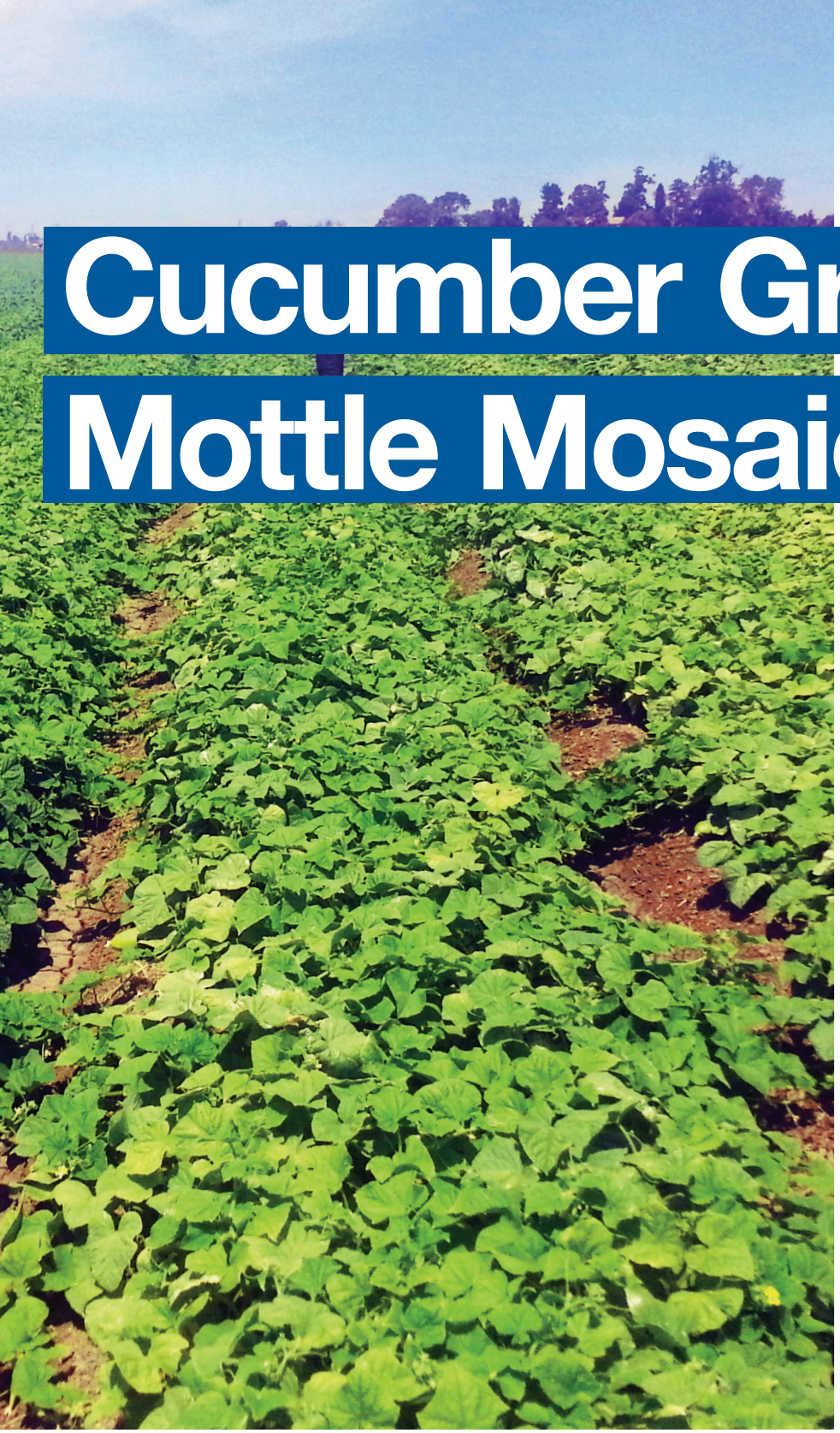






Cucumber Green Mottle Mosaic Virus

Photo credits (clockwise from left): HM.CLAUSE, Inc., Monsanto Vegetable Seeds, HM.CLAUSE, Inc.

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A Seed Production and Commercial Growers Guide

This educational pamphlet is intended to provide commercial and seed crop cucurbit growers with information about CGMMV and to provide recommendations for reducing the risk of occurrence of the disease in their crop productions. Contributors to this brochure include academic and industry CGMMV experts. It is sponsored and distributed by the American Seed Trade Association (ASTA), and was initiated as a response to the 2013 occurence of the disease in California. This is the first reported incident of CGMMV in the United States. Date: April 2014.

About CGMMV

Cucumber green mottle mosaic virus (CGMMV) which was originally described in 1935, was first reported in the U.S. in a melon seed production field in California in the summer of 2013. However, CGMMV, and the disease it causes has long been known in Europe, Asia, the Middle East, and more recently in Canada. CGMMV is a member of the genus tobamovirus, which also includes the well-known tobacco mosaic virus (TMV). TMV has an extremely wide host range that includes tomato, pepper, and tobacco. However, CGMMV is considered to have a narrow host range which is primarily limited to cucurbit species, including watermelon, melon, cucumber, pumpkin, squash, gourds, etc. In some environments and production cultures CGMMV can be especially problematic due to the ease with which it is transmitted and its stability and long viability in plant debris, in soil or on greenhouse or equipment surfaces. The damage it causes to the host plant and fruit can be extensive, resulting in substantial yield losses. Therefore, it is an important threat to fresh market, export and cucurbit seed industries in the areas where CGMMV is known to occur.



Typical early CGMMV symptoms on greenhouse cucumber plants.



Healthy greenhouse cucumber plants

Causes and Sources

Cucumber green mottle mosaic is caused by the cucumber green mottle mosaic virus (CGMMV), which is a rod-shaped, microscopic (300 nm long x 18 nm wide) particle. The virus is easily sap and seed transmissible, and it survives for long periods in infected crop debris. Transmission in seed has been most frequently reported in cucumber but it can occur in other species as well. The virus can be introduced into a crop production in many ways, but contaminated seed and soil are among the most common. The virus can only infect plants through wounds, and can survive and disseminate by several means, including:

- Infection of roots can occur in soil that is contaminated with infective plant debris. The virus can spread through root to root contact.
- CGMMV can spread in water or in nutrient solutions in soilless culture.
- Spread of the virus can occur very readily by mechanical transfer, especially in protected or high input culture systems where plants are pruned, staked, handled or touched frequently. In open field productions machinery used for cultivation or weed control can spread the virus.
- Infected rootstock plants can serve as source for outbreaks if plants are grafted.
- Seed harvested from infected plants may carry CGMMV, and transmission can be 20% or more.
- Some weed species such as squirting cucumber (*Echallium elaterium*) can have symptomless infection.
- The virus can survive for long periods on greenhouse or equipment surfaces or on tools.
- It has been reported that CGMMV was transmitted in pollen in artificial pollination under greenhouse conditions, but it has yet to be confirmed that spread occurs via pollen naturally in the field.
- To date no insect has been proven to vector CGMMV. It is theoretically possible that some chewing insects could vector the virus, but this has not been confirmed experimentally.

How to Diagnose CGMMV

Plant Symptoms



Seedlings: Symptoms may be indistinct or difficult to recognize as being caused by a virus on young seedlings. In severe infections cotyledons may become yellow but more often symptoms are not seen until the 1st or 2nd leaf stage. Plants with suspicious symptoms should be tested using acceptable CGMMV detection methods.



Leaf: CGMMV symptoms (mottling and mosaic on leaves, fruit mottling and distortion) can be confused with those caused by many other cucurbit viruses, making diagnosis based exclusively on visual symptoms unreliable. Early symptoms include vein clearing and crumpling on young leaves while mature leaves become bleached and chlorotic. There are different strains of the virus and depending on strain, mild to severe leaf distortion can occur, with leaf mottling and blistering, and plant stunting. Leaf distortion can be more severe at low temperatures when plants grow more slowly. The onset of symptoms after exposure varies according to host, amount of inoculum and environmental conditions but with cucumber symptoms can appear 7-14 days after infection.



Leaf symptoms on field grown melon leaves.



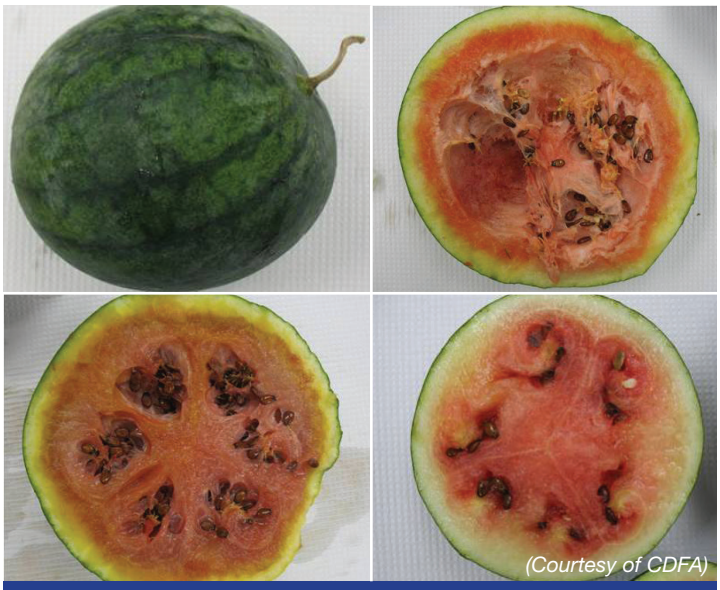
Mottling on mature watermelon leaves.



Fruit: These may be symptomless, at least externally, or can become severely spotted or streaked and distorted, especially during high temperatures. In some cases, fruit that show no external symptoms may be internally discolored or necrotic. This seems to be especially pronounced in watermelon.



Mottling and discoloration caused by CGMMV on cucumber fruit. Affected fruit will not be marketable.



External (top left) and internal symptoms on watermelon fruit caused by severe CGMMV infection.

Factors Influencing Symptom Expression

Environment (temperature, lighting)

Generally, a lower temperature early in the spring growing season with low light intensity tends to result in more severe symptoms than in the hot summer.

Strain of the virus

Some strains of CGMMV can induce more severe symptoms than others. Research in Europe is evaluating using a mild strain of the virus for cross-protection in greenhouse cucumber production.

Growing conditions

A protected culture production system with many more hands-on activities such as de-leafing, crop training and fruit picking tends to be prone for virus spread due to the ease of mechanical transmission of this virus.

Tissue type (leaf, fruit)

Symptoms of mottling mosaic and distortion are generally more visible on young growing leaves. In severely infected plants, fruit symptoms can occur, especially in cucumber and watermelon.

Host (species, variety)

All cucurbits are hosts to CGMMV but cucumber is more susceptible than other cucurbits with intense symptom expression on the infected plants. Currently, some CGMMV resistant cucumber cultivars are available commercially. Further investigations are needed to identify sources of genetic resistance for other cucurbit species.

Time of infection (crop stage)

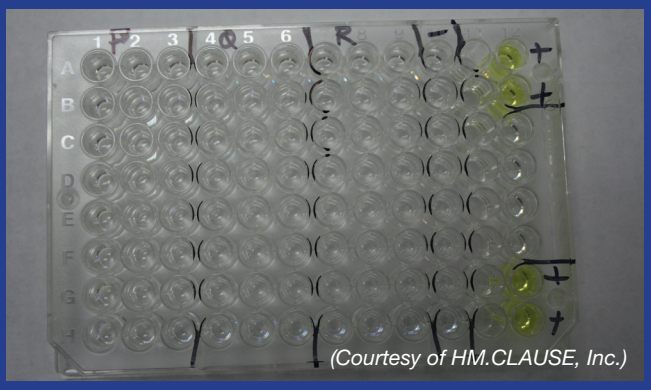
There is generally lower virus titer in an early Spring season crop than the late season fall crop. In protected culture, a new crop after greenhouse strip-cleaning has lower chance of infection than the subsequent crop because with the later crop inoculum could have built up earlier on the structure, carts, tools and clothing.

Growth stage (seedling-adult)

An early infection at the young seedling stage would generate more intense symptoms, resulting in greater yield losses. Thus, it is advisable to minimize unnecessary handling of plants in the first few weeks after transplanting. Adult plants are more tolerant to virus infection.

Laboratory or Field Detection Tests

Enzyme linked immunosorbent assay (ELISA) detects specific virus coat protein, and is commonly used to test seed for viruses.



ELISA seed assay test plate, showing positive (dark colored) control wells.

- ❒ Reverse transcription Polymerase Chain Reaction (RT-PCR) detects virus RNA.
- ❒ Real-time RT-PCR, also detects virus RNA.
- ❒ Loop-mediated isothermal amplification (LAMP) offers an alternative to PCR with shorter reaction time and without as much sample preparation.
- ❒ Bioassay through mechanical inoculation on host plant detects viable virus. The reaction on inoculated plants can vary depending on virus strain and environmental conditions. Therefore, additional research is needed to determine which strain(s) of virus can be detected through inoculation of specific hosts under which conditions.
- ❒ ImmunoStrip (www.agdia.com, no. ISK 45700) is a dip-stick type test that allows for rapid onsite detection of all CGMMV strains.



Positive ImmunoStrip reaction for CGMMV

Recommendations for CGMMV Control

Seed Use

❒ Like other tobamoviruses, CGMMV is thought to be found primarily on the surface, and to a lesser extent internally, in the seed and can maintain its infectivity for years.

❒ All sources of seed (experimental, parent seed, trial varieties or commercial, or if grafting is performed, the seed of the rootstock and scion) should be tested and found to be "negative" or with "no evidence" of CGMMV using an appropriate seed sampling and testing method. Note that these results help ensure, but do not guarantee that the seed lot is free of CGMMV. Typically the sample would be at least 1,500 – 2,000 seeds or 10% of the total for smaller lots.

❒ Tests should be conducted by a reliable and recognized laboratory with seed testing experience. Testing labs could use the National Seed Health System (http://www.seed health.org/index.html) (NSHS, test accreditation pending), or International Seed Testing Association (ISTA) validated method (https://www.seedtest.org/upload/cms/user/SH-07-026-2014.pdf).

❒ It is recommended that seed lots that have not been tested for CGMMV either not be used, or be planted in isolation so that the crop cannot serve as a source of infection for others if the pathogen is in the seed.

❒ Several common seed treatment methods (including thermotherapy at 72C for 3 days, chemotherapy in 10% trisodium phosphate or a combination of both) have some effect on CGMMV infectivity in seeds. However, the completeness in deactivation of CGMMV infectivity in seeds should be confirmed through bioassay.

> Successful management of CGMMV requires that everyone in the production chain from seed to commercial production must do their part. Following the practices mentioned below will help reduce the chance of introduction and spread of the virus, and associated crop losses.

Transplant Production

❒ Use seed from a reputable producer that has been adequately tested for CGMMV.

❒ Regularly inspect seedlings, beginning at about the two true leaf stage of growth. Note that symptoms of CGMMV most likely will be subtle and difficult to recognize when plants are small. To date there are no reported incidences of CGMMV being found in cucurbit transplant production greenhouses in the US, but it is still judicious to take appropriate precautions.

❒ If the virus is detected the most prudent course of action would be to destroy all plants within a minimum of 3-5 feet beyond the outermost symptomatic seedling. It is recommended that the presence of CGMMV on suspect plants be confirmed through appropriate lab tests before taking plant destruction actions.

❒ The virus is efficiently transmitted through mechanical means and requires a wound to infect plants. Worker's hands, clothing and tools can spread the virus to healthy plants during de-leafing, crop handling, fruit harvesting, or after packaging of infected fruit.

❒ Sterilize or destroy all plant trays that contained infected plants prior to reuse. Several products, including potassium peroxymonosulfate (Virkon S) or freshly prepared 0.5% sodium hypochlorite (NaOCl) bleach, can be effective disinfectants. In protected productions the use of Non-Fat Dry Milk ((NFDM) 3.5% protein) could prevent spread of several tobamoviruses and therefore could be effective against CGMMV.

Transplant Production (cont'd)

❒ Inspect areas surrounding the transplant greenhouse or growing facility and destroy all cucurbit weeds that could serve as a host for the virus.

❒ CGMMV can easily spread in the grafting process. Ensure that tools are sterilized by dipping in 2% Virkon or freshly prepared 10% bleach. Be sure to rinse tools and hands afterwards to remove residue. Inspect grafted plants often for the presence of the symptoms.

❒ It is strongly recommended that nurseries/transplant operations implement a comprehensive hygiene program which includes the use of disinfectants/sanitizers and protective clothing for workers and visitors.





Good sanitation prior to entering (for inspection, etc.) a commercial seed production field (as depicted with the broccoli field) would involve sanitization of boots, clothing, hands and implements

Commercial Production Growers: Open field

❒ Use seed from a reputable producer that has been adequately tested for CGMMV. If transplants are used, ensure that they are inspected for the disease prior to planting.

❒ Follow good sanitation and cultural practices which include controlling weeds, especially cucurbit species that border fields, and chewing insects as a precaution.

❒ Inspect fields for symptoms at regular intervals. Take plant tissue samples and have a diagnostic analysis completed on suspect plants.

Commercial Production Growers: Protected culture

❒ Use seed from a reputable producer that has been adequately tested with no evidence for CGMMV.

❒ Seed treatment on a CGMMV-contaminated seed lot with chemotherapy with 10% trisodium phosphate or thermotherapy at 72C for 3 days has been reported in scientific literature to have some effect against CGMMV infectivity with no major adverse effect on seed germination.

❒ Prevent cross-contamination between greenhouses in different zones and from packing houses by using disinfectants at each entrance. Only personnel with clean clothing and disinfected equipment should be allowed to enter a cleaned greenhouse.

❒ Follow hygiene practices by washing hands with soap or disinfectants after handling plants.

❒ Minimize additional crop handling in the first few weeks after transplanting.

❒ In hydroponic production systems, avoid recirculation of the nutrient solution for several weeks after transplanting because CGMMV has been shown to maintain its infectivity in irrigation water.

Commercial Production Growers: Protected culture (cont'd)

❒ The growing plants should be inspected by experienced technicians for early disease symptoms, confirmation tests on suspect plants conducted by lab analysis and diseased plants carefully removed and placed in a container (plastic bag, etc.) for disposal. Take care to avoid touching the container to remaining plants in the greenhouse during removal.

❒ Sanitize cutting tools after each plant with disinfectants (such as 2% Virkon, freshly prepared 10% bleach solution or NFDM in de-leafing, fruit picking, pruning and other crop plant handling activities.

❒ Thoroughly clean and disinfect the greenhouse at the end of a crop season.

Seed Increase Growers

❒ Use seed from a reputable producer that has been adequately tested for CGMMV.

❒ Inspect planters for cleanliness and any plant debris prior to planting.

❒ Inspect fields for symptoms of CGMMV and other diseases and pests as needed during the crop production cycle.

❒ When conducting field inspections it is recommended that shoes, clothing and hands be disinfected between fields, and any field suspected of having CGMMV be inspected last.

❒ Fields should be walked for inspection in a systematic pattern to increase the chances of finding isolated disease outbreaks.

❒ All equipment should be power washed with a high pressure cleaner at seed harvest. The harvester should be inspected and re-cleaned if necessary.

❒ All seed washers should be cleaned with a high pressure washer, inspected and re-cleaned if debris or seed from a previous crop remain.

❒ Seed dryers should be thoroughly cleaned out and swept clean between lots and then re-inspected. Seed cleaning/ conditioning equipment should be cleaned and inspected between seed lots.Insects have not been proven to vector CGMMV but controlling chewing insects such as cucumber beetle as a part of a good pest control program could be of benefit.



Cucurbit seed harvest equipment.



Cucurbit seed drying equipment.

What to do if CGMMV is Suspected

An easy to use, rapid diagnostic kit from Agdia®, the ImmunoStrip® is commercially available. This type of test can be useful for developing a preliminary diagnosis, but a more thorough, comprehensive evaluation for confirmation is recommended. Take a sample of symptomatic tissue to the local extension office, state university or private diagnostic laboratory for the confirmation.

Question and Answers

What makes CGMMV such a problematic disease for greenhouse cucurbit produce production?

Because of many hands-on activities associated with greenhouse cucumber and other cucurbit production, CGMMV outbreaks have been observed frequently in greenhouse cucumber production in some global locations (not yet in the U.S.). A primary source of CGMMV infection is from contaminated seed or seedlings and a secondary source is from mechanical spread through hands-on activities. The potential for pollen transmission of CGMMV is not well understood, so it will be important to discern the possible role of bumble bees and other pollinators in CGMMV spread.

What is the most susceptible cucurbit to CGMMV?

Resistance has been identified in cucumber, and functions by reducing virus multiplication and spread in the plant. There are a limited number of commercial varieties in the heated greenhouse market (primarily Europe and Canada) that use this resistance. All other cucurbit crops, with the exception of some varieties used as rootstocks in grafting, are susceptible to CGMMV infection. Cucumber that does not have this resistance can be very susceptible and the infected plants typically develop mottle mosaic symptoms. Melon and watermelon are susceptible too, followed by squash and pumpkins. Bottle gourd, which is commonly used as a rootstock, is very susceptible to CGMMV. Screening cucurbit germplasm to identify sources of resistance is a first step in developing CGMMV resistant cultivars through plant breeding.



Heavily infected bottle gourd plant.

Is the incorporation of plant resistance a solution to control CGMMV?

The ideal solution in managing tobamovirus diseases is to use a disease resistant cultivar. There is only limited public information on cucurbit germplasm with resistance to CGMMV. Some transgenic materials with CGMMV resistance have been developed, but their application will be not easy. Therefore, additional screening of the USDA germplasm of cucumber and other cucurbits for resistance is necessary.

Does a seed sanitation method exist to control CGMMV?

There are reports that seed treatments with thermotherapy at 72C for 3 days or chemotherapy with 10% trisodium phosphate can be effective. However, such treatments may only reduce the virus titer, and thus only reduce virus infectivity and not eradicate the virus. Therefore, it is necessary to conduct bioassay to verify the deactivation of the virus infection from treatment. Additional efforts in searching for better chemical or treatment procedures may be necessary.

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In cooperation with

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HM.CLAUSE, Inc.

Additional sources of information on CGMMV:

CABI Crop Protection Compendium (http://www.cabi.org/cpc/)

Kim SM, Nam SH, Lee JM, Yim KO, Kim KH, 2003. Destruction of Cucumber greenmottle mosaic virus by heat treatment and rapid detection of virus inactivation by RT-PCR. Molecules and Cells 16, 338-42.

Ling, K.-S., Li, R. and Zhang, W. 2014. First Report of Cucumber green mottle mosaic virus Infecting Greenhouse Cucumber in Canada. Plant Dis. 98:000, 2014; published online as http://dx.doi.org/10.1094/PDIS-09-13-0996-PDN.

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