# Grower Summary

## PE 033/ PE 033a

Tomato brown rugose fruit virus: survival of the virus and efficacy of disinfection approaches

Final 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/).

*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.
- ToBRFV was destroyed on plastic trays soaked in hot water for 5 min at 90°C. A soak in hot water at 70°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°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.

| Surface | Disinfectant | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 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