Original thinking... applied

AHDB project PE 033:
Tomato brown rugose fruit virus: Survival and disinfection

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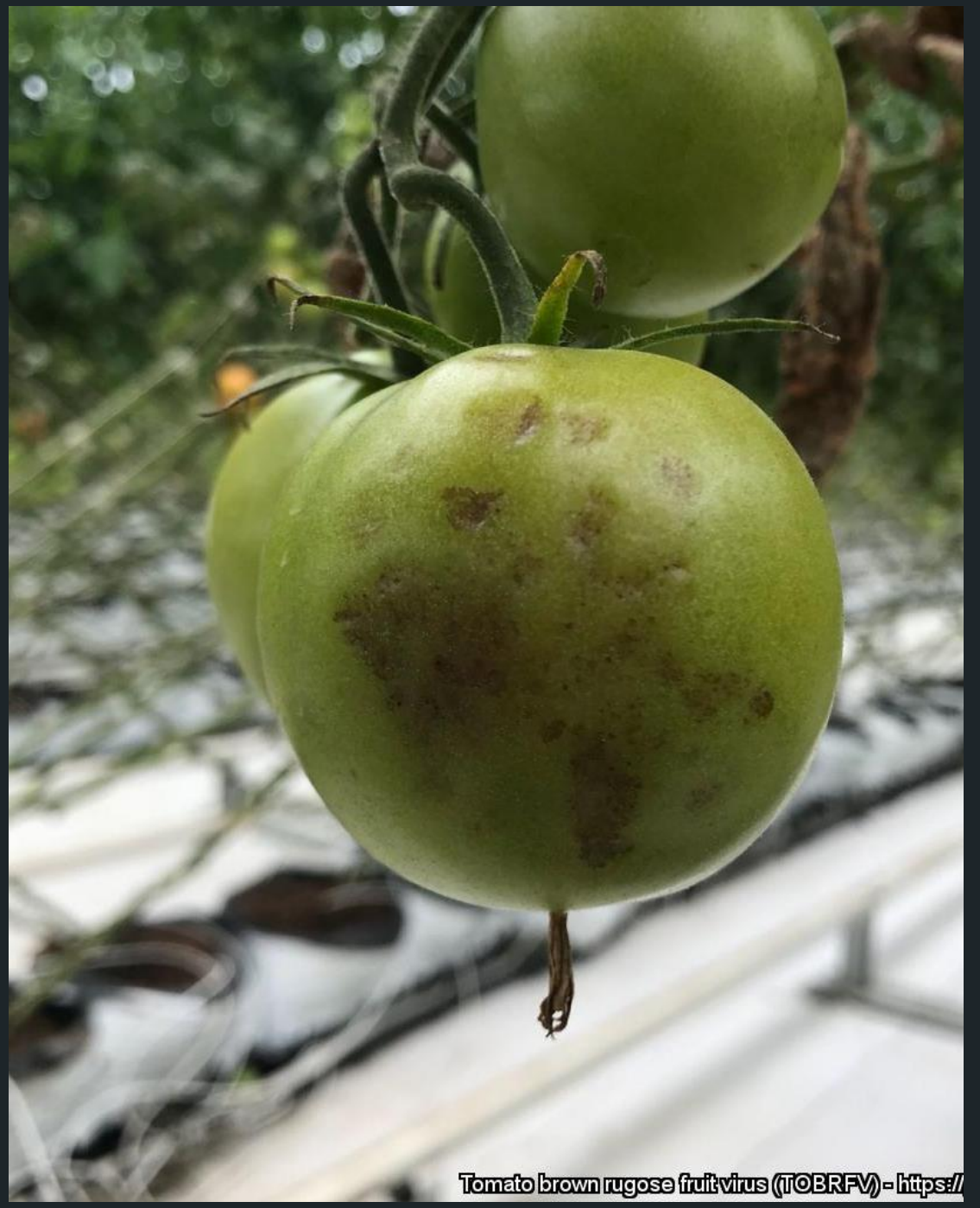


Tomato brown rugose fruit virus

- Virus can overcome TMV resistance genes in tomato
- Spread through mechanical contact such as direct plant to plant contact, tools, clothing, bumblebees
- Spreads rapidly within crop
- Seed transmission implicated
- Once plants are infected they cannot be ‘cured’
- Good hygiene measures minimise spread and limit impact should an outbreak occur
- Also recorded on Pepper crops

Source: A. Dombrovsky/EPPO

Source: S Davino/EPPO

ToBRFV an emerging problem

Middle East:

- First outbreak recorded in Israel (2014), now present in all tomato growing regions.
- Also reported in Jordan (2015)

Americas:

- Mexico (2018), California, USA (2018 – Considered Eradicated)

Europe:

- Italy (2018), Germany (2018 – Eradicated), **Turkey (2019), China (2019)**
- **UK (July, 2019)**
- Unconfirmed reports from Belgium and Netherlands (2019 – source Seedquest.com)

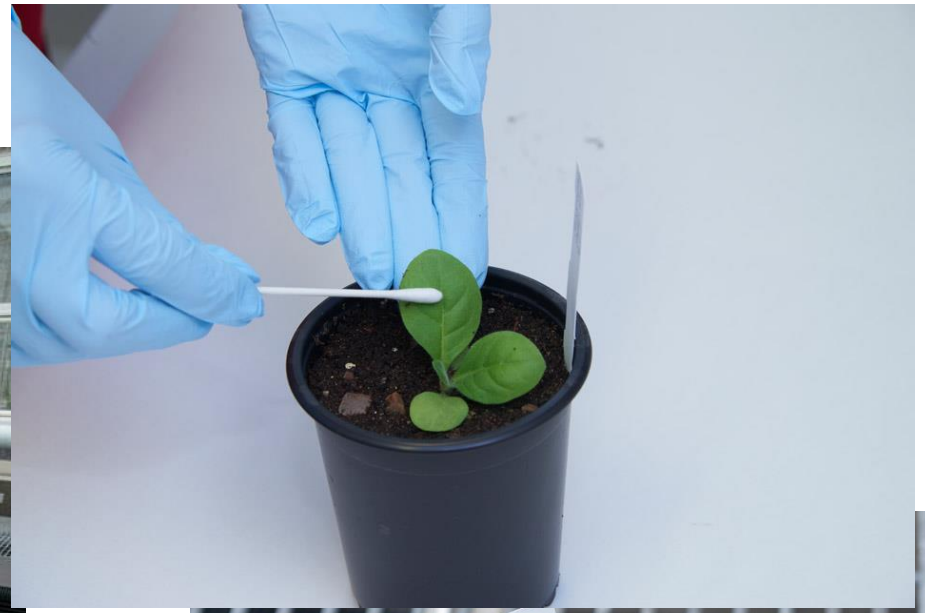
Aims of ToBRFV survival & disinfection project

To provide information on the efficacy of preventative hygiene measures & disinfection to minimise the risks posed by tomato brown rugose fruit virus.

Investigate:

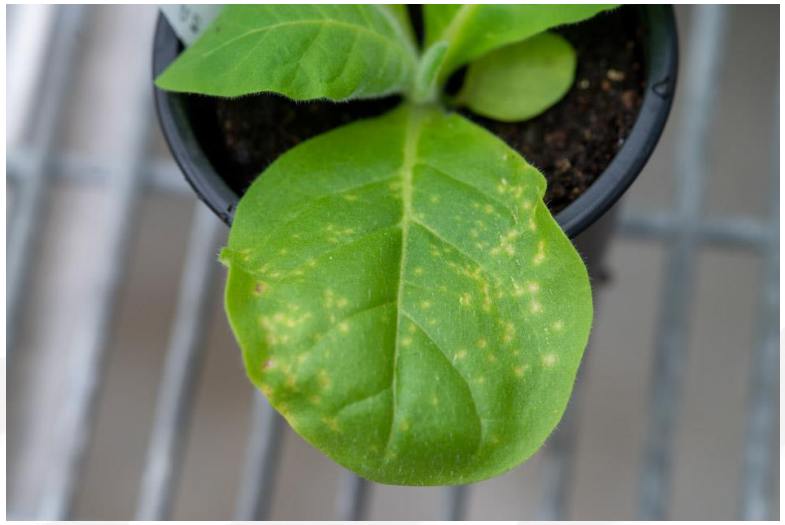
- Survival of ToBRFV on skin and gloves
- Handwashing to reduce the risk of contamination in the glasshouse
- Hot water treatment of contaminated trays
- Survival of the virus on glasshouse surfaces and tools
- Efficacy of disinfection approaches on glasshouse surfaces and tools

Experimental set-up



Survival on skin and gloves

- Hands/gloves contaminated with **infected sap**
- Hands rubbed with cotton bud, and then this is 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



Survival on skin and gloves

Surface	15 mins	30 min	45 min	1 hour	1 hr 30 mins	2 Hours
Skin	+	+	+	+	+	+
Gloves	+	+	+	+	+	+

- This was repeated with just **contact** transmission from infected leaf. The same results were seen (survived for 2 hours +).

Efficacy of Handwashing

- Hands contaminated with **infected leaf**
- Hands washed for **30 seconds (water, + soap, + medicated hand wash, hand wash + gel)**
- 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

Surface	30 second water wash	30 second wash with soap	30 second wash with med. hand wash	30 second wash with med hand wash + gel
Skin	+	+	+	+

Efficacy of handwashing

- Handwashing experiment repeated
- Hands contaminated with **infected leaf**
- Hands washed for **I minute (water, + soap, + medicated hand wash, hand wash + gel)**

Surface	1 minute water wash	1 minute wash with soap	1 minute wash with med. hand wash	1 minute wash with med hand wash + gel
Skin	-	-	+	-

- ToBRFV survived 1 minute washing with water and medicated hand wash.
- Removal of virus may be due to the rubbing action.
- Recommend wearing gloves and changing often.

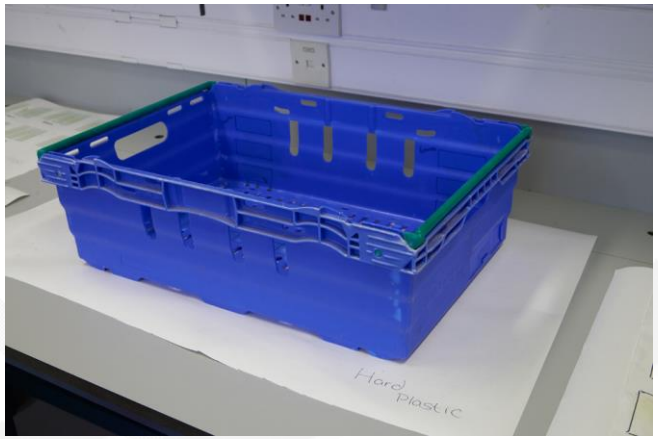
Hot water treatment of trays + Disinfection

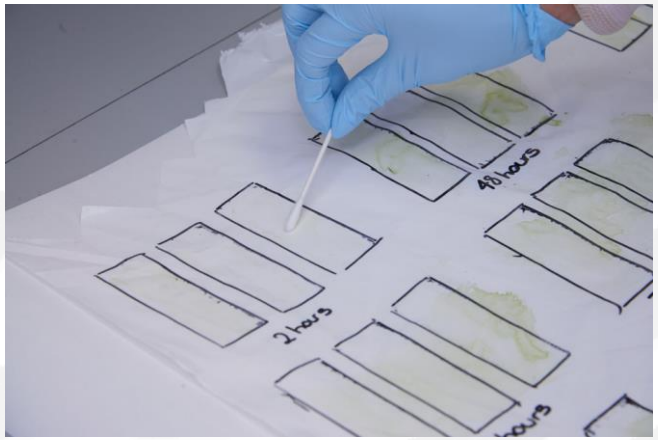
- Tray sections contaminated with **infected sap**
- Swabbed pre-treatment (onto test plants)
- Treatments for 5 mins at 70^{O}C and 90^{O}C
- Swab post heat treatment then sprayed with Virkon, left for 1 minute and re-swabbed.
- After (max) 3 weeks test plants tested by ELISA for the presence of ToBRFV

Treatment	Pre-treatment	5 min soak	After soak + virkon
70^{O}C	+	+	-
90^{O}C	+	-	-

Survival on glasshouse surfaces

- Different surfaces contaminated with **infected sap**
- Surfaces: glass, concrete, aluminium, hard plastic (trays), polythene & stainless steel
- Swabbed at different time periods (onto test plants): 2 hrs, 8 hrs, 48 hours, 7 days, 4 weeks.....
- After (max) 3 weeks test plants tested by ELISA for the presence of ToBRFV





Survival on glasshouse surfaces

Surface	2 hrs	8 hrs	24 hrs	7 days	4 weeks
Glass	+	+	+	+	+
Concrete	+	+	+	+	-
Aluminium	+	+	+	+	+
Hard plastic	+	+	+	+	+
Polythene	+	+	+	+	+
Stainless steel	+	+	+	+	+

- Testing carrying on to 3 and 6 months
- Experiment to be repeated

Efficacy of disinfectants

- A range of disinfection treatments being tested, including **Virkon S**, **sodium hypochlorite**, **TSOP**, **Meno Florades, Huwa-san** and **Jet 5**. Used at recommended rate.
- 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.
- Testing in progress.

- Many thanks to Certis, Royal Brinkman and Roamtechnology for providing the Jet 5, Menno Florades and Huwa-san.

UK outbreak

- Affected glasshouse cleared voluntarily
- Glasshouse clean up: Formaline, Bleach, Hydrogen peroxide
- Service areas: Virkon, Bleach and Menno florades
- Swabs taken post clean up from glass, concrete, aluminium, plastic & ‘other’
- Swabs inoculated onto test plants and tested by ELISA
- No ToBRFV detected

Conclusion and further work

- Survival of AT LEAST 2 hours on skin and gloves
- Survival through ‘quick’ hand wash (30 seconds), including medicated handwash.
- After washing for 1 minute with water and medicated hand wash virus still survived. Recommend wearing gloves and changing often.
- Survival on trays through 70^{0}C wash for 5 minutes.
- Likely to be denatured at 90^{0}C/5 minutes.
- Virkon appears to be effective
- Survival on all surfaces tested for at least 4 weeks (except concrete, 7+ days)
- Disinfection trials in progress.