



## **March 2021 – update - PEST Report** (5<sup>th</sup> follow-up pest report of November 2019)

### **1.1 Official confirmation of Tomato brown rugose fruit virus (ToBRFV) in *Solanum lycopersicum* at 32 professional fruit production locations (closed conditions) since the first outbreak report of 17 October 2019.**

#### **1.2 Executive summary**

Following the first suspicion, national measures for ToBRFV were implemented on October 4th 2019, as based on the measures of EU implementing decision 2019/615 which entered into force on 1 November 2019.

#### **This update (fifth update):**

#### **Fruit production companies**

At the present time there are 23 professional fruit production locations where measures are in force. Since the last report the virus was confirmed at 2 more companies, totalling 32 production locations in total. One of these recent findings at fruit growers is linked to an earlier suspicion of a plant grower (see pest report December 2020). Investigations are ongoing. For 4 of these companies measures are on hold, in view of cultivation of non-host plants. For 5 five locations eradication was confirmed to be successful following sampling and testing of the new crop. For 8 locations eradication was not successful and the virus was again confirmed in the new crop.

Eradication measures can remain in force at production locations for a prolonged time. In cases whereby a crop is planted other than tomato or capsicum, companies are subject to retesting if in future again tomato or Capsicum would be cultivated.

The total area where measures are in force (23 production locations) totals 436.8 ha. Total area where the virus has been detected earlier totals 32 production locations (476.6 ha). For five production locations the virus has been eradicated (25.7 ha).

#### **Tracing investigations seed lots**

Most seed lots used by fruit growers where the virus was confirmed have been sampled and tested. This resulted in four positive seed lots. However, it was not possible to confirm whether these seeds had caused the infection of the fruit crop, since sequencing of the virus was not possible.

#### **Tracing investigations plant growers**

At one grower measures were imposed on the basis of positive testing outcomes of the young plants recently delivered to two fruit growers. Measures imposed at the plant grower are as follows:

- (i) Suspected or infested sites and all related sites were blocked and inspected
- (ii) Work protocols and hygiene standards were examined
- (iii) All sites: sampling of plants in case of symptoms
- (iv) All lots from sites where infested lot(s) originated have been subjected to visual inspection and sampling.
- (v) If all lots tested negative at a production site, plants could be delivered in The Netherlands after approval by the fruit grower.

### **Possible source of the outbreaks**

The source of the outbreaks is not known. Based on genome sequencing analysis it is concluded that probably at least 3 different introductions have occurred. Thus far positively tested seed lots could not be linked to specific outbreaks of fruit growers.

The organism is regulated as part of EU Commission implementing regulation 2020/1191.

Identity of the pest (scientific name) Tomato brown rugose fruit virus.

Categorization of the pest EU Commission implementing regulation 2020/1191.

Location: municipalities:

'Westland' (8 findings), 'Hollands Kroon' (5 findings), 'Brielle' (1 finding), 'Reimerswaal' (1 finding), 'Haarlemmermeer' (1 finding), 'Horst aan de Maas' (1 finding), 'Lansingerland' (1 finding), 'Zuidplas' (1 finding), 'Steenbergen' (1 finding), 'Goeree-Overflakkee' (1 finding), 'Westvoorne' (1 finding), 'Noordoostpolder' (1 finding)

Reason of the notification: Update report.

How the pest was found (1) pest related official survey;

Information on the infested area, severity and source of the outbreak – summary

### **Update information:**

Fruit growers reported losses varying between 5-30%. Some companies reported less than 5% loss. Other companies had to remove a crop and start a new crop. Extra costs of measures for official waste destruction amount to approximately Euro 5,000 – 10,000 per hectare. In addition extra costs are needed for hygiene measures.

At the first company visual symptoms were observed on approximately 8% of plants in a fruit production site of 2.8 ha. Fruits of affected plants showed a delay in ripening.

At other companies damage is variable, ranging from no damage to similar impact as for the first company.

### Official phytosanitary measures

Measures are aimed at eradication and preventing new introductions and spread of ToBRFV. At affected fruit production sites, strict hygiene measures are applied including restricting access, disinfection or replacement of clothing, machines, equipment, surfaces and packaging material. In particular use of the disinfection agent potassium peroxymonosulfate is recommended.

For any fruits harvested from the production place, specific hygiene measures are applied both at the fruit production place as well as the packing station, including cleaning and disinfection of packaging material.

Following removal of the crop, cleaning and disinfection of the greenhouse, the production site will be monitored, including testing at least six months after planting, to verify absence of the virus in the succeeding crop, before measures are lifted.

Specific surveillance has been intensified targeting fruit companies based on information from fruit growers, packaging stations, plant growers and private labs. Seed batches of related seed is tested as part of ongoing trace-back measures. In several cases this resulted in a positive finding of seed, which were notified in Europhyt.

<b>1.3 Type of notification</b>	(3) Update notification
<b>2.1 Single Authority</b>	Notification from the National Plant Protection Organization of the Netherlands – Netherlands Food and Consumer Product Safety Authority
<b>2.2 Official contact</b>	M.B. de Hoop. +31651584878 Email: <a href="mailto:m.b.dehoop@nvwa.nl">m.b.dehoop@nvwa.nl</a>
<b>3. Location of presence of harmful organism</b>	Municipalities: 'Westland' (8 findings), 'Hollands Kroon' (5 findings), 'Brielle' (1 finding), 'Reimerswaal' (1 finding), 'Haarlemmermeer' (1 finding), 'Horst aan de Maas' (1 finding), 'Lansingerland' (1 finding), 'Zuidplas' (1 finding), 'Steenbergen' (1 finding), 'Goeree-Overflakkee' (1 finding), 'Westvoorne' (1 finding), 'Noordoostpolder' (1 finding)
3.2 Map of the location.	
<b>4. Reason of the notification and pest status</b>	(1) First presence of the harmful organism
<b>4.3 Previous Pest status</b>	Transient: actionable, under eradication.
<b>4.4 Current Pest status</b>	Present: actionable, under eradication
<b>5. Information relating to the finding.</b>	(1) pest related official survey.
5.2 Date of finding.	Suspicious symptoms at the first production site were observed on October 1, 2019.
5.3 Sampling for laboratory analysis	<p>A representative sample of at least 200 plants per production site (or lot in case of growers of plants for planting). For example for 100 rows, at least 2 plants per row. Samples comprised of young leaves from the top of the plant (for plants for planting) or of the calyx (sepals on top of the fruit)</p> <p>A seed sample consists of at least 3,000 seeds per lot and consists of 'untreated', i.e. non-pelleted and non-coated seeds. Pelleted seeds are not considered suitable for testing.</p>

	Subsamples for RT-PCR consist of 1,000 seeds maximum, in line with 2020/1191.
5.4 Laboratory	Ms Floor Peeters Tel: +31 6 151 041 53 Email: f.m.peeters@nvw.nl National Reference Centre - NPPO of the Netherlands
5.5 Diagnostic method.	For the first screening, a real-time RT-PCR test is used based on the primers and probes described in the ISF testing protocol. Since 15 August 2020, following a first positive testing outcome, a second PCR based on Menzel and Winter is used for confirmation, in line with 2020/1191. Illumina sequencing is still used as a 'research tool' to monitor the occurrence of sequence variation between ToBRFV isolates from different locations and in time. See also: <a href="https://www.biorxiv.org/content/10.1101/2020.06.02.129395v1">https://www.biorxiv.org/content/10.1101/2020.06.02.129395v1</a> and <a href="https://www.youtube.com/watch?v=ivJ3cJmj0BI">https://www.youtube.com/watch?v=ivJ3cJmj0BI</a>
5.6 Date of official confirmation of the harmful organism's identity	The suspicion of ToBRFV was confirmed by DAS-ELISA and real-time PCR testing results on 7 October 2019. Definite identification of ToBRFV was determined on 17 October 2019.
<b>6. Information related to the area, severity of the finding and source of the finding</b>	The total area where measures are in force (23 production locations) totals 436.8 ha. Total area where the virus has been detected earlier totals 32 production locations (476.6 ha). For five production locations the virus has been eradicated (25.7 ha).
6.2. Characteristics of the infested area and its vicinity.	(3) Physically closed conditions (3.1) greenhouse; Plants for planting for fruit production of <i>Solanum lycopersicum</i> <i>Plants for planting for delivery to professional fruit growers.</i>
6.3. Host plants in the infested area and its vicinity.	There are many professional fruit production companies of <i>Solanum lycopersicum</i> and <i>Capsicum</i> spp. in the immediate vicinity.
6.4. Infested plant(s), plant product(s) and other object(s).	Fruit production company of <i>Solanum lycopersicum</i> .
6.5. Vectors present in the area.	
6.6. Severity of the outbreak.	<b>Update information:</b> Fruit growers reported losses varying between 5-30%. Some companies reported less than 5% loss. Other companies had to remove a crop and start a new crop. Costs of measures for

official waste destruction amount to approximately Euro 5,000 – 10,000 per hectare. In addition extra costs are needed for hygiene measures.

For the first grower in 2019, visual symptoms were observed on approximately 8% of plants in a fruit production site of 2.8 ha. Some fruits of affected plants showed a delay in ripening. Some plants are also infected with Pepino mosaic virus. Symptoms may also be caused by this virus or a mixed infection of this virus together with ToBRFV. At other companies damage is variable, ranging from limited damage to similar impact as for the first company.



Figure 1: Some fruits of ToBRFV and PepMV-affected tomato plants showed a delay in ripening.  
(NPPO of the Netherlands, 20191007)



Figure 2: Mosaic symptoms in the young leaves of ToBRFV and PepMV-affected tomato plants (NPPO of the Netherlands, 20191007)



Figure 3: Narrowing of young leaves, typical symptoms of several tobamoviruses in tomato plants. (NPPO of the Netherlands, 20191007)

6.7. Source of the outbreak.

The source of the outbreaks is not known. Based on genome sequencing analysis it is concluded that probably at least 3

	different introductions have occurred. Thus far positively tested seed lots could not be linked to specific outbreaks of fruit growers.
<b>7. Official phytosanitary measures</b>	
7.1. Adoption of official phytosanitary measures.	<p>Measures are aimed at eradication and preventing new introductions and spread of ToBRFV. At affected fruit production sites, strict hygiene measures are applied including restricting access, disinfection or replacement of clothing, machines, equipment, surfaces and packaging material. In particular use of the disinfection agent potassium peroxymonosulfate is recommended.</p> <p>For any fruits harvested from the production place, specific hygiene measures are applied both at the fruit production place as well as the packing station, including cleaning and disinfection of packaging material.</p> <p>Following removal of the crop, cleaning and disinfection of the greenhouse, the production site will be monitored, including testing at least six months after planting, to verify absence of the virus in the succeeding crop, before measures are lifted. Specific surveillance has been intensified targeting fruit companies based on information from fruit growers, packaging stations, plant growers and private labs. Seed batches of related seed is tested as part of ongoing trace-back measures. In several cases this resulted in a positive finding of seed, which were notified in Europhyt.</p>
7.2. Date of adoption of the official phytosanitary measures. In case of temporary measures, indication of their expected duration.	4 October 2019.
7.4. Objective of the official phytosanitary measures.	(1) eradication;
7.5. Measures affecting the movement of goods. Indication of one of the following options	As of 4 October 2019, every suspicion of the presence of the virus should be reported by professional operators (including laboratories) to the NPPO. National measures have been implemented as of this date in line with EU Implementing Decision 2019/1615 and subsequently EU regulation 2020/1191.

7.6. Specific surveys.	Specific surveillance has been intensified targeting fruit companies based on information from fruit growers, packaging stations, plant growers and private labs. Seed batches of related seed are tested as part of ongoing trace-back measures. In several cases this resulted in a positive finding of seed which has been notified in Europhyt.
<b>8. Pest risk analysis/assessment</b>	Pest risk analysis is not required (harmful organism is listed in Annex II of Regulation 2019/2072, or is subject to measures adopted pursuant to Article 30 of Regulation 2016/2031).
<b>9. Links to relevant websites, other sources of information.</b>	<a href="https://english.nvwa.nl/topics/pest-reporting/contents/pest-reports">https://english.nvwa.nl/topics/pest-reporting/contents/pest-reports</a>