BRITISH GROWERS ASSOCIATION TOMATO CONFERENCE

Tomato brown rugose fruit virus: where do we go from here?

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Original thinking... applied

Tomato brown rugose fruit virus: where do we go from here?

Adrian Fox

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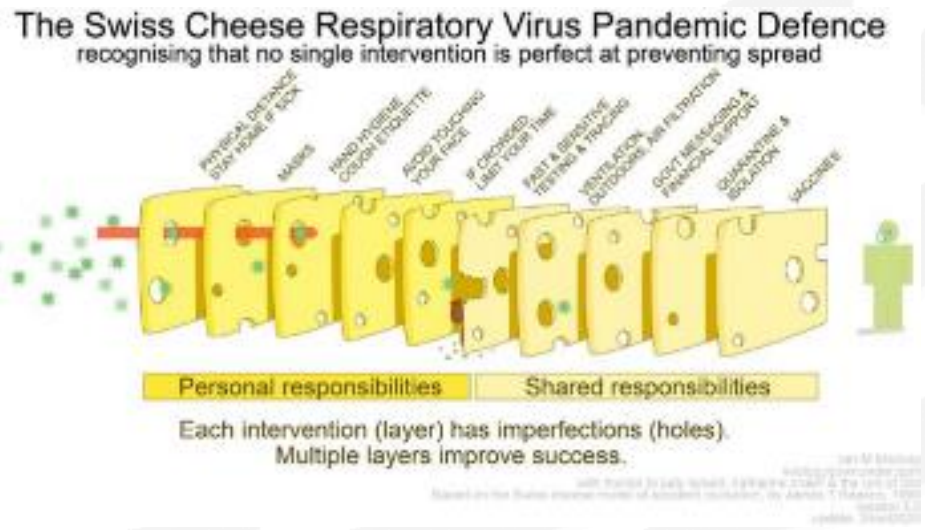
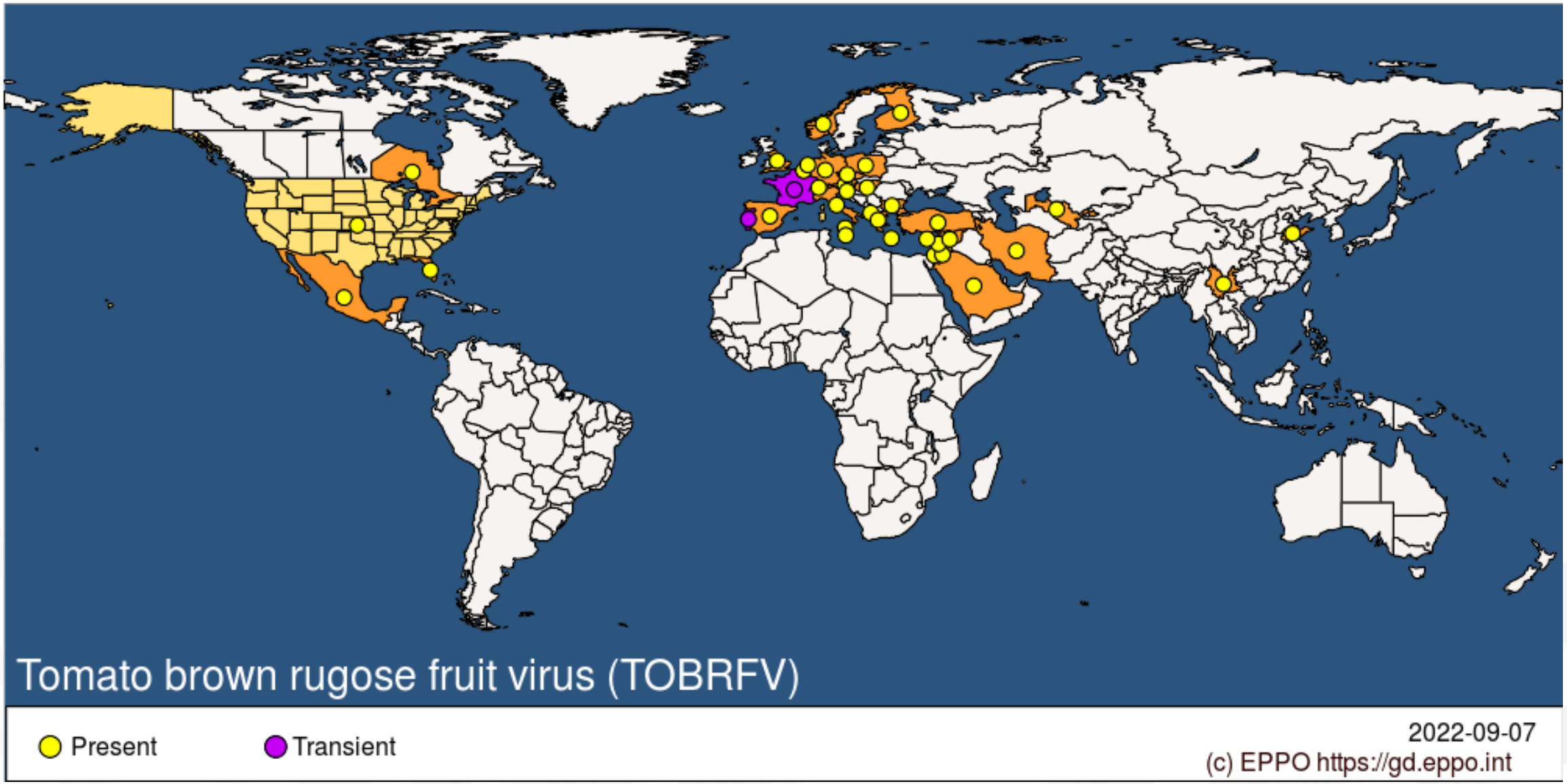
Tomato brown rugose fruit virus

- Virus can overcome TMV resistance genes in tomato
- First recorded in Jordan and Israel 2014-2015
    - Also recorded on pepper
- Rapidly spread through direct plant to plant contact, handling, tools, clothing, bumblebees…+
- Seed transmission demonstrated
- Good hygiene measures minimise spread and limit impact should an outbreak occur



The current global situation…



The UK coordinated response

UK ToBRFV steering group

- Formed late 2018 (after outbreak in Germany)
- Key stakeholders from industry, research, regulation, inspection, knowledge exchange/extension
  - Initially chaired by AHDB, now led from British Tomato Growers.
- Aims:
  - Monitor UK and international situation
  - Discuss UK position and response
  - Identify research gaps
  - Update on research outcomes
  - Coordinate comms

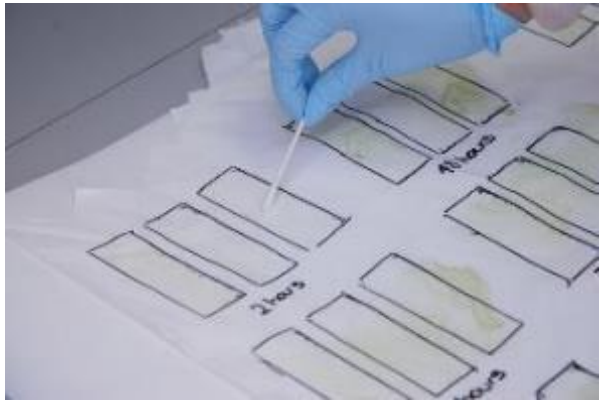
Currently UK status

- UK has reduced from 5 outbreaks (2020) to a single recurrent outbreak under eradication action (2022)

Key research questions…

- How do we improve detection strategies?
- Once we have an outbreak how do we get rid of it?

AHDB PE033/a Survival and disinfection



Surface	2 hrs	8 hrs	24 hrs	7 days	4 weeks	3 Months	6 Months
Glass	+	+	+	+	+	(+)	(+)
Concrete	+	+	+	+	-	(+)	-
Aluminium	+	+	+	+	(+)	-	-
Hard plastic	+	+	+	+	+	+	(+)
Polythene	+	+	+	+	+	+	(+)
Stainless steel	+	+	+	+	+	(+)	-

+ = Virus survival in all repetitions; (+) = Virus survival in some repetitions (inconsistent); - = Virus denatured; * = 1st rep only completed

Disinfectants tested:

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 (Fogging)	Hydrogen Peroxide	50%	25%	12.5%
Huwa San TR 50 (Surface)	Hydrogen Peroxide	50%	6%	3%
TSOP	Trisodium orthophosphate		10%	10%
Sodium hypochlorite	Sodium hypochlorite	10,000 ppm	20 ml in 500 ml water	400ppm
Unifect G	Glutaraldehyde & quaternary ammonium compounds		1:25	
Virocid	Glutaraldehyde & quaternary ammonium compounds		1%	

AHDB PE033/a : Efficacy of disinfectants

Surface	Disinfectant 60 minute treatment																	
	Menno Florades		Jet 5		Sodium hypochlorite		Virkon S		Huwa San 3% ai		Huwa San 12.5% ai		TSOP		Unifect G		Virocid	
	Rep 1	Rep 2	Rep 1	Rep 2	Rep 1	Rep 2	Rep 1	Rep 2	Rep1	Rep2	Rep 1	Rep 2	Rep 1	Rep 2	Rep1	Rep 2	Rep 1	Rep2
Glass	-	-	+	2 of 3	1 of 3	-	-	-	2 of 3	2 of 3	-	-	1 of 3	1 of 3	-	-	-	-
Concrete	1of 3	3 of 3	2 of 3	-	-	-	-	2 of 3	1 of 3	2 of 3	3 of 3	3 of 3	2 of 3	2 of 3	-	-	-	-
Aluminium	-	-	2 of 3	1 of 3	-	-	-	-	2 of 3	2 of 3	-	-	2 of 3	2 of 3	-	-	-	-
Hard Plastic	-	1 of 3	-	1 of 3	-	-	-	-	2 of 3	2 of 3	-	-	2 of 3	-	-	-	-	-
Polythene	-	-	2 of 3	-	1 of 3	-	-	-	-	+	-	-	2 of 3	1 of 3	-	-	-	-
Stainless steel	-	-	+	+	-	2 of 3	-	-	-	2 of 3	-	-	2 of 3	2 of 3	-	-	-	-

- **Virkon has similar efficacy at 20 minute exposure**
- **Unifect G has efficacy at 10 minutes exposure**
- **Menno Florades gives total efficacy after 16 hours exposure**

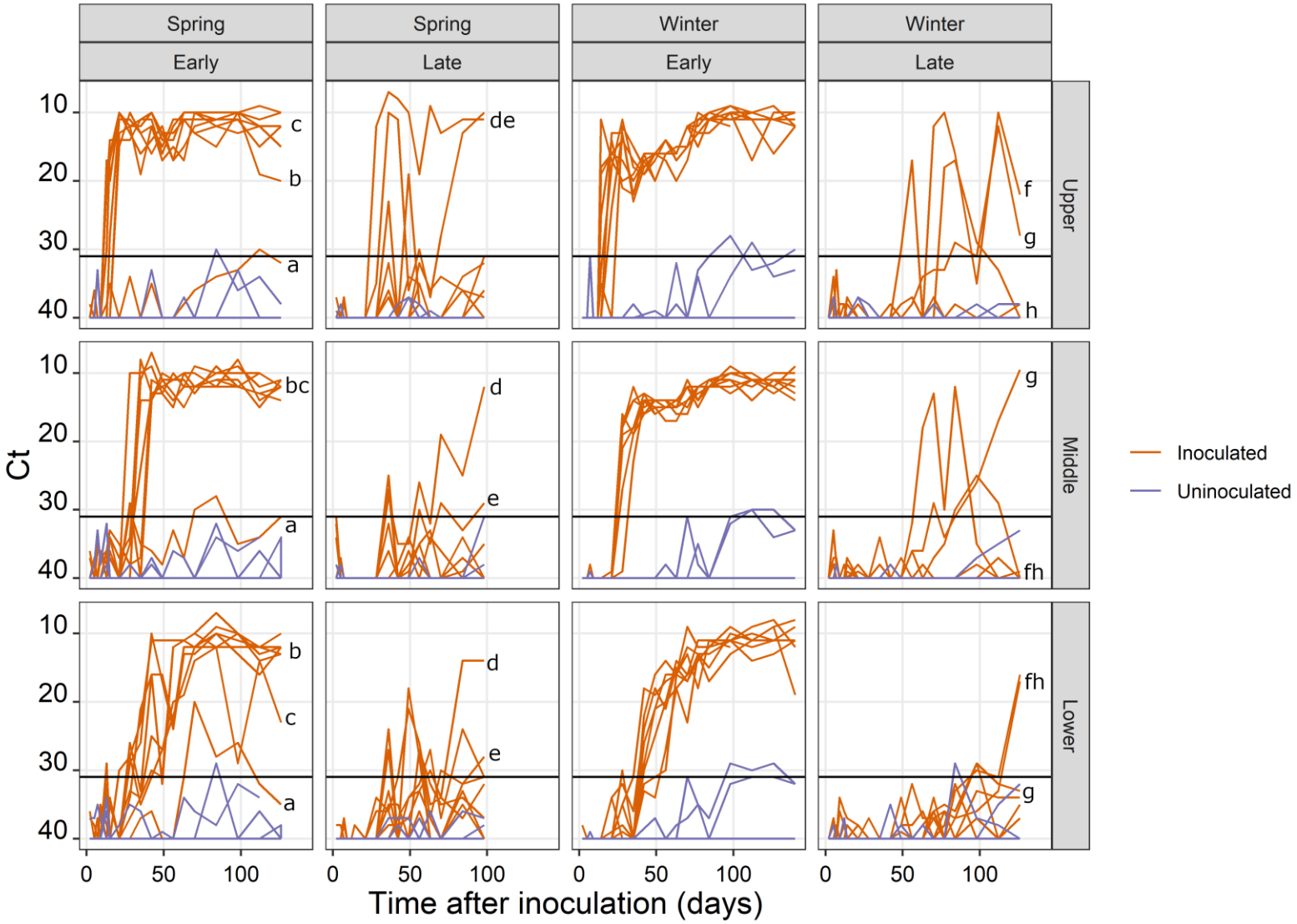
Improving detection approaches

➢ **Improving sampling**

- Latent surveys:
  - 200 leaves from XXX,000 plants
    - Needle in a haystack?
      - Statistics support these numbers but work still needed on agreed “best practice” for sampling for reliable detection
- 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 RT-qPCR tests, which could not be confirmed through a secondary method
  - Use of cut-offs/thresholds…?

➢ **Exploiting alternative technologies**

PE034: Variability in detection across from leaves



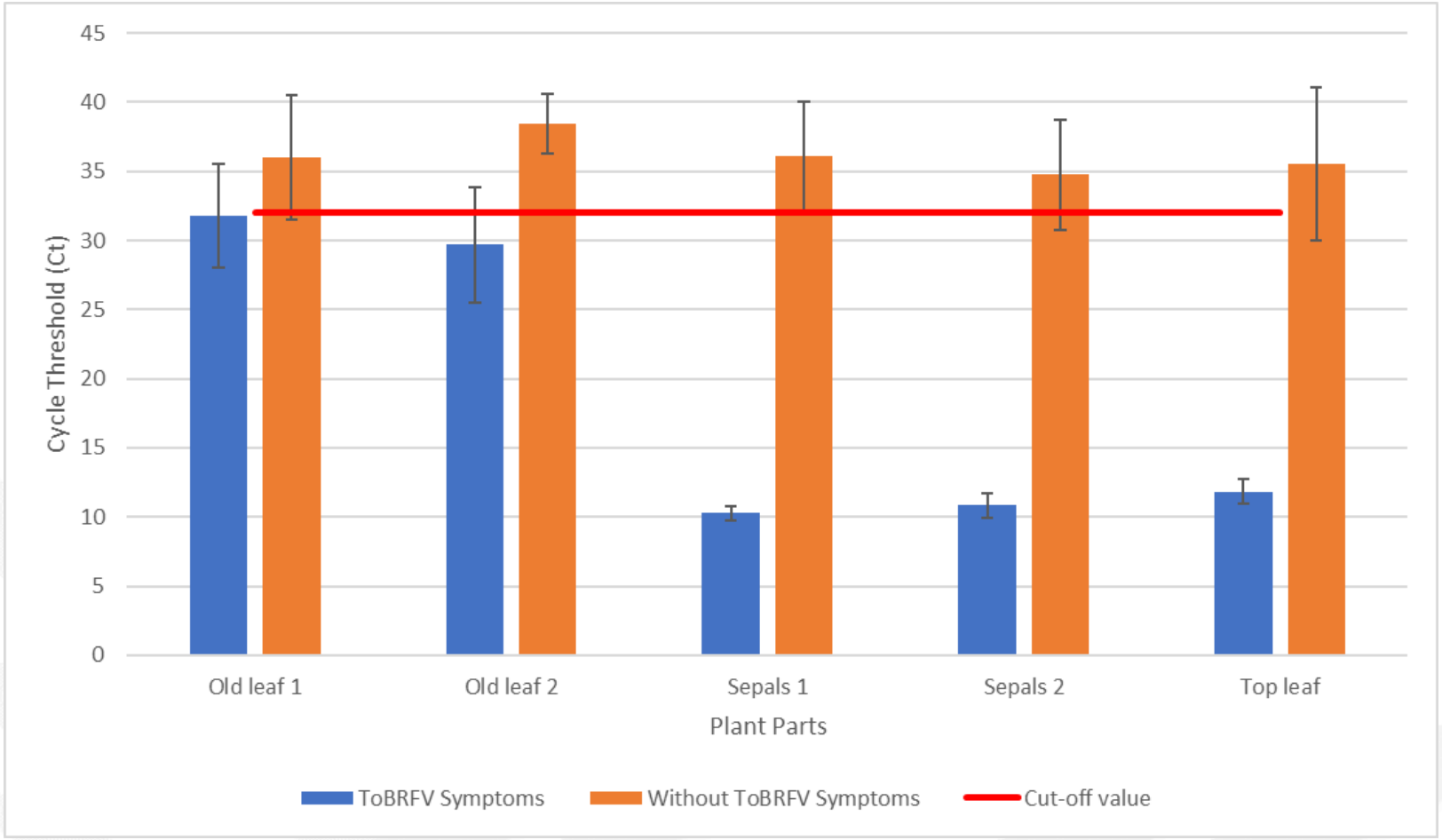
Skelton et al (in prep)

PE034: Comparison of first detection from different plant parts (days post inoculation)

Infection time	Crop	Sample site	Leaf	Sepal	Fruit
Early	Spring	Lower	13	56	56
Early	Spring	Middle	28	63	63
Early	Spring	Upper	13	70	126
Early	Winter	Lower	28	77	77
Early	Winter	Middle	28	77	77
Early	Winter	Upper	14	77	112
Late	Spring	Lower	36	14	21
Late	Spring	Middle	2[a]	21	14
Late	Spring	Upper	28	21	21
Late	Winter	Lower	98	14	35
Late	Winter	Middle	63	35	35
Late	Winter	Upper	49	35	Inf

Skelton et al (in prep)

Same question, different approach:
(Data courtesy of M. Botermans, NVWA, NL)



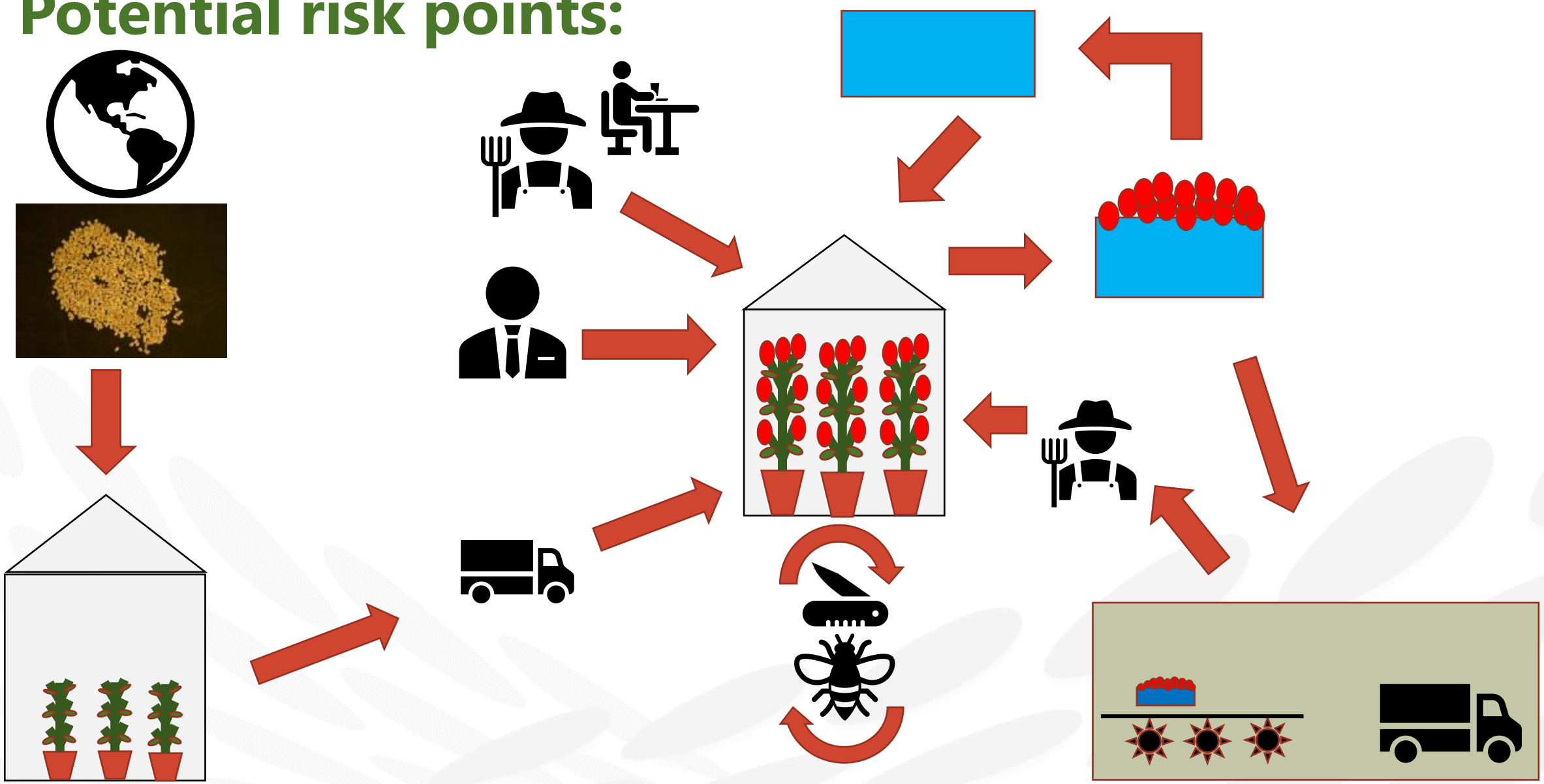
Skelton et al (in prep)

Change to plant sampling advice:

- In crops prior to the development of fruit trusses, sampling should focus on leaves from the top of the plant

- In crops following fruit setting, a sampling regime should take leaves from the tops of plants, however, an additional sample of sepals and/or fruit should also be taken

Potential risk points:



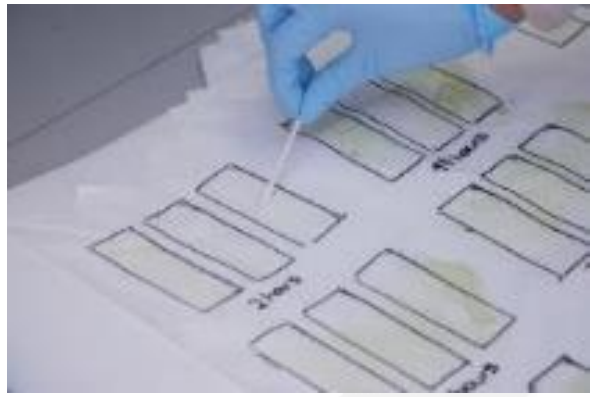
Exploiting alternative technologies and strategies

Environmental monitoring may provide an alternative approach to plant testing?

- LAMP – "Isothermal" amplification method
  - Amenable for crude extractions
  - Portable
    - Onsite testing?

  - Non-invasive approaches to identify potential sites for further investigation
    - Monitoring large areas without using plants
      - Swab testing?
      - (Irrigation water monitoring?)

➢ Joint AHDB-Defra funded project to evaluate LAMP
  - AHDB PE035
  - Defra Future Proofing Plant Health



INEXTVIR

Stakeholder Workshop

People?
- Hands?
- Clothing/shoes
- Mobiles
- Specs

Canteen
Tables/Surfaces
Coffee machines/kettle
Fridge doors
Door handles

Surfaces
- E.g. “managers” cars
- Transport in/out
- Materials/consumables

Onsite Accommodation

Office
Computer terminals
Labour registration terminals (on entry to g/house)

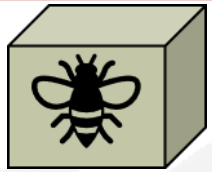
Plant intake

Glasshouse

Fruit Dispatch

Equipment
-crates
-pull chains/doors
- Trolleys
- disposable gloves/PPE

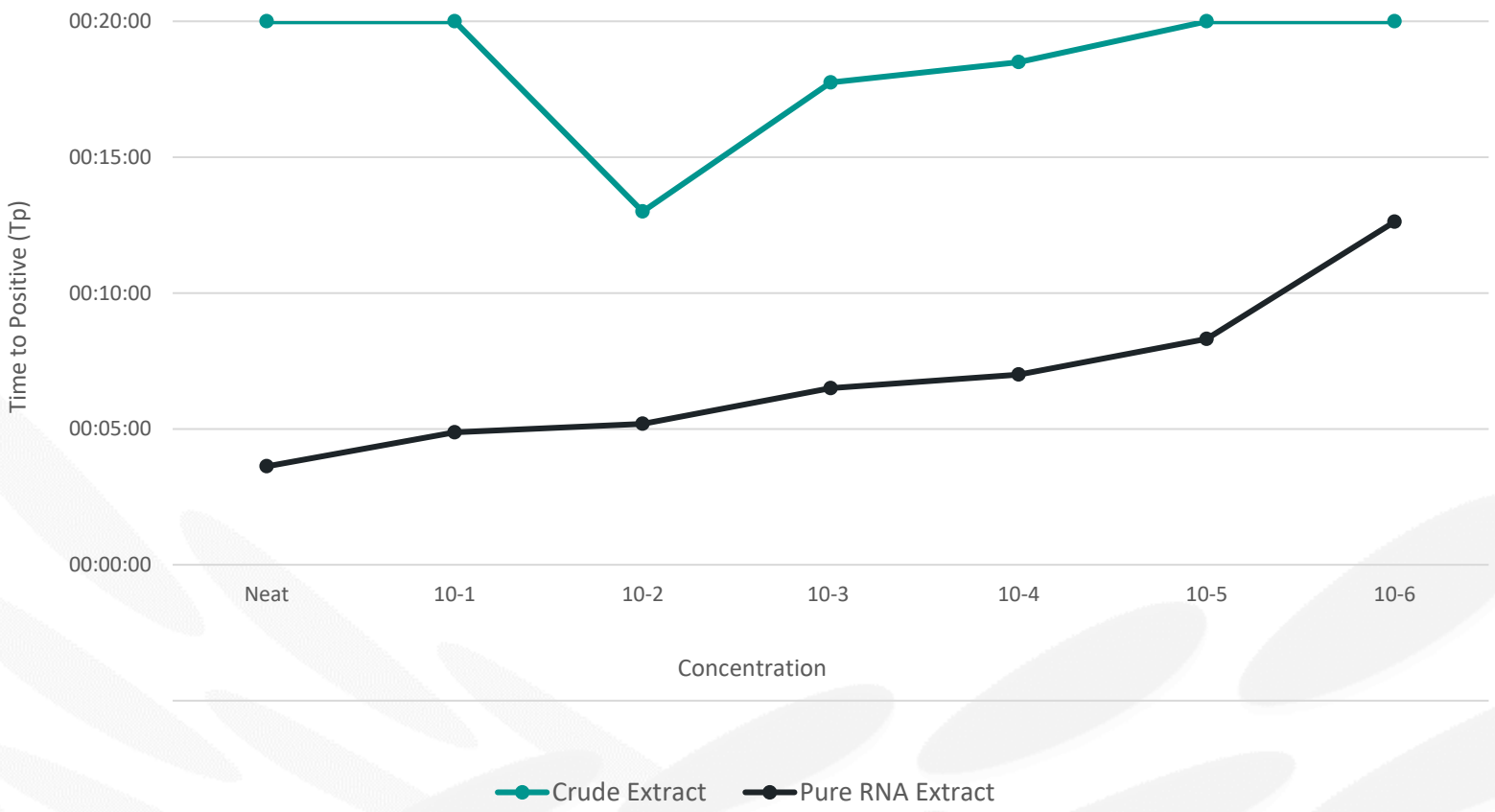
Tools
-scissors
- Cleaning materials



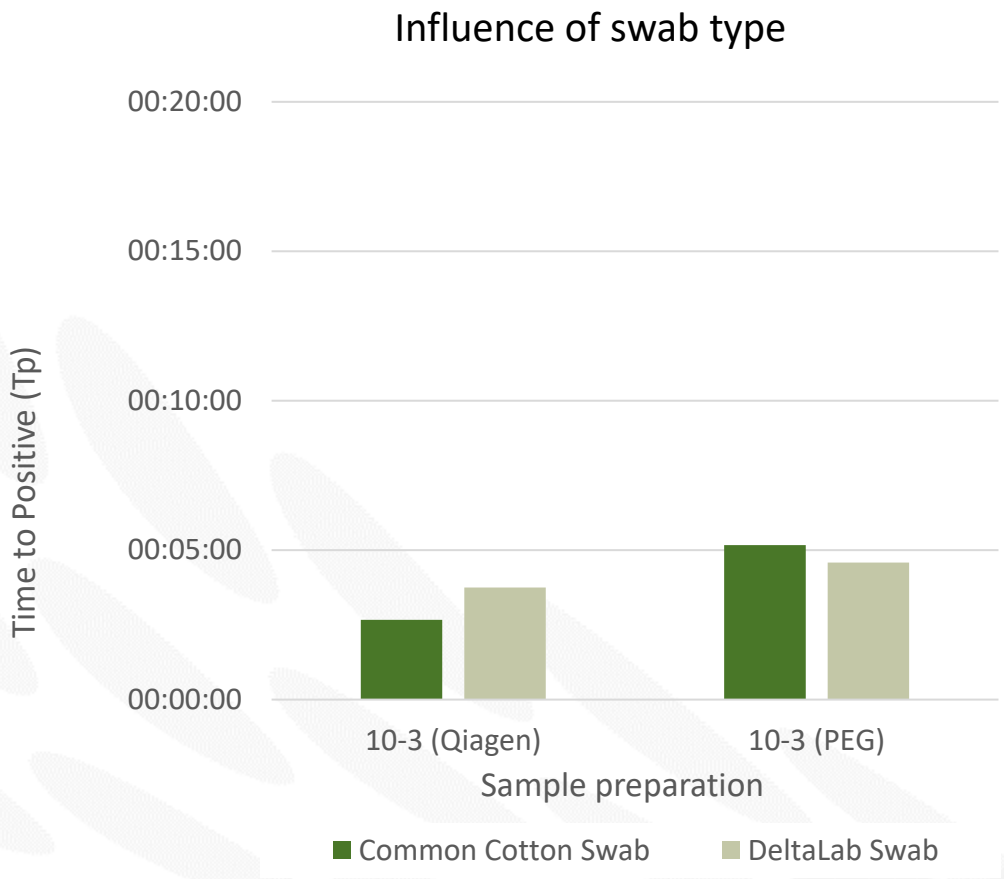
Irrigation

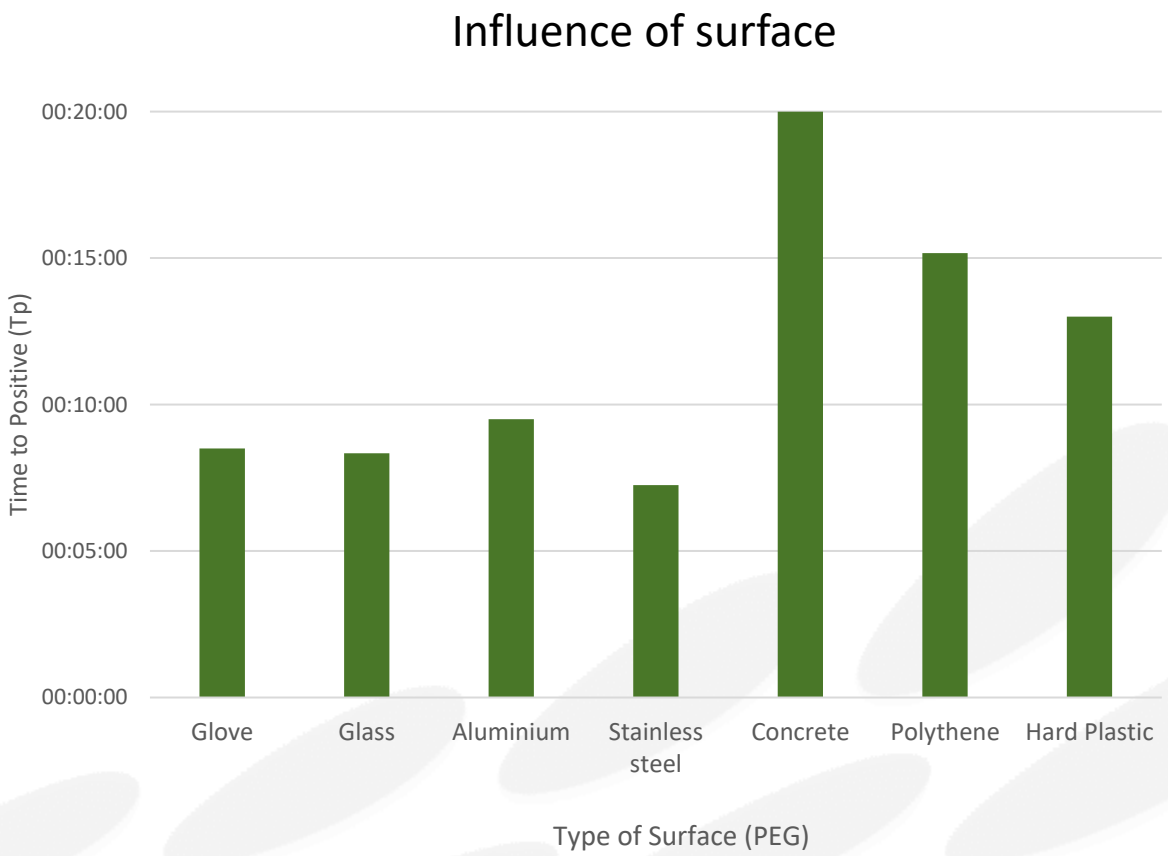
“visitors”
- Contractors (tools)
- students

Validation of LAMP... sample preparation



Validation of LAMP... matrix effects





Swabbing the UK ToBRFV outbreak site

		real-time PCR	LAMP (RNA extract)		
		Ct Ave	Tp	Ta	
Glasshouse 1	Top of light	35.36	19:15	84.45	
	Fan	22.09	6:45	84.3	
	Under Gutter	20.86	6:00	84.15	
	Behind whiteboard	33.55	/	/	Negative
	Top of Cable tray	20.25	6:30	84.53	
	Bee Box 1	22.91	8:30	82.52	
	Inside panel door	23.39	8:15	82.82	
	socket lid	25.57	8:15	84.5	
	Pheromone trap	31.63	19:15	84.35	
Glasshouse 2	Roof structure G2	17.36	6:15	83.42	
	Hive G2	16.98	5:15	84.61	
	Top light G2	25.33	7:00	84.41	
	Fan G2	17.54	4:45	84.67	
	Under gutter	16.76	5:00	84.47	
People…	Tyvek Suit	16.23	4:45	84.44	
	Grower Phone	31.18	/	/	Negative
	Grower Glove	19.80	5:15	84.52	
	Neg - H2O	40	/	/	Negative
	Pos - ToBRFV+	11.21	5:45	85.32	
	Bee box 2 - PEG	n/a	16:30	84.7	
	NFT Water		/	/	Negative
	Substrate Water		/	/	Negative

qPCR v RNA extract LAMP v Crude LAMP

- Glasshouse cubicle used for survival, disinfection and detection studies
- Multiple swab testing strategies to directly compared
    - Real-time RT-PCR positives from EVERY surface
    - LoD of LAMP on RNA extracts around 31Ct (real-time RT-PCR)
        - ~ equivalent sensitivity of conventional
    - vs LAMP/RNA extract ~ 60% detection
    - vs LAMP Crude extract ~ 25% detection

➢ Swab testing by real-time RT-PCR appears reliable

➢ Environmental virus residues <u>EVERYWHERE</u> in both glasshouses tested!

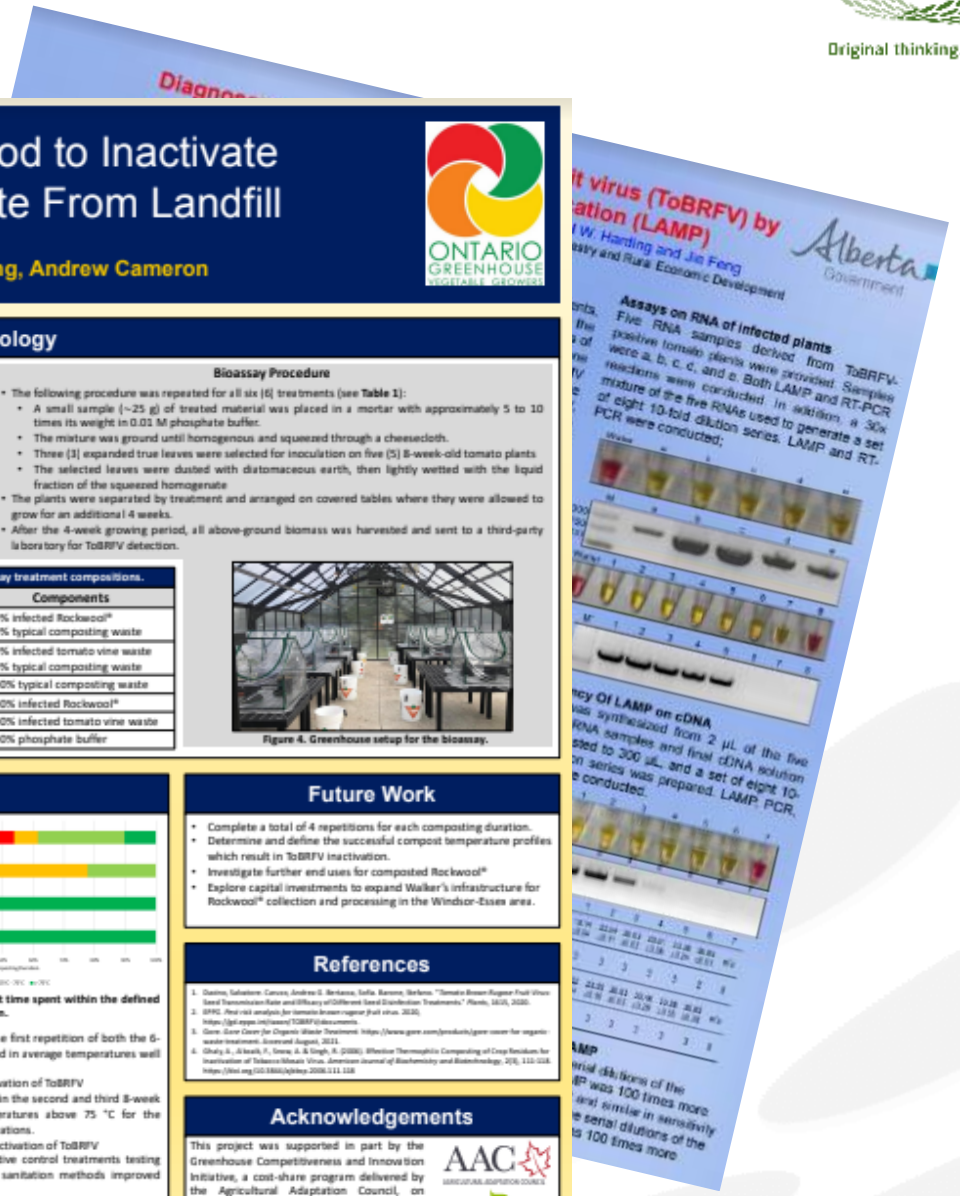
	Real-time RT-PCR				
	RNA extract	LAMP			
		RNA Extract		Crude (PEG)	
Sample type	Avg Ct	Tp	Ta	Tp	Ta
window 1	28.43	00:10:45	84.51	/	/
window 2	28.27	00:11:15	84.52	/	/
window 3	29.11	00:11:15	84.86	/	/
Bench edging - face out	34.37	/	/	/	/
Bench edging - face in	32.24	/	/	/	/
ladder	25.93	00:08:30	84.66	00:10:45	84.61
wall 1	34.88	/	/	/	/
wall 2	28.15	00:12:15	84.72	/	/
floor	26.96	/	/	00:04:45	84.46
plant pot 1	26.93	00:06:30	84.77	/	/
plant pot2	26.77	00:06:45	84.58	/	/
plant pot black tray	21.08	00:05:45	84.66	00:14:45	84.61
Stand - leg	32.25	/	/	/	/
Stand - middle bar	29.47	00:18:15	84.44	/	/
Stand - grid panel	18.89	00:05:30	/	00:06:30	84.26
Glove	36.70	/	/	/	/
Tyvek sleeve	36.60	/	/	/	/
Plastic apron	31.06	00:16:15	84.79	/	/
H2O	40.00	-	-	-	-
ToBRFV + (avg)	22.98	00:06:00	85.3	00:06:23	85.15

International Perspectives

International Perspectives

- ToBRFV International Research Symposium, Ontario, Canada
  - Delayed from April 2020 due to COVID
- 120 Representatives from Europe, Israel, Jordan, Mexico, USA, Canada
- Growers, Extension services, Policy, Global Research and Diagnostics providers, Consultants, Seed Industry, Disinfectant companies (e.g. Menno, Virocid)
- 17 keynote talks – Epidemiology, Detection and Surveillance, Management, Resistance, Disinfection, Impacts, Insurance, Regulation (impact of USDA regs)
- 15 posters covering diagnostic approaches, resistance, disinfection, composting

Key messages: Posters



Static Aerated Composting as a Method to Inactivate ToBRFV and Divert Greenhouse Waste From Landfill

Caleb Fretz, Erin Agro, Carly Lacy, Lisa Immel, Emily Skelding, Andrew Cameron

Research Objective

To determine the efficacy of Walker's static aerated composting process in rendering the Tomato Brown Rugose Fruit Virus inactive in spent Rockwool® and infected tomato vines.

Background

Tomato brown rugose fruit virus (ToBRFV) is a highly virulent and damaging pathogen that infects multiple varieties of tomatoes and peppers. Since its 2014 discovery in Israel, ToBRFV has rapidly spread across the globe. Confirmed cases have been reported throughout North America and Europe.[1] In response to the threat of destructive outbreaks, distinguishing virus prone locations has encouraged municipalities and greenhouses to securely dispose of end-of-crop-cycle plant waste and growing media. Although disposal methods such as landfills or incineration are effective, their non re-newable nature is unsustainable and poses many environmental consequences.[2] As such, it is critical that alternative discarding methods are developed to ensure materials are properly recovered and recycled.

Figure 1. The proportion of waste management strategies used for greenhouse waste streams in southwestern Ontario. Data is based on an internal waste audit questionnaire in which 109 producers of tomatoes, cucumbers, and peppers participated.

Walker believes that their existing GORE® composting system could be an effective method to stop ToBRFV and create a circular economy for greenhouse waste. The covers used in the GORE® system are unique as they retain heat, humidity, contaminants, and odours while simultaneously discharging carbon dioxide (see **Figure 2**).[3] One hypothesis regarding virus inactivation involves the tobacco mosaic virus (TMV), a close relative of ToBRFV. Since GORE® system temperatures regularly exceed 65°C, a threshold proven to disable TMV, it could theoretically have an effect on ToBRFV.[4] This project will study the survival rate of ToBRFV in spent Rockwool® and tomato vines when incorporated with typical source separated organic (SSO) waste and processed through the GORE® system.

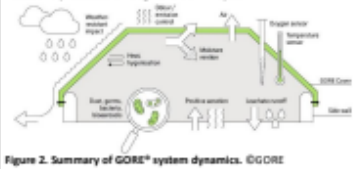


Figure 2. Summary of GORE® system dynamics. ©GORE

Methodology

Composting Procedure

- Infected (ToBRFV) Rockwool® and tomato vine waste from OMAFRA were obtained.
- Three (3) replicates of each treatment (see **Table 1**) were confined in onion bags surrounded by SSO and placed in mesh bags. The bags were then buried near the center of the GORE® cell as it was being filled under standard procedures (see **Figure 3**).
- The GORE® cell was operated under the standard procedure and the bags remained buried for either 6 weeks or 8 weeks to compost.
- After the composting duration, the bags were removed and the three (3) replicates were combined to form one (1) representative sample for each treatment.
- Surrounding material was collected for each of the replicates to form a representative composted control sample (see **Table 1**).
- To measure the presence of ToBRFV after composting, bioassays were performed on tomato plants with the composted waste (see **Bioassay Procedure**).

Figure 3. A composting treatment being placed in a GORE® cell.

Bioassay Procedure

- The following procedure was repeated for all six (6) treatments (see **Table 1**):
  - A small sample (~25 g) of treated material was placed in a mortar with approximately 5 to 10 times its weight in 0.01 M phosphate buffer.
  - The mixture was ground until homogenous and squeezed through a cheesecloth.
  - Three (3) expanded true leaves were selected for inoculation on five (5) 8-week-old tomato plants
  - The selected leaves were dusted with diatomaceous earth, then lightly wetted with the liquid fraction of the squeezed homogenate
- The plants were separated by treatment and arranged on covered tables where they were allowed to grow for an additional 4 weeks.
- After the 4-week growing period, all above-ground biomass was harvested and sent to a third-party laboratory for ToBRFV detection.

Table 1. Composting and bioassay treatment compositions.

Treatment	Components
Composted Rockwool®	- 20% infected Rockwool® - 80% typical composting waste
Composted Vine	- 20% infected tomato vine waste - 80% typical composting waste
Composted Control	- 100% typical composting waste
Positive Rockwool® Control	- 100% infected Rockwool®
Positive Vine Control	- 100% infected tomato vine waste
Negative Control	- 100% phosphate buffer

Figure 4. Greenhouse setup for the bioassay.

Results

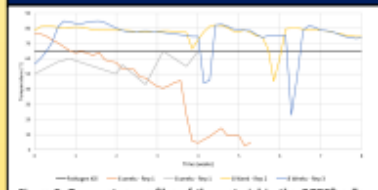


Figure 5. Temperature profiles of the material in the GORE® cells across the treatment time of each repetition.

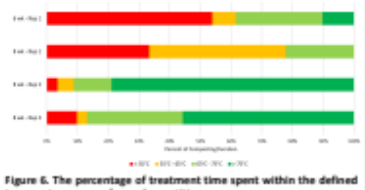


Figure 6. The percentage of treatment time spent within the defined temperature ranges for each repetition.

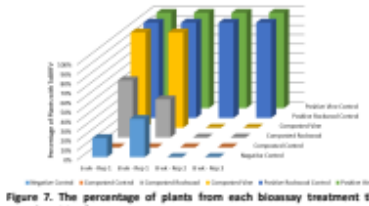


Figure 7. The percentage of plants from each bioassay treatment that tested positive for ToBRFV.

- Poor GORE® performance in the first repetition of both the 6-week and 8-week trials resulted in average temperatures well below 65 °C.
  - Resulted in partial inactivation of ToBRFV
- Standard GORE® performance in the second and third 8-week repetitions resulted in temperatures above 75 °C for the majority of the composting durations.
  - Resulted in complete inactivation of ToBRFV
- The decreasing trend of negative control treatments testing positive for ToBRFV suggests sanitation methods improved over the course of the trial.
- Bioassay results for the composted control treatments suggest the virus did not spread beyond the mesh bags during composting.

Future Work

- Complete a total of 4 repetitions for each composting duration.
- Determine and define the successful compost temperature profiles which result in ToBRFV inactivation.
- Investigate further end uses for composted Rockwool®
- Explore capital investments to expand Walker's infrastructure for Rockwool® collection and processing in the Windsor-Essex area.

References

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3. Gore. Cover Cover for Organic Waste Treatment. https://www.gore.com/products/gore-cover-for-organic-waste-treatment. Accessed August, 2021.
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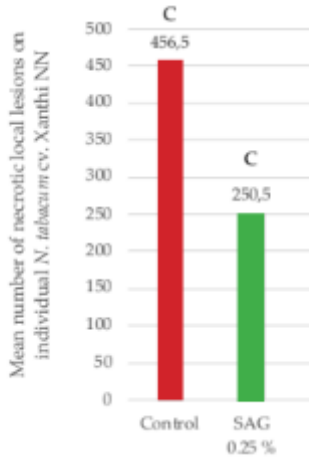
Acknowledgements

This project was supported in part by the Greenhouse Competitiveness and Innovation Initiative, a cost-share program delivered by the Agricultural Adaptation Council, on behalf of the Ontario Ministry of Agriculture, Food and Rural Affairs (OMAFRA). Contributions were also made by Rijk Zwaan.

Key messages: Talks

fera
Original thinking... applied

CLEANING OF CONTAMINAT

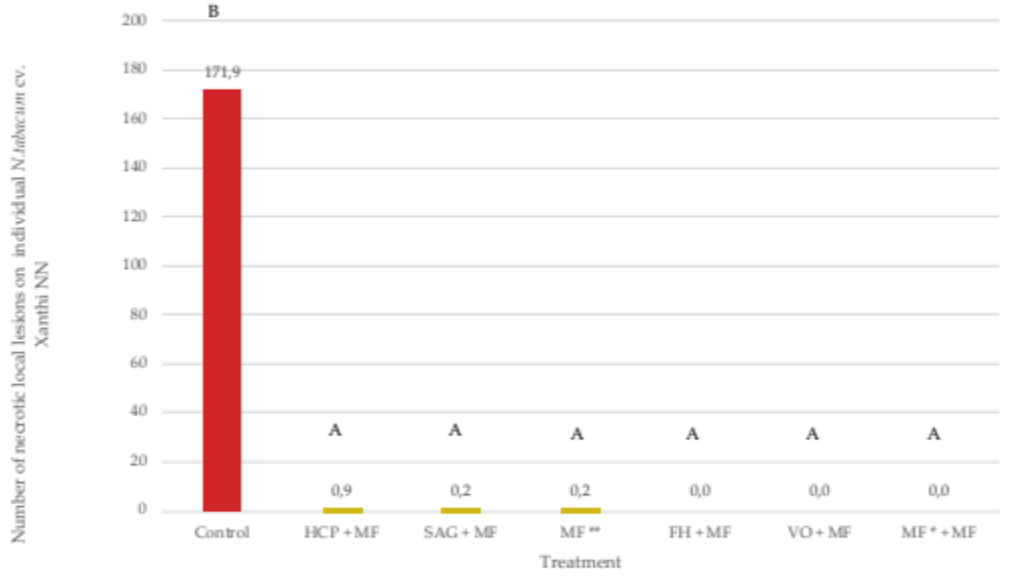


Letters based on pairwise treatment comparisons using gen
Bonferroni correction). n=24. 'Control'= Fabric contaminated
'MF'= Menno Florades; 'FH'= Fadex H+; 'HCP'= Hortisept C

Jens Ehlers ToBRFV Research Symposium, Toror

DECONTAMINATION OF CLEANING SOLUTIONS

HUMBOLDT-UNIVERSITÄT ZU BERLIN



Letters based on pairwise treatment comparisons using generalized linear mixed model analysis of count data (alpha=0.05, with Bonferroni correction). n=24. 'Control'= Deionized water with ToBRFV-contaminated fabric; 'SAG'=Spee Activ Gel; 'VO'= Vanish Oxi Action Gel; 'MF'= Menno Florades; 'FH'= Fadex H+; 'HCP'= Hortisept Clean Plus. *10 min contact time; **4 h contact time.

- The addition of **Menno Florades** (4 %) at a contact time of 16h reliably inactivated ToBRFV in all treated CS
- Important to take the pH value of the solution into account when adding Menno Florades (HCP solution pH 12)

18

Jens Ehlers ToBRFV Research Symposium, Toronto

August, 17th-18th, 2022

HUMBOLDT-UNIVERSITÄT ZU BERLIN

- Cleaning solutions (CS) get contaminated with ToBRFV
- CS of control and household products are highly infectious
- CS of agricultural detergents and the disinfectant only pose a very slight risk of infection

17

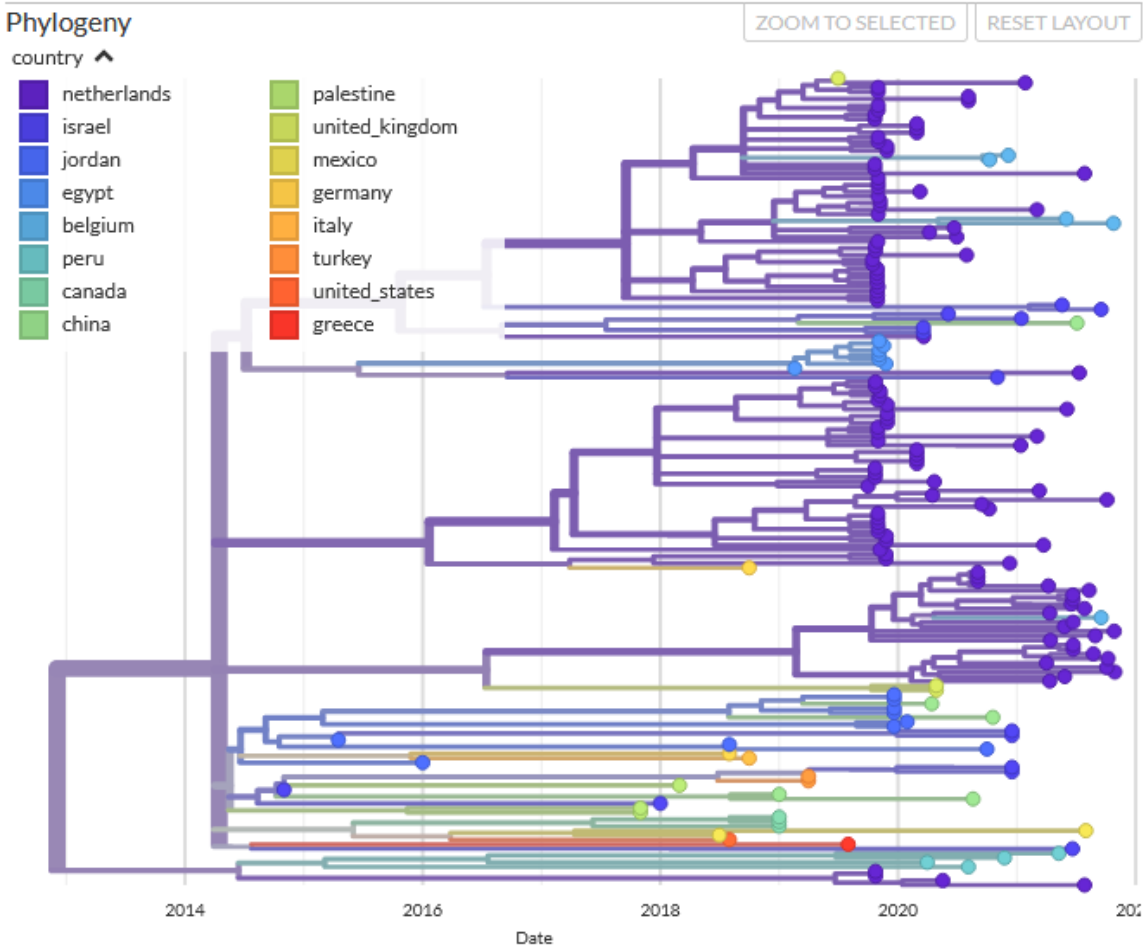
August, 17th-18th, 2022

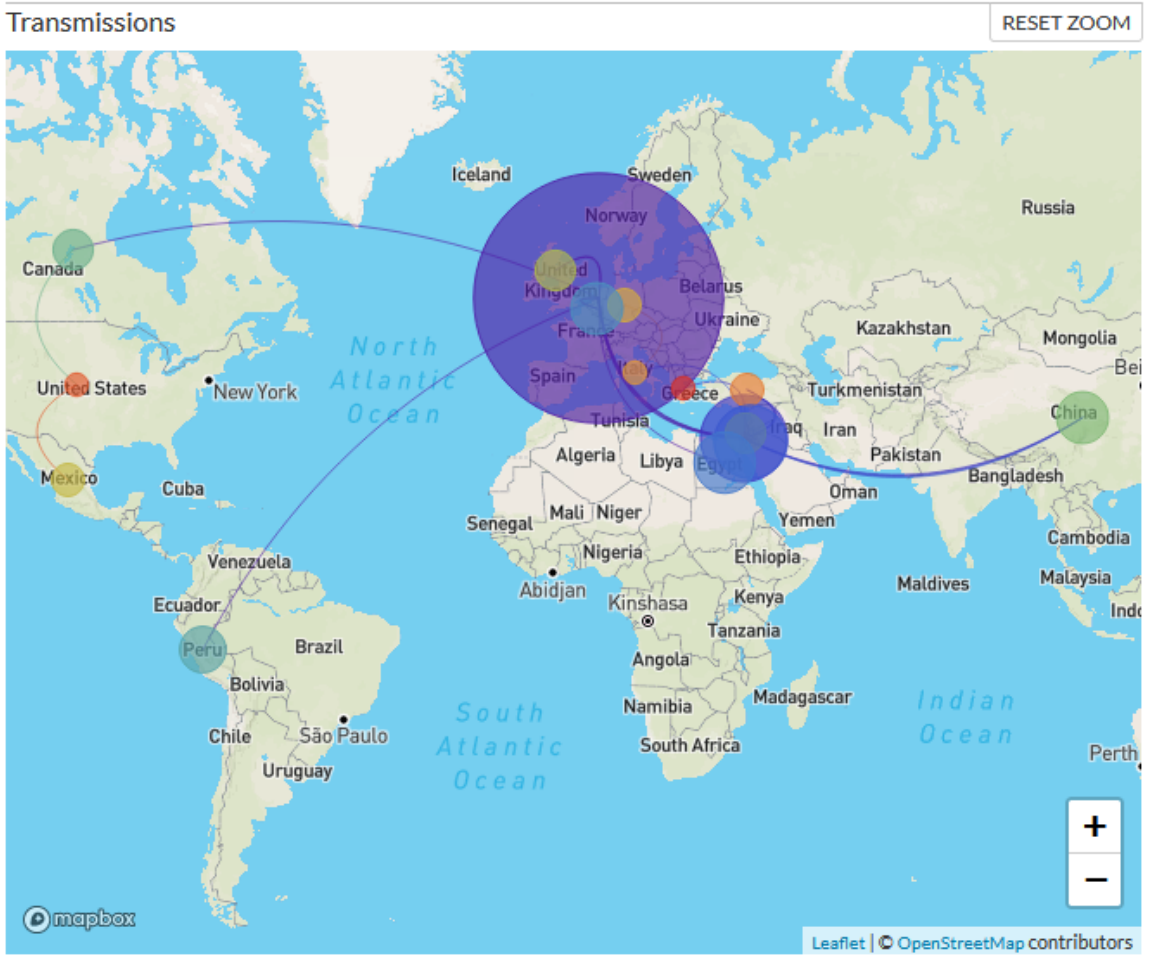
ToBRFV- Track and Trace

Real-time tracking of Tomato brown rugose fruit virus (ToBRFV) outbreaks using Nextstrain (v3)

Maintained by Bart T.L.H. van de Vossenberg and Michael Visser.

Showing 179 of 179 genomes sampled between Nov 2014 and Oct 2021.





Botermans et al (in review) Tomato brown rugose fruit virus Nextstrain build version 3: rise of a novel clade

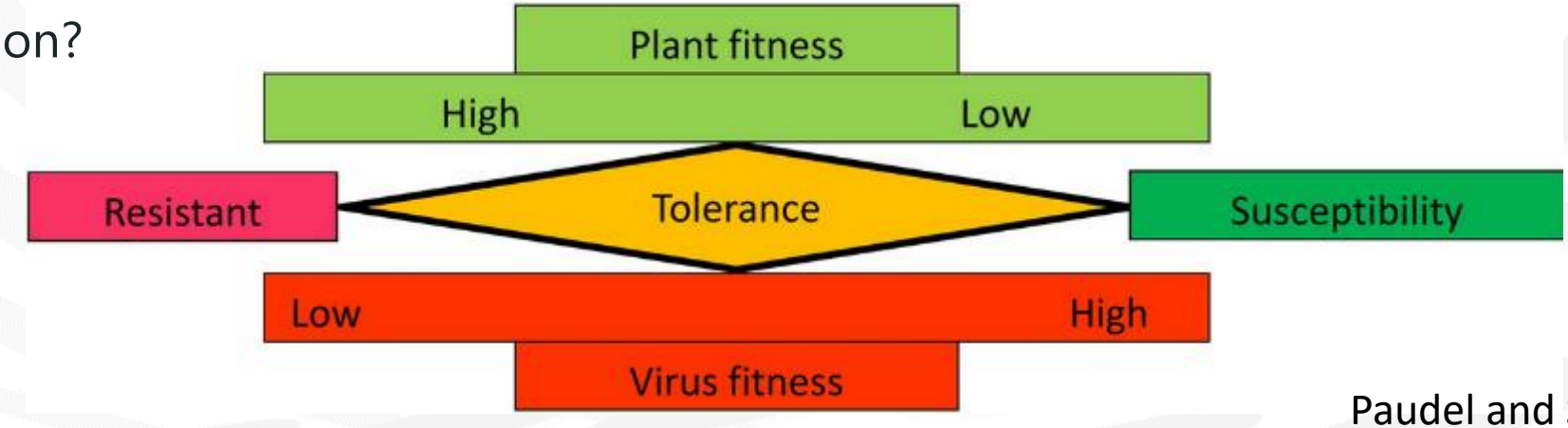
Future outlook

Breeding for resistance

- Conventional approaches – screening germplasm of tomato and other Solanaceae (e.g. wild relatives)
- CRISPR technology
- Seed companies bringing varieties to market in near future:

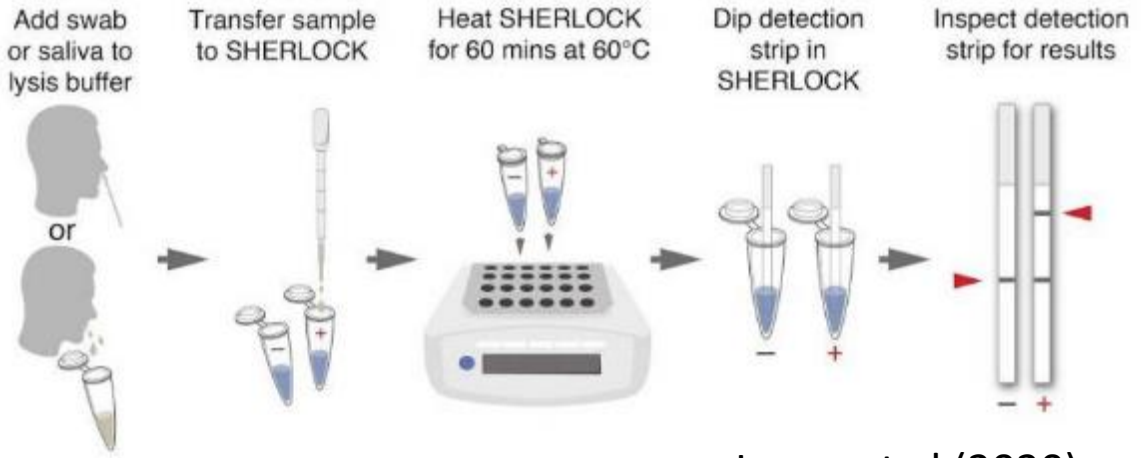
  HR – "High Resistance"

  IR – "Intermediate Resistance"
- Some of these appear to be marketing terms rather than based on clear definitions of Resistance and Tolerance
- Suppression?



Paudel and Sanfacon (2018)

(Near) Future diagnostics?



Joung et al (2020)

CRISPR based diagnostics

- Potential for rapid, specific diagnostics
- Amenable to in field testing E.g. LFD or dipstick
- Amplification step can be isothermal e.g. LAMP, RPA
- Already at/near market for several human pathogens
    - E.g. SHERLOCK (**S**pecific **H**igh-Sensitivity **E**nzymatic **R**eporter Un**LOCK**ing
- ToBRFV proof of concept in progress @ Fera

Decentralised High-Throughput Sequencing?

- small, portable
- Rapid “real-time” base calling, long chain reads (intact genomes?)
- Already in use in many applications
- RT- step poses a challenge
- Validation needed

What next from Fera on ToBRFV?

Understanding the impact of environmental residues…

- Sources of environmental residues
  - “dust” and plant debris
- Detection and viability
  - Risk for carry over infections?
  - Simple/onsite RNA extraction?

Detection and survival of ToBRFV in soils

- Euphresco project starting from November ’23
  - 17 partners, (UK lead)
  - 12 countries plus International Seed Federation
- Detection, Survival and infection from plant debris in soil?
- Handling green waste?

IPPC diagnostic protocol for ToBRFV

- Drafting team has started working on international diagnostic standard

Acknowledgements

Fera

Virology

- Anna Skelton
- Aimee Fowkes
- Kinda Alraiss
- Rachel Hodgson

Validation

- Yue Lin Loh

Statistical Analysis

- Roy Macarthur

NVWA, Netherlands

- Marleen Botermans
- Jerom van Gemert

Newcastle University

- Neil Boonham

NIB, Slovenia

- Olivera Maksimovic