Nepovirus Tomato black ring virus

Scientific Name
Nepovirus Tomato black ring virus

Figure 1: Raspberry leaf (cv. Malling Exploit) infected with TBRV. A.T. Jones, Scottish Crop Research Institute (SCRI).

Synonyms
None

Common Name(s)
Virus: **Tomato black ring virus (TBRV)**, bean ringspot virus, lettuce ringspot virus**,** potato pseudo-aucuba virus, and tomato black ring nepovirus.

Disease: Tomato black ring, ringspot of bean**,** ringspot of beet**,** ringspot of lettuce, potato bouquet, potato pseudo-aucuba, and yellow vein of celery.

Type of Pest
Virus

Taxonomic Position
Class: Not assigned, **Order:** *Picornavirales*, **Family:** *Secoviridae*

Reason for Inclusion in Manual
Additional Pest of Concern - 2017

Background Information
Tomato black ring virus (TBRV) is a member of the genus *Nepovirus* (*Secoviridae: Comovirinae*) and infects a wide range of economically important crop species as well as many weed and ornamental species (Harrison, 1957). The genus *Nepovirus* was among the original groups of viruses recognized by the International Committee on Taxonomy of Viruses (ICTV) (Sanfaçon, 2009). Nematode transmission was one of the original defining characteristics of this genus, but nepoviruses are currently classified based on the organization of the RNA genome (Sanfaçon, 2009).

Tomato black ring virus was first described in the United Kingdom (UK) in 1946 (Smith, 1946). The virus was initially isolated from a ripe tomato fruit that showed lesions in the skin. The tomato was also infected with two other viruses, however, and TBRV alone was not linked to the fruit lesions. Despite the name, TBRV is rarely found in tomato, and tomato is not considered an economically important host of this virus (Martelli and Taylor, 1990).

Pest Description

Nepoviruses, which have isometric particles of 26-30 nm with sharp hexagonal outlines (Fig. 2), are distinguished from related genera based on their single large coat protein (CP) (Sanfaçon, 2009). Nepoviruses are divided into three subgroups (A, B, and C) based on the length and packaging of RNA 2, sequence similarities, and serological properties (Sanfaçon, 2009). Tomato black ring virus belongs to subgroup B of the genus. Like other nematode-transmitted viruses, the host range of TBRV is very broad. The complete nucleotide sequence of the virus has been determined and confirms a close relationship of TBRV with *Grapevine chrome mosaic virus* and *Cycas necrotic stunt virus* (Digiaro et al., 2015).

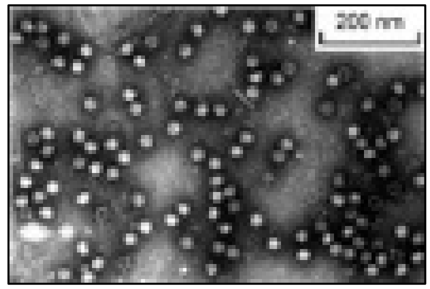

Figure 2: Electron micrograph showing purified particles of TBRV (top) Courtesy of SCRI.

Biology and Ecology

TBRV is transmitted by the soil-inhabiting nematodes *Longidorus attenuatus* and *L. elongatus* (Fig. 3) (Harrison et al., 1961; EFSA, 2013). These nematodes feed ectoparasitically on the roots of host plants with their long mouth stylets. The mechanism of retention and transmission of TBRV in nematodes is not well understood, but nepoviruses transmitted by *Longidorus* spp. are usually associated with the odontostyle (stylet) (Sanfaçon, 2009). Acquired nepoviruses typically remain infective in *Longidorus* spp. hosts for up to nine weeks (Sanfaçon, 2009). Nepoviruses are generally restricted by the distribution of their nematode vectors. Due to the restricted movement of nematodes, the spread of nematode-transmitted viruses through an infected field is often slow and occurs in patches (Fig. 5) (Sanfaçon, 2009). TBRV infection can cause few or no symptoms in the early stages of infection (Converse, 1987). In addition to nematode transmission, TBRV is also transmissible by pollen and seed (Lister and Murant, 1967).

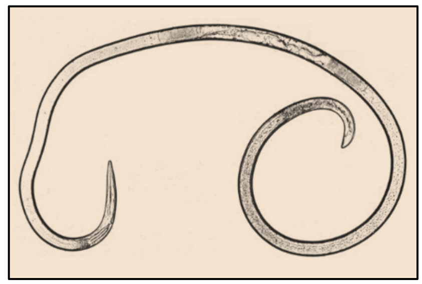

Figure 3: An adult specimen of the virus vector nematode *Longidorus elongatus*. Courtesy of SCRI.

Tomato black ring virus is durable and can remain infective for long periods of time. In laboratory experiments, the virus was found to remain infective after a ten minute exposure to 58ºC (136ºF) but failed to infect after exposure to 62ºC (144ºF) (Smith, 1946). After six years in storage, TBRV-infected seed of *Capsella bursa-pastoris* (shepherd's purse) and *Stellaria media* (common chickweed) germinated to produce virus-infected seedlings (Lister and Murant, 1967).

Figure 4: TBRV infected strawberry leaves. Chlorotic streaking in cv. Auchincruive Climax (left), chlorotic spots and mottling in cv Talisman (middle), and ringspots in cv. Talisman (right). Courtesy of A. T. Jones, SCRI.

Symptoms/Signs

<u>Strawberry (Fig. 4, 5):</u> Symptoms in *Fragaria vesca* (wild/ European strawberry) plants include leaf blotching and necrotic ring spots (Martin and Tzanetakis, 2006). In general, streaks/blotches of chlorosis and chlorotic spots are observed. Necrotic spots are observed in some cultivars. In some susceptible cultivars, stunting and dwarfing is often observed as symptoms progress and plants eventually die. In tolerant cultivars leaves and plants may appear to recover or be symptomless but those plants still contain the virus (Converse, 1987).

Symptoms in commercial strawberry vary depending upon the cultivar and the time of year. Numerous strawberry cultivars, including ‘Talisman’, ‘Huxley’, and ‘Auchincruive Climax’ were found to be susceptible to TBRV infection (Converse, 1987). The susceptibility of commercially grown North American strawberry cultivars to TBRV, however, is not well known. Strawberry cultivars used by commercial growers in North America change frequently, and strawberry varieties tend to be locally adapted and respond strongly to local day length and temperature conditions (Hokanson and Finn, 2000).

Figure 5: Strawberries in eastern Scotland affected by tomato black ring and raspberry ringspot viruses. Courtesy of SCRI.

In Europe, TBRV is often found in mixed infections with raspberry ringspot virus (RpRSV) in strawberry (Converse et al., 1987). Both of these viruses are vectored by *L. elongatus*, and they exhibit similar symptoms in strawberry (Martin and Tzanetakis, 2006). Damage is greater when both viruses are present in infected strawberry (Converse et al., 1987; Martelli and Taylor, 1990).

Raspberry (Fig. 1): Susceptible cultivars react to infections with chlorotic mottling, vein yellowing, yellow speckling, yellow or chlorotic ringspot, leaf curling, reduced vigor, stunting, and deformation of fruit (Martelli and Taylor, 1990). While damaging outbreaks of TBRV have previously been reported in raspberry (Harrison et al., 1961), effective control measures have greatly reduced the impact of TBRV in this host (Converse, 1987).

Other hosts:

- **Artichoke:** Symptoms include systemic chlorotic ringspot of the leaves, decreased head weight, and fewer heads per plant (Gallitelli et al., 2004).

- **Celery: "**An intense chrome-yellow vein banding, restricted in some leaves to a few flecks, but forming extensive yellow areas around the major veins in others, and sometimes involving the entire leaf lamina" (Hollings, 1965).

- **Potato:** Symptoms include leaf necrosis and stunted shoots (Harrison, 1959). Infection is rare, however, and transmission of the virus through tubers does not occur (Hooker, 1981).

Symptoms of numerous additional hosts, including bean, carrot, cucumber, pea, spinach, and zucchini have also been studied. Common symptoms in these hosts include: chlorotic spotting, mosaic symptoms, necrotic lesions, ring spots or pattern lines, and deformed fruit (see Appendix B for images of symptomatic plants (minor and experimental hosts)) (Pospieszny et al. 2004; Šneideris et al., 2012; Šneideris and Staniulis 2014).

Pest Importance

In highly sensitive strawberry cultivars, crops can be rendered valueless by TBRV infection in 1-2 years (Converse, 1987). Strawberry is a high-value specialty crop in the United States. In 2014, strawberry was planted on 59,895 acres in the United States, and the value of strawberry production was over $2.8 billion (USDA-NASS, 2016).

Several other crops which are susceptible to TBRV infection are economically important in the United States. Raspberry was grown on 18,050 acres in 2014, and over 90,000 fresh tons were produced with a value of over $388 million. Artichoke was planted on 6,500 acres in 2015, and the total harvest was valued at $72.8 million. Celery was planted on 30,500 acres in 2015, and the total harvest was valued at $456 million (USDA-NASS, 2016).

Since 1957, there have been reports of significant damage caused by TBRV infection to several other important hosts. Whereas these reports are mostly old and infrequent, they underscore the potential for this virus to cause damage in a wide range of economically important host plants. One report showed a disease incidence of 50% in an infected celery field (Hollings, 1965). Another report showed incidence of 5-39% in heavily infected potato fields (Harrison, 1959). Yield loss in an infected artichoke field was reported to be 40% (Gallitelli et al., 2004). The infection rate in one grape vineyard, in contrast, was found to be only 0.95% (Kominek, 2008).

Tomato black ring virus is listed as a harmful organism in 64 countries (USDA-PCIT, 2016). Many of these countries are important trading partners including: Brazil, Canada, China, France, Germany, India, Japan, Mexico, Taiwan, and the UK. There may be trade implications with these countries if TBRV becomes established in the United States.

Known Hosts

Major hosts: *Fragaria* spp. (strawberry) (Harrison et al., 1961; Converse 1987)

Minor hosts: *Allium ascalonicum* (shallot), *Allium cepa* (onion), *Apium graveolens* (celery), *Arctium zuppu*, *Asparagus officinalis* (asparagus), *Beta vulgaris* (beet), *Brassica napobrassica* (swede), *Brassica napus* (rape), *Brassica oleracea* (cabbage), *Brassica rapa* (turnip), *Capsella bursa-pastoris* (shepherd's purse), *Capsicum annum* (chile pepper), *Cerastium vulgatum* (big chickweed), *Clematis* spp. (clematis), *Crocus* spp. (crocus), *Cucumis sativus* (cucumber), *Cucurbita pepo* (zucchini), *Cynara cardunculus* (artichoke), *Cynara scolymus* (globe artichoke), *Daucus carota* (carrot), *Fumria oficinalis* (fumitory), *Geranium dissectum* (cutleaf geranium), *Heracleum sphondylium* (hogweed), *Hosta* spp. (hosta), *Lamium amplexicaule* (henbit deadnettle), *Lamprocapnos spectabilis (bleeding hearth),* *Latuca sativa* (lettuce), *Lolium perenne* (perennial ryegrass), *Lycopsis arvensis* (wild bugloss), *Medicago sativa* (alfalfa), *Myosotis arvensis* (field forget-me-not), *Narcissus psuedo-narcissus* (daffodil), *Pastinaca sativa* (parsnip), *Pelargonium* spp. (geranium), *Petroselinum crispum* (parsley), *Phlox* spp. (phlox), *Polygonum spp.* (knotgrass), *Prunus dulcis* (almond), *Prunus persica* (peach), *Pyrus* spp. (pear), *Rheum rhabarbarum* (rhubarb), *Ribes* spp. (currant), *Robina pseudoacacia* (black locust), *Rubus idaeus* (raspberry)*, *Rubus* spp. (blackberry)*, *Sambucus nigra* (elder), *Senecio vulgaris* (groundsel), *Solanum lycopersicum* (tomato), *Solanum tuberosum* (potato), *Sonchus asper* (sow thistle), *Sorbus aucuparia* (mountain ash), *Spergula arvensis* (corn spurry), *Spinacia oleracea* (spinach), *Stellaria media* (common chickenweed), *Taraxacum officinale* (common dandelion), *Trifolium repens* (white clover), *Tulipa geseneriana* (tulip), *Tussilago farfara* (coltsfoot), *Urtica urens* (annual nettle), *Veronica agrestis* (speedwell), *Veronica persica*, and *Vitis vinifera* (grapevine) (Smith, 1946; Harrison, 1957, 1959; Harrison et al., 1961; Hollings, 1965; Lister and Murant, 1967; Converse, 1987; Gallitelli et al., 2004; Pospieszny et al., 2004; Pospieszny and Borodynko, 2005; Kominek, 2008; Šneideris et al., 2012; EFSA, 2013; Šneideris and Staniulis 2014).

Experimental hosts: *Amaranthus caudatus* (amaranth), *Avena sativa* (oat), *Calendula officinalis* (English marigold), *Chenopodium album* (lamb's quarters), *C. amaranticolor* (chenopodium), *C. murale* (nettle-leaved goosefoot), *C. quinoa* (quinoa), *Datura stramonium* (jimsonweed), *Emilia sagittata*, *Glycine max* (soybean), *Gomphrena globosa* (globe amaranth), *Lathyrus odoratus* (sweet pea), *Nicandra physalodes* (apple of Peru), *Nicotiana* spp. (tobacco), *Petunia hybrida* (petunia), *Phaseolus vulgaris* (French bean), *Poa annua* (annual meadow grass), *Portulaca oleracea* (little hogweed), *Tetragonia tetragonioides* (New Zealand spinach), *Triticum* spp. (wheat), *Vicia faba* (fava bean),*Vigna sinensis* (black eyed pea), and *Vigna unguiculata* (cowpea) (Harrison, 1957; Hollings, 1965; Lister and Murant, 1967; Converse, 1987; Martelli and Taylor, 1990; Pospieszny et al., 2004).

***Note:** Blackberry (*Rubus* spp.) is reported as a 'main host' of TBRV in CABI (2015b). However, there are no other confirmed reports of blackberry as a host of this virus. Raspberry is also reported as a 'main host' (CABI, 2015b), but early reports of damage are not specific (Harrison et al., 1961), and virtually no reports of significant damage to raspberry have been published in the last 50 years.

Known Vectors (or associated insects

Longidorus attenuatus and *L. elongates* are the vectors of TBRV (Harrison et al., 1961; EFSA, 2013).

Known Distribution

Europe: Albania, Austria, Belarus, Belgium, Bulgaria, Croatia, Czech Republic, Finland, France, Germany, Greece, Hungary, Ireland, Lithuania, Moldova, Netherlands, Norway, Poland, Russia, Serbia, Sweden, Switzerland, Turkey, and the United Kingdom (UK). **Asia:** India and Japan (Harrison, 1957; Brunt et al., 1996; Gangl et al., 2011; Šneideris et al., 2012; EFSA, 2013; CABI, 2015b; EPPO, 2015).

Tomato black ring virus has been intercepted in Brazil, Canada, Kenya, Portugal, and the United States, but the virus is not known to be established in these countries (CABI, 2015b; EPPO, 2015). In addition, TBRV has been intercepted on plant material which reportedly originated from Spain, Colombia, and Nigeria (Chalam et al., 2008; EPPO, 2015), but the virus has not been confirmed to be present in those countries. There are also unreliable records of TBRV in Chile, China, Denmark, Italy, Morocco, and Romania (EPPO, 2015).

Pathway

The most likely pathway of entry for TBRV is through movement of infected plant material, seed, or the nematode vector. The interception records of TBRV in numerous countries demonstrate the portability of this virus. Many hosts of the virus are symptomless, and other hosts do not exhibit symptoms for up to a year after infection, creating the possibility of inadvertent shipment of infected plant material (Converse, 1987).

The import of seed from numerous plant hosts of TBRV is currently allowed, including: *Allium cepa*, *Apium graveolens*, *Brassica* spp., *Cynara scolymus, Daucus carota*, *Fragaria* spp., and *Vigna* spp. (USDA, 2016). The import of *Rubus* spp. propagative material is also currently allowed from the United Kingdom (UK) (USDA, 2016). Since 2006, there have been 19 shipments of *Rubus* spp. plant material from the UK totaling 957 plant units (AQAS, 2016).

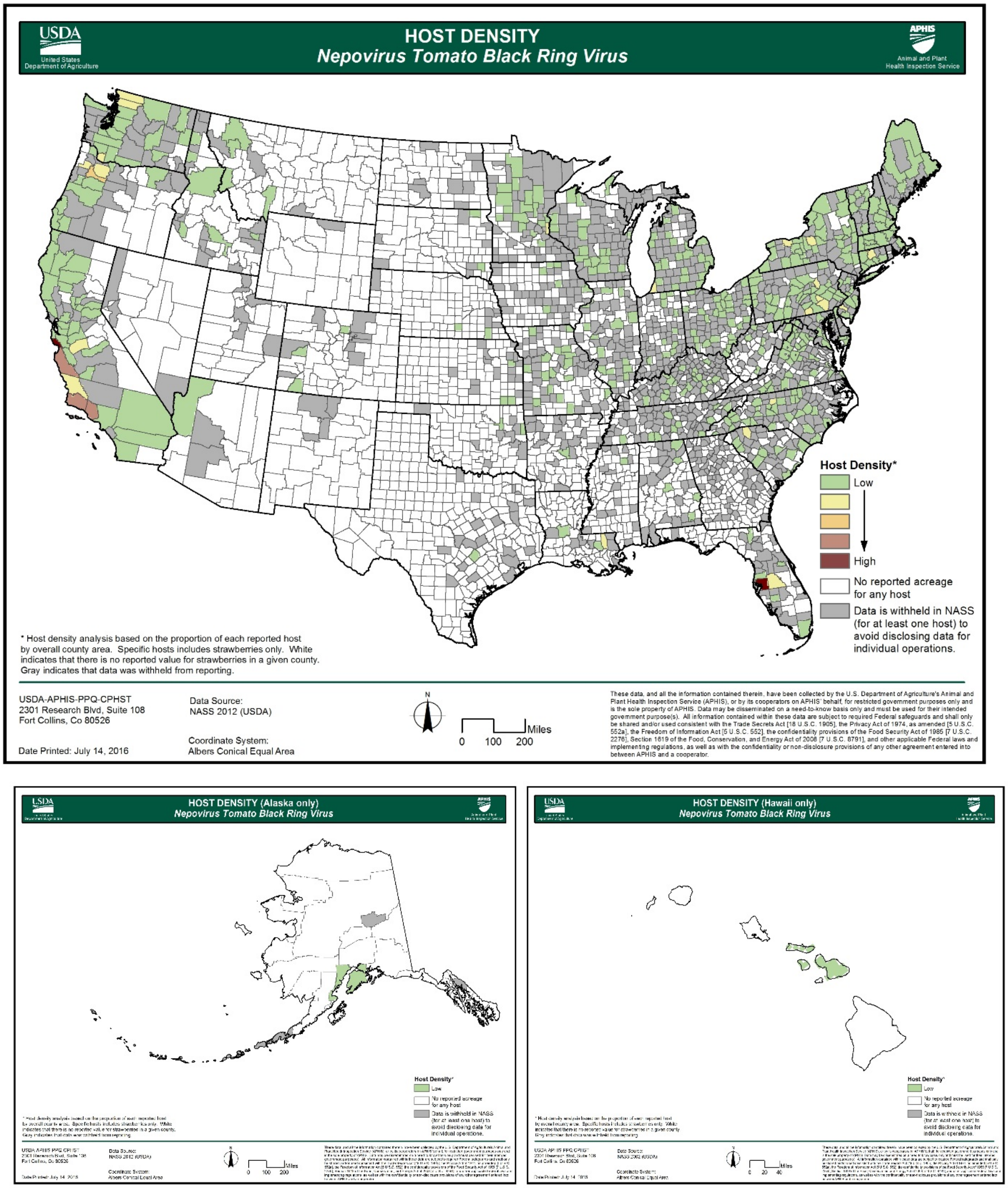

Figure 6. Combined distribution map for *Nepovirus Tomato black ring virus* within the continental United States and Alaska and Hawaii. Values represent combined host density low to high for strawberry. Map courtesy of USDA-APHIS-PPQ-CPHST.

Several countries have intercepted TBRV or traced a new national record of TBRV to imported plant material that was infected with TBRV. There was a detection of TBRV in Canada in infected grapevine imported from Europe in the 1980s. The infected plant material was destroyed, and the virus has not been found in Canada since (EPPO, 2015). In the UK, TBRV was intercepted on imported *Pelargonium* spp. (geranium) which reportedly came from Spain (EPPO, 1998). In Portugal, TBRV was also detected on imported *Pelargonium* spp. (EPPO, 2015). The source of the interception in the United States is unknown (CABI, 2015b).

Since 2006, there have been interceptions of *Fragaria* spp. plant material intended for propagation from Poland (22), Belarus (3), Switzerland (3), Turkey (3), Bulgaria (2), Hungary (2), France (1), India (1), Japan (1), Netherlands (1), and Russia (1). These interceptions occurred in mail, permit cargo, and baggage, and many of them contained soil (AQAS, 2016). There have also been interceptions of *Rubus* spp. plant material intended for propagation from Poland (9), Japan (5), Russia (3), Serbia (2), Belarus (1), Hungary (1), Turkey (1), and UK (1) (AQAS, 2016). Since 2012, there have been four interceptions of *Longidorus* spp. at U.S. ports of entry. Two of these interceptions came on baggage from Poland, a country known to have both TBRV and *Longidorus elongatus* (EPPO, nd).

Potential Distribution within the United States

The nematode vector, *Longidorus elongatus*, is present in the United States (Arkansas, California, Oregon) (CABI, 2015a; Chitambar, 2015). There are also many hosts of TBRV which are present throughout the United States. For example, strawberry is primarily grown in California, which accounts for about 90% of total production in the United States (USDA-NASS, 2016). Florida accounts for roughly 6.5% of U.S. strawberry production. Other states with 1,000 or more acres of strawberry cultivation in 2014 are: Oregon, Washington, North Carolina, and New York (USDA-NASS, 2016).

About half of all U.S. commercial raspberry production occurs in Washington. Significant raspberry cultivation also occurs in California and Oregon (USDA-NASS, 2016). Virtually all commercial artichoke cultivation takes place in California (USDA-NASS, 2016). About 95% of commercial celery production also occurs in California with the remainder occurring in Michigan (USDA-NASS, 2016).

A recent host distribution map for TBRV developed by USDA-APHIS-PPQ-CPHST (Fig. 6) identifies areas of high host acreage based on the acreage of strawberry, the only host currently identified in this datasheet as a major host where economic damage has been consistently reported. This map illustrates that though there are counties in nearly all states with a low level of risk, California, Florida, and Oregon have counties with the highest level of risk for TBRV 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 strawberry in the continental United States and Alaska and Hawaii are provided in the Appendix at the end of the document.

Survey

Approved Method for Pest Surveillance*: The CAPS-approved survey method is a random sample of host material.

Many of the hosts of TBRV are asymptomatic (not showing symptoms) but still contain the virus. Green leaves of mature plants should be sampled randomly irrespective of whether they are showing symptoms.

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

Literature-Based Methods:
Outbreaks of disease caused by TBRV tend to occur in patches ranging from a few square meters to a few hectares in diameter, with patches reflecting the horizontal distribution of the nematode vector in the soil (Converse, 1987). When infection arises from the use of infected planting material, however, infected plants are typically distributed randomly throughout the field (Converse, 1987).

Visual symptoms of TBRV infection are described for strawberry and for some other hosts (see 'symptoms/signs' section). The survey methods used for this virus is visual surveying for symptomatic host material or random/stratified sampling of all host material regardless of symptom expression.

Ward et al. (2009) surveyed *Allium* spp. for several viruses including TBRV by collecting leaves from fields of commercial crops. For sampling, each field was walked in a "W" pattern and 50-100 leaves were collected randomly irrespective of whether they were showing symptoms.

Key Diagnostics

Approved Method for Pest Surveillance*:
Serological for screening and molecular for confirmation.

Screening: A TBRV-specific ELISA kit is commercially available (Neogen Europe Ltd, http://plant.neogeneurope.com/prodtype.asp?strPageHistory=search&numSearhStartRecord=1&CAT_ID=275). However, this ELISA was found to cross react with other nepoviruses and failed to identify one of six tested strains of TBRV (Harper et al., 2011).

Agdia offers testing services for TBRV in the United States via DAS ELISA (https://orders.agdia.com/), but reagents do not appear to be available from Agdia. The ELISA reagents, however, are available from other companies, including Bioreba, which is represented by Eurofin Labs in the United States (http://www.eurofinsus.com/). AC

Diagnostics, based out of Fayettville, AR, recently began to offer a DAS ELISA testing kit and reagents for TBRV (http://www.acdiainc.com/tbrv.htm).

Bioreba also offers a DAS ELISA testing kit for TBRV (http://www.bioreba.ch/). The product description mentions that "the virus shows quite some antigenic variation between strains", suggesting that the kit might not detect all isolates of TBRV. Bioreba's kit uses a mix of antibodies to grapevine and potato virus isolates, and different extraction buffers are recommended depending on the host material.

Confirmation: Work instructions (WI-B-T-G-23 and WI-B-T-G-24) were developed by the CPHST Beltsville Lab. These work instructions were developed using a synthetic target (a mini gene) spiked into healthy plant RNA.

Note: These work instructions require additional validation by CPHST Beltsville using field collected plant samples before work instructions will be made available to the CAPS community.

Detection of *Tomato black ring virus* (TBRV) using a Multiplex Reverse-Transcription (RT) Conventional PCR (Work Instruction: WI-B-T-G-23)
TBRV RNA2 sequences available in National Center for Biotechnology Information (NCBI) nucleotide databases (NC-004440, AY157944 and X80831) were used to design the virus-specific forward primer PTBRV2f and reverse primer PTBRV486mr, a modified version of the published reverse primer TBRV-sp-a (Digiaro et al. 2007). The TBRV assay targets a segment of the capsid protein gene on RNA2 using TBRV-specific primers (PTBRV2f and PTBRV486mr) multiplexed with Nad5 plant gene-specific primers (Menzel et al., 2002) as an internal control in a two-step RT-PCR using a commercial kit.

Detection of *Tomato black ring virus* (TBRV) using a Multiplex RT quantitative PCR (qPCR) in a Cepheid SmartCycler (Work Instruction: WI-B-T-G-24)
The TBRV assay targets a segment of the capsid protein gene on RNA2 using TBRV-specific primers (TBRV2f and TBRV4r) and TaqMan FAM probe (TBRV125p), developed by the Beltsville Lab, multiplexed with plant gene (Nad5) specific primers and TaqMan TET probe (Nad5p1) as an internal control in a two-step RT qPCR using a commercial kit.

*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:
Digiaro et al., (2007) developed a one-step RT-PCR with primers specific to TBRV. This assay was tested by Harper et al. (2011) and found to be effective. Wei and Clover (2008) developed a nepovirus group-B specific one-step RT-PCR, which was successfully used by Šneideris et al., (2012) to identify TBRV in Lithuania. However, this protocol failed to detect one of six tested strains of TBRV when tested by Harper et al. (2011). In addition, degenerate primers RT-

PCR developed by Wei and Clover (2008) could be used for identification of the suspect samples via RT-PCR and sequencing. Harper et al. (2011) developed a one-step real-time RT-PCR that can detect TBRV with high sensitivity and does not cross-react with other common nepoviruses.

Šneideris and Staniulis (2014) developed primers which amplify the full copy of the TBRV coat protein gene sequence in a two-step RT-PCR. These primers successfully identified twelve different strains of TBRV.

A TBRV specific reverse transcription loop-mediated isothermal amplification assay (RT-LAMP) assay was also recently described by Hasiów-Jaroszewska et al. (2015). This protocol was developed using TBRV isolates from 16 different hosts, most which came from Poland. The authors claim the RT-LAMP assay is 100-fold more sensitive than RT-PCR protocols in detecting TBRV.

Easily Confused Species

The number of viruses known to infect strawberry has grown significantly in the last decade (Tzanetakis and Martin, 2013). Other nematode-transmitted viruses which infect strawberry include: strawberry latent ringspot virus (SLRSV), arabis mosaic virus (ArMV), raspberry ringspot virus (RpRSV), and tomato ringspot virus (ToRSV) (Tzanetakis and Martin, 2013). In Europe, TBRV is often found in mixed infections with RpRSV. Both of these viruses are vectored by *L. elongatus*, and they exhibit similar symptoms in strawberry (Martin and Tzanetakis, 2006).

In addition to strawberry, numerous viruses are known to infect raspberry (Martelli and Taylor, 1990), celery (Martelli and Taylor, 1990), artichoke (Gallitelli et al., 2004), and potato (Harrison et al., 1961).

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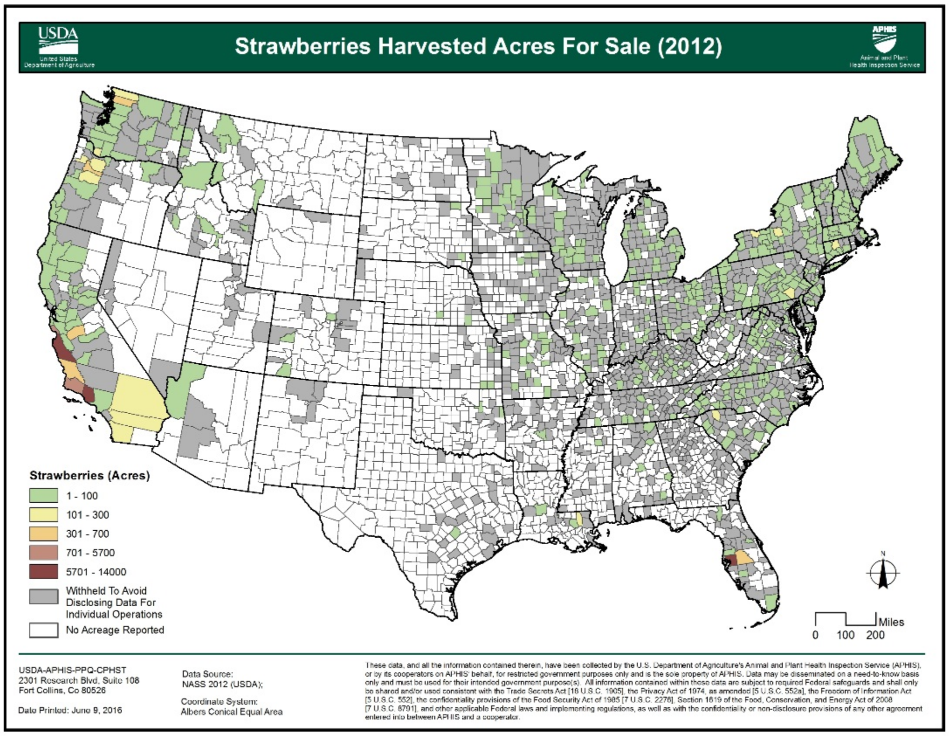
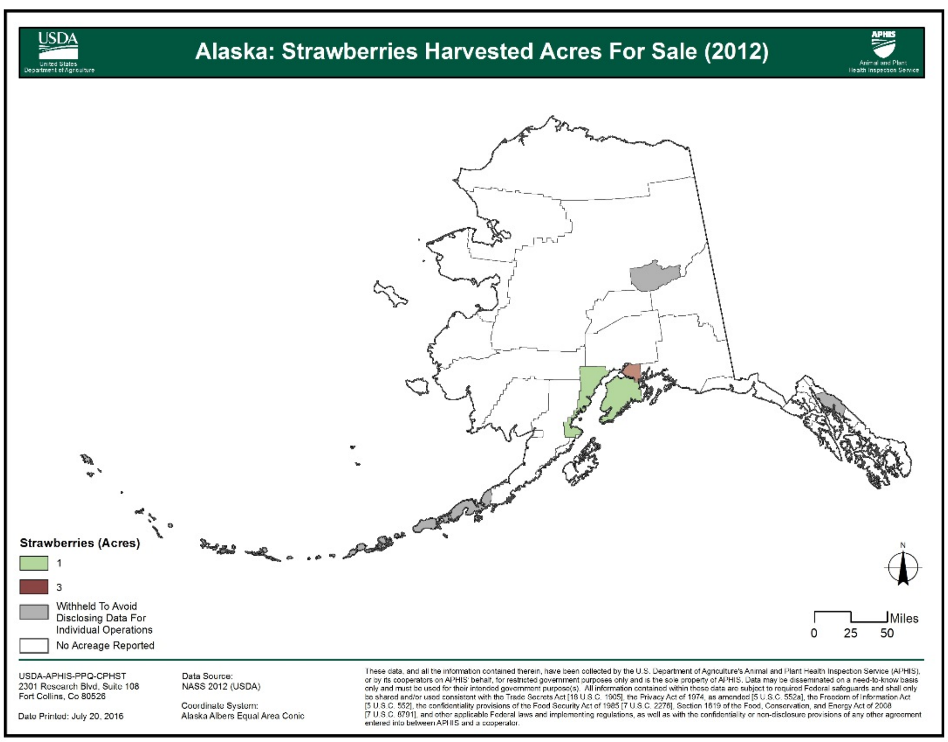
July 2016: Datasheet sent out for subject matter expert review.

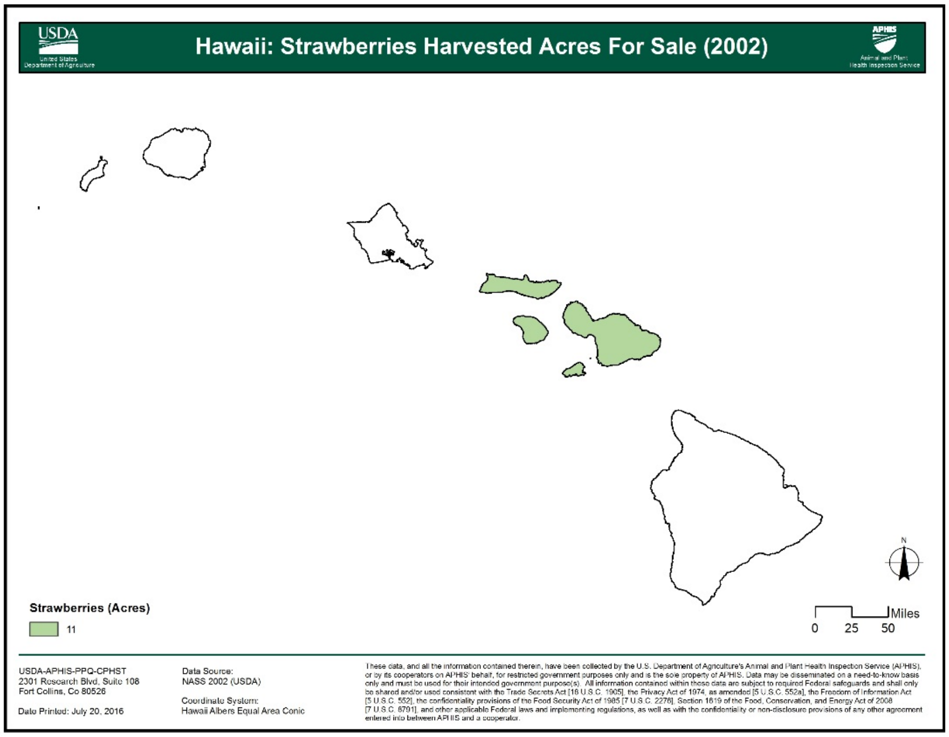
October 2016: Datasheet posted to CAPS Resource and Collaboration site.

Appendix A: Individual Host Maps for *Nepovirus Tomato black ring*

Individual host maps were prepared for the major hosts of *Nepovirus Tomato black ring virus*. At this time, only strawberry is identified as a major host of TBRV.






USDA
United States Department of Agriculture
Hawaii: Strawberries Harvested Acres For Sale (2002)
APHIS
Animal and Plant Health Inspection Service
N
Strawberries (Acres)
11
Miles
0
25
50
USDA-APHIS-PPQ-CPHST
2301 Research Blvd, Suite 108
Fort Collins, Co 80526
Date Printed: July 20, 2016
Data Source:
NASS 2002 (USDA)
Coordinate System:
Hawaii Albers Equal Area Conic
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Appendix B

Figure 7: Local necrotic lesions and ringspots *in Nicotiana tabacum* cv. Xanthi infected with TBRV and showing recovery in new leaves. Courtesy of SCRI.

Figure 8: Symptoms of TBRV on carrot, cv. Panther. Plant Protection Service, Wangeningen, NL.

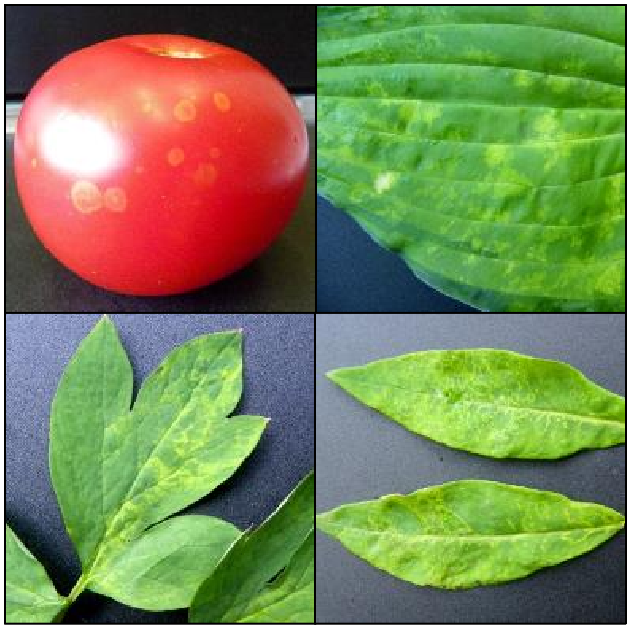

Figure 9. Symptoms of tomato black ring virus (TBRV): Top left – tomato fruit. Top right: hosta. Bottom left: bleeding hearth. Bottom right: phlox. Courtesy of Dontas Šneideris.

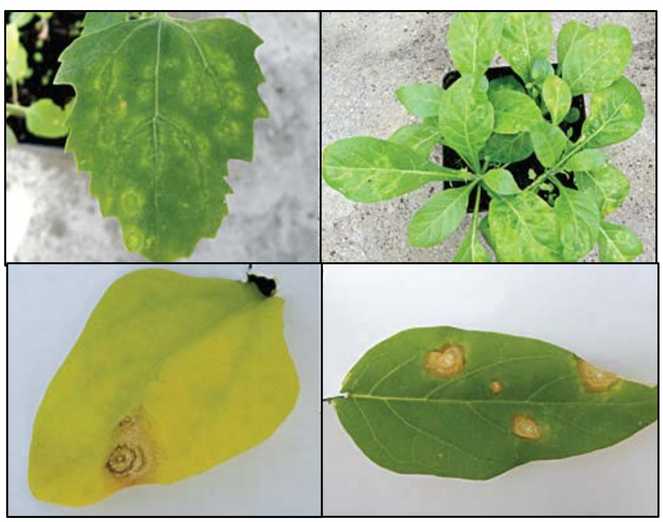

Figure 10. Symptoms of TBRV infection on experimental hosts. Top left: Quinoa leaf (*C. quinoa*). Top right: *Nicotiana debneyi*. Bottom left: leaf of New Zealand spinach (*Tetragonia tetragonioides*). Bottom right: Jimsonweed (*Datura stramonium*) with necrotic ringspots. Courtesy of Dontas Šneideris.

Figure 11. Symptoms of TBRV infection: A) mosaics on *Robinia pseudoacacia* B) mosaics on leaves and ringspots on *Cucurbita pepo* fruits C) mosaics and ringspots on *Nicotiana tabacum* cv. Xanthi D) necrosis on *Solanum lycopersicum*. Image courtesy of Beata Hasiów-Jaroszewska.