Project title:	Tomato brown rugose fruit virus: survival of the virus and efficacy of disinfection approaches
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Date project ended:	16 July 2021

DISCLAIMER

This project has been conducted for research and development purposes. The research evaluated a range of products used for general disinfection purposes (hand sanitisation; cleansing and disinfection of glasshouse surfaces). No endorsement or recommendation of named products is intended nor is any criticism implied of alternative, untested products.

The products named in this report are not necessarily authorised as biocides across all UK cropping situations and mention of a product does not constitute a recommendation for its use against specific plant pathogens. Biocidal and plant protection products must only be used in accordance with the authorised conditions of use.

Any product marketed for use specifically against Tomato Brown Rugose Fruit Virus (ToBRFV) or any other plant pest/disease would require an authorisation under the Plant Protection Products Regulations/Regulation (EC) 1107/2009 before they are placed on the market for this use.

Regular changes occur in the authorisation status of biocides and plant protection products. For the most up to date information, please check with your professional supplier, BASIS registered adviser or the Chemical Regulation Division (CRD) of HSE (https://www.hse.gov.uk/crd/).

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The results and conclusions in this report are based on an investigation conducted over a one-year period. The conditions under which the experiments were carried out and the results have been reported in detail and with accuracy. However, because of the biological nature of the work it must be borne in mind that different circumstances and conditions could produce different results. Therefore, care must be taken with interpretation of the results, especially if they are used as the basis for commercial product recommendations.

AUTHENTICATION

We declare that this work was done under our supervision according to the procedures described herein and that the report represents a true and accurate record of the results obtained.

Anna Skelton

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Signature  Date 22/09/2021

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CONTENTS

GROWER SUMMARY

Headlines

- ToBRFV can survive on hands and gloves for at least 2 hours.
- Hand-washing is of limited use against ToBRFV but remains essential to prevent spread of other contact transmitted pathogens.
- ToBRFV survived on all glasshouse surfaces tested for at least 7 days, and in some cases for over 6 months.
- ToBRFV was destroyed on plastic trays soaked in hot water for 5 min at 90^{o}C. A soak in hot water at 70^{o}C for 5 min was insufficient alone to kill the virus but was effective when trays were sprayed with Virkon (1% ai, 1 min contact time) after the heat treatment.
- The thermal inactivation point for ToBRFV is 90^{o}C. This confirms the hot water treatment results and shows the inactivation is due to the heat treatment and not a washing effect of the water.
- Unifect G (1:25 dilution, 10 min duration) and Virocid (1%, 1 hour duration) was effective against ToBRFV on all surfaces tested. Virkon (1% ai, 20 min treatment duration), Menno Florades (0.36% ai, 16 hours contact time) and Huwa San (12.5% ai, 1 hour treatment duration) inactivated ToBRFV on all glasshouse surfaces tested except concrete. Menno Florades (0.36% ai, 1 hour contact time) also inactivated ToBRFV on most surfaces tested (except for concrete and one replicate for hard plastic).
- Since inactive viral RNA can still be detected by PCR following effective disinfection measures, official swab testing is no longer recommended after crop clean up or for declaring eradication. It can however, still provide extra reassurance to growers that the virus is absent and be used as a management tool.

Background

Tomato brown rugose fruit virus (ToBRFV) is an emerging contact transmitted virus related to tobacco mosaic virus (TMV) and tomato mosaic virus (ToMV). The virus was first described from tomato crops in Israel in 2014, where the virus spread in tomato greenhouses almost nationwide within the period of one year after the first outbreak reports. The virus was then reported from Jordan (2015), and has since been reported in several European countries, China, USA and Mexico. In June 2019, ToBRFV was first reported in the UK. In the UK,

voluntary eradication action was taken to try to limit the impact and the spread of the virus and eradication of the virus was confirmed. In 2020 there were further outbreaks of ToBRFV in the UK at different nurseries. Produce imports into the UK present the risk of further introductions through infected seed, plants for planting and on fruit from infected plants.

Unlike TMV and ToMV, ToBRFV can overcome the *Tm*-2^2 resistance gene in tomatoes. There is currently work ongoing to develop varieties with intermediate resistance to ToBRFV. The virus is thought to be robust (environmentally stable, including under ambient UV), and due to limited information, current preventative hygiene and disinfection approaches are based on strategies to control and eradicate other contact transmissible pathogens. As with other tobamoviruses, ToBRFV is seed transmitted via seed coat contamination, however, there are reports of effective seed treatment. There have also been reports that the virus can be transmitted by bumblebees during pollination.

The recent emergence of this pathogen means there is a lack of specific information on the epidemiology of the virus. Currently, advice for control of the pathogen is being formulated by extrapolation from information given for similar viruses (TMV/ToMV) and other contact transmissible pathogens of glasshouse crops. The aim of this project is to try to close the knowledge gaps on survival of the virus and potential disinfection approaches. This information will allow better formulation of advice to growers to implement both as prophylactic measures and in the event of an outbreak to try to mitigate the impact and spread of the virus.

Summary

The aims of this project were to investigate the following with specific reference to ToBRFV:

1. Survival of ToBRFV on skin and gloves
2. Handwashing to reduce the risk of contamination in the glasshouse
3. Survival of the virus on glasshouse surfaces and tools
4. Efficacy of disinfection approaches on glasshouse surfaces and tools
5. Ct values of swab samples taken from different surfaces after treatment with Unifect G and Virkon.
6. Hot water treatment of contaminated picking trays
7. Thermal inactivation of ToBRFV (funded under the Defra-Fera Long Term Service Agreement)

Experimental set up

The general experimental approach was to contaminate a range of representative glasshouse surfaces either by coating with sap from infected plants, or by lightly rubbing with an infected leaf. Subsequently these surfaces were rubbed with a damp cotton wool swab, and swabs were then rubbed onto test plants of *Nicotiana tabacum*, an experimental host of ToBRFV. Plants were left for up to 3 weeks to allow symptoms of infection to develop, and infection was then confirmed using ELISA testing. Swabs were taken after initial contamination to show that initial inoculum was present. In the case of survival studies further swabs were taken at specified time points. In the case of handwashing and disinfection studies further swabs were taken post-treatment.

All experiments were carried out on 3 plants per treatment, and all experiments were performed in duplicate at different time points to see whether results could be consistently generated. In each case a non-treated control was also included.

For all tables the following applies:

+ = positive result by ELISA, indicating the virus is viable (all 3 reps for both experiments were positive)

- = negative result by ELISA, indicating the virus is not viable (all 3 reps for both experiments were negative)

(+) = positive result by ELISA, indicating the virus is positive, for 1 of the 2 experiments only

x/3 = number out of 3 plants positive by ELISA, indicating whether the virus is viable or not

Full experimental details are provided in the Science Section.

1. *Survival on skin and gloves*

ToBRFV survived on both skin and gloves for the full experimental exposure period (2 hours), highlighting the robustness of the virus and the potential for transfer of the virus via human activity when working.

2. *Hand washing to reduce contamination risk*

The results (Table 1) show that any form of handwashing for an extended period may have some effect on reducing ToBRFV levels, however, this is not a reliable method of ensuring the virus will be removed or denatured. The only treatment which appears to be effective was a 1-minute wash with the product NZYM Rugo. Ensuring a thorough wash for 1 minute will

be a challenge on a commercial nursery and the advice to growers should be that the most reliable method to avoid cross-contamination in the glasshouse is to use disposable gloves. These should be changed as frequently as the task dictates, either on a zonal basis, such as between rows, or between tasks. Handwashing, however, remains suitable to prevent spread of other contact transmitted pathogens.

Table 1. Combined results of multiple handwashing experiments. ELISA results of test plants swabbed from ToBRFV contaminated hands after washing using water, water plus handwash / soap treatments, Enno Rapid, Mydis or Nzym Rugo.

Surface	Time	Water	Water plus treatments	Enno Rapid	Mydis	Nzym Rugo
Skin (hands)	30 seconds	(+)	(+)	(+)	+	(+)
	1 minute	(+)	(+)	(+)	N/A	-

(+) = Virus survival in some repetitions (inconsistent)

3. Survival of the virus on glasshouse surfaces and tools

Results from virus survival experiments confirmed that ToBRFV is environmentally stable for extended periods on a range of common glasshouse surfaces (Table 2). The implication is that hard plastics, such as picking crates, should be routinely treated to reduce the risk of cross-contamination between fruit and growing crops (See *Section 6: Efficacy of hot water treatment combined with disinfection*). Survival of ToBRFV on concrete looks variable, possibly a reflection of an uneven surface allowing the virus to harbour.

Table 2. ELISA results of test plants swabbed from surfaces contaminated with ToBRFV **infected sap** at different time periods.

	Time since contamination of surface									
Surface	2 hours	8 hours	24 hours	48 hours	7 days	2 weeks	3 weeks	4 weeks	3 months	6 months
Glass	+	+	+	+	+	N/A	N/A	+	(+)	(+)
Concrete	+	+	+	+	+	-	-	-	(+)	-
Aluminium	+	+	+	+	+	N/A	N/A	1/3 3/3	-	-
Hard Plastic	+	+	+	+	+	N/A	N/A	+	+	(+)
Polythene	+	+	+	+	+	N/A	N/A	+	+	(+)
Stainless steel	+	+	+	+	+	N/A	N/A	+	(+)	-

4. Efficacy of disinfection approaches on glasshouse surfaces and tools

None of the disinfectants tested (Menno Florades, Jet 5, Sodium hypochlorite & Virkon) gave control of ToBRFV at 1 minute exposure times. Subsequent trials of disinfectants (Tables 3 and 4) focused on a 60-minute exposure. Virkon-S, Virocid and Huwa San (12.5%) effectively denatured ToBRFV after 60 minutes exposure except on concrete. Menno Florades was also mainly effective at a 1-hour contact time on all surfaces except concrete.

Sodium hypochlorite was partially effective at denaturing ToBRFV on polythene, glass and stainless steel and was effective against ToBRFV on other surfaces. Jet 5 and TSOP were ineffective on most surfaces.

Table 3. Disinfectants tests against ToBRFV

Product	Active ingredient	% active in formulated product	Product dilution used for trial	% active
Virkon S	Potassium peroxymonosulfate		I tablet in 500 ml water	1%
Menno Florades	Benzoic acid	9%	4% applied as a foam	0.36%
Jet 5	Peroxyacetic Acid	5%	1:125	0.04%
Huwa San TR 50	Hydrogen Peroxide	50%	25%	12.5%
Huwa San TR 50	Hydrogen Peroxide	50%	6%	3%
TSOP	Trisodium orthophosphate		10%	10%
Sodium hypochlorite	Sodium hypochlorite	Approx. 10,000 ppm	20 ml in 500 ml water	400ppm
Unifect G	Glutaraldehyde & quaternary ammonium compounds		1:25	
Virocid	Glutaraldehyde & quaternary ammonium compounds		1%	

Table 4. ELISA results of test plants swabbed from surfaces contaminated with ToBRFV **infected sap 60 minutes** after being sprayed with disinfectant.

	Disinfectant											
Surface	Menno Florades		Jet 5		Sodium hypochlorite		Virkon S		Huwa San 12.5% ai		TSOP	
	Rep 1	Rep 2	Rep 1	Rep 2	Rep 1	Rep 2	Rep 1	Rep 2	Rep 1	Rep 2	Rep 1	Rep 2
Glass	-	-	+	2/3	1/3	-	-	-	-	-	1/3	1/3
Concrete	1/3	3/3	2/3	-	-	-	-	2/3	3/3	3/3	2/3	2/3
Aluminium	-	-	2/3	1/3	-	-	-	-	-	-	2/3	2/3
Hard Plastic	-	1/3	-	1/3	-	-	-	-	-	-	2/3	-
Polythene	-	-	2/3	-	1/3	-	-	-	-	-	2/3	1/3
Stainless steel	-	-	+	+	-	2/3	-	-	-	-	2/3	2/3

In the project extension, (PE 033a) further trials were conducted on different products, and different contact times (Tables 3, 5, 6 & 7). In summary:

- Unifect G (1:25) was effective against ToBRFV on all surfaces tested, at 10 minutes and 1 hour contact time.
- Virocid (1%, 1 hour contact time) was also effective on all surfaces tested.
- Virkon S (1%) was only partially effective against ToBRFV at 10 minutes contact time. Virkon was then tested at a 20-minute contact time and was effective on all surfaces except concrete, as recorded for Virkon applied for 1 hour at the same concentration.
- Menno Florades (4%, foam, 16-hour contact time) was effective against ToBRFV except on concrete.
- Huwa San (a.i. 3%) was ineffective against ToBRFV at 1 hour contact time or 16 hours contact time. Previous testing of Huwa San at 12.5% active ingredient (1 hour) had shown that Huwa San was effective against ToBRFV except on concrete.

Table 5. ELISA results of test plants swabbed from surfaces contaminated with ToBRFV **infected sap 60 minutes** after being sprayed with disinfectant.

	Unifect G		Virocid		Huwa San 3%	
Surface	Rep1	Rep 2	Rep 1	Rep2	Rep1	Rep2
Glass	-	-	-	-	2/3	2/3
Concrete	-	-	-	-	1/3	2/3
Aluminium	-	-	-	-	2/3	2/3
Hard Plastic	-	-	-	-	2/3	2/3
Polythene	-	-	-	-	-	+
Stainless steel	-	-	-	-	-	2/3

Table 6. ELISA results of test plants swabbed from surfaces contaminated with ToBRFV **infected sap 10 minutes or 20 minutes** after being sprayed with disinfectant.

	Unifect G **10 minutes**		Virkon **10 minutes**		Virkon **20 minutes**	
Surface	Rep1	Rep 2	Rep 1	Rep2	Rep1	Rep2
Glass	-	-	-	2/3	-	-
Concrete	-	-	-	-	+	+
Aluminium	-	-	2/3	-	-	-
Hard Plastic	-	-	1/3	-	-	-
Polythene	-	-	-	-	-	-
Stainless steel	-	-	2/3	1/3	-	-

Table 7. ELISA results of test plants swabbed from surfaces contaminated with ToBRFV **infected sap 16 hours** after being sprayed with disinfectant.

	Menno Florades		**Huwa San 3% ai**	
Surface	Rep1	Rep 2	Rep 1	Rep2
Glass	-	-	1/3	-
Concrete	2/3	2/3	1/3	+
Aluminium	-	-	-	1/3
Hard Plastic	-	-	2/3	2/3
Polythene	-	-	-	+
Stainless steel	-	-	+	2/3

5. *Cycle threshold (Ct) values of swab samples taken from different surfaces after treatment with Unifect G and Virkon.*

With real time PCR, the technology works by driving a biochemical reaction amplifying the presence of viral RNA segments through a number of heating and cooling cycles, and detection is via fluorescence produced during this reaction. The point at which fluorescence is detectable is often termed the “Ct value”. Ct values can be used to give an idea of the level of virus present (the lower the Ct value, the more viral RNA detected).

As glutaraldehyde can be used as a fixing agent there was a concern that Unifect G (active ingredient glutaraldehyde) may preserve inactivated viral RNA giving a positive result by real time PCR, even though the virus is no longer biologically active.

To investigate this, swabs were taken from different surfaces contaminated with ToBRFV infected sap before and after being sprayed with Virkon and Unifect G (see Table 3 for the rates used). The nucleic acid was extracted from swabs and tested by real time PCR for ToBRFV.

ELISA tests showed that ToBRFV was biologically inactive following treatment with Unifect G, and partially inactive after treatment with Virkon. However, Ct values obtained after treatment of the different surfaces with disinfectants Unifect G and Virkon were similar to those of the positive controls. This indicated that viral RNA that is no longer biologically active, can still be detected from swabs using real time PCR, following treatment by both Unifect G and Virkon. The results demonstrate that this phenomenon is not just limited to Unifect G (as a glutaraldehyde) but occurs with other disinfectant compounds.

Swab testing can provide extra assurance to growers that the virus is absent and as a management tool, but it is no longer recommended that official swab testing is carried out after crop clean up or for declaring eradication because ToBRFV can still be detected despite being inactivated.

6. *Efficacy of hot water treatment combined with disinfection*

One area of immediate concern for growers is the circulation of plastic crates within the industry. Given the stability and survival of the virus these could act as a potential source of infection into glasshouses. The aim of this aspect of the work was to investigate the efficacy of hot water treatment.

Hot water treatment at 70^{O}C alone does not give adequate control of the virus, but at 90^{O}C the virus was destroyed (Table 8). At 70^{O}C a short treatment with Virkon was required, but this may indicate the added value of a combination treatment between hot water/washing and disinfectant.

Table 8. ELISA results of test plants swabbed from plastic trays contaminated with ToBRFV **infected sap** before soaking, after soaking at different temperature and after spraying with Virkon (1 % a.i, 1 minute contact time)

Temperature of water	Pre-treatment	5 minute soak	After soak + Virkon
70^{O}C	+	+	-
90^{O}C	+	-	-

7. Thermal inactivation of ToBRFV.

Plastic trays are now being steamed by some growers at 95^{0}C for approximately 40 minutes. Although hot water treatment of plastic trays at 90^{o}C has been shown to be effective at inactivating ToBRFV, the thermal inactivation of ToBRFV was investigated to see if the inactivation was due to just the heat treatment or also a washing effect of the water.

Ground ToBRFV infected tomato sap (1:10 dilution), in an Eppendorf tube, was soaked for 5 minutes at various temperatures and then checked for transmissibility.

The thermal inactivation point for ToBRFV is 90^{o}C. These results show the inactivation is due to the heat treatment and not a washing effect of the water (Table 9).

Table 9. ELISA results of test plants swabbed with ToBRFV infected sap after soaking for 5 minutes at various temperatures.

Temperature (5 minute soak)	ELISA result	Comments
70 ^{o}C	+	Many lesions seen on each test plant
80 ^{o}C	+	Few lesions seen on each test plant
85 ^{o}C	2/3+	Only 1 lesion seen on each of 2 test plants
90 ^{o}C	-	No lesions seen
95 ^{o}C	-	No lesions seen

Financial Benefits

- Tomato brown rugose fruit virus has the potential to infect 100% of an infected crop as at present there are no available tomato varieties resistant to ToBRFV. There is currently work ongoing to develop varieties with intermediate resistance.
- It was identified in the UK for the first time in 2019, has potential to lead to total crop loss, with potential costs of £500k/ha for loss of a crop. Stricter hygiene measures now required to prevent the disease have significant additional costs to individual businesses
- Following the UK outbreak, a quick response on hygiene measures research and awareness of these amongst UK industry may have contributed to limiting disease spread and costs associated with an outbreak of ToBRFV.

Action Points

Given the nature of the virus, growers should follow hygiene best practice and risk assessment guidelines for their business as given on the AHDB Knowledge-library page for ToBRFV : https://ahdb.org.uk/knowledge-library/tomato-brown-rugose-fruit-virus.

Use disposable gloves: Virus can survive on hands and gloves for at least 2 hours. Disposable gloves should be used and changed regularly.

Hand washing: Is of limited use against ToBRFV with generally at least a 1-minute wash required to remove the virus, which is not practical. However, handwashing will help reduce the spread of other contact transmitted pathogens**.**

Efficacy of disinfection approaches on glasshouse surfaces and tools: Unifect G (1:25, 10 minute duration), Virkon (1 % ai, 20 minute duration), Virocid (1%, 1 hour duration) Huwa San (12.5% ai, 1 h duration) and Menno Florades (0.36% ai, 1 hour duration) are effective for ToBRFV deactivation on a range of glasshouse surfaces. However, only Unifect G (1:25, 10 minute duration), Virocid (1%, 1 hour duration) and sodium hypochlorite (400ppm, 1 hour duration) gave effective control of ToBRFV on concrete.

Hot water treatment of contaminated picking trays: Soaking ToBRFV contaminated plastic picking trays in hot water for 5 min at 90^{o}C will denature the virus. Soaking the trays at 70^{o}C for 5 min is insufficient alone to kill the virus but is effective when trays are sprayed with Virkon (1% ai, 1 minute duration) after the heat treatment. The confirmed thermal inactivation point for ToBRFV is 90^{o}C.

Swab testing: Swab testing can provide extra assurance to growers that the virus is absent and as a management tool, but it is no longer recommended that official swab testing is carried out after crop clean up or for declaring eradication.

Reporting of suspected outbreaks: Please note, it is a statutory requirement for any suspected outbreaks of a viroid or virus in a crop, or any other non-native plant pest, to be reported to the relevant authority.

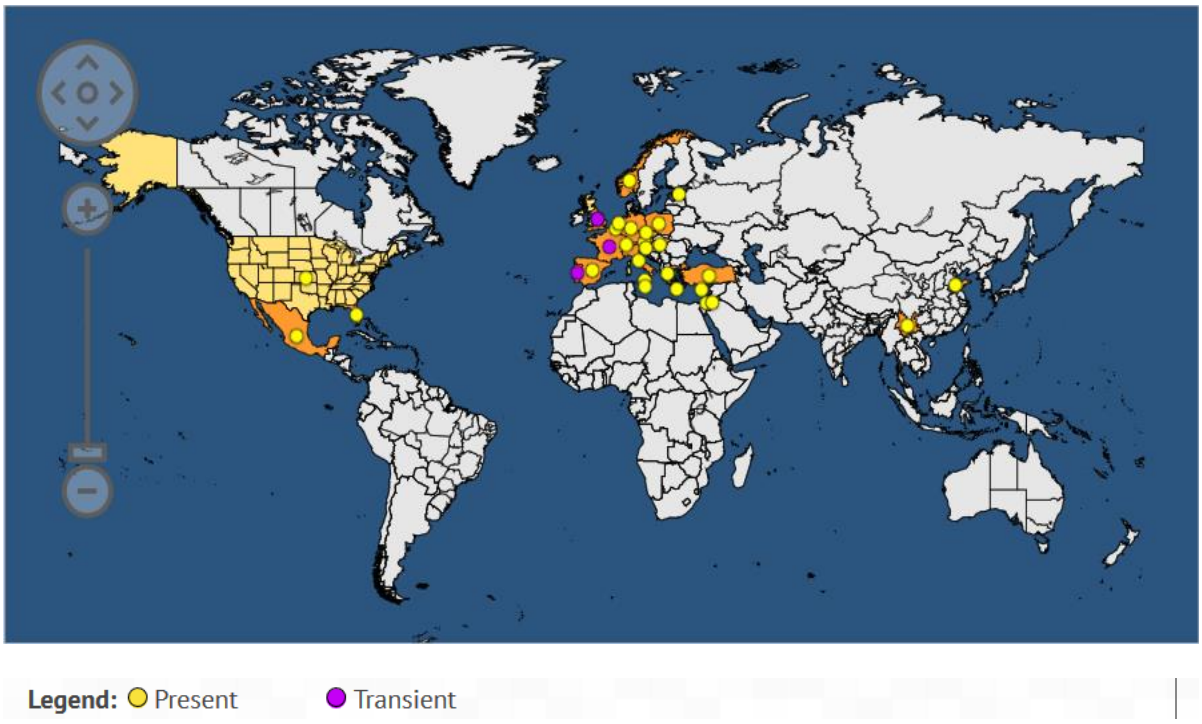
- For England and Wales, contact your local APHA Plant Health and Seeds Inspector, or the PHSI Headquarters, Sand Hutton, York.
  Tel: 0300 1000 313.
  Email: planthealth.info@apha.gsi.gov.uk.
- For Scotland, contact the Scottish Government's Horticulture and Marketing Unit:
  Email: hort.marketing@gov.scot
- For Northern Ireland, contact the DAERA Plant Health Inspection Branch:
  Tel: 0300 200 7847
  Email: planthealth@daera-ni.gov.uk

SCIENCE SECTION

Introduction

Tomato brown rugose fruit virus (ToBRFV) is an emerging contact transmitted virus related to tobacco mosaic virus (TMV) and tomato mosaic virus (ToMV). The virus was first described from tomato crops in Israel in 2014, where the virus spread in tomato glasshouses almost nationwide within the period of one year after the first outbreak reports. The virus was then reported from Jordan (2015), and has since been reported in several European countries, China, USA and Mexico. In June 2019, ToBRFV was first reported in the UK where voluntary eradication action was taken to try to limit the impact and the spread of the virus, and eradication of the virus was confirmed. In 2020 there were further outbreaks of ToBRFV in the UK at different nurseries. No new sites have been affected to date in 2021 (July).

Map showing the distribution of ToBRFV, Sept 2021 (source: EPPO global database)



Tomato and pepper are the main hosts of ToBRFV but other species can be infected (Table 1).

Table 1. Hosts of tomato brown rugose fruit virus (source: EPPO global database)

Species	Classification
Solanum lycopersicum	Major
Capsicum annuum	None (if L-gene containing cultivars) Major (if no L-genes present)
Chenopodiastrum murale	Artificial, confirmed as natural host in Israel (Dombrovsky, pers. com. 2019)
Chenopodium bengalense	Artificial
Chenopodium quinoa	Artificial
Nicotiana benthamiana	Artificial
Nicotiana clevelandii	Artificial
Nicotiana glutinosa	Artificial
Nicotiana tabacum	Artificial
Petunia x hybrida	Artificial
Solanum nigrum	Artificial and as natural host in Israel (Dombrovsky, pers. comm. 2019)
Solanum melongena	ToBRFV only detected in seed lots not on plant material

Unlike TMV and ToMV, ToBRFV can overcome the *Tm*-2^2 resistance gene in tomatoes. There is currently work ongoing to develop varieties with intermediate resistance to ToBRFV. The virus is thought to be robust (environmentally stable) and due to limited information, current preventative hygiene and disinfection approaches are based on strategies to control and eradicate other contact transmissible pathogens. As with other tobamoviruses, ToBRFV is seed transmitted via seed coat contamination, however, there are reports of effective seed treatment (Davino et al, 2020). There have also been reports that the virus can be transmitted by bumblebees during pollination.

Common symptoms in younger leaves are mosaics, puckering and in some cases leaves may be narrow. Necrotic streaks may occur on the stems. Fruit from ToBRFV-infected plants

may mature irregularly and can be mottled with yellow or brown spots making fruit unmarketable. These symptoms are similar to those seen with other viruses (EPPO Global database).

The aim of this project is to provide information for industry on the efficacy of preventative hygiene measures and disinfection to minimise the risks posed by tomato brown rugose fruit virus.

The objectives were to investigate with specific reference to ToBRFV:

- Survival of ToBRFV on skin and gloves
- Handwashing to reduce the risk of contamination in the glasshouse
- Survival of the virus on glasshouse surfaces and tools
- Efficacy of disinfection approaches on glasshouse surfaces and tools
- Detection of ToBRFV by PCR using swab samples taken from disinfected surfaces
- Hot water treatment of contaminated picking trays
- Thermal inactivation of ToBRFV (funded under the Defra-Fera Long Term Service Agreement)

The objectives of the project were formulated based on a balance between potential efficacy of approach, practicality of application in a glasshouse environment, and practicality of investigating a highly transmissible, robust plant pathogen with limited knowledge on disinfection approaches at the start of the project. For example, extended handwashing times were discounted due to the challenges of implementing and monitoring these in practical application in a working glasshouse. The choice of surfaces for treatment were identified as representative of common surfaces in a glasshouse environment. The choice of products, both for handwashing and disinfection were guided by what was commercially available and to give a representative range of disinfection active ingredients. Disinfection treatments were as per manufacturer's recommendations. The rationale for treatment times used being an attempt to balance the minimum exposure time for control across these different surfaces. Following the poor performance of treatments at one minute exposure, one hour was trialled, and then those products which were successful at one hour were investigated further at lower exposure times.

In some cases, experimental approaches were limited by restrictions arising from laboratory and experimental considerations. For example, the approaches taken for thermal inactivation work, rather than steam inactivation, were dictated by the challenges posed by developing a trial method for accurately assessing the efficacy of steam inactivation.

Materials and methods

Bioassay for determination of viable virus

In each experiment described below the presence of viable virus was demonstrated by biological assay onto test plants. Cotton buds, soaked in phosphate buffer pH7 containing celite, a mild abrasive powder, were used to take swabs from different surfaces. Swabs were taken by rubbing the surface with the cotton bud and then these were gently rubbed onto leaves of *Nicotiana tabacum* plants (approx. 5 weeks from sowing), covered with a perforated polypropylene bag to avoid cross contamination and placed in a glasshouse at 20 to 25ºC for 2 to 3 weeks. *N. tabacum* is a test plant that is susceptible to ToBRFV and rapidly shows symptoms on the inoculated leaves. Five weeks is the optimum plant age for inoculation as there are sufficient leaves to inoculate and developing symptoms can be observed.

For each variable e.g. surface and time, three swabs were taken and three test plants inoculated. After this time, the inoculated leaves (previously marked by a hole from a pipette tip) were removed and tested by ELISA for ToBRFV using antisera from DSMZ, Germany, according to the manufacturers' instructions. While ELISA is not as sensitive as PCR, it was considered sufficiently sensitive for detection of ToBRFV in this situation where the virus had been bio-amplified in the test plants. In addition, use of ELISA was more appropriate for the number of samples being tested.

All experiments were carried out in duplicate. The level of replication used is typical for this type of study. The duplicate run each experiment was considered essential given the variability of data that was sometimes encountered.

Survival on skin and gloves

ToBRFV infected tomato leaf was collected 2 to 3 weeks after inoculation and confirmed positive by ELISA. The infected leaves were ground in water (1:5 dilution) and the sap was rubbed onto a bare hand and a gloved hand (nitrile glove). The bare hand and gloved hand were swabbed at 15 minute intervals up to 1 hour and then 30 minute intervals up to 2 hours. These swabs were inoculated onto *Nicotiana tabacum* test plants and after 2 to 3 weeks the plants were tested by ELISA for ToBRFV.

The above was repeated, except instead of using ground sap of ToBRFV infected leaves, the infected leaves were simply rubbed onto the hands and gloved hands.

Hand washing to reduce contamination risk

ToBRFV infected tomato leaf was collected 2 to 3 weeks after inoculation and rubbed onto hands. To account for potential differences in hand surfaces, two different members of staff of different ages, one male, one female, were selected to carry out experiments. As a positive control, swabs were taken from the hands before washing and inoculated onto *N. tabacum* test plants. The hands were then washed for 30 seconds or 1 minute using the following washes:

- Water only
- Water & soap
- Water & medicated hand wash (Hibiscrub)
- Water & medicated hand wash (Hibiscrub), followed by an alcohol gel
- Enno Rapid (hand gel)
- Nzym Rugo (hand gel)
- Mydis (hand gel)

Swabs were then taken from the hands and inoculated onto test plants. The test plants were tested by ELISA for ToBRFV 2 to 3 weeks after inoculation. Results of this work are presented in Tables 5 to 8.

Survival on glasshouse surfaces

A range of glasshouse surfaces (glass, concrete, aluminium, hard plastic, polythene and stainless steel) were contaminated with ToBRFV infected leaf sap (1:5 dilution with water). A picking crate from a tomato grower was used as the hard plastic. The surfaces were kept at ambient temperature and swabs were taken at different time periods (ranging from 2 hours to 6 months) and inoculated onto test plants. The test plants were tested by ELISA for ToBRFV 2 to 3 weeks after inoculation.

Efficacy of disinfection approaches

As for the survival on glasshouse surfaces experiment, the six surfaces were contaminated with ToBRFV infected leaf sap. Once the sap on the surfaces was dry, as a positive control, swabs were taken from the surfaces and inoculated onto test plants, to show the virus was viable. The surfaces were then sprayed with a disinfectant, at the recommended rate, and left for different contact times (1 minute, 10 minutes, 20 minutes, 1 hour or 16 hours) before

swabs were taken and inoculated onto test plants. The test plants were tested by ELISA for ToBRFV 2 to 3 weeks after inoculation. Disinfectants tested are shown in Table 2.

Table 2. Disinfectants tests against ToBRFV

Product	Active ingredient	% active in formulated product	Product dilution used for trial	% active
Virkon S	Potassium peroxymonosulfate		I tablet in 500 ml water	1%
Menno Florades	Benzoic acid	9%	4% applied as a foam	0.36%
Jet 5	Peroxyacetic Acid	5%	1:125	0.04%
Huwa San TR 50	Hydrogen Peroxide	50%	25%	12.5%
Huwa San TR 50	Hydrogen Peroxide	50%	6%	3%
TSOP	Trisodium orthophosphate		10%	10%
Sodium hypochlorite	Sodium hypochlorite	Approx. 10,000 ppm	20 ml in 500 ml water	400 ppm
Unifect G	Glutaraldehyde & quaternary ammonium compounds		1:25	
Virocid	Glutaraldehyde & quaternary ammonium compounds		1%	

Cycle threshold (Ct) values of swab samples taken from different surfaces after treatment with Unifect G and Virkon.

Swabs were taken from different surfaces contaminated with ToBRFV infected sap before and after being sprayed with Virkon and Unifect G (see Table 2 for the rates used). The surfaces (glass, concrete, aluminium, hard plastic [trays], polythene and stainless steel) were swabbed at 10 minutes after application of disinfectant. The nucleic acid was extracted from swabs using Qiagen kits, tested by real time PCR for ToBRFV and Ct values recorded.

Efficacy of hot water treatment combined with disinfection

Sections of a hard plastic glasshouse tray were contaminated with ToBRFV infected sap and left to dry. Swabs were taken from the tray sections and inoculated onto healthy test plants. The tray sections were then soaked in hot water at either 70ºC or 90ºC for 5 minutes. After soaking, swabs were taken and inoculated onto test plants and then the tray sections were sprayed with 1% Virkon S (recommended rate) and left for 1 minute. Again, swabs were taken

and all test plants were tested for ToBRFV by ELISA after 2 weeks if showing symptoms. If no symptoms were evident, plants were left a further week before testing at 3 weeks.

Thermal inactivation of ToBRFV

Ground ToBRFV infected tomato sap (1:10 dilution), in an Eppendorf, was soaked for 5 minutes at various temperatures (70^{o}C to 95^{o}C) in a water bath. Cotton buds were dipped in each Eppendorf, and then rubbed onto test plants to check for transmissibility. After 3 weeks the test plants were tested by ELISA for the presence of ToBRFV.

Results

For all tables the following applies:

+ = positive result by ELISA, indicating the virus was viable (all 3 reps for both experiments were positive)

- = negative result by ELISA, indicating the virus was not viable (all 3 reps for both experiments were negative)

(+) = positive result by ELISA, indicating the virus was positive, for 1 of the 2 experiments only

x/3 = number out of 3 plants positive by ELISA, indicating whether the virus was viable or not

Survival on skin and gloves

Results show that ToBRFV can survive on both hands and gloves for at least 2 hours (Tables 3 and 4). This was the same for both ground-up infected sap and from rubbing infected leaves onto hands or gloves.

Table 3. ELISA results of test plants swabbed from skin and gloves after being contaminated with ToBRFV **infected sap**.

	Time (minutes) after contamination with ToBRFV						
Surface	0	15	30	45	60	90	120
Skin	+	+	+	+	+	+	+
Gloves	+	+	+	+	+	+	+

Table 4. ELISA results of test plants swabbed from skin and gloves after contaminating by rubbing ToBRFV **infected leaves**.

	Time (minutes) after contamination with ToBRFV						
Surface	0	15	30	45	60	90	120
Skin	+	+	+	+	+	+	+
Gloves	+	+	+	+	+	+	+

Hand washing to reduce contamination risk

Results from a series of experiments done to test hand washing techniques and products (as they became available) are shown in Tables 5-8.

In Experiment 1, all the handwashing treatments tested (water, water and soap, water and medicated soap and water, medicated soap and gel) with a 30 second wash were ineffective at removing all the virus. After a 1-minute wash, all the treatments were effective at controlling ToBRFV except the medicated hand wash with water.

Table 5. ELISA results of test plants swabbed from ToBRFV contaminated hands after washing using different treatments (Experiment 1)

	Hand wash							
Length of wash	Water only		Water & soap		Water & medicated hand wash (Hibiscrub)		Water & medicated hand wash, followed by gel	
	Rep1	Rep2	Rep1	Rep2	Rep1	Rep2	Rep1	Rep2
30 seconds	3/3	3/3	1/3	2/3	1/3	3/3	3/3	2/3
1 minute	0/3	0/3	0/3	0/3	2/3	2/3	0/3	0/3

In Experiment 2, it appeared that Enno Rapid was an effective hand wash against ToBRFV, at both 30 seconds and 1 minute (Table 6), however, the results for the water only wash

differed from the results obtained previously (Table 5) and therefore, it was decided to repeat this experiment to check results.

Table 6. ELISA results of test plants swabbed from ToBRFV contaminated hands after washing using water and Enno Rapid (Experiment 2)

	Hand wash			
Length of wash	Water only		Enno Rapid	
	Rep1	Rep2	Rep1	Rep2
30 seconds	0/3	0/3	0/3	0/3
1 minute	2/3	0/3	0/3	0/3

The results from Experiment 3 show that at 30 seconds none of the treatments (water, Enno Rapid and Nzym Rugo) were effective against ToBRFV (Table 7). With a 1-minute treatment, Nzym Rugo was effective but Enno Rapid did not give effective control.

Table 7. ELISA results of test plants swabbed from ToBRFV contaminated hands after washing using water, Enno Rapid and Nzym Rugo (Experiment 3).

	Hand wash					
Length of wash	Water only		Enno Rapid		Nzym Rugo	
	Rep1	Rep2	Rep1	Rep2	Rep1	Rep2
30 seconds	2/3	2/3	3/3	3/3	1/3	2/3
1 minute	0/3	2/3	2/3	1/3	0/3	0/3

As part of the extension to the project (PE 033a) Mydis hand gel was also tested at 30 seconds but did not give effective control of ToBRFV (Experiment 4) (Table 8).

Table 8. Combined results from handwashing Experiments 1-4. ELISA results of test plants swabbed from ToBRFV contaminated hands after washing using water, water plus treatments, Enno Rapid, Nzym Rugo and Mydis.

Surface	Time	Water	Water plus treatments (Expt 1)	Enno Rapid	Mydis	Nzym Rugo
Skin (hands)	30 seconds	(+)	(+)	(+)	+	(+)
	1 minute	(+)	(+)	(+)	N/A	-

(+) = Virus survival in some repetitions (inconsistent)

- = Virus did not survive

+ = Virus survived

These results show that the results of hand washing are very variable and are further considered in the Discussion section below.

Survival on glasshouse surfaces

ToBRFV remained infective on all surfaces tested for at least 7 days and was infective on some of the surfaces (glass, hard plastic and polythene) for at least 6 months (Table 9).

The results of a first experiment showed that ToBRFV was no longer viable on concrete at 4 weeks, therefore, for the second experiment, swabs were also taken at 2 and 3 weeks for concrete. These results were also negative for ToBRFV, as were the 4-week results, suggesting the virus did not survive on concrete for much more than 7 days. Results from 3 months, however, (2nd experiment) show that ToBRFV is still infective at 3 months, suggesting survival of ToBRFV on concrete is variable, possibly a reflection of an uneven surface allowing virus to harbour in.

Table 9. ELISA results of test plants swabbed from surfaces contaminated with ToBRFV infected sap at different time periods.

	Time since contamination of surface									
Surface	2 h	8 h	24 h	48 h	1 week	2 weeks	3 weeks	4 weeks	3 months	6 months
Glass	+	+	+	+	+	N/A	N/A	+	(+)	(+)
Concrete	+	+	+	+	+	-	-	-	(+)	-
Aluminium	+	+	+	+	+	N/A	N/A	1/3 3/3	-	-
Hard Plastic	+	+	+	+	+	N/A	N/A	+	+	(+)
Polythene	+	+	+	+	+	N/A	N/A	+	+	(+)
Stainless steel	+	+	+	+	+	N/A	N/A	+	(+)	-

Efficacy of disinfection approaches

ToBRFV remained viable after 1 min treatments with a range of disinfectants (at recommended rates) on all glasshouse surfaces tested (Table 10). As the range of disinfectants tested did not appear to be effective against ToBRFV at 1 minute, it was decided to discontinue testing of the other disinfectants at this contact time and to investigate longer duration contact times (1 hour). However, not all the positive controls (swabs taken from the different surfaces before spraying with the disinfectant and inoculated onto test plants) were positive for the 1-hour experiment. Therefore, the original results for the 1-hour contact times were considered unreliable and are not presented here.

The 1-hour duration testing was repeated and showed that Virkon-S, and Huwa San gave effective denaturing of ToBRFV after 60 minutes exposure except on concrete (Table 11). Menno Florades was also mainly effective at a 1-hour contact time on all surfaces except concrete. Note that the Huwa San concentration used (12.5%) for surface disinfection in this experiment was selected based on communication from the manufacturer but actually relates to the recommended rate to disinfect empty glasshouses during crop change using large nebulizing systems (cold foggers).

Sodium hypochlorite was partially effective at denaturing ToBRFV on polythene, glass and stainless steel and was effective against ToBRFV on other surfaces. Jet 5 and TSOP were ineffective on most surfaces.

Table 10. ELISA results of test plants swabbed from surfaces contaminated with ToBRFV infected sap **1 minute** after being sprayed with disinfectant.

	Disinfectant					
Surface	Menno Florades	Jet 5	Sodium hypochlorite	Virkon S	Huwa San	TSOP
Glass	+	+	+	+	N/A	N/A
Concrete	+	+	+	+	N/A	N/A
Aluminium	+	+	+	+	N/A	N/A
Hard Plastic	+	+	+	+	N/A	N/A
Polythene	+	+	+	+	N/A	N/A
Stainless steel	+	+	+	+	N/A	N/A

N/A = Treatment not tried at this exposure time/surface combination.

Table 11. ELISA results of test plants swabbed from surfaces contaminated with ToBRFV infected sap **60 minutes** after being sprayed with disinfectant.

	Disinfectant											
Surface	Menno Florades		Jet 5		Sodium hypochlorite		Virkon S		Huwa San 12.5% ai		TSOP	
	Rep 1	Rep 2	Rep 1	Rep 2	Rep 1	Rep 2	Rep 1	Rep 2	Rep 1	Rep 2	Rep 1	Rep 2
Glass	-	-	+	2/3	1/3	-	-	-	-	-	1/3	1/3
Concrete	1/3	3/3	2/3	-	-	-	-	2/3	3/3	3/3	2/3	2/3
Aluminium	-	-	2/3	1/3	-	-	-	-	-	-	2/3	2/3
Hard Plastic	-	1/3	-	1/3	-	-	-	-	-	-	2/3	-
Polythene	-	-	2/3	-	1/3	-	-	-	-	-	2/3	1/3
Stainless steel	-	-	+	+	-	2/3	-	-	-	-	2/3	2/3

As part of the extension to the project (PE 033a) further trials were done to investigate different disinfectant products, rates and contact times (Tables 12, 13 and 14).

- Unifect G (1:25) was effective against ToBRFV on all surfaces tested, at 10 minutes and 1 hour contact time.
- Virocid (1%, 1 hour contact time) was also effective on all surfaces tested.
- Virkon S (1%) was only partially effective against ToBRFV at 10 minutes contact time. For some of the positive samples only one lesion was seen on the test plants, suggesting that a slightly longer contact time would be effective. Therefore, Virkon S was subsequently tested for 20 min and was effective on all surfaces except concrete. These results are the same as for Virkon S for 1 hour at the same concentration.
- Menno Florades (4%, foam, 16 hour contact time) was effective against ToBRFV except on concrete.
- Huwa San was ineffective at 1 h or 16 h contact time when re-tested at the rate recommended for surface disinfection (a.i. 3%). This was in contrast to the previous result when Huwa San was applied as a surface disinfectant for 1 h (albeit at the recommended fogging rate of 12.5% a.i) and was effective against ToBRFV except on concrete.

Table 12. ELISA results of test plants swabbed from surfaces contaminated with ToBRFV infected sap **60 minutes** after being sprayed with disinfectant.

	Unifect G		Virocid		Huwa San 3%	
Surface	Rep1	Rep 2	Rep 1	Rep2	Rep1	Rep2
Glass	-	-	-	-	2/3	2/3
Concrete	-	-	-	-	1/3	2/3
Aluminium	-	-	-	-	2/3	2/3
Hard Plastic	-	-	-	-	2/3	2/3
Polythene	-	-	-	-	-	+
Stainless steel	-	-	-	-	-	2/3

Table 13. ELISA results of test plants swabbed from surfaces contaminated with ToBRFV infected sap **10 minutes or 20 minutes** after being sprayed with disinfectant.

	Unifect G **10 minutes**		Virkon **10 minutes**		Virkon **20 minutes**	
Surface	Rep1	Rep 2	Rep 1	Rep2	Rep1	Rep2
Glass	-	-	-	2/3	-	-
Concrete	-	-	-	-	+	+
Aluminium	-	-	2/3	-	-	-
Hard Plastic	-	-	1/3	-	-	-
Polythene	-	-	-	-	-	-
Stainless steel	-	-	2/3	1/3	-	-

Table 14. ELISA results of test plants swabbed from surfaces contaminated with ToBRFV infected sap **16 hours** after being sprayed with disinfectant.

	Menno Florades		**Huwa San 3% ai**	
Surface	Rep1	Rep 2	Rep 1	Rep2
Glass	-	-	1/3	-
Concrete	2/3	2/3	1/3	+
Aluminium	-	-	-	1/3
Hard Plastic	-	-	2/3	2/3
Polythene	-	-	-	+
Stainless steel	-	-	+	2/3

Ct values of swab samples taken from different surfaces after treatment with Unifect G and Virkon.

With real time PCR, the technology works by driving a biochemical reaction amplifying the presence of viral RNA segments through a number of heating and cooling cycles, and detection is via fluorescence produced during this reaction. The point at which fluorescence is detectable is often termed the “Ct value”.

Previous ELISA tests indicated that ToBRFV was biologically inactive following treatment with Unifect G, and partially inactive after treatment with Virkon (Table 13). With real time PCR, Ct values can be used to give an idea of the level of virus remaining after disinfection (the lower the Ct value the more viral RNA detected). The Ct values obtained after treatment of the different surfaces with disinfectants Virkon S and Unifect G were similar to those of the positive controls (Tables 15 and 16). The ELISA results of test plants swabbed from surfaces contaminated with ToBRFV infected sap 10 minutes after being sprayed with Virkon S and Unifect G (from Table 13) are shown for comparison.

The Ct values obtained demonstrate that viral RNA that does not appear to be biologically active, can still be detected using swabs, by real time PCR, following treatment by both Unifect G and Virkon.

Table 15. Ct values of swabs taken from surfaces contaminated with ToBRFV infected sap 10 minutes after being sprayed with Virkon S. Ct values can be used to give an idea of the level of virus present (the lower the Ct value the more viral RNA detected).

	Virkon				
Surface	Ct values			ELISA results of test plants	
	Positive control (before sprayed with disinfectant)	Rep 1	Rep 2	Positive control (before sprayed with disinfectant)	After 10 minute contact time with Virkon S
Glass	12	10	12	+	-
Concrete	12	12	11	+	-
Aluminium	11	12	11	+	2/3
Hard Plastic	12	12	11	+	1/3
Polythene	11	12	13	+	-
Stainless steel	12	13	11	+	2/3

Table 16. Ct values of swabs taken from surfaces contaminated with ToBRFV infected sap 10 minutes after being sprayed with Unifect G. Ct values can be used to give an idea of the level of virus present (the lower the Ct value the more viral RNA detected).

	Unifect G				
Surface	Ct values			ELISA results of test plants	
	Positive control (before sprayed with disinfectant)	Rep 1	Rep 2	Positive control (before sprayed with disinfectant)	After 10-minute contact time with Unifect G
Glass	12	18	15	+	-
Concrete	10	16	15	+	-
Aluminium	11	15	14	+	-
Hard Plastic	13	15	16	+	-
Polythene	12	15	16	+	-
Stainless steel	12	14	15	+	-

Efficacy of hot water treatment combined with disinfection

A 5-minute treatment of a contaminated plastic tray with water at 90^{o}C was effective in eliminating ToBRFV (Table 17). Soaking the contaminated tray for 5 minutes at 70^{o}C did not denature the virus but was effective when the trays were sprayed with 1% Virkon (1-minute contact time) after the heat treatment. From the results of the disinfection work in this project it is known that a 1-minute contact time with Virkon alone and no previous soaking does not stop ToBRFV being viable.

Table 17. ELISA results of test plants swabbed from plastic trays contaminated with ToBRFV infected sap before soaking after soaking at different temperature and after spraying with Virkon

Temperature of water	Pre-treatment	5 minute soak	After soak + Virkon
70^{O}C	+	+	-
90^{O}C	+	-	-

Thermal inactivation of ToBRFV

From heating tomato sap infected with ToBRFV (in Epindorf tubes) at different temperatures in a water bath, it was shown that the thermal inactivation point for ToBRFV is 90^{o}C (Table 18). This confirms previous hot water treatment results, where ToBRFV was unviable after soaking contaminated trays in hot water for 5 min at 90^{o}C but was still viable after a soak at 70 ^{o}C for 5 min. These results show the inactivation is due to the heat treatment and not a washing effect of the water.

Table 18. ELISA results of test plants swabbed with ToBRFV infected sap after soaking for 5 minutes at various temperatures.

Temperature (5-minute soak)	ELISA result	Comments
70 ^{o}C	+	Many lesions seen on each test plant
80 ^{o}C	+	Few lesions seen on each test plant
85 ^{o}C	2/3+	Only 1 lesion seen on each of 2 test plants
90 ^{o}C	-	No lesions seen
95 ^{o}C	-	No lesions seen

Discussion

ToBRFV has been shown to survive for at least 2 hours on both hands and gloves, therefore, if the hands of workers became contaminated with the virus e.g. from fruit imported to the site for packaging, from a random infected plant or nursery ‘touch points’, the virus could spread quickly through a crop. If gloves are worn they should be changed regularly to prevent spread of the virus.

The results of the hand washing experiments are very variable, even when repeating the same washing conditions. This may be due to different levels of virus picked up on the hands from rubbing infected leaves, or different hand washing techniques by individuals. In general, the results show that handwashing is unreliable and to get thorough elimination of the virus, washing for over 30 seconds is required. This demonstrates the difficulties in managing the spread of this particularly persistent virus. In some cases, washing the hands for 1 minute removes infectious virus; soap and water or Nzym Rugo appear to be effective after a 1 minute contact time, as in some cases does just water. This may be due more to the physical washing action than the product used. However, 1 minute handwashing is not practical and would be difficult to enforce, therefore, from these and the survival experiments it would be recommended to wear gloves and change them as often is necessary. This should be determined by carrying out a task specific risk assessment. Hand-washing is of limited use against ToBRFV but is still recommended to prevent spread of other contact transmitted pathogens eg. *Clavibacter* sp.

The virus survives on some glasshouse surfaces for at least 6 months, therefore, once the virus contaminates a surface it has the potential to spread the virus for a long period of time. Once an outbreak of ToBRFV occurs, normal glasshouse working practices can quickly spread the virus via movements of contaminated tools and equipment (e.g. during plant cutting, on workers hands and clothing, via picking carts and crates and on glasshouse structures).

The results for survival on concrete were variable (positive at 7 days, negative at 14 to 28 days and then positive at 3 months) maybe due to the rough surface, making it harder to remove the virus by contact.

None of the disinfectants tested (Menno Florades, Jet 5, Sodium hypochlorite and Virkon S) were effective against ToBRFV at a 1-minute contact time. Unifect G (1:25) was effective against ToBRFV on all surfaces tested, at 10 minutes and 1 hour contact time. This is the dilution recommended by the manufacturer for high levels of contamination. Virocid (1%, 1 hour contact time) was also effective on all surfaces tested. Virkon (1%) was only partially effective at 10 minutes contact time but effective on all surfaces, except concrete, at 20 minutes and 1 hour contact time.

Menno Florades also looks to be mainly effective at a 1-hour contact time, and effective at 16 hour contact time, on all surfaces except concrete. These results suggest concrete could be a difficult surface to disinfect once contaminated with ToBRFV infected leaf sap. Sodium hypochlorite is partially effective at denaturing ToBRFV on polythene, glass and stainless

steel and is effective against ToBRFV on other surfaces. Jet 5 and TSOP were ineffective on most surfaces.

Huwa San (a.i. 3%) was ineffective against ToBRFV at 1 hour contact time and 16 hours contact time. This is the concentration recommended for surface disinfection. Testing of Huwa San at 12.5% active ingredient (1 hour) showed that Huwa San was effective against ToBRFV except on concrete but this was achieved using the concentration recommended to disinfect empty glasshouses during crop change using large nebulizing systems (cold foggers).

It must be noted that ground infected sap was added to each surface and this may be an artificially high amount of virus. Also, the disinfectants tested have not all been used at the recommended contact times, as the aim was to find a contact time that was useful in as many situations as possible. Equipment such as picking carts and hand tools (e.g. pruning knives) should all be cleaned and disinfected routinely. Tools should ideally be disinfected during pruning activities between individual plants. Equipment should be cleaned and disinfected at least between crops.

Viral RNA that does not appear to be biologically active can still be detected using swabs, by real time PCR, following treatment by both Unifect G and Virkon. As glutaraldehyde can be used as a fixing agent there was a concern that Unifect G (active ingredient glutaraldehyde) may preserve inactivated viral RNA giving a positive result by real time PCR, even though the virus is no longer biologically active. The results demonstrate that this phenomenon is not just limited to Unifect G (as a glutaraldehyde) but occurs with other disinfectant compounds.

Swab testing can provide extra assurance to growers that the virus is absent and as a management tool. Due to ToBRFV detection after disinfection despite inactivation, it is no longer recommended that official swab testing by PCR is carried out after crop clean up or for declaring eradication. If swab testing is carried out in the event of an outbreak after clean-up, it would be recommended to get swabs tested for tomato brown rugose fruit virus by inoculation onto test plants to give more confidence in the clean-up procedure.

Initially, results from the 1-hour contact time disinfection experiments were unreliable because the positive controls were not consistently positive. The positive controls were test plants inoculated with swabs taken from the different ToBRFV contaminated surfaces before the surfaces were sprayed with disinfectant. As the virus has been shown to survive on all surfaces for at least 7 days and up to 6 months, it was very unusual that the controls were not positive after less than an hour on each surface. The most likely explanation for this is the light levels in the glasshouse where the test plants were kept after inoculation. These test plants were kept in the glasshouse with LED lights in December when the general light levels were very low. The International Seed Federation protocol on detection of ToBRFV in seed

recommends at least 12 hours of light for inoculated test plants. These plants did receive 12 hours of light but the LED lights may not have given a suitable light level. In subsequent re-testing metal halide growth lights were used.

Soaking of plastic trays in hot water at 90^0C for 5 minutes was shown to be an effective way of controlling the virus, however soaking at 70^0C was not effective. This hot water soaking can be used for treating plastic trays coming onto site to prevent the introduction of the virus. Hot water treatment was used as a small-scale methodology to test temperature effects on ToBRFV. Commercially, plastic trays are now being steamed by some growers at 95^0C for approximately 40 minutes. There was a small risk that the soak in hot water did not mirror steaming, as soaking may have the physical effect of washing rather than just heating. However, the thermal inactivation point for ToBRFV was found to be 90°C, which is similar to other tobamoviruses for example cucumber green mottle mosaic virus in sap is inactivated by 10 minutes at 90^0C (Brunt et al, 1996). This confirms previous hot water treatment results, where ToBRFV was shown not to be viable after soaking contaminated trays in hot water for 5 min at 90°C but was still viable after a soak at 70°C for 5 min. The thermal inactivation data show the inactivation is due to the heat treatment and not a washing effect of the water.

Conclusions

Use disposable gloves: Virus can survive on hands and gloves for at least 2 hours. Disposable gloves should be used and changed regularly.

Hand washing: Is of limited use against ToBRFV with generally at least a 1-minute wash required to remove the virus, which is not practical on a commercial nursery. However, handwashing will help reduce the spread of other contact transmitted pathogens.

Survival on glasshouse surfaces: ToBRFV can survive on all surfaces tested for at least 7 days and for longer than 6 months in some cases.

Efficacy of disinfection approaches on glasshouse surfaces and tools: None of the disinfectants were effective against ToBRFV at 1 minute contact time. Unifect G (1:25, 10 minute duration), Virkon (1 % ai, 20 minute duration), Virocid (1%, 1 hour duration) Huwa San (12.5% ai, 1 h duration) and Menno Florades (0.36% ai, 1 hour duration) were effective for ToBRFV inactivation on a range of glasshouse surfaces. However, only Unifect G (1:25, 10 minute duration), Virocid (1%, 1 hour duration) and sodium hypochlorite (400 ppm, 1 hour duration) gave effective control of ToBRFV on concrete.

Swab testing: Swab testing can provide extra assurance to growers that the virus is absent and as a management tool, but it is no longer recommended that official swab testing is carried out after crop clean up or for declaring eradication because ToBRFV can still be detected despite being inactivated.

Hot water treatment of contaminated picking trays: ToBRFV was denatured on trays soaked in hot water for 5 min at 90ºC. A soak in hot water at 70ºC for 5 min was insufficient alone to kill the virus but was effective when trays were sprayed with Virkon after the heat treatment. The confirmed thermal inactivation point for ToBRFV is 90ºC.

Further information on the hygiene best practice is available from the AHDB ToBRFV webpages in the AHDB knowledge library.

References

Brunt AA, Crabtree K, Dallwitz MJ, Gibbs AJ & Watson L, 1996. Viruses of Plants. Descriptions and lists from the VIDE database.

Davino S, Giovanni Caruso A, Bertacca S, Barone S & Panno S, 2020. Tomato brown rugose fruit virus: Seed transmission rate and efficacy of different seed disinfection treatments. Plants, 9(11), 1615.

Knowledge and Technology Transfer

Presentations:

- Tomato growers conference, Coventry, UK (September 2019)
- The work was referenced in a presentation to the G20 MACS (Agricultural chief scientists) workshop on transboundary plant pests, Tsukuba, Japan (December 2019)
- Ontario glasshouse growers research workshop, Toronto, Canada (postponed, potentially May 2022)
- Tomato growers conference, UK (online September 2020)
- University of California, School of Agriculture and Natural resources, Tomato disease workshop (online, November 2020)
- AAB ToBRFV and CGMMV workshop (online, December 2020)

- East-West Seeds annual conference (online, December 2020)
- UK Plant Health Symposium (online, March 2021)

Literature:

- AHDB Website knowledge library content
- Additionally, the work has been referenced in the following publications:
    - EPPO PRA on tomato brown rugose fruit virus
    - Defra contingency plan on tomato brown rugose fruit virus
    - Defra plant pest factsheet on tomato brown rugose fruit virus

Other resources:

- AHDB ToBRFV Webinars: Two webinars were conducted regarding the virus and the work being carried out on the virus. (online, March 2019 and February 2020)
- Fortnightly/monthly contributions to ToBRFV steering group discussions
- AHDB protected vining crops webinar (online, March 2021)

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