Tospovirus Groundnut bud necrosis virus

Scientific Name
Tospovirus Groundnut bud necrosis virus

Synonyms
Groundnut bud necrosis (GBNV) bud blight disease, bud necrosis disease (BND), bud necrosis virus, groundnut bud necrosis disease, groundnut bud necrosis tospovirus, mung bean leaf curl disease, peanut bud necrosis, peanut bud necrosis tospovirus, peanut bud necrosis virus (PBNV).

Type of Pest
Virus

Taxonomic Position
Class: Not assigned, **Order:** Not assigned, **Family:** Bunyaviridae

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

Figure 1. Wilting of GBNV infected tomato plants (right) compared to healthy tomato plants (left). Courtesy of Naidu Rayapati, Washington State University.

Background Information
Groundnut bud necrosis (GBNV) was first described in India in 1968 and later determined to be caused by infection of tospovirus (Jain et al., 2005). Tospoviruses (family *Bunyaviridae,* genus *Tospovirus*) are among the most important plant virus groups in the world and infect a wide range of economically important crop plants (Mandal et al., 2012). Until 1990, Tomato Spotted Wilt Virus (TSWV), the namesake virus of the tospovirus genus, was considered to be the only species of tospovirus. However, in 1992 it was suggested based on serology that bud necrosis of groundnut was caused by a tospovirus different from TSWV, and the virus was named *Groundnut bud necrosis virus* (GBNV) (Mandal et al., 2012). Tospovirus species are often distinguished from each other based on the amino acid sequence of the nucleocapsid (N) gene (Mandal et al., 2012).

Pest Description
Tospoviruses are quasispherical (80–110 nm in diameter) enveloped isometric RNA viruses with a tripartite genome containing small (S), medium (M), and large (L) segments of single stranded RNA (Bhat et al., 2002). The complete genome of GBNV (type isolate, groundnut) has been sequenced, and it consisted of three linear single-stranded RNA molecules, the large (L, 8.9 kb), the Medium (M, 4.8 kb), and the small (S, 3.05 kb) RNAs. The L RNA is entirely of negative polarity, with one open reading

frame located on the viral complementary strand encoding the viral polymerase of the 330 kDa L-protein. The M RNA molecule encodes a 34.3 kDa movement protein (NSm) in the viral sense and a 127.3 kDa precursor to the two viral membrane glycoproteins, Gn and Gc, in the viral complementary sense (Satyanaryana et al., 1996). The S RNA molecule encodes the 49.5 kDa nonstructural protein (NSs) in the virus sense and the 30.6 kDa nucleocapsid (N) protein in the virus complementary sense. The NSs protein of GBNV has been characterized as a bifunctional enzyme containing RNA stimulated ATPase and 5' phosphatase activities, which possibly participates in suppression of the host defense mechanism (Mandal et al., 2012). Comparative sequence analysis of GBNV isolates from groundnut, mung bean, and tomato revealed that the genome of the M RNA was considerably different in their intergenic regions (56 to 89% sequence identity) and Gn/Gc protein regions (Mandal et al., 2012).

Biology and Ecology

Tospoviruses are transmitted by thrips (Thysanoptera) insects (Fig. 2) in a circulative and propagative manner (Bhat et al., 2002; Mandal et al., 2012). A unique feature of the thrips – tospovirus relationship is that only adults which acquired the virus at the first larval stage are able to transmit the virus (Sakimura, 1963). Larval and adult thrips both transmit GBNV in a persistent manner (Manjunatha, 2008). A survey by Gopal et al. (2011) found that GBNV incidence was higher in rainy season crops than in post-rainy season crops.

Figure 2. *Thrips palmi*, a known vector of GBNV (Florida Division of Plant Industry Archive, FDACS, Bugwood.org).

Tospoviruses are not seedborne, and it is assumed that the primary spread of these viruses is by thrips coming from other crops or weeds, whereas secondary spread takes place from infected plants within the same field (Mandal et al., 2012). The primary sources of GBNV include a range of solanaceous and fabaceous hosts which can sustain viral infection and support thrips vector multiplication (Mandal et al., 2012). Spread of this virus in peanut/groundnut, however, is mostly monocyclic, and disease incidence in this host depends on infection by thrips that acquire the virus from other crops or weed hosts (Mandal et al., 2012).

Symptoms/Signs

In general, symptoms of GBNV infection, which include chlorosis, mottling, lesions, stunted growth, necrotic rings, and bud necrosis, are very similar regardless of the infected host or virus isolate (Mandal et al., 2012).

In peanut (Fig. 3): “Initially, mild chlorotic spots appear on young quadrifoliate leaves, and subsequently necrosis and chlorotic rings develop. In rainy and post-rainy seasons, necrosis of the terminal bud is the main characteristic symptom. Secondary symptoms such as stunting, axillary shoot proliferation, and malformation of leaflets are common. Plants infected early are bushy, stunted, and die prematurely. If plants older than one

Figure 3. Rings and chlorotic spots, stunting, and necrosis of terminal bud of peanut. Courtesy of B.V. Bhaskara Reddy, Acharya N G Ranga Agricultural University.

month are infected, the symptoms are restricted to a few branches only" (Mandal et al., 2012).

In tomato (Fig. 1, 4): Symptoms include necrotic rings on leaf**,** stem necrosis**,** concentric rings and patchy color on fruit (Manjunatha, 2008; Mandal et al., 2012). Necrosis of the foliage often leads to collapse of a stem or of the whole plant resembling symptoms of blight. In general, tomato plants infected at an early stage often collapse and die (Akram et al., 2012).

In potato (Fig. 5): Symptoms of infection are characterized by stem/petiole necrosis, foliar spotting/deformation/necrosis and stunting of the plant (Kaushal et al., 2010). Necrosis of foliage often leads to collapse of a stem or the entire plant. In general, potato plants infected at an early stage often collapse and die (Akram et al., 2012; Pundhir et al., 2012).

In bean, pea, and other legumes (Fig. 6, 7, Appendix B): GBNV infection induces chlorotic and necrotic spots on leaves, browning in veins, stem necrosis, ring spots on fruits and pods (Akram and Naimuddin, 2010; Akram et al., 2012).

Pest Importance

Total annual losses due to GBNV in Asia are estimated at more than $89 million (Pappu, 1997). Disease incidence in tomato of over 90% has been reported in India (Manjunatha, 2008; Mandal et al., 2012). Yield losses of 80% have been reported in infected peanut fields (Manjunatha, 2008). Stem necrosis in potato caused by GBNV is also known to occur in India, and disease incidence of 50-90% and losses of up to 29% have also been reported there (Mandal et al., 2012; Pundhir et al., 2012). Additionally, disease incidence in bean cultivars of up to 20% has been reported (Akram et al., 2012).

Figure 4. Symptoms of GBNV infection in tomato compared to a healthy tomato (middle). Courtesy of Naidu Rayapati.

Numerous crops, including peanut, potato, tomato, and beans are important to the United States and vulnerable to GBNV infection. In 2015, peanut was grown on 1.625 million acres, and over 6.2 billion pounds of peanuts were produced (USDA-NASS, 2016). Tomato was planted on 97,500 acres, and the value of the harvest was over $1.2 billion (USDA-NASS, 2016). Potato was planted on 1,065,000 acres, and over 49 billion pounds of potatoes were produced (USDA-NASS, 2016). Snap beans were planted on 77,600 acres, and the value of the harvest was over $236 million (USDA-NASS, 2016).

At the genus level, tospoviruses are listed as a harmful organism in the following countries: Australia, Georgia, Japan, and Nauru (USDA-PCIT, 2016). There may be trade implications with these countries if GBNV becomes established in the United States.

Known Hosts

Major hosts: *Arachis* spp. (groundnut), *Arachis hypogaea* (peanut), *Solanum lycopersicum* (tomato), and *Solanum tuberosum* (potato) (Akram et al., 2004, 2012; Manjunatha, 2008; Kaushal et al., 2010; Mandal et al., 2012).

Minor hosts: *Acalypha indica* (copperleaf), *Acanthospermum hispidum* (bristly starbur), *Ageratum conyzoides* (tropic ageratum), *Allium cepa* (onion), *Alysicarpus* spp. (moneywort), *Amaranthus* spp. (amaranthus), *Calotropis gigantea* (giant milkweed), *Citrullus vulgaris* (watermelon), *Commelina benghalensis* (Benghal dayflower), *Colocasia esculenta* (taro), *Corchorus capsularis* (white jute), *Corchorus trilocularis* (jew's mallow), *Cucumis sativus* (cucumber), *Eclipta alba* (false daisy), *Glycine max* (soybean), *Jasminum sambac* (jasmine), *Lagascea mollis* (silkleaf), *Lochnera pusilla*,

Phaseolus vulgaris (bean), *Physalis minima* (cape gooseberry), *Pisum sativum* (pea), *Sesamum indicum* (sesame), *Sesbania rostrata* (sesbania), *Vigna mungo* (black gram, urd-bean), *Vigna radiata* (mung bean), *Vigna triloba,* and *Vigna unguiculata* (cowpea) (Bhat et al., 2002; Jain et al., 2004, 2007; Manjunatha, 2008; Akram and Naimuddin, 2010; Gopal et al., 2011; Siviprasad et al., 2011ab; Sujitha et al., 2012; CABI, 2014).

Figure 5: Necrotic spots on GBNV infected potato leaves. Courtesy of Mohammad Asnar, Bihar Agriculture University.

Experimental hosts: *Alternanthera sessilis* (sessile joyweed), *Beta vulgaris* (beet), *Cajanus cajan* (pigeon-pea), *Canavalia ensiformis* (jackbean), *Capsicum* spp. (pepper), *Cassia* spp. (cassia), *Chenopodium album* (lambsquarters), *Chenopodium quinoa* (quinoa), *Citrullus lanatus* (watermelon), *Crotalaria juncea* (sunn hemp), *Cucumis melo* (cantaloupe), *Datura stratamonium* (jimsonweed), *Dolichos uniflorus* (horsegram), *Emilia sonchifolia* (lilac tasselflower), *Gomphrena globosa* (globe amaranth), *Luffa acutangula* (sinkwa towelsponge), *Nicotiana* spp. (tobacco), *Petunia hybrida* (petunia), *Phaseolus lunatus* (lima bean), *Physalis floridana* (husk-tomato), *Vinca rosea* (periwinkle), and *Zinnia elegans* (zinnia) (Golnaraghi et al., 2002; Jain et al., 2005; Manjunatha, 2008).

Known Vectors (or associated insects)

Frankliniella fusca (tobacco thrips), *F. occidentalis* (western flower thrips), *F. schultzei* (common blossom thrips), *Scirtothrips dorsalis* (chilli thrips), *Thrips palmi* (melon thrips), and *T. tabaci* (onion thrips) are known vectors of GBNV (Manjunatha, 2008; Pranav and Krishnaraj, 2010).

Known Distribution

Asia: Bangladesh, China, India, Indonesia, Iran, Nepal, Pakistan, Sri Lanka, Thailand, and Vietnam (Golnaraghi et al., 2002; Jain et al., 2004; Manjunatha, 2008; Akhter et al., 2012).

Pathway

The most likely pathway of entry for GBNV is by transport of infected plant material or infected insect vectors. The import of *Arachis* and *Solanum* spp. plant material for propagation is generally prohibited (USDA, 2015), but interceptions of such plant material do occur. Since 2006, there were interceptions of *Solanum lycopersicum* plant material intended for propagation from China (14), India (12), Thailand (1), and Vietnam (1) (AQAS, 2016).

Since 2006, the insect vector *Thrips palmi* has been intercepted at U.S. ports of entry 2,423 times from countries where GBNV is known to be present. These interceptions came from Thailand (2,350), India (43), China (16), Vietnam (11), Nepal (2), and Bangladesh (1) (AQAS, 2016). Interceptions of *Thrips palmi* have occurred at ports of entry throughout the entire United States on baggage, mail, permit cargo, and general cargo (AQAS, 2016). The majority of the *T. palmi* interceptions occurred on *Dendrobium* spp. cut flowers. (AQAS, 2016). The genus *Dendrobium* encompasses many species of orchids. Other unidentified *Thrips* spp. have also been intercepted 156 times from known host countries since 2006 (AQAS, 2016).

Frankliniella spp. are also known to vector GBNV (Pranav and Krishnaraj, 2010). There were 122 interceptions of *Frankliniella* spp. from eight different countries which have GBNV since 2006, including: China (53), India (33), Thailand (23), Vietnam (6), Nepal (3), Sri Lanka (2), Bangladesh (1), and Pakistan (1) (AQAS, 2016).

Potential Distribution within the United States

Two thirds of all U.S. commercial tomato acreage is found in California and Florida (USDA-NASS, 2016). Other states with significant commercial tomato cultivation include: Ohio, Tennessee, North Carolina, New Jersey, New York, Michigan, South Carolina, Georgia, and Virginia (USDA-NASS, 2016). Tomato is also popular with home gardeners nationwide.

Georgia is the largest peanut producing state and accounts for over half of all commercial peanut production in the United States. The other top commercial peanut producing states are Alabama, Florida, Texas, North Carolina, South Carolina, Mississippi, and Virginia (USDA-NASS, 2016).

Potato is grown throughout the United States in backyard gardens and is grown commercially. Idaho and Washington account for roughly half of the U.S. commercial potato acreage. Other states with significant commercial potato cultivation include: North Dakota, Wisconsin, Colorado, Maine, Michigan, Minnesota, and Oregon (USDA-NASS, 2016).

Snap beans are also grown commercially in numerous states. The top states for planting acreage of snap beans in 2015 were: Florida, Georgia, New York, California, Tennessee, and North Carolina (USDA-NASS, 2016).

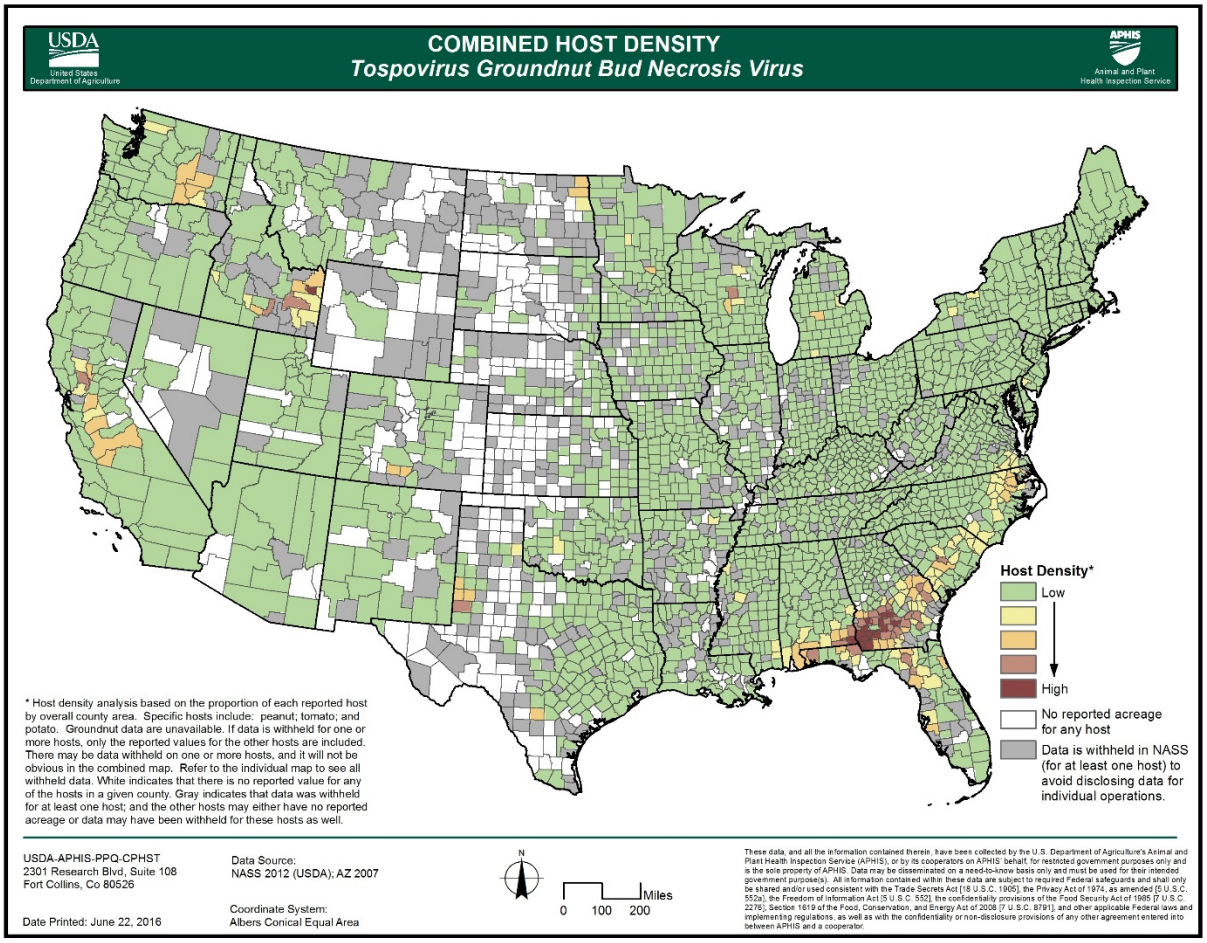

Figure 8. Combined distribution map for *Tospovirus Groundnut bud necrosis virus* within the continental United States. Values represent combined host density low to high (peanut, potato, and tomato). Map courtesy of USDA-APHIS-PPQ-CPHST.

In addition to many GBNV hosts, the following vectors of GBNV are all present in the United States: *Frankliniella fusca*, *F. occidentalis*, *F. schultzei*, *Scirtothrips dorsalis*, *Thrips palmi*, and *T. tabaci* (Reitz et al., 2011; NISIC, 2016). If GBNV becomes established in the United States, chances of spread through vector transmission are high.

A recent combined host distribution map for GBNV developed by USDA-APHIS-PPQ-CPHST (Fig. 8) identifies areas of high host acreage based on the combined acreage of major hosts peanut, potato, and tomato. Although multiple *Arachis* spp. (groundnut) are susceptible to GBNV, information was only available for *Arachis hypogaea* (peanut). This map illustrates that though there are counties in nearly all states with a low level of risk. Alabama, California, Colorado, Florida, Georgia, Idaho, Michigan, Minnesota, North Carolina, North Dakota, South Carolina, Texas, Washington, and Wisconsin, however, have counties with the highest level of risk for GBNV 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 peanut, potato (continental, Alaska, and Hawaii), and tomato are provided in the Appendix at the end of the document.

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/.

Key Diagnostics

Approved Method for Pest Surveillance*:

Serological: A field-based screening method/assay (ELISA) is commercially available for GBNV (at the serogroup IV level) from Agdia (https://orders.agdia.com/agdia-set-wsmv-and-gbnv-alkphos-sra-61500).

Note: This ELISA kit detects both *Watermelon silver mottle virus* (WSMoV) (not known to occur in the United States) and *Groundnut bud necrosis virus* and (GBNV) at the same time. The ELISA kit is specific to test WSMoV, GBNV and other potential serogroup IV of tospoviruses. In addition, it gives no cross-reaction with other tospoviruses known to occur in the United States such as *Impatiens necrotic spot virus* (INSV), *Tomato spotted wilt virus* (TSWV), *Groundnut ringspot virus* (GRSV), *Tomato chlorotic spot virus* (TCSV), or *Iris yellow spot virus* (IYSV).

It is possible that the specific ELISA kit for WSMoV and GBNV will cross-react with *Capsicum chlorosis virus (*CaCV) and Watermelon bud necrosis virus (WBNV). Although this specific ELISA kit could possibly react with CaCV and WBNV, in addition to the desired GBNV, these viruses (CaCV and WBNV) are not present in the United States. Hence, the cross-reactions with close-related tospoviruses in the same serogroup (IV) during the survey are not a major concern.

Confirmation, however, requires a combination of molecular techniques.

Optional: Agdia has specific ELISA or ImmunoStrip methods for other tospoviruses in other serogroups such as *Impatiens necrotic spot virus* (INSV), *Iris yellow spot virus* (IYSV), *Groundnut ringspot virus* (GRSV), *Tomato chlorotic spot virus* (TCSV), and **Tomato spotted wilt virus** (TSWV). The serological assays for INSV, IYSV, GRSV, TCSV, and TSWV could be helpful to identify the causal agent of similar diseases on the same host plant. In addition, Agdia provides a Tospovirus group-specific PCR, which may be helpful to confirm that the causal agent of the disease belongs to tospovirus group.

*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 numerous publications which describe serological/ molecular diagnostic methods for GBNV.

The following are some examples:

Jain et al. (2005) created a high titre polyclonal antiserum to the nucleocapsid protein of GBNV that can successfully detect GBNV infection via a DAC-ELISA in a wide range of hosts, including cowpea, mung bean, soybean, tomato, and urd bean. However, this assay could not distinguish among the different tospoviruses in serogroup IV, such as *Watermelon bud necrosis virus* (WBNV) and *Capsicum chlorosis virus* (CaCV). Since GBNV and WBNV have some common solanaceous hosts (including tomato and pepper), mixed infection of these two viruses is possible (Jain, personal communication, 2016).

Akram et al. (2004) identified GBNV using an RT-PCR. The primers were developed based on the sequence of the movement protein (NSm) of the virus. Pundhir et al., (2012) developed an RT-PCR using primers targeting the nucleocapsid protein of GBNV. Kaushal et al. (2010) developed a print capture RT-PCR (PC-RT-PCR) which diagnosed GBNV infection in potato and in thrips insect vector tissue.

Maheshwari et al. (2015) developed a single chain variable fragment (scFv) for specific diagnosis of GBNV. This scFv was able to detect GBNV via DAC-ELISA and immunocapture RT-PCR (IC-RT-PCR). The IC-RT-PCR was able to distinguish GBNV from the closely related (WBNV).

Easily Confused Species

There are several other tospovirus species which infect the same hosts as GBNV, and at least two are present in the United States. For example, *Groundnut ringspot virus* (GRSV) and *Tomato spotted wilt virus* (TSWV) cause similar symptoms in tomato, are present in the United States, and are also vectored by *Frankliniella occidentalis* and *F. schultzei* (Webster et al,. 2010; Reitz et al., 2011).

Other tospovirus species which infect solanaceous hosts and are also vectored by thrips include: *Capsicum chlorosis virus* (CaCV), *Groundnut yellow spot virus* (GYSV), *Peanut chlorotic fan-spot virus* (PCFV), *Tomato chlorotic spot virus* (TCSV), *Tomato yellow ring virus* (TYRV), and *Watermelon bud necrosis virus* (WBNV) (Reitz et al., 2011; Jain, personal communication, 2016). Molecular identification is necessary for confirmation of GBNV.

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Reviewers:

Dr. B. V. Bhaskara Reddy, ANG Ranga Agril University, Tirupati, India
Dr. Rakesh Jain, Indian Agricultural Research Institute (IARI), New Delhi, India

Dr. Vessela Mavrodieva, USDA-APHIS-PPQ-CPHST, Beltsville, MD

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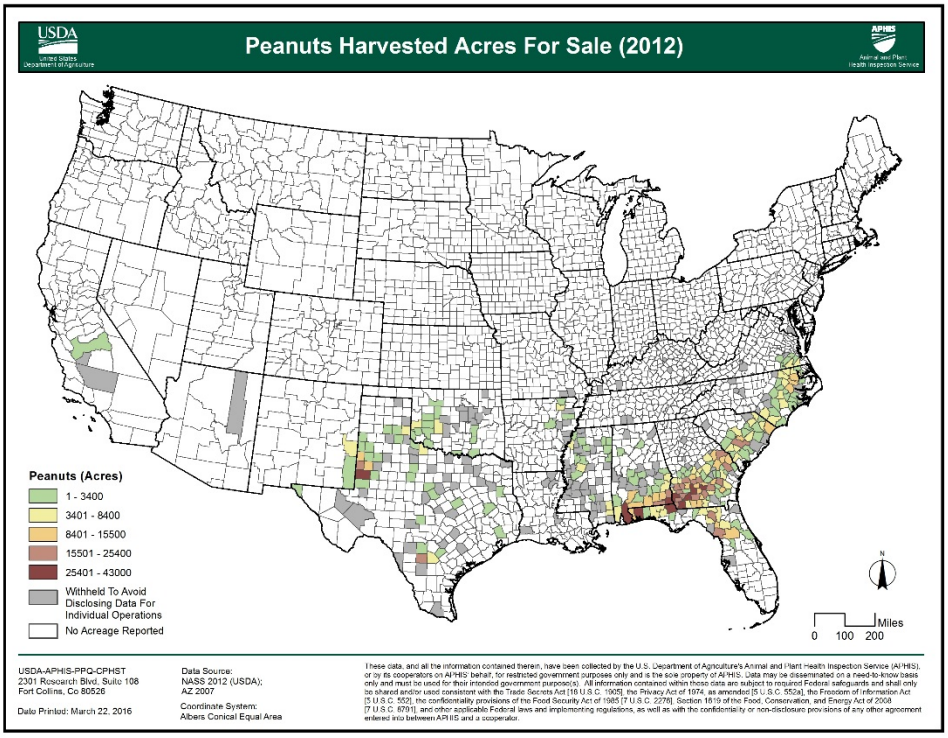
Appendix A: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Animal and Plant Health Inspection Service
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Coordinate System:
Albers Conical Equal Area
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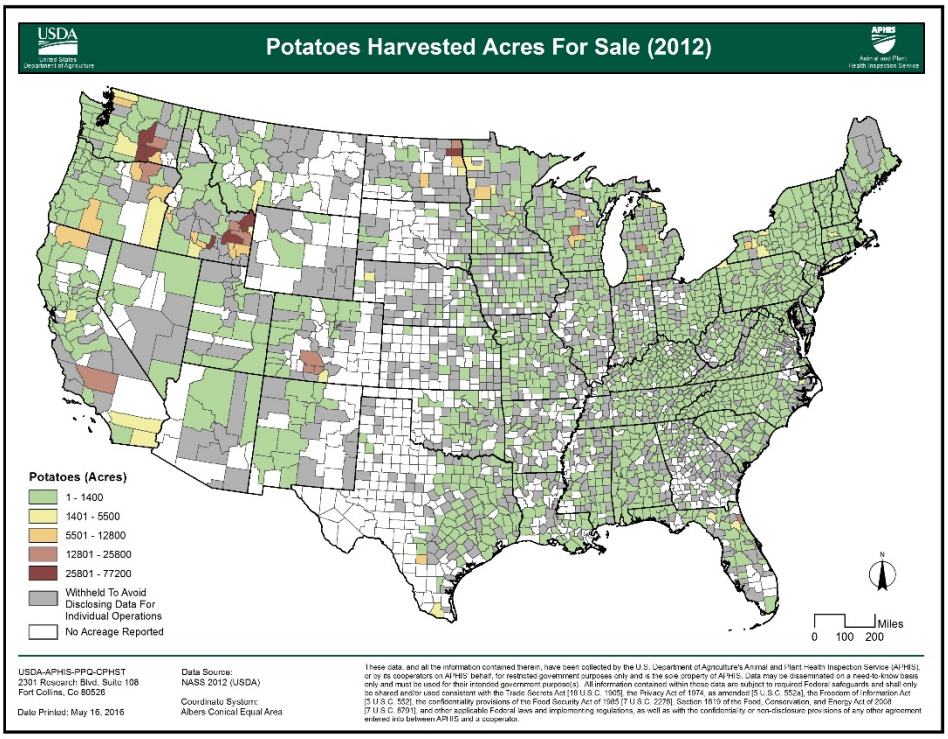
USDA
United States Department of Agriculture
Potatoes Harvested Acres For Sale (2012)
APHIS
Animal and Plant Health Inspection Service
Potatoes (Acres)
1 - 1400
1401 - 5500
5501 - 12800
12801 - 25800
25801 - 77200
Withheld To Avoid Disclosing Data For Individual Operations
No Acreage Reported
N
0 100 200
Miles
USDA-APHIS-PPQ-CPHST
2301 Research Blvd, Suite 108
Fort Collins, Co 80526
Data Printed: May 16, 2016
Data Source:
NASS 2012 (USDA)
Coordinate System:
Albers Conical Equal Area
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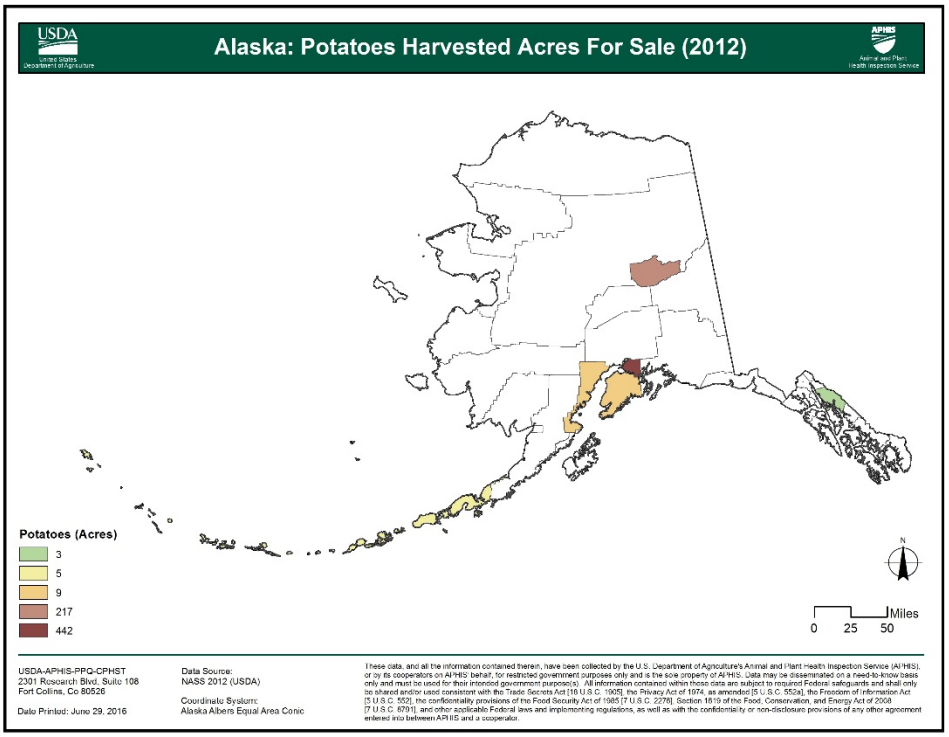
USDA
United States Department of Agriculture
Alaska: Potatoes Harvested Acres For Sale (2012)
APHIS
Animal and Plant Health Inspection Service
Potatoes (Acres)
3
5
9
217
442
N
0 25 50 Miles
USDA-APHIS-PPQ-CPHST
2301 Research Blvd, Suite 108
Fort Collins, Co 80526
Date Printed: June 29, 2016
Data Source:
NASS 2012 (USDA)
Coordinate System:
Alaska Albers Equal Area Conic
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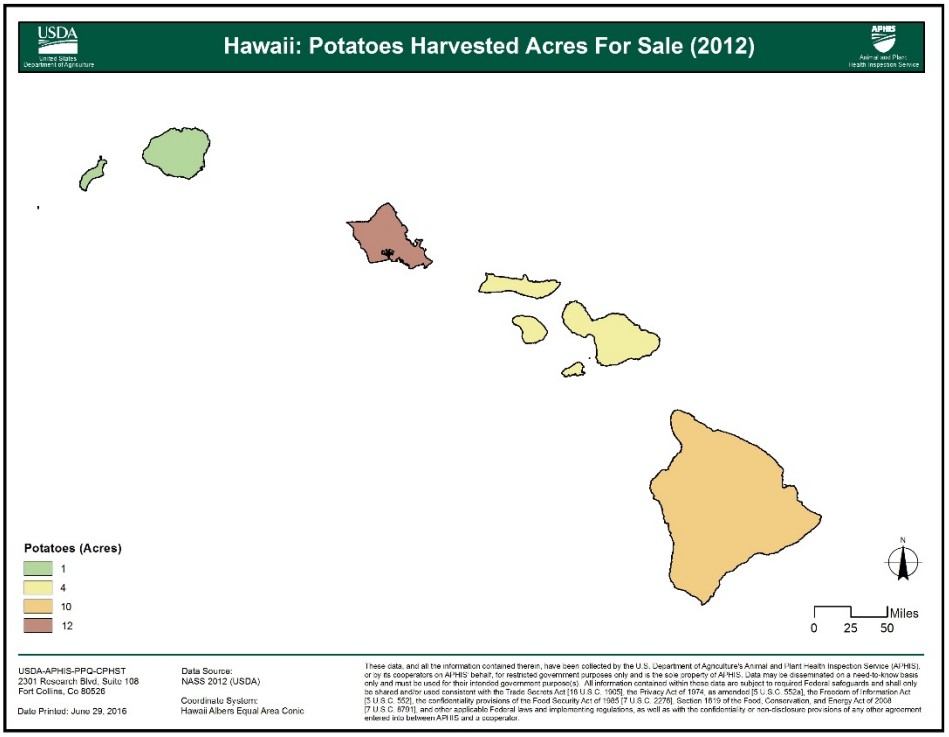
USDA
United States Department of Agriculture
Hawaii: Potatoes Harvested Acres For Sale (2012)
APHIS
Animal and Plant Health Inspection Service
Potatoes (Acres)
1
4
10
12
N
0 25 50 Miles
USDA-APHIS-PPQ-CPHST
2301 Research Blvd, Suite 108
Fort Collins, Co 80526
Date Printed: June 29, 2016
Data Source:
NASS 2012 (USDA)
Coordinate System:
Hawaii Albers Equal Area Conic
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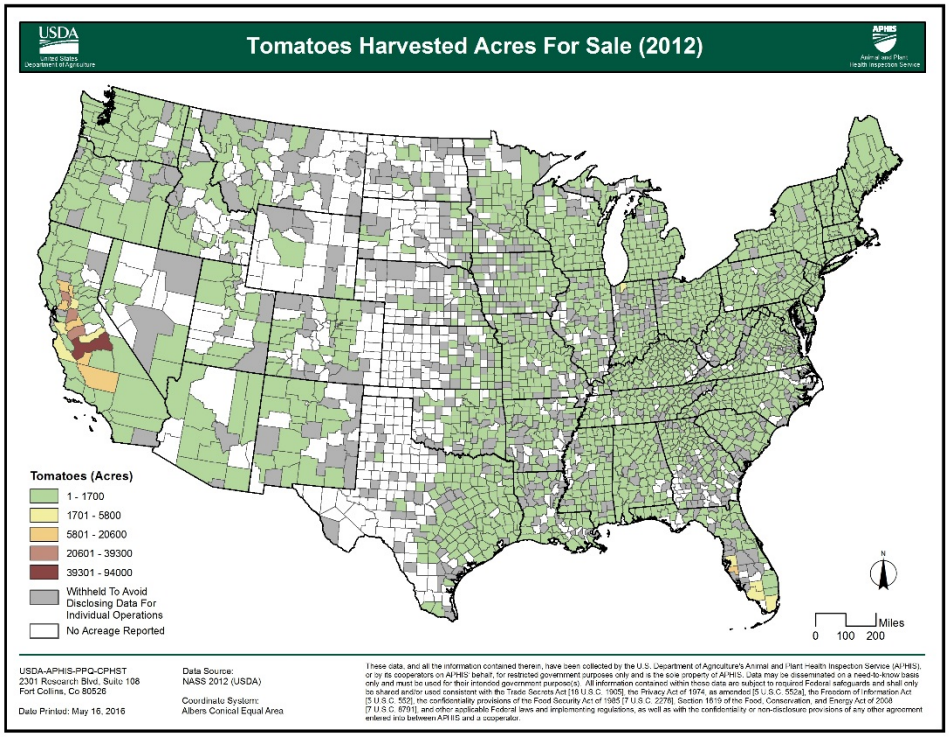
USDA
United States Department of Agriculture
Tomatoes Harvested Acres For Sale (2012)
APHIS
Animal and Plant Health Inspection Service
Tomatoes (Acres)
1 - 1700
1701 - 5800
5801 - 20600
20601 - 39300
39301 - 94000
Withheld To Avoid Disclosing Data For Individual Operations
No Acreage Reported
N
Miles
0 100 200
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
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Appendix B:

Figure 6. *Vigna* spp. infected with GBNV. Courtesy of Mohd Akram, Indian Institute of Pulses Research.

Figure 7. *Pisum sativum* (pea) infected with GBNV. Courtesy of Mohd Akram.