Department
for Environment
Food & Rural Affairs

Pest specific plant health response plan: Outbreaks of tomato brown rugose fruit virus

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

This document is also available on our website at:

https://planthealthportal.defra.gov.uk/pests-and-diseases/contingency-planning/

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Contents

1. Introduction and scope 4

2. Summary of threat 4

3. Risk assessments 5

4. Actions to prevent outbreaks 6

5. Response 8

Official action to be taken following the suspicion or confirmation of ToBRFV on imported plants, fruit and seed e.g. at a packhouse 8

Official action to be taken following the suspicion of a *ToBRFV* outbreak 9

Official action to be taken following the confirmation of an outbreak 13

6. Criteria for declaring eradication / change of policy 16

7. Evaluation and review of the contingency plan 16

8. Appendix A 17

9. References 28

10. Authors and reviewers 31

1. Introduction and scope

1.1. This pest specific response plan has been prepared by the Defra Risk and Policy 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 and Wales 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 and Bee Health in England* (https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/593508/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 China, France, Greece, Italy, Mexico, the Netherlands, Poland, Spain, Turkey, the UK and the USA (EPPO Reporting Service, 2019a, c, d, h, i, j, k, l, m, n, o, p, 2020a, b, c, d, e). There has also been an outbreak of the virus in Germany, but this has been eradicated (EPPO Reporting Service, 2019f, g).

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). Following the application of Commission Implementing Decision 2019/1615 in November 2019, there are measures to reduce the likelihood of ToBRFV being introduced into the EU on seeds and plants for planting. However, EPPO (2019b) concluded that these measures are not sufficient to prevent entry on seeds and plants for planting. There are also no EU measures for fruit, leaving open the possibility of the virus spreading onto a growing crop if infected fruit is packed on the same premises as that crop (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 has been confirmed at five other sites (EPPO Reporting Service, 2020d, e). Phytosanitary measures to eradicate the virus are being taken in all cases (EPPO Reporting Service, 2020d, e).

2.7. As of April 2020, there have been eleven interceptions of the virus on tomato and pepper seed in the UK (eight interceptions on *S. lycopersicum*, one on *S. pimpinellifolium*, and two on *Capsicum*) that have been published on Europhyt. Eight of the interceptions originated from Israel, and the other interceptions originated from China, Thailand and the US.

3. Risk assessments

3.1. ToBRFV has an unmitigated and mitigated UK Plant Health Risk Register score of 48, which is a moderately high rating. These scores are reviewed as and when new information becomes available (https://secure.fera.defra.gov.uk/phiw/riskRegister/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 has been included on the EPPO Alert list and has therefore been highlighted by EPPO as potentially presenting a risk.

4.2. 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.

Prohibitions

4.3. In Annex VI of EU regulation 2019/2072 (https://eur-lex.europa.eu/eli/reg_impl/2019/2072/oj), plants for planting of Solanaceae (which includes *S. lycopersicum* and *Capsicum* spp.), other than seeds and the plants covered by Annex VI 15, 16, or 17, are prohibited from third countries, other than Albania, Algeria, Andorra, Armenia, Azerbaijan, Belarus, Bosnia and Herzegovina, Canary Islands, Egypt, Faeroe Islands, Georgia, Iceland, Israel, Jordan, Lebanon, Libya, Liechtenstein, Moldova, Monaco, Montenegro, Morocco, North Macedonia, Norway, Russia (only the following parts: Central Federal District (Tsentralny federalny okrug), Northwestern Federal District (Severo-Zapadny federalny okrug), Southern Federal District (Yuzhny federalny okrug), North Caucasian Federal District (Severo-Kavkazsky federalny okrug) and Volga Federal District (Privolzhsky federalny okrug)), San Marino, Serbia, Switzerland, Syria, Tunisia, Turkey and Ukraine.

4.4. Prohibited solanaceous plants can be imported and held under a plant health authorisation in quarantine conditions (usually for research purposes). Once work on the plants has been completed, destruction of the plants is normally required. However, given adequate testing, the plants can, in some cases, be released from the terms of the authorisation if they are shown to be free of pests and pathogens.

Phytosanitary certificates

4.5. In Annex XI Part A of EU regulation 2019/2072, a phytosanitary certificate is required for the following hosts of ToBRFV upon introduction into the EU:

- Plants for planting (of all hosts), other than seeds, from third countries other than Switzerland
- Parts of plants, other than fruits, of *S. lycopersicum*, from third countries other than Switzerland
- Fruits of Solanaceae from third countries other than Switzerland
- Seeds of *Capsicum* spp. from third countries other than Switzerland

Plant passports

4.6. In Annex XIII of EU regulation 2019/20172, a plant passport is required for the following hosts of ToBRFV when moved within the EU:

- All plants for planting, other than seeds

- Seed of *C. annuum* and *S. lycopersicum*, if it is moved within the scope of application of Directive 2002/55/EC (vegetable marketing directive covering all tomato seed and most pepper seed), and for which specific RNQPs have been listed according to Article 37(2) of Regulation 2016/2031 in Annex IV

- Seed of *C. annuum*, if it is moved within the scope of application of Directive 98/56/EC (ornamental marketing directive covering pepper seed not covered by 2002/55/EC), and for which specific RNQPs have been listed according to Article 37(2) of Regulation 2016/2031 in Annex IV

Commission Implementing Decision 2019/1615 to prevent the introduction into, and spread within, the Union of ToBRFV (https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:32019D1615)

This Decision must be followed by all EU member states, but how the decision is implemented is agreed at the national level.

4.7. The introduction into, and movement within, the Union of ToBRFV shall be prohibited.

4.8. All consignments of *S. lycopersicum* and *C. annuum* shall be officially checked at the point of entry into the Union or at the place of destination.

4.9. Member states shall ensure that any person, which may have plants infected with ToBRFV, are immediately informed of:

- The presence or suspected presence of ToBRFV

- The possible consequences and risks

- The measures to be taken to prevent the establishment and spread of ToBRFV

4.10. Member states shall conduct annual surveys for the presence of ToBRFV on host plants in their territory.

4.11. Requirements for introducing *S. lycopersicum* and *C. annuum* into the Union:

- Plants for planting (including seed) shall originate in a third country free of ToBRFV, as established by the National Plant Protection Organisation (NPPO), in accordance with the relevant International Standards for Phytosanitary Measures; or

- Plants for planting (including seed) shall originate in an area free from ToBRFV, as established by the NPPO, in accordance with the relevant International Standards for Phytosanitary Measures; or

- Plants for planting (other than seed) shall be produced in a production site which is registered and supervised by the NPPO in the country of origin and known to be free from ToBRFV on the basis of official inspections carried out at the appropriate time to detect the virus. These plants shall also derive from seed which either originates in areas free from ToBRFV or has undergone testing for ToBRFV on a representative sample using appropriate methods, and has been found to be free from ToBRFV; or

- Seed shall be officially sampled and tested for ToBRFV on a representative sample using appropriate methods, and found to be free from ToBRFV

4.12. Requirements for moving *S. lycopersicum* and *C. annuum* within the Union:

- Plants for planting (including seed) shall originate in an area free from ToBRFV, as established by the NPPO, in accordance with the relevant International Standards for Phytosanitary Measures; or

- Plants for planting (other than seed) shall originate in a production site where ToBRFV is known not to occur on the basis of official inspections carried out at the appropriate time to detect the virus. These plants shall also derive from seed which either originates in areas free from ToBRFV or has undergone testing for ToBRFV on a representative sample using appropriate methods, and has been found to be free from ToBRFV; or

- Seed shall be officially sampled and tested for ToBRFV on a representative sample using appropriate methods, and it shall have been found to be free from ToBRFV

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 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 Fera Science Ltd, 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 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.

5.3. A Europhyt notification should be made upon confirmation of an interception of ToBRFV (but see exception for fruit in 5.4).

5.4. The risk posed by fruit infected with ToBRFV will be assessed on a case-by-case basis. If the fruit poses no immediate risk to sites growing *S. lycopersicum* or *C. annuum* and is going for retail, then this is permitted, but all precautions should be followed to avoid contact of the infected fruit with equipment, material, staff etc. that enter production or propagation sites. As statutory action is not being taken in these cases, a notification will be sent directly by Plant Health Policy to the relevant NPPO.

5.5. In the event that all or part of an actionable consignment has not been held and 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 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 AHDB ToBRFV webpage is also a good source of information (https://ahdb.org.uk/knowledge-library/tomato-brown-rugose-fruit-virus).

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, a Contingency Core Group (CCG), 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 at strategic and operational levels. This may include gathering more information on the suspected outbreak, notification of ministers and senior officials, and agreeing a communications strategy in consultation with the ToBRFV steering group.

5.9. The CCG will set an alert status, which will take account of 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. A black alert status refers to a significant plant pest with potential for limited geographical spread, while a white alert status refers to a non-significant plant pest with potential for limited geographical spread. 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 commander. An Incident Management Team (IMT) meeting, chaired by the Incident Commander, 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.

Precautionary measures (by the grower)

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://ahdb.org.uk/knowledge-library/tomato-brown-rugose-fruit-virus).

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. Information obtained regarding the origins of infected consignments should be used to locate other related and therefore potentially infected consignments. The relevant NPPO of the exporting country should be contacted and sent delivery notes by Plant Health Policy. Information should also be obtained on the destination to which suspect consignments, including plants, seed and fruit, have been sent.

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/static/media/files/publication/en/2019/02/ISPM_06_2018_En_Surveillance_2018-05-20_PostCPM13.pdf):

- 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 (see Appendix III the Defra *Generic Contingency Plan for Plant and Bee Health in England*). As much of

this information should be gathered prior to the CCG as possible, but where not all of this information can be gathered in time, the most relevant information should be prioritised. The rest of the information can be gathered after the CCG.

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. 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 APHA inspector 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 APHA inspector 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 re-extracted and tested using a second real-time RT-PCR to confirm the result.

Criteria for determining an outbreak

5.29. 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. If it is found to have spread or likely to have spread beyond its original consignment, for example if the virus is found across multiple lots in a glasshouse or packhouse, then an outbreak should 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, EU 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 authorities, 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://ahdb.org.uk/knowledge-library/tomato-brown-rugose-fruit-virus).

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 either the PHSI or the Risk and Horizon Scanning team.

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 and fruit that may be sold directly to retail/wholesale. Fruit should not be moved to other production sites for packing unless there are suitable hygiene measures in place to prevent infection of growing crops. This should be decided by the IMT/management team. If the fruit is moved off site for packing it should be ensured that there is no risk of spread to other production sites from the reuse of packaging used for transport of infected fruit under statutory plant health notice. A notice will also be issued to sites where infected fruit is sent prior to being moved for retail/wholesale.

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.

- 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 in spring, with a possible follow up inspection carried out later in the season if no symptoms are seen, to check the following crop for symptoms of ToBRFV. The optimum time for inspection at propagation sites may vary. 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://ahdb.org.uk/knowledge-library/tomato-brown-rugose-fruit-virus).

6. Criteria for declaring eradication / change of policy

6.1. ToBRFV can be declared eradicated (by the Chief Plant Health Officer) if it has not been found following inspection and sampling of the new crop after an appropriate host crop-free period.

7. Evaluation and review of the contingency plan

7.1. The Defra *Generic Contingency Plan for Plant and Bee Health in England* requires that the pest specific plan is reviewed following an outbreak. This pest specific contingency plan should also be reviewed regularly in order to take account of 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-2^2 resistance gene, which gives resistance against TMV and ToMV (Luria *et al.*, 2017). While in pepper, ToBRFV seems unable to break the L^1, L^3 and L^4 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).

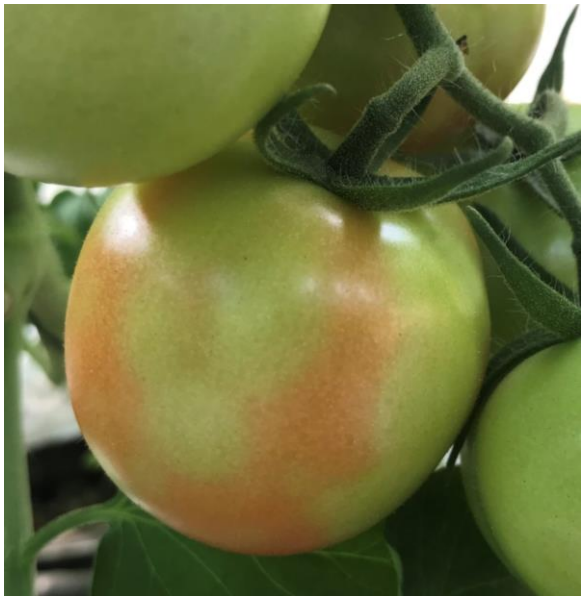

Figure 4. Fruit discoloration (Courtesy of Neil Giltrap).

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 Cambrón-crisantos *et al.* (2018), but these have yet to be fully validated.

Distribution

Table 1. Distribution of ToBRFV.

(P) present, (W) widespread, (L) localized, (O) occasionally present, (D) reported in the past, no longer present, (E) eradicated, (I) absent, intercepted only		
COUNTRY/REGION	**DISTRIBUTION** (see codes above)	**REFERENCES**: please write (name, date) citation here and include full bibliographic details in reference list
ASIA	P	
CHINA	P	EPPO Reporting Service (2019a); Yan *et al.* (2019)
Shandong	P	EPPO Reporting Service (2019a); Yan *et al.* (2019)
ISRAEL	P	Alkowni *et al.* (2019); EPPO (2019a); Levitzky et al. (2017); Luria *et al.* (2017)
JORDAN	P	Salem *et al.* (2016)
AFRICA	Absent	
NORTH AMERICA	P	
MEXICO	L	Camacho-Beltrán *et al.* (2019); Cambrón-Crisantos *et al.* (2018); EPPO Reporting Service (2019c, d)
USA	P	EPPO Reporting Service (2020a); Ling *et al.* (2019)
CENTRAL AMERICA & THE CARIBBEAN	Absent	
SOUTH AMERICA	Absent	
EUROPE	P	
FRANCE	L	EPPO Reporting Service (2020b)
GERMANY	E	EPPO Reporting Service (2019f, g); Menzel *et al.* (2019)
GREECE	P	EPPO Reporting Service (2019h)
Kriti	P	EPPO Reporting Service (2019h)
ITALY	L	EPPO Reporting Service (2019i, j, k, l); Panno *et al.* (2019)
Sicily	L	EPPO Reporting Service (2019i, k); Panno *et al.* (2019)
NETHERLANDS	L	EPPO Reporting Service (2019m)
POLAND	P	EPPO Reporting Service (2020c)
SPAIN	L	EPPO Reporting Service (2019n)

TURKEY	P	EPPO Reporting Service (2019o); Fidan *et al.* (2019)
UK	L	EPPO Reporting Service (2019p, 2020d, e); Skelton *et al.* (2019)
England	L	EPPO Reporting Service (2019p, 2020d, e); Skelton *et al.* (2019)
OCEANIA	Absent	

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 China, France, Greece, Italy, Mexico, the Netherlands, Poland, Spain, Turkey, the UK and the USA (EPPO Reporting Service, 2019a, c, d, h, i, j, k, l, m, n, o, p, 2020a, b, c, d, e). There has also been an outbreak of the virus in Germany, but this has been eradicated (EPPO Reporting Service, 2019f, g).

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 have been applied to minimise movement of the virus into and within Mexico. These include 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).

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 has been confirmed at five other sites (EPPO Reporting Service, 2020d, e). Phytosanitary measures to eradicate the virus are being taken in all cases (EPPO Reporting Service, 2020d, e).

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 are being carried out in the outbreak area (EPPO Reporting Service, 2019h).

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 are being carried out and surveillance undertaken (EPPO Reporting Service, 2019m).

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 are being applied and surveillance undertaken (EPPO Reporting Service, 2019n).

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 are being 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).

Phytosanitary status

In accordance with Commission Implementing Decision 2019/1615 (emergency decision), the introduction into, and movement within, the EU, of ToBRFV is prohibited. 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 and Italy (EPPO, 2019b; Tomassoli, personal communication, 2019). In accordance with Commission Implementing Decision 2019/1615, seed of *S. lycopersicum* and *C. annuum* imported into, or moved within, the EU must be accompanied by a phytosanitary certificate (or plant passport) and either originate from an area free of the virus or have been officially sampled and tested negative for the virus.

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 the EU from third countries other than those in Europe and the Mediterranean (Point 18, Annex IV, Commission Implementing Regulation 2019/2072). Commission Implementing Decision 2019/1615 also requires that plants for planting of *S. lycopersicum* and *C. annuum* imported into, or moved within, the EU must be accompanied by a phytosanitary certificate (or plant passport) and either originate from an area free of the virus or originate from a registered production site known to be free of the virus on the basis of inspection, and derive from seeds that originate from an area free of the virus or from seeds that have been tested negative for the virus.

While the measures for seed and plants for planting reduce the likelihood of ToBRFV being introduced into the EU, they do not eliminate the risk completely, as ToBRFV is an emerging pest and may be present in more countries than are currently known, the level of testing is not specified and a low level of seed testing, which may not pick up the virus, could be carried out, and there are no restrictions on *Capsicum* species, other than *C. annuum*, which are potential hosts of the virus.

Fruit

The virus has been intercepted on fruit in the 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). Despite the risk, however, there are currently no restrictions on the importation into, or movement within, the EU of tomato and pepper fruit.

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 L^1, L^3 and L^4 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 L^1, L^3 and L^4 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).

9. References

Alkowni, R., Alabdallah, O., and Fadda, Z. (2019) Molecular identification of tomato brown rugose fruit virus in tomato in Palestine. Journal of Plant Pathology. 101, 719-723.

ASTA (2018) Questions & Answers on the new Tobamovirus: Tomato Brown Rugose Fruit Virus (ToBRFV). ASTA (American Seed Trade Association). 1–5.

Avni, B., Gelbart, D., Sufrin-Ringwald, T., Zinger, A., Chen, L., Machbash, Z., Bekelman, I., Segoli, M., Dombrovsky, A., Kamenetsky, R., Levin, I. and Lapidot, M. (2020) Tomato genetic resistance to tobamoviruses is compromised. Acta Horticulturae. (in press).

Camacho-Beltrán, E., Pérez-Villarreal, A. and Leyva-López, N. A. (2019) Occurrence of Tomato brown rugose fruit virus infecting tomato crops in Mexico. Plant Disease 103, 1440.

Cambrón-Crisantos, J. M., Rodríguez-Mendoza, J., Valencia-Luna, J. B., Alcasio-Rangel, S., García-Ávila, C. J., López-Buenfil, J. A. and Ochoa-Martínez, D. L. (2018) First report of Tomato brown rugose fruit virus (ToBRFV) in Michoacan, Mexico. Revista Mexicana de Fitopatología 37, 1-8.

Dombrovsky, A. and Smith, E. (2017) Seed Transmission of Tobamoviruses: Aspects of Global Disease Distribution. In Advances in Seed Biology. Vol. Chapter 12, 233–260.

EPPO (2019a) *Tomato brown rugose fruit virus* (TOBRFV) [Online]. Available: https://gd.eppo.int/taxon/TOBRFV/distribution. Accessed: 18/01/2020.

EPPO (2019b) Draft pest risk analysis for *Tomato brown rugose fruit virus* (Tobamovirus) (in press).

EPPO Reporting Service (2010) Isolated finding of Thaumatotibia (Cryptophlebia) leucotreta on Capsicum chinensis in the Netherlands [Online]. Available: https://gd.eppo.int/reporting/article-321. Accessed: 18/01/2020.

EPPO Reporting Service (2019a) First report of tomato brown rugose fruit virus in China [Online]. Available: https://gd.eppo.int/reporting/article-6573. Accessed: 18/01/2020.
EPPO Reporting Service (2019c) First report of tomato brown rugose fruit virus in Mexico [Online]. Available: https://gd.eppo.int/reporting/article-6444. Accessed: 18/01/2020.
EPPO Reporting Service (2019d) Update of the situation of Tomato brown rugose fruit virus in Mexico [Online]. Available: https://gd.eppo.int/reporting/article-6622. Accessed: 18/01/2020.
EPPO Reporting Service (2019e) New data on quarantine pests and pests on the EPPO Alert List [Online]. Available: https://gd.eppo.int/reporting/article-6457. Accessed: 18/01/2020.
EPPO Reporting Service (2019f) First report of tomato brown rugose fruit virus in Germany [Online]. Available: https://gd.eppo.int/reporting/article-6442. Accessed: 18/01/2020.
EPPO Reporting Service (2019g) Tomato brown rugose fruit virus eradicated from Germany [Online]. Available: https://gd.eppo.int/reporting/article-6575. Accessed: 18/01/2020.
EPPO Reporting Service (2019h) First report of tomato brown rugose fruit virus in Greece [Online]. Available: https://gd.eppo.int/reporting/article-6640. Accessed: 18/01/2020.
EPPO Reporting Service (2019i) First report of tomato brown rugose fruit virus in Italy (Sicilia) [Online]. Available: https://gd.eppo.int/reporting/article-6443. Accessed: 18/01/2020.
EPPO Reporting Service (2019j) New outbreak of tomato brown rugose fruit virus in Italy (Piemonte) [Online]. Available: https://gd.eppo.int/reporting/article-6554. Accessed: 18/01/2020.
EPPO Reporting Service (2019k) Update on the situation of tomato brown rugose fruit virus in Sicilia (Italy) [Online]. Available: https://gd.eppo.int/reporting/article-6574. Accessed: 18/01/2020.
EPPO Reporting Service (2019l) Tomato brown rugose fruit virus eradicated from Piemonte (Italy) [Online]. Available: https://gd.eppo.int/reporting/article-6621. Accessed: 18/01/2020.
EPPO Reporting Service (2019m) First report of tomato brown rugose fruit virus in the Netherlands [Online]. Available: https://gd.eppo.int/reporting/article-6639. Accessed: 18/01/2020.
EPPO Reporting Service (2019n) First report of tomato brown rugose fruit virus in Spain [Online]. Available: https://gd.eppo.int/reporting/article-6668. Accessed: 18/01/2020.
EPPO Reporting Service (2019o) First report of tomato brown rugose fruit virus in Turkey [Online]. Available: https://gd.eppo.int/reporting/article-6553. Accessed: 18/01/2020.
EPPO Reporting Service (2019p) First report of tomato brown rugose fruit virus in the United Kingdom [Online]. Available: https://gd.eppo.int/reporting/article-6593. Accessed: 18/01/2020.
EPPO Reporting Service (2020a) Update of the situation of tomato brown rugose fruit virus in the USA [Online]. Available: https://gd.eppo.int/reporting/article-6802. Accessed: 14/07/2020.
EPPO Reporting Service (2020b) First report of tomato brown rugose fruit virus in France [Online]. Available: https://gd.eppo.int/reporting/article-6715. Accessed: 24/03/2020.
EPPO Reporting Service (2020c) First report of tomato brown rugose fruit virus in Poland [Online]. Available: https://gd.eppo.int/reporting/article-6800. Accessed: 14/07/2020.
EPPO Reporting Service (2020d) New outbreaks of tomato brown rugose fruit virus in the United Kingdom [Online]. Available: https://gd.eppo.int/reporting/article-6756. Accessed: 14/07/2020.
EPPO Reporting Service (2020e) Update of the situation of tomato brown rugose fruit virus in the United Kingdom [Online]. Available: https://gd.eppo.int/reporting/article-6801. Accessed: 14/07/2020.
EU (2019) Interceptions of commodities imported into the EU or Switzerland with harmful organism(s) [Online]. Available: https://ec.europa.eu/food/plant/plant_health_biosecurity/europhyt/interceptions_en. Accessed: November 2019.

Europool System (2019) ToBRFV Statement. (april), 1–8.

FDACS (2019) Virus in Mexican tomatoes causing concern, USDA action needed [Online]. Available: https://www.fdacs.gov/News-Events/Press-Releases/2019-Press-Releases/Virus-in-Mexican-Tomatoes-Causing-Concern-USDA-Action-Needed. Accessed: 18/01/2020.

Fidan, H., Sarikaya, P. and Calis, O. (2019) First report of Tomato brown rugose fruit virus on tomato in Turkey. New Disease Reports. 39, 18.

ICTV (2019) Genus: Tobamovirus [Online]. Available: https://talk.ictvonline.org/ictv-reports/ictv_online_report/positive-sense-rna-viruses/w/virgaviridae/672/genus-tobamovirus. Accessed: 18/01/2020.

Levitzky, N., Smith, E., Lachman, O., Luria, N., Mizrahi, Y., Bakelman, H., Sela, N., Laskar, O., Milrot, E., and 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.

Ling, K. S., Tian, T., Gurung, S., Salati, R., Gilliard A (2019) First report of Tomato brown rugose fruit virus infecting greenhouse tomato in the United States. Plant Disease 103, 1439.

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. and Dombrovsky, A. (2017) A new Israeli Tobamovirus isolate Infects tomato plants harboring Tm-22 Resistance Genes. PLoS ONE 12(1): e0170429.

Menzel, W., Knierim, D., Winter, S., Hamacher, J. and Heupel, M. (2019) First report of tomato brown rugose fruit virus infecting tomato in Germany. New Disease Reports. 39, 1.

Netherlands hygiene protocol (2019) Brief ToBRFV hygiene protocol STAFF [Online]. Available: https://www.tuinbouwalert.nl/content/docs/Dossiers/ToBRFV/Kort_hygi%C3%ABneprotocol/Kort_hygie%CC%88neprotocol_Tomaat_voor_Personeel_A3_ENGELS.pdf. Accessed: 18/01/2020.

OGVG (2019) Ontario Greenhouse Vegetable Growers (OGVG) communiqué to members [Online]. Available: http://thegrower.org/news/tomato-brown-rugose-fruit-virus-identified-ontario. Accessed: 18/01/2020.

Panno, S., Garuso, A. G. and Davino, S. (2019) First report of Tomato brown rugose fruit virus on tomato crops in Italy. Plant Disease. 103, 1443.

Salem, N., Mansour, A., Ciuffo, M., Falk, B. W., Turina, M. (2016) A new tobamovirus infecting tomato crops in Jordan. Archives of Virology. 161, 503-506.

Salem, N. M., Cao, M. J., Odeh, S., Turina, M. and Tahzima, R. (2019) First report of tobacco mild green mosaic virus and tomato brown rugose fruit virus infecting *Capsicum annuum* in Jordan. APS Publication. 1–3.

Scandella, D. (2019) Production de tomates dans les jardins potagers CTIFL. Les études économiques. (CTIFL. Dir).

Skelton, A., Buxton-Kirk, A., Ward, R., Harju, V., Frew, L., Fowkes, A., Long, M., Negus, A., Forde, S., Adams, I. P., Pufal, H., McGreig, S., Weekes, R. and Fox, A. (2019) First report of Tomato brown rugose fruit virus in tomato in the United Kingdom. New Disease Reports. 40, 12.

Smith, E., Luria, N., Reingold, V., Frenkel, O., Koren, A., Klein, E., Bekelman, H. and Lachman, O. (2019) Aspects in tobamovirus management in modern agriculture : Cucumber green mottle mosaic virus. Acta Horticulture. 1257, 1–8.

Tomassoli, L., Rosaria D'Anna, S. V. and Davino, S. (2019) Express Pest Risk Analysis: Tomato brown rugose fruit virus - ToBRFV.

Wilstermann, A. and Ziebell, H. (2018) Express – PRA1 for Tomato brown rugose fruit virus.

Wilstermann, A. and Ziebell, H. (2019) Tomato brown rugose fruit virus (ToBRFV). JKI Data Sheets – Plant Diseases and Diagnosis [Online]. Available:

https://www.openagrar.de/servlets/MCRFileNodeServlet/openagrar_derivate_00022213/Ziebell%20_ToBRFV%20englisch.pdf. Accessed: 18/01/2020.

Yan, Z-Y, Ma, H-Y, Han, S-L, Genc, C., Tian, Y-P and Li, X-D. (2019) First report of *Tomato brown rugose fruit virus* infecting tomato in China. Plant Disease. 103, 2973.

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