



Original thinking... applied

Determining the biological relevance of molecular detections*

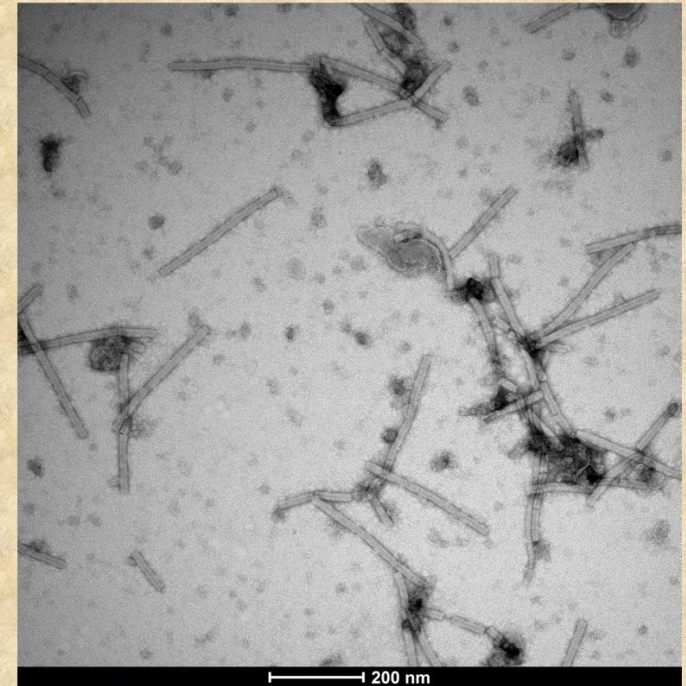
Adrian Fox

Fera Science Ltd

*other detection methods are available, associated risks may increase or decrease

WANTED

Dead or Alive



\$ 1,000,000

Virus diagnostic development – 1898 - 2024



Source <http://wellcomeimages.org>



Increasing validation requirements



Source: https://commons.wikimedia.org/wiki/File:Ouchterlony_Double_Diffusion.JPG



Lets be precise...

- “**positive**” and “**negative**” are interpretations of diagnostic data.
- **Bioassay** – the presence of viable, transmissible virus
- **ELISA** – presence of target proteins e.g. viral coat protein
- **PCR** - presence of fragments of target nucleic acid (may be quite large fragments...)
- **qPCR** – even smaller fragments of target nucleic acid
- **HTS** – lots and lots of fragments of nucleic acid

Each of these methods has advantages and disadvantages in a diagnostic workflow!

Detection Methods – e.g. ToBRFV EPPO PM7/146

Recommended test

Molecular test



2 x Conventional PCR

4 x Real-time PCR

“Other test”...

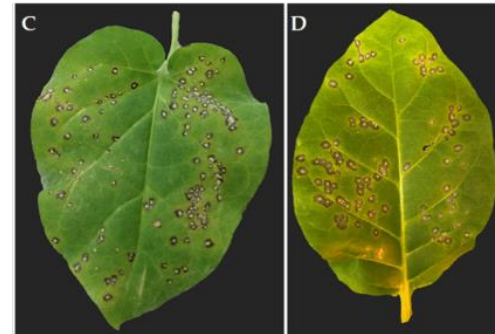
Serological test



ELISA

- Symptomatic plants only

Bioassay



Tobacco species

- Local lesion assay
- Viability!

Sequencing



Determining a positive and negative

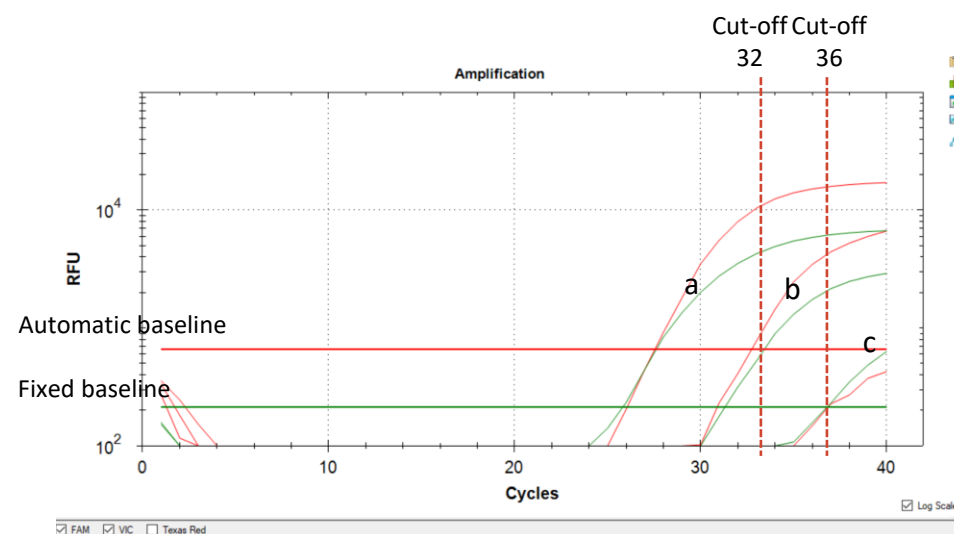
Harmonization difficult even when same tests used:

- Differences between the test “results” between laboratories (especially seeds!)
 - Technical differences
 - Sample preparation, humans, equipment, reagents...
 - Interpretation of (slight) differences in Ct/Cq-values
 - Cut-off values (if used)
 - Context?
 - Leaf, seed, water, swab?
- What does your validation data tell you?

COMMISSION IMPLEMENTING REGULATION (EU) 2020/1191

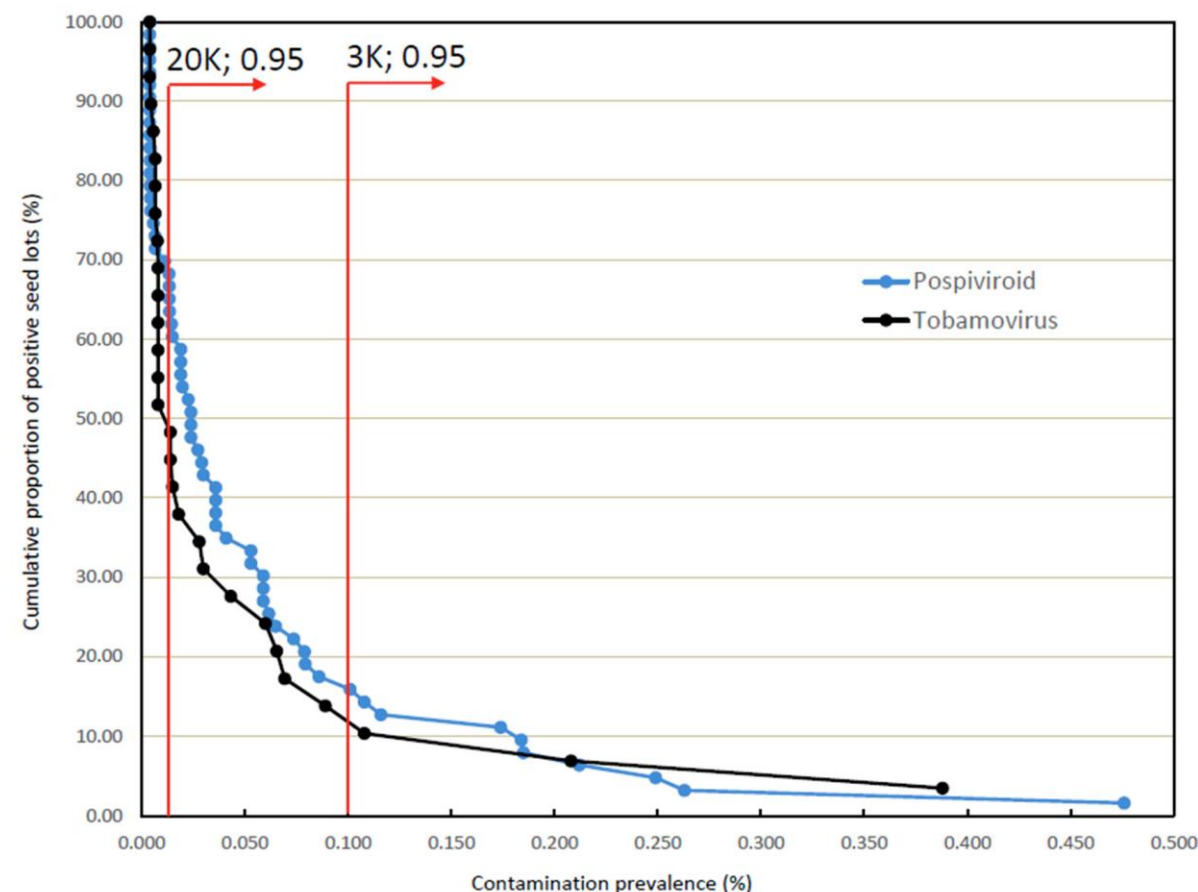
of 11 August 2020

establishing measures to prevent the introduction into and the spread within the Union of Tomato brown rugose fruit virus (ToBRFV) and repealing Implementing Decision (EU) 2019/1615



How relevant is your sample?

Number of units in lot	P = 95% (confidence level)					P = 99% (confidence level)				
	% level of detection × efficacy of detection					% level of detection × efficacy of detection				
	5	2	1	0.5	0.1	5	2	1	0.5	0.1
25	24*	-	-	-	-	25*	-	-	-	-
50	39*	48	-	-	-	45*	50	-	-	-
100	45	78	95	-	-	59	90	99	-	-
200	51	105	155	190	-	73	136	180	198	-
300	54	117	189	285*	-	78	160	235	297*	-
400	55	124	211	311	-	81	174	273	360	-
500	56	129	225	388*	-	83	183	300	450*	-
600	56	132	235	379	-	84	190	321	470	-
700	57	134	243	442*	-	85	195	336	549*	-
800	57	136	249	421	-	85	199	349	546	-
900	57	137	254	474*	-	86	202	359	615*	-
1 000	57	138	258	450	950	86	204	368	601	990
2 000	58	143	277	517	1553	88	216	410	737	1800
3 000	58	145	284	542	1895	89	220	425	792	2353
4 000	58	146	288	556	2108	89	222	433	821	2735
5 000	59	147	290	564	2253	89	223	438	840	3009
6 000	59	147	291	569	2358	90	224	442	852	3214
7 000	59	147	292	573	2437	90	225	444	861	3373
8 000	59	147	293	576	2498	90	225	446	868	3500
9 000	59	148	294	579	2548	90	226	447	874	3604
10 000	59	148	294	581	2588	90	226	448	878	3689
20 000	59	148	296	589	2781	90	227	453	898	4112
30 000	59	148	297	592	2850	90	228	455	905	4268
40 000	59	149	297	594	2885	90	228	456	909	4348
50 000	59	149	298	595	2907	90	228	457	911	4398
60 000	59	149	298	595	2921	90	228	457	912	4431
70 000	59	149	298	596	2932	90	228	457	913	4455
80 000	59	149	298	596	2939	90	228	457	914	4473
90 000	59	149	298	596	2945	90	228	458	915	4488
100 000	59	149	298	596	2950	90	228	458	915	4499
200 000+	59	149	298	597	2972	90	228	458	917	4551



How relevant is a molecular result?



Detection of *Tomato brown rugose fruit virus* (ToBRFV) in Tomato and Pepper Seed by SE-qPCR

Validation Report, March 2020

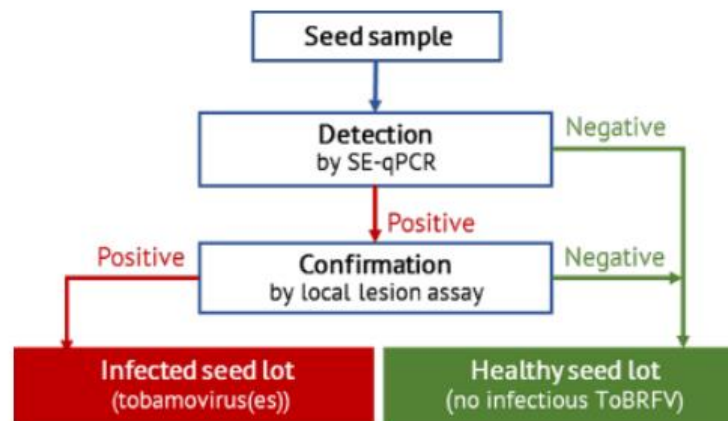


Figure 2. Method process flow

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ORIGINAL ARTICLE

Plant Pathology WILEY

Detection of tomato brown rugose fruit virus in environmental residues: The importance of contextualizing test results

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Abstract

Tomato brown rugose fruit virus (ToBRFV) is regulated as a quarantine pest in many countries worldwide. To assess whether ToBRFV is present in cultivations, plants or seed lots, testing is required. The interpretation of test results, however, can be challenging. Reverse transcription-quantitative (real-time) PCR results, even though considered "positive", may not always signify plant infection or indicate the presence of infectious virus, but could be due to the presence of viral residues in the environment. Here, case studies from the Netherlands, Belgium, and the United Kingdom address questions regarding the detection of ToBRFV in various settings, and the infectiousness of ToBRFV-positive samples. These exploratory analyses demonstrate widespread detection of ToBRFV in diverse samples and environments. ToBRFV was detected inside and around greenhouses with no prior history of ToBRFV infection, and on different materials and surfaces, including those that were untouched by individuals, plants or objects. This suggested the dispersal of viral residues through aerosols. ToBRFV or its residues were more often detected in regions with nearby tomato

What is the purpose of a sample?

ISPM 31:

Sampling of consignments is done for inspection and/or testing in order to:

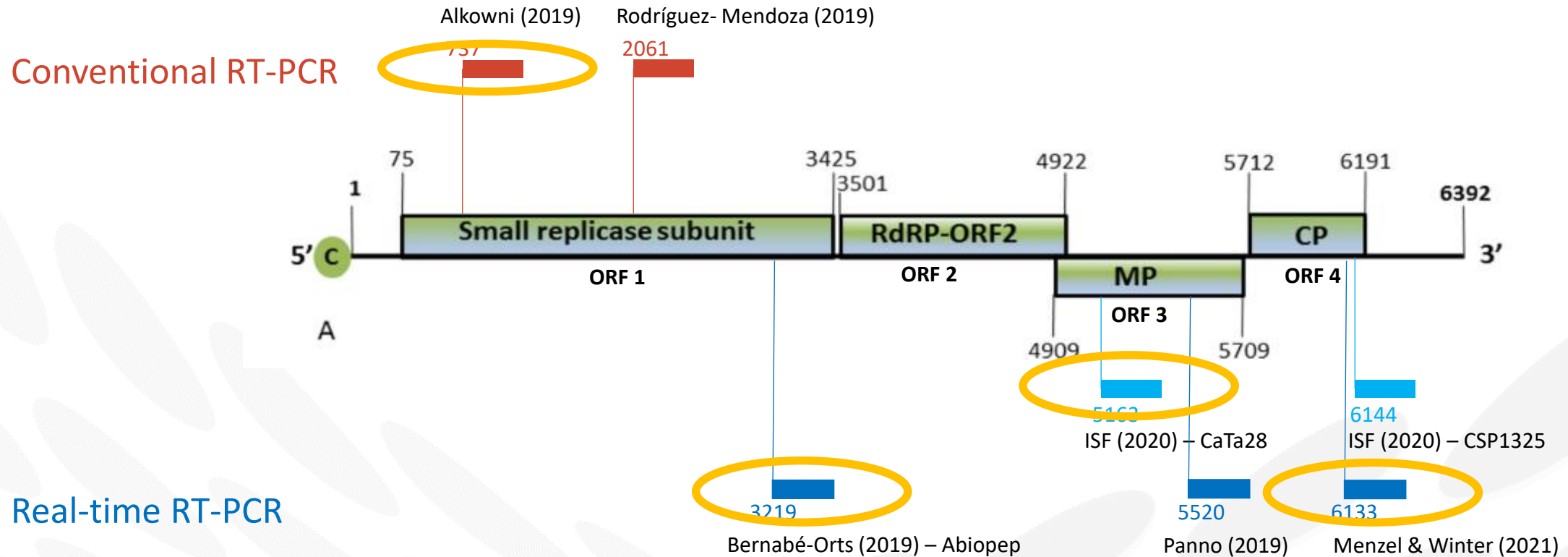
- **detect regulated pests**
- provide assurance that the number of regulated pests or infested units in a consignment does not exceed the specified tolerance level for the pest
- **provide assurance of the general phytosanitary condition of a consignment**
- detect organisms for which a phytosanitary risk has not yet been determined
- **optimize the probability of detecting specific regulated pests**
- maximize the use of available sampling resources
- gather other information such as for monitoring of a pathway
- **verify compliance with phytosanitary requirements**
- determine the proportion of the consignment infested.

How does this sit within legislation?

Notification of the presence or suspected presence of certain plant pests

42.—(1) The occupier or other person in charge of premises who knows or suspects that any plant pest to which this article applies is present on the premises, or any other person who, in the course of his duties or business, **becomes aware or suspicious of the presence of such plant pest** on any premises, shall immediately give notice to the Secretary of State or an inspector of the presence or suspected presence of such plant pest and shall as soon as reasonably practicable after giving such notice confirm it in writing.

Molecular Tests used at Fera



How relevant is your bioassay?

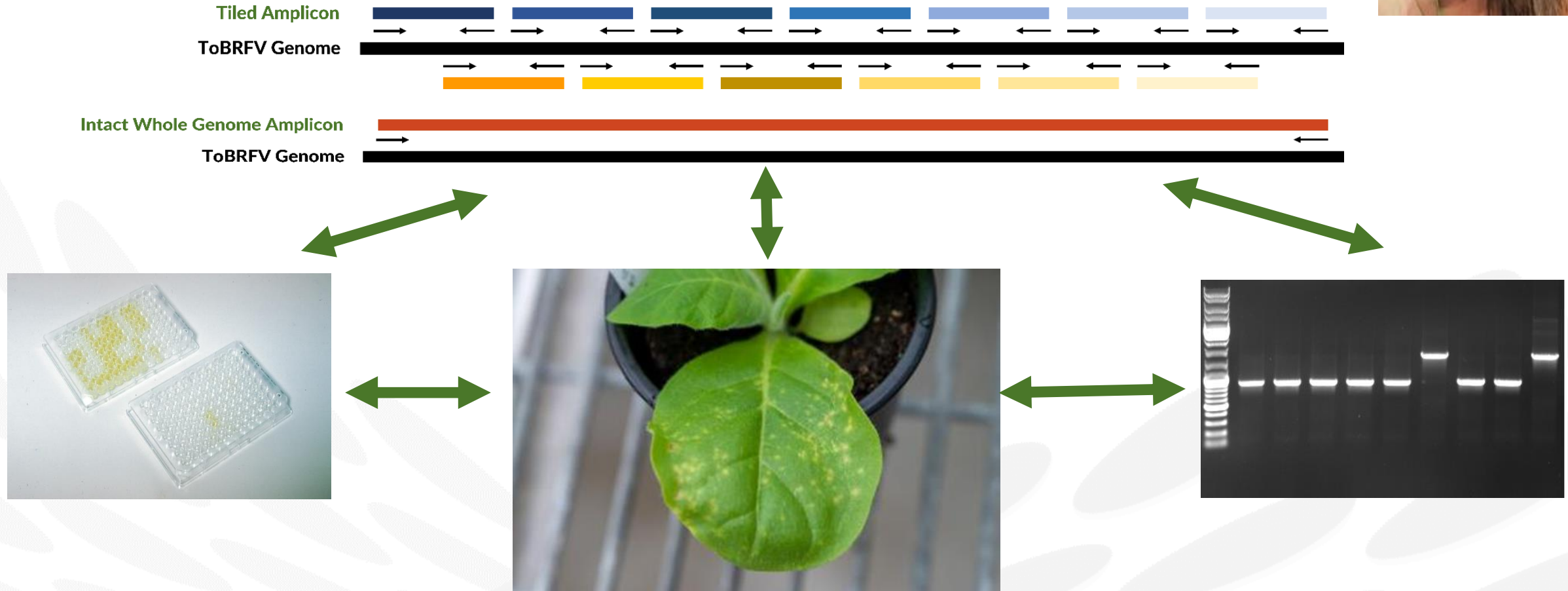
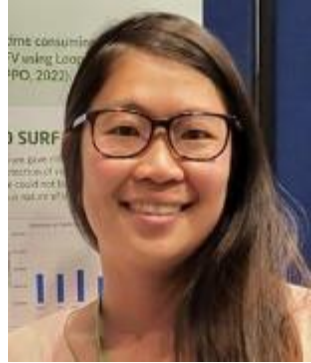


≠



What is the biological relevance of your detection?

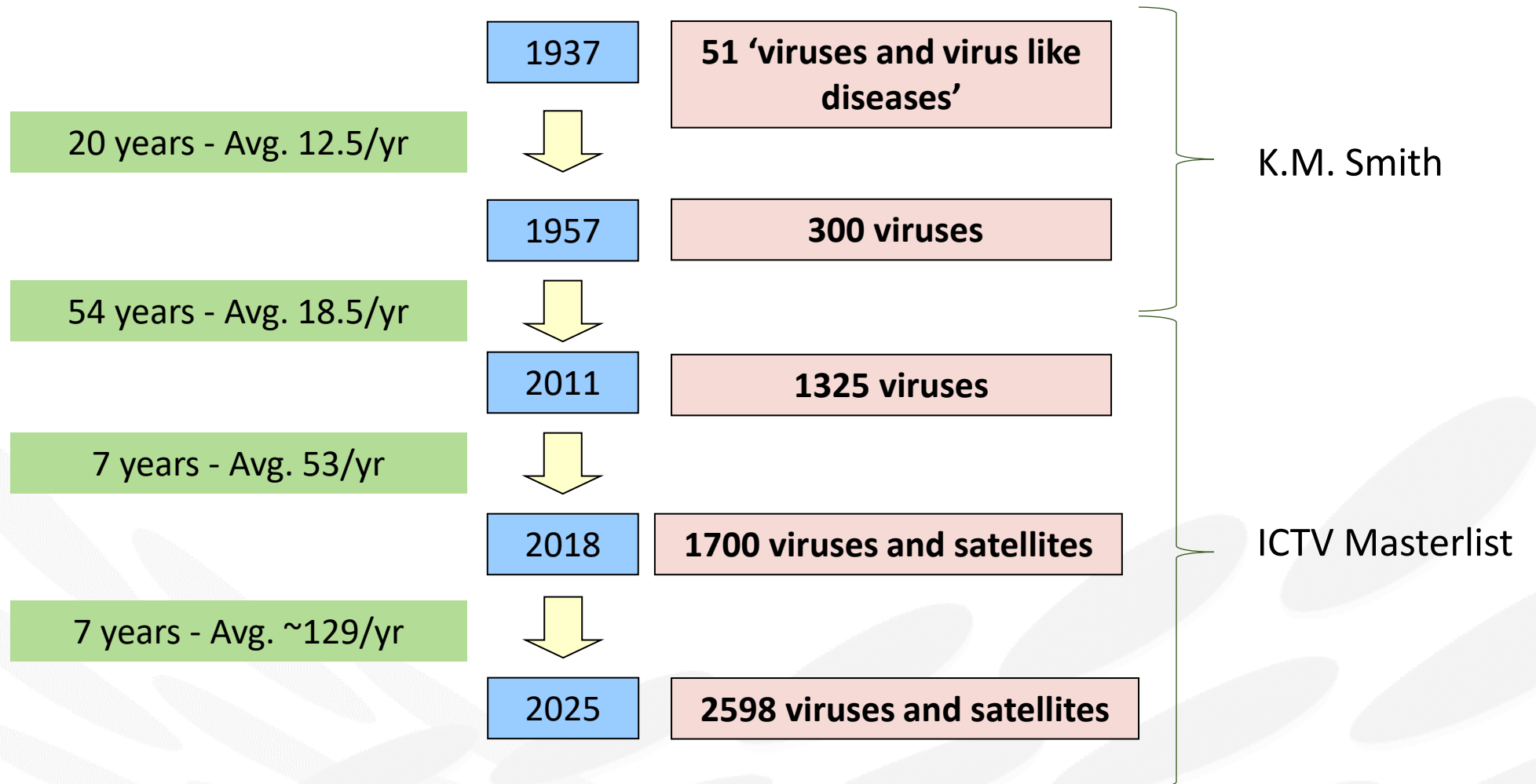
(Defra PhD)



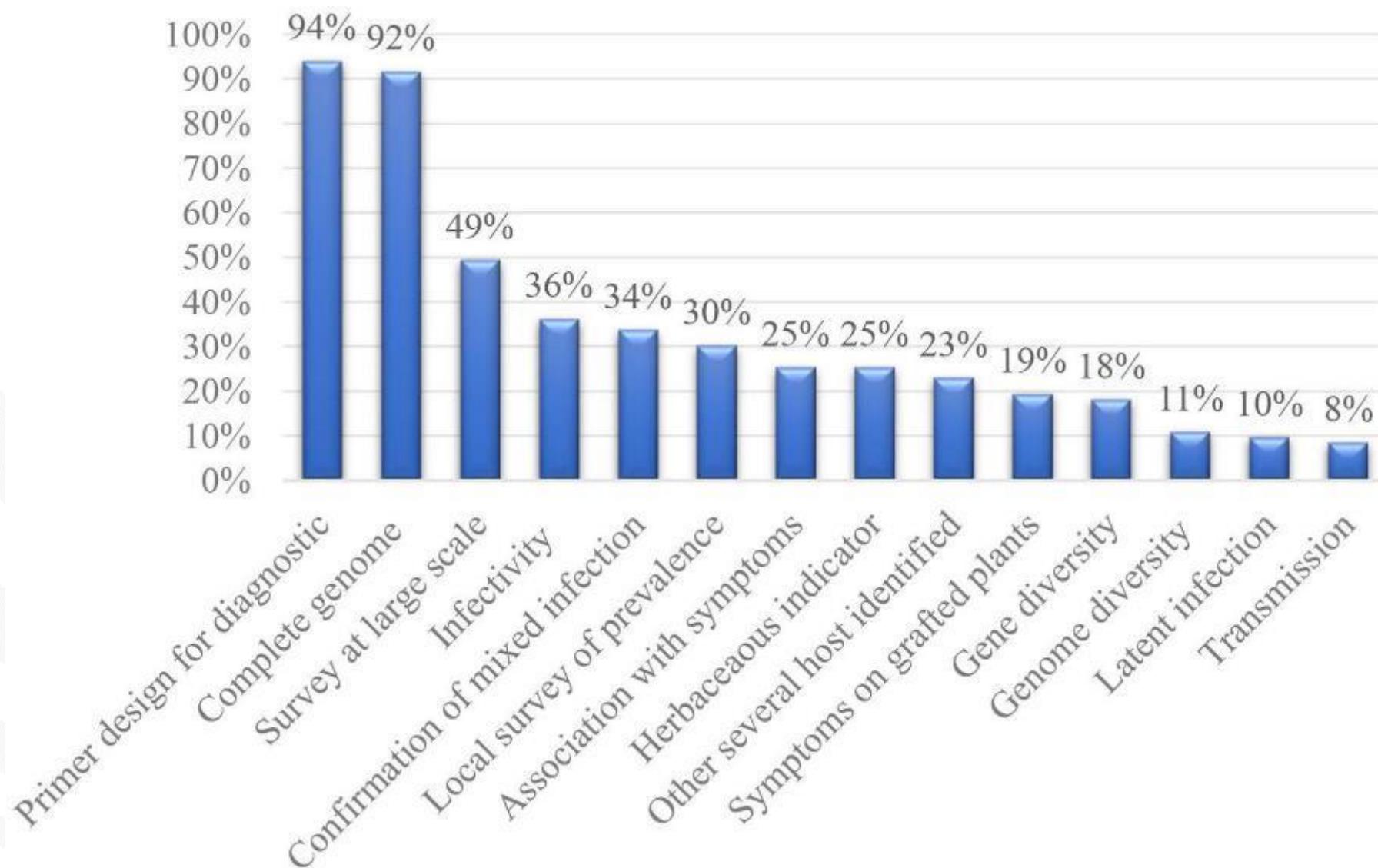
Biological relevance in the age of HTS...

(What does biological relevance ACTUALLY mean....)

The pace of discovery...

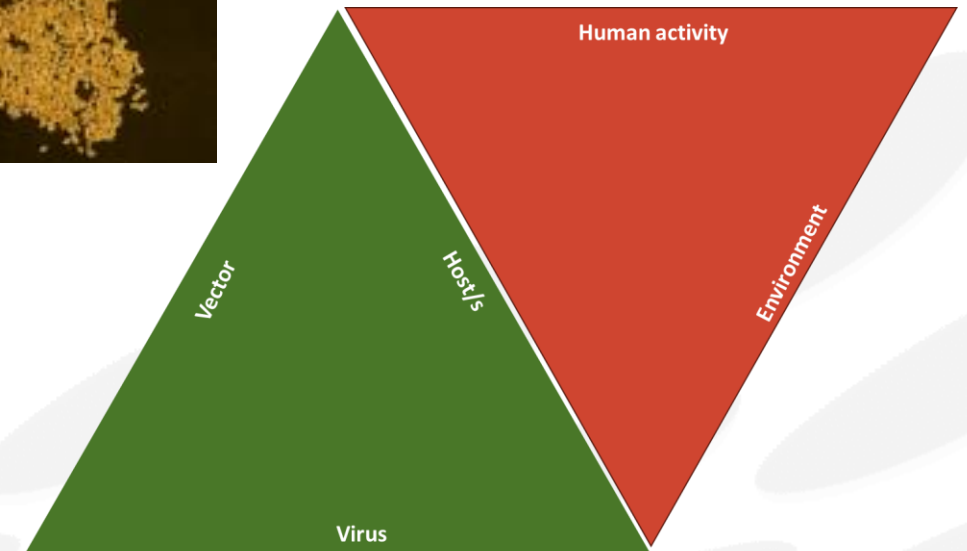
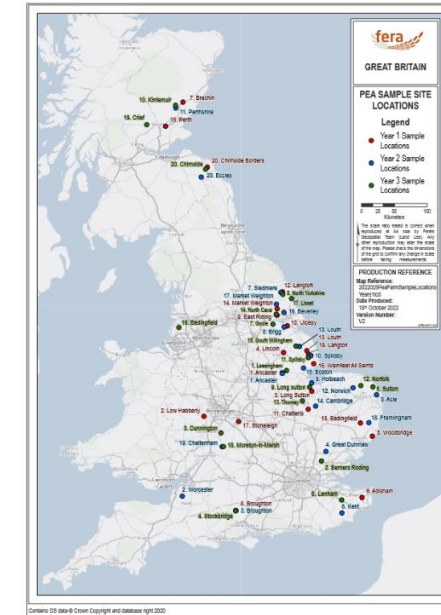
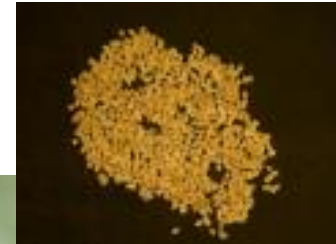


The emerging biological desert...



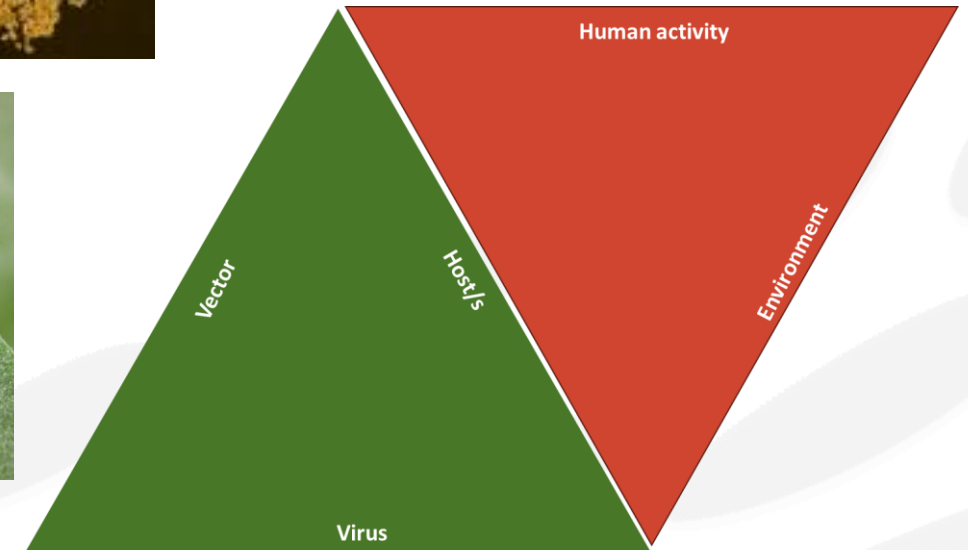
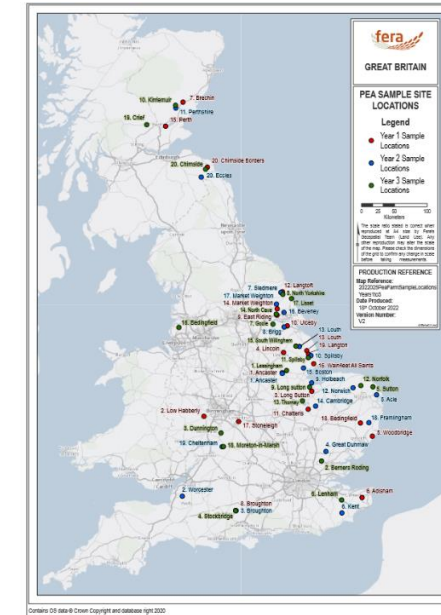
Essential questions to support pest risk analysis:

- Presence
 - Prevalence
 - Distribution
- Impact
- Host range
- Transmission
- Pathways



The challenges...

- Costs
- Time
- Staff resource
 - Inspectors/sampling
 - Interdisciplinarity
- Glasshouse availability
- Skills
- Biology...
- Prioritisation...



The “Massart-Fox Sweet Spot” for biological characterisation

Importance

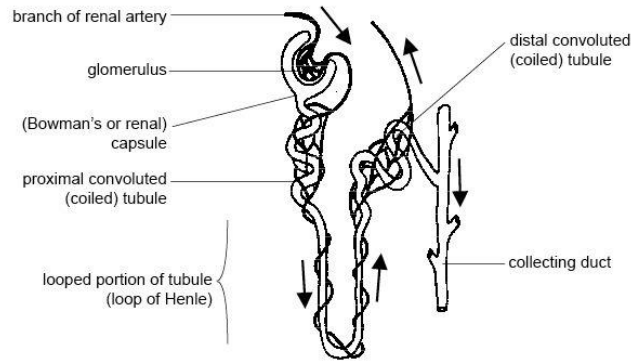
ToBRFV is HERE

Are there alternative ways to approach the question of
biological characterisation?

own...

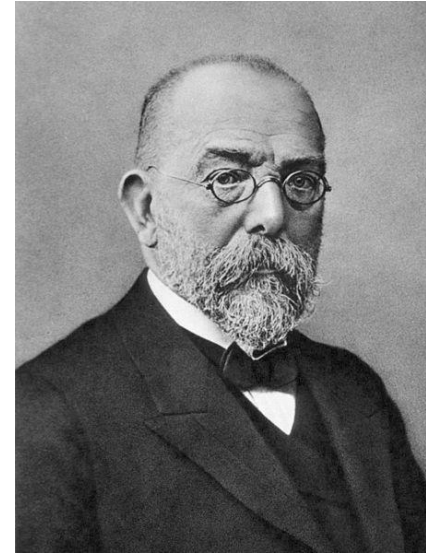
play with

A brief history of causal association...



Friedrich Gustav Jakob Henle (1809-1885)

- Described a looped portion of renal tubule
- 1840 - "On Miasmata and Contagie"
 - Stipulated criteria for inferring causation



Heinrich Hermann Robert Koch (1843-1910)

- Described anthrax, tuberculosis, and cholera
- Microscopy pioneer (e.g. Oil immersion)
- Developed bacterial culturing on agar (with his assistant Julius Petri...)
- 1884 then **1890 – Formulated “postulates” on determining aetiology with a set experimental approach**

...parasites never behave as accidental saprophytes but in the manner in which well-known pathogenic bacteria act. Therefore, **we are justified in stating that if only the first two conditions of the rules of proof are fulfilled**, i.e., if the regular and exclusive occurrence of the parasite is demonstrated, **the causal relationship between parasite and disease is validly established.**

Robert Koch, 1891

At the time when they were formulated Koch's postulates were essential for the progress of knowledge of infectious diseases; but progress having left behind old rules requires new ones which some day without doubt will also be declared obsolete. **Thus, in regard to certain diseases, particularly those caused by viruses, the blind adherence to Koch's postulates may act as a hindrance instead of an aid.**

Thomas M. Rivers, 1937

#foxpostulates

- Based on concepts from Bradford-Hill, 1965
- **Adapts observed and epidemiological evidence to infer causal association**
 - Relevant data can be gathered during survey
 - Not limited to one pathogen-one disease
 - Allows a range of factors to be considered
- **Not a strict framework**
 - Designed to encourage rigour in approach rather than a “tick list”
- **How to report uncertainty?**

Criteria	Suggested approaches
Experiment	An isolate should be inoculated into an uninfected host and observed for symptom development (if possible)
Strength	Based on field/glasshouse observation. HTS and statistical analysis of affected and symptomless individuals. Account for polymicrobial effects and latent infections.
Consistency	Is the relationship as strong at multiple geographic locations and/or at different times.
Coherence and Plausibility	Are there any confounding factors? Are there similar effects reported in other pathosystems to support the conclusions?

Is environmental contamination biologically relevant?



Detection of Infectious Tomato Mosaic Tobamovirus in Fog and Clouds

J. D. Castello, D. K. Lakshman, S. M. Tavantzis, S. O. Rogers, G. D. Bachand, R. Jagels, J. Carlisle, and Y. Liu

First, fourth, fifth, and eighth authors: professor, associate professor, and graduate research assistants, respectively, State University of New York, College of Environmental Science & Forestry, Faculty of Environmental & Forest Biology, 1 Forestry Dr., Syracuse 13210-2788; second and third authors: associate scientist and professor, respectively, University of Maine, Department of Plant Biology & Pathology, 5722 Deering Hall, Orono 04469-5722; sixth and seventh authors: professor and chair, and assistant scientist, respectively, University of Maine, Department of Forest Ecosystem Science, 5755 Nutting Hall, Orono 04469-5755.

Supported in part by grants from the McIntire-Stennis Cooperative Forestry Research Program of the USDA and USDA-NRICGP award 93-37101-8837 to J. D. Castello, the Hatch CSRS Program of the USDA to S. M. Tavantzis, and The Andrew W. Mellon Foundation to R. Jagels.

We thank D. Wolfe and S. Roychowdhury, Atmospheric Sciences Research Center, Whiteface Mountain Field Station, for collection of cloud samples; C. Long and J. O'Day for technical assistance; D. Leopold, J. Hamacher, V. Jacobi, W. Powell, M. Schaedle, and S. Silbiger for reviews of earlier drafts of the manuscript.

Accepted for publication 17 August 1995.

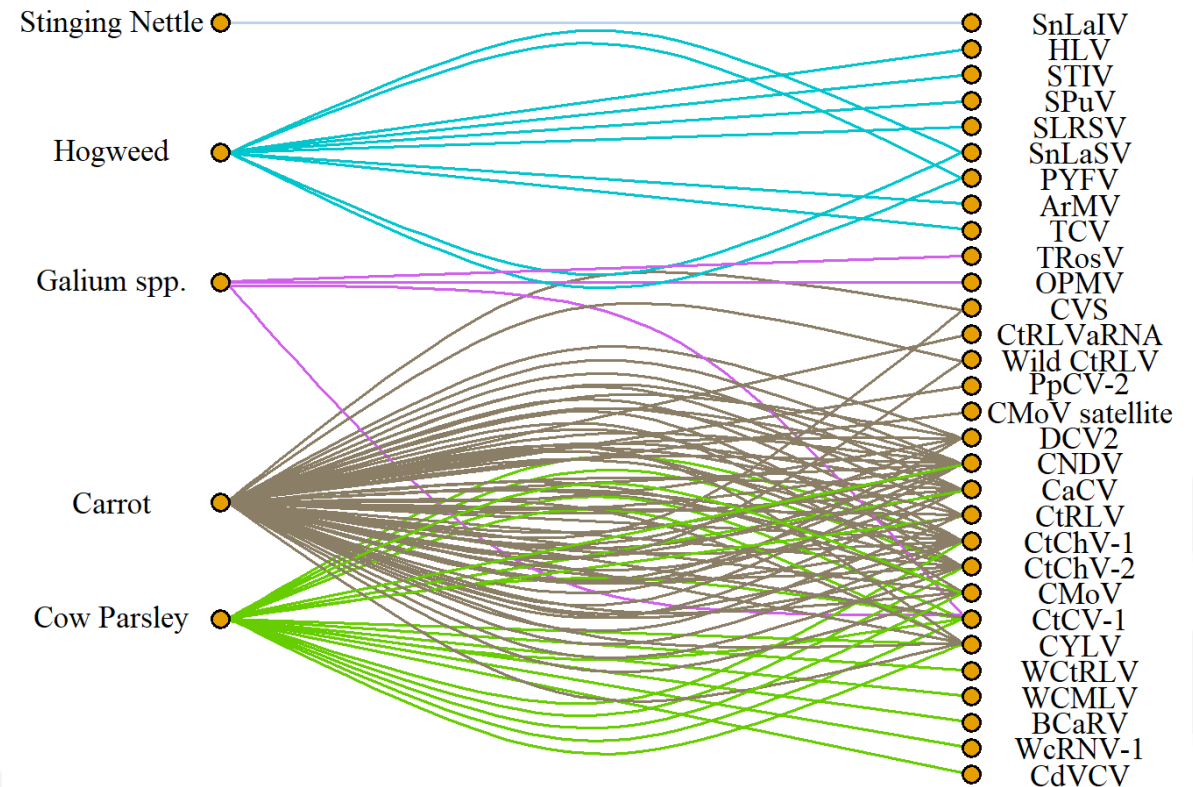


	real-time PCR	LAMP (RNA extract)		
	Ct Ave	Tp	Ta	
Top of light	35.36	19:15	84.45	

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

Using virome studies and network ecology to support PRA?

- Could “virome study” approaches be adapted to inform potential pathways and transmission routes?
 - Reveal new viruses
 - Rigour of context data?
- **How to deal with uncertainty?**
 - Host data?
 - Presence/Distribution?
 - Looking for potential transmission pathways?



Source: Defra Future Proofing Plant Health

How to risk assess an “electronic” virus?



Virology
Volume 582, May 2023, Pages 106-113



Short Notes

Symptomatic, widespread, and inconspicuous: new detection of tomato fruit blotch virus

Broadening
diversity of

V. Kavi Sidharthan^a

Show more ▾

+ Add to Mendeley

<https://doi.org/10.1016/j.virol.2023.106-113>

Sidharthan

- SRA search
- 22
- Broadening diversity of trees, aquatics, cereals

LAURE
LIVIER

roscope,

Need to “ground truth” these detections...

Are they a risk?

Can we use these data to support risk analysis?

- Potential novel hosts : Potato and Sweet potato

Plant disease records can have a significant impact on trade

- Some countries concerned about malicious use of shared SRA data

Research portfolio

