

United States Department of Agriculture

Animal and Plant Health Inspection Service

Plant Protection and Quarantine

### New Pest Response Guidelines

Cucumber green mottle mosaic virus

CGMMV



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### Introduction

Chapter

The purpose of this NPRG is to provide the basic information likely to be needed by the initial PPQ response team in the first 30 to 60 days following a detection of *Cucumber green mottle mosaic virus* (CGMMV) in the United States.

These guidelines for CGMMV include the following:

- Summary of relevant pest biology
- Guide to identification or screening for the pest in the field based on damage
- Preliminary method for conducting a delimiting survey
- Preliminary method for collecting seed samples for testing
- Summary of known potential control and management options
- Summary of knowledge gaps

**Note:** This document is based on the best information available at the time of development; however, at the time of an actual emergency, new scientific and technical information may be identified. In addition, each pest incursion has unique, site-specific characteristics that are impossible to predict. Therefore, this document should be considered only as a general guideline. As the pest situation evolves and new information is gathered, the responses implemented, including survey protocols, may need to be modified from the original recommendations.

### Pest at a Glance

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Chapter

#### **Pest Summary**

#### **Justification**

CGMMV is considered to be transient and under official control in the United States (El-Lissy, 2014). The purpose of this NPRG is to provide guidance for the initial PPQ response team should CGMMV be detected in a new area.

Damage caused by CGMMV can be severe, resulting in substantial yield and quality losses depending upon the host infected (Liu et al., 2014; Reingold et al., 2015; Fletcher et al., 1969; Nilsson, 1977; Zhou et al., 2008). The most severe yield losses result from early infections, while late infections have little effect (Fletcher et al., 1969). Preventing the entry and establishment of CGMMV in new areas is critical for disease management (Dombrovsky et al., 2017).

#### Economically Important Crops at Risk in the United States

White or wax gourd (*Benincasa hispida*), watermelon (*Citrullus lanatus*), West Indian gherkin (*Cucumis anguria*), melon and cantaloupe (*C. melo*), garden cucumber (*C. sativus*), winter squash and pumpkin (*Cucurbita maxima*), crookneck squash (*Cu. moschata*), field pumpkin and zucchini (*Cu. pepo*), bottle gourd (*Lagenaria siceraria*), ridged gourd or sinkwa towelsponge (*Luffa acutangula*), smooth loofah gourd or sponge gourd (*L. cylindrica*), bitter gourd or balsampear (*Momordica charantia*), and snake gourd (*Trichosanthes cucumerina*) (Dombrovsky et al., 2017).

#### **Key Information**

- CGMMV is generally introduced into new areas or fields via contaminated seed or soil or infected transplant seedlings.
- Once introduced, CGMMV is easily spread to susceptible plants by mechanical means and normal agricultural practices.
- CGMMV can be asymptomatic in some hosts, such as pumpkin and

certain melon cultivars.

• Look-alike pathogens are present in the United States; plant foliage, fruit, or seeds must be tested to identify CGMMV.

#### Chapter

### **Pest Overview**

**Pest Information** 

#### **Scientific Name**

• Cucumber green mottle mosaic virus

#### **Taxonomic Position**

♦ Virgaviridae: Tobamovirus

#### Synonym(s)

- bottlegourd Indian mosaic virus
- cucumber green mottle mosaic tobamovirus
- cucumber green mottle mosaic watermelon strain (W)
- cucumber mottle virus
- cucumber virus 2
- cucumber virus 3
- cucumber virus 4
- cucumis virus 2
- tobacco mosaic virus watermelon strain-W

#### **Common Names**

♦ CGMMV

#### **Hosts**

**Table 3-1** List of reported plant hosts of *Cucumber green mottle mosaic virus*; (\*) indicates a species not known to occur in the United States (NRCS, 2018) and (<sup>a</sup>) indicates a weed host.

Scientific name	Common name	References
Benincasa hispida	White gourd, wax gourd	Dombrovsky et al., 2017
Citrullus lanatus	Watermelon	Dombrovsky et al., 2017
Cucumis anguria	West Indian gherkin	Dombrovsky et al., 2017

Cucumis melo	Melon, cantaloupe	Dombrovsky et al., 2017
Cucumis sativus	Garden cucumber	Dombrovsky et al., 2017
Cucurbita maxima	Winter squash, pumpkin	Dombrovsky et al., 2017
Cucurbita moschata	Crookneck squash	Dombrovsky et al., 2017
Cucurbita pepo	Field pumpkin, zucchini	Dombrovsky et al., 2017
Lagenaria siceraria	Bottle gourd	Dombrovsky et al., 2017
Luffa acutangula	Ridged gourd, sinkwa towelsponge	Dombrovsky et al., 2017
Luffa cylindrica	Smooth loofah gourd, sponge gourd	Dombrovsky et al., 2017
Momordica charantia	Bitter gourd, balsampear	Dombrovsky et al., 2017
Trichosanthes cucumerina	Snake gourd	Dombrovsky et al., 2017
Amaranthus blitoides <sup>a</sup>	Spreading amaranth	Dombrovsky et al., 2017
Amaranthus graecizans <sup>a</sup>	Prostrate pigweed	Shargil et al., 2017
Amaranthus muricatus <sup>a</sup>	African amaranth	Shargil et al., 2017
Amaranthus retroflexus <sup>a</sup>	Redroot pigweed	Dombrovsky et al., 2017
Amaranthus viridisª	Slender amaranth	Dombrovsky et al., 2017
Chenopodium album <sup>a</sup>	Lambsquarters	Dombrovsky et al., 2017
Citrullus colocynthis <sup>a</sup>	Colocynth	Dombrovsky et al., 2017
Chrozophora tinctoriaª	Dyer's croton, giradol	Shargil et al., 2017
Ecballium elateriumª	Squirting cucumber	Shargil et al., 2017
Heracleum moellendorffii*ª	Persian hogweed	Dombrovsky et al., 2017
Heliotropium europaeumª	European heliotrope	Dombrovsky et al., 2017
Moluccella laevis <sup>a</sup>	Bells of Ireland, shellflower	Shargil et al., 2017
Mukia maderaspatana*ª	Erinkanyaba okí-òka	Dombrovsky et al., 2017
Portulaca oleraceaª	Purslane, little hogweed	Dombrovsky et al., 2017
Solanum nigrumª	Black nightshade	Dombrovsky et al., 2017
Withania somniferaª	Indian ginseng, withania	Shargil et al., 2017

#### **Dispersal**

#### **Natural Movement**

CGMMV is seedborne in cucurbits (Dombrovsky et al., 2017), with transmission rates varying by host (Al-Tamimi et al., 2009; Wu et al., 2011; Li et al., 2016; Reingold et al., 2016a)(Figure 3-1).

CGMMV can survive in and spread from contaminated irrigation water, recirculated greenhouse water, soil, and plant debris (Reingold et al., 2016a; Reingold et al., 2015; Antignus et al., 2005; Dombrovsky et al., 2017; Li et al., 2016); the pathogen can persist in soil for up to 2-years (Dombrovsky et al., 2017). The virus is transmitted by plant-to-plant contact during normal growth (Rao and Varma, 1984). Honeybees (*Apis mellifera*) can spread CGMMV while foraging (Darzi et al., 2018; Tran-Nguyen et al., 2015). It can be pollen-transmitted from infected cucumbers under experimental conditions only (Liu et al., 2014); cucumber leaf beetles (*Raphidopalpa feveicollis*) may also be vectors (Rao and Varma, 1984), although this hasn't been proven.



**Figure 3-1.** The complex disease cycle of *Cucumber green mottle mosaic virus* (CGMMV) in cucurbit crop plants (Dombrovsky et al., 2017). Red arrows represent major infection events. Gray arrows represent the survival of virus infectivity in soil, weeds, planting trays, etc.

#### **Human-Assisted Spread**

CGMMV has been introduced to new areas via infected transplants received from contaminated nurseries (Dombrovsky et al., 2017). CGMMV is very stable and can remain infectious on contaminated surfaces and in infested plant debris or soil for several months to several years (Antignus, 2012; Hollings et al., 1975; Reingold et al., 2016a; (Dombrovsky et al., 2017)). Therefore, it is easily spread by mechanical means, agricultural practices (Reingold et al., 2016a), and plant-to-plant contact (Dombrovsky et al., 2017). CGMMV can survive on pruning equipment, clothing, boxes, crates, hands, and machinery (Hollings et al., 1975;

Liu et al., 2014), and is readily transmitted when contaminated items come in contact with susceptible hosts (Reingold et al., 2016a).

#### Chapter

# Pest Identification and Damage

#### **Species Description/Morphology**

CGMMV can be identified using electron microscopy (Hollings et al., 1975; CABI, 2018), serology (Agdia, 2016; AgDia, 2018; Shang et al., 2011) and molecular methods (Mandal et al., 2008; Li et al., 2013). The International Seed Testing Association (ISTA) has developed an internationally recognized seed testing protocol (ISTA, 2014), but, Australia and others have since raised concerns about the probability of detecting low levels of infection with this method (Falk et al., 2016; DAWR, 2017). U.S. regulatory agencies use serology and molecular methods to identify CGMMV-infected cucurbits.

#### Signs and Symptoms

CGMMV symptom development is affected by the environmental conditions, viral strain, and host developmental stage at the time of infection (Dombrovsky et al., 2017); foliar symptoms may take six or more weeks to develop after seed sowing (Li et al., 2016). Typical symptoms include leaf mottling, leaf mosaic, and fruit mottling or distortion (Reingold et al., 2015). However, foliage and fruit symptoms vary among cucurbit species and cultivars, and plants may have asymptomatic infections.

- **Cucumber-** Green mottling occurs on young leaves and fruit surfaces; infected plants may collapse (Dombrovsky et al., 2017)(Figure 4-1).
- Watermelon- Young plants display mottling and mosaic on leaves and may develop brown necrotic lesions on the stems and peduncles. Foliage eventually develops a bleached appearance and wilts. Runners or whole plants may die prematurely. In some cases, foliar symptoms may fade as plants mature or may be masked in field-grown plants (Reingold et al., 2013). Fruit are often malformed, with the internal flesh becoming spongy or rotten and developing a yellow or dirty red discoloration (Dombrovsky

et al., 2017)(Figure 4-2).

- Melon- Young leaves initially develop mottle and mosaic symptoms that often disappear once foliage matures. Fruits develop varying degrees of malformation, mottling, and surface netting; some melon cultivars are asymptomatic (Rajamony et al., 1987; Rajamony et al., 1990; Sugiyama et al., 2006)(Figure 4-3).
- **Pumpkin, squash, and zucchini-** Infected foliage is usually asymptomatic, although leaf mottling and mosaic occasionally occur. Pumpkin fruits are always asymptomatic, while squash and zucchinis are sometimes externally asymptomatic but internally discolored and necrotic (Dombrovsky et al., 2017).
- Weeds- In general, non-cucurbitaceous weeds are asymptomatic (Shargil et al., 2017; Tran-Nguyen et al., 2015).



Figure 4-1. Foliar symptoms on cucumbers infected with CGMMV (T. Pittman, University of California – Davis).



Figure 4-2. Symptomatic watermelon fruit (above) and leaves (below) from plants infected with CGMMV (T. Pittman, University of California- Davis).



Figure 4-3. Foliar symptoms of CGMMV on cantaloupe (T. Pittman, University of California – Davis).

#### **Similar Species**

It is difficult to identify CGMMV based on symptoms alone because other viruses cause similar symptoms in cucurbits. These include *Kyuri green mottle mosaic virus* (Francki et al., 1986), *Cucumber fruit mottle mosaic virus* (Antignus et al., 2001), *Zucchini green mottle mosaic virus* (Ryn et al., 2000), *Cucumber mottle mosaic virus* (Orita et al., 2007), *Watermelon green mottle mosaic virus* (Cheng et al., 2018), *Cucumber mosaic virus*, *Alfalfa mosaic virus, Watermelon mosaic virus*, and *Squash mosaic virus*, *Alfalfa mosaic virus, Watermelon mosaic virus*, and *Squash mosaic virus*, *Alfalfa mosaic virus, Watermelon mosaic virus*, and *Squash mosaic virus* occur within the United States (CABI, 2018; Falk et al., 2016).

### Survey

## 5

Chapter

#### Introduction

Use *Chapter 5 Survey Procedures* as a guide to conducting detection and delimitation surveys for CGMMV in fruit and seed production areas. While some survey components are the same, the approach and design of each survey is different based on the survey goal. Detection surveys are designed to determine if CGMMV is present or absent in a defined area. Thus, surveys are conducted in areas and at times where disease may be most to be visible. In contrast, delimitation surveys are designed to determine the geographic distribution of a pest. Surveys begin at a known detection site and sampling is done around that site to determine how widespread the disease is in a specified area.

Before starting, determine whether you need a detection or delimitation survey, and follow the appropriate survey protocol. The protocols have been written such that each protocol may be removed from the NPRG and shared with surveyors.

#### **Detection Survey**

Perform this survey if CGMMV is not present. Detection surveys ascertain the presence or absence of a pest in an area where it is not known to occur. Surveys can be broad in scope over large areas (e.g. multiple fields, farms, or states), or may be restricted to small focused areas such as a field or a greenhouse.

The survey will generate either positive (pathogen detected) or negative (pathogen not detected) results. Negative detection survey results are **not** sufficient to declare an area free of CGMMV. They are, however, valuable for informing future surveys by providing clues about the mode of dispersal, temporal occurrence, or effect of industry practices, particularly when considered with results from similar areas.

#### **Timing of Survey**

Survey timing depends upon the pathogen life cycle, the plant growth stage when infection is likely to occur, and the ecological parameters that support pathogen dispersal. Visually surveying for CGMMV can be a challenge as symptoms may not appear until six weeks or more after seed sowing and may fade as plants mature; certain hosts may also be asymptomatic. If possible, the survey should be conducted during a time when infection is most likely to be apparent.

**In greenhouses-** Virus symptoms may not be readily expressed in young transplants. Transplants should be inspected within 1-wk of their projected shipping date. If any symptoms are noted during inspection, collect a sample from the suspect plant(s) and from its immediate neighbors (i.e., the leaves of neighboring plant(s) that are in immediate contact with the suspect plant).

**In the field-** Begin surveying six weeks after seed sowing. Early in the growing season, surveyors should focus on identifying plants exhibiting foliar symptoms. If any symptoms are noted during inspection, samples should be collected from the symptomatic plant and neighboring plants within 2.5 m (8 ft). If no symptoms are present, samples should be randomly collected from within the field.

Later in the growing season, surveyors should focus on detecting plants exhibiting fruit symptoms. Crops such as watermelon, melon, and cucumber should express such symptoms (see Chapter 4). For these crops, samples should be collected from the symptomatic plant and neighboring plants within 2.5 meters of the suspect plant. For crops, such as pumpkin, squash, and zucchini, fruit will appear symptomless externally. For these crops, samples should be randomly collected from within the field.

**Seed-** Infected seeds appear asymptomatic. Samples must be collected postharvest, but prior to any seed disinfestation or treatment activities as such treatments can impact our ability to detect CGMMV.

#### **Survey Techniques for Detection**

#### Survey Preparation, Sanitization and Clean-Up

This section provides information to aid personnel in preparing to conduct a survey, procedures to follow during a survey, and instructions for proper cleaning and sanitizing of supplies and equipment after the survey is finished.

- 1. Prior to beginning a survey, determine whether any pesticide applications have recently occurred that would render it unsafe to inspect the plants. Contact the property owner or manager and ask if a re-entry period is in effect due to pesticide application. Look for posted signs indicating recent pesticide applications, particularly in commercial fields or nurseries.
- 2. Determine whether quarantines for other pests or crops are in effect for the survey area. Comply with all quarantine requirements.
- **3.** When visiting an area to conduct surveys or take samples, take strict measures to prevent transmission of CGMMV or other pests between fields during inspections.
- 4. Use disposable protective clothing, gloves and footwear, and change them before entering and as you leave each site. Upon exiting the field, the disposable clothing and gloves should be placed immediately into collection bags.
- 5. Confirm that equipment, tools and footwear are clean and sanitized.
  - a. When taking plant samples, disinfect tools, including the handles, with 10.0 percent hypochlorite solution (bleach).
  - b. Briefly spray to runoff or immerse each tool in bleach and allow it to airdry. This practice effectively inactivates plant pathogens and prevents their spread.
  - c. Disinfect footwear or dispose of foot covers prior to leaving a site.
  - d. Use a high pressure delivery system, such as a steam pressure wash system, to disinfect large pieces of equipment, storage areas or bins.
    Vehicles and equipment should be cleaned with a detergent or disinfectant during pressure washing to ensure deactivation of the virus.

#### **Visual survey**

Conduct a visual inspection by searching for plants with typical CGMMV

symptoms as follows (see also Section 4).

Collect samples from all symptomatic plants and submit them for testing. The type of tissue collected will depend on when you conduct the survey.

Two basic principles should govern your inspection and sampling processes:

**1. CGMMV cannot be diagnosed by a visual inspection of symptoms alone;** only laboratory testing can provide a definitive diagnosis.

### **2.** If you have any doubt as to whether the symptoms observed could be caused by CGMMV, collect a sample.

The following symptoms can typically be found on hosts infected with CGMMV:

- Cucumber
  - Foliage: Green mottling on young leaves, collapsed plants;
  - Fruit: Green mottling on fruit surfaces;
- Watermelon
  - Foliage: Leaf mottling and mosaic symptoms in young plants, brown necrotic lesions on stems and peduncles, foliage bleached and wilted, dead runners or whole plants;
  - Fruit: Malformed, internal flesh spongy or rotten with a yellow or dirty red discoloration;
- Melon
  - Foliage: Mottle and mosaic symptoms on young leaves,mature leaves may be asymptomatic;
  - Fruit: Malformed, mottled, or with surface netting, some may be asymptomatic;
- Pumpkin, squash, and zucchini
  - Foliage: Usually asymptomatic, although leaf mottling and mosaic may occur;
  - Fruit: Pumpkin fruits are asymptomatic, squash and zucchini fruits are sometimes externally symptomless but internally discolored and necrotic.

#### **Field Sample Collection**

Survey task forces should include an experienced survey specialist or plant pathologist familiar with CGMMV and the symptoms it causes.

- 1. Surveyors visiting sites to obtain samples should collect the following information:
  - Date of collection or observations

- Collector's name
- Grower's field identification numbers (grower can provide this information)
- Full name of business, institution, or agency
- Full mailing address, including country
- Type of property (commercial nursery, natural field, orchard, vineyard, or residence)
- GPS coordinates of the host plant and property
- Host plant species and specific crop plant variety, if applicable
- Presence or absence of the pest
- Observations of signs and symptoms
- Percentage of the field displaying disease symptoms
- General conditions or any other relevant information
- 2. Samples to collect:
  - Foliar samples: A sample consists of five symptomatic leaflets from a single plant. Use a pruner to remove and collect the leaflets. Place all leaflets into a single re-sealable plastic bag, label the bag as described above, and then place the sample bag into a second, re-sealable plastic bag. Multiple samples from the same field or greenhouse may be placed into the second bag. The pruner and your gloves must be sanitized with a disinfectant between each plant. Preferentially collect foliar samples.
  - Fruit samples: Collect these samples only if the foliage has already dried down or died. To examine fruit, cut the fruit open with a knife or similar instrument. If symptoms are observed, cut a disk from the symptomatic fruit, place it into a single, re-sealable plastic bag, label the bag as described above, and then place into a second, re-sealable plastic bag. Multiple samples from the same field or greenhouse may be placed into the second bag. The knife and your gloves must be sanitized with a disinfectant between each plant.

#### Seed Sample Collection

The survey task force should include a certified seed sampler or experienced survey specialist. Within the detection survey area, randomly choose the seed production fields to be surveyed. Collect the seed samples post-harvest, but prior to any planned seed treatments.

Surveyors visiting sites to obtain samples should collect the following information for each seed sample:

• Date of collection or observations

- Collector's name
- Seed lot number
- Seed lot volume
- Full name of business, institution, or agency
- Full mailing address, including country
- Origin(s) of seed lot(s). including physical location(s), field- or nurserygrown, and country of origin
- Host plant species and specific crop plant variety, if applicable
- General conditions or any other relevant information

CGMMV-infected seeds are rarely symptomatic. Take the following actions to collect a representative sample from the seed lot:

- 1. Using a disposable cup, such as a 3- or 5-oz paper Dixie cup, obtain a sample of the seed lot. Five to 40 subsamples should be collected from each seed lot (Table 5-1)(Kruse et al., 2004).
  - a. For large seed lots, collect the required volume for that host (Table 5-1).
  - b. For small seed lots from which only a few seeds are available, collect 20 percent of the seed lot (Dombrovsky et al., 2017).
- 2. Combine the subsamples into a single, labeled plastic bag. Include the disposable cup in the sample bag. Do not re-use the disposable cup.
- 3. Record the required information on the sample bag.
- **4.** Double bag the samples and deliver them to a diagnostic laboratory within 48-hr.

Table 5-1	Required nu	mber of seed subsamples to collect to detect CGMMV in a seed	
production field post-harvest (modified from Kruse et al., 2004).			
Lot size		Number of subsamples to collect	

Lot size	Number of subsamples to collect
up to 500 kg	five (5)
501-3,000 kg	one subsample for each 300 kg, but no less than five (5)
3,001-20,000 kg	On subsample for each 500 kg, but no less than ten (10)
>20,001 kg	one subsample for each 700 kg, but no less than forty (40)

**Table 5-2**Required seed sample sizes for hosts of *Cucumber green mottle mosaic*virus (7 CFR § 201.46, 2000).

Host	Seeds per gram	Required sample size
Cucumber	40	100 grams (4 ounces = 0.5 cups)
West India gherkin	153	27 grams (1 ounce = 0.125 cups)
Melon	45	89 grams (3 ounces = 0.33 cups)
Pumpkin	5	800 grams (28 ounces = 3.25 cups)
Squash	14	286 grams (10 ounces = 1.25 cups )
Watermelon	11	364 grams (13 ounces = 1.67 cups)

#### **Delimiting Survey**

After confirming a CGMMV detection in a new area, conduct a delimiting survey to define the geographic extent of the pest. Surveys after an initial U.S. detection should be most intensive around the known positive detection(s), including adjacent fields, **any associated fields**<sup>\*</sup> and any potentially infected areas discovered through trace-back and trace-forward investigations.

\* Agricultural fields that share ownership, tendency, seed, seed source, drainage or runoff, farm machinery, personnel, or other elements of shared cultural practices with the infested fields that could allow spread of inoculum. A field or land that has been exposed as a result of unauthorized deposit of soil from infested fields is also considered an associated field.

CGMMV may infect fields and greenhouses used for seed or for fruit production. The method for surveying and collecting samples from fields and greenhouses is approximately the same. For seed production fields within the delimitation area, seeds should also be tested for CGMMV after harvest (see Seed Sample Collection).

#### **Delimitation Area**

The extent of the delimitation area will be determined by the following factors:

- The number and size of associated and adjacent cucurbit fields or greenhouses;
- Shared use of farm equipment and/or personnel between fields/greenhouses;
- Trace out investigations;
- Flow of irrigation or water from the infested field(s).

For the delimitation survey, the entire field should be inspected along with any weeds that are present within or neighboring (i.e., in immediate foliar contact with the suspect cucurbit plants) the field.

Seed production crops in the delimitation area must undergo two inspections: 1) a field inspection during the growing season (targeting young plants and leaves), and 2) seed sampling after harvest.

Within greenhouses, visually inspect all plants for virus symptoms. If any symptoms are noted during inspection, collect a sample from the suspect plant(s) and from its immediate neighbors (i.e., the leaves of neighboring plant(s) that are in immediate contact with the suspect plant).

In the field, visually inspect all plants for virus symptoms. If surveying early in

the growing season, focus on identifying plants exhibiting foliar symptoms. If any symptoms are noted during inspection, samples should be collected from the symptomatic plants and neighboring plants within 2.5 meters (8 feet). If no symptoms are present, samples should be randomly collected from within the field. If surveying late in the growing season, inspect plants for symptomatic foliage and fruit crops such as watermelon, melon, and cucumber should express symptoms (see Chapter 4). For these crops, samples should be collected from the symptomatic plants and neighboring plants within 2.5 meters of the suspect plant. For crops, such as pumpkin, squash, and zucchini, fruit will appear symptomless externally and samples should be randomly collected from within the field.

#### **Survey Techniques for Delimitation**

#### Survey Preparation, Sanitization and Clean-Up

This section provides information to aid personnel in preparing to conduct a survey, procedures to follow during a survey, and instructions for proper cleaning and sanitizing of supplies and equipment after the survey is finished.

- Prior to beginning a survey, determine whether any pesticide applications have recently occurred that would render it unsafe to inspect the plants. Contact the property owner or manager and ask if a re-entry period is in effect due to pesticide application. Look for posted signs indicating recent pesticide applications, particularly in commercial fields or nurseries.
- 2. Determine whether quarantines for other pests or crops are in effect for the survey area. Comply with all quarantine requirements.
- **3.** When visiting an area to conduct surveys or take samples, take strict measures to prevent transmission of CGMMV or other pests between fields during inspections.
- 4. Use disposable protective clothing, gloves and footwear, and change them before entering and as you leave each site. Upon exiting the field, the disposable clothing and gloves should be placed immediately into collection bags.
- 5. Confirm that equipment, tools and footwear are clean and sanitized.
  - a. When taking plant samples, disinfect tools, including the handles, with 10.0 percent hypochlorite solution (bleach).
  - b. Briefly spray to runoff or immerse each tool in bleach and allow it to airdry. This practice effectively inactivates plant pathogens and prevents their spread.
  - c. Disinfect footwear or dispose of foot covers prior to leaving a site.

d. Use a high pressure delivery system, such as a steam pressure wash system, to disinfect large pieces of equipment, storage areas or bins.
Vehicles and equipment should be cleaned with a detergent or disinfectant during pressure washing to ensure deactivation of the virus.

#### **Delimitation Procedure**

Begin the delimiting survey by conducting a thorough visual inspection of the field(s), searching for plants with typical CGMMV symptoms as follows (see also Chapter 4).

Two basic principles should govern your inspection and sampling processes:

**1. CGMMV cannot be diagnosed by a visual inspection of symptoms alone;** only laboratory testing can provide a definitive diagnosis.

### 2. If you have any doubt as to whether the symptoms observed could be caused by CGMMV, collect a sample.

Collect a minimum of 40 samples from symptomatic plants in each associated field and each fields identified by trace-forward and trace-back investigations. If few to no symptomatic plants are observed, samples should be randomly collected from throughout the field to maximize the probability of detection (for an example, see Figure 5-1).



Figure 5-1. Stratified random sampling demonstrating uniform quadrants and sample sites.

The following symptoms can typically be found on hosts infected with CGMMV:

- Cucumber
  - Foliage: Green mottling on young leaves, collapsed plants;
  - Fruit: Green mottling on fruit surfaces;
- Watermelon
  - Foliage: Leaf mottling and mosaic symptoms in young plants, brown necrotic lesions on stems and peduncles, foliage bleached and wilted, dead runners or whole plants;
  - Fruit: Malformed, internal flesh spongy or rotten with a yellow or dirty red discoloration;
- Melon
  - Foliage: Mottle and mosaic symptoms on young leaves,mature leaves may be asymptomatic;
  - Fruit: Malformed, mottled, or with surface netting, some may be asymptomatic;
- Pumpkin, squash, and zucchini

- Foliage: Usually asymptomatic, although leaf mottling and mosaic may occur;
- Fruit: Pumpkin fruits are asymptomatic, squash and zucchini fruits are sometimes externally symptomless but internally discolored and necrotic.

#### Field Sample Collection

Survey task forces should include an experienced survey specialist or plant pathologist familiar with CGMMV and the symptoms it causes.

- 2. Surveyors visiting sites to obtain samples should collect the following information:
  - Date of collection or observations
  - Collector's name
  - Grower's field identification numbers (grower can provide this information)
  - Full name of business, institution, or agency
  - Full mailing address, including country
  - Type of property (commercial nursery, natural field, orchard, vineyard, or residence)
  - GPS coordinates of the host plant and property
  - Host plant species and specific crop plant variety, if applicable
  - Presence or absence of the pest
  - Observations of signs and symptoms
  - Percentage of the field displaying disease symptoms
  - General conditions or any other relevant information
- 2. Samples to collect:
  - Foliar samples: A sample consists of five symptomatic leaflets from a single plant. Use a pruner to remove and collect the leaflets. Place all leaflets into a single re-sealable plastic bag, label the bag as described above, and then place the sample bag into a second, re-sealable plastic bag. Multiple samples from the same field or greenhouse may be placed into the second bag. The pruner and your gloves must be sanitized with a disinfectant between each plant. Preferentially collect foliar samples.
  - Fruit samples: Collect these samples only if the foliage has already dried down or died. To examine fruit, cut the fruit open with a knife or similar instrument. If symptoms are observed, cut a disk from the symptomatic fruit, place it into a single, re-sealable plastic bag, label the bag as described above, and then place into a second, re-sealable

plastic bag. Multiple samples from the same field or greenhouse may be placed into the second bag. The knife and your gloves must be sanitized with a disinfectant between each plant.

#### Seed Sample Collection

The survey task force should include a certified seed sampler or experienced survey specialist. Seed samples should be collected from: 1) the original seed lot(s) sown in the CGMMV-infested field or greenhouse (if any seed remains), and, 2) seed lots that were harvested from seed production fields or greenhouses within the delimitation area.

Surveyors visiting sites to place holds or obtain samples should collect the following information for each seed sample:

- Date of collection or observations
- Collector's name
- Seed lot number
- Seed lot volume
- Full name of business, institution, or agency
- Full mailing address, including country
- Origin(s) of seed lot(s), including physical location(s), field- or nurserygrown, and country of origin
- Host plant species and specific crop plant variety, if applicable
- General conditions or any other relevant information

CGMMV-infected seeds are rarely symptomatic. Take the following actions to collect a representative sample from the seed lot:

- 1. Using a disposable cup, such as a 3- or 5-oz paper Dixie cup), obtain a sample of the seed lot. Five to 40 subsamples should be collected from each seed lot (Table 5-1)(Kruse et al., 2004).
  - a. For large seed lots, collect the required volume for that host (Table 5-1).
  - b. For small seed lots from which only a few seeds are available, collect 20 percent of the seed lot (Dombrovsky et al., 2017).
- 2. Combine the subsamples into a single, labeled plastic bag. Include the disposable cup in the sample bag. Do not re-use the disposable cup.
- **3.** Record the required information on the sample bag.
- **4.** Double bag the samples and deliver them within 48-hr to a diagnostic laboratory.

**Table 5-3** Required number of seed subsamples to collect, based on post-harvest seedlot size, from seed production fields in the delimitation area (modified from Kruse et al.,2004).

Lot size (kg)	Number of subsamples to collect	
1 to 100	Three (3)	
101-200	Six (6)	
201-300	Nine (9)	
301-400	Twelve (12)	
401-500	Twelve (12)	
501-600	Twelve (12)	
601-700	Fourteen (14)	
701-800	Sixteen (16)	
801-1,500	Sixteen (16)	
1,501-3,000	Sixteen (16)	
3,001-5,900	Twenty (20)	
>5,900	Thirty (30)	

**Table 5-2**Required seed sample sizes for hosts of *Cucumber green mottle mosaic*virus (7 CFR § 201.46, 2000).

Host	Seeds per gram	Required sample size
Cucumber	40	100 grams (4 ounces = 0.5 cups)
West India gherkin	153	27 grams (1 ounce = < 0.25 cups)
Melon	45	89 grams (3 ounces = 0.33 cups)
Pumpkin	5	800 grams (28 ounces = 3.25 cups)
Squash	14	286 grams (10 ounces = 1.25 cups )
Watermelon	11	364 grams (13 ounces = 1.67 cups)

#### Chapter

### **Control Options**

6

#### **Overview**

The complex epidemiology of this pathogen, including multiple modes of transmission and persistence in the soil, along with a lack of chemical treatment options for plants, fields, and greenhouses, makes eradication and control difficult.

PPQ decision-makers may use this information after a detection to eradicate or suppress CGMMV. The efficacy and feasibility of eradication will depend on the pest situation at the time of detection.

#### **Current Practices in Place in the United States**

Several companies that import cucurbit seed from foreign origins participate in the (National Seed Health Accreditation Pilot Program). Signatories to this program agree to test seed for CGMMV prior to importation and self-report positive detections to the National Seed Health System/PPQ. Positive seed lots are then destroyed prior to planting.

#### **Eradication Options**

#### **Host Removal**

All infected plant material must be removed and properly disposed of using APHIS-approved disposal methods. Cut, rake and burn all plant material in infected fields. If burning is not an option, disking of fields is an acceptable alternative to facilitate microbial decomposition of plant material. Disc fallow would be preferable to chemical fallow to speed breakdown of crop residues by microbial action. Alternatively, personnel may remove infected material and transport it to a landfill (load should be fully enclosed) for deep burial. Equipment must be pressure washed with detergent after use following the sanitation guidelines below:

#### Follow a two-step process for field or farm equipment:

- **1. Equipment must be power washed or steam cleaned** to remove all soil and debris before it leaves infested areas; **AND**;
- **2. All surfaces must be disinfected.** Surfaces must be exposed to the disinfectant for at least **15-minutes** using one of the following chemistries:
  - Sodium hypochlorite (Bleach): Active ingredient (AI): 6%; Dose: 5000 ppm. pH of the solution must be between pH 4 and 7 for efficacy. Add the same amount of acetic acid (white vinegar) as the sodium hypochlorite. Follow Label;
  - Potassium peroxymonosulfate + Sodium chloride: EPA reg. no. 71654-6; A.I.; 21.4 + 1.5%; Equivalent to 9.75% available chlorine; Dose: 2% (wt./vol.) or Powder, 2.7 oz./gal. water; Tablet, 16 tablets/gal. water; Sachets, 2 sachets/ gal. water; Follow Label;
  - Hydrogen dioxide + peroxyacetic acid: EPA reg. no. 70299-12; A.I.; 27.1 + 2 %; Dose: 1:50 or 2.5 fl. oz./gal; Follow Label.

To prevent infection of new host material, infested field(s) should remain free of CGMMV hosts for at least two (2) years. During this time, aggressive weed control must be practiced to prevent volunteer cucurbit and broadleaf weed host growth, and sanitation measures must be taken for equipment or personnel entering the fields to reduce the likelihood of spreading CGMMV. Alternatively, infested fields may be planted with non-hosts or remain fallow for two (2) years, again imposing vigilant control of volunteer cucurbit plants and susceptible weed hosts.

Fields should be subject to testing for the presence of CGMMV before they are returned to cucurbit production. This can be accomplished during the non-host crop or fallow period by using sentinel plants (susceptible hosts) distributed throughout the fields.

#### **Chemical Control**

At this time, no chemical controls are available to treat CGMMV infected plants in fields or greenhouses.

#### **Other Options**

#### Seed treatment

Planting seed that has tested CGMMV-free is the best way to prevent the virus's introduction into new areas (Dombrovsky et al., 2017). CGMMV is present on the surface of and within infected seeds (Reingold et al., 2015). The only way to completely eliminate CGMMV from infected seed is to destroy the seed.

Should CGMMV become established in an area, the following treatments may limit further spread via seed. However, none of them will completely eradicate CGMMV from infested seed:

- Heat treatment- Heating dried bottle gourd seeds to 72°C for 72hours eliminated CGMMV contamination (Kim, 2000; Kim et al., 2003). The same treatment reduced, but did not eliminate, CGMMV from contaminated melon and cucumber seeds (Reingold et al., 2015). Heat treatment is most efficient with fresh seeds (Macias, 2000);
- Chemical treatment- Treating infected cucumber and melon seeds for 30-minutes with 10 percent trisodium phosphate (TSP) reduced, but did not eliminate CGMMV contamination (Reingold et al., 2015). The following chemicals may be used to disinfect seed surfaces: 0.5-1.0 percent hydrochloric acid, 0.3-0.5 percent sodium hypochlorite, and 10 percent sodium phosphate (Macias, 2000);
- **Combination treatments-** Soaking seeds in 10% TSP for 30-minutes and then heating the seeds to 72°C for 72-hours reduced CGMMV contamination more than either treatment alone (Reingold et al., 2015).

#### **Alternative Control Techniques**

#### **Preventative Measures**

Because CGMMV is a seedborne pathogen, management should begin with the seed. Early detection and identification are critical to prevent introduction of CGMMV.

- Use seed and transplants from reputable sources. Make sure that seed is tested and declared free of CGMMV. Do not use untested seed (Dombrovsky et al., 2017; Park et al., 2005). Inspect all transplants for symptoms before planting.
- For transplant production, monitor seedlings regularly and test any suspected to be infected with CGMMV. If the virus is detected,

destroy all plants within three to five feet of the symptomatic seedlings (ASTA, 2018) to prevent further spread of the virus.

- During grafting operations, follow strict sanitation practices for all work surfaces and tools (Dombrovsky et al., 2017). The most effective disinfectants include nonfat dried milk (NFDM, 20 percent weight/volume plus 0.1 percent Tween 20), potassium peroxymonosulfate and sodium chloride (2 percent solution), benzalkonium chloride (50 percent solution), and sodium hypochlorite (0.6 percent solution) (Dombrovsky et al., 2017; Falk et al., 2016).
- Institute hygiene worker training programs that include sanitation of boots, clothing, hands, and tools (Dombrovsky et al., 2017).

#### Measures for reducing spread

- Keep records of seed and transplant sources so they can be traced if needed (Falk et al., 2016).
- Disinfect all agricultural machinery and equipment before moving between fields and farms (Dombrovsky et al., 2017).
- To prevent contamination from visitors and their vehicles, limit access to a secure place near the entrance to the farm; this site should have cleaning and disinfection facilities (Dombrovsky et al., 2017).
- Eliminate potential CGMMV reservoirs such as volunteer plants and alternate host weeds, particularly any weeds in the cucurbit family (Dombrovsky et al., 2017).
- Minimize crop handling and other procedures that may wound the plants, especially early in the growing season, as the most severe yield losses result from early infections (Dombrovsky et al., 2017).
- Institute hygiene and worker training programs that include sanitation of boots, clothing, hands, and tools in production operations (Reingold et al., 2016b).
- Scout plants in the field for symptoms of CGMMV infection; take tissue samples and have diagnostic testing done on suspect plants. Train field workers and fruit pickers to report any unusual-looking plants or fruit (ASTA, 2018; Dombrovsky et al., 2017; Falk et al., 2016).
- Follow a crop rotation strategy with two to three years between cucurbitaceous crops (Park et al., 2010; Falk et al., 2016; Dombrovsky et al., 2017).

#### **Biological Control**

At this time, no biological control options are available for CGMMV.

#### **Host Resistance**

As a long-term strategy for managing CGMMV, breeders are trying to identify resistance genes in cucurbit cultivars. Researchers in Israel and elsewhere have identified resistance genes in wild and cultivated muskmelons (*Cucumis myriocarpus, C. africanus, C. figarei, C. meeusii, C. zeyheri, C. ficifolius, C. melo* var. *momordica, C. melo* 'Kachri' and 'Chang Bougi')(Rajamony et al., 1987; Sugiyama et al., 2006). Only *C. figarei* was immune to infection, while the others were asymptomatic carriers of CGMMV (Rajamony et al., 1987; Sugiyama et al., 2006). However, no commercial cultivars have been developed yet.

### **Research Needs**

New technology, research or assessment is needed to:

- Develop an assay that will differentiate between deactivated CGMMV and infectious CGMMV;
- Determine how CGMMV is transmitted from infested seed to seedlings;
- Identify new disease management options for CGMMV, including identifying weed hosts in the United States.

Chapter

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