



United States  
Department of  
Agriculture

Animal and  
Plant Health  
Inspection  
Service

Plant Protection  
and Quarantine

# New Pest Response Guidelines

*Tobamovirus: Tomato brown rugose fruit virus*



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# Introduction

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Plant Protection and Quarantine (PPQ) develops New Pest Response Guidelines (NPRGs) in preparation for potential future pest introductions. This document is based on the best information available at the time of development and may not reflect the latest state of knowledge at the time the pest is detected. In addition, the PPQ response must be tailored to the specific circumstances of each pest introduction event, which cannot be predicted. Therefore, this document provides only general guidelines, to be used as a basis for developing a situation-specific response plan at the time a new pest is detected.

# Pest Overview

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## Key Information

- ◆ *Tomato brown rugose fruit virus* (ToBRFV) was first identified in Jordan in 2015 (Salem et al., 2016) and has since been reported from numerous other countries.
  - ◆ Its main hosts are tomato (*Solanum lycopersicum* L.) and sweet pepper (*Capsicum annuum* L.) (Salem et al., 2016; Salem et al., 2019).
  - ◆ ToBRFV is primarily a problem in greenhouses. There is no reported case of ToBRFV outbreak in open field tomato production.
  - ◆ Once in a production system, this virus cannot be controlled with chemicals. The only options in these cases are destruction of the plant material and disinfection.
  - ◆ ToBRFV is easily transmitted mechanically through tools, equipment, clothing, workers' hands, plant to plant contact, and in crop debris in soil (Broadbent, 1976; Broadbent and Fletcher, 1963; Dey, 2019).
  - ◆ ToBRFV has been detected in tomato, pepper and hot pepper seeds lots (NVWA, 2020).
  - ◆ The virus can also be spread by the bumble bee *Bombus terrestris* (Linnaeus), which is not present in the United States (Levitzky et al., 2019). We do not know if pollinators present in the United States can spread the virus.
  - ◆ Tobamovirus particles are highly stable (Dombrovsky and Smith, 2017) and can survive for months to years outside of a living host (Broadbent, 1976; Broadbent and Fletcher, 1963).
  - ◆ The virus appears to have an incubation period of 4-30 days depending on hosts and growing conditions. It is systemic, especially in established plants (Ling, 2019a).
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# Taxonomy

## Scientific Name

- ◆ *Tobamovirus: Tomato brown rugose fruit virus*

## Taxonomic Position

- ◆ Viruses : Virgaviridae

## Synonym(s)

- ◆ None

## Common Name(s)

- ◆ *Tomato brown rugose fruit virus*
- ◆ **ToBRFV**

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## Hosts

ToBRFV natural hosts ([Table 2-1](#)) and experimental hosts ([Table 2-2](#)) are listed below.

**Table 2-1** List of natural hosts of ToBRFV

Scientific name	Common name	References
<i>Capsicum annuum</i> L.	sweet pepper	Salem et al., 2019
<i>Capsicum</i> spp.	pepper	NCBI, 2019
<i>Physalis angulata</i> L.	cut-leaf ground-cherry	Duarte et al., 2019
<i>Solanum lycopersicum</i> L.	tomato	Salem et al., 2016
<i>Solanum melongena</i> L. <sup>1</sup>	eggplant	EPPO, 2019 Miss, 2019

<sup>1</sup> A positive sample for ToBRFV was detected in eggplant in Sinaloa, Mexico in December 2018 (EPPO, 2019). Luria et al., 2017, however, tested eggplant and found it not to be a host.

**Table 2-2** List of experimental hosts of ToBRFV

Scientific name	Common name	References
<i>Chenopodium giganteum</i> D. Don (= <i>Chenopodium amaranticolor</i> (Coste & A. Reyn.) Coste & Reyn.)	tree-spinach	Luria et al., 2017
<i>Chenopodium murale</i> (L.) S. Fuentes et al. (= <i>Chenopodium</i> <i>murale</i> L.)	nettle-leaf goosefoot	Luria et al., 2017
<i>Chenopodium quinoa</i> Willd.	quinoa	Luria et al., 2017
<i>Nicotiana benthamiana</i> Domin		Luria et al., 2017
<i>Nicotiana clevelandii</i> A. Gray	Cleveland's tobacco	Luria et al., 2017

<i>Nicotiana glutinosa</i> L.	tobacco	Luria et al., 2017
<i>Nicotiana tabacum</i> L.	tobacco	Luria et al., 2017
<i>Petunia ×atkinsiana</i> (Sweet) D. Don ex W. H. Baxter (= <i>Petunia ×hybrida</i> hort. ex E. Vilm.)	petunia	Luria et al., 2017
<i>Solanum nigrum</i> L. <sup>1</sup>	black nightshade	Luria et al., 2017

<sup>1</sup> Asymptomatic experimental host (Luria et al., 2017)

## Dispersal

### Human-Assisted Spread

Tobamoviruses can be transmitted mechanically through contaminated tools (pruning), farm equipment (Broadbent, 1976), clothing, workers' hands, plant to plant contact, and propagation materials (cuttings, grafting) (Broadbent and Fletcher, 1963; Dey, 2019). They can survive for years in crop debris in soil (Broadbent, 1976) and spread in irrigation water (Smith and Dombrovsky, 2019). Infected seed coats attached to young plants (7-14 days old) can increase transmission of the virus between plants during transplanting of the seedlings (Broadbent, 1965).

### Natural Dispersal

ToBRFV is seed-borne (Dombrovsky, 2019). Seeds lots from tomato, pepper and hot pepper have tested positive for ToBRFV (NVWA, 2020). While transmission of tobamoviruses from seed to seedling is low, mechanical transmission occurs during handling of contaminated seeds while sowing (Smith and Dombrovsky, 2019).

Levitzky et al. (2019) conducted studies in green-/net-houses in Europe and determined that the bumble bee *Bombus terrestris* (Linnaeus) can transmit ToBRFV from contaminated hives to healthy tomato plants during pollination. Transmission of ToBRFV may occur through the adherence of pollen grains attached to the bumble bee's abdomen, mechanically by its vibrating bodies or through the transfer of sap on its mandibles (Levitzky et al., 2019). In the United States, a different bumble bee species, *Bombus impatiens* Cresson, is mass-reared for pollination in greenhouse tomato production in the United States and has been shown to vector the *Pepino mosaic virus* between tomato plants (Shipp et al., 2008) and between tomato and nightshade (*Solanum dulcamara* L.) (Stobbs and Greig, 2014). Further research is needed to determine if *B. impatiens* or other U.S.-bred pollinators can spread the virus.

# Pest Identification

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## Species ID/Diagnostic

Serological methods can be used to detect the presence of tobamoviruses in infected plant materials, and molecular methods are used to confirm the identity of ToBRFV. For seed testing, the International Seed Testing Association has developed an internationally recognized protocol (ISTA, 2019) <https://seedhealth.org/wp-content/uploads/2019/04/ISTARules2019SHmethods7-028.pdf>.

### Serological

- ◆ Agdia ImmunoStrip® for *Tobacco mosaic virus* (TMV) cross-reacts with other tobamoviruses and will give a positive reaction for ToBRFV (Agdia, 2019). Molecular testing is needed following this test for regulatory confirmations of ToBRFV.

### Molecular

- ◆ ToBRFV cannot be diagnosed by a visual inspection of symptoms alone. Symptoms of this virus are very similar to those caused by other tobamoviruses (Alkowni et al., 2019) including *Tomato mosaic virus* and TMV, both of which affect tomato and pepper (Semini, 2017, 2018).
- ◆ ToBRFV can be detected by using a real-time PCR test (Ling et al., 2019; Panno et al., 2019).
- ◆ Recently, a loop-mediated isothermal amplification (LAMP)-based assay to detect ToBRFV has been developed (Sarkes et al., 2020).

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## Signs and Symptoms

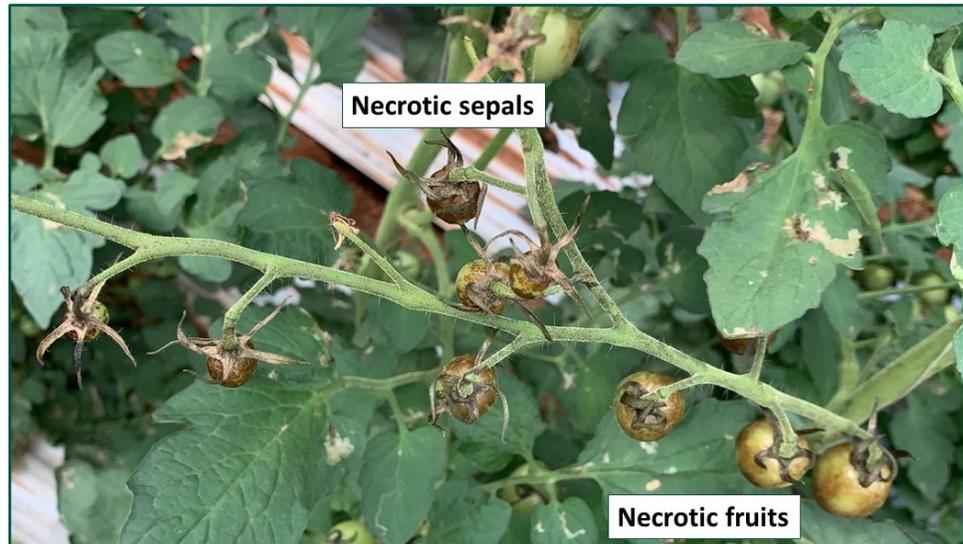
Symptoms on tomato and pepper are similar (Cambron-Crisantos et al., 2018; Luria et al., 2017). The following are important factors to consider:

- ◆ Some infected plants do not necessarily express symptoms (ASTA, 2019).

- ◆ Tomato variety can affect symptom expression (Luria et al., 2017).
- ◆ Severity and occurrence of symptoms vary with the age of the plant at the time of infection. Plants infected at a younger age will develop the most severe symptoms (Fig. 3-1) (DeRuiter, 2019; Dombrovsky et al., 2017).
- ◆ Symptom expression is also influenced by growing conditions (light and temperature), nutritional status, and fruit load (DeRuiter, 2019).
- ◆ Systemic symptoms may appear as early as 4 days post-infection (Luria et al., 2017) but may take as long as 30 days (Panno et al., 2019). Therefore, do not assume asymptomatic plants are not infected (ASTA, 2019).

The following symptoms are found on hosts infected with ToBRFV (Fig. 3-2):

- ◆ Leaves exhibit a mild to severe chlorotic (yellow) mosaic pattern (cover image), wrinkling, mottling (blotching), and occasional narrowing (Fidan et al., 2019; Luria et al., 2017).
- ◆ Fruits display chlorotic, brown, and necrotic areas and can be rough and deformed (cover image), (Figs. 3-1, 3-2) (Cambron-Crisantos et al., 2018; Fidan et al., 2019). In pepper, fruits display similar symptoms (Fig. 3-3) but can also exhibit green grooves (Cambron-Crisantos et al., 2018).
- ◆ Calyces, peduncle, sepals, and petioles develop necrotic spots, which can lead to fruit drop (Davino, 2019b; Fidan et al., 2019; Luria et al., 2017). Stems can also exhibit necrosis (Davino, 2019b) (Fig. 3-4).



**Figure 3-1** Necrotic areas on young tomato fruits and sepals due to infection with ToBRFV (image credit Salvatore Walter Davino, University of Palermo)



**Figure 3-2** Tomato plants naturally infected with ToBRFV; (A-C) leaves of tomato cv. Mose exhibiting symptomatic mosaic patterns; (C) narrowing of leaves; (D) dried peduncles and calyces of tomato cv. Shiran; (E) calyces, pedicle, and petioles displaying necrotic symptoms on tomato cv. Ikram; (F) tomato cv. Mose fruit with yellow spots; (G-I) various symptoms on tomato cv. Odelia fruits; (G) typical disease symptoms; (H) mixed symptoms of tomatoes infected with ToBRFV and *Tomato spotted wilt virus*; and (I) symptoms of ToBRFV found at Sde-Nitzan, Israel (image credit, Luria et al., 2017)



**Figure 3-3** Pepper fruits with yellow blotching due to ToBRFV (image credit Raed Alkowni, An-Najah National University)



**Figure 3-4** Necrotic areas on tomato stems and sepals due to infection with ToBRFV (image credit Salvatore Walter Davino, University of Palermo)

# Delimitation Survey

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## Delimitation Area

Delimitation surveys are carried out to determine the extent of the infected area once an infection has been confirmed. Generally speaking, if ToBRFV has been confirmed on a plant in a greenhouse, all plants in the greenhouse should be removed and autoclaved. However, under certain conditions, it may be permissible to destroy only a portion of the plants. This may be the case if repeated molecular diagnostics reveal negative results in the remaining plants in a greenhouse where positive plants were previously found. In such instances, a delimitation survey may be used to determine how far the virus infection has spread and which plants need to be destroyed.

Talk to the people working with the infected plant(s) to determine which activities may have resulted in the initial infection or transmitted the virus to nearby healthy plants. This information is vital because ToBRFV is mechanically transmitted and asymptomatic in early infections. Consider the following:

- ◆ The source of the infected plant(s);
- ◆ Activities that involve touching infected plants, such as transplanting, pruning, or harvesting;
- ◆ Whether tools were used on multiple plants or across multiple rows;
- ◆ How often neighboring plants are touching; and
- ◆ Which direction workers may have traveled while performing these activities.

Sanitation is key to preventing the rapid spread of ToBRFV during surveys in the greenhouse and/or an open field. **Any contact with an infected plant can transmit this virus.** Change gloves after touching an infected plant and disinfect any tools that have been used to collect samples.

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## Timing of Surveys

Symptoms may appear anywhere between 4 and 30 days after infection (Luria et

al., 2017; Panno et al., 2019). This variability makes it difficult to determine the best time to conduct ToBRFV surveys, as plants may become infected at different times, and therefore, symptom expression will also vary. Therefore, survey tomato and pepper seedlings as early as possible for infection and observe plants at least weekly for symptoms, especially in greenhouse production (Garza, 2018, 2019; Ling, 2019b). Since this virus is mechanically transmitted, survey areas where equipment and people are most likely to be in contact with plant material, especially in entry and exit areas in fields.

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## Survey Techniques for Delimitation

### Survey Preparation, Sanitization and Clean-Up

1. Prior to beginning a survey, determine whether there have been recent pesticide applications that would render it unsafe to inspect the plants. Contact the property owner or manager and ask if there is a re-entry period in effect due to pesticide application. Look for posted signs indicating recent pesticide applications, particularly in commercial fields or nurseries.
2. Determine whether quarantines for other pests or crops are in effect for the survey area. Comply with all quarantine requirements.
3. Take strict measures to prevent contamination by ToBRFV or other pests between properties during inspections.
  - a. Designate a clean area where transport vehicles can park. Make sure this area is not located near infected fields or greenhouses.
  - b. Use disposable protective clothing, gloves and footwear, and change them before entering each site.
  - c. Confirm that equipment, tools, and footwear are clean and sanitized after each use.
  - d. Disinfect used tool(s) with any of the following disinfectants (Li et al., 2015):
    - a. 10% bleach solution, 1 part bleach (any commercial bleach) to 9 parts water
    - b. 21.4% potassium peroxymonosulfate plus 1.5% sodium chloride applied at 2.0% (20 g/L or 0.17 lb/gal)
    - c. 20% solution (wt/vol) of Non-fat dry milk plus 0.1% polysorbate nonionic surfactant
  - e. Thoroughly spray tools with or immerse the cutting portion of the tool(s) in the disinfectant and allow to air-dry to prevent the spread of this virus.
  - f. Change gloves after touching an infected or suspected infected plant.
  - g. Disinfect vehicles and large equipment (e.g., storage areas and bins).

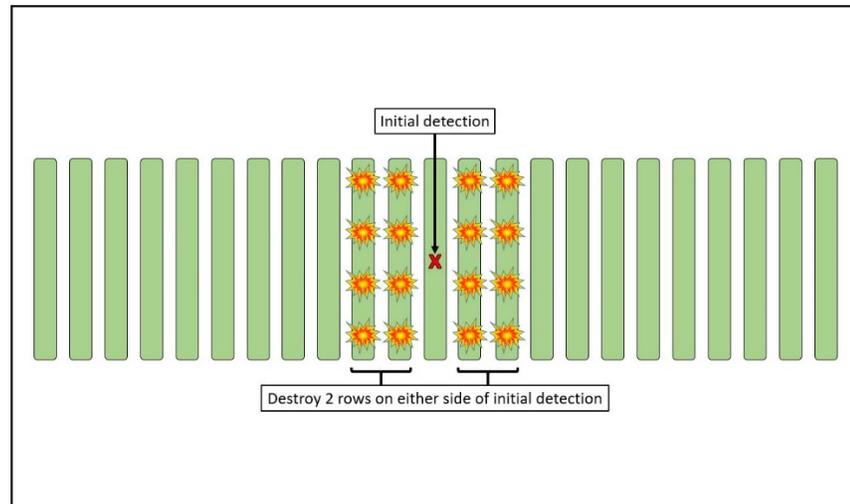
## Visual Inspection

Visually search for plants with symptoms of ToBRFV (see [Signs and Symptoms](#)). Since this virus is mechanically transmitted, visually inspect areas where equipment and people are most likely to be in contact with plant material, especially in entry and exit areas in fields.

## Delimitation Survey

### Greenhouse

1. Remove and autoclave all plants in the row containing the infected plant(s) and at least two rows on either side ([Fig. 4-1](#)). Carefully remove infected and suspected infected plants and place them in large plastic garbage bags to be autoclaved (Gilbertson, 2019b). Remember to change gloves and disinfect any tools used to remove plants.



**Figure 4-1** After an initial detection, destroy the row with the infected plant as well as the two rows on either side

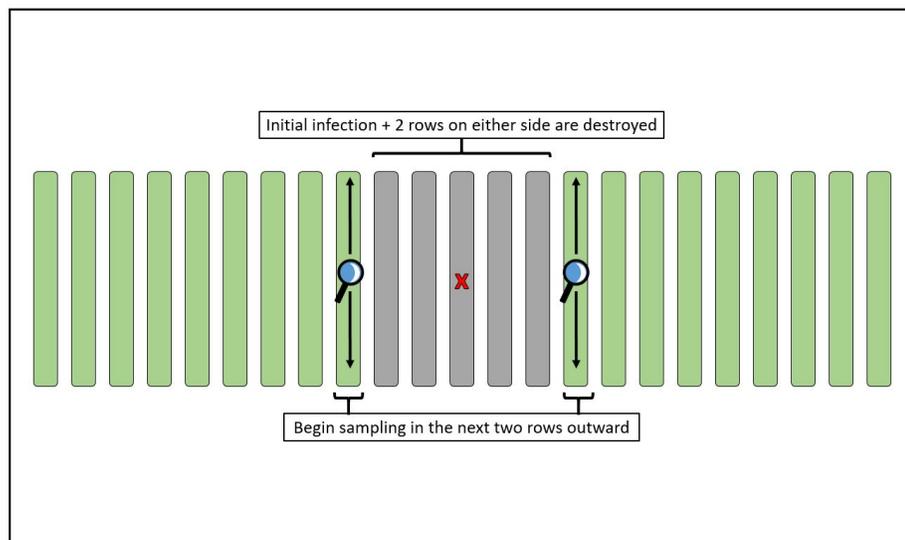
2. After these five rows have been destroyed, begin conducting a delimitation survey to determine the boundary of the virus spread, or the core infected area. The delimitation survey will begin in the two rows next to the five rows removed in step 2 ([Fig. 4-2](#)).
3. Using ImmunoStrip<sup>®</sup> for TMV, determine if the remaining rows contain any tobamovirus-positive plant(s) ([Fig. 4-2](#)) (Ling, 2019a).
4. Consider each row a survey unit. The approximate number of plants in the survey unit will determine the number of random samples collected.
  - a. For example, if there are 100 plants per row, collect a minimum of 59 samples in that row to detect a 5% infection rate with a 99%

confidence interval (Table 4-1).

**Table 4-1** Minimum sample collected to detect a 5% infection rate based on survey unit size (IPPC, 2016)

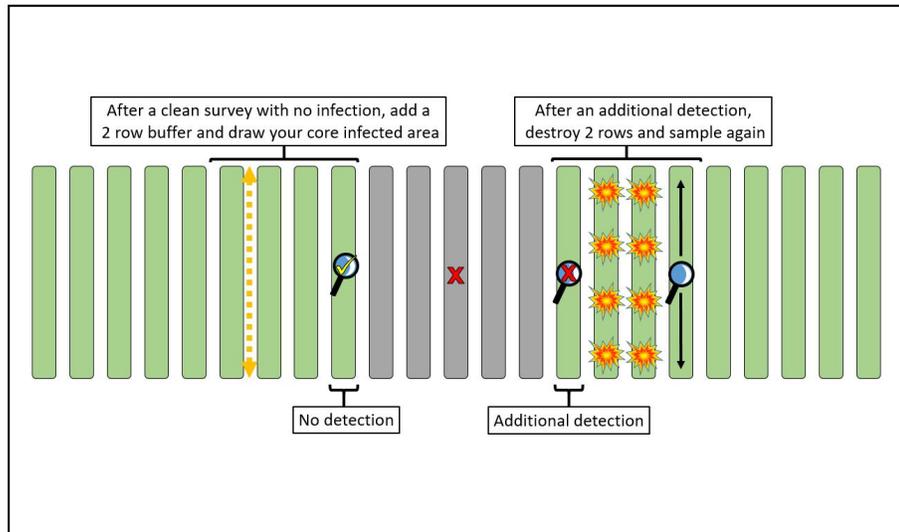
Number of plants in a survey unit	Number of samples needed
25	25
50	45
100	59
200	73
300	78
400	81
500	83
600	84
700–800	85
900-1000	86
2000	88
3000-5000	89
6000 and above	90

<sup>1</sup> We recommend a 99% confidence interval, with a 5% level of detection. If a lower level of confidence interval is desired, then refer to Table 1 in IPPC, 2016.



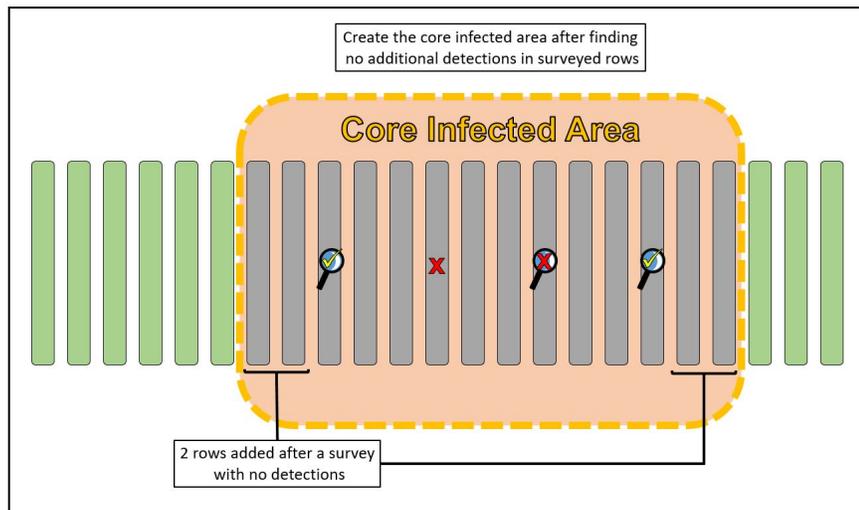
**Figure 4-2** Beginning sampling for the delimitation survey

5. If a positive plant(s) is found, stop sampling.
6. Confirm ToBRFV infection via molecular diagnostics.
7. If positive, repeat the instructions in step 2. Remember to change gloves and disinfect tools used to remove plants.
8. Continue sampling until no positive plant(s) is detected. Once no positive plants are found in the two rows next to a row that has been destroyed, continue sampling for two additional rows (Fig. 4-3).



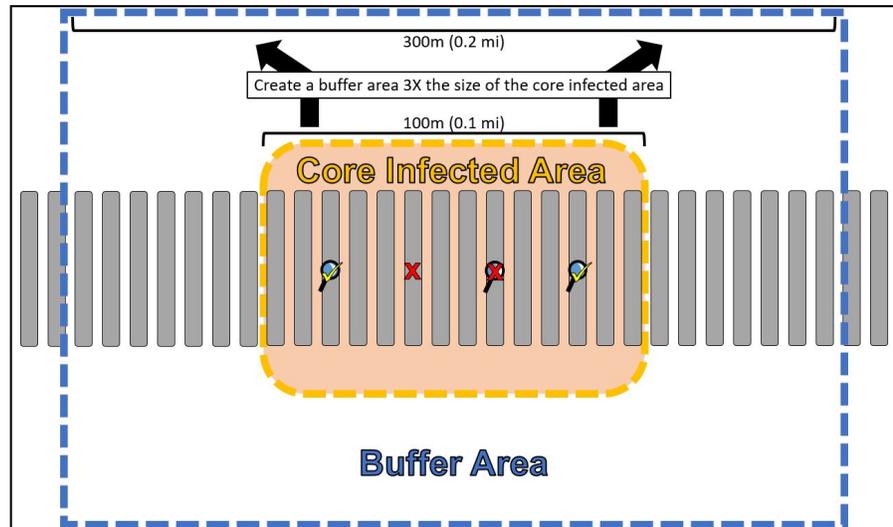
**Figure 4-3** Continuing the survey with a positive detection versus no additional detection

9. If you find no positive plants in these two rows, consider this boundary as the outer limit of your core infected area (Fig. 4-4).



**Figure 4-4** Determining the outer limit of the core infected area

10. Next determine the buffer zone, which will be 3x the diameter of the core infected area (Fig. 4-5).



**Figure 4-5** Creating the buffer zone, which is 3X the diameter of the core infected area. All plants in this area should be considered infected

11. Send all positive samples to a diagnostic laboratory to confirm infection with ToBRFV.
12. Once infection with ToBRFV is confirmed, remove and autoclave all plants within the core infected area and the buffer zone even if they do not show symptoms or test positive.
13. Quarantine the remaining section of the greenhouse and monitor plants at least weekly for symptoms. The incubation period for this virus ranges from 4-30 days (Luria et al., 2017; Panno et al., 2019). Therefore, randomly sample plants for infection for at least a month, continuing to follow sanitation protocols.

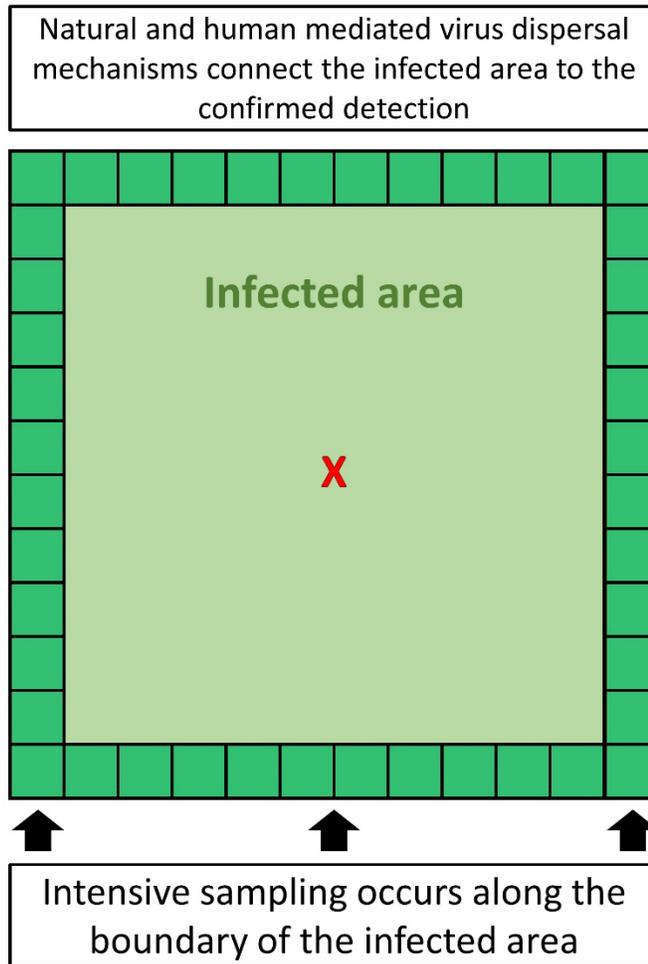
### Field

- ◆ For the field delimitation survey, the infected area must be defined before the survey can take place. The infected area includes the initial confirmed detection and any adjacent or continuous tomato production at risk for virus transmission. This area has a high likelihood of virus transmission.
- ◆ Survey sampling will begin at the outer edge of the infected area and expand outward depending on the location of infected plants found near the survey periphery.
- ◆ The size of the infected area cannot be accurately predicted beforehand, so we suggest delineating the infected area at the time of detection using information from growers and knowledge of the most likely mechanisms for natural and human-mediated virus dispersal.
- ◆ To fully delimit the infection, survey sampling will begin at the outer edge of the initial infected area.

- If infected plants are located along the outer edge, the survey will expand outward. If no infected plants are located, surveys in the buffer zone will commence.
- With no detections along the edge of the initial infected area or in the buffer zone, the initial infected area will be considered the fully delimited infected area.

### *Surveying Along the Boundary of the Infected Area*

1. To help delineate the infected area, collect production information from growers.
  - ◆ Discuss the irrigation practices, tool and equipment use, worker movement, and the amount of contact between adjacent plants near the confirmed infection.
  - ◆ Identify areas adjacent to the initial detection where plants are physically touching, share irrigation, share tools and equipment, or are handled by shared workers.
2. Define the infected area by drawing survey boundary lines along natural or man-made borders that surround the confirmed infection and any plants that are either touching or share human-mediated practices (i.e. recycled irrigation, tools and equipment, or workers) with the confirmed infection.
  - ◆ Because this virus is easily transmitted through contact, this area may be very large.
  - ◆ Plants within the infected area are at high risk of virus transmission and should be quarantined.
  - ◆ After the initial detection, continue monitoring within the infected area weekly for symptomatic plants or infected asymptomatic plants using visual inspections and testing with diagnostic immunostrips.
3. Once the infected area has been defined, divide the area extending outward along the infected area boundary into square survey units. Depending on the size of the infected area, these squares will vary in size. The maximum size of the survey units should be one hectare (100 m × 100 m squares, ~2.5 acres) (Fig. 4-6).



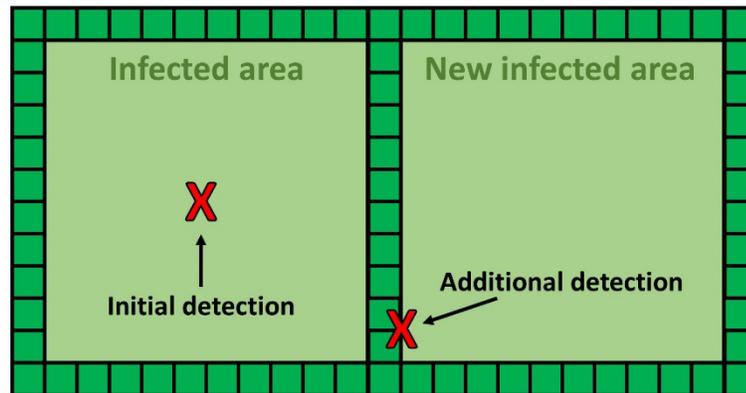
**Figure 4-6** Infected area and boundary survey diagram

2. Each grid square created in step 3 is considered a survey unit and should be sampled systematically. Use the hypergeometric sampling table ([Table 4-2](#)) to determine the number of samples needed per survey unit. Then use [Sampling Plant Part](#) for sampling instructions.

**Table 4-2** Number of plant samples to collect based on the plant population in each field (95% confidence limit of detecting a 1% infestation rate) (Ausvet, 2019)

Total # of plants in field	Total # of plants to sample	# of pooled subsamples	Samples per pooled subsample
25	25	1	25
50	50	1	50
100	100	2	50
200	175	4	50
300	210	5	50
400	235	5	50
500	250	5	50
600	265	6	50
700	270	6	50
800	275	6	50
900	280	6	50
1000	285	6	50
2000	300	7	50
3000	315	7	50
4000+	330	7	50

3. If the region is not easily divided into a grid, use natural boundaries or roads to divide the area into individual survey units.
4. When a new detection is made along the boundary of the infected area, define the new infected area following step 1 (Fig. 4-7). Continue sampling along the boundary of the infected area(s) until no infected plants are found.

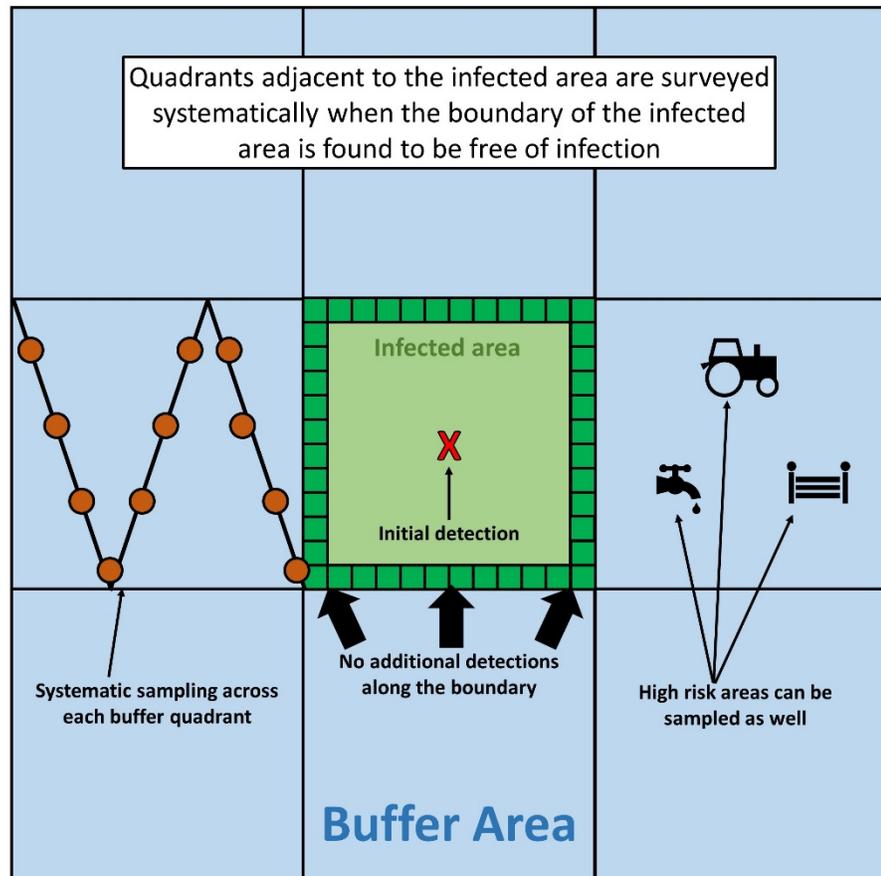


Upon an additional detection, the new infected area is defined as the entire area surrounding the 2<sup>nd</sup> detection that shares some mechanism for virus transmission.

**Figure 4-7** Creating a new infected area based on an additional detection

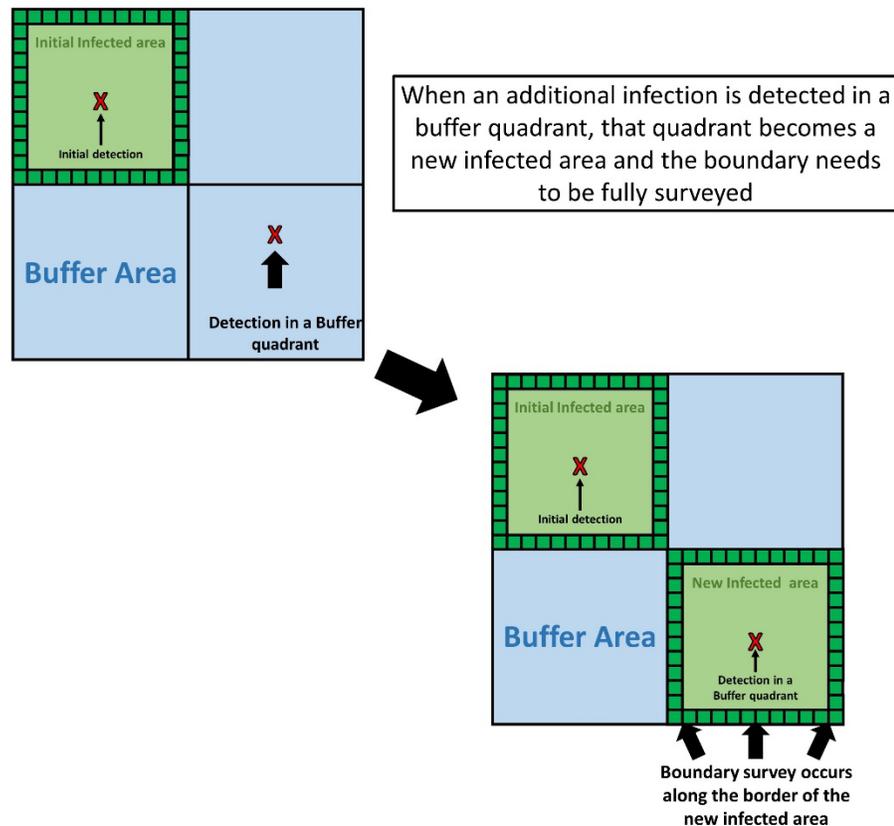
### *Surveying the Buffer Area*

1. Once the boundary around the infected area is fully surveyed and no additional detections have been found, conduct the buffer survey.
2. The buffer area is defined as any region directly adjacent to the infected area that has the potential for virus transmission via natural and human mediated dispersal mechanisms.
  - ◆ Discuss with growers the irrigation practices, tool and equipment use, worker movement, and the amount of contact between adjacent plants in operations adjacent to the infected area.
  - ◆ Any plants that are physically touching, share irrigation, share tools and equipment, or are handled by shared workers within a region adjacent to the infected area should be grouped into buffer survey quadrants.
  - ◆ Divide into distinct buffer survey quadrants all regions adjacent to the infected area that do not share any potential means for transmitting the virus.
3. Sample systematically in each buffer survey quadrant ([Fig. 4-8](#)). Use an estimate of the number of plants in each buffer survey quadrant and the hypergeometric sampling table ([Table 4-2](#)) to determine the number of samples needed per buffer survey quadrant. Then use [Sampling Plant Part](#) for sampling instructions.



**Figure 4-8** Defining the buffer area and surveying within the buffer area

4. High risk areas within the buffer area can be sampled as well. Areas that are likely to be at a high risk for infection with ToBRFV include the following:
  - ◆ Entry and exit areas for equipment
  - ◆ Areas where human activity is more likely
  - ◆ Areas near irrigation and/or drainage



**Figure 4-9** Expanding the delimitation survey when a new detection is made in one of the buffer quadrants

5. When a new detection is made within one of the buffer quadrants, it will become a new infected area, and the boundary will need to be surveyed as described in steps 1-5 of the previous section (Fig. 4-9). Positive detections in the buffer may greatly increase the total size of the delimitation survey.

## Sampling

- ◆ Because ToBRFV is mechanically transmitted, it is vital to change gloves between sample collections and limit touching of plants.
- ◆ Diagnostic laboratory studies must be conducted to determine if ToBRFV is present in a plant(s) and in a seed(s).
- ◆ If there is any doubt as to whether the seed lot originated from a potentially infected plant(s) or could have been the source of an infected plant(s), collect a sample.

## Plant Part

### Leaves

1. Wear gloves when collecting samples.
2. Collect two to three young leaves from a symptomatic plant (Gilbertson, 2019a). If you cannot collect enough symptomatic leaves to fulfill the sample amount indicated in [Table 4-1](#), collect leaves from asymptomatic plants.
  - a. Invert a plastic resealable bag over your hand and use it to pluck two to three young leaves from the plant.
  - b. Pull the plastic resealable bag off your hand and over the leaf, then seal the bag. **Do not remove the leaf from the plastic resealable bag after it has been sealed.**
  - c. Remember to change gloves and disinfect any tools that may have touched the plant. \*Note: if only the baggie has touched the plant, then changing gloves and disinfecting tools is not required. It is recommended, however, to frequently disinfect or change gloves during the sampling process.
  - d. Use a new bag for each leaf sample.
  - e. Label each bag with the following:
    - i. Date sample was collected
    - ii. Greenhouse identifier or growers field identification number
    - iii. Subsample number
    - iv. Collector's identification
    - v. General location in the greenhouse
  - f. Up to 50 plant samples (50 individual bags) can be pooled and placed into a larger, 1-gallon plastic resealable bag. **Pool together samples that are located within adjoining areas of the greenhouse.** This will aid in determining the exact locations of any positive plant(s) that will need to be removed.
    - i. Label the larger 1 gallon plastic resealable bag as the pooled number indicated in [Tables 4-1](#) and [4-2](#).
    - ii. Double bag each sample and place a hard copy of [PPQ Form 391](#) inside the outer bag.
3. Keep plant tissue refrigerated until shipment.
4. When preparing to ship the samples, place the double-bagged samples in a heavy styrofoam container with a few ice blocks. Do not place the samples directly on the ice blocks. Use newspaper or other means to prevent direct contact.
5. Prior to shipping samples, submitters should contact S&T–Beltsville by e-mail at:

[APHIS-PPQCPHSTBeltsvilleSampleDiagnostics@aphis.usda.gov](mailto:APHIS-PPQCPHSTBeltsvilleSampleDiagnostics@aphis.usda.gov)

[PPQ.Domestic.Diagnostic.Coordinator@aphis.usda.gov](mailto:PPQ.Domestic.Diagnostic.Coordinator@aphis.usda.gov)

- i. Include in the e-mail the tracking number, number of samples to be shipped, the suspected plant pathogen, and an electronic attachment of [PPQ Form 391](#).
  - b. Seal all seams of the shipping container with shipping tape.
  - c. Ship via overnight express courier (FedEx, UPS, etc.) Monday-Thursday. Do not ship on Fridays.
6. Send samples to:  
Sample Diagnostics  
Attn: John Bienapfl  
USDA-APHIS-PPQ-S&T  
B-580, BARC-East  
Powder Mill Road  
Beltsville, MD 20705-2350

### *Fruits*

Collecting fruit samples is not recommended when leaf tissue is available. If there is not an adequate amount of fresh leaf material available to complete the survey because the foliage has already dried up or died, or the leaf tissue is not accessible to the surveyor, then fruit may be collected.

- A. If symptoms are observed, cut a disk from the symptomatic fruit, place it into a single, resealable plastic bag, label the bag as described above, and then place it into a second, resealable plastic bag.
- B. Multiple individually labeled bags with fruit samples from the same field or greenhouse may be placed into the second bag.
- C. The knife and your gloves must be sanitized with a disinfectant between each plant.

### **Seeds**

Surveyors visiting greenhouses should obtain seed samples after a positive detection of ToBRFV.

Take the following actions to collect a representative sample from the seed lot:

1. Samples from individual lots **MUST** be kept separate.
2. Collect seed samples based on a percentage of the total number of seeds per lot ([Table 4-3](#)).
3. Take the appropriate number of spoonfuls of seed (sub-samples) from each

seed lot as per [Table 4-3](#) and based on the approximate number of seeds remaining in the lot.

- a. Use a clean (new) sampling spoon per seed lot to avoid cross-contamination.
  - b. Place the spoon into the seed bag and extract the appropriate amount of seeds.
  - c. Pour the seeds into a clean plastic resealable bag ([Fig. 4-10](#)) and repeat for as many spoonfuls as are required per [Table 4-3](#).
  - d. After the last spoonful for the seed lot, insert the spoon into the bag and seal the bag. The sample should include the seeds and the spoon.
  - e. Label each bag with the following:
    - i. Sample type
    - ii. Seed lot number
    - iii. Variety (if known)
    - iv. Date sample collected
    - v. Greenhouse identifier
    - vi. Subsample number
    - vii. Collector's identification
    - viii. Any other pertinent identification information
  - f. Place the bagged seed samples in a large plastic resealable bag(s) with a copy of [PPQ Form 391](#) – Specimens for detection.
4. Ship the seed samples to S&T–Beltsville via UPS/FedEx; the preferred route is UPS. Send samples to:

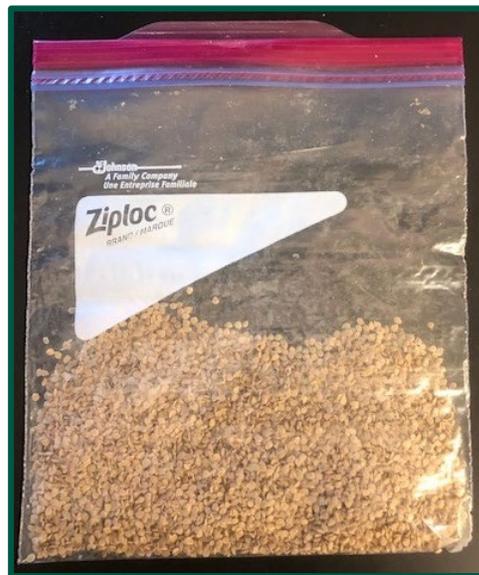
#### Sample Diagnostics

Attn: Vessela Mavrodieva or Deric Picton  
USDA–APHIS–PPQ–S&T Beltsville Lab  
Bldg. 580, BARC-East,  
Powder Mill Rd, Beltsville, MD 20705  
Phone 301-313-9214 or 301-313-9200

**Table 4-3** Sample size for seed lots

Lot size (N = # of seeds)	Sample size (# of seeds) for testing	# teaspoons for testing
500	100	0.25
1,000	200	0.5
1,500	300	0.75
2,000	400	1.0
2,500	500	1.25
3,000	600	1.5
3,500	700	1.75
4,000	800	2.0
4,500	900	2.25
5,000	1,000	2.75
7,500	1,500	3.75
10,000	2,000	5.0
15,000	2,500	6.25
20,000	2,800	7.0

<sup>1</sup> For lots larger than 20,000 seeds a sample size of 3000 is required to be tested.



**Figure 4-10** One quart plastic resealable bag containing a sample of approximately 10 spoonfuls  $\approx$  16 grams of tomato seed sampled

# Eradication and Control Options

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## Overview

This information can be used by PPQ decision-makers after a detection to assess the suitability of potential actions to eradicate, contain, or suppress ToBRFV. The efficacy and feasibility of each control option depends on the pest situation at the time of detection. Factors including detection location (e.g., natural or urban environment, agricultural crops, greenhouses, nurseries), area of spread, the climatic region, the time of year, the phenology of the host, and current practices already in place contribute to determining whether a particular control option is appropriate.

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## Eradication Options

### Host Removal

#### Greenhouse and Field

Remove all infected plant material and properly dispose of it using APHIS-approved disposal methods. Destroy or dispose of all plant material that can be reasonably removed from the field. This includes cull piles and other plant debris. A heat treatment (steam/autoclave) with an internal temperature of 100 °C (212 °F) for a minimum of 30 minutes should be applied to all plant material (i.e. plant parts, soil) that may contain or is suspected of containing ToBRFV. All material can then be buried in a landfill. The heat produced in compost piles may not be sufficient to destroy ToBRFV (USDA-APHIS-PPQ-S&T, 2019).

#### Cultural Control and Sanitary Measures

In addition to host removal, follow the measures listed below.

To reduce the risk of spreading ToBRFV within the greenhouse, nursery, or field, use the following strict sanitation and preventative practices (Agriculture Victoria, 2019; ASTA, 2019; Darzi et al., 2018; Davino, 2019a; Euroseeds, n.a.; Levitzky

et al., 2019; Persley and Gambley, 2010; Smith and Dombrovsky, 2019; Verhoeven, 2019):

- ◆ Use certified seed, seedling, and virus-free graft material.
- ◆ Treat each infected area (greenhouse, nursery, or field) as a separate unit:
  - Limit facility access to include only authorized personnel.
  - Wear only clean clothing. Clothing can become contaminated through exposure in infected greenhouses, nurseries, and fields. Wash all clothing in hot water with soap prior to wearing again.
  - Do not move protective clothing (overalls, gloves, head cap, and shoe covers) and tools from one area to another. Store them at each site.
    - If possible, use disposable protective clothing.
    - Pull off gloves from the wrist upwards so that the glove ends up inside out.
    - Put all clothing into a hermetically sealed bag prior to exiting the area for washing or disposal.
    - Put disposable clothing in the appropriate bin for immediate destruction.
  - Use disinfectant mats at entrances for footwear and wheeled equipment.
    - Disinfect footwear before entering and leaving infected areas.
  - Sanitize tools after use on each plant.
  - Wash hands with soap or disinfectants before and after handling plants and before and after donning gloves.
- ◆ Minimize movement between sites and move from a non-infected area to an infected area.
- ◆ This virus can survive in water. Disinfect drains, water storage areas and irrigation water.
- ◆ When removing plants and plant material, do not touch plants or surfaces in greenhouse or nurseries.
  - Turn off irrigation water a day prior to plant removal to decrease the risk of sap transfer.
  - Incinerate all infected and suspected infected plants and plant material.
  - Destroy or sterilize all plant trays that come into contact with infected or suspected infected plants and plant material.
- ◆ Do not bring anything into an infected or suspected infected area that is not needed (i.e. jewelry, watches, phones, laptops, etc.). Everything will need to be disinfected before exiting the site.

- ◆ Clean glasses with alcohol tissues when leaving infected areas.
- ◆ Use any of the following disinfectants on all tools, trolleys (including wheels), machinery, work areas, and anything else that may have come into contact with infected plants and plant material (Li et al., 2015):
  - 10% bleach solution, 1 part bleach (any commercial bleach) to 9 parts water
  - Use 21.4% potassium peroxymonosulfate plus 1.5% sodium chloride applied at 2.0% (20 g/L) (20 g/L or 0.17 lb/gal)
  - 20% solution (wt/vol) of non-fat dry milk plus 0.1% polysorbate nonionic surfactant
- ◆ Researchers are conducting studies on the efficacy of disinfection methods and ToBRFV survival.
  - Interim results indicate that this virus will survive on skin and gloves for longer than 2 hours and on bare hands washed with water and medicated hand wash for one minute (Skelton, 2019).
  - ToBRFV can also survive on glass, hard plastic, stainless steel and polythene for at least 3 months, on aluminum for at least 1 month, and on concrete for up to 3 months, but this varies because in some cases the virus did not survive 2 weeks (Skelton, 2020).
  - Skelton (2019) was able to determine that this virus cannot survive on trays soaked in hot water at 90 °C (194 °F) for 5 minutes.
- ◆ Best practice recommendation for *Cucumber green mottle mosaic virus* is to avoid the use of bee hives in greenhouses if infection is found prior to flowering. Consider following the same protocol for ToBRFV.
- ◆ Use naïve (young) bee hives for pollination.
- ◆ Rotate with non-solanaceous crops every two to three years.

**Important:** Sanitize all equipment and tools between pruning and cutting. See [Survey Preparation, Sanitization and Clean-Up](#) for more information.

## Chemical Control

No chemical controls are available for plants infected with ToBRFV.

Soil remediation studies using short-duration steaming with exothermic chemicals, such as potassium hydroxide, decreased the infectivity of TMV below 3.0 % (Luvisi et al., 2015). Similar treatments could possibly be applied to ToBRFV.

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# Environmental Compliance

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## Introduction

Use *Appendix A* as a guide to environmental regulations pertinent to *Tomato brown rugose fruit virus*.

## Overview

Program managers of Federal emergency response or domestic pest control programs must ensure that their programs comply with all Federal Acts and Executive Orders pertaining to the environment, as applicable. Two primary Federal Acts, the National Environmental Policy Act (NEPA) and the Endangered Species Act (ESA), often require the development of significant documentation before program actions may commence. Environmental and Risk Analysis Services (ERAS), a unit of APHIS' Policy and Program Development Staff (PPD), is available to provide guidance and advice to program managers and prepare drafts of applicable environmental documentation. In preparing draft NEPA documentation ERAS may also perform and incorporate assessments that pertain to other Acts and Executive Orders, described below, as part of the NEPA process. The Environmental Compliance Team (ECT), a part of PPQ's Emergency Domestic Programs (EDP), assists ERAS in development of documents and implements any environmental monitoring. Program leadership is strongly advised to consult with ERAS and/or ECT early in the development of a program in order to conduct a preliminary review of applicable environmental statutes and to ensure timely compliance.

Environmental monitoring of APHIS pest control activities may be required as part of compliance with environmental statutes, as requested by program managers, or as suggested to address concerns with controversial activities. Monitoring may be conducted with regards to worker exposure, pesticide quality assurance and control, off-site chemical deposition, or program efficacy. Different tools and techniques are used depending on the monitoring goals and control techniques used in the program. Staff from ECT will work with the program manager to develop an environmental monitoring plan, conduct training to implement the plan, provide day-to-day guidance on monitoring, and provide an

interpretive report of monitoring activities.

The following is list of pertinent laws and Executive Orders:

**National Environmental Policy Act (NEPA)** – NEPA requires all Federal agencies to examine whether their actions may significantly affect the quality of the human environment. The purpose of NEPA is to inform the decision-maker prior to taking action and to inform the public of the decision. Actions that are excluded from this examination, actions that normally require an Environmental Assessment, and actions that normally require Environmental Impact Statements are codified in APHIS’ NEPA Implementing Procedures located in 7 CFR 372.5.

The three types of NEPA documentation are:

### **1. Categorical Exclusion**

Categorical exclusions are classes of actions that do not have a significant effect on the quality of the human environment and for which neither an environmental assessment (EA) nor an environmental impact statement (EIS) is required. Generally, the means through which adverse environmental impacts may be avoided or minimized have actually been built into the actions themselves (see 7 CFR 372.5(c)).

### **2. Environmental Assessment (EA)**

An EA is a public document that succinctly presents information and analysis for the decision-maker of the proposed action. An EA can lead to the preparation of an environmental impact statement (EIS), a finding of no significant impact (FONSI), or the abandonment of a proposed action.

### **3. Environmental Impact Statement (EIS)**

In the event that a major Federal action may significantly affect the quality of the human environment (adverse or beneficial), or, the proposed action may result in public controversy, an EIS is prepared.

**Endangered Species Act (ESA)** – This statute requires that programs consider their potential effects on federally protected species. The ESA requires programs to identify protected species and their habitat in or near program areas and documentation of how adverse effects to these species will be avoided. The documentation may require review and approval by the U.S. Fish and Wildlife Service and the National Marine Fisheries Service before program activities can begin. Knowingly violating this law can lead to criminal charges against individual staff members and program managers.

**Migratory Bird Treaty Act** – This statute requires that programs avoid harm to over 800 endemic bird species, eggs, and their nests. In some cases, permits may be available to capture birds, which require coordination with the U.S. Fish and Wildlife Service.

**Clean Water Act** – This statute requires various permits for work in wetlands and for potential discharges of program chemicals into water. This may require coordination with the Environmental Protection Agency, individual states, and the Army Corps of Engineers. Such permits would be required even if the pesticide label allows for direct application to water.

**Tribal Consultation** – This Executive Order requires formal government to government communication and interaction if a program might have substantial direct effects on any federally-recognized Indian Nation. This process is often incorrectly included as part of the NEPA process, but must be completed prior to general public involvement under NEPA. Staff should be cognizant of the conflict that could arise when proposed federal actions intersect with tribal sovereignty. Tribal consultation is designed to identify and avoid such potential conflict.

**National Historic Preservation Act** – This statute requires programs to consider potential impacts on historic properties (such as buildings and archaeological sites) and requires coordination with local State Historic Preservation Offices. Documentation under this act involves inventorying the project area for historic properties and determining what effects, if any, the project may have on historic properties. This process may require public involvement and comment prior to the start of program activities.

**Coastal Zone Management Act** – This statute requires coordination with states where programs may impact Coastal Zone Management Plans. Federal activities that may affect coastal resources are evaluated through a process called “federal consistency”. This process allows the public, local governments, Tribes, and state agencies an opportunity to review the federal action. The federal consistency process is administered individually by states with Coastal Zone Management Plans.

**Environmental Justice** – This Executive Order requires consideration of program impacts on minority and economically disadvantaged populations. Compliance is usually achieved within the NEPA documentation for a project. Programs are required to given consider if the actions might disproportionately impact minority or economically disadvantaged populations, and if so, how such impact will be avoided.

**Protection of Children** – This Executive Order requires federal agencies to

identify, assess, and address environmental health risks and safety risks that may disproportionately affect children. If such a risk is identified, then measures must be described and implemented to minimize such risks.

# Comments and Feedback

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## Considerations

The NPRG production team focuses on the technical scientific methods found in the literature that may be useful to the agency when taking action on a new pest. We attempt to balance scientific rigor with program feasibility but are not involved in the decision making processes. In order to improve our ability to balance what we find in the literature with what is possible in the field, we would like you to answer the following questions after using this document. Please email a copy of the NPRG after answering the questions below to [PPQ.NPRG@usda.gov](mailto:PPQ.NPRG@usda.gov).

Name:

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Contact (please include contact information if you would like us to reach out to you in response to your comments):

Which parts of the NPRG did your group find most useful?

Which parts of the NPRG were not useful, or were unclear?

What could be added to future NPRGs to make them more useful?

If there is not enough space for your responses to the questions above, or if you have specific feedback about this NPRG, please use track changes or insert comments into the document itself.

Thank you!

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## Cover Image

Infected tomato fruits can be rough, deformed, brown, necrotic, and wrinkled, while leaves are mottled or mosaic (image credit José Antonio Garzón-Tiznado, Universidad Autónoma de Sinaloa)