



Tomato Brown Rugose Virus (ToBRFV) session and panel

Adrian Fox (Fera Science Ltd); Martijn van Stee (Enza Zaden); Paul Simmonds (Rijk Zwaan); Matthew Everatt (Defra); Nick Arnst (La Serra)



ToBRFV resistance

Martijn van Stee
Enza Zaden

HREZ High Resistance to ToBRFV

How does HREZ work?

British Tomato Conference | September 2023 | Martijn van Stee, Enza Zaden



ENZA ZADEN





Martijn van Stee

Crop Breeding Manager Tomato

ENZA ZADEN





How did we start the journey to find high resistance against ToBRFV?

Screening of thousands of wild tomato plants which led to identifying the resistance gene



Solanum lycopersicum



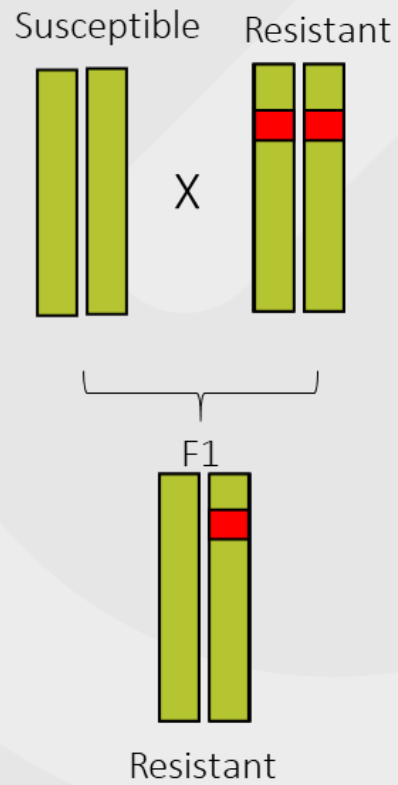
Solanum habrochaites

Single, dominant gene giving **high resistance**

Member of gene family that includes *Tm-2²* gene

Introgression in cultivated tomato plants

Traditional breeding techniques used



Not any resistance, but **High Resistance**.

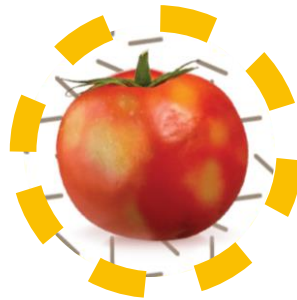
1,000,000 times less virus in HREZ plant under normal virus pressure

No transmission to other plants under normal virus pressure



High Resistance (HR)

- Highly restricts the accumulation of the virus.
- No spread of the virus in the crop.
- Yield is unaffected under normal virus pressure



Intermediate Resistance (IR)

- Virus propagation is delayed.
- Presence of virus particles in the plant or fruits.
- The crop can show symptoms of the virus in the leaf and/or fruits.
- Yield can be affected.



No resistance

- Virus multiplies to a high level in the plant.
- Yellow spots and wrinkled patches on plant & fruits.
- Presence of virus particles in the plant or fruits.
- Yield is affected.

You'll be able to recognize it by a new mark of confidence.

HIGH RESISTANCE ToBRFV
HREZ
ENZA ZADEN

High Resistance
and
High performing

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How does the HREZ resistance mechanism work?

Our HREZ is based on a **hypersensitive reaction**. This means that, when in contact with the virus, surrounding cells die.

This mechanism will actively block the virus from multiplying and spreading.



There are similarities between **ToBRFV** and **ToMV**.



In terms of:

- Hypersensitive reaction.
- ToMV-HR stopped the spread.
- The Tm-2² gene eradicated the ToMV virus over time.



Now we can grow.

- Over 500 HREZ hectares in the Benelux.
- Over 1000 HREZ hectares worldwide.

Paul Grootscholten, Agrocare:

"We currently grow almost only HREZ varieties here. One variety that is not yet resistant will also be replaced next year. It's really great for us to be able to focus fully on cultivation again now."



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Key lessons learned for successful crops.

HIGH RESISTANCE ToBRFV
HREZ
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Strict hygiene.



Use only HREZ varieties in one greenhouse.



Use HREZ rootstocks.





Thank you.

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Any information provided by Enza Zaden in this presentation as to the quality (including but not limited to viability, germination, seed size, mechanical or genetic purity, seed health) or performance of its products applies only to the results obtained by Enza Zaden at the time of the test with the specific seed sample used and under the conditions in which the test was performed. Any such information does not constitute an express or implied warranty by Enza Zaden as to the quality or performance of its products.

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ToBRFV Research update

Adrian Fox

Fera Science Ltd



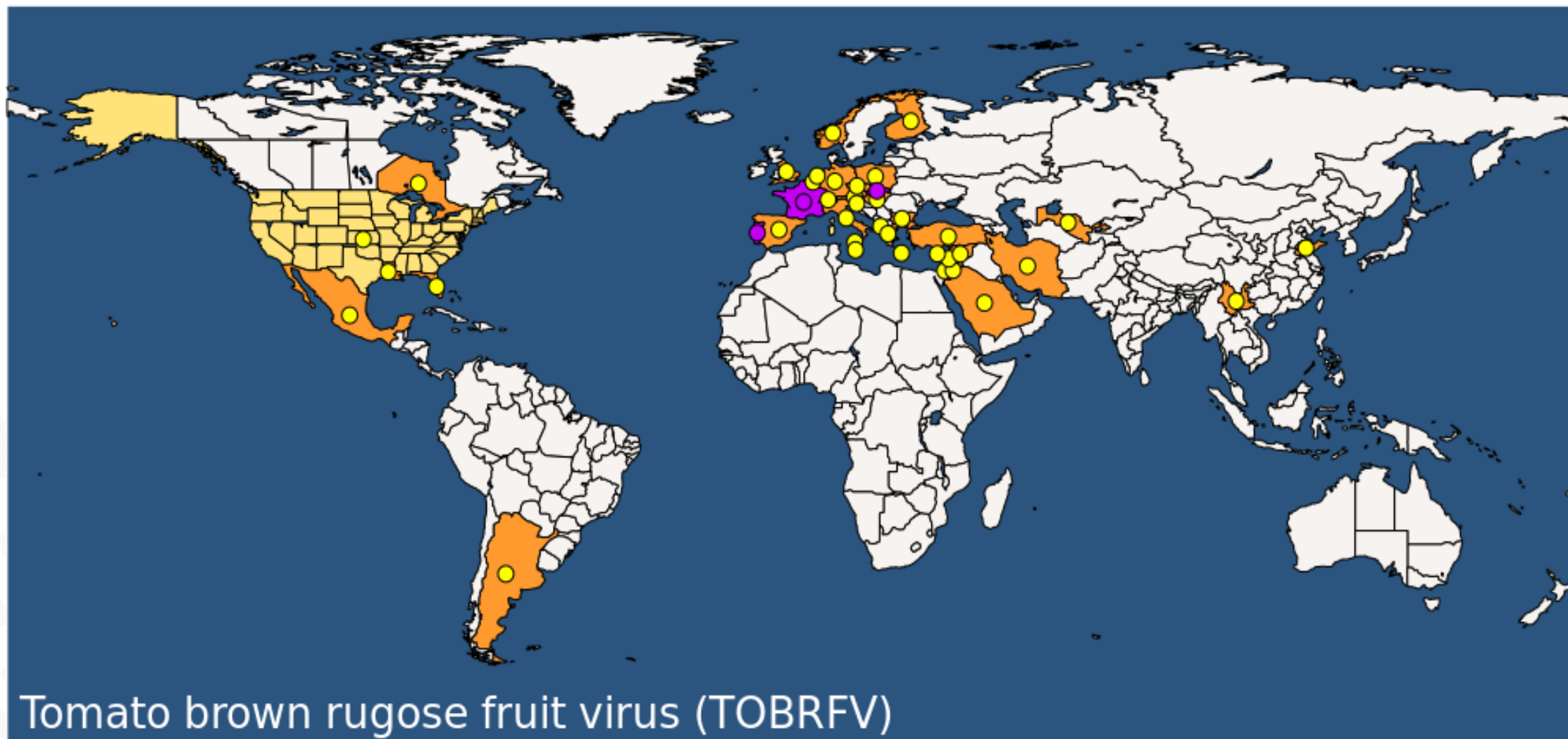
Original thinking... applied

Research update: Improving strategies for ToBRFV surveillance in tomato fruit production

Adrian Fox



Current global distribution



● Present

● Transient

2023-09-11

(c) EPPO <https://gd.eppo.int>

ToBRFV current situation - UK

- UK surveillance indicates 3 sites currently infected and under official plant health controls
- “North” – Infection detected towards end of 2022 growing cycle.
 - Glasshouse switched to cucumber production.
 - “cross protection” ToBRFV genotype
- “Midlands” - Ongoing re-infection since 2020.
 - Infection initially early in cycle, thought to be via packhouse contamination
 - Currently testing negative
- “South” – Infection detected spring 2023, early in crop cycle
 - Grower maintaining crop
 - Eradication action required before next crop



Research questions:

Is the current approach of plant focused surveillance fit for purpose?

- How do we optimise plant sampling?
- Are there better ways of performing surveillance?
- Are we conducting ToBRFV surveillance in 2023 with a 2018 mindset?

Plant sampling and testing

Initially: XXX leaves (plants) from XXX,000 plants

- Statistics support these numbers but work still needed on agreed “best practice” for sampling for reliable detection

BUT:

- Some strong positives detected with no symptoms at time of sampling.
 - **Symptoms are not a reliable measure of infection**
- Some weak positives (“high Ct”), across multiple real-time PCR tests, which could not be confirmed through a secondary method
 - **Biological issue, Environmental residues, or laboratory contamination?**
 - Experienced labs take extreme precautions against laboratory contamination:
 - Separation of work (spatial and temporal)
 - Disposable PPE, bench covers,
 - Prophylactic, routine disinfection
 - Routine swab testing



Optimising plant sampling

- Idea from Samuel, 1934
- Work carried out at Fera, UK, and NIVIP and WUR, NL.
- Inoculation experiments
 - Young plants (~ 4 weeks post emergence,
 - Plants entering production (~8 weeks)
 - Mature plants (~17 weeks)
- Outbreak site sampling
 - Unknown timing of infection

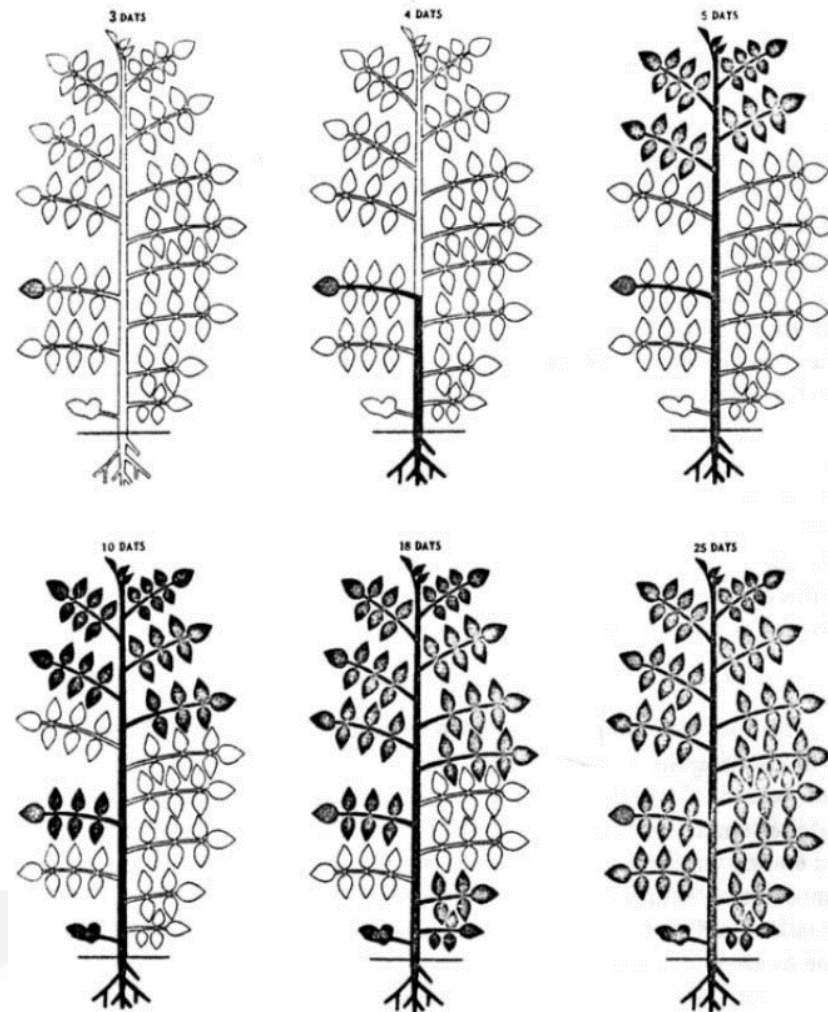
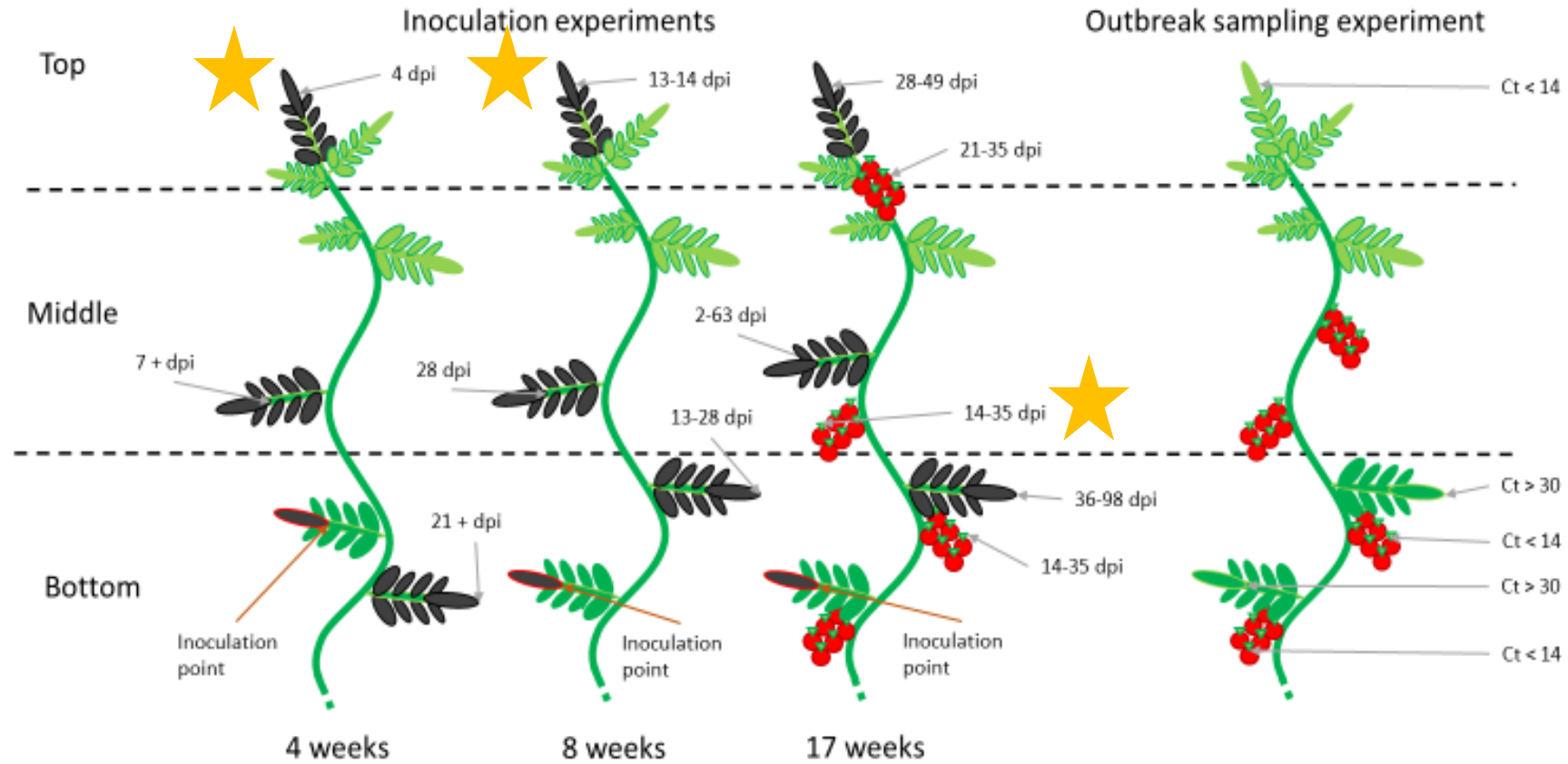


Figure reproduced from Samuel 1934, *Ann. App. Biol.*

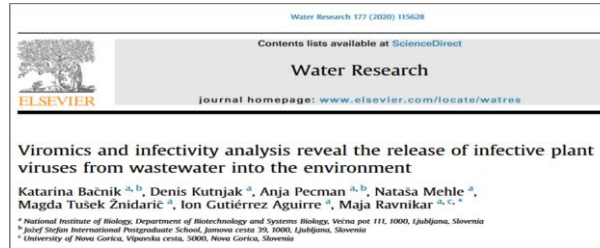
Sample sepals where available...



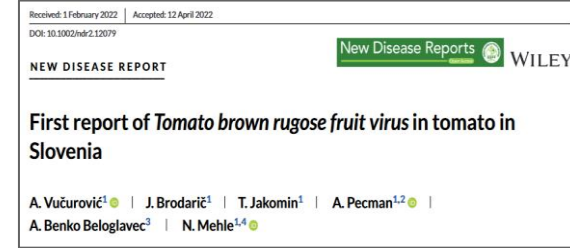
Source of water contamination with ToBRFV?



Mehle & Ravnikar, Water Research 2012, 46(16): 4902-4917

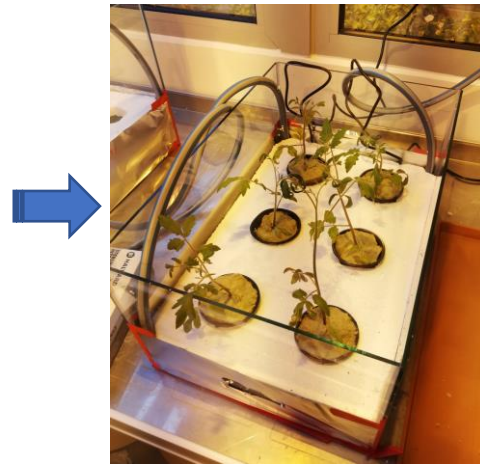


Slovenia 2017: ToBRFV detected in wastewater



Slovenia 2021: ToBRFV detected in tomato

Can ToBRFV be released from infected plants into water (nutrient solution) through the roots?



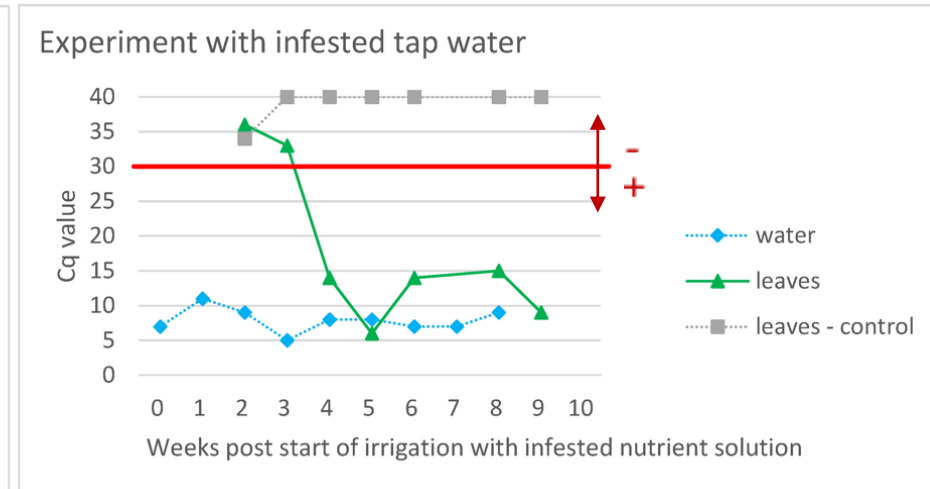
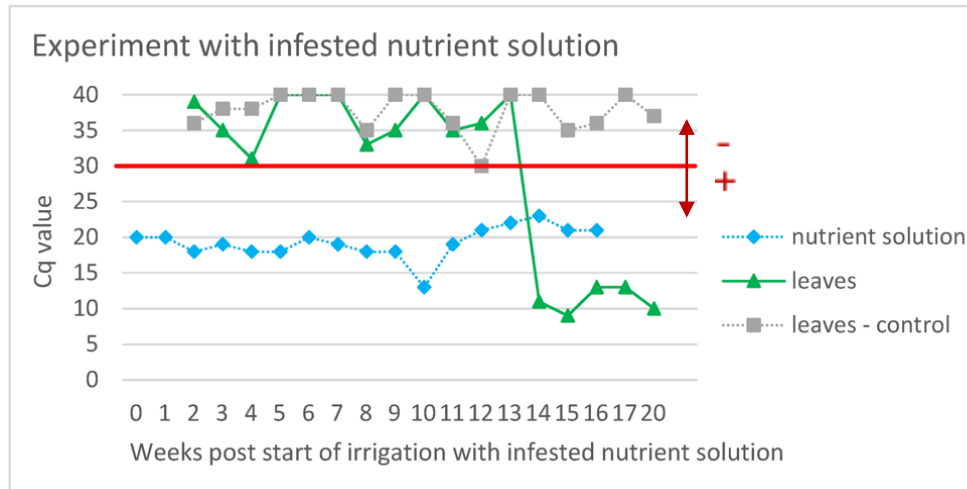
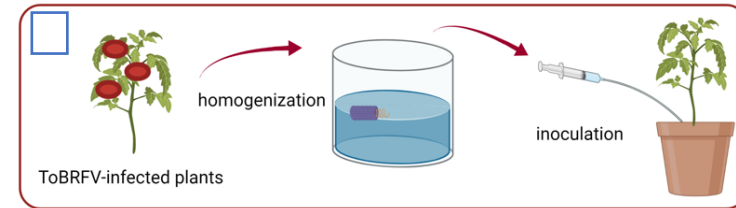
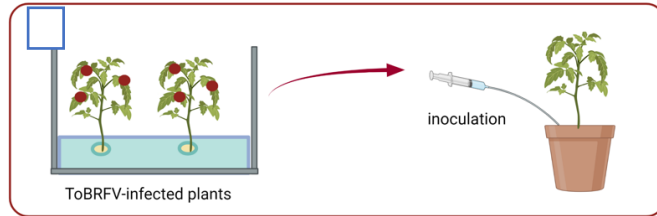
week	RT-qPCR* (Cq)
0	undet
1	25
2	25
3	23
4	21
5	19
6	19
7	17
8	15
9	16



✓ Virus particles released from the roots are infectious

Can plant infection occur when irrigating with ToBRFV contaminated water?

Evaluation of ToBRFV infection in tomato plants grown in substrate irrigated with ToBRFV-infested nutrient solution/tap water

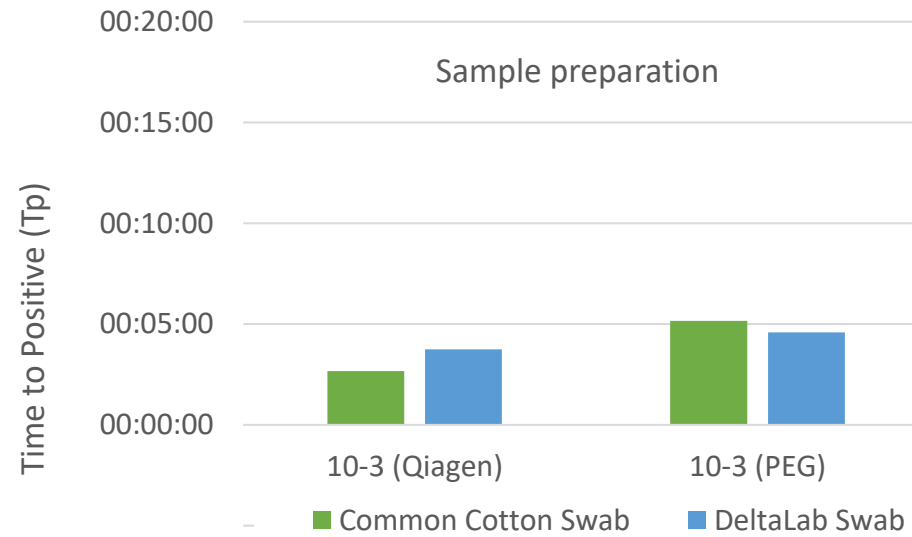
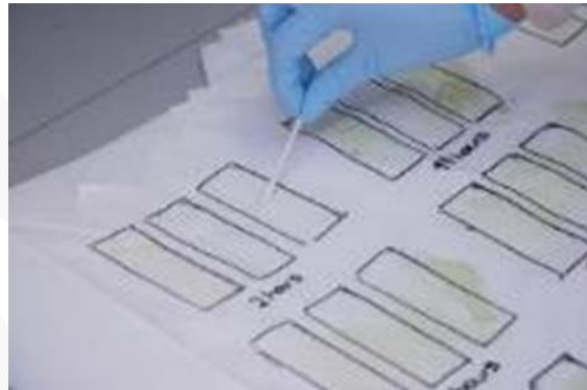


Mehle et al.,
Frontiers in Plant Science 2023,
doi.org/10.3389/fpls.2023.1187920

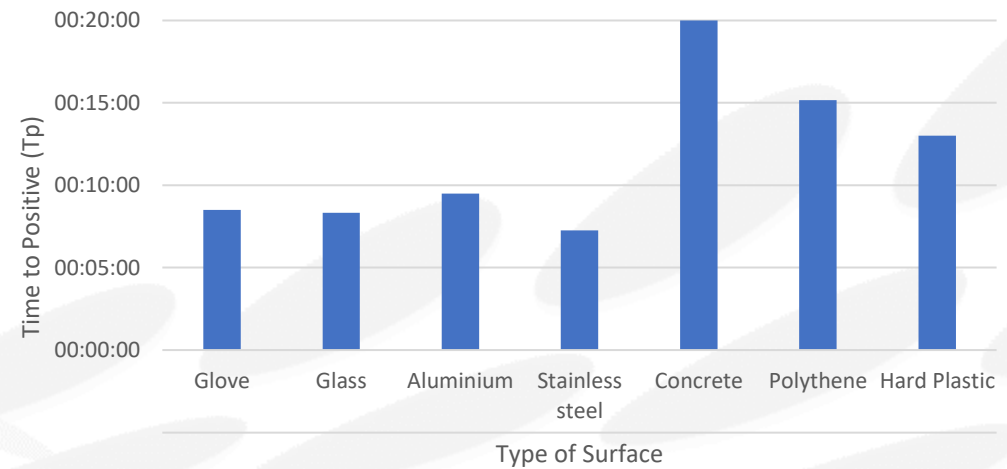
Future plans:
similar experiments with ToMMV

T **BAM** <http://projects.nib.si/tobamo/>
(L4-3179)

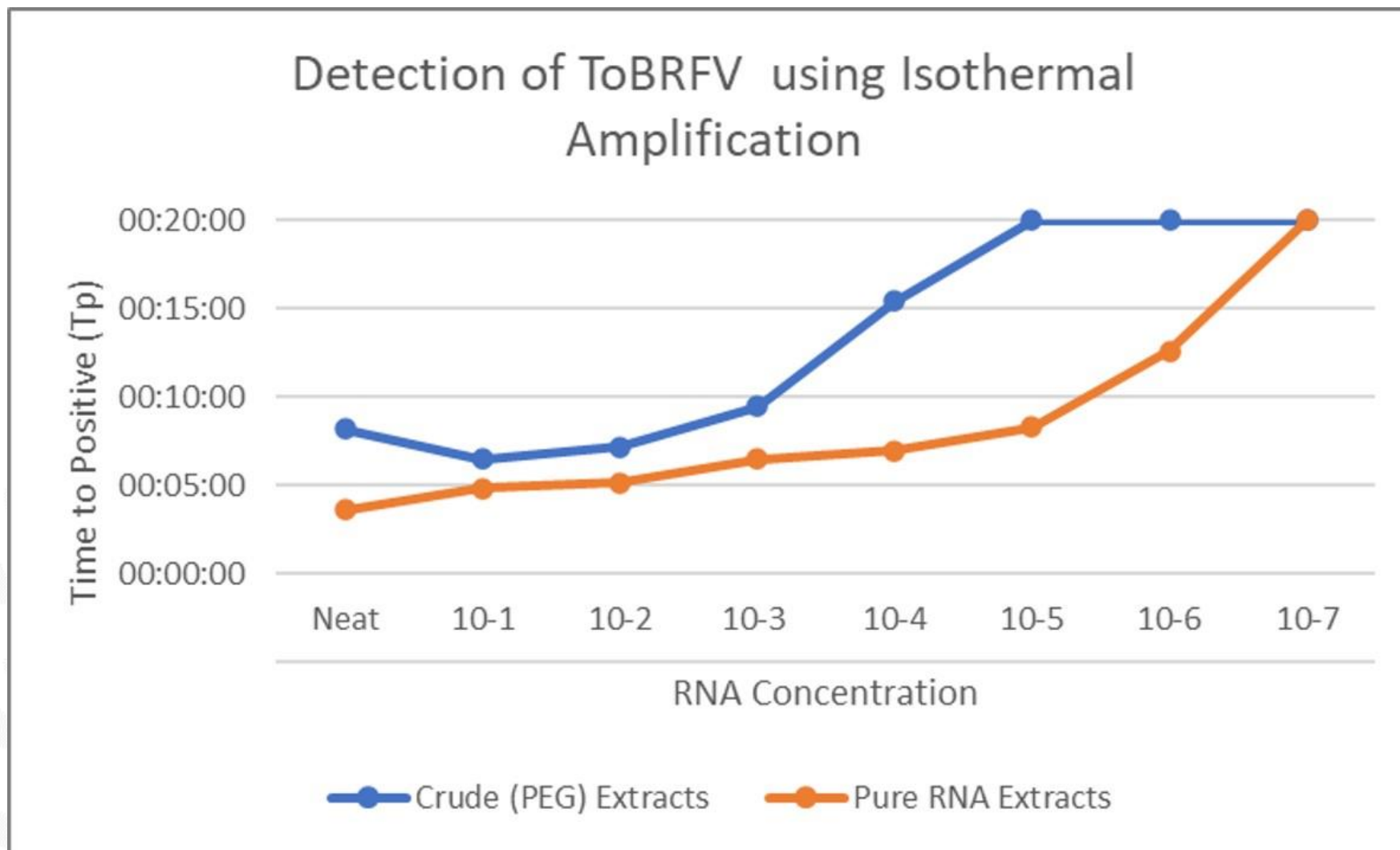
Swabs: validation for on-site testing with LAMP?



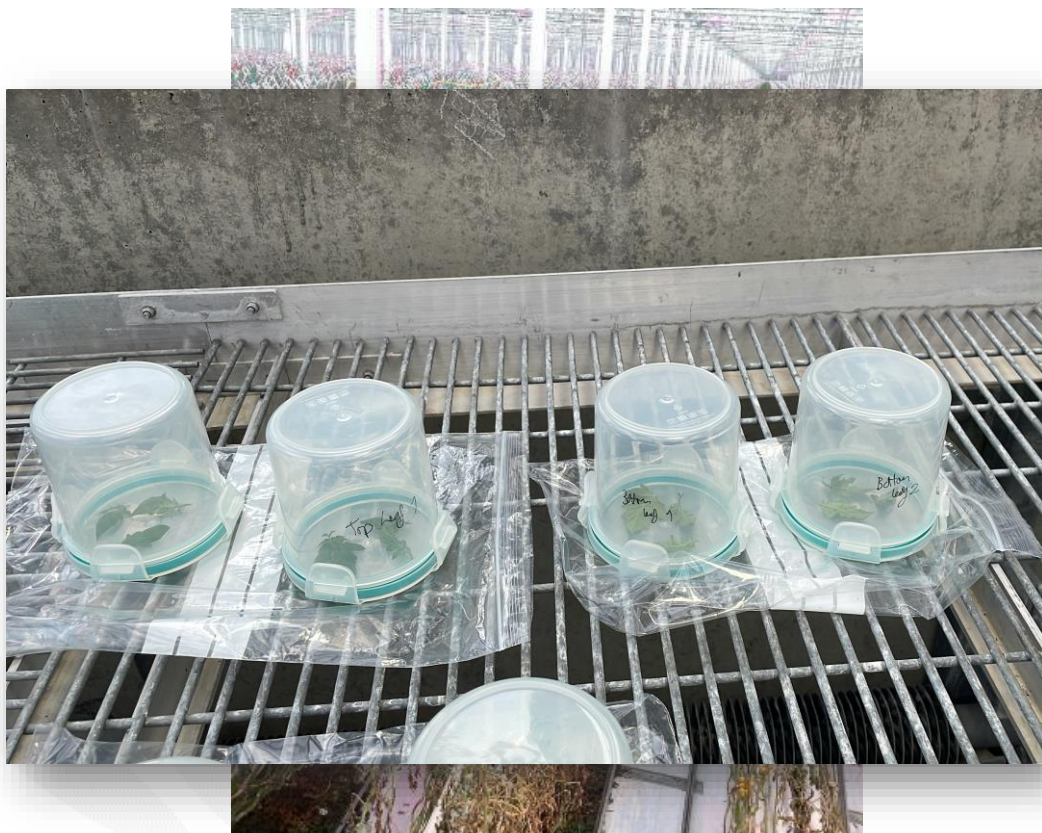
Detection of ToBRFV from glasshouse surfaces



Optimising crude extraction for in field use



Understanding sources of environmental contamination

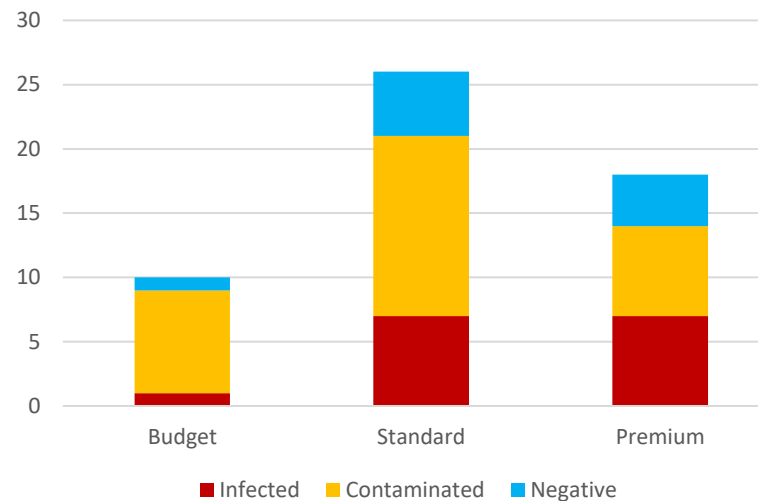
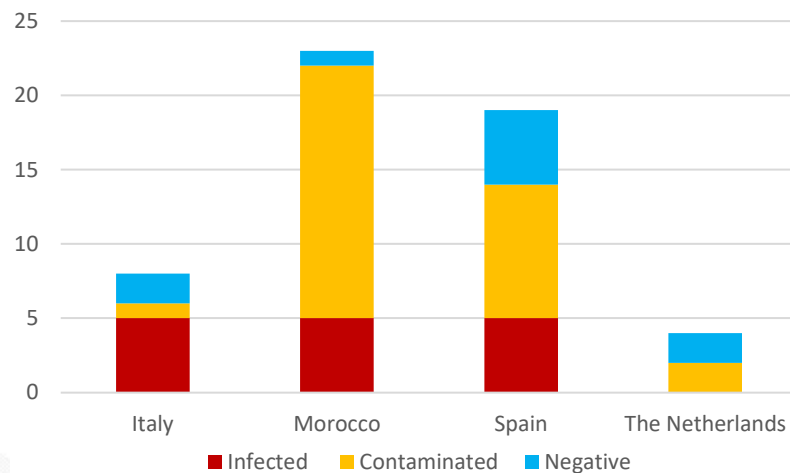


	real-time PCR	LAMP (RNA extract)		
	Ct Ave	Tp	Ta	
Top of light	35.36	19:15	84.45	
Bee box 2 - PEG	n/a	16:30	84.7	
NFT Water		/	/	Negative
Substrate Water		/	/	Negative

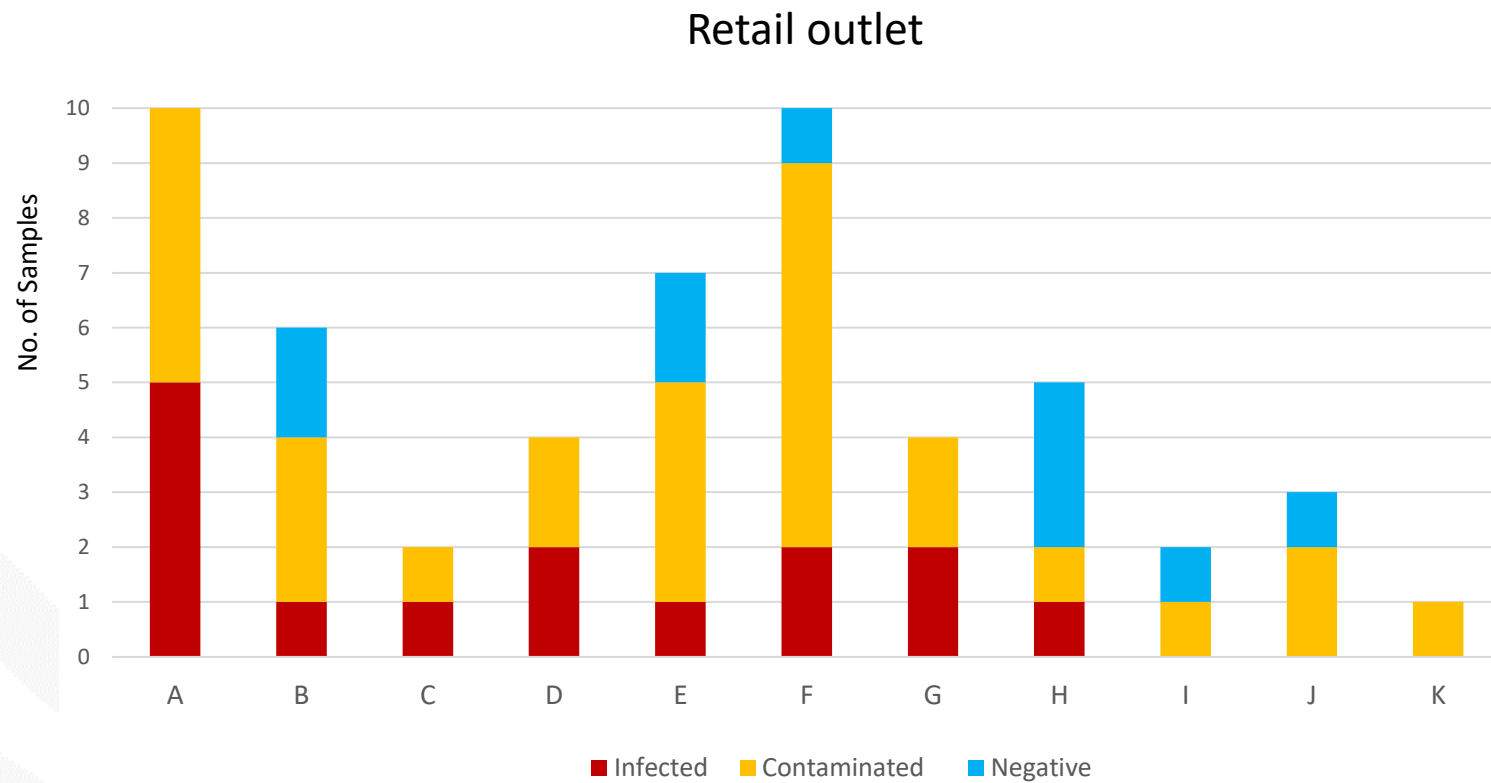


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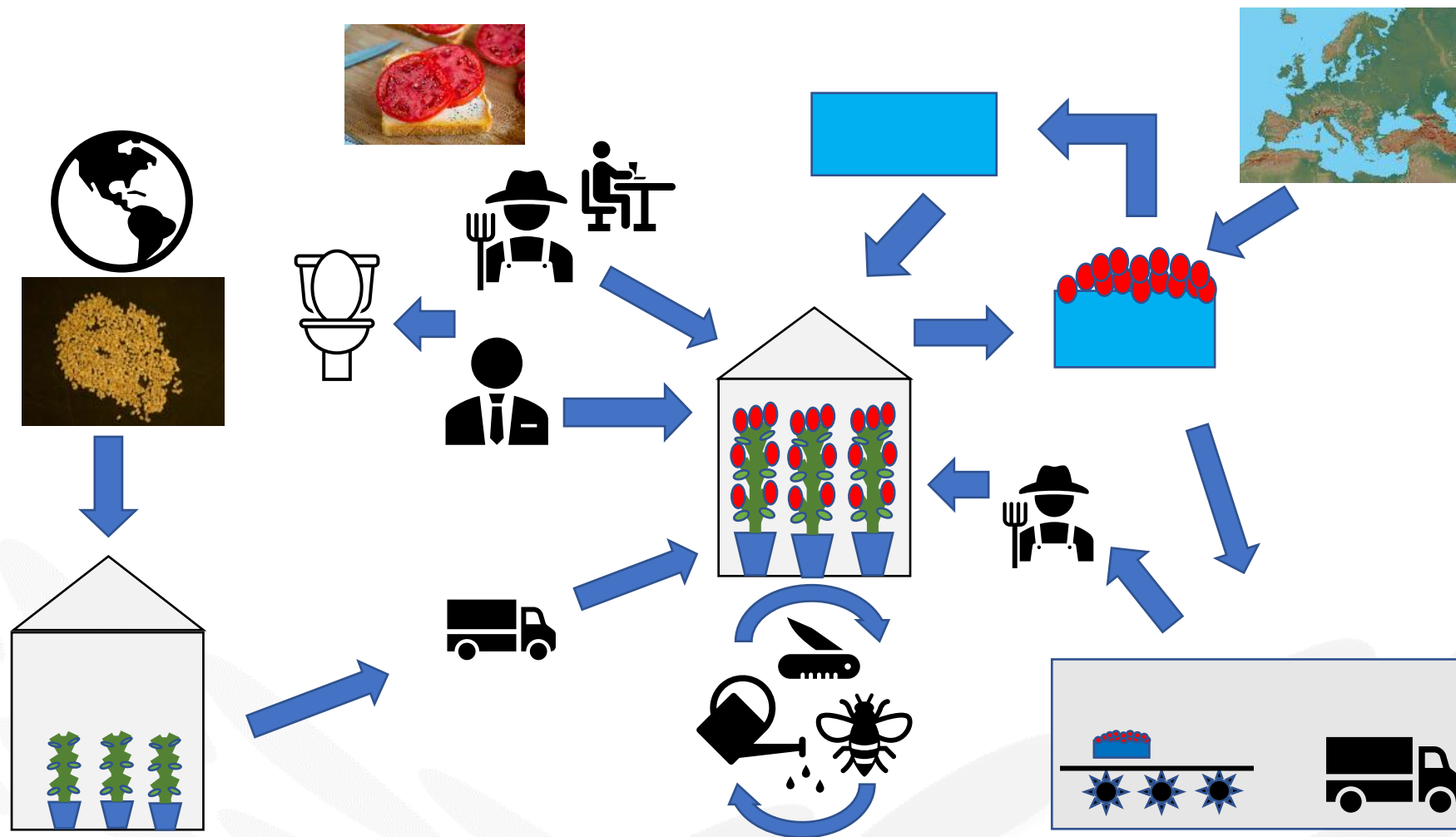
Does import fruit pose a risk for commercial production?



How widely distributed is infected fruit?

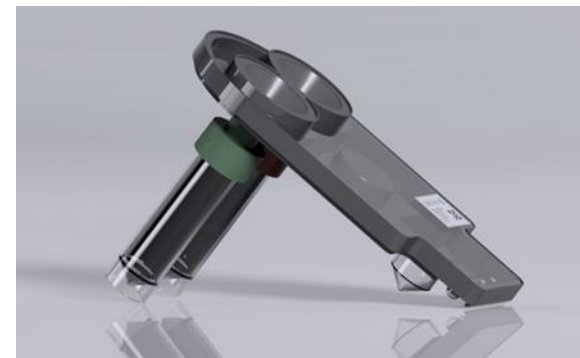


Considering relative risks within surveillance



Future developments

- Need for grower focused research to answer key questions about ongoing management of ToBRFV (and beyond?)
 - **Grower monitoring** – Diagnostic solutions for on-site testing building on previous work
 - Packhouse fruit?
 - Routine surveillance of environmental sources?
 - How to test?
 - Where to test?
 - What to test?
 - “One health” diagnostics – other targets of interest?
 - **Resistance management strategies**



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ENIGMA

A fera led collaborative R&D model 

Acknowledgements

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Department
for Environment
Food & Rural Affairs



Netherlands Food and Consumer
Product Safety Authority
Ministry of Agriculture,
Nature and Food Quality



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NATIONAL INSTITUTE OF BIOLOGY



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RESEARCH INSTITUTE



ToBRFV UK regulation and policy

Matthew Everatt

Defra



Department
for Environment
Food & Rural Affairs

ToBRFV: UK regulation and policy

Matthew Everatt
Defra, York, UK

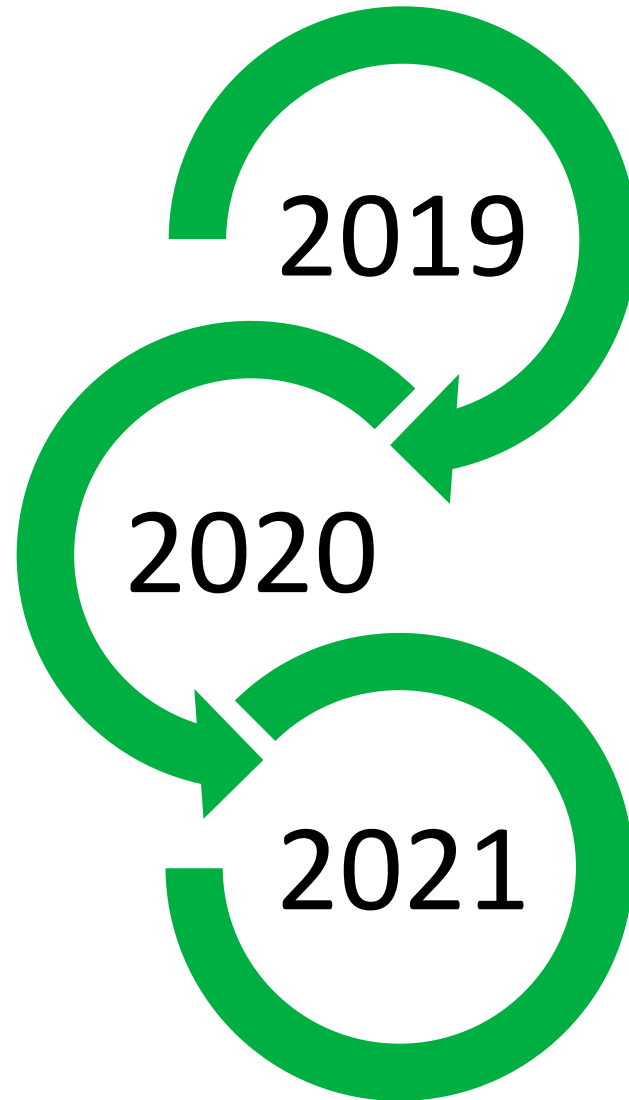


Forestry Commission
England



Environment
Agency

Background



First EU regulation based on Italian Pest Risk Analysis

Revised EU regulation – stronger measures

EU regulation transposed into GB legislation

The Plant Health (Phytosanitary Conditions) (Amendment) (EU Exit) Regulations 2020

Schedule 1 – List of GB quarantine pests

Schedule 2 – List of GB provisional quarantine pests

Schedule 3 – List of pest free area quarantine pests and GB pest free areas

Schedule 4 – List of regulated non-quarantine pests

Schedule 5 – Measures for regulated non-quarantine pests

Schedule 6 – Prohibitions

Schedule 7 – Import measures for third countries

Schedule 8 – Measures for movement within GB

Schedule 9 – Pest free area measures

Schedule 10 – Plants, plant products and other objects requiring a phytosanitary certificate

ToBRFV as a GB quarantine pest

5

Feasible and effective measures are available to prevent entry, establishment and spread

4

Would cause unacceptable economic, environmental or social impacts



3

Capable of entering, establishing and spreading within GB

1

The identity of the organism is established

2

Not present in GB or not widely distributed in GB

ToBRFV as a GB quarantine pest

- Prohibited from being introduced into, or moved within, GB
- Professional operators and persons other than professional operators must notify the competent authority if ToBRFV is suspected or confirmed
- Actions should be taken to prevent the spread of ToBRFV
- Annual official surveillance



GB prohibitions

Entry	Plants and plant products	Countries
18.	Plants for planting of <i>Solanaceae</i> other than seeds and the plants specified in entries 15, 16 and 17	Any third country other than: Albania, Algeria, Andorra, Armenia, Azerbaijan, Belarus, Bosnia and Herzegovina, Canary Islands, Egypt, EU Member States, Faroe Islands, Georgia, Iceland, Israel, Jordan, Lebanon, Libya, Liechtenstein, Moldova, Monaco, Montenegro, Morocco, North Macedonia, Norway, Russia (only the following parts: Central Federal District (Tsentralny federalny okrug), Northwestern Federal District (Severo-Zapadny federalny okrug), Southern Federal District (Yuzhny federalny okrug), North Caucasian Federal District (Severo-Kavkazsky federalny okrug) and Volga Federal District (Privolzhsky federalny okrug)), San Marino, Serbia, Switzerland, Syria, Tunisia, Turkey and Ukraine

GB measures 2023: hosts that apply



Tomato (*Solanum lycopersicum*)



Pepper (*Capsicum spp.*)

* But resistant varieties are out of scope for import and movement requirements

GB measures 2023: requirements for industry (seeds)

Movement **into** GB

Seeds

Must be accompanied by a phytosanitary certificate and fulfil the following requirements:

- Mother plants have been produced in a place of production that is free of ToBRFV, based on official inspections
- The seeds or the mother plants have been sampled and tested for the virus (and are negative)

Movement **within** GB

Seeds

Must be accompanied by a plant passport and fulfil the following requirements:

- Mother plants have been produced in a place of production that is free of ToBRFV, based on official inspections
- The seeds or the mother plants have been sampled and tested for the virus (and are negative)

GB measures 2023: seed derogation

Seeds that had been harvested prior to 15th August 2020 do not require an inspection of mother plants, but instead can be imported if:

the seeds or their mother plants have undergone official sampling and testing for tomato brown rugose fruit virus and have been found, according to those tests, to be free from that pest

This will be embedded into legislation next year.

GB measures 2023: seed imports

All tomato and pepper seed from third countries (including the EU) will be held and tested at the border (*if consignments meet size requirements)

- From January 2021 – July 2023, tomato and pepper seed represented 87.5% of all seed interceptions (slightly down from 92.5% from July 2020 – June 2021)
- ToBRFV represented 11.6% of all seed interceptions (significantly down from 79% from July 2020 – June 2021)
- Previously, EU seed was not held, but there have been findings of ToBRFV on seed from EU countries which, because consignments were not held, have caused complications down the line
- Given the risk of imports from the EU, there is justification to hold seed in line with other third countries

GB measures 2023: requirements for industry (plants for planting other than seed)

Movement into GB	Movement within GB
<p>Plants for planting</p> <p>Tomato and pepper plants must be accompanied by a phytosanitary certificate and fulfil the following requirements:</p> <ul style="list-style-type: none">• Derive from seeds that have been sampled and tested for ToBRFV (and are negative)• Originate in a registered production site known to be free of the virus on the basis of official inspection, and in the case of symptoms, the plants have been sampled and tested for the virus (and are negative)	<p>Plants for planting</p> <p>Tomato and pepper plants must be accompanied by a plant passport and fulfil the following requirements:</p> <ul style="list-style-type: none">• Derive from seeds that have been sampled and tested for ToBRFV (and are negative)• Originate in a production site known to be free of the virus on the basis of official inspection, and in the case of symptoms, the plants have been sampled and tested for the virus (and are negative)

GB measures 2023: sampling and testing

Sampling and testing regimes have been prescribed in GB legislation

- Seed sampling based on ISPM 31 Methodologies for sampling of consignments
- Plant sampling based on collection of leaves
- Testing requirements based on RT-PCR

Tomato fruit

- Fruit is allowed to be moved to retail or wholesale
- If possibly infected fruit is moved to other premises, such as packhouses, adequate hygiene measures should be in place to prevent cross-infection
- Key is good biosecurity to prevent the introduction of the virus from fruit



UK outbreak management

Department
for Environment
Food & Rural Affairs

Generic Contingency Plan for Plant Health in England

January 2022

Department
for Environment
Food & Rural Affairs

**Pest specific plant health response plan:
Outbreaks of tomato brown rugose fruit virus**




Figure 1. Tomato infected with tomato brown rugose fruit virus.

1

UK outbreak management



- Minimising spread to other tomato and pepper propagation and production premises
 - Restricting movement
 - Good hygiene
-

- Removal of the crop
 - Cleaning and disinfection
-

- Monitoring of new crop for a full crop cycle

ToBRFV review

The European Union (EU) will be holding a review of ToBRFV in 2024



The European and Mediterranean Plant Protection Organisation will be reviewing the ToBRFV pest risk analysis within the next year



Thank you for listening

