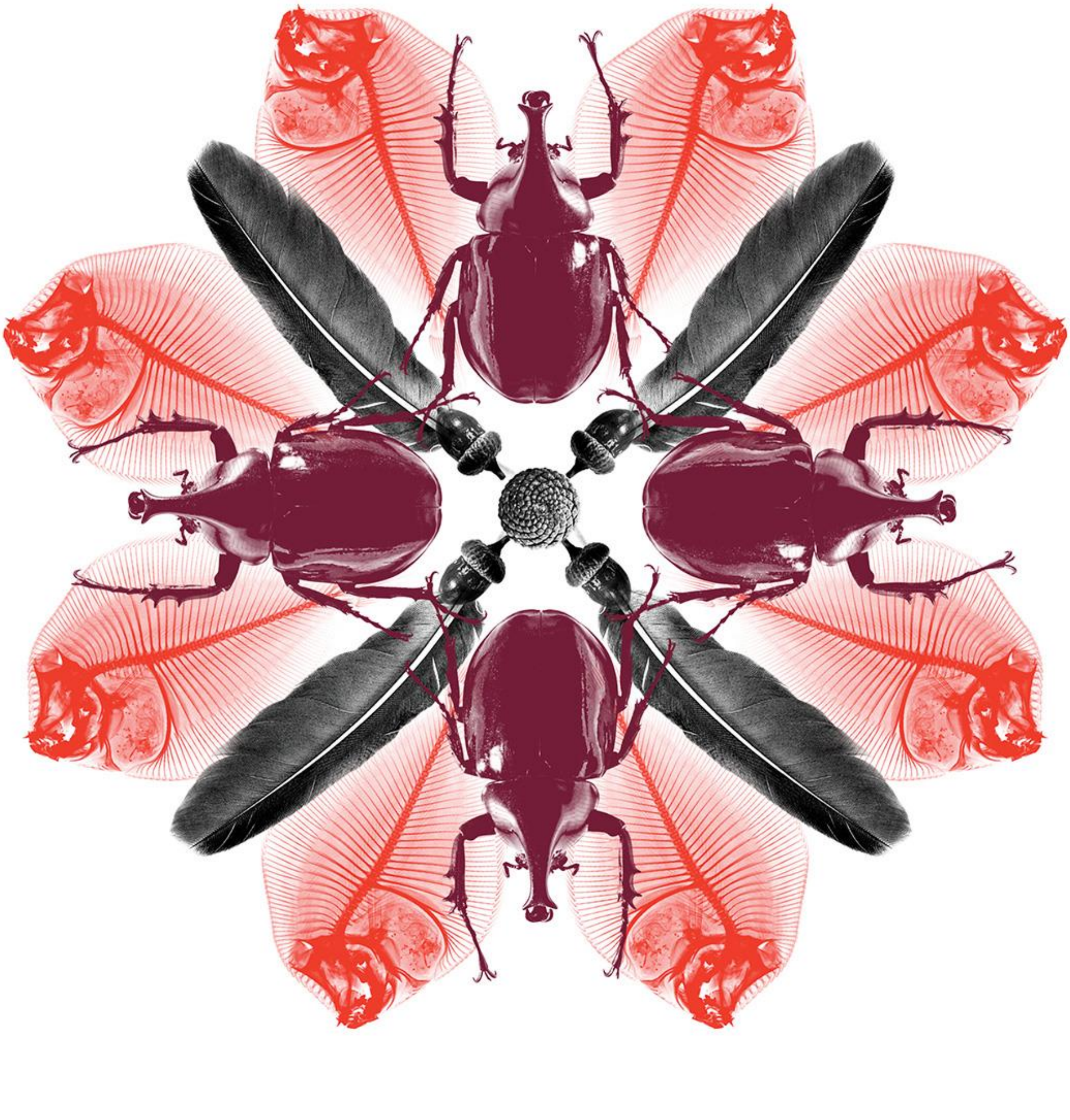
Final pest risk analysis for *Cucumber green mottle mosaic virus* (CGMMV)

November 2017



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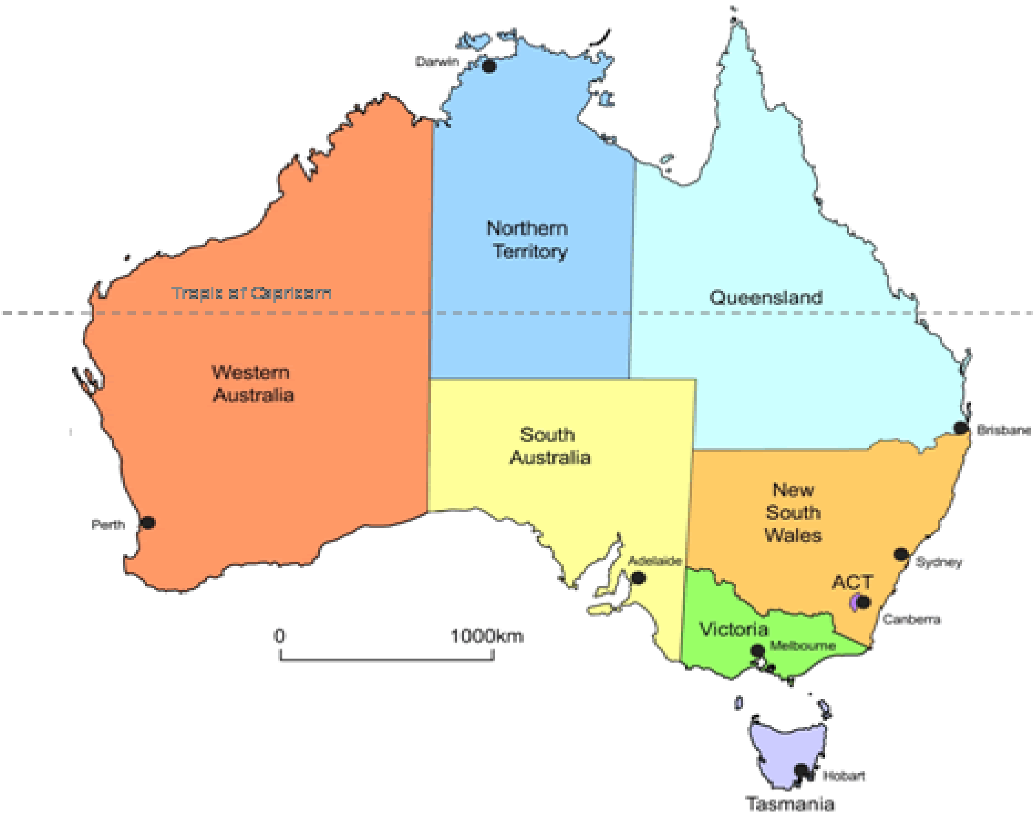
Contents

Figures

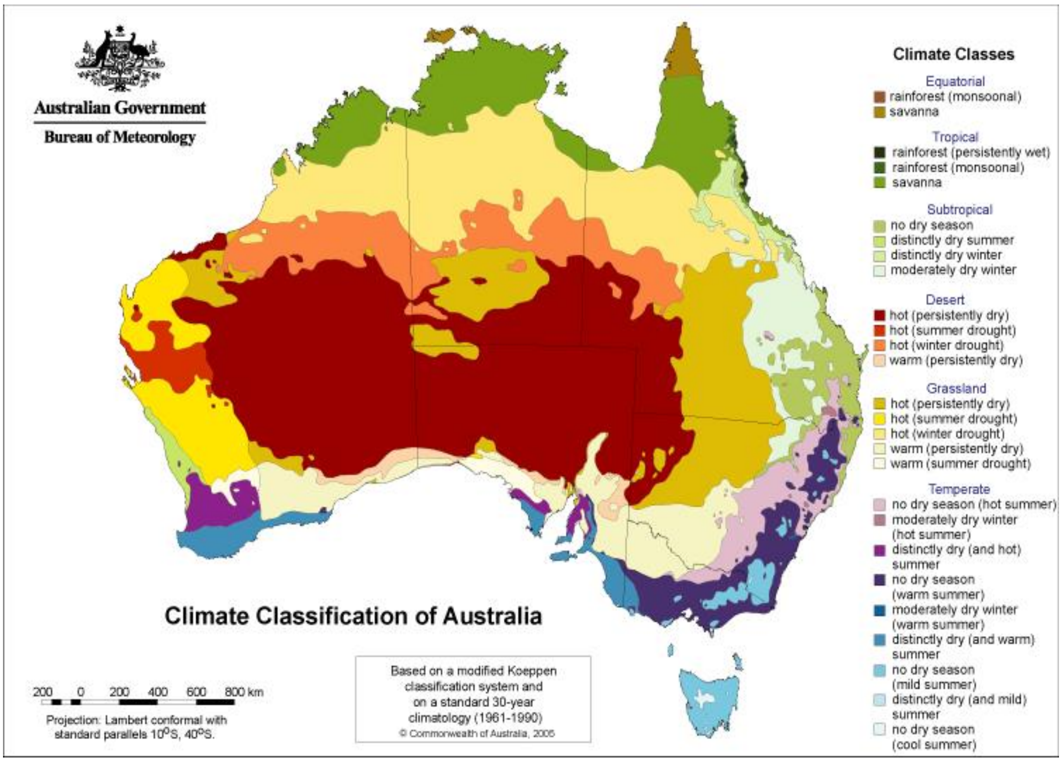
Maps

Tables

Map 1 Map of Australia



Map 2 A guide to Australia's bio-climatic zones



Acronyms and abbreviations

Term or abbreviation	Definition
ACT	Australian Capital Territory
ALOP	Appropriate level of protection
BICON	Australia’s Biosecurity Import Conditions System
BIRA	Biosecurity Import Risk Analysis
DEDJTR	Victorian Government Department of Economic Development, Jobs, Transport and Resources
DPIF	Northern Territory Government Department of Primary Industries and Fisheries
ELISA	Enzyme-Linked Immunosorbent Assay
FAO	Food and Agriculture Organization of the United Nations
IPC	International Phytosanitary Certificate
IPPC	International Plant Protection Convention
ISPM	International Standard For Phytosanitary Measures
ISTA	International Seed Testing Association
CGMMV	*Cucumber green mottle mosaic virus*
NSW	New South Wales
NPPO	National Plant Protection Organisation
NT	Northern Territory
PEQ	Post-entry quarantine
PCR	Polymerase Chain Reaction
PRA	Pest risk analysis
QLD	Queensland
SA	South Australia
SEM	Scanning Electron Microscopy
SPS Agreement	WTO agreement on the Application of Sanitary and Phytosanitary Measures
TAS	Tasmania
TEM	Transmission Electron Microscopy
the department	The Australian Government Department of Agriculture and Water Resources
VIC	Victoria
WA	Western Australia
WTO	World Trade Organization

Summary

The Australian Government Department of Agriculture and Water Resources (the department) initiated this pest risk analysis (PRA) in response to the introduction of emergency measures against *Cucumber green mottle mosaic virus* (CGMMV). This virus was detected in September 2014 in the Northern Territory, Australia and declared a quarantine pathogen. Delimiting surveys were initiated for all cucurbit growing areas in the Northern Territory. Australia introduced emergency measures in October 2014 to mitigate the risk of any further introductions of CGMMV into Australia. Although there have been several subsequent incidents of CGMMV in Australia, substantial resources are being invested in its eradication, containment and management, as appropriate. Therefore, CGMMV is considered under official control, and continues to be a quarantine pest for Australia.

The International Plant Protection Convention (IPPC) and the 'World Trade Organisation Agreement on the Application of Sanitary and Phytosanitary Measures' (SPS Agreement) require that phytosanitary measures against the introduction of new pests be technically justified. The IPPC's International Standards for Phytosanitary Measures (ISPM) No. 1 acknowledges that countries may take appropriate emergency action on a pest posing a potential threat to its territories, however, it also requires that the action be evaluated as soon as possible to justify its continuance. This PRA meets Australia's international obligations to review the emergency phytosanitary measures on cucurbit seeds associated with CGMMV (*Citrullus lanatus*, *Cucumis melo*, *Cucumis sativus*, *Cucurbita maxima*, *Cucurbita moschata*, *Cucurbita pepo*, *Lagenaria siceraria*, *Trichosanthes cucumerina* and any hybrid of these species).

The scope of this PRA was limited to reviewing the emergency measures for imports of cucurbit seeds associated with CGMMV and recommending appropriate import conditions. The continued suspension of importation of host nursery stock and tissue cultures was outside the scope of this PRA.

The department considers that the emergency measures are adequate to mitigate the risk posed by CGMMV associated with host seeds. The recommended minor changes to the emergency measures include an option for small seed lots to be tested off-shore, and for acceptance of the use of a Polymerase Chain Reaction (PCR) testing method approved by the department.

The recommended import conditions for cucurbit seeds associated with CGMMV (*Citrullus lanatus*, *Cucumis melo*, *Cucumis sativus*, *Cucurbita maxima*, *Cucurbita moschata, Cucurbita pepo*, *Lagenaria siceraria*, *Trichosanthes cucumerina* and any hybrid of these species) are summarised below.

- Testing—mandatory off-shore or on-shore testing by International Seed Testing Association (ISTA) validated Enzyme-Linked Immunosorbent Assay (ELISA) or a PCR method approved by the department on a sample size of 9,400 seeds (or 20 per cent for small seed lots) to verify freedom from CGMMV; AND
- Certification— seed lots tested off-shore must be accompanied by an official government Phytosanitary Certificate endorsed with the following additional declaration:

    'The consignment of [*botanical name* (*Genus species*)] was tested by ELISA [*insert laboratory name and report number*] on a sample size of 9,400 seeds (or 20 per cent of small seed lots) and found free from CGMMV'; OR

    'The consignment of [botanical name (Genus species)] was tested by PCR [insert

laboratory name and report number] on a sample size of 9,400 seeds (or 20 per cent of small seed lots) and found free from CGMMV'; AND

- On-arrival inspection—seed lots must be inspected on arrival to verify freedom from live insects, soil, disease symptoms, prohibited seeds, other plant material (for example, leaf, stem material, fruit pulp and pod material), animal material (for example, animal faeces and feathers) and any other extraneous contamination of quarantine concern.

The department considers that the recommended risk management measures will be adequate to mitigate the risks posed by CGMMV associated with host cucurbit seeds.

The department has considered stakeholders comments and made several changes to the 'Draft pest risk analysis for *Cucumber green mottle mosaic virus* (CGMMV)'. However, these changes have no impact on the recommended risk management measures.

1 Introduction

1.1 Australia's biosecurity policy framework

Australia's biosecurity policies aim to protect Australia against the risks that may arise from exotic pests entering, establishing and spreading in Australia, thereby threatening Australia's unique flora and fauna, as well as those agricultural industries that are relatively free from serious pests.

The risk analysis process is an important part of Australia's biosecurity policies. It enables the Australian Government to formally consider the level of biosecurity risk that may be associated with proposals to import goods into Australia. If the biosecurity risks do not achieve the appropriate level of protection (ALOP) for Australia, risk management measures are proposed to reduce the risks to an acceptable level. If the risks cannot be reduced to an acceptable level, the goods will not be imported into Australia until suitable measures are identified.

Successive Australian Governments have maintained a stringent, but not a zero risk, approach to the management of biosecurity risks. This approach is expressed in terms of the ALOP for Australia, which is defined in the *Biosecurity Act 2015* as providing a high level of protection aimed at reducing risk to a very low level, but not to zero.

Australia's risk analyses are undertaken by the Australian Government Department of Agriculture and Water Resources (the department) using technical and scientific experts in relevant fields, and involve consultation with stakeholders at various stages during the process.

Risk analyses may take the form of a biosecurity import risk analysis (BIRA) or a non-regulated risk analysis (such as scientific review of existing policy and import conditions, pest-specific assessments, weed risk assessments, biological control agent assessments or scientific advice).

Further information about Australia's biosecurity framework is provided in the *Biosecurity Import Risk Analysis Guidelines 2016* located on the Australian Government Department of Agriculture and Water Resources website.

1.2 This risk analysis

The department undertook this risk analysis to meet Australia's obligations under the International Plant Protection Convention (IPPC) and International Standard For Phytosanitary Measures (ISPM) No. 1: *Phytosanitary principles for the protection of plants and the application of phytosanitary measures in international trade* (FAO 2016a) to review emergency phytosanitary measures introduced to manage the risk of introducing *Cucumber green mottle mosaic virus* (CGMMV) into Australia. Australia introduced emergency measures for host propagative material and notified trading partners of the emergency measures in October 2014 through a World Trade Organization Sanitary and Phytosanitary (WTO SPS) notification (G/SPS/N/AUS/347). These emergency measures were amended in December 2015 to include the seeds of additional cucurbit hosts (G/SPS/N/AUS/347/Add.3).

The IPPC and the World Trade Organisation Agreement on the Application of Sanitary and Phytosanitary Measures (SPS Agreement), require that any measure against the introduction and spread of new pests must be justified by a science-based assessment, such as a PRA. The department published a 'Draft pest risk analysis for *Cucumber green mottle mosaic virus* (CGMMV)' in April 2016 to provide justification for the introduction of emergency measures

(G/SPS/N/AUS/384). This is the final report that takes into account stakeholders' comments received on the draft report.

1.2.1 Background

The association of CGMMV with crops in the Cucurbitaceae family (cucurbits) was first reported in the 1930s from the United Kingdom (Ainsworth 1935; Ugaki et al. 1991). Since then, the virus has spread among cucurbit crops worldwide, causing severe disease symptoms and yield losses (Reingold et al. 2015; Reingold et al. 2013; Shang et al. 2011). The virus systemically infects cucurbit hosts (Moreno, Thompson & Garcia-Arenal 2004), and is seed-borne and seed-transmitted in several cucurbit species (Liu et al. 2014; Reingold et al. 2015; Yoon et al. 2008). The association of CGMMV with seed is likely to have contributed to its spread worldwide. For example, the introduction of CGMMV into Japan in 1966 was linked to *Lagenaria siceraria* seed imported from India (Kobayashi 1990; Tochihara & Komuro 1974).

Australia was free of CGMMV, however in September 2014, the virus was detected in commercial watermelon farms near Katherine and Darwin, in the Northern Territory. CGMMV was declared a quarantine pathogen and delimiting surveys were initiated for all cucurbit growing areas in the Northern Territory. The detection of CGMMV in the Northern Territory triggered the introduction of emergency measures in October 2014 to manage the risk of further introductions of CGMMV into Australia. In April 2015, CGMMV was found on a single property near Charters Towers in Queensland and the infected property remains under quarantine. The emergency measures were amended in December 2015 to update the host list of CGMMV in response to the detection of the virus in seeds of additional hosts.

Prior to the introduction of emergency measures, the importation of cucurbit crop propagative material (tissue cultures, nursery stock and seeds) was permitted into Australia without any specific disease testing. The imposed emergency measures require testing of seeds of cucurbit hosts in which CGMMV is known to be seed-borne. In addition, the importation of nursery stock and tissue cultures of natural hosts of CGMMV is suspended under the emergency measures.

Under ISPM No. 1: *Phytosanitary principles for the protection of plants and the application of phytosanitary measures in international trade* (FAO 2016a), Australia is required to review emergency phytosanitary measures and conduct a science-based assessment, such as a PRA, to justify the continuance of the measures. Therefore, Australia initiated this risk analysis to assess the emergency measures put in place in October 2014 to manage CGMMV associated with cucurbit seeds.

In February 2016, the Northern Territory government announced the formal revocation of the CGMMV quarantine zone and introduction of new regulations to manage the virus. However, these new regulations prohibit the export of seeds, seedlings and bee hives from the Northern Territory without approval of the Northern Territory Chief Inspector of Plant Health, and require growers to have an auditable farm biosecurity plan for the purpose of controlling CGMMV. Therefore, CGMMV remained under official control in Australia, with risk management measures implemented to contain the virus.

In 2016, CGMMV was detected on cucumber farms near Geraldton (July), Carnarvon (August), Perth (September) and Kununurra (September) in Western Australia. The Government of

Western Australia is assisting growers to develop farm biosecurity plans to prevent further spread of CGMMV and to manage the disease on infected properties.

In February 2017, CGMMV was detected on cucumber farms near Bundaberg (Queensland). Biosecurity Queensland is providing technical advice on biosecurity and control measures to growers, assisting them to develop and implement a plan for eradicating the virus from their production areas, and minimising the risk of spreading the virus to the wider industry.

1.2.2 Scope

The scope of this PRA was limited to:

- assessing the risk of introducing CGMMV associated with cucurbit seeds intended for sowing;
- reviewing and evaluating the existing management measures (including emergency measures) for identified risks; and
- recommending amended or additional risk management measures where appropriate.

The current suspension of importation of tissue cultures and nursery stock of known CGMMV hosts was outside the scope of this review. The justification of emergency measures for suspension of host tissue cultures and nursery stock imports will be reviewed at a later date.

1.2.3 Existing import conditions

International import conditions

Prior to the introduction of emergency measures for cucurbit seeds against CGMMV, seed lines of permitted species of cucurbitaceous crops were permitted entry from all sources subject to the department's standard seed for sowing import conditions.

Standard seed for sowing import conditions

The standard import conditions for seed intended for sowing stipulate that:

- each shipment must be packed in clean, new packaging and be clearly labelled with the full botanical name of the species.
- where the seed lot weight is greater than 10 kilograms and the seed size is less than 8 mm in diameter, mandatory sampling of the seed consignment, in accordance with International Seed Testing Association (ISTA) protocols be used to establish that the consignment is free from weed seed contamination. This testing may be performed at department approved ISTA laboratories overseas or on arrival at Australian accredited facilities.
- where seed lots are less than or equal to 10 kilograms in weight, or contain seeds greater than 8 mm in diameter, they will be subject to visual inspection by a biosecurity officer on arrival into Australia. The biosecurity officer will inspect the consignment for freedom from live insects, soil, disease symptoms, contaminant seed, other plant material (for example, leaf and stem material, fruit pulp, and pod material), animal material (for example, animal faeces and feathers) and any other extraneous contamination of biosecurity concern.
- All seed consignments imported into Australia for all end uses must meet the departmental standards for seed contamination and tolerances.

Following inspection, and provided the above conditions are met, the consignment can then be released from biosecurity control without any further requirements.

Since October 2014, in addition to the standard seed for sowing conditions, seeds of cucurbit hosts in which CGMMV is known to be seed-borne are subject to:

Large seed lots

- Testing—mandatory off-shore or on-shore testing by Enzyme-Linked Immunosorbent Assay (ELISA) following the protocol of ISTA 7-026 (ISTA 2014) on a sample size of 9,400 seeds to verify freedom from CGMMV; AND
- Certification— seed lots tested off-shore must be accompanied by an official government Phytosanitary Certificate endorsed with the following additional declaration:

  'The consignment of [*botanical name* (*genus/species*)] was tested by ELISA [*insert laboratory report number*] on a sample size of 9,400 seeds following the protocol of the ISTA 7-026 and found free from CGMMV'.

Small seed lots

- Testing—mandatory on-shore testing by ELISA following the protocol of ISTA 7-026 on a sample size of 20 per cent of the seed lot by weight (to a maximum sample of 9,400 seeds) to verify freedom from CGMMV.

Domestic arrangements

The Australian Government is responsible for regulating the movement of goods such as plants and plant products into and out of Australia. However, the state and territory governments are responsible for plant health controls within their individual jurisdiction. Legislation relating to resource management or plant health may be used by state and territory government agencies to control interstate movement of plants and their products. Once plants and plant products have been cleared by Australian Government biosecurity officers, they may be subject to interstate movement conditions. It is the importer's responsibility to identify, and ensure compliance with all requirements.

1.2.4 Consultation

The department consults stakeholders through the public release of a draft report for comment. The draft pest risk analysis for '*Cucumber green mottle mosaic virus*' was released for a 30 day stakeholder consultation period on 8 April 2016. All submissions were carefully considered and where relevant, changes were made to the final report. A summary of major stakeholder comments and responses to them is contained in Appendix A.

The department worked closely with industry stakeholders during the development of the emergency measures to manage the risk and to minimise disruptions to trade and crop production. Prior to introducing emergency measures against CGMMV, the department consulted with the seed industry including through AUSVEG, the Australian Seed Federation (ASF), representatives of domestic and international seed companies and the Australian Melon Association. A summarised chronology of this consultation is provided here.

9 October 2014	The department held a teleconference with industry to discuss the proposed emergency measures for the importation of known CGMMV host cucurbit seeds.

14 October 2014	The department held a teleconference with industry to discuss the proposed testing requirements for the importation of known CGMMV host cucurbit seeds.
22 October 2014	The department held a teleconference with industry to notify stakeholders of the immediate implementation of emergency measures for known CGMMV host cucurbit seeds and tissue cultures.
23 October 2014	Australia notified trading partners of the emergency measures on 23 October 2014 through a World Trade Organization Sanitary and Phytosanitary (WTO SPS) notification (G/SPS/N/AUS/347). The notification reported that the emergency measures for known CGMMV host cucurbit seeds (mandatory seed testing using ELISA) were to be implemented in three phases to minimise trade disruption. **Phase 1**: Allowing entry of seeds in transit exported prior to 22 October 2014. However, the consignment had to be accompanied by a Phytosanitary Certificate or equivalent supporting document stating that the seed had been tested in accordance with the ISTA protocol 7-026 using a minimum sample size of 2,000 seeds. **Phase 2**: Seeds to be ELISA tested for CGMMV using 9,400 seeds using ISTA 7-026. **Phase 3:** Seeds to be subject to PCR testing using 9,400 seeds, replacing ELISA testing.
5 November 2014	Australia notified trading partners through a WTO SPS addendum notification that Phase 1 had expired and was replaced with Phase 2 (G/SPS/N/AUS/347/Add.1).
20 January 2015	Australia notified trading partners through a WTO SPS addendum notification that Phase 2 was to remain in place until further notice (G/SPS/N/AUS/347/Add.2).
27 August 2015	The department notified industry stakeholders on the progress of the CGMMV PRA at the Australian Seed Federation Annual Conference in Toowoomba, Australia.
11 December 2015	The department notified Australian industry stakeholders on the impending amendment of the emergency measures to include seeds for sowing of *Cucurbita maxima*, *Cucurbita moschata* and hybrids with host parentage.
18 December 2015	Australia notified international trading partners that the emergency measures against CGMMV would now apply to seeds for sowing of *Cucurbita maxima*, *Cucurbita moschata* and hybrids with host parentage. (G/SPS/N/AUS/347/Add.3).
8 April 2016	The department released the 'Draft pest risk analysis for *Cucumber green mottle mosaic virus* (CGMMV)' for a 30 day stakeholder comment period (G/SPS/N/AUS/384).

4 May 2016	The department extended the stakeholder consultation period for a further 30 days (G/SPS/N/AUS/384/Add.1).
8 June 2016	Closing date for the department to receive comments from domestic and international stakeholders on the draft PRA.

2 Method for pest risk analysis

This chapter sets out the method used for the pest risk analysis (PRA) in this report. The Australian Government Department of Agriculture and Water Resources has conducted this PRA in accordance with the International Standards for Phytosanitary Measures (ISPMs), including ISPM No. 2: *Framework for pest risk analysis* (FAO 2016b) and ISPM No. 11: *Pest risk analysis for quarantine pests* (FAO 2016f) that have been developed under the SPS Agreement (WTO 1995).

A PRA is 'the process of evaluating biological or other scientific and economic evidence to determine whether an organism is a pest, whether it should be regulated, and the strength of any phytosanitary measures to be taken against it' (FAO 2016d). A pest is 'any species, strain or biotype of plant, animal, or pathogenic agent injurious to plants or plant products' (FAO 2016d).

Biosecurity risk consists of two major components: the likelihood of a pest entering, establishing and spreading in Australia from imports, and the consequences should this happen. These two components are combined to give an overall estimate of the risk.

Unrestricted risk is estimated taking into account the existing commercial production practices of the exporting country and knowledge that, on arrival in Australia, the department will verify the consignment received is as described on the commercial documents and its integrity has been maintained.

Restricted risk is the estimated final risk of importation with phytosanitary measure(s) applied. A phytosanitary measure is 'any legislation, regulation or official procedure having the purpose to prevent the introduction and/or spread of quarantine pests, or to limit the economic impact of regulated non-quarantine pests' (FAO 2016d).

A glossary of the terms used in this document is provided at the end of this report.

The PRA is conducted in the following three consecutive stages: initiation, pest risk assessment and pest risk management.

2.1 Stage 1 Initiation

The initiation of a risk analysis involves identifying the pest(s) and pathway(s) that should be considered for risk analysis in relation to the identified PRA area. According to ISPM No. 2 (FAO 2016b), a PRA process may be initiated as a result of:

- identification of a pathway that presents a potential pest risk (a means of pest introduction or spread);
- identification of a pest that may require phytosanitary measures (a pest may have been detected or intercepted, a request made to import it or it may have been reported elsewhere);
- review or revision of existing phytosanitary policies and priorities; or
- identification of an organism not previously known to be a pest.

Australia introduced emergency measures in October 2014 after the detection of CGMMV in melon crops in the Northern Territory. In accordance with ISPM No. 2 (FAO 2016b), this PRA was initiated by the department as a basis for review and possible revision of the emergency measures introduced by Australia for importation of seeds of cucurbit hosts in which CGMMV is known to be seed-borne. In Australia, CGMMV has been regulated as a quarantine pest since October 2014.

In the context of this PRA, the seeds of *Citrullus lanatus*, *Cucumis melo*, *Cucumis sativus*, *Cucurbita maxima*, *Cucurbita moschata*, *Cucurbita pepo, Lagenaria siceraria*, *Trichosanthes cucumerina* and any hybrid of these species are assessed to be a potential import 'pathway' by which CGMMV can enter Australia.

In the context of this PRA, the 'PRA area' is defined as the whole of Australia.

2.2 Stage 2 Pest risk assessment

A pest risk assessment (for quarantine pests) is the 'evaluation of the probability of the introduction and spread of a pest and of the magnitude of the associated potential economic consequences' (FAO 2016d).

The following three consecutive steps were used in the pest risk assessment:

2.2.1 Pest categorisation

Pest categorisation identifies pests that have the potential to be on the commodity, may become quarantine pests for Australia, and that require a pest risk assessment. A 'quarantine pest' is a pest of potential economic importance to the area endangered thereby and not yet present there, or present but not widely distributed and being officially controlled (FAO 2016d).

The process of pest categorisation is summarised by ISPM No. 11 (FAO 2016f) as being a screening procedure for potential pest speciesbased on the following criteria:

- identity of the pest;
- presence or absence in the PRA area;
- regulatory status;
- potential for establishment and spread in the PRA area; and
- potential for economic, environmental and social consequences in the PRA area.

2.2.2 Assessment of the probability of entry, establishment and spread

Details of how to assess the 'probability of entry', 'probability of establishment' and 'probability of spread' of a pest are given in ISPM No. 11 (FAO 2016f). The SPS Agreement (WTO 1995) uses the term likelihood rather than probability for these estimates. In qualitative PRAs, the department uses the term 'likelihood' for the descriptors it uses for its estimates of likelihood of entry, establishment and spread. The use of the term 'probability' is limited to the direct quotation of ISPM definitions.

A summary of this process is given below, followed by a description of the qualitative methodology used in this PRA.

Likelihood of entry

The likelihood of entry describes the likelihood that a quarantine pest will enter Australia as a result of trade in a given commodity, be distributed in a viable state in the PRA area and subsequently be transferred to a host. Assessing the likelihood of entry requires an analysis of each of the pathways with which a pest may be associated, from its origin to distribution in the PRA area.

For the purpose of considering the likelihood of entry, the department divides this step into two components:

- **Likelihood of importation**—the likelihood that a pest will arrive in Australia when a given commodity is imported.
- **Likelihood of distribution**—the likelihood that the pest will be distributed, as a result of the processing, sale or disposal of the commodity, in the PRA area and subsequently transfer to a susceptible part of a host.

Factors to be considered in the likelihood of importation may include:

- distribution and incidence of the pest in the source area
- occurrence of the pest in a life-stage that would be associated with the commodity
- mode of trade (for example, bulk, packed)
- volume and frequency of movement of the commodity along each pathway
- seasonal timing of imports
- pest management, cultural and commercial procedures applied at the place of origin
- speed of transport and conditions of storage compared with the duration of the lifecycle of the pest
- vulnerability of the life-stages of the pest during transport or storage
- incidence of the pest likely to be associated with a consignment
- commercial procedures (for example, refrigeration) applied to consignments during transport and storage in the country of origin, and during transport to Australia.

Factors to be considered in the likelihood of distribution may include:

- commercial procedures (for example, refrigeration) applied to consignments during distribution in Australia
- dispersal mechanisms of the pest, including vectors, to allow movement from the pathway to a host
- whether the imported commodity is to be sent to a few or many destination points in the PRA area
- proximity of entry, transit and destination points to hosts
- time of year at which import takes place
- intended use of the commodity (for example, for planting, processing or consumption)
- risks from by-products and waste.

Likelihood of establishment

Establishment is defined as the 'perpetuation for the foreseeable future, of a pest within an area after entry' (FAO 2016d). In order to estimate the likelihood of establishment of a pest, reliable biological information (lifecycle, host range, epidemiology and survival) is obtained from the areas where the pest currently occurs. The situation in the PRA area can then be compared with that in the areas where it currently occurs and expert judgement used to assess the likelihood of establishment.

Factors to be considered in the likelihood of establishment in the PRA area may include:

- availability of hosts, alternative hosts and vectors
- suitability of the environment
- reproductive strategy and potential for adaptation
- minimum population needed for establishment
- cultural practices and control measures.

Likelihood of spread

Spread is defined as 'the expansion of the geographical distribution of a pest within an area' (FAO 2016d). The likelihood of spread considers the factors relevant to the movement of the pest, after establishment on a host plant or plants, to other susceptible host plants of the same or different species in other areas. In order to estimate the likelihood of spread of the pest, reliable biological information is obtained from areas where the pest currently occurs. The situation in the PRA area is then carefully compared with that in the areas where the pest currently occurs and expert judgement used to assess the likelihood of spread.

Factors to be considered in the likelihood of spread may include:

- suitability of the natural and/or managed environment for natural spread of the pest
- presence of natural barriers
- potential for movement with commodities, conveyances or by vectors
- intended use of the commodity
- potential vectors of the pest in the PRA area
- potential natural enemies of the pest in the PRA area.

Assigning likelihoods for entry, establishment and spread

Likelihoods are assigned to each step of entry, establishment and spread. Six descriptors are used: high, moderate, low, very low, extremely low and negligible. Definitions for these descriptors and their indicative probability ranges are given in Table 1. The indicative ranges are only provided to illustrate the boundaries of the descriptors and are not used beyond this purpose in qualitative PRAs. These indicative likelihood ranges provide guidance to the risk analyst and promotes consistency between different pest risk assessments.

Table 1 Nomenclature of likelihoods

Likelihood	Descriptive definition	Indicative range
High	The event would be very likely to occur	0.7 < to ≤ 1
Moderate	The event would occur with an even likelihood	0.3 < to ≤ 0.7
Low	The event would be unlikely to occur	0.05 < to ≤ 0.3
Very low	The event would be very unlikely to occur	0.001 < to ≤ 0.05
Extremely low	The event would be extremely unlikely to occur	0.000001 < to ≤ 0.001
Negligible	The event would almost certainly not occur	0 < to ≤ 0.000001

Combining likelihoods

The likelihood of entry is determined by combining the likelihood that the pest will be imported into the PRA area and the likelihood that the pest will be distributed within the PRA area, using a matrix of rules (Table 2). This matrix is then used to combine the likelihood of entry and the likelihood of establishment, and then the likelihood of entry and establishment is combined with the likelihood of spread to determine the overall likelihood of entry, establishment and spread.

Table 2 Matrix of rules for combining likelihoods

	High	Moderate	Low	Very low	Extremely low	Negligible
High	High	Moderate	Low	Very low	Extremely low	Negligible
Moderate		Low	Low	Very low	Extremely low	Negligible
Low			Very low	Very low	Extremely low	Negligible
Very low				Extremely low	Extremely low	Negligible
Extremely low					Negligible	Negligible
Negligible						Negligible

For example, if the likelihood of importation is assigned a descriptor of 'low' and the likelihood of distribution is assigned a descriptor of 'moderate', then they are combined to give a likelihood of 'low' for entry. The likelihood for entry is then combined with the likelihood assigned for establishment of 'high' to give a likelihood for entry and establishment of 'low'. The likelihood for entry and establishment is then combined with the likelihood assigned for spread of 'very low' to give the overall likelihood for entry, establishment and spread of 'very low'. This can be summarised as:

importation x distribution = entry [E] **low x moderate = low**

entry x establishment = [EE] **low x high = low**

[EE] x spread = [EES] **low x very low = very low**

Time and volume of trade

One factor affecting the likelihood of entry is the volume and duration of trade. If all other conditions remain the same, the overall likelihood of entry will increase as time passes and the overall volume of trade increases.

The department normally considers the likelihood of entry on the basis of the estimated volume of one year's trade. However, in case of a high risk propagative commodity the volume of trade is restricted to certain numbers. Therefore, other factors listed in ISPM No. 11 (FAO 2016f) may not be relevant to propagative material of a high risk commodity.

2.2.3 Assessment of potential consequences

The objective of the consequence assessment is to provide a structured and transparent analysis of the potential consequences if the pest or disease agents were to enter, establish and spread in Australia. The assessment considers direct and indirect pest effects and their economic and environmental consequences. The requirements for assessing potential consequences are given in Article 5.3 of the SPS Agreement (WTO 1995), ISPM No. 5 (FAO 2016d) and ISPM No. 11 (FAO 2016f).

Direct pest effects are considered in the context of the effects on:

- plant life or health
- other aspects of the environment.

Indirect pest effects are considered in the context of the effects on:

- eradication, control
- domestic trade
- international trade
- environment.

For each of these six criteria, the consequences were estimated over four geographic levels, defined as:

Local—an aggregate of households or enterprises (a rural community, a town or a local government area).

District—a geographically or geopolitically associated collection of aggregates (generally a recognised section of a state or territory, such as 'Far North Queensland').

Regional—a geographically or geopolitically associated collection of districts in a geographic area (generally a state or territory, although there may be exceptions with larger states such as Western Australia).

National—Australia wide (Australian mainland states and territories and Tasmania).

For each criterion, the magnitude of the potential consequence at each of these levels was described using four categories, defined as:

Indiscernible—pest impact unlikely to be noticeable.

Minor significance—expected to lead to a minor increase in mortality/morbidity of hosts or a minor decrease in production but not expected to threaten the economic viability of production. Expected to decrease the value of non-commercial criteria but not threaten the criterion's intrinsic value. Effects would generally be reversible.

Significant—expected to threaten the economic viability of production through a moderate increase in mortality/morbidity of hosts, or a moderate decrease in production. Expected to significantly diminish or threaten the intrinsic value of non-commercial criteria. Effects may not be reversible.

Major significance—expected to threaten the economic viability through a large increase in mortality/morbidity of hosts, or a large decrease in production. Expected to severely or irreversibly damage the intrinsic 'value' of non-commercial criteria.

The estimates of the magnitude of the potential consequences over the four geographic levels were translated into a qualitative impact score (A–G) using Table 3. For example, a consequence with a magnitude of 'significant' at the 'district' level will have a consequence impact score of D.

Table 3 Decision rules for determining the consequence impact score based on the magnitude of consequences at four geographic scales

	Geographic scale			
Magnitude	Local	District	Region	Nation
Indiscernible	A	A	A	A
Minor significance	B	C	D	E
Significant	C	D	E	F
Major significance	D	E	F	G

Note: In earlier qualitative PRAs, the scale for the impact scores went from A to F and did not explicitly allow for the rating 'indiscernible' at all four levels. This combination might be applicable for some criteria. In this report, the impact scale of A to F has been changed to become B–G and a new lowest category A ('indiscernible' at all four levels) was added. The rules for combining impacts in Table 2.4 were adjusted accordingly.

The overall consequence for each pest is achieved by combining the qualitative impact scores (A–G) for each direct and indirect consequence using a series of decision rules (Table 4). These rules are mutually exclusive, and are assessed in numerical order until one applies.

Table 4 Decision rules for determining the overall consequence rating for each pest

Rule	The impact scores for consequences of direct and indirect criteria	Overall consequence rating
1	Any criterion has an impact of 'G'; or more than one criterion has an impact of 'F'; or a single criterion has an impact of 'F' and each remaining criterion an 'E'.	Extreme
2	A single criterion has an impact of 'F'; or all criteria have an impact of 'E'.	High
3	One or more criteria have an impact of 'E'; or all criteria have an impact of 'D'.	Moderate
4	One or more criteria have an impact of 'D'; or all criteria have an impact of 'C'.	Low
5	One or more criteria have an impact of 'C'; or all criteria have an impact of 'B'.	Very Low
6	One or more but not all criteria have an impact of 'B', and all remaining criteria have an impact of 'A'.	Negligible

2.2.4 Estimation of the unrestricted risk

Once the assessment of the likelihood of entry, establishment and spread for potential consequences are completed, the unrestricted risk can be determined for each pest or group of pests. This is determined by using a risk estimation matrix (Table 5) to combine the estimates of the likelihood of entry, establishment and spread and the overall consequences of pest establishment and spread. Therefore, risk is the combination of likelihood and consequence.

When interpreting the risk estimation matrix, note the descriptors for each axis are similar (for example, low, moderate and high) but the vertical axis refers to likelihood and the horizontal axis refers to consequences. Accordingly, a 'low' likelihood combined with 'high' consequences, is not the same as a 'high' likelihood combined with 'low' consequences—the matrix is not symmetrical. For example, the former combination would give an unrestricted risk rating of 'moderate', whereas the latter would be rated as a 'low' unrestricted risk.

Table 5 Risk estimation matrix

Likelihood of pest entry, establishment and spread	Consequences of pest entry, establishment and spread					
	Negligible	Very low	Low	Moderate	High	Extreme
High	Negligible risk	Very low risk	Low risk	Moderate risk	High risk	Extreme risk
Moderate	Negligible risk	Very low risk	Low risk	Moderate risk	High risk	Extreme risk
Low	Negligible risk	Negligible risk	Very low risk	Low risk	Moderate risk	High risk
Very low	Negligible risk	Negligible risk	Negligible risk	Very low risk	Low risk	Moderate risk
Extremely low	Negligible risk	Negligible risk	Negligible risk	Negligible risk	Very low risk	Low risk
Negligible	Negligible risk	Negligible risk	Negligible risk	Negligible risk	Negligible risk	Very low risk

2.2.5 The appropriate level of protection (ALOP) for Australia

The SPS Agreement defines the concept of an 'appropriate level of sanitary or phytosanitary protection (ALOP)' as the level of protection deemed appropriate by a WTO Member establishing a sanitary or phytosanitary measure to protect human, animal or plant life or health within its territory (WTO 1995).

Like many other countries, Australia expresses its ALOP in qualitative terms. The ALOP for Australia, which reflects community expectations through government policy, is currently expressed as providing a high level of sanitary or phytosanitary protection aimed at reducing risk to a very low level, but not to zero. The band of cells in Table 5 marked 'very low risk' represents the maximum acceptable ALOP threshold for Australia.

2.3 Stage 3 Pest risk management

Pest risk management describes the process of identifying and implementing phytosanitary measures to manage risks to achieve the ALOP for Australia, while ensuring that any negative effects on trade are minimised.

The conclusions from the pest risk assessment process are used to decide whether risk management is required and if so, the appropriate measures to be used. Where the unrestricted risk estimate does not achieve the ALOP for Australia, risk management measures are required to reduce this risk to a very low level. The guiding principle for risk management is to manage risk to achieve the ALOP for Australia. The effectiveness of any proposed phytosanitary measures (or combination of measures) is evaluated, using the same approach as used to evaluate the unrestricted risk, to ensure the restricted risk for the relevant pest or pests achieves the ALOP for Australia.

Examples of risk management measures which may be applied to propagative material consignments include:

- Import from pest-free areas only (ISPM No. 4, 10)—the establishment and use of a pest-free area by a National Plant Protection Organisation (NPPO) provides for the export of plants from the exporting country to the importing country without the need for application of additional phytosanitary measures when certain requirements are met.
- Testing for freedom from regulated pests—this is a practical measure for pests which do not produce visible symptoms on plants.
- Inspection and certification (ISPM No. 7, 12, 23)—the exporting country may be asked to inspect the shipment and certify that the shipment is free from regulated pests before export.
- Pre-entry or post-entry quarantine—the importing country may define certain control conditions, inspection and possible treatment of shipments upon their entry into the country. Pre- or post-entry quarantine of dormant cuttings, seeds and tissue cultures (*in vitro* plantlets) can help avoid the introduction of new viruses or allied pathogens into the importing countries.
- Removal of the pest from the consignment by treatment or other methods—the importing country may specify chemical or physical treatments that must be applied to the consignment before it may be imported.
- Prohibition of commodities—the importing country may prohibit the commodity if no satisfactory measure can be found.

In some cases, more than one risk management measure may be required in order to reduce the pest risk to an acceptable level.

3 The pathogen

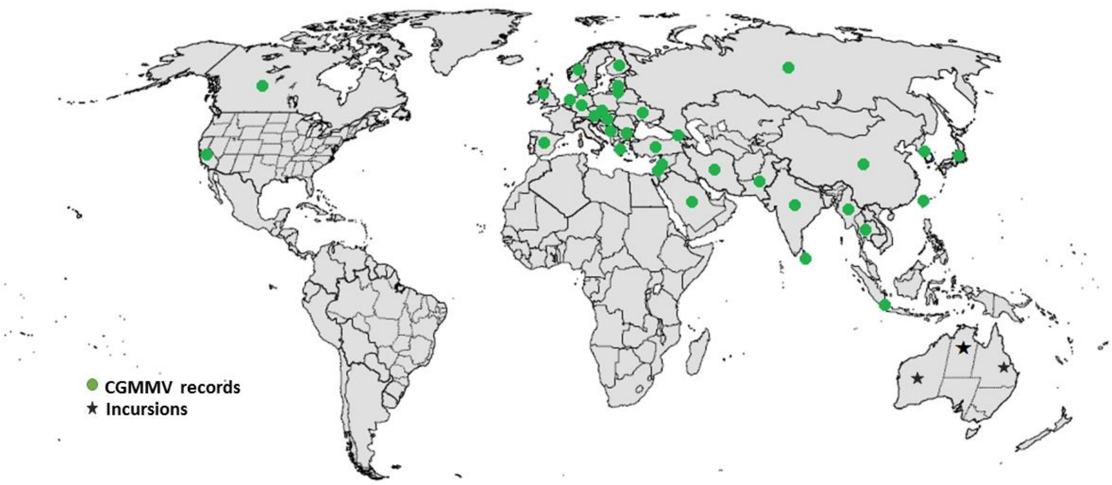
Cucumber green mottle mosaic virus (CGMMV) belongs to the genus *Tobamovirus* in the family Virgaviridae (Adams, Antoniw & Kreuze 2009). CGMMV was first described in the 1930s in cucumber and was named *Cucumber virus 3* and *Cucumber virus 4* (Ainsworth 1935; Ugaki et al. 1991). CGMMV is a rod-shaped virus (Lecoq & Desbiez 2012) and contains a single-stranded RNA genome (Hollings, Komuro & Tochihara 1975).

Many strains and isolates of CGMMV have been reported in the literature. Different authors have described CGMMV strains based on geographic distribution, host range, symptoms on natural host plants, serological methods, and molecular methods, or by differential responses on indicator plants such as *Chenopodium amaranticolor* and *Datura stramonium* (Antignus et al. 2001; Boubourakas et al. 2004; Hollings, Komuro & Tochihara 1975). Some isolates formerly classified as strains of CGMMV are now accepted as being separate virus species. For instance, Yodo strain (CGMMV-Y) and Cucumber strain (CGMMV-C or CGMMV-Cu) were both previously considered to be strains of CGMMV, but are now considered to be strains of *Kyuri green mottle mosaic virus* (KGMMV) (Antignus et al. 2001; Varveri, Vassilakos & Bem 2002).

3.1 Geographical distribution of CGMMV

CGMMV was first reported in the United Kingdom in the 1930s (Ainsworth 1935; Ugaki et al. 1991). Since then, CGMMV has spread among cucurbit crops worldwide (Reingold et al. 2015) and is now reported to occur on most continents and in over 30 countries (Map 3).

Map 3 Global occurrence of CGMMV



In September 2014, the presence of CGMMV was confirmed in the Northern Territory, Australia on commercial watermelon farms (Government of Queensland 2017; Northern Territory

Goverment 2017). In April 2015, CGMMV was detected on a single cucurbit farm in Queensland; and the infected property remains under quarantine.

In February 2016, the Northern Territory government announced the formal revocation of the CGMMV quarantine zone. However, simultaneously implemented regulations now prohibit the export of seeds, seedlings and bee hives from the Northern Territory without approval from the Northern Territory Chief Inspector of Plant Health, and require growers to have an auditable farm biosecurity plan for the purpose of controlling CGMMV.

In 2016, CGMMV was detected on cucumber farms near Geraldton (July), Carnarvon (August), Perth (September) and Kununurra (September) in Western Australia. The Government of Western Australia is assisting growers to develop farm biosecurity plans to prevent further spread of CGMMV and to manage the disease on infected properties.

In February 2017, CGMMV was detected on cucumber farms near Bundaberg. Biosecurity Queensland is providing technical advice on biosecurity and control measures to growers, and assisting them to develop and implement a plan for eradicating the virus from their production areas and minimising the risk of spreading the virus to the wider industry.

Given the isolated occurrences of the virus and contining measures to prevent its spread, CGMMV is considered to be under official control in Australia.

3.2 Biology of CGMMV

CGMMV infects host plants systemically (Inouye et al. 1967; Komuro et al. 1971) and causes structural changes in infected tissues (Hatta et al. 1971; Hatta & Ushiyama 1973). During infection of cucumber plants, CGMMV moves systemically through the phloem (Simon-Buela & Garcia-Arenal 1999) and xylem (Moreno, Thompson & Garcia-Arenal 2004). CGMMV persists in seeds (Reingold et al. 2015; Tian et al. 2014), infected plant debris (Choi 2001; Liu et al. 2014) and infected plant material in the soil (Hollings, Komuro & Tochihara 1975; Rao & Varma 1984). CGMMV is also able to be mechanically transmitted, and infect host plants through wounds caused by pruning or harvesting (Hollings, Komuro & Tochihara 1975; Lecoq & Desbiez 2012).

3.2.1 Hosts of CGMMV

The host range of CGMMV includes many species within the Cucurbitaceae family of plants (Hollings, Komuro & Tochihara 1975). The Cucurbitaceae family comprises 122 genera and approximately 960 species (Mabberley 2008); most of these species are not commonly cultivated (Freeman et al. 2015; McCormack 2005). According to published evidence, the majority of species within the Cucurbitaceae are not known to be natural hosts of CGMMV. Furthermore, although this virus is reported to infect many cucurbit species, it is only known to be associated with the seeds of *Citrullus lanatus*, *Cucumis melo*, *Cucumis sativus*, *Cucurbita maxima*, *Cucurbita maxima* x *Cucurbita moschata*, *Cucurbita moschata*, *Cucurbita pepo*, *Lagenaria siceraria* and *Trichosanthes cucumerina* (Table 6).

In recent years, CGMMV has been detected in an increasing number of non-cucurbit host plants, particularly weeds. It has been reported to naturally occur in non-cucurbit species from the families Amaranthaceae, Apiaceae, Boraginaceae, Chenopodiaceae, Portulacaceae and Solanaceae (Boubourakas et al. 2004; Cho, Kim & Jeon 2015). To date, CGMMV is not known to be associated with seeds of non-cucurbit species. The known natural cucurbit and non-cucurbit hosts of CGMMV are listed in Table 6.

Table 6 Natural hosts of CGMMV and association of the virus with host seeds

Scientific name	Common name (s)	Natural host of CGMMV	Is CGMMV seed-borne?
CROPS			
Benincasa hispida (Thunberg) Cogniaux [Cucurbitaceae]	Ash gourd, Wax gourd, White gourd	Yes (Noda et al. 1993)	No evidence of CGMMV being seed-borne in this species.
Citrullus lanatus (Thunberg) Matsumura & Nakai [Cucurbitaceae]	Watermelon	Yes (Lee et al. 1990; Reingold et al. 2015; Yoon et al. 2008)	Yes (Lee et al. 1990; Yoon et al. 2008)
Cucumis anguria Linnaeus [Cucurbitaceae]	Bur cucumber, West Indian gherkin	Yes (Rashmi et al. 2005)	No evidence of CGMMV being seed-borne in this species.
Cucumis melo Linnaeus [Cucurbitaceae]	Cantaloupe, Melon	Yes (Reingold et al. 2015; Sugiyama, Ohara & Sakata 2006; Tian et al. 2014)	Yes (Reingold et al. 2015; Tian et al. 2014)
Cucumis sativus Linnaeus [Cucurbitaceae]	Cucumber	Yes (Liu et al. 2014)	Yes (Faris-Mukhayyish & Makkouk 1983; Liu et al. 2014)
Cucurbita maxima Duchesne [Cucurbitaceae]	Buttercup squash, Kabotcha, Pumpkin	Yes (Stephen West [NT Department of Primary Industry and Fisheries (DPIF)] 2015, pers. comm., 9 September)	Yes (Fiona Constable [Department of Economic Development, Jobs, Transport and Resources, Victoria (DEDJTR)] 2015, pers. comm., 2 December).
Cucurbita maxima Duchesne x *Cucurbita moschata* Duchesne [Cucurbitaceae]	Kabotcha, Pumpkin	Yes (Fiona Constable [DEDJTR] 2015, pers. comm., 2 December)	Yes (Fiona Constable [DEDJTR] 2015, pers. comm., 2 December).
Cucurbita moschata Duchesne [Cucurbitaceae]	Butternut pumpkin, Butternut squash, Calabaza pumpkin , Pumpkin, Tropical pumpkin	Yes (Noda et al. 1993; Zhang, Li & Li 2009)	Yes (Fiona Constable [DEDJTR] 2015, pers. comm., 2 December).
Cucurbita pepo Linnaeus [Cucurbitaceae]	Acorn squash, Pumpkin, Summer squash, Zucchini	Yes (Al-Tamimi, Kawas & Mansour 2009; Qin et al. 2005)	Yes (Al-Tamimi, Kawas & Mansour 2009; Kwon et al. 2014)
Lagenaria siceraria (Molina) Standley [Cucurbitaceae]	Bottle gourd, Calabash, Korean native gourd, Long melon	Yes (Boubourakas et al. 2004; Zhang, Li & Li 2009)	Yes (Boubourakas et al. 2004; Choi 2001)
Luffa acutangula (Linnaeus) Roxburgh [Cucurbitaceae]	Angled luffa, Chinese okra, Ridged gourd	Yes (Noda et al. 1993; Sharma et al. 2014)	No evidence of CGMMV being seed-borne in this species.
Luffa cylindrica (Linnaeus) Roemer	Smooth loofah gourd	Yes (Noda et al. 1993)	No evidence of

Scientific name	Common name (s)	Natural host of CGMMV	Is CGMMV seed-borne?
[Cucurbitaceae]			CGMMV being seed-borne in this species.
Momordica charantia Linnaeus [Cucurbitaceae]	Balsam apple, Balsam pear, Bitter gourd, Bitter melon	Yes (Noda et al. 1993; Pandey & Joshi 1989)	No evidence of CGMMV being seed-borne in this species.
Trichosanthes cucumerina Linnaeus [Cucurbitaceae]	Serpent gourd, Snake gourd	Yes (Nagendran et al. 2015)	Yes (Ariyaratne, Weeraratne & Ranatunge 2005)
WEEDS			
Amaranthus blitoides Watson [Amaranthaceae]	Mat amaranth, Prostrate amaranth	Yes (Boubourakas et al. 2004)	No evidence of CGMMV being seed-borne in these species.
Amaranthus retroflexus Linnaeus [Amaranthaceae]	Redroot amaranth, Redroot pigweed	Yes (Boubourakas et al. 2004)	
Amaranthus viridis Linnaeus [Amaranthaceae]	Green amaranthus	Yes (Stephen West [NT DPIF] 2015, pers. comm., 9 September)	
Chenopodium album Linnaeus [Chenopodiaceae]	Fat hen	Yes (Boubourakas et al. 2004)	No evidence of CGMMV being seed-borne in this species.
Ecballium elaterium (Linnaeus) Richard [Cucurbitaceae]	Squirting cucumber	Yes (Antignus et al. 1990)	No evidence of CGMMV being seed-borne in this species.
Heliotropium europaeum Linnaeus [Boraginaceae]	Common heliotrope	Yes (Boubourakas et al. 2004)	No evidence of CGMMV being seed-borne in this species.
Heracleum moellendorffii Hance [Apiaceae]	Duan mao du huo, Eosuri	Yes (Cho, Kim & Jeon 2015)	No evidence of CGMMV being seed-borne in this species.
Portulaca oleracea Linnaeus [Portulacaceae]	Parsley, Pigweed, Purslane	Yes (Boubourakas et al. 2004)	No evidence of CGMMV being seed-borne in this species.
Solanum nigrum Linnaeus [Solanaceae]	Blackberry nightshade, Black nightshade	Yes (Boubourakas et al. 2004)	No evidence of CGMMV being seed-borne in this species.

3.2.2 Spread of CGMMV

CGMMV is capable of natural spread in protected environments (such as greenhouses) and in the field, and also through human activities such as pruning and harvesting.

Short-distance spread

The natural short-distance spread of CGMMV mainly occurs through physical contact with contaminated water, contaminated soil or infected plant materials or debris (Broadbent & Fletcher 1963; Choi 2001; Reingold et al. 2015).

- CGMMV is very stable and can survive for long periods on equipment and tools (ASTA 2014). The virus may be spread through standard cultivation practices and harvesting (Lecoq & Desbiez 2012), when plants are handled by farm personnel, or when contaminated equipment or machinery is used (Boubourakas et al. 2004; Reingold et al. 2015).
- Spread of CGMMV can also occur through leaf contact between healthy and infected plants (Lecoq & Desbiez 2012).
- CGMMV has been found in surface and irrigation water, contact with which can lead to plant infection (Dorst 1988; Vani & Varma 1993). CGMMV can spread to new host plants through the recirculation of nutrient solutions (Büttner, Marquardt & Schickedanz 1995).
- CGMMV can survive for long periods on plant debris. In soil contaminated by infected plant debris, CGMMV may spread over short-distances through contact via roots (Hollings, Komuro & Tochihara 1975). For example, 18 per cent transmission of CGMMV was recorded in *L. siceraria* plants sown in soil mixed with infected plant debris (Rao & Varma 1984).

Long-distance spread

Long-distance spread of CGMMV via human-assisted means can be rapid, and the commercial movement of infected seeds or seedlings for planting is likely to be a significant pathway for the spread of the virus (Kobayashi 1990; Lecoq & Katis 2014; Liu et al. 2014). Other possible pathways for long-distance spread through human-assisted means include infested soil and debris contaminating seeds (Lecoq & Katis 2014).

- The seed trade is a major potential pathway for the worldwide spread of exotic pathogens, including CGMMV. The introduction of CGMMV into Japan in 1966 was linked to *Lagenaria siceraria* seed imported from India (Kobayashi 1990; Tochihara & Komuro 1974). Choi (2001) concluded that virus-contaminated bottle gourd seeds were the major cause of disease transmission to watermelon growing regions in Korea in 1998. The rapid spread of CGMMV throughout the cucurbit-growing areas of the world is likely to be associated with the trade of host cucurbit seeds.
- CGMMV is known to be seed-borne in *Citrullus lanatus*, *Cucumis sativus*, *Cucumis melo, Cucurbita maxima, Cucurbita maxima* x *Cucurbita moschata, Cucurbita moschata, Cucurbita pepo*, *Lagenaria siceraria* and *Trichosanthes cucumerina* (Fiona Constable [DEDJTR] 2015, pers. comm., 2 December)(Choi 2001; Lee et al. 1990; Liu et al. 2014).
- Seed to seedling transmission of CGMMV has been demonstrated in several host species. Reported transmission rates vary widely, from less than 0.1 per cent up to 76.7 per cent (Broadbent 1976; Lecoq & Desbiez 2012; Liu et al. 2014; Rast & Stijger 1987; Reingold et al. 2016). However, rates of transmission reported in most publications were relatively low commonly around 1 per cent; in contrast Liu et al. (2014) reported a rate of up to 76.7 per cent.
- CGMMV transmission through seeds and soil, even at very low levels, may initiate the disease cycle (Choi 2001).

Other potential means of spread

- **Pollen**—CGMMV has been detected in the pollen of bottle gourd (Rao & Varma 1984), however, natural spread by pollen has not been proven. Under experimental conditions, the artificial pollination of cucumber flowers with pollen infected with CGMMV resulted in the infection of up to 51.2 per cent of fruits. Seed samples from infected fruits also tested positive for the virus (Liu et al. 2014). Infection through pollen could result in a systemic infection in mature plants and be passed on to the next generation via infected seeds. Therefore, pollen transmission could play a role in the epidemiology and management of CGMMV (Liu et al. 2014; Mink 1993). However, more information is required to establish that spread occurs naturally via pollen.
- **Insects**—No CGMMV transmission was observed in trials using aphids (*Aphis gossypii* and *Myzus persicae*) and cucumber leaf beetles (*Aulacophora femoralis*) as candidate vectors (Hollings, Komuro & Tochihara 1975). However, Rao and Verma (1984) reported that cucumber leaf beetles are potential vectors of CGMMV; in their transmission studies cucumber leaf beetles transmitted CGMMV directly to 10 per cent of tested plants (Rao & Varma 1984). Infective particles of CGMMV were also detected in the regurgitated fluid and excreta of cucumber leaf beetles (Rao & Varma 1984). Therefore, cucumber leaf beetles may be a potential vector for short-distance spread of CGMMV in some countries.

3.3 Symptoms of CGMMV

CGMMV can cause a range of symptoms in host plants and may also infect plants symptomlessly. In cucurbits, CGMMV symptoms typically include leaf mottling, mosaic symptoms and fruit mottling or distortion (Reingold et al. 2015). The virus may also symptomatically infect some non-cucurbit crops. For instance, *Heracleum moellendorfii* (Apiaceae) plants naturally infected with CGMMV have shown leaf mottling and mosaic symptoms (Cho, Kim & Jeon 2015). CGMMV symptoms may vary with tissue type, growth stage, isolate, strain, season, host and environmental conditions (ASTA 2014).

This review focuses on symptoms that CGMMV typically causes on the natural host species (*Citrullus lanatus*, *Cucumis melo*, *Cucumis sativus*, *Cucurbita maxima*, *Cucurbita moschata*, *Cucurbita pepo*, *Lagenaria siceraria* and *Trichosanthes cucumerina*) that are known to carry the virus in seed.

3.3.1 Symptoms caused by CGMMV on *Citrullus lanatus*

Symptoms of CGMMV on *Citrullus lanatus* (watermelon) plants generally include vein clearing and crumpling of young leaves, mottling or mosaic patterns, a pale-yellow or bleached appearance on mature leaves (Figure 1A), and necrotic spots on the peduncle (Figure 1B) (Reingold et al. 2013). Fruit symptoms may include yellowing of the normally pink to red flesh, especially on its outer perimeter (Figure 1C), and the development of internal cavities and areas that prematurely soften and become mushy (Figure 1C and D) (ASTA 2014; Department of Primary Industry and Fisheries 2017).

Variability in CGMMV symptoms on watermelon plants has been reported. For example, Boubourakas et al. (2004) described three types of CGMMV symptoms on grafted watermelon crops in Greece. The first type consisted of asymptomatic leaves and the regular appearance of necrotic lesions on the fruit peduncle (Figure 1B). Fruits at maturity showed whitish-yellow discoloration of the peripheral region and water-soaked inner pulp (Figure 1C and D). The second type of symptoms occurred more frequently and consisted of chlorotic leaf spots, which later coalesced to form characteristic irregular chlorotic patterns on leaves. The third type of symptoms consisted of chlorotic leaf patterns followed by pedicel necrosis (Boubourakas et al. 2004).

Figure 1 CGMMV symptoms on watermelon: (A) leaf mottling and chlorotic mosaic symptoms; (B) peduncle necrosis; (C) whitish-yellow discoloration of the peripheral region of fruit; (D) water-soaked inner pulp of fruit

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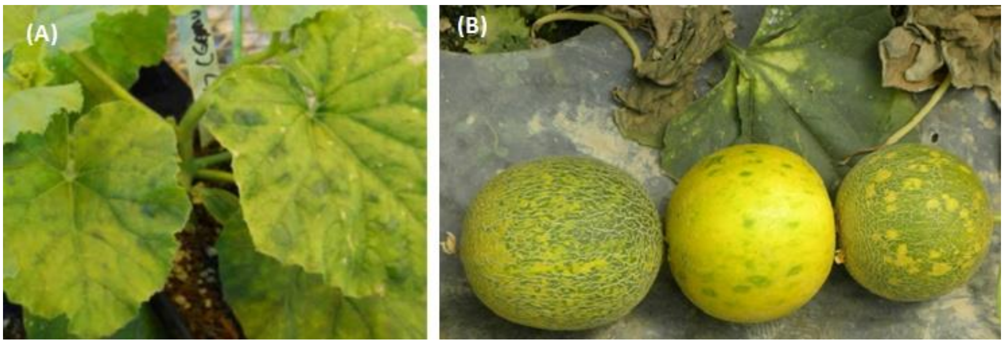
3.3.2 Symptoms caused by CGMMV on *Cucumis melo*

CGMMV causes mosaic and wrinkling, green vein-banding and stunting of *Cucumis melo* (melon) crops in the field. In the greenhouse, symptoms can include mild chlorosis, mosaic, vein-banding, and deformed leaves with blisters in older leaves (Figure 2A) (AMA 2015; Raychaudhuri & Varma 1978). Infected fruit may display mottled symptoms as shown in Figure 2B (AMA 2015).

Figure 2 CGMMV symptoms on melon leaves and fruits: (A) mild chlorosis and mosaic symptoms on leaves; (B) mottle on fruits



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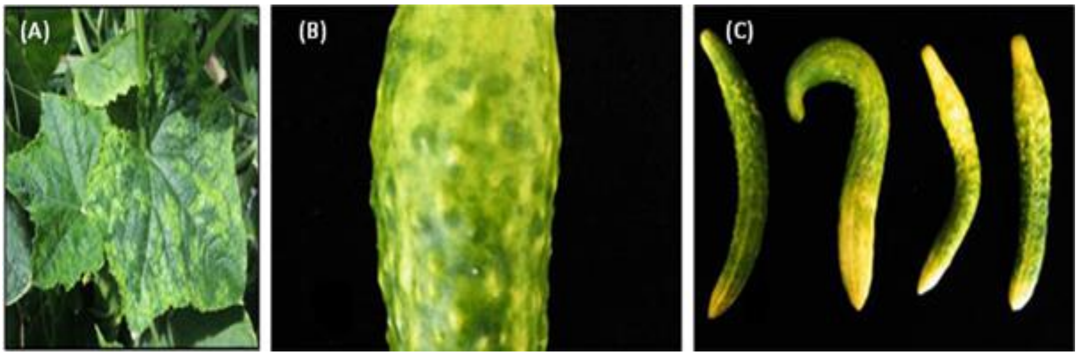
3.3.3 Symptoms caused by CGMMV on *Cucumis sativus*

CGMMV causes a range of symptoms in *Cucumis sativus* (cucumber), with symptoms varying between strains and isolates (Hollings, Komuro & Tochihara 1975). Leaf mottling, blistering and distortion (Figure 3A), and stunted growth have been observed on cucumbers infected with type strain CV3 (AMA 2015; Hollings, Komuro & Tochihara 1975; Ling, Li & Zhang 2014). While the type strain does not typically cause fruit symptoms in cucumber (Hollings, Komuro & Tochihara 1975), some strains can cause severe fruit mottling and distortion (Liu et al. 2014) (Figure 3B and C). The Indian bottle gourd strain C can cause blister-mottle, stunting and crop losses (Hollings, Komuro & Tochihara 1975). Several Greek isolates showed symptoms of higher severity and had a wider host range compared with other described CGMMV isolates (Boubourakas et al. 2004).

Figure 3 CGMMV symptoms on cucumber leaves and fruits: (A) leaf mottling and chlorosis; (B) fruit blistering and mottling; (C) fruit distortion



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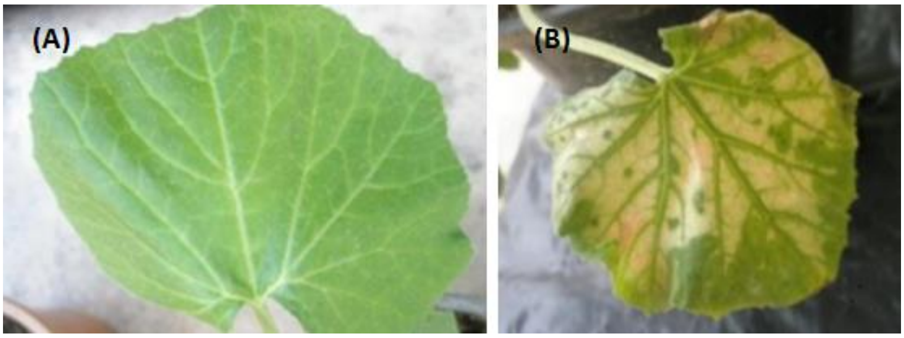
3.3.4 Symptoms caused by CGMMV on other hosts

CGMMV is reported to infect different species of pumpkin, including *Cucurbita maxima, Cucurbita moschata*, *Cucurbita maxima* x *Cucurbita moschata* and *Cucurbita pepo*. CGMMV is reported to cause systemic mosaic symptoms in *Cucurbita moschata* (Zhou et al. 2008). Under experimental conditions, symptomless CGMMV infection of *Cucurbita moschata* has also been reported (Moradi & Jafarpour 2010).

The pumpkin species *Cucurbita maxima* has recently been reported to be a natural host of CGMMV (Stephen West [DPIF] 2015, pers. comm., 9 September). The virus was reported to be associated with *C. maxima* seeds for the first time in December 2015 (Fiona Constable [DEDJTR] 2015, pers. comm., 2 December). The symptoms of this virus in *C. maxima* are not reported.

In *Cucurbita pepo* (pumpkin, squash and zucchini), CGMMV is reported to cause systemic chlorotic spots, systemic mosaic symptoms, leaf mottle and leaf vein yellowing symptoms (Nontajak et al. 2014; Qin et al. 2005) (Figure 4A and B). The virus has been detected in the seed of symptomless *Cucurbita pepo* fruit (Al-Tamimi, Kawas & Mansour 2009).

Figure 4 CGMMV symptoms in zucchini: (A) mild mosaic leaf symptoms; (B) internal vein yellowing leaf symptoms



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CGMMV is also known to infect different gourd species. In bottle gourd (*Lagenaria siceraria*), CGMMV symptoms vary from leaf mosaic and mottle symptoms (Kim et al. 2010) (Figure 5) to systemic mosaic symptoms (Mandal et al. 2008; Zhang, Li & Li 2009). Symptoms on serpent gourd (*Trichosanthes cucumerina*) include leaf mosaic, leaf mottling, reduced leaf size and phylloid flowers (Nagendran et al. 2015).

Figure 5 CGMMV mottle and mosaic symptoms on a bottle gourd leaf

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4 Pest risk assessment

This risk assessment was initiated to fulfil Australia's obligations under the IPPC and ISPM No. 1 (FAO 2016a) to review the emergency phytosanitary measures that Australia introduced for *Cucumber green mottle mosaic virus* (CGMMV) in October 2014 and amended in December 2015. CGMMV is identified as a quarantine pest for Australia because it:

- has a restricted distribution in Australia and is under official control;
- has the potential for entry, establishment and spread in Australia, has the potential to be introduced into Australia on the seed pathway (Choi 2001; Liu et al. 2014), has established in areas with a wide range of climatic conditions (CABI 2017; Tian et al. 2014), and can spread under natural conditions and by human activities (Kobayashi 1990; Tochihara & Komuro 1974); and
- is an economically important pathogen (Nilsson 1977; Reingold et al. 2015).

4.1 *Cucumber green mottle mosaic virus* (CGMMV)

CGMMV is associated with several species of cultivated cucurbit crops (Hollings, Komuro & Tochihara 1975; Lecoq & Desbiez 2012). However, it is only reported to be seed-borne in *Citrullus lanatus* (Lee et al. 1990; Yoon et al. 2008), *Cucumis melo* (Reingold et al. 2015; Tian et al. 2014), *Cucumis sativus* (Faris-Mukhayyish & Makkouk 1983; Liu et al. 2014), *Cucurbita maxima, Cucurbita maxima* x *Cucurbita moschata, Cucurbita moschata* (Fiona Constable [DEDJTR] 2015, pers. comm., 2 December), *Cucurbita pepo* (Al-Tamimi, Kawas & Mansour 2009; Kwon et al. 2014), *Lagenaria siceraria* (Boubourakas et al. 2004; Choi 2001) and *Trichosanthes cucumerina* (Ariyaratne, Weeraratne & Ranatunge 2005). Seeds of these species and their hybrids therefore constitute a potential pathway for the introduction of CGMMV into Australia.

4.1.1 Likelihood of entry

The likelihood of entry is divided for assessment purposes into the likelihood of importation (the likelihood that CGMMV will be imported when host seed is imported) and the likelihood of distribution (the likelihood that CGMMV will be transferred to a suitable host).

Likelihood of importation

The likelihood that CGMMV will arrive in Australia with host cucurbit seeds intended for sowing is assessed as HIGH.

This assessment reflects the fact that CGMMV is seed-borne, and can be expected to remain viable during transport and storage. In addition, Australia imports a large volume of cucurbit seeds for sowing each year for planting.

Association of the pest with the pathway

It is highly likely that CGMMV is associated with the pathway.

- CGMMV is known to be seed-borne in *Citrullus lanatus* (Lee et al. 1990; Yoon et al. 2008), *Cucumis melo* (Reingold et al. 2015; Tian et al. 2014), *Cucumis sativus* (Faris-Mukhayyish & Makkouk 1983; Liu et al. 2014), *Cucurbita maxima, Cucurbita moschata* (Fiona Constable [DEDJTR] 2015, pers. comm., 2 December), *Cucurbita pepo* (Al-Tamimi, Kawas & Mansour

2009; Kwon et al. 2014), *Lagenaria siceraria* (Boubourakas et al. 2004; Choi 2001) and *Trichosanthes cucumerina* (Ariyaratne, Weeraratne & Ranatunge 2005).

- CGMMV also has been found to be associated with seeds of the hybrid *Cucurbita maxima* x *Cucurbita moschata* (Fiona Constable [DEDJTR] 2015, pers. comm., 2 December), and therefore has the potential to be associated with seeds of hybrids of other seed-borne hosts of CGMMV.
- The association of CGMMV with cucurbit seeds is reported to be mainly external to the germplasm (Lecoq & Desbiez 2012). The highly stable nature of the virus helps it remain infectious in dried seed coats, from which it can subsequently infect plantlets during germination (Lecoq & Katis 2014). CGMMV is, however, also found in the inner tissue of infected seeds (Reingold et al. 2015).
- Global trade in seeds and the associated movement of seeds across borders is likely to have played a significant role in the introduction of CGMMV into geographically distant areas.
  - CGMMV was first reported in the 1930s in the United Kingdom (UK) (Ainsworth 1935; Ugaki et al. 1991) and has since been found to infect cucurbit crops worldwide (Reingold et al. 2015).
  - CGMMV was presumed to have been introduced by infected bottle gourd (*Lagenaria siceraria*) seeds into Japan in 1966 (Kobayashi 1990; Tochihara & Komuro 1974).
  - An outbreak of CGMMV in the watermelon growing areas of Korea was considered to be caused by the introduction of contaminated bottle gourd seeds from China (Choi 2001).

Ability of the pest to survive transport and storage

It is highly likely that CGMMV will survive storage and transport.

- CGMMV is highly likely to survive during transport and storage since the primary conditions for its survival are fulfilled by the presence of viable seeds and maintenance of environmental conditions conducive to seed survival. Host cucurbit seeds are also packaged and shipped to areas conducive to their survival. The handling of the seed is highly unlikely to be detrimental to the survival of CGMMV.
- CGMMV can survive in an infective state on the surface of seeds for months to a few years without invading the inner tissues of the seeds (Reingold et al. 2015). In addition to association with the seed coat, CGMMV has been detected in the inner tissue of CGMMV-infected seeds (Reingold et al. 2015) which may further assist the survival of the virus during transport and storage.
- Transport and storage of seeds is done at low temperatures, and these conditions are not expected to affect the viability of CGMMV. Hollings et al. (1975) reported that CGMMV is very stable and, when stored at 2 °C, can retain nearly all serological activity for at least six years.

Ability of the pest to survive existing pest management procedures

It is highly likely that CGMMV will survive existing pest management procedures.

- There are a few effective control strategies for the protection of cucurbit crops against natural infection by CGMMV. Cultural practices, including the use of CGMMV-free seeds, removal and destruction of all infected host material, disinfection of equipment, tools and clothes, and strict control on the movement of potentially infected material from affected farms are critical factors in managing CGMMV.
- Currently, most commercial seed lots are subject to dry heat or chemical disinfection treatments prior to marketing (Lecoq & Desbiez 2012). However, there is conflicting

evidence on the effectiveness of heat and chemical treatments in eliminating CGMMV from host seeds.

- Some reports indicate that the virus can be completely eliminated using dry heat treatment. For example, Lecoq & Desbiez (2012) and Lecoq & Katis (2014) reported that CGMMV could be eliminated from seed treated at 70 °C for 24 to 72 hours.
- Other studies have found that heat and chemical treatments are ineffective in eliminating the virus from seeds. For example, Reingold et al. (2015) found that heat treatment of seeds at 72 °C for 72 hours did not eliminate CGMMV from seeds, which had an infection rate of 66.6 per cent after treatment. Additionally, Reingold et al. (2015) found that all infected seeds treated with 10 per cent trisodium phosphate (TSP) still contained the entire viral genome after treatment, and had an infection rate of 83.3 per cent after treatment. While the combination of treating seeds with 10 per cent TSP and heat treatment was more effective in disinfesting CGMMV from seeds than either individual treatment, the infection rate was still 31.5 per cent after both treatments (Reingold et al. 2015).

Likelihood of distribution

The likelihood that CGMMV will be distributed within Australia in a viable state with imported cucurbit host seeds intended for sowing and be transferred to a suitable host is assessed as HIGH.

This assessment reflects the facts that cucurbit seeds for sowing are commercially distributed throughout Australia, and seed to seedling transmission has been demonstrated for CGMMV in multiple hosts. CGMMV is very stable and can be transmitted mechanically. This transmission mechanism provides a means by which the virus can infect a new host plant; in many instances the germinating seedling is the new host.

Distribution of the imported commodity in the PRA area

It is highly likely that imported cucurbit host seeds and resultant seedlings will be distributed across Australia.

- Association of the virus with cucurbit seeds provides the opportunity for CGMMV to be widely distributed through the trade of seeds across Australia.
- CGMMV is likely to survive on and infect cucurbit propagative material which will be transported, stored and maintained under conditions that are unlikely to have an adverse impact on the virus. Asymptomatic infected plants that develop from infected seeds may be overlooked and sold to commercial producers and households.

Ability of the pest to move from the pathway to a suitable host

It is highly likely that CGMMV will be transferred to a suitable host.

- CGMMV is seed-borne and seed transmissible in some cucurbit hosts (Choi 2001; Lee et al. 1990; Liu et al. 2014). Therefore, CGMMV arriving in Australia with imported infected cucurbit seeds for sowing is already associated with a suitable host. Consequently, there is no need for CGMMV to move from the seed pathway to another host.
- Cucurbit seeds are imported specifically for the purpose of propagation and infected cucurbit seeds are therefore likely to be sown in suitable habitats at multiple locations throughout Australia. The distribution of infected cucurbit seeds commercially will facilitate the distribution of CGMMV.

- If an infected seed results in an infected seedling, CGMMV is likely to transfer to other hosts by mechanical transmission. CGMMV is very stable and can survive for long periods on equipment and on tools (ASTA 2014). The virus may be transferred to other plants through standard cultivation practices and harvesting (Lecoq & Desbiez 2012), when plants are handled by farm personnel, and when infected equipment or machinery is used (Boubourakas et al. 2004; Reingold et al. 2015).
- Cucurbit seeds for commercial production are likely to be propagated in large numbers in the field or glasshouse, therefore, multiple hosts are likely to be available in close proximity to the infected plant. Consequently, CGMMV could transfer through leaf contact between healthy and infected plants (Lecoq & Desbiez 2012).
- CGMMV can survive for long periods on plant debris, contact with which can assist its spread. CGMMV may spread over short distances through contact via roots in soil contaminated by infected plant debris (Hollings, Komuro & Tochihara 1975). For example, 18 per cent infection with CGMMV was reported in *Lagenaria siceraria* plants sown in soil mixed with infected plant debris (Rao & Varma 1984).
- CGMMV has been found in irrigation water, contact with which can lead to plant infection. Spread of CGMMV through surface water and water for irrigation has been reported (Dorst 1988; Vani & Varma 1993). CGMMV is also reported to be able to be spread to new host plants through the recirculation of nutrient solutions (Büttner, Marquardt & Schickedanz 1995).
- Insect vectors may help transfer CGMMV from infected to healthy plants. Hollings et al. (1975) were unable to demonstrate transmission of CGMMV by *Myzus persicae*, *Aphis gossypii* and *Aulacophora femoralis*. However, a later study demonstrated CGMMV transmission by *A. femoralis* (Rao & Varma 1984). While *A. femoralis* is not present in Australia, it is not known if related species that are present in Australia (for example, *A. abdominalis* and *A. hilaris*) might play a role in transferring CGMMV to healthy host plants.
- Suitable hosts for CGMMV are widely distributed across Australia. CGMMV mainly infects plants within the Cucurbitaceae family (Hollings, Komuro & Tochihara 1975), including crops such as cucumber, gourd, loofah, melon, pumpkin, squash, watermelon and zucchini (Liu et al. 2014; Qin et al. 2005; Reingold et al. 2015). These species are widely distributed throughout Australia, with many commercial farms, semi-rural properties and residential properties in metropolitan areas growing these vegetables.
- A growing number of weeds have been reported as natural hosts of CGMMV, including species within the families Amaranthaceae, Apiaceae, Boraginaceae, Chenopodiaceae, Cucurbitaceae, Portulacaceae and Solanaceae (Antignus et al. 1990; Boubourakas et al. 2004; Cho, Kim & Jeon 2015). Many of these weeds are widely distributed throughout Australia.

Overall likelihood of entry

The overall likelihood of entry of CGMMV is determined by combining the likelihood of importation with the likelihood of distribution using the matrix of rules for combining qualitative likelihoods (Table 2).

The likelihood that CGMMV will enter Australia with imported seed intended for sowing and transfer to a suitable host is assessed as HIGH.

4.1.2 Likelihood of establishment

The likelihood that CGMMV, having entered on imported seeds for sowing, will establish within Australia, based on a comparison of factors in the source and destination areas considered pertinent to its survival and reproduction, is assessed as HIGH.

This assessment reflects the extensive planting of cucurbit crops in Australia, the deliberate introduction and establishment of plants grown from imported seeds, the transmission of CGMMV from infected seeds to seedlings, wide distribution of suitable hosts, and broad availability of suitable climates in Australia.

Availability of suitable hosts, alternative hosts and vectors in the PRA area

It is highly likely that CGMMV will establish in Australia because of broad availability of host plant species.

- CGMMV is seed-borne, seed-transmissible (Choi 2001; Liu et al. 2014) and infects host plants systemically (Moreno, Thompson & Garcia-Arenal 2004). Therefore, the introduction and establishment of plants from imported host cucurbit seeds promotes the establishment of CGMMV with the propagative material.
- Host plant seeds are likely to be maintained in places with similar climates to the area of production. Climatic conditions are expected to favour the establishment of the pathogen.
- Seeds are intended for propagation and are deliberately introduced, distributed and assisted to establish. Seeds will be sown and the resultant seedlings will be maintained in a suitable habitat, potentially in substantial numbers and for an indeterminate period of time. Therefore, the introduction and establishment of plants from imported seeds in essence establishes CGMMV associated with the propagative material.
- Natural hosts of CGMMV are widely cultivated throughout Australia, with many commercial, semi-rural and residential properties in metropolitan areas growing host crops. Therefore, the ready availability of host material throughout Australia will help establishment of CGMMV.
- Alternative hosts include weed species (Boubourakas et al. 2004), some of which are widely distributed in Australia. Contact between infected cucurbits and weed species will help transfer CGMMV from its cucurbit crop host to an alternative host. Consequently, weed hosts may help to establish CGMMV.
- A latent period of CGMMV infection before visible symptoms appear (up to 14 days) has been reported in cucumber (Smith 1972) and may hinder non-detection of the virus. Asymptomatic leaves and symptomless fruits are also often observed in CGMMV-infected cucurbit hosts (Hollings, Komuro & Tochihara 1975; Liu et al. 2014). Therefore, CGMMV may have ample time to establish in new areas before being detected.
- CGMMV is effectively transmitted by mechanical means such as hands, clothing and tools (Broadbent & Fletcher 1963; Reingold et al. 2015). The virus is very stable and can survive for long periods on equipment surfaces and tools (ASTA 2014). Therefore, it is highly likely that mechanical transmission will spread the virus from infected plants to healthy host plants.
- Vectors can play an important role in the transmission of viruses to new host plants. However, CGMMV, like other mechanically- or sap-transmitted viruses, is capable of moving to new host plants through direct contact. Therefore, the virus has the potential to establish and spread without vectors.

- Establishment of CGMMV could be aided by the presence of insect vectors in the PRA area. The cucumber leaf beetle (*Aulacophora femoralis*) has been shown to transmit CGMMV, and infective virus particles were detected in regurgitated fluid and excreta (Rao & Varma 1984). While *A. femoralis* is not present in Australia, it is not known if related species that are present in Australia (for example, *A. abdominalis* and *A. hilaris*) might play a role in transferring CGMMV to healthy host plants.

Suitability of the environment

It is highly likely that climatic conditions in Australia will be suitable for establishment of CGMMV.

- CGMMV has established in areas with a wide range of climatic conditions (Map 3). The current reported distribution of CGMMV indicates that climatic conditions would be suitable for its establishment in outdoor crops in parts of Australia.
- The origin of CGMMV is not known and is difficult to determine; however, this virus was first reported from the UK (Ainsworth 1935). Subsequently, CGMMV has been reported to occur in most continents and over 30 countries, including but not limited to China, Finland, Germany, Greece, India, Israel, Japan, Korea, Pakistan, Saudi Arabia and Spain (Francki, Hu & Palukaitis 1986; Komuro et al. 1968; Lee et al. 1990; Lee 1996; Shang et al. 2011). The climatic regions across its known range are diverse and there are similar climatic regions in parts of Australia that would be suitable for the establishment of CGMMV.
- In September 2014, CGMMV was confirmed to occur in commercial field-grown watermelon farms near Katherine and Darwin, in the Northern Territory, Australia, demonstrating that parts of Australia have suitable environmental conditions for the survival of this virus. Subsequent outbreaks elsewhere in Australia further support this conclusion.
- Intensive cultivation, high plant density and frequent crop handling are likely to facilitate the spread of mechanically transmitted tobamoviruses, including CGMMV, in greenhouse-grown host crops.

The reproductive strategy and survival of the pest

CGMMV has a suitable reproductive strategy for establishment in Australia.

- Viruses in general are obligate parasites and reproduce within host cells (Persley, Cooke & House 2010). CGMMV multiplies and survives in the tissues of infected host plants (Moreno, Thompson & Garcia-Arenal 2004), however, it is not reliant on a living host for survival.
- CGMMV is able to survive long periods in plant debris and soil without a living host (Reingold et al. 2015). Transmission of the virus via roots in soil contaminated with infected plant debris has been reported (Hollings, Komuro & Tochihara 1975; Rao & Varma 1984).
- Generally, plant viruses rapidly lose their infectivity in seed coats during the process of seed drying, however, CGMMV is very stable and can remain infectious in dried seed coats for a long period of time (Lecoq & Katis 2014). CGMMV can survive in an infective state on the surface of seeds for months to a few years without invading the inner tissues of the seeds (Reingold et al. 2015).
- In addition to association with the seed coat, CGMMV has been detected in the inner tissue (endopleura) of CGMMV-infected seeds (Reingold et al. 2015). Infection of the inner tissues of seeds may assist in the survival and establishment of this virus.
- New cucurbit seeds produced by the progeny of infected imported cucurbit seeds will assist in the continued survival and establishment of CGMMV.

4.1.3 Likelihood of spread

The likelihood that CGMMV, having entered with seeds for sowing and established, will spread in Australia, based on a comparison of factors in the source and destination areas considered pertinent to the expansion of the geographic distribution of the pest is assessed as MODERATE.

This is because CGMMV is very stable and can be transmitted in infected plants mechanically, via infected tools and machinery which come in contact with the virus. Long distance dispersal of this virus could also occur through the movement of infected propagative material.

The suitability of the natural or managed environment for natural spread

The environment in Australia is suitable for natural spread of CGMMV.

- CGMMV was first reported in the UK in the 1930s (Ainsworth 1935; Ugaki et al. 1991) and since then it has been recorded in many countries, including but not limited to China, Finland, Germany, Greece, India, Israel, Japan, Korea, Pakistan, Saudi Arabia and Spain (Komuro et al. 1968; Lee et al. 1990; Lee 1996; Shang et al. 2011). The climatic regions across this range are diverse, and there are similar climatic regions in parts of Australia that would be suitable for the establishment and spread of CGMMV.
- In September 2014, CGMMV was confirmed to occur in commercial watermelon farms near Katherine and Darwin, in the Northern Territory, Australia, demonstrating that parts of Australia have suitable environmental conditions for the survival of this virus.
- The managed environment in cucurbit farms, nurseries, propagation houses and private gardens are all favourable for the natural spread of CGMMV, as host plants are abundantly available. Plants are often closely placed, which will favour the natural spread of CGMMV through plant to plant contact and mechanical transmission.
- Intensive cultivation, high plant density and frequent crop handling may facilitate the spread of CGMMV in greenhouse-grown cucurbit crops. Therefore, greenhouse-grown cucurbit crops may be highly vulnerable to this mechanically transmitted virus.
- CGMMV is very stable and can survive for long periods on equipment and on tools (ASTA 2014). The virus can be spread through standard cultivation practices and harvesting (Lecoq & Desbiez 2012), when plants are handled by farm personnel, and when infected equipment or machinery is used (Boubourakas et al. 2004; Reingold et al. 2015). The observed pattern of spread in cultivated crops is commonly along a planting row, which indicates that mechanical transmission plays a major role in the spread of CGMMV to new hosts once the virus has been introduced into an area. On this basis it is apparent that, crop management practices can assist CGMMV spread.
- CGMMV can survive for long periods of time on plant debris. CGMMV may spread locally through contact via roots in soil contaminated by infected plant debris (Hollings, Komuro & Tochihara 1975).
- CGMMV is seed-borne and seed-transmissible (Choi 2001; Liu et al. 2014). This increases its dispersal potential over time and distance, and is an important aspect of its epidemiology (Liu et al. 2014).
- CGMMV can spread through contaminated irrigation water or nutrient solutions (Dorst 1988; Lecoq & Desbiez 2012) in natural and managed environments.

Presence of natural barriers

The presence of natural barriers in Australia is unlikely to have any influence on the spread of CGMMV.

- Cucurbit crops are grown across Australia, with long distances often separating commercial production fields. This would be likely to aid in the containment of CGMMV if establishment were to occur in an isolated cucurbit growing region.
- While pollen transmission may play a role in the spread of CGMMV (Liu et al. 2014), there is no confirmed report that CGMMV can spread naturally via pollen. Under experimental conditions, artificial pollination of cucumber flowers with pollen from CGMMV infected plants resulted in up to 51.2 per cent fruit infection. Seed from infected fruit were also positive for the virus (Liu et al. 2014). Therefore, pollen may play a role in spreading CGMMV to other areas.
- CGMMV has spread from its initial detection in the UK (Ainsworth 1935; Ugaki et al. 1991) to Asia, Europe, the Mediterranean region and North America (CABI 2017; Ling, Li & Zhang 2014; Tian et al. 2014). This suggests that the virus is capable of overcoming natural barriers through means of dispersal such as human-assisted transport of infected seeds.
- It is not known if insect vectors could assist the distribution of CGMMV across Australia. Cucumber leaf beetles (*Aulacophora femoralis*) are probable vectors of CGMMV, but are not recorded in Australia; however, two species of *Aulacophora* (*A. abdominalis* and *A. hilaris*) have been recorded feeding on commercial cucurbit crops in Australia (Napier 2009). Therefore, these beetles may be vectors of CGMMV that might assist its spread.

Potential for movement with commodities or conveyances

There is a potential for domestic trade to facilitate the spread of CGMMV.

- Spread of CGMMV via human-assisted means can be rapid and significant through the commercial movement of infected seeds or seedlings for planting (Kobayashi 1990; Lecoq & Katis 2014; Liu et al. 2014). Other possible pathways for spread through human-assisted means include the movement of contaminated soil and debris (Lecoq & Katis 2014).
- As visual symptoms of CGMMV may not be present and in the absence of specific testing regimes, infected propagative material could easily be moved into new areas. The introduction of infected planting material establishes the pathogen in new areas and unregulated movement will accelerate the spread of CGMMV.
- Increased trade of seed within and between countries has led to new opportunities for plant pathogens to be moved to new areas or countries (Dehnen-Schmutz et al. 2010; Stenlid et al. 2011; Wingfield, Slippers & Wingfield 2010). Since it was first reported in the 1930s from the UK, CGMMV has spread to many countries, potentially through the cucurbit seed trade*.* CGMMV is thought to have been introduced to Japan through *Lagenaria siceraria* seeds imported from India (Kobayashi 1990; Tochihara & Komuro 1974).

4.1.4 Overall likelihood of entry, establishment and spread

The overall likelihood of entry, establishment and spread is determined by combining the likelihoods of entry, establishment and spread using the 'matrix of rules' for combining qualitative likelihoods shown in Table 2.

The overall likelihood that CGMMV will enter Australia on host cucurbit seeds, be distributed in a viable state to susceptible hosts, establish in that area and subsequently spread within Australia is assessed as MODERATE.

4.1.5 Consequences

The potential consequences of the introduction and spread of CGMMV in Australia have been estimated according to the methods described in Table 3. In assessing the potential impact of CGMMV in Australia, the economic losses caused by this pathogen overseas were considered. Reasoning for these ratings is provided below:

Criterion	Estimate and rationale
Direct	
Plant life or health	E—Major significance at the district level The direct impact of CGMMV on plant life or health would be of major significance at the district level, significant at the regional level, and of minor significance at the national level, which has an impact score of 'E'. This is because the impact would be expected to threaten economic viability through a large decrease in production of infected crops at the district level of a state or territory. CGMMV has a significant effect on the health, life and yield of economically important cucurbit species such as cucumber, melon, pumpkin, watermelon, and zucchini (Liu et al. 2014; Reingold et al. 2015; Vani & Varma 1993). A variety of CGMMV symptoms are described in the literature, including light-yellow to green leaf spots, vein clearing, deformation of young leaves, leaf-bleaching, chlorotic mottling, stunted growth, necrosis, fruit mottling and fruit distortion (AMA 2015; ASTA 2014; Boubourakas et al. 2004; Hollings, Komuro & Tochihara 1975; Ling, Li & Zhang 2014; Reingold et al. 2015). CGMMV is generally reported to cause yield losses of 15 per cent in cucurbit crops (Shang et al. 2011), however, significantly higher yield losses are reported in some crops (Zhou et al. 2008). • In cucumber, CGMMV infection is reported to cause 15 per cent yield reduction (Reingold et al. 2015). Nilsson (1977) reported a crop loss of up to 33 per cent of first grade cucumber fruits. Fruit quality can also be severely affected and the associated financial loss has been calculated to be in the range of 2 to 24 per cent (Nilsson 1977; Reingold et al. 2015). • Watermelon yield losses of up to 48 per cent have been reported (Zhou et al. 2008). Watermelon fruit symptoms can occur simultaneously with necrotic spots on the peduncle, but obvious symptoms are often only revealed after harvest (Reingold et al. 2013). Symptoms on watermelon fruit include exocarp deterioration and mesocarp rotting and yellowing, while the inner pulp can be water soaked and without dietary value (Reingold et al. 2013; Zhou et al. 2008). This renders the fruit unmarketable and can cause severe economic losses to growers (Reingold et al. 2013).
Other aspects of the environment	B—Minor significance at the local level The direct impact of CGMMV on the environment would be of minor significance at the local level, and indiscernible at the district, regional and national levels, which has an impact score of 'B'. There may be some impact on insect or vertebrate animal species that feed on infected host plants due to the reduced availability or vigour of these host plants.
Indirect	
Eradication, control, etc.	E—Major significance at the district level The indirect impact of CGMMV on eradication and control would be of major significance at the district level, significant at the regional level, and of minor significance at the national level, which has an impact score of 'E'. This is because the impact would be expected to threaten economic viability through a large increase in costs for containment, eradication and control at a district level. Containment and eradication processes are costly, and would also cause disruption to Australia's agribusiness and associated trades at the district level. The ability of CGMMV to survive for long periods in plant debris and soil without a living host (Reingold et al. 2015), combined with its potential human-assisted spread (trade in propagative material), is likely to make this a difficult pathogen to eradicate. • There are no effective agri-chemicals available for the control of CGMMV in host crops. Once the virus is established, CGMMV is difficult to eradicate as it can survive for long

Criterion	Estimate and rationale
	periods on plant debris in the soil. It can also survive on alternate hosts, including various cucurbit and non-cucurbit weeds. • Removal and destruction of all infected hosts may help in the management of the disease. However, this is likely to be labour intensive and significantly increase production costs. Furthermore, CGMMV can survive in soil and debris, so the removal of host plants may not eliminate CGMMV from the growing area. • CGMMV can spread through mechanical transmission (Lecoq & Desbiez 2012). Therefore, strict controls for the movement of potentially contaminated equipment and people from affected farms, and increased use of labour and consumables for washing and disinfection, are likely to be required to manage the spread of the virus, which would increase production costs.
Domestic trade	D—Significant at the district level • The indirect impact of CGMMV on domestic trade would be of major significance at the local level, significant at the district level, and of minor significance at the regional level, which has an impact score of ‘D’. This is because the impact would be expected to threaten economic viability through a reduction of trade or loss of domestic markets at the district level. Biosecurity measures would be enforced to prevent the movement of plant material out of the incursion area, which would have significant economic impact on plant industries and businesses at the district level. The presence of CGMMV is likely to result in domestic movement restrictions for cucurbit propagative material. Interstate restrictions on seeds, nursery stock and other plant products may lead to a loss of markets, which would be likely to require industry adjustment.
International trade	D—Significant at the district level • The indirect impact of CGMMV on international trade would be of major significance at the local level, significant at the district level, and of minor significance at the regional level, which has an impact score of ‘D’. This is because the impact would be expected to threaten economic viability through loss of trade and export markets at the district level. Several countries have established quarantine policies and protocols against plant material from areas known to have CGMMV. The establishment of CGMMV in Australia may therefore reduce access to international markets and result in additional requirements to achieve phytosanitary conformity, which will impose a cost burden.
Environmental and non-commercial	B—Minor significance at the local level. • The indirect impact of CGMMV on the environment would be of minor significance at the local level, and indiscernible at the district, regional and national levels, which has an impact score of ‘B’. This is because the introduction of CGMMV may result in increased use of consumables for washing and disinfection of infected equipment, tools and machinery to manage the spread of the virus, which may have some negative impacts on the environment. • The potential use of herbicides to control alternate weed hosts may have a negative impact on native plants. Any broad-scale chemical treatments directed against a possible insect vector may have some negative impacts on native insects.

Based on the decision rules described in Table 4, that is, where the potential consequences of a pest with respect to one or more criteria have an impact of ‘E’, the overall consequences are estimated to be MODERATE.

4.1.6 Unrestricted risk estimate

Unrestricted risk is the result of combining the likelihoods of entry, establishment and spread with the outcome of overall consequences. Likelihood and consequences are combined using the risk estimation matrix (Table 5) and results are shown in Table 7.

Table 7 Unrestricted risk estimates of CGMMV for seed pathway

Overall likelihood of entry, establishment and spread	MODERATE
Consequences	MODERATE
Unrestricted risk	MODERATE

The unrestricted risk estimate for CGMMV has been assessed as Moderate, which does not achieve the ALOP for Australia. Therefore, risk management measures against CGMMV are required to achieve the ALOP for Australia.

5 Pest risk management

Cucumber green mottle mosaic virus (CGMMV) presents an unrestricted risk that does not achieve the appropriate level of protection (ALOP) for Australia. Therefore, risk management measures are required to reduce the estimated risk to a level of very low, and to achieve the ALOP for Australia. The recommended risk management measures are described in this chapter.

5.1 Recommended risk management measures

Australia considers that the emergency measures introduced in October 2014, and amended in December 2015, are adequate to mitigate the risk posed by CGMMV associated with seeds of the Cucurbitaceae family. Therefore, the emergency measures, with some minor amendments, are recommended to become the standard conditions for the importation of cucurbit seeds for sowing associated with CGMMV. The recommended minor changes to the emergency measures include an option for small seed lots to be tested off-shore, and acceptance of a Polymerase Chain Reaction (PCR) testing method approved by the Australian Department of Agriculture and Water Resources (the department).

NPPOs that propose to use PCR testing to verify freedom from CGMMV must provide the department with an appropriate submission describing the details of the PCR testing method, for its consideration.

The recommended import conditions for seeds of cucurbit hosts in which CGMMV is known to be seed-borne (*Cucumis sativus*, *Cucumis melo*, *Cucurbita maxima*, *Cucurbita moschata*, *Cucurbita pepo*, *Citrullus lanatus*, *Lagenaria siceraria*, *Trichosanthes cucumerina* and all hybrids of these species) are provided below.

5.1.1 Import conditions

- **Testing**—mandatory off-shore or on-shore testing by International Seed Testing Association (ISTA) validated Enzyme-Linked Immunosorbent Assay (ELISA) or a PCR method approved by the department on a sample size of 9,400 seeds (or 20 per cent for small seed lots) to verify freedom from CGMMV; AND
- **Certification**—seed lots tested off-shore must be accompanied by an official government Phytosanitary Certificate endorsed with the following additional declaration:
    'The consignment of [*botanical name* (*Genus species*)] was tested by ELISA [*insert laboratory name and report number*] on a sample size of 9,400 seeds (or 20 per cent of small seed lots) and found free from CGMMV'; OR
    'The consignment of [botanical name (Genus species)] was tested by PCR [insert laboratory name and report number] on a sample size of 9,400 seeds (or 20 per cent of small seed lots) and found free from CGMMV'; AND
- **On-arrival inspection**—seed lots must be inspected on arrival to verify freedom from live insects, soil, disease symptoms, prohibited seeds, other plant material (for example, leaf, stem material, fruit pulp and pod material), animal material (for example, animal faeces and feathers) and any other extraneous contamination of quarantine concern.

For the purposes of these import conditions, small seed lots of specified cucurbit species are defined by seed lot weight (Table 8). The recommended risk management measures for the importation of seeds subject to these import conditions are summarised in Table 9.

Table 8 Small seed lot weights for specified cucurbit seeds

Scientific name	Common name	Seed lot weight
Cucumis sativus	Cucumber	2 kg or less
Cucumis melo	Melon (Rockmelon & Honeydew)	2 kg or less
Citrullus lanatus	Watermelon	3 kg or less
Cucurbita maxima	Pumpkin	10 kg or less
Cucurbita moschata	Pumpkin	10 kg or less
Cucurbita pepo	Zucchini, Squash, Pumpkin	10 kg or less
Lagenaria siceraria	Bottle gourd	5 kg or less
Trichosanthes cucumerina	Serpent gourd	5 kg or less

Table 9 Risk management measures for the importation of specified cucurbit seeds for sowing

Recommended risk mitigation measures	Cucurbit seeds for sowing
Pre-border	
Off-shore testing	Yes* (ELISA or PCR)
Phytosanitary Certification	Yes* (if testing conducted off-shore)
Border	
On-arrival inspection	Yes
On-shore testing	Yes* (if seeds are not tested off-shore)

* Cucurbit seeds for sowing are subject to mandatory off-shore or on-shore testing. A Phytosanitary Certificate is only required for consignments that have been subject to off-shore testing.

5.2 Consideration of alternative measures

Consistent with the principle of equivalence detailed in ISPM 1 (FAO 2016a), the Australian Government Department of Agriculture and Water Resources will consider any alternative measure proposed by a NPPO, providing that it manages CGMMV to achieve the ALOP for Australia. Evaluation of such measures or treatments will require a technical submission from the NPPO that details the proposed treatment, including data from suitable treatment trials to demonstrate efficacy.

5.2.1 Sourcing seeds from pest free areas (country freedom)

The establishment and use of a pest free area (PFA) by a NPPO provides assurance that specific pests are not present in a delimited geographic area. The delimitation of a PFA should be relevant to the biology of the pest concerned.

The requirements for establishing PFAs are set out in ISPM 4 (FAO 2016c). This ISPM identifies a PFA as being 'an area in which a specific pest does not occur as demonstrated by scientific evidence and in which, where appropriate, this condition is being officially maintained'. A PFA may concern all or part of several countries and it is managed by the NPPO of the exporting country. The establishment and use of a PFA by a NPPO allows for the export of plants and other regulated articles from the exporting country to the importing country without the need for the application of additional phytosanitary measures, when certain requirements are met.

Requirements for a NPPO to establish and maintain a PFA include:

- systems to establish freedom (general surveillance, specific surveys);
- phytosanitary measures to maintain freedom (regulatory actions, routine monitoring, extension advice to producers); and
- checks to verify freedom has been maintained.

NPPOs that propose to use area freedom as a measure for managing risks posed by CGMMV must provide the Department of Agriculture and Water Resources with an appropriate submission demonstrating area freedom for its consideration.

5.2.2 Sourcing seeds from pest-free places of production

Requirements for establishing pest-free places of production are set out in ISPM 10 (FAO 2016e). The concept of 'pest freedom' allows exporting countries to provide assurance to importing countries that plants, plant products and other regulated articles are free from a specific pest or pests and meet the phytosanitary requirements of the importing country. Where a defined portion of a place of production is managed as a separate unit and can be maintained pest free, it may be regarded as a pest-free production site.

Requirements for a NPPO to establish and maintain a pest-free place of production or a pest-free production site as a risk management measure by the NPPO include:

- systems to establish pest freedom;
- systems to maintain pest freedom;
- verification that pest freedom has been attained or maintained; and
- product identity, consignment integrity and phytosanitary security.

Where necessary, a pest-free place of production or a pest-free production site also includes the establishment and maintenance of an appropriate buffer zone.

Administrative activities required to support a pest-free place of production or pest-free production site involve documentation of the system and the maintenance of adequate records concerning the measures taken. Review and audit procedures undertaken by the NPPO are essential to support assurance of pest freedom and for system appraisal. Bilateral agreements or arrangements may also be needed.

NPPOs that propose to use pest-free places of production as a measure to manage the risk posed by CGMMV must provide the Department of Agriculture and Water Resources with an appropriate submission demonstrating pest-free place of production status, for its consideration.

5.2.3 Sourcing seeds produced under a systems approach

ISPM 14 (FAO 2016g) provides guidelines on the use of systems approaches to manage pest risk. According to ISPM 14 (FAO 2016g), 'a systems approach requires the integration of different measures, at least two of which act independently, with a cumulative effect' that achieves the appropriate level of protection.

Systems approaches could provide an alternative to relying on a single measure to achieve the ALOP of an importing country, or be used where no single measure is available. Systems approaches are often tailored to specific commodity–pest–origin combinations and may be developed and implemented collaboratively by exporting and importing countries. The importing country selects the appropriate approach after considering technical requirements, minimal impact, transparency, non-discrimination, equivalence and operational feasibility.

NPPOs that propose to use a systems approach as a measure for managing risks posed by CGMMV must provide the Department of Agriculture and Water Resources with an appropriate submission describing their preferred systems approach and rationale, for its consideration.

5.3 Review of policy

The Department of Agriculture and Water Resources reserves the right to review the import policy when it has reason to believe that the pest or phytosanitary status has changed.

6 Conclusion

The findings of this pest risk analysis are based on a comprehensive analysis of relevant scientific literature on *Cucumber green mottle mosaic virus* (CGMMV). CGMMV meets the International Plant Protection Convention (IPPC) definition of a quarantine pest, that is, being of 'potential economic importance to the area endangered thereby and not yet present there, or present but not widely distributed and being officially controlled' (FAO 2016d). The PRA provides technical justification that the virus meets the IPPC definition of a quarantine pest, and that the introduction of emergency measures was in accordance with international phytosanitary standards.

The Department of Agriculture and Water Resources (the department) considers that the existing emergency measures are adequate to achieve an appropriate level of protection against CGMMV associated with cucurbit seeds for sowing. Therefore, these emergency measures are recommended to become the standard conditions for importation of seeds of cucurbit hosts in which CGMMV is known to be seed-borne into Australia. The recommended minor changes to the emergency measures include an option for small seed lots to be tested off-shore, and acceptance of an alternative Polymerase Chain Reaction (PCR) testing method approved by the department.

Appendix A: Stakeholders comments' on the draft pest risk analysis (PRA)

The Australian Government Department of Agriculture and Water Resources circulated the draft pest risk analysis for *Cucumber green mottle mosaic virus* (CGMMV) in April 2016 for stakeholder consultation (G/SPS/N/AUS/384). The department received several written responses from stakeholders, including industry representatives, trading partners and state and territory governments. A summary of the responses to issues raised by stakeholders is provided below.

Issues raised by stakeholders in response to the draft PRA

Cucumber green mottle mosaic virus (CGMMV) taxonomy and biology

Taxonomic classification of *Kyuri green mottle mosaic virus* (KGMMV)

Stakeholders sought clarification on the taxonomy of KGMMV, particularly regarding whether the Australian Government considers KGMMV as a strain of CGMMV.

The department notes that several closely related species and strains of CGMMV are reported to infect cucurbits globally, which has caused taxonomic confusion. The cucumber strain of CGMMV (CGMMV-C or CGMMV-Cu) which was described in Japan causes severe fruit distortion in cucumber and induces local lesions in *Datura stramonium* (Antignus et al. 2001). This strain was considered by Francki et al. (1986) as a distinct virus and was named KGMMV based on serological and molecular hybridisation analysis. The department recognises KGMMV as a distinct virus species, based on the current acceptance of KGMMV as a distinct species of the genus *Tobamovirus* by the International Committee on Taxonomy of Viruses.

This PRA is limited to CGMMV, therefore all other cucurbit-infecting tobamoviruses, including KGMMV, are out of the scope of this PRA. The department is currently reviewing the import conditions for vegetable seeds, including cucurbit seeds, and all other cucurbit-infecting tobamoviruses of concern will be considered. When finalised, the draft cucurbit seed review will be released for stakeholder consultation. Trading partners will be notified of its release through a World Trade Organization Sanitary and Phytosanitary notification.

Cucurbit hosts in which CGMMV is known to be seed-borne

Stakeholders have sought further clarification on the status of *Cucurbita maxima*, *Cucurbita moschata* and *Cucurbita pepo* as hosts in which CGMMV is seed-borne.

The evidence that CGMMV is seed-borne in *Cucurbita maxima* and *Cucurbita moschata* was a personal communication from the Department of Economic Development, Jobs, Transport and Resources Victoria (DEDJTR). The DEDJTR state laboratory is approved by the Australian Government for testing imported seeds for CGMMV. DEDJTR advised the department that it has detected CGMMV in imported seeds of *Cucurbita maxima*, *Cucurbita maxima* x *Cucurbita moschata* and *Cucurbita moschata* using Australia's approved seed testing method.

With regard to *Cucurbita pepo*, two scientific papers have reported that CGMMV is seed-borne in this host. These papers have been peer-reviewed and published in reputable scientific journals (Al-Tamimi, Kawas & Mansour 2009; Kwon et al. 2014). Based on that evidence, Australia considers *Cucurbita pepo* to be a host in which CGMMV is seed-borne. The department will review the list of hosts in which CGMMV is known to be seed-borne as new scientific information becomes available.

Status of CGMMV in Australia

Stakeholders have questioned the quarantine status of CGMMV in Australia.

In September 2014, the presence of CGMMV was confirmed in the Northern Territory, Australia on commercial watermelon farms and quarantine measures were immediately imposed (Government of Queensland 2017; Northern Territory Goverment 2017). In February 2016, the Northern Territory government formally revoked the CGMMV quarantine zone and implemented new risk management measures to contain the spread of CGMMV. The new measures prohibit the export of seeds, seedlings and bee hives from the Northern Territory without approval from the Northern Territory Chief Inspector of Plant Health, and require growers to have an auditable farm biosecurity plan for the purpose of controlling CGMMV.

In April 2015, CGMMV was detected in a single cucurbit farm in Queensland and the infected property remains under quarantine. In February 2017, CGMMV was further detected on cucumber farms near Bundaberg, in Queensland. Biosecurity Queensland is providing technical advice on biosecurity and control measures to growers, and assisting them to develop and implement plans for eradicating the virus from their production areas, and thereby mitigating and minimising the risk of spreading the virus to the wider state and national industry.

In 2016, CGMMV was detected on cucumber farms near Geraldton (July), Carnarvon (August), Perth (September) and Kununurra (September) in Western Australia. The Western Australian Department of Agriculture and Food is assisting growers to develop biosecurity plans to prevent further spread of CGMMV and to manage the disease on infected properties.

According to ISPM No. 5, a quarantine pest is defined as ‘a pest of potential economic importance to the area endangered thereby and not yet present there, or present but not widely distributed and being officially controlled’ (FAO 2016d). CGMMV has a limited distribution in Australia, and substantial resources are being invested in its eradication, containment and management, as appropriate. Therefore, CGMMV meets the requirements for a pest under official control, and remains a quarantine pest for Australia.

Proposed import conditions

Sample size of large seed lots

Stakeholders have suggested that the sample size of 9,400 seeds required by Australia is high, and that a sample size of 2,000 seeds would be sufficient to detect CGMMV in host cucurbit seeds.

The department acknowledges that the ISTA method uses a sample size of minimum 2,000 seeds and achieves a 95 per cent level of confidence for detecting infected seeds, if the infected seeds make up at least 0.15 per cent of a seed lot. However, departmental specialists have examined the statistical level of confidence for a sample size of 2,000 seeds and found that it reduces appreciably if the level of contamination is much smaller than 0.15 per cent.

The sample size of 9,400 seeds was chosen for several reasons, including empirical findings and statistical and operational reasons, consistent with ISPM No. 31 (FAO 2016h). ISPM No. 31

indicates that statistical estimation most appropriately uses a hypergeometric distribution. Using a hypergeometric distribution and a seed lot size of 100,000 seeds, when 9,400 seeds are tested there is a 99 per cent confidence level for detection of an infection rate of 0.05 per cent (one infected seed in 2,000 seeds). When 2,000 seeds of a 100,000 seed lot are tested, this confidence level drops to 63 per cent for detection of an infection rate of 0.05 per cent. Table 10 illustrates the additional reliability achieved by sampling 9,400 seeds.

Table 10 Effect of sample size on confidence levels for detecting infected seeds

Number of infected seeds in 100,000 seed lot	Confidence level of detecting infection using a 2,000 seed sample	Confidence level of detecting infection using a 9,400 seed sample
150	0.951814	1.0
140	0.941009	0.999999
130	0.927782	0.999997
120	0.911591	0.999993
110	0.891773	0.999981
100	0.867514	0.999949
90	0.837822	0.999862
80	0.801479	0.999629
70	0.756997	0.999005
60	0.702554	0.997328
50	0.635921	0.992824
40	0.554371	0.980735
30	0.454564	0.948281
20	0.332418	0.861172
10	0.182935	0.627384

Tests for other seed-borne pathogens are conducted on samples of between 10,000 and 50,000 seeds to detect low levels of infection. For example, testing samples of 30,000 crucifer seeds is the industry standard for detecting *Xanthomonas campestris* pv. *campestris*, and 10,000 to 20,000 carrot seeds for detecting *Xanthomonas campestris* pv. *carotae* (APHIS 2001; De Boer, Elphinstone & Saddler 2007). Testing samples of 20,000 bean seeds is the standard for detecting *Pseudomonas syringae* pv. *phaseolicola* (Agarwal & Sinclair 1996). A test of 10,000 to 50,000 tomato seeds is used to detect *Clavibacter michiganensis* subsp. *michiganensis* (van Vaerenbergh et al. 2013). A test of 30,000 lettuce seeds is used to detect *Lettuce mosaic virus* (APHIS 2001). Australian laboratories test tomato and capsicum seeds for viroids using samples of 20,000 seeds. Samples of 20,000 seeds are also used by Australian laboratories to detect '*Candidatus* Liberibacter solanacearum' in carrot seeds.

It is expected that some infected seed lots will contain very few infected seeds and consist mostly of healthy seeds. To detect the infected seeds in these lots, substantial seed samples need to be tested (as indicated in table 10). Any infected seed can introduce the pathogen to new areas. Therefore, tests for seed-borne pathogens are frequently conducted on samples of higher numbers.

Furthermore, seed lots with very low levels of infection have been detected on-shore after emergency measures were implemented. It is unlikely that a sample size of 2,000 seeds would have been sufficient to detect these low levels of infection. Therefore, a sample size of 9,400 seeds is considered necessary to reduce the risk of CGMMV to a very low level, as required to achieve the appropriate level of protection (ALOP) for Australia.

Sample size of small seed lots

Stakeholders have suggested that the sample size of 20 per cent for small seed lots is quite large and a five per cent sampling rate is adequate to detect CGMMV in host cucurbit seeds.

The department developed import conditions for small seed lots following consultation with industry stakeholders. If the small seed lots definition is met, the importer has the option to test a sample size of 20 per cent per small seed lot or to test 9,400 seeds per small seed lot. The sample size of 20 per cent is generally calculated by weight, however, it can be calculated from the number of seeds if the lot is very small.

The department acknowledges that lowering the percentage of seed to be tested in small seed lots may enable some seed companies to harmonise their testing requirements and trial more seed varieties in Australia. However, as explained above for large seed lots, tests using a smaller sample size may fail to detect a low level of CGMMV infection and therefore may potentially provide a pathway for the introduction of the virus. Therefore, Australia considers a sample size of 20 per cent for small seed lots is justified.

Glossary

Term or abbreviation	Definition
Additional declaration	A statement that is required by an importing country to be entered on a Phytosanitary Certificate and which provides specific additional information on a consignment in relation to regulated pests (FAO 2016d).
Appropriate level of protection (ALOP)	The level of protection deemed appropriate by the Member establishing a sanitary or phytosanitary measure to protect human, animal or plant life or health within its territory (WTO 1995).
Appropriate level of protection (ALOP) for Australia	The *Biosecurity Act 2015* defines the appropriate level of protection (or ALOP) for Australia as a high level of sanitary and phytosanitary protection aimed at reducing biosecurity risks to very low, but not to zero.
Area	An officially defined country, part of a country or all or parts of several countries (FAO 2016d).
Area of low pest prevalence	An area, whether all of a country, part of a country, or all or parts of several countries, as identified by the competent authorities, in which a specific pest occurs at low levels and which is subject to effective surveillance, control or eradication measures (FAO 2016d).
Biosecurity	The prevention of the entry, establishment or spread of unwanted pests and infectious disease agents to protect human, animal or plant health or life, and the environment.
Biosecurity import risk analysis (BIRA)	The *Biosecurity Act 2015* defines a BIRA as an evaluation of the level of biosecurity risk associated with particular goods, or a particular class of goods, that may be imported, or proposed to be imported, into Australian territory, including, if necessary, the identification of conditions that must be met to manage the level of biosecurity risk associated with the goods, or the class of goods, to a level that achieves the ALOP for Australia. The risk analysis process is regulated under legislation.
Biosecurity risk	The *Biosecurity Act 2015* refers to biosecurity risk as the likelihood of a disease or pest entering, establishing or spreading in Australian territory, and the potential for the disease or pest causing harm to human, animal or plant health, the environment, economic or community activities.
Consignment	A quantity of plants, plant products or other articles being moved from one country to another and covered, when required, by a single phytosanitary certificate. A consignment may be composed of one or more commodities or lots (FAO 2016d).
Control (of a pest)	Suppression, containment or eradication of a pest population (FAO 2016d).
Endangered area	An area where ecological factors favour the establishment of a pest whose presence in the area will result in economically important loss (FAO 2016d).
Entry (of a pest)	Movement of a pest into an area where it is not yet present, or present but not widely distributed and being officially controlled (FAO 2016d).
Equivalence (of phytosanitary terms)	The situation where, for a specified pest, different phytosanitary measures achieve a contracting party's appropriate level of protection (FAO 2016d).
Establishment (of a pest)	Perpetuation, for the foreseeable future, of a pest within an area after entry (FAO 2016d).
Goods	The *Biosecurity Act 2015* defines goods as an animal, a plant (whether moveable or not), a sample or specimen of a disease agent, a pest, mail or any other article, substance or thing (including, but not limited to, any kind of moveable property).
Host	An organism that harbours a parasite, mutual partner, or commensal partner, typically providing nourishment and shelter.

Term or abbreviation	Definition
Host range	Species capable, under natural conditions, of sustaining a specific pest or other organism (FAO 2016d).
Import permit	An official document authorising the importation of a commodity in accordance with specified phytosanitary import requirements (FAO 2016d).
Infection	The internal 'endophytic' colonisation of a plant, or plant organ, and is generally associated with the development of disease symptoms as the integrity of cells and/or biological processes are disrupted.
Infestation (of a commodity)	Presence in a commodity of a living pest of the plant or plant product concerned. Infestation includes infection (FAO 2016d).
Inspection	Official visual examination of plants, plant products or other regulated articles to determine if pests are present or to determine compliance with phytosanitary regulations (FAO 2016d).
Intended use	Declared purpose for which plants, plant products, or other regulated articles are imported, produced or used (FAO 2016d).
Interception (of a pest)	The detection of a pest during inspection or testing of an imported consignment (FAO 2016d).
International Plant Protection Convention (IPPC)	The IPPC is an international plant health agreement, established in 1952, that aims to protect cultivated and wild plants by preventing the introduction and spread of pests. The IPPC provides an international framework for plant protection that includes developing International Standards for Phytosanitary Measures (ISPMs) for safeguarding plant resources.
International Standard for Phytosanitary Measures (ISPM)	An international standard adopted by the Conference of the Food and Agriculture Organization, the Interim Commission on Phytosanitary Measures or the Commission on Phytosanitary Measures, established under the IPCC (FAO 2016d).
Introduction (of a pest)	The entry of a pest resulting in its establishment (FAO 2016d).
Life cycle	Cyclical progression of stages in the growth and development of an organism (plant, animal, or pathogen) that occur between the appearance and reappearance of the same stage of the organism (Shurtleff & Averre 1997).
Lot	A number of units of a single commodity, identifiable by its homogeneity of composition, origin et cetera, forming part of a consignment (FAO 2016d).
National Plant Protection Organization (NPPO)	Official service established by a government to discharge the functions specified by the IPPC (FAO 2016d).
Non-regulated risk analysis	Refers to the process for conducting a risk analysis that is not regulated under legislation (Biosecurity import risk analysis guidelines 2016).
Official control	The active enforcement of mandatory phytosanitary regulations and the application of mandatory phytosanitary procedures with the objective of eradication or containment of quarantine pests or for the management of regulated non-quarantine pests (FAO 2016d).
Pathogen	A biological agent that can cause disease to its host.
Pathway	Any means that allows the entry or spread of a pest (FAO 2016d).
Pest	Any species, strain or biotype of plant, animal or pathogenic agent injurious to plants or plant products (FAO 2016d).
Pest categorisation	The process for determining whether a pest has the characteristics of a quarantine pest or regulated non-quarantine pest (FAO 2016d).
Pest free area (PFA)	An area in which a specific pest does not occur as demonstrated by scientific evidence and in which, where appropriate, this condition is being officially maintained (FAO 2016d).
Pest free place of production	Place of production in which a specific pest does not occur as demonstrated by scientific evidence and in which, where appropriate, this condition is being officially

Term or abbreviation	Definition
	maintained for a defined period (FAO 2016d).
Pest free production site	A defined portion of a place of production in which a specific pest does not occur as demonstrated by scientific evidence and in which, where appropriate, this condition is being officially maintained for a defined period and that is managed as a separate unit in the same way as a pest free place of production (FAO 2016d).
Pest risk analysis (PRA)	The process of evaluating biological or other scientific and economic evidence to determine whether an organism is a pest, whether it should be regulated, and the strength of any phytosanitary measures to be taken against it (FAO 2016d).
Pest risk assessment (for quarantine pests)	Evaluation of the probability of the introduction and spread of a pest and of the magnitude of the associated potential economic consequences (FAO 2016d).
Pest risk assessment (for regulated non-quarantine pests)	Evaluation of the probability that a pest in plants for planting affects the intended use of those plants with an economically unacceptable impact (FAO 2016d).
Pest risk management (for quarantine pests)	Evaluation and selection of options to reduce the risk of introduction and spread of a pest (FAO 2016d).
Pest risk management (for regulated non-quarantine pests)	Evaluation and selection of options to reduce the risk that a pest in plants for planting causes an economically unacceptable impact on the intended use of those plants (FAO 2016d).
Pest status (in an area)	Presence or absence, at the present time, of a pest in an area, including where appropriate its distribution, as officially determined using expert judgement on the basis of current and historical pest records and other information (FAO 2016d).
Phytosanitary Certificate	An official paper document or its official electronic equivalent, consistent with the model of certificates of the IPPC, attesting that a consignment meets phytosanitary import requirements (FAO 2016d).
Phytosanitary certification	Use of phytosanitary procedures leading to the issue of a Phytosanitary Certificate (FAO 2016d).
Phytosanitary measure	Phytosanitary relates to the health of plants. Any legislation, regulation or official procedure having the purpose to prevent the introduction and/or spread of quarantine pests, or to limit the economic impact of regulated non-quarantine pests (FAO 2016d). In this risk analysis the term 'phytosanitary measure' and 'risk management measure' may be used interchangeably.
Phytosanitary procedure	Any official method for implementing phytosanitary measures including the performance of inspections, tests, surveillance or treatments in connection with regulated pests (FAO 2016d).
Phytosanitary regulation	An official rule to prevent the introduction and/or spread of quarantine pests, or to limit the economic impact of regulated non-quarantine pests, including establishment of procedures for phytosanitary certification (FAO 2016d).
PRA area	Area in relation to which a pest risk analysis is conducted (FAO 2016d).
Quarantine	Official confinement of regulated articles for observation and research or for further inspection, testing or treatment (FAO 2016d).
Quarantine pest	A pest of potential economic importance to the area endangered thereby and not yet present there, or present but not widely distributed and being officially controlled (FAO 2016d).
Regulated article	Any plant, plant product, storage place, packaging, conveyance, container, soil and any other organism, object or material capable of harbouring or spreading pests, deemed to require phytosanitary measures, particularly where international transportation is involved (FAO 2016d).
Restricted risk	Restricted risk is the risk estimate when risk management measures are applied.
Risk analysis	Refers to the technical or scientific process for assessing the level of biosecurity risk associated with the goods, or the class of goods, and if necessary, the identification

Term or abbreviation	Definition
	of conditions that must be met to manage the level of biosecurity risk associated with the goods, or class of goods to a level that achieves the ALOP for Australia.
Risk management measure	Are conditions that must be met to manage the level of biosecurity risk associated with the goods or the class of goods, to a level that achieves the ALOP for Australia. In this risk analysis, the term 'risk management measure' and 'phytosanitary measure' may be used interchangeably.
Spread (of a pest)	Expansion of the geographical distribution of a pest within an area (FAO 2016d).
SPS Agreement	WTO Agreement on the Application of Sanitary and Phytosanitary Measures.
Stakeholders	Government agencies, individuals, community or industry groups or organisations, whether in Australia or overseas, including the proponent/applicant for a specific proposal, who have an interest in the policy issues.
Surveillance	An official process which collects and records data on pest occurrence or absence by surveying, monitoring or other procedures (FAO 2016d).
Systems approach(es)	The integration of different risk management measures, at least two of which act independently, and which cumulatively achieve the appropriate level of protection against regulated pests.
Tissue culture	The products of 'an in vitro technique of cultivating (propagating) cells, tissues, or organs in a sterile synthetic medium' (Shurtleff & Averre 1997); comprising plant cells, tissues or organs, sterile synthetic medium, and the vessel in which cells have been propagated.
Treatment	Official procedure for the killing, inactivation or removal of pests, or for rendering pests infertile or for devitalisation (FAO 2016d).
Unrestricted risk	Unrestricted risk estimates apply in the absence of risk management measures.
Vector	An organism that does not cause disease itself, but which causes infection by conveying pathogens from one host to another.
Viable	Alive, able to germinate or capable of growth.

References

Adams, MJ, Antoniw, JF & Kreuze, J 2009, 'Virgaviridae: a new family of rod-shaped plant viruses', *Archive of Virology*, vol. 154, pp. 1967–72.

Agarwal, VK & Sinclair, JB 1996, *Principles of Seed Pathology*, 2 edn, CRC Press, Boca Raton.

Ainsworth, CG 1935, 'Mosaic disease of the cucumber', *Annals of Applied Biology*, vol. 22, pp. 55–67.

Al-Tamimi, N, Kawas, H & Mansour, A 2009, 'Seed transmission viruses in squash seeds (*Cucurbita pepo*) in Southern Syria and Jordan Valley', *Jordan Journal of Agricultural Sciences*, vol. 5, pp. 497–506.

AMA 2015 '*Cucumber green mottle mosaic virus* – CGMMV signs and symptoms', available at melonsaustralia.org.au/news/CGMMV-identification.

Antignus, Y, Pearlsman, M, Ben-Yoseph, R & Cohen, S 1990, 'Occurrence of a variant of *Cucumber green mottle mosaic virus* in Israel', *Phytoparasitica*, vol. 18, no. 1, pp. 50–6.

Antignus, Y, Wang, Y, Pearlsman, M, Lachman, O, Lavi, N & Gal-On, A 2001, 'Biological and molecular characterization of a new cucurbit-infecting Tobamovirus', *Phytopathology*, vol. 91, pp. 565–71.

APHIS 2001 'Seed health testing and phytosanitary field inspection methods reference manual B (RM-B)', National Seed Health System, Animal and Plant Health Inspection Service, USA, available at aphis.usda.gov.

Ariyaratne, I, Weeraratne, WG & Ranatunge, RKR 2005, 'Identification of a new mosaic virus disease of snake gourd in Sri Lanka', *Annals of the Sri Lanka Department of Agriculture*, vol. 7, pp. 13–21.

ASTA 2014 '*Cucumber green mottle mosaic virus* – a seed production and commercial growers guide', available at eurofinsus.com/media/162072/cucumber-green-mottle-mosaic-virus.pdf.

Boubourakas, IN, Hatziloukas, E, Antignus, Y & Katis, NI 2004, 'Etiology of leaf chlorosis and deterioration of the fruit interior of watermelon plants', *Journal of Phytopathology*, vol. 152, pp. 580–8.

Broadbent, L 1976, 'Epidemiology and control of *Tomato mosaic virus*', *Annual Review of Phytopathology*, vol. 14, no. 1, pp. 75–96.

Broadbent, L & Fletcher, JT 1963, 'The epidemiology of tomato mosaic: IV. Persistence of virus on clothing and glasshouse structures', *Annals of Applied Biology*, vol. 52, pp. 233–41.

Büttner, C, Marquardt, K & Schickedanz, F 1995, 'Studies on transmission of *Cucumber mosaic virus* (CMV) and *Cucumber green mottle mosaic virus* (CGMMV) by nutrient solutions of ebb and flow irrigation systems', *Gartenbauwissenschaft*, vol. 60, pp. 109–14.

CABI 2017, 'Crop Protection Compendium', CAB International, Wallingford, UK, available at http://www.cabi.org/cpc/, accessed 2017.

Cho, SY, Kim, YS & Jeon, YH 2015, 'First report of *Cucumber green mottle mosaic virus* infecting *Heracleum moellendorffii* in Korea', *Plant disease*, vol. 99, p. 897.

Choi, GS 2001, 'Occurrence of two *Tobamovirus* diseases in cucurbits and control measures in Korea', *Plant Pathology Journal*, vol. 17, pp. 243–8.

De Boer, SH, Elphinstone, JG & Saddler, GS 2007 *Molecular detection strategies for phytopathogenic bacteria*, Wallingford, CABI, Wallingford.

Dehnen-Schmutz, K, Holdenrieder, O, Jeger, MJ & Pautasso, M 2010, 'Structural change in the international horticultural industry: some implications for plant health', *Scientia Horticulturae*, vol. 125, pp. 1–15.

Department of Primary Industry and Fisheries, NTG 2017 'Infomation sheet: *Cucumber green mottle mosaic virus*', available at nt.gov.au/d/cgmmv/index.cfm?header=CGMMV Fact Sheet.

Dorst, HJM 1988, 'Surface water as source in the spread of *Cucumber green mottle mosaic virus*', *Netherlands Journal of Agricultural Science*, vol. 36, pp. 291–9.

FAO 2016a, *International Standards for Phytosanitary Measures (ISPM) no. 1: Phytosanitary principles for the protection of plants and the application of phytosanitary measures in international trade*, Secretariat of the International Plant Protection Convention, Food and Agriculture Organization of the United Nations, Rome, Italy, available at https://www.ippc.int/en/core-activities/standards-setting/ispms/.

-- -- 2016b, *International Standards for Phytosanitary Measures (ISPM) no. 2: Framework for pest risk analysis*, Secretariat of the International Plant Protection Convention, Food and Agriculture Organization of the United Nations, Rome, Italy, available at https://www.ippc.int/en/core-activities/standards-setting/ispms/.

-- -- 2016c, *International Standards for Phytosanitary Measures (ISPM) no. 4: Requirements for the establishment of pest free areas*, Secretariat of the International Plant Protection Convention, Food and Agriculture Organization of the United Nations, Rome, Italy, available at https://www.ippc.int/en/core-activities/standards-setting/ispms/.

-- -- 2016d, *International Standards for Phytosanitary Measures (ISPM) no. 5: Glossary of phytosanitary terms*, Secretariat of the International Plant Protection Convention, Food and Agriculture Organization of the United Nations, Rome, Italy, available at https://www.ippc.int/en/core-activities/standards-setting/ispms/.

-- -- 2016e, *International Standards for Phytosanitary Measures (ISPM) no. 10: Requirements for the establishment of pest free places of production and pest free production sites*, Secretariat of the

International Plant Protection Convention, Food and Agriculture Organization of the United Nations, Rome, Italy, available at https://www.ippc.int/en/core-activities/standards-setting/ispms/.

-- -- 2016f, *International Standards for Phytosanitary Measures (ISPM) no. 11: Pest risk analysis for quarantine pests*, Secretariat of the International Plant Protection Convention, Food and Agriculture Organization of the United Nations, Rome, Italy, available at https://www.ippc.int/en/core-activities/standards-setting/ispms/.

-- -- 2016g, *International Standards for Phytosanitary Measures (ISPM) no. 14: The use of integrated measures in a systems approach for pest risk management*, Secretariat of the International Plant Protection Convention, Food and Agriculture Organization of the United Nations, Rome, Italy, available at https://www.ippc.int/en/core-activities/standards-setting/ispms/.

-- -- 2016h, *International Standards for Phytosanitary Measures (ISPM) no. 31: Methodologies for sampling of consignments* Secretariat of the International Plant Protection Convention, Food and Agriculture Organization of the United Nations, Rome, Italy, available at https://www.ippc.int/en/core-activities/standards-setting/ispms/.

Faris-Mukhayyish, S & Makkouk, KM 1983, 'Detection of four seedborne plant viruses by the enzyme-linked immunosorbent assay (ELISA)', *Phytopathologische Zeitschrift Journal of Phytopathology*, vol. 106, pp. 108–14.

Francki, RIB, Hu, J & Palukaitis, P 1986, 'Taxonomy of cucurbit-infecting *Tobamovirus* as determined by serological and molecular hybridization analyses', *Intervirology*, vol. 26, pp. 156–63.

Freeman, JH, McAvoy, EJ, Dittmar, PJ, Ozores-Hampton, M, Paret, M, Wang, Q, Miller, CF & Webb, SE 2015 'Cucurbit Production', available at edis.ifas.ufl.edu/pdffiles/CV/CV12300.pdf.

Government of Queensland 2017 *Cucumber green mottle mosaic virus*, Department of Agriculture and Fisheries, Queensland, available at daf.qld.gov.au/plants/health-pests-diseases/a-z-significant/cucumber-green-mottle-mosaic-virus.

Hatta, T, Nakamoto, T, Takagi, Y & Ushiyama, R 1971, 'Cytological abnormalities of mitochondria induced by infection with *Cucumber green mottle mosaic virus*', *Virology*, vol. 45, pp. 292–7.

Hatta, T & Ushiyama, R 1973, 'Mitochondrial vesiculation associated with *Cucumber green mottle mosaic virus*-infected plants', *Journal of General Virology*, vol. 21, pp. 9–17.

Hollings, N, Komuro, Y & Tochihara, H 1975 '*Cucumber green mottle mosaic virus*, Descriptions of Plant Viruses ', available at dpvweb.net/dpv/showdpv.php?dpvno=154.

Inouye, T, Inouye, N, Asatani, M & Mitsuhata, K 1967, 'Studies on *Cucumber green mottle mosaic virus* in Japan', *Nogaku Kenkyu*, vol. 51, pp. 175–86.

ISTA 2014 *Detection of Squash mosaic virus, Cucumber green mottle mosaic virus and Melon necrotic spot virus in cucurbits*, International rules for seed testing, ISTA, Bassersdorf, Switzerland.

Kim, OK, Mizutani, T, Natsuaki, KT, Lee, KW & Soe, K 2010, 'First report and the genetic variability of *Cucumber green mottle mosaic virus* occurring in bottle gourd in Myanmar', *Journal of Phytopathology*, vol. 158, pp. 572–5.

Kobayashi, T 1990, 'Impact of seedborne pathogens on quarantine in Japan', *Seed Science and Technology*, vol. 18, pp. 427–33.

Komuro, Y, Tochihara, H, Fukatsu, R, Nagai, Y & Yoneyama, S 1968, '*Cucumber green mottle mosaic virus* on watermelon in Chiba and Ibaraki Prefectures' (in Japanese), *Annals of the Phytopathological Society of Japan*, vol. 34, p. 377.

-- -- 1971, '*Cucumber green mottle mosaic virus* (watermelon strain) in watermelon and its relation to the fruit deterioration known as `Konnyaku' disease', *Annals of the Phytopathological Society of Japan*, vol. 37, pp. 34–42.

Kurowski, C, Conn, K, Lutton, J & Rosenberger, S 2015 'Cucurbit disease field guide', available at seminisus.s3.amazonaws.com/wp-content/uploads/2015/01/Disease-Guide-Final-012015.pdf.

Kwon, JY, Hong, JS, Kim, MJ, Choi, SH, Min, BE, Song, EG, Kim, HH & Ryu, RH 2014, 'Simultaneous multiplex PCR detection of seven cucurbit-infecting viruses', *Journal of Virological Methods*, vol. 206, pp. 133–9.

Lecoq, H & Desbiez, C 2012, 'Viruses of cucurbit crops in the Mediterranean region: an ever-changing picture', *Advances in Virus Research*, vol. 84, pp. 67–126.

Lecoq, H & Katis, N 2014, 'Control of cucurbit viruses', *Advances in Virus Research*, vol. 90, pp. 255–95.

Lee, KW, Lee, BC, Park, HC & Lee, YS 1990, 'Occurrence of *Cucumber green mottle mosaic virus* disease watermelon in Korea', *Korean Journal of Plant Pathology*, vol. 6, pp. 250–5.

Lee, KY 1996, 'Current occurance and control of CGMMV konjak disease', *Plant Disease and Agriculture*, vol. 2, pp. 38–9.

Ling, KS, Li, R & Zhang, W 2014, 'First report of *Cucumber green mottle mosaic virus* infecting greenhouse cucumber in Canada', *Plant Disease*, vol. 98, p. 701.

Liu, HW, Luo, LX, Li, JQ, Liu, PF, Chen, XY & Hao, JJ 2014, 'Pollen and seed transmission of *Cucumber green mottle mosaic virus* in cucumber', *Plant Pathology*, vol. 63, pp. 72–7.

Mabberley, DJ 2008, *Mabberley's plant-book: a portable dictionary of plants, their classifications and uses*, Cambridge University Press, UK.

Mandal, S, Mandal, B, Mohd, Q, Haq, R & Varma, A 2008, 'Properties, diagnosis and management of *Cucumber green mottle mosaic virus*', *Plant Virus*, vol. 2, pp. 25–35.

McCormack, JH 2005 'Cucurbit seed production: an organic seed production manual for seed growers in the Mid-Atlantic and Southern U.S.', available at carolinafarmstewards.org/wp-content/uploads/2012/05/CucurbitSeedProductionver1.4.pdf.

Mink, GI 1993, 'Pollen and seed-transmitted viruses and viroids', *Annual Review of Phytopathology*, vol. 31, pp. 375–402.

Moradi, Z & Jafarpour, B 2010, 'Mixed infections of *Watermelon mosaic potyvirus* and *Cucumber green mottle mosaic tobamovirus* in cucurbit hosts', *Plant Protection Journal*, vol. 2, pp. 353–65.

Moreno, IM, Thompson, JR & Garcia-Arenal, F 2004, 'Analysis of the systemic colonization of cucumber plants by *Cucumber green mottle mosaic virus*', *Journal of General Virology*, vol. 85, pp. 749–59.

Nagendran, K, Aravintharaj, R, Mohankumar, S, Manoranjitham, SK, Naidu, RA & Karthikeyan, G 2015, 'First report of *Cucumber green mottle mosaic virus* in snake gourd (*Trichosanthes cucumerina*) in India', *Plant Disease*, vol. 99, p. 559.

Napier, T 2009 *Insect pests of cucurbit vegetables*, Department of Primary Industries, available at dpi.nsw.gov.au/__data/assets/pdf_file/0005/290219/insect-pests-of-cucurbit-vegetables.pdf.

Nilsson, B 1977, 'Study of *Green mottle mosaic virus* on cucumber', *Nordisk Jordbrugsforskning*, vol. 59, pp. 513–9.

Noda, C, Kittipakorn, K, Inchan, P, Wanapee, L & Deema, N 1993, 'Distribution of cucurbit viruses and reactions of some cucurbit species to certain viruses', paper presented at Proceedings of the 31st Kasetsart University Annual Conference: Plants, Bangkok, Thailand.

Nontajak, S, Pulyasevi, S, Jonglaekha, N & Smitamana, P 2014, 'Detection of *Cucumber green mottle mosaic tobamovirus* in three growth stages of Japanese cucumber in the highland area of Northern Thailand', *Journal of Agricultural Technology*, vol. 10, pp. 277–87.

Northern Territory Goverment 2017 *Cucumber green mottle mosaic virus*, Northern Territory Government, available at nt.gov.au/d/cgmmv/.

Pandey, S & Joshi, RD 1989, 'Effect of cucumis virus-3 infection on chlorophyll content, chloroplast number and chlorophyllase activity of bitter gourd', *Indian Phytopathology*, vol. 42, pp. 549–50.

Persley, D, Cooke, T & House, S 2010, *Diseases of vegetable crops in Australia*, CSIRO Publishing, Collingwood, Victoria, Australia.

Qin, BX, Cai, JH, Liu, ZM, Chen, YH, Zhu, GN & Huang, FX 2005, 'Preliminary identification of a *Cucumber green mottle mosaic virus* infecting pumpkin', *Plant Quarantine*, vol. 4, pp. 198–200.

Rao, ALN & Varma, A 1984, 'Transmission studies with *Cucumber green mottle mosaic virus*', *Phytopathologische Zeitschrift*, vol. 109, pp. 325–31.

Rashmi, CM, Reddy, CNL, Praveen, HM & Nagaraju, N 2005, 'Natural occurrence of *Cucumber green mottle mosaic virus* on gherkin (*Cucumis anguria* L.)', *Environment and Ecology*, vol. 23, pp. 781–4.

Rast, ATB & Stijger, CCMM 1987, 'Disinfection of pepper seed infected with different strains of capsicum mosaic virus by trisodium phosphate and dry heat treatment', *Plant Pathology*, vol. 36, no. 4, pp. 583–8.

Raychaudhuri, M & Varma, A 1978, 'Mosaic disease of muskmelon, caused by a minor variant of *Cucumber green mottle mosaic virus*', *Phytopathologische Zeitschrift*, vol. 93, pp. 120–5.

Reingold, P, Lachman, O, Blaosov, E & Dombrovsky, A 2015, 'Seed disinfestion treatments do not sufficiently eliminate the infectivity of *Cucumber green mottle mosaic virus* (CGMMV) on cucurbit seeds', *Plant Pathology*, vol. 64, pp. 245–55.

Reingold, V, Lachman, O, Belausov, E, Koren, A, Mor, N & Dombrovsky, A 2016, 'Epidemiological study of *Cucumber green mottle mosaic virus* in greenhouses enables reduction of disease damage in cucurbit production', *Annals of Applied Biology*, vol. 168, pp. 29–40.

Reingold, V, Lachman, O, Koren, A & Dombrovsky, A 2013, 'First report of *Cucumber green mottle mosaic virus* (CGMMV) symptoms in watermelon used for the discrimination of non-marketable fruits in Israeli commercial fields', *New Disease Reports*, vol. 18, p. 11.

Shang, H, Xie, Y, Zhou, X, Qian, Y & Wu, J 2011, 'Monoclonal antibody-based serological methods for detection of *Cucumber green mottle mosaic virus*', *Virology Journal*, vol. 8, p. 228.

Sharma, P, Verma, RK, Mishra, R, Sahu, AK, Choudhary, DK & Gaur, RK 2014, 'First report of *Cucumber green mottle mosaic virus* association with the leaf green mosaic disease of vegetable crop, *Luffa acutangula* L', *Acta Virologica*, vol. 58, pp. 103–4.

Shurtleff, MC & Averre, CW 1997, *Glossary of Plant-Pathological Terms*, American Phytopathology Society Press, St. Paul, Minnesota.

Simon-Buela, L & Garcia-Arenal, F 1999, 'Virus particles of *Cucumber green mottle mosaic tobamovirus* move systemically in the phloem of infected cucumber plants', *Molecular Plant-Microbe Interactions*, vol. 12, pp. 112–8.

Smith, KM 1972, *A textbook of plant virus diseases*, Prentice Hall Press, London, UK.

Stenlid, J, Oliva, J, Boberg, JB & Hopkins, AJM 2011, 'Emerging diseases in European forest ecosystems and responses in society', *Forests*, vol. 2, pp. 486–504.

Sugiyama, M, Ohara, T & Sakata, Y 2006, 'A new source of resistance to *Cucumber green mottle mosaic virus* in melon', *Journal of the Japanese Society for Horticultural Science*, vol. 75, pp. 469–75.

Tian, T, Posis, K, Maroon-Lango, CJ, Mavrodieva, V, Haymes, S, Pitman, TL & Falk, BW 2014, 'First report of *Cucumber green mottle mosaic virus* on melon in the United States', *Plant Disease*, vol. 98, p. 1163.

Tochihara, H & Komuro, Y 1974, 'Infectivity test and serological relationships among various isolates of *Cucumber green mottle mosaic virus*; some deduction of the invasion route of the virus into Japan', *Annals of the Phytopathological Society of Japan*, vol. 40, pp. 52–8.

Ugaki, M, Tomiyama, M, Kakutani, T, Hidaka, S, Kiguchi, T, Nagata, R, Sato, T, Motoyoshi, F & Nishiguchi, M 1991, 'The complete nucleotide sequence of *Cucumber green mottle mosaic virus* (SH strain) genomic RNA', *Journal of General Virology*, vol. 72, pp. 1487–95.

van Vaerenbergh, J, Bergsma-Vlami, M, Elphinstone, J, Stefani, E & Olivier, V 2013, '*Clavibacter michiganensis* subsp. *michiganensis*', *Bulletin OEPP/EPPO Bulletin*, vol. 43, pp. 46–67.

Vani, S & Varma, A 1993, 'Properties of *Cucumber green mottle mosaic virus* isolated from water of River Jamuna', *Indian Phytopathology*, vol. 46, pp. 118–22.

Varveri, C, Vassilakos, N & Bem, F 2002, 'Characterization and detection of *Cucumber green mottle mosaic virus* in Greece', *Phytoparasitica*, vol. 30, pp. 493–501.

Wingfield, MJ, Slippers, B & Wingfield, BD 2010, 'Novel associations between pathogens, insects and tree species threaten world forests', *New Zealand Journal of Forestry Science*, vol. 40, pp. 95–103.

WTO 1995, *Agreement on the application of sanitary and phytosanitary measures*, World Trade Organization, Geneva, available at wto.org/english/docs_e/legal_e/15-sps.pdf.

Yoon, JY, Choi, GS, Choi, SK, Hong, JS, Choi, JK, Kim, W, Lee, GP & Ryu, KH 2008, 'Molecular and biological diversities of *Cucumber green mottle mosaic virus* from cucurbitaceous crops in Korea', *Journal of Phytopathology*, vol. 156, pp. 408–12.

Zhang, YJ, Li, GF & Li, MF 2009, 'Occurrence of *Cucumber green mottle mosaic virus* on cucurbitaceous plants in China', *Plant Disease*, vol. 93, p. 200.

Zhou, LL, Wu, YH, Zhao, XX, Li, LM, Cai, M, Wang, L & Wang, WH 2008, 'The biological characteristics of *Cucumber green mottle mosaic virus* and its effects on yield and quality of watermelon', *Journal of Shenyang Agricultural University*, vol. 39, pp. 417–22.