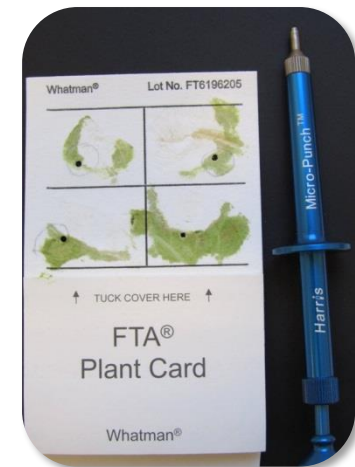




EuroBlight tracking of evolving European populations of *Phytophthora infestans* as an aid to late blight management

David Cooke

Jens G Hansen, Poul Lassen, Alison Lees,
Geert Kessel+ *many partners*



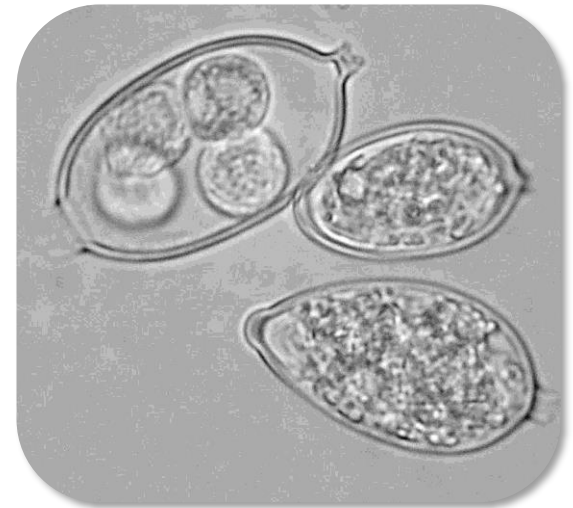
3rd AsiaBlight International Meeting, Beijing 25-27 Oct 2019



Objectives

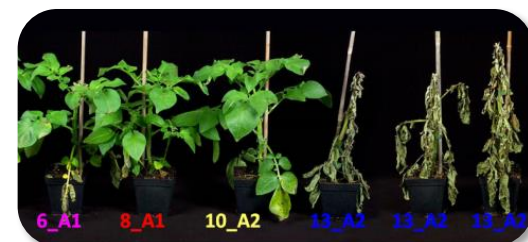
- How EuroBlight database benefits growers/industry?
- How it works - mechanisms?
- Share our experiences
- Continue to support AsiaBlight
- Learn from AsiaBlight network experiences

- Key to network success
 - **TRUST**
 - **CO-OPERATION**
 - **COMMITMENT**
 - **STANDARDISATION**
 - **MUTUAL GAIN**



Need to study population change

- Environmental response (RH and °C) – DSS
- Fungicide insensitivity – rapid evolution
- Fitness, Aggressiveness, Virulence
- Survival
- Rate of evolution



My links with research in Asia

- **Ying Li** – 12-plex SSR development
- **Sanjoy Guha Roy** – broad collaboration
- **Buddhi Sharma** - Nepal SSR analysis
- **Mei Guo** – China SSR analysis
- **Kwangsoo Cho** – South Korea SSR analysis
- **Louise Cooke** and many collaborators FTA analysis
- **Dolf DeBoer & Birte Komolong** - PNG & Australasia
- **Mohammad Rashidul Islam** – Bangladesh SSR analysis
- **Huub Schepers/Geert Kessel**- WUR projects

European sponsors/contributors/collaborators

Commercial



Academic/state research

ACVNPT *
BASF SE
Belchim Crop Prot.
Certis
Cheminova
CropSolutions
CUConsulting
Emsland Group
Germicopa SAS*
HZPC Holland B.V.
Neiker
Nordisk Alkali
PCA
Profytodsd
Staphyt
Syngenta/ ChinaChem
SynTech Research
UPL

ADAMA
Agrifirm
Agriphar
Bayer CropScience AG
Corteva



+Many more global teams

AFBI
Agricultural Institute of Slovenia
AHDB Potatoes and FAB Scouts
Aarhus University*
ARVALIS-Institut du Végétal*
Bar-Ilan University
Bayerische Landesanstalt für Landwirtschaft
Centre Wallon de Recherches Agronomiques
Estonian University of Life Sciences*
INRA*
Inst. Plant Protection & Environment, Serbia
The James Hutton Institute*
NIBIO, Norway*
Wageningen University
Plant Breeding & Acclimatization Inst. (IHAR)
Swedish University of Agricultural Sciences
TEAGASC

* Partners in the IPMBlight2.0 project



EucaBlight themes

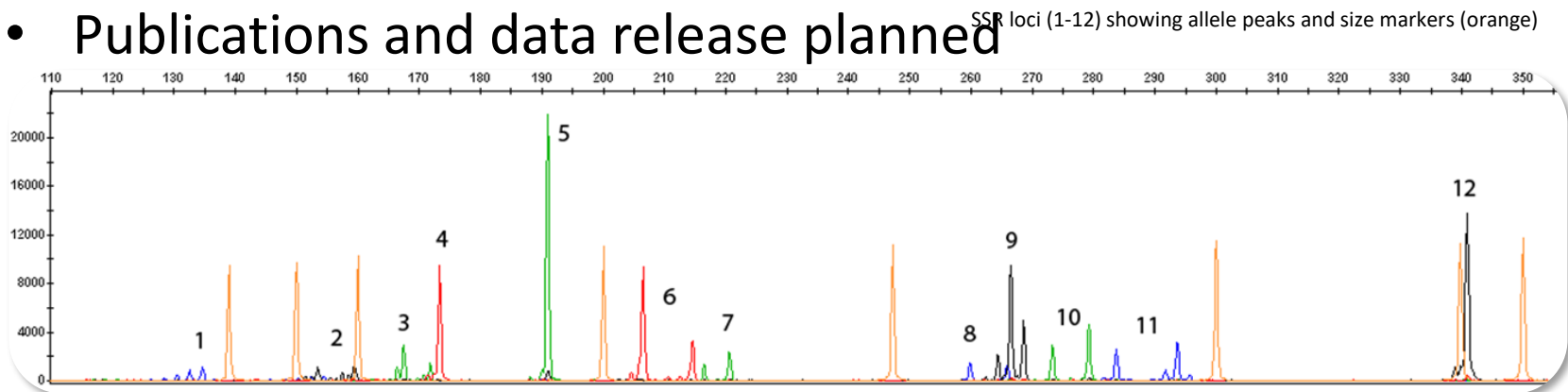
- **Tracking the potato blight pathogens** in a global context - Tools, network infrastructures and activities
- **Stewardship of host resistance genes and active ingredients** - develop and adopt innovative and sustainable control strategies on regional scales
- **Education, advocacy and communication** - taking into account different scales and stakeholders



Monitoring methods



- Scouts monitoring crops provide samples
- Outbreak data recorded
- Active lesions sampled
- DNA fingerprinted using 12-plex SSRs (Li *et al.* 2013)
 - Hutton, INRA, IHAR, WUR – strong co-operation
- Genotypes defined – QC and to EuroBlight database
- Data mapped on www.euroblight.net + annual summary
- Publications and data release planned



Sampling



- Scouts issued with sample forms and FTA cards
 - Costs €25 per sample (covers costs - not profit-making)
 - Cards pre-labelled with organisation and year. e.g. Syngenta19001
 - Some groups collect isolates – needed for phenotyping
- Outbreak data (e.g. location, crop type, cultivar) recorded
 - Sampling not prescriptive – aim for broad geographical spread
 - AHDB GB – 4-8 lesions per crop across outbreak
 - Companies sample efficacy trials at lower frequency
 - Avoid excess sampling in one location – creates bias



Use of FTA cards



- Fresh, sporulating lesion onto FTA card
- Air dry at room temp. store & post
- 12 year old cards OK
- Handle carefully - stop cross-contamination

Protocol will be made available via web page

Database

- Custom-built & managed by Jens Hansen and Poul Lassen (Aarhus University, Denmark)
- Synergy with their Wheat Rust database
- David Cooke – population data manager
 - Co-ordinate FTA card provision
 - Collate cards at end of each season
 - Run SSR analysis (& receive other group's data)
 - Quality control data 2 stages
 - Step 1 Sample data
 - Step 2 SSR data
- Raw data never shared without owner permission



AARHUS
UNIVERSITY

PLB Toolbox

16K samples with genotype data in EU database

POTATO LATE BLIGHT TOOLBOX

Home Samples Trials Partners

Welcome David Cooke [logout](#)

SAMPLE OVERVIEW

Data Samples Genotypes by SSR Mating type Virulence Without results

Continent Europe Africa & West/Central Asia South/East Asia South America Oceania

Country	Total	2019	2018	2017	2016	2015	2014	2013	2012	2011	2010	2009	2008	2007	2006	2005	2004	2003	2002	2001	2000	1999	1998	1997	1996	Older
Austria	106		2					4											100							
Belarus	45		1		5		29	10																		
Belgium	1571		98	74	169	97	187	75	193	58	72	35	24	54	26	13	16	15	32	105	97	82	49			
Bulgaria	17				2	3	6	6																		
Croatia	5		2				3																			
Cyprus	1							1																		
Czech Republic	380		3	6	7		7	5							80	177	64	31								
Denmark	1136	228				64	37	126	114	87				23	1			65								
England	5967		115	397	363	97	403	188	451	61	101	306	590	901	801	56	98	47					336	630		26
Estonia	1026			24	107	1	68	54	49	51	41				103	103	144	84	89	83	13	12				
Finland	2981			6	7	5	4	4							87			234		210	675	457	538	602	16	136
France	4762		236	260	230	116	222	185				12	125	331	382	303	444	72	136	213	160	332	233	123	156	491
Germany	439		3	94	86	87	62	40				3		53				3	1		7					
Greece	44		3	3	6	14	4			14																
Hungary	309		12	3	4										56	67			93	27	3		36	5	1	2
Ireland	150		1				1								1											147
Italy	43		5	4	2		2	13				6													10	1
Jersey	1								1																	
Latvia	183			1	5			52	44	48	33															
Lithuania	172			5	5		40	53	25	27	17															
Netherlands	2579		125	249	195	79	149	84				12		15		1	25	109			481				353	702

Data upload steps

POTATO LATE BLIGHT TOOLBOX

Home Samples Trials Partners

Welcome David Cooke [logout](#)

GENOTYPES

Unpublished Published for project partners Published for data suppliers Published for all Genotype map Import Loci, alleles and types

Date format dd.mm.yyyy File format Comma

New samples Change previous created samples New genotype results Change previous created genotype results

CREATE SAMPLE INPUT TEMPLATE FILE

Select data owner Alison Lees Select country Argentina Select year 2019 Select number of samples 10

Create sample file

UPLOAD AND IMPORT SAMPLE INPUT FILE

CSV sample file (file size limit is 1 MB) Select

Upload

Enter new Sample data (makes db key)

Change Sample data

Link in new SSR data

Change SSR data

Sample data entry (csv)

	Owner	Sample ID	Date	Collector	Host	Cultivar	ISO Country code	Location	Latitude	Longitude	Outbreak type
--	-------	-----------	------	-----------	------	----------	------------------	----------	----------	-----------	---------------

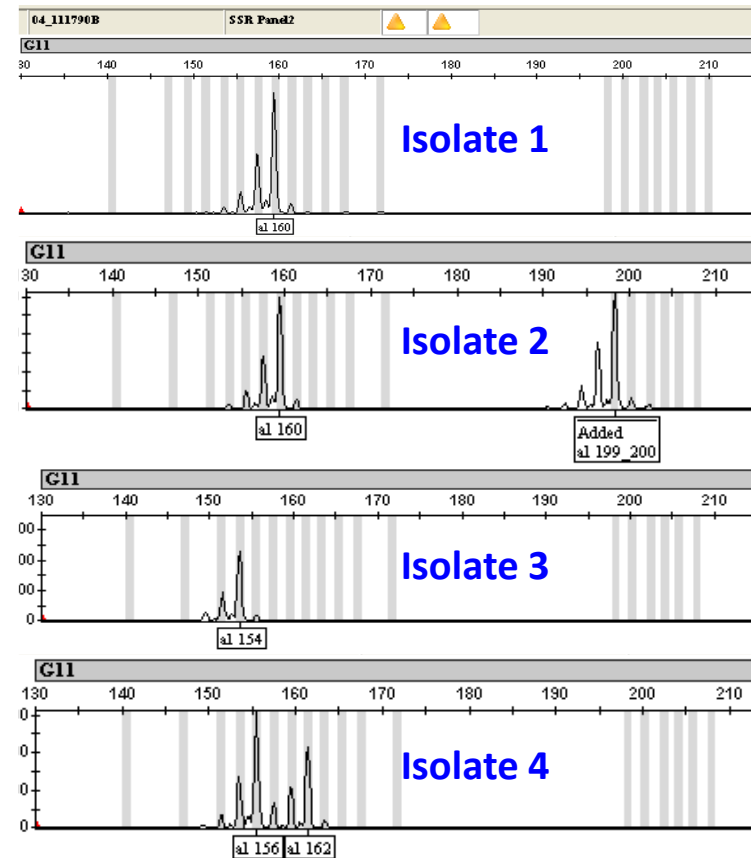
	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R
1	No.	Owner	Sample code	Sample	Collecti	Collecti	Collecti	Collector	Institution	Host	Cultivar	Country	Region	Location	Latitude	Longitude	Site	Comment
2	integer	string[4]	string[100]	acronym	integer	integer	integer	string[100]	string[100]	acronym	string[100]	string[2]	string[50]	string[20]	float	float	acronym	text
3	1	DCO	A01700018B	L	2018	4	13	Graeme SI	The James I	potato	Desiree	EN	Kent	CT7	51.3718	1.30256	Dump	
4	2	DCO	A01700018C	L	2018	4	13	Graeme SI	The James I	potato	Desiree	EN	Kent	CT7	51.3718	1.30256	Dump	
5	3	DCO	A01700018D	L	2018	4	13	Graeme SI	The James I	potato	Desiree	EN	Kent	CT7	51.3718	1.30256	Dump	
6	4	DCO	A01700026A	L	2018	4	17	Graeme SI	The James I	potato	Desiree	EN	Kent	CT6	51.3643	1.12831	Dump	
7	5	DCO	A01700026B	L	2018	4	17	Graeme SI	The James I	potato	Desiree	EN	Kent	CT6	51.3643	1.12831	Dump	
8	6	DCO	A01700034A	L	2018	4	17	Graeme SI	The James I	potato	King Edwa	EN	Kent	CT7	51.3718	1.30256	Dump	
9	7	DCO	A01700034B	L	2018	4	17	Graeme SI	The James I	potato	King Edwa	EN	Kent	CT7	51.3718	1.30256	Dump	
10	8	DCO	A01700034C	L	2018	4	17	Graeme SI	The James I	potato	King Edwa	EN	Kent	CT7	51.3718	1.30256	Dump	
11	9	DCO	A01700034D	L	2018	4	17	Graeme SI	The James I	potato	King Edwa	EN	Kent	CT7	51.3718	1.30256	Dump	
12	10	DCO	W00002495A	L	2018	5	30	Stephen M	The James I	potato	Lady Rose	WA	Pembrok	SA73	51.7172	-5.01581	Conventional	
13	11	DCO	W00002495B	L	2018	5	30	Stephen M	The James I	potato	Lady Rose	WA	Pembrok	SA73	51.7172	-5.01581	Conventional	
14	12	DCO	W00002495C	L	2018	5	30	Stephen M	The James I	potato	Lady Rose	WA	Pembrok	SA73	51.7172	-5.01581	Conventional	
15	13	DCO	W00002495D	L	2018	5	30	Stephen M	The James I	potato	Lady Rose	WA	Pembrok	SA73	51.7172	-5.01581	Conventional	
16	14	DCO	W00002503A	L	2018	6	10	Douglas S	The James I	potato		EN	Cornwall	TR12	50.0448	-5.18429	Garden	
17	15	DCO	W00002503B	L	2018	6	10	Douglas S	The James I	potato		EN	Cornwall	TR12	50.0448	-5.18429	Garden	
18	16	DCO	W00002503C	L	2018	6	10	Douglas S	The James I	potato		EN	Cornwall	TR12	50.0448	-5.18429	Garden	
19	17	DCO	W00002503D	L	2018	6	10	Douglas S	The James I	potato		EN	Cornwall	TR12	50.0448	-5.18429	Garden	
20	18	DCO	W00002552B	L	2018	6	4	David Har	The James I	potato	Charlotte	EN	Cornwall	TR19	50.1019	-5.62632	Conventional	
21	19	DCO	W00002552C	L	2018	6	4	David Har	The James I	potato	Charlotte	EN	Cornwall	TR19	50.1019	-5.62632	Conventional	

Genotypic marker – Simple Sequence Repeats



ABI 3730 capillary sequencer

Marker G11



Journal of Microbiological Methods 92 (2013) 316–322

Contents lists available at SciVerse ScienceDirect



Journal of Microbiological Methods

journal homepage: www.elsevier.com/locate/jmicmeth



Efficient multiplex simple sequence repeat genotyping of the oomycete plant pathogen *Phytophthora infestans*

Ying Li ^{a,c,d}, David E.L. Cooke ^{b,*}, Evert Jacobsen ^c, Theo van der Lee ^{d,*}

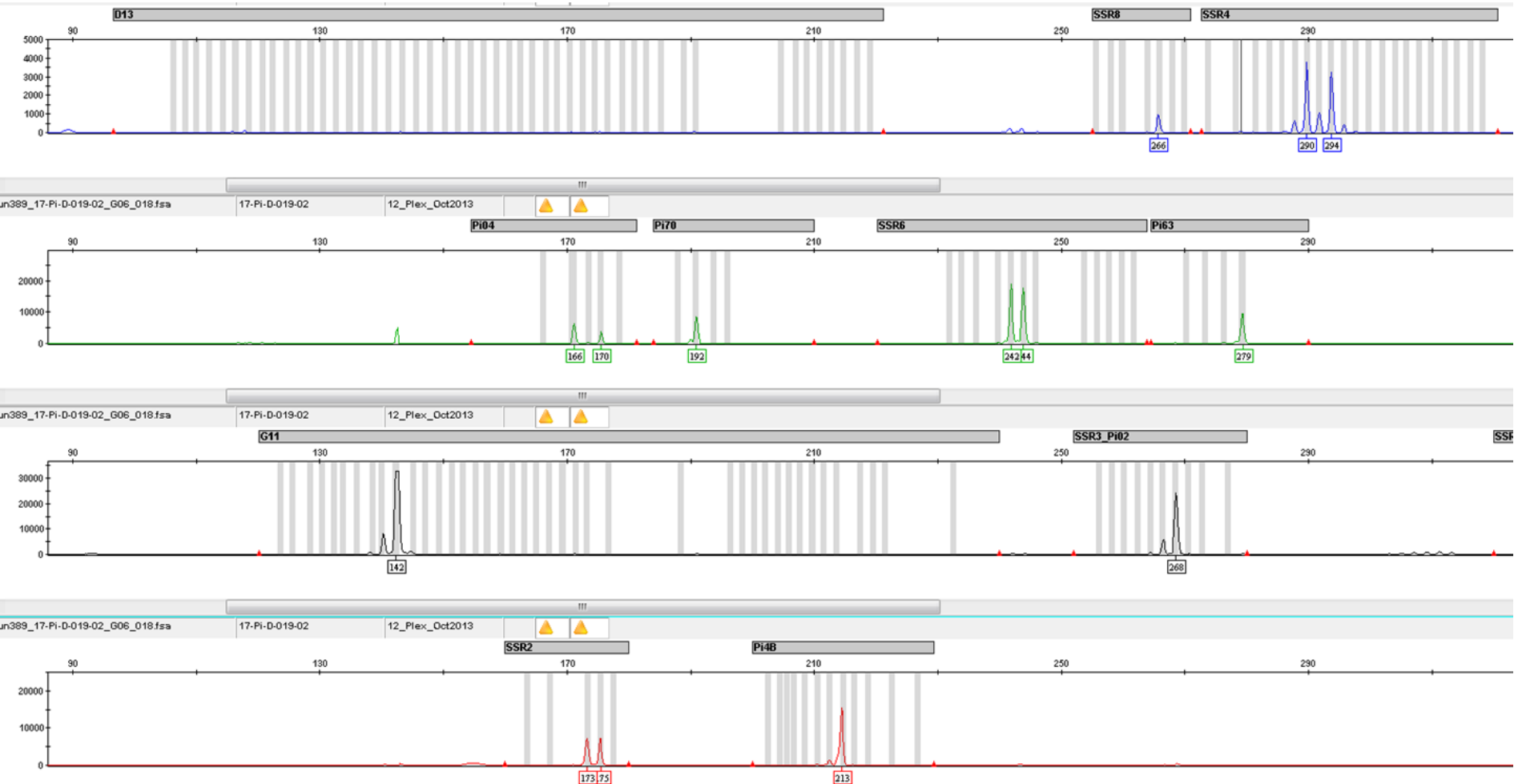
^a Institute of Vegetables and Flowers, Chinese Academy of Agricultural Sciences, Beijing, 100081, China

^b The James Hutton Institute, Invergowrie, Dundee, DD2 5DA, UK

^c Plant Breeding, Wageningen UR, P.O. Box 386, 6708 PB Wageningen, The Netherlands

^d Bio-Interaction and Plant Health, Plant Research International B.V., Wageningen UR, P.O. Box 16, 6700 AA Wageningen, The Netherlands

SSR amplification

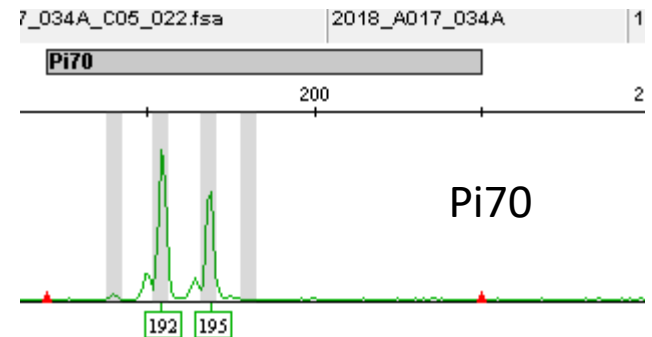
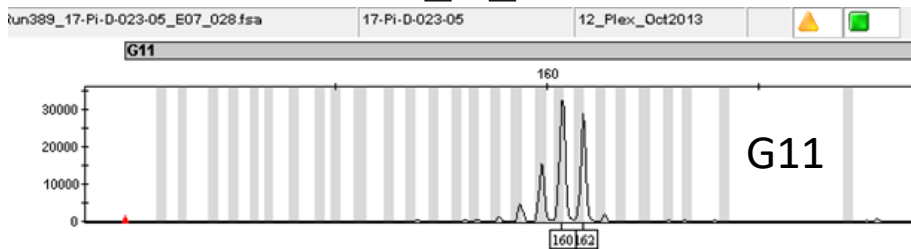
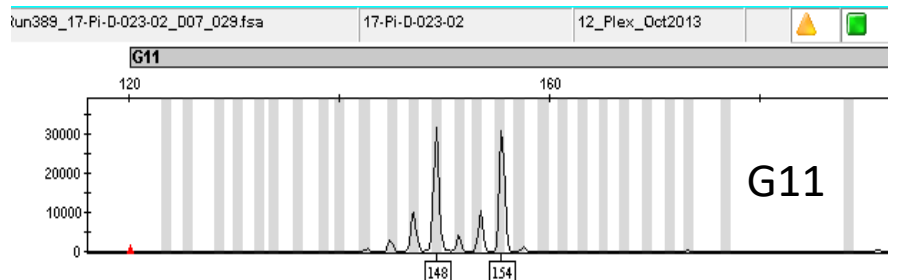


- 12 SSR loci amplified in single PCR



SSR scoring

- Key labs trained – Hutton, WUR, INRA, IHAR
- 12 SSR loci (2 tri-& 10 dinucleotide rpts)
- Allele numbers per loci 4-40
- Allele name standardisation is critical – bins help this – sharing of bin profiles
- Allele-specific pattern of stutter peaks



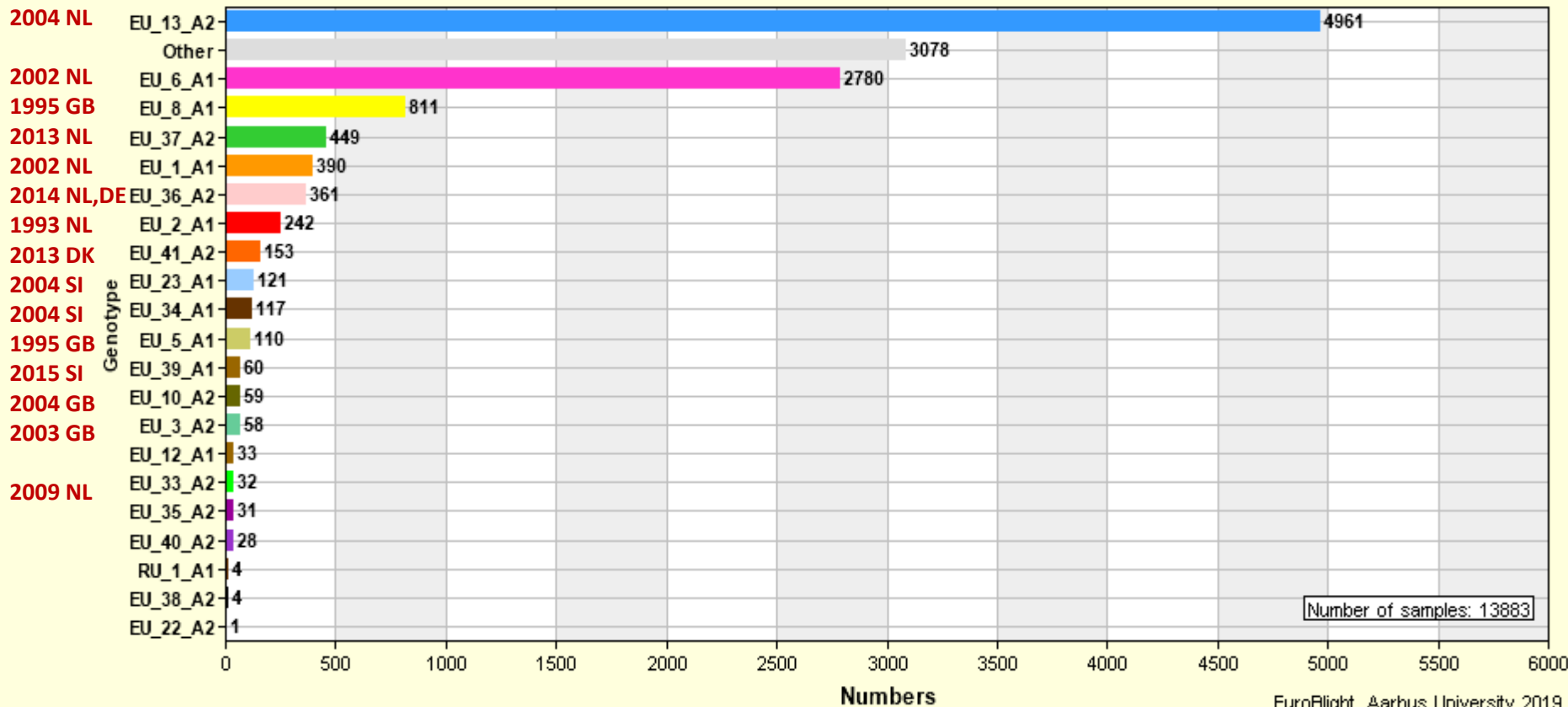
SSR data entry

- Excel sheet generated by database
- Data added in standard format

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R
1	Collection	SampleID	ResultID	ResultGen	SSRLocusS	SampleCode	Owner	Year	Cultivar	Country	Location	Collection	Laborat	SampleFile	Genotype	Locus Pi02	Locus Pi02	Locus Pi02
2	NO EDIT	NO EDIT	NO EDIT	NO EDIT	NO EDIT	NO EDIT	NO EDIT	NO EDIT	NO EDIT	NO EDIT	NO EDIT	NO EDIT	acronym	string[200	acronym	Allele 1	Allele 2	Allele 3
3	47197	1	1	1	2 A01700018B	DCO	2018	Desiree	EN	CT7	13.4.2018	JHI	Run380_2	EU_37_A2	268			
4	47197	2	1	1	2 A01700018C	DCO	2018	Desiree	EN	CT7	13.4.2018	JHI	Run380_2	EU_6_A1	258	266	268	
5	47197	3	1	1	2 A01700018D	DCO	2018	Desiree	EN	CT7	13.4.2018	JHI	Run380_2	EU_6_A1	258	266	268	
6	47198	1	1	1	2 A01700026A	DCO	2018	Desiree	EN	CT6	17.4.2018	JHI	Run380_2	EU_36_A2	268			
7	47198	2	1	1	2 A01700026B	DCO	2018	Desiree	EN	CT6	17.4.2018	JHI	Run380_2	EU_36_A2	268			
8	47199	1	1	1	2 A01700034A	DCO	2018	King Edwa	EN	CT7	17.4.2018	JHI	Run380_2	EU_36_A2	268			
9	47199	2	1	1	2 A01700034B	DCO	2018	King Edwa	EN	CT7	17.4.2018	JHI	Run380_2	EU_36_A2	268			
10	47199	3	1	1	2 A01700034C	DCO	2018	King Edwa	EN	CT7	17.4.2018	JHI	Run380_2	EU_36_A2	268			
11	47199	4	1	1	2 A01700034D	DCO	2018	King Edwa	EN	CT7	17.4.2018	JHI	Run380_2	EU_36_A2	268			
12	47212	1	1	1	2 A01700067B	DCO	2018	Desiree	EN	CT7	29.6.2018	JHI	Run384_A	EU_36_A2	268			
13	47212	2	1	1	2 A01700067C	DCO	2018	Desiree	EN	CT7	29.6.2018	JHI	Run384_A	EU_36_A2	268			
14	47212	3	1	1	2 A01700067D	DCO	2018	Desiree	EN	CT7	29.6.2018	JHI	Run384_A	EU_36_A2	268			
15	47200	1	1	1	2 W00002495A	DCO	2018	Lady Rose	WA	SA73	30.5.2018	JHI	Run383_2	EU_6_A1	258	266	268	
16	47200	2	1	1	2 W00002495B	DCO	2018	Lady Rose	WA	SA73	30.5.2018	JHI	Run383_2	EU_6_A1	258	266	268	

Introducing the clones 2004-2018

Numbers of genotypes sampled
 from Belarus, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, England, Estonia, Finland, France, Germany
 Greece, Hungary, Ireland, Italy, Latvia, Lithuania, Netherlands, Northern Ireland, Norway, Poland, Portugal, Romania
 Russian Federation, Scotland, Serbia, Slovakia, Slovenia, Spain, Sweden, Switzerland, Ukraine, Wales
 in 2018, 2017, 2016, 2015, 2014, 2013, 2012, 2011, 2010, 2009, 2008, 2007, 2006, 2005, 2004

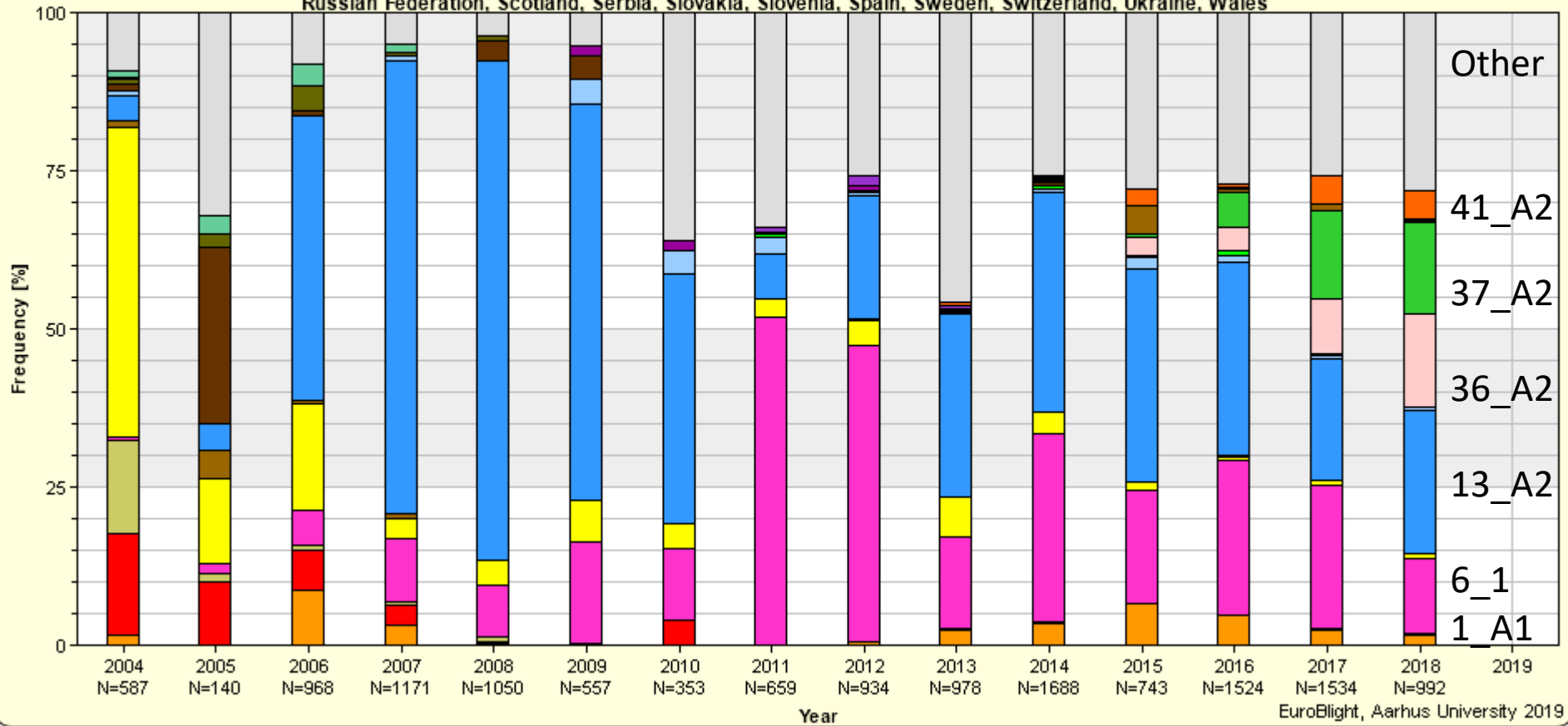


EuroBlight, Aarhus University 2019

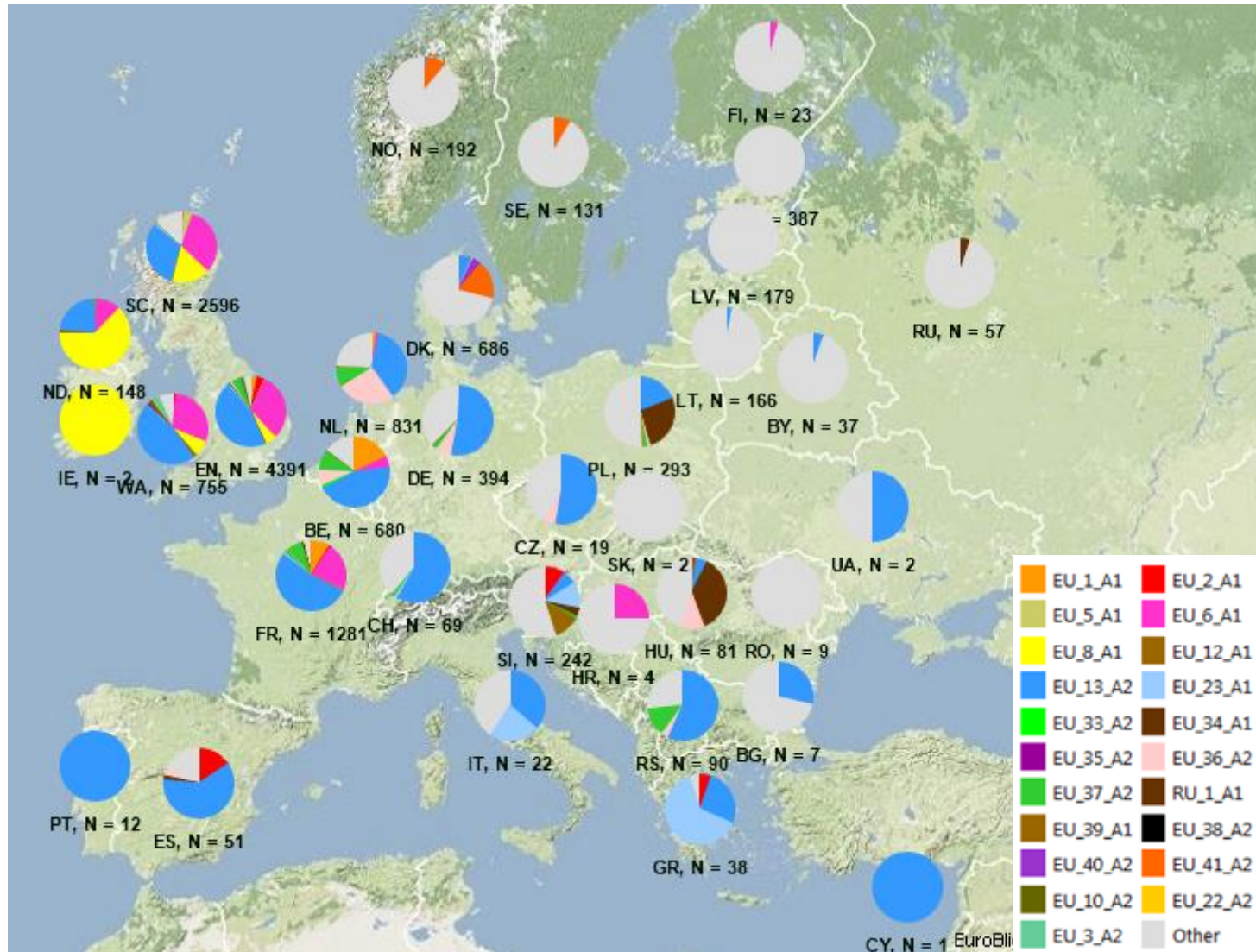


EU *P. infestans* genotype change

Frequency of genotypes
 from Belarus, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, England, Estonia, Finland, France, Germany
 Greece, Hungary, Ireland, Italy, Latvia, Lithuania, Netherlands, Northern Ireland, Norway, Poland, Portugal, Romania
 Russian Federation, Scotland, Serbia, Slovakia, Slovenia, Spain, Sweden, Switzerland, Ukraine, Wales

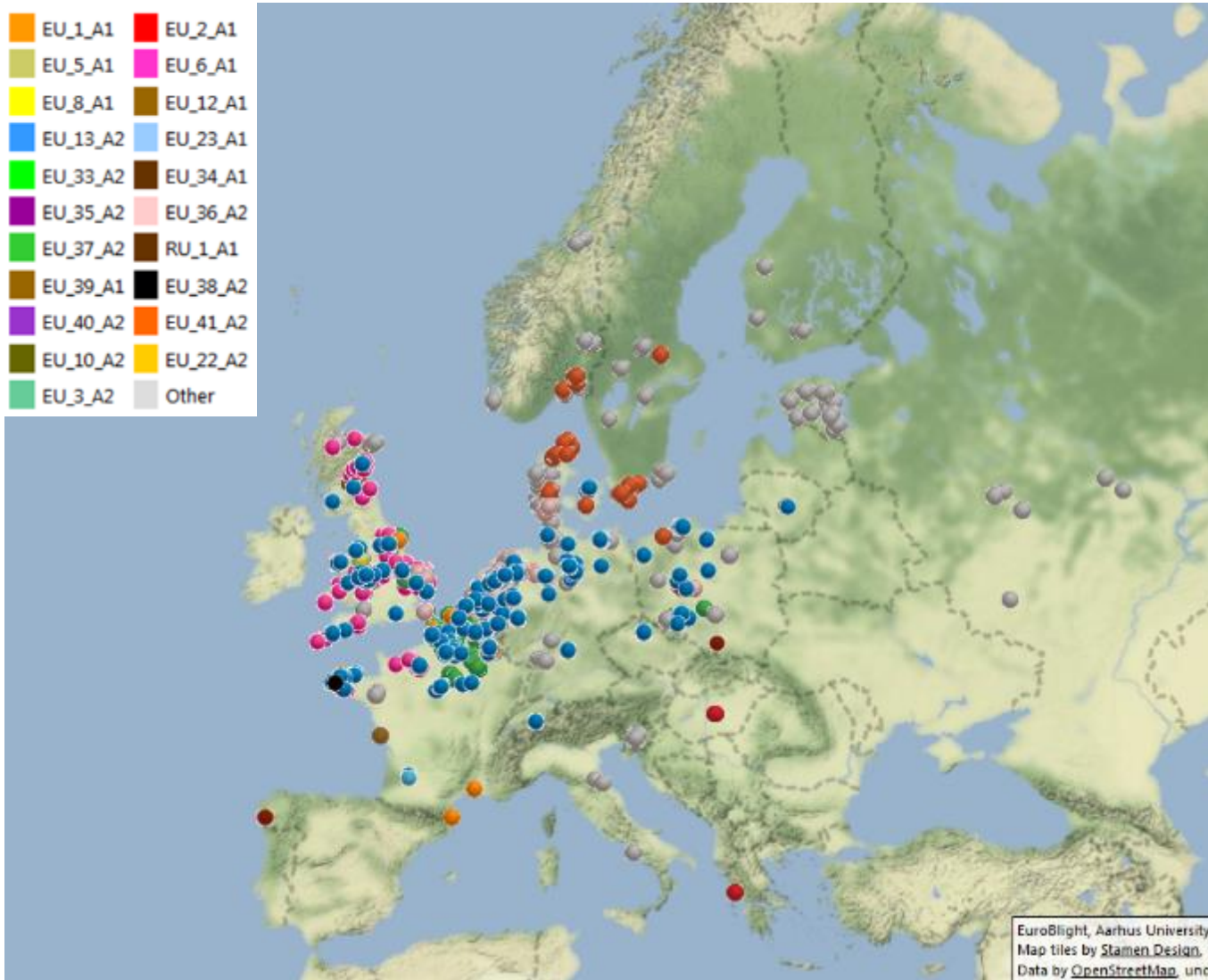


2013-2018 summary



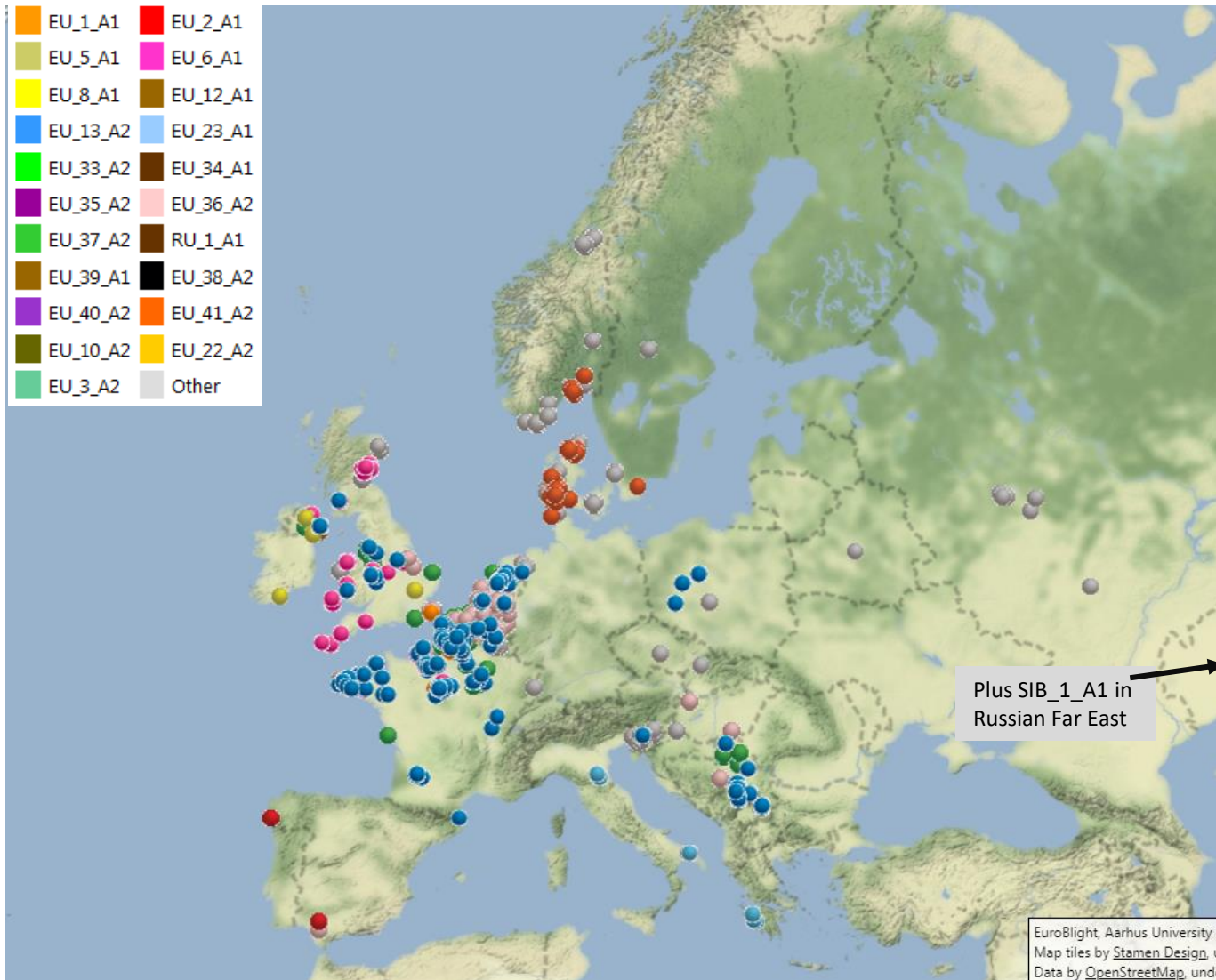
- >8000 samples from 34 countries

2017 samples



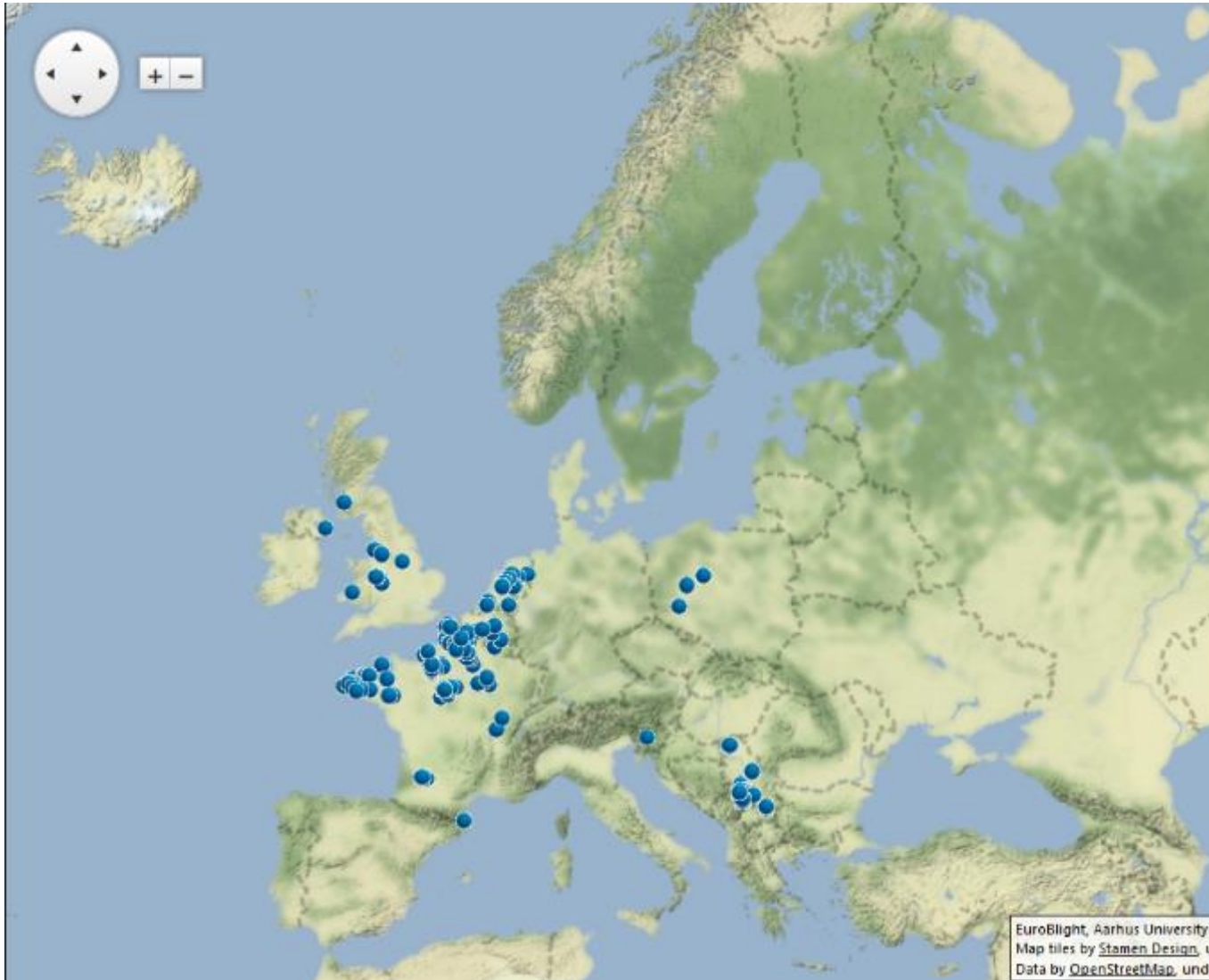
- 1534 samples from 22 countries

2018 samples

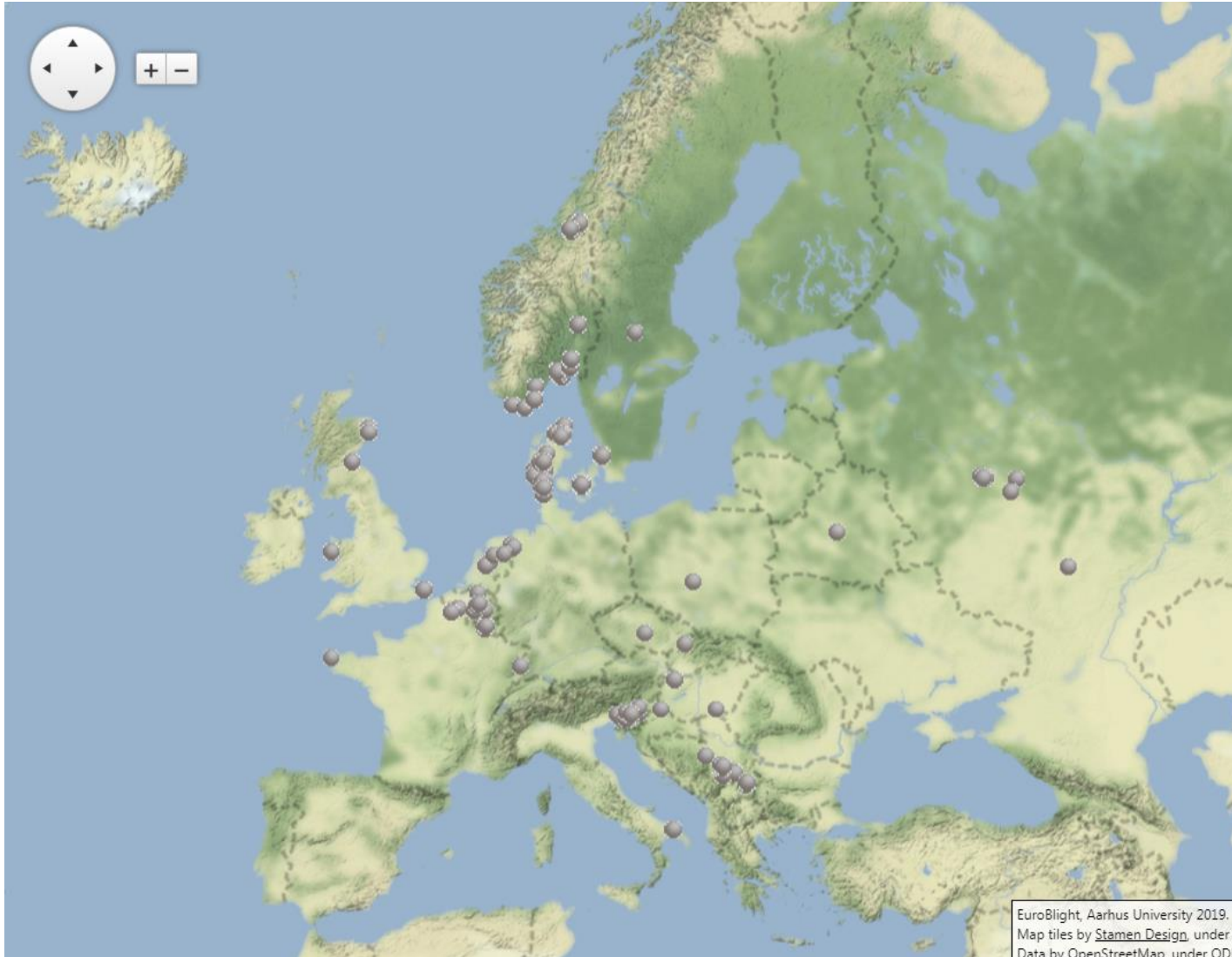


- 979 samples from 23 countries

2018 samples – EU_13_A2



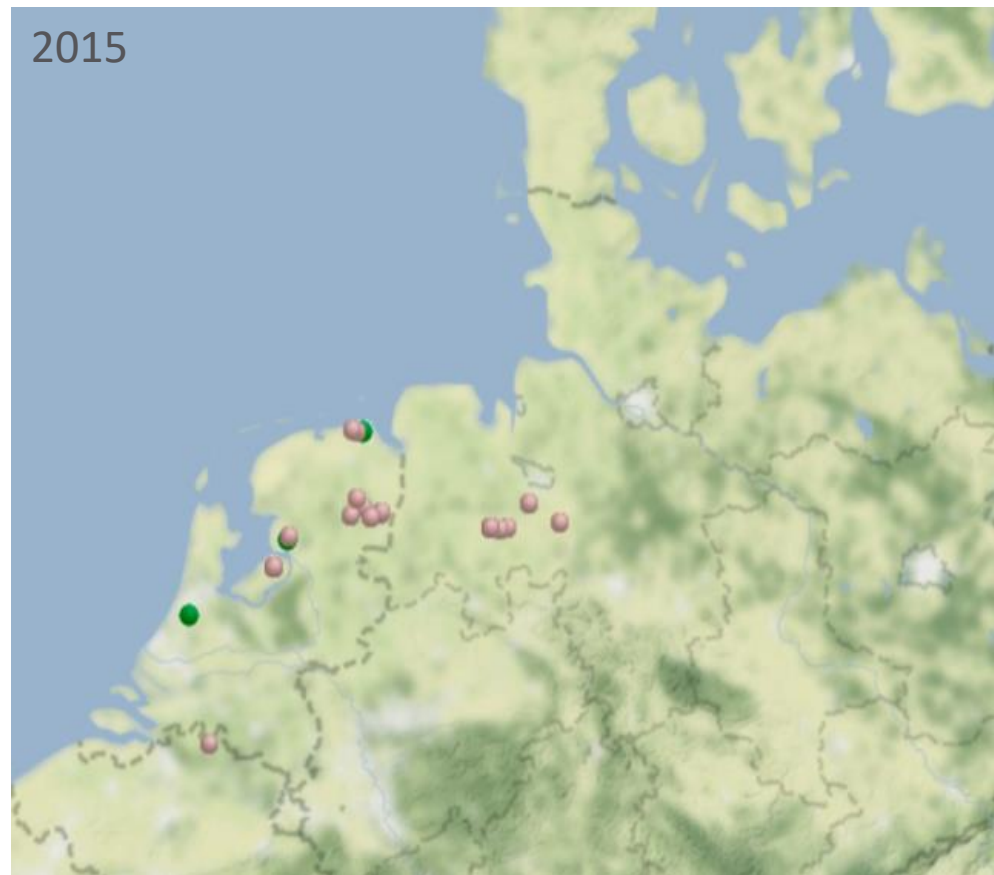
2018 samples – ‘Other’



Timeline of two new clones



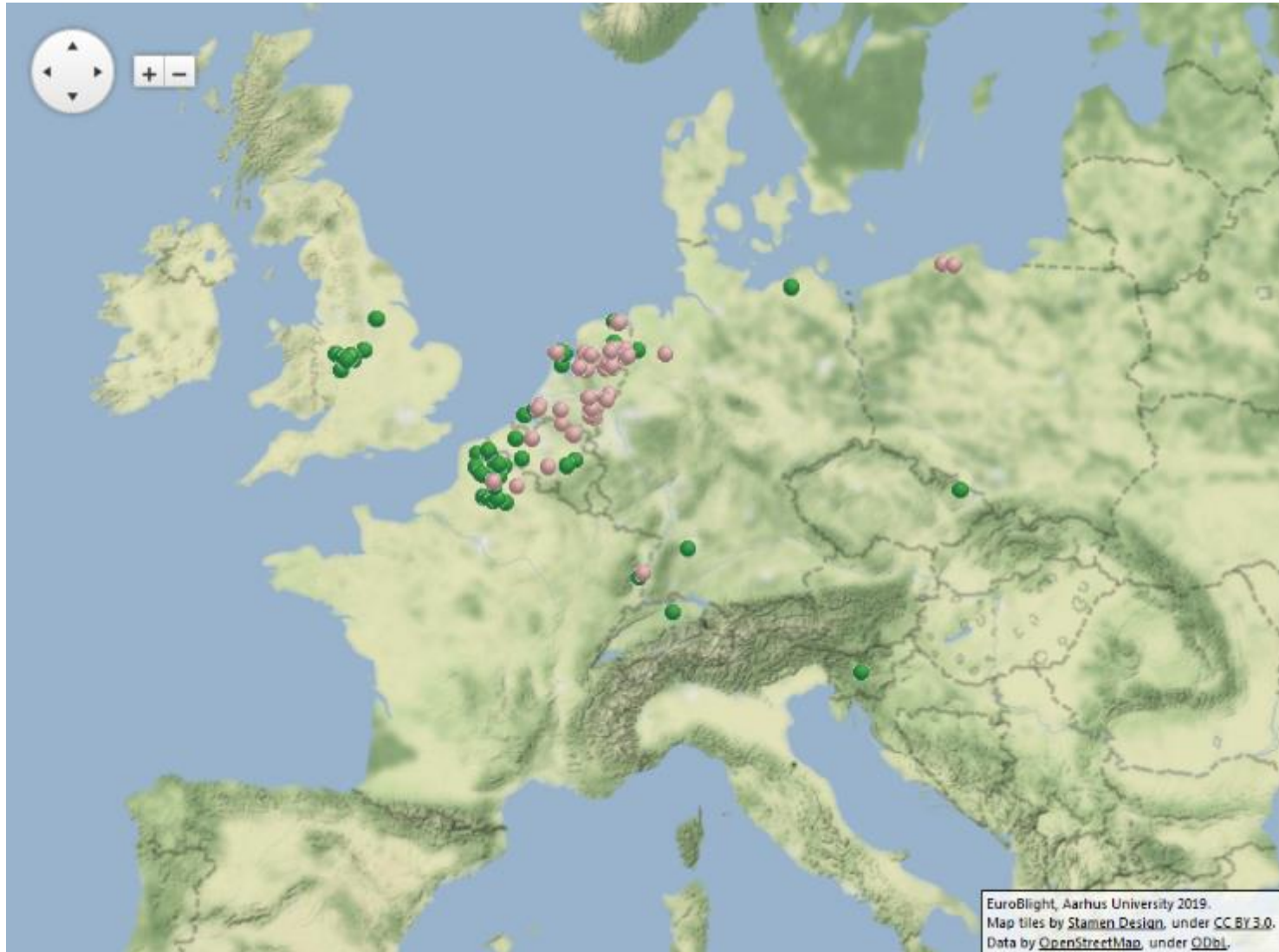
The James
Hutton
Institute



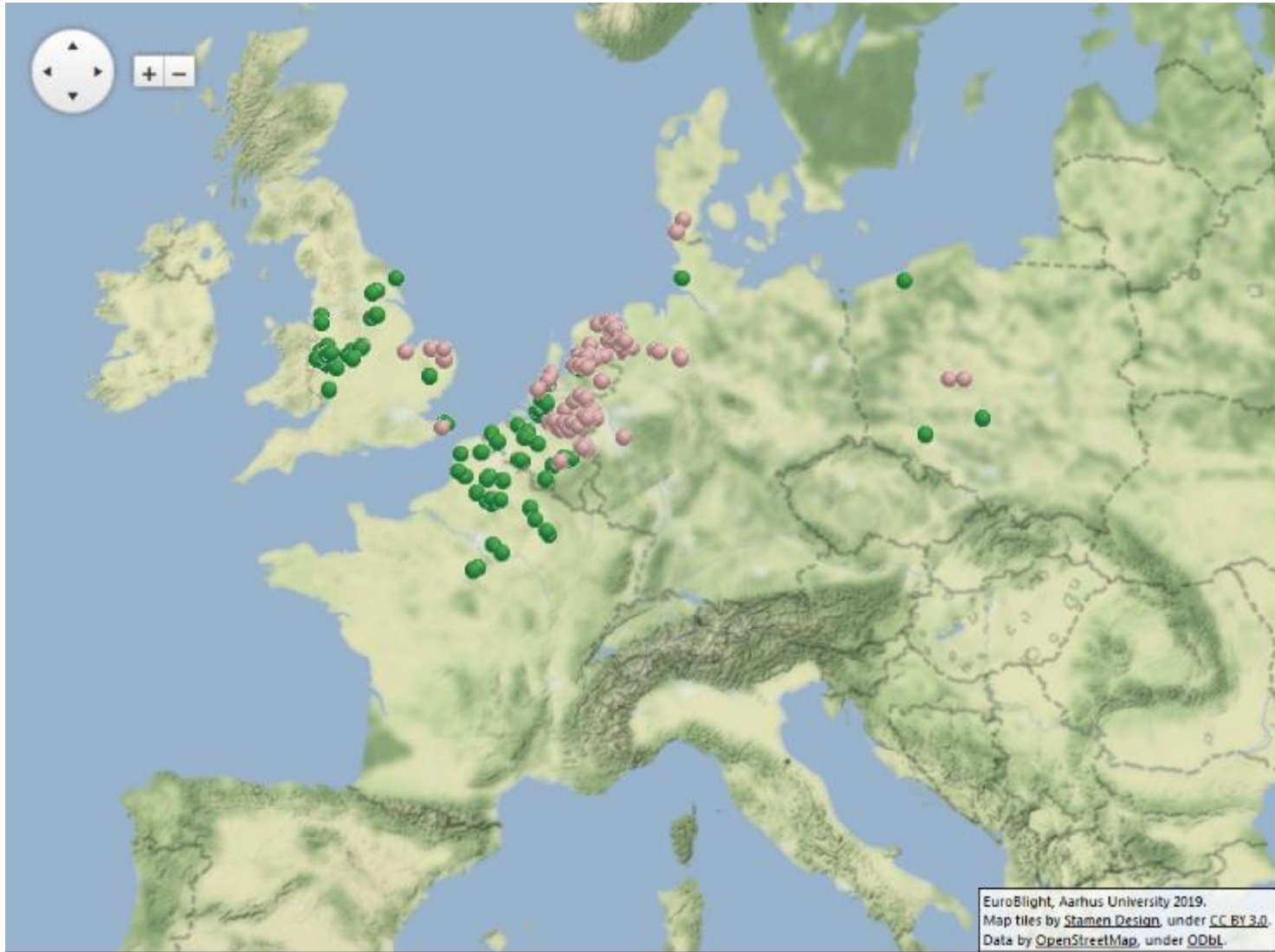
EU_36_A2

EU_37_A2

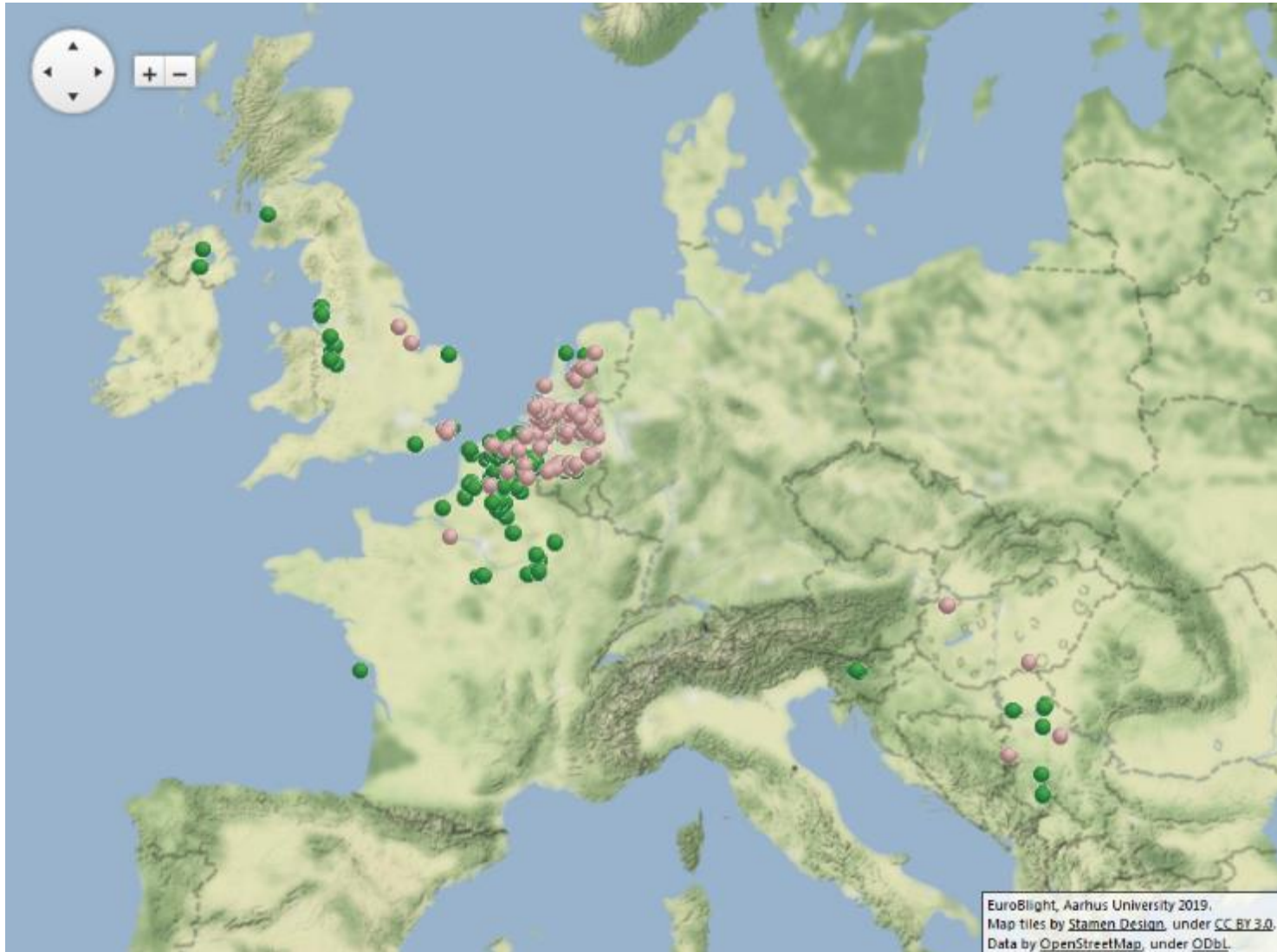
2016 36_A2 & 37_A2



2017 36 & 37_A2



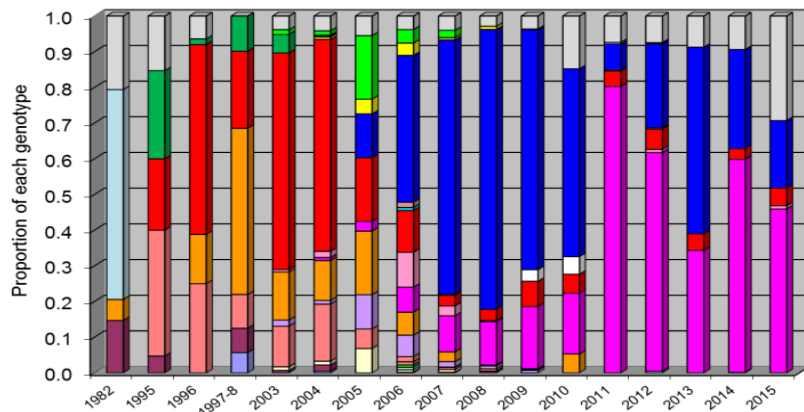
2018 36_A2 & 37_A2



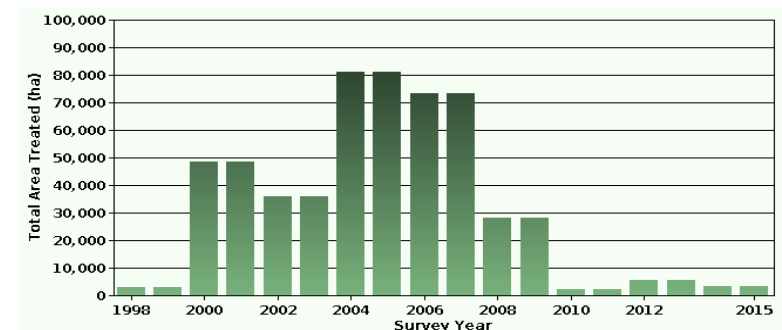
- What does it all mean to growers with crops to protect?

Population change and blight management

- Fungicide resistance management important
- EU_13_A2 emerged 2004, resistant to metalaxyl
- Metalaxyl use fell dramatically in UK

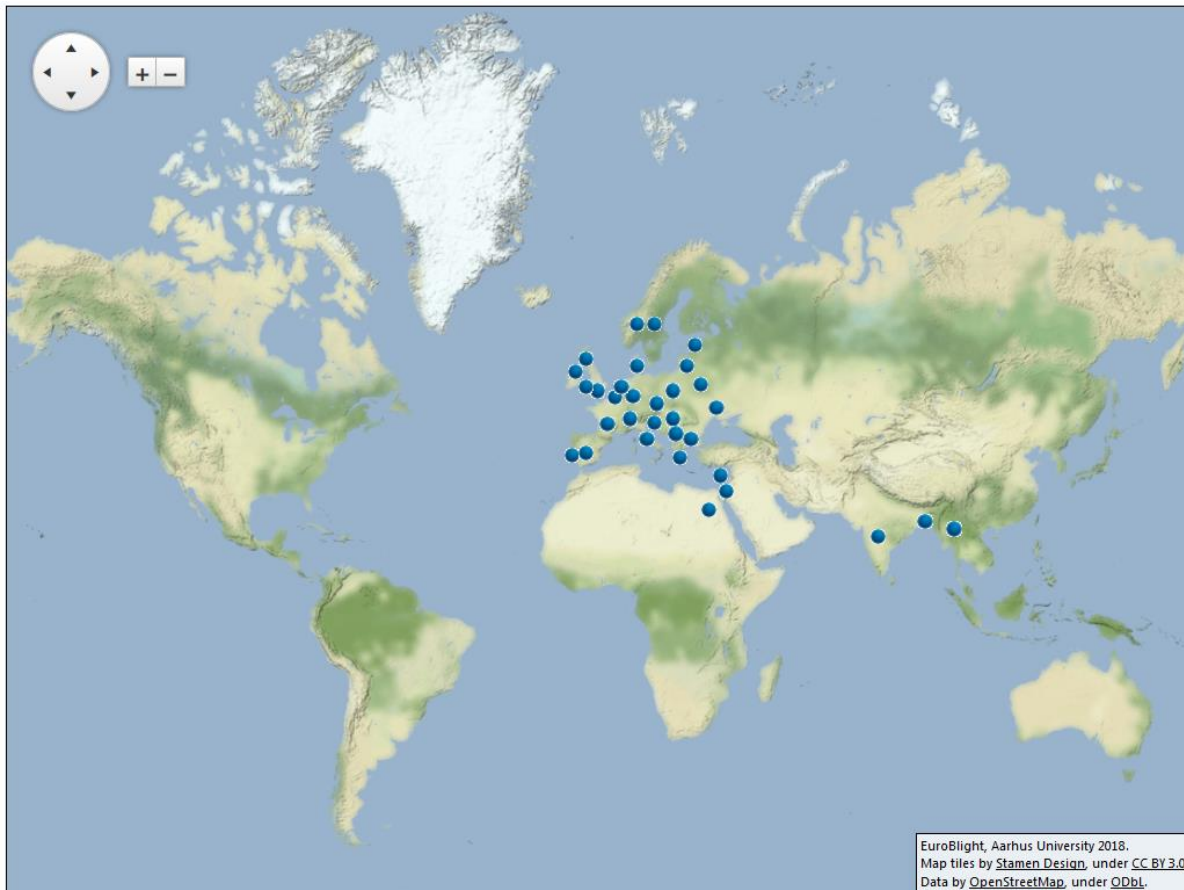


Total Area Treated¹ (ha) of Metalaxyl-M applied to Potatoes in Great Britain



UK Pesticide Usage Surveys – Fera Science Ltd, UK

13_A2 global distribution



- 13_A2 also in;
 - Egypt (Sherif el Ganainy)
 - China (Ying Li)
 - Bangladesh (Geert Kessel)
 - Nepal (Buddhi Sharma)
 - India (Pallem Chowdappa and Sanjoy Guhar Roy)
 - Myanmar (WUR)
 - Israel (Yigal Cohen)
- Advice to growers not always given rapidly enough

Fluazinam insensitivity

- **EU_33_A2** and **EU_37_A2** show insensitivity to fluazinam

Eur J Plant Pathol

<https://doi.org/10.1007/s10658-018-1430-y>



CrossMark

Reduced efficacy of fluazinam against *Phytophthora infestans* in the Netherlands

**H. T. A. M. Schepers • G. J. T. Kessel • F. Lucca •
M. G. Förch • G. B. M. van den Bosch • C. G. Topper •
A. Evenhuis**

UK industry awareness campaign



Dark Green 37: Coming to a field near you

BY JOHN SWIRE ON OCTOBER 26, 2017

CROPS, NEWS, POTATOES

The emergence of a new strain of potato late blight (*Phytophthora infestans*) with resistance to fluazinam, one of the most commonly used blight fungicides, is raising concern among agronomists.

Fungicide resistance warning for new potato blight strain

Friday 30 June 2017 14:58

Richard Allison

A reduced sensitivity to a key blight fungicide is being partly blamed for the spread of a new strain of the potato disease across Europe, with UK farmers urged to alternate their fungicide actives this season. The Dark Green 37 (EU-37) strain of blight was first detected in the Netherlands in 2013 and it has now spread to England, German, Belgium and north-west France. See also: How spud growers will benefit from blight forecasts Worryingly, this strain of the most important [...]



© Tim Scrivener

Blight actives feel the strain



Late blight pressure and a flurry of activity from blight scouts gives an early indication that new blight strain 37_A2 is on the rise. *CPM* reports.

By Lucy de la Pasture

For the first time, blight genotyping has been carried out in 'real time' this season by David Cooke at James Hutton Institute (JHI). The monitoring has been carried out following the spread of the blight strain 37_A2 (dark green) in the Netherlands (where it was first found in 2013), Germany, Belgium, and NW France where isolates of have shown a reduced sensitivity to fluazinam.

Over the summer, there've been 15 findings (up to 25 Sept) of the new blight strain, reported for the first time in the UK in 2016 in a very small number of samples.



EuroBlight Population Genetics Platform

Potato Late Blight
Toolbox



POPPR
Population Genetics in R

Zhian Kamvar & Nik Grünwald

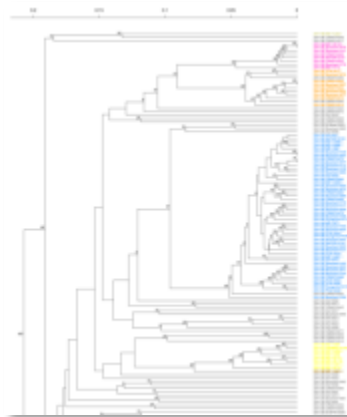
Kamvar et al. 2015. *Frontiers in Genetics* 6:208



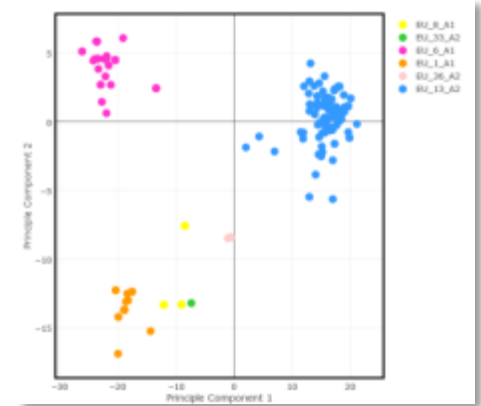
Population
Statistics



Minimum
Spanning
Tree



Phylogenetic
Tree



Principal
Component
Analysis



2018 Genetic diversity - PCA

PCA with Bruvo distance as input

Choose x axis

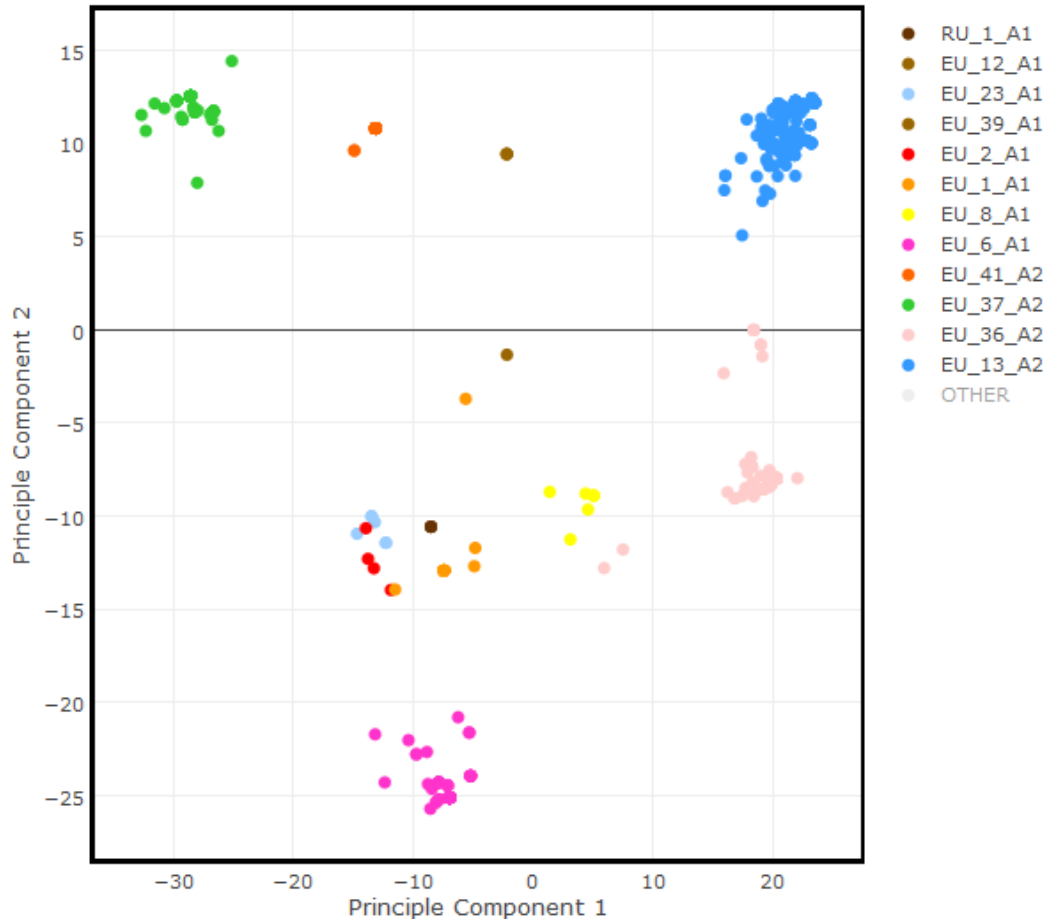
1

Choose y axis

2

Choose z axis

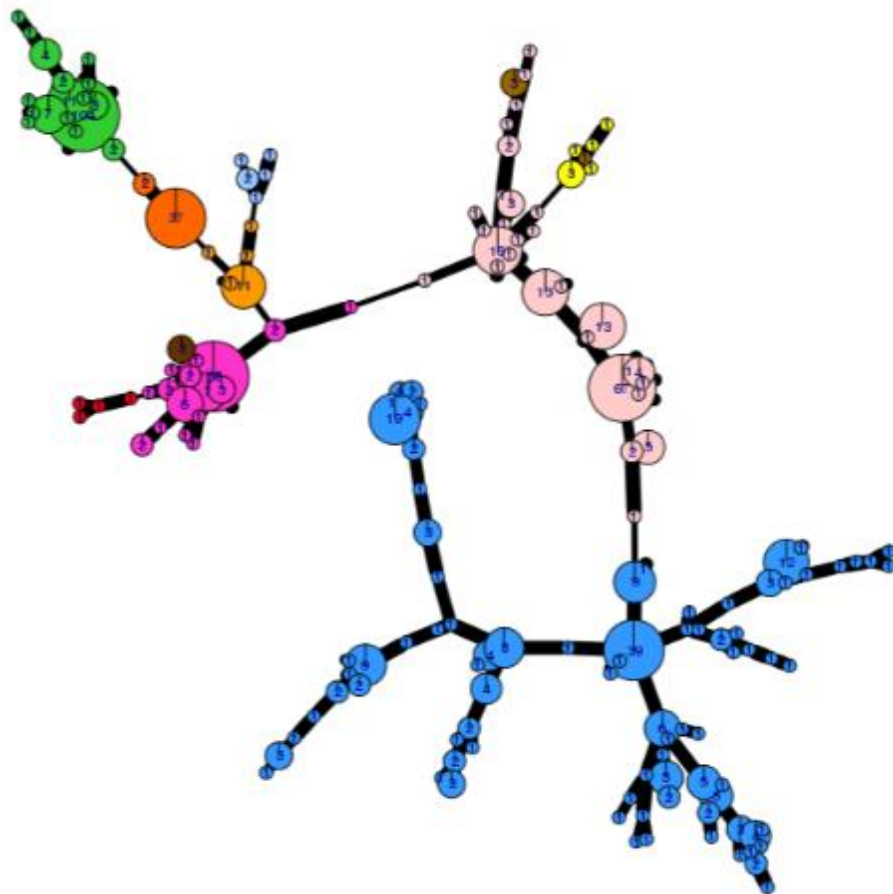
3



- 2018 Principle Coordinate Analysis (minus 'Other' samples)
- Distinct genetic clusters representing the major clones
- Minor variation within clones explains spread of points
- PCA an imperfect tool as some overlap of points inevitable



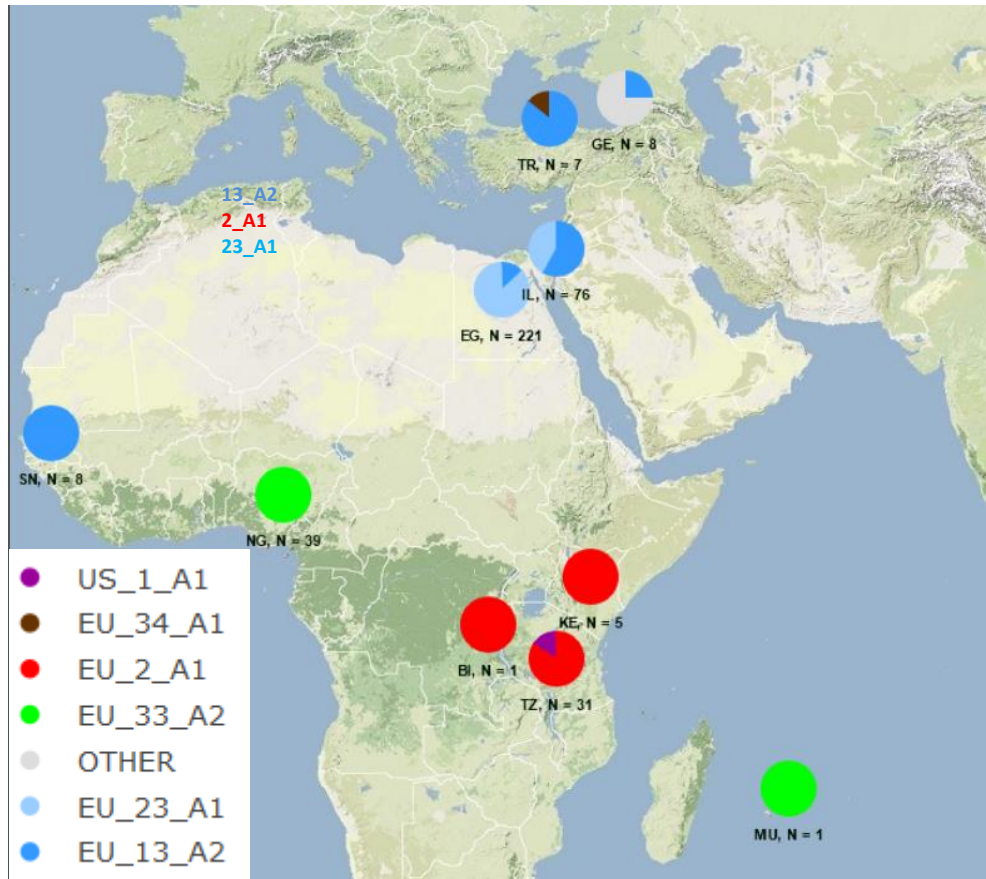
2018 Genetic diversity - MSN



- Minimum Spanning Network of 2018 samples (**clones only**)
- Node size proportional to the number of samples & coloured by clone
- Edge thickness proportional to genetic distance. Dark thick line genetically close - thinner lines between clones genetically distant
- 37_A2 diploid and null locus so less diverse
- Age of EU_13_A2 apparent with 84 variant MLGs in 224 samples



Global context



- Senegal 13_A2 present in 2019 (Chris Ursell CUConsulting)
- 33_A2 present in Nigeria (Emmanuel Nnadi, Plateau State University, Bokkos)
- East Africa remains free of contemporary EU lineages
- Anne Njoroge hoping to run AfricaBlight
- 13_A2 also present in Turkey, Egypt and Georgia)

Conclusions

- **New insights** on pathogen diversity
- Dominance of few clones across large areas of European crops means EU growers/industry **share management challenges**
- **New clones** (EU_36_A2, EU_37_A2 and EU_41_A2) continued to spread in 2018 - **displacing older genotypes**
- Phenotypic data is helping industry manage blight
- Primary inoculum is locally generated and spread. Better **management of inoculum sources** would aid management
- ‘Other’ populations highly diverse, ephemeral and most likely the result of sexual oospore germination. Drivers?
- **High genetic diversity increases the risk of blight** management problems - evolving virulence against novel host resistance genes and reduced sensitivity to fungicide active ingredients
- **Collaboration** with partner networks is strengthening

