-	-	-	-	-	-	-	-
Section 4 – Shoot	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Table 6. CGMMV Positive watermelon sections

	Watermelon		
#	1	2	3
Section 1 – Roots	+	+	+
Section 2 – Shoot	+	+	+
Section 3 – Shoot	+	+	+

Sectioning of Wild Gooseberry indicated that CGMMV was present in 3/4 of the selected weeds. However, the level of infection/titre of the virus is likely to be relatively low as positive results were from qPCR with an average of Cycle threshold (Ct) 32. A similar result was also observed in all other positive sections from each of the weeds/grasses. When these results are compared to the sectioned watermelons, in which an average qPCR of Ct 9 was recorded, CGMMV may be able to initially infect the inoculated leaf/area. There may however, be little if any movement of the virus throughout the weeds after this point. When observing the weeds following inoculation, often the inoculated leaf would fall off within 1 – 2 weeks post inoculation. Inoculated watermelon leaves were in most cases, still attached at the end of the trial.

Field collected Crowsfoot and caltrop tested positive using one CGMMV PCR test but CGMMV was not confirmed in caltrop and only a short CGMMV sequence fragment was identified in Crowsfoot grass. These results indicate only fragments of the virus and unclear whether they are true hosts of the virus.

The weeds trial in WA found that inoculated leaves of each plant tested positive for CGMMV. However, CGMMV was not detected in the other leaves and seed pods tested. Samples tested by RT-

PCR and electron microscopy and again, only the leaf blades of inoculated leaves were positive. This implied that the CGMMV infection was localized and did not move through and replicate in the weed host or the seed pots of the two *Chenopodium* species. Investigations into the seed transmission in Afghan melon were conducted. Flowers of infected afghan melon were pollinated and fruit allowed to mature, the infected fruit showed mild mottling of the rind. The seeds of mature fruit were collected and planted, while the peduncle, rind and flesh of the fruit was confirmed infected with CGMMV. A total of 65 seedlings (to date) was tested by ELISA at 28 days post transplanting and no transmission of CGMMV found. Further pollination of luffa plants and production of seeds from infected plants was unsuccessful.

<u>Understand virus persistence in different soils under different environmental conditions by year 2 of project</u>

Previous field trial work in the NT showed that CGMMV remains viable after at least 12 months without host plants. Further work was conducted where clean watermelon seeds were sown directly into pots containing soil with CGMMV, taken from positive control pots from a previous trial. The positive plants were removed and roots left remaining before seeds were directly sown. Plants were tested at eight weeks to identify the presence of CGMMV. A temperature-controlled trial where positive soil samples were incubated at 36C and 72C over time was tested. After incubation, the soil was used for plant bioassays and immunocapture to purify CGMMV particles. Results from PCR assays as well as immunocapture were inconsistent. Work thus far using the immunocapture protocol shows that it is unreliable when using soil which is not heavily infested but works with infected leaf.

<u>Temperature controlled testing of CGMMV infected soil</u>

CGMMV had been found to survive for up to 12 months in a range of NT soils with differing conditions. Previous research had suggested temperatures of over 60C for a period of 3+ days (72+ hours), can completely remove all viable CGMMV. Avgelis & Manios (1992), were able to remove all viable virus at a temperature of 72C after just 2 days. Soil was collected from previous pot trials in which the host plants were manually infected with the virus and left to grow for a period of eight weeks. Testing of these plants indicated a high level of virus present. The infected soil was assessed at two different temperatures, 72°C and 36°C with bioassays conducted at two weeks post-incubation. PCR's were then performed using CGMMV specific RT-PCR assays and RT-qPCR assay. Immunocapture was performed on soil at 1, 2 and 4 weeks post-incubation.

The results of the bioassay are inconclusive due to the poor infection rate in the positive control pots (0 hour) in which only 1 of 6 was confirmed as being CGMMV positive (Table 7). At 36°C no plants were observed to be positive in any of the tests conducted, while only 1 plant at 72°C was confirmed as CGMMV positive. Reasons for this result may include an initially low titre of virus in the collected soil samples or possible degradation of the virus between collection and beginning of the temperature trial. As the plants in the original soil were manually inoculated via their leaves, the virus may not have moved into the root system, contamination of the soil may have been a result of infected plant debris falling onto the soil.

Table 7. Bioassays of CGMMV soil incubated at 36°C and 72°C for 2 weeks.

Temperature	RNA Subunit	Coat Protein	Movement Protein	RT-qPCR	CGMMV
Positive Control (0 hrs)	1/6	0/6	1/6	2/6	1/6
36°C	0/6	0/6	0/6	0/6	0/6
72°C	1/6	1/6	1/6	1/6	1/6

The immunocapture results are also inconclusive with no positive results for the positive control soil tested at 0 hours (Table 8). While five of the six soil samples at 36°C and four of the six samples at 72°C collected at 4 weeks post-incubation tested positive for CGMMV.

Table 8. Immunocapture of soil incubated at 36°C and 72°C for 1, 2 and 4 weeks

Sample	Positive in Immunocapture
Positive Control (0 hour)	0/6
36°C 1 Week	0/6
72°C 1 Week	1/6
36°C 2 Week	1/6
72°C 2 Week	3/6
36°C 4 Week	5/6
72°C 4 Week	4/6

In the absence of soil, the PCR assay showed that the protocol was sensitive to detect 1:1,000 fraction with Ct values of 27.5 compared with neat inoculum with soil at Ct 23.4. Further dilutions in soil failed to detect CGMMV indicating the levels were below detection levels. This suggests that the immunocapture protocol may be useful for heavily infested soils.

In their evaluation of the immunocapture protocol in QLD, they adjusted the pH to help improve the protocol. However, no CGMMV was detected in any of the soil samples, with the spiked controls working correctly. As this soil is from infested properties, there is no CGMMV in the soil tested, the virus binds to the soil and the assay is unable to disassociate them, or the assay is not sensitive enough to detect the amount of CGMMV particles released. The Agdia antisera could detect CGMMV to a 1:30 dilution (in healthy sap), and the Prime Diagnostic antisera could detect a 1:100 dilution (in healthy sap) with an acceptable signal to background in cucurbit tissue. Higher backgrounds for both tests have been observed with non-cucurbit hosts, and sample bulking levels have to take this into account. Bulking of 10 leaf samples per well is well within the detection range of the prime diagnostic kit, allowing 400 samples per plate to be tested in mass screening.

Validation work in WA resulted in similar findings where comparative experiments to investigate seedlings transplanted into potting mix contaminated with CGMMV infected resulted in 11/100 plants becoming infected. When seeds were directly sown into the potting mix with CGMMV sap, it was found that CGMMV only remained infectious in soil for two weeks. This finding differs from field trial results in the NT and also literature and needs further investigation. In addition, a high clay content ORIA soil type was included, only 1 plant in high clay and potting mix was infected at 12 weeks but not time 0 or after 24 weeks. The 24 and 36 weeks experiments were repeated and there was 1 plant at 36 weeks that was still infective. A range of immunocapture protocols and IC-RTPCR was tested and also showed that the immunocapture protocol was inefficient in detecting CGMMV in soil. A 150g sample of heavily infected soil (soil of an infected plant with the upper portions of the plant removed – contained all the roots, in which CGMMV was easily detected by ELISA). When the protocol was used on the infected soils, the result was negative; however, a 0.1g sample of soil/root material tested by regular ELISA using specific CGMMV antibodies was clearly positive – the immunocapture method lacks sensitivity, in addition it is very laborious to use. Discontinued work using the immunocapture method as impractical for routine use and failed to detect CGMMV in infected soil whereas it was detected by ELISA.

Higher transmission seen by transplanting indicating damage of the seedling roots was important in enabling transmission, but direct seeding also caused a significant number of plants to become infected. Transmission rate decreased rapidly during storage of soil, with infection reducing by 90% within 4 weeks (Table 9) (48 % at 0 weeks vs. 4% at 4 weeks when using transplants). No transmission seen at 24 or 36 weeks of soil storage but cannot be excluded given the small number of plants used (Table 10).

Table 9. Transmission of CGMMV when cucumbers were added to infected soil by different methods

Planting Method[A]	Contamination source[B]	Infected plants at 21 dpi[C]
Direct Seeding	Fresh infected sap	8 of 100
Direct seeding	Fresh infected roots	3 of 100
Transplanted	Fresh infected sap	11 of 100
Transplanted	Fresh infected roots	48 of 98

[A]'Reko' cucumbers were either directly seeded in contaminated soil (direct seeding) or were transplanted when the first true leaf was emerging (transplanted).
[B]Soil contaminated with either sap extracted from leaves and stems of 'Reko' cucumbers (100g contaminated plant material per 1kg of soil extracted in 1L of water) or using the root and soil material of contaminated plants.
[C]Infection of plants was confirmed by ELISA at 21 dpi to the virus in upper leaves.

Table 10. Persistence of CGMMV in soil under ambient conditions

Length of storage[A]	Infected at 21 dpi (by sap)[B]	Infected at 21 dpi (by roots)[C]
0 weeks	11 of 100	48 of 98
2 weeks	4 of 48	23 of 100
4 weeks	2 of 19	4 of 100
12 weeks	0 of 20	1 of 102
24 weeks	0 of 24	0 of 20
36 weeks	0 of 24	0 of 24
24 weeks#	0 of 20	0 of 112
36 weeks#	0 of 24	1 of 104

[A]Soil was stored under ambient temperatures in the greenhouse (average daily maximum of 20 ^{o}C) with no water applied to the soil. At each time point 'Reko' cucumbers were transplanted into contaminated soil at the emergence of the first true leaf.
[B]Infection of cucumbers was tested at 21 dpi by ELISA of upper leaves. The soil used was contaminated with 100g of plant material homogenized in 1L of water per kg of soil used.
[C] Infection of cucumbers was tested at 21 dpi by ELISA of upper leaves. The soil used contained the infected roots of infected 'Reko' cucumbers and was used without the addition of any healthy soil.
24 weeks and 36 weeks was repeated

Further validation work on the immunocapture protocol was investigated in WA and included

Comparison of assays to detect CGMMV in soil containing infective roots

- A soil/root mixture using roots of highly infected cucumber plants tested by several different methods
- Immunocapture protocol (Version 3, supplied by the NT) followed closely with 20g aliquot of soil tested, as well as plant leaf positive and negative controls.
- Second protocol developed in-house using 1g of same root/soil samples extracted in PBS-T buffer with ball bearings at 22 Hz and tested by traditional ELISA (Agdia antisera), RT-PCR (Qiagen RNEasy and Promega RT-PCR) or IC-RT-PCR.
- IC-RT-PCR involved binding CGMMV antibody to the side of PCR tubes adapted from a previously published protocol (Kamenova & Adkins, 2004) followed by standard RT-PCR.

Development of a Multiplex RPA assay

It was decided that a multiplex assay targeting the cucurbit infecting potyviruses (PRSV, ZYMV and WMV) as well as CGMMV would be a valuable addition to in-field diagnostics. As a trial case, the focus is on PRSV and would be expanded into the other viruses. For virus capture, magnetic beads were constructed from Protein G conjugated paramagnetic particles and CGMMV antibodies (prime) and PRSV antibodies (Sediag). For the detection reagent, completely random DNA sequence without any homology to anything in the public gene databases was synthesised (Integrated DNA technologies, Coralville, Iowa, USA). A number of RPA primers were designed for this DNA sequence and the best performing primers in a 10 min RPA assay were put into a second fragment of synthetic DNA with more appropriate lengths and amplification primer design. Probes were designed for lateral flow detection with 5' biotin labels, internal exonuclease IV sites, and 3' amplification blocks. Reverse primers were labelled with 5' FAM for DNA1 and 5' digoxigenin for DNA2. Fragments of the DNA were amplified with 5' amine labelled primers and covalently attached to carboxylic acid groups Protein A via EDC or EDC/sNHS covalent coupling (Figure 3). A five times excess of DNA to protein was used to limit protein A:protein A coupling. The coupled reagents were tested via a lateral flow RPA assay (Figure 4) with both reagents being able to be successfully detected in single and multiplex.

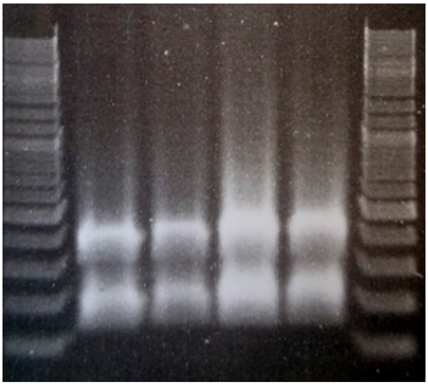


Figure 3. Coupling of detection DNA1 and Detection DNA2 to protein A. Lane 1: DNA1 EDC, Lane 2:DNA1 EDC/sNHS, Lane 3: DNA2 EDC, Lane 4 DNA2 EDC/sNHS. Lower band is uncoupled DNA, and higher band is Protein A:DNA fusion.

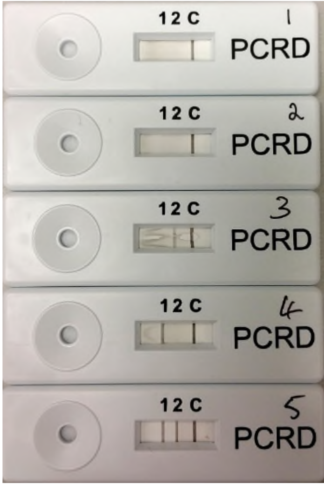


Figure 4. Testing of the detection reagents. Multiplex RPA assay were run for 15 minutes and 1:100 dilution of the detection reagent. 1: No Reaction, 2: No template, 3: DNA2 only, 4: DNA1 only, 5: Both DNA1 and DNA2. Both reactions worked well, though DNA2 is a little weaker than DNA1.

The protein A:DNA1 was combined with anti-CGMMV antibody, and protein A:DNA2 was combined with anti-PRSV antibody. Lyophilised and powdered healthy cucumber, CGMMV-infected and PRSV-infected plant material (1 mg each) was added to 0.5 mL of blocking buffer (PBS-Tween + 1.5% PVA) plus 5 µL of mixed CGMMV and PRSV magnetic beads. Combined detection reagent (2 µL per sample) was added to the samples and allowed to bind for 5 minutes. Magnetic beads were removed and washed in 1 ml of PBS-tween for 30 seconds then removed into 0.2 mL tubes for the multiplex RPA assay. The product was diluted 1:20 to run on the lateral flow strips (Figure 5).

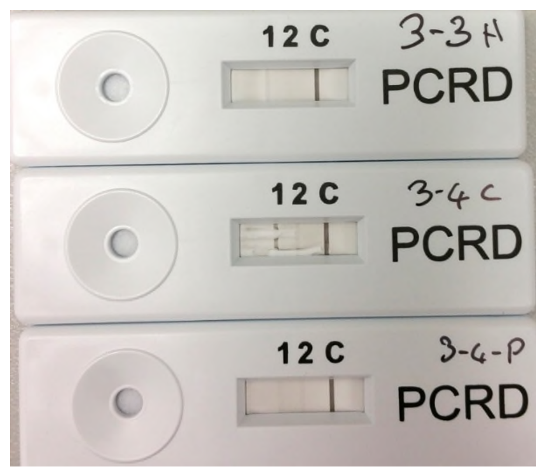


Figure 5. Multiplex RPA testing of plant material. 3-3H Healthy Cucumber plant material. 3-4 C CGMMV plant material. 3-4 P PRSV plant material. Band can be seen for CGMMV, but not for PRSV.

The assay did not detect PRSV, but did detect CGMMV successfully. A number of optimisations are carried out, most notably the extraction buffer that has been recently found to be sub-optimal for CGMMV antibody work. Unfortunately the results for the RPA-assay was not reproducible and needs further optimization to bypass the false positive results from the cross binding reactions that was encountered. It would appear that chemicals to covalently link the protein A to the antibodies (both for the magnetic beads and detection reagents) failed to stop possible cross binding between the detection reagents and to make a more stable reaction.

There were false positives in the immuno-RPA, thought to be due to the protein A DNA detection method. Alternative attachments methods for the DNA to the antibody were investigated. Capture antibodies were directly attached to the COOH- functionalised magnetic particles via EDC + sNHS coupling at pH 8 to target the n-terminus for attachment. These capture beads were via RT-PCR for CGMMV and PRSV, following a one minute binding of the viruses to the antibody coated beads, and two thirty second washes. Both beads were found to bind both viruses successfully. To create the detection reagent a number of different approaches were tried (Table 11). Direct amine to amine attachment was tried with the cross linker DMP, but was unsuccessful due to the large amount of antibody to antibody attachment. Attachment of biotin to the antibody via a (+)-Biotin N-hydroxysuccinimide ester, was successful, and trialled further in experiments, though alternatives were also sought, as the three step creation of the detection reagent was cumbersome for a rapid diagnostic test. Attachment of alkyne-labelled DNA via a copper-catalysed Azide-alkyne Cycloaddition (CuAAC or 'Click chemistry') was tried. The carbohydrate side chains of the antibodies were reduced and the azide functional group added via UDP-galNAz (N-azidoacetylgalactosamine-tetraacylated with the ß1,4-galactosyltranferase mutant Y289L-Gal-T1 enzyme. The Cu1+ ions required for the click reaction was created by reducing copper sulfate with sodium ascorbate and the ligand TBTA (tris-(benzyltriazolylmethyl)amine)and stabilised by TCEP (tris–(2-carboxyethyl)-phosphine). Labelling with this method worked, but at a insufficient level for the detection reagent. Attachment of the amine labelled DNA was attempted through maleimide functionalisation of reduced disulfide linkages created by incubating with the reducing agent TCEP (to break the antibody subunits in half). This method was not successful in creating a labelled antibody. Reduction

of the antibodies to yield viable half antibodies can be a difficult procedure. The final method of attachment of the DNA to the antibodies tried was the indirect attachment of the amine labelled DNA via the spacer bis-carboxy PEG. The PEG was functionalised by EDC/sNHS then added in excess to the DNA. This was ethanol purified away from the excess PEG, and refunctionalized with a 5 fold excess of EDC/sNHS again. This was again purified, and added to the antibody in pH 8 phosphate buffer to target the antibodies N-terminus. There is good labelling of the antibody with the DNA, with a minor fraction of the antibodies containing two DNA labels.

Table 11. Attachment methods for generation of immune-RPA detection reagents

Method	Antibody Functional Group	DNA functional group	Spacer	(Cross)linker	Labelling Successful
1 Direct amine to amine	Amines	Amine	-	DMP(dimethyl pimelimidate)	No
2 Biotin reporter	Amine to biotin	Biotin		Strepavidin	Yes
3 Click Chemistry	Azide	Alkyne	-	CuAAC reaction	No
4 Maleimide	Thiol	Amine	-	sNHS-maleimide	No
5 Indirect Amine to amine	Amines	Amine	Poly(ethylene glycol) bis(carboxymethyl) ether	EDC/sNHS	Yes

- Using Version 3 IC-PCR protocol virus was only detectable in infected leaf material and not in heavily infected soil (Figure 6).
- However, using standard RT-PCR protocol, the virus was clearly detectable in both infected soil samples.
- Virus was also detectable in soil samples using ELISA and same soil/PBS-T extracts.
- Sensitivity of the IC-PCR method appears lower than traditional RT-PCR protocols, however another factor may be that the amount of virus released from infected roots is lower when using an orbital shaker (IC-PCR protocol) versus ball bearings and a tissue homogenizer (RT-PCR).

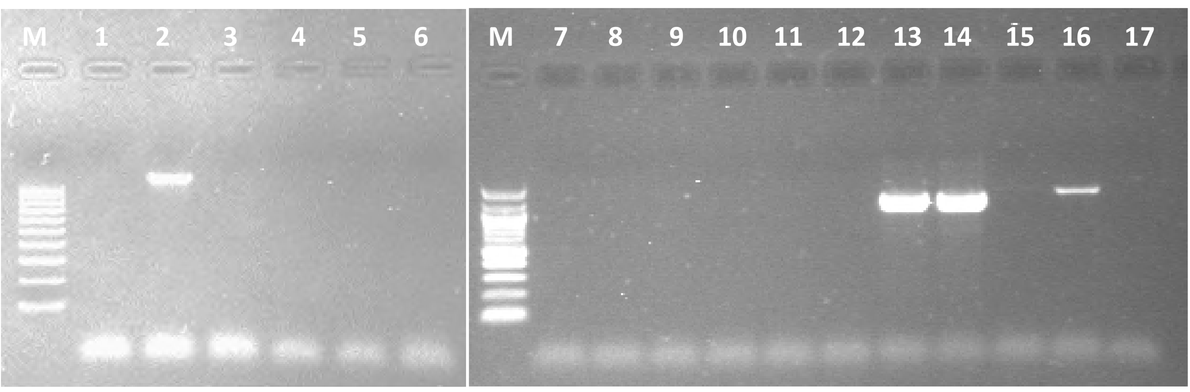


Figure 6 Detection of CGMMV by immunocapture RT-PCR (Version 3, 1-6) and conventional RT-PCR (7-17). M: 100bp ladder; 1. Non template PCR control; 2. Infected leaf sample; 3. Healthy leaf sample; 4. Infected soil; 5. Soil and healthy roots; 6. Soil only; 7. Water non-template control A; 8. Water non-template control B; 9. Soil only A; 10. Soil only B; 11. Soil and healthy roots A; 12. Soil and healthy roots B; 13. Infected soil A; 14. Infected soil B; 15. PCR non template control; 16. PCR positive control.

Persistence of CGMMV in soil research in WA had been conducted as part of the project "Resolving the critical disease threats to the Western Australian cucurbit industry from new and previous incursions of damaging cucurbit viruses" and information included here to add value to our understanding of how CGMMV persists in the soil and its biology.

Potting mix contaminated with CGMMV infective sap

Expt 1 - When 100 cucumber 'Reko F1' seedlings were transplanted into potting mix contaminated with CGMMV infected sap (50g of leaf material homogenized in water and mixed with 5kg of soil), 11 of 100 plants became infected.

Expt 2 - When 12 cucumber 'Reko F1' seeds were transplanted into potting mix contaminated with CGMMV infective sap (as above) after 0, 2 and 4 weeks, a total of 12, 4 and 0 seedlings became infected respectively. There were 4 replicates. This indicated CGMMV may only remain infectious in soil for 2 weeks. None of the cucumber plants growing in the healthy potting mix became infected (Table 12).

Table 12. Results from experiment 2 (potting mix with CGMMV sap)

Time (weeks)	Healthy[A]	Infected with sap[B]
0	0,0,0,0	4,8,0,0
2	0,0,0,0	1,2,1,0
4	0,0,0,0	0,0,0,0

[A]Number of plants out of 12 which became infected when transplanted into healthy potting mix
[B]Number of plants out of 12 which became infected when transplanted into sap infected potting mix.

The finding that the CGMMV infective sap may not be infectious after two weeks is interesting and need further investigation. As the longevity of CGMMV particles is documented to be very stable in soil for many months without host plant. In the NT, the field trials showed that the CGMMV was still infective after at least 12 months without host plants (but it is not clear how long the fields were without plant debris – roots in the soil).

Soil contaminated with CGMMV infected roots

When 10 cucumber plants were direct sown or transplanted into high clay content ORIA soil (ex. Kununurra) or potting mix containing CGMMV infected roots for 0, 12 or 24 weeks, a total of 2 plants became infected at one time point (12 weeks, Table 13). There were 2 replicates. None of the cucumber plants growing in the healthy potting mix or ORIA soil became infected.

Table 13. Results from pot trial with high clay soils.

Time (weeks)	Healthy Potting mix	Infected potting mix	Healthy ORIA	Infected ORIA
0	0,0	0,0	0,0	0,0
12	0,0	0,1	0,0	1,0
24	0,0	0,0	0,0	0,0

- Number of plants out of 10 which became infected/replicate.
- Cucumber direct seeded at 0 weeks, and transplanted seedlings used at 12 and 24 weeks.

Application of disinfectants

The efficacy of two common disinfectants, Virkon and Chlorine, were assessed for their ability to kill CGMMV particles using three contact times (Table 14).

Table 14: PCR results of Watermelon and tobacco plants inoculated with CGMMV mixed with either Virkon or Chlorine with contact times of 30 seconds, 1 minute and 5 minutes. For each, there were five plants for each treatment.

Treatment	PCR Results			
2% Virkon	**RNA Replicase Subunit**	**Coat Protein**	**Movement Protein**	**RT-qPCR**
Watermelon/Tobacco 30s	-	-	-	-
Watermelon/Tobacco 1m	-	-	-	-
Watermelon/Tobacco 5m	-	-	-	-
1% Chlorine	**RNA Replicase Subunit**	**Coat Protein**	**Movement Protein**	**RT-qPCR**
Watermelon/Tobacco 30s	-	**+**	-	-
Watermelon/Tobacco 1m	-	**+**	-	-
Watermelon/Tobacco 5m	-	**+**	-	-
Positive Control	**+**	**+**	**+**	**+**
Negative Control	-	-	-	-

When inoculum is treated with either Virkon or Chlorine at the recommended concentrations, there was no establishment of the virus when comparing it to the positive control. Although there are positive PCR results (Coat Protein) for the tobacco inoculated with 1% Chlorine at all time-points, sequencing results identified that host plant was co-amplifying in this assay and not CGMMV. To confirm the effectiveness of the disinfectants, all tobacco plants inoculated with either Virkon or Chlorine at 30 seconds, were sampled individually and tested for the presence of CGMMV (Table 15).

Table 15. PCR results of tobacco inoculated with CGMMV mixed with 2% Virkon or 1% Chlorine after 30 seconds of contact time.

	PCR Results (Positive/Total Plants)			
Treatment	**RNA Replicase Subunit**	**Coat Protein**	**Movement Protein**	**qPCR**
2% Virkon	1/5	4/5	1/5	1/5
1% Chlorine	0/5	4/5	0/5	0/5
Positive Control	5/5	5/5	5/5	5/5
Negative Control	0/5	0/5	0/5	0/5

All of the tobacco plants inoculated with CGMMV and 1% Chlorine after 30 seconds tested PCR negative for the virus for RNA Replicase Subunit, Movement Protein and qPCR. Four of five plants inoculated with CGMMV and 1% Chlorine or 2% Virkon tested PCR positive for the Coat Protein, sequencing of the Coat Protein identified that host plant was co-amplified and not CGMMV. One of five plants inoculated with CGMMV and 2% Virkon, tested PCR positive for the virus when tested for RNA Replicase Subunit, Movement Protein and qPCR. In this case the same plant tested positive to all three tests, which may indicate a reintroduction of the virus, but to confirm the viability, a bioassay using the material was conducted. The use of Virkon and Chlorine at their respective concentrations, appear to work as quickly as 30 seconds to reduce the viability of CGMMV.

National Diagnostic protocol validated

The CGMMV diagnostic protocol that was developed in the NT as a part of the CGMMV outbreak was circulated and used by all project collaborators. However further work on the RT-qPCR assay is needed. Diagnostic work within the NT, VIC and WA has identified that the current two RT-qPCR assays available has the potential for false positives results depending on the cucurbit hosts and weed hosts tested and the threshold cycles as determined in the diagnostic laboratory. Thus, as more CGMMV whole genomes are publically available, it would be possible to develop new primer and probes. This takes additional funds and not possible within the current project time frame.

INFORMATION SHEET

Cucumber Green Mottle Mosaic Virus (CGMMV)

BACKGROUND

Cucumber green mottle mosaic virus (CGMMV) is a plant disease which was considered exotic to Australia up until September 2014, when it was detected on melon crops in the Northern Territory.

Subsequently it was detected in Queensland in melon crops in April 2015 and then in cucumber crops in Western Australia in July 2016. CGMMV also occurs in Europe, Asia, the Middle East, some parts of the USA, and Canada.

The virus infects fruit and vegetables belonging to the family Cucurbitaceae; including watermelon, cucumber, melons, zucchini, pumpkin, squash, bitter gourd, and bottle gourd and has been found in Cucurbitaceae weeds.

There are at least five strains of the virus, and symptoms can vary between hosts. Other mosaic diseases, caused by potyviruses, are known to occur in Australia and express somewhat similar symptoms. This makes it difficult to visually identify CGMMV, which can only be conclusively established by laboratory testing.

Infected watermelon plants may appear stunted with a bleached appearance, created by mosaic-like mottling on the leaves . Affected plants may also wilt and then runners, or the whole plant, may die prematurely. Symptoms on fruit can include fruit abortion, yellowing, dirty red discolouration and decomposition of the flesh of the fruit. Infection may also cause fruit malformation. The combined effects of CGMMV can result in substantial crop losses.

TRANSMISSION

CGMMV can be easily spread and may remain viable for an extended period in plant debris and soil, or on vehicles, equipment and tools.

The virus can be introduced into a crop in many ways, but contaminated seed and soil are among the most common. It can readily infect plants and survive and spread by several means, including:

- Infection of roots in soil that is contaminated with infected plant debris and can spread through root-to-root contact.
- In water or in nutrient solutions in soil-less culture.
- By mechanical transfer, especially in protected or high-input culture systems where plants are frequently pruned, staked, handled or touched. This can occur via contaminated machinery, clothing, or even the hands of persons who have come in contact with infected plants.
- Packaging materials such as bins used for harvesting, storage or marketing fruit. Recycling of packaging materials should be avoided.
- In field production by machinery, pickers, and possibly by birds and other wildlife in the crop.
- Infected rootstock plants and grafts.
- Seed harvested from infected plants.

The virus can remain dormant within the seed coat and entry of the virus into the plant normally occurs through entry of the virus into plant cells through plant wounds. Preliminary findings to date suggest that bees may play a role in the transmission of CGMMV.

SYMPTOMS

Seedlings

Typical CGMMV symptoms can be mistaken for similar symptoms caused by other cucurbit viruses. This renders visual identification of CGMMV as unreliable.

Symptoms on young seedlings may be indistinct or difficult to recognize as being caused by a virus. In severe infections embryonic leaves may become yellow, but symptoms may not be apparent until more mature leaves emerge.

Leaf

On young leaves, vein clearing and crumpling may be apparent, while mature leaves may display mottling or mosaic patterns, or be pale, yellow, or yellow-white.

Fruit

Fruit may be symptomless—at least externally—or can become severely spotted or streaked and distorted, especially during high temperatures. In some cases, fruit showing no external symptoms may be internally discoloured or necrotic. This can be especially pronounced in watermelon .

This project has been funded by Horticulture Innovation Australia Limited using the research and development Australian vegetable levy and funds from the Australian Government.

CGMMV watermelon fruit yellowing

CGMMV watermelon leaf mottling

Watermelon flesh breakdown

MANAGEMENT OF CGMMV IN THE NORTHERN TERRITORY

As a trade sensitive pest, affected industries and governments from all states, territories and the Commonwealth have agreed to a national plan for managing CGMMV in Australia to prevent spread, reduce impacts on currently affected regions and mitigate trade impacts. A copy of the plan can be found at www.nt.gov.au and search for CGMMV.

Growers and government in the Northern Territory (NT) are working together to manage and contain CGMMV to areas of current infestation and reduce its spread. Restrictions apply to the movement of plant material, seeds, soil, machinery and bee hives from the NT. Growers are required to have farm biosecurity plans which may be audited annually by NT Quarantine.

If you suspect the presence of CGMMV call the hotline number listed below.

FOR MORE INFORMATION

Please phone the

Exotic Plant Pest Hotline on

1800 084 881

or contact

NT Farmers on **08 8983 3233**

or idm@ntfarmers.org.au

BIOSECURITY

Farm biosecurity plans should identify risks of transmission of CGMMV onto and off the property and measures growers have implemented to address those risks. Such measures may include restricting farm visitor access, minimising entry and exit of vehicles, using footbaths upon entry and exit to the property, and cleaning and disinfecting tools and machinery.

Other biosecurity practices that will help limit the spread of CGMMV include:

- Sterilization of vehicles, equipment, plant trays, tools and footwear with potassium peroxymonosulfate or freshly prepared 1% sodium hypochlorite (NaOCl) bleach.
- Disposal on site of suspect plants and crop residues by burning or deep burial.
- Removal of weeds that may harbour viruses in and around cucurbit crops.
- Developing a biosecurity plan for your farm.

A template for a CGMMV Farm Biosecurity Plan can be found at www.farmbiosecurity.com.au.

For assistance in completing your farm biosecurity plan, please contact NT Farmers on 08 8983 3233 or idm@ntfarmers.org.au

NT FARMERS

Farm Biosecurity Plan Template for CGMMV and NT Cucurbit farms

Business name ....

Farm Address ....

Contact ....

Office ....

Mobile ....

Email ....

Completed by ....

Signed .... Date ....../....../......

WARNING

FARM BIOSECURITY IN PLACE

Please contact the office before entering.

Do not enter property without prior approval.
Keep to roadways and laneways.
Do not enter growing areas.

AUSVEG · Plant Health Australia · EnviroVeg · Horticulture Innovation Australia

INFORMATION SHEET

Symptoms of Cucumber Green Mottle Mosaic Virus (CGMMV)

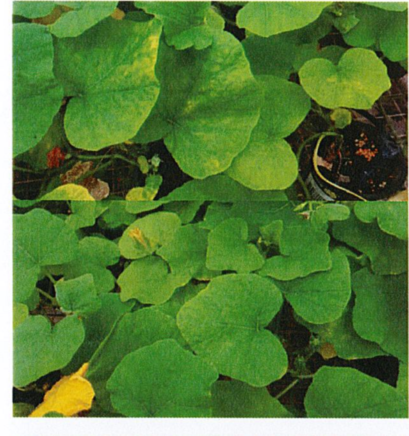
VARYING SYMPTOMS

Three of the most common crops grown in the Northern Territory; watermelon, cucumber and pumpkin, show varying symtomology not only within each crop species but also between species. A consideration when looking for symptoms is whether the crops are grown in the ground and in the open, or in pots under shade structures.

Within pots, the symptoms are often severe, with very detailed mottling, while in the field, symptoms in watermelon and pumpkin can vary from subtle to severe, often making it difficult to observe and distinguish between other diseases and nutritional problems.

Cucumber

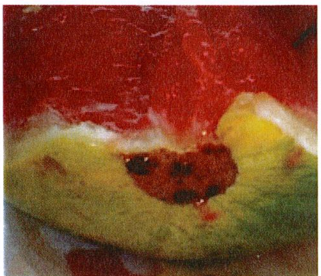
Pumpkin



Watermelon

The fruits rarely show symptoms on the outside, however browning and lesions on the peduncle (stalk) has been noted. When an infected fruit is dissected, the internal structure is sponge like with a meat-like texture and is not suitable for market.

Above: examples of lesions on the watermelon penduncle



Above: examples of infected watermelon fruit

For further details, contact **Lucy Tran-Nguyen**, DPIR Principal Molecular Scientist on **(08) 8999 2235**

This project has been funded by Hort Innovation using the 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

INFORMATION SHEET

Symptoms of Cucumber Green Mottle Mosaic Virus

Cucumber green mottle mosaic virus (CGMMV) is a tobamovirus capable of infecting cucurbit, Asian vegetables and melon crops.

Identifying CGMMV within crops can be difficult early on as visual symptoms may not be observed until 2-6 weeks following infection. This is also dependent upon factors including; initial titre of the virus, temperature during infection and cultivar and species of host which can influence the level or load of symptomology.

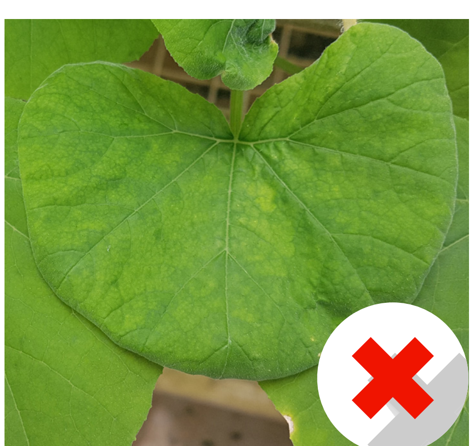
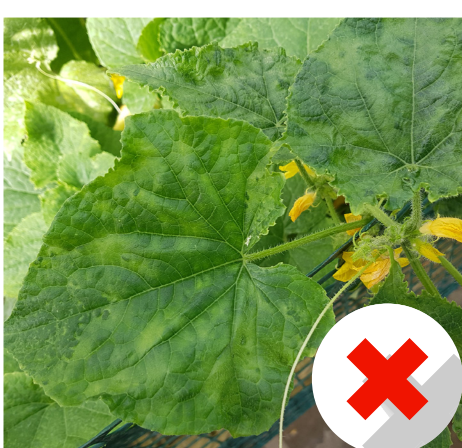
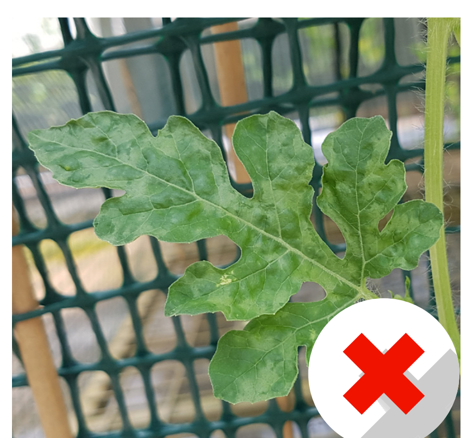
SYMPTOMS

Mosaic mottling of leaf material is the most common symptom in an infection and often the only symptom. This can be confused with Potyvirus, which also causes similar symptomology in the leaf material of cucurbits.

A consideration when looking for symptoms is whether the crops are grown in the ground and in the open, or in pots under shade structures. Within pots, the symptoms are often severe, with very detailed mottling, while in the field, symptoms in watermelon and pumpkin can vary from subtle to severe, often making it difficult to observe and distinguish between other diseases and nutritional problems.

Please see examples of healthy and infected plants below:

Pumpkin | **Cucumber** | **Watermelon**

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INFORMATION SHEET

Symptoms of Cucumber Green Mottle Mosaic Virus (CGMMV)

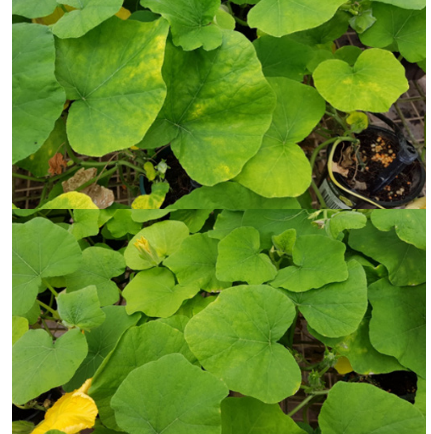
VARYING SYMPTOMS

Three of the most common crops grown in the Northern Territory; watermelon, cucumber and pumpkin, show varying symtomology not only within each crop species but also between species. A consideration when looking for symptoms is whether the crops are grown in the ground and in the open, or in pots under shade structures.

Within pots, the symptoms are often severe, with very detailed mottling, while in the field, symptoms in watermelon and pumpkin can vary from subtle to severe, often making it difficult to observe and distinguish between other diseases and nutritional problems.

Cucumber

Pumpkin



Watermelon

The fruits rarely show symptoms on the outside, however browning and lesions on the peduncle (stalk) has been noted. When an infected fruit is dissected, the internal structure is sponge like with a meat-like texture and is not suitable for market.

Above: examples of lesions on the watermelon penduncle

Above: examples of infected watermelon fruit

For further details, contact **Lucy Tran-Nguyen**, DPIR Principal Molecular Scientist on **(08) 8999 2235**

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Cucumber Green Mottle Mosaic Virus (CGMMV) SYMPTOMS AND DAMAGE

The hosts of CGMMV include cucumber, bottle gourd, melons, pumpkin, squash, watermelon, zucchini and other species. In 2014, discovery of CGMMV in Katherine prompted a biosecurity emergency response focused on eradication. Since this time, CGMMV was detected in Queensland in April 2015 and in Western Australia in July 2016. Nationally, it is accepted that CGMMV is endemic in NT and WA and still under quarantine in QLD.

Symptoms can vary between plant species and sometimes can be difficult to diagnose without laboratory testing. The most common symptoms can be observed on the leaves or fruit.

Symptoms on leaves
- Mottling and mosaic
- Blistering or bubbling
- Vein clearing
- Leaf distortion

Symptoms on fruit
- Often no external symptoms
- Spotted and distorted
- Internally discoloured and rotting
- Uneven ripening

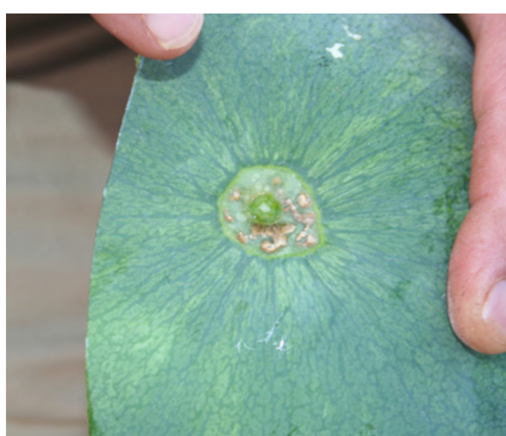
Fruit with yellow patches in flesh.
(most extreme case observed)

Rotten/mushy flesh.

Fruit with internal breakdown and cavities.
Note the patch of rotten/mushy flesh, whilst the rest of the flesh is still relatively normal.

Leaf mottling.
Photo taken overseas © Monsanto

Fruit with necrotic patches on stalk.



Necrotic area on fruit peduncle.

This project has been funded by Horticulture Innovation Australia Limited using the research and development Australian vegetable levy and funds from the Australian Government.

For more information phone the Exotic Plant Pest Hotline on 1800 084 881 or visit www.nt.gov.au

INFORMATION SHEET

Non-hosts of Cucumber Green Mottle Mosaic Virus

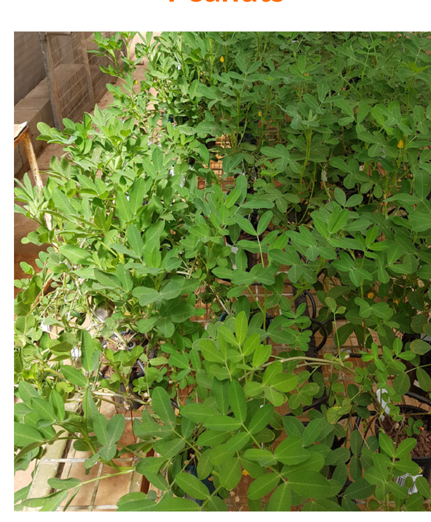
Cucumber green mottle mosaic virus (CGMMV) is a plant disease that is found in cucurbits (e.g. watermelon, cucumber and pumpkin) and a number of common weed species. A range of vegetable species and cover crops have been identified as non-hosts of the virus.

Research on the survival of CGMMV in soil, free from host plants and weeds, has indicated that the virus can survive for at least 12 months. With this knowledge and in consultation from Northern Territory Farmers Association, a range of vegetable species and cover crops were selected for testing to identify whether they could be hosts of CGMMV. As there are two distinct seasons in the Northern Territory (NT), dry (d) and wet (w), crops for each of the seasons were investigated. These crops included; sweetcorn (d), snake bean (d), okra (w), capsicum (d), peanuts (w) and sorghum (w). Research identified that these crops are not hosts of the virus, nor do they harbour it for further spread. This may offer an alternative crop for affected growers in the NT and nationally.

Capsicum

Okra

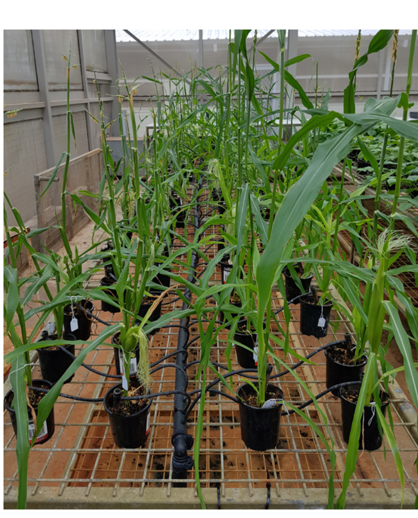
Peanuts



Snake bean

Sorghum

Sweetcorn



For further details, contact **Lucy Tran-Nguyen**, DPIR Principal Molecular Scientist on **(08) 8999 2235**

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NORTHERN TERRITORY GOVERNMENT

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INFORMATION SHEET

Weed hosts of Cucumber Green Mottle Mosaic Virus

Cucumber green mottle mosaic virus (CGMMV) is a plant disease that is found in cucurbits (e.g. watermelon, cucumber and pumpkin) and a number of common weed species.

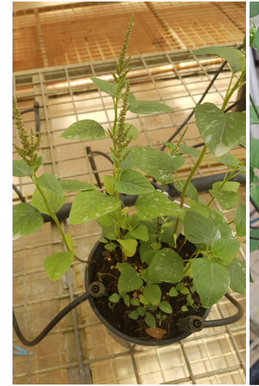
A number of weeds and grasses have been identified as hosts of CGMMV following diagnostic surveys between 2015 and 2017. Weeds common to cucurbit growing areas have been opportunistically collected close to previously infested properties and tested for the virus. These surveys have detected the virus in weeds and grasses not tested before, indicating a potentially larger weed host range than first reported.

SUSPECTED WEED HOSTS OF CGMMV

From continued surveys conducted since the initial detections of CGMMV in the Northern Territory, a number of weeds and grasses have been identified as potential hosts. Unlike crop hosts, identified weeds and grasses are not reported to show any symptoms, making it more difficult to determine if CGMMV is present.

Weed species commonly found in cucurbit growing areas are currently being investigated further to determine if the selected weeds and grasses are true hosts of the virus and to identify if any host reactions are identifiable.

Common Name	Scientific Name
Amaranth	*Amaranthus viridis*
Black Nightshade	*Solanum nigrum*
Caltrop	*Tribulus terrestris*
Crowfoot Grass	*Eleusine indica*
Pigweed	*Portulaca oleracea*
Sabi Grass	*Urochloa mosambicensis*
Wild Gooseberry	*Physalis minima*



Amaranth

Black Nightshade

Caltrop

Pigweed

Sabi Grass

Wild Gooseberry

For further details, contact **Lucy Tran-Nguyen**, DPIR Principal Molecular Scientist on **(08) 8999 2235**

VEGETABLE FUND

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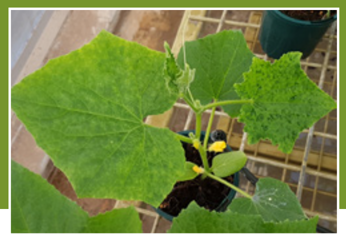
www.nt.gov.au

VG15013 – Improved Management options for *Cucumber Green Mottle Mosaic Virus* (CGMMV)



The Northern Territory Government leads a national project titled "VG15013 Improved management options for *Cucumber green mottle mosaic virus*" funded by Horticulture Innovation Australia using vegetable industry levy and funds from the Australian Government. The key research areas of the project are to

1. Determine the importance of weed and non-hosts of CGMMV in disease epidemiology.
2. Examine the potential for in-field diagnostics to assist rapid detection of the virus on farms known/suspected to be infected with CGMMV.
3. Develop multilingual communication and extension materials to assist with management options to cucurbit growers including on-farm biosecurity protocols.

The three year project commenced in February 2016 and progress to date includes conducting weed surveys in regions previously infested with CGMMV in the NT, and cucurbit growing regions in WA, QLD, NSW and VIC (depending on production periods). In consultation with NT Farmers Association, a non-host list was compiled to determine alternative crops that could be grown in CGMMV infested soils. A technique to purify CGMMV particles from soil was initiated with a small-scale experiment from leaf material and water using magnetic beads coated with CGMMV antibodies. The research group has also been conducting research into the link between honey bees and CGMMV. In addition, the NTG funded project to investigate CGMMV persistence in soil was completed.

Preliminary findings to date show a range of weed species do harbor CGMMV and it is recommended that growers maintain weed control on their properties as part of their farm management. Pot trials using soils collected from infested properties from different growing regions within the NT has persistent CGMMV in the soil in the absence of any cucurbit hosts. Honey bee hive surveys were conducted on bees and bee products from hives in the Darwin, Katherine and Ti Tree areas were conducted from October 2014 and December 2014. Further surveys were conducted in Katherine/Mataranka region in February 2015, Aug/Sept 2015 and April 2016. A small number of newly emerged bees, brood, wax and propolis was tested. CGMMV was detected in all samples tested but viability plant testing determined that there was only live virus in pollen and honey thus far.

A preliminary bee field trial was conducted in late 2015 to determine whether bees are able to transfer CGMMV to virus free cucurbit plants. Only flowers sampled from cucurbit plants available to the bees returned positive results for the virus and the leaves remained virus free. Plants that were excluded from bees remained virus free throughout the trial. These results suggest that bees or any other insect pollinators may be able to transmit the virus. A larger scale bee trial is planned to understand the risk of moving hives between properties and regions.

Extension activities include growers meetings and stakeholder engagement plan with consultation with key stakeholders have been developed and currently awaiting approval from HIA.

This project has been funded by Horticulture Innovation Australia Limited using the research and development Australian vegetable levy and funds from the Australian Government.

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INFORMATION SHEET

VG15013 Improved Management options for Cucumber Green Mottle Mosaic Virus (CGMMV)

CGMMV AND EUROPEAN HONEY BEES: RESEARCH UPDATE - FEBRUARY 2018

Cucumber green mottle mosaic virus (CGMMV) is a plant disease which was exotic to Australia until September 2014.

There is strong evidence that honey bees can introduce CGMMV into clean cucurbit plants. Trials in Israel have shown that bees are able to transfer CGMMV from infected cucurbit plants to clean cucurbit plants in a shade house under specific conditions (Darzi et al 2017). Two honey bee field trials have been conducted in the Northern Territory and each time, CGMMV was found in the flowers but not the leaves thus suggesting an introduction by pollinators.

Hive products from the Northern Territory and Queensland have been tested for the presence and viability of CGMMV. All hive products (adult bees and brood, honey, pollen, empty cells, propolis) have been shown to contain CGMMV. Of those samples tested pollen, honey and adult bees have the highest prevalence of CGMMV. The viability of CGMMV in hive products has been tested. So far, viable virus (capable of causing infection in plants) has been isolated from pollen, honey and adult bees.

It is not known how long CGMMV remains viable inside bee hives. Viable samples of CGMMV have been collected from bee hives in the Northern Territory and Queensland in 2017, but we suspect that the source of this virus is a recent reintroduction rather than the virus persisting over years. Pollen samples from hive product testing have been reserved for future work to determine what plant species the CGMMV is coming from.

The Hort Innovation VG15013 project team is currently finalising a sampling protocol for the detection of CGMMV in bee hives. It is likely that this protocol will recommend taking small samples (e.g. three bees, three pollen cells) from multiple hives within an apiary.

We do not understand how bees move CGMMV around in the environment. The crucial question is, can honey bees move live virus out of their hive to infect clean plants? This would present a significant risk if managed pollinators are exposed to the virus and then moved between locations. We are pursuing opportunities to continue this work.

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(1) 244-251.

For further information please contact:
Project leader: Dr. Lucy Tran-Nguyen
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Department of Primary Industry and Resources
E: lucy.tran-nguyen@nt.gov.au
P: 08 8999 2235

This project has been funded by Horticulture Innovation Australia Limited using the research and development Australian vegetable levy and funds from the Australian Government.

INFORMATION SHEET FOR APIARIES AND BEEKEEPERS

Management practices to minimise Cucumber Green Mottle Mosaic Virus in European honey bee hives

CGMMV AND BEE HIVES

Cucumber green mottle mosaic virus (CGMMV) is a plant disease that is found in cucurbits (e.g. watermelon, cucumber and pumpkin) and a number of common weed species.

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 we have no evidence that CGMMV affects the health of bee hives. There is some evidence that bees are able to move CGMMV infective material from CGMMV positive plants to healthy plants and thus transmit the virus.

GOOD APIARY MANAGEMENT

Apiary management requires vigilance of the health of hives. Good biosecurity practices to ensure hive health include; regularly checking brood production and appearance, honey production and worker bee behaviour and appearance. Other practices that maintain hive hygiene include:

- quarantining and isolating new entrants to the apiary. For bee diseases this is typically 4-6 weeks
- clean all equipment between hives or loads of hives. If possible, have separate equipment between loads
- store equipment and consumables on the apiary in such a fashion that bees cannot access it
- hive components should only be interchangeable within a load
- honey supers should be separated at the extraction plant and not interchangeable between loads
- the extraction plant and hive equipment should be cleaned between loads to ensure all wax and honey debris is removed. Typically this is done using hot water or steam cleaning.

PRINCIPLES OF CGMMV MANAGEMENT

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 hives and increase the likelihood of being able to trace detections back to the source. A variety of management practises are used, and may involve separation of single hives, separation of loads of hives or even the separation of entire apiaries into distinct units.

The principles of apiary management are the same, no matter what type of management system you adopt. Principles of apiary management are:

- physical separation to prevent and minimise possible CGMMV spread, changing frames and spinning off honey immediately after a known exposure to CGMMV positive plants
- use of biosecurity practices to minimise the introduction of CGMMV e.g. not working crops known to be CGMMV positive and resting hives at 3-5km away from known CGMMV positive sites
- keeping concise and accurate records, to enable trace back to determine the source of a disease.

Specific management practices are context specific and can be developed to suit commercial or individual needs.

VEGETABLE FUND

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INFORMATION SHEET FOR APIARIES AND BEEKEEPERS

Management practices to minimise Cucumber Green Mottle Mosaic Virus (CGMMV) in European honey bee hives

MANAGEMENT PRACTICES

Management practices for CGMMV require the continuous implementation of biosecurity measures.

ENSURE:

- clear permanent marking and identification of hives (individually or in loads) and their components
- accurate and concise keeping of records for all apiary activities
- you have a clear understanding on the how management systems operate
- you understand how bees and hives are exposed to CGMMV
- a 3-5 km separation of possible CGMMV infected hives and CGMMV free hives
- hives that contain CGMMV are attended to last in the workflow, and that you use separate hive tools and bee keeping gear for these hives
- restrict movement of people, vehicles and animals to hives that you suspect contain CGMMV
- you do not neglect hives, or equipment associated with hives suspected to contain CGMMV. They may act as a reservoir
- the apiary and pollination sites are kept free from weeds that may act as reservoir hosts for CGMMV.

VISIT OUR WEBSITE FOR FURTHER INFORMATION

https://nt.gov.au/industry/agriculture/food-crops-plants-and-quarantine/cucumber-green-mottle-mosaic-virus

https://dpir.nt.gov.au/primary-industry/primary-industry-strategies-projects-and-research/plant-industries-research

If you have any questions, please contact the Exotic Plant Pest Hotline on 1800 084 881.

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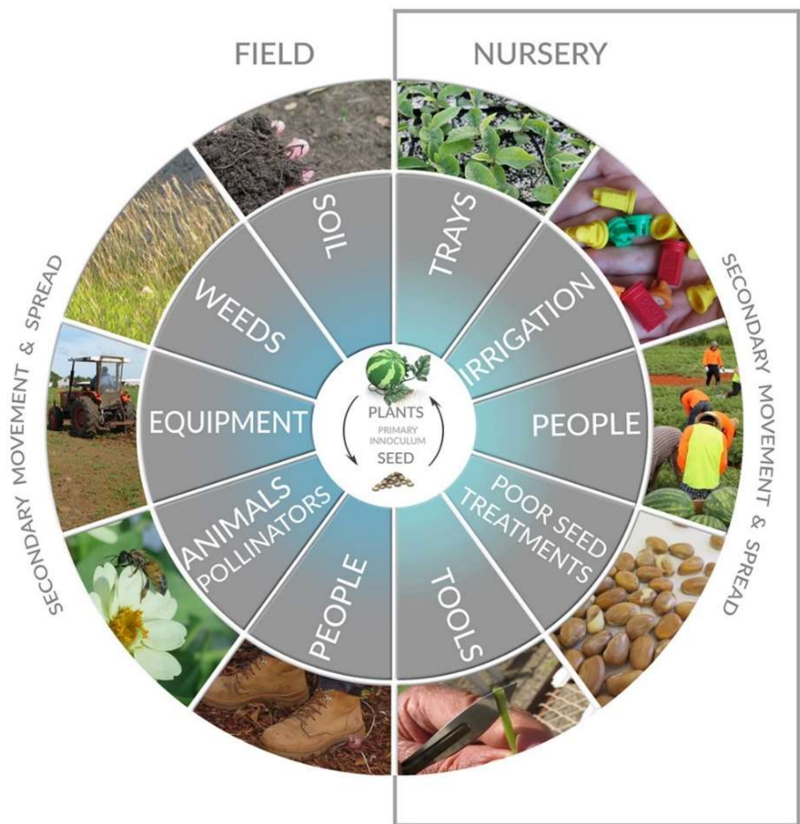
CGMMV: improved management options

Cucumber green mottle mosaic virus (CGMMV) is a tobamovirus that can infect cucurbit plants and is responsible for significant economic losses worldwide.

Introduction

There are several strains of CGMMV worldwide and the primary avenue for spread is through contaminated seed. This provides an infection route between countries and new uninfected cucurbit growing areas.

CGMMV is a highly stable particle that can persist on plant debris, soil, water and seed. Transmission in the ground occurs when seedlings come into contact with contaminated plant debris, soil, machinery, water, seedlings and packing material. See the graphic below for details on methods for movement and spread.



For more information read: Dombrovsky, A., Tran-Nguyen, L.T.T., Jones R.A.C. (2017). *Cucumber green mottle mosaic virus*: Rapidly Increasing Global Distribution, Etiology, Epidemiology, and Management. Annual Review of Phytopathology. 55:231–56.

Weeds and grasses identified as potential hosts of CGMMV

In glasshouse trials and field surveys, a number of weeds and grasses have been identified as potential hosts of CGMMV. These plants do not show any physical symptoms, making it more difficult to determine if CGMMV is present. See the following table for more information and links to weed descriptions.

Scientific name	Common name
Solanum nigrum	Black nightshade
Amaranthus viridis	Amaranth
Portulaca oleracea	Pigweed
Urochloa mosambicensis	Sabi Grass
Physalis angulata	Wild Gooseberry
Eleusine indica	Crowfoot Grass
Tribulus terrestris	Caltrop

More information

Dr Lucy Tran-Nguyen, DPIR

Phone: +61 8 8999 2235

Email: Lucy.Tran-Nguyen@nt.gov.au

Web

Non-hosts of CGMMV

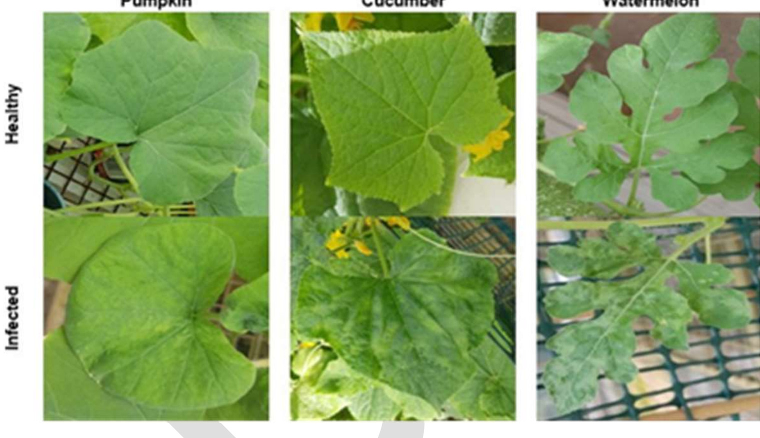
A range of vegetable and cover crop species were selected for testing to identify whether they are hosts of CGMMV. Dry and wet season crops were tested including:

- sweetcorn
- snake bean
- okra
- capsicum
- peanuts
- sorghum.

Research has identified that these crops are not hosts of the virus, nor do they harbour it for further spread. Sorghum is the most widely used wet season cover crop in the Northern Territory, it is not a host nor will it enable persistence of the virus in the environment.

Signs and symptoms

Identifying CGMMV within crops can be difficult early on as visual symptoms may not be observed or distinguishable from other viruses until two-six weeks following infection. This is also dependent upon factors including, initial titre of the virus, temperature during infection, cultivar and species of host which can influence level/load of symptomology.



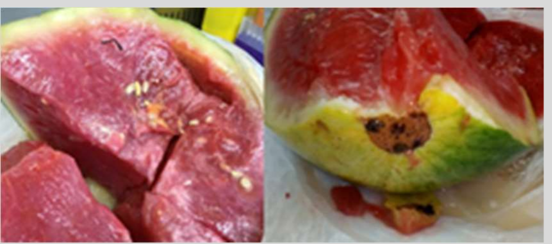
Role of bees and the persistence of CGMMV in honey bee hives

There is strong evidence that honey bees can introduce CGMMV into clean cucurbit plants. Trials in Israel have shown that bees are able to transfer CGMMV from infected cucurbit plants to clean cucurbit plants in a shade house under specific conditions.[1]

All hive products (adult bees and brood, honey, pollen, empty cells, propolis) from the Northern Territory and Queensland trials have been shown to contain CGMMV. The pollen, honey and adult bees have the highest prevalence of the virus. The

Symptoms

Melons rarely show symptoms on the outside, however browning and lesions on the peduncle may indicate infection.



When an infected fruit is cut open, the internal structure is sponge like with a meat texture. In this case, fruit is not suitable for sale.

Persistence in honey bee hives

Two field trials were conducted in the Northern Territory to assess the role of bees in transmitting the virus. On each occasion, CGMMV was found on the flowers but not the leaves, suggesting that pollinators can introduce the virus into uninfected areas.

CGMMV is typically found on the flower indicating transmission by bees/pollinators

viability of CGMMV in hive products has been tested. So far, viable virus (capable of causing infection in plants) has been isolated from pollen, honey and adult bees. It is not currently known how long CGMMV remains viable inside bee hives.

For more information read: 1 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(1) 244-251.

Good apiary management

Viable CGMMV found in hives from pollen, honey and adult bees.

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 their health. There is some evidence that bees are able to move the virus from CGMMV positive plants to healthy plants and thus transmit the virus but it is unclear whether transmission is also due to mechanical means.

Apiary management requires vigilance of the health of hives. Good biosecurity practices to ensure hive health include:

- regularly checking brood production and appearance
- honey production and worker bee behaviour and appearance.

Other practices that maintain hive hygiene include:

- quarantining and isolating new entrants to the apiary. For bee diseases this is typically four-six weeks
- clean all equipment between hives or loads of hives. If possible, have separate equipment between loads
- store equipment and consumables on the apiary in such a fashion that bees cannot access it
- hive components should only be interchangeable within a load
- honey supers should be separated at the extraction plant and not interchangeable between loads
- the extraction plant and hive equipment should be cleaned between loads to ensure all wax and honey debris is removed. Typically this is done using hot water or steam cleaning.

Improving diagnostics for plant and seed material

Research efforts have improved the speed and accuracy of CGMMV diagnostics. The project validated a new dipstick test kit which is now commercially available. This test has some cross sensitivity with papaya ringspot virus (PRSV) but provides a fast and accurate in field solution.

Biosecurity considerations

Farm biosecurity plans should identify risks of transmission of CGMMV onto and off the property and measures growers have implemented to address those risks. Such measures may include:

- restricting farm visitor access
- minimising entry and exit of vehicles
- using footbaths upon entry and exit to the property
- cleaning and disinfecting tools and machinery.
- only plant seeds that have been treated using the 9400 seed standard. Visit the Pest risk analysis for CGMMV webpage for more information on this treatment.
- do not share seeds
- practice good hygiene; Come Clean, Go Clean

Other biosecurity practices that will help limit the spread of CGMMV include:

- sterilisation of vehicles, equipment, plant trays, tools and footwear with potassium peroxymonosulfate or freshly prepared 1% sodium hypochlorite (NaOCl) bleach or 2% Virkon™ S.
- disposal on site of suspect plants and crop residues by burning or deep burial.
- removal of weeds that may harbour viruses in and around cucurbit crops.
- developing a biosecurity plan for your farm. A template for a CGMMV Farm Biosecurity Plan can be found at the melons Australia website.

Understanding CGMMV biology in contaminated soil

CGMMV can persist in the soil for at least 12 months and longer if infected plant debris is present.

It is recommended infested areas are kept weed free of potential hosts (cucurbits and weeds) to ensure the lifecycle of the virus articles ends. This process can take more than 12 months. In the USA, it is recommended that infested soils are left to fallow for three years.

Planting in contaminated soil increases the risk of an infection in the seedlings which can then subsequently infect nearby plants by mechanical means.

DRAFT

This project has been funded by Hort Innovation using the 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

Farm Biosecurity Plan Template for CGMMV and NT Cucurbit farms

Business name ……………………………………………………………………

Farm Address ………………………………………………………………………

Contact ………………………………………………………………………

Office ………………………………………………………………………

Mobile ………………………………………………………………………

Email ………………………………………………………………………

Completed by ……………………………………………………………………..

Signed …………………………….. Date ……/……/……

WARNING

FARM BIOSECURITY IN PLACE

Please contact the office before entering.

Do not enter property without prior approval.
Keep to roadways and laneways.
Do not enter growing areas.

AUSVEG Plant Health Australia EnviroVeg Horticulture Innovation Australia

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Farm Map- please show

- Growing area
- Wash down point for entry to the clean farm, location of footbath
- Access for visitors, deliveries, pick ups
- Any Domestic Areas - sheds and dwellings
- Roads, Gates and Fences

Farm Map

Major Risks	Actions	In place/ completed			Comment
		✓	X	N/A	
Signs and gates	Biosecurity signs in place Gates shut and lockable Phone number on the sign Parking area signs in place Multiple access points identified and clearly signed or locked Open fields clearly signed				
Seeds and seedlings	Certified or own “clean” seeds used Seedlings from registered nursery Seed and seedling register completed				
Staff	Staff trained, and record completed Clothes and equipment cleaned regularly Boots and secateurs used only on farm Footbath available (recipe is on the back of this page)				

Major Risks	Actions	In place/ completed			Comment
		✓	X	N/A	
Visitors	Park in designated area Contact farmer before coming on farm Use footbath before entering growing area Visitors don't bring plant material on farm Visitors instructed on farms biosecurity measures				
Machinery	Concrete or gravel wash down area with run-off away from growing area Clean any machinery or vehicles coming onto the growing area or leaving the farm Complete register of machinery coming onto the farm Use on-farm only vehicles if possible for transport around the growing area				
Weeds	Know the host weeds for CGMMV Remove host weeds where possible Monitor for volunteer host plants Monitor wash down area for host weeds or plants				

Major Risks	Actions	In place/ completed			Comment
		✓	X	N/A	
Animals and Birds	Fences and nets inspected and maintained Reduce food sources by managing waste produce Dump waste away from the growing area Open field crops clearly singed				
Pests and Disease	Know which pests spread disease Monitor crops regularly for disease symptoms Know the symptoms of CGMMV (picture is Lebanese cucumber leaf with CGMMV)				
Deliveries and pick ups	All deliveries and produce pickups are done at the shed. Field crates and bins washed and disinfected regularly Wooden pallets are cleaned before going into the farm area and stored on hard surface				

Major Risks	Actions	In place/ completed			Comment
		✓	X	N/A	
Waste	Old crops are sprayed out and removed Waste produce is disposed of correctly away from the growing area Other waste management practices to keep farm area clean				
Response to Infection	Isolate the infected crop area and restrict movement to the area Get the crop tested for CGMMV If positive notify NT Quarantine Spray out and remove infected dead crop material Plant a non-host cover crop Monitor other host crops closely Test the soil before replanting				

1% Chlorine Solution using domestic bleach products (42g/L active chlorine)

250ml of bleach per l Litre of water

1% Chlorine Solution using pool liquid chlorine products (125g/L active chlorine)

80ml of liquid chlorine solution per l Litre of water

Registers

Seed and seedling register

Crop	Variety	Seed Company	Batch Number	Certified CGMMV tested		Date purchased	Date Planted
				✓	X		

Machinery movement register

Date & Time	Machinery/ vehicle	Identification	From where	Intended use	Checked by	Signature

Visitors Register

Date	Name	Company	Reason for visit	Time in	Time out	Induction	
						✓	X

CGMMV Monitoring record

Date	Crop and location	Inspected by	Unknown or suspect detection of CGMMV	Number of plants affected	Notified NT Quarantine 8999 2118		Action taken
					✓	X	

Staff Training record

Date	Employee / worker	Farm Biosecurity training		Trainer	Comments
		✓	X		

V5 12/5/2016

Farm Biosecurity Plan Template for CGMMV and NT Cucurbit farms

Bảng mẩu quy hoạch An toàn sinh hoc trang trại đối phó bệnh CGMMV cho trang trại bầu bí dưa

Business name/tên doanh nghiệp ……………………………………………………………………

Farm Address/địa chỉ trang trại……………………………………………………

Contact/người liên hệ ………………………………………………………

Office/văn phòng ……………………………………………………

Mobile/số di động ………………………………………………………

Email ………………………………………………………………………

Completed by/người lập bảng ………………………………………………

Signed/ký tên …………… Date/Ngày ……/……/……

WARNING

FARM BIOSECURITY IN PLACE

Please contact the office before entering.

Do not enter property without prior approval.
Keep to roadways and laneways.
Do not enter growing areas.

AUSVEG Plant Health AUSTRALIA EnviroVeg Horticulture Innovation Australia

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Farm Map- please show / Sơ đồ trang trại – nên có các chi tiết sau:

- Growing area / Khu sản xuất
- Wash down point for entry to the clean farm, location of footbath/ điểm tẩy rửa, nơi đặt khay tẩy trùng giày
- Access for visitors, deliveries, pick ups/điểm tập kết khách, người và phương tiện giao nhận hàng
- Any Domestic Areas - sheds and dwellings/các khu vực sinh hoạt gia đình
- Roads, Gates and Fences/ đường đi, cổng, hang rào

Farm Map/Sơ đồ trang trại

Major Risks / Nguy cơ chính	**Actions/hành động**	**In place/ completed Đã có/đã hoàn thành**			**Comment/ghi chú**
		✓	**X**	**N/A**	
Signs and gates/Biển báo và cổng chính	Biosecurity signs in place/Biển báo ATSH Gates shut and lockable/Cổng luôn đóng và có thể khóa Phone number on the sign/Có số điện thoại trên biển báo Parking area signs in place/có chỉ dẫn nơi đậu xe				
Seeds and seedlings/hột giống và cây giống	Certified or own “clean” seeds used/Sử dụng hột giống có chứng nhận hoặc hột “sạch”tự sản xuất Seedlings from registered nursery/cây giống từ vườn ươm có chứng nhận Seed and seedling register completed/hoàn thành đăng ký cây giống hột giống				
Staff/nhân công	Staff trained and record completed/hoàn thành huấn luyện nhân công và sổ ghi chép				

<table>
<tr><td></td><td>Clothes and equipment cleaned regularly/thường xuyên làm sạch quần áo và dụng cụ

Boots and secateurs used only on farm/Ủng và dao kéo chỉ sử dụng trong trang trại

Footbath available (recipe is on the back of this page) có khay tẩy trùng giày – xem hướng dẫn phía sau</td><td></td><td></td><td></td><td></td></tr>
<tr><td>Major Risks</td><td>Actions</td><td colspan="3">In place/ completed</td><td>Comment</td></tr>
<tr><td></td><td></td><td>✓</td><td>X</td><td>N/A</td><td></td></tr>
<tr><td>Visitors/khách</td><td>Park in designated area/đậu xe đúng chổ

Contact farmer before coming on farm/liên hệ trước khi đến

Use footbath before entering growing area/tẩy trùng giày trước khi vào khu sản xuất

Visitors don’t bring plant material on farm/không mang cây cỏ vào trang trại

Visitors instructed on farms biosecurity measures/được hướng dẫn về các biện pháp ATSH</td><td></td><td></td><td></td><td></td></tr>
<tr><td>Machinery/phương tiện-xe cộ</td><td>Concrete or gravel wash down area with run-off away from growing area/có sân xi măng hoặc đá sỏi rửa xe với đường nước chảy cách xa khu sản xuất</td><td></td><td></td><td></td><td></td></tr>
</table>

	Clean any machinery or vehicles coming onto the growing area or leaving the farm/Rửa sạch mọi phương tiện ra vào khu sản xuất Complete register of machinery coming onto the farm/Ghi chép mọi phương tiện ra vào Use on-farm only vehicles if possible for transport around the growing area/Nếu được thì nên có phương tiện chỉ sử dụng riêng trong phạm vi trang trại				
Weeds/cỏ dại	Know the host weeds for CGMMV/Biết các loại cỏ dại có thể nhiễm CGMMV Remove host weeds where possible/Dọn sạch cỏ dại Monitor for volunteer host plants/Lưu ý theo dõi cây rài tự mọc trong vườn Monitor wash down area for host weeds or plants/ Lưu ý theo dõi cây cỏ dại có thể nhiễm CGMMV quanh bải rửa xe				
Major Risks	**Actions**	**In place/ completed**			**Comment**
		✓	**X**	**N/A**	
Animals and Birds/thú vật chim chóc	Fences and nets inspected maintained/kiểm tra tu bổ hang rào, lưới che				

	Reduce food sources by managing waste produce/hạn chế nguồn thức ăn bằng cách che đậy kỹ rác thải Dump waste away from the growing area/bỏ rác thải xa khu sản xuất				
Pests and Disease/sâu bệnh	Know which pests spread disease/biết được sâu hại nào lây truyền bệnh Monitor crops regularly for disease symptoms/thường xuyên theo dõi cây trồng để phát hiện triệu chứng bệnh Know the symptoms of CGMMV (picture is Lebanese cucumber leaf with CGMMV)/hiểu biết triệu chứng bệnh (trong hình là triệu chứng bệnh CGMMV trên lá cây dưa Lebanese)				
Deliveries and pick ups/Giao nhận hàng	All deliveries and produce pickups are done at the shed./Tất cả công việc giao nhận hàng đều ở tại nhà xưởng (shed) Field crates and bins washed and disinfected regularly/Thường xuyên làm sạch bin, kết...				

	Wooden pallets are cleaned before going into the farm area and stored on hard surface/ thường xuyên làm sạch Ba - lệt gổ ra vào trang trại và giử trên nền xi măng				
Major Risks	**Actions**	**In place/ completed**			**Comment**
		✓	**X**	**N/A**	
Waste / rác thải	Old crops are sprayed out and removed/Cây trồng hết mùa vụ được phun thuốc và dọn khỏi vườn Waste produce is disposed of correctly away from the growing area/Rác xác bả cây trồng được thu gom tiêu hủy đúng cách và cách xa nơi trồng Other waste management practices to keep farm area clean/Thực hành thu gom, quản lý rác thải sinh hoạt cho trang trại luôn sạch sẻ				
Response to Infection/ Ứng phó với hoa màu nhiễm bệnh	Isolate the infected crop area and restrict movement to the area/Cô lập khu bị nhiễm bệnh và cấm di chuyển ra vào khu vực này Get the crop tested for CGMMV/ Lấy mẩu giám định bệnh CGMMV If positive notify NT Quarantine/Nếu giám định có bệnh phải báo cơ quan kiểm dịch Spray out and remove infected dead crop material/Phun thuốc và dọn sạch cây bị nhiễm				

	Plant a non-host cover crop/ Trồng các loại hoa màu khác không nhiễm bệnh này Monitor other host crops closely/ Theo dõi chặc chẻ các loại cây trồng khác cùng bị nhiễm bệnh này Test the soil before replanting/Thử mẩu đất trước khi trồng vụ mới.				

	1% Chlorine Solution using domestic bleach products (42g/L active chlorine)/ Dung dịch Chlorine nồng độ 1% dùng thuốc tẩy rửa gia dụng (loại 42g/L chlorine nguyên chất)

	1% Chlorine Solution using pool liquid chlorine products (125g/L active chlorine)/ Dung dịch Chlorine nồng độ 1% dùng thuốc tẩy rửa hồ bơi (loại 125g/L chlorine nguyên chất)

Registers/Đăng ký

Seed and seedling register/Dăng ký hột giống, cây giống

Crop/loại cây trồng	Variety/tên giống	Seed Company/công ty giống	Batch Number/số lô sản xuất	Certified CGMMV tested/Chứng nhận đã kiểm tra CGMMV		Date purchased/Ngày mua hàng	Date Planted/Ngày gieo trồng
				✓	X		

Machinery movement register/ Đăng ký di chuyển máy móc, xe cộ

Date & Time Ngày giờ	Machinery/ Vehicle/Loại phương tiện	Identification/Nhận dạng	From where/Nơi xuất phát	Intended use/Mục đích sử dụng	Checked by/Người kiểm tra	Signature/Ký tên

Visitors Register/ Khách đăng ký

Date/Ngày	Name/Họ tên	Company/Công ty	Reason for visit/Mục đích viếng thăm	Time in/Vào lúc	Time out/Ra lúc	Induction/được hướng dẫn	
						✓	X

CGMMV Monitoring record/Ghi chép theo dõi bệnh CGMMV

Date / Ngày	Crop and location/Loại cây trồng và nơi trồng	Inspected by/Người giám định	Unknown or suspect detection of CGMMV/Không nhận rỏ hoặc nghi ngờ có CGMMV	Number of plants affected/Số cây nhiễm bệnh	Notified NT Quarantine /Báo cáo cơ quan kiểm dịch theo số…		Action taken/Biện pháp xử lý đã thực hiện
					✓	X	

Staff Training record/Ghi chép huấn luyện nhân công

Date/Ngày	Employee / worker Tên nhân công	Farm Biosecurity training/Huấn luyện ATSH		Trainer/Người dạy	Comments/Ghi chú
		✓	X		

V5 12/5/2016

គំរូផែនការជីវសុវត្ថិភាពនៃកសិដ្ឋានសម្រាប់កសិដ្ឋានផ្លែល្ពៅ NT និង CGMMV

ឈ្មោះអាជីវកម្ម ....

អាសយដ្ឋានកសិដ្ឋាន ....

ទំនាក់ទំនង ....

ការិយាល័យ ....

ទូរសព្ទដៃ ....

អ៊ីមែល ....

បំពេញដោយ ....

ចុះហត្ថលេខា .... កាលបរិច្ឆេទ ....../....../......

សូមប្រយ័ត្ន

ជីវសុវត្ថិភាពនៃកសិដ្ឋានដែលត្រូវអនុវត្ត

សមទាក់ទងការិយាល័យសិនមនពេលចលកសិដាន។

សូមកុំចូលក្នុងកសិដ្ឋានដោយគ្មានការអនុញ្ញាតជាមុន។

សូមកុំចេញពីផ្លូវថ្នល់ នឹងច្រកផ្លូវ។

សមកំចលទៅកងកន្លែងដាំដុះ។

AUSVEG | Plant Health Australia | EnviroVeg | Horticulture Innovation Australia

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ផែនទីកសិដ្ឋាន- សូមបង្ហាញ

- តំបន់ដាំដុះ
- ចំណុចលាងសម្អាត ដើម្បីចូលទៅកសិដ្ឋានស្អាត ទីតាំងនៃកន្លែងសម្អាតជើង
- កន្លែងសម្រាប់ភ្ញៀវចូលទស្សនា កន្លែងដឹកជញ្ជូន និងកន្លែងប្រមូលផល
- តំបន់ណាមួយក្នុងកសិដ្ឋាន - រោង និងលំនៅដ្ឋាន
- ផ្លូវ ទ្វារ និងរបង

ផែនទីកសិដ្ឋាន

ហានិភ័យចម្បង	សកម្មភាព	ត្រូវអនុវត្ត/បានបញ្ចប់			មតិយោបល់
		✓	X	គ្មាន	
សញ្ញា និងទ្វារ	សញ្ញាជីវសុវត្ថិភាពដែលត្រូវអនុវត្ត ទ្វារបិទ និងអាចចាក់សោបាន លេខទូរសព្ទនៅលើសញ្ញា សញ្ញាកន្លែងចំណតដែលត្រូវអនុវត្ត ច្រកចូលច្រើនកន្លែងដែលត្រូវកំណត់ និងដាក់សញ្ញាឱ្យបានច្បាស់លាស់ ឬត្រូវចាក់សោ ទីវាលចំហត្រូវដាក់សញ្ញាឱ្យបានច្បាស់លាស់				
គ្រាប់ពូជ និងកូនរុក្ខជាតិ	ប្រើគ្រាប់ពូជដែលបានបញ្ជាក់ត្រឹមត្រូវ ឬគ្រាប់ពូជ "ស្អាត" ផ្ទាល់ខ្លួន កូនរុក្ខជាតិពីកន្លែងបណ្ដុះកូនរុក្ខជាតិដែលបានចុះបញ្ជី បានបំពេញការចុះបញ្ជីគ្រាប់ពូជ និងកូនរុក្ខជាតិ				
បុគ្គលិក	បានបំពេញបុគ្គលិកដែលត្រូវបានបណ្ដុះបណ្ដាល និងកំណត់ត្រា សំលៀកបំពាក់ និងឧបករណ៍ដែលត្រូវសម្អាតជាប្រចាំ ស្បែកជើងកវែង និងកន្ត្រៃកាត់មែកឈើ ដែលត្រូវប្រើសម្រាប់តែនៅកសិដ្ឋានប៉ុណ្ណោះ មានកន្លែងលាងសម្អាតជើង (រូបមន្តទឹកលាងសម្អាតនៅខាងក្រោយទំព័រនេះ)				

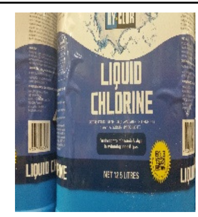
ហានិភ័យចម្លង	សកម្មភាព	ត្រូវអនុវត្ត/បានបញ្ចប់			មតិយោបល់
		✓	X	គ្មាន	
ភ្ញៀវទស្សនា	ចតនៅក្នុងតំបន់ដែលបានកំណត់ សូមទាក់ទងទៅកសិករ មុនពេលចូលកសិដ្ឋាន ធ្វើការលាងសម្អាតជើង មុនពេលចូលតំបន់ដាំដុះ ភ្ញៀវទស្សនាមិនត្រូវយកបំណែករុក្ខជាតិនៅលើកសិដ្ឋានទេ ភ្ញៀវទស្សនាដែលត្រូវបានណែនាំអំពីវិធានការជីវសុវត្ថិភាពនៃកសិដ្ឋាន				
គ្រឿងចក្រ	កន្លែងលាងសម្អាតបេតុង ឬក្រួសដោយបង្ហូរចេញឆ្ងាយពីតំបន់ដាំដុះ លាងសម្អាតគ្រឿងចក្រ ឬយានយន្តដែលចូលមកក្នុងតំបន់ដាំដុះ ឬ ចាកចេញពីកសិដ្ឋាន បំពេញការចុះបញ្ជីគ្រឿងចក្រដែលចូលមកក្នុងកសិដ្ឋាន បើអាច ត្រូវប្រើតែយានយន្តសម្រាប់កសិដ្ឋានប៉ុណ្ណោះ សម្រាប់ការដឹកជញ្ជូននៅជុំវិញតំបន់ដាំដុះ				
ស្មៅ	ត្រូវស្គាល់ស្មៅចង្រៃចំពោះ CGMMV ដកស្មៅចង្រៃចេញតាមដែលអាចធ្វើបាន ត្រូវពិនិត្យរកមើលរុក្ខជាតិចង្រៃមិនបានការ ត្រូវត្រួតពិនិត្យកន្លែងលាងសម្អាត កុំឱ្យមានស្មៅ ឬរុក្ខជាតិចង្រៃ				

ហានិភ័យចម្លង	សកម្មភាព	ត្រូវអនុវត្ត/បានបញ្ចប់			មតិយោបល់
		✓	X	គ្មាន	
សត្វ និងបក្សី	របង និងសំណាញ់ដែលត្រូវត្រួតពិនិត្យ និងថែរក្សា កាត់បន្ថយប្រភពអាហារដោយការគ្រប់គ្រងផលិតផលសំណល់ ចាក់ចោលកាកសំណល់ឲ្យឆ្ងាយពីតំបន់ដាំដុះ ដំណាំនៅទីវាលចំហត្រូវដាក់សញ្ញាឲ្យបានច្បាស់លាស់				
សត្វល្អិត និងជំងឺ	ត្រូវស្គាល់សត្វល្អិតនានាដែលបង្កជំងឺដល់ដំណាំ ត្រួតពិនិត្យដំណាំឲ្យបានទៀងទាត់ ដើម្បីរកមើលរោគសញ្ញានៃជំងឺ ត្រូវស្គាល់ពីរោគសញ្ញារបស់ CGMMV (រូបភាពនៃស្លឹកត្រសក់លីបង់ដែលមានមេរោគ CGMMV)				
ការដឹកជញ្ជូន និងការប្រមូលផល	រាល់ការដឹកជញ្ជូន និងការប្រមូលផលត្រូវបានធ្វើនៅឯរោង។ ធុង និងកំប៉ុងត្រូវលាងសម្អាត និងសម្លាប់មេរោគជាទៀងទាត់ ក្តារឈើត្រូវសម្អាត មុនពេលចូលទៅក្នុងតំបន់កសិដ្ឋាន និងរក្សាទុកនៅលើផ្ទៃរឹង				

ហានិភ័យចម្លង	សកម្មភាព	ត្រូវអនុវត្ត/បានបញ្ចប់			មតិយោបល់
		✓	X	គ្មាន	
កាកសំណល់	ដំណាំចាស់ត្រូវបាញ់ថ្នាំសម្លាប់ និងយកចេញ ផលិតផលសំណល់ត្រូវបានបោះចោលយ៉ាងត្រឹមត្រូវឆ្ងាយពីតំបន់ដាំដុះ ការអនុវត្តការគ្រប់គ្រងកាកសំណល់ផ្សេងៗទៀតដើម្បីរក្សាឲ្យតំបន់កសិដ្ឋានបានស្អាត				
ការឆ្លើយតបទៅនឹងការឆ្លងមេរោគ	បំបែកតំបន់ដំណាំដែលមានមេរោគពីតំបន់ដទៃទៀត ហើយដាក់កំហិតសកម្មភាពនៅលើតំបន់នោះ យកដំណាំនោះទៅធ្វើតេស្តរកមេរោគ CGMMV បើលទ្ធផលវិជ្ជមាន ត្រូវជូនដំណឹងទៅ NT Quarantine បាញ់ថ្នាំសម្លាប់ និងយកបំណែកដំណាំមានមេរោគដែលងាប់ចេញ ដាំដំណាំដែលគ្មានមេរោគលើដីនោះ ត្រួតពិនិត្យដំណាំដែលមានមេរោគផ្សេងទៀតឲ្យបានល្អិតល្អន់ ធ្វើតេស្តដី មុនពេលដាំឡើងវិញ				

សូលុយស្យុងក្លរីន 1% ដោយប្រើផលិតផលសម្អាតក្នុងស្រុក (42g/L នៃក្លរីនសកម្ម)

250ml នៃសារធាតុសម្អាតក្នុងមួយលីត្រទឹក



សូលុយស្យុងក្លរីន 1% ដោយប្រើផលិតផលក្លរីនរាវក្នុងអាងទឹក (125g/L នៃក្លរីនសកម្ម)

សូលុយស្យុងក្លរីនរាវ 80ml ក្នុងមួយលីត្រទឹក

ការចុះបញ្ជី

ការចុះបញ្ជីគ្រាប់ពូជ និងកូនរុក្ខជាតិ

ដំណាំ	ពូជដំណាំ	ក្រុមហ៊ុនលក់គ្រាប់ពូជ	លេខបាច់	បានធ្វើតេស្ត CGMMV ដែលបានបញ្ជាក់		កាលបរិច្ឆេទបានទិញ	កាលបរិច្ឆេទបានដាំ
				✓	X		

ការចុះបញ្ជីចលនាគ្រឿងចក្រ

កាលបរិច្ឆេទ និងពេលវេលា	គ្រឿងចក្រ / យានយន្ត	អត្តសញ្ញាណ	មកពី	បំណងនៃការប្រើប្រាស់	បានពិនិត្យដោយ	ហត្ថលេខា

ការចុះឈ្មោះភ្ញៀវទស្សនា

កាលបរិច្ឆេទ	ឈ្មោះ	ក្រុមហ៊ុន	មូលហេតុនៃការមកទស្សនា	ពេលចូល	ពេលចេញ	ការបញ្ជូល	
						✓	X

កំណត់ត្រាការត្រួតពិនិត្យមេរោគ CGMMV

កាលបរិច្ឆេទ	ដំណាំ និងទីតាំង	បានត្រួតពិនិត្យដោយ	ការរកឃើញមេរោគ CGMMV ដែលមិនស្គាល់ ឬសង្ស័យ	ចំនួនរុក្ខជាតិដែលរងផលប៉ះពាល់	បានជូនដំណឹង ទៅ NT Quarantine 8999 2118		វិធានការដែលត្រូវអនុវត្ត
					✓	X	

កំណត់ត្រាការបណ្តុះបណ្តាលបុគ្គលិក

កាលបរិច្ឆេទ	និយោជិក/កម្មករ	ការបណ្តុះបណ្តាលអំពីជីវសុវត្ថិភាពនៃកសិដ្ឋាន		គ្រូបណ្តុះបណ្តាល	មតិយោបល់
		✓	X		