

EPPO Datasheet: *Tobamovirus fructirugosum*

Last updated: 2020-12-11

IDENTITY

Preferred name: *Tobamovirus fructirugosum*

Taxonomic position: Viruses and viroids: Riboviria: Orthornavirae: Kitrinoviricota: Alsuviricetes: Martellivirales: Virgaviridae

Other scientific names: *ToBRFV*, *Tomato brown rugose fruit virus*
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EPPO Categorization: A2 list, Alert list (formerly)
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EU Categorization: Emergency measures

EPPO Code: TOBRFV



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HOSTS

Tomato (*Solanum lycopersicum*) and pepper (*Capsicum annuum*) are the only confirmed natural hosts of tomato brown rugose fruit virus (ToBRFV). This virus has overcome the long-lasting resistance genes (*Tm-1*, *Tm-2/Tm-2²*) that provide resistance in tomato to several tobamoviruses such as tomato mosaic virus (ToMV) and tobacco mosaic virus (TMV). These genes are currently used for protection in most commercial tomato cultivars (APS, 2014; Luria *et al.*, 2017). Pepper plants (*Capsicum* spp.) have genes/alleles^[1] providing resistance against tobamovirus spp. infections, which are designated *L* genes/alleles and have been introduced into pepper cultivars. There is no report of systemic infection in pepper varieties (*Capsicum* spp.) harbouring *L* resistance genes/alleles, even when crops are produced in very close proximity to infected tomato fields (EPPO, 2020). However, recent research points to breaking of resistance in *Capsicum* varieties harbouring some *L* resistance genes/alleles (Fidan, 2020) and this conclusion may need to be reconsidered when additional research is made available.

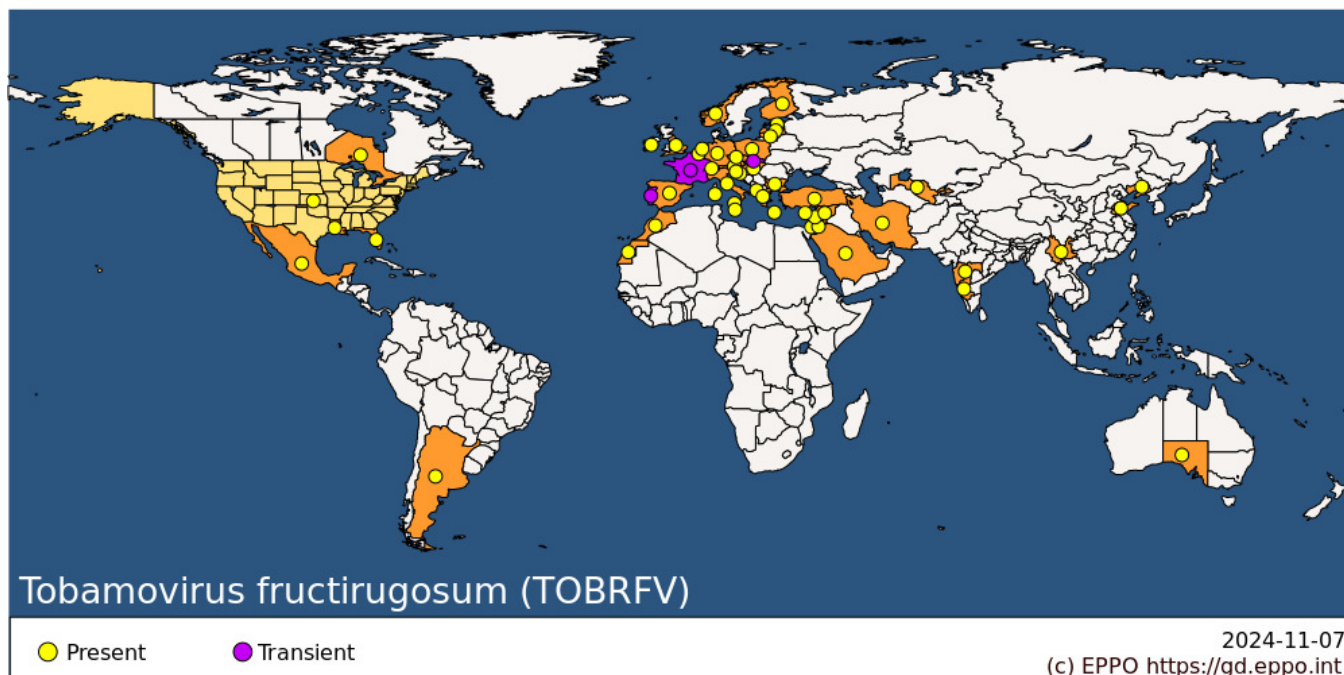
ToBRFV was also detected infecting naturally growing weed species (*Chenopodium murale* and *Solanum nigrum*) in infected tomato crops in Israel (Dombrovsky, *pers. comm.*, 2019). In inoculation trials, various plants proved to be artificial hosts.

[1] Remark: It is not known for certain whether differences in resistance are caused by allelic variants of the same gene, by presence/absence of different genes or by variations in different homologous genes. In this datasheet, the different variants existing in pepper will be referred to as *L* resistance genes/alleles.

Host list: *Amaranthus retroflexus*, *Beta vulgaris* subsp. *maritima*, *Capsicum annuum*, *Capsicum*, *Chenopodium murale*, *Convolvulus arvensis*, *Corchorus olitorius*, *Erigeron canadensis*, *Malva parviflora*, *Oxalis corniculata*, *Polycarpon tetraphyllum*, *Portulaca oleracea*, *Solanum elaeagnifolium*, *Solanum lycopersicum*, *Solanum nigrum*, *Taraxacum officinale*, *Veronica syriaca*

GEOGRAPHICAL DISTRIBUTION

The virus was first reported in 2016 from tomato plants grown in greenhouses in Jordan in 2015 (Salem *et al.*, 2016). Thus, limited information is available on its distribution. Prior to this, in 2014, an outbreak of a new disease infecting resistant tomato cultivars grown in net houses was observed in Southern Israel and was later determined to be caused by the Israeli isolate of ToBRFV with high genomic sequence identity to the Jordan isolate (Luria *et al.*, 2017). As specific detection tests are only recent and considering the interceptions on infected seed in international trade, the pest may be present in countries where it has not been reported yet. The virus is considered transient under eradication in a number of countries such as Cyprus, France, the Netherlands, Spain and the United Kingdom.



EPPO Region: Albania, Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Estonia, Finland, France (mainland), Germany, Greece (mainland, Kriti), Hungary, Ireland, Israel, Italy (mainland, Sardegna, Sicilia), Jordan, Latvia, Lithuania, Malta, Morocco, Netherlands, Norway, Poland, Portugal (mainland), Slovakia, Slovenia, Spain (mainland), Switzerland, Türkiye, United Kingdom (England), Uzbekistan

Africa: Morocco, Western Sahara

Asia: China (Liaoning, Shandong, Yunnan), India (Karnataka, Maharashtra), Iran, Israel, Jordan, Lebanon, Saudi Arabia, Syria, Uzbekistan

North America: Canada (Ontario), Mexico, United States of America (Florida, Louisiana)

South America: Argentina

Oceania: Australia (South Australia)

BIOLOGY

The viral particles of tobamoviruses are extremely stable and they are easily mechanically transmitted from plant to plant through common cultural practices (e.g. worker's hands, clothes, tools including knives, equipment including trellising ropes, tractor paths in open fields) and through circulating water (e.g. in the case of hydroponic tomato crops) (Broadbent, 1976; Dombrovsky & Smith, 2017).

Infectivity of tobamoviruses is preserved in seeds for up to several years (Dombrovsky & Smith, 2017). ToBRFV, as is the case for most tobamoviruses, contaminates the seed coat of the host plant, but not the embryo (Dombrovsky, *pers. comm.*, 2019). However, some tobamoviruses such as cucumber green mottle mosaic virus (CGMMV) can be found in the perisperm-endosperm envelope. Consequently, this cannot be excluded for ToBRFV.

Most of the tobamoviruses display a very low percentage of seed-to-seedling transmission or no transmission at all. The viruses attached to the seed coat are very rarely transmitted during germination, but they may enter the embryo during germination via small wounds or be transmitted to the roots if they are wounded during transplantation (Dombrovsky & Smith, 2017). Limited experimentation has been performed on the seed-to-seedling transmission for ToBRFV. Even with a very low level of seed-to-seedling transmission, the contribution of one infected seedling to an epidemic may be significant.

Cell-to-cell movement of tobamoviruses within plants occurs via plasmodesmata aided by the viral movement protein. Longer distance movement within the plant hosts occurs via the phloem and requires the viral replicase (Dombrovsky & Smith, 2017).

Tobamoviruses have no known natural vectors (Adams *et al.*, 2016). However, mechanical transmission of ToBRFV

is possible via bumble-bee colonies (*Bombus terrestris*) used for pollination from a greenhouse infected by ToBRFV to an uninfected greenhouse (Levitzky *et al.*, 2019). Other tobamoviruses are known to be transmitted by other animals such as birds (Broadbent, 1976; Peters *et al.*, 2012).

Tobamoviruses can survive outside of the host on inert (e.g. cardboard, pallet, transport material, tools, clothes, vehicles, stakes) and biological surfaces (e.g. human hands, plant remnants, pollinator insects) as well as in nutrient film solutions and soil for months without losing their virulence (Li *et al.*, 2016; Smith *et al.*, 2019). As part of an experiment conducted in Fera (GB) on sap or leaf rubbing contamination, it was shown that ToBRFV can survive on skin and gloves for at least 2 hours, and in dried sap on greenhouse surfaces (glass, aluminium, steel, hard plastic, polythene sheeting) for at least 4 weeks (period of the experiment). Lower survival is observed after one week on concrete.

DETECTION AND IDENTIFICATION

Symptoms

ToBRFV has a wide range of symptoms. Leaf symptoms often first appear in the young shoots at the top of the plant. Other viruses such as pepino mosaic virus (PepMV), physostegia chlorotic mottle virus (PhCMoV), ToMV and TMV cause similar (non-specific) leaf and fruit symptoms or may be confused with ToBRFV symptoms (Alkowni *et al.*, 2019). For plants, such as seedlings, there are not always external signs of infection. The following symptoms may be observed on tomato infected with ToBRFV (AHDB, 2019; Cambrón-Crisantos *et al.*, 2018; Dombrovsky & Smith, 2017; Salem *et al.*, 2016).

Leaves

Chlorosis, mosaic pattern (chlorotic/pale patches) and mottling are often observed on younger leaves in the head and side shoots. Younger leaves may also be crumpled, puckered or deformed. Narrowing of leaves (needle-like symptoms) is occasionally observed on tomato. Blistering of the leaf surface is observed. Leaves may wilt, followed by yellowing and death of complete plants.

Pedicle (stem), calyces, and petioles

Brown necrotic lesions may appear.

Fruits

Chlorotic (yellow) spotting and marbling of fruit can appear to be similar to infection with PepMV. Young fruits may be deformed and have uneven ripening. Dark colouration spots may be observed on green fruits. Brown rugose (wrinkled) patches are rarely observed. The number of fruits per branch may be reduced.

Pepper varieties not harbouring *L* resistance genes/alleles infected by ToBRFV are often subject to mixed infections. Symptoms observed on pepper require further investigation (e.g. infected plants should be tested for the absence of any co-infection with other viruses) before they can be reliably described. In pepper plants cultivated in ToBRFV-contaminated soil from previously grown infected tomato plants, especially in temperatures above 30°C, the hypersensitivity response included necrotic lesions on roots and stems resulting in inhibited plant growth sometimes leading to the death of the plant (Luria *et al.*, 2017).

Morphology

Tobamoviruses consist of a single stranded RNA-molecule 6.3 to 6.6 kb long, arranged in four open reading frames (ORFs), which is located in a crinkled cylindrical capsid (ICTV, 2019).

Detection and inspection methods

Inspection

Visual inspection will generally allow detection of symptoms in susceptible varieties during the growing season. However, some varieties are symptomless, infection can remain symptomless under certain growing conditions and plantlets are usually symptomless when they are traded (before 8 weeks / 7 leaves) (EPPO, 2020). Symptoms are not visible on seeds. A low level of symptomatic fruits may not be detected (e.g. when symptomatic fruits have been removed during sorting, making the visual inspection less reliable in term of detection).

Detection

Generic RT-PCR tests such as Letschert *et al.*, 2002, Levitzky *et al.*, 2019, Li *et al.*, 2018, Menzel *et al.*, 2019 may be used for screening but they also detect other tobamoviruses. Specific molecular tests described in the identification section thereafter may also be used for the detection of ToBRFV. A technique for the detection of plant viruses that relies on the serological method enzyme-linked immunosorbent assay (ELISA) was adapted successfully for the detection of tobamoviruses. ELISA is considered to be a robust technique and enables the detection of viral capsid protein subunits of tobamoviruses. Commercial serological kits are available; however, these ELISA kits are not species-specific (Dombrovsky & Smith, 2017; Tomassoli *et al.*, 2019); ToBRFV antisera were found to cross-react with other tobamoviruses. In general, analytical sensitivity of bioassay is known to be lower than for ELISA and molecular tests. However, experience in laboratories in the region indicates that it may be used for detection from symptomatic material.

Identification

Several specific molecular tests have been described for the identification of ToBRFV (Alkowni *et al.*, 2019; ISF, 2020; Ling *et al.*, 2019; Luria *et al.*, 2017; Panno *et al.*, 2019; Rodríguez-Mendoza *et al.*, 2019). Sequencing may be performed to identify ToBRFV after amplification by generic tobamoviruses primers (see Generic RT-PCR tests in the detection section). High Throughput Sequencing technologies may be used to obtain complete or almost complete genome sequences, which can be analysed for identification of a virus isolate. An EPPO diagnostic protocol for ToBRFV is in preparation.

PATHWAYS FOR MOVEMENT

Local spread, as well as entry from countries where the virus occurs, will mainly be linked to human assisted mechanical transmission of the pathogen, as well as the movement of infected tomato and pepper plants i.e. seeds, plants for planting (excluding seeds) and fresh fruits.

Containers used to transport infected fruits (even when empty) moved between countries, and persons working in places producing host plants or fixing greenhouses travelling internationally are other possible pathways.

It is assumed that natural dispersal (e.g. with water, pollinating insects and birds) of ToBRFV will generally remain within the same production area, where suitable hosts are available.

PEST SIGNIFICANCE

Economic impact

So far, economic damage has been reported on tomato plants and pepper (Cambrón-Crisantos *et al.*, 2018). ToBRFV is of special concern because of its ability to overcome resistance of the *Tm-2/Tm-2²* (and *Tm-1*) resistance genes in tomato (Luria *et al.*, 2017). The virus can infect up to 100% of the plants in a crop and cause 30-70% loss of tomato yield on plants (FDACS, 2019). Infection can also significantly reduce plant vigour thereby reducing the length of the production period during which tomato fruits are harvested (e.g. 8-10 fruit clusters usually harvested in Israel, instead of 24-30 clusters before the disease was established) (EPPO, 2020). Due to the symptoms, the fruits of infected plants lose market value or are unmarketable. Infections may also on occasion lead to premature death of the plant. However, the intensity of symptoms seems to vary according to varieties, management practices and climatic conditions.

In addition to direct crop losses (impact on yield and on the reduction in quality of fruit), the economic impact is due to the cost of applying hygiene measures, and to the loss of export market for seed and plantlets. In some cases, the grower may have to switch to non-host plants that may be less profitable. A higher impact is expected in intensive glasshouse production areas than in open fields. The impact for field crops is not well documented but is likely to be lower because there is usually less handling of the crop, and therefore the spread within the crop is less likely, and fruit quality is usually less important (e.g. fruits grown for processing).

Control

No chemical treatment can be used to cure infected plants. Control measures applied to eradicate, contain, or limit the impact of the disease in host crops are classical measures against tobamoviruses, and include:

Restriction of access to the production site.

Disposal of infested plant lots, associated plant debris and other material: Remove and dispose of all host plants in the infested site, associated plant debris, and other material, such as string and growing media using appropriate disposal methods (including burning, deep burial or steaming). Composting is insufficient for the secure inactivation of the virus (Richter *et al.*, 2019).

Sanitation and physical methods: After infested plants are destroyed, sanitation measures are required (Richter *et al.*, 2019; Tomassoli *et al.*, 2019). Substrates or nutrients solution, protective clothing, tools and containers should not be moved from infested production sites to areas with healthy plants. The disinfection of hands, pots, tools and equipment is possible with disinfectants with virucidal effect (Richter *et al.*, 2019; Wilstermann & Ziebell, 2019). Additional hygiene measures include installing a disinfectant mat at the entrance of the greenhouse, cleaning the greenhouse with water and detergent to remove traces of organic matter, disinfecting all the surfaces of the greenhouse, disinfection of soil using solarization in the south of the EPPO region or chlorine treatment, not growing host plants for at least one year and controlling weeds. Non-metallic equipment is to be disinfected with bleach.

Use of ToBRFV free planting material: Virus-free seeds and planting material should be used. Unlike for other Tobamoviruses, so far, no ToBRFV-resistant tomato cultivars are available. Resistant cultivars of *Capsicum* exist but may be more expensive, or not suitable for the market of the grower. Research activities to develop new resistance genes/alleles are ongoing.

Removal of (bumble) beehives: Colonies of bumble bees (*Bombus terrestris*) that had contact with infected plants need to be destroyed and replaced.

Phytosanitary risk

Tomato and pepper are important crops in the EPPO region. ToBRFV can establish in the whole EPPO region wherever tomato and pepper are grown and is likely to cause economic impact at least in crops in protected conditions. It is regulated in many countries worldwide in relation to plants for planting. It can have negative impacts on the production and trade of host fruits, as well as of seed and other plants for planting. Eradication is only considered possible if the outbreak is detected early and strict measures are taken.

PHYTOSANITARY MEASURES

ToBRFV was added to the EPPO Alert List in January 2019 and to the EPPO A2 List of pests recommended for regulation as quarantine pests in 2020. It is a quarantine pest for the European Union (EU, 2019) and other EPPO member countries (ONSSA, 2019; WTO, 2019). Phytosanitary measures to prevent the introduction of ToBRFV are recommended in the pest risk analysis (PRA) performed by EPPO in 2019 (EPPO, 2020) and these are as follows. Imported *S. lycopersicum* and *Capsicum* sp. plants for planting (excluding seeds and pollen) should originate from a pest free production site for ToBRFV established according to EPPO Standard PM 5/8 *Guidelines on the phytosanitary measure 'Plants grown under complete physical isolation'* (EPPO, 2016), or should be of varieties known to be fully resistant to ToBRFV infection. In addition, these plants should be traded in new or disinfected trays. Seeds should be produced in a pest free production site for ToBRFV established according to EPPO Standard

PM 5/8, produced from parent plants that have been inspected and tested, be of varieties known to be fully resistant to ToBRFV, or have been directly tested. Fresh fruits of *S. lycopersicum* and *Capsicum* sp. should be imported with a phytosanitary certificate certifying that they are free from ToBRFV. In addition to the measures to be implemented by the exporting countries, importing countries are encouraged not to store or repack fruits at destination in facilities that also grow host fruits, or in facilities that also pack local tomato or pepper fruits. Disinfection of used containers (including trays), tools and equipment to prevent entry of ToBRFV in facilities that grow host fruits should be encouraged. The application by personnel working in such facilities of hygiene protocols appropriate for ToBRFV should also be encouraged.

The Good Seed and Plant Practices (GSPP) system already sets a number of standards for the production of tomato seed and plants for planting (GSPP, 2013) with the aim of minimising the risk posed by the seed-transmitted bacterial pathogen *Clavibacter michiganensis* subsp. *michiganensis*, which causes bacterial canker of tomatoes. The GSPP system incorporates phytosanitary measures which would contribute to decreasing the risk of ToBRFV infection. The use of this protocol is a possible way to implement PM 5/8 requirements.

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Datasheet history

This datasheet was first published in the EPPO Bulletin in 2020. It is now maintained in an electronic format in the EPPO Global Database. The sections on 'Identity', 'Hosts', and 'Geographical distribution' are automatically updated from the database. For other sections, the date of last revision is indicated on the right.

EPPO (2020) Datasheets on pests recommended for regulations. Tomato brown rugose fruit virus. *EPPO Bulletin* **50** (3), 529-534. <https://doi.org/10.1111/epp.12711>