

AHDB Research Project: PE 033 (Fera Science Ltd)

Interim results - March 2020

Tomato brown rugose fruit virus: survival and disinfection

Survival on skin and gloves

Headline

ToBRFV remained infective for at least 2 hours on skin and gloves (confirmed in two trials by different methods).

Trial summary:

- Hands/gloves were contaminated with infected sap or infected leaf
- Hands were rubbed with cotton bud, and then rubbed onto test plants at time intervals to check for transmissibility
- After (max) 3 weeks test plants tested by ELISA for the presence of ToBRFV

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	+	+	+	+	+	+	+

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

Table 2. 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	+	+	+	+	+	+	+

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

Hand washing to reduce contamination risk

Headlines

- *In trial 1, a range of handwashing treatments for 30 seconds (water, + soap, + medicated handwash, + medicated hand wash and gel) were ineffective in preventing virus spread. After a longer treatment (1 minute), all the treatments (except water + medicated handwash) were effective in eliminating the virus.*
- *In trial 2, there was no spread of ToBRFV after handwashes for 30 seconds or 1 minute with Enno Rapid. This trial was repeated to check for 'false negatives' as results for a 'water only' treatment varied from trial 1.*
- *In trial 3 Enno Rapid and Nzym Rugo were ineffective in preventing virus spread at 30 seconds but Nzym Rugo was effective at 1 minute*
- *In general handwashing is not very effective at removing ToBRFV with, in most instances, a 1minute wash required to remove the virus, which is not very practical.*

Trial summary:

- Hands were contaminated with **infected leaf**
- Hands washed for 30 seconds and 1 minute (water, water + soap, water + medicated hand wash, water + medicated hand wash + gel, Enno Rapid)
- Hands swabbed with cotton bud and then cotton bud used to inoculate test plant
- After (max) 3 weeks test plants tested by ELISA for the presence of ToBRFV

Table 3. ELISA results of test plants swabbed from ToBRFV contaminated hands after washing using different treatments

Length of wash	Hand wash							
	Water only		Water & soap		Water & medicated hand wash		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

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

Table 4. ELISA results of test plants swabbed from ToBRFV contaminated hands after washing using water and Enno Rapid (This testing has been repeated as the water results for 30 seconds did not match the previous results)

Length of wash	Hand 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

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

Table 5. ELISA results of test plants swabbed from ToBRFV contaminated hands after washing using water, Enno Rapid and Nzym Rugo.

Length of wash	Hand 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

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

Survival on glasshouse surfaces

Headlines

- *ToBRFV survived for at least 3 months on most surfaces, including hard plastic, polythene, glass and stainless steel (test ongoing)*
- *ToBRFV survived for at least 1 month on aluminium*
- *ToBRFV can survive on concrete up to 3 months, however, this looks variable as in some cases the virus did not survive 2 weeks.*

Trial summary:

- Different surfaces were contaminated with infected sap
- Surfaces swabbed at different time periods (onto test plants): 2 hrs, 8 hrs, 48 hours, 7 days, 4 weeks, 3 months, 6 months
- After (max) 3 weeks test plants tested by ELISA for the presence of ToBRFV
- For the second replicate experiment due to the ToBRFV not surviving on concrete at 4 weeks, swabs were also taken from concrete at 2 and 3 weeks.

Table 6. ELISA results of test plants swabbed from surfaces contaminated with ToBRFV infected sap at different time periods. 6 months still in progress.

Surface	Time since contamination of 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	+	(+)	

+ = positive result by ELISA, indicating the virus is viable

- = negative result by ELISA, indicating the virus is not viable

(+) = positive result by ELISA, indicating the virus is viable, for 1 of the 2 survival experiments only (all 3 reps for the 1 experiment were positive)

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

Efficacy of disinfection approaches

Headlines

- *ToBRFV remained viable after 1 min treatments with a range of disinfectants (at recommended rates) on all glasshouse surfaces tested.*
- *Longer duration disinfectant treatments (1 hour) are now being tested.*
- *Initial results (1 rep only) suggest Virkon is effective against ToBRFV at 1 hour contact time but Jet 5 (1 rep only) does not look effective on most surfaces.*
- *Virkon and Huwa san (1 rep only) appears to be effective against ToBRFV at 1 hour contact time except on concrete. Initial results (1 rep only) suggest sodium hypochlorite is partially effective against ToBRFV on polythene and glass and is effective against ToBRFV on other surfaces. Jet 5 (1 rep only) does not look to be effective on most surfaces.*

Trial summary:

- A range of disinfection treatments were used at the recommended rate (except Huwa San: used at 25%) on different glasshouse surfaces contaminated with ToBRFV infected sap.
- Surfaces: glass, concrete, aluminium, hard plastic (trays), polythene & stainless steel.
- Swabbed at **1 minute** after application onto test plants.
- After (max) 3 weeks test plants tested by ELISA for the presence of ToBRFV.
- As the virus was still viable with a 1 minute contact time with all the disinfectants tested, the experiment was repeated with a **1 hour** contact time.

Table 7. ELISA results of test plants swabbed from surfaces contaminated with ToBRFV **infected sap** 1 minute and 60 minutes after being sprayed with disinfectant. **1 hour contact work in progress**

		Disinfectant													
		Menno Florades		Jet 5		Sodium hypochlorite		Virkon S			Huwa San		TSOP		
Surface	Contact time (mins)	1	60	1	60*	1	60*	1	60	60 (rep 1)	60 (rep 2)	1	60*	1	60
Glass		+		+	+	+	1/3	+	-	-		N/A	-	N/A	
Concrete		+		+	2/3	+	-	+	-	2/3		N/A	3/3	N/A	
Aluminium		+		+	2/3	+	-	+	-	-		N/A	-	N/A	
Hard Plastic		+		+	-	+	-	+	-	-		N/A	-	N/A	
Polythene		+		+	2/3	+	1/3	+	-	-		N/A	-	N/A	
Stainless steel		+		+	+	+	-	+	-	-		N/A	-	N/A	

+ = 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)

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

N/A not being tested

60* only 1 rep has been carried out, testing to be repeated.

Hot water treatment of plastic trays and disinfection

Headlines

- *ToBRFV was eliminated from trays soaked in hot water for 5 min at 90°C*

- *A hot water soak (70°C for 5 min) was insufficient alone to kill the virus but was effective when trays were sprayed with Virkon after the heat treatment.*

Trial summary:

- Tray sections were contaminated with **infected sap**
- Swabs were taken pre-treatment (onto test plants)
- Treatments for 5 mins at 70°C and 90°C
- Swabs taken post heat treatment then sprayed with Virkon, left for 1 minute and re-swabbed.
- After (max) 3 weeks test plants tested by ELISA for the presence of ToBRFV

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

Temperature of water	Pre-treatment	5 minute soak	After soak + Virkon
70°C	+	+	-
90°C	+	-	-

+ = 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)