

Pest specific plant health response plan:

Outbreaks of tomato brown rugose fruit virus (ToBRFV)



Figure 1. Tomato infected with tomato brown rugose fruit virus (courtesy of Neil Giltrap).

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Any enquiries regarding this document should be sent to us at:

The UK Chief Plant Health Officer

Department for Environment, Food and Rural Affairs

Room 11G32

York Biotech Campus

Sand Hutton

York

YO41 1LZ

Email: plantpestsrisks@defra.gov.uk

www.gov.uk/defra

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Executive summary

	Background			
Regulation	GB Quarantine pest			
Key Hosts	Tomatoes and peppers			
Distribution	Austria, Belgium, Bulgaria, China, Czech Republic, Estonia, France, Greece, Hungary, Italy, Malta, Mexico, the Netherlands, Norway, Poland, Portugal, Slovenia, Spain, Switzerland, Turkey, UK, USA and Uzbekistan			
Key pathways	Plants for planting including seed			
Industries at risk	Tomato and pepper growers			
Symptoms (2.3)*	 Mosaic patterning on leaves Leaf deformation Wilting and chlorosis of plant Necrosis of pedicels, calyxes, petioles and flowers Discolouration, deformation and necrosis of fruit 			
Surveillance				
Demarcated zones (5.33)	The infected premises (i.e. the infected glasshouse and its vicinity). Other premises in which staff/growers have visited or worked in, premises in which stock has been sent or received, and/or any other premises where there is a perceived risk may also be included.			
Surveillance activities (5.17-5.20)	Visual surveillance and symptomatic and asymptomatic sampling.			
Response measures				
	Response measures			
Interceptions (5.1-5.6)	 The consignment (excluding fruit) should be held until a diagnosis is made, Tracing exercises carried out where required. UKPHINs notification to be made Fruit is permitted to move to retail/wholesale, or to packhouses if 			
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(5.1-5.6) Outbreaks	 The consignment (excluding fruit) should be held until a diagnosis is made, Tracing exercises carried out where required. UKPHINs notification to be made Fruit is permitted to move to retail/wholesale, or to packhouses if adequate hygiene measures are in place Movement restrictions Removal and destruction of infected and at risk plants Post-crop clean up measures Key control measures 			
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* Numbers refer to relevant points in the plan

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1. Introduction and scope

- 1.1. This pest specific response plan has been prepared by the Defra Risk and Horizon Scanning team. It describes how the Plant Health Service for England will respond if an infection of tomato brown rugose fruit virus (ToBRFV) is discovered on imported plants, fruit and seed, and in a growing crop.
- 1.2. The plant health authorities in Northern Ireland, Scotland, Wales and the Crown Dependencies have been consulted on this plan and will use it as the basis for the action they will take in the event of ToBRFV being detected in their territories.
- 1.3. This document will be used in conjunction with the Defra Contingency Plan for Plant Health in England (https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/5935 08/generic-contingency-plan-plant-bee-health-england.pdf), which gives details of the teams and organisations involved in pest response in England, their responsibilities and governance. It also describes how these teams and organisations work together in the event of an outbreak of a plant health pest.
- 1.4. The aims of this response plan are to facilitate the containment and eradication of ToBRFV and to make stakeholders aware of the planned actions.

2. Summary of threat

- 2.1. ToBRFV was first observed in Israel in 2014, and in Jordan in the following year (EPPO, 2019a; Salem *et al.*, 2016). Since then, the virus has been officially reported from Austria, Belgium, Bulgaria, China, Czech Republic, Estonia, France, Greece, Hungary, Italy, Malta, Mexico, the Netherlands, Norway, Poland, Portugal, Slovenia, Spain, Switzerland, Turkey, the UK, the USA and Uzbekistan (EPPO Reporting Service, 2019a, c, d, h p, 2020a e, 2021 a l). Outbreaks of the virus have been eradicated in Germany and the UK (EPPO Reporting Service, 2019f, g, 2022).
- 2.2. Confirmed natural hosts of ToBRFV include tomato (Solanum lycopersicum) and pepper (Capsicum annuum) (Luria et al., 2017; Salem et al., 2016, 2019). The virus has also been detected in natural weed species Chenopodium murale (nettle-leaved goosefoot) and Solanum nigrum (black nightshade) in Israel, and experimentally in Chenopodium amaranticolor, C. quinoa (quinoa), Nicotiana bethamiana (benth), N. clevelandii (Cleveland's tobacco), N. glutinosa (tobacco), N. tabacum (cultivated tobacco) and Petunia hybrida (petunia) (Dombrovsky personal communication, 2019; Luria et al., 2017).

- 2.3. Symptoms of the virus include mosaic patterning and deformation of leaves, necrosis of pedicels, calyces, petioles and flowers, and discoloration, deformation and necrosis of fruit (EPPO, 2019b). In severe cases, ToBRFV may lead to the wilting and yellowing, and eventually the death, of the plant (EPPO, 2019b; Wilstermann and Ziebell, 2019).
- 2.4. ToBRFV can infect up to 100% of a crop and cause yield losses of between 25 and 70% (Alkowni *et al.*, 2019; Avni *et al.*, 2020; FDACS, 2019; Salem *et al.*, 2016). These yield losses are the result of symptomatic fruit being unmarketable and the production period being shorter for less vigorous infected crops (EPPO, 2019b). Other economic costs, aside from direct yield losses, include hygiene and eradication costs, export costs, and the costs of switching to a non-host crop in a specialised tomato and/or pepper production facility (EPPO, 2019b). There are also potential social impacts for gardens and allotments, as well as for temporary workers in tomato and pepper production if not as many jobs are generated during the growing season (EPPO, 2019b).
- 2.5. The main pathways for long distance spread of the virus are seed, plants for planting and fruit (EPPO, 2019b). The virus can spread locally by mechanical transmission on people, equipment, machinery, bees and via plant to plant contact, as well as in soil, water and nutrient film solutions (EPPO, 2019b; Levitsky et al., 2019; Luria et al., 2017; Smith et al., 2019). And as for other tobamoviruses, the virus can remain stable on a number of different surfaces for weeks and months (Skelton unpublished data, 2019).
- 2.6. In the UK, the first outbreak of ToBRFV was in a tomato glasshouse in Kent in July 2019 (EPPO Reporting Service, 2019p). Phytosanitary measures, including the removal and destruction of all tomato plants, the disinfection of the glasshouse, and a 14-week period of plant freedom, were taken to eradicate the virus (EPPO Reporting Service, 2019p). This outbreak has now been declared eradicated (EPPO Reporting Service, 2020e). However, as of July 2020, ToBRFV had been confirmed at five other sites (EPPO Reporting Service, 2020d, e). Phytosanitary measures to eradicate the virus were taken in all cases (EPPO Reporting Service, 2020d, e), and the pest was declared eradicated from the UK in January 2022. However, one UK site has since become re-infected and eradication measures are being applied at this outbreak site.
- 2.7. As of April 2020, there have been many interceptions of the virus on tomato and pepper seed in the UK from a variety of countries including Bulgaria, Chile, China, India, Indonesia, Israel, Lithuania, the Netherlands, Peru, Spain and the USA. Additionally, a further seven interceptions have been made on fruit six on tomatoes from the Netherlands and one on peppers from Spain.

3. Risk assessments

- 3.1. ToBRFV has an unmitigated and mitigated UK Plant Health Risk Register score of 48. Overall scores range from 1 (very low risk) to 125 (very high risk). These scores are reviewed as and when new information becomes available (https://planthealthportal.defra.gov.uk/pests-and-diseases/uk-plant-health-risk-register/viewPestRisks.cfm?cslref=28537).
- 3.2. Pest risk analyses have been carried out by EPPO, Germany and Italy (EPPO, 2019b; Tomassoli *et al.*, 2019; Wilstermann and Ziebell, 2018). These analyses concluded that ToBRFV has the potential to establish and cause significant economic damage to tomato crops in their respective regions.

4. Actions to prevent outbreaks

- 4.1. ToBRFV is listed in <u>Schedule 1</u> of <u>The Plant Health (Phytosanitary Conditions)</u> (<u>Amendment) (EU Exit) Regulations 2020</u>. Schedule 1 is the list of GB quarantine pests that are absent from GB and as such they are prohibited from being introduced into, moved within or held, multiplied or released into GB. Further pest and host specific requirements are listed in <u>Schedule 7</u>.
- 4.2. In addition to the above measures, there is a derogation of the import requirements for tomato and pepper seed harvested prior to 15th August 2020 to allow import of this seed from third countries without the need for an inspection of mother plants until 31st December 2022. This seed must still be sampled and tested for ToBRFV in line with the Plant Health (Phytosanitary Conditions) (Amendment) (EU Exit) Regulations 2020.
- 4.3. ToBRFV is an EPPO A2 listed pest and is therefore recommended for regulation by EPPO member countries, despite being present in the EPPO region.
- 4.4. The Plant Health Service should be aware of the measures described in this plan and be trained in responding to an outbreak of ToBRFV. It is important that capabilities in detection, diagnosis, and risk management are available.

5. Response

Official action to be taken following the suspicion or confirmation of ToBRFV on imported plants, fruit and seed e.g. at a packhouse

- 5.1. If ToBRFV is suspected by the Plant Health and Seeds Inspectorate (PHSI) to be on a consignment of plants and seeds moving in trade, the PHSI should hold the consignment at the point of entry (PoE) until a diagnosis is made. Other consignments that are at risk of cross-contamination should also be held pending a risk assessment on whether cross-contamination has or could have potentially occurred. Samples should be sent in by the PHSI to Plant Clinic, Fera Science Ltd, York Biotech Campus, Sand Hutton, York, YO41 1LZ (01904 462000) in a sealed bag or container, within at least two other layers of containment, which are not liable to be crushed during transit.
- 5.2. If ToBRFV is confirmed, the PHSI should advise the client of the action that needs to be taken by way of an official plant health notice (but see exception for fruit in 5.4). The consignment of plants and seeds should be destroyed by either incineration, deep burial or another approved method (see 5.51-5.52). This is most likely to occur for seeds which are tested at the point of entry. The measures in 5.1 and 5.2 do not apply to fruit.
- 5.3. A UKPHINS (UK Plant Health Interception Notification System) notification should be made upon confirmation of an interception of ToBRFV (but see exception for fruit in 5.4). UKPHINS is the IT system for recording findings and non-compliance in order to maintain records and to notify other National Plant Protection Organisations (NPPO) of plant health issues.
- 5.4. The risk posed by fruit infected with ToBRFV is lower and as such statutory notices should be issued if ToBRFV is suspected on fruit, permitting the fruit to move direct to retail or wholesale prior to confirmation. If the fruit is being moved to other premises, such as packhouses, adequate hygiene measures should be in place to prevent cross-infection. Guidelines on hygiene measures should be placed on the notice.
- 5.5. If all or part of an actionable consignment has been distributed to other premises prior to diagnosis, trace forward and trace back inspections should take place upon suspicion or confirmation of ToBRFV. Details of recent past and future consignments from the same grower/supplier should also be obtained for tracing purposes.

5.6. A Defra pest factsheet to raise awareness of ToBRFV and its symptoms should be distributed to packers/processors and importers where ToBRFV has been found, and to those associated with the infected premises.

The pest factsheet can be found on the Plant Health Portal (https://planthealthportal.defra.gov.uk/assets/factsheets/ToBRFV-factsheet-v4.pdf).

Further information on ToBRFV can be found on the AHDB website (https://horticulture.ahdb.org.uk/knowledge-library/tomato-brown-rugose-fruit-virus-tobrfv).

Official action to be taken following the suspicion of a ToBRFV outbreak

- 5.7. Suspected outbreaks are generally being treated as business as usual (BAU) by the PHSI and are not being escalated further.
- 5.8. However, each new outbreak will be assessed on a case by case basis and, in particular situations, an Outbreak Triage Group (OTG), chaired by the Chief Plant Health Officer (CPHO) or their deputy and including specialists from APHA, Defra and other organisations, may need to be set up to assess the risk and decide on a suitable response
- 5.9. The OTG will set an alert status, which will consider the specific nature of the outbreak. Under most scenarios, a suspected outbreak of ToBRFV in a protected tomato or pepper crop is likely to be given either a black or white alert status. These alert levels, in order of increasing severity, are white, black, amber and red (more details on these levels can be found in table 2 of the Defra Generic Contingency Plan for Plant Health in England. A black alert status refers to a significant plant pest with potential for limited geographical spread leading to moderate economic, environmental or social impacts, while a white alert status refers to a plant pest which does not require statutory action or that can be managed as part of routine plant health activities (e.g. a pest with a management SOP). In the case of a black alert, the CCG will decide who will be the control authority (likely APHA), and the control authority will then nominate an Incident Controller. An Incident Management Team (IMT) meeting, chaired by the Incident Controllerr, will subsequently convene to produce an Incident Action Plan (IAP). See the Defra Generic Contingency Plan for Plant and Bee Health in England for full details. In the case of a white alert, an IMT will not be required and management of the outbreak can be coordinated outside of the formal outbreak management process.

Restrictions on movement of plants, plant products, material, equipment and machinery to and from the place of production

- 5.10. ToBRFV is associated with plants for planting (including seed), fruit, and living parts of plants (EPPO, 2019b), so these should be prevented from leaving the affected glasshouse (and wider site if considered a risk), other than for destruction by deep burial, incineration or another approved method (see 5.51-5.52).
- 5.11. The virus can also be transmitted mechanically on material, equipment and machinery, and can survive for long periods outside of the plant (Luria et al., 2017; Skelton unpublished data, 2019). Movement of material, equipment and machinery between infected and non-infected areas should therefore be restricted. If, however, movement of any such items is necessary, they should be thoroughly cleaned and disinfected at the designated outbreak site.
- 5.12. The movement of personnel into an infected area should be restricted, especially during the early investigation phase and/or if ToBRFV is detected. Movement of personnel between packing and production sites, and to other production sites, should also be restricted, and strict hygiene practices put in place where movement is essential.

General biosecurity advice and advisory measures for growers

- 5.13. Given the potential for ToBRFV to be transmitted mechanically, good hygiene practice, as described in EPPO (2019b) should be followed as below, both prophylactically and following suspicion/confirmation of the virus:
 - Training staff to recognise symptoms of ToBRFV and to use good hygiene practices
 - Monitoring of the crop for symptoms of ToBRFV
 - Assigning equipment and workers to particular sections of the glasshouse, and ensuring workers pass through a hygiene lock upon entry and exit of each section
 - Maintaining the working direction. If human-assisted spread of a pathogen occurs, it will occur in the direction that the human is working. Working in the same direction reduces the extent of the spread and allows measures to be carried out in a more concentrated way.
 - Washing and disinfecting equipment. This should be done between every row and ideally between individual plants, but at least between crops.
 - Using disposable gloves, which should be replaced regularly

- Using disposable clothing, which should only be used when entering the glasshouse or section of the glasshouse and removed upon leaving the glasshouse or section and not reused
- Prohibiting the consumption of fresh tomato and pepper on site e.g. in sandwiches
- Prohibiting the introduction of ornamental plants on site e.g. *Petunia*, which is an experimental host
- Restricting the introduction of items, such as jewellery, watches and phones
 into the glasshouse (Netherlands hygiene protocol, 2019). If a phone must be
 brought into the glasshouse, it must be placed in a sealed plastic disinfected
 cover (Netherlands hygiene protocol, 2019).
- Double bagging samples taken and disinfecting the outer packaging (Netherlands hygiene protocol, 2019)
- Cleaning and disinfection of the glasshouse following the removal of plants.
 There is an AHDB funded project looking at the efficacy of disinfectants
 (https://horticulture.ahdb.org.uk/knowledge-library/tomato-brown-rugose-fruit-virus-tobrfv).
- 5.14. Volunteer plants and weeds, such as *Chenopodium murale* and *Solanum nigrum*, may act as reservoirs for ToBRFV. Controlling these plants within and around glasshouses reduces the chance of the crop becoming infected and reduces the risk of survival and persistence of the pest in the event of an outbreak. Volunteer plants and weeds can be controlled mechanically (e.g. hoeing), chemically (e.g. herbicides), and manually (e.g. roguing).
- 5.15. Any fallen fruit and other debris that may harbour the virus should also be regularly removed and destroyed (see 5.51-5.52).

Preliminary trace forward / trace backward

- 5.16. If an infected consignment is considered as being the source of the suspect outbreak, investigations regarding the origins of infected consignments will be undertaken to locate other related and therefore potentially infected consignments moving to and from the site. If applicable the relevant NPPO should be contacted.
- 5.17. In addition to tracing investigations relating to consignments, trace forward/back investigations linked to equipment, machinery and personnel in the infected premise should also be made by the PHSI.

Confirming a new outbreak

How to survey to determine whether there is an outbreak

- 5.18. The following information should be gathered by the PHSI on the suspicion of an infection of ToBRFV, in accordance with ISPM 6; guidelines for surveillance (https://www.ippc.int/en/publications/615/):
 - The origin of the host plants and associated pathways (e.g. mechanical transmission routes), date of planting and plans for the date of succeeding crops
 - Any previous history of ToBRFV finds on site, in linked premises (e.g. packhouse) or neighbouring tomato or pepper production sites
 - Details of other premises or destinations where the potentially infected host plants/products have been sent
 - The growing system being used, i.e. rockwool, nutrient film technique or soil grown
 - Details of how waste material is disposed of
 - The layout of the premises and surrounding area, including a map of the fields/cropping/buildings, at risk growers, and details of neighbouring crops, especially any commercial or non-commercial hosts in glasshouses
 - Details of the host and rootstock variety, growth stage and any other relevant information
 - Description of the surrounding habitat, including all hosts e.g. Solanum weeds
 - Area and level of infection, including a description of symptoms (photos should be taken, but the camera/phone should be cleaned/disinfected following appropriate biosecurity protocols) and the location within the affected premise e.g. whether it is widespread across the planting, clustered in hotspots, or whether it is related to specific operations
 - The date and time the sample was taken
 - Current treatments/controls in place including the use of prophylactic treatments for pepino mosaic virus
 - Details of the movement of people, equipment, machinery etc. to and from the infected area
 - Cultural, biosecurity and working practices

- The name, address, email and telephone number of the person who found the pest and/or its symptoms, and the business owner
- 5.19. This information should be included on the plant pest investigation template.
- 5.20. Further to information gathering, samples of other plants, particularly those that are symptomatic, but possibly also those that are asymptomatic, should be taken to confirm the extent of the outbreak e.g. in associated glasshouses. This initial survey will be used to determine if it is an isolated finding or an established outbreak.
- 5.21. Finance for the surveys will depend on the individual circumstances of the outbreak and will normally be determined by Defra policy and the PHSI.

Sampling

- 5.22. Plants can be visually inspected for mosaic patterning and deformation of leaves, necrosis of pedicels, calyces, petioles and flowers, and discoloration, deformation and necrosis of fruit (EPPO, 2019b). However, the virus cannot be confirmed by symptoms alone due to its similarities with other viruses, such as related tobamoviruses and the potexvirus *Pepino mosaic virus* (Wilstermann and Ziebell, 2019).
- 5.23. Following the identification of a suspect plant, symptomatic parts of the plant (e.g. leaves, fruit and stems) should be placed in a sealed bag or container, with at least two other layers of containment, which are not liable to be crushed during transit. Plants should be handled with gloves. It is advisable to separate a lot of plants into zones, with gloves being changed between these zones, and to move from asymptomatic to symptomatic plants, to reduce spread across a lot. Gloves and other disposable items should be destroyed (via incineration or deep burial) following use.
- 5.24. Each sample should be labelled with full details of the sample number, location (including location within the premises), variety and suspect pest, and sent for confirmatory diagnosis as in point 5.1.

Diagnostic procedures

5.25. On arrival in the laboratory, samples will initially be screened for the presence of ToBRFV using an appropriate method. Real-time RT-PCR will be used for symptomatic plant material or fruit, seed for compliance testing and asymptomatic plant material from outbreak sites. The real-time RT-PCR method has been adapted from the International Seed Federation ISHI-veg protocol using CATA primers/probe (https://www.worldseed.org/wpcontent/uploads/2020/03/Tomato-ToBRFV_2020.03.pdf) and supplemented using the Menzel and Winter (in press)

- primer set. Symptomatic plant material or fruit may also be tested using ELISA as per DSMZ manufacturer's instructions.
- 5.26. The sample size of seed consignments for compliance testing will be 3000 seeds, unless it is a smaller consignment, in which case the sample size will be determined on a case by case basis.
- 5.27. In the event of a positive screening test, a preliminary result will be issued to the PHSI and the Defra Risk and Horizon Scanning team.
- 5.28. A confirmatory diagnosis will then be carried out using conventional PCR primers (Levitsky et al., 2019), and sequence analysis of the PCR product. The final confirmed result will then be reported to the PHSI and the Defra Risk and Horizon Scanning team. Confirmation will take about 10 days. For samples where the initial CT value is above 30 (the limit for conventional PCR), sub-samples will be reextracted and tested using a second real-time RT-PCR to confirm the result.

Criteria for determining an outbreak

5.29. An outbreak will be declared if there is confirmed evidence that ToBRFV is present and has spread or is likely to have spread beyond its original consignment, for example if the virus is found across multiple lots in a glasshouse or packhouse. If ToBRFV is detected at a port or confined to a particular consignment with no risk of spread, then an outbreak should not be declared.

Official Action to be taken following the confirmation of an outbreak

5.30. The scale of the outbreak will determine the size and nature of the IMT/management team and action.

Communication

- 5.31. The IMT/management team will assess the risks and communicate details to the IPPC and EPPO, in accordance with ISPM 17: pest reporting (https://www.ippc.int/en/publications/606/), as well as within Government to Ministers, senior officials and other government departments, devolved administrations, and agencies (e.g., the Environment Agency) on a regular basis as appropriate; and to stakeholders (e.g. relevant trade bodies).
- 5.32. A pest factsheet to raise awareness of ToBRFV and its symptoms should be distributed to packers/processors and importers where ToBRFV has been found, and to those associated with the infected premises. The factsheet may be sent to

other premises as appropriate. The AHDB ToBRFV webpage is also a good source of information (https://horticulture.ahdb.org.uk/knowledge-library/tomato-brown-rugose-fruit-virus-tobrfv).

Demarcated zones

- 5.33. After an outbreak has been confirmed, a demarcated area should be established that includes the infected premises (i.e. the infected glasshouse and its vicinity). Other premises in which staff/growers have visited or worked in, premises in which stock has been sent or received, and/or any other premises where there is a perceived risk, should be demarcated for surveillance.
- 5.34. If required, initial maps of outbreak sites should be produced by officials.
- 5.35. All areas of the infected premises and any suspect sites should be visually inspected, and any suspect samples should be sent for diagnosis. Asymptomatic samples should also be sent for diagnosis as appropriate.
- 5.36. The demarcated area should be adjusted in response to further findings.

Pest Management procedures

Propagation site

5.37. Host plants should not be moved off site, with the exception of plants being moved for destruction under statutory plant health notice. The crop should be immediately removed, and actions taken as per 5.44 – 5.47.

Production site

- 5.38. Host plants should not be moved off site, with the exception of :
 - plants being moved for destruction under statutory plant health notice
 - fruit that is being sold directly to retail/wholesale.
 - fruit that is moving to other production sites for packing under statutory plant health notice, provided there are deemed to be suitable hygiene measures in place to prevent infection of growing crops.
- 5.39. Plants exhibiting severe symptoms (indicating high levels of infection) should be cut at the stem base to kill the plants. Any fruit removed should be disposed of safely (by incineration, deep burial or another approved method as in 5.51-5.52), using good hygiene practices. It is advised that the cut plants are left *in situ* until the end of the growing season and not handled, but the grower could remove the plants earlier than this.

- 5.40. Precautionary measures described in points 5.13 5.15 should be followed.
- 5.41. Where pollinator beehives are used, action to minimise the spread of bumblebees will be taken on a case by case basis. If other growers' glasshouses with host crops are at risk of being infected by ToBRFV carried by bumblebees from the affected glasshouse, the beehives may need to be removed and destroyed and/or vents sealed (closed or covered in mesh) to prevent entry and exit of bumblebees.
- 5.42. If there are other glasshouses growing host crops on the same site or on adjacent sites, these should be regularly monitored for symptoms of the virus, and any suspect symptoms notified to the PHSI. Depending on the situation, sampling and testing of asymptomatic material may also be appropriate.
- 5.43. At the end of the harvesting period of the infected crop, the crop should be removed and actions taken as per 5.44 5.47.

Post-crop clean up

- 5.44. The following should be carried out under statutory notice:
 - Removal of all the infected crop and associated plant debris. This can be disposed of by incineration, deep burial or another approved method (5.51 – 5.52).
 - Once the infected crop has been removed, all remaining material e.g. string, plastic flooring, and growing media, should be destroyed by incineration, deep burial or another approved method, or recycled. Rockwool / coconut coir / other growing media can be recycled for non-horticultural use. For production systems that grow plants in soil, the soil may not be able to be removed and destroyed.
 - All areas of the glasshouse (aside from soil) should subsequently be cleaned
 with water and detergent to remove traces of organic matter, and then
 disinfected using appropriate disinfectants. Cleaning of surfaces prior to
 disinfection is essential as many disinfectants are inactivated by the presence
 of organic matter.
 - Measures should be taken to prevent the germination of self-sown tomato and pepper seeds prior to the introduction of a new crop e.g. using herbicide or salt treatment on areas where self-sown plants are likely to occur.
 - Prior to any pesticides being used the risk posed by the pesticide to people and the environment will be assessed.
 - Any applications should be made following the advice on the product label and be in accordance with HSE guidance. In some cases there

may be a requirement to carry out a Local Environment Risk Assessment for Pesticides (LERAP) depending on the product used and the situation of the finding.

- Water is also a potential route of transmission (EPPO, 2019b). As a
 precaution, the irrigation system should be decontaminated and cleaned out
 as per manufacturer guidelines at the end of the season. Water for
 hydroponic and irrigation systems should subsequently come from sources
 free from the virus, and, if possible, water should not be mixed between
 infected and non-infected lots.
- 5.45. Before the introduction of a new host crop, there should be a host crop-free period. The length of this period will be determined by the IMT/management team in discussion with the grower. During this period, the grower may want to take swabs of regularly used surfaces and have them tested (including the use of bioassay to ensure the virus is viable) to provide them with information on the presence of the virus. Swabs will not be carried out officially and are simply a management tool for the grower. If the plants are grown in soil, a longer crop free period may be required if soil cannot be removed, as the virus can persist in the soil for long periods. EPPO (2019b) advises a minimum of 1 year. Alternatively, the removal of soil (if possible e.g. if plants are potted) could be used in consultation with the IMT/management team. Growers may opt to use a break crop, such as cucumber.
- 5.46. After the new host crop has been planted, regular monitoring should be carried out to ensure that self-sown seedlings or potential host weeds are not growing in or in close proximity to the glasshouse. If any are found, these should be removed wearing disposable gloves, and both plants and gloves disposed of by incineration, deep burial or another approved method. Early removal is important, as self-sown seedlings from the infected crop can transfer ToBRFV to the next crop.
- 5.47. An official inspection at production sites should be conducted at an appropriate time for symptom development, with a possible follow up inspection carried out later in the growing season if no symptoms are seen, to check the following crop for symptoms of ToBRFV. Samples will also be taken of asymptomatic host material.

Measures to be taken in the case of detection of infection in fruit after harvest (e.g. during processing/packaging and grading)

- 5.48. The following should be designated as infected:
 - The lot from which the sample was taken
 - The waste from the infected lot, such as processed waste

- The equipment and other articles (e.g. machinery and packing material) which have been in contact with the lot
- The glasshouse where the lot was grown
- 5.49. Areas where potentially infected equipment, waste, and other articles, have been used should be inspected.
- 5.50. Refer to the pest management procedures section if ToBRFV is found in a glasshouse.

Disposal plan

- 5.51. The primary means of disposing of infected material and plants is through incineration (licensed) and deep burial. Deep burial may be done at an approved landfill site, or on the site or nearby farm, if practical and in agreement with the local Environment Agency. Incineration must comply with appropriate waste management regulations. If the material has to be moved off the premises, it should be contained within at least two sealed layers, if possible (e.g. small plant within two plastic bags) under statutory plant health notice.
- 5.52. Aside from incineration and deep burial, other viable methods of destruction may include anaerobic digestion, composting and recycling (e.g. of Rockwool slabs for non-horticultural use). However, these and any other methods should be agreed by the IMT/management team.
- 5.53. All objects designated as infected, such as equipment, machinery and storage facilities that may be contaminated with infected plant material or other items should be thoroughly cleaned and disinfected. This should be carried out at the outbreak site in agreement with a Plant Health and Seeds Inspector. Any waste material generated should be bagged and sent for deep burial, incineration or another approved method. There is an AHDB funded project looking at the efficacy of disinfectants, which can be drawn on for appropriate disinfectant recommendations (https://horticulture.ahdb.org.uk/knowledge-library/tomato-brown-rugose-fruit-virus-tobrfv).

6. Criteria for declaring eradication / change of policy

6.1. ToBRFV can be declared eradicated (by the Chief Plant Health Officer) after a full cycle of the next host crop, if it has not been found following inspection and sampling of the new crop. In cases where no crop, or a non-host crop, is intended

to be grown in the affected glasshouse(s) for more than one year following the outbreak and clean up, eradication can be declared one year after eradication action, without the need for follow up inspection and sampling. As a grower may not know immediately what they intend to plant more than one year following the outbreak, the decision on whether eradication can be declared will be made at least one year after eradication action.

7. Evaluation and review of the contingency plan

- 7.1. This pest specific contingency plan should be reviewed regularly in order to consider any changes in legislation, control procedures, pesticides, sampling and diagnosis methods, and any other relevant amendments.
- 7.2. Lessons should be identified during and after any outbreak (of ToBRFV or other pest), including what went well and what did not. These should be included in any review of the contingency plan leading to continuous improvement of the plan and response to outbreaks.

8. Appendix A

Data sheet for Tomato brown rugose fruit virus

Identity

PREFERRED SCIENTIFIC NAME	AUTHOR (taxonomic authority)
Tomato brown rugose fruit virus	ICTV accepted

SUPERKINGDOM: Viruses

FAMILY: Virgaviridae

GENUS: Tobamovirus

COMMON NAMES

Virus rugoso del tomate (ES)

Jordan-Virus (DE)

Notes on taxonomy, nomenclature and morphology

Tomato brown rugose fruit virus (ToBRFV), which was first described in 2016 (Salem *et al.*, 2016) is one of 37 Tobamoviruses (ICTV, 2019). Other viruses within the genus include tobacco mosaic virus (TMV), tomato mosaic virus (ToMV) and cucumber green mottle mosaic virus (CGMMV).

Tobamoviruses consist of a single stranded RNA molecule enclosed within a crinkled cylindrical capsid (Luria *et al.*, 2017). The RNA molecule is composed of four reading frames or ORFs. ORF1 and 2 encode non-structural proteins that form the replicase complex, ORF3 encodes the non-structural movement protein, and ORF4 encodes the coat protein (Luria *et al.*, 2017).

Biology and ecology

Life history

Like most other tobamoviruses, ToBRFV does not infect the embryo of a seed, and instead contaminates the seed coat (Dombrovsky personal communication, 2019). In this way, ToBRFV can be preserved on the seed for several years (Dombrovsky and Smith, 2017).

Seed-to-seedling transmission is very low for most tobamoviruses, as transmission often fails when the seed coat separates from the seedlings. There is no published evidence of ToBRFV passing from the seed to seedling, though it is highly suspected to occur (Dombrovsky personal communication, 2019).

In plants, small wounds allow ToBRFV to enter and reproduce (Dombrovsky and Smith, 2017). The virus moves from cell to cell by way of its movement protein, and moves longer distances within the plant by way of its replicase complex (Dombrovsky and Smith, 2017; EPPO, 2019b).

Outside of the plant, ToBRFV can survive for long periods on a number of surfaces; the virus can survive on hard plastic and polythene for at least 6 months, on glass and stainless steel for at least 3 months, on aluminium for at least 4 weeks, on concrete for at least 1 week, and on skin and gloves for at least 2 hours (Skelton unpublished data, 2019). ToBRFV may also survive in clay for years (Dombrovsky unpublished data, 2019). This is supported by the related tobamovirus ToMV remaining infective in dry soil and powdered leaf debris for 2 years (EPPO, 2019b). ToMV's survival was heavily influenced by the moisture content, however, and in moist soil, infectivity of ToMV was lost within a month (EPPO, 2019b).

Hosts/crops affected

Confirmed natural hosts of ToBRFV include tomato (*Solanum lycopersicum*) and pepper (*Capsicum annuum*) (Luria *et al.*, 2017; Salem *et al.*, 2016, 2019). The virus has also been detected in natural weed species *Chenopodium murale* and *Solanum nigrum* in Israel (Dombrovsky, personal communication, 2019). Experimentally, ToBRFV has been demonstrated to infect *Chenopodium amaranticolor*, *C. quinoa*, *Nicotiana bethamiana*, *N. clevelandii*, *N. glutinosa*, *N. tabacum* and *Petunia hybrida* (Luria *et al.*, 2017). Transmission has been attempted in potato (*Solanum tuberosum* cv. Nicola, cv. Sephora, cv. Georgina, cv. Regina and cv. Mozart) as well, but infection did not occur (Luria *et al.*, 2017; Dombrovsky, personal communication, 2019).

In tomato, ToBRFV can break the Tm-2² resistance gene, which gives resistance against TMV and ToMV (Luria *et al.*, 2017). While in pepper, ToBRFV seems unable to break the L¹, L³ and L⁴ resistance genes, which give resistance against tobamoviruses (EPPO, 2019b).

Plant stage affected

ToBRFV affects the fruiting stage and the vegetative growing stage.

Plant parts affected

ToBRFV affects the leaves, pedicel, calyces, petioles and fruit.

Symptoms/signs - description

Whole plant

Infection by ToBRFV may lead to wilting and yellowing, and eventually the death, of the plant (EPPO, 2019b; Wilstermann and Ziebell, 2019).

Leaves

Symptoms include light to strong mosaic patterning, deformation (e.g. puckering and narrowing), blistering, and smaller or wilted leaves (EPPO, 2019b; Tomassoli *et al.*, 2019; Wilstermann and Ziebell, 2018).

Pedicel (stem), calyces, petioles and flowers

Necrosis may be observed (EPPO, 2019b; Fidan et al., 2019; Tomassoli et al., 2019).

Fruit

In spite of the virus's name, brown rugose symptoms are rarely seen, and fruit generally suffers from discoloration (e.g. chlorotic marbling and dark spots), uneven ripening, deformation, small fruit, and necrosis (EPPO, 2019b; Fidan *et al.*, 2019; Tomassoli *et al.*, 2019; Wilstermann and Ziebell, 2018)



Figure 2. Leaf deformation (Courtesy of Neil Giltrap).



Figure 3. Leaf deformation (Courtesy of Neil Giltrap).



Figure 4. Fruit discoloration (Courtesy of Neil Giltrap).



Figure 5. Fruit discoloration (Courtesy of Neil Giltrap).

Detection and inspection methods

ToBRFV cannot be confirmed from its symptoms alone, as its symptoms bear many similarities to other viruses, including the tobamoviruses TMV and ToMV, and the potexvirus *Pepino mosaic virus* (Wilstermann and Ziebell, 2019). Molecular analysis is therefore required.

An enzyme-linked immunosorbent assay (ELISA) can be used to detect tobamoviruses, including ToBRFV, but it cannot be used to detect ToBRFV specifically, as it cross-reacts with other tobamoviruses (Dombrovsky and Smith, 2017; Tomassoli *et al.*, 2019).

Instead, ToBRFV is identified using universal generic primers in RT-PCR followed by sequencing of the amplicons. The whole genome has been sequenced for the German, Italian, Israeli and Jordan isolates (Alkowni *et al.*, 2019; Luria *et al.*, 2017; Panno *et al.*, 2019; Salem *et al.*, 2016) and genome sequences from China, Germany, Italy, Israel, Jordan, Mexico, Turkey and the UK have been deposited in the GenBank database (https://www.ncbi.nlm.nih.gov/genbank/). Specific primers for ToBRFV have also been developed for RT-PCR, such as those developed by Alkowni *et al.* (2019) and Cambróncrisantos *et al.* (2018), but these have yet to be fully validated.

Distribution

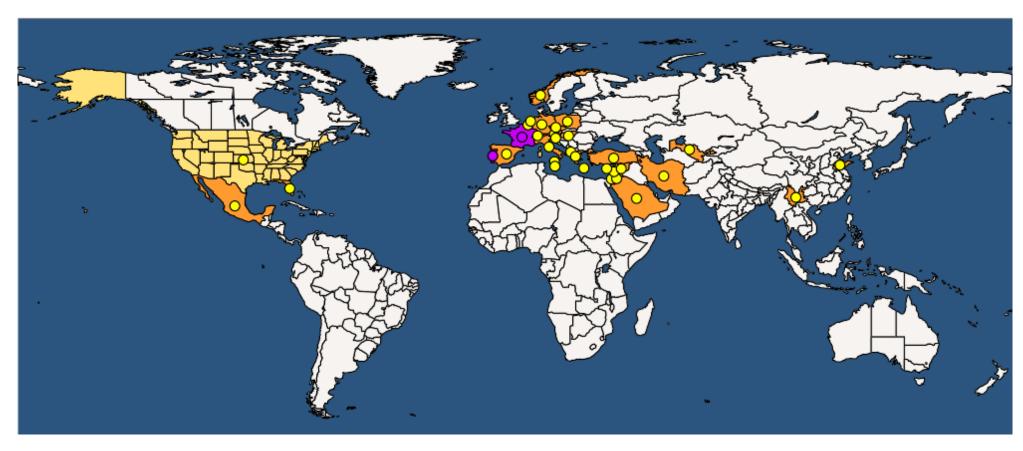


Figure 2. ToBRFV distribution as of May 2022. (Source: EPPO Global database). The link below provides up to date distribution data. https://gd.eppo.int/taxon/TOBRFV/distribution

History of introduction and spread

Global spread

ToBRFV was first observed in Israel in 2014, and in Jordan in the following year (EPPO, 2019a; Salem *et al.*, 2016). Since then, the virus has been officially reported from Austria, Belgium, Bulgaria, China, Czech Republic, Estonia, France, Greece, Hungary, Italy, Malta, Mexico, the Netherlands, Norway, Poland, Portugal, Slovenia, Spain, Switzerland, Turkey, the UK, the USA and Uzbekistan (EPPO Reporting Service, 2019a, c, d, h - p, 2020a - e, 2021 a - I). Outbreaks of the virus have been eradicated in Germany and the UK (EPPO Reporting Service, 2019f, g, 2022).

ToBRFV is likely to be more widely distributed than is currently known, as it is an emerging pest, having only been formally described in 2016, and not all countries have access to the appropriate molecular tests to identify the virus (EPPO, 2019b). This is supported by unconfirmed reports of the virus in Canada, Chile, Ethiopia, Saudi Arabia, and Sudan, as well as confirmation of the virus in imports to Mexico from Canada, Guatemala, India, Kenya, Morocco, Peru, Thailand and Vietnam, and confirmation that seed grown on to produce infected plants in Sicily came from France and Peru (ASTA, 2018; EPPO, 2019b; OGVG, 2019; Tomassoli, personal communication, 2019).

Israel and Palestine

The virus was first observed in tomato crops in Ohad (southern Israel) in 2014, and by February 2015, it had been found in a further three areas in the south: Melilot, Beit Ezra and Achituv (EPPO, 2019a). It has now been detected in the Ramat Negev region, the Arava valley, the Beit Shea area and, most recently, in Palestine (EPPO, 2019a).

Jordan

In April 2015, ToBRFV was observed affecting almost 100% of a greenhouse tomato crop (Salem *et al.*, 2016).

Mexico

As early as 2017, there was evidence of the virus spreading mechanically in tomato crops in Baja California Sur (Camacho-Beltrán *et al.*, 2019), and by February 2019, 117 outbreaks had been found in 20 states (Aguascalientes, Baja California, Baja California Sur, Chiapas, Chihuahua, Coahuila, Colima, Durango, Guanajuato, Hidalgo, Jalisco, Michoacán, Morelos, Puebla, San Luis Potosi, Sinaloa, Sonora, Tamaulipas, Yucatán, Zacatecas) (EPPO Reporting Service, 2019d). Phytosanitary measures were applied to minimise movement of the virus into and within Mexico. These included measures on the importation of cuttings, plants, seeds and seedlings of tomato, pepper and aubergine, and the national regulation of propagative material of these plants (EPPO Reporting Service, 2019d).

Germany

ToBRFV was recorded in seven greenhouses growing tomatoes in North Rhine-Westphalia in July 2018 (EPPO Reporting Service, 2019f; Menzel *et al.*, 2018). On average, around 10% of the plants were symptomatic (EPPO Reporting Service, 2019f). Phytosanitary measures, including the clearing and destruction of tomato plants in the affected greenhouses, and the disinfection of the greenhouses and materials used in tomato production or in the clearance of the greenhouses, were applied to eradicate the virus (EPPO Reporting Service, 2019f). In the following year, new plants in the greenhouses were sampled for the virus. The virus was not found and the outbreak was declared eradicated (EPPO Reporting Service, 2019g).

USA

In September 2018, the virus was recorded in a tomato greenhouse in Santa Barbara County, California (EPPO Reporting Service, 2019e). All of the infected and symptomatic plants were destroyed, and the outbreak was declared eradicated (EPPO Reporting Service, 2019e). ToBRFV was detected again, however, in commercial tomato greenhouses in winter 2019-20 (EPPO Reporting Service, 2020a).

Italy

ToBRFV was first identified in one tomato greenhouse, affecting 10% of plants, in the municipality of Ipsica, Sicily, in December 2018 (EPPO Reporting Service, 2019i, j). Following surveys, a further seven locations harbouring the virus were identified (EPPO Reporting Service, 2019k). As in Germany and the USA, phytosanitary measures have been applied to eradicate the virus (EPPO Reporting Service, 2019k). ToBRFV was also found in a greenhouse, affecting 15% of plants, in the municipality of Bra, Cuneo Province, Piedmont, in May 2019 (EPPO Reporting Service, 2019j). This outbreak is now considered to be eradicated after the application of phytosanitary measures (EPPO Reporting Service, 2019l). In 2020 the virus was confirmed in 11 additional sites in the Ragusa and Siracusa provinces and a further 3 findings in commercial tomato greenhouses was confirmed in 2021 in the provinces of Caltanissetta and Scicli. Additionally findings were made on the mainland in Toscana in 2020 and Apulia in 2021, all within commercial tomato greenhouses (EPPO Reporting Service, 2021p).

Turkey

Symptoms of the virus were first recorded on 20% of tomato plants in a greenhouse in Demre in January 2019 (EPPO Reporting Service, 2019o). Phytosanitary measures have been applied (EPPO Reporting Service, 2019o).

China

ToBRFV was observed in around 50% of tomato plants in 3 greenhouses in Yucheng, Shandong Province, in 2019 (EPPO Reporting Service, 2019a).

UK

ToBRFV was confirmed in a tomato glasshouse in Kent in July 2019 (EPPO Reporting Service, 2019p). Phytosanitary measures were taken to eradicate the virus. These included the removal and destruction of all the tomato plants, the disinfection of the glasshouse, and a 14 week period of plant freedom (EPPO Reporting Service, 2019p). This outbreak has now been declared eradicated (EPPO Reporting Service, 2020e). However, as of July 2020, ToBRFV had been confirmed at five other sites (EPPO Reporting Service, 2020d, e). Phytosanitary measures to eradicate the virus were taken in all cases and the virus was declared eradicated from the UK in 2022 (EPPO Reporting Service, 2020d, e, 2022). However, one UK site has since become re-infected and eradication measures are being applied at this outbreak site.

Greece

In August 2019, symptoms of ToBRFV were observed on the island of Crete in the regional unit of Chania (EPPO Reporting Service, 2019h). The plants were destroyed and surveys were carried out in the outbreak area (EPPO Reporting Service, 2019h). Following this, further findings have been made in Crete and on the mainland in the Peloponnese region, Central Greece, East Macedonia, Thrace and Attica in field grown and protected tomato crops (EPPO Reporting Service, 2020f).

The Netherlands

Symptoms of ToBRFV were observed in around 8% of plants at one site in the municipality of Westland in October 2019 (EPPO Reporting Service, 2019m). Phytosanitary measures were carried out and surveillance undertaken (EPPO Reporting Service, 2019m). Since October 2019, ToBRFV had been detected in 32 companies where it was eradicated from five and a further four have switched to production of non-host crops. In eight sites, eradication has not been successful and reinfection has been confirmed (EPPO Reporting Service, 2020g, 2021m).

Spain

In October 2019, ToBRFV was observed in 0.5% of tomato plants in a greenhouse in the municipality of Vícar, Almería province, Andalucía (EPPO Reporting Service, 2019n). Phytosanitary measures were applied and surveillance undertaken (EPPO Reporting Service, 2019n). It was later detected in the neighbouring municipalities of Almeria, Vicar and El Ejido following surveys in October 2020 (EPPO Reporting Service, 2021n).

France

In January 2020, symptomatic tomato plants were observed in a 2.4 ha greenhouse in the Bretagne region (EPPO Reporting Service, 2020b). Phytosanitary measures were carried out, including the destruction of plants and growing media, and the disinfection of facilities (EPPO Reporting Service, 2020b).

Poland

In April 2020, ToBRFV was confirmed in a greenhouse in the municipality of Barczewo (EPPO Reporting Service, 2020c). It was eradicated but has since been declared in the provinces of Podkarpackie and Michałowice in tomato and pepper seed lots respectively. Infected seeds were destroyed but a further finding was made in 4 lots of tomato in the province of Łódzkie in February 2021 (EPPO Reporting Service, 2021o).

Czech Republic

In August 2020, the virus was detected in a greenhouse of a breeding station of a seed company in a seed crop of *Capsicum annuum*. Official measures including prohibitions on movement and delimiting surveys were carried out and eradication measures were applied (EPPO Reporting Service, 2021a).

Belgium

In August 2020, the virus was detected in a single tomato plant in a University greenhouse, following tracing exercises carried out by the Dutch NPPO. All the plants and seeds were destroyed but the virus was detected again in December 2020 in a commercial tomato greenhouse which had sourced Dutch plantlets (EPPO Reporting Service, 2021b)...

Uzbekistan

In October 2020, the virus was detected in a commercial tomato greenhouse in the Ferghana region and a further finding was made in another tomato greenhouse in the region of Davlatobod in 2021. Official measures are being taken to control the virus (EPPO Reporting Service, 2021k).

Malta

ToBRFV was found in April 2021 at a greenhouse in Xewkija and a further two greenhouses in Mgarr producing tomatoes. All infected plants were destroyed (EPPO Reporting Service, 2021c).

Hungary

In May 2021, the virus was detected in two tomato greenhouses in the municipalities of Lébény and Forráskút. Eradication measures were implemented (EPPO Reporting Service, 2021d).

Norway

In May 2021, the virus was detected in tomato plants in a small producer in the municipality of Vestfold. Eradication measures were implemented (EPPO Reporting Service, 2021e).

Bulgaria

In June 2021, ToBRFV was detected in a tomato greenhouse in the municipality of Mezdra. Eradication measures were implemented, and infected plants were destroyed (EPPO Reporting Service, 2021d).

Austria

In June, 2021, the virus was detected in a commercial tomato greenhouse in the municipality of Münchendorf. Eradication measures were implemented including the destruction of all plants, fruits and growing medium (EPPO Reporting Service, 2021f).

Estonia

A finding of the virus in a commercial tomato greenhouse in the municipality of Saue vald was confirmed in July 2021. Eradication measures were implemented, and fruit had been allowed to be marketed for food (EPPO Reporting Service, 2021g). Eradication was declared in April 2022 following the termination of all agricultural activities at the production site (EPPO Reporting Service, 2022b).

Slovenia

In July 2021, ToBRFV was detected in a commercial tomato greenhouse in the municipality of Grosuplje. Eradication measures were implemented, and non-symptomatic fruit has been allowed to be marketed (EPPO Reporting Service, 2021h).

Switzerland

In July 2021, ToBRFV was detected in a commercial tomato greenhouse in the region of Ostschweiz. Eradication measures were implemented, and non-symptomatic fruit has been allowed to be marketed, if it is packed at the production site (EPPO Reporting Service, 2021i).

Portugal

ToBRFV was detected during annual surveys in August 2021 at two nurseries in the Algarve region. The virus was detected in seeds originating from China and Israel, some of which were used to produce plantlets. Eradication measures were implemented, and tracing exercises were carried out on potentially infected plantlets (EPPO Reporting Service, 2021j).

Phytosanitary status

ToBRFV is a GB Quarantine Pest. It is also an A2 listed pest for the EPPO region and is therefore recommended for regulation by EPPO member countries, despite its presence in the EPPO region. The virus is also present on several other phytosanitary lists (see table 2).

Table 2. Global phytosanitary categorisation of ToBRFV (EPPO, 2019).

Country/NPPO/RPPO	List	Year of addition			
AFRICA					
South Africa		2019 (year measures reported)			
AMERICAS					
Argentina	A1 list	2019			
Chile		2019 (year measures reported)			
Mexico		2019 (year measures reported)			
USA		2019 (year measures reported)			
ASIA					
Republic of Korea		2019 (year measures reported)			
Thailand		2019 (year measures reported)			
OCEANIA					
Australia		2019 (year measures reported)			
New Zealand		2019 (year measures reported)			
RPPO					
EPPO	Alert list	2019			
EU	Emergency measures	2019			

Means of movement and dispersal

Long distance spread

There are three main pathways of ToBRFV into the UK: on seed, on plants for planting, and on fruit.

Seeds and plants for planting

The virus can be preserved on the seed coat for several years (Dombrovsky and Smith, 2017), and while seed to seedling transmission has not been demonstrated for ToBRFV, it is highly likely to occur (Dombrovsky personal communication, 2019). Evidence of the

virus moving in seed has also been confirmed in Mexico, Italy and the UK (EPPO, 2019b; Tomassoli, personal communication, 2019).

Although there have been no official interceptions of the virus on plants for planting, this pathway is believed to be the cause of outbreaks in Germany and the UK (EPPO, 2019b). Plants for planting of Solanaceae are prohibited from entering GB from third countries other than those in Europe and the Mediterranean (Point 18, Annex 6A, The Plant Health (Phytosanitary Conditions) (Amendment) (EU Exit) Regulations 2020). This Regulation also requires that plants for planting of *S. lycopersicum* and *Capsicum* spp. and seeds of *S. lycopersicum* and *C. annuum* intended for planting meet requirements to prevent the introduction of ToBRFV (Point 7, Annex 7B and Point 2, Annex 8B), as well as requiring a plant passport for internal movements (Point 2, Annex 13).

Fruit

The virus has been intercepted on fruit in the UK, EU and the USA (EU, 2019; FDACS, 2019), and may be able to contaminate other fruit or containers if the infected fruit becomes damaged and sap is released (EPPO, 2019b). On growing sites, where imported fruit is stored or packed on the same site, there is the potential for the virus to be transmitted mechanically from infected fruit to the growing crop. Other pests, including PepMV, *Tuta absoluta* and *Thaumatotibea leucotreta*, have been shown to spread in similar situations (EPPO, 2010). Seeds of fruit imported for consumption may also be used for propagation (by amateur growers), and lead to the development of infected plants (EPPO, 2019b). As the risk of this pathway is deemed to be low there are currently no restrictions on the importation into, or movement within, GB of tomato and pepper fruit, as it is considered to be disproportionate.

Other pathways

Further pathways of long-distance spread include the movement of the virus on artificial materials, such as trays, tools, equipment and vehicles, and by people (EPPO, 2019b). The Europool System, for example, moves more than 1 billion rented trays per year and these can be moved between countries (Europool System, 2019). While trays in the Europool System are cleaned, disinfected and tested to ensure they are free of ToBRFV, there are likely to be other companies that transport materials internationally that do not clean and disinfect their materials to the same standard (EPPO, 2019b). People involved with the maintenance of glasshouses, or agronomists, visitors etc. also have the potential to mechanically transmit ToBRFV from infected to uninfected premises (EPPO, 2019b).

Local spread

The principle method of local spread is mechanical transmission, via people, equipment, machinery, and plant to plant contact, and is considered to be one of the main reasons behind the spread of ToBRFV in Israel (Luria *et al.*, 2017).

Significantly, bumblebees (*Bombus terrestris*), which are used for pollination of tomatoes worldwide, have been demonstrated to mechanically transmit the virus (Levitsky *et al.*,

2019). When bumblebee hives from ToBRFV infected glasshouses were placed into uninfected glasshouses, some of the tomato plants from the uninfected glasshouses became infected with the virus. Upon closer inspection, the virus was shown to coat the abdomen of the bumblebees, and therefore seems to be spread mechanically when the bumblebees vibrate their bodies during pollination.

ToBRFV can also move in soil, water, and nutrient film solutions (EPPO, 2019b; Smith *et al.*, 2019).

Control

Cultural controls and sanitary methods

Resistance

There are currently no resistant varieties of tomato (EPPO, 2019b), but research to develop new resistant varieties is being carried out (Lapidot M. personal communication, 2019). Peppers harbouring the L¹, L³ and L⁴ genes appear to be resistant to ToBRFV (Luria *et al.*, 2017).

Prevention

The best means of prevention is the use of virus free planting material (EPPO, 2019b).

Hygiene practices

The only means of removing the virus from a crop is by destroying the plants and following good hygiene practice. Good hygiene practices are listed below:

- Sorting and/or packing of plant produce from other sites should be restricted (EPPO, 2019b)
- Training staff to recognise symptoms of ToBRFV and to use good hygiene practices (EPPO, 2019b)
- Monitoring of the crop for symptoms of ToBRFV (EPPO, 2019b)
- Removing weeds, which may act as reservoirs for the disease, and animals, such as mice and birds, which could aid the spread of the virus (EPPO, 2019b)
- Assigning equipment and workers to particular sections of the glasshouse, and ensuring workers pass through a hygiene lock upon entry and exit of each section (EPPO, 2019b).
- Restricting the movement of containers, substrate, nutrient solutions etc. from infected premises (EPPO, 2019b)
- Restricting the movement of staff between packing and production sites, and to other production sites (EPPO, 2019b; Netherlands hygiene protocol, 2019)
- Washing and disinfecting equipment. This should be done between every row and ideally between individual plants, but at least between crops (EPPO, 2019b). There are a few disinfectants with viricidal activity, including Virkon S, but these

disinfectants have not yet been validated against ToBRFV (EPPO, 2019b). It is important that any organic matter is removed before using a disinfectant. If possible, equipment and containers can be heat treated to remove ToBRFV. Five minutes at 90°C and five minutes at 70°C + Virkon S can remove the virus (Fox personal communication, 2019).

- Using disposable gloves (EPPO, 2019b). A suitable hand washing procedure has yet to be validated.
- Disinfecting shoes using a spray or sanitary mat (EPPO, 2019b). Appropriate disinfection has yet to be validated. An alternative would be to use overshoes (EPPO, 2019b).
- Using disposable clothing, which should only be used when entering the
 glasshouse or section of the glasshouse, and removed upon leaving the
 glasshouse or section and not reused (EPPO, 2019b). Alternatively, clothing
 could be provided that is only used in the glasshouse or section and is regularly
 laundered at high temperature (EPPO, 2019b).
- Prohibiting the consumption of fresh tomato and pepper on site e.g. in sandwiches (EPPO, 2019b)
- Prohibiting the introduction of ornamental plants on site e.g. *Petunia*, which is an experimental host (EPPO, 2019b)
- Restricting the introduction of items, such as jewellery, watches and phones into the glasshouse (Netherlands hygiene protocol, 2019). If a phone must be brought into the glasshouse, it must be placed in a sealed plastic disinfected cover (Netherlands hygiene protocol, 2019). Glasses could be wiped with a disinfectant (Netherlands hygiene protocol, 2019).
- Double bagging samples taken and disinfecting the outer packaging (Netherlands hygiene protocol, 2019)
- Cleaning and disinfection of the glasshouse following the removal of plants (EPPO, 2019b)

Biological control

There are no biological controls available for ToBRFV.

Chemical control

There are no chemical controls available for ToBRFV.

Impacts

Economic impact

ToBRFV can infect up to 100% of a crop and cause yield losses between 25 and 70% (Alkowni *et al.*, 2019; Avni *et al.*, 2020; FDACS, 2019; Salem *et al.*, 2016). These yield losses are the result of symptomatic fruit being unmarketable and the shortening of the production period, as plants reduce in vigour and die prematurely (EPPO, 2019b). In

Israel, the shortening of the production period has meant that, in some cases, two crops are grown per year instead of one, increasing production costs (EPPO, 2019b).

The susceptibility of the crop is dependent on the variety, cultural practices and the climate (EPPO, 2019b). Tomatoes are more likely to be susceptible than peppers, for instance, as there are no tomato varieties known to be resistant to the virus, while in pepper, plants harbouring the L¹, L³ and L⁴ genes appear to be resistant to ToBRFV (Luria *et al.*, 2017). Glasshouse crops are also expected to be more susceptible than outdoor crops, as there is likely to be more handling and spread via mechanical transmission (Tomassoli *et al.*, 2019).

Aside from direct yield losses caused by the virus, other costs include:

- Hygiene and eradication costs e.g. Italy have reportedly spent €58,000 on eradication of the virus and €270,000 on compensating growers (Tomassoli personal communication, 2019)
- Export costs, due to heightened restrictions from the importing country (EPPO, 2019b)
- Costs of switching to a non-host crop, particularly for specialised tomato production premises, which have invested heavily in their facilities, equipment and staff (EPPO, 2019b).

Environmental impact

No environmental impact has been recorded. Hosts in the wider environment have so far only been infected experimentally (EPPO, 2019b).

Social impact

There are potential social impacts on garden and allotment tomatoes and peppers (EPPO, 2019b). This may be more significant in certain countries; in France, for instance, more tomatoes are produced in gardens (400,000 tonnes per year) than are purchased (371,000 tonnes per year) (Scandella, 2019). There may also be an impact for temporary workers in tomato and pepper production if not as many jobs are generated (EPPO, 2019b).

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10. Authors and reviewers

Authors:

Original: Matthew Everatt (Defra) (2020)

Revised by: Simon Honey (Defra) (2022)

Reviewers:

Adrian Fox (Fera Science Ltd.)

Melanie Tuffen (Defra)

Dominic Eyre (Defra)

Sharon Matthews-Berry (Defra)

Jane Barbrook (APHA)