Tomato brown rugose fruit virus (ToBRFV) - pest status in the EU, likelihood of eradication and evaluation against RNQP-criteria

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Abstract

Tomato brown rugose fruit virus (ToBRFV) is a tobamovirus that was first described from Jordan in 2016. Since then, it has been reported from many countries worldwide including 17 member states of the European Union (EU). Economically important hosts of ToBRFV are tomato (*Solanum lycopersicum*) and pepper (*Capsicum* spp.). In the EU, outbreaks have mostly been reported in tomato. There are currently no tomato cultivars available that are fully resistant against ToBRFV. In *Capsicum*, symptomatic plants have only incidentally been reported in the EU. *Capsicum* cultivars are available that harbour L-resistance genes/alleles that are considered effective but uncertainty exists if they confer full resistance under all conditions. ToBRFV is regulated by emergency measures (Commission implementing regulation (EU) 2020/1191, amended by Commission implementing regulation (EU) 2021/74 and Commission Implementing regulation (EU) 2021/1809) which apply until 31 May 2023. ToBRFV is present in the EU and eradication is unlikely to be achieved. The virus can be transmitted by seeds but also easily by contact. Seeds and seedlings are considered a significant pathway, especially when growers take hygiene measures to prevent the introduction of the virus into the production site. The presence of ToBRFV on seeds and seedlings of tomato plants and susceptible pepper plants is assessed to have an unacceptable economic impact as regards to the intended use of those plants for planting. Feasible and effective measures are available to prevent its presence on seeds and seedlings of tomato and susceptible pepper cultivars. ToBRFV meets all criteria to be considered as a RNQP except that it is currently subject to temporarily measures adopted pursuant to Article 30(1) of EU Regulation 2016/2031.

1. Introduction

Tomato brown rugose fruit virus (ToBRFV) is a relatively new tobamovirus that was first described from Jordan in 2016 (Salem et al., 2016). The virus had already been found in Israel in 2014 but at that time it was still an undescribed species (Maayan et al., 2018). ToBRFV infects tomato (*Solanum lycopersicum* L.) and pepper (*Capsicum* L.) and especially poses a threat to tomato production because it breaks known resistance genes (*Tm-1, Tm-2, Tm-2*^{2}) that are effective against other tobamoviruses that can infect tomato such as tobacco mosaic virus (TMV) and tomato mosaic virus (ToMV) (Maayan et al., 2018).

In 2018, outbreaks of ToBRFV in tomato were reported from the EU member states Germany and Italy followed by reports from the Netherlands, Greece and Spain in 2019, from Belgium, Cyprus, Czech Republic, France, Poland and Cyprus in 2020 and from Austria, Malta, Bulgaria, Hungary, Slovenia, Estonia and Portugal in 2021 (Table 1). Since 1 November 2019, the virus has been

regulated in the EU by emergency measures which were strengthened in August 2020, January 2021 and October 2021 (Commission implementing decision (EU) 2019/1615[1] replaced by Commission implementing regulation (EU) 2020/1191[2] in August 2020, amended by Commission implementing regulation (EU) 2021/74[3] in August 2020 and Commission implementing regulation (EU) 2021/1809[4] in October 2021).

Pest risk analyses for ToBRFV have been prepared by several member states and the European and Mediterranean Plant Protection Organization (EPPO, 2020b). These studies all concluded that the virus poses a high phytosanitary risk for these member states, the EU or EPPO-region. Consequently, measures were recommended to prevent introduction of ToBRFV. However, since the first finding of ToBRFV in the EU, the virus has been reported from many more EU member states and may already have established in some parts. Emergency measures are temporary and to decide on a possible (dis)continuation of the measures an evaluation of the efficacy of these measures and possible alternatives (i.e. regulation as a Union regulated non-quarantine pest (RNQP)) is needed. This document, therefore, addresses the following questions:

1) what is the current pest status of ToBRFV in the EU?
2) can eradication be achieved in the EU?
3) what are the consequences for listing as a Union quarantine pest?
4) does ToBRFV fulfill the criteria of a RNQP and which management options are available?

2. Pest status in the EU

2.1 European Union

ToBRFV is 'under eradication' or 'under official control' in 16 member states and eradicated in one member state (Table 1). In many member states the virus has officially been confirmed at only one or a few locations but in some member states, e.g. Greece, Italy and the Netherlands, the virus appears to have a wider distribution. In general, ToBRFV may have a wider distribution in the EU than officially known especially because of asymptomatic infections (see sections 2.2 – 2.4).

Eradication measures taken by member states include removal of the crop followed by cleaning and disinfection of the greenhouse. Most findings have been reported from greenhouses producing fresh tomatoes. Most member states allow harvest and trade of fruit from infested greenhouses under strict conditions until the next crop cycle (see pest reports in EPPO (2021a)). Production areas and production values of fresh tomatoes in the different EU member states are provided in Annex II. There have been fewer outbreaks in *Capsicum* crops probably because mainly *L* resistant peppers are used for commercial production in the EU (EPPO, 2020b).

Detailed information on the pest situation in Greece, Italy, the Netherlands and Spain is provided in sections 2.2 – 2.4.

[1] COMMISSION IMPLEMENTING DECISION (EU) 2019/1615 of 26 September 2019 establishing emergency measures to prevent the introduction into and the spread within the Union of Tomato brown rugose fruit virus (ToBRFV). OJ 250, 30.9.2019, p. 91-94.
[2] COMMISSION IMPLEMENTING REGULATION (EU) 2020/1191 of 11 August 2020 establishing measures to prevent the introduction into and the spread within the Union of Tomato brown rugose fruit virus (ToBRFV) and repealing Implementing Decision (EU) 2019/1615. OJ L 262, 12.8.2020, p. 6–13.
[3] COMMISSION IMPLEMENTING REGULATION (EU) 2021/74 of 26 January 2021 amending Implementing Regulation (EU) 2020/1191 on measures to prevent the introduction into and the spread within the Union of Tomato brown rugose fruit virus (ToBRFV). OJ L 27, 27.1.2021, p. 15-17.
[4] COMMISSION IMPLEMENTING REGULATION (EU) 2021/1809 of 13 October 2021 amending Implementing Regulation (EU) 2020/1191 on measures to prevent the introduction into and the spread within the Union of Tomato brown rugose fruit virus (ToBRFV)

Table 1. Pest status of ToBRFV in EU member states where the virus has been detected

Member state	**Pest status declared by NPPO[1]**	**References of pest reports**
Austria	Present, under eradication	(EPPO, 2021b)
Belgium	Present, under official control	(EPPO, 2021c)
Bulgaria	Absent, pest eradicated[2]	(EPPO, 2021m;2021l)
Cyprus	Present, under eradication	(EPPO, 2020f)
Czech Republic	Present, under eradication	(EPPO, 2020g)
Estonia	Present, under eradication	(EPPO, 2021j)
France	Transient, under eradication	(EPPO, 2020h)
Germany	Present, under eradication	(EPPO, 2020i)
Greece	Present, under eradication	(EPPO, 2020j;2020a)
Hungary	Present, under eradication	(EPPO, 2021h)
Italy	Present, under eradication	(EPPO, 2019c;2019a;2020k;2021n)
Malta	Present, under eradication	(EPPO, 2021d)
Netherlands	Present, under eradication	(NPPO-NL, 2019;2021b)
Poland	Present, under eradication	(EPPO, 2020d;2021e)
Portugal	Transient, under eradication	(EPPO, 2021f)
Slovenia	Present, under eradication	(EPPO, 2021g)
Spain	Present, under eradication	(EPPO, 2019b;2020c;2021k)

[1] Source: EPPO (2021i).

[2] The pest had been detected in one greenhouse of 500 m^2.

2.2 Greece

2.2.1 Situation

After the first outbreak was detected in the Regional Unit of Chania (September 2019), several outbreaks of ToBRFV were detected in greenhouses that mainly produce tomato fruit and in greenhouses that produce tomato seedlings in several Regions of Greece (Peloponnese, Central Greece, East Macedonia, Thrace, Attica) in 2020. The occurrence of ToBRFV in pepper plant cultivation seems very limited in Greece. ToBRFV has thus far been detected in one greenhouse pepper fruit crop (without any symptoms), in the Regional Unit of Drama.

In all aforementioned cases of ToBRFV an official investigation was carried out by the Regional phytosanitary authorities which included tracing back the origin of the seeds from which the plants had been grown. It turned out that the seeds came from third countries directly or through another EU member state. Greece does not have tomato and pepper seed production units and all the quantities of seed required for seedling production are imported.

In 2021, ToBRFV has been found on the island of Crete in the Regional Unit of Lasithi.

In line with Implementing Regulation (EU) 2020/1191, a survey program is carried out at greenhouse units for production of seedlings and units for production of tomato and pepper fruits, as well as at outdoor cultivation of tomato. In 2020, 206 sites were visited in 12 regions; samples were taken from each site and samples from 24 sites in 5 different regions tested positive.

2.2.2 Eradication measures

For all outbreaks, guidelines were given by the NPPO of Greece for the implementation of strict hygiene measures. The measures were implemented immediately and concerned the use of disposable clothes by the staff, disinfection of tools and machines, immediate removal of plants with symptoms associated with ToBRFV and their destruction by deep burial or burning. After the cropping cycle, the crop residues were completely removed and the greenhouses disinfected. The soil was treated by solarization to lower the virus concentration and recommendations were made for crop rotation with a crop not affected by ToBRFV.

Guidelines have been given by the NPPO of Greece to the country's phytosanitary services for sampling 100% of tomato and pepper seed lots from other EU Member States if they had not been tested for ToBRFV by real time PCR, as well as for carrying out phytosanitary controls (physical checks and sampling) on a 100% basis of tomato and pepper seed lots originating from third countries.

2.3 Italy

2.3.1 Situation

Following the first report of ToBRFV in Italy in October 2018 (Panno et al., 2019a), when ToBRFV was found in a greenhouse used for tomato cultivation and in four nurseries in Sicily, new findings were reported at 45 tomato producing companies (Panno et al., 2020b). In 2020, an official survey was carried out at 43 producers. Crops were visually inspected, and 277 samples were taken from plants with suspicious symptoms. This survey resulted in 32 positive samples from nine different greenhouses. In other investigations, ToBRFV was detected in asymptomatic plants. Hence the virus could be more widespread than officially known. ToBRFV has been eradicated in some sites in Sicily, but the Sicilian production area is highly specialized for tomato and non-host crops have been rarely cultivated after an infected tomato crop.

Surveys are continuing in 2021. Three new outbreaks in tomato cultivations were detected in Sicily: two in the province of Caltanissetta and one in open field in the province of Agrigento. Eradication measures have been prescribed to the farmer: removal and destruction of all plants by burning or deep burial within 15 days, and ban on the cultivation of species susceptible to ToBRFV for a period of not less than one year. Official inspection at the Sicilian nurseries from which the seedlings originated, did not result in any positive samples. Following an interception by the NPPO of Malta, ToBRFV was found in a protected nursery in tomato and pepper plants and tomato seeds. All plants of the infected lots and infected seeds were destroyed and the site was subjected to eradication measures. The movement of plants was temporarily banned. Subsequent inspections, sampling and testing gave negative results and this outbreak is now considered eradicated.

As regards the numerous outbreaks detected, ToBRFV is now considered distributed in most of the principal tomato production area of Sicily.

In 2019, an outbreak occurred in Piedmont where the production of fresh tomatoes is restricted to a few municipalities. The virus was found in one greenhouse (30,000 m^2) and 15% of plants had symptoms associated with ToBRFV. The applied eradication measures were successful.

In 2020, after communication by the National competent authority concerning the delivery of seeds from the Netherlands that could be infested with the virus, several Italian Regions performed a trace back survey during which several nurseries and fruit production cultivations were inspected for symptoms of ToBRFV and samples were taken for further analysis. In most cases the cropping cycle had already ended and seeds, seedlings and plants were not available anymore. Only in Tuscany, plants in two greenhouses (2400 m^2 in total) were found infected and the virus is under eradication.

In 2021, an outbreak has been identified in Apulia in two adjacent greenhouses producing tomato and pepper fruit, respectively. Only the tomato plants were symptomatic. The pepper plants were asymptomatic. Several pepper cultivars were grown in the greenhouse; samples had been pooled and it was not possible to identify which cultivars were infected. The greenhouses were subjected to eradication measures including destruction of all plants. A trace back survey did not result in any findings of ToBRFV in seedlings at the nursery of origin.

2.3.2 Eradication measures

At outbreak sites, hygiene measures are promptly applied with restricted access of personnel using disposable clothing, disinfection of tools and machineries while the symptomatic plants and those plants surrounding the symptomatic ones are removed and destroyed by burning. In case of symptomatic plants with an incidence of more than 50% in a greenhouse, the entire crop must be destroyed. In the latter case, the greenhouses are cleaned and disinfected, and the soil is treated by solarization. In case of soilless crops, the growing media must be destroyed and the irrigation system disinfected. Whenever possible, a non-host crop is cultivated before switching back to tomato or alternatively solarization is carried out. Recently, an "Action Plan" (June, 2021) has been made by the Sicilian Region which provides specific measures for the different protected crops:

- soilless cultivation: the replanting of susceptible species to ToBRFV can be done after the cultivation of non-host species (e.g. cucurbits) or, alternatively, the absence of cultivation for a period of not less than two months;
- soil cultivation: as an alternative to soil solarization, the replanting of a species (*Solanum lycopersicum*, *Capsicum annuum*) susceptible to ToBRFV is allowed after a minimum interval of one year with cultivation of non-host species (e.g. cucurbits).

After the above measures, the cultivation of tomatoes and peppers is allowed in the greenhouse and the first host crop is inspected for any symptoms of ToBRFV.

Eradication measures were successful in Piedmont where fresh tomato production covers restricted areas and the greenhouse system is also adapted to grow non-host plants for crop rotation. In Tuscany, ToBRFV is still under eradication; non-host are grown and sentinel tomato plants are used to determine if the measures are successful. In the specialized and intensive production areas in Sicily, eradication measures appear to be less effective, probably due to a high chance of re-infestation by connections with other production sites during harvesting and trade of tomatoes.

To prevent further spread of ToBRFV, the Regional competent authorities have recommended all operators to apply hygiene measures. Some of the recommendations are to clean and change clothes regularly and to use antiviral disinfectants for hand and hand-tools, to use disinfectant mats at the entrance of greenhouse and to use cleaned, disinfected harvesting boxes and separated premises for packing of tomatoes from production sites. Currently, all Italian Regions are carrying out surveys at nurseries and fruit production sites by sampling asymptomatic leaf material and testing by real-time RT-PCR according to Regulation (EU) 2020/1191.

2.4 Netherlands

2.4.1 Situation

ToBRFV was first detected in October 2019 (NPPO-NL, 2019) but results from trace-back surveys including information from growers indicated that the virus had probably been present for one or two years in the Netherlands already. After the first official finding more tomato greenhouses were found to be infested. Based on the genomic diversity of ToBRFV isolates the virus had likely been introduced several times (Van de Vossenberg et al., 2020). A trace back investigation was conducted after each finding but in most cases it was not possible to identify the source of the infestation.

In January 2021, the virus had been detected on 28 tomato production locations (468.1 ha), in March 2021 at 32 production locations (476.6 ha) and in September 2021 (situation on 13 September) at 41 locations (NPPO-NL, 2021a;2021b; NVWA, 2021a). Outbreaks of ToBRFV have so far been eradicated at nine locations by removal of the crop and hygiene measures, but was re-introduced at one of these nine locations. Four locations switched to a non-host crop, but most locations still grow tomatoes with facilities highly specialized for tomato production. On 13 September 2021, 29 locations were still under control covering approximately 25% of the total tomato acreage in the Netherlands (1872 ha in 2020[5]). Note that an entire production location which may cover tens of ha of tomato plants in different greenhouse compartments is under official control even if only one sample from one compartment is found infected. An exception is made if the production location is clearly divided into physically separated units between which the spread of the virus can be excluded due to the application of very strict hygiene measures. Most production locations do, however, not have such a strict division in units.

The total number of production locations that is infested may be higher than officially known because the virus can be present in symptomless crops (it has been detected in crops with no apparent symptoms) and not all tomato greenhouses have been inspected and sampled by the NPPO. Except in the case of trace-back or trace-forward surveys, official surveys are based on visual inspections and samples are only taken in case of suspicious symptoms. Moreover, production locations are very large, ranging from 4 up to 70 ha, and during an inspection only a limited number of plants can be visually inspected. Many findings have been made after growers

[5] Source: : https://opendata.cbs.nl/statline/#/CBS/nl/dataset/37738/table?fromstatweb

had reported the possible presence of the virus at their premises based on symptoms (and positive test results from a private laboratory).

Thus far, the virus has only been detected in tomato plants/crops grown in soilless culture and no *Capsicum* crops have been found infected despite being grown in the same areas. ToBRFV was once detected in a (symptomless) pepper crop in a greenhouse in which a ToBRFV-infected tomato crop had previously been growing. However, this test result was most likely due to contamination of the sample (NVWA, unpublished results)[6].

Scientific information on resistance of *Capsicum* against ToBRFV is limited but cultivars carrying *L3*/*L4* resistance genes/alleles appear to be fully resistant (NVWA, 2021b). According to information from stakeholders, more than 99% of the total acreage of *Capsicum* in the Netherlands is grown with cultivars carrying *L3*/*L4* resistance genes/alleles. Thus, the virus seems to mainly pose a threat to tomato cultivation in the Netherlands.

2.4.2 Eradication measures

After a ToBRFV find, growers do not have to remove the entire crop immediately but are still allowed to harvest and trade tomato fruit until crop rotation. Hygiene measures at the packing station and greenhouse are, however, obligatory to prevent spread of the virus to other greenhouses. At the greenhouse, growers are obliged to establish a hygiene plan and at the end of the growing period measures must be taken to eradicate the virus. These measures include removal of the crop and substrate (or steaming of the substrate), followed by cleaning and disinfection of the greenhouse. Any materials that cannot be properly cleaned and disinfected should be discarded. Six months after planting of a new crop, the NPPO inspects the crop and takes samples from 200 plants for real-time RT-PCR testing to verify whether eradication was successful and measures can be lifted. Visual inspection alone is not considered sufficient because symptomless infections may occur.

Thus far (13 September 2021), ToBRFV has been detected at 41 tomato production locations (see 2.4.1). In many cases, eradication measures taken during crop rotation were unsuccessful and/or the virus had been reintroduced before the new crop was inspected and sampled. At those locations, measures were extended for another cropping cycle and the total number of greenhouses that are under official control has only been increasing since the first official finding in October 2019 (see also section 2.4.1).

2.5 Spain

2.5.1. Situation

Tomato fruit production

ToBRFV was detected for the first time in Spain in one tomato fruit greenhouse (1.38 ha) located in the municipality of Vícar (Almería) in November 2019. Due to the investigations carried out as a result of this finding, ToBRFV was detected in two other tomato fruit greenhouses located in Vícar (1.36 ha) and El Ejido (Almeria, 0.50 ha) in December 2019. The crops had been grown from the same seed lot as the first crop found infected. Surveys were also carried out in other tomato fruit greenhouses owned by the same producers, resulting in the finding of two more infested tomato fruit greenhouses (1.13 and 0.99 ha) in Vícar.

In February 2020, ToBRFV was found in another tomato fruit greenhouse (0.89 ha) located in Vícar, with a crop grown from the same seed lot as the crop found infected in November 2019 (see above). In June 2020, the presence of the virus was confirmed in one more tomato fruit greenhouse (0.51 ha) located in Vícar.

[6] Due to the very low ToBRFV concentration, the test results were regarded suspicious and re-sampling of 200 pepper plants (harbouring *L3*/*L4* genes/alleles) was undertaken. Gloves were changed every row and extra care was given to only touch plants (no materials or surfaces). In these samples ToBRFV was not detected.

Research centre
In June 2020, ToBRFV was detected at a Research Centre located in the municipality of El Ejido (Almería), in one facility that produces *Capsicum* seeds for research purposes.

Foundation
In October 2020, the virus was detected in a tomato sample taken in a greenhouse belonging to a Foundation located in the municipality of Almería. The seeds from which the plants had been grown did not originate from any commercial operator, but came from the Foundation's research lines obtained through crosses between varieties from its own seed bank. Therefore, the most likely source of infection was mechanical transmission by workers or work tools.

Pepper seed production
In December 2020, ToBRFV was detected in one facility of *Capsicum* mother plants for the production of commercial seeds (0.67 ha) in the municipality of Vícar (Almería). Another finding was in one facility of *Capsicum* mother plants (690 plants) belonging to the same professional operator as the previous finding, but in this case used for production of seeds for research purposes.

In January 2021, ToBRFV was detected in four greenhouses or production fields with *Capsicum* mother plants (6,920 plants) for the production of seeds, located in the municipality of El Ejido (Almería). Also in this case, it is not known (yet) whether the *Capsicum* cultivar(s) carried *L*-resistance genes/alleles.

2.5.2 Eradication measures

Tomato fruit production
First outbreak: all plants in the greenhouses were removed from four of the seven greenhouses found infested. The plants were stored in airtight containers inside the greenhouse and destroyed under official control. Weeds were removed and the greenhouse, tools etc. were disinfected. Bumble bees boxes were treated and removed. The soil was disinfested by soil solarization. During the actions access to the greenhouses was prohibited. In the other three greenhouses, any symptomatic plants and the ones nearby were removed and destroyed under official control. After the destruction of these plants, virus symptoms were not observed in these greenhouses which were exhaustively monitored through visual inspections by the competent authority during the remainder of the growing season. Additionally, the following measures were compulsory:
- before growing Solanaceae plants, the soil had to be disinfected by means of solarization and the greenhouse had to be closed for at least 30 days to increase the temperature. In case plants had been grown on artificial substrate, this substrate had to be replaced;
- before the beginning of the following crop, disinfection of the structure of the greenhouse;
- before the beginning of the following crop, all the growing tools (plastics, tutors, rings, etc.), boxes and pallets had to be replaced or disinfected.

In addition to these measures for infested greenhouses, Resolution of 24 January 2020 of Junta de Andalucia establishes preventive measures for all the Autonomous Region of Andalucía, as well as recommendations for implementing phytosanitary measures.

Research centre
Regarding the outbreak in the Research centre in June 2020: no plant passports were issued and all pepper seeds were placed on hold. A sampling procedure was designed for the 3,495 seed lots to identify which of them were contaminated. All seeds from the 41 lots that made up the positive composite sample were destroyed. The remaining lots (3,454) were tested and the results were negative. Preventive measures were laid down for the greenhouse, belonging to the research centre, where these seeds were grown and collected in spring 2019 and investigations on the origin of these seeds were carried out. Once the production of the next generation will be completed, these seeds will be tested.

Foundation
More than 90% of the tomato plants were symptomatic and the destruction of all the plants present in the greenhouse was ordered. Plant debris was managed through an authorized manager, and it was prohibited to use it for composting. Precautionary phytosanitary measures were imposed for the other greenhouses at the same location growing host plants of ToBRFV, and samples were taken in all of them (all tested negative). Regarding the tomato seed research lines the competent authority carried out an inspection with sampling of all the research lines (29 in total) to check whether they were free from ToBRFV.

Pepper seed production
The pepper (*Capsicum*) mother plants were placed on hold and all symptomatic plants were destroyed. Regarding the pepper research lines, the entire stock (4.79 kg in 16 batches) was placed on hold, until further testing had been carried out to determine which lines were infested (and need to be destroyed) and which were not. The professional operator was obliged to establish an action procedure that must be approved by the Almería Plant Health Department.

3. Likelihood of eradication

The experiences (see section 2) show that eradication can be achieved at the level of a production site but that eradication is less likely in tomato production areas with multiple infestations. Eradication measures can probably become more effective by imposing stricter measures, e.g. obligatory cultivation of a non-host after removal of the infected crop or a minimum interval between two host crops. However, given the high percentage of infested greenhouses in some areas in the EU such measures would have massive impacts on the tomato production in those areas, e.g. in the Netherlands and Sicily.

In the Netherlands, approximately 25% of the total tomato acreage is currently under official control for ToBRFV (see section 2.4 for details). If this acreage (approximately 500 ha) must be replaced by a non-host or a resistant host, growers may opt for cucumber or *Capsicum* (using cultivars harbouring *L3*/*L4* genes/alleles). These fruit vegetable crops are also grown on a relatively large acreage in the Netherlands (on 595 and 1532 ha, respectively)[7]. Other greenhouse crops that may be grown instead are grown on a much smaller acreage in the Netherlands (e.g. eggplant on 109 ha, melon and *Phaseolus* bean on less than 10 ha). Any increase in acreage of these crops may already lead to a significant decrease of the prices that a grower will receive for the products. Therefore, these crops are not a realistic alternative except maybe for a few hectares. In case the 500 ha would be replaced by cucumber and *Capsicum*, the prices of these products are expected to decrease significantly due to the strong increase in supply making the production unprofitable. In addition, 25% of the tomato production corresponds to approximately 230 million kg of tomatoes (total production in the Netherlands: 910 million kg; source: https://opendata.cbs.nl/statline/). When replacing this acreage by another crop this would have a massive impact on the supply of fresh tomatoes in the Netherlands with a total production value of approximately 750 million euro (Annex II). In addition, Dutch tomato producers are highly specialized and growing another crop, like cucumber or *Capsicum* involves additional costs. For cucumber and *Capsicum* this would require an investment of approximately 10 and 20% of the original purchase price of the greenhouse; currently approximately 110 euro per m^2. Harvesting, sorting and packing systems must be adapted as well as energy screens and long-term energy contracts (different crops have different energy requirements). Any artificial light systems will become worthless or needs adaptation because *Capsicum* fruit is not (yet) produced with artificial light and cucumber tolerates much less light than tomato. Finally, the cultivation of another crop will require other skills from the grower and the staff and different crops require different number of staff members (information on costs for replacing tomato by another crop from the Fresh Produce Centre (https://freshproducecentre.com/) and Glastuinbouw Nederland (https://www.glastuinbouwnederland.nl/english/), July, 2021).

In Sicily, ToBRFV is now considered distributed in most of the principal tomato production area of Sicily and replacing tomatoes in every infested greenhouse would have a massive impact on the tomato supply. The Sicilian fresh tomato production is highly specialized (10,748 ha with a

[7] Figures from 2020; source: https://opendata.cbs.nl/statline/#/CBS/nl/dataset/37738/table?fromstatweb

production of more than 300 million kg; the total Italian production is about 580 million kg – source: http://dati.istat.it/).

Stricter measures, especially the obligatory cultivation of a non-host or resistant crop, will increase the chance of eradication at the level of the production site. However, it is still uncertain if such measures will finally lead to complete eradication of the virus from a production area. Infestations can go undetected (for some time) because of asymptomatic infections (see for example section 2.3) and these infections can be a source for further spread of the virus through human activities. In addition, spread of ToBRFV by bumble bees has been shown within a greenhouse (Levitzky et al., 2019) and spread by bumble bees may also be possible between greenhouses especially when they are located close to each other. For these reasons and to ensure eradication, the cultivation of susceptible hosts should be prohibited in the entire production area for one or even several years because it is uncertain how long the virus can remain viable on surfaces, on clothes and in soil. Such measures would probably have long lasting effects on the economy in the areas and are not considered feasible. It is, therefore, concluded that eradication in the EU is unlikely to be achieved in at least some of the tomato fruit producing areas.

4. Consequences for listing as a Union Quarantine pest

Listing of ToBRFV as a Union Quarantine pest (listing in Annex IIB of Implementing Regulation (EU) 2019/2072) would mean that fruit from infested greenhouses may no longer be traded. Article 5 point 1 of Regulation (EU) 2016/2031[8] states that "*A Union quarantine pest shall not be introduced into, moved within, or held, multiplied or released in, the Union territory*." This is also reflected in article 2 of (EU) 2020/1191. Tomato fruit is known to contain high virus titers and may also act as a pathway for spread (EPPO, 2020b). Continued regulation of ToBRFV within the EU may thus require more specific measures for fruits.

Currently, most member states allow trade of fruit from infested greenhouses (see section 2). Some do not allow trade of fruit from symptomatic plants but this is not considered sufficiently effective to fully prevent spread of the virus by fruit because asymptomatic infections can occur (EPPO, 2020b). In addition, symptoms caused by ToBRFV may resemble those caused by other viruses. Thus, requiring removal of all infected plants in a tomato crop may involve testing of hundreds of individual plants. Some production locations are very large consisting of several greenhouses that are connected to each other. Such locations may cover more than 50 ha of greenhouses. Any finding of ToBRFV at such a location would require even more tests. Infected non-symptomatic plants may not all be detected and those plants may thus remain in the greenhouse and act as a source for further spread of the virus. Moreover, testing of non-symptomatic plants in one infested greenhouse in the Netherlands resulted in inconclusive PCR-results (i.e. Ct-values that were just below or above the threshold value) and it remains still uncertain if these plants were infected or only contaminated by non-infectious virus particles (NPPO of the Netherlands, unpublished results). Besides the challenges in assessing if ToBRFV is present at a greenhouse, or not, there is also a high chance that the virus is spread within the greenhouse when infected plants are removed. The virus may also remain on surfaces, tools etc. that are difficult to clean and disinfect when a crop is still present in the greenhouse. For these reasons, removal of only the infected plants is not considered a feasible approach and the entire crop should be removed from an infested greenhouse to ensure that no infected tomato fruit is traded. However, tomato crops produce for nearly one year before being replaced by a new one and removal of the entire crop during the season will have major financial consequences for the grower. Listing of ToBRFV as a Union Quarantine pest would, therefore, have a massive impact for tomato production in areas where the virus is prevalent (see also section 3). In areas where the virus is prevalent, growers may even stop producing tomatoes because the financial risk of a ToBRFV-infection may be too high.

[8] REGULATION (EU) 2016/2031 OF THE EUROPEAN PARLIAMENT OF THE COUNCIL of 26 October 2016 on protective measures against pests of plants, amending Regulations (EU) No 228/2013, (EU) No 652/2014 and (EU) No 1143/2014 of the European Parliament and of the Council and repealing Council Directives 69/464/EEC, 74/647/EEC, 93/85/EEC, 98/57/EC, 2000/29/EC, 2006/91/EC and 2007/33/EC. OJ L. 317, 23.11.2016, p. 4-104.

5. Evaluation against RNQP-criteria

5.1 Methodologies

In order to qualify as a Union regulated non-quarantine pests (RNQP), the organism should meet all of the following criteria (Regulation (EU) 2016/2031, article 36):

(i) its identity should be clear,
(ii) it should be present in the Union territory,
(iii) it should be transmitted mainly through specific plants for planting, and
(iv) its presence on those plants for planting has an unacceptable economic impact, as regards the intended use of those plants for planting,
(v) feasible and effective measures are available to prevent its presence on the plants for planting concerned.

One additional RNQP-criterion is the regulatory status of the pest: "*it is not a Union quarantine pest or a pest subject to measures adopted pursuant to Article 30(1)*". ToBRFV is currently regulated by emergency measures pursuant Article 30(1) and the present document evaluates whether the pest fulfils all other criteria to be listed as a RNQP.

For criterion (iii) (transmitted mainly through plants for planting), the methodology described by Picard et al. (2017) was used. This methodology was also used to recommend listing of pests as RNQPs in the EU when the initial list of RNQPs was established in 2019. This methodology evaluates whether the 'plants for planting – pathway' is a significant pathway compared to other pathways in areas where the pest is present, taking into account measures the grower has available to control the other pathways.

Several pest risk analyses (PRAs) for ToBRFV have recently been conducted including a very comprehensive one by EPPO (2020b). Therefore, no extensive literature search was conducted and much information was taken from the EPPO-PRA (EPPO, 2020b). However, to include new relevant information, a literature search was conducted in CABabstracts and Agricola via Ovid (search term: tomato AND brown AND rugose AND fruit AND virus) focusing on papers that have been published since 2020 and were not included in the EPPO-PRA. Information about the worldwide pest distribution was retrieved from the EPPO Global Database (EPPO, 2021b), from recent literature and internet (Google search). Detailed information on the virus, its taxonomy, life cycle, host plants etc. is already provided by EPPO (2020b) and is not repeated here.

5.2 Evaluation

5.2.1 Identity of the pest (criterion i)

Tomato brown rugose fruit virus (ToBRFV) is a relatively recently discovered virus (family: Virgaviridae; genus: *Tobamovirus*) of which the identity is well established (EPPO, 2020b).

Conclusion: ToBRFV fulfills the RNQP-criterion (i) of having a clear identity.

5.2.2 Pest distribution (criterion ii)

ToBRFV is at least present on three continents (Table 2). ToBRFV has been intercepted on seeds or fruit from many countries that have not officially reported the presence of the virus. These interceptions suggest that ToBRFV has a wider distribution worldwide than officially known. ToBRFV is present in the EU where it is unlikely to be eradicated (see sections 2 and 3).

Conclusion: ToBRFV fulfills the RNQP-criterion (ii) of being present in the Union territory.

Table 2. Worldwide reports on findings of tomato brown rugose fruit virus including interceptions on seeds and fruit by other countries (source: EPPO (2021i) unless stated otherwise).

Continent	**Country**
Africa	
	Egypt (absent, earlier record considered invalid by NPPO)[1]
	Ethiopia (interception)[2]
America	
	Dominican Republic (interception)[2]
	Guatemala (interception)[2]
	Mexico
	Peru (interception)[2]
	United States of America
Asia	
	China
	India (interception)[2]
	Iran (Ghorbani et al., 2021)
	Israel
	Japan (interception)[2]
	Jordan
	Saudi Arabia (Sabra et al., 2021)
	Syria
	Taiwan (interception)[2]
	Thailand (interception)[2]
	Turkey
Europe	
	Austria
	Belgium
	Bulgaria (absent, pest eradicated)
	Cyprus
	Czech Republic
	Estonia
	France
	Germany
	Greece
	Hungary
	Italy
	Malta
	Netherlands
	Norway
	Poland
	Portugal
	Slovenia
	Spain
	Switzerland
	United Kingdom
Oceania	
	Australia (interception)[2]

[1] Reported by Amer & Mahmoud (2020) but the NPPO of Egypt considered the record invalid (EPPO, 2020e).
[2] Intercepted on seeds or fruit that had been imported from the country; no official report of its presence in the country.

5.2.3 Pathways for spread (criterion iii)

5.2.3.1 Natural spread

In greenhouses, natural spread of ToBRFV (via water and pollinating insects) is assumed to be limited to spread within the same crop. Pollinating insects but also birds may enter and leave greenhouses but there are currently no indications that this is a major pathway of spread. In open

fields, spread to neighboring fields by natural mechanisms may be more likely (less physical constraints) but human assisted spread including trade of infected plants for planting is considered far more important (EPPO, 2020b).

5.2.3.2 Human assisted spread (including trade)

ToBRFV is highly persistent and local spread will be mainly linked to human-assisted mechanical transmission of the virus (EPPO, 2020b). The virus can also be transmitted through seeds (Davino et al., 2020) and trade of seeds and seedlings is considered to be the main pathway of spread over longer distances (EPPO, 2020b). The pathway of 'plants for planting' becomes more significant, also in areas where the virus is present, when growers take strict hygiene measures to prevent introduction of the virus through mechanical transmission, i.e. by implementing strict rules for workers, visitors, machinery and tools for entering the greenhouse. For these reasons, plants for planting (seeds and seedlings) is considered a significant pathway compared to other pathways, also in areas where the virus is present.

The virus can also be spread through infected tomato fruit but this pathway is considered less important than the plants for planting pathway as long as the fruit is not sorted and packed at other tomato/capsicum production locations (EPPO, 2020b). Packing stations that sort and pack fruit from multiple production locations can take hygiene measures to prevent the spread of the virus by cleaning and disinfection of reusable crates and by cleaning and disinfection of transport vehicles that are used to transport the fruit from the production locations to the packing station. In the Netherlands for example, packing stations follow a strict hygiene protocol and although a large acreage is infested (see section 2) there are no indications that the virus has spread through the packing stations (based on genomic diversity of isolates found at production locations using the same packing station).

Conclusion: ToBRFV fulfills RNQP-criterion (iii) as trade of seeds and seedlings is considered a significant pathway compared to other pathways.

5.2.4 Potential economic impact (criterion iv)

5.2.4.1 Tomato

ToBRFV-infections in tomato can cause fruit yield reduction (in total kg/plant) and reduction of fruit size; symptomatic fruits lose market value or are unmarketable. Because all tomato cultivars are susceptible ToBRFV can have a high economic impact on tomato fruit production (EPPO, 2020b). However, environmental conditions and tomato genotype influence the severity of disease symptoms and the impact of virus infections: "*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 titer: testing still shows that the plant is highly infected* (Dombrovsky, pers. comm., 2019 in (EPPO, 2020b)). DeRuiter (2019) states that the most severe symptoms are seen on plants infected at a young stage. No evidence has, however, been found that infection at a younger plant stage leads to higher yield losses during the entire growing period of the plant. A crop can (temporally) exhibit severe symptoms under "stressful" events as is the case for viruses in general, (e.g. planting, snow cover on greenhouse roof, change in microclimate or light regime). However, yield losses are generally expected to be higher when ToBRFV is introduced early during the cropping period because more plants are likely to become infected than in situations where the virus is introduced by mechanical transmission at a later stage.

The potential impact caused by ToBRFV for tomatoes grown in the open field (mainly processing tomatoes and fresh tomatoes produced for the local market) has been assessed to be lower than for tomatoes grown in greenhouses due to less handling of the crop, and thus a lower spread rate of the virus within the crop (EPPO, 2020b). In addition, quality standards for these fruits are lower and growers can more easily grow alternative crops on the same plot (EPPO, 2020b).

ToBRFV-infections may increase the impact of other virus infections in tomato. Results of Klap et al. (2020) suggest that infections by mild isolates of pepino mosaic virus (PepMV), could result in symptoms characteristic of PepMV aggressive strains when preceded by a ToBRFV infection.

5.2.4.2 Pepper (Capsicum)

Severe symptoms have been reported for pepper. However, EPPO (2020b) concluded that the potential impact for pepper is likely to be minor because cultivars that are mostly used in the EPPO region are assumed to harbour *L* resistance genes/alleles against tobamoviruses that are effective against ToBRFV. However, EPPO (2020b) also noted that some recent research indicated breaking of some *L* resistance genes/alleles and that the conclusion "*may need to be reconsidered when additional publications are made available*". Luria et al. (2017) found a hypersensitivity response in cultivars carrying *L1, L3* or *L4* genes/alleles. However, they also found ToBRFV to be "*capable of infecting L1,3,4 resistant pepper plants when cultivated on contaminated soil from previous growing cycle in high temperatures above 30°C*". According to Fidan et al. (2021) neither of the *L1,2,3,4* genes confer resistance to ToBRFV: in experiments, plants carrying *L1* or *L2* gave similar symptoms as plants lacking any *L* gene; *L3* and *L4* were not effective at temperatures of 32°C or above. However, Dutch seed companies were not aware of any resistance breaking in commercial *Capsicum* cultivars carrying *L1, L3* or *L4* (TuinbouwAlert, 2019). In the EU, very few outbreaks with symptomatic plants have so far been reported in *Capsicum*. One outbreak with symptomatic plants was reported in a pepper crop in Sicily not harbouring any *L* resistance genes/alleles (Panno et al., 2020a). More findings of ToBRFV have been reported in pepper crops in the EU, but symptoms were (mostly) not observed or mentioned and it was not indicated if the plants carried L-genes. In August 2020, the Czech Republic reported ToBRFV in a seed crop of *C. annuum* grown from breeding material; the plants were symptomless (EPPO, 2020g). In Spain, asymptomatic *Capsicum* mother plants (seed production) tested positive for ToBRFV (EPPO, 2021k). In Italy (Apulia) in one greenhouse asymptomatic pepper plants tested positive (EPPO, 2021d). In Greece one asymptomatic fruit crop tested positive. Thus, most detections in pepper reported in the EU concerned asymptomatic plants. In the Netherlands, pepper crops are grown in close proximity to tomato crops and thus far no outbreaks have been reported in pepper although many outbreaks have been found in tomato crops (see also section 2.4.1). For these reasons, the potential impact for pepper is still assessed to be minor for the EU (medium uncertainty); incidentally (locally) severe yield losses may occur in cases where susceptible cultivars are grown. There is, however, uncertainty to which extent *Capsicum* plants harbouring *L* resistance genes/alleles are fully resistant against ToBRFV.

5.2.4.2 Control options

Tomato growers can reduce the impact of ToBRFV (and other pathogens that are mainly spread in a crop by mechanical transmission) by taking strict hygiene measures to prevent introduction of the virus in the greenhouse and, once the virus is present, to reduce spread of the virus within the crop. This can be achieved by blocking access to rows with virus infected plants, by allowing each worker to only enter certain rows, and by using different harvesting tools (knives/scissors) for each row, etc. Measures to prevent spread within the crop can be taken as a precaution because ToBRFV may already be present for some time before being noticed.

The use of tolerant and resistant tomato cultivars may decrease the impact of ToBRFV-infections in the (near) future. Zinger et al. (2021) recently screened 160 tomato genotypes, 29 of which were tolerant including eight cultivated lines or hybrids. These genotypes showed no symptoms but virus levels were as high as in sensitive cultivars. One genotype displayed resistance but it should be noted that breeding for cultivars that are fully resistant against ToBRFV will take some time.

Conclusion: ToBRFV fulfills RNQP-criterion (iv) as its presence on tomato plants and susceptible pepper plants has an unacceptable economic impact as regards the intended use of those plants for planting.

5.2.5 Available measures for plants for planting (criterion v)

EPPO (2020b) has evaluated pest risk management options for seeds and seedlings and they are summarized below.

5.2.5.1 Seeds

Visual inspection of the mother plants. Because plants can be infected without showing symptoms this measure should only be used in combination with other measures.

Testing of mother plants at the place of production. Mother plants can be tested for the presence of ToBRFV; several PCR-based tests have been validated. This strategy is sufficiently effective as a standalone measure but its efficacy is strongly influenced by sample size (number of mother plants tested) and timing of the sampling. It is important that sampling of the mother plants takes place shortly before harvesting of the seeds to take into account any late infections that may occur. Testing of the mother plants in combination with visual inspections of these plants is currently one of the options in the EU-regulation (Implementing regulation (EU) 2020/1191, articles 7 and 9).

Resistant cultivars. Currently, there are no tomato cultivars known that are fully resistant against the virus. *Capsicum* cultivars that are known to be resistant are exempted from the EU-regulation (Implementing regulation (EU) 2020/1191, article 2 point 2). It should, however, be emphasized that hybrid seeds of resistant cultivars may become infested if the mother line is susceptible and resistance is introduced through the father line. In addition, the seeds may become contaminated with the virus during extraction and cleaning of the seeds. However, these infestations will not lead to infected plants as long as the resistance is not broken.

Pest free production site. A pest free production site can be achieved by complete physical protection and strict hygiene measures. However, pest freedom should still be verified by testing (see 'Testing at place of production'). In general, strict hygiene measure should be applied for the production of tomato and *Capsicum* seeds to prevent introduction of pathogens that can be spread mechanically.

Pest free area. Theoretically, this option is very effective but tobamoviruses are known to spread rapidly even between continents (Dombrovsky & Smith, 2017) and the virus may be present in an area long before its official detection. ToBRFV has been detected in seeds and fruits originating in countries from which the virus has not been reported (Table 2). Therefore, testing of mother plants or seeds would still be needed.

Testing of seeds. Validated PCR-based tests are available for tomato and *Capsicum* seeds and the efficacy of the method (the percentage of infested seeds that can be detected with a high reliability) is mainly determined by sample size. This method in combination with visual inspections of the mother plants is currently one of the options in the EU-regulation (Implementing regulation (EU) 2020/1191, articles 7 and 9). One major drawback of this measure is that PCR-based tests do not distinguish between infectious and non-infectious virus. Thus, a positive test result does not necessarily mean presence of infectious virus. However, there is currently no evidence that bioassays are sufficiently effective to detect low virus concentrations.

Treatment of seeds. Recently, two studies have been published on disinfection treatments (thermal and chemical) of seeds (Davino et al., 2020; Samarah et al., 2020). Both studies show that seed treatments, especially chemical treatments, can reduce the probability of seed-borne infections by ToBRFV. However, the numbers of seeds tested were too low to conclude with a high level of confidence that these treatments are 100% effective. In addition, ToBRFV may also be present in inner tissue of seeds (Davino et al., 2020) which also raises the possibility that disinfection treatments are not fully effective. Additional studies, using higher numbers of seeds would be required to obtain a higher level of confidence regarding the effectivity of these treatments (see Annex I for an evaluation of these two studies). Still, treatment of seed lots prior to seed testing may be an option for seed lots with low levels of virus-contamination.

5.2.5.2 Seedlings

Visual inspection of seedlings. Infected young plants for planting can be symptomless and visual inspection is not effective (EPPO, 2020b).

Testing of seedlings. Virus titers in young plants that have been infected via seed may be too low for reliable detection (EPPO, 2020b). For grafted plants testing may be more effective: the plants are grown for a longer period and have a higher chance to become infected through mechanical transmission. However, testing as standalone measure is also not considered to be sufficiently effective for grafted plants (EPPO, 2020b).

Resistant cultivars. As for seeds (section 5.5.1).

Pest free production site. Starting with seeds free of ToBRFV in combination with a pest free production site is the most effective measure to prevent young plants from becoming infected with ToBRFV. A pest free production site can be achieved by physical protection and strict hygiene measures. 'Complete' physical protection is not considered necessary because the probability that the virus would enter with pollinators through ventilation openings is assessed to be very low as long as no flowering plant are present in the greenhouse (EPPO, 2020b). However, pest freedom will be difficult to check because visual inspections and testing are not very effective. Production sites could, however, be audited for proper implementation of hygiene protocols and seeds used to raise the seedlings or their mother plants should be tested and found free of the virus.

Pest free area. As for seeds (section 5.5.1)

Conclusion: ToBRFV fulfills RNQP-criterion (v) as feasible and effective measures are available to prevent its presence on the plants for planting concerned.

5.3 Overall conclusion

ToBRFV fulfills all criteria for inclusion as a RNQP (as soon as the current emergency measures have expired or have been lifted). Possible threshold and measures for plants for planting for RNQP-regulation would be:

Plants for planting	**Threshold**	**Measure**
Seeds of *Solanum lycopersicum* L., *Solanum lycopersicum* L. hybrids, *Capsicum* L. other than cultivars which are known to be resistant to ToBRFV	0%	The seeds or their mother plants have been subject to official testing for ToBRFV on a representative sample and using appropriate PCR-based methods, and have been found in these tests, free from the pest
Plants for planting other than seeds of *Solanum lycopersicum* L., *Solanum lycopersicum* L. hybrids, *Capsicum* L. other than cultivars which are known to be resistant to ToBRFV	0%	The plants have been grown from seeds which have been subject to official testing or their mother plants have been subject to official testing for ToBRFV on a representative sample and using appropriate PCR-based methods, and have been found in these tests, free from the pest, and strict hygiene measures have been taken at the production site to prevent entry of the virus

Annex I Efficacy of seed disinfection

Summary of published data

Samarah et al. (2020) tested hydrochloric acid (HCl), trisodium phosphate (TSP), dry heat and hydropriming and a combination of these on seed germination and elimination of ToBRFV using DAS-ELISA and a bioassay (local lesion assay). Four replicates of 250 seeds each were used per treatment. DAS-ELISA and bioassay results were negative for treatments including HCl or TSP. HCl and TSP alone did not negatively affect seed quality; HCl even improved seed quality (Table 1). Samarah et al. (2020) do not provide any indication on the transmission of non-treated seeds and verification on whether transmission is prevented by the tested treatments. The current EU regulation specifies that seeds are to be tested using real time RT-PCR, but this testing method was not included, so there was no indication on whether the treatments eliminate ToBRFV to the level that the seeds are no longer RT-PCR positive.

Davino et al. (2020) tested four dry heat treatments and four chemical treatments (TSP, hydrogen peroxide, a combination of HCl and sodium hypochlorite (NaOCl) and NaOCl alone) on seed germination and ToBRFV (Tabel 2).. Seeds used for the experiments were extracted from fruit from infected plants and washed in a sieve for 10 min (not entirely clear how the seeds were washed but probably with water).The presence of ToBRFV in these seeds was confirmed by testing a sample of 250 seeds using realtime RT-PCR (Panno et al., 2019b) which resulted in a Ct value of 14. ToBRFV seed transmission rate to tomato seedlings was established to be 1.8% based on positive signals via realtime RT-PCR on a sample of 500 third true leaf samples. For each of the heat and chemical treatments samples of 100 seeds were used. After treatment, seeds were germinated and percentage germination was recorded after 7 and 14 days. Each seedling was homogenized and tested by realtime RT-PCR. Homogenized seedlings that tested positive were grouped (10 per group) and inoculated onto tomato plants. The combination of HCl and NaOCl resulted in 0% germination 14 days after treatment and its effect on ToBRFV was therefore not determined. After the treatment with 2.5% NaOCl for 15 min ToBRFV could not be detected in realtime Rt-PCR. In the remaining six treatments, ToBRFV could still be detected by realtime RT-PCR. In all treatments but one (65°C – 120 h) the bioassay was negative (Table 2). No control treatment was reported in this experiment. In addition, Davino et al. (2020) tested 500 seeds with the TSP treatment and compared these to non-treated seeds. The cotyledons and third true leaves from these seeds were separately tested with RT-PCR in 50 samples from 10 plant each. All 50 bulk samples tested positive for cotyledons of non-treated seeds, whereas 1/50 tested positive for TSP treated seeds. For third true leaves, 2 and 0 bulk samples tested positive for non-treated and TSP treated seeds, respectively.

Table 1. Effect of different seed treatments on tomato seed quality and level of infestation with ToBRFV; summary of results by (Samarah et al., 2020)

Treatment	**Seed quality**	**Disinfection rate**
2% HCl-30 min	Positive	100%
10% TSP- 3 h	Not significant	100%
Dry heat 72°C-72 h	Negative	0%
Hydropriming-3h (control)	Positive	0%

- Per treatment four replicates with 250 seeds each
- HCl = Hydrochloric acid
- TSP = trisodium phosphate

Table 2. Effect of different seed treatments on tomato seed quality and level of infestation with ToBRFV; summary of results by (Davino et al., 2020)

Treatment	**Seed germination (%)**		**Positive RT-qPCR-results (% seedlings)**	**Ct-values**	**Biossay**
	7 dpt	14 dpt			
65°C – 120 h	100	-	100	23.43-28.38	2/10
70°C – 96 h	89	100	100	24.54-26.45	0/10
75°C – 48 h	67	100	80	20.07-26.04	0/8
80°C – 24 h	100	-	60	25.83-27.68	0/6
10% TSP – 3 h	94	100	3	25.83-27.68	0/3
4% H_2O_2- 30 min	100	-	100	24.94-27.31	0/6
2% HCl+1.5% NaOCl-24 h	0	0	ND	-	ND
2.5% NaOCl-15 min	88	100	0	-	ND

- dpt = days post treatment; ND = not determined
- Per treatment 100 seeds were used. Seedlings that tested positive (RT-PCR; (Panno et al., 2019b)) were grouped in lots of ten seedlings except in the TSP-treatment where the three seedlings that tested positive were kept separate, homogenized and used as inoculum in the bioassay.
- Bioassay: plants positive/total number of inoculated plants (no control treatment reported)

Discussion

Both studies show that seed treatments, especially chemical treatments, can reduce the probability of seed-borne infections by ToBRFV. Davino et al. (2020) concluded that all treatments can be used to disinfect seeds except the 65°C heat treatment but that the sodium hypochlorite treatment is advisable because the virus was not detectable any more by PCR after treatment. However, the numbers of seeds tested were too low to conclude with a high level of confidence that these treatments were 100% effective. For example, the established seed transmission of 1.8% in untreated seeds and 0% in TSP treated seeds in samples of 500 seeds by Davino et al. (2020) implies that you are 95% confident that transmission has dropped to 0.6% or below in the treated seeds and 50% confident that transmission had dropped to 0.14% or below. In a field or greenhouse with ~30.000 plants this yields 50% confidence that 42 plants or less are infected. Moreover, the efficacy might depend on the seed batch, e.g. level of contamination and time of infection of the parental lines. Indeed, a seed disinfection study with another tobamovirus (cucumber green mottle mosaic virus; CGMMV) showed that such treatments are not always effective, possibly by the presence of virus particles in the inner tissues of the seed (Reingold et al., 2015). ToBRFV may also be present in inner tissue of seeds (Davino et al., 2020) which raises the possibility that disinfection treatments are not fully effective.

According to current EU-legislation (Implementing Regulation 2020/1191), seeds must be produced at a pest free production site, but once a site has become contaminated it can be very difficult to eradicate the virus. Therefore, treatment of seed lots (with a low level of infestation) prior to testing may be an alternative option. This option may prevent the destruction of valuable (large) seed lots in which ToBRFV is detected (at low concentrations).

Conclusion

In conclusion, seed disinfection treatments will reduce the probability of seedborne infections by ToBRFV, but it is uncertain if such treatments will always be 100% effective. Additional studies, using higher numbers of seeds would be required to obtain a higher level of confidence regarding the effectivity of these treatments. Treatment of seed lots prior to seed testing, still requiring a negative PCR-result, may be an option for seed lots with low levels of virus-contamination.

Annex II Production area and production value of fresh tomatoes

Tomatoes for fresh consumption: cultivation area and production value in EU member states in 2017 - 2019

	Production area (unit: 1000 hectares)			Production value (unit: million euro)		
	2017	2018	2019	2017	2018	2019
Austria	0.18	0.20	0.20	44.16	46.67	52.76
Belgium	0.52	0.55	0.57	196.14	183.15	204.58
Bulgaria	5.01	4.52	5.15	62.34	52.19	54.4
Croatia	0.45	0.49	0.32	29.84	22.42	24.38
Cyprus	0.26	0.29	0.28	11.16	12.63	13.25
Czech Republic	0.24	0.30	0.16	4.05	11.69	15.09
Denmark	0.03	0.03	0.03	29.73	27.06	30.67
Estonia	0.00	0.00	0.00	4.34	5.12	9.91
Finland	0.11	0.10	0.09	66.52	72.91	66.23
France	5.75	5.74	5.66	635.67	536.73	590.04
Germany	0.37	0.40	0.39	138.8	152.58	158.95
Greece	13.32	16.02	15.01	334.76	387.54	439.12
Hungary	2.19	2.50	2.41	78.02	87.8	89.7
Ireland	0.01	0.01	0.01	8.5	9.58	9.56
Italy	92.67	97.17	91.41	1019.72	915.14	1148.6
Latvia	0.00	0.00	0.00	6.29	6.54	6.77
Lithuania	0.55	0.57	0.56	:	:	:
Luxembourg	0.00	0.00	0.00	0.04	0.02	0.05
Malta	0.00	0.00	0.00	3.76	3.42	3.59
Netherlands	1.79	1.79	1.80	769.2	658.46	744.64
Poland	12.64	13.11	13.50	1041.72	1085.43	1254.62
Portugal	20.87	15.83	16.13	170.18	173.07	172.52
Romania	22.21	22.97	23.78	484.97	542.62	599.89
Slovakia	0.60	0.59	0.48	33.57	14.8	15.41
Slovenia	0.20	0.19	0.22	11.29	11.05	13.89
Spain	60.85	56.13	56.94	1804.58	1496.02	1527.91
Sweden	0.04	0.04	0.04	20.74	26.39	19.84

Source: Eurostat – extraction date 04.06.2021.
‘:’ no data available

References

Richtlijn 2000/29/EG van de Raad van 8 mei 2000 betreffende de beschermende maatregelen tegen het binnenbrengen en de verspreiding in de Gemeenschap van voor planten en voor plantaardige producten schadelijke organismen,

Amer M & Mahmoud S, 2020. First report of Tomato brown rugose fruit virus on tomato in Egypt. New Disease Reports, 41 (24).

Davino S, Caruso AG, Bertacca S, Barone S & Panno S, 2020. Tomato Brown Rugose Fruit Virus: Seed Transmission Rate and Efficacy of Different Seed Disinfection Treatments. Plants, 9 (11), 1615.

DeRuiter, 2019. Tomato brown rugose fruit virus. Cultivation-insights. Available online: https://www.deruiterseeds.com/en-ca/resources/cultivation-insights/tomato-brown-rugose-fruit-virus.html

Dombrovsky A & Smith E, 2017. Seed Transmission of Tobamoviruses: Aspects of Global Disease Distribution. In, Advances in Seed Biology. InTech.

EPPO, 2019a. Update on the situation of Tomato brown rugose fruit virus in Sicilia (Italy). EPPO Reporting Service, 2019/144.

EPPO, 2019b. First report of Tomato brown rugose fruit virus in Spain EPPO Reporting Service, 2019/238.

EPPO, 2019c. Tomato brown rugose fruit virus eradicated from Piemonte (Italy). EPPO Reporting Service, 2019/191.

EPPO, 2020a. New outbreaks of tomato brown rugose fruit virus report in Greece. EPPO Reporting Service, 2020/079.

EPPO, 2020b. Pest Risk Analysis for Tomato brown rugose fruit virus (Tobamovirus). European and Mediterranean Plant Protection Organisation. Available online: https://gd.eppo.int/taxon/TOBRFV/documents

EPPO, 2020c. Update on the situation of tomato brown rugose fruit virus in Spain. EPPO Reporting Service, 2020/039.

EPPO, 2020d. Eradication of tomato brown rugose fruit virus in Poland EPPO Reporting Service, 2020/200.

EPPO, 2020e. Tomato brown rugose fruit virus does not occur in Egypt EPPO Reporting Service, 2020/125.

EPPO, 2020f. First report of tomato brown rugose fruit virus in Cyprus EPPO Reporting Service, 2020/173.

EPPO, 2020g. First report of tomato brown rugose fruit virus in the Czech Republic. EPPO Reporting Service, 2020/223.

EPPO, 2020h. First report of tomato brown rugose fruit virus in France EPPO Reporting Service, 2020/037.

EPPO, 2020i. New finding of tomato brown rugose fruit virus in Germany EPPO Reporting Service, 2020/199.

EPPO, 2020j. Update on the situation of tomato brown rugose fruit virus in Greece. EPPO Reporting Service, 2020/224.

EPPO, 2020k. Findings of tomato brown rugose fruit virus on Capsicum in the EPPO region EPPO Reporting Service, 2020/080.

EPPO, 2021a. Tomato brown rugose fruit virus - reporting [Web page]. EPPO Global Database. Available online: https://gd.eppo.int/taxon/TOBRFV/reporting [Accessed: 15.10.2021].

EPPO, 2021b. First report of tomato brown rugose fruit virus in Austria. EPPO Reporting Service, 2021/159.

EPPO, 2021c. First report of tomato brown rugose fruit virus in Belgium EPPO Reporting Service, 2021/017.

EPPO, 2021d. First report of tomato brown rugose fruit virus in Malta. EPPO Reporting Service, 2021/106.

EPPO, 2021e. Update on the situation of tomato brown rugose fruit virus in Poland. EPPO Reporting Service, 2021/087.
EPPO, 2021f. First report of tomato brown rugose fruit virus in Portugal. EPPO Reporting Service, 2021/196.
EPPO, 2021g. First report of tomato brown rugose fruit virus in Slovenia. EPPO Reporting Service, 2021/177.
EPPO, 2021h. First report of tomato brown rugose fruit virus in Hungary. EPPO Reporting Service, 2021/134.
EPPO, 2021i. Tomato brown rugose fruit virus - Distribution (last updated 2021-09-30) [Web page]. European and Mediterranean Plant Protection Organisation. Available online: https://gd.eppo.int/taxon/TOBRFV/distribution [Accessed: 06.10.2021].
EPPO, 2021j. First report of tomato brown rugose fruit virus in Estonia. EPPO Reporting Service, 2021/176.
EPPO, 2021k. Update on the situation of tomato brown rugose fruit virus in Spain. EPPO Reporting Service, 2021/018.
EPPO, 2021l. New data on quarantine pests and pests of the EPPO Alert List EPPO Reporting Service, 2021/146.
EPPO, 2021m. First report of tomato brown rugose fruit virus in Bulgaria. EPPO Reporting Service, 2021/135.
EPPO, 2021n. Update on the situation of tomato brown rugose fruit virus in Italy. EPPO Reporting Service, 2021/137.
Fidan H, Sarikaya P, Yildiz K, Topkaya B, Erkis G & Calis O, 2021. Robust molecular detection of the new Tomato brown rugose fruit virus in infected tomato and pepper plants from Turkey. Journal of integrative agriculture, 20 (8), 2170-2179.
Ghorbani A, Rostami M, Seifi S & Izadpanah K, 2021. First report of Tomato brown rugose fruit virus in greenhouse tomato in Iran. New Disease Reports, 44 (2), e12040. Available online: https://doi.org/https://doi.org/10.1002/ndr2.12040
Klap C, Luria N, Smith E, Hadad L, Bakelman E, Sela N, Belausov E, Lachman O, Leibman D & Dombrovsky A, 2020. Tomato Brown Rugose Fruit Virus Contributes to Enhanced Pepino Mosaic Virus Titers in Tomato Plants. Viruses, 12, 8.
Levitzky N, Smith E, Lachman O, Luria N, Mizrahi Y, Bakelman H, Sela N, Laskar O, Milrot E & Dombrovsky A, 2019. The bumblebee Bombus terrestris carries a primary inoculum of Tomato brown rugose fruit virus contributing to disease spread in tomatoes. PLoS ONE, 14 (1), e0210871.
Luria N, Smith E, Reingold V, Bekelman I, Lapidot M, Levin I, Elad N, Tam Y, Sela N, Abu-Ras A, Ezra N, Haberman A, Yitzhak L, Lachman O & Dombrovsky A, 2017. A new Israeli Tobamovirus isolate infects tomato plants harboring Tm-2² resistance genes. PLoS ONE, 12 (1).
Maayan Y, Pandaranayaka EPJ, Srivastava DA, Lapidot M, Levin I, Dombrovsky A & Harel A, 2018. Using genomic analysis to identify tomato Tm-2 resistance-breaking mutations and their underlying evolutionary path in a new and emerging Tobamovirus. Archives of Virology, 163 (7), 1863-1875. Available online: https://doi.org/http://dx.doi.org/10.1007/s00705-018-3819-5
NPPO-NL, 2019. Official confirmation of Tomato brown rugose fruit virus (ToBRFV) in Solanum lycopersicum at two professional fruit production companies (closed conditions). Netherlands Food and Consumer Product Safety Authority, the Netherlands. Available online: https://english.nvwa.nl/topics/pest-reporting/documents/plant/plant-health/pest-reporting/documents/official-confirmation-of-tomato-brown-rugose-fruit-virus-tobrfv-in-solanum-lycopersicum-at-two-professional-fruit-production-companies-closed-conditions
NPPO-NL, 2021a. Official confirmation of Tomato brown rugose fruit virus (ToBRFV) in Solanum lycopersicum at 28 professional fruit production locations (closed conditions) since the first outbreak report of 17 October 2019. Netherlands Food and Consumer Product Safety Authority, the Netherlands. Available online: https://english.nvwa.nl/topics/pest-

reporting/documents/plant/plant-health/pest-reporting/documents/pest-reporting-tobrfv-january-2021
NPPO-NL, 2021b. Official confirmation of Tomato brown rugose fruit virus (ToBRFV) in Solanum lycopersicum at 32 professional fruit production locations (closed conditions) since the first outbreak report of 17 October 2019. Netherlands Food and Consumer Product Safety Authority, the Netherlands. Available online: {NPPO-NL, 2019 #1925}
NVWA, 2021a. Vondsten ToBRFV [Web page]. Nederlandse Voedsel- en Warenautoriteit. Available online: https://www.nvwa.nl/onderwerpen/tomato-brown-rugose-fruit-virus-tobrfv/vondsten-tobrfv [Accessed: 14.09.2021].
NVWA, 2021b. Import en handel binnen EU met zaden resistente paprika's [Web page]. Nederlandse Voedsel- en Warenautoriteit. Available online: https://www.nvwa.nl/onderwerpen/tomato-brown-rugose-fruit-virus-tobrfv/import-en-handel-binnen-eu-met-zaden-resistente-paprikas [Accessed: 03-06-2021].
Panno S, Caruso A, Blanco G & Davino S, 2020a. First report of Tomato brown rugose fruit virus infecting sweet pepper in Italy.
Panno S, Caruso A & Davino S, 2019a. First report of tomato brown rugose fruit virus on tomato crops in Italy. Plant Disease, 103 (6), 1443-1443.
Panno S, Caruso AG, Barone S, Lo Bosco G, Rangel EA & Davino S, 2020b. Spread of Tomato Brown Rugose Fruit Virus in Sicily and Evaluation of the Spatiotemporal Dispersion in Experimental Conditions. Agronomy, 10 (6), 834.
Panno S, Ruiz-Ruiz S, Caruso AG, Alfaro-Fernandez A, San Ambrosio MIF & Davino S, 2019b. Real-time reverse transcription polymerase chain reaction development for rapid detection of Tomato brown rugose fruit virus and comparison with other techniques. PeerJ, 7, e7928.
Picard C, Ward M, Benko-Beloglavec A, Matthews-Berry S, Karadjova O, Pietsch M & Van Der Gaag D, 2017. A methodology for preparing a list of recommended regulated non-quarantine pests (RNQP s). EPPO Bulletin, 47 (3), 551-558.
Reingold V, Lachman O, Blaosov E & Dombrovsky A, 2015. Seed disinfection treatments do not sufficiently eliminate the infectivity of Cucumber green mottle mosaic virus (CGMMV) on cucurbit seeds. Plant Pathology, 64 (2), 245-255.
Sabra A, Al Saleh MA, Alshahwan IM & Amer MA, 2021. First Report of Tomato Brown Rugose Fruit Virus Infecting Tomato Crop in Saudi Arabia. Plant Disease. Available online: https://doi.org/10.1094/pdis-05-21-1065-pdn
Salem N, Mansour A, Ciuffo M, Falk BW & Turina M, 2016. A new tobamovirus infecting tomato crops in Jordan. Archives of Virology, 161 (2), 503-506. Available online: https://doi.org/http://dx.doi.org/10.1007/s00705-015-2677-7
Samarah N, Sulaiman A, Salem N & Turina M, 2020. Disinfection treatments eliminated tomato brown rugose fruit virus in tomato seeds. European Journal of Plant Pathology, 1-10.
TuinbouwAlert, 2019. Statement ToBRFV-resistentie bij paprikarassen. Plantum Available online: https://www.tuinbouwalert.nl/dossiers/tobrfv-in-tomatengewas/
Van de Vossenberg BT, Visser M, Bruinsma M, Koenraadt HM, Westenberg M & Botermans M, 2020. Real-time tracking of Tomato brown rugose fruit virus (ToBRFV) outbreaks in the Netherlands using Nextstrain. bioRxiv.
Zinger A, Lapidot M, Harel A, Doron-Faigenboim A, Gelbart D & Levin I, 2021. Identification and Mapping of Tomato Genome Loci Controlling Tolerance and Resistance to Tomato Brown Rugose Fruit Virus. Plants, 10 (1), 179.