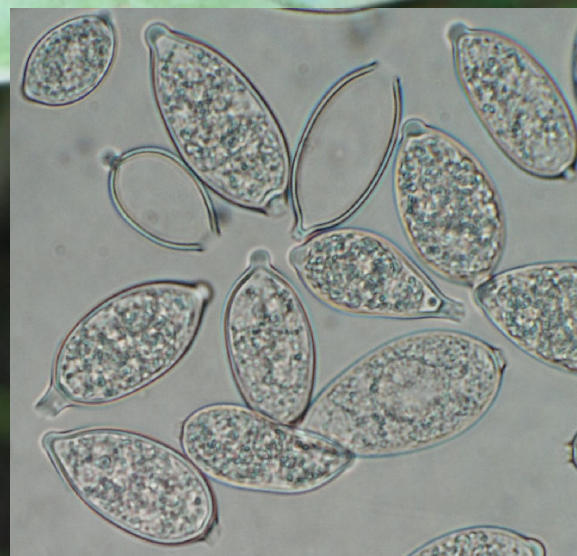
Eucablight – one year on:
an update on European blight populations

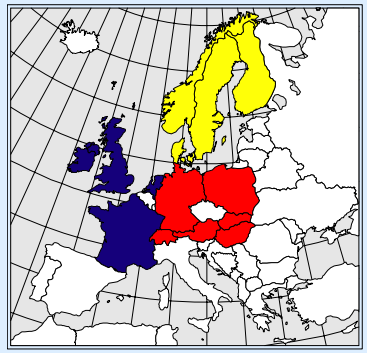
David Cooke
Björn Andersson
Jozsef Bakonyi
Jens Grønbech Hansen
Poul Lassen
Alison Lees
All data submitters



Overview

- **Introduction – Eucablight project aims**
- **What elements of *P. infestans* biology are important**
- **What contributions Eucablight pathogen database can (and cannot) make**
- **Eucablight overview**
  - **data collection & progress since Tallinn**
  - **need for pathogen genotyping (SSR) data**
  - **data interpretation and presentation**
- **Key examples of the data collected**
- **Conclusions & future plans**

Main aims of pathogen section of Eucablight project



WP1

'... establish a **comprehensive network** on the population biology of *P.infestans* across Europe'

WP2

Collect and collate data (pheno and genotype) on existing/past *P. infestans* collections

WP4

4.1 **Collate and review existing methods** for assessing variation in *P. infestans* populations and to test, standardise and publish these methods in a www database

4.2 To create a **European isolate database** detailing existing data on isolate variation using new data as assessed by the methods developed in Objective 4.1

4.3 **Training** course on agreed and adopted methods

4.4 Pan-European **interpretation** of changing population structure in *P. infestans*

WP5

Integration of all derived data to benefit of control strategies

Linking *P. infestans* biology and blight management

1. **Where, when and how blight infection starts**
   - primary inoculum
2. **Rate of infection and spread**
   - foliar
   - tuber
3. **Control options**
   - fungicide efficacy
   - host resistance
4. **Survival**
   - cull piles
   - volunteers
   - solanaceous weeds
   - oospores
5. **Changing pathogen population**
   - Immigration
   - Evolution – mutation & recombination

Integration of all data into practical management advice

Data collected -1

P. infestans VARIATION - Type

Phenotype	Mating type Virulence Fungicide resistance
Genotype	Isozyme RG57 mtDNA SSR AFLP SNP SEQ

Data collected - 2

VARIATION - Other factors

Host	Tomato Potato Others Weeds
Management	Variety Fungicide Crop type Seed trade etc

Data collected -3

VARIATION - Scales

	Fine Scale	Coarse Scale
Spatial	Leaf	Continent
Temporal	Weeks	Years

Data analysis options

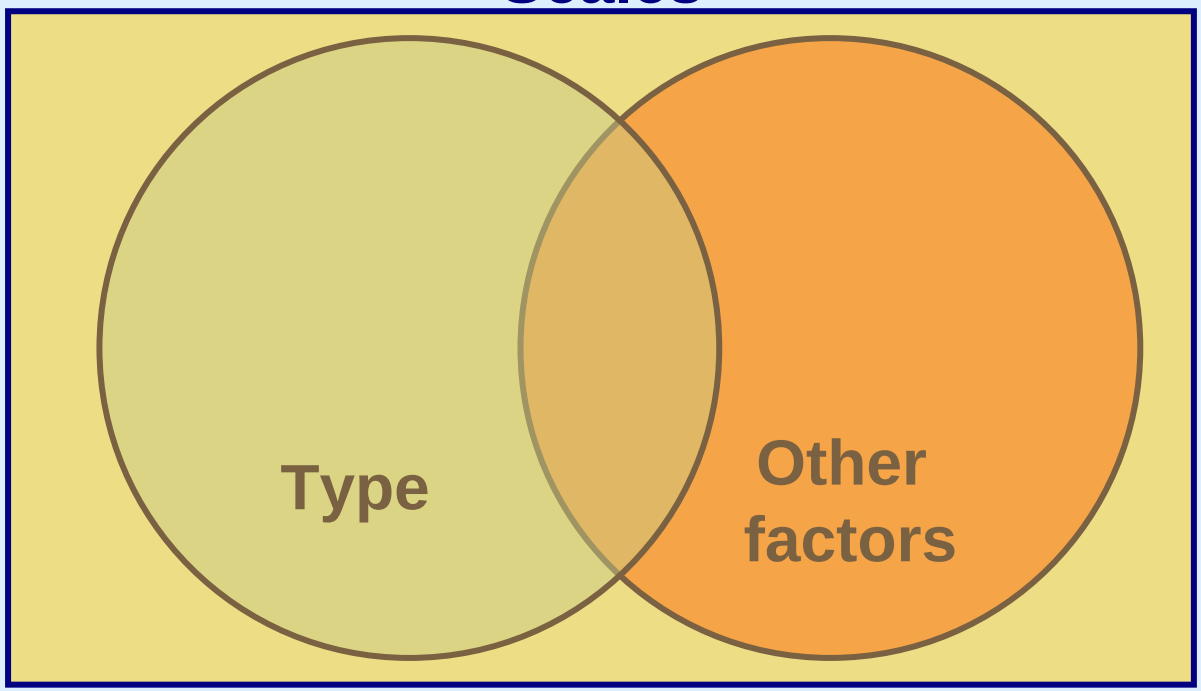
Multiple options for data analysis

Depends on how complete the data set is

Fundamental and applied biology

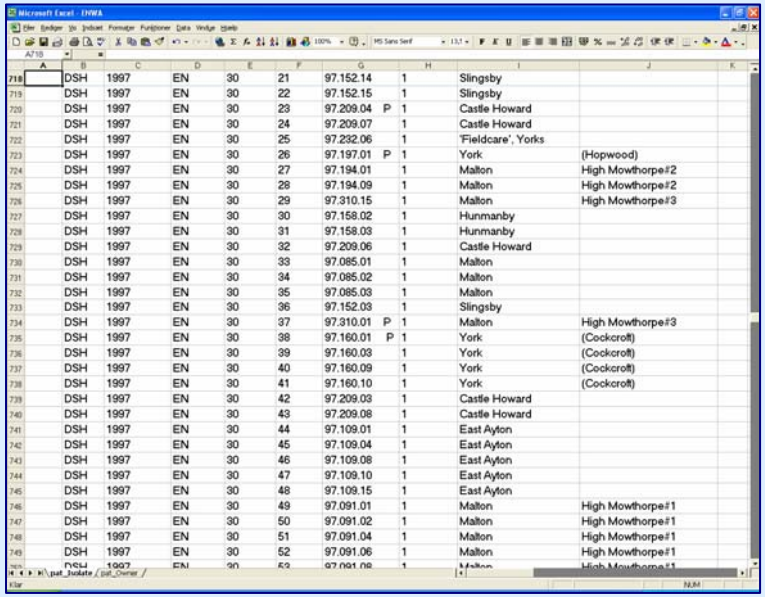
Linkage between two is strong

Scales



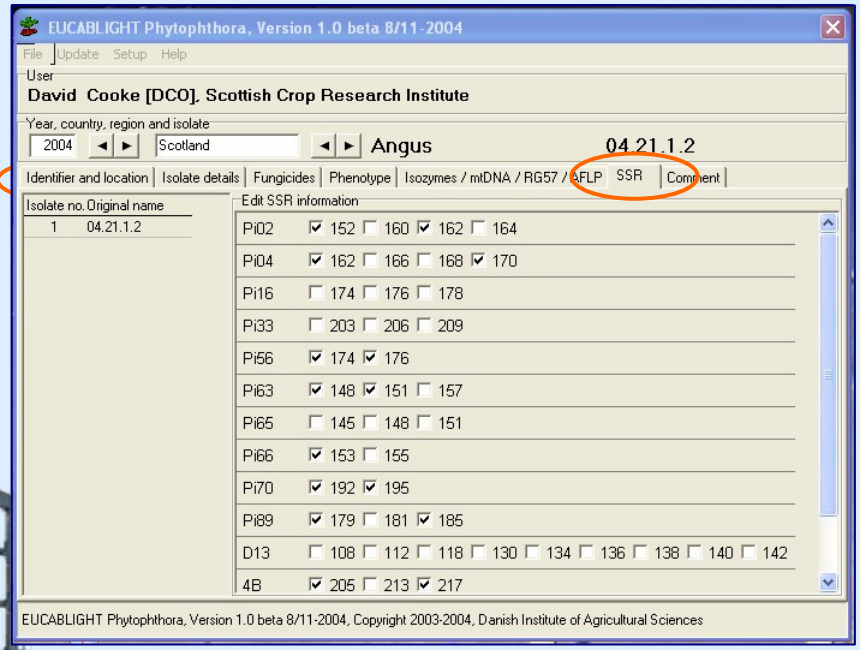
Pathogen database

”Old” pathogen data



718	DSH	1997	EN	30	21	97.152.14	1	Slingsby	
719	DSH	1997	EN	30	22	97.152.15	1	Slingsby	
720	DSH	1997	EN	30	23	97.209.04	P 1	Castle Howard	
721	DSH	1997	EN	30	24	97.209.07	1	Castle Howard	
722	DSH	1997	EN	30	25	97.232.06	1	'Fieldcare', Yorks	
723	DSH	1997	EN	30	26	97.197.01	P 1	York	(Hopwood)
724	DSH	1997	EN	30	27	97.194.01	1	Malton	High Mowthorpe#2
725	DSH	1997	EN	30	28	97.194.09	1	Malton	High Mowthorpe#2
726	DSH	1997	EN	30	29	97.310.15	1	Malton	High Mowthorpe#3
727	DSH	1997	EN	30	30	97.158.02	1	Hunmanby	
728	DSH	1997	EN	30	31	97.158.03	1	Hunmanby	
729	DSH	1997	EN	30	32	97.209.06	1	Castle Howard	
730	DSH	1997	EN	30	33	97.085.01	1	Malton	
731	DSH	1997	EN	30	34	97.085.02	1	Malton	
732	DSH	1997	EN	30	35	97.085.03	1	Malton	
733	DSH	1997	EN	30	36	97.152.03	1	Slingsby	
734	DSH	1997	EN	30	37	97.310.01	P 1	Malton	High Mowthorpe#3
735	DSH	1997	EN	30	38	97.160.01	P 1	York	(Cockcroft)
736	DSH	1997	EN	30	39	97.160.03	1	York	(Cockcroft)
737	DSH	1997	EN	30	40	97.160.09	1	York	(Cockcroft)
738	DSH	1997	EN	30	41	97.160.10	1	York	(Cockcroft)
739	DSH	1997	EN	30	42	97.209.03	1	Castle Howard	
740	DSH	1997	EN	30	43	97.209.08	1	Castle Howard	
741	DSH	1997	EN	30	44	97.109.01	1	East Ayton	
742	DSH	1997	EN	30	45	97.109.04	1	East Ayton	
743	DSH	1997	EN	30	46	97.109.08	1	East Ayton	
744	DSH	1997	EN	30	47	97.109.10	1	East Ayton	
745	DSH	1997	EN	30	48	97.109.15	1	East Ayton	
746	DSH	1997	EN	30	49	97.091.01	1	Malton	High Mowthorpe#1
747	DSH	1997	EN	30	50	97.091.02	1	Malton	High Mowthorpe#1
748	DSH	1997	EN	30	51	97.091.04	1	Malton	High Mowthorpe#1
749	DSH	1997	EN	30	52	97.091.06	1	Malton	High Mowthorpe#1

”New” pathogen data



Server at DIAS

Pathogen data overview

www.eucablight.org

Tallinn Oct 2005	12,300
Rennes Jan 2006	13,600
NJF Nov 2006	15,000
Bologna May 2007	15,500

Welcome David Cooke

EUCABLIGHT
Potato Late Blight Network For Europe

02 May 2007

Overview | Graphic analysis | Genotype analysis | Virulence analysis | Documentation | SSR analysis | Map
Pie | My isolates

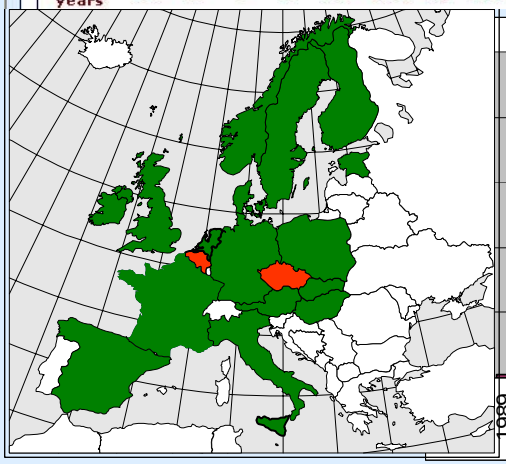
Pathogen overview

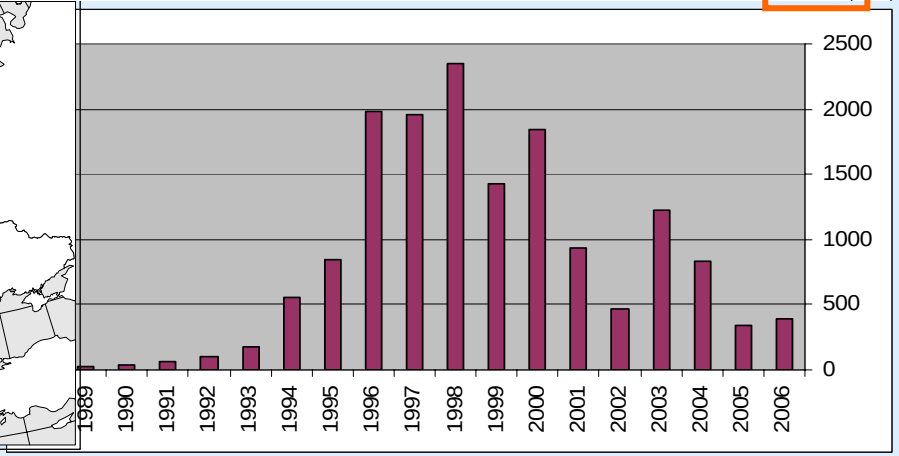
Select one or more traits and press the show button. Help

☐ Mating type ☐ Metalaxyl resistance ☐ Aggressiveness ☐ Virulence ☐ mtDNA ☐ AFLP ☐ Isozyme ☐ SSR ☐ All

Show

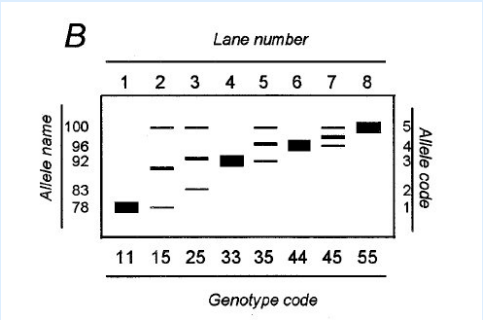
Country Year	AT	BE	DE	DK	EE	EN	ES	FI	FR	HU	IE	IT	MA	NI	NL	NO	PL	SC	SE	SK	WA	All countries
2006					103			87		56	1			20				125				392
2005					103					67				54		21	90					335
2004					144									24	25	115	46	456		26		836
2003				65	84			234	7					40	109	331	22	216	88	26		1222
2002	100				89				75	93				58			30			26		471
2001					83			210	112	27				38			149		277	36		932
2000			7		13			675	84	3			42	26	481	197	155		163			1846
1999					12			457	216				25	35		269	149		258			1421
1998		46				336		538	73	36				78		678	256	22	263		25	2351
1997						630		602	86	5				53		167	147	215			48	1953
1996						143	10	16	156	1	147	10	7	195	353	493	189	171			97	1988
1995						26	12	1	135					114	383	1	1	152			16	841
1994							12	117	87				1	64	278		1					560
1993								3	69	1				60	41							174
1992								15	83					4								102
1991									56	1		1										58
1990									35													35
1989									22													22
1988									4													4
All years	100	46	7	65	631	1135	34	2955	1300	290	148	11	75	863	1670	2272	1235	1357	1049	114	186	15543





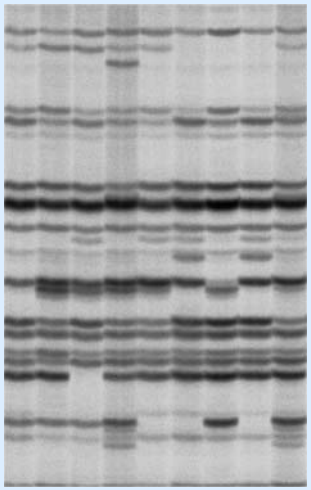
Tools for estimating genetic diversity

Isozymes

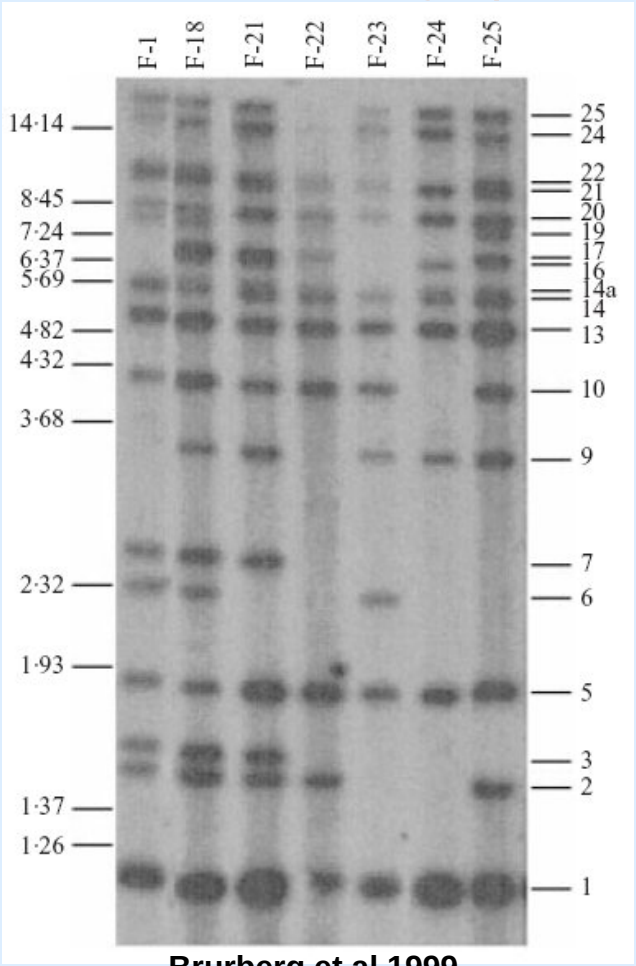


Forbes et al 1998

AFLPs

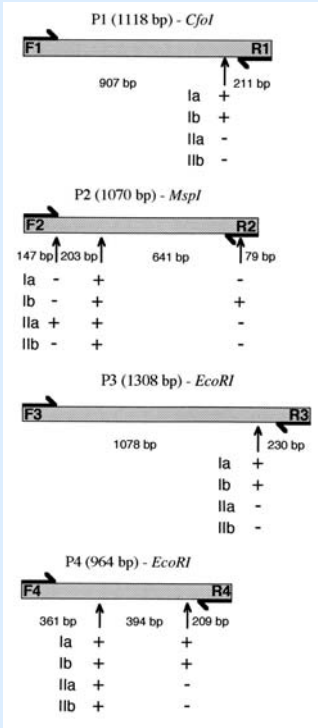


RG57 RFLP fingerprint



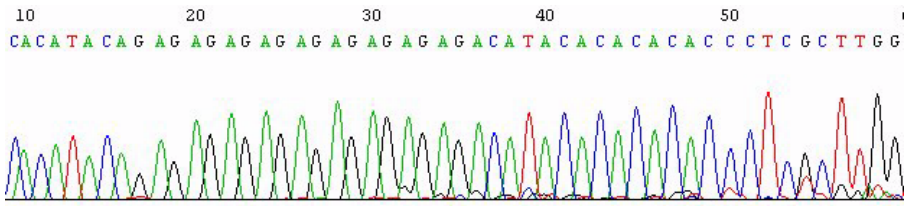
Brurberg et al 1999

mtDNA haplotype



38Kb
95% coding
Slow evolving
4 haplotypes world-wide

Simple Sequence Repeats



TCGACCCACGCGTNNGCCCACGCGTCCGGAAGCAGCAGCCTCCGTGCAAGACGATCA

Fwd Primer →

TGCTAGGTCTGAGACTTGC***AGAACTACCGCCCGAGAC***AATTCGACCGAGCGGTGTAG

SSR 10 X 'TG' repeat

CTGAGTACTACTCACGGAGCTTTGAGAGAGAGAGAGAGAGAGAGCGTGTGGAGCTTC

← **Rev Primer**

GTGGTCTTCGCGCACCTTGCGCTCGTACAAGATGGTGGA***ATGTTCTTGTGACCATCC***
AACCCTGTCCAGCGCTCTCTACAAGGCGATATTGACACTATTGATGTTGCTGTTAAG
GCCACTACTGCTAAGCAACGTGAAGGAACCGAGGAAAGTGGTCAAAGCTCTACGAAG

Important features

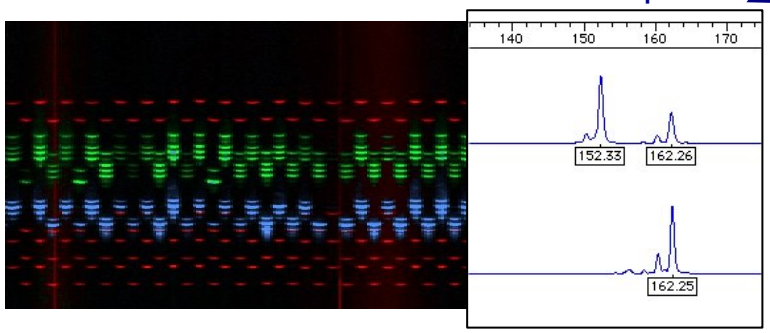
- Similar to human forensics
- Objective – easy to compare lab to lab
- Specific
- Both alleles scored
- Can be run on leaf material
- Good resolution

PCR amplification

e.g.162 bp product

Accurate sizing

n.b diploid



11 markers/loci =
(many potential combinations!)

How does SSR data look in practice?

				Pi02		G11		Pi33		Pi04		Pi4B		Pi16	
1				Pi02	Pi02	G11	G11	Pi33	Pi33	Pi04	Pi04	Pi4B	Pi4B	Pi16	Pi16
2	Seed	Kelso	M.Piper	162	162	156	162	203	203	166	170	205	217	176	178
3	Seed	Kelso	M.Piper	162	162	156	162	203	203	166	170	205	217	176	178
4	Seed	Kelso	M.Piper	162	162	156	162	203	203	166	170	205	217	176	178
5	Seed	Kelso	M.Piper	162	162	156	162	203	203	166	170	205	217	176	178
6	Seed	Kelso	M.Piper	162	162	156	162	203	203	166	170	205	217	176	178
7	Seed	Kelso	M.Piper	162	162	156	162	203	203	166	170	205	217	176	178
8	Seed	Kelso	M.Piper	162	162	156	162	203	203	166	170	205	217	176	178
9	Seed	Maize of Gallery	M.Piper	162	162	166	166	203	203	166	170	205	217	176	178
10	Seed	Maize of Gallery	M.Piper	162	162	166	166	203	203	166	170	205	217	176	178
11	Seed	Maize of Gallery	M.Piper	162	162	166	166	203	203	166	170	205	217	176	178
12	Seed	Maize of Gallery	M.Piper	162	162	166	166	203	203	166	170	205	217	176	178
13	Seed	Maize of Gallery	M.Piper	162	162	166	166	203	203	166	170	205	217	176	178
14	Seed	Maize of Gallery	M.Piper	162	162	166	166	203	203	166	170	205	217	176	178
15	Seed	Maize of Gallery	M.Piper	162	162	166	166	203	203	166	170	205	217	176	178
16	Seed	Maize of Gallery	M.Piper	162	162	166	166	203	203	166	170	205	217	176	178
17	Seed	Maize of Gallery	M.Piper	162	162	166	166	203	203	166	170	205	217	176	178
18	Ware	Sprouslee	P.Javelin	152	162	154	156	203	203	166	170	217	217	176	178
19	Ware	Sprouslee	P.Javelin	152	162	154	156	203	203	166	170	217	217	176	178
20	Ware	Sprouslee	P.Javelin	152	162	154	156	203	203	166	170	217	217	176	178
21	Ware	Sprouslee	P.Javelin	152	162	154	156	203	203	166	170	217	217	176	178
22	Ware	Sprouslee	P.Javelin	152	162	154	156	203	203	166	170	217	217	176	178
23	Ware	Sprouslee	P.Javelin	152	162	154	156	203	203	166	170	217	217	176	178
24	Ware	Sprouslee	P.Javelin	152	162	154	156	203	203	166	170	217	217	176	178
25	Ware	Sprouslee	P.Javelin	152	162	154	156	203	203	166	170	217	217	176	178

Outbreak 1

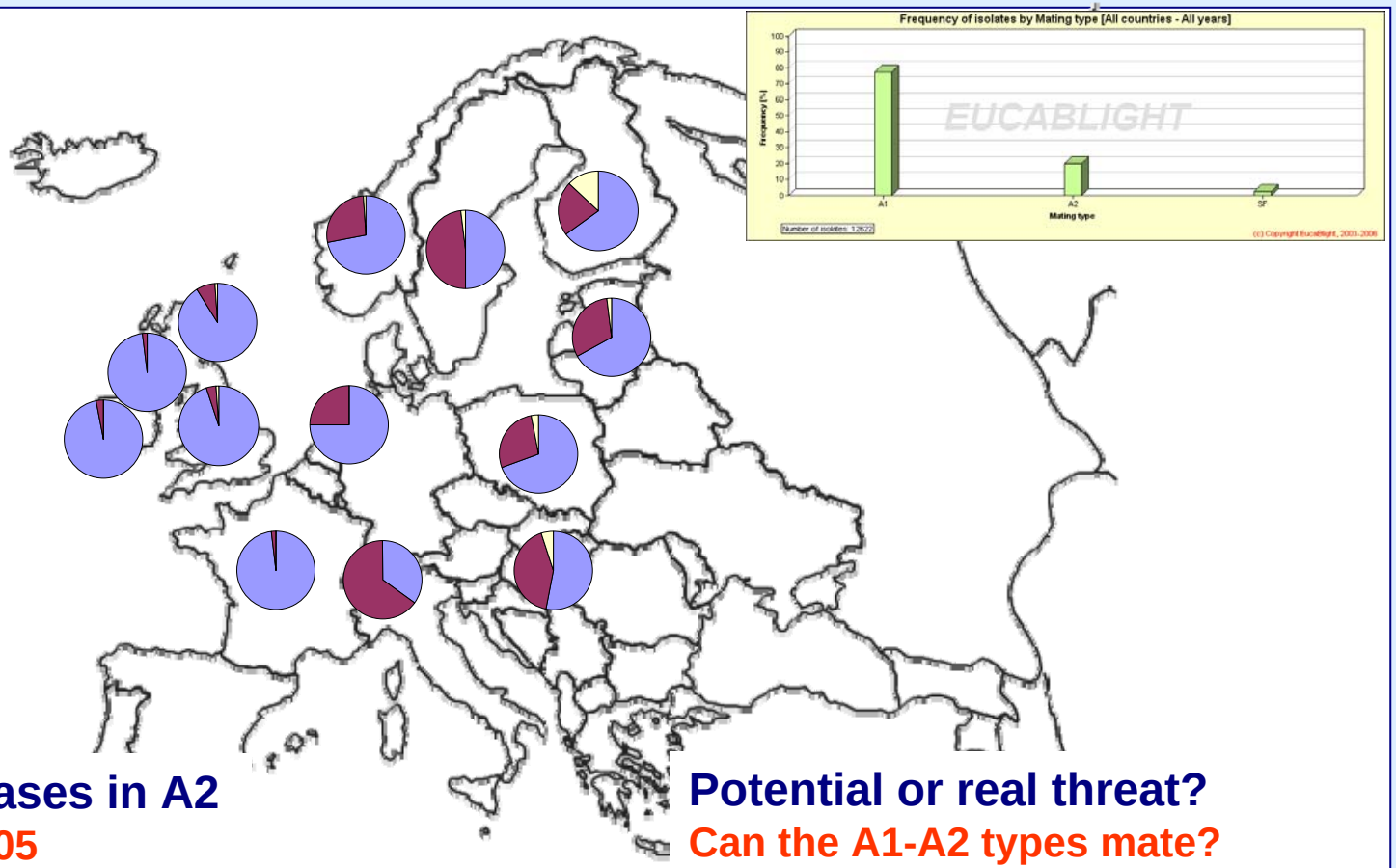
Outbreak 2

Outbreak 3

Main aspects

1. **Where, when and how blight infection starts**
   - **primary inoculum**
2. Rate of infection and spread
   - foliar
   - tuber
3. Control options
   - fungicide efficacy
   - host resistance
4. **Survival**
   1. **cull piles**
   2. **volunteers**
   3. **solanaceous weeds**
   4. **oospores**
5. Changing pathogen population?
   1. Immigration
   2. Evolution

Distribution of mating types



Recent increases in A2

N. France – 2005

Netherlands - 2005

UK – 2005 & 2006

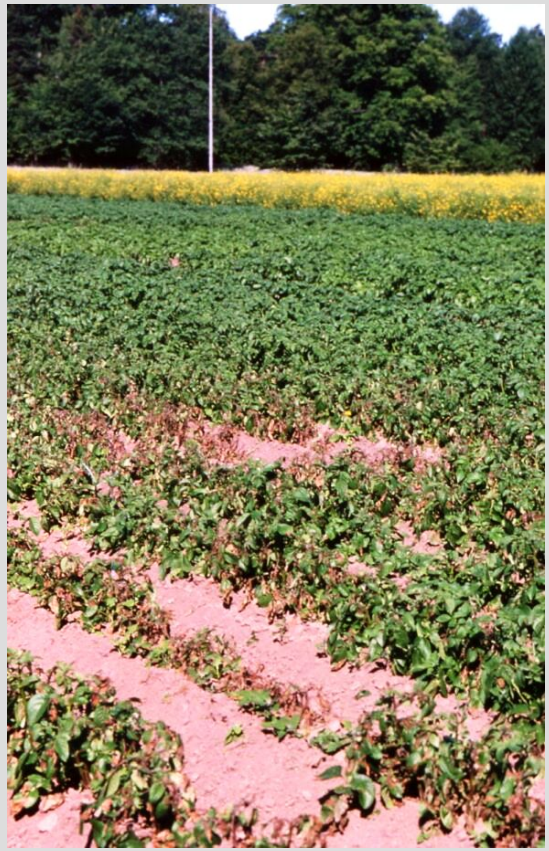
Potential or real threat?

Can the A1-A2 types mate?

Role as primary inoculum?

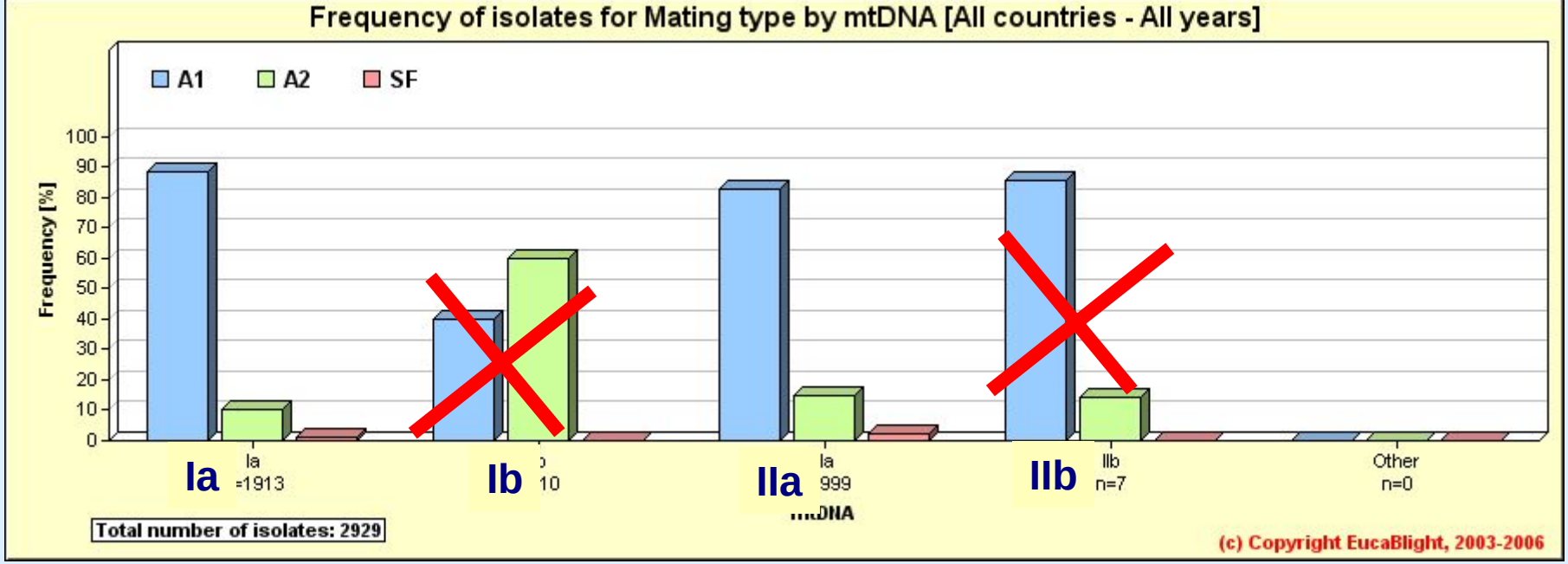
What types of A1 and A2 present?

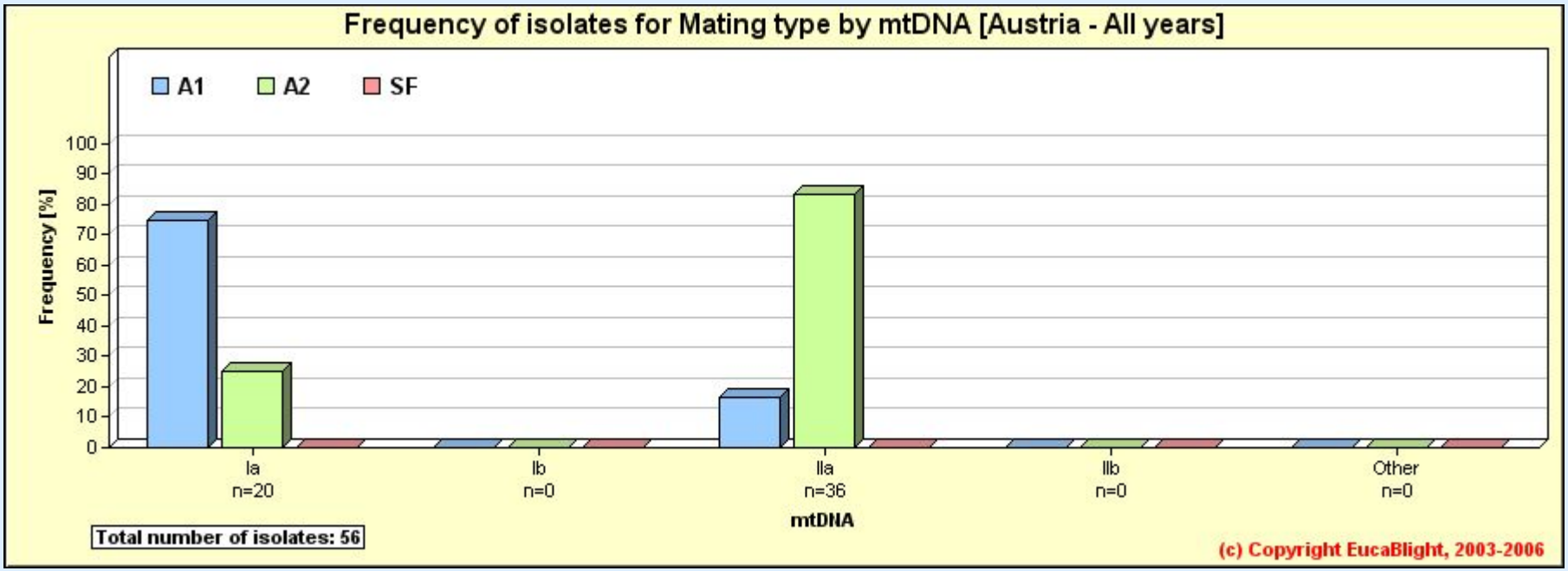
Evidence of oospore derived epidemics?



Sudden death of localised patches of plants
Björn Andersson (SLU, Sweden)

Hannukala *et al* Finland (Plant. Path. 2007)

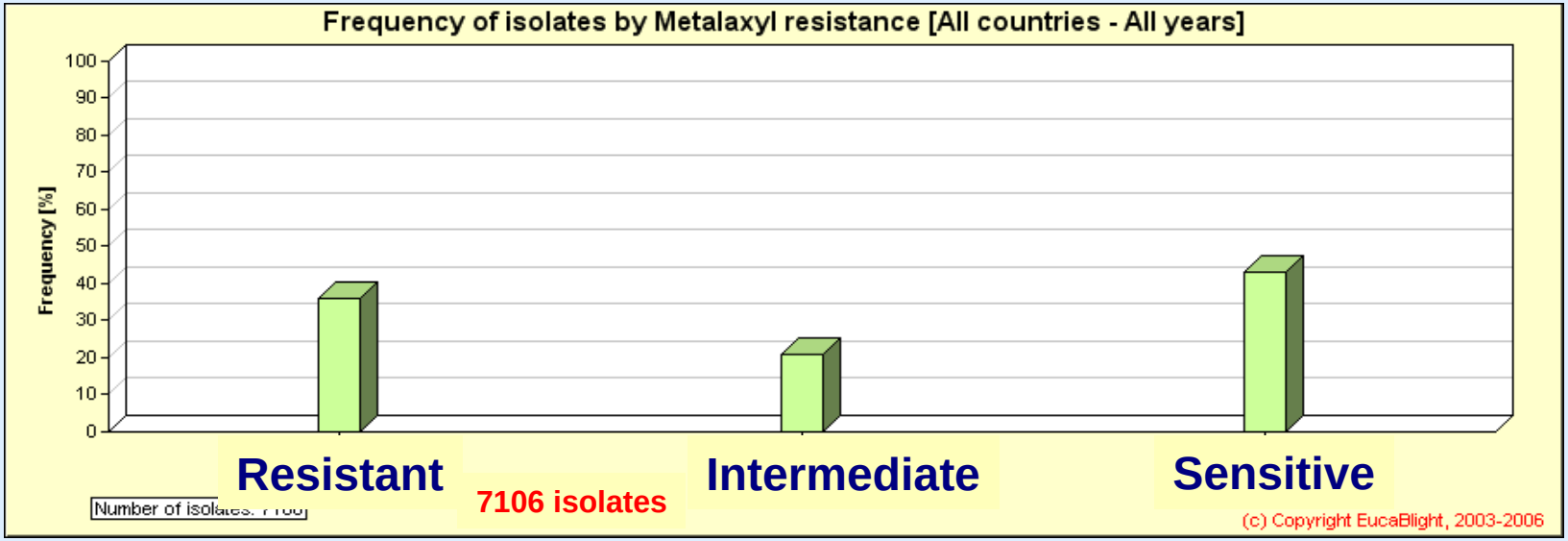

Frequency of isolates for Mating type by mtDNA [All countries - All years]
A1
A2
SF
Frequency [%]
100
90
80
70
60
50
40
30
20
10
0
Ia
Ib
IIa
IIb
Ia
=1913
10
Ia
999
IIb
n=7
Other
n=0
mtDNA
Total number of isolates: 2929
(c) Copyright EucaBlight, 2003-2006

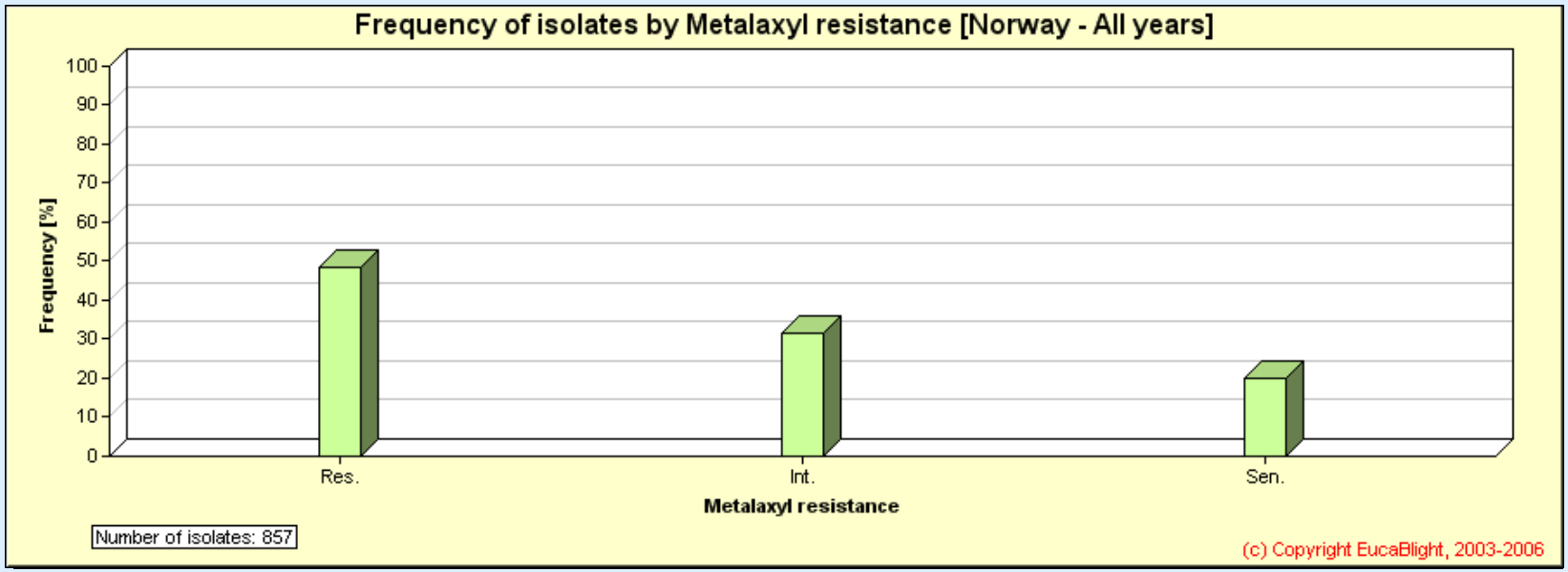

Frequency of isolates for Mating type by mtDNA [Austria - All years]
A1
A2
SF
Frequency [%]
100
90
80
70
60
50
40
30
20
10
0
Ia
n=20
Ib
n=0
IIa
n=36
IIb
n=0
Other
n=0
mtDNA
Total number of isolates: 56
(c) Copyright EucaBlight, 2003-2006

Main aspects

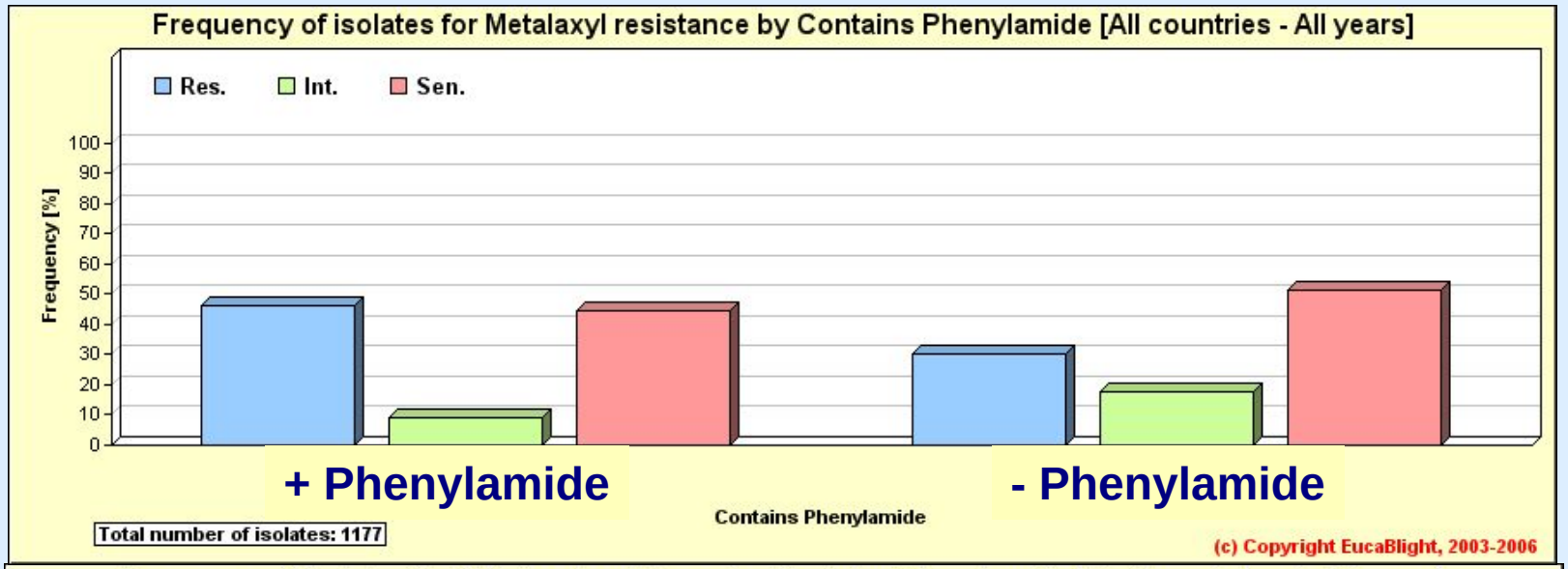
1. Where, when and how blight infection starts
   - primary inoculum
2. Rate of infection and spread – **not covered in eucablight**
   - foliar
   - tuber
3. **Control options**
   - **fungicide efficacy**
   - **host resistance**
4. Survival
   1. cull piles
   2. volunteers
   3. solanaceous weeds
   4. oospores
5. Changing?
   1. Immigration
   2. Evolution

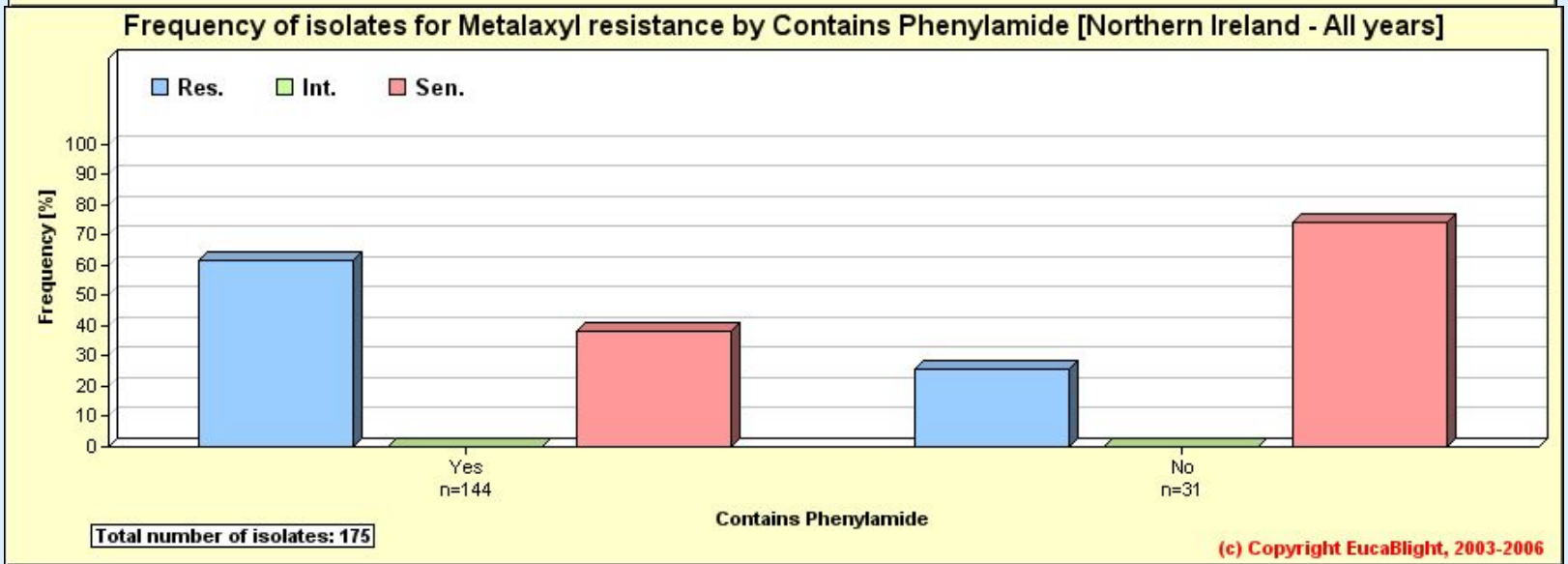
Metalaxyl resistance



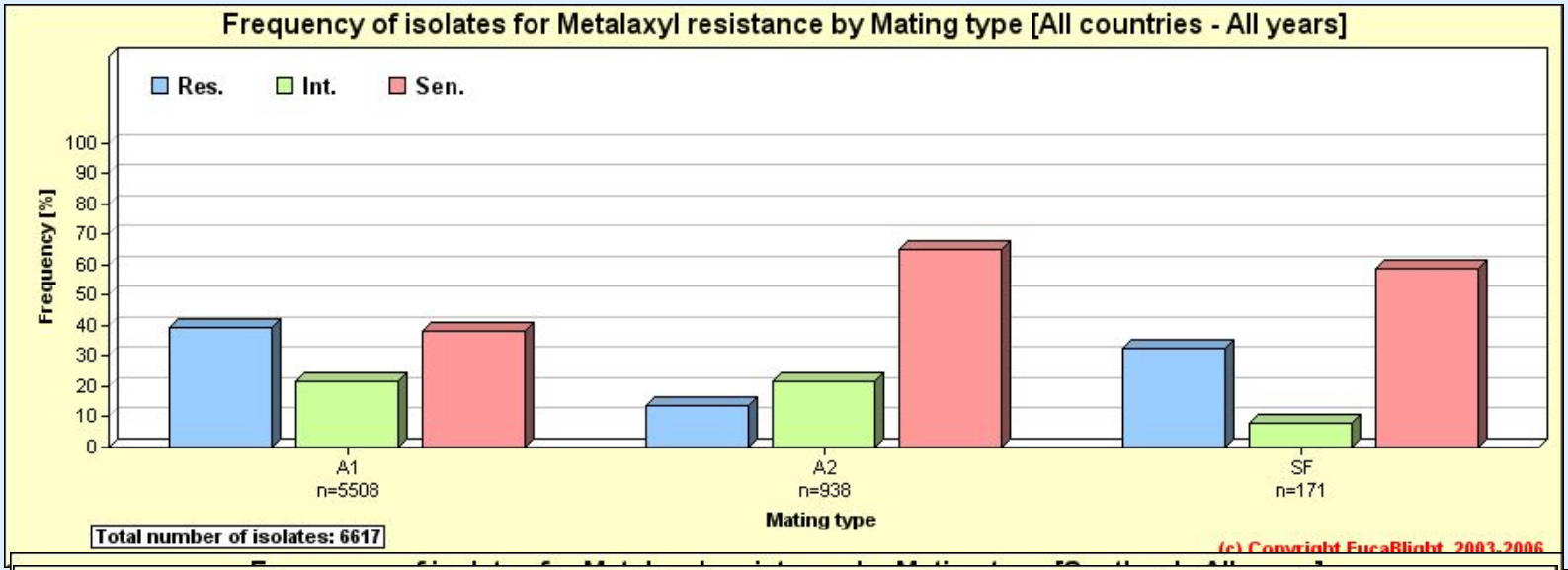


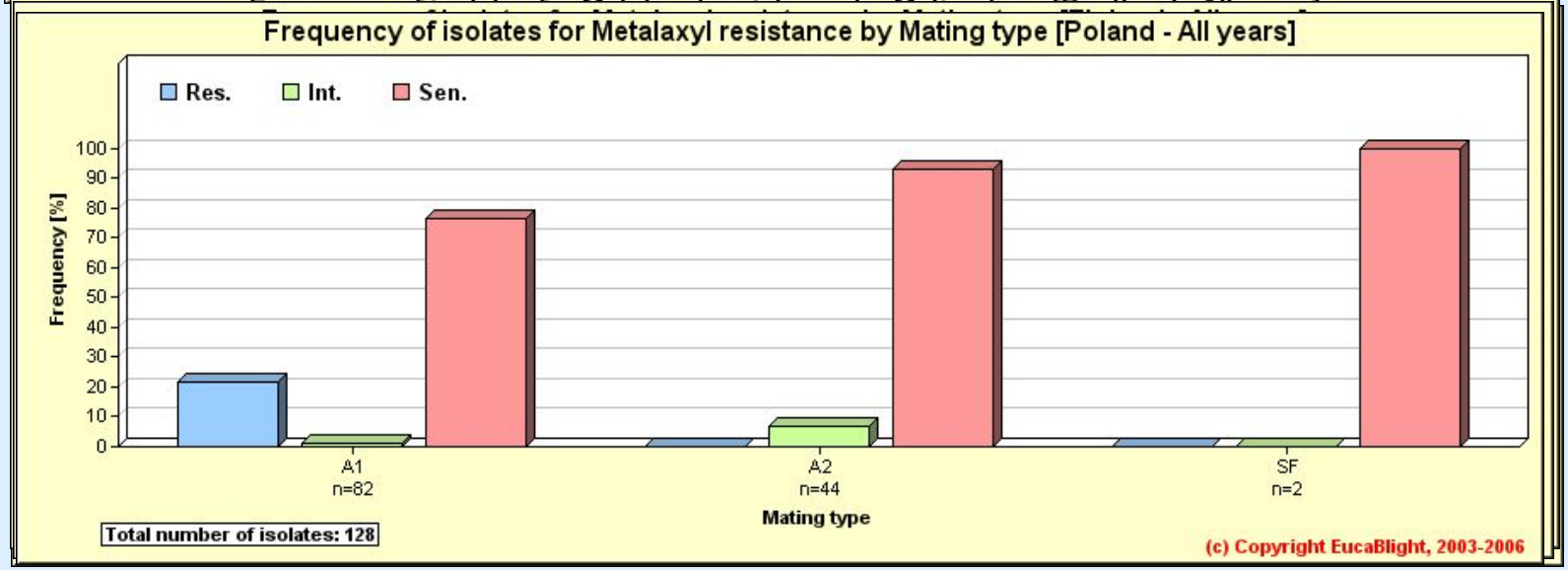
Metalaxyl resistance in relation to metalaxyl application



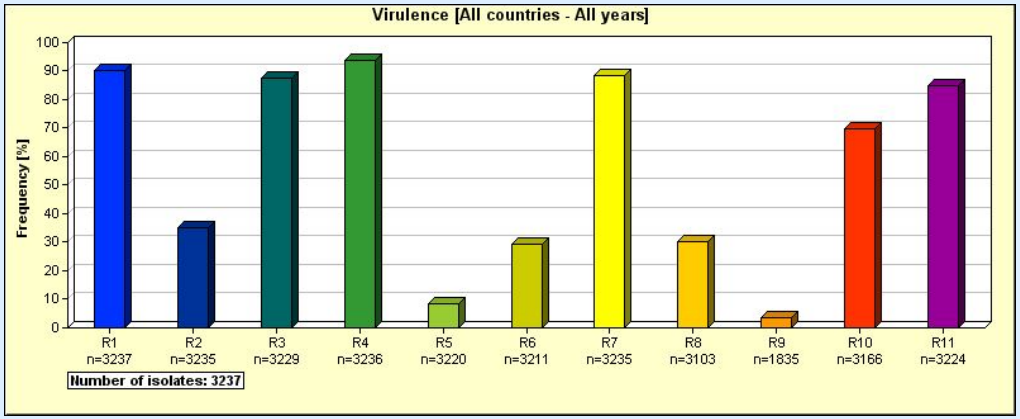


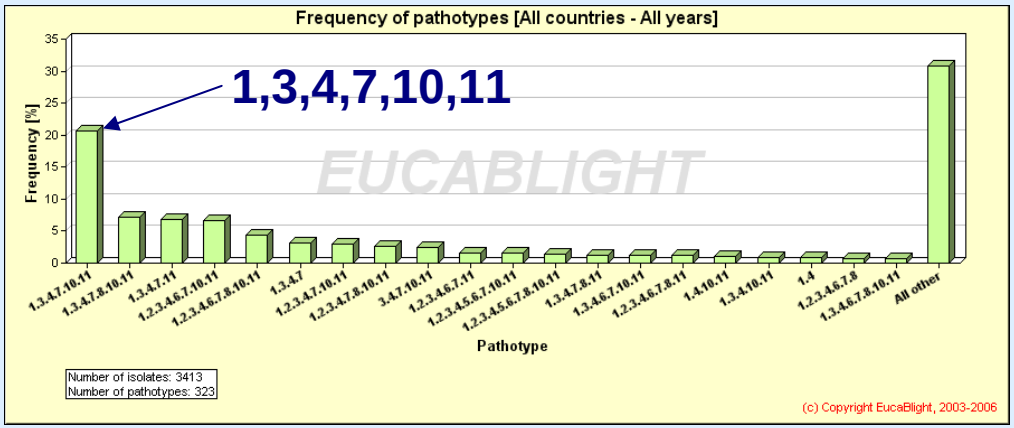
Metalaxyl resistance vs mating type

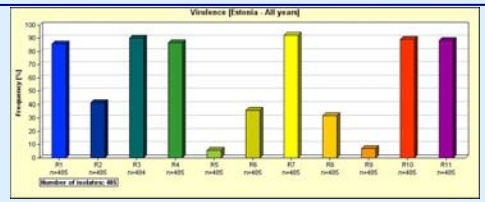




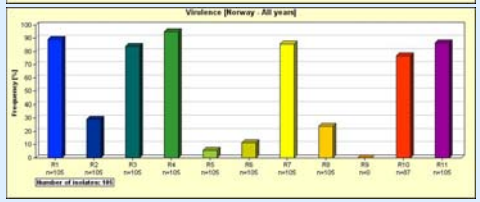
Virulence



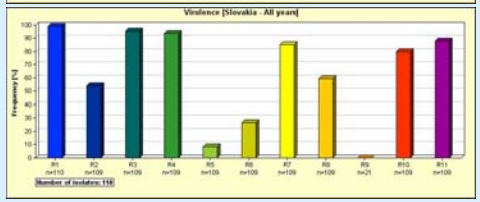




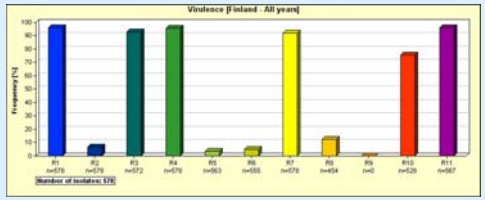
EE



NO



SK



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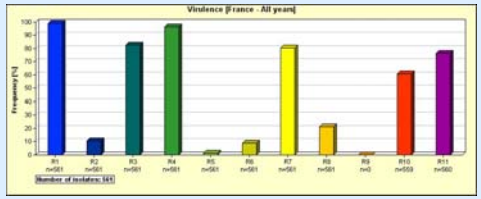


FR

Could R-genes be used to control blight?

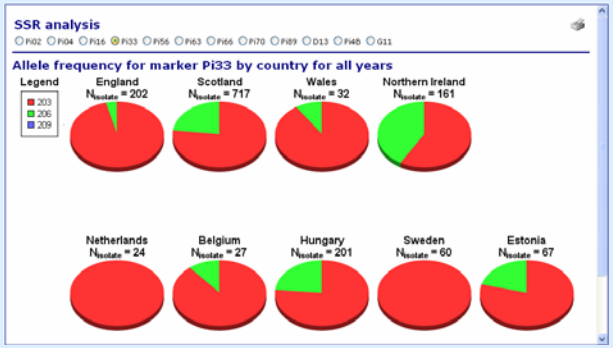
Not 1,3,4,7,10,11

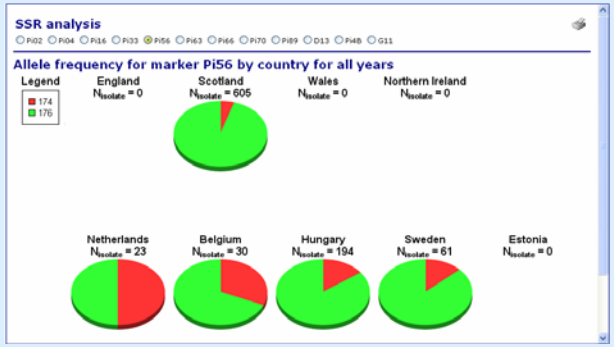
More research on others (R5,R6,R8,R9) needed to understand why virulence frequency lower.

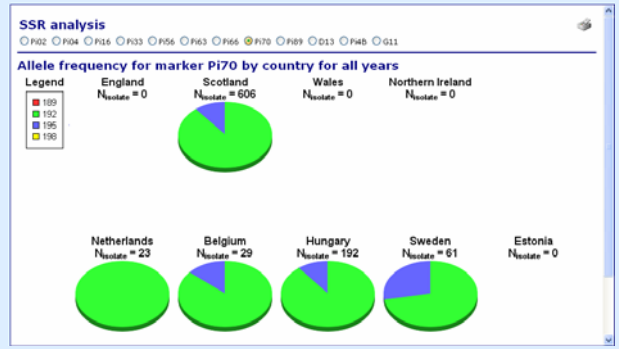
Main aspects

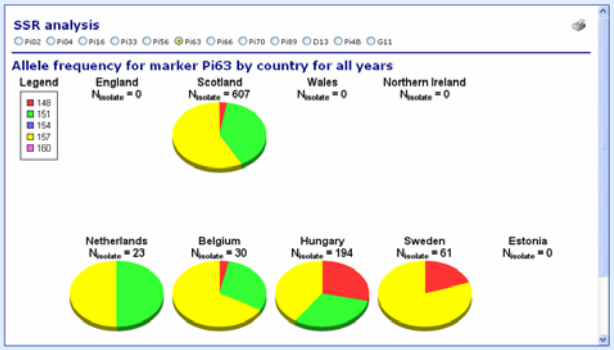
1. Where, when and how blight infection starts
   - primary inoculum
2. Rate of infection and spread
   - foliar
   - tuber
3. Control options
   - fungicide efficacy
   - host resistance
4. Survival
   - cull piles
   - volunteers
   - solanaceous weeds
   - oospores
5. **Changing pathogen population?**
   - **Immigration**
   - **Evolution – mutation & recombination**

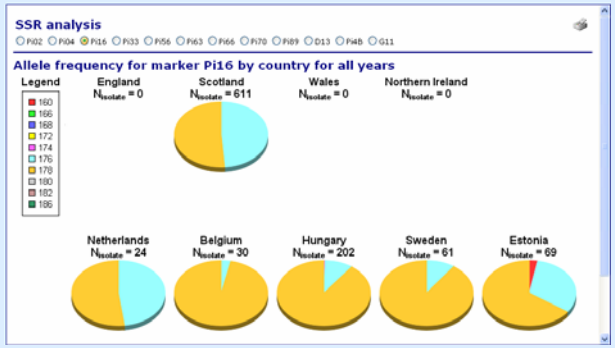
SSR allele distribution

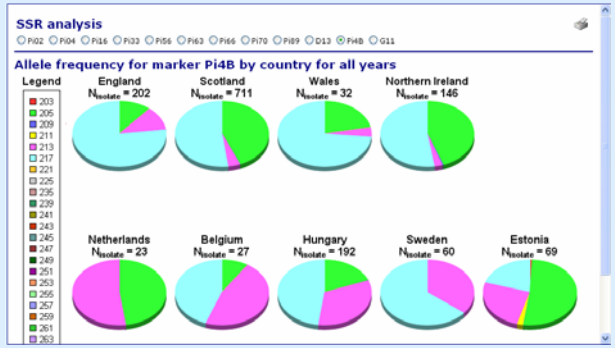


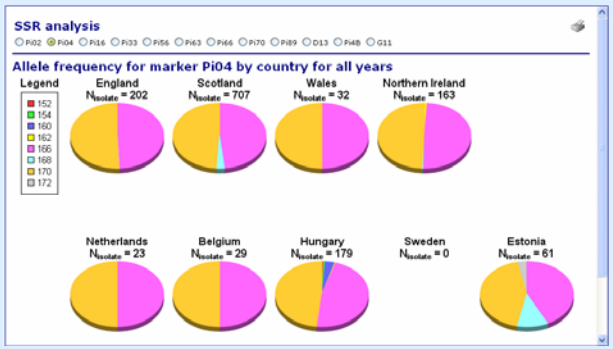


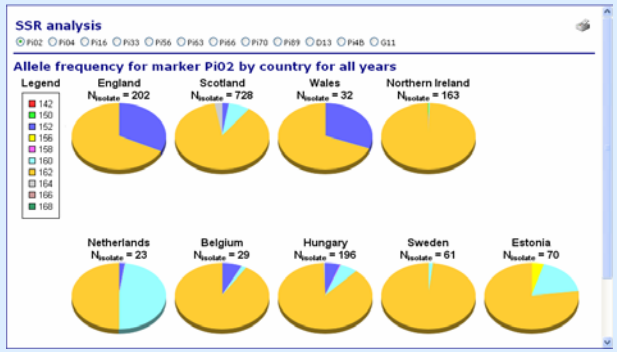


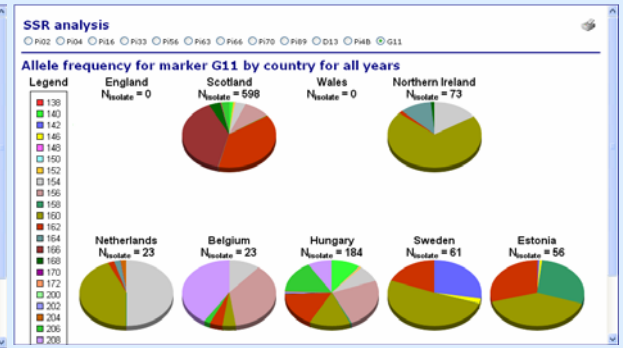




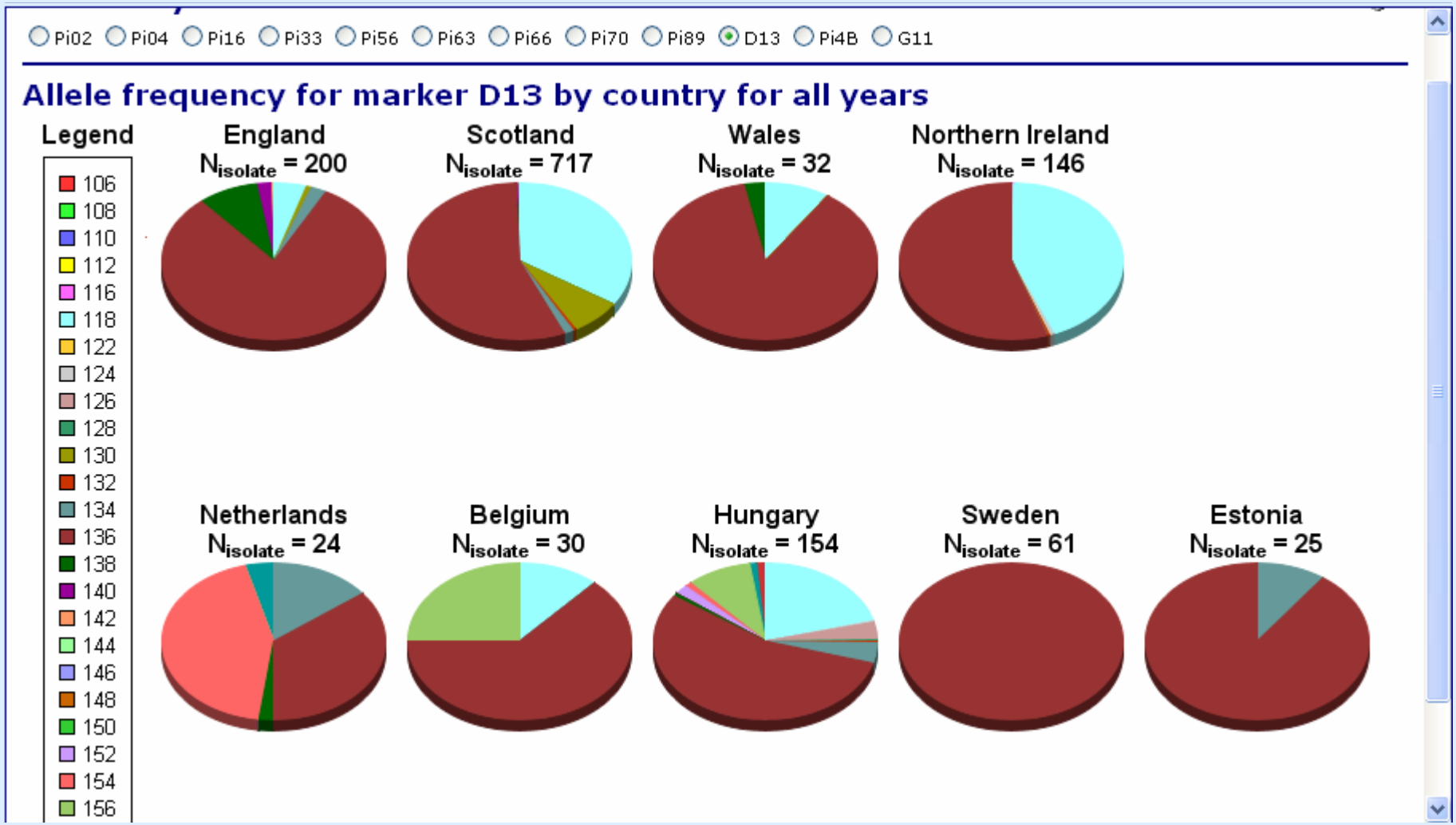




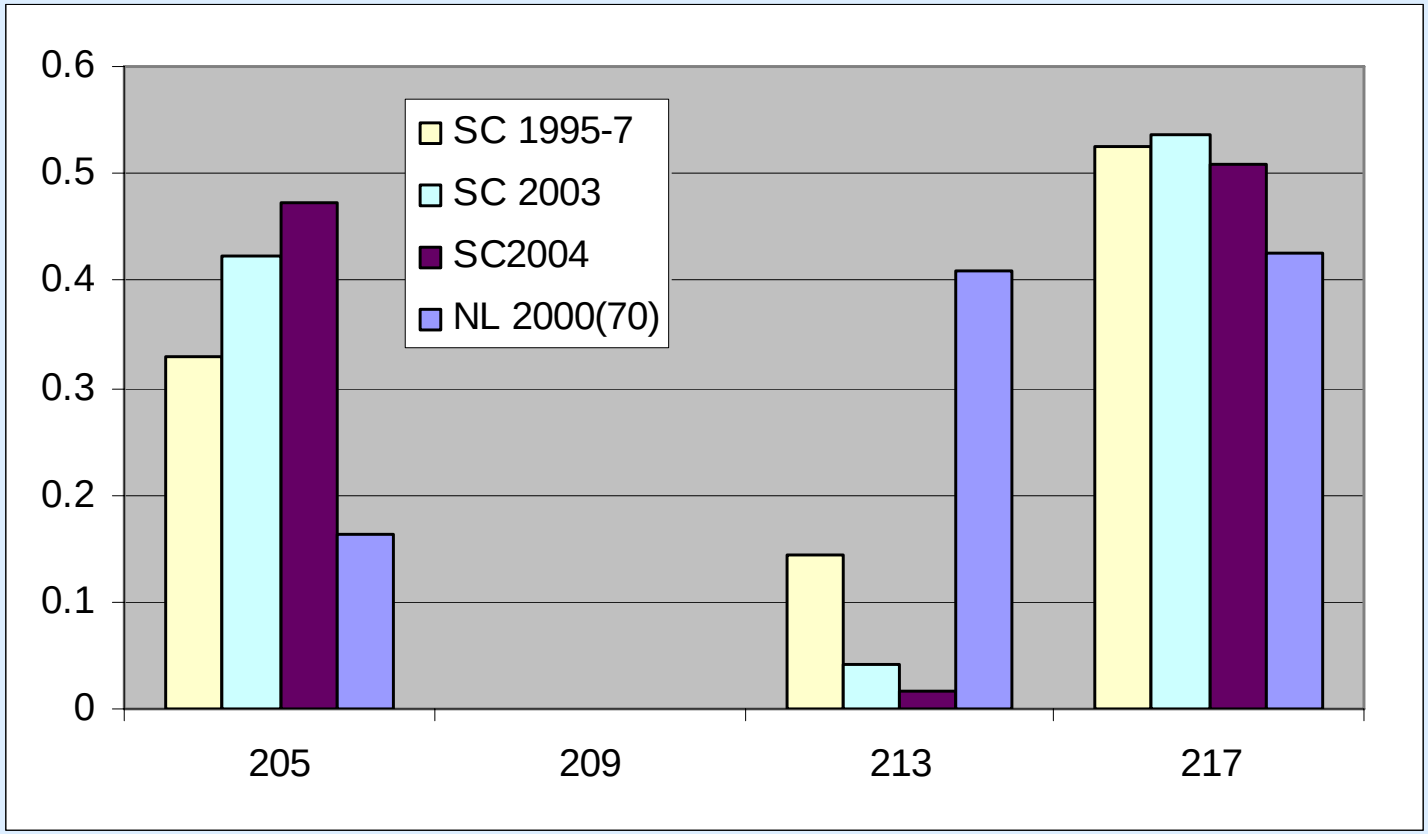




Regional patterns in populations (allele frequencies)



SSR allele change



System updates

- Expansion to include South and Central America
- Improvements to efficiency of data transfer
- Addition of sequence data option

Conclusions & Future plans

- Unique resource to help understand pathogen population change on a range of scales
  (thanks to all data submitters and DIAS)
- *P. infestans* population differs from country to country
- Association between factors observed (e.g. fungicide resistance and mating type)
- New insights into pathogen change emerging

- Database updates and more interpretation at local and EU scale required
- Need to link data on population change with the cause of that change. Identify factors that 'push' or 'pull' population change (e.g. increased aggressiveness and fitness?).
- Exploitation of host resistance (GM-based?) – success of such a strategy will depend on understanding pathogen populations
- Expanding dataset beyond Europe to set context of EU populations