



Original thinking... applied

Using collections to support pest risk assessment of novel and unusual pests discovered through HTS

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Overview

- Diagnostics as a driver of new species discovery
 - Developing HTS for frontline sample diagnosis
- Using herbaria samples to investigate pathogen origin and pathways of introduction
- What do we mean by 'historic samples'
- My interest in historic samples...
- Sharing results – the final hurdle?

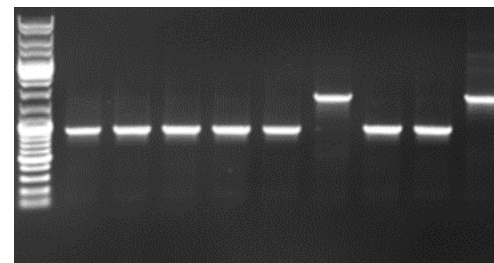
Long road of diagnostic development



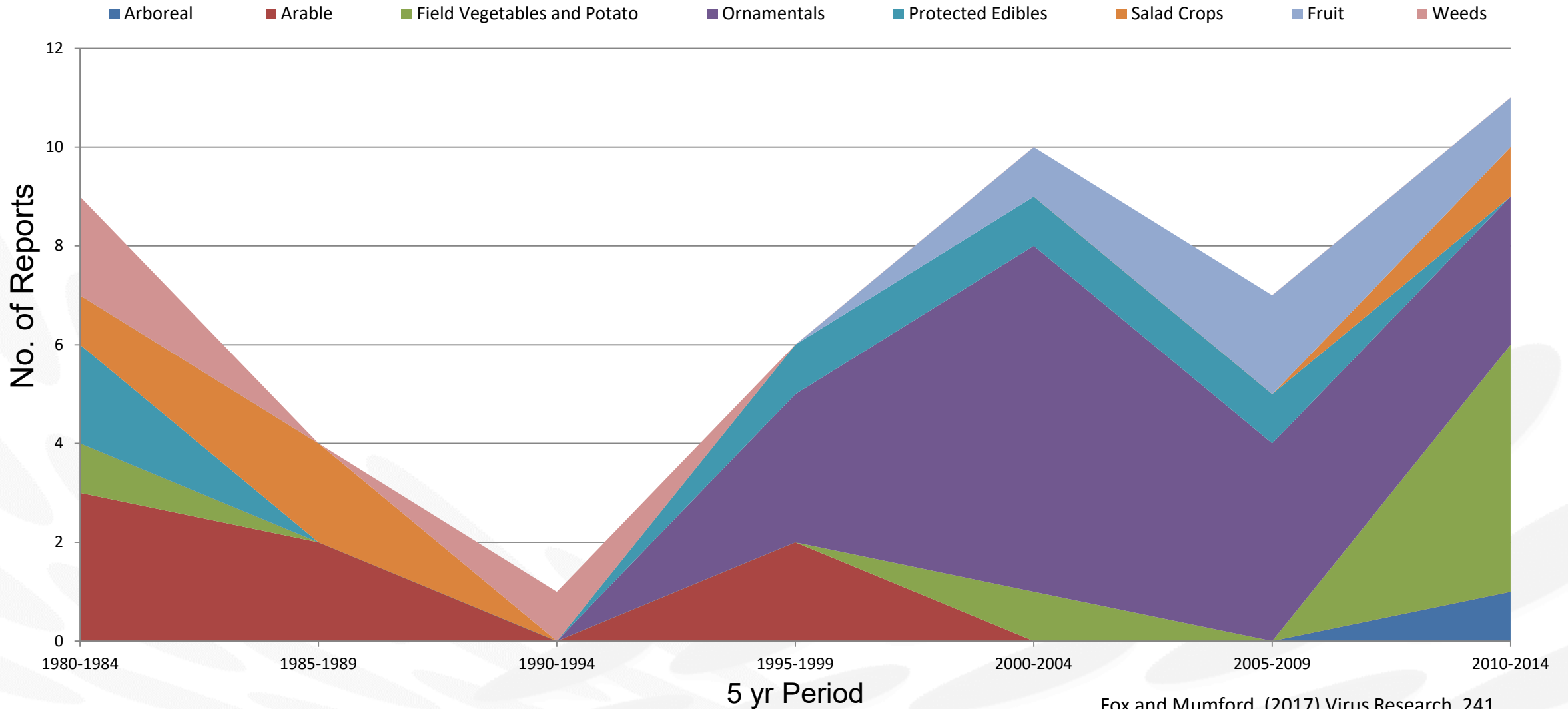
Source <http://wellcomeimages.org>



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



Factors driving virus discovery (UK 1980-2014)



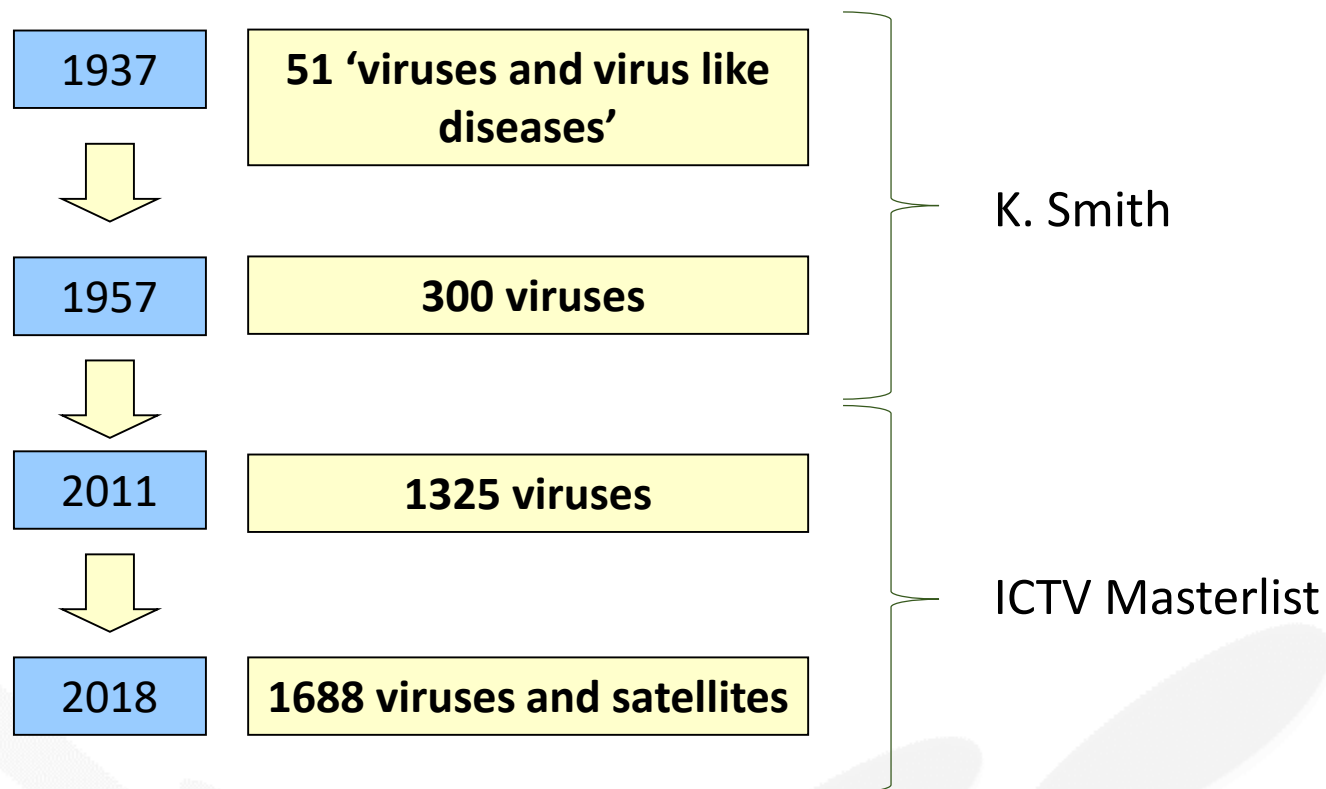
HTS in plant pathology

- 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



Known viruses - the tip of the iceberg?



Known viruses - the tip of the iceberg?

	Crop	samples	known plant viruses	Novel plant viruses	total samples
Farm 1	maize	4	Maize chlorotic mottle virus, Sugarcane mosaic virus, Maize yellow dwarf mosaic virus	Tombusvirus, Carmovirus, Foveavirus, Closterovirus, betaflexivirus, positive strand ssRNA virus	30
	others	26	bean common mosaic virus, Beet pseudoyellows virus, Maize yellow dwarf mosaic virus, SCMV, Potato virus S	Caulimoviridae virus, Chrysovirus, Crinivirus, Potyvirus(es), Tombusvirus, unclassified ssRNA positive strand virus, Varicosavirus, Filoviridae virus	
Farm 2	maize	9	12	chrysovirus, polerovirus, unclassified	29
	others	20		Shakumavirus, Maize yellow dwarf mosaic virus, Chrysovirus, Waikavirus associated virus, polerovirus, positive strand	
Farm 3	maize	6	Maize chlorotic mottle virus, Maize yellow dwarf mosaic virus	Badnavirus, polerovirus associated RNA, Tymoviridae virus	26
	others	20	Turnip mosaic virus,	Badnavirus, Chrysovirus, Cytorhabdovirus, unclassified ssRNA positive strand virus	
Farm 4	maize	4	Maize chlorotic mottle virus, Maize yellow dwarf mosaic virus	none	30
	others	26	Banana streak virus, Apple stem grooving virus, Citrus tristeza virus	Badnavirus, potyvirus(es), Tombusvirus, Rhabdoviridae virus, positive strand ssRNA virus, unclassified virus, Varicosavirus	
				total	115

Adams et al (2018), EJPP, 152(4)

Mining historic collections...

Herbaria are a major frontier for species discovery

Daniel P. Bebber^a, Mark A. Carine^b, John R. I. Wood^c, Alexandra H. Wortley^d, David J. Harris^d, Ghilleen T. Prance^e, Gerrit Davidse^f, Jay Paige^f, Terry D. Pennington^e, Norman K. B. Robson^b, and Robert W. Scotland^{c,1}

^aEarthwatch Institute, Oxford OX2 7DE, United Kingdom; ^bDepartment of Botany, Natural History Museum, London SW7 5BD, United Kingdom; ^cDepartment of Plant Sciences, University of Oxford, Oxford OX1 3RB, United Kingdom; ^dRoyal Botanic Garden Edinburgh, Edinburgh EH3 5LR, United Kingdom; ^eRoyal Botanic Gardens, Kew, Richmond, Surrey TW93AB, United Kingdom; and ^fMissouri Botanical Garden, St. Louis, MO 63166-0299

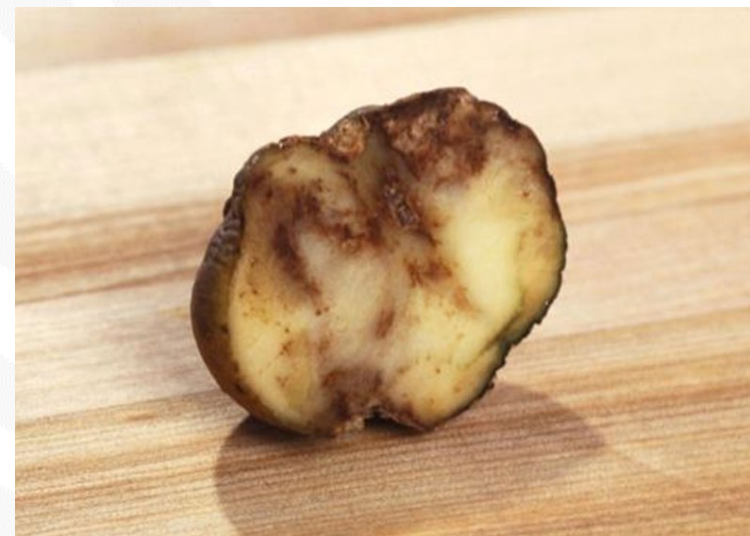
Edited by David B. Wake, University of California, Berkeley, CA, and approved November 2, 2010 (received for review August 11, 2010)

Despite the importance of species discovery, the processes including collecting, recognizing, and describing new species are poorly understood. Data are presented for flowering plants, measuring quantitatively the lag between the date a specimen of a new species was collected for the first time and when it was subsequently described and published. The data from our sample of new species published between 1970 and 2010 show that only 16% were described within five years of being collected for the first time. The description of the remaining 84% involved much older specimens, with nearly one-quarter of new species descriptions involving specimens >50 y old. Extrapolation of these results suggest that, of the estimated 70,000 species still to be described, more than half already have been collected and are stored in herbaria. Effort, funding, and research focus should, therefore, be directed as much to examining extant herbarium material as collecting new material in the field.

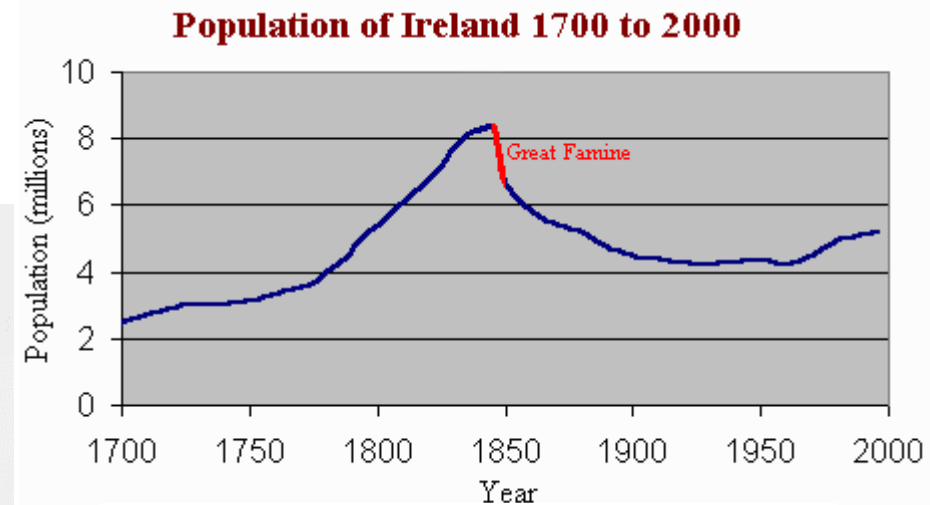
for monographs (Fig. 24). The combined data had a median *I* of 23–25 y, with only 14.4–16.9% (95% confidence interval) of species being described within 5 y of collection. This result emphasizes the relative importance of older collections for the discovery of new species of flowering plant.

The difference in the distribution of *I* between *Kew Bulletin* and monographs was statistically significant (Cox proportional hazards model; $P < 0.001$), with *Kew Bulletin* collection having a 9.6–33.8% (95% confidence interval) greater rate of description and, thus, smaller *I*, than the monographs. This difference was reflected in later *C* for the *Kew Bulletin* data (interquartile range 1938–1979 vs. 1931–1975 for monographs). Cox models showed that the description rate increased by 7.5–8.0% per year (95% confidence interval), i.e., more recently collected specimens had a greater chance of being described. Comparing modeled description rates for specimens collected in a given year

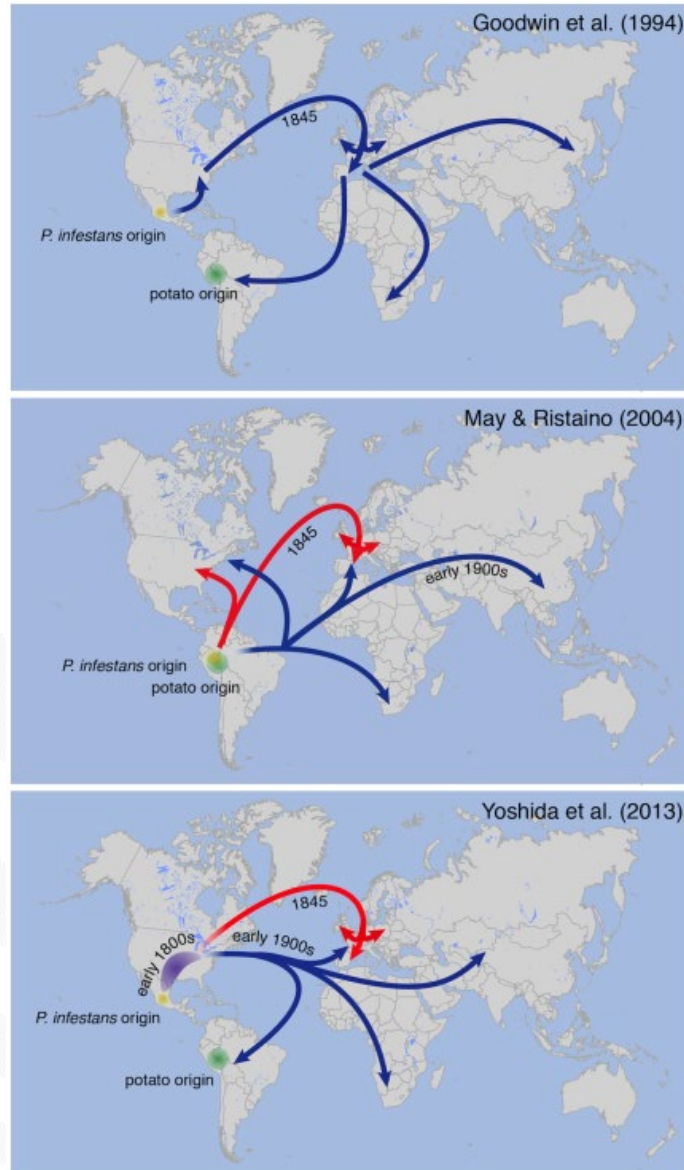
Historical perspectives – Potato Famine



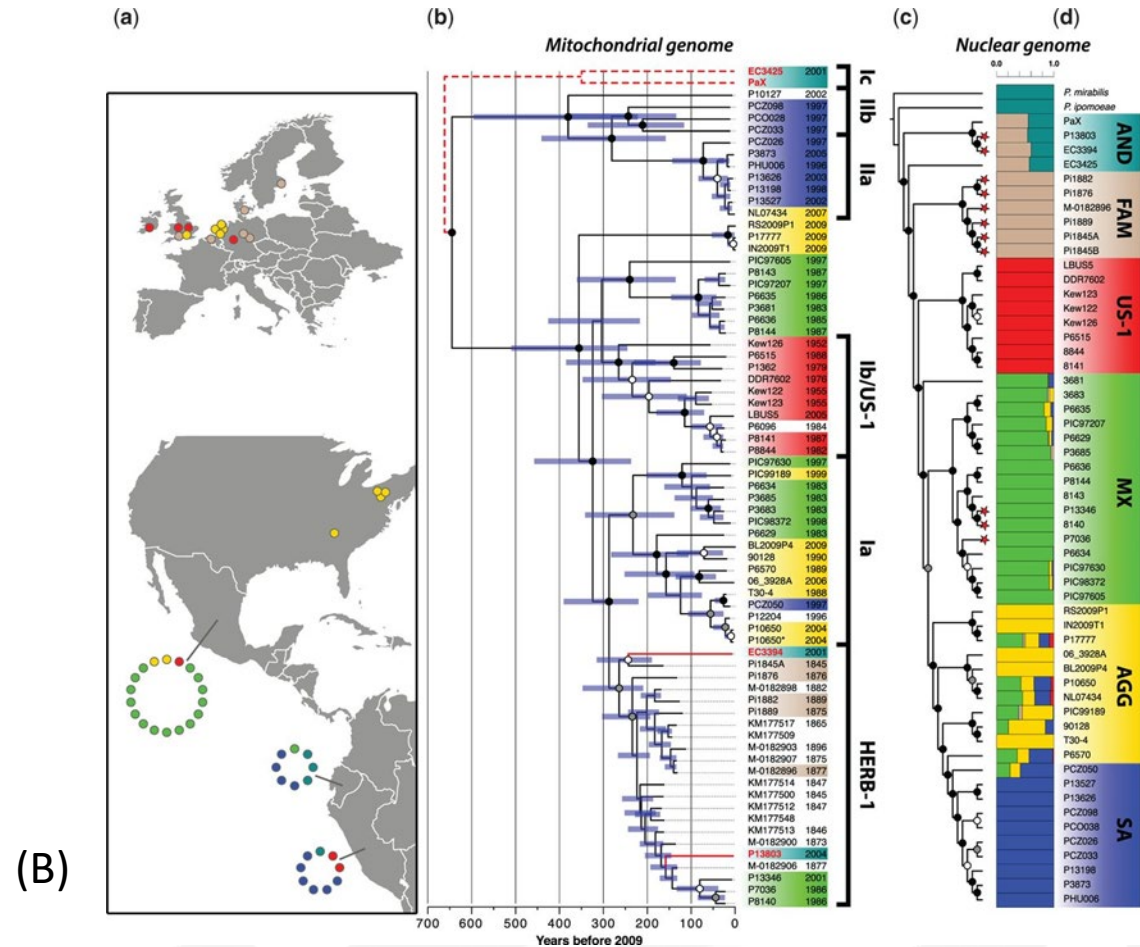
Skibbereen
by James Mahony, 1847



Investigate origins of outbreaks and pathways



(A)



(B)

(A) Yoshida et al (2014) *PLoS Path* 10(4) e1004028

(B) Martin et al (2015) *Mol Biol and Evolution* 33(2)

What do we mean by 'Historic Isolates'

Viruses have been recovered from ancient nucleic acids

Hepatitis B

- 7000 yrs old
 - Krause

But:

Archaeological record

- 'Fortunate' findings
 - Amenable storage conditions
- Single data point, no biology
 - Difficult to infer context beyond archaeology

Zea mays chrysovirus 1

- 1000 yrs old
 - Peyam

Isolate collections and herbaria:

- Well preserved
- Multiple isolates
- Supporting contextual and biological data (hopefully)

Barley stripe mosaic virus

- 750 yrs old
 - Smith et al (2014) Sci. Reports



Picture courtesy Marilyn Roossinck

Our interest started with an email and a tin...

- **From:** Roger Jones
Sent: 26 July 2014 05:55
To: Neil Boonham; Rick Mumford
Subject: RE: Virus isolates/ Visit to FERA in late September

Dear Neil and Rick

I will be visiting Europe at the end of September Would it be possible for me to visit FERA for a day early in the week after that. The main objective would be to discuss how best to proceed with the deep sequencing of the isolates I left behind in England in 1986.



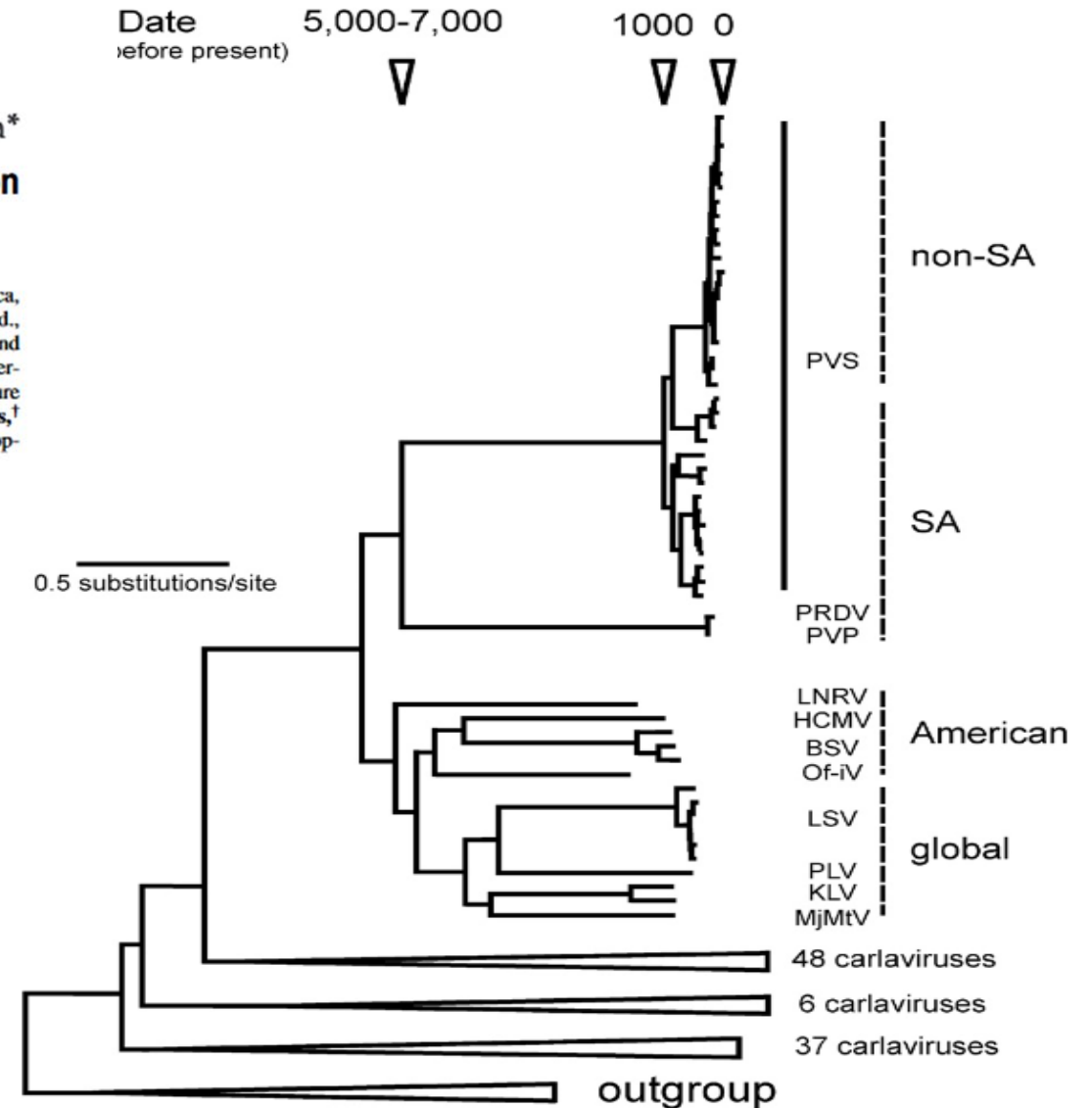
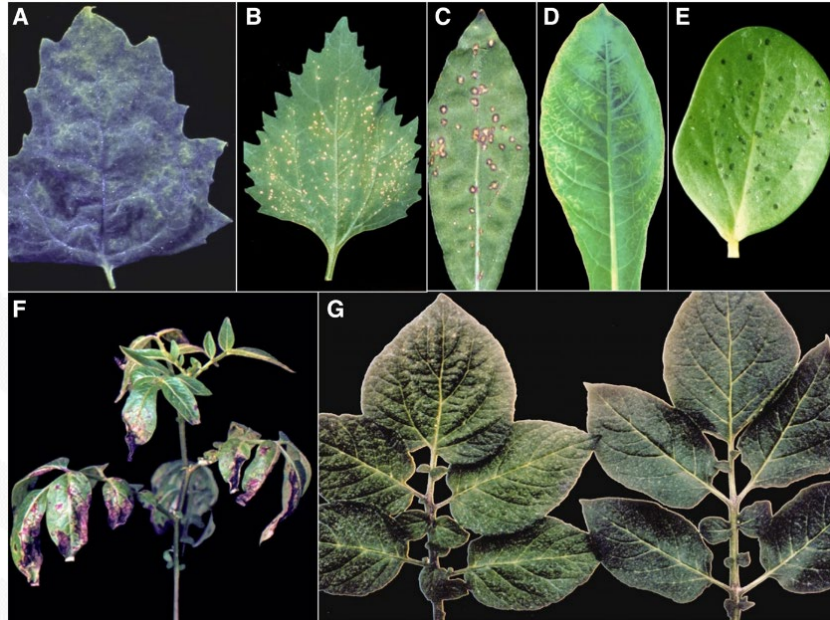
Investigating phylo-dynamics of potato viruses

Plant Disease • 2018 • 102:869-885 • <https://doi.org/10.1094/PDIS-09-17-1414-RE>

e-Xtra*

The Biology and Phylogenetics of *Potato virus S* Isolates from the Andean Region of South America

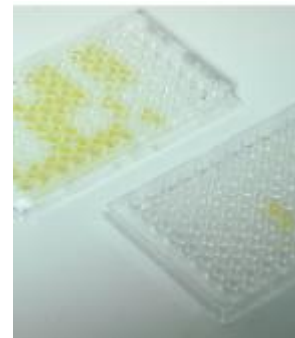
Franklin W. Santillan, Departamento de Fitopatología, Universidad Nacional Agraria, La Molina, Lima, Peru; and Universidad de Cuenca, Cuenca, Azuay, Ecuador; **Cesar E. Fribourg**, Departamento de Fitopatología, Universidad Nacional Agraria, Peru; **Ian P. Adams**, Fera Ltd., Sand Hutton, York, UK; **Adrian J. Gibbs**, Emeritus Faculty, Australian National University, ACT, Australia; **Neil Boonham**, Fera Ltd.; and Institute for Agrifood Research Innovations, Newcastle University, Newcastle upon Tyne, UK; **Monica A. Kehoe**, Diagnostic Laboratory Services, Department of Primary Industries and Regional Development, South Perth, WA, Australia; **Solomon Maina**, Department of Agriculture and Environment and Institute of Agriculture, University of Western Australia, Crawley, Perth, WA, Australia; and **Roger A. C. Jones**,[†] Institute of Agriculture, University of Western Australia; and Crop Protection Branch, Department of Primary Industries and Regional Development, South Perth, WA, Australia



Ulluco: HTS in an outbreak reveals novel viruses



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

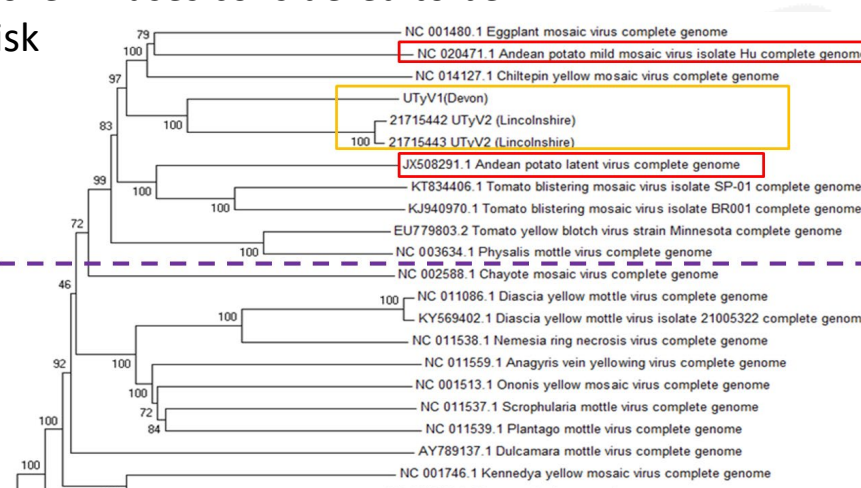


Initial screen suggests presence of quarantine viruses but this could not be confirmed



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

Two novel viruses considered to be high risk



Brussels, XXX
SANTE/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 iso

...resco...



Microbiology
Resource Announcements

GENOME SEQUENCES



Complete Genomic Sequence of the Potyvirus *Mashua Virus Y*, Obtained from a 33-Year-Old Mashua (*Tropaeolum tuberosum*) Sample

Ian P. Adams,^a Adrian Fox,^a Nell Boonham,^{a,b} Roger A. C. Jones^{c,d}

^aFera, Sand Hutton, York, United Kingdom

^bSchool of Natural and Environmental Sciences, Newcastle University, Newcastle upon Tyne, United Kingdom

^cInstitute of Agriculture, Faculty of Science, University of Western Australia, Crawley, Western Australia, Australia

^dDepartment of Primary Industries and Regional Development, South Perth, Western Australia, Australia

ABSTRACT We present the complete genomic sequence of a new potyvirus we tentatively call *Mashua virus Y* (MasVY), first isolated in 1984 from a plant of the Andean tuber crop mashua (*Tropaeolum tuberosum*, family *Tropaeolaceae*). There was a 70% nucleotide identity between MasVY and a genomic sequence of *Verbena virus Y*.

of RNA...

From a sample collected during a 1977 survey of...
3,600 m in the Comas Valley, Department of Junin in the Peruvian...
was isolated from plants of potato (*Solanum tuberosum* subsp. *andigena*) showing
bright yellow leaf markings. The virus was mechanically transmissible to 44 species
from 7 plant families. However, it proved difficult to reestablish systemic infection with
it in potato plants, so it may predominantly infect potato roots and rarely invade the



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SOCIETY FOR
MICROBIOLOGY



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Abstract
isolate
compar
nucleotide

ABSTRACT
isolate
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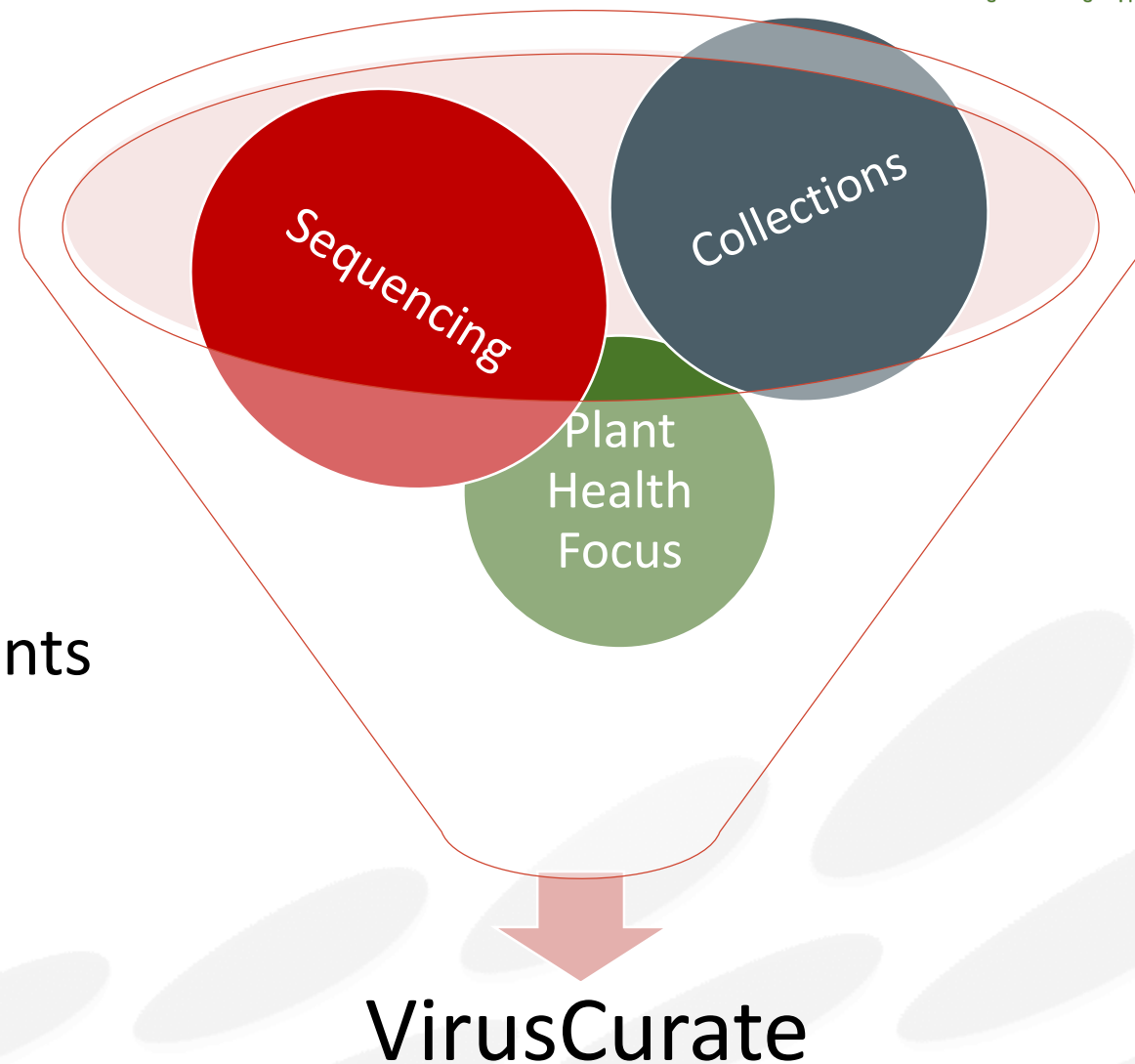
Abstract
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Downloaded from <http://r>

VirusCurate

- 12 Partners(Currently)
 - 8 Countries
- Generate sequence for unsequenced species
- Focus on regulatory relevance
- Harmonize minimum requirements for reporting
- Streamline data sharing and reporting



Informing the need for action: *Plantago* potexviruses (and others...)

- **Plantago asiatica mosaic virus (PIAMV)**
 - Detected in *Lillium* in UK (2012, 2018)
 - Virus not known to be present in UK
 - Reported worldwide in lily production
 - Losses up to 80% reported in commercial production
- **Plantain virus X (PIVX)**
 - previously reported from UK(1981)
 - Samples collected in 1970's from Cambridge and Wellesbourne. Widespread at time of reporting
 - No supporting sequence: at risk of removal from ICTV master list
- **Are these the same species?**



Informing the need for pest listing: Non-European virus of fruits

- **Actinidia virus X (AcVX)**
 - Reported from *Actinidia* sp. (Kiwifruit) in 2011
 - Generally latent/asymptomatic
 - Distribution thought to be limited to New Zealand

- Intercepted on entry to Canada in (2016)
- *Ribes nigrum* - Origin Unknown
- Plants showing chlorosis and leaf deformity

- **Non-European virus of Ribes?**



Barriers to progress: sharing and reporting

- Fera
 - Single sample testing....(30 MiSeq runs)
 - **190** near-complete viral genomes
- Virome studies?
 - "Area de Conservación Guanacaste" Project
 - "**2600** distinct viruses"
 - Tall Grass Prairie Reserve Project
 - "**300** distinct viruses, 18 known viral species"
- Suitable outlet?
 - Time?
 - Cost?

Reporting options – NCBI Genbank?

I keep six honest serving-men,
(They taught me all I knew);
Their names are **What** and **Why** and **When**
and **How** and **Where** and **Who**



Rudyard Kipling

A reporting solution?

- Disease note style reporting
 - C. 300 Words
 - Contextual data
 - Includes Genbank accession
 - Provide DOI for reference
 - Edited by community of experts
 - Low input, quick turnaround
 - Open access; Online; (Free)



Summary

- HTS offers unparalleled potential for detection and diagnosis of plant pathogens
- Historic isolates may hold unique data on novel or unusual species
- Data associated with historic isolates can support plant health risk assessment and actions
 - Pathways analysis
 - Distribution data
 - Biological data (Host range, Impact, Transmission)
- Disseminating new findings remains a barrier

Acknowledgements

Fera, UK

- Ian Adams
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University of Warwick, UK

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University of Western Australia

- Roger Jones

USDA-ARS

- John Hammond

NPPO, Netherlands

- Marleen Botermans

Wageningen University, Netherlands

- Martin Verbeek



Department
for Environment
Food & Rural Affairs



Euphresco

