EUROPEAN AND MEDITERRANEAN PLANT PROTECTION ORGANIZATION
ORGANISATION EUROPEENNE ET MEDITERRANEENNE POUR LA PROTECTION DES PLANTES

Pest Risk Analysis for

Tobamovirus fructirugosum (tomato brown rugose fruit virus) (Tobamovirus)

A. Dombrovsky – EPPO Global Database (EPPO Code: TOBRFV) – Fruit symptoms, Mexico (2019)

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The risk assessment follows EPPO standard PM 5/5(1) *Decision-Support Scheme for an Express Pest Risk Analysis* (available at http://archives.eppo.int/EPPOStandards/pra.htm), as recommended by the Panel on Phytosanitary Measures. Pest risk management (detailed in ANNEX 1 and ANNEX 2) was conducted according to the EPPO Decision-support scheme for quarantine pests PM 5/3(5). The risk assessment uses the terminology defined in ISPM 5 *Glossary of Phytosanitary Terms* (available at https://www.ippc.int/index.php).

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Based on the first version of this PRA, ToBRFV was added to the EPPO A2 List of pests recommended for regulation as quarantine pests in 2020. Measures for plants for planting (including seeds but excluding pollen) and fresh fruits of *Solanum lycopersicum* and *Capsicum* spp. were recommended. In addition to the measures to be implemented by the exporting countries, importing countries were encouraged to implement additional measures.
The update from 2024 concluded that recommendation as A2 pest was still justified as there are EPPO countries where the pest is not present or widespread. Depending on pest situation, the Panel on Phytosanitary Measures considered that EPPO countries could apply some of the measures for seeds and young plants in another regulatory framework (e.g. RNQP, ISPM 16, 21).

Pest Risk Analysis for
Tobamovirus fructirugosum (tomato brown rugose fruit virus) (***Tobamovirus***)

PRA area: EPPO region
Prepared by: Expert Working Group (EWG) on *Tomato brown rugose fruit virus*
Date: The EWG met in 2019-11-18/21. The text was further reviewed and amended following comments by EPPO core members and the EPPO Panel on Phytosanitary Measures (2020-03, see below).
In December 2024, the text was amended based on an analysis of new scientific literature and unpublished scientific data on ToBRFV carried out by EPPO Secretariat and reviewed by the EWG (enlarged to other participants) in 2024-02-7/8 and by the EPPO Panel on Phytosanitary Measures in 2024-03-19/21. Outdated information was deleted. The amended part (except when purely editorial) is marked in light green.

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All personal communications in this PRA were obtained in July 2019-May 2020 from the following experts and updated in 2024: Acal M. (SENASICA, Direccion General de Sanidad Vegetal, Mexico), Albakri M. (Ministry in charge of Agriculture, Jordan), Botermans M. (NVWA, the Netherlands), Chan Hon Tong A. (Ministry in charge of Agriculture, France), Davino S. W. (University of Palermo, Italy), Desulauze J.-Y. (VILMORIN SA, France), Dombrovsky A. (Agricultural Research Organization, Volcani Center, Israel), Ertas, H. (Ministry of Agriculture and Forestry, Türkiye), Fox A. (Fera Science Ltd., United Kingdom), Garcia C. (SENASICA, Direccion General de Sanidad Vegetal, Mexico), Gentit P. (ANSES, France), Giltrap N. (DEFRA, United Kingdom), Hanssen I. (DCM, Belgium), Koenraadt H. (Naktuinbouw, the Netherlands), Lapidot M. (Agricultural Research Organization, Volcani Center, Israel), Li X. (Shandong Agricultural University, China), Lybeert H. (HM. Clause, France), Ramirez y Ramirez F. (SENASICA, Direccion General de Sanidad Vegetal, Mexico), Schenk M. (NVWA, the Netherlands), Scholz-Döbelin H (Landwirtschaftskammer Nordrhein-Westfalen, Germany), Steffek R (Austrian Agency for Health and Food Safety), Tomassoli L. (CREA, Italy), Turina M. (Istituto per la Protezione Sostenibile delle Piante, CNR, Italy), van der Krieken S. (VALTO, the Netherlands), Vogel E. (DCM and Scientia Terrae, Belgium; EU Horizon 2020 Project VIRTIGATION).

The first draft of the PRA was prepared by the EPPO Secretariat.

Ratings of likelihoods and levels of uncertainties were made during the meeting of the EWG in 2019. These ratings are based on evidence provided in the PRA and on discussions in the group. Each EWG member provided a rating and a level of uncertainty anonymously and proposals were then discussed together in order to reach a final decision. Such a procedure is known as the Delphi technique (Schrader et al., 2010).

Following the EWG, the PRA was further reviewed by the following core members: Avendaño Garcia N and Guitian Castrillon JM (with the help of Fernandez Gallego MM), Gachet E, Hannunen S, MacLeod A, Montecchio L, Steffek R, Üstün N and Van Der Gaag DJ. Additional comments were also received during the process from Fox A and Scholz-Döbelin H.

The PRA, in particular the section on risk management, was reviewed and amended by the EPPO Panel on Phytosanitary Measures on 2020-03. EPPO Working Party on Phytosanitary Regulations and Council agreed that *Tomato brown rugose fruit virus* should be added to the A2 List of pests recommended for regulation as quarantine pests in 2020.
The updated version was reviewed and amended by the EPPO Panel on Phytosanitary Measures on 2024-03. EPPO Working Party on Phytosanitary Regulations and Council agreed that for ToBRFV recommendation as A2 pest was still justified in 2024, as there are EPPO countries where the pest is not present or widespread. Depending on pest situation, the Panel on Phytosanitary Measures considered that EPPO countries could apply some of the measures for seeds and young plants in another regulatory framework (e.g. RNQP, ISPM 16, 21).should be kept in the A2 List of pests recommended for regulation as quarantine pests in 2024.
The main new information after literature review since 2020 and EWG meeting were:

- The enlarged distribution of ToBRFV in the EPPO area
- The demonstration of efficiency of hygiene practices, disinfection and treatment
- The recent availability of tomato varieties with some resistance level to ToBRFV

CONTENTS

Summary of the Pest Risk Analysis for *Tobamovirus fructirugosum (Tobamovirus, Virgaviridae)*

PRA area: *EPPO region* (Albania, Algeria, Austria, Azerbaijan, Belarus, Belgium, Bosnia and Herzegovina, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Georgia, Germany, Greece, Guernsey, Hungary, Ireland, Israel, Italy, Jersey, Jordan, Kazakhstan, Kyrgyzstan, Latvia, Lithuania, Luxembourg, The Republic of North Macedonia, Malta, Moldova, Montenegro, Morocco, Netherlands, Norway, Poland, Portugal, Romania, Russia, Serbia, Slovakia, Slovenia, Spain, Sweden, Switzerland, Tunisia, Turkey, Ukraine, United Kingdom, Uzbekistan)

Describe the endangered area:
ToBRFV could establish in the whole EPPO region wherever tomatoes are grown and is likely to cause economic impact. The area where tomatoes are grown covers the area where peppers are grown,thus far no impact is recorded on peppers, due to resistance of most pepper varieties..

Main conclusions
Entry: the pest has already entered many times in the EPPO region with different pathways (infected fruits and seeds) and has greatly increased its distribution since 2020. The probability of further entry was considered as high with a low uncertainty, the highest ratings being seeds of pepper and tomato; plants for planting (excluding seeds and pollen); fruits stored or repacked at destination in facilities that also grow host fruits, or repacked at destination in facilities that also pack local fruits; used containers, tools, equipment and conveyance vehicles; as well as persons working in place of production of host plants. It should be noted that, from mid-2019, several EPPO countries (e.g. the EU countries, Morocco, Turkey) have introduced requirements for some of these pathways which have reduced the risk of further entry into these countries.

Establishment: Establishment of ToBRFV is very likely to occur indoors in the EPPO region (with a low uncertainty) as established populations have already been reported in these conditions in the PRA area (e.g. in Israel and Jordan, as well as in Greece, Italy, and the Netherlands). Under protected conditions, conditions are favourable to the development of the crops and therefore also of the virus. The chance of the virus surviving eradication programmes is dependent on early management, the intensity of tomato production and of the quantity of ToBRFV infested plants in a certain area. Establishment of ToBRFV is likely to occur outdoors in the EPPO region (with a moderate uncertainty) where host plants are grown. However, the proportion of infected plants may remain low when the management practices are less favourable to the spread of the virus within the crops.

The magnitude of spread was rated very high with a low uncertainty. The pest could spread by natural dispersal (e.g. with pollinating insects and birds) in a production area or wider via human-assisted mechanical transmission by workers, visitors, tools and equipment (including plastic containers used for the transport of fresh tomatoes) as well as with the trade of plants for planting, seed and fruit.

Impact (economic and social) is likely to be high with a moderate uncertainty. The virus causes major concerns for growers of tomato. ToBRFV overcame the *Tm-1* and *Tm-2/Tm-2*[2] tomato resistance genes to tobamoviruses, reducing the vigour of the plant, causing yield losses and virus symptoms making the fruits downgraded or unmarketable. The virus can significantly reduce plant vigour and, under certain conditions, may cause premature death of the plants. To date, impact on pepper production is not important. The EWG considered that in countries of the PRA area where in 2024 ToBRFV, is present, costs are still significant for surveillance, outbreak declaration, eradication and hygiene measures. Eradication is not always possible. The chance of the virus surviving eradication programmes is dependent on local measures, early management, the intensity of tomato production and of the quantity of ToBRFV infested plants in a certain area. New resistant varieties could minimize the impacts.

The EWG considered that phytosanitary measures to prevent further introductions should be recommended for *Solanum lycopersicum* and *Capsicum* species.

The Panel on Phytosanitary Measures noted that the situation of this pest has evolved with an increase in the area of distribution of ToBRFV (in 2020 outbreaks were present in 9 EPPO countries whereas in 2024, 31 EPPO countries were concerned.). It also noted that with hygiene measures, varieties with a certain level of resistance on the market and seed testing, a reduction of this impact is expected. The EWG considered that recommendation as A2 pest was still justified as there are EPPO countries where the pest is not present or widespread. Depending on pest situation, the Panel on Phytosanitary Measures considered that EPPO countries could apply some of the measures in another regulatory framework (e.g. RNQP, ISPM 16, 21).

Phytosanitary Measures to reduce the probability of entry: Risk management options are considered for host seeds, plants for planting (excluding seeds and pollen), fresh fruits, used containers, tools, equipment and conveyance vehicles associated with the host production and supply chain, as well as persons working in place of production of host plants.

In addition, hygiene practices have been shown to be efficient at the plant production level and for treatment/disinfection of greenhouse surfaces, irrigation systems, seeds, soil and disposal of crop material.

Phytosanitary risk for the *endangered area* *(Individual ratings for likelihood of entry and establishment, and for magnitude of spread and impact are provided in the document)*	High ☒	Moderate ☐	Low ☐
Level of uncertainty of assessment *(See Section 17 for a justification of the rating. Individual ratings of uncertainty of entry, establishment, spread and impact are provided in the document)*	High ☐	Moderate ☐	Low ☒

Other recommendations: The EWG made recommendations (detailed in section 18) related to surveys in producing and importing countries, studies on resistance level of tomato and pepper varieties, development and validation of methods to evaluate infectivity potential and/or genome integrity and assess efficiency of treatments.

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Stage 1. Initiation

Reason for performing and updating the PRA: *Tobamovirus fructirugosum* (genus *Tobamovirus* - ToBRFV) was first observed in 2014 and 2015 on tomatoes in Israel and Jordan, and then outbreaks occurred in China, Mexico, the USA, and several EPPO countries. The virus causes major concerns for growers of tomato and pepper as it reduces the vigour of the plant, causes yield losses and virus symptoms make the fruits unmarketable. Therefore, ToBRFV was added to the EPPO Alert List in January 2019. In March 2019, the Panel on Phytosanitary Measures suggested ToBRFV as the highest priority for an EPPO PRA in 2019.
Rapid risk assessments for ToBRFV have already been published in France (ANSES, 2020), Germany (JKI, 2019), Italy (Tomassoli *et al.*, 2019), the United Kingdom (DEFRA, 2019) and have been performed in Mexico (Acal, pers. comm., 2019). Because ToBRFV was relatively new to science, limited scientific information had been published. It was considered that organizing an Expert Working Group preparing a PRA would allow to share lot of experience which has not yet been published and could be shared for the benefit of the whole EPPO region.

In 2023, the Working Party on Phytosanitary Regulations, following a request from the European Commission, agreed that the EPPO Secretariat should analyse the recent scientific literature and unpublished scientific data on ToBRFV so that the Panel on Phytosanitary Measures could discuss whether major changes have occurred and if so, if these would impact the conclusion of the PRA. Following this discussion, the Panel decided to update the PRA.

PRA area: EPPO region in 2024 (map at https://www.eppo.int/ABOUT_EPPO/eppo_members)

Stage 2. Pest risk assessment

1. Taxonomy

Taxonomic classification. Kingdom: Viruses and Viroids; Classification: Viruses; Family: *Virgaviridae*; Genus: *Tobamovirus*; Species: *Tobamovirus fructirugosum*
Virus name: tomato brown rugose fruit virus (ToBRFV)
In this PRA, the new ICTV guidelines on virus taxonomy have been followed for the capitalisation and the italicisation of the virus and disease name (ICTV, 2020)

Synonyms. There is no synonym.

Common names.
Virus rugoso del tomate (ES), Jordan-Virus (DE), Brown virus (NL, BE)

2. Pest overview

Because ToBRFV is relatively new to science, limited published scientific information is available. When data is missing, non-published data and data on other scientifically well-researched tobamoviruses such as tobacco mosaic virus (TMV), tomato mosaic virus (ToMV) and cucumber green mottle mosaic virus (CGMMV) have been used to provide information for the assessment of the risk of ToBRFV (including that on seed transmission and measures).

2.1 Morphology

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

- ORF1 and ORF2 are separated by a leaky stop codon and encode non-structural proteins that form the replicase complex,
- ORF3 on the large subgenomic RNA encodes the nonstructural movement protein (MP),
- ORF4 on the small subgenomic RNA encodes the coat protein (CP) of 17 to 18 kDa (Luria *et al.*, 2017).

A high number of ToBRFV isolates have been sequenced showing a low genetic diversity (Abrahamian *et al*., 2022; Botermans *et al.*, 2023; Celik *et al.*, 2022; Chanda *et al*., 2020; Vossenberg *et al.*, 2020 and 2021).

2.2 Life cycle

The viral particles of tobamoviruses are extremely stable and infectivity is preserved in seeds for up to several years (Dombrovsky & Smith, 2017).

ToBRFV contaminates seed coats in tomato and pepper (Davino *et al*., 2020; Salem *et al*., 2022b; Eldan *et al*., 2022) and very low infection rates of root and cotyledons were observed in seeds (Davino *et al*., 2020). Seed contamination depends on the time of infection of the mother plant (e.g: the earlier the infection of the plant, the more chances of seed contamination) and several harvesting periods increase the risk of infection of the mother plant (A. Dombrovsky, pers. com., 2024).
ToBRFV displays a very low percentage of seed-to-seedling transmission, from 0.08% to 2.8% (Davino et al., 2020; Salem et al., 2022a; 2022b) and up to 9% (Vargas et al., 2023). Zhang *et al*. (2022) suggests that seeds may establish initial infection foci and further spread would be by mechanical contact. Even with a very low level of seed-to-seedling transmission, the contribution of one infected seedling to an epidemic may be significant in areas where the virus is absent. In experiments in IL, 2 out of 8000 plants originating from contaminated seeds were infected by

ToBRFV (A. Dombrovsky, pers. com., 2024). The EWG considered that percentage of seed-to-seedling transmission cited in literature may be overestimated compared to other tobamoviruses (Dombrovsky et al., 2017).
Upon infection, large quantities of new viruses are being reproduced. Cell-to-cell movement within plants occurs via plasmodesmata aided by the viral movement protein. Long distance movement within plant hosts occurs via the phloem and requires a viral replicase (Dombrovsky & Smith, 2017).

Tobamoviruses are also mechanically transmitted from plant to plant through common cultural practices causing wounds or microlesions (e.g. hands, clothes, tools including knives, equipment including trellising ropes, movement of tractors and other machinery in production fields) and through circulating water (e.g. in the case of hydroponic tomato crops) (Broadbent, 1976; Dombrovsky & Smith, 2017). Mechanical transmission has been demonstrated for ToBRFV on tomato and pepper plants (Panno *et al.*, 2019b).
ToBRFV shows a very high rate of spread due to crop handling in high-intensity production system greenhouses where, starting with very few initially infected plants, infection can approach 100% (González-Concha *et al.*, 2021).
ToBRFV invades the root system during the first week of infection (Vaisman et al., 2022). The roots of infected plants have been confirmed as a source of infectious ToBRFV particles in water, and contaminated water can lead to plant infection (Mehle *et al*., 2023). Soil-mediated ToBRFV infection has been confirmed but low (3%) (Klein *et al*., 2023). Both these infection routes are less successful in comparison to mechanical transmission (E. Vogel, pers. comm., 2024).
Aerial irrigation (e.g. when using sprinklers) can cause injury to plants that can facilitate infection. This has been demonstrated for CGMMV (Dombrovsky *et al.*, 2015).

Tobamoviruses have no known natural vectors (Adams *et al.*, 2016). However, Levitzky *et al.* (2019) demonstrated that transmission of ToBRFV is possible via bumblebee colonies (*Bombus terrestris*) used for pollination within a greenhouse infected by ToBRFV, as well as to an uninfected greenhouse. ToBRFV may adhere to the pollen grains attached to the bumblebees or be present in crude sap on their bodies and mandibles. The bumblebees may transmit the virus by causing wounds when using their mandibles to grasp the anther cone, or microlesions when vibrating bodies (Levitzky *et al.*, 2019; Velthuis & van Doorn, 2006). *Tuta absoluta* can have a role in spreading the inoculum and mechanically infecting new healthy plants through micro wounds (Caruso et al., 2024).

Some tobamoviruses, such as CGMMV, may also be pollen transmitted in absence of pollinators (Liu *et al.*, 2014). Pollen grains from infected plants with ToBRFV contain infective virus but no infection occurred in an experiment consisting of the hand-pollination of tomato plants with pollen harvested from plants infected with ToBRFV, Pollen germination assays showed that ToBRFV-infected pollen did not germinate (Avni et al., 2022).

Tobamoviruses can survive outside of the host on many surfaces (Section 2.3).

2.3 Survival out of living host plants
Tobamoviruses can survive outside of the host on inert (e.g. cardboard, pallet, transport material, tools, clothes, vehicles, stakes) and biological surfaces (e.g. hands, plants remnants, pollinators insects) as well as in nutrient film solutions and soil for months without losing their virulence (Li *et al.*, 2016; Smith *et al.*, 2019).
Tobamoviruses may be found in extreme conditions: ToMV can be present at high altitude, in fog of an arid area, in spring water as well as in cigarettes and in the mouth of a person smoking (Castello et al., 1999, 1995). Other tobamoviruses such as pepper mild mottle virus (PMMoV) are known to be used as indicators of water quality (Kitajima et al., 2018).

FERA (2021b) published results showing, based on bioassays and ELISA detection, that ToBRFV can survive on hands and gloves for at least 2 hours and on glasshouse surfaces for at least 7 days and in some cases over 6 months, while hands washing was of limited use (Skelton et al., 2023c).
Ehlers *et al*. (2023) and other authors confirmed contamination by infectious ToBRFV (PCR detection and indexing on *Nicotiana. tabacum* cv. *xanthi*) of greenhouse, packaging material, clothes, and accommodation of workers. They stressed the spread from an infected greenhouse with no hygiene gate and necessary hygiene measures.
Experiments were conducted in the Netherlands in cucumber production in a greenhouse with a previous ToBRFV outbreak on tomato and in another greenhouse with no previous tomato cultivation. ToBRFV was detected in high quantities on cucumber plants (non-host plant) by real-time RT-PCR, on documents, soil, and swabs from different parts of the greenhouse. The bioassays carried out by inoculating indexing plants with the cucumber leaf extracts were negative (Giesbers, personal communication July 2023), indicating that detection seems to be due to surface contamination. Experiments conducted in the Netherlands and Spain in dust collected with a dust catcher showed detection of ToBRFV (Rijk Zwaan, 2023), indicating that it is possible to detect environmental RNA of ToBRFV in air samples. This does not confirm survival of ToBRFV on non-host plants but raises the question of detection of contaminants and of the biological relevance of such detections (i.e: if the virus detected is still infectious).

Survival of ToBRFV in soil was demonstrated for up to 5 months (last experimentation date) (Dombrovsky et al, 2022) and 79 days in dry earth pile (last experimentation date) or 184 days in wet earth pile (infectivity was reduced to 0 after 205 days) (Molad et al., 2024)

Mehle *et al*. (2023) showed that infectious ToBRFV was detected in drain water of a contaminated greenhouse and surface water, and that it remained infective in water stored at room temperature for up to four weeks while RNA was detected for a longer period. This confirms what was known for other tobamoviruses (such as PMMoV) which are used as indicators of water quality.

2.4 Temperature requirements

ToBRFV reproduces in host plants grown in protected conditions as well as outdoor (SENASICA, 2019b).
Tobamoviruses can overwinter in soil (Smith *et al*., 2019). For example, CGMMV was shown to overwinter in soil sample with debris of infected plant at a depth between 10 and 30 cm with an average air temperature of 1°C and a minimum air temperature of -9°C without losing its virulence (Li *et al*., 2016).
There are no published experiments assessing the survival of ToBRFV at very low or high temperatures in soil, growing media or other material (e.g. surfaces in the greenhouses). Recent research conducted at Fera (GB) suggests that treatment of trays in a waterbath at 70°C for 5 minutes is ineffective, but 90°C will inactivate the virus (Skelton et al., 2023c). This paper validated the inactivation of the virus at 90°C in sap inactivation experiments. Symptoms during the winter season in Israel may be significantly milder (e.g. around 14°C in greenhouses, not lower than 10°C).

2.5 Nature of the damage

Nature of the damages includes the decrease in the quantity and quality of tomato fruit production. The typology of symptoms ranges from a pale decolouration to necrotic lesions. Contrary to what the name suggests, brown rugose symptoms are rarely seen in fruits. Infections of specific tomato varieties with ToBRFV may lead to complete wilting of the plant (Hanssen, pers. comm., 2019; Scholz-Döblin & Leucker, 2019; Wilstermann & Ziebell, 2019) (ANNEX 3. Figure 1B).

Update 2024: Reduction of yield can be high: 25-40% reduction of the average tomato fruit weight (Gonzalez-Concha *et al*., 2022) and 15-55% of yield reduction (Avni *et al*., 2021). The root biomass and elongation are affected (Vaisman *et al*., 2022). In susceptible pepper varieties, reduction of the size of the plant, of the number of internodes, of the size of the fruits and number of seeds is observed (Ortiz-Martinez, 2021), 85% of the plants were infected in a greenhouse (Panno et al., 2020a).

Symptoms of infection

ToBRFV has a wide range of symptoms, however it can also occur without symptoms. As observed for TMV (Samuel, 1934), 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 and may be confused with ToBRFV symptoms (Alkowni *et al.*, 2019). In a number of EPPO countries (e.g. Belgium, Germany, the Netherlands, United Kingdom), tomato plants are inoculated (i.e. vaccinated) with mild strain(s) of PepMV in an early growth stage at the fruit production site to prevent infection by more severe strains (this technique is called 'cross protection') (Agüero *et al.*, 2018; Hanssen, pers. comm., 2019). In co-infections with Israeli isolates of PepMV and ToBRFV, symptoms were increased, and a reduction of plant height was observed (Klap et al., 2020b),) when ToBRFV infection preceded PepMV infection, showing synergic effects. However, synergistic effects were not observed when ToBRFV was co-infected with two Spanish PepMV isolates (Hernando & Aranda, 2023). In the EU Horizon 2020 research project VIRTIGATION, it was observed that mixed infections of ToBRFV with aggressive PepMV isolates were much more devastating for the plant than a combination of ToBRFV with a mild PepMV isolate (Vos, 2023).

The following virus symptoms (see also pictures in ANNEX 3) may be observed on tomato (*Solanum lycopersicum*) infected with ToBRFV (AHDB Horticulture, 2019; Cambrón-Crisantos *et al.*, 2018; Dombrovsky & Smith, 2017; Salem *et al.*, 2016):

- **Leaves or plants:**
  - Chlorosis, mosaic pattern (chlorotic/pale patches) and mottling on younger leaves in the head and side shoots (often observed),
  - Crumpling, puckering or deformation of young leaves,
  - Narrowing of leaves (needle-like symptoms) (occasionally observed),
  - Blistering of leaf surface,
  - Wilting of leaves, followed by yellowing and plant death,
  - Infected plants can be symptomless
- **Pedicle (stem), calyx (sepals), and petioles:**
  - Brown necrotic lesions,
- **Fruits:**
  - Chlorotic (yellow) spotting and marbling of fruits (can appear to be similar to infection with PepMV),
  - Deformation and uneven ripening of young fruits (e.g. individual fruit can be red in some parts and showing green stripes, blotches or patches in other parts). The uneven ripening seen on fruits infected by ToBRFV is in general more severe than for PepMV,
  - Orange fruits not turning red (variety Juanita in Germany, Scholz-Döbelin, pers. comm., 2020).
  - Dark coloured (necrotic) spots on green fruits,
  - Brown rugose (wrinkled) patches (rarely observed),
  - Reduced number of fruits per branch or fruit size.

Skelton et al. (2023b) showed that fruit symptoms are not a reliable indication of infection status.
If the above symptoms are observed in a tomato variety harbouring tobamovirus resistance genes (e.g. *Tm-2*^{2}), it is likely that ToBRFV is present and so confirmatory testing should be performed.

Based on the pictures of symptoms from Mexico (SADER & SENASICA, 2019b), pepper shows relatively similar symptoms to those described for tomato but more severe necrosis is observed on fruits. However, pepper varieties not harbouring *L* resistance genes/alleles (see '*Resistance*') infected by ToBRFV are often subject to mixed infections (SADER & SENASICA, 2019b). Therefore, the observed symptoms are likely due to combinations of viruses (e.g. Salem *et al.*, 2019). Symptoms of a hypersensitive response on pepper harbouring the *L* resistance genes/alleles are shown in ANNEX 3, figure 3.

Additional pictures are available in EPPO Global Database:
https://gd.eppo.int/taxon/TOBRFV/photos

Resistance
In tomato, long lasting resistance genes (*Tm-1*, *Tm-2*/*Tm-2*^{2}) have been identified that provide complete resistance to several tobamoviruses such as ToMV and TMV. These genes have been crossed from wild into cultivated tomato varieties and are currently used for protection in most commercial cultivars (APS, 2014; Luria *et al.*, 2017). However, ToBRFV has overcome all these resistance genes.
No commercial varieties are currently known to be fully resistant to ToBRFV.

Twenty-six tolerant *Solanum* accessions were identified with a 0-20% Disease Severity Index (DSI) and presence of virus confirmed by RT-PCR and bioassays (Jewehan *et al*., 2021). Kabas *et al*. (2022) identified 3 accessions with a low disease severity. Jewehan *et al*., (2021) also identified a high level of resistance in 3 *S. ochranthum* accessions, showing no symptoms and detection of ToBRFV only in inoculated leaves. Two other accessions, found with a low DSI and virus content at an early stage, recovered with no further detection of the virus. In a further study Jewehan *et al*. (2022a), identified segregating resistant accessions, 9 of *S. habrochaites* and one of *S. peruvianum*, with plants with no symptoms and ToBRFV detection by real-time RT-PCR. These plants expressed symptoms at 33°C. A mutant ToBRFV strain breaking the resistance of these accessions was identified (Jewehan *et al*., 2022b). Zinger *et al*. (2021) identified 29 tolerant accessions (no symptoms and high viral levels) and one resistant one (no symptoms and extremely low viral level). Nunhems (2021) described plants with improved Tobamovirus resistance in a patent.
Zisi *et al.* (2024), observed virus symptoms on leaves and fruits of newly introduced ToBRFV-resistant varieties under high virus pressure, concluded on a resistance breaking ToBRFV isolate, and identified the causal point mutation.

The EWG noted that there is no official protocol for assessing resistance of varieties and different terms are used in literature or industry communication: resistance, tolerance, intermediate resistance, without describing the level of symptom expression and virus load of the plants. A CPVO co-funded project is currently evaluating protocols and a set of varieties to define these levels of resistance (P. Gentit, pers. comm., 2024).
Research on alternative approaches to conventional resistance strategy is still going on: Ishikawa et al. (2022) used CRISPR/Cas9 to knock out TOM1 genes in tomato and obtained quadruple mutants with no capsid detection nor disease symptoms. The same technique was used by Kravchik et al. (2022) with double mutants showing a lower accumulation of the coat protein and RNA than in the wild type and no symptoms. Resistance to ToBRFV was obtained by chemical mutagenesis (Vilmorin & Cie, 2022) and recombinant techniques by modification of the Tm2^2 gene and patented (Volcani Center, 2022)

In pepper plants harbouring *L* resistance genes/alleles cultivated in ToBRFV-contaminated soil, the hypersensitivity response included necrotic lesions on roots and stems. This resulted in inhibited plant growth and sometimes lead to the death of the plant. This has only been observed in soil from previously grown infected tomato plants, especially in temperatures above 30°C (Luria *et al.*, 2017).

Update 2024: Fidan *et al*. (2021) observed symptoms on pepper plants carrying L^1 and L^2 resistance genes while L^3 and L^4 genes conferred resistance. This resistance was broken at 32°C or above. Eldan *et al*. (2022) observed a transient leaf infection in L^1, L^3 and L^4 inoculated plants in early and late stages (140 dpi), fruits were ToBRFV positive but symptomless, and except from two L^4 cultivars, seeds were not infected. The ToBRFV positive seeds of the two L^4 cultivars were negative in a bioassay (which raises the question of biological relevance). At the last stage of harvest, fruits were no longer infected. The authors concluded that there is a dissociation between systemic infection and disease manifestations which should alert growers about possible sources of infection. Fidan *et al.* (2022), observed symptoms and detected ToBRFV in L^3 and L^4 inoculated plants kept at or below 32°C in a growth chamber. To date, despite the presence of ToBRFV in pepper production areas, no relevant impact on pepper production has been reported.

As commented on in EU (2023a), the new articles raise the question as to whether any tomato or pepper varieties are fully resistant (with no virus load).

Additional considerations on symptom expression
Plants infected with ToBRFV do not show physical symptoms immediately. Systemic symptoms developed in tomato cultivars at 12–23 days post inoculation (Luria *et al.*, 2017; Panno *et al.*, 2019a), but this can be shorter (4-5 days) in tomato varieties not harbouring any resistance gene (Gentit, pers. comm., 2019).

In Israel, the Netherlands and Italy, symptoms on young tomato seedlings have never been seen in nurseries producing young plants for planting (Dombrovsky, pers. comm., 2019; Tomassoli, pers. comm., 2019 and 2024).

Symptoms from soil infection appear only after a minimum of 3 to 4 weeks in Israel (Dombrovsky, pers. comm., 2019).

Symptoms from infection by ToBRFV-contaminated water appear after 2 – 6 months (Mehle et al., 2023).

After artificial inoculation, petunia and certain weeds are symptomless hosts (Luria *et al.*, 2017).

For tomato and pepper, symptoms are cultivar-dependant. Some cultivars present more symptoms on fruits, and other cultivars present more symptoms on leaves (Scholz-Döbelin & Leucker, 2019). Environmental conditions such as light, temperature, nutrient deficiency may influence symptomology. Under Israeli conditions, some tomato varieties (including Israelian leading varieties) are known to be symptomless all over the year (Dombrovsky, pers. comm., 2019).

2.6 Detection and identification methods

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.*, 2019a; 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). The whole genome of German, Italian, Jordan and the Israelian isolates were sequenced (Alkowni *et al.*, 2019; Luria *et al.*, 2017; Panno *et al.*, 2019b; Salem *et al.*, 2016). Genome sequences from China, Germany, Italy, Israel, Jordan, Mexico, Turkey and the United-Kingdom are available in GenBank (https://www.ncbi.nlm.nih.gov/genbank/) and of Belgium and the Netherlands (Nextstrain). These sequences are very similar: they vary between 98 to 99.9% homology.

High Throughput Sequencing technologies may be used for obtaining complete or almost complete genome sequences, which analysis can be used for identification of a virus isolate.

Update on detection and identification in 2024
An EPPO diagnostic protocol has been published in 2020 and revised in 2022 (EPPO, 2022). A sampling scheme has been agreed in the EU emergency measures (EU, 2023).
CRISPR-based methods (Alon *et al*., 2021; Bernabé *et al*., 2022), LAMP (FERA, 2021a; Rizzo *et al*., 2021; Sarkes *et al*., 2020), targeted nanopore sequencing (Kubaa *et al*., 2023), ELISA, Immunostrips and AmplifyRP XRT (Eads *et al*., 2023), immunochromatographic strips (ZhiYong *et al*., 2022) and many PCR tests (Chanda *et al*., 2021a; Fidan *et al*., 2021; Giesbers *et al*., 2021; Luigi *et al*., 2022; Magana-Alvarez *et al*., 2021; Menzel & Winter, 2021; Nolasco-Garcia *et al*., 2020; Rizzo *et al*., 2021; Tiberini *et al*., 2022; ZhiYong *et al*., 2021) have been developed or validated for detection and identification of ToBRFV, that could be considered for an update of the EPPO diagnostic protocol.

Salem *et al*. (2022b) compared bioassays, ELISA and RT-PCR on naturally infected seeds, with correlated results, showing that viruses detected by ELISA and RT-PCR were infectious.

FERA (2021a) performed detection on swabs from surfaces treated with different products and showed that viral RNA that is no longer biologically active can still be detected with real-time RT-PCR. This information, combined with detection in dust, and surface contamination and environment residues, raises the question of detection of contaminants and of the biological relevance of such detections.

For detection on plants, Skelton *et al*. (2023b) determined the organs to be sampled for better detection depending on the crop stage, highlighting the importance of sampling calyx material once trusses are formed.
Artificial intelligence combined with remote sensing is currently under progress in Türkiye for early detection on plants based on symptoms (H. Ertas, pers. comm., 2024,)

3. Is the pest a vector?

Yes ☐ **No** ✓

4. Is a vector needed for pest entry or spread?

Yes ☐ **No** ✓

Tobamoviruses have no known natural vectors (Adams *et al.*, 2016). ToBRFV spread mainly mechanically and via seeds. However, bumblebees and *Tuta absoluta* have been shown to be able to mechanically transmit the virus from infected plants to healthy plants (Section 2.2).

5. Regulatory status of the pest

In the EPPO region, Turkey has required since 2019-03-15 an additional declaration at import stating that consignments of *S. lycopersicum* and *Capsicum* seeds have been analysed by means of RT-PCR methods and have been found to be free from the virus (WTO, 2019g). Morocco also requires that an additional declaration at import states that *S. lycopersicum* seeds have been analysed for ToBRFV (ONSSA, 2019).

The EU has agreed emergency measures against ToBRFV (Commission implementing decision (EU) 2019/1615 (EU, 2019a)). The measures have applied since 2019-11-01 and specify the following requirements for import of *S. lycopersicum* and *C. annuum* plants for planting and seed:
Requirements for seeds:
-When moved into the EU, seeds must be accompanied by a phytosanitary certificate and fulfil one of the following requirements:

- Originate in a country free of the virus
- Originate in an area free of the virus
- Have been officially sampled and tested negative for the virus

-When moved within the EU, seeds must be accompanied by a plant passport and fulfil one of the following requirements:

- Originate in an area free of the virus
- Have been officially sampled and tested negative for the virus

Requirements for seedlings (plants for planting other than seed)
-When moved into the EU, plants must be accompanied by a phytosanitary certificate and fulfil one of the following requirements:

- Originate in a country free of the virus
- Originate in an area free of the virus
- Originate in a registered production site known to be free of the virus on the basis of inspection, and derive from seeds that originate from an area free of the virus or from seeds that have undergone testing for the virus

-When moved within the EU, plants must be accompanied by a plant passport and fulfil one of the following requirements:

- Originate in an area free of the virus
- Originate in a production site known to be free of the virus on the basis of inspection, and derive from seeds that originate from an area free of the virus or from seeds that have undergone testing for the virus

As of November 2019, emergency and regular measures on ToBRFV have also already been introduced by Argentina[1] (WTO, 2019h), Australia[2] (BICON, 2019; WTO, 2019c, 2019b), Chile[3] (Ministerio del interior y seguridad publica, 2019; WTO, 2019d), Korea (Republic of) (WTO,

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[1] Consignments of *S. lycopersicum* and *C. annuum* plants imported in Argentina should be tested for ToBRFV, since 2019-10;
[2] Consignments of *S. lycopersicum* and *Capsicum* seeds imported in Australia should be tested for ToBRFV, since 2019-03;
[3] Consignments of *S. lycopersicum* and *C. annuum* seeds imported in Chile should have been analysed and come from a nursery inspected and analysed for ToBRFV, since 2019-09;

2019a)[4], Mexico[5] (SADER & SENASICA, 2019a), New Zealand (WTO, 2019e)[6], South-Africa[7] (Levi, pers. comm., 2019 according to Import Permit), Thailand[8] (WTO, 2019f, 2019i) and the USA[9] (USDA, 2019). Pending a risk analysis, USA also requires that tomato and pepper fruits from Canada (because of re-export from Mexico), Israel, Mexico and the Netherlands have to be inspected for the absence of disease symptoms at the point of origin prior to export (USDA, 2019). Israel is regulating tobamoviruses for the import of tomato and pepper seeds (MARD-PPIS, 2009). In Mexico, ToBRFV is a Regulated Non-Quarantine pest (SENASICA, 2019c). In most cases, testing of seeds is required (see footnotes 3-11).
ToBRFV was not found in the lists of regulated pests for any other countries. However, the information presented in this document is not exhaustive as the situation is evolving rapidly, and ToBRFV may be regulated in more countries.

These measures were updated in the Commission implementing decision (EU) 2023/1032 (EU, 2023). For seed movement within the EU and introduction into the EU, pest free production sites are required for mother plants, supplemented by sampling and testing of the seeds. For plants for planting and seeds, a confirmation of resistance of the varieties which are known to be resistant to ToBRFV is stated in “Additional Declaration “of the phytosanitary certificate.

6. Distribution

The virus was only first reported in a scientific paper in 2016 (Salem *et al.*, 2016).
ToBRFV was initially isolated from tomato plants grown in greenhouses in Jordan in 2015 (Salem *et al.*, 2016). Prior to this, in 2014, an outbreak of a new disease infecting resistant tomato cultivars grown in screenhouses was observed in Southern Israel and was later determined to be caused by ToBRFV (Luria *et al.*, 2017). ToBRFV was later reported from China, Greece, Italy, Mexico, The Netherlands, Spain, Turkey and the United Kingdom (Table 1 and Figure 1) and as well as in Germany and in the USA where it was eradicated. ToBRFV was intercepted on infected seeds and fruits in international trade from countries where there was no record of the pest as well as unofficial communications of disease presence (see below the tables) (Oladokun *et al.*, 2019).

Update on distribution in 2024 (Fig.1)
EPPO Global Database (EPPO GD) provides updated information on the distribution of ToBRFV with supporting references.
Compared to the situation reported in the PRA in 2020, ToBRFV has been reported additionally as present in Argentina, Albania, Austria, Belgium, Bulgaria, Canada, Cyprus, the Czech Republic, Finland, Germany, Hungary, Iran, Ireland, Lebanon, Malta, Morocco, Norway, Poland, Slovenia, Saudi Arabia, Syria, Switzerland, the USA and Uzbekistan. It has also been recently identified in Peru (Rodriguez-Grados et al., 2024) and India. The EU (2023) reported a notable increase of

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[4] ToBRFV has been added to the quarantine pest list, since 2019-11;
[5] Seeds, plants, seedlings and cuttings of *S. lycopersicum*, *Capsicum* sp. and *S. melongena* imported in Mexico should be tested for ToBRFV, since 2019-01;
[6] Consignments of *S. lycopersicum* and *Capsicum* seeds imported in New Zealand should come from a Pest-free area or be tested for ToBRFV, since 2019-03;
[7] Consignments of *S. lycopersicum* and *C. annum* seeds imported in South-Africa should come from a Country of production which is free from ToBRFV or has been tested using the existing commercial ELISA seed test (ISTA 7-028) on a sample size of 3,000 seeds (or 20 per cent for small seed lots) and found free from ToBRFV, since August 2019;
[8] Consignments of *S. lycopersicum* and *Capsicum* seeds imported in Thailand should come from a Pest free area, a Pest free place of production, a Pest free production site or be tested for ToBRFV (on sample of 3,000 seeds or at least 10% of the lot as a small seed lot), since 2019-07;
[9] Lots of *S. lycopersicum* and *Capsicum* propagative material (including plants for planting, seeds, grafts, obscured seed, and cuttings) imported from all countries in the USA must be accompanied by a Phytosanitary Certificate or a re-export Phytosanitary Certificate with an additional declaration certifying that the country of origin is free from ToBRFV or that a representative sample has been officially tested and found free of ToBRFV. An additional option for small lots or breeder lines consists in the testing of all mother plants no more than 10 days prior to fruit harvest and found free of ToBRFV. Regulation in place since 2019-11;

notification of ToBRFV outbreaks from 2020 to 2021, and a spread that continued in 2022 with new notifications. They also reported a low number of reports about successful eradication.

Van der Gaag et al (2021), report that in some EU member states (Greece, Italy and the Netherlands) ToBRFV has been detected in several sites, showing a wider distribution, with areas where eradication measures were not successful (Sicily, Netherlands). They conclude that eradication can be achieved at the level of a production site but that it is less likely in tomato production areas with multiple infestations. They also conclude that this pest fulfils the criteria of an RNQP for EU. In Italy, in 2023, ToBRFV was detected in four new Regions. Since October 2020, 5 of the 18 outbreaks have been eradicated (3 regard seed Companies) (L. Tomassoli, pers. comm., 2024). In contrast, in France distribution is limited, but with an increase of the number of outbreaks (ANSES, 2023).

Table 1. Distribution of *Tobamovirus fructirugosum* as of March 2020 and updated as for March 2024.[The latest update of the geographical distribution is available at https://gd.eppo.int/taxon/TOBRFV/distribution]

Region	Country	References and comments
Africa	**Morocco**	In 2 regions, under official control (EPPO Global database, 2024)
America	**Mexico**	In tomato and pepper crops indoor and outdoor.
	Argentina	First found in December 2022 in tomato greenhouses belonging to three different growers in Santa Lucía and Lavalle (Corrientes), (EPPO Global database, 2024)
	Canada	Tomato fruits imported from Canada and first report in Ontario in 2019 (EPPO Global database, 2024)
	USA	Several isolated detections of ToBRFV in US commercial greenhouses in winter 2019-2020 (no details on location) (EPPO Global database, 2024)
Asia	**China**	In 3 tomato crops under greenhouse conditions. Shandong Province (Yan *et al.*, 2019) and seeds and plants (EPPO Global database, 2024).
	India	In fruit samples (EPPO Global database, 2024)
	Iran	In tomato and pepper plants (EPPO Global database, 2024)
	Lebanon	In sweet pepper (EPPO Global database, 2024)
	Saudi Arabia	In tomato plants in greenhouse (EPPO Global database, 2024)
	Syria	Widespread (EPPO Global database, 2024)
	Uzbekistan	In several districts (EPPO Global database, 2024)
EPPO region	**Albania**	In tomato plants in greenhouse (EPPO Global database, 2024)
	Austria	In tomato plants in greenhouse, under eradication (EPPO Global database, 2024)
	Belgium	In tomato plants in greenhouse, in several arrondissements, under eradication (EPPO Global database, 2024)
	Bulgaria	In Smolyan oblast, and in Pazardzhik oblast in June 2022. Under eradication. (EPPO Global database, 2024)
	Cyprus	In some parts of the member state (EPPO Global database, 2024)
	Czech Republic	In tomato fruit production and pepper seed company. Under eradication. (EPPO Global database, 2024)
	Ireland	crop contained within a site with physical protection, under eradication (2024-04), (EPPO Global database, 2024)
	Finland	In tomato plants in greenhouse, under eradication (EPPO Global database, 2024)
	France	In tomato crops under greenhouse conditions in Finistère, Bretagne. Under eradication (NPPO of France, 2020).
	Germany	Outbreaks in different locations (EPPO Global database, 2024)
	Greece	In tomato crops producing fruits, under greenhouse conditions. First found in Crete in 2019-10. It was also detected on the mainland, in the Peloponnese. Under eradication (EPPO, 2019a, 2020b).
	Hungary	In tomato plants in two greenhouses, under eradication (EPPO Global database, 2024)
	Israel	A first outbreak was reported in 2014 in tomato crops in Israel (Luria *et al.*, 2017). Now established under greenhouse conditions.
	Italy	In tomato and sweet pepper crops producing fruits (the sweet pepper variety was not harbouring the *L* resistance genes/alleles (Davino, pers. comm., 2020)) and tomato nurseries producing tomato seedlings, both under greenhouse conditions. Under eradication in Sicily (EPPO, 2019i; Panno *et al.*, 2020, 2019°) and eradicated in Piedmont (EPPO, 2019g). Detected in Sardegna on tomato in greenhouse (EPPO Global database, 2024)
	Jordan	Isolated in 2015 (Salem *et al.*, 2016). The virus was reported in 2015 in additional tomato production sites in the Jordan Valley as well as in the Northern part of the country (Salem, pers. comm., 2019). Also found in pepper crops (Salem *et al.*, 2019) not harbouring *L* resistance genes/alleles.
	Malta	In tomato greenhouses, under eradication (EPPO Global database, 2024)
	Netherlands	First found in Westland in 2019-10, under eradication (EPPO, 2019d) In 2020-02, 17 outbreaks in tomato crops, under greenhouses conditions in Westland, Hollands Kroon, Brielle and Reimerswaal (EPPO, 2020c). In 2021 23 sites from 12 municipalities (EPPO Global database, 2024)
	Norway	One location, under eradication (EPPO Global database, 2024)
	Poland	In seeds and plants for planting of tomato, under eradication (EPPO Global database, 2024)

Region	Country	References and comments
	Slovenia	One location, under eradication (EPPO Global database, 2024)
	Spain	In tomato crops, under greenhouses conditions. Under eradication (EPPO, 2019c), only in some parts of the Member State concerned under eradication (2022-01) (EPPO Global database, 2024)
	Switzerland	One location, under eradication (EPPO Global database, 2024)
	United Kingdom	First detected in 2019-07 in Kent, and later in Worcestershire, in tomato crops under greenhouse conditions. Under eradication (EPPO, 2019e, 2020a; Skelton *et al.*, 2019)
	Türkyie	In tomato crops, under greenhouses conditions. Demre (Fidan *et al.*, 2019). Fruits imported during several years from Turkey into Israel have been tested positive with ToBRFV (Dombrovsky, unpublished data, 2019).

Unofficial reports of pest presence

In addition to the information provided in Table 1 and **Erreur ! Source du renvoi introuvable.**, the American seed trade association (ASTA) states that the virus has also been confirmed in Saudi Arabia (ASTA, 2018). Likely occurrences have been reported but not confirmed in Chile, Ethiopia and Sudan (ASTA, 2018). The Ontario Greenhouse Vegetable Growers states that ToBRFV is present in Ontario (Canada) (OGVG, 2019). However, no official pest reports by NPPOs or scientific articles are available to confirm this information.

Tomato and pepper seeds imported from more than 14 different countries in Africa (Kenya), America (Canada, Guatemala, Peru, USA), Asia (China, India, Thailand, Vietnam) and in the EPPO region (France, Israel, Morocco, the Netherlands and Spain), were tested positive for ToBRFV in Mexico (SADER & SENASICA, 2019b). Trace-back analysis showed that the seeds from France that tested positive in Mexico were imported from Thailand (Lopez-Buenfil, 2019) through Spain (Chan Hon Tong, pers. comm., 2019) and the seeds from Spain that tested positive in Mexico were also imported from Thailand (Lopez-Buenfil, 2019). Infected tomato plants in Sicily were grown from seeds imported from France as well as from Peru (Tomassoli, pers. comm., 2019). Infected tomato fruits from Egypt have been intercepted by the NPPO at import in the Netherlands (EU, 2019c), but the presence in Egypt has not been officially confirmed by the Egyptian NPPO.

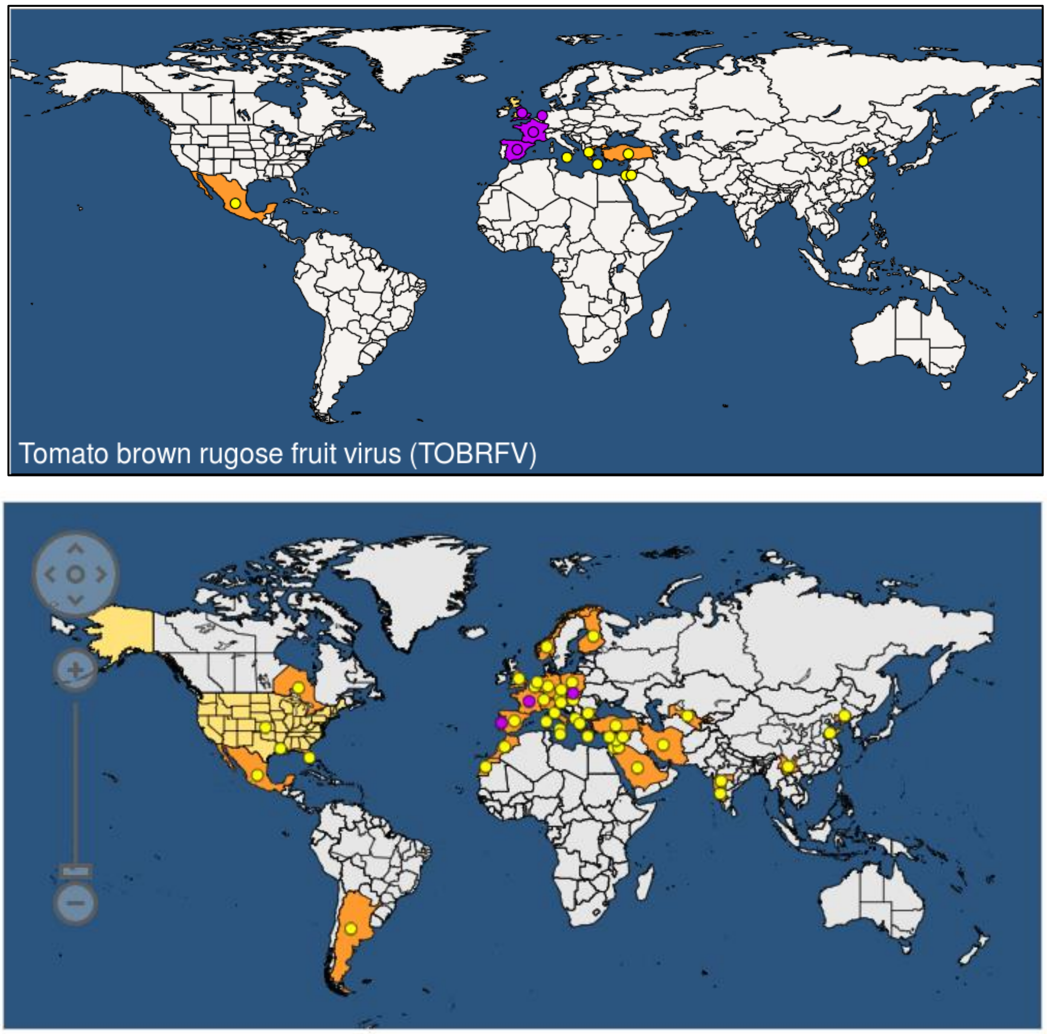


Figure 1. Distribution of tomato brown rugose fruit virus (top as of March 2020, bottom as March 2024), prepared by the EPPO Secretariat, using https://gd.eppo.int/). In orange: States where ToBRFV occurs. In purple: States where ToBRFV is transient.

7. Host plants and their distribution in the PRA area

Host plants

Tomato (*S. lycopersicum*) and pepper (*C. annuum*) are the only crops which are confirmed natural hosts of ToBRFV (Luria *et al.*, 2017; NAPPO, 2018; Salem *et al.*, 2016, 2019). In experimental host range determination studies conducted in Turkey, some cucumber, squash, eggplant and potato varieties inoculated with ToBRFV were not infected with ToBRFV (H. Ertas, pers. comm., 2024).ToBRFV was also detected in natural weeds species (e.g.: *Chenopodium murale* and *Solanum nigrum)* in Israel (Salem et al., 2022a). In studies conducted in greenhouse tomato and pepper production areas in Turkey, weed species *Amaranthus spinosus, Galium aparine* and *Trifolium resupinatum* were found to be infected with ToBRFV (H. Ertas, pers. comm., 2024)
In inoculation trials, various plants proved to be artificial hosts (Table 2).

Table 2. Hosts of tomato brown rugose fruit virus in production conditions and inoculation trials (as of March 2020)

Host	Presence in PRA area (Yes/No/Not known)	Comments	References for host status
Confirmed hosts			
Capsicum annuum	Yes, widely grown		Luria *et al.*, 2017; Salem *et al.*, 2019
Capsicum sp.	Yes, widely grown	No detail on the species other than *C. annuum* which have been found infected in Mexico.	In addition to *C. annuum*, several Mexican publications are mentioning that Chili pepper and peppers are infected without mentioning the species (SADER & SENASICA, 2019a)
Solanum lycopersicum	Yes, widely grown		Luria *et al.*, 2017
Chenopodium (=*Chenopodiastrum*) *murale*	Yes, wild/weed	Detected in natural conditions in Israel (may display symptoms of discoloration).	In experimental (Luria *et al.*, 2017) and in natural conditions (Dombrovsky, pers. comm., 2019)
Solanum nigrum	Yes, wild/weed	Asymptomatic or with mild mottling in experimental conditions. Detected in natural conditions in Israel (often with no symptoms, rarely with mosaic symptoms).	In experimental (Luria *et al.*, 2017) and in natural conditions (Dombrovsky, pers. comm., 2019)
Experimental hosts (inoculation trials)			
Chenopodium. amaranticolor	Yes, wild/weed	Experimental host. Asymptomatic	Luria *et al.*, 2017
C. quinoa	Yes, experimentally (Pawlowski, 2018)	Experimental host. Asymptomatic (Luria *et al.*, 2017) or with local and necrotic lesions (Alkowni *et al.*, 2019)	Alkowni *et al.*, 2019; Luria *et al.*, 2017
Nicotiana benthamiana	No, model organism	Experimental host. Symptomatic	Luria *et al.*, 2017
N. clevelandii	No, wild/weed in America	Experimental host.	Luria *et al.*, 2017
N. glutinosa	Yes, in tobacco hybrids, model organism	Experimental host.	Luria *et al.*, 2017
N. tabacum	Yes, as crop	Experimental host. Symptomatic (Luria *et al.*, 2017). Infection of tobacco is followed by a HR response (Dombrovsky, pers. comm., 2019)	Luria *et al.*, 2017

Host	Presence in PRA area (Yes/No/Not known)	Comments	References for host status
Petunia hybrida	Yes, as ornamental	Experimental host. Asymptomatic	Luria *et al.*, 2017

To date, the only confirmed natural hosts grown in the PRA area are *C. annuum* and *S. lycopersicum*. However, in addition to *S. lycopersicum,* it is proposed to consider for this PRA that all *Capsicum* spp. (including *C. annuum*, *C. chinense* and *C. frutescens*) are hosts because the three species are closely related; and hybrids are commonly bred and distributed globally. In addition, seeds of the three *Capsicum* species within the complex are similar in appearance and thus difficult to distinguish. Seed traders usually only identify seeds using the common name '*Capsicum*'.

Doubtful records:

- *Solanum melongena* (aubergine) is mentioned as host in Mexico only because of two positive detections (Garcia, pers. comm., 2019; SENASICA, 2019b). Garcia (pers. comm., 2019) explained to the EWG, that the detection was performed on imported seeds by PCR (SENASICA, 2019a). In the laboratory, after sowing seeds from one of these two seed lots, young *S. melongena* plants tested positive by PCR but did not show any symptoms. The plants were destroyed after 6 weeks (Garcia, pers. comm., 2019). The Expert Working Group considered that this positive result may be due to contamination by contact rather than a systemic infection resulting from seed transmission. Alternatively, it might be due to contamination in the laboratory during testing. Furthermore, Luria *et al.* (2017) and Panno *et al.* (2019b) could not transmit the virus to *S. melongena* during inoculation trials. The EWG concluded that there is not enough evidence to consider aubergine as a host plant in this PRA. The EWG in 2024 still considers that there is no strong evidence to consider *S. melongena* as a host plant.
- *Brassica oleracea* (cauliflower) is mentioned as a host only in one case in Mexico with no description of symptoms (SENASICA, 2019c). Replying to a question of the EWG, it was mentioned that the cauliflower crop showed unusual symptoms and when tested, it was positive for ToBRFV. The crop was grown in open air, in close proximity to an infected tomato field. It was not possible to further confirm the original positive result on *B. oleracea* with further testing (Garcia, pers. comm., 2019). The EWG concluded that there is not enough evidence to consider cauliflower as a host plant and that the positive test may be due to a contamination via workers.

Plants tested and confirmed as non-host

In trials, it was not possible to transmit the virus to potatoes (*Solanum tuberosum* cv. Nicola) (Luria *et al.*, 2017). This was later confirmed on other potato cultivars commonly grown in Israel, cv. Sephora, cv. Georgina, cv. Regina and cv. Mozart (Dombrovsky, pers. comm., 2019).

Update on host range in 2024

EPPO GD provides updated information on host plants with supporting references for each species (https://gd.eppo.int/taxon/TOBRFV/hosts). There are a number of new experimental hosts detailed in EPPO GD which were not in the original PRA. The same applies for wild/weed hosts, which could act as natural reservoirs of ToBRFV (Salem *et al*., 2022a; Matzrafi *et al*., 2023). No new cultivated hosts of agricultural importance have been described.

8. Pathways for entry

ToBRFV can be transported in live plants (Luria *et al*., 2017). Tobamoviruses are transmitted by mechanical contact and are capable of preserving infectivity on seeds and contaminated soil (Section 2.4).

In this Section, only peppers and tomatoes are considered as host plants[10].

The following pathways for entry from countries where ToBRFV is present into or spread within the EPPO region are discussed in this PRA. Pathways in bold are described and evaluated in Section 8.1; other pathways were considered very unlikely for reasons stated in Section 8.2.

- **Seeds of tomato and pepper**
- **Plants for planting (excluding seeds and pollen) of tomato and pepper**
- **Fresh fruits of tomato and pepper**
- **Used containers, tools, equipment and conveyance vehicles associated with the tomato and pepper production and supply chain**
- **Persons working in a place of production of host plants**
- Soil and growing media as such
- Natural spread
- Pollinating insects used in host fruit production
- Pollen of host plants
- Processed and dried fruits of tomato and pepper
- Soil or growing media attached to non-host plants
- In 2024 the EWG considered water based on new publications cited in the PRA update (not detailed in Section 8.2).

Seeds and plants for planting of experimental host plants, cut flowers of *Petunia* and dried leaves of tobacco are not further considered as pathways for entry as there is no evidence that they are susceptible to natural infection. However, even if this was the case, the probability of transfer from cut flowers or dried tobacco leaves to a suitable host plant in the EPPO region would be very low and therefore the probability of entry very low with low uncertainty. The import of solanaceous plants (such as *Petunia*) is already prohibited in many EPPO countries. In case of confirmation of natural infection of other experimental hosts, additional pathways may need to be considered to re-evaluate the risk of entry.

8.1 Pathways investigated in detail

ToBRFV was found in host plants for planting and is associated with host seeds. During production of host plants for planting and especially the growing of plants for fruit production, the virus is transmitted very quickly mechanically by the intensive and repeated handling of the plants. ToBRFV can survive on many surfaces and may be transmitted from these to host plants. In the case of substrate-free cultivation, by analogy with other viruses, it is considered that the virus may be transmitted from plant to plant in nutrient solutions (Büttner *et al.*, 1995a, 1995b).
All the pathways are considered for *Capsicum* spp. and *S. lycopersicum* from areas where the pest has been reported to be present to the EPPO region. Experimental hosts are not considered as a potential pathway in this PRA as there is no evidence that they may be naturally infected in the area of origin. Host seeds are studied in Table 3 and host plants for planting in Table 4. Fruits, soil and growing media, mechanical transmission and natural spread are discussed after these tables.

Examples of prohibition and inspection are given for some EPPO countries (the regulations of all EPPO countries were not analysed in this express PRA). Similarly, the current phytosanitary requirements of EPPO countries in place on the different pathways are not detailed in this PRA (although some were taken into account when looking at management options). EPPO countries

[10] Situation on 2020-06-19: Recent research points to breaking of resistance in *Capsicum* varieties harbouring some *L* resistance genes/alleles. Consequently, this may need to be reconsidered when additional publications are made available.

would have to check whether their current requirements are appropriate to help prevent the introduction of the pest.

Table 3. Host seeds

Pathway	Host seeds
Coverage	• Seeds of host plants
Pathway prohibited in the PRA area?	No
Pathway subject to a plant health inspection at import?	Yes, in some EPPO countries. For example, in the EU, phytosanitary certificate for imported seeds of *S. lycopersicum* and *C. annuum*. Since 2019-11, emergency measures on ToBRFV in the EU impose that *S. lycopersicum* and *C. annuum* seeds either originate in a third country free from ToBRFV, in a pest-free area (PFA); or that a representative sample has been officially tested and found free from the pest.. These measures were updated in the Commission implementing decision (EU) 2023/1032 (EU, 2023). For seed movement within the EU and introduction into the EU, pest free production sites are required for mother plants, supplemented by sampling and testing of the seeds. For plants for planting and seeds, a confirmation of resistance of the varieties which are known to be resistant to ToBRFV is stated in "Additional Declaration "of the phytosanitary certificate. Turkey is requiring since 2019-03-15 that consignments of *S. lycopersicum* and *Capsicum* seeds have been analysed by means of RT-PCR method and found free from the virus. Morocco is also requiring the testing of tomato seeds to be imported. The EWG had no further information on the requirements for other non-EU EPPO countries.
Pest already intercepted?	Update in 2024: ToBRFV infection over the period 2019-2021, was studied by Dall *et al*. (2023) on 659 small seed lots (intended for breeding, seed production or field trials) of tomato and 228 of capsicum. Thirty-two small seed lots tested positive using conventional RT-PCR and Sanger sequencing, whereas the 118 (larger) commercial seed lots all tested negative. In Italy, 7.28% of analyzed seeds lots were positive for ToBRFV (Panno *et al*., 2020). Investigations performed by Euroseeds, based on data from 15 seed companies, compared the number of seed lots imported and the interceptions reported in TRACES. For 2020, 2021 and 2022 respectively there were 14467, 12193 and 11352 tomato or pepper seed lots imported and 100, 87 and 67 interceptions (Euroseeds, 2022). The EU (2023) showed that numerous seed lots were found to be contaminated at import. Interception in EU countries were made on seed originating both from EU and non-EU countries (EPPO, 2021). In 2019, about 1000 commercial tomato seed lots were tested in Sicily with 30 seeds per lots, and 25 lots were found infected (Davino, pers. comm., 2019). In 2019, 34 commercial tomato seed lots (3000 seeds tested per lot) were tested by bioassay in Israel and 7 of these lots were found infected by ToBRFV (Levi, pers. comm., 2019). ToBRFV has been intercepted in the Netherlands on *Capsicum* seeds from Israel (snack pepper [sweet pepper in snack size] and hot pepper, and no *L* resistance genes/alleles for two of them, third unknown) (NVWA, 2020; EU, 2020a; Schenk, pers. comm., 2020).

Pathway	Host seeds
	ToBRFV was intercepted in Mexico on 60 tomato and pepper seed lots (out of 165 tested in 2018) from more than 14 different countries in Africa (Kenya), America (Canada, Guatemala, Peru, USA), Asia (China, India, Thailand, Vietnam) and in the EPPO region (France, Israel, Morocco, the Netherlands and Spain), (SADER & SENASICA, 2019b; SENASICA, 2019c). The seeds from France that tested positive in Mexico were imported from Thailand (Lopez-Buenfil, 2019) through Spain (Chan Hon Tong, pers. comm., 2019) and the seeds from Spain that tested positive in Mexico were also imported from Thailand (Lopez-Buenfil, 2019). ToBRFV was intercepted twice in December 2019 in the Netherlands on pepper seed from Spain, but these seeds were of Chinese origin according to their owner (sweet pepper [regular block pepper]. No information about the presence of *L* resistance genes/alleles). The United Kingdom also reported in 2020 interceptions on *Capsicum* seeds, one from China (via Italy) and one from Israel (EU, 2020b, 2020a; Schenk, pers. comm. 2020). As previous diagnostic tests (e.g. ELISA) did not delineate ToBRFV, previous interceptions of ToBRFV are likely to have been mistakenly diagnosed as ToMV or other tobamoviruses. It should be noted that practices to combat the disease are evolving, and the seed industry is taking measures to help guarantee the absence of contamination in seed lots (e.g. more systematic cleaning, treatment and testing of seeds) (Hanssen *et al.*, 2010b; ISF, 2020).
Plants concerned	*S. lycopersicum* and *Capsicum* spp..
Most likely stages that may be associated	ToBRFV particles may be found on the seed coat and in a very low percentage of cases in the embryo. Seed transmission has been demonstrated for ToBRFV (Davino *et al*., 2020, Salem *et al*., 2022a, Salem *et al*., 2022b; Vargas *et al*., 2023). Matsushita et al. (2024) showed that ToBRFV was detected on the seed coats of contaminated tomato and bell pepper seeds, but not on eggplant seed coats. Seeds of bell peppers transmitted ToBRFV at higher rates than tomato seeds, but a bell pepper cultivar that has resistance gene L3 was not systemically infected, and its seeds did not harbor the virus. ToBRFV infection over the period 2019-2021, was studied by Dall *et al*. (2023) on 659 small seed lots (intended for breeding, seed increase or field trials) of tomato and 228 of capsicum. Thirty-two small seed lots tested positive using conventional RT-PCR and Sanger sequencing, whereas the 118 (larger) commercial seed lots all tested negative. In Italy, 7.28% of analyzed seeds lots were positive for ToBRFV (Panno *et al*., 2020) until May 2019, whereas after this date no positive lots were reported. ..
Important factors for association with the pathway	Usually, seeds of varieties intended for glasshouse fruit production (high-value varieties) are produced indoor, while open pollinated seeds (non-hybrids) of public varieties or seeds of varieties intended for industry (lower value varieties) are produced outdoor (Lybeert, pers. comm., 2020). However, low value hybrid seeds are sometimes also produced outdoor (e.g. in the Gansu province, China or in Thailand) with several manipulations (Desulauze, pers. comm., 2020). As the virus is reported to be more prevalent indoor than outdoor (presumably due to more manipulations indoor vs. outdoor production), the risk of association with the seed varieties intended for glasshouse fruit production is likely to be higher. However, more

Pathway	Host seeds

quality controls e.g. more testing are performed on high value seeds. The industry is also using more and more hybrid varieties (Desulauze, pers. comm., 2020). The EWG did not separate the risk of association with the seed pathway depending on the intended use of the varieties.

The association of the pest with the pathway is difficult to assess as the following factors may affect the association of the pest with the seeds:

1 - It is difficult to traceback the geographical origin of seed lots. Individual consignments of hybrid seeds are often composed of mixed lots of seeds produced in several countries of origin, which increases the probability of association of the virus with the seed and the presence of infected seed in the individual seed lots derived from the bulked seed.

The production of some hybrid tomato seed lots typically involves activities in several countries performed (Figure 2) by major international seed companies (Bai & Lindhout, 2007; Mordor Intelligence, 2018). This sector is experiencing a high degree of concentration, as 95% of the EU market is in the hands of only five companies, which control 45% of the tomato varieties (Mammana, 2014). Plant lines used to produce hybrid seeds are often grown, selected and multiplied in two or three countries successively (AGDAWR, 2018; ISF, 2017). Larger seed companies typically contract out the production and multiplication processes to farmers, farmers' associations or private firms, often in countries with low production costs (AGDAWR, 2018).

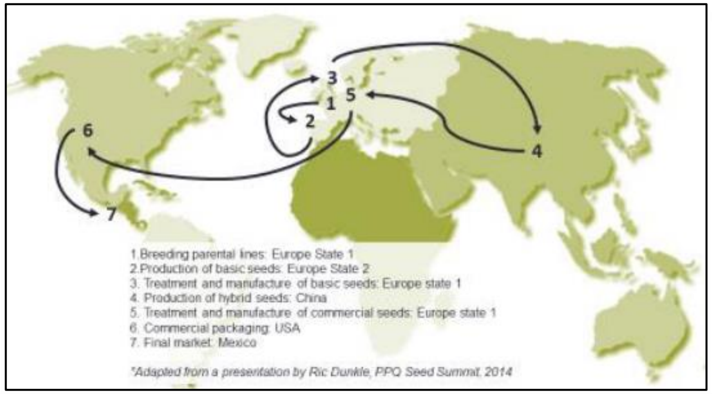


Figure 2. Seeds, a complicated trade model (ISF, 2017)

Throughout this process, the mother plants of the parental lines and basic seeds (or the seeds themselves) will be tested repeatedly for the presence of relevant pathogens.

During processing and shipment, tomato seed from one lot[11] is commonly divided up into a series of batches. Batches may be handled and packaged in different ways for different end-users. Each time a batch is divided, treated or repackaged, the batch and its derivatives are usually assigned new batch numbers (AGDAWR, 2018). After production, tomato seed batches are transhipped by airfreight via other countries. As an example, some tomato seeds sent to Australia are transhipped through France, Israel, Japan, the Netherlands or the USA (AGDAWR, 2018). Portions of a lot are sold to fruit production growers and nurseries in many countries (AGDAWR, 2018). Before the seeds from these international companies are distributed to the final user, they will be tested for relevant pathogens.

The International Seed Federation (ISF) has also recognised that phytosanitary certification of seed can be challenging because the final destination

[11] a production lot originates in one farm or field and is produced in one season.

Pathway	Host seeds
	of the seed may not be known when the seed is produced (ISF, 2017). **2–- By growing plant lines in different places, the plant lines may be exposed to a greater risk of infection by ToBRFV.** In China, the places where seed crops are grown outside change relatively often, as tomato crops for seed production are typically rotated every year in response to pest and pathogen pressure, which may expose them to infection by viruses (EU Commission, 2019; Gould, 1992). Moreover, the location of crops will change as the farm businesses, who are independent from the seed trading businesses, make decisions about subcontracted seed production every year (Venkateswarlu, 2007). However, seed production for the high-tech tomato production in the EU is largely done following GSPP (Good Seed and Plant Practices, https://gspp.eu/) standards, so under insect-proof greenhouses with high hygiene standards. The majority of tomato and pepper seeds imported from Guatemala, Israel, Kenya, Tanzania (countries evaluated during EU commission audits; EU Commission, 2019) are produced in high-tech insect-proof greenhouses. **3–- Combining seeds from different sources into a single consignment may also introduce infected seeds to healthy seed lots.** Different from GSPP tomato seeds, combining seeds of different origins into a single lot may introduce infected seeds to healthy commercial lots. Seed lots that include very small numbers of infected seeds may be difficult to detect depending on the number of infected seeds and on the sensitivity of the detection method. However, the commercial lots can be mixed and homogenised to provide a homogeneous germination level. Combining of vegetable production seed lots is a commercial practice (Bello & Bradford, 2016; ISF, 2017) for certain commodities (e.g spinach) but not for high-tech tomato production in the EU. However, assured traceability is one of the key requirements in GSPP, so the combining of seed lots is unlikely to occur in seed lots for high-tech tomato production following this certification scheme. **4 – The seed production process involves many steps and manipulations which may be a source of mechanical transmission and spread of the disease.** The tomato seed production process begins with plant breeding and involves the production of parent lines which are usually hybridised to produce the seed. Hybridisation involves emasculation of flowers and pollination by hand (Cheema & Dhaliwal 2005). This process has the potential to mechanically transmit and spread ToBRFV (Figure 3).  **Figure 3.** The emasculation process, involving removal of the staminate cone (courtesy H. Bolcan) (APS, 2014) **5 – The seed production process involves regular cleaning, heat and chemical treatments which may reduce the association of the virus with the seed.** After harvesting the fruit, the seed is extracted and cleaned by separating the seed from the pulp. Typically, the pulp is fermented for several hours, washed with an acidic solution, and then washed with water several times. This washing is efficient to decrease the association with some viruses.

Pathway	Host seeds
	This extraction process may be done on the place of production. Seeds are often treated which will decrease the probability of association with the seed pathway: ✓ Heat treatments of dry seeds for 2-4 days at 70°C to 76°C are commonly used to eliminate external and internal viruses, apparently without affecting seed germination (APS, 2014; Dombrovsky & Smith, 2017). The optimal time for seed treatment by thermotherapy using dry heat against another tomato tobamovirus, TMV, is 70°C during 24 h (Silva *et al.*, 2011). ✓ Chemical treatments are also commonly used on large scale. The most commonly used treatment in commercial seed production is 10% Trisodium phosphate (Na_3PO_4) which is known to eliminate tobamoviruses such as TMV and ToMV (APS, 2014; Córdoba-Sellés *et al.*, 2007; Dombrovsky & Smith, 2017) . Treatment with a 10% solution of trisodium phosphate for at least 15 min is considered to eliminate external viruses, apparently without affecting seed germination (APS, 2014). However, Lapidot (pers. comm., 2019) showed that 30 min treatment was needed for ToBFRV. Other disinfection methods used in commercial seed include the use of 1-9% hydrochloric acid (HCl), 1-5% calcium hypochlorite ($Ca(OCl)_2$), 1-3% sodium hypochlorite (NaOCl) and tetramethylthiuram disulphide ($(CH_3)_2NCSS_2CSN(CH_3)_2$). However, this treatment is sometimes performed after shipment to a facility in another country (AGDAWR, 2018). **Update on seed treatment in 2024** Efficiency of seed treatment has been studied. Davino *et al*. (2020) showed that 2% of seed coats samples were contaminated with ToBRFV after a 10% trisodium phosphate (TSP) seed treatment for 180 min, compared to 100% for untreated seeds. They performed different seed treatments based on a thermal procedure that for some of them affected germination and showed positive ToBRFV results by real-time RT-PCR. These positive samples' extracts were mechanically inoculated in tomato plants that were analyzed 30 days post inoculation and gave negative real-time RT-PCR except for one treatment at a lower temperature of 65°C. The more efficient treatments were based on a 2.5% hypochlorite solution with all samples tested after treatment by real-time RT-PCR being negative, and on a trisodium phosphate treatment with 3 samples out of 100 tested positive by real-time RT-PCR. These positive samples' extracts were mechanically inoculated in tomato plants that were analyzed 30 days post inoculation and gave negative real-time RT-PCR results, raising the question about how to carry out detection. Samarah *et al*. (2021) obtained a 100% disinfection rate with 2% a HCl treatment for 30 min or a 10% TSP treatment for 3h. Salem *et al*. (2022b) treated a sample of 100% infected tomato seeds with HCl and showed that treated seeds were all negative by DAS-ELISA and RT-PCR. Zamora-Macorra et al. (2023) showed that a 3% sodium hypochlorite solution was effective as a seed disinfection treatment. **6 – Existing field inspections and seed-lots testing for the detection of other pests prior to export reduces the risk of association** - Exporting countries commonly visually inspect seed crops to check that certain pests are not present in the crop (AGDAWR, 2018). However, careful visual examination of the crops may not enable an inspector to detect the presence of ToBRFV as some varieties are symptomless. Infection can also remain symptomless under certain growing conditions. - Seed companies often test tomato seed-lots for the presence of tobamoviruses as part of their quality system (De Ruiter, 2019).

Pathway	Host seeds
Survival during transport and storage	**Survival of the virus in the seed coat:** After extraction and treatment, seed lots may be stored for several years (AGDAWR, 2018) but this is unlikely to significantly affect the survival of the virus. Cucumber seeds inoculated with CGMMV were found infected at 85% after four years (Dombrovsky, pers. comm., 2019). Survival would be more limited by the impact of storage on seed germination.
Trade	Almost all the imported seed is thought to be first generation (F1) hybrid[12] seed produced by cross-pollination (hybridisation) of parental lines (AGDAWR, 2018). Trade data available in Eurostat (i.e. into the EU) cover all '*vegetable seeds for sowing (excluding salad beet or beetroot* 'Beta vulgaris *var.* conditiva')' (EU CN code 12099180). It is therefore not possible to obtain detailed data for tomato and pepper. Data was extracted for years 2014 to 2018 (Table 1 of ANNEX 6 and below). Additional information on the main trade of small vegetable seeds is available in Figure 1a and 1b of ANNEX 6. Within the countries known to be infected by ToBRFV, Italy and China are major suppliers of vegetable seeds in the EU. Regular imports of vegetable seeds occur from Israel, main importers being the Netherlands (8700 to 36900 kg per year), Spain (1000 to 3700 kg per year) and Italy (500 to 8500 kg per year). The Netherlands is the main EU vegetable seeds importer from Mexico (2700 to 6300 kg per year) (Figure 1a and 1b of ANNEX 6). China is the main provider of tomato and pepper seeds to France (up to 23 600 kg/year of tomato seeds and 10 400 kg/year of pepper seeds during the period 2014-2019) (ANNEX 7, Table 1 and 2) as well as in Italy (ANNEX 7, Table 3 and 4). China is therefore assumed to be an important provider of tomato and pepper seeds to the whole of the EPPO region.
Transfer to a host	Seed transmission to the seedling rates range from 0.08 to 2.8% (Davino *et al*., 2020, Salem *et al*., 2022a, Salem *et al*., 2022b) and can be up to 9% (Vargas *et al*., 2023). However, even a very low percentage of disease transmission can result in a small infection focus and this infection can then be spread very rapidly by mechanical contacts within the growing crop (Dombrovsky & Smith, 2017). The EWG considered that percentage of seed-to-seedling transmission cited in literature may be overestimated compared to other tobamoviruses (Dombrovsky *et al*., 2017). As the virus load on seed coat (especially untreated seeds) can be high, handling of seeds and further manipulations of plants in a nursery by a worker could potentially lead to infections.

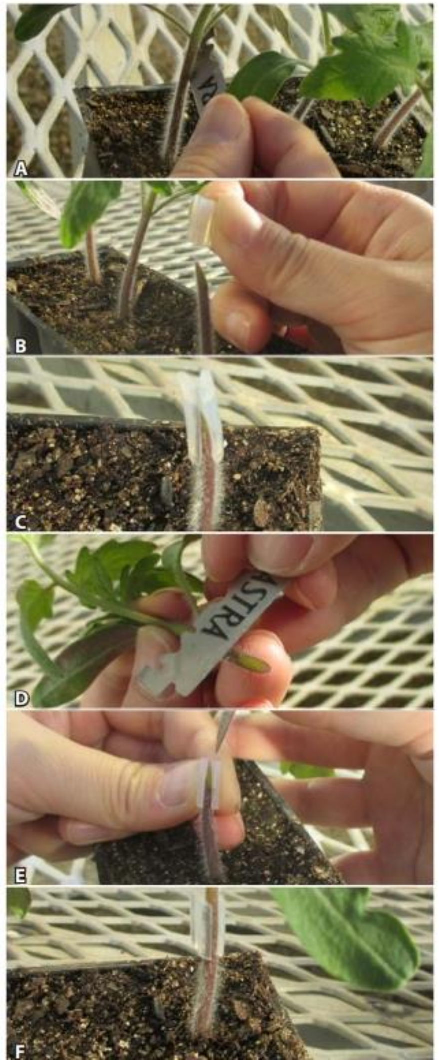
[12] Tomato hybrids are reported to have better vigour, uniformity, disease resistance and stress tolerance, and to have desirable horticultural traits including early fruiting, longer shelf life and consistent yield.

Pathway	Host seeds
Likelihood of entry and uncertainty	**Tomato seeds:** High likelihood (presence of ToBRFV in the seed coat, other tobamoviruses are known to be seed transmitted, there is low genetic diversity of the virus over a wide geographical range, a very low transmission rate is sufficient to cause infections in new areas, trace-back experience in Sicilia, underestimation of the ToBRFV distribution) with a moderate uncertainty (relative importance of other pathways in the current distribution of ToBRFV, biological relevance linked to detection test). However, the likelihood could decrease due to voluntary and regulatory measures and treatments which are implemented by the industry. **Pepper seeds (varieties which do not harbour *L* resistance genes/alleles):** High likelihood with high uncertainty (less data available on pepper). USDA (2023b) rated the likelihood of ToBRFV being introduced [into the United States] on imported tomato and pepper seeds and plants as high.

Table 4. Host plants for planting (except seeds, pollen)

Pathway	Host plants for planting (except seeds, pollen)
Coverage	• Plants for planting of host plants, grafted or not. This pathway does not include seeds and pollen, but includes seedlings, rootstocks and scions.
Pathway prohibited in the PRA area?	Partly. In the EU, import of plants of *S. lycopersicum* and *Capsicum* intended for planting, other than seeds, is prohibited from third countries, other than European and Mediterranean countries[13], according to Annex VI point 18 of Regulation (EU) 2019/2072 (EU, 2019b). Since 2019-11, emergency measures on ToBRFV in the EU (Section 5) impose that *S. lycopersicum* and *C. annuum* plants for planting other than seeds either originate in a third country free from ToBRFV, in a pest-free area, or in a pest-free production site with the plants deriving from seeds which originate from a pest-free area or have been tested. These measures were updated in the Commission implementing decision (EU) 2023/1032 (EU, 2023) imposing that plants for planting originate from seeds which comply to the requirements of the implementing decision (see above), that they have been produced in a production site which is known to be free from ToBRFV; a confirmation of resistance of the varieties which are known to be resistant to ToBRFV is stated in "Additional Declaration "of the phytosanitary certificate. .
Pathway subject to a plant health inspection at import?	Yes, in some EPPO countries. For example, in the EU, following emergency measures. It may be noted that other phytosanitary requirements already applied to tomato and pepper plants: phytosanitary certificate and inspection requirement for chrysanthemum stem necrosis virus, *Keiferia lycopersicella*, tomato yellow leaf curl virus (for *S. lycopersicum*); and *Ralstonia solanacearum*, '*Candidatus* Phytoplasma solani' (for *C. annuum* and *S. lycopersicum*). However, symptoms on young seedlings are usually not visible at import (Section 2.5).
Pest already intercepted?	Yes (EPPO, 2021).
Plants concerned	*S. lycopersicum* and *Capsicum* spp..
Most likely stages that may be associated	The virus may be present in the plant, as well as in the growing media associated with the plants.
Important factors for association	Transplants are produced from seeds. The use of high-quality seeds certified as disease free is essential to reduce the risk of association with the pathway.

[13] Albania, Algeria, Andorra, Armenia, Azerbaijan, Belarus, Bosnia and Herzegovina, Canary Islands, Egypt, Faeroe Islands, Georgia, Iceland, Israel, Jordan, Lebanon, Libya, Liechtenstein, Moldova, Monaco, Montenegro, Morocco, North Macedonia, Norway, Russia (only the following parts: Central Federal District (Tsentralny federalny okrug), Northwestern Federal District (Severo-Zapadny federalny okrug), Southern Federal District (Yuzhny federalny okrug), North Caucasian Federal District (Severo-Kavkazsky federalny okrug) and Volga Federal District (Privolzhsky federalny okrug)), San Marino, Serbia, Switzerland, Syria, Tunisia, Turkey and Ukraine

Pathway	Host plants for planting (except seeds, pollen)
with the pathway	Tomato transplants are usually produced in trays. This is probably similar for pepper transplants. Production techniques and practices of transplants may influence the risk of association with the pathway: **1–- Grafting increases the probability of association with the plants for planting** Grafting of tomato transplants (Figure 4) has become an important cultivation practice for greenhouse tomato fruit production systems (Singh *et al.*, 2017). Limited information on compatibility with open-field tomato cultivars is available but vegetable grafting is also gaining interest in open-field and high tunnel tomato production (OECD, 2017). The grafting technique is widely used on a commercial scale (e.g. in Spain, nearly 90% of tomato plants are grafted (Penella *et al.*, 2017); in Italy, 40% of the plants used for the commercial tomato production are grafted (Tomassoli, pers. comm., 2019), whereas only 12% are grafted in Turkey (Yetişir, 2017)). Grafting is mainly performed to improve the vigour of the plant. There are a variety of grafting techniques, but the most widely adopted method worldwide for grafted tomato production is tube grafting (OECD, 2017). Tomato is grafted on different species (e.g. *S. lycopersicum*, *S. melongena*, *S. lycopersicum* × *S. habrochaites*) (GEVES, 2019; Yetişir, 2017). Pepper is less commonly grafted (e.g. In Italy, only 10% of the pepper plants are grafted (Tomassoli, pers. comm., 2019)) because of the lack of interesting rootstocks. Grafting of pepper is mainly performed on rootstocks of the same species, *C. annuum*. Transmission of diseases in the production process of grafted seedlings occurs more easily than in regular seedling production. This is because some diseases can be transmitted from seedling to seedling by cutting tools. For CGMMV, the use of grafting knives can infect the four following grafted plants (Reingold *et al.*, 2015). In addition, the cut surfaces of both the rootstock and the scion are entry points for pathogens, and high relative humidity and ambient temperature in the healing chambers promote the spread of diseases (Yetişir, 2017). Handling during the grafting process is also an important factor. Grafting is an additional possible source of infection by ToBRFV. **2 – Plantlets are likely to be symptomless** Symptoms on non-grafted young plantlets would usually not be visible in the plant production nursery (section 2.5) because they are traded earlier than grafted plantlets.  **Figure 4.** The procedure for splice grafting tomato plants (Guan & Hallett, 2016).

Pathway	Host plants for planting (except seeds, pollen)
	3 – Water supply increases the probability of association with the plants for planting. Containerized production of tomato transplants is usually performed with abundant supply of quality water. Irrigation is performed with overhead (usually with an automated watering system) and ebb and flow (or ebb and flood). When producing transplants, water may be chlorinated (1 ppm chlorine) (APS, 2014) but this is probably not sufficient to inactivate tobamoviruses (Li *et al.*, 2015). It is assumed to be the same for pepper transplant production. Aerial irrigation can cause injury to plants that facilitate infections and circulating water can be a source infection (Section 2.2). **4–- The re-use of trays increases the probability of association with the plants for planting.** Transplant trays may be cleaned and sterilized before reuse to avoid the build-up of soilborne pathogens, by washing with soap and water, followed by a chemical dip in either quaternary ammonium (2-5%) or sodium hypochlorite (1-2%) solution for at least 20 min. Trays are rinsed in clean water and dried before reuse. Steam sterilization at 70-82°C for 40-60 min may be used to eliminate tray-borne pathogens, but purchasing sterilization equipment can be costly, and this can contribute to the deterioration of the trays (APS, 2014). Ongoing experiments show that 5 minutes soak at 90°C or 5 minutes soak at 70°C + Virkon S seems to be highly effective (Fox, pers. comm., 2019). When not appropriately cleaned and sterilized, the re-use of transplant trays is a source of infection by contact, which increases the probability of association with the plants for planting.
Survival during transport and storage	Plantlets are fragile and can only travel during a few days. They are generally transported in trucks. Therefore, this limits the distance plants can be traded. The temperature of transport and storage allow survival of the ToBRFV.
Trade	In the EU, transplants used to establish fruiting crops in a country are often grown in the same country. In some EU countries however, plants are introduced from other EU countries, mainly originating from the Netherlands (Werkman & Sansford, 2010). Importing plants from other countries seems to be common practice nowadays. For example, most of plants imported to the United Kingdom and Belgium are produced in the Netherlands. In the Rheinland (west part of Germany, most important area for German tomato production), most tomato and pepper plants for planting are imported from the Netherlands (except plants for organic production) (Scholz-Döbelin, pers. comm.). In Austria, commercial tomato growers buy in general young plantlets from abroad. Local production of plants (from seeds) is only for the hobby sector (Steffek, pers. comm., 2020). One company in the Netherlands (Beekenkamp) produces over 600 million young plants per year of tomato, pepper and other vegetables in more than 30 hectares of greenhouses (https://www.beekenkamp.nl/plants/en/tomato-nursery/). Tens of thousands of tomato and pepper plants for planting from Spain are exported every year to France and Portugal (Guitian-Castrillon, pers. comm., 2019), as well as a more limited number of tomato plants from the United Kingdom to France (Gentit, pers. comm., 2019).
Transfer to a host	Since tomato and Capsicum plants are directly introduced at production sites, it is very likely that infected plants will aid transfer of ToBRFV to suitable hosts.

Pathway	Host plants for planting (except seeds, pollen)
Likelihood of entry and uncertainty	**Host plants for planting (except seeds, pollen):** High likelihood (more manipulations than seed production, ToBRFV reproduces in the plant, possible contaminations within the nurseries, testing is less reliable because of the difficulty to test ten thousands of plants, one infected plant would cause an outbreak, DE, FR and UK outbreaks are suspected to be linked to infected plants for planting) with a low uncertainty (No uncertainty on the transmission for the plant pathway. Other sources of uncertainty: pest distribution in the EPPO region, unknown trade of plants to northern African and eastern European countries).

Table 5. Host fruits

Pathway	Host fruits for fresh market
Coverage	Tomato and pepper fruits are sold for the fresh market or processed into many different products (puree, paste, peeled, canned, chutney, ketchup, juice, dried etc.). This pathway only focuses on tomato and pepper fruits for the fresh market and includes containers (e.g. trays, boxes and packaging) when associated with fruits. For processed fruits, see section 8.2.
Pathway prohibited in the PRA area?	No
Pathway subject to a plant health inspection at import?	Yes, in some EPPO countries. For example, in the EU, a phytosanitary certificate is required to import fruits of *S. lycopersicum* and *Capsicum*. However depending on the origin, a reduced frequency check may be applied (https://ec.europa.eu/food/sites/food/files/plant/docs/ph_biosec_trade-non-eu_prods-recom-reduced-ph-checks_2019.pdf).
Pest already intercepted?	Yes. Infected fruits from Egypt have been intercepted in the Netherlands (EU, 2019c). ToBRFV has been detected in fruits from Mexico during inspections in Florida (USA) (FDACS, 2019). Skelton *et al.* (2023b) performed a study on 54 tomato retail trade tomato fruit samples and showed 27.8% to be infected (low Ct values) and 53.7% to be contaminated (high Cq values) by ToBRFV. Yilmaz *et al.* (2023) showed that in several grocery stores in Florida, 86.5% of fruit samples were infected by ToBRFV alone (73% mixed infected by ToBRFV and PepMV). The EWG considered that the high level of imported infected fruits in retail could indicate a higher level of plant infection than notified.
Plants concerned	*S. lycopersicum* and *Capsicum* not harbouring *L* resistance genes/alleles.
Most likely stages that may be associated	ToBRFV may be found in all plant parts, including fruit. Tomato fruit can have a very high viral load (Hanssen, pers. comm., 2019; Skelton *et al.*, 2023b), Yilmaz *et al.*, 2023) and survive outside of living plant (section 2.3). ToBRFV may also remain infectious on packaging material that comes in contact with infected plants or fruits.
Important factors for association with the pathway	Fruits from infected crops are likely to be infected. **1–Manipulations increase the risk of association with fruits. Association with fruits is likely to be higher for indoor production compared to outdoor production.** All manipulations during fruit production (plant tying, pruning, removing of plant suckers), harvesting (e.g. the use of manual or mechanical harvesting), will increase the risk of association with the pathway, because of the risk of mechanical transmission. As more manipulations are expected for intensive indoor production rather than for outdoor production, association with the pathway is considered higher for indoor production. **2 – Sorting out process**

Pathway	Host fruits for fresh market
	When symptoms are visible, fruits will be downgraded and may not be traded anymore for fresh consumption (i.e. rather processed for use as tomato sauce, paste etc.). However, asymptomatic fruits are still likely to be present in the consignment and infection will not be evident at inspection. **3 – Cultivation of seed trial lines close to fruit production.** Trial lines are subject to more limited seed testing than commercial varieties (Dombrovsky, pers. comm., 2019). Transfer could occur because of the cultivation of trial lines or trial lots of tomato seed close to fruit production. Fruit production businesses and seed businesses collaborate to grow trial lines to determine their suitability. Tomato trial lines are usually grown at the same time and in the same place as larger fruit production crops. Van Brunschot *et al.* (2014) have reported trial lines infected with pospiviroids. Therefore, trial lines can be a source of infection for tomato crops (AGDAWR, 2018). In such case, infection of fruits would require a combination of different pathways (e.g. seed infection of the trial seeds and mechanical transmission).
Survival during transport and storage	As the pathogen is being transported in living plant material, there is high probability that the virus will survive transport and storage. The virus may also survive during transport and storage on packaging material.
Trade	Although tomatoes are mostly traded within the EPPO region and between neighbouring countries, an intercontinental trade of tomato exists, including trade from small producing and exporting countries. The total volume of imports of fresh tomato fruit from non-EPPO countries represents a small part of the imports of EPPO countries (only 3% when considering maximum estimated quantities) (EPPO, 2015). Mexico (1st exporter in the world with 1 748 858 t), Netherlands (2nd exporter with 992 601 t), Turkey (6th exporter with 485 963 t), Jordan (7th exporter with 361 439 t), China (12th exporter with 206 343 t) and Italy (13th exporter with 104 937 t) belong to the top 30 tomato producers in the world (ANNEX 8). During the period 2001-2010, Mexico exported a maximum of 2785 tonnes/year to EPPO countries (data from FAOSTAT analysed by EPPO, 2015). Spain, Mexico, the Netherlands, Israel and Jordan are major exporters of *Capsicum* fruits in the world (Workman, 2019).
Transfer to a host	Release of sap from vine tomatoes during handling is likely to occur and therefore contaminate containers and equipment (Dombrovsky, pers. comm., 2019). Contamination of containers or other fruits by the fruit themselves will only occur via damaged fruits where sap is released (Klap *et al.*, 2020a). The presence of the tomato peduncle may also cause damage to other fruits during grading, which may release sap. Peppers are always sold with a peduncle. Pepper fruit are less susceptible to physical damage and consequential sap release than ripe tomato fruit. When imported fruit is stored or repacked at destination in facilities that also grow tomatoes, consignments can be present for several days at packing stations. In that specific case, ToBRFV may be mechanically transmitted from imported infected fruits to a suitable host plant if workers and machinery are shared between the packing area and the production site. The practice of using the same facilities for imported fruit and for production was shown as a factor facilitating transfer of pests in the past in tomato and pepper supply chains (e.g. PepMV, *Tuta absoluta*,

Pathway	Host fruits for fresh market
	Thaumatotibia leucotreta) (EPPO, 2010). This may be changing as a result of these recent pest introductions but there is no evidence of this for the whole EPPO region (EPPO, 2015). Another possibility allowing transfer, is ToBRFV contamination of workers hands with infested fruits (bought e.g. in a supermarket), when workers are involved in a place of production of host plants for planting or fruit. This can only be avoided if strict hygiene measures are applied on site. Finally, fruits imported for consumption may be inappropriately used for propagation by amateur gardeners or small professional farmers. Fruits could also be discarded or partially composted, and the fruits with seeds can germinate (volunteers). In each case the resulting plants may be infected. However, this is more likely to occur in domestic situations and not in major crop production sites. Imported tomato fruits can be stored or repacked at destination in facilities that also grow tomatoes. Imported tomato fruits can also be repacked at destination in facilities that also pack local fruits, and transfer may occur via contaminated material (e.g. containers of imported tomato fruits reused locally in tomato production sites to harvest local tomatoes). Update in 2024: Transmission by fruits has been shown to be possible when fruits were damaged but not when they are intact (Klap *et al*., 2020a). USDA (2023a) performed an assessment of the risk of introduction via infected fruits and concluded that despite high import volumes, imports of tomato and pepper fruit for consumption are unlikely to introduce ToBRFV into the United States. Fruit consumption by workers on production site has been suspected in some outbreaks in France (P. Gentit, 2024 pers. com.). In the UK, as 100% of seed lots are tested, there have been suspicions of outbreaks linked to fruits, but with no evidence yet (A. Fox, pers com, 2024).
Likelihood of entry and uncertainty	**Host fruits already packed for final consumer before export:** very low likelihood with a low uncertainty (no possibility of transfer except with workers eating these fruits). In 2024, in line with new information, the EWG considered a low likelihood with a medium uncertainty (linked with level of infection of fruits but transfer equivalent as during the PRA) **Host fruits stored or repacked at destination in facilities that also grow host fruits, or repacked at destination in facilities that also pack local fruits:** high likelihood (fruits only visually examined for the presence of pests, workers may not be aware of the risk, similar cases demonstrated for pepino mosaic virus, trade of fruits is much larger than trade of plants for planting. The likelihood of entry would be higher for host fruits stored or repacked at destination in facilities that also grow host fruits than for repacking at destination in facilities that also pack local fruits) with a moderate uncertainty (volume repacked because of European Standards vs. volume already packed before export, the use of high hygiene standards in some EPPO countries).

Pathway	Host fruits for fresh market
	When imported fruit is not stored or repacked at destination in facilities that also grow tomatoes (e.g. already packed for the final consumer before export), or repacked at destination in facilities that also pack local fruits, fruits are considered to be an unlikely pathway of entry.

Used containers, tools, equipment and conveyance vehicles associated with the tomato and pepper production and supply chain
Note: Containers used to transport fruits and trays used to transport plants for planting are considered together with the fruit and plants for planting pathways.

Tobamovirus may remain viable for a long time on surfaces. International movement of the virus and transfer to host plants is likely to happen only if the containers, tools and equipment have previously been associated with the tomato and pepper production and supply chain.
Used containers could be moved without fruits between countries, e.g. Europool System is moving more than 1 billion trays a year which are not owned by users but rented[14] (Europool System, 2019). Containers provided by Europool System are cleaned, disinfected and tested using a validated protocol for ToBRFV. However, such protocols are probably not applied for all used containers, tools, equipment and conveyance vehicles associated with the tomato and pepper production and supply chain.

Likelihood of entry by mechanical transmission: medium; *Uncertainty*: high (volume treated vs. untreated)

Persons working in place of production of host plants
Persons working in a place of production may have been in contact with infected or contaminated material (e.g. seasonal workers).
Persons installing and fixing greenhouses as well as trellising workers may travel internationally and were suspected to be a source of contamination for PepMV in the EU.
Other people, such as technical teams, experts and crop advisors traveling around and visiting different places of production can introduce ToBRFV. Indeed, it is thought that one of the main reasons for the rapid dissemination of ToBRFV in Israel was the very large number of people who visited the first outbreak (Dombrovsky, pers. comm., 2019).
Detection of ToBRFV on hands, gloves, clothes and accommodation of workers has been shown (FERA, 2021b; Ehlers *et al*.,2024).

Likelihood of entry by workers: low (this pathway is less likely at international level than for local and regional spread); *Uncertainty*: moderate

8.2 Unlikely pathways: very low likelihood of entry

- **Soil and growing media as such.**

This pathway is intended to cover growing media with or without a component of organic material.

The virus may be present in soil and growing media if infected plants have been in contact with them. Transfer to the roots of a host plant is possible through soil and plant debris: the infectivity of tobamoviruses is preserved in plant debris and in contaminated soil and clay soil for months to years (Dombrovsky & Smith, 2017; Smith & Dombrovsky, 2019). Some tobamoviruses are resistant to denaturing even in compost, but this will also depend on specific conditions including moisture content, temperature, length of time etc. (AHDB, 2019b).

Professional growers may use a wide variety of soils, the most suitable for greenhouse tomatoes being those classified as loams, sandy loams, and some silty loams, all with a high organic-matter content (Papadopoulos, 1991). Nowadays, intensive greenhouse production of tomato involves the use of hydroponics, a production system in which the roots are irrigated with water containing a mix of essential nutrients while sustained in a substrate of inert material or the same solution instead of soil (OECD, 2017).

[14] Videos explaining how recycled containers are provided by Europool System are available at https://youtu.be/JFxY5mOqKHc and at https://www.youtube.com/watch?v=L__BBxSoEzk.

The import of soil and growing media is usually regulated in the EPPO region (e.g. soil and growing media as such from all third countries other than Switzerland, cannot be imported in the EU according to Annex VI point 19 and 20 of Commission Implementing Regulation (EU) 2019/2072 (EU, 2019b)). If soil or growing media in which infected plants were previously grown or composted, is imported, it may be a pathway for entry of the pest. However, this is considered very unlikely for growing media for professional use. In particular, because of the difficulty of achieving the correct composition (i.e. peat, perlite, vermiculite, fertilizer, etc.), new and small tomato transplant growers use commercial pre-blended soilless mix (APS, 2014) which would present much less risk because they are not deemed to be recycled. When re-using growing media for tomato production, this would be done within a production site or locally. It is very unlikely that growers will reuse growing media that has already been used in another country or by another producer to grow tomato.

Uncertainty: low

- **Natural spread, from countries where ToBRFV occurs to EPPO countries where it does not occur** (over the next 10 years in absence of eradication/control measures).

No official measures against ToBRFV are applied in some EPPO countries (e.g. Israel, Jordan). However, most of the production of tomato and pepper in Israel and Jordan are under protected conditions and the virus is more prevalent indoor than outdoor in the EPPO region. Even if insects present in an infected production site may have the capacity to fly long distances (e.g. more than 10 km in search of desirable floral rewards for *Apis mellifera*), it is assumed that only a small number of these insects (e.g. 'scout' bees) fly longer distances, and it is well known that bees tend to forage within 2.0 km of their hive if there are attractive floral resource in the vicinity (Hagler *et al.*, 2011). Other tobamoviruses are known to be transmitted by other animals such as birds (Broadbent, 1976; Peters *et al.*, 2012). Like for pollinating insects, it is assumed that their role will be limited compared to human-assisted pathways between different production areas. Natural spread from these countries to the rest of the EPPO region via pollinating insects and other animals is therefore considered to be an unlikely pathway.

Uncertainty: low

- **Pollinating insects** (from third countries) **used in host fruit production.**

Tomato flowers are typically pollinated by wind and/or bees in open fields and bumblebees in greenhouses or screenhouses. Before the 1990's, pollination was performed in tomato greenhouse conditions mechanically by vibrating the plants or with hormones. Nowadays, pollination in greenhouse tomato crops is commonly performed with bumblebees (Velthuis & van Doorn, 2006).

Tomato crops under greenhouse is the main agricultural crop that bumblebees pollinate: Worldwide, this involves about 95% of all bumblebee sales. Up to 50 bumblebee colonies are used per hectare of fruit production during the growing season (Velthuis & van Doorn, 2006). Import of pollinating insects (including bumblebees) is often restricted in the EPPO region. E.g. in the European Union, imports of bees are restricted in order to prevent the introduction of two exotic pests, which are absent from the EU, the small hive beetle (*Aethina tumida*) and the Tropilaelaps mite (*Tropilaelaps* spp.). Only cages of queen bees (*Apis mellifera* and *Bombus* spp.) and containers of bumblebees can be imported from a list of third countries which includes Israel and Mexico, but not Jordan (EU, 2017a, 2017b). Most of the market share is covered by the 3 companies (Biobest (BE), Koppert Biological Systems (NL) and Bunting Brinkman Bees (NL)), having rearing facilities not only in their homeland, but also in other countries and on other continents, usually under their own name (Velthuis & van Doorn, 2006).

Movement within the EU is possible with a health certificate. Although a large quantity of commercially produced bumblebees is moved within the PRA area, the probability of movement of ToBRFV with traded bumblebees is estimated as very unlikely because the probability of association is very unlikely. This is because bumblebees are produced on a diet of bee-collected pollen and sugar water (Velthuis & van Doorn, 2006; Werkman & Sansford, 2010). Therefore, the movement of pollinating insects are not considered to pose a risk of entry into the PRA area from distant third countries. The main risk from bumblebees is associated with spread within an infected area.

Uncertainty: low

- **Pollen of host plants**

ToBRFV is present in pollen but does not infect the plant (Avni *et al*., 2022).

Uncertainty: low

- **Processed and dried fruits of tomato and pepper.**

The virus may survive in dried fruits or products made from tomatoes and/or peppers for several months. However, it is very unlikely that ToBRFV will transfer from processed and dried fruits to commercial production of tomato and pepper. High temperatures in some processes may also denature the virus (e.g. canning).

Uncertainty: low

- **Soil or growing media attached to non-host plants**

It is very unlikely that non-host plants for planting will be grown in growing media previously used to grow tomato or pepper. Secondly, it is unlikely that they will be replanted in a field where host plants are grown.

Uncertainty: low

8.3 Overall rating of the likelihood of entry

For all pathways and at the scale of the PRA area, it is considered that the current phytosanitary requirements in place are not enough to prevent further introductions of ToBRFV into the EPPO region. There are prohibitions on the import of tomato and pepper plants for planting in some countries, but this is not considered enough. ToBRFV has already been reported into the EPPO region. However, it is not known whether these reports are linked to new introductions into the EPPO region or to spread from EPPO countries. The seed industry is taking measures to help guarantee the absence of contamination in seed lots (e.g. more systematic cleaning, treatment and testing of seeds), and the import of plants for planting from outside the EPPO region is limited.

By combining the assessments from the individual pathways considered, the EWG concluded that the overall likelihood of entry is high with a low uncertainty (i.e. the likelihood varies between high and very high).

Rating of the likelihood of entry	*Very low* ☐	*Low* ☐	*Moderate* ☐	*High* ☒	*Very high* ☐
Rating of uncertainty			*Low* ☒	*Moderate* ☐	*High* ☐

9. Likelihood of establishment outdoors in the PRA area

9.1 Climatic suitability

The biological functions of viruses are strongly integrated with those of their host plants and there is no indication that their requirements in terms of environment are substantially different from those of their host plants.

Tomato plants are sensitive to cold, especially to low night temperatures, and are a warm season crop; however, they may tolerate some variation. CAL (2007) indicates optimal temperatures of 21-32°C with a tolerance for exposure to temperatures below 12.7°C or above 37.7°C for short periods (remark: tomato transplants can tolerate a temperature as low as 5°C for 2-3h (APS, 2014)). In desert areas where relative humidity is low, tomato plants are grown with additional irrigation. Tomato is cultivated outdoors as far north as Alaska or similar northern locations, especially in gardens (EPPO, 2015).

Peppers also require warm temperatures. Optimum germination temperature is about 27°C. In seedlings, the optimum day temperature is 24 to 29°C, and the optimum night temperature 10 to 16°C. By the time the plant is flowering, best temperature for fruit set is between 18 and 27 °C. Plants will not set fruits well during periods of extended hot weather (McCormack, 2006).

Tomato and pepper are grown in a wide range of climates throughout the PRA area. In the northern part of the PRA area, tomato and pepper are only grown commercially under active protected cultivation (with heating) (Section 10). However, tomato and pepper plants are also grown outdoors in domestic gardens in the summer. In warmer areas in the southern part of the PRA area, tomato and pepper may be commercially grown outdoors as well as under passive greenhouse (without heating).

In Mexico, outbreaks were found both in the open field and in greenhouses (about 56% from open field, and 32% from greenhouses (SENASICA, 2019c)).

Climatic conditions would therefore probably not limit establishment of ToBRFV in the PRA area and it is considered that the pest could establish wherever suitable hosts are produced in such conditions.

9.2 Host plants

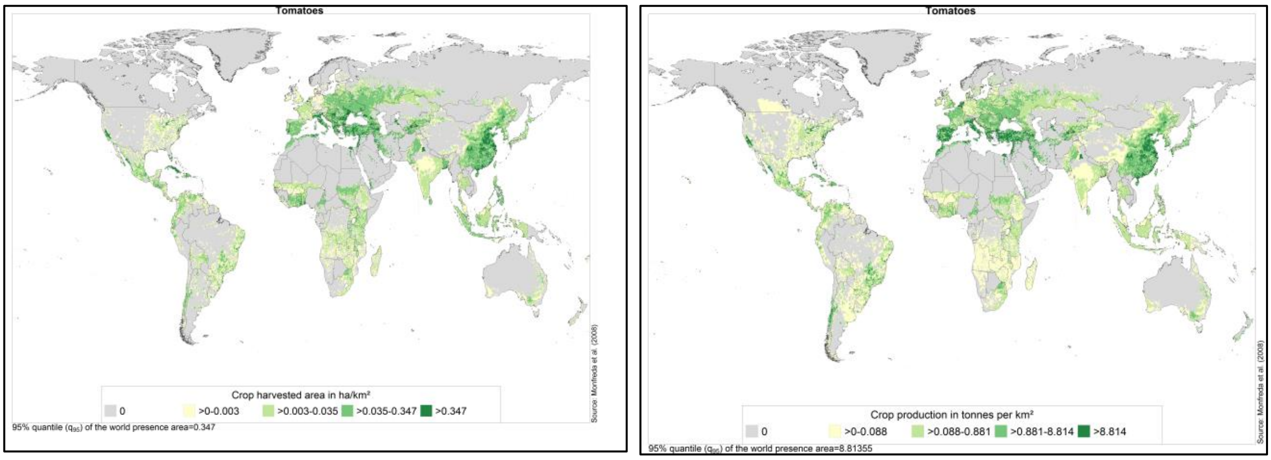
So far only tomato and pepper are reported as crops naturally infected by ToBRFV.

Tomato

Tomato (*S. lycopersicum*) is grown worldwide outdoors and under protected conditions (Figure 5), commercially and in gardens. Under cultivation, tomato is grown as an annual crop. It is one of the most widely grown and eaten food crops in the world, with an annual global production of about 170.7 million tons in 2014 from an area of 5 million hectares (FAOSTAT 2014 analysed by Singh *et al.*, 2017).

In Germany and other countries in Northern and Central Europe, large quantities of tomato plants are cultivated, mostly in greenhouses for the production fruits (Section 10). Furthermore, there are half-yearly outdoor cultivations in private gardens, on balconies or in private greenhouses (JKI, 2019). In southern Europe, fresh tomatoes can be produced in greenhouses or in open field (in Italy, 9-10% of tomato is produced indoor, representing 7 229 hectares and 535 564 tonnes of fruit in 2018 (ISTAT database)).

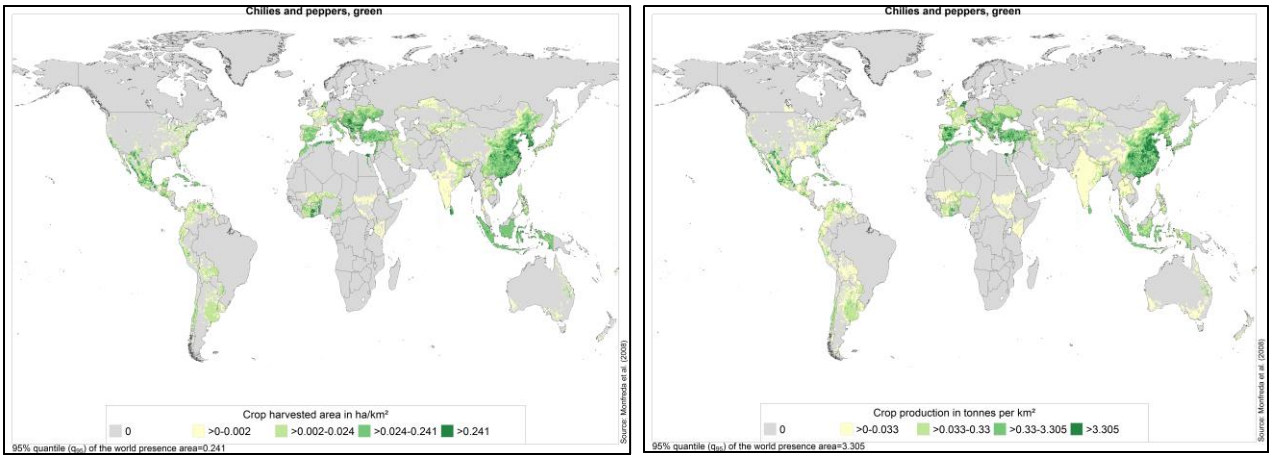
Figure 5. Tomato production in the world, both indoor and outdoor, in 2000 (left: harvested area in ha/km^2; right: tonnes per km^2) (Monfreda *et al.*, 2008)



Pepper

Peppers (*Capsicum* spp.) is grown worldwide outdoors and under protected conditions (Figure 6), commercially and in gardens. In southern Europe, fresh peppers can be produced in greenhouses or in open field (In Italy, 30% of pepper is produced indoor, representing 1976 hectares and 7897 tonnes of fruit in 2018 (ISTAT database)). Mainly *L* resistant peppers are grown commercially in the EU and Israel. However, this may not be the case for varieties used by amateurs: when bought at garden centres/supermarkets for bioassays, pepper and hot pepper varieties used by amateurs were not found to harbour *L* resistance genes/alleles (Tomassoli, pers. comm., 2019).

Figure 6. Chillies and peppers production in the world, both indoor and outdoor, in 2000 (left: harvested area in ha/km^2; right: tonnes per km^2) (Monfreda *et al.*, 2008)



Other plants

Plants that possibly could serve as a reservoir are also present outdoors in the EPPO region. It was shown that weeds, e.g. *C. murale* and *S. nigra*, were infected in natural field conditions in Israel and Jordan. Other examples for potential reservoir plants are tobacco, *Chenopodium quinoa* (in Germany only cultivated in small quantities (JKI, 2019)), and garden petunia (*Petunia hybrida*; important ornamental).

9.3 Biological considerations

ToBRFV can persist until the following crop in plant debris and overwinter in soil. Volunteer tomato seedlings are likely to carry-over into the next crop. These two factors increase the likelihood of establishment.

ToBRFV is easily mechanically transmitted. However, as there is much less handling in tomato and pepper crops grown outdoors compared to crop indoors, it is less likely that one infected plant in a

field will infect many other plants. Even if weeds may serve as reservoir, transfer to host crops is limited because there are no vectors.
The growing period for tomato and pepper outdoors is shorter than indoors, which will not allow a high inoculum to build up. Infected plants may not show symptoms.

9.4 Overall rating of the likelihood of establishment outdoors
ToBRFV has a high likelihood of establishment outdoors where host plants are grown. However, the number of infected plants may remain low.

Rating of the likelihood of establishment outdoors	*Very low* ☐	*Low* ☐	*Moderate* ☐	*High* ☒	*Very high* ☐
Rating of uncertainty			*Low* ☐	*Moderate* ☒	*High* ☐

Uncertainty: less data available for the EPPO region on growing practices outdoor compared to indoor production (e.g. rotations), no example of establishment outdoor in the EPPO region

10. Likelihood of establishment in protected conditions in the PRA area
This category ('protected conditions') includes production technologies such as: glasshouses, screenhouses, tunnels and covered fields. Tomatoes may be produced under active or passive greenhouses (i.e. with or without heating) (Section 9.1). Although there are many types of growing systems for greenhouse tomatoes, the two principal cropping systems are two crops per year and one crop per year (OECD, 2017) without rotation.

Given that host plants of ToBRFV can be grown in all EPPO countries in protected conditions, the whole PRA area is considered as having an environment suitable for ToBRFV.

When host plants are grown under protected conditions, conditions are favourable to the development of the crops and therefore of the virus. Since tomato and pepper are crops where crop-handling procedures are very intensive there is a high risk of mechanical spread.

Several outbreaks have already been reported in the PRA area in protected conditions. In some countries, eradication was not considered possible and ToBRFV is considered established in protected conditions in e.g. Israel and Jordan.

If new growing media is used for each growing period, the likelihood of establishment would be lower. The used media may be recycled (Diara *et al.*, 2012) in conditions that allow the survival of ToBRFV.

The chance of the virus surviving eradication programmes is dependent on the intensity of tomato production in a certain area. Infections in isolated greenhouses are more easily eradicated than infections in greenhouses in dense production areas.

Update in 2024
Gaag *et al*. (2021) report that in three EU-member states (Greece, Italy and the Netherlands) ToBRFV has been detected in several sites, showing a wider distribution, with areas where eradication measures were not fully successful (Sicily, Netherlands). They conclude that eradication can be achieved at the level of a production site but that it is less likely in tomato production areas with multiple infestations.
In contrast, in France distribution is limited, but with a progression of the number of outbreaks (ANSES, 2023).
EU (2023) reported a notable increase of notification of ToBRFV outbreaks from 2020 to 2021, and a spread that continued in 2022 with new notifications. They also reported a limited number of reports about successful eradication.

The EWG considered that the chance of the virus surviving eradication programmes is dependent on early eradication, the intensity of tomato production and of the quantity of ToBRFV infested plants in a certain area.

Rating of the likelihood of establishment in protected conditions	*Very low* ☐	*Low* ☐	*Moderate* ☐	*High* ☐	*Very high* ☒
Rating of uncertainty			*Low* ☒	*Moderate* ☐	*High* ☐

11. Spread in the PRA area

Natural spread
It is assumed that the 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.

Human-assisted spread
Locally, spread will mainly be linked to human-assisted mechanical transmission of the pathogen by workers, visitors, tools and equipment (Section 2.2). This is considered as one of the reasons for the rapid spread of the disease in Israel, in addition to infected plants for planting (Luria *et al.*, 2017) and fruits (Lapidot, pers. comm., 2019).
ToBRFV shows a very high rate of spread in greenhouses where, starting with very few initially infected plants, infection can approach 100% (González-Concha *et al*., 2021)

Reusable plastic containers are often used for the transport of fresh tomatoes to packing stations or auction markets locally but also between countries within the EPPO region (see also section 8.1). In the outbreak in Germany, it is considered that the use of plastic trays may have distributed the virus between different production sites. Swaps taken from plastic trays tested for ToBRFV were positive (Ziebell, pers. comm., 2019). The EWG considered that not all containers will be submitted to adequate cleaning and disinfection in the EPPO region. It may be noted that recent research in the UK showed that water temperature of 70°C for 5 minutes was not enough to inactivate the virus and recommended a temperature of 90°C for 5 minutes. However, large commercial companies handling reusable plastic containers have recently validated their cleaning and disinfection protocols to guarantee that their containers are free from ToBRFV (Europool System, 2019; IFCO, 2019). This cleaning procedure includes the use of detergent, hydrogen peroxid and peracetic acid. Other devices have also been recently developed to remove ToBRFV from containers[15].

Workers may have their hands contaminated by ToBRFV after working in an infected crop, handling infected fruit (e.g. for repacking), or after consuming contaminated fresh host fruits. The virus may also remain infectious in dried fruits of tomato and pepper for several months. If workers consume these products, they may carry the virus on their hands and transmit it to crops. This can only be avoided if strict hygiene measures are applied on site. Ehlers *et al*. (2023) FERA (2021b), based on ELISA detection, showed that ToBRFV can survive on hands and gloves for at least 2 hours and on glasshouse surfaces for at least 7 days and in some cases over 6 months, while hands washing was of limited use (Skelton *et al*., 2023c).

For processing tomatoes, the same harvester can be used between different locations and different owners, facilitating the spread of ToBRFV through soil and plant debris.

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[15] e.g. https://www.hortidaily.com/article/9112000/disinfection-unit-to-fight-the-spread-of-the-tobrf-virus/, https://www.hortidaily.com/article/9139375/steam-sterilisation-unit-developed-to-fight-tobrfv/.

Non-professional tomato growers may use their self-made compost to grow tomatoes, which may be a source of infection if infected tomato fruits were previously composted.

At longer distances, the pest could be transported in seeds, seedlings and fruits (Section 8). There is a large trade of seeds and seedlings between EPPO countries (e.g. from the Netherlands).

Rating of the magnitude of spread, in absence of eradication measures	*Very low* ☐	*Low* ☐	*Moderate* ☐	*High* ☐	*Very high* ☒
Rating of uncertainty			*Low* ☒	*Moderate* ☐	*High* ☐

The Panel on Phytosanitary measures noted that in 2024, hygiene measures and regulation have been implemented that could lower the spread.

12. Impact in the current area of distribution

12.1 Nature of the damage: See details in section 2.5.
Economic damage has mainly been reported on tomato plants. In Mexico, economic impact is also reported on pepper (*C. annuum*) (Cambrón-Crisantos *et al.*, 2018). 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).

The virus caused systemic infection of all tomato cultivars including the ones harbouring resistance genes (*Tm-1*, *Tm-2*/*Tm-2*^{2}) as certified by the Tomato Genetic Resource Center (TGRC) (Luria *et al.*, 2017). Reduction of yield may be high: 25-40% reduction of the average tomato fruit weight (González-Concha *et al*., 2022) and 15-55% of yield reduction (Avni *et al*., 2021). The root biomass and elongation are affected (Vaisman *et al*., 2022). In pepper, reduction of the size of the plant, of the number of internodes, of the size of the fruits and number of seeds is observed (Ortiz-Martinez, 2021), 85% of the plants were infected in a greenhouse (Panno et al., 2020a). Due to the symptoms, the fruits of infested plants lose market value or are unmarketable.
Infection can also significantly reduce plant vigour thereby reducing the length of the production period during which tomato fruits are harvested. Any reduction in the length of a crop's production season can have real impact on the profitability of the individual crop and tomato production in general. Infections may also on occasions lead to premature death of the plant. However, the intensity of symptoms seems to vary according to varieties and can also be affected by cultural practices and climatic conditions.
In addition to direct crop losses, the economic impact is associated to the cost of applying hygiene measures, and to the impact on the export market for seed, plantlets and fruits (inspection and testing, complete market losses). In some cases, the grower may have to switch to non-host plants that may be less profitable. This can be especially problematic for highly specialised tomato growers who have invested heavily in their facilities, equipment and labour force to produce, pack and market tomatoes. The Panel on Phytosanitary measures noted that with new varieties with a certain level of resistance, impact can be reduced in tomato production in infected areas.

12.2 Impact in countries where ToBRFV is considered to be established and relatively widespread
In countries where no eradication measures have been applied immediately after the first detection, ToBRFV has very rapidly spread to the whole country.

In Israel, after a first outbreak reported in 2014 in tomato crops cultivars (*S. lycopersicum* cv. Mose & Ikram) and in absence of eradication measures, the virus spread in tomato greenhouses almost nationwide within the period of one year, probably through the practice of visiting agronomists and professional inspectors or by transfer of non-tested contaminated seeds or seedlings (Luria *et al.*, 2017) and fruits (Lapidot, pers. comm., 2019). Sometimes, tomato plants were found infected at a rate of up to 100% (e.g. Cvs 'Ikram' and 'Azmeer' from different regions of Palestine), with

symptomatic plants exhibiting mild to severe symptoms (Alkowni *et al.*, 2019). In the first outbreak, yellow spotted fruits were estimated to amount to 10 to 15% of the total fruit of each symptomatic plant (Luria *et al.*, 2017).
Before the disease was established, tomato was grown for 24 up to 30 fruit clusters (8-9 months). Since the disease has become more prevalent, it has significantly reduced the vigour of the plant and the fruit quality. The quality loss becomes more and more problematic from the 4^{th}-5^{th} cluster onwards. This can result in the shortening of the growing period and generally only 8 to 10 clusters are harvested. However, this would depend on the climatic conditions and the cultural practices: the number of harvested clusters may be even more reduced when high temperatures occur during the summer period. Symptomatic fruits from the last clusters with strong symptoms may be sold at a lower value. Instead of growing tomato for nine months, two crops may be grown over one year (4-5 months for each growing period) increasing production costs. Rotation with non-host crops is sometimes applied, which allows to decrease the inoculum pressure (e.g. cleaning of the greenhouse, control of weeds, removal of plant debris). Since ToBRFV is widespread in Israel, the use of grafted plants has decreased (source: exchanges during the technical visit, 2019).
During the winter period in Israel, symptoms are milder, and the impact is lower on some varieties. It is sometimes even difficult to see the symptoms on fruits and in the upper part of the plants on these varieties. However, this does not contribute to a decrease of virus titre: testing still shows that the plant is highly infected (Dombrovsky, pers. comm., 2019).
In Israel, no impact is observed on *Capsicum* spp. This is due to the fact that only varieties harbouring *L* resistance genes/alleles are grown commercially (Dombrovsky, pers. comm., 2019).

In Jordan, a tomato (*S. lycopersicum* cv. Candela) crop grown in greenhouses showed in April 2015 strong brown rugose symptoms on fruits that greatly affected the market value of the crop. Disease incidence was close to 100 % (Salem *et al.*, 2016). Since this first detection, there has been no official survey and no attempt to eradicate the pest. Jordan seed export has been affected (Albakri, pers. comm., 2019). ToBRFV has also been reported on *C. annuum* (Salem *et al.*, 2019) not harbouring *L* resistance genes/alleles.

In Mexico, severe mosaic, blistering and leaf distortion symptoms were reported in July 2018 in tomato plants in protected greenhouse (Camacho-Beltrán *et al.*, 2018). In one year, the disease has spread to most Mexican regions producing tomatoes and is considered a threat for fruit production. It is found both in indoor and outdoor production. Severe symptoms have been observed on tomato and pepper fruits (SENASICA, 2019c). In Baja-California, tomato fruits are not grown anymore after the 10^{th} cluster (Dombrovsky, pers. comm., 2019). The PRA conducted by Mexico considered that ToBRFV could have a social impact for workers, because tomato production generates many temporary jobs (SADER & SENASICA, 2018). Mixed infections have been often reported in tomato (e.g. with PepMV) and pepper (e.g. with PMMoV), which make the evaluation of the impact of ToBRFV difficult. Additional costs are foreseen due to the mandatory visual examination of fruits before exportation to the USA, which is a major export market for Mexico.

In 2024, the EWG had no report of impact on pepper.

12.3 Impact in countries where ToBRFV is eradicated, transient under eradication or present in a restricted distribution

In countries where strict eradication measures have been immediately applied, the costs of the measures as well as reputational damage are considered to be the main impact for the producers.

- **Outside the EPPO region:**

In China, symptoms on plants and fruits were reported in April 2019 on about 50% of the plants in 3 tomato greenhouses (~1 acre, about 4000m^2) (Yan *et al.*, 2019). The diseased plants were collected and burned (Li, pers. comm., 2019).

In the USA (southern California), severe disease outbreak, including mosaic, mottling, and plant stunting was observed in September 2018 on grafted tomato plants in a greenhouse (~8 acres, about 3.2 ha^2) (Ling *et al.*, 2019). All the plants have been destroyed on a voluntary basis, which is what represented the main cost for the producer.

- **In the EPPO region:**

In 2020:

In Germany, ToBRFV was confirmed in 2018 in 7 greenhouses growing tomatoes in North Rhine-Westphalia causing unusual fruit and leaf symptoms and death of plants (JKI, 2019; Menzel *et al.*, 2019). Eradication measures were immediately applied, with extensive sampling performed in June 2019. The pest is now considered to be eradicated (EPPO, 2019h).

In Italy, the presence of ToBRFV was first reported in January 2019 in Sicilia (Ispica municipality, Ragusa province), in one greenhouse (2000 m^2) growing tomato. About 10% of plants were infected but symptoms were not severe (EPPO, 2019b). In March 2019, a total of 5 new infected greenhouses producing tomato fruits (15% plants infected without severe symptoms) and 2 nurseries producing tomato seedlings under protected conditions (6 lots of seedlings representing 6000 plants, and 7 lots of seeds, imported from France and Peru) were found infected (EPPO, 2019i; Tomassoli *et al.*, 2019). In May 2019, suspected symptoms of ToBRFV were confirmed in Piedmont (municipality of Bra, Cuneo Province) in a greenhouse (30 000 m^2) producing tomato in hydroponic cultivation. About 15% of plants were showing symptoms of viral disease, but no severe symptoms on fruits were observed (EPPO, 2019g). In January 2020, the presence of ToBRFV was reported again in Sicilia (Ragusa province), in a greenhouse where ToBRFV was reported in 2019 and then used to grow red sweet pepper. In total, 85% of the plants showed virus-like symptoms (Panno *et al.*, 2020). Eradication measures are being applied (EPPO, 2019g, 2019b, 2019i) representing the main costs for the producers. The cost of eradication of the outbreak in Piedmont was about 58 000 € for destruction and removal of plants, cleaning and disinfection, others (inspection and tests). Compensating the value of the destroyed plants was estimated about 270 000 € (Tomassoli, pers. comm., 2019).

In the Netherlands, within the 13 confirmed cases, some of the growers switched to non-host plants and decided to grow cucumber after an infection by ToBRFV (Botermans, pers. comm., 2019).

In Turkey, the presence of ToBRFV was confirmed in a greenhouse growing tomato plants in Demre, near Antalya. Fruit and leaf symptoms were observed. A total of approximately 20% of the total area of 0.7 ha was diseased (Fidan *et al.*, 2019). Main costs consisted of the demarcation of the infected area and the application of eradication measures. Further surveys are being conducted in tomato and pepper production in the entire country (EPPO, 2019f).

In the United-Kingdom, the presence of ToBRFV was confirmed in a glasshouse producing tomato fruits in Kent (8 ha, circa 100,000 plants). Main costs consist of the eradication measures which were voluntary applied by the growers, consisting of the removal of all plants from the glasshouse, cleaning and disinfection, followed by a 14-week period of the glasshouse being kept clear of plants (EPPO, 2019d; Giltrap, pers. comm., 2019).

This information in 2020 is outdated for the EPPO region and outside of EPPO region, information of the situation in 2024 in the EPPO region can be found in section 6 and 10. The EWG considered that in countries of the PRA area where in 2024 ToBRFV is present, costs are still significant for surveillance, outbreak declaration, eradication and hygiene measures. Eradication is not always possible. The chance of the virus surviving eradication programmes is dependent on local measures, early management, the intensity of tomato production and of the quantity of ToBRFV infested plants in a certain area. New resistant varieties could reduce the impacts.

12.4 Existing control measures

Control measures should be applied to limit the impact of the disease in host crops. Typical measures against tobamoviruses are as follows:

Use of virus-free planting material
Virus-free seeds and plants for planting should be used. Seeds are often treated (see table 4 *Host seeds* in section 8.1, subsection *Important factors for association with the pathway*).

Disinfection of soil before planting
In Israel, one or two days before planting tomato plantlets for fruit production, the soil is treated with a chlorine solution, normally applied through the irrigation system, to prevent early infection from the contaminated soil (Mor *et al.*, 2017).
Soil disinfection with chlorine-based and chlorinated tri-sodium phosphate treatments allowed a reduction of ToBRFV infection rate (Dombrovsky *et al*., 2022). A reduction of infection was also obtained with root coating treatments (Klein *et al*., 2023).

Uprooting symptomatic plants
When detected early in the growing period, symptomatic plants may be uprooted or cut at the base (and left in situ so they dry out) to limit further contamination.

Destruction of infected crops
At the end of the growing period, infected crops should be disposed of safely by methods such as incineration (waste incineration) or deep burial (Dombrovsky, pers. comm., 2019).

Sanitation of the structure and cleaning of the material
After infected crops are destroyed, sanitization 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 infected production sites to healthy plants. The disinfection of hands, pots and tools is possible with disinfectants with virucide effect (Richter *et al.*, 2019; Scholz-Döbelin & Leucker, 2019; Wilstermann & Ziebell, 2019) (Section 16).
In the Arava region (Israel), all growers apply a crop-free period of at least 1.5 months. During this period mandatory sanitation treatments such as solarization and chlorine treatments are employed. These sanitation rules are applied for different crops and pests so as to enable the exportation of fruits to Europe and the USA to occur (Dombrovsky, pers. comm., 2019). Cleaning and disinfecting is also performed during this period.
Colonies of bumblebees (*Bombus terrestris*) that had contact with infested plants are removed and disposed of safely.

Update on existing control measures in 2024

Several disinfectants were tested on glasshouse surfaces and tools (FERA, 2021b; Skelton *et al*., 2023c; ANSES, 2023) and proved efficient except on concrete after an exposure of 60 min. They also showed efficacy of hot water treatment at 90°C, or at 70°C combined with spraying with Potassium peroxymonosulfate 1%, on plastic crates. Chanda *et al*. (2021b) identified 4 disinfectants out of 16 tested which had 90-100% efficacy. Ling *et al*., 2022 identified 4 disinfectants which could completely deactivate ToBRFV after max. 15 min exposure. Ehlers *et al*. (2022a) identified 2 detergents and 1 disinfectant which had more than 99% efficacy to remove ToBRFV from fabrics. Under practical conditions, Ehlers *et al*. (2022b) underlines the need to scrape/wipe shoes for a sufficient time on the disinfecting mat, as the removal of the virus is mainly physical. One disinfectant achieved complete inactivation of the virus in a disinfection mat (Ehlers *et al*., 2022b). ANSES, 2023 studied composting and did not conclude on its possible use to control ToBRFV.
Elicitors and biostimulants have been tested in plants infected by ToBRFV (Ortiz *et al*., 2022). Leaves of *Combretum micranthum* (Iobbi *et al*., 2022) and garlic (Iobbi *et al*., 2023) extracts have shown some antiviral activity.

12.5 Rating of the magnitude of impact and uncertainty

The rating is performed for tomato in countries where ToBRFV is considered to be widespread (Israel, Jordan and Mexico):

Rating of the magnitude of impact in the current area of distribution	*Very low* ☐	*Low* ☐	*Moderate* ☐	*High* ☒	*Very high* ☐
Rating of uncertainty			*Low* ☒	*Moderate* ☐	*High* ☐

The magnitude of impact was rated as high because the crop can still be grown, but with high reduction of yield and quality and for a shorter growing period.
The EWG did not propose any rating for pepper because of very limited data on damage on pepper caused by ToBRFV. Different situations are observed (Symptoms observed in Mexico vs. no symptoms but varieties with *L* resistance genes/alleles used in Israel) and because data on yield and quality impacts in Mexico are more limited than in tomato.

13. Potential impact in the PRA area

Will impacts be largely the same as in the current area of distribution? In tomatoes, Yes; in pepper, Yes

The cultivation of tomatoes for the production of seeds, plants and fruits is economically important in the EPPO region:
Production of vegetable seeds (including tomato seeds) represent more than 78 000 tonnes for a value of about 800 million EUR in the EU (EPPO, 2018). The annual value of the worldwide tomato seed market is about one billion euros (Mordor Intelligence, 2018). The four main tomato seed producers in the EPPO region are (by alphabetical order) France, Italy, the Netherlands and Spain (ANNEX 5). However, these countries export more tomato seeds than what is produced on their territories: e.g. in France, in 2017-2018, only 10 to 18 ha of tomato were grown for seed production (including 8.2 to 14.2 ha in greenhouses), 3.7 to 5.5 ha of pepper and chili pepper for seed production (including 2.8 to 5 ha in greenhouses) (non-published data provided by the French Interprofessional Organisation for Seeds and Plants[16] (GNIS)).
The production and trade of plants for planting is also a large market (see Table 5). France also produce 128 to 136 million tomato plants for planting (including about 5 million organic plants) and 14 to 16 million pepper and chili pepper plants for planting (including about 1 million organic plants) (non-published data provided by GNIS). The regulation of ToBRFV as a quarantine pest in several countries worldwide will impact on the export of tomato and pepper seeds and plants for planting from EPPO countries and will result in additional costs (e.g. surveillance and testing costs). Any outbreak in these producing countries would have a reputational impact on their market.
In some countries (e.g. the Netherlands), the production of seedlings is carried out by a small number of very large and very specialized growers. Growers of fruits do not produce their own seedlings. In the event of a suspicion or an outbreak occurring at such a large seedling producer, this could interrupt the supply of young plants to a very large number of fruit producers and at worst leave them with no alternative supplier. Another consequence could be the increase of the price of seedlings.

The top 30 tomato fruit producers in the world include 15 EPPO countries. In order of importance of production (in tonnes), these are: Turkey, Italy, Spain, Uzbekistan, Russia, Ukraine, Tunisia, Portugal, Morocco, Greece, Romania, Netherlands, Algeria, Jordan and Poland (EPPO, 2015).

ToBRFV is of special concern because of its ability to overcome resistance of the *Tm-1* and *Tm-2/Tm-2*^{2} tomato resistance alleles (Luria *et al.*, 2017). However, the *L*^{1}, *L*^{3} and *L*^{4} genes/alleles in pepper

[16] https://www.gnis.fr/en/

have been shown to provide resistance to ToBRFV. In pepper varieties harbouring these *L* resistance genes/alleles, ToBRFV may induce hypersensitivity response, under high inoculum pressure (e.g. when growing peppers after an infected tomato crop), especially in hot temperatures above 30°C (Luria *et al.*, 2017). As most of commercial varieties of pepper grown in the EPPO region harbour *L* resistance genes/alleles, impact on pepper is likely to be minor.

The stability and infectious nature of ToBRFV via mechanical transmission is likely to result in a high damage in the production of tomato fruits, as well as in the production of plantlets. The virus can be spread rapidly by workers on tools and equipment during the handling of plants, with infection most likely occurring when seedlings are thinned in nurseries or transplanted, plus transmission through contaminated seed, soil and circulating water (see 11. Spread in the PRA area). In the event of an infection, prophylactic measures to be set up by growers are very costly.

Economic impact of ToBRFV may be due to its direct impact on yield and on the reduction in quality of fruit. It should be noted that even a small yield loss can result in significant impact for individual growers. Since it has been shown that ToBRFV can affect fruit quality and therefore may result in fewer fruit achieving Class 1 fruit status, the level of economic loss will depend upon differences in market price and marketing between fruit classes within the EPPO region.

If the reduction of the productive growing period caused by ToBRFV would be similar to what occurs in Israel (removal of the crop after harvesting the 8^{th} to 10^{th} cluster), tomato production is likely to be no longer profitable in some important tomato production areas (e.g. in Spain) where the crop is currently grown for at least 8 to 10 consecutive months (until the 24^{th} up to the 30^{th} cluster is harvested). There is currently no alternative and profitable crop that can be grown in these areas due to water salinity (very adequate for high-quality tomatoes but not for other crops) and because of the period of the year during which tomato production is often carried out (wintertime, from October to April). In contrast to Israelian tomato fruit production, which is mainly for the local market, such areas in Spain produce tomatoes during winter and mainly for international markets. The high-quality fruit standards at export may even shorten the production period. Exports markets may be lost because of fruit discolouration, deformation and because of the risk of contamination. Additional costs may be necessary for the handling of infested fruits.

Mixed infections with established viruses (e.g. in tomato with TMV, ToMV, TYLCV, PepMV) might occur with a risk of synergism, or even recombination events, which might lead to new viral disease problems in the future (as noted by Hanssen *et al.*, 2010 for emerging viruses). However, experiments consisting in infecting tomato plants with mild PepMV strains and four weeks later infecting the same plants with ToBRFV did not show any difference of impact compared to those only infected with ToBRFV (van der Krieken, pers. comm., 2019). Additionally, synergistic effects were not observed when ToBRFV was co-infected with two Spanish PepMV isolates (Hernando & Aranda, 2023). In the EU Horizon 2020 research project VIRTIGATION, it was observed that mixed infections of ToBRFV with aggressive PepMV isolates were much more devastating for the plant than a combination of ToBRFV with a mild PepMV isolate (Vos, 2023). Menzel *et al.* (2019) purified ToBRFV from a mixed infection with PepMV by passage on *Chenopodium murale*. The tomato plants subsequently inoculated with *C. murale* plant sap showed the same leaf symptoms as those originally observed with the mixed infection.
A first infection of pepper plants with another virus may allow a second infection with ToBRFV usually blocked by *L* resistance genes/alleles.
A first infection of tomato plants with ToBRFV may also allow a second infection by another tobamovirus usually blocked by resistance genes (*Tm-1*, *Tm-2*/$Tm\text{-}2^2$).

A higher impact is expected in intensive glasshouse production areas (e.g. Ragusa province, Sicilia, compared to the northern Italy) (Tomassoli *et al.*, 2019). The impact for open field crops is not well documented but is likely to be lower because there is less handling of the crop, and therefore the spread within the crop is less likely, and fruit quality is usually less important as the fruits are grown mainly for processing or local market. Growers also tend to be less specialised so can grow alternative crops and have not invested heavily in glasshouses or other infrastructure and facilities to grow a tomato crop.

Climate conditions may influence the impact to be observed in the PRA area (Section 12.2. Israel). In the Northern part of the EPPO region, direct damage may be lower. However, impact could still be very significant because of highly specialized methods of production and facilities. Quality marketing standards for the fruit are usually also higher in Northern Europe.

Complete wilting of infected tomato plant was observed in the German outbreak but has not been observed in Israel. This might be due to indirect impact of ToBRFV (e.g. secondary pathogens attacking the diseased plant). If these symptoms were to be more frequent, this would increase the potential impact of the pest in the PRA area.

The virus may also cause a social impact as the tomato production and supply chain is an important source of employment in the EPPO region, and amateur growing is important for some populations. In France, the total volume of tomatoes produced by gardeners (about 400 000 tonnes per year) is higher than the total annual volume purchased (about 371 000 tonnes per year) (Scandella, 2019). Home/urban gardening and the use of tomato and pepper as ornamentals may be impacted if infected with ToBRFV.

There is no record of impact on pepper (L. Tomassoli, pers. com. 2024).

Research activities to develop new resistance genes and tolerant varieties are already ongoing(Jewehan *et al*., 2021 ; Kabas *et al*., 2022 ; Jewehan *et al*., 2022a ; Zinger *et al*., 2021 ; Nunhems, 2021) ; Vilmorin et cie, 2019) and new varieties claiming resistance to ToBRFV are developed and already commercially available. The EWG noticed that resistance levels need to be clearly defined in terms of symptom expression and virus load, and that no official list of resistant varieties exist for pepper and tomato.

Rating of the magnitude of potential impact in the next 5 years in 2020	*Very low* ☐	*Low* ☐	*Moderate* ☐	*High* ☒	*Very high* ☐
Rating of uncertainty			*Low* ☐	*Moderate* ☒	*High* ☐

Uncertainty: influence of the climate conditions, range of growing practices, susceptibility of cultivars, length of time needed to develop cost effective control measures.

The EWG in 2024 estimated that control measures in place (hygiene measures, resistant varieties and seed testing) would in the future minimize the impact of ToBRFV. The Panel on Phytosanitary Measures considered that the situation has changed compared to 2020 with hygiene measures, varieties with a certain level of resistance on the market and seed testing, allowing a reduction of this impact.

Rating of the magnitude of potential impact in the next 5 years in 2024	*Very low* ☐	*Low* ☐	*Moderate* ☒	*High* ☐	*Very high* ☐
Rating of uncertainty			*Low* ☐	*Moderate* ☒	*High* ☐

Uncertainty: influence of the climate conditions, range of growing practices, susceptibility of cultivars, occurrence of resistance breaking strains, length of time needed to develop cost effective control measures.

14. Identification of the endangered area

ToBRFV could establish in the whole EPPO region wherever tomatoes are grown and is likely to cause economic impact. The area where tomatoes are grown covers the area where peppers are grown, although most registered pepper varieties harbour *L* resistance genes/alleles and will not be at risk.

15. Overall assessment of risk

Summary of ratings:

	likelihood	**Uncertainty**
Entry (overall)	**High**	**Low**
Seeds of - tomato - pepper when not harbouring *L* resistance genes/alleles[17]	 High High	 Moderate High
Plants for planting (excluding seeds, pollen) of tomato and pepper	High	Low
Fresh fruits of tomato and pepper not harbouring *L* resistance genes/alleles - already packed for final consumer before export - stored or repacked at destination in facilities that also grow host fruits, or repacked at destination in facilities that also pack local fruits	 Very low High	 Low Moderate
Used containers, tools, equipment and conveyance vehicles associated with the tomato and pepper production and supply chain	Moderate	High
Persons working in place of production of host plants who have been in contact with infected or contaminated material	Low	Moderate
Soil and growing media as such	Very low	Low
Natural spread	Very low	Low
Pollinating insects used in host fruit production	Very low	Low
Pollen of host plants	Very low	Low
Processed and dried fruits of tomato and pepper	Very low	Low
Soil or growing media attached to non-host plants	Very low	Low
Establishment outdoors	**High**	**Moderate**
Establishment indoors	**Very high**	**Low**
Spread	**Very high**	**Low**
Magnitude of impact in the current area of distribution	High	Low
Magnitude of potential impact in the next 5 years as in 2020	High	Moderate
Magnitude of potential impact in the next 5 years as in 2024	Moderate	Moderate

Entry: the pest has already entered several times in the EPPO region with different pathways (infected fruits and seeds) and is very likely to be present in more EPPO countries than where it is reported. The probability of further entry was considered as high with a low uncertainty, the highest ratings being seeds of pepper (when not harbouring *L* resistance genes/alleles) and tomato; plants for planting (excluding seeds and pollen); fruits stored or repacked at destination in facilities that also grow host

[17] Situation on 2020-06-19: Recent research points to breaking of resistance in *Capsicum* varieties harbouring some *L* resistance genes/alleles. Consequently, this may need to be reconsidered when additional publications are made available.

fruits, or repacked at destination in facilities that also pack local fruits; used containers, tools, equipment and conveyance vehicles; as well as persons working in place of production of host plants. It should be noted that, from mid-2019, several EPPO countries (e.g. the EU countries, Morocco, Turkey) have introduced requirements for some of these pathways which will reduce the risk of further entry into these countries.

Establishment: Establishment of ToBRFV is very likely to occur indoors in the EPPO region (with a low uncertainty) as established populations have already been reported in these conditions in the PRA area (e.g. in Israel and Jordan). Under protected conditions, conditions are favourable to the development of the crops and therefore also of the virus. The chance of the virus surviving eradication programmes is dependent on the intensity of tomato production in a certain area. Establishment of ToBRFV is likely to occur outdoors in the EPPO region (with a moderate uncertainty) where host plants are grown.

The magnitude of spread was rated very high with a low uncertainty. The pest could spread locally by natural dispersal (e.g. with pollinating insects and birds) within a production area or more widely via human-assisted mechanical transmission by workers, visitors, tools and equipment (including plastic containers used for the transport of fresh tomatoes) as well as with the trade of plants for planting, seed and fruit.

Impact (economic and social) is likely to be high with a moderate uncertainty. The virus causes major concerns for growers of tomato. ToBRFV overcame the *Tm-1* and *Tm-2/Tm-2*^{2} tomato resistance genes to tobamoviruses, reducing the vigour of the plant, causing yield losses and virus symptoms making the fruits downgraded or unmarketable. The virus can significantly reduce plant vigour and, under certain conditions, may cause premature death of the plants. Impact on pepper is likely to be minor because varieties produced in the EPPO region are assumed to be mostly harbouring *L* resistance genes/alleles to tobamoviruses.

The EWG considered that phytosanitary measures to prevent further introductions should be recommended for *S. lycopersicum* and *Capsicum* species.

The Panel on Phytosanitary Measures noted that the situation of this pest has evolved with an increase in the area of distribution of ToBRFV (in 2020 outbreaks were present in 9 EPPO countries whereas in 2024, 31 EPPO countries were concerned). It also noted that with hygiene measures, varieties with a certain level of resistance on the market and seed testing, a reduction of this impact is expected.
The EWG considered that recommendation as A2 pest was still justified as there are EPPO countries where the pest is not present or widespread. Depending on pest situation, the Panel on Phytosanitary Measures considered that countries could apply some of the measures in another regulatory framework (e.g. RNQP, ISPM 16, 21)

Stage 3. Pest risk management

16. Phytosanitary measures

16.1 Measures on individual pathways

Measures are recommended for host seeds, host plants for planting (excluding seeds and pollen) and fresh fruits (for details see ANNEX 1).

The EWG recommended that measures should apply to *S. lycopersicum* and *Capsicum* spp. and that new host species should be added if they are shown to host ToBRFV naturally (Section 7).

Possible pathways *(in order of importance)*	**Measures identified for the exporting country (see ANNEX 1 for details)**
Plants for planting (excluding seeds, pollen) of *Solanum lycopersicum* and *Capsicum* spp.	Plants should be produced in 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', [ANNEX 2 of this PRA lists the specific conditions for ToBRFV such as the use of seeds free from ToBRFV, hygiene conditions…].* AND Plants should be traded in new or disinfected trays[2]
Seeds of *Solanum lycopersicum* and *Capsicum* spp.	A - Seeds should be produced in 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' [ANNEX 2 of this PRA lists the specific conditions for ToBRFV such as the use of seeds free from ToBRFV, hygiene conditions…]* or B - Seeds should be produced from parent plants that have been inspected and tested and seed lots have been kept separated from any other source of infestation (seeds or plants): - The parent plants should be regularly inspected during the growing period. If suspicious symptoms are seen, testing for ToBRFV should have been undertaken and plants found free from ToBRFV. - In addition, a/ When all the harvested seeds from the parent plants are combined to form a single lot of seeds, parent plants should be tested as practically close as possible to the harvesting of the last fruits for seed production. Sampling for testing should be performed to achieve a 99% confidence level to detect an infection level of 2% (see ISPM 31). Plants should be found free from ToBRFV. b/ When batches of seeds harvested from the parent plants are moved before the end of seed production, parent plants should be regularly tested during the harvesting period of fruit for seed production. Sampling for each testing should be performed to achieve a 99% confidence level to detect an infection level of 2% (see ISPM 31). Plants should be found free from ToBRFV. or

Possible pathways *(in order of importance)*	Measures identified for the exporting country (see ANNEX 1 for details)
	C - The seed lot has been tested (number of seeds depending on the size of the seed lot, with a maximum of 1000 seeds per subsample for PCR methods (EU, 2023b, based on ISPM 31)). The test should be indicated on the phytosanitary certificate. For small seed lots, means of verification of the pest status of the lot should be agreed by bilateral agreement with the importing country. *[Remark: However, the EWG and the Panel on Phytosanitary Measures considered that this option D provides a higher level of protection than option B.]*
Fresh fruits of *Solanum lycopersicum* and *Capsicum* spp.	Phytosanitary Certificate only

Knowledge of the origin of seed lots is essential, and this information should be clearly recorded and documented.

In addition to the measures to be implemented by the exporting countries, the Working Party encourages importing countries to implement the following measure:

Fruits are not stored or repacked at destination in facilities that also grow host fruits, or repacked at destination 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.

Remark:

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 (e.g. isolation of the seed and seedling production location from the environment; prevention of infection by managing different risk factors: water, people, propagation material, materials; constant monitoring during the growing season; check before delivery; independent audits; training of the staff; traceability requirements). The GSPP certification process is implemented by 44 companies (tomato seed/plant producers with 102 production sites across more than 20 countries, see https://www.gspp.eu/accredited-companies-sites). However, only a proportion of internationally traded tomato seed is already produced under the GSPP system, because of the associated costs.

Notes on the measures that were considered but not considered suitable by the Panel on Phytosanitary Measures

1-Feasibility of establishing a pest free area (PFA). The EWG discussed the requirements for establishing a PFA for ToBRFV:

- To establish and maintain the PFA, detailed surveys and monitoring should be conducted in the area since the beginning of the preceding growing period and continued every year.
- Surveys should include high risk locations, such as places where potentially infested material may have been imported.
- There should be restrictions on the movement of host material (e.g. seeds, seedlings, fruits) into the PFA, and into the area surrounding the PFA, especially the area between the PFA and

the closest area of known infestation. Containers that are used to grow the plants should be new or disinfected.

The EWG and the Panel on Phytosanitary Measures considered that this option was currently impossible to apply in most situations, because:

- The current distribution of ToBRFV is very uncertain and very likely to be underestimated. The origin of the disease is unknown, the virus has only been reported and regulated recently, confirmation requires molecular tests which are not widely used. There have been several new reports of presence made during the last year.
- Seed lots from different origins are mixed, so there is a risk of introduction with seeds or young plants grown from the seeds each season,
- Tomato plants are extensively grown by amateurs in many countries worldwide,
- There is a high risk of mechanical transmission
- Infected fruits are known to move around and are considered to be a pathway of introduction (including in amateur production),
- Host plants can be asymptomatic and requires targeted testing to confirm the presence of the virus.

The PFA is considered to be difficult to establish and would provide less guaranty of pest freedom than pest free production site and/or testing. Therefore, the EPPO Panel on Phytosanitary Measures did not recommend this option.

2-Requirements for fresh fruits. In addition to the PFA option discussed in the paragraph above, the EWG recommended the following risk management options:

- Fruits have been produced in a pest-free production site for ToBRFV under complete physical isolation according to EPPO Standard PM 5/8, or
- or
- Fruits are already packed for final consumer before export, or
- Fruits are not stored or repacked at destination in facilities that also grow host fruits, or repacked at destination in facilities that also pack local tomato or pepper fruits[18]

When the Panel on Phytosanitary Measures reviewed the measures, they agreed that the last option was the most effective when implemented by the importing countries and recommended only this measure to be applied. Therefore, the Panel did not recommend any special requirements for exporting countries on fruits, except the use of a phytosanitary certificate which should guaranty the absence of the pest (in particular the absence of symptoms).

16.2 Good hygiene practices in greenhouses

Tobamovirus could survive for months in clothes, plant remnants, substrates and on tools (DEEDI, 2017; FERA, 2021b). Strict hygiene practices should be applied during the seed, plant and fruit production (Li *et al.*, 2015; Ehlers *et al.*, 2023) in greenhouses to limit the risk of association with these pathways and several practices have been shown efficient (FERA, 2021b; Skelton et al., 2023c ; Chanda *et al.*, 2021b ; Ling *et al.*, 2022; Ehlers *et al.*, 2022b). These precautions should be applied routinely.

ANSES (2023) recommended treatment and destruction measures for plant material (incineration, burial) and listed available active substances and commercial products inactivating ToBRFV (benzoic acid, glutaraldehyde with quaternary ammonium salts, sodium hypochlorite and potassium peroxymonosulfate).

Additional recommendations are available in a protocol used in the Netherlands[19] (Vermunt & van Marrewijk, 2019) :

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[18] this option allows the entry of infected fruit but impose measures on the importing country to prevent transfer to host crops.

[19] https://www.tuinbouwalert.nl/content/docs/Dossiers/ToBRFV/Hygi%C3%ABneprotocol_ToBRFV_en_Clavibacter_in_tomaat.pdf

Good hygiene practices at the place of production:

- **Sorting/packing of plant produce from other locations should be prohibited**

- **Train staff to recognise plant diseases and to employ best practices for a high health crop**
  Staff (e.g. growers, workers) should be trained in basic symptom recognition and hygiene measures to contain outbreaks of pathogens when they occur, including best practice for plant handling etc. They should be educated about the severity of ToBRFV and the consequences of an outbreak in the place of production.

- **Early and continuous monitoring of the crop, to identify any early symptoms, to identify the cause and where appropriate remove the plant(s)**

Good hygiene practices at the site of production:

- **Maintaining the place of production free of weeds and animals**
  The place of production should be free of weeds, insects potentially damaging the plants, birds, and other small animals (e.g. mice).

- **Compartmentation of the production / use of hygiene locks**
  Access should be limited to people working in the specific crop/greenhouse. This will minimise the risk of inadvertent introduction and spread around the greenhouse production facility via human activity.
  Workers, equipment and tools should be assigned to certain parts of the greenhouses to avoid any introduction and spread of ToBRFV:
    - Equipment (including protective clothing) and tools should not leave the production part it has been assigned to.
    - Workers should enter/leave each greenhouse part through a hygiene lock. The washing and disinfecting shoes, hands etc. should be performed in these hygiene locks.
    - Substrates or nutrients solution and containers should not be moved from these parts of the greenhouses to other parts.

  Ideally staff should not be moving between packing and production facilities.

- **Use of pest-free irrigation water**
  Water used in irrigation systems should be disinfected, except if groundwater or rainwater is used.
  Remark: Infectivity after conventional wastewater treatment has been demonstrated for a number of plant viruses from the genus Tobamovirus (family Virgaviridae), including ToBRFV (Bacnik *et al.*, 2020).
  A very low pH, lower than 2 (very acidic) is effective for virus deposition (Vermunt & van Marrewijk, 2019).
  Ehlers et al. (2022a) showed efficiency of several cleaning products and commercial agricultural detergents on water.

- **Washing and disinfecting tools and equipment with an authorized disinfectant with virucide effect**
  Equipment and tools which come into contact with an infected plant can act as a source of virus for onward transmission. Equipment such as picking carts, sprayers, and hand tools (e.g. pruning knives) should all be cleaned and disinfected routinely. Tools should ideally be disinfected during pruning activities between individual plants. Equipment should be cleaned and disinfected at least between crops.
  As several disinfectants marketed as providing a virucide effect are shown to have a limited effect on viruses (Marcussen & Meyer-Kahsnitz, 1990), only authorized products should be

used. Products such as Virkon S, solutions of stabilized Chlorine or diluted Hypochlorite may be used. However, these products are not always effective alone and their efficacy would depend on the concentration, the surface, the contact time, the contamination (e.g. contamination with highly infected sap may be more difficult to wash and disinfect) and the presence of organic matter. Thorough rinsing and cleaning to remove e.g. organic matter, dust and plant debris should be done before disinfection.

Several disinfectants were tested on glasshouse surfaces and tools (FERA, 2021b; Skelton et al., 2023c, ANSES, 2023) and proved efficient except on concrete after an incubation of 60 min. They also showed efficacy of hot water treatment at 90°C, or at 70°C combined with spraying with Potassium peroxymonosulfate 1%, on plastic crates. Chanda *et al*. (2021b) identified 4 disinfectants out of 16 tested which had 90-100% efficacy. Ling *et al*., 2022 identified 4 disinfectants which could completely deactivate ToBRFV after max. 15 min exposure. Ehlers *et al*. (2022a) identified 2 detergents and 1 disinfectant which had more than 99% efficacy to remove ToBRFV from fabrics.

- **Washing hands with a disinfectant, alcalic soap or use disposable gloves**
  FERA (2021b) published results showing, based on ELISA detection, that ToBRFV can survive on hands and gloves for at least 2 hours and on glasshouse surfaces for at least 7 days and in some cases over 6 months, while hands washing was of limited use (Skelton et al., 2023c).

- **Washing and disinfecting shoes or use overshoes**
  After washing:
    - shoes should be disinfected with an authorized disinfectant (see *Washing and disinfecting tools and equipment*),
    - a sanitary mat with solutions may be used. The sanitary mats should never get dry. The following active substances are commonly used for solutions in sanitary mats: trisodium phosphate, sodium hypochlorite, formulation of stabilized chlorine, Virkon S and Potassium hydroxide.

  An alternative would consist in the use of disposable overshoes.

- **Using dedicated or disposable clothes**
  Workers should use company clothing (which stays at the company and is washed onsite) or disposable clothing, such as a disposable overall, to minimise introduction and spread of the pathogen. These should be put on when entering a greenhouse and should be disposed of on leaving the greenhouse and not reused. If this is not feasible for full-time staff, then they should be issued clothing to wear only in the greenhouse which is then regularly (e.g. weekly) laundered at high temperature. Growers could use clothing in different colours to denote workers from different parts of the site to prevent cross contamination. If it is necessary to use a phone in the growing area, it must be placed and kept in a plastic bag.

- **Prohibiting consumption of tomato and pepper fruits in the premises**
  Growers and employees eating fruits of tomato and pepper (e.g. sandwiches with tomato or pepper inside, processed food with tomato or pepper such as tabasco). which may be contaminated could inadvertently pass on the virus. Ideally, this prohibition should include any food.

- **Prohibiting the presence of ornamentals in the production site**
  Petunia is an experimental asymptomatic host. The presence of ornamentals should be avoided within the site of production.

At the end of the cropping period, a thorough cleaning should be performed, and plants should be destroyed with a proper industrial composting or treatment system (e.g. steam).
These precautions should be applied routinely.

16.3 Eradication and containment

Eradication and containment measures should involve surveillance (visual examination and testing), destruction of infected plants, as well as strict hygiene measures. No chemical treatment can be used to cure infected plants.

The EWG considered that eradication was possible indoors and whilst potentially more challenging in the case of an outdoor outbreak, eradication would still be possible if infection is detected early.

Update in 2024: Gaag et al. (2021), report that in some EU-member states (Greece, Italy and the Netherlands) ToBRFV has been detected in several sites, showing a wider distribution, with areas where eradication measures were not fully successful (Sicily, Netherlands) (and Toscany, Sardinia, L. Tomassoli, 2024, pers. com.). They conclude that eradication can be achieved at the level of a production site but that it is less likely in tomato production areas with multiple infestations. In contrast, in France distribution is limited, but with a progression of the number of outbreaks (ANSES, 2023). EU (2023) reported that "*Once outbreaks are identified, strict hygiene and other measures have been put in place to prevent the spread of ToBRFV within the production site. Eradication was successfully achieved in some outbreaks. However, in many cases the work is ongoing*". In UK with low levels of inoculum and low levels of outbreaks, eradication has been possible (A. Fox, 2024 pers. com.).

In the event of an outbreak indoors, the EWG considered that the following measures should be applied, by order of application, to achieve eradication:

1- Immediate action at the infested site of production (all host plants in the site of production should be considered as infected).

- Restrict access to the site of production
- Apply hygiene measures (e.g. disinfect all possibly infected tools and equipment, install a disinfectant mat at the entrance of the greenhouse)
- Remove (bumble)beehives in the greenhouse (and destroy them)
- Remove and dispose of all host plants in the greenhouse, associated plant debris, and other material, such as string and growing media using appropriate disposal methods (including burning, deep burial or steaming)
- Clean the greenhouse with water and detergent to remove traces of organic matter
- Disinfect all the surfaces of the greenhouse (see section 16.2 on good hygiene practices)

2- Further action at the place of production:

- Inspect and test other tomato and capsicum crops. If they test positive, the outbreak area should be extended, and similar measures applied as in the initial outbreak area.
- Conduct trace back and trace forward studies (e.g. investigate where the seed/plants for planting came from, where the workers have been, whether host material was moved to other places of production)

3- After the infected crop has been removed and the greenhouse and tools have been completely cleaned and disinfected, it is possible to grow a non-host crop (e.g. cucumber) on new growing medium. The crop should be monitored to prevent any self-sown tomato (and pepper) seedlings or solanaceous weeds growing in or near to the greenhouse.

A new crop of tomato (or pepper) may be established, using a new growing medium, after a host crop-free period. The length of this host crop-free period should be considered taking into account the individual circumstances of the outbreak and allow a thorough cleansing and disinfection of the greenhouse (e.g. 2 months in Germany was successful for eradication in combination with disinfection measures). Official inspections should be performed on the new crop over the full growing period. Methods concerning substrate disinfection such as solarization, for countries located in the south of the EPPO region, and steaming are being researched in the EU Horizon 2020 project VIRTIGATION.

For crops grown in soil (e.g. for organic production), the soil may be disinfected using solarization in the south of the EPPO region or chlorine treatment (Dombrovsky, 2022) or steaming (ANSES, 2023 citing Luvisi et al., 2015). However, chlorine treatments are not allowed in organic production in some countries (Scholz-Döbelin, pers. comm., 2020). Alternatively, a gap of minimum 1 year without host plants (and controlling any solanaceous weeds) should be applied. Maintaining the soil moist could help eradicating the virus (Section 2.3).

In the event of an outbreak in an open field, the EWG considered that the following measures should be applied to achieve eradication:

1- Immediate action at the infested place of production

- Restrict access to the place of production
- Consider other fields which have shared machinery/equipment/workers with the infected field as "probably contaminated" and perform inspection and testing on host crops. If host crops test negative, there should be no restriction on cropping for the following year.
- Clean and disinfect the material and equipment that has been used in the infected field during the growing period, as well as the storage area for host material. A procedure similar to EPPO Standard PM 10/1 *Disinfection procedures in potato production* may be used.
- Establish a buffer zone including the susceptible host crops in the immediate vicinity of the outbreak as well as the host weeds and tomato volunteers.
- Destroy all plants for the infected field on site (e.g. by incineration or steaming) or remove them and dispose of them safely (e.g. by deep burial and incineration). Composting may be insufficient for the secure inactivation of the virus (Richter *et al.*, 2019).
- Clean and disinfect the equipment that was used for the destruction.
- Conduct trace back and trace forward studies (e.g. investigate where the seed/plants for planting came from, whether reusable containers were shared with other places of production, whether host material was moved to other places of production)

2- After the infected crop has been removed

- No host crop should be grown in the infested field for at least 1 year. Solanaceous weeds and tomato/pepper volunteers should be controlled during the same period.
- Official inspections should be performed during the full growing period on the new host crop as well as in the vicinity (in particular any field of host crops)
- Official inspections should be performed on the first host crop grown in the site.

17. Uncertainty

Main sources of uncertainty within the risk assessment are linked to the

- Level of resistance of new tomato and pepper varieties, and occurrence of resistance breaking isolates.
- Accuracy between sensitivity of detection and seed to seedling transmission/infectivity potential, biological relevance. Efficiency of seed disinfection and methods to evaluate infectivity potential and/or genome integrity.
- How infected pepper seeds can impact further (or in vicinity) tomato production

18. Remarks

- Survey should be continued in countries producing tomato and pepper seeds, fruits and plants. Testing imported fruits may also provide useful information about the current distribution of ToBRFV.
- Countries importing high risk material from infested area should continue conduct surveys.
- Pest reporting is still encouraged (e.g. by conditioning eventual financial compensations to this reporting obligation).
- Further studies on resistance level of tomato and pepper varieties should be conducted

- Develop and validate methods to evaluate infectivity potential and/or genome integrity and assess efficiency of treatments

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ANNEX 1. Consideration of pest risk management options

The table below summarizes the consideration of possible measures for the pathways '*seeds*', '*plants for planting*', '*fresh fruits not for processing*'.

For measures, *Capsicum* and *S. lycopersicum* are considered as potential host plants.
When a measure is considered appropriate, it is noted "*yes*", or "*yes, in combination*" if it should be combined with other measures in a systems approach (see after the table). "*No*" indicates that a measure is not considered appropriate. A short justification is included. Elements that are common to several pathways are in bold.

For fresh fruits, there is only a risk of entry when fruits are stored or repacked at destination in facilities that also grow host fruits or repacked at destination in facilities that also pack local fruits. Options presented below are the options discussed by the EWG. However, the Panel on Phytosanitary Measures recommended that the importing EPPO countries should be responsible for separating the storage and repacking of imported fruits with the local fruit production. When this measure is applied by the importing countries, the two identified standalone options consisting in making a difference between resistant and non-resistant cultivars at import, or to propose to the exporting country the option that fruits are produced under complete physical isolation, are no more necessary.

Option	**Host seeds**	**Host plants for planting (except seeds)**	**Fresh host fruits, not for processing,** stored or repacked at destination in facilities that also grow host fruits, or repacked at destination in facilities that also pack local fruits
Existing measures in EPPO countries	Partly, see Section 8.	Partly, See section 8.	No, See section 8.
Options at the place of production			
Visual inspection at place of production	Yes, in combination* (for measures marked with '*', see after the table). Plants should be free from any symptom of virus infection. If symptoms are seen, plants should be tested for ToBRFV (see below). Detection by visual inspection is unlikely to be completely effective at the place of production (in plants used to produce seeds) and needs to be used within a systems approach.	No Infected young plants for planting are symptomless up to at least the stage of 7 leaves (based on the experience in Israel and Mexico). Plants are normally traded before symptoms become evident. Therefore, an inspection at the place of production is unlikely to reliably detect infected plants.	Yes, in combination*. The absence of symptoms is not sufficient to guarantee pest freedom. Even when plants do not present any symptoms of the disease, the level of inoculum of the virus in the plants may be high. However, removing symptomatic plants early in the growing season may help limiting further mechanical transmission of ToBRFV within the greenhouse and therefore the number of infected fruits. However, by the time symptoms become visible spread will already have occurred.

Option	Host seeds	Host plants for planting (except seeds)	**Fresh host fruits, not for processing,** stored or repacked at destination in facilities that also grow host fruits, or repacked at destination in facilities that also pack local fruits
	Some tomato varieties are known not to show symptoms of ToBRFV infection		
Testing at place of production	Yes Tests exist (Section 2.6) to detect the virus in the parental plants (i.e. plants that produce the final seeds). Seeds should be produced from parent plants that have been inspected and tested: the parent plants should be regularly inspected during the growing period. If suspicious symptoms are seen, testing for ToBRFV should have been undertaken and plants found free from ToBRFV. In addition, - A/ When all the harvested seeds from the parent plants are combined to form a single lot of seeds, parent plants should have been tested as practically close as possible to the harvesting of the last fruits for seed production. Random sampling for testing should be performed, to achieve a 99% confidence level to detect an infection level of 2% (see ISPM 31; FAO, 2016). Plants should have been found free from ToBRFV. - B/ When batches of seeds harvested from the parent plants are moved before the end of seed production, parent plants of each lot should be regularly tested during the harvesting period of fruit for seed production. Random sampling of each lot for each testing should be performed to achieve a 99% confidence level to detect an	Yes, in combination, for grafted plants* Tests exist to detect the virus in plants. Plants for planting may be tested at the place of production before being traded and infested plant lots removed. Testing young plantlets was carried out routinely for other viruses such as TYLCV when it was regulated (Guitian Castrillon, pers. comm. 2019). Leaf samples from several plants can be pooled to reduce the cost of testing, but virus concentration may be below the detection limit of the test. The EWG considered that the amount of virus in the seedling is likely to be low, when the infection is caused by seed-to-seedling transmission and would be difficult to detect. For grafted plants, testing would be more relevant (plants for planting are grown for one month longer and subject to more manipulation, which could result in mechanical infection. The titre of virus in grafted plants could be higher than with seed-to-seedling transmission).	Yes, in combination* Tests exist to detect the virus in plants. Plants may be tested if symptoms are observed and infected plants removed. However, a low level of infection may not cause symptoms and therefore may not be detected. Some tomato cultivars are known to be symptomless, but fruits may carry a high titre of virus. A representative asymptomatic testing of the plants would be too costly.

Option	Host seeds	Host plants for planting (except seeds)	**Fresh host fruits, not for processing,** stored or repacked at destination in facilities that also grow host fruits, or repacked at destination in facilities that also pack local fruits
	infection level of 2% (see ISPM 31; FAO, 2016)). Plants should be found free from ToBRFV. Indeed, companies either require that the totality of the seeds harvested from a parent plant field is moved as a unique seed lot (e.g. Clause, France), or allow that different batches are harvested and traded all along the harvesting period of fruits for seed production (e.g. 4 to 12 lots may be harvested and traded from a single glasshouse in the Netherlands) (Lybeert, pers. comm., 2020) Regular testing during the harvesting period of fruit for seed production is common practice in some companies in Israel. Testing of the parent plants two weeks before the fruit for seed production are harvested is already applied in seed selection (Lybeert, pers. comm., 2020). EWG considers that testing the parent plants at the place of production provides a better guarantee than direct testing of the seeds.		
Treatment of crop	**No. The virus cannot be destroyed by pesticides in the crop.**	**No. The virus cannot be destroyed by pesticides in the crop.** As for seed	No. The virus cannot be destroyed by pesticides in the crop. Cross-protection is used in tomato production to protect them for virulent strains of PepMV. Unauthorized applications of an isolate of ToBRFV as a cross-protection product has been reported (NWVA, 2024, Botermans et al., 2023) .
Resistant cultivars	**There is no harmonized official list of**	**There is no harmonized official list of**	**There is no harmonized official list of resistant varieties yet.**

Option	Host seeds	Host plants for planting (except seeds)	**Fresh host fruits, not for processing,** stored or repacked at destination in facilities that also grow host fruits, or repacked at destination in facilities that also pack local fruits
	resistant varieties yet. **There are currently no fully resistant (no virus load) tomato and pepper cultivars to ToBRFV. However, the work on the development of resistance and tolerance to ToBRFV in tomato plants has already been initiated.**	**resistant varieties yet.** As for seeds.	As for seeds.
Growing the crop in glasshouses/ screenhouses	Not relevant**,** because of the risk of mechanical transmission. Remark: A recent EU report noted that the majority of tomato and pepper seeds imported in the EU are from Guatemala, Israel, Kenya, Tanzania are produced in high-tech insect-proof greenhouses, but that in China, there are produced in open-air (EU Commission, 2019).	Not relevant, because of the risk of mechanical transmission.	No. The risk to produce infected fruit is higher in protected conditions
Specified age/size of plant, growth stage or time of year of harvest	Not relevant. The virus is present in the whole plant/at all growth stage	Not relevant. The virus is present in the whole plant/at all growth stage. Plants for planting are symptomless at the growth stage usually traded for such a commodity.	Not relevant. The virus is present in the whole plant/at all growth stage
Produced in a certification scheme	Yes under the conditions outlined below In particular, initial seeds should be tested and found free for ToBRFV. Parental plants should be tested close to fruit harvest to verify that they are pest-free. Regular inspections should be conducted	Yes. In particular, initial seeds used to produce seedlings should be tested and found free for ToBRFV. Use of good practices under this certification scheme will contribute to decrease the risk of association with the pathway.	Not relevant. Certification schemes are used for plants for planting.

Option	Host seeds	Host plants for planting (except seeds)	**Fresh host fruits, not for processing,** stored or repacked at destination in facilities that also grow host fruits, or repacked at destination in facilities that also pack local fruits
	during the production and any plant showing suspicious symptom should be tested. Good practices should be applied. The production should be performed under strict hygiene measures (Section 16.2), and in appropriate isolation conditions similarly to the production in a pest free production site following EPPO Standard PM 5/8 '*Plants grown under complete physical isolation*' (see below and ANNEX 2). The use of the GSPP protocol is a possible way to implement PM 5/8 requirements.	The production should be performed under strict hygiene measures (Section 16.2), and in appropriate isolation conditions, similarly to the GSPP protocol.	
Pest free production site	**Yes, if grown according to EPPO Standard PM 5/8 under complete physical isolation (ANNEX 2).** **Although, the virus is not vectored by sucking insects, chewing insects may transmit ToBRFV. A glass structure (or equivalent solid material), a plastic structure (such as polyethylene) or nets should be required to provide complete physical isolation to prevent presence of any insect, birds, mice etc. that could damage the plants. This includes nets/mesh to cover vents in the greenhouse.** **Strict hygiene measures should be taken to guarantee a pest-free production site.** **The pest-free production site should be free of weeds (visual inspection and**	**Yes, if grown according to EPPO Standard PM 5/8 under complete physical isolation (ANNEX 2).** As for seed. However, as young plants do not flower and are not attractive for pollinators, the EWG considered that the risk of opening greenhouse windows for ventilation was acceptable provided no flowering of any host plants within the greenhouse occurs.	**Yes, if grown according to EPPO Standard PM 5/8 under complete physical isolation (ANNEX 2).** As for seed

Option	Host seeds	Host plants for planting (except seeds)	**Fresh host fruits, not for processing,** stored or repacked at destination in facilities that also grow host fruits, or repacked at destination in facilities that also pack local fruits
	herbicide treatments) to decrease the risk of infection.		
Pest free place of production	**Yes, if all the individual sites of production are under complete physical isolation according to EPPO Standard PM 5/8 (ANNEX 2).** **Strict hygiene measures should be taken to guarantee a pest-free place of production.** **Maintaining the place of production free of weeds (visual inspection and herbicide treatments) would help decrease the risk of infection.** **Use of pest free place of production outdoors with a buffer zone is considered possible in principle. But requirements to prevent possible introduction with human movement (mechanical transmission), birds, bumblebees etc. would be difficult to apply in practice. Tomato plants are extensively grown by amateur growers and can be a potential source of infection.**	**Yes, if all the individual sites of production are under complete physical isolation according to EPPO Standard PM 5/8 (ANNEX 2).** See the seed pathway However, as young plants do not flower and are not attractive for pollinators, the EWG considered that the risk of opening greenhouse windows for ventilation was acceptable provided no flowering of any host plants within the greenhouse occurs.	**Yes, if all the individual sites of production are under complete physical isolation according to EPPO Standard PM 5/8 (ANNEX 2).** See the seed pathway
Pest-free area	**Yes, theoretically possible** • **To establish and maintain the PFA, detailed surveys and monitoring should be conducted in the area since the beginning of the preceding growing period and continued every year.**	Yes, theoretically. As for seeds.	Yes, theoretically. As for seeds.

Option	Host seeds	Host plants for planting (except seeds)	**Fresh host fruits, not for processing,** stored or repacked at destination in facilities that also grow host fruits, or repacked at destination in facilities that also pack local fruits
	• **Surveys should include high risk locations, such as places where potentially infested material may have been imported.** • **There should be restrictions on the movement of host material (e.g. seeds, seedlings, fruits) into the PFA, and into the area surrounding the PFA, especially the area between the PFA and the closest area of known infestation.** **BUT** **It is considered impossible to apply in most situations.** **- The current distribution is uncertain and likely underestimated (the origin of the disease is unknown, the virus has only been reported and regulated recently, and several new reports of presence have been made during the last year),** **- There is a high risk of mechanical transmission,** **- Seed lots from different origins are mixed, so there is a risk of introduction with seeds or young plants grown from the seeds each season,** **- Infected fruits are known to move around and are considered to be an important pathway of introduction,** **- Tomato plants are extensively grown by amateurs in many countries worldwide,**		

Option	Host seeds	Host plants for planting (except seeds)	**Fresh host fruits, not for processing,** stored or repacked at destination in facilities that also grow host fruits, or repacked at destination in facilities that also pack local fruits
	- Host plants can be asymptomatic and requires targeted testing to confirm the presence of the virus, **The PFA is considered to be difficult to establish and would provide less guaranty than testing. The Panel on Phytosanitary Measures agreed that the PFA option should not be recommended for EPPO countries.**		
Options after harvest, at pre-clearance or during transport			
Visual inspection of consignment	Not relevant. Symptoms are not visible on seeds.	No. It is unlikely to detect infected plants as most are symptomless when they are traded (i.e. before 8 weeks/ 7 leaves).	Yes, in combination*. Inspection may detect symptoms but will not guarantee detection. 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). Some tomato varieties are known to be symptomless, but fruits may carry a high titre of virus.
Testing of commodity	Yes, for seed. Tests allow to detect the virus in seeds. An EPPO Diagnostic protocol has been published and an IPPC one is under preparation. An ISHI-Veg protocol has been agreed (ISF, 2020) for the detection of infectious ToBRFV in tomato and pepper seeds. This protocol requires 3000 seeds to be tested.	Yes, in combination* for plants. However, a very large number of plants should be tested. Valitest Project and the EU reference laboratory for Virology are going to validate the protocol to be used in the future. An EPPO diagnostic protocol has been published and an IPPC one is under preparation.	Yes, in combination*. It is in principle possible to test fruits to detect the virus. If symptoms are observed, a sample may be tested to verify the presence of the virus. However, to reliably guarantee pest-freedom of the consignment, a very high number of fruits would have to be tested, which is unlikely to be cost effective

Option	**Host seeds**	**Host plants for planting (except seeds)**	**Fresh host fruits, not for processing,** stored or repacked at destination in facilities that also grow host fruits, or repacked at destination in facilities that also pack local fruits
	For the time being, the EWG recommended applying this protocol and using minimum 3000 seeds per lot. This provides 95% of probability of detecting 0.1% of infection. Testing a smaller number of seeds provides a lower probability of detection. Remark: Australia requires a sample size of 20 000 seed (or 20 per cent for small seed lots), which give a higher probability to detect an infection (99% probability of detection 0.02% of infection).		
Treatment of the consignment	Yes, in combination*. Davino et al. (2020) showed that the more efficient treatments were based on a 2.5% hypochlorite solution and on a trisodium phosphate treatment. Samarah et al. (2021) obtained a 100% disinfection rate with 2% a HCl treatment for 30 min or a 10% TSP treatment for 3h. Salem et al. (2022b) treated with HCl a sample of 100% infected tomato seeds and showed that treated seeds were all negative by DAS ELISA and RT-PCR. Zamora-Macorra et al. (2023) showed that a 3% sodium hypochlorite solution was effective as a seed disinfection treatment.	No. Heat treatment will affect the viability of the plants.	No. The pest is a virus and treatment would destroy the fruit.
Pest only on certain parts of plant/plant product, which can	No. Virus is on the seeds	No. Virus is in the plants	No Virus is in the fruit.

Option	Host seeds	Host plants for planting (except seeds)	**Fresh host fruits, not for processing,** stored or repacked at destination in facilities that also grow host fruits, or repacked at destination in facilities that also pack local fruits
be removed			
Prevention of infestation by packing/handling method	No. Sorting infected seeds is not possible. The mixing of seed lots from different origins poses serious problems for traceability. The EWG recommend that the origin of all seed lots in a consignment is clearly recorded and documented.	Yes, in combination*. To prevent contamination, only new or cleaned and disinfected packing material should be used.	Yes, in combination*. For quality reasons, it is likely that symptomatic fruits will be sorted out when preparing fruit lots for export. To prevent contamination, only new or cleaned and disinfected packing material should be used.
Options that can be implemented after entry of consignments			
Pre or Post-entry quarantine	No Infestation in seeds would need testing to be detected during post-entry quarantine (see '*Testing of the commodity*').	No. Plants for planting of tomato and pepper should be replanted rapidly and cannot be kept in post-entry quarantine.	Not applicable to fruit.
Limited distribution of consignments in time and/or space or limited use	Not relevant The use of a seed cannot be limited to reduce the probability of entry	Not relevant. The use of plantlets cannot be limited to reduce the probability of entry. The virus can be present all year around in plants.	Not relevant. Already excluded from the pathway Fruits should not be imported to packing houses where these co-exist with host fruit production sites, or strict hygiene practices should be required to prevent transfer of the virus from infested fruits to the production area.
Only surveillance and eradication in the importing country	**No.** **Detection is difficult, the pest may be detected only once established and the pest would be very difficult to eradicate**.	**No.** As for seeds	**No.** As for seeds

*The EWG considered whether the measures identified above as 'Yes in combination' (listed below) could be combined to achieve a suitable level of security. This was not possible for all these commodities.

Host seeds	Host plants for planting (except seeds, pollen)	Host fruits not for processing
Visual inspection at place of production	Testing at place of production for grafted plants	Visual inspection at place of production
Treatment of the consignment	Prevention of infestation by packing/handling method	Testing at place of production
	Testing of the commodity	Visual inspection of consignment
		Testing of the commodity
		Prevention of infestation by packing/handling method

ANNEX 2. Guidelines on the phytosanitary measure 'Plants grown under complete physical isolation' for ToBRFV, adapted from PM 5/8 (1) (EPPO, 2016)

Facilities should be approved by the NPPO according to the criteria detailed below.

General measures that need to be implemented to guarantee and maintain pest freedom status, and to ensure that the measure 'complete physical isolation' will be effective

- The structure should be free from ToBRFV before starting the production.
- Access to the structure should be limited to trained and authorized personnel.
- All the host plants for planting for production that enter the structure should be free of ToBRFV and freedom should be verified prior to introduction (see Section 16.1 of this document).
- Other plants or plant products that could potentially carry ToBRFV should not be introduced into the structure (e.g. host fruits for packing, storage or consumption by staff).
- Growing media or any material (e.g. trays and boxes) likely to carry ToBRFV which are introduced into the structure should also be free from ToBRFV. The risk of movement of ToBRFV with machineries and working tools in the structure should be mitigated by using dedicated machineries and tools, and/or cleaning and disinfection (Section 16.2).
- Traceability of any plant for planting and growing media that are introduced should be guaranteed.
- The risk of entry and movement of ToBRFV with the personnel working in the structure should be mitigated by using dedicated clothes, different working clothes or footwear in different areas, disinfecting hands, shoes and tools (Section 16.2).
- The risk of entry of ToBRFV with animals (insects, mice, birds) should be prevented by using a glass structure (or equivalent solid material), a plastic structure (such as polyethylene) or a net of a suitable mesh size to exclude the entry of these animals. As the main risk is deemed to be with pollinating insects when plants are flowering, the EWG considered that for the production of young plants, the risk of opening greenhouse windows for ventilation is acceptable provided no flowering of any host plants within the greenhouse occurs.
- Pest-free irrigation water should be used.
- The entire structure should be inspected regularly to ensure physical integrity, in particular following meteorological events. These inspections should be recorded.
- Regular inspections of all plants for signs and symptoms of ToBRFV infection being produced under complete physical isolation should be carried out during the growing period to monitor any possible breach in the system. This should include laboratory testing of plants showing suspicious symptoms and of asymptomatic plants (ISPM 31 provides useful information on the number of plants to be sampled). Inspections should be recorded.

Good production practices described in Section 16.2 such as regular sanitation of the site of production (e.g. absence of weeds and cleaning or disinfection of the whole site of production at the end of production period) are also recommended. Establishment of a hygiene lock with a footbath or a foot mat at the entrance is also recommended.

Consequences of a breach

In the event of a breach (e.g. if ToBRFV is detected within the structure or there is physical damage to the integrity of the structure), plants grown within the structure should no longer be considered as free from ToBRFV. The NPPO should be notified. It is the responsibility of the NPPO to decide on the appropriate corrective action.

ANNEX 3. Symptoms of tomato brown rugose fruit virus (ToBRFV) in tomato and pepper plants and fruits (all pictures available in EPPO Global Database)

Figure 1. Symptoms of tomato brown rugose fruit virus (ToBRFV) in infected tomato plants

A - Narrowing (needle-like symptoms) and blistering of the surface of tomato leaves. Courtesy: Camille Picard (EPPO)

B - Wilting of leaves, followed by yellowing and plant death observed in Germany. Courtesy: Heike Scholz-Döbelin (LWK NRW)

C - Mosaic symptoms on tomato leaves Courtesy: Camille Picard (EPPO)

D - Necrosis of the sepals on young tomato fruit. Courtesy: Prof. Salvatore Davino

Additional pictures are available in EPPO Global Database at https://gd.eppo.int/taxon/TOBRFV/photos

Figure 2. Symptoms of tomato brown rugose fruit virus (ToBRFV) in infected tomato fruits

A - Typical fruit symptoms with yellow spots
Courtesy: Dr Aviv Dombrovsky

B – Dark coloured (necrotic) spots on green fruits
Courtesy: Diana Godínez

C - Marbling of fruit and delay in ripening
Courtesy: Dr Aviv Dombrovsky

D - Reduced number of fruits per branch
Courtesy: Diana Godínez

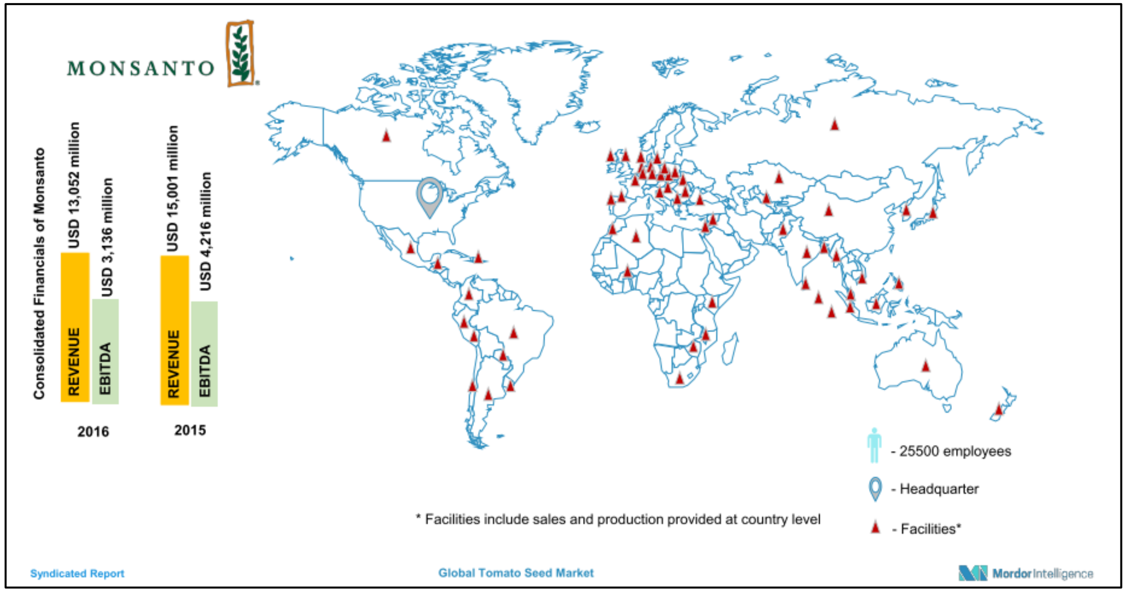
Additional pictures are available in EPPO Global Database at https://gd.eppo.int/taxon/TOBRFV/photos

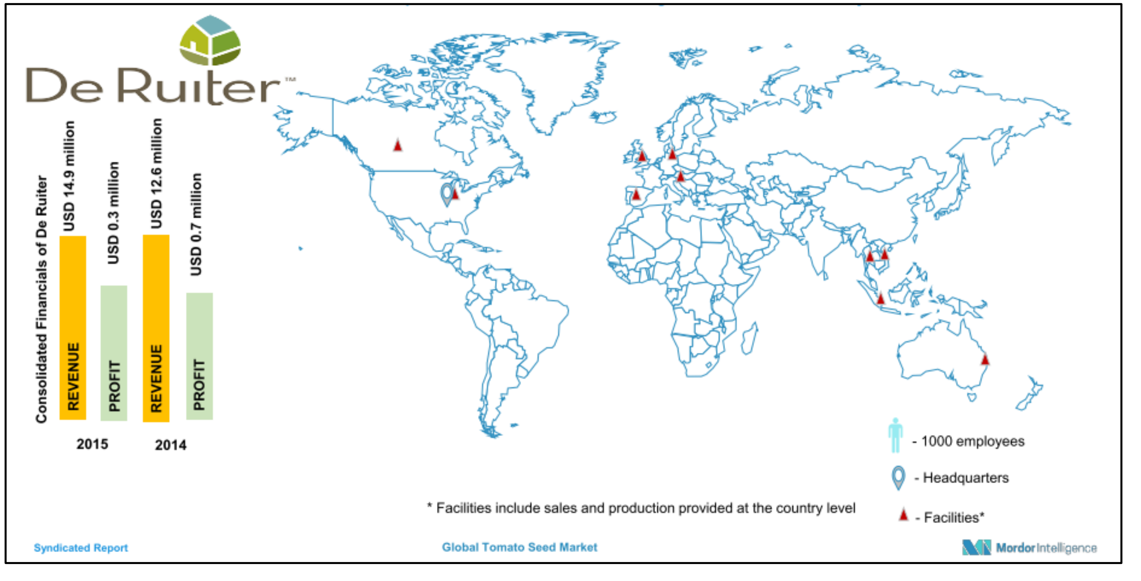
Figure 3. Symptoms of a hypersensitive response (HR) to infection by tomato brown rugose fruit virus (ToBRFV) in pepper plants harbouring *L* resistance genes/alleles (Luria *et al.*, 2017).

Legend : (A-D) Symptoms developed following sap-mechanical inoculation of leaves showing **(A)** necrotic lesions; **(B)** yellowing; **(C, D)** leaf necrosis. **(E-G)** HR symptoms developed following mechanical inoculation of roots showing necrotic spots on stems and growth reduction.

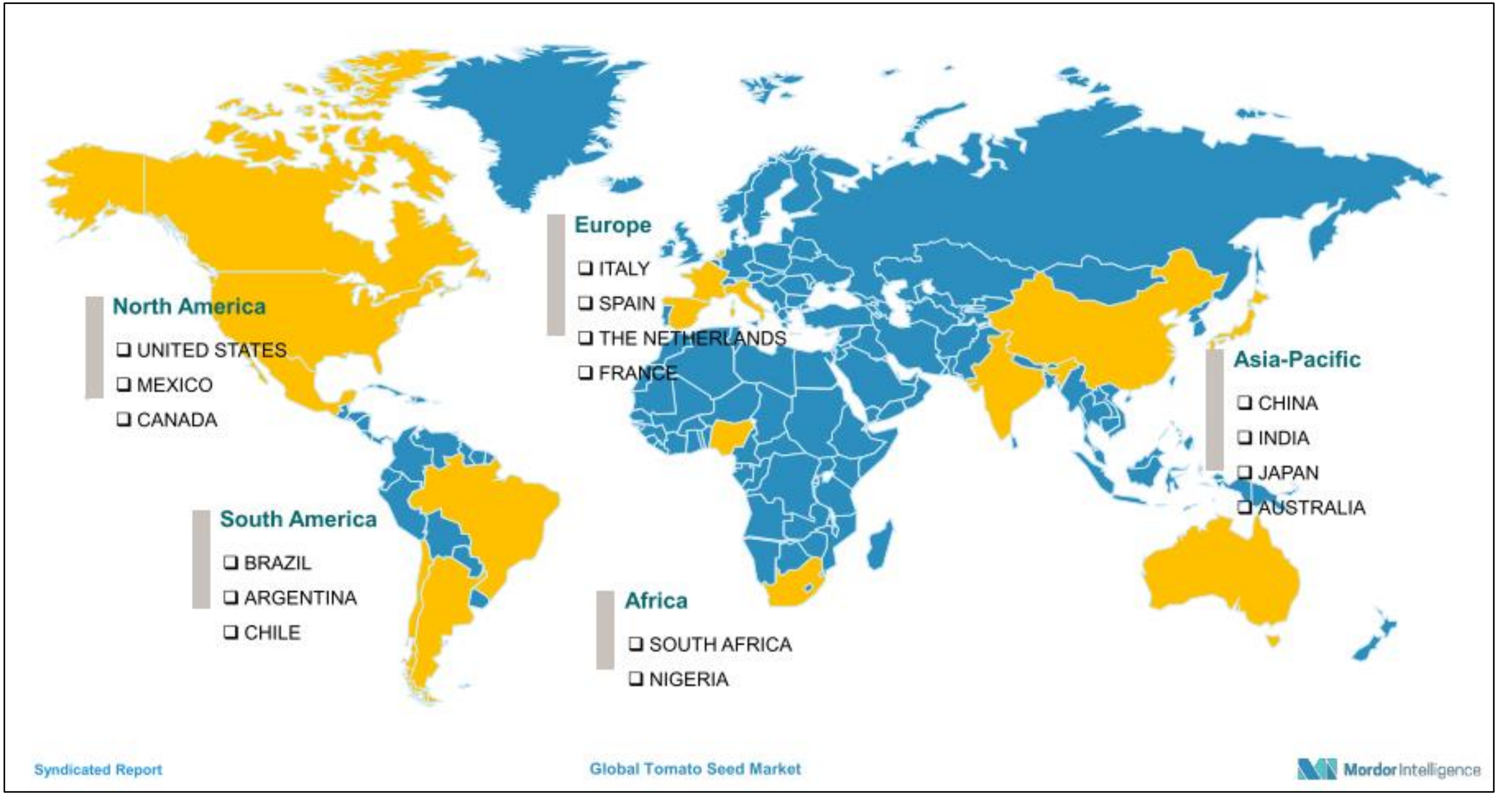
ANNEX 4. Profiles of International seed companies involved in the tomato seed production

Example of Monsanto (now Bayer), and De Ruiter (one of the two subsidiaries specialized in the tomato seed segment, under the authority of Monsanto (now Bayer)) (Mordor Intelligence, 2018)





ANNEX 5. ***Overview of the main countries producing tomato seed in the world*** (Mordor Intelligence, 2018). Legend: these countries are indicated in orange on the map. Other countries are in blue.



ANNEX 6. Import of vegetable seeds

Table 1a. Eurostat - Import of '*vegetable seeds for sowing (excluding salad beet or beetroot* 'Beta vulgaris *var*. conditiva')' (EU CN code 12099180) from countries known to be infected by ToBRFV into EU members in 2014-2018. Data extracted on 2019-05-03. Unit: 100kg of vegetable seeds. ':0' indicates that positive imports between 0 and 0.5*100kg have been reported. ':' indicates that no import or no data is available.

Partner	China					Israel					Italy					Jordan					Mexico					Turkey				
Reporter/Period	**2014**	**2015**	**2016**	**2017**	**2018**	**2014**	**2015**	**2016**	**2017**	**2018**	**2014**	**2015**	**2016**	**2017**	**2018**	**2014**	**2015**	**2016**	**2017**	**2018**	**2014**	**2015**	**2016**	**2017**	**2018**	**2014**	**2015**	**2016**	**2017**	**2018**
AUSTRIA	0	0	0	:	:	:	0	:	:	0	115	846	975	388	576	:	:	:	:	:	:	:	:	:	0	:	:	:	:	:
BELGIUM	626	1 224	502	995	1 000	4	24	:	0	:	21	114	161	121	96	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:
BULGARIA	:	:	5	45	:	1	1	0	1	0	177	224	204	210	106	:	:	230	:	:	:	:	:	:	:	3	871	0	:	3 709
CYPRUS	1	1	0	1	0	15	7	10	9	8	134	69	339	54	23	:	:	:	:	:	:	:	:	:	:	:	:	:	:	0
CZECH REPUBLIC	90	75	196	71	100	:	:	:	:	:	1 724	1 957	2 095	1 571	1 499	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:
GERMANY	636	176	195	368	234	2	5	4	5	3	2 564	1 806	2 177	2 188	1 853	:	2	:	:	:	:	:	:	:	:	256	45	73	241	49
DENMARK	170	413	77	86	21	0	0	0	1	0	470	262	204	480	176	:	:	0	:	0	:	:	:	:	:	:	:	:	:	:
ESTONIA	:	:	0	:	0	0	0	0	0	:	:	19	59	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:
SPAIN	1 664	842	1 430	385	883	19	10	13	19	37	951	3 188	3 808	8 025	723	2	3	3	2	3	0	1	0	0	0	168	0	6	1	3
FINLAND	:	:	:	:	:	:	:	:	:	:	:	1	:	27	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:
FRANCE	1 238	2 094	1 471	796	711	7	4	5	5	5	2 333	2 189	3 683	3 730	2 744	7	8	2	1	3	2	0	0	0	7	18	2	2	0	5
UNITED KINGDOM	38	76	50	139	163	2	1	2	4	3	3 556	4 784	2 913	476	847	0	0	0	0	0	:	:	:	:	:	45	0	0	:	:
GREECE	1 474	913	372	411	293	16	15	21	5	9	3 860	3 118	2 757	2 835	3 525	:	:	:	:	:	:	:	0	:	0	537	338	315	260	859
HUNGARY	73	505	359	28	28	3	3	0	2	0	597	213	206	475	411	:	:	:	:	0	:	:	:	:	:	2	1	1	148	1
IRELAND	0	0	0	0	0	0	:	:	0	:	:	:	:	66	114	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:
ITALY	9 905	4 421	3 748	1 390	2 573	3	2	32	74	85	:	:	:	:	:	1	2	1	2	4	0	9	0	0	0	24	48	149	177	174
LITHUANIA	:	650	400	220	415	:	:	:	:	:	527	384	693	1 090	1 277	:	:	:	:	:	:	:	:	:	:	:	:	0	:	:
LUXEMBOURG	:	:	:	:	:	:	:	:	:	:	0	0	0	5	8	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:
LATVIA	:	:	:	:	:	:	:	:	:	:	49	6 429	501	205	393	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:
MALTA	:	0	30	0	0	0	0	1	0	0	1 113	1 198	175	74	397	0	:	:	:	:	:	:	:	:	:	:	:	0	:	0
NETHERLANDS	7 333	5 287	5 427	6 051	6 224	87	369	182	228	185	5 150	4 116	4 360	5 265	5 419	0	2	4	11	11	63	32	27	49	57	330	353	243	342	230
POLAND	113	41	55	128	222	0	0	0	0	0	672	521	1 250	785	1 233	:	:	:	:	:	:	:	:	0	:	:	:	:	:	0
PORTUGAL	15	25	16	17	10	0	:	0	:	0	1 169	912	804	1 430	952	:	:	:	:	:	:	:	:	:	:	:	0	:	:	0
ROMANIA	3 605	3 706	3 959	214	2 482	1	1	3	1	92	1 478	1 852	2 628	3 812	5 791	:	:	:	:	:	:	:	:	:	:	:	0	0	0	0
SWEDEN	0	0	0	1	0	18	12	19	14	14	81	65	81	175	186	:	:	:	0	0	:	:	15	:	:	:	:	:	:	:
SLOVENIA	480	0	0	:	0	0	:	0	:	:	1 627	1 765	9 262	2 544	3 083	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:
SLOVAKIA	6	6	23	3	6	:	:	:	:	:	7	10	20	10	16	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:
CROATIA	:	0	0	1	0	:	:	:	:	:	1 587	3 241	2 865	2 311	1 423	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:
Total	**27 467**	**20 455**	**18 315**	**11 350**	**15 365**	**178**	**454**	**292**	**368**	**441**	**29 962**	**39 283**	**42 220**	**38 352**	**32 871**	**10**	**17**	**240**	**16**	**21**	**65**	**42**	**42**	**49**	**64**	**1 383**	**1 658**	**789**	**1 169**	**5 030**

Figure 1a. Main trade of small vegetable seeds from EU and NAFTA in 2017 (in million euros) (Source: GNIS, https://www.gnis.fr/en/)

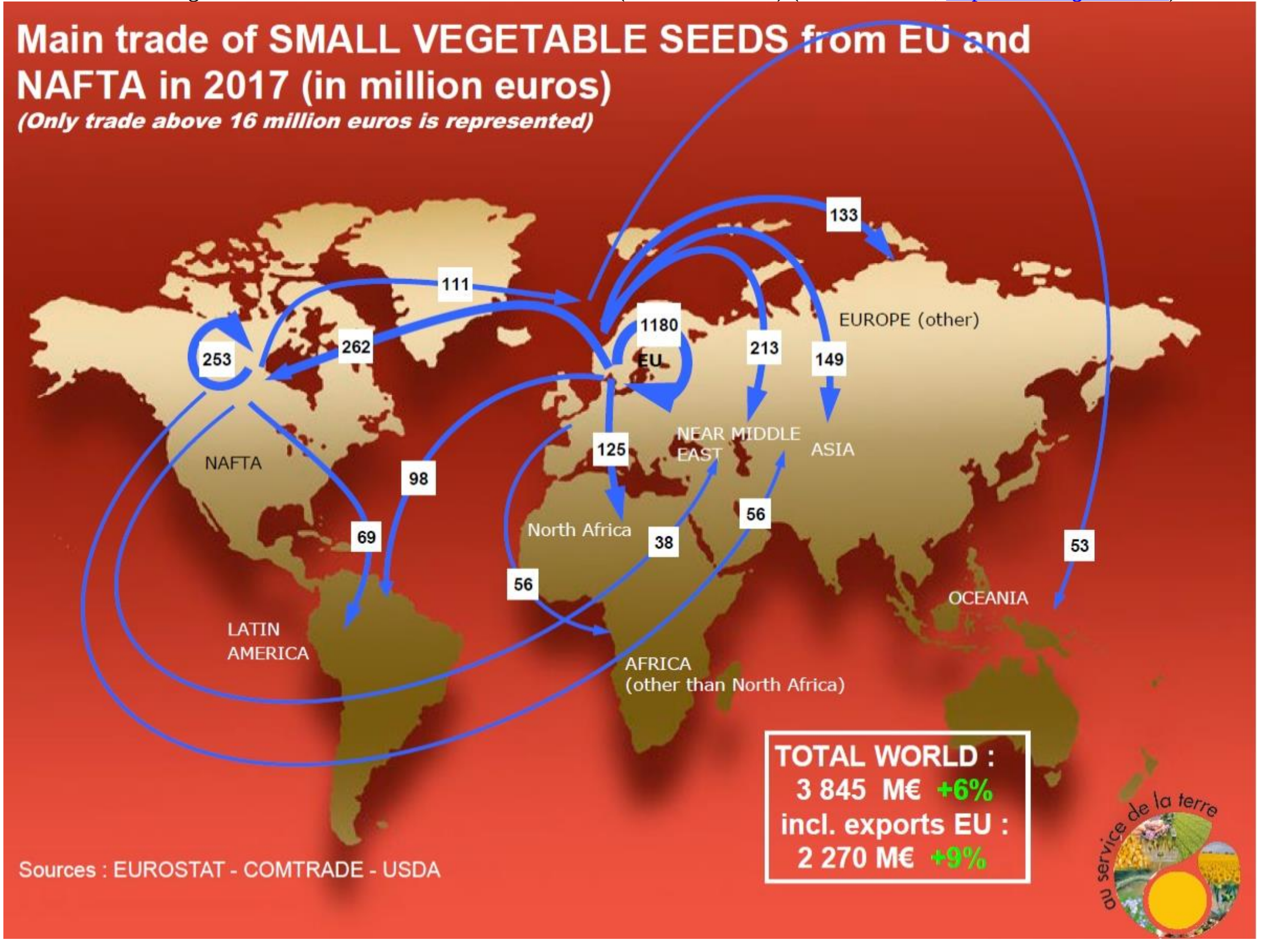
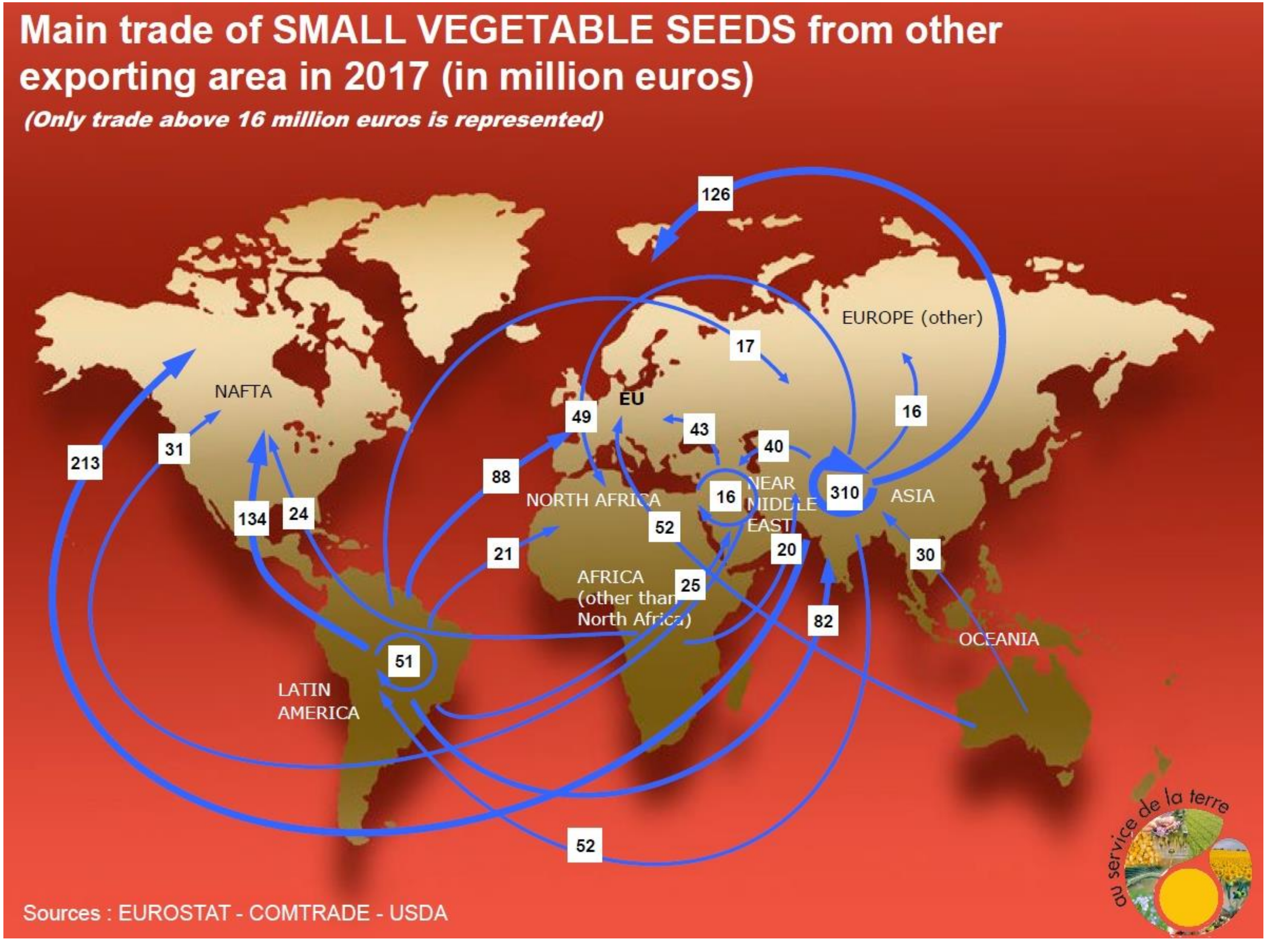


Figure 1b. Main trade of small vegetable seeds from other exporting areas in 2017 (in million euros) (Source: GNIS, https://www.gnis.fr/en/)



ANNEX 7. Import of tomato and pepper seeds

Table 1. FRANCE (GNIS, https://www.gnis.fr/en/) - Import of tomato seeds from third countries into France in 2012-2019. Data extracted on 2019-07-09. Unit: 100kg of seeds. Countries are ordered by total volume imported on the period 2012-2019. ‘0.00’ indicates that positive imports between 0 and 0.05*100kg have been reported. ‘ ’ indicates that no import or no data is available.

Reporter/Period	2012-2013	2013-2014	2014-2015	2015-2016	2016-2017	2017-2018	2018-2019
VIETNAM	2.87	605.27	4.65	3.95	1.76	3.76	2.28
CHINA	235.89	139.41	29.02	11.92	1.64	1.60	7.20
GUATEMALA	0.08	1.43	245.23	0.00	0.60	0.12	0.00
INDIA	10.61	24.81	26.53	28.37	9.77	0.78	0.59
BRAZIL	5.38	1.10	1.59	75.10	0.05	0.02	0.00
THAILAND	4.48	0.42	1.28	24.09	0.61	0.84	0.84
ISRAEL	0.46	8.72	0.23	6.72	0.19	0.24	0.11
CHILE	0.47	2.46	0.99	0.87	0.88	0.74	0.44
USA	0.18	1.26	0.60	0.78	0.17	0.52	0.75
MADAGASCAR	0.00			1.55	0.10	0.04	0.06
TURKEY	0.46	0.58	0.15	0.07	0.06	0.13	0.03
SENEGAL	0.91		0.10		0.15	0.06	
TAIWAN	0.45	0.05	0.06	0.06	0.12	0.02	0.12
KENYA			0.01		0.00	0.00	0.80
SOUTH AFRICA	0.00	0.00	0.04	0.03	0.02	0.06	0.39
MEXICO	0.50		0.00	0.00	0.01	0.00	0.01
PERU	0.00	0.00	0.01	0.24	0.06	0.00	0.03
MOROCCO		0.06	0.09	0.01	0.02	0.06	0.05
SWITZERLAND	0.04	0.01	0.01	0.01		0.04	0.04
THE NETHERLANDS	0.10						
SPAIN					0.00		0.09
BOLIVIA	0.00	0.00	0.00	0.00	0.00	0.07	0.00
DOMINICA							0.03
STE LUCIA							0.03
JORDAN		0.01	0.01	0.00			
JAPAN	0.01		0.00	0.00		0.00	0.00
TANZANIA			0.01				
TOTAL :	**262.89**	**785.60**	**310.61**	**153.77**	**16.19**	**9.11**	**13.89**

Table 2. FRANCE (GNIS) - Import of *Capsicum* seeds from third countries into France in 2012-2019. Data extracted on 2019-07-09. Unit: 100kg of seeds. Countries are ordered by total volume imported on the period 2012-2019. '0.00' indicates that positive imports between 0 and 0.05*100kg have been reported. ' ' indicates that no import or no data is available.

Partner	**France**						
Reporter/Period	**2012-2013**	**2013-2014**	**2014-2015**	**2015-2016**	**2016-2017**	**2017-2018**	**2018-2019**
CHINA	104.31	76.25	39.09	52.7	60.08	95.25	48.61
THAILAND	14.42	9.06	17.54	22.58	22.03	36.59	16.97
INDIA	19.15	1.66	9.76	11	11.28	11.41	38.32
MADAGASCAR				0.03	8.05	19.11	0.21
VIETNAM	2.3	3.76	4.79	2.3	3.71	5.44	2.05
PERU	2.23	13.16	1.67	0.88	1.93	1.8	1.03
CHILE	2.03	4.34	2.97	1.82	1.47	8.61	1.41
USA	0.07	0.26	4.31	1.61	0.05	0.32	0.61
BRAZIL	4.16	0.05	0.67	0.04	0.23	1.24	0.51
GUATEMALA	0			0.18	0.45	0.42	1.05
BENIN				0.07	0.11	1	
BURKINA FASO					0.85	0.14	
COSTA-RICA		0.01		0.01	0.53	0.1	0.01
MOROCCO	0.54	0.05		0	0	0	0.01
TUNISIA		0.6					
ISRAEL	0.02	0.04	0.06	0.05	0.03	0.09	0.08
SENEGAL	0.01			0	0.13	0.09	0.06
MEXICO	0	0	0.02	0.01	0.05	0.02	0
JORDAN			0.09		0	0	
TURKEY	0	0.03	0.03	0	0	0.01	0.02
SPAIN				0	0	0.06	0
MYANMAR	0.05						
SWITZERLAND				0.03			
VENEZUELA	0.02						
TOTAL:	**149.33**	**109.27**	**80.98**	**93.33**	**110.99**	**181.71**	**110.93**

Table 3. ITALY (NPPO)– Total import of tomato seeds from non-EU countries into Italy (regions Campania, Emilia Romagna, Lombardia, Sicilia and Veneto) in 2015-2019. Data collected on 2019-10-30. Unit: 100kg of seeds. Countries are ordered by total volume imported on the period.

Partner	Italy
Reporter/Period	**2015- 2019**
CHINA	54.96
INDIA	9.11
ISRAEL	3.39
USA	1.46
THAILAND	0.82
VIETNAM	0.69
MEXICO	0.60
TAIWAN	0.25
UGANDA	0.15
KENYA	0.05
KOREA, REPUBLIC OF	0.02
TURKEY	0.01
GUATEMALA	0.00
JAPAN	0.00

Table 4. ITALY (NPPO) – Total import of *Capsicum* seeds from non-EU countries into Italy (regions Campania, Emilia Romagna, Lombardia, Sicilia and Veneto) in 2015-2019. Data collected on 2019-10-30. Unit: 100kg of seeds. Countries are ordered by total volume imported on the period.

Partner	Italy
Reporter/Period	**2015- 2019**
CHINA	21.75
THAILAND	12.38
UNITED STATES	3.11
INDIA	1.84
SERBIA	1.25
TURKEY	0.80
BRAZIL	0.60
VIETNAM	0.47
ISRAEL	0.05
TAIWAN	0.04
JAPAN	0.03
MEXICO	0.00

ANNEX 8. Export of tomato fruits

Table 1. FAO STAT – Total export of tomato fruits in 2016 by Country. Data extracted on 2019-07-09. Unit: tonnes of fruits.

Country	Value (tonnes)
Mexico	1748858
Netherlands	992601
Spain	911106
Morocco	524907
Turkey	485963
Jordan	361439
India	247990
France	247053
Belgium	222297
United States of America	208628
China	206343
China, mainland	206311
Canada	192618
Portugal	125725
Italy	104937
Azerbaijan	98333
Poland	94939
Belarus	86303
Afghanistan	81206
Albania	63701
Egypt	62617
Malaysia	51498
Guatemala	50800
Honduras	48278
Uzbekistan	41373
Greece	41322
Syrian Arab Republic	40435
Lithuania	37442
Armenia	36043
Ukraine	35482
South Africa	29074
Tunisia	26544
Pakistan	24792
Iran (Islamic Republic of)	24650
North Macedonia	24018
Germany	18094
Saudi Arabia	15900
Oman	14155
Burkina Faso	12610
Tajikistan	12100
Serbia	11534
Austria	11480
Czechia	10938
Ethiopia	10261
Senegal	9817
Russian Federation	8982
Dominican Republic	8493
Kuwait	8128
Slovenia	7877
Croatia	7456
Argentina	6270
United Arab Emirates	5377
Israel	5181
Slovakia	5000
Nicaragua	4799
Brazil	4755
Cameroon	4392
Bulgaria	4307
Guinea	4000
Republic of Korea	3798
New Zealand	3704
United Kingdom	3636
Liberia	3487
Latvia	3472
Georgia	3034
Republic of Moldova	2953
Kazakhstan	2846
Hungary	2540
Sweden	2427
Guinea-Bissau	2325
Côte d'Ivoire	2185
Thailand	1876
Turkmenistan	1838
Sierra Leone	1283
Central African Republic	1217
Peru	1171
Ireland	1063
Denmark	907
Bosnia and Herzegovina	873
Australia	748
Luxembourg	575
Namibia	546
United Republic of Tanzania	546
Libya	526
Romania	376
Singapore	371
Viet Nam	359
Yemen	331
Venezuela (Bolivarian Republic of)	316
Indonesia	312
Lebanon	303
Nigeria	301
Kyrgyzstan	259
Colombia	208
Estonia	191
Burundi	162
Costa Rica	162
Uganda	145
Algeria	130
Somalia	118
Guyana	115
Bahrain	106
Zimbabwe	102
Switzerland	96
Madagascar	82
Eswatini (Swaziland)	75
Angola	47
Benin	45
Bangladesh	34
Chile	25
China, Taiwan Province of	20
Finland	17
Zambia	17
Japan	15
China, Hong Kong SAR	12
Palestine	12
Kenya	11
Norway	11
Iceland	6
Niger	6
Uruguay	6
Barbados	5
Botswana	4
Suriname	4
Trinidad and Tobago	4
Fiji	3
Cyprus	2
Antigua and Barbuda	1
Bermuda	1
Ghana	1
Montenegro	1
Saint Vincent and the Grenadines	1
Sri Lanka	1
Brunei Darussalam	0
Cayman Islands	0
Democratic Republic of the Congo	0
El Salvador	0
Jamaica	0
Lao People's Democratic Republic	0
Mauritius	0
Myanmar	0
Nepal	0
Panama	0
Papua New Guinea	0
Philippines	0
Rwanda	0
Sudan	0