Project title:	Tomato brown rugose fruit virus: Survival of the virus and efficacy of disinfection approaches
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Date project commenced:	03 June 2019

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.

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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.
- Virkon (1% ai, 1 hour treatment duration) and Huwa San (12.5% ai, I hour treatment duration) killed ToBRFV on all glasshouse surfaces tested except concrete. Menno Florades (0.36% ai, 1 hour contact time) also killed ToBRFV on most surfaces tested (except for concrete and one replicate for hard plastic).
- ToBRFV was destroyed on plastic 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 (1% ai, 1 min contact time) after the heat treatment.

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 has since been reported from Jordan (2015), Mexico (both tomatoes and pepper), USA (eradicated) and several areas of Italy, including the island of Sicily. It has been eradicated in Germany following outbreaks in several glasshouses. In the last year there have been reports from Turkey, China, Greece, and, in June 2019, the first report 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 has now been confirmed. In 2020 there have been further outbreaks of ToBRFV in the UK at different nurseries. The virus has also been reported to be present in the Netherlands, Spain and Italy, with produce imports into the UK presenting 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 and at present there is no reported tomato 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, it is also thought that ToBRFV is seed transmitted. There have been reports that the virus can be transmitted by bumblebees during pollination.

The recent emergence of his 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. Hot water treatment of contaminated picking trays

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 as specified below.

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

1. *Survival on skin and gloves*

This was investigated by exposing skin and gloved hands (Nitrile type disposable gloves) to infected sap, and also by rubbing infected leaves. Results are shown in Tables 1 and 2. In both cases the virus 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.

Table 1. 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 2. ELISA results of test plants swabbed from skin and gloves after contaminating by rubbing with ToBRFV **infected leaves**.

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

2. *Hand washing to reduce contamination risk*

Hands were rubbed with leaves from ToBRFV infected tomato plants. 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. 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

Results for this investigation are given in Table 3.

Subsequently further treatments were investigated using the same approach as above but the specific treatments investigated were:

- Water (Control)
- Enno Rapid (hand gel)
- Nzym Rugo (hand gel)

Each treatment was tested for 30 seconds and 1 minute, results are presented in Table 4.

Table 3. Combined results of multiple handwashing experiments. ELISA results of test plants swabbed from ToBRFV contaminated hands after washing using water, Enno Rapid and Nzym Rugo.

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

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

The results of handwashing indicate that any form of handwashing for an extended period may have some effect on reducing inoculum 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 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.

3. *Survival of the virus on glasshouse surfaces and tools*

A range of common materials used in glasshouses were surface contaminated with sap from infected tomato plants. These were left at room temperature for up to 6 months and periodically swabbed to ascertain the length of time virus will survive on surfaces,

Table 4. 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	+	(+)	-

These results confirm the assumption that, as with other tobamoviruses, ToBRFV is environmentally stable for extended periods on a range of common glasshouse surfaces. The implication of this would be 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 5: Hot water disinfection of plastic crates*). Survival of ToBRFV on concrete looks to be variable, possibly a reflection of an uneven surface allowing the virus to harbour.

4. *Efficacy of disinfection approaches on glasshouse surfaces and tools*

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 either 1 minute or 1 hour 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 were:

Table 5. 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%
TSOP	Trisodium orthophosphate		10%	10%
Sodium hypochlorite	Sodium hypochlorite	Approx. 10,000 ppm	20 ml in 500 ml water	400ppm

Table 6. 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 7. 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		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

Initially disinfectants were tested for short exposure efficacy, with a one-minute exposure time. The disinfectants tested at one-minute were Menno Florades, Jet 5, Sodium hypochlorite and Virkon S. No disinfectant gave control of ToBRFV at 1 minute exposure times. Additional disinfectants (Huwa San and TSOP) were not trialled at one minute. Subsequent trials have focused on a 60 minute exposure. Virkon-S, and Huwa San appear to give effective denaturing of ToBRFV after 60 minutes exposure except on concrete. Menno Florades also looks to be mainly effective at a 1 hour contact time on all surfaces except concrete.

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 do not look to be effective on most surfaces.

Further trials are being conducted on different products. The results of these will be reported via AHDB when available.

5. 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. Pieces of plastic crate were contaminated with infected plant sap and then a 'pre-treatment' swab was taken to ensure the virus treatment on surfaces was infectious. These were then soaked at either 70^{O}C or 90^{O}C for 5 minutes and were re-swabbed. Surfaces were then treated with Virkon at short exposure time and re-swabbed to ensure that the virus had been denatured.

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	+	-	-

These data indicate that treatment at 70^{O}C alone does not give adequate control of the virus, but at 90^{O}C the virus was destroyed. 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.

The results presented here indicate that ToBRFV is robust and survives for extended periods on both skin and gloves, and on a range of glasshouse surfaces. At present the only treatments that appear to work in isolation on surfaces are either heating to 90^{O}C for 5 minutes, or a 1 hour exposure to Virkon-S, but further work is ongoing to investigate these longer exposure times across a range of other disinfectants.

Financial Benefits

- Tomato brown rugose fruit virus, has the potential to infect 100% of an infected crop as there is no genetic resistance to the virus in tomato.
- 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: Virkon (1 % ai, 1 h duration) and Huwa San (12.5% ai, 1 h duration) and Menno Florades (0.36% ai, 1 hour duration) are effective for ToBRFV kill on a range of glasshouse surfaces. No product gave effective control of ToBRFV on concrete except sodium hypochlorite (400ppm, 1 hour duration).

Hot water treatment of contaminated picking trays: Soaking ToBRFV contaminated plastic picking trays in hot water for 5 min at 90°C will denature the virus. Soaking the trays at 70°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.

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.

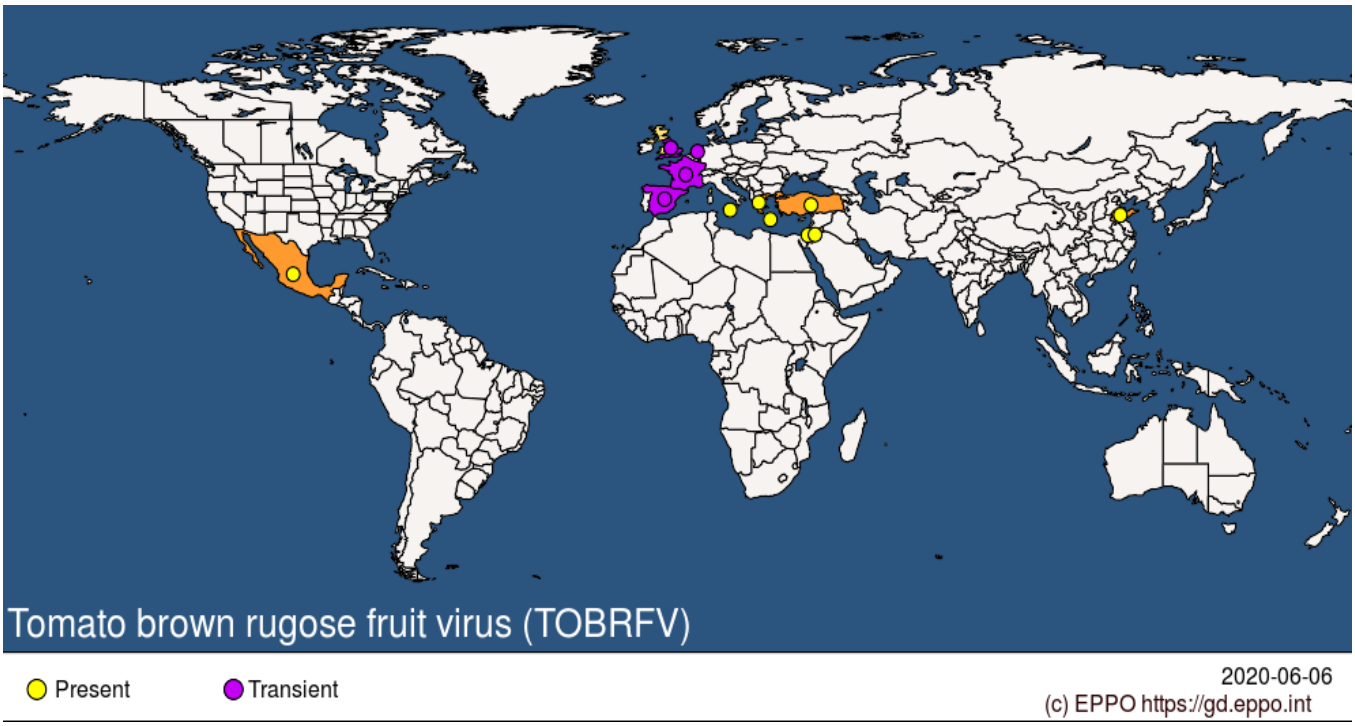
- For England and Wales, contact your local APHA Plant Health and Seeds Inspector, or the PHSI Headquarters, Sand Hutton, York.
  Tel: 0300 1000 313.
  Email: planthealth.info@apha.gsi.gov.uk.
- For Scotland, contact the Scottish Government's Horticulture and Marketing Unit:
  Email: hort.marketing@gov.scot
- 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 greenhouses almost nationwide within the period of one year after the first outbreak reports. The virus has since been reported from Jordan (2015), Mexico (both tomatoes and pepper), USA (eradicated) and several areas of Italy, including the island of Sicily. It has been eradicated in Germany following outbreaks in several glasshouses. In the last year there have been reports from Turkey, China, Greece, Netherlands, Spain and in June, 2019, the first report 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 has now been confirmed. In 2020 there have been further outbreaks of ToBRFV in the UK at different nurseries.

Map showing the distribution of ToBRFV (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 and at present there is no reported tomato 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 it is also thought that ToBRFV is seed transmitted. There have been recent 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 are known to 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 aims of this project 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
- Hot water treatment of contaminated picking trays

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 bread 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 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.

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 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)

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. 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 bioamplified in the test plants. In addition, use of ELISA was more appropriate for the number of samples being tested.

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 either 1 minute or 1 hour 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%
TSOP	Trisodium orthophosphate		10%	10%
Sodium hypochlorite	Sodium hypochlorite	Approx. 10,000 ppm	20 ml in 500 ml water	400 ppm

Further disinfectants will be tested in subsequent work.

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 70°C or 90°C 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.

Results

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

Survival on skin and 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	+	+	+	+	+	+	+

These results show that ToBRFV can survive on both hands and gloves for at least 2 hours. This result is the same for both ground up infected sap and from rubbing infected leaves onto the hands or gloves.

Hand washing to reduce contamination risk

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

	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 this first handwashing experiment, 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. At 1 minute all the treatments were effective at controlling ToBRFV except the medicated hand wash with water.

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

	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

In this experiment it appears that Enno Rapid is an effective hand wash against ToBRFV, at both 30 seconds and 1 minute, however, the results for the water only wash differ from the results obtained previously (Table 5) and therefore, it was decided to repeat this experiment (Table 7).

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

	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

The results from this experiment show that at 30 seconds none of the treatments (water, Enno Rapid and Nzym Rugo) are effective against ToBRFV. With a 1 minute treatment, Nzym rugo appears to be effective but Enno Rapid does not appear to give effective control.

Table 8. Combined results of multiple handwashing experiments. ELISA results of test plants swabbed from ToBRFV contaminated hands after washing using water, Enno Rapid and Nzym Rugo.

Surface	Time	Water	Enno Rapid	Nzym Rugo
Skin (hands)	30 seconds	+*	+*	+*
	1 minute	+*	+*	-

+* = Virus survival in some repetitions (inconsistent)

-= Virus did not survive

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

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 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	+	(+)	-

ToBRFV remained infective on all surfaces tested for at least 7 days and can remain infective on some of the surfaces for at least 6 months.

The results of the 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, however, the 3 month results for the 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.

Efficacy of disinfection approaches

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. Some 1hour contact time work is still in progress.

	Disinfectant											
Surface	Menno Florades		Jet 5		Sodium hypochlorite		Virkon S		Huwa San		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

ToBRFV remained viable after 1 min treatments with a range of disinfectants (at recommended rates) on all glasshouse surfaces tested. 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 not reliable and are not presented here.

This testing has been repeated. The results show that Virkon-S, and Huwa San appear to give effective denaturing of ToBRFV after 60 minutes exposure except on concrete. Menno Florades also looks to be mainly effective at a 1 hour contact time on all surfaces except concrete.

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 do not look to be effective on most surfaces.

Efficacy of hot water treatment combined with disinfection

Table 12. 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	+	-	-

A 5 minute treatment, of the contaminated plastic tray, with water at 90ºC was effective in eliminating ToBRFV. Soaking the contaminated tray for 5 minutes at 70ºC 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 disinfection work it is known that a 1 minute contact time with Virkon alone and no previous soaking does not stop ToBRFV being viable.

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 or from a random infected plant, 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.

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. Virkon-S, and Huwa San appear to give effective denaturing of ToBRFV after 60 minutes exposure except on concrete. Menno Florades also looks to be mainly effective at a 1 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 do not look to be effective on most surfaces. 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 used 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. Further disinfection work is planned which will give more information on disinfectants to use and contact times needed.

In the event of an outbreak after clean-up, it would be recommended to take swabs from various surfaces from around the glasshouse and get them tested for tomato brown rugose fruit virus by inoculation onto test plants. This will 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. Whilst specific data on thermal inactivation of ToBRFV is not available, other tobamoviruses are known to be inactivated at high temperature, for example cucumber green mottle mosaic virus in sap is inactivated by 10 minutes at 90^0C. Commercially, plastic trays are now being steamed by some growers at 95^0C for approximately 40 minutes. There is a small risk that the soak in hot water does not mirror steaming, as soaking may have the physical effect of washing rather than just heating. However, heat will be a major part of the effect, with these results showing that the 70^0C treatment repeatedly did not work, so soaking alone is not sufficient.

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. 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 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. Virkon, Huwa San and Menno Florades appear to be effective at a 1 hour contact time except on concrete.

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.

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

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 October 2020)

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. (March 2019 and February 2020)
- Fortnightly contributions to ToBRFV steering group discussions

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