Tobamovirus Cucumber green mottle mosaic virus

Scientific Name
Tobamovirus Cucumber green mottle mosaic virus

Synonyms
None

Virus/viroid Name(s)
***Cucumber green mottle mosaic virus* (CGMMV)**,
Bottlegourd Indian mosaic virus, CuGMMV
Cucumber green mottle mosaic watermelon strain W,
Cucumber mottle virus, Cucumber virus 3
Cucumber virus 4, Cucumis virus 2, Tobacco mosaic virus watermelon strain – W, White break virus

Type of Pest
Virus

Taxonomic Position
Class: Unassigned, **Order:** Unassigned, **Family:** Virgaviridae

Figure 1. Green mottling symptoms on cucumber leaves. Kai Ling, USDA-ARS.

Reason for Inclusion in Manual
Pests of Economic and Environmental Concern Listing 2017

Background Information
Tobamoviruses are known to cause severe crop diseases and are responsible for significant economic losses worldwide in a wide range of plant species (Reingold et al., 2016). *Cucumber green mottle mosaic virus* (CGMMV) was first described in the 1930s as Cucumber virus 3 in the United Kingdom (UK) (Ainsworth, 1935) and has since spread among cucurbit crops worldwide and caused significant economic losses (Reingold et al., 2015). This virus was detected in the western hemisphere for the first time in 2013, when it was found in both Canada (Ling et al., 2014) and the United States (Tian et al., 2014). The find of CGMMV in the United States is considered a regulatory incident with the infested fields under the control of the California Department of Food and Agriculture (CDFA) (NPAG, 2013).

Pest Description
Tobamoviruses are characterized by their unique rod-shaped viral particles, ~300 nm in length, that include a single positive-sense strand of RNA, ~6.4 kb (Tan et al., 2000; Yoon et al., 2008) that encodes replication proteins (126 and 180 kDa), a movement protein (MP, 30 kDa), and a coat protein (CP, 17 kDa) (Dorokhov et

Figure 2. Top: Typical early CGMMV symptoms on greenhouse cucumber leaves. Middle: CGMMV infection on greenhouse cucumber. Bottom: CGMMV symptoms, which are not always obvious, in cucumber fruit. Kai Ling, USDA-ARS.

Figure 3: Symptoms of CGMMV infection in infected squash (left) and cucumber (right). Courtesy of Seminis Vegetable Seeds, Inc.

al., 1994). Tobamovirus particles are known to be very stable and can persist for long periods without a living host in plant debris, soil, or on seed surfaces (Reingold et al., 2015).

Numerous different isolates or strains of CGMMV have been characterized from different countries in Asia and Europe (Shang et al., 2011). Different strains of CGMMV may vary in their cucurbit host range and experimental host reactions (NPAG, 2013).

Biology and Ecology

Tobamoviruses, including CGMMV, are not known to be transmitted by any insect vector, but they are efficiently transmitted by mechanical means, including workers' hands, clothing, and tools (Broadbent & Fletcher, 1963). In addition to mechanical transmission, CGMMV is also transmissible in seed (Reingold et al., 2015), sap (ASTA, 2014), pollen (Liu et al., 2013), and water (NPAG, 2013). Although the rate of natural virus transmission through seed is relatively low, the ease of mechanical transmission of CGMMV from a contaminated seedling to adjacent plants, especially in propagation houses, makes this virus very contagious (Ellhouze et al., 2016)

After infecting a few cells in a susceptible host, CGMMV then colonizes the plant tissues through the vascular system, primarily the phloem (NPAG, 2013). The viral coat protein of CGMMV has been visualized in several infected host plant tissues, including: phloem, xylem, trichomes, and grasping tendrils (Reingold et al., 2016). The severity of CGMMV infection can vary depending on the growing season. Generally, infection is more

severe early in spring in lower temperature and light intensity conditions (ASTA, 2014). Yield loss is reduced significantly when infection is delayed for six weeks (Fletcher, 1969).

In 2005, CGMMV was unexpectedly found in Antarctica near a Ukrainian research station (Polischuk et al., 2007). It is currently unknown how the virus arrived there, but this discovery is a testament to the capability of CGMMV to travel long distances and survive in harsh weather conditions.

Figure 4. CGMMV symptoms on infected bottle gourd leaves. Kai Ling, USDA-ARS.

Symptoms/Signs

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 symptoms are usually not seen until the first or second leaf stage" (ASTA, 2014).

Leaf: Early symptoms include vein clearing (the disappearance of green color in or around leaf veins) and crumpling on young leaves, while mature leaves become bleached and chlorotic (Fig. 1, 2) (ASTA, 2014; Reingold et al., 2016). 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. Symptoms in cucumber leaves can appear within 7 to 14 days after infection.

Fruit: Typical symptoms in infected fruit include green mottling, spotting, and streaking (Fig. 3) (ASTA, 2014; Reingold et al., 2016). Infected fruit, however, do not always show symptoms (Fig. 2) (ASTA, 2014). In some cases, fruit that show no external symptoms may be internally discolored or necrotic. This seems to be especially pronounced in watermelon (Fig. 6) (ASTA, 2014; Tesoreiro et al., 2016).

Other symptoms in cucumber: In the early infection, young leaves of infected cucumber plants displayed light green mottle and blisters. The infected plants were stunted in growth, with darker green blisters and green mottle mosaic symptoms on mature leaves (Ling et al., 2014). Root systems in inoculated cucumber seedlings are underdeveloped compared to healthy seedlings (Fletcher, 1969).

Figure 5: Top. Damage to the fruit stalk (left) and necrosis on the fruit peduncle (right) in infected watermelon. Bottom. Leaf mottling in infected watermelon (left) and Infected watermelon compared to a healthy melon (right). Courtesy of Barry Conde, NT Dept Prim Ind & Fish, Australia.

Other symptoms in watermelon: In addition to leaf mottling, symptoms include damage to the fruit stalk, necrosis on the fruit peduncle, and underdeveloped fruit (Fig. 6, 7) (ASTA, 2014; Tesoriero et al., 2016).

Pest Importance

The unique property of stability combined with high infectivity has elevated the economic importance of CGMMV (ASTA, 2014; Ellouze et al., 2016). A small number of infected plants in a cucumber greenhouse can eventually lead to the spread of CGMMV to the entire crop, and infection may occur either in the current crop or in subsequent crops (Ellouze et al., 2016). Seed treatment is a commonly used measure to control CGMMV, but treatments using heat and/or chemicals are not totally effective at de-activating the virus (Reingold et al., 2015).

Figure 6: Stunted fruit ripening (left) and rotten flesh (right) in infected watermelon. Courtesy of Barry Conde, NT Dept Prim Ind & Fish, Australia.

In severe cases of infected greenhouses in Canada, diseased cucumber plants were widely distributed inside the greenhouse, resulting in estimated yield losses of 10 to 15% (Ling et al., 2014). Similar yield losses of about 15% have been reported in cucurbit crops elsewhere (Shang et al., 2011). In Israel, infection rates surpassed 90% in some greenhouses, and total losses in some watermelon fields were reported there in 2013 (Reingold et al., 2016).

CGMMV is known to infect numerous crops which are important in the United States. In 2015, watermelon was grown on 115,750 acres in the United States, and the total value of the watermelon crop was $483 million. Cucumbers were grown on 37,980 acres, and the total value of the cucumber crop was $177 million. Squash was grown on 38,690 acres, and the total value of the squash harvest was $174 million (USDA-NASS, 2016).

Cucumber green mottle mosaic virus is listed as a harmful organism in the following countries: Chile, China, Colombia, Ecuador, French Polynesia, Georgia, Honduras, Indonesia, Israel, Japan, New Caledonia, New Zealand, Paraguay, Peru, Taiwan, Thailand, and Timor-Leste (USDA-PCIT, 2016). There may be trade implications with these countries if this virus becomes established in the United States.

Known Hosts

Major hosts: *Citrullus lanatus* (watermelon) *C. vulgaris* (watermelon), *Cucumis melo* (melon), *C. sativus* (cucumber), *Cucurbita* spp. (squash), and *Lagenaria siceraria* (bottle gourd) (Varveri et al., 2002; Kim et al., 2003; Boubourakas et al., 2004; Yoon et al., 2008; NPAG, 2013; Reingold et al., 2013; Tesoreiro et al., 2016).

Minor hosts: *Amaranthus blitoides* (matweed), *A. retroflexus* (amaranth) *Barbilophozia* spp. (liverwort), *Chenopodium album* (lambsquarters; fathen), *Cucumis anguria* (West Indian gherkin), *Deschampsia antarctica* (Antarctic hair grass), *Ecballium elaterium* (squirting cucumber), *Heliotropium europaeum* (heliotrope), *Heracleum moellendorffii*

(eosuri), *Luffa acutangula* (loofah), *Polytrichum* spp. (haircap moss), *Portulaca oleracea* (purslane), *Solanum nigrum* (nightshade), and *Trichosanthes cucumerina* (snake gourd) (Varveri et al., 2002; Polischuk et al., 2007; Cho et al., 2015; NPAG, 2013).

Experimental Hosts: *Chenopodium amaranticolor* (lambsquarter), *Chenopodium quiñoa* (quiñoa), *Datura stramonium* (jimsonweed), *Momordica charantia* (bitter gourd), *Nicotiana benthamiana*, *Physalis floridana* (husk tomato), *Portulaca oleracea* (purslane), *Solanum lycopersicum* (reported as *Lycopersicum esculentum* (tomato)), and *Tetragonia expansa* (New Zealand spinach) (Boubourakas et al., 2004; Yoon et al., 2008; NPAG, 2013; Reingold et al., 2015).

Note: Tobacco (*Nicotiana tabacum*) is reported as an experimental host of CGMMV (Yoon et al., 2008), but this host is not systemically infected by the virus. Local lesions are produced in inoculated *N. tabacum,* but CGMMV is very unlikely to establish in this crop (Ling, 2016, personal communication).

Known Vectors (or associated insects)

Cucumber green mottle mosaic virus is not known to be vectored, nor is it known to vector any pathogens.

Known Distribution

Africa: Zambia, Zimbabwe**. Antarctica:** Argentine Islands. **Asia:** China, India, Indonesia, Iran, Israel, Japan, Jordan, Lebanon, Myanmar, Pakistan, Saudi Arabia, Sri Lanka, Syria, Taiwan, Turkey. **Europe:** Austria, Bulgaria, Czech Republic, Denmark, Finland, Germany, Greece, Hungary, Latvia, Lithuania, Netherlands, Romania, Russia, Spain, Sweden, United Kingdom, Ukraine, Yugoslavia (former). **Oceania:** Australia (Northern Territory). **North America:** Canada (Alberta), United States (California)* (Ainsworth, 1935; Varveri et al., 2002; Boubourakas et al., 2004; Polischuk et al., 2007; Yoon et al., 2008; Al-Tamimi et al., 2009; Shang et al., 2011; NPAG, 2013; Reingold et al., 2013; Ling et al., 2014; Nematollahi et al., 2014; Tian et al., 2014; CABI, 2016; Tesoreiro et al., 2016)

*Note: The find of CGMMV in the United States is considered a regulatory incident with the infested fields under the control of the California Department of Food and Agriculture (CDFA) (NPAG, 2013).

Pathway

The most likely pathway of CGMMV entry into the United States is through the transport of infected seed. Treatments of seed using heat, trisodium phosphate (TSP), and a combination of the two, were all determined to be insufficient at eliminating the infectivity of the CGMMV in cucurbit seeds (Reingold et al., 2015). Other possible pathways include infected planting material or soil (Reingold et al., 2013), infected shipping containers (NPAG, 2013), and pollinators moving infected pollen (Liu et al., 2013).

In 2013, CGMMV was detected for the first time in the United States, in adjacent melon and cucumber fields in California. The seed lot for the melon field was found to contain CGMMV and was likely the source of infection in California (Tian et al., 2014). The source country of this infected seed is under investigation. The CGMMV isolate found in Canada was 99% identical to strains found in Asia, suggesting Asian origin of the isolate (Ling et al., 2014). The isolate found in the United States, however, showed far less homology to Asian strains (92%) (Tian et al., 2014), suggesting a possible different geographic origin.

Cucumis melo (melon) is a major host of CGMMV, and there are currently no restrictions on the import of propagules of this plant species. While seeds are subject to FSA-V regulations, there are no other import restrictions (USDA, 2016). *Citrullus lanatus* (watermelon) and *Cucurbita* spp. (squash), two other major hosts of CGMMV, also have no import restrictions other than the FSA-V regulation (USDA, 2016). Propagules of all of these hosts are regularly imported from countries known to have CGMMV (AQAS, 2016). Since 2006, there were 560 shipments of *Cucurbita* spp. propagative material from China, and these shipments contained a combined total of over 955,000 kg of seed. There were also shipments of *Cucurbita* spp. seed from Japan (249), India (40), and Spain (11) during this timeframe (AQAS, 2016). In addition, there have been shipments of *Citrullus* spp. propagative material from the following host countries since 2006: China (61), India (22), and Japan (22) (AQAS, 2016). There have also been 312 shipments of *Cucumis melo* propagative material from China since 2006, containing a combined total of over 245,000 kg of seed. There have been many shipments of *Cucumis melo* seed from other known host countries since 2003, including: Spain (167), Japan (160), India (97), Turkey (31), Germany (16), Russia (5), and the United Kingdom (4) (AQAS, 2016).

Potential Distribution within the United States

Based on the current known distribution of CGMMV, the virus is likely to survive outdoors in plant hardiness zones 4-12, which encompass virtually the entire continental United States (NPAG, 2013). CGMMV may also be found in any plant hardiness zone in greenhouses. Cucurbit crops are grown commercially and in backyard gardens throughout the United States (NPAG, 2013). *Citrullus* spp., *Cucumis* spp., and *Cucurbita* spp. are all widely distributed throughout the United States (BONAP, 2015).

A recent combined host distribution map for CGMMV developed by USDA-APHIS-PPQ-CPHST (Fig. 7) identifies areas of high host acreage based on the combined acreage of cucumber, squash, and watermelon. This map illustrates that though there are counties in nearly all states with a low level of risk. California, Florida, Georgia, Indiana, Maryland, Michigan, Missouri, New Jersey, New York, North Carolina, Texas, and Wisconsin, however, have counties with the highest level of risk for CGMMV based on host density. The host distribution maps are based on county level data. To combine host data for pest-specific analyses, CPHST normalizes the data by dividing the total host present in a county by overall county area (acres of host in county/ total acres of county). This yields host by county area and allows CPHST to properly combine host distributions without the skewing effects of overall county size. For example, 500 acres

of broccoli grown in Tulare County, CA can now be compared to 500 acres of broccoli grown in Scott County, AR. The individual host acreage maps for cucumber, squash, and watermelon are provided in the Appendix at the end of the document.

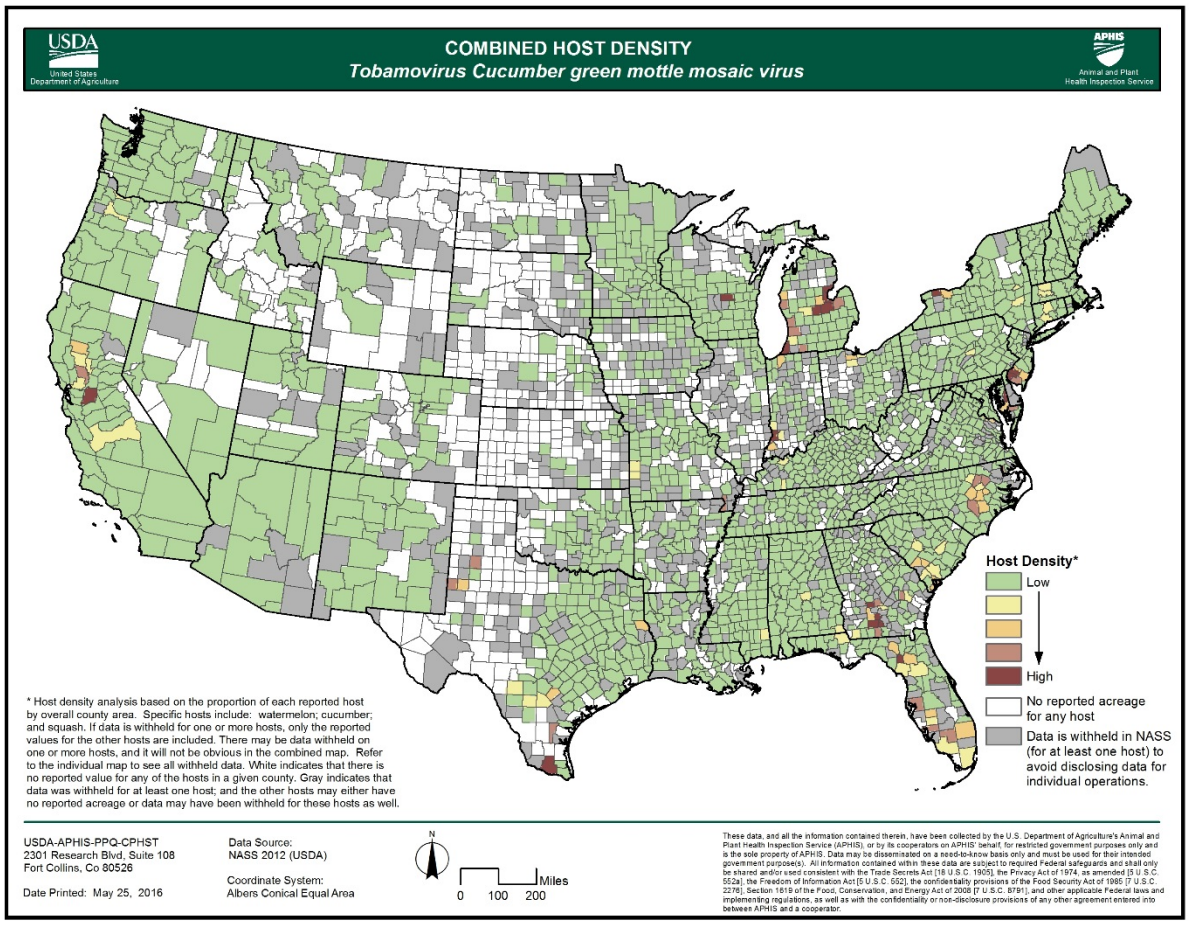

Figure 7. Combined distribution map for *Tobamovirus Cucumber green mottle mosaic virus* within the continental United States. Values represent combined host density low to high (cucumber, squash, and watermelon). Map courtesy of USDA-APHIS-PPQ-CPHST.

Survey

Approved Method for Pest Surveillance*:

The CAPS-approved survey method is visual survey for symptomatic host material.

*For the most up-to-date methods for survey and identification, see Approved Methods on the CAPS Resource and Collaboration Site, at http://caps.ceris.purdue.edu/.

Literature-Based Methods:

Characteristic symptoms, including a mosaic and mottling of leaves and fruit, can indicate CGMMV infection, although the cause of the symptoms must be confirmed by molecular or serological methods (ASTA, 2014).

Key Diagnostics

Approved Method for Pest Surveillance*:

Serological: There are several field-based screening aids available for CGMMV surveying. An immunoStrip test is available from Agdia that does not cross react with other known viruses that infect cucurbits (no. ISK 45700). Another test kit is available from Hangzhou Keen Biotech Co., Ltd. http://www.keenbio.net/pdes.asp?id=1). These tests may be used on suspect symptomatic host material. At least two DAS-ELISA kits are also commercially available for CGMMV detection in symptomatic leaves (LOEWE®FAST Kit CGMMV and Agdia ELISA Reagent Set for CGMMV).

Note: Agdia serological tools may not detect all isolates of CGMMV. Confirmation requires a combination of molecular techniques and sequencing.

*For the most up-to-date methods for survey and identification, see Approved Methods on the CAPS Resource and Collaboration Site, at http://caps.ceris.purdue.edu/.

Literature-Based Methods:

There are many published molecular diagnostic methods which can detect CGMMV. For example, the virus can be detected by conventional RT-PCR, and primers using the highly conserved regions of CGMMV movement protein (MP) and coat protein (CP) can detect different strains of the virus. Numerous different sets of primers have been designed for CGMMV detection by RT-PCR (Varveri et al., 2002; Boubourakas et al. 2004; Ling et al., 2014; Reingold et al., 2015).

Li et al. (2013) developed a one-step reverse transcription loop-mediated isothermal amplification (RT-LAMP) for the detection of CGMMV in watermelon plants or seeds.

Shang et al. (2011) describe five serological methods for detection of CGMMV, including: Antigen-coated plate enzyme-linked immunosorbent assay (ACP-ELISA), triple antibody sandwich enzyme-linked immunosorbent assay (TAS-ELISA), Dot-immunobinding assay (DBIA), direct tissue blot immunoassay (DTBIA), and immunocapture reverse transcriptase polymerase chain reaction (IC-RT-PCR).

Easily Confused Species

Symptoms of CGMMV can be confused with those caused by many other cucurbit viruses, making diagnosis based exclusively on visual symptoms unreliable (ASTA, 2014). Mixed infections with other viruses are common. In a study in the Middle East, several other viruses were found in squash and watermelon fields which contained CGMMV including: *Arabis mosaic virus* (ArMV), *Cucumber Mosaic virus* (CMV), *Squash leaf curl virus* (SLCV), *Squash mosaic virus* (SqMV), *Watermelon mosaic virus* (WMV), and *Zucchini yellow mosaic virus* (ZYMV) (Al-Tamimi et al., 2009). Mixed infections containing CGMMV and *Melon necrotic spot virus* (MNSV) were reported in cucumber in Canada (Ling et al., 2014). Melons infected with CGMMV in California were also found to be infected with SqMV (Tian et al., 2014). Molecular confirmation is necessary to identify CGMMV.

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Reviewers:

- Kai Ling, U.S. Vegetable Seed Laboratory, USDA-ARS
- Vessela Mavrodieva and the CPHST Beltsville lab, USDA-APHIS-PPQ-CPHST, Beltsville, MD

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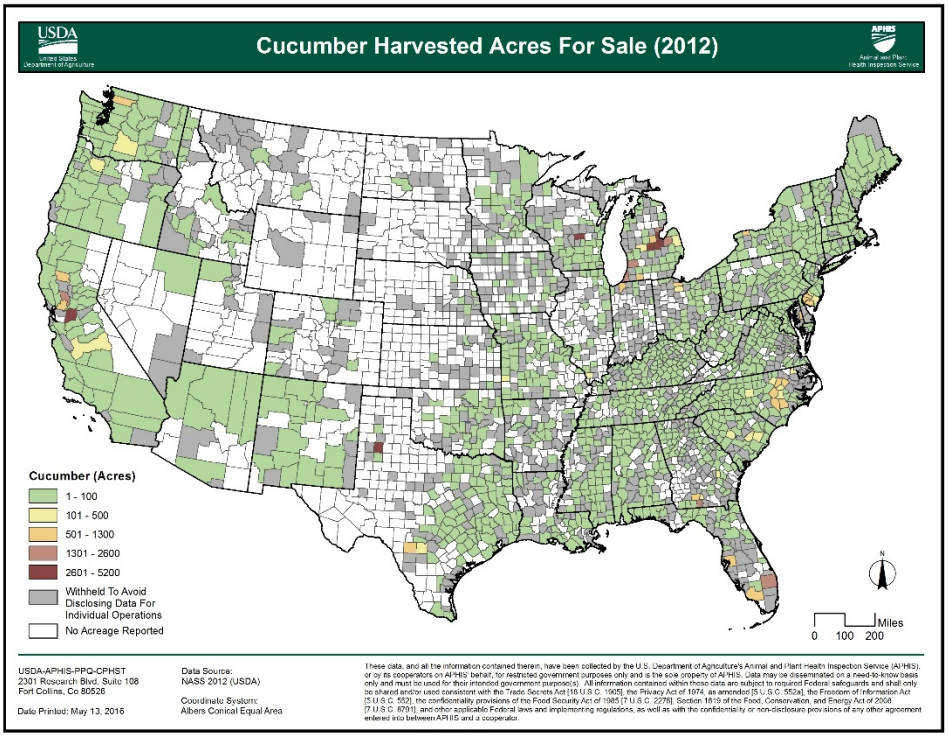
Appendix:


USDA
United States Department of Agriculture
Cucumber Harvested Acres For Sale (2012)
APHIS
Animal and Plant Health Inspection Service
Cucumber (Acres)
1 - 100
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1301 - 2600
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Withheld To Avoid Disclosing Data For Individual Operations
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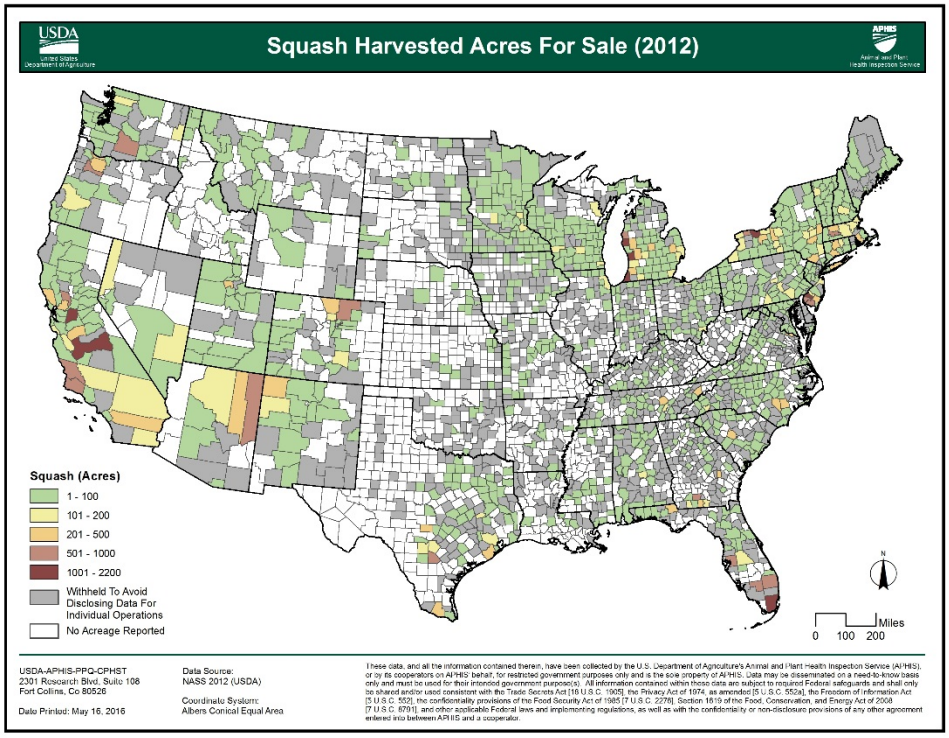
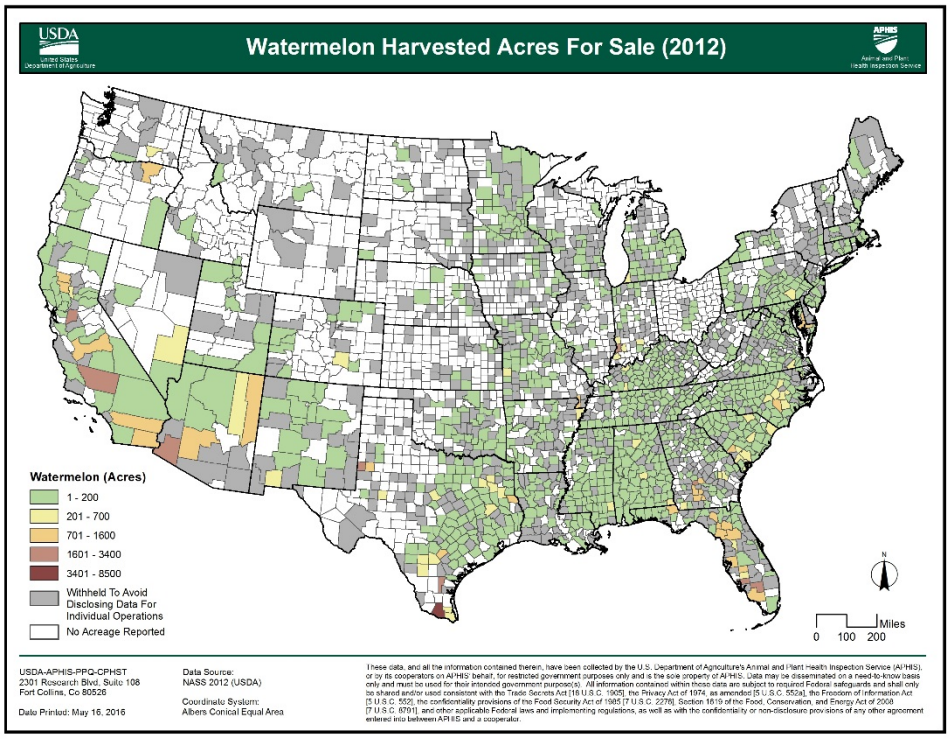

USDA
United States Department of Agriculture
Squash Harvested Acres For Sale (2012)
APHIS
Animal and Plant Health Inspection Service
Squash (Acres)
1 - 100
101 - 200
201 - 500
501 - 1000
1001 - 2200
Withheld To Avoid Disclosing Data For Individual Operations
No Acreage Reported
N
0 100 200 Miles
USDA-APHIS-PPQ-CPHST
2301 Research Blvd, Suite 108
Fort Collins, Co 80526
Date Printed: May 16, 2016
Data Source:
NASS 2012 (USDA)
Coordinate System:
Albers Conical Equal Area
These data, and all the information contained therein, have been collected by the U.S. Department of Agriculture's Animal and Plant Health Inspection Service (APHIS), or by its cooperators on APHIS' behalf, for restricted government purposes only and is the sole property of APHIS. Data may be disseminated on a need-to-know basis only and must be used for their intended government purpose(s). All information contained within these data are subject to required Federal safeguards and shall only be shared and/or used consistent with the Trade Secrets Act [18 U.S.C. 1905], the Privacy Act of 1974, as amended [5 U.S.C. 552a], the Freedom of Information Act [5 U.S.C. 552], the confidentiality provisions of the Food Security Act of 1985 [7 U.S.C. 2276], Section 1619 of the Food, Conservation, and Energy Act of 2008 [7 U.S.C. 8791], and other applicable Federal laws and implementing regulations, as well as with the confidentiality or non-disclosure provisions of any other agreement entered into between APHIS and a cooperator.


USDA
United States Department of Agriculture
Watermelon Harvested Acres For Sale (2012)
APHIS
Animal and Plant Health Inspection Service
Watermelon (Acres)
1 - 200
201 - 700
701 - 1600
1601 - 3400
3401 - 8500
Withheld To Avoid Disclosing Data For Individual Operations
No Acreage Reported
N
0 100 200
Miles
USDA-APHIS-PPQ-CPHST
2301 Research Blvd. Suite 108
Fort Collins, Co 80526
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