

TOMATO BROWN RUGOSE FRUIT VIRUS (ToBRFV):

Q&A ON THE NEW TOBAMOVIRUS:
TOMATO BROWN RUGOSE
FRUIT VIRUS (ToBRFV)



INTRODUCTION

Tomato brown rugose fruit virus (ToBRFV) was first discovered in late 2014. It subsequently was found on tomato in Jordan (2015), then in Mexico and is quickly being identified in other countries worldwide.

OBJECTIVE

As ToBRFV continues to spread to solanaceous crops (especially peppers and tomatoes) globally, it is essential for us to understand:

1. How to detect the virus
2. Its detrimental impact
3. Prevention and mitigation

PURPOSE

This document is a Q&A for informational purposes only in order to assist seed companies and growers in taking precautions and preventative steps to help minimize the risks associated with the disease, especially in high input production systems.

VIRUS HISTORY & SPREAD

Where else has the virus been affecting growers?

ToBRFV has been confirmed in the United States (California), Mexico, Germany, Italy (Sicily), Saudi Arabia, Israel, Jordan and Turkey. Likely occurrences have been reported but not confirmed in Chile, Ethiopia, Sudan and the Netherlands.



CHARACTERISTICS & RESISTANCE

Tobamoviruses, as a group, share many similar characteristics. How does this virus compare to those previously described on tomato?

There are several tobamoviruses, including *Tobacco Mosaic Virus* (TMV) and *Tomato Mosaic Virus* (ToMV), in addition to ToBRFV that infect tomato. However, one of the distinguishing and problematic characteristics of **ToBRFV is its ability to overcome all known genetic resistances in tomato, including the Tm-2² gene, and cause severe fruit symptoms on otherwise resistant tomato varieties** (see images page 3).

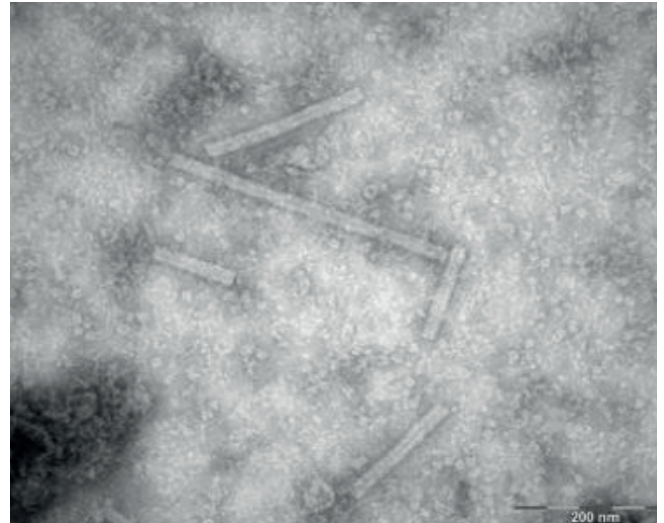
Pepper is another primary host for ToBRFV. The L genes for resistance to TMV and PMMoV (*Pepper mild mottle virus*) in pepper currently seem to hold up to ToBRFV. Peppers without the resistance gene(s) are highly susceptible to the virus (see images page 3).

In both tomato and susceptible pepper, mechanical transmission of the virus occurs extremely easily. Therefore, caution is advised in order to help avoid the possible transfer of the virus from infected to healthy plants. This commonly occurs via mechanical means such as touching and manipulation of infected plants. The virus can be spread between pepper and tomato plants, as well as plants of the same crop type, especially in transplant productions or in crop production systems in which plants are regularly handled. The virus can also spread via infected fruits.

ToBRFV is similar to other tobamoviruses in that the virus particles are long, symmetrical rods. Therefore, it cannot be distinguished by observation by electron microscopy. It also has many other characteristics similar to those of other tobamoviruses, including being **very stable** and **very infectious**.

Are there any resistant tomato varieties?

Tomato F1 cultivars that are highly resistant (HR) to ToMV and TMV can be severely affected by this new virus. The known resistance genes do not protect against



Tobamoviruses are long, symmetrical rod viruses

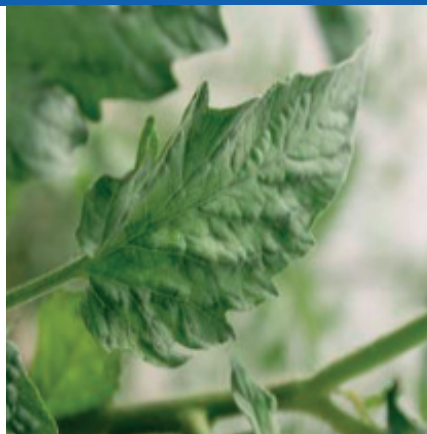
ToBRFV, and only preventative crop management and sanitation practices will aide in preventing introduction. or mitigating the spread of the virus and allow for the production of a salable crop.

Note that it has been reported that some tomato varieties can develop a high virus level without physically expressing symptoms. Infected plants of these varieties can then unwittingly become a source for spread of ToBRFV infection to healthy tomato or susceptible pepper plants.

How long will the seed industry need to develop a resistant variety?

Seed companies are diligently working on identifying and introducing resistance to the virus in new varieties. However, it could take several years until new resistant varieties are introduced, so it is essential to keep strict sanitary protocols to prevent development and further spread of the virus.

LEAF: Symptoms caused by ToBRFV include bubbling and mosaic on leaves of susceptible pepper, and fern leaf and mosaic of tomato.



CALYX: Symptoms include distinct discoloration (browning) of the veins of the calyx in an early stage of tomato fruit development or drying out and browning of the end of the calyx tips.



FRUIT: Fruit on infected tomato plants may be undersized with a rough surface, or complete fruit abortion may occur. Fruit coloration is affected with symptoms such as blotching, pale color and/or brown necrotic spots occurring. The number of fruits produced is reduced and fruits could be unmarketable or reduced in quality. Necrosis can occur on susceptible pepper fruit.



TO HELP MANAGE THE RISK FROM ToBRFV

What do the symptoms look like in tomato?

The symptoms of ToBRFV are similar to those that occur on leaves, the fruit calyx and the fruit of TMV and ToMV infected susceptible tomato plants (see images page 2). These symptoms, when they occur on varieties with the Tm resistance gene(s), strongly suggest the presence of ToBRFV. Additionally, symptoms caused by this virus may be much more severe, especially on the fruit.

What are some suggestions if ToBRFV infection is suspected?

- First, check if the symptoms match with typical symptoms of tobamovirus.
- It would then be prudent to isolate suspected plants and surrounding plants (at least 1.5 meters suggested), and to initially handle them through sanitary precautions in the same way as would be done with known tobamovirus infections.
- If tobamovirus symptoms occur on a variety with known resistance, it increases the likelihood that it could be ToBRFV. However further tests would be needed for verification. It has been reported that

the Agdia immunostrip for TMV will give a positive reaction for ToBRFV. This can be used as a prescreen, but confirmation must then be done with additional laboratory based identification testing.

How will commercial tomato varieties be affected?

Even Tomato F1 cultivars with high resistance (HR) to ToMV and TMV can be severely affected by this new virus. There is some indication that ToBRFV may cause more severe symptoms on some varieties vs. others. It is possible that a variety may be infected in which symptoms are mild, or not present, but the plants may still have a high concentration of the virus. Also, note that as is typical in a tobamovirus infection, symptoms may vary depending on environmental and growing conditions, especially light and temperature.

Can growers use chemicals to cure infected plants?

No chemicals can be used to cure an infected plant; however precautions such as the use of disinfectants to sanitize surfaces and implements can be helpful in controlling the spread of the virus.

Can ToBRFV be found on the seed?

ToBRFV, like other tobamoviruses, can occur in association with tomato and pepper seed. It is thought to be found primarily on the seed surface, and to a lesser extent internally in the seed, and can maintain its infectivity for years. By testing all sources of seed (experimental, parent, trial varieties or commercial), or if grafting is performed with tomato, the seed of the rootstock and scion, it is possible to detect the presence of living and pathogenic virus. An appropriate sampling and testing method should be used. The International Seed Health Initiative (ISHI-Veg) method

for detection of tobamoviruses, detects ToBRFV. It is a local lesion assay which involves inoculation of indicator tobacco plant leaves with tomato and pepper seed, respectively, ground in buffer (<https://www.worldseed.org/our-work/phytosanitary-matters/seed-health/isshi-veg-protocols/>). In addition, ELISA can be used as a pre-screen to evaluate seedlots for the presence of ToBRFV. It detects the presence of virus coat protein but does not determine if the virus is viable or not. Therefore, a positive result with ELISA should be followed with a confirmatory test such as the local lesion assay.

SUGGESTIONS TO TRY AND LIMIT THE SPREAD OF ToBRFV:

What can be done in the transplant nursery?

- Inspect transplants, especially prior to grafting (if used) weekly for visible symptoms of ToBRFV. Note that symptoms may not be visible on infected young plants. However, If infected plants are detected in the transplant nursery, it is possible that other nearby seedlings are infected as well. In this case, it is recommended to confirm the presence of ToBRFV, and then to eliminate all plants within a minimum of 1.5 meters beyond the outermost symptomatic plant because it is possible that these plants also have been infected, but have not yet developed symptoms.
- Be careful not to touch other surfaces during the plant removal process. Cutting off irrigation to the plants to be removed a day prior to the process will result in a lower risk of sap transfer and inspection spread. After removal, dispose of these plants by incineration. Sterilize or destroy all plant trays that contained infected plants. All crop debris and substrate must be removed and buried or incinerated in a manner to avoid airborne debris.
- Work areas, tools or machinery must be cleaned and disinfected. Several products, including potassium peroxy-monosulfate (Virkon S) or freshly prepared 0.5% sodium hypochlorite (NaOCl) bleach, can be effective disinfectants. In protected productions the use of Non-Fat Dry Milk (NFDM, 3.5% protein) helps prevent spread of tobamoviruses.

During cultivation

Healthy crop:

- Only enter the crop with clean (washed) clothes.
- Prevent any unnecessary visits and **do not touch plants** if not absolutely needed.
- Be aware that **clothes could be contaminated** during the eating of tomatoes at home, or through exposure in infected nurseries or greenhouses.
- Follow good hygiene practices by **washing hands with soap or disinfectants before and after handling plants. Use a small nail brush to improve the hand washing process.**

- Preferably use protective clothing **that will stay in the greenhouse after use.**
- Clean work boots and/or shoes with **disinfectant before entering and after leaving the greenhouse.**
- **Sanitize cutting tools and other implements** after each plant with disinfectant.
- Thoroughly **clean and disinfect** the greenhouse at the end of a crop season.

Infected crop:

If an infected plant is detected (confirmation of the diagnosis to be done by a specialized laboratory).

It is recommended:

- To carefully **remove** symptomatic plants and **destroy** them by burying or incineration in a manner to avoid airborne debris.
- To treat each infected greenhouse as a separate unit. Use specific protective clothing (lab coat and gloves) and tools and store them in the greenhouse. Do not move them to another greenhouse. Start every day with cleaned clothes. Wash all clothes in hot water and soap before using them again.
- To wash hands with soap. Small tools can be dipped in a **Non-Fat Dry Milk (NFDM) 3.5% protein** solution. Milk has the advantages of being effective, safe and inexpensive.
- To limit access to the facilities to authorized personnel only.
- To prevent spread in the greenhouse, the area surrounding the one from which infected plants have been removed **should be worked last.**
- Do not assume that asymptomatic plants are not infected. It takes several days from initial infection for symptoms to develop. Also, some varieties may be asymptomatic despite infection by the virus.
- If possible, adjust the daily operations based on the sanitary status of the greenhouses by **avoiding moving from an infected greenhouse or field to a non- infected one.**
- At the end of cultivation make sure that all materials, and the greenhouses are cleaned and disinfected.

HOW LONG WILL THE VIRUS HAVE AN IMPACT?

Tobamoviruses are very stable and can survive for long periods in infected crop debris, in the soil or on contaminated surfaces. On surfaces such as a bench tops, survival could be weeks to months (see ASTA CGMMV Bulletin) and in infected plant debris, survival can be for as long as the infected debris remains intact. Spread of the virus can occur very readily by mechanical

transfer, especially in protected or high input culture systems where plants are pruned, staked, handled or touched frequently. In open field productions machinery used for cultivation or weed control can spread the virus, and there are some reports that tobamoviruses can spread in irrigation water.

SUMMARY: KEY POINTS ABOUT ToBRFV

1. ToBRFV, is a highly virulent very aggressive virus that can cause severe infection on tomatoes with resistance genes including Tm-2², and susceptible peppers that lack the L resistance genes.
2. This virus can spread quickly and easily by mechanic transmission, especially under intensive production practices.
3. Symptoms may vary by variety, and in some cases, infected varieties may be asymptomatic. Typically, infected plants have fruit with severe symptoms.
4. Leaf symptoms include distortion, shoestring and fern leaf; calyx symptoms include browning of the veins. Affected fruit may be aborted or small with blotching or brown spots.
5. The virus behaves similarly to other tobamoviruses such as TMV or ToMV, but the symptoms (especially in the fruit) may be much more severe.
6. The virus can VERY easily be moved from plant to plant by workers or even from root to root contact. Personnel coming from an infected greenhouse can introduce the virus if proper sanitation measures are not in place.
7. ToBRFV is very stable and can survive for long periods in infected debris, in the soil or on contaminated surfaces.
8. Do not rely on genetic resistance to tobamovirus to provide control, especially with tomato. Strict sanitation measures must be implemented including using clean clothing, tools and implements, stakes, etc.
9. Symptomatic plants can be removed and destroyed but ONLY very carefully, being sure not to touch any other plants or surfaces. Do not move from infected to clean greenhouses. Approach each production as if there is no resistance to this highly transmittable and damaging tobamovirus.
10. If you find plants with tobamovirus symptoms, especially if the variety has genetic resistance, obtain a professional diagnosis for confirmation.
11. Overall, best practices for prevention are essential. Workers should wear protective clothing when moving between greenhouses, especially disposable coats and gloves. Even if the virus has not been detected, this should be standard procedure.