



Original thinking... applied

New frontiers in plant movement: The use of high throughput sequencing in plant virology

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Overview

- Diagnostics as a driver of new species discovery
 - What technologies are available?
 - Developing high throughput sequencing (HTS) for frontline sample diagnosis
- Applying HTS in plant health
 - Investigating causation
 - New trade pathways and niche crops
 - Surveillance and the importance of baseline data
- What's next?

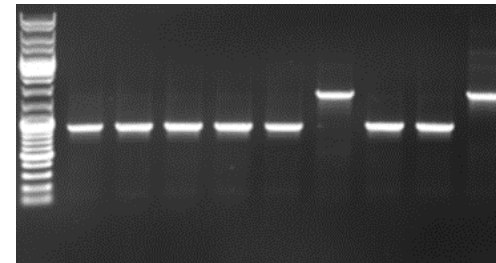
Long road of diagnostic development



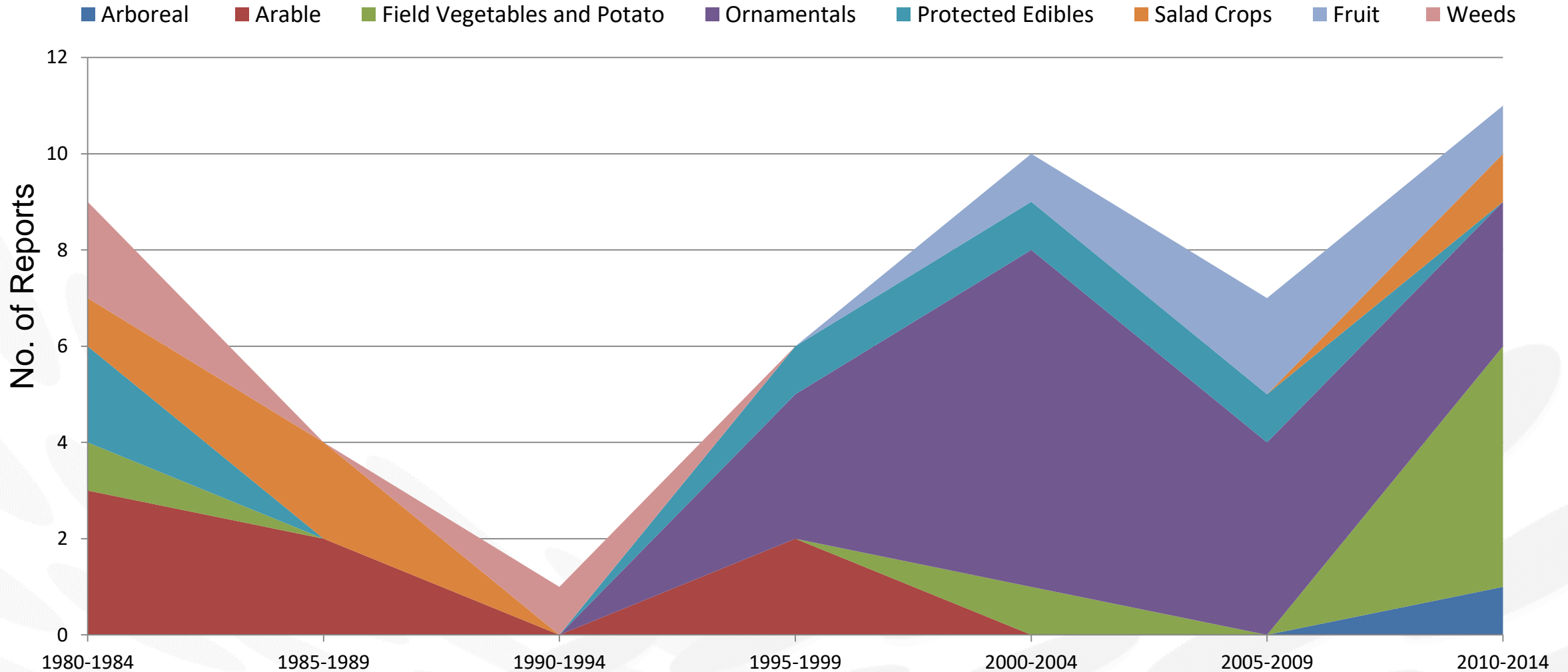
Source <http://wellcomeimages.org>



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



Factors driving virus discovery (UK 1980-2014)



5 yr Period

Fox and Mumford, (2017) Virus Research, 241

High throughput sequencing in plant pathology

- Range of platforms and approaches...
- Key applications investigated:
 - HTS informed diagnostics
 - Unknown aetiology
 - 'Megaplex' screening
 - Improving targeted diagnostics
 - Disease monitoring (population genetics)
- Few studies on:
 - Equivalence
 - Standardisation
 - Validation
 - Controls

International plant health authorities have concerns about reporting of findings from 'stand alone' use of technology



Developing HTS for frontline virus diagnostics

Sample Assessment

- Symptomatic sample assessment and/or
- ELISA/PCR screen positive (generally a generic test positive)
- Negative screen for knowns, but recognised virus in host with no other test available
- Negative screen for knowns/expected, but bioassay shows symptoms

HTS

- Standardised protocol for sample preparation and testing
- Samples spiked with suite of synthetic RNA controls
- 'Negative' tobacco included in run
- Quality check of data
- De novo assembly – initial quality check of assembled sequence
- Reference mapping
- Initial taxonomic assignment of findings

Confirmation

- Known virus: Existing test available – Where possible use a different biological principle (ELISA?); **as a minimum use previously published primers**
- Known virus: No existing test available – Design primers to consensus sequence
- Novel virus – Design primers to sequenced isolate



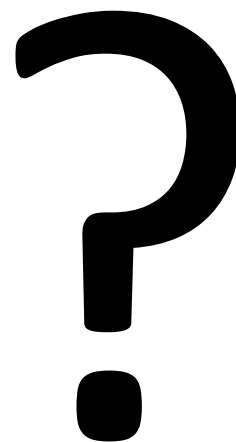
Where to put the HTS in the workflow...



Screening



Confirmation



'no-eyed deer'



Difficult cases

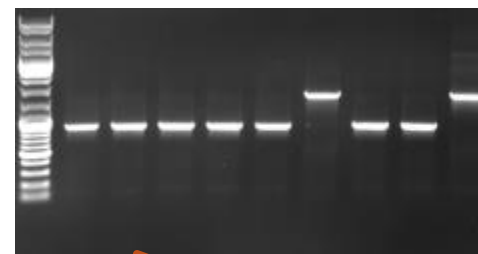


Surveillance/inspection

Unknown aetiology : Viral necrosis of carrots (AHDB FV382a)



Stocks of carrots rejected due to the presence of internal necrosis linked to viruses.

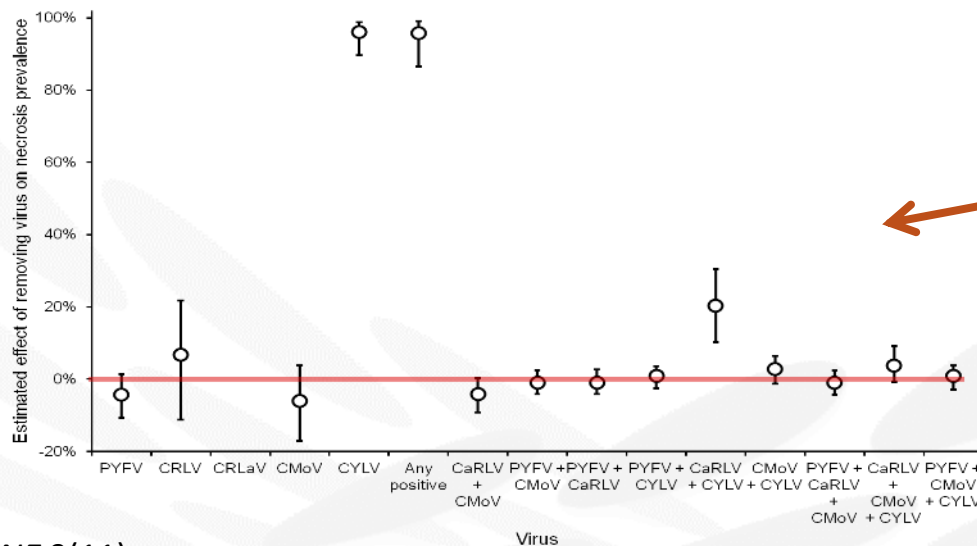


Conventional PCR of affected & unaffected carrots showed no association with expected viruses



MiSeq sequencing identified *Carrot yellow leaf virus* in necrotic samples. Sequencing also detected a Closterovirus (CtCV-1) and a Torradovirus (CaTV-1) plus several other viruses which were new to science.

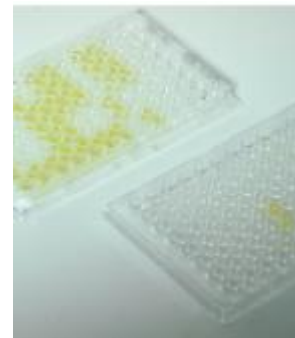
Real-time PCR demonstrated a strong association between CYLV and necrosis, suggesting that removal of this virus would reduce necrosis by 96%



Ulluco: HTS in an outbreak reveals novel viruses (Defra)



Summer 2017: NPPO notified of crop of *U. tuberosus* being grown for seed without certification

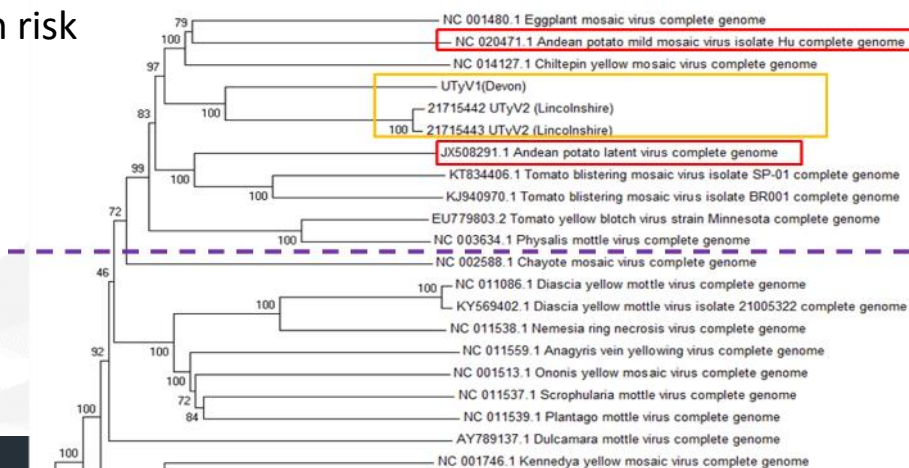


Initial screen suggests presence of quarantine viruses but this could not be confirmed



Genetic sequencing revealed 6 viruses new to science and non-native pathogens.

Two novel viruses considered to be high risk



Brussels, XXX
SANT/10766/2018
[...](2018) XXX draft

COMMISSION IMPLEMENTING REGULATION (EU) .../...

of XXX

establishing a provisional list of high risk plants, plant products or other objects, within the meaning of Article 42 of Regulation (EU) 2016/2031 and a list of plants for which phytosanitary certificates are not required for introduction into the Union, within the meaning of Article 73 of that Regulation

2019: ulluco included in European legislation as a high-risk plant species

New sequences

Old viruses?

Fox et al (2019) ELISA	Fox et al (2019) HTS	Previous reports from Ullucus (No Sequence data reported)	Source
Andean potato latent virus	Ullucus tymovirus 1 (Devon) Ullucus tymovirus 2 (Lincolnshire)	Andean potato latent virus	Lizaragga et al, 1996
Potato leaf roll virus	Ullucus polerovirus 1	Potato leafroll virus	Lizaragga et al, 1996 b
Potyvirus / Potato virus Y	Ullucus potyvirus 1	Ullucus mosaic virus	Brunt et al, 1982
	Papaya mosaic virus-Ulluco strain	Papaya mosaic virus - Ulluco strain	Brunt et al, 1982
	Ullucus tobamovirus 1	TMV-U Ullucus mild mottle virus	Brunt et al, 1982 Offei et al, 1995
	Ullucus comovirus 1, 2, ?	Ullucus virus C (UVC)	Brunt et al, 1982 b
	Broad bean wilt virus 1 + 2		No previous record

Historic is -lat... supporti...

GENOME SEQUENCES

CO...

- Links with shopping
- Support
 - (P)
 - F
 - M

Archives of Virology
<https://doi.org/10.1007/s00705-019-04395-7>
 BRIEF REPORT



Genomic sequence and host range studies reveal considerable variation within the species *Arracacha virus B*

Roger A. C. Jones^{1,2} · Adrian Fox³ · Neil Boonham⁴ · Ian P. Adams^{3,4}

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Abstract

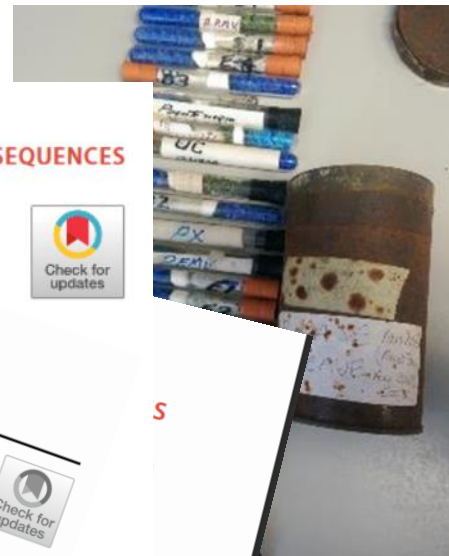
Arracacha virus B type (AVB-T) and oca (AVB-O) strains from arracacha (*Arracacia xanthorrhiza*) and oca (*Oxalis tuberosa*) samples collected in 1975 and two additional isolates obtained from arracacha (AVB-PX) and potato (AVB-6A) in Peru in 1976 and 1978, respectively, were studied. In its host responses and serological properties, AVB-PX most resembled AVB-T, whereas AVB-6A most resembled AVB-O. Complete genomic sequences of the RNA-1 and RNA-2 of each isolate were determined. AVB-PX and AVB-6A shared 70% nucleotide identity with AVB-T and AVB-O, respectively. AVB-PX and AVB-6A shared 70% nucleotide identity between MasVY and a genomic sequence of *Verbeke virus* (AVB-V) and 79% nucleotide identity between MasVY and a genomic sequence of *Arracacha virus Y* (ArVY). AVB-PX and AVB-6A shared 79% nucleotide identity with those of ArVY. AVB-PX and AVB-6A shared 79% nucleotide identity with those of ArVY. AVB-PX and AVB-6A shared 79% nucleotide identity with those of ArVY.

ABSTRACT We present the complete genomic sequence of a novel virus tentatively call *Mashua virus Y* (MasVY), first isolated in 1976 in Peruvian Andean tuber crop mashua (*Tropaeolum tuberosum*, family Tropaeolaceae). MasVY shared 70% nucleotide identity with those of *Verbeke virus* (AVB-V) and 79% nucleotide identity with those of *Arracacha virus Y* (ArVY). MasVY shared 79% nucleotide identity with those of ArVY. MasVY shared 79% nucleotide identity with those of ArVY.

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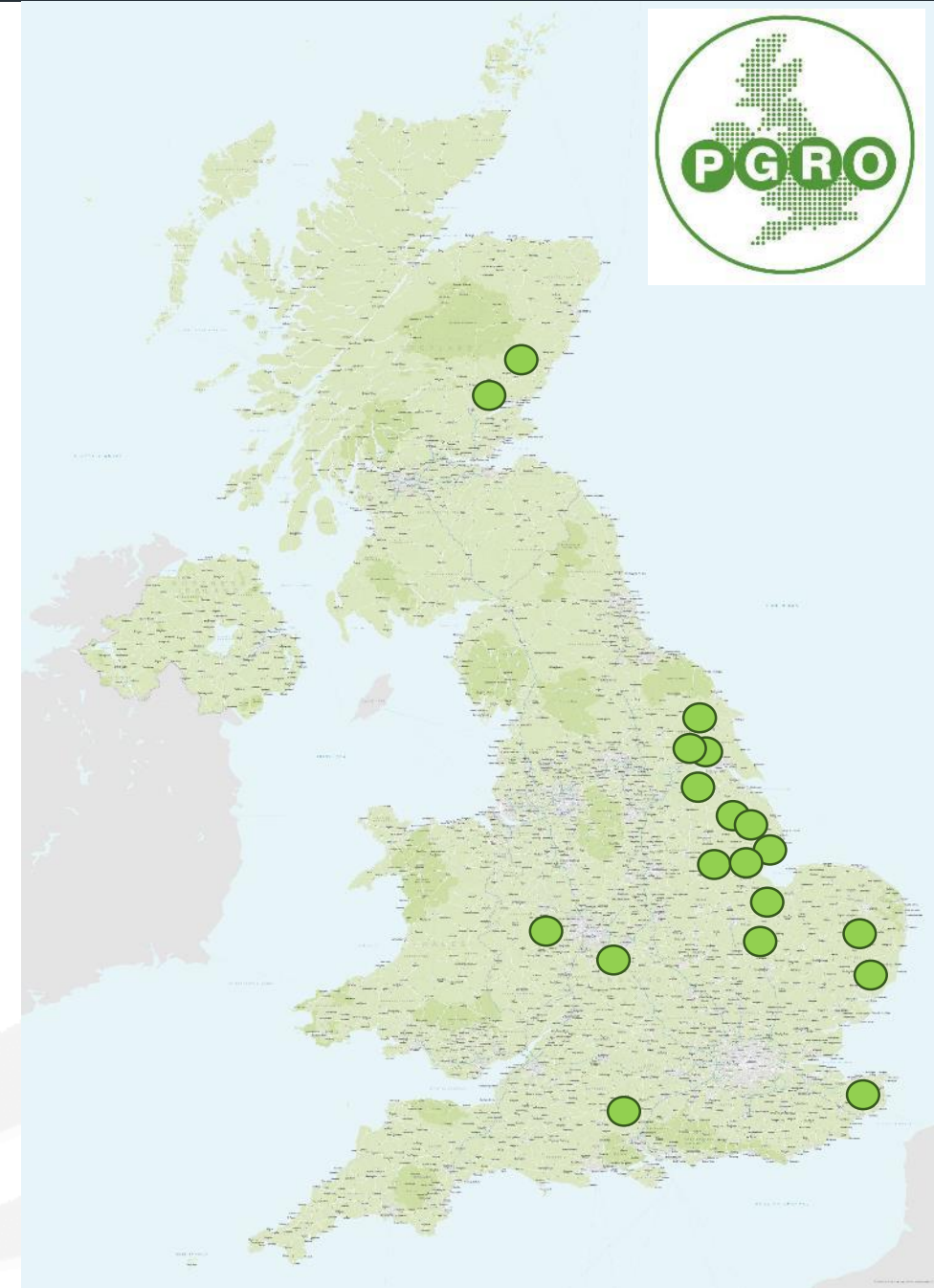


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Using HTS for field survey: Pea viruses in the UK (AHDB FV 459)

- 3 yr AHDB funded project
- PGRO as collaborative partner
- 20 field crops per year
 - National coverage
 - 1 visit per field
 - Bulk 'random' sample for HTS screening and virus incidence
 - Symptomatic individual plants
 - Yield loss (if possible...)

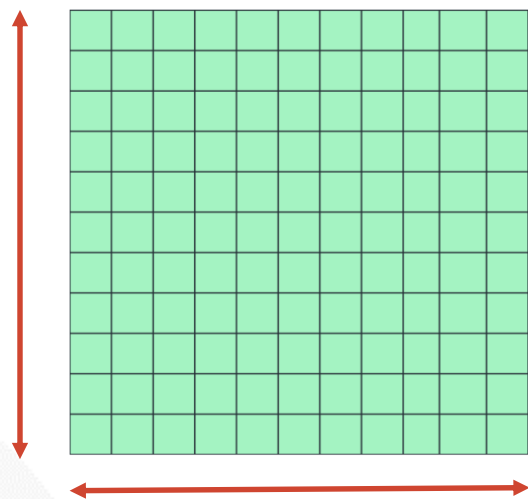


Using peas as a model crop

- Over 120 viruses known to infect peas
 - 43 recorded from natural infections
 - 27 viruses recorded from UK that will infect peas
 - 7 recorded from UK pea crops
- No recent surveillance work in the UK
 - Most recent work in 1970's and many reports based on symptomatic disease descriptions
- Major issues in Germany with emerging viruses of legumes (nanoviruses)
 - Pea necrotic yellow dwarf virus



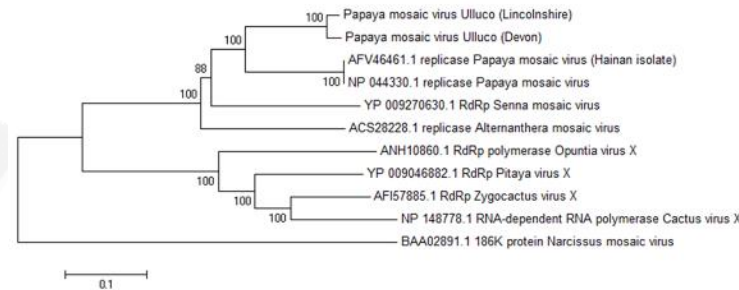
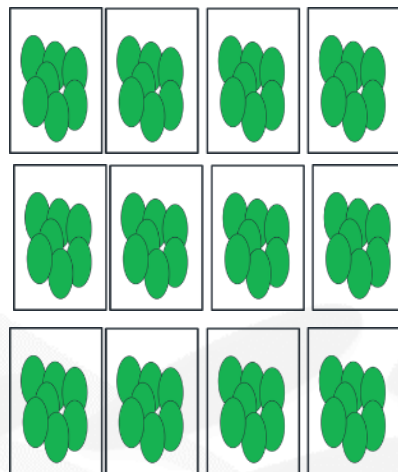
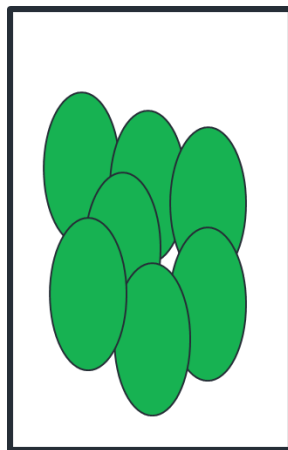
Generic surveillance approach



100m x 100m



X%





Overview of year 1

- 20 crops sampled in year 1 from East of England up to Central Scotland
- 121 'random' leaves per bulked sequencing sample, plus C.30 individual symptomatic plants
- 7 viruses detected:
 - Pea enation mosaic virus 1 (PEMV1), *Enamovirus*
 - Pea enation mosaic virus 2 (PEMV2), *Umbravirus*
 - Pea enation mosaic virus satellite
 - Pea seed-borne mosaic virus (PSbMV), *Potyvirus*
 - Bean yellow mosaic virus (BYMV), *Potyvirus*
 - Turnip yellows virus (TuYV), *Luteovirus* – **New host record for UK**
 - Soybean dwarf virus (SbDV), *Luteovirus* – **First record for UK**
- 'Control' Samples stored since 2007 have tested positive for TuYV and SbDV....



First year results...

- 13 / 20 (65%) crops with virus infection
- 12/20 crops infected with TuYV
- 5/20 crops single infection (TuYV)
- SbDV First record for UK
- Early crops less infection and fewer viruses

Sample	HTS Candidate viruses	TuYV	PEMV1	SbDV
		Estimate	Estimate	Estimate
1	Negative			
2	Negative			
3	Negative			
4	Negative			
5	Negative			
6	TuYV	12.46		
7	Negative			
8	PEMV1 PEMV2		27.44	
9	TuYV	1.71		
10	TuYV	6.76		
11	TuYV	60.62		
12	TuYV PEMV2	9.7	0.85	
13	TuYV PEMV2	21.8	0	
14	TuYV PEMV2 SbDV	93.33		1.71
15	TuYV PEMV1 PEMV2 SbDV	2.64	0.85	4.53
16	TuYV PEMV1 PEMV2 PEMV Sat	8	3.72	
17	TuYV	6.98		
18	TuYV PEMV Sat	93.33		
19	TuYV PEMV2	80.01		
20	TuYV PEMV1 PEMV2 PEMV Sat	14.29	30.09	

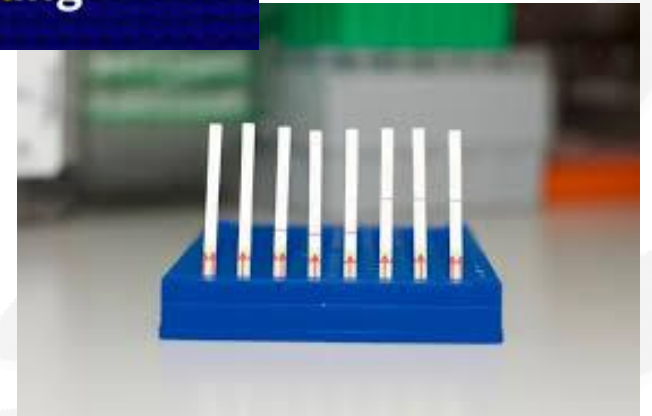
What's next?

iKnife : GC-MS linked to a cauterising scalpel



SHERLOCK : CRISPR Cas13 based
isothermal nucleic acid detection

SHERLOCK
Specific High-sensitivity
Enzymatic Reporter
unLOCKing



Summary

- HTS offers unparalleled potential for detection and diagnosis of plant pathogens
- Moving beyond research into frontline 'routine' diagnostics
 - Increased number of 'novel' pathogens
 - How do we assess risk of the novel?
 - Increased focus on baseline data (What is already here?)
- New technologies could revolutionise rapid detection
 - Less invasive, more rapid, non-targeted
 - Sensitivity and specificity of targeted 'in-field' diagnostics

Acknowledgements

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