Survey of 2006 Blight populations

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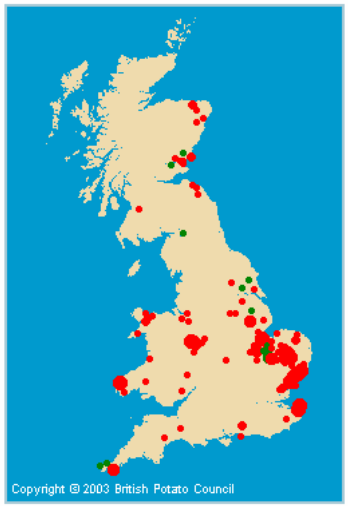
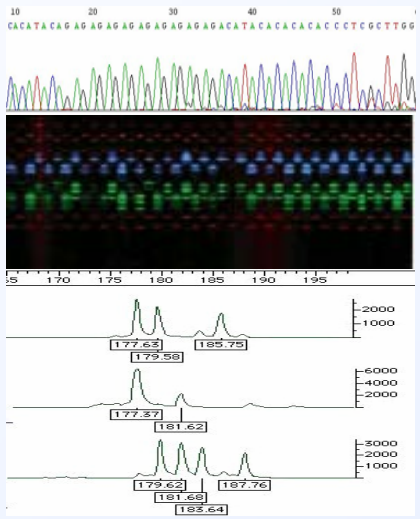
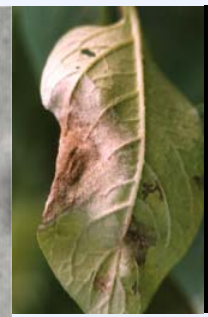
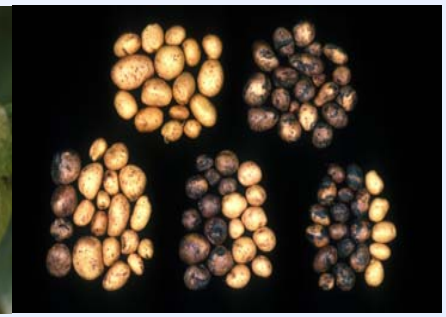
SCOUTS









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Project aims

1. **Determine levels of A2 mating type of *P. infestans* in 2006, 2007 and 2008 potato crops in GB.**

2. **Examine specific early outbreaks to determine the likely source of inoculum with particular emphasis on the roles of oospores and seed-borne blight over the 2006, 2007 and 2008 seasons.**

3. **Determine genetic diversity of GB *P. infestans* populations in 2006, 2007 and 2008 seasons and compare to that in previous years and other states.**

4. **Analysis of oospore production and survival under GB conditions.**

5. **Assess the implications of and risks arising from the observed *P. infestans* population structure and oospore prevalence and survival (incorporating information from other studies).**

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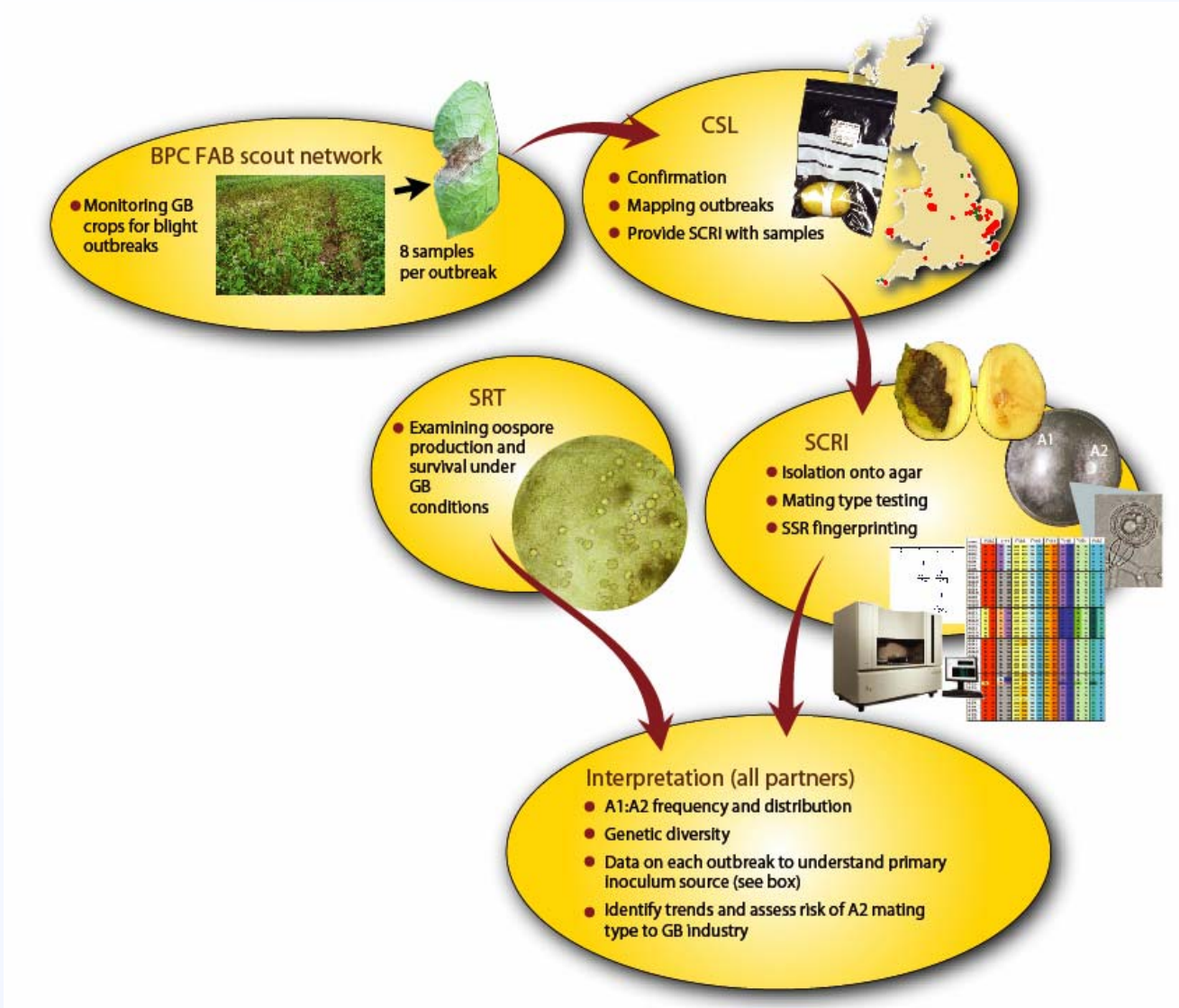
Project Outline (2006-8)



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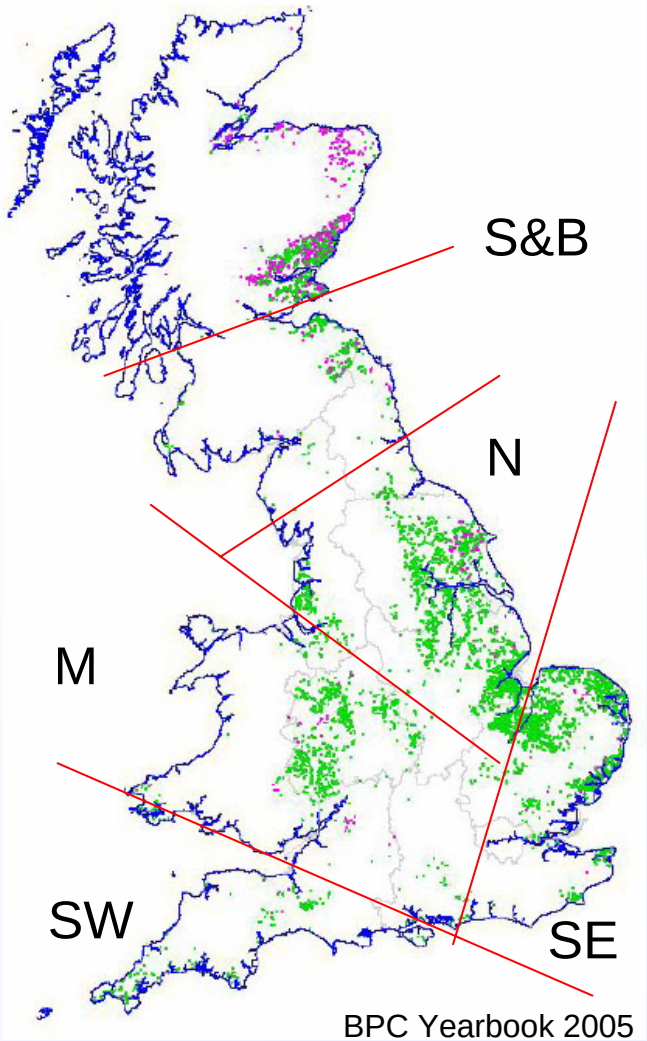
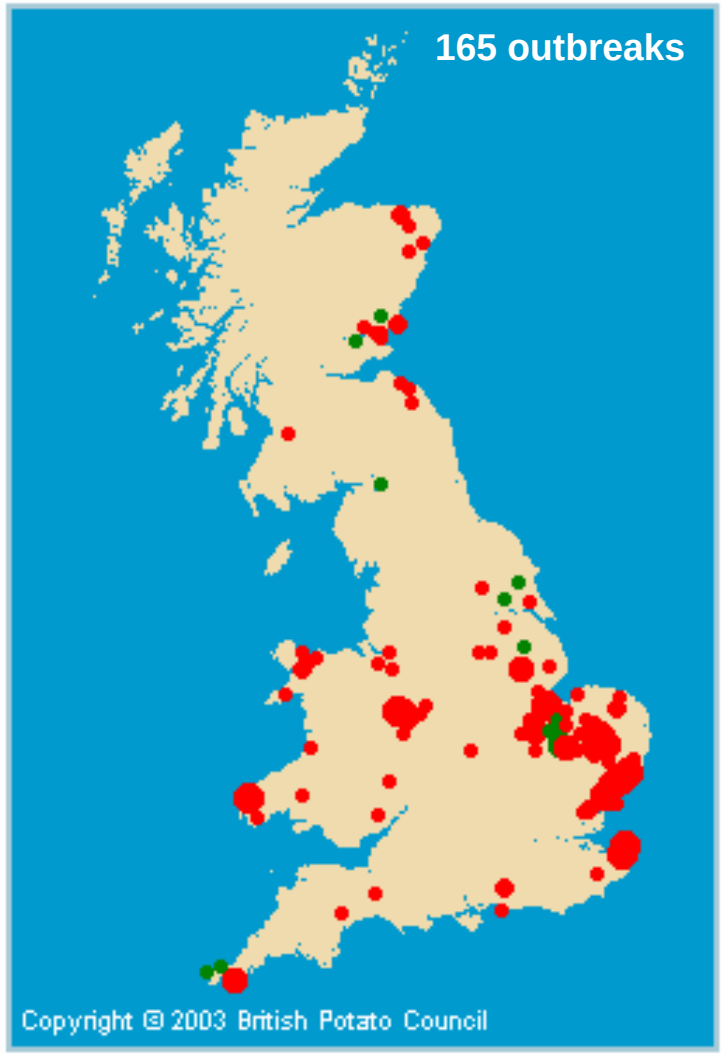
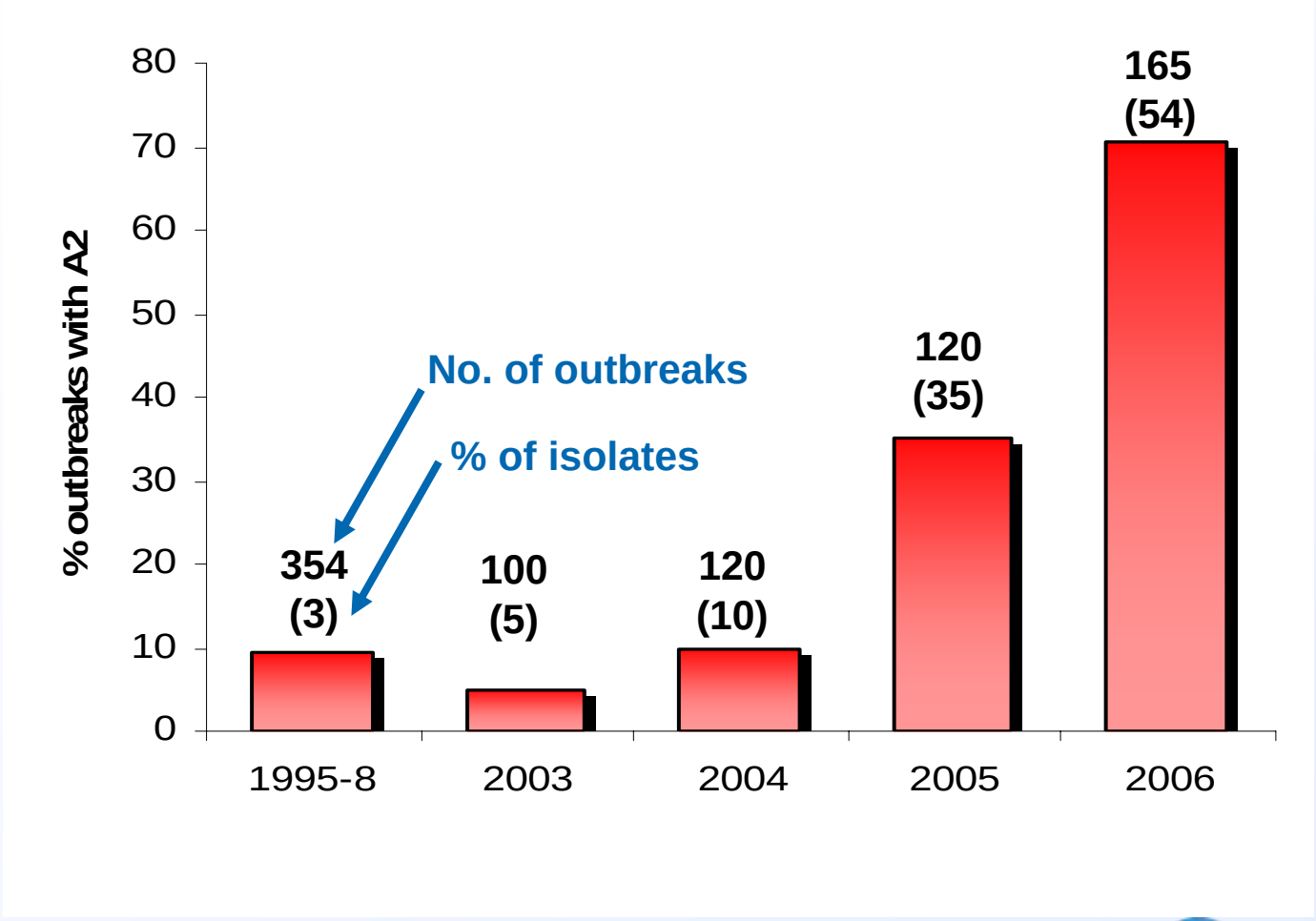
Sample distribution



BPC Yearbook 2005



2006: A2 increased



22% of outbreaks of mixed mating type

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Questions raised

- **More detail on the A2**
  - When and where has change occurred?
  - One type of A2 or a range?
  - Why the increase (mutation – fungicide R – ↑aggressive)?
- **Is it only the A2 type that's changed?**
- **Origins ?**
  - Seed?
  - Airborne?
  - Populations in other EU countries?
- **Implications ?**
  - Risk of oospore formation?
  - Evidence of oospores & early outbreaks?
  - Host resistance breakdown?
  - Fungicide efficacy?
  - Increase in diversity?

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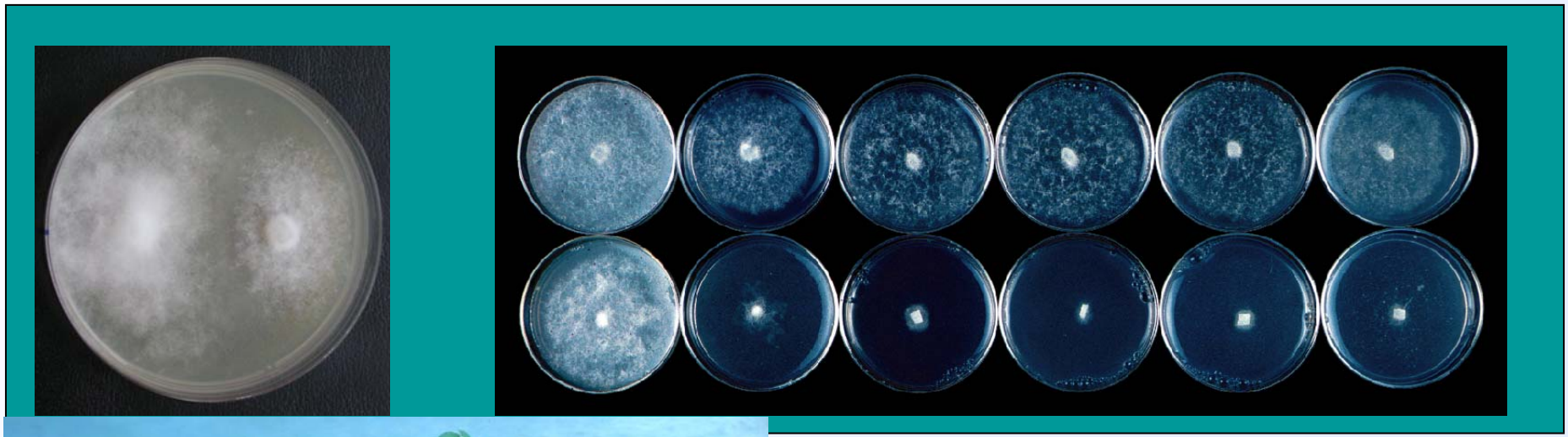
Phenotypic Markers

- Mating type
- Fungicide resistance
- Virulence

Insufficient features to discriminate isolates to detail needed
Time consuming
Expensive



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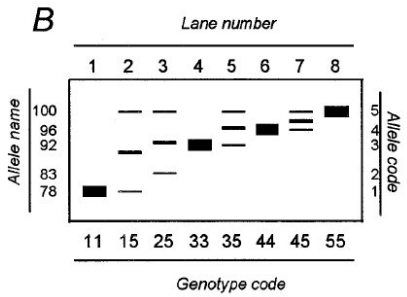
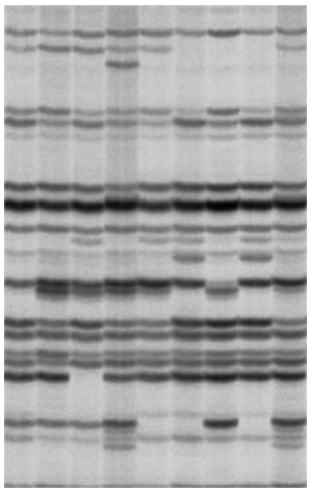
Tools for estimating genetic diversity

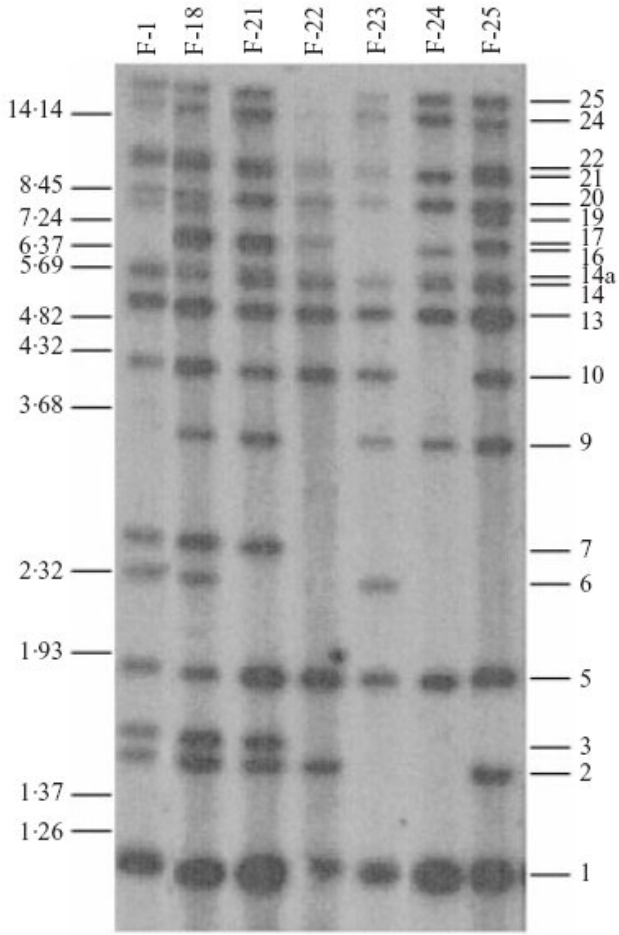
Isozymes

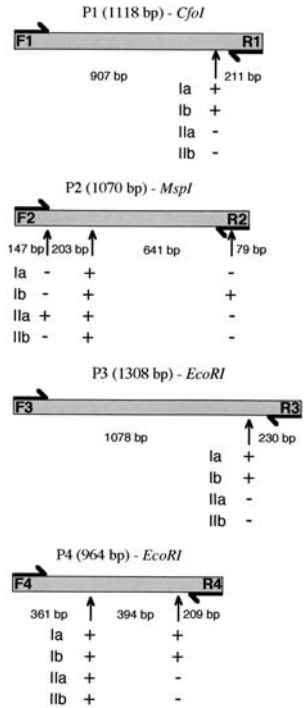


AFLPs



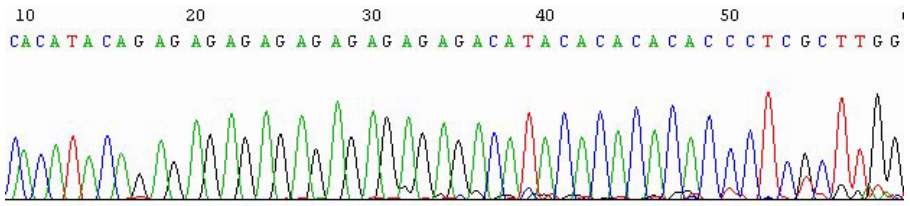
RG57 RFLP fingerprint





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

Simple Sequence Repeats



TCGACCCACGCGTNNGCCCACGCGTCCGGAAGCAGCAGCCTCCGTGCAAGACGATCA

Fwd Primer →

TGCTAGGTCTGAGACTTGC***AGAACTACCGCCCGAGAC***AATTCGACCGAGCGGTGTAG

SSR 10 X 'TG' repeat

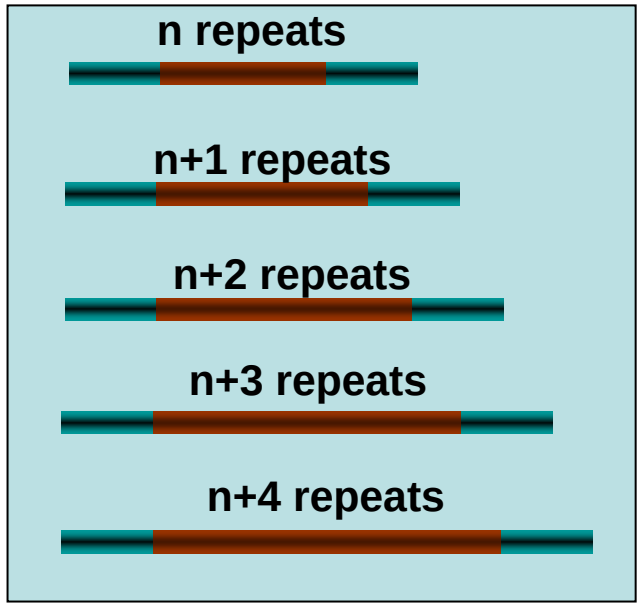
CTGAGTACTACTCACGGAGCTTTGAGAGAGAGAGAGAGAGAGAGCGTGTGGAGCTTC

← **Rev Primer**

GTGGTCTTCGCGCACCTTGCGCTCGTACAAGATGGTGGA***ATGTTCTTGTGACCATCC***

AACCCTGTCCAGCGCTCTCTACAAGGCGATATTGACACTATTGATGTTGCTGTTAAG

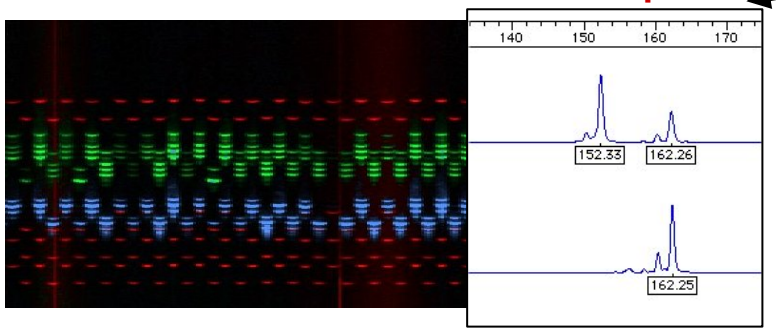
GCCACTACTGCTAAGCAACGTGAAGGAACCGAGGAAAGTGGTCAAAGCTCTACGAAG



PCR
(e.g.162 bp product)

Accurate sizing

n.b diploid



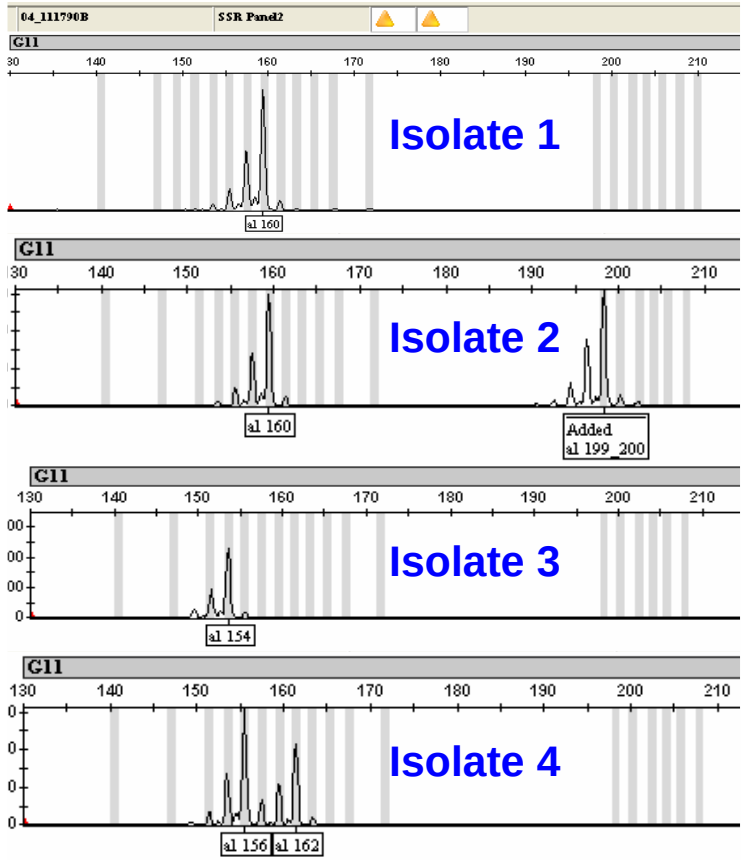
11 markers/loci currently available at SCRI (many potential combinations!)

Genotypic marker – Simple Sequence Repeats

ABI 3730 capillary sequencer

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	X	Y
1																									
2	Isolate	Pi02		G11		Pi33		Pi16		Pi04		Pi4B		Pi56		Pi63		Pi66		Pi70		Pi89		D13	
3	03.01.1.2	162	162	156	162	203	203	176	178	166	170	205	217	176	176	157	157	228	230	192	192	179	181	136	136
4	03.01.3.1	162	162	156	162	203	203	176	178	166	170	205	217	176	176	157	157	228	230	192	192	179	181	136	136
5	03.01.3.2	162	162	156	162	203	203	176	178	166	170	205	217	176	176	157	157	228	230	192	192	179	181	136	136
6	03.01.3.3	162	162	156	162	203	203	176	178	166	170	205	217	176	176	157	157	228	230	192	192	179	181	136	136
7	03.01.4.1	162	162	156	162	203	203	176	178	166	170	205	217	176	176	157	157	228	230	192	192	179	181	136	136
8	03.01.4.3	162	162	156	162	203	203	176	178	166	170	205	217	176	176	157	157	228	230	192	192	179	181	136	136
9	03.01.5.2	162	162	156	162	203	203	176	178	166	170	205	217	176	176	157	157	228	230	192	192	179	181	136	136
10	03.02.1.2	162	162	166	166	203	203	176	178	166	170	205	217	176	176	151	157	228	228	192	192	179	181	118	136
11	03.02.2.2	162	162	166	166	203	203	176	178	166	170	205	217	176	176	151	157	228	228	192	192	179	181	118	136
12	03.02.2.3	162	162	166	166	203	203	176	178	166	170	205	217	176	176	151	157	228	228	192	192	179	181	118	136
13	03.02.3.1	162	162	166	166	203	203	176	178	166	170	205	217	176	176	151	157	228	228	192	192	179	181	118	136
14	03.02.4.1	162	162	166	166	203	203	176	178	166	170	205	217	176	176	151	157	228	228	192	192	179	181	118	136
15	03.02.4.3	162	162	166	166	203	203	176	178	166	170	205	217	176	176	151	157	228	228	192	192	179	181	118	136
16	03.02.5.1	162	162	166	166	203	203	176	178	166	170	205	217	176	176	151	157	228	228	192	192	179	181	118	136
17	03.02.5.2	162	162	166	166	203	203	176	178	166	170	205	217	176	176	151	157	228	228	192	192	179	181	118	136
18	03.02.5.3	162	162	166	166	203	203	176	178	166	170	205	217	176	176	151	157	228	228	192	192	179	181	118	136
19	03.03.1.1	152	162	154	156	203	203	176	178	166	170	217	217	174	176	148	151	228	230	192	195	179	179	136	136
20	03.03.1.2	152	162	154	156	203	203	176	178	166	170	217	217	174	176	148	151	228	230	192	195	179	179	136	136
21	03.03.1.3	152	162	154	156	203	203	176	178	166	170	217	217	174	176	148	151	228	230	192	195	179	179	136	136
22	03.03.2.2	152	162	154	156	203	203	176	178	166	170	217	217	174	176	148	151	228	230	192	195	179	179	136	136
23	03.03.2.3	152	162	154	156	203	203	176	178	166	170	217	217	174	176	148	151	228	230	192	195	179	179	136	136
24	03.03.3.1	152	162	154	156	203	203	176	178	166	170	217	217	174	176	148	151	228	230	192	195	179	179	136	136
25	03.03.3.2	152	162	154	156	203	203	176	178	166	170	217	217	174	176	148	151	228	230	192	195	179	179	136	136
26	03.03.3.3	152	162	154	156	203	203	176	178	166	170	217	217	174	176	148	151	228	230	192	195	179	179	136	136
27	03.04.1.1	162	162	166	166	203	206	176	178	166	170	205	217	176	176	151	157	228	228	192	192	179	181	118	136
28	03.04.1.2	162	162	166	166	203	206	176	178	166	170	205	217	176	176	151	157	228	228	192	192	179	181	118	136
29	03.04.2.1	162	162	166	166	203	203	176	178	166	170	205	217	176	176	151	157	228	228	192	192	179	181	118	132
30	03.04.2.2	162	162	166	166	203	203	176	178	166	170	205	217	176	176	151	157	228	228	192	192	179	181	118	136
31	03.04.3.1	162	162	166	166	203	203	176	178	166	170	205	217	176	176	151	157	228	228	192	192	179	181	118	132
32	03.04.3.3	162	162	166	166	203	203	176	178	166	170	205	217	176	176	151	157	228	228	192	192	179	181	118	132
33	03.04.4.1	162	162	166	166	203	203	176	178	166	170	205	217	176	176	151	157	228	228	192	192	179	181	118	132
34	03.04.5.2	162	162	166	166	203	203	176	178	166	170	205	217	176	176	151	157	228	228	192	192	179	181	118	136
35	03.04.5.3	162	162	166	166	203	203	176	178	166	170	205	217	176	176	151	151	228	228	192	192	179	181	118	136
36	03.17.1.2	162	162	166	166	203	206	176	178	166	170	205	217	176	176	151	157	228	228	192	192	179	181	118	136
37	03.17.2.1	162	162	162	162	203	206	176	178	166	170	205	217	176	176	151	157	228	228	192	192	179	181	118	136
38	03.17.2.2	152	162	154	156	203	203	176	178	166	170	217	217	174	176	148	151	228	228	192	195	179	179	136	136
39	03.17.2.3	162	162	166	166	203	206	176	178	166	170	205	217	176	176	151	157	228	228	192	192	179	181	118	136

Marker G11



RG57 and SSR results do match

SSR type	RG57 type		Pi02	Pi02	D13	D13	Pi33	Pi33	Pi04	Pi04	Pi4B	Pi4B	Pi16	Pi16	G11	G11	G11	Pi56	Pi56	Pi63	Pi63	Pi70	Pi70	Pi89	Pi89
13_A2	blue	**A2**	160	162	136	154	203	203	166	170	205	213	176	178	154	160	0	174	176	151	157	192	192	179	179
3_A2	green	**A2**	162	162	118	136	203	203	166	170	213	213	176	178	154	160	0	176	176	148	157	192	195	179	179
10_A2	yellow	**A2**	162	162	136	136	203	203	166	170	213	217	178	178	162	208	0	176	176	151	157	192	192	181	181
22_A2	RF40	**A2**	162	162	136	136	203	203	166	170	213	217	176	178	140	156	162	174	176	151	157	192	195	179	181

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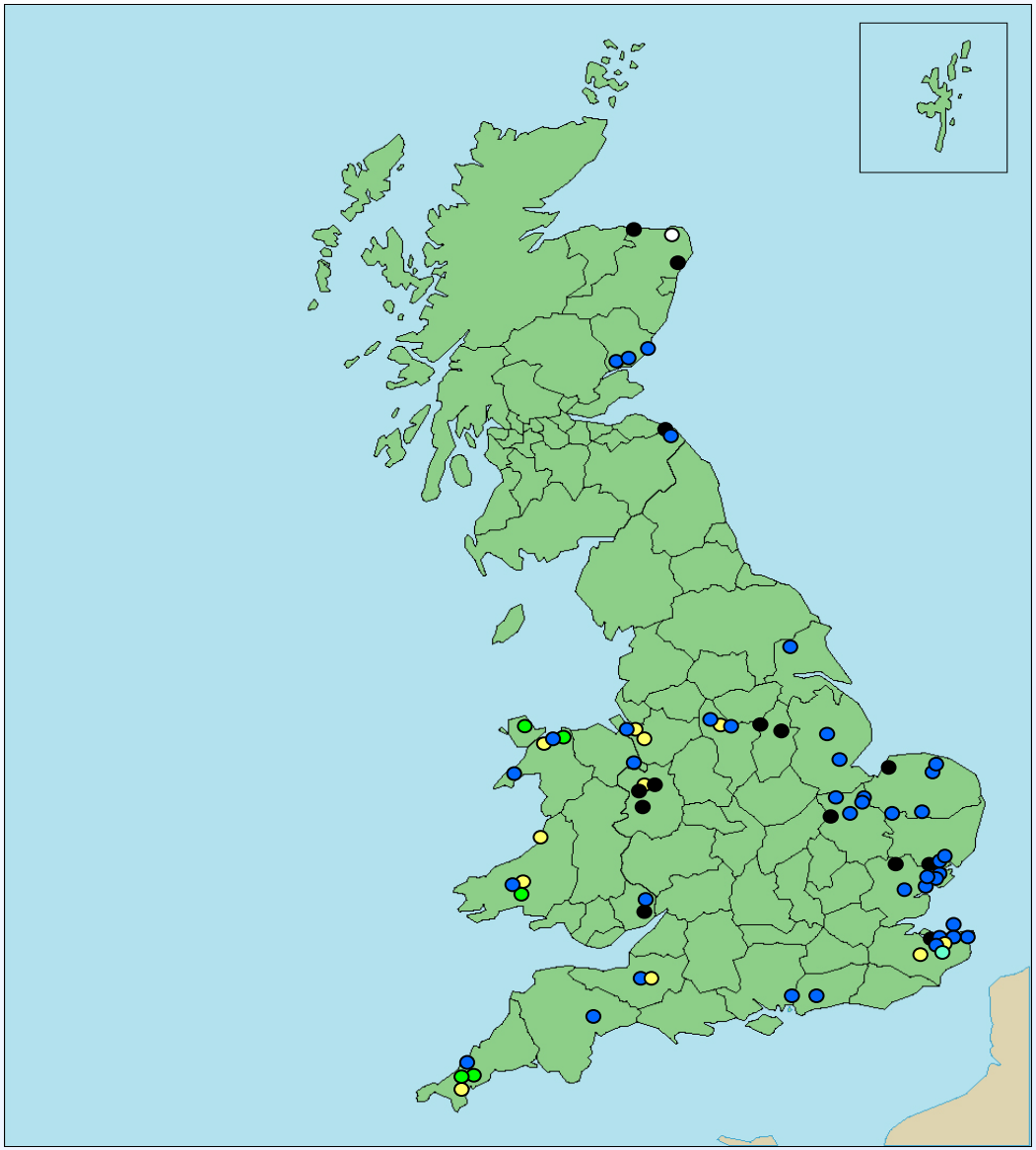
Spatial distribution of A2 types: 2006



Most of increase relates to blue type

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Origins of new A2 type?

- Long present in GB and increased in frequency?
- A mutation of an existing clone?
- Recent entry via seed, airborne spores or other means?

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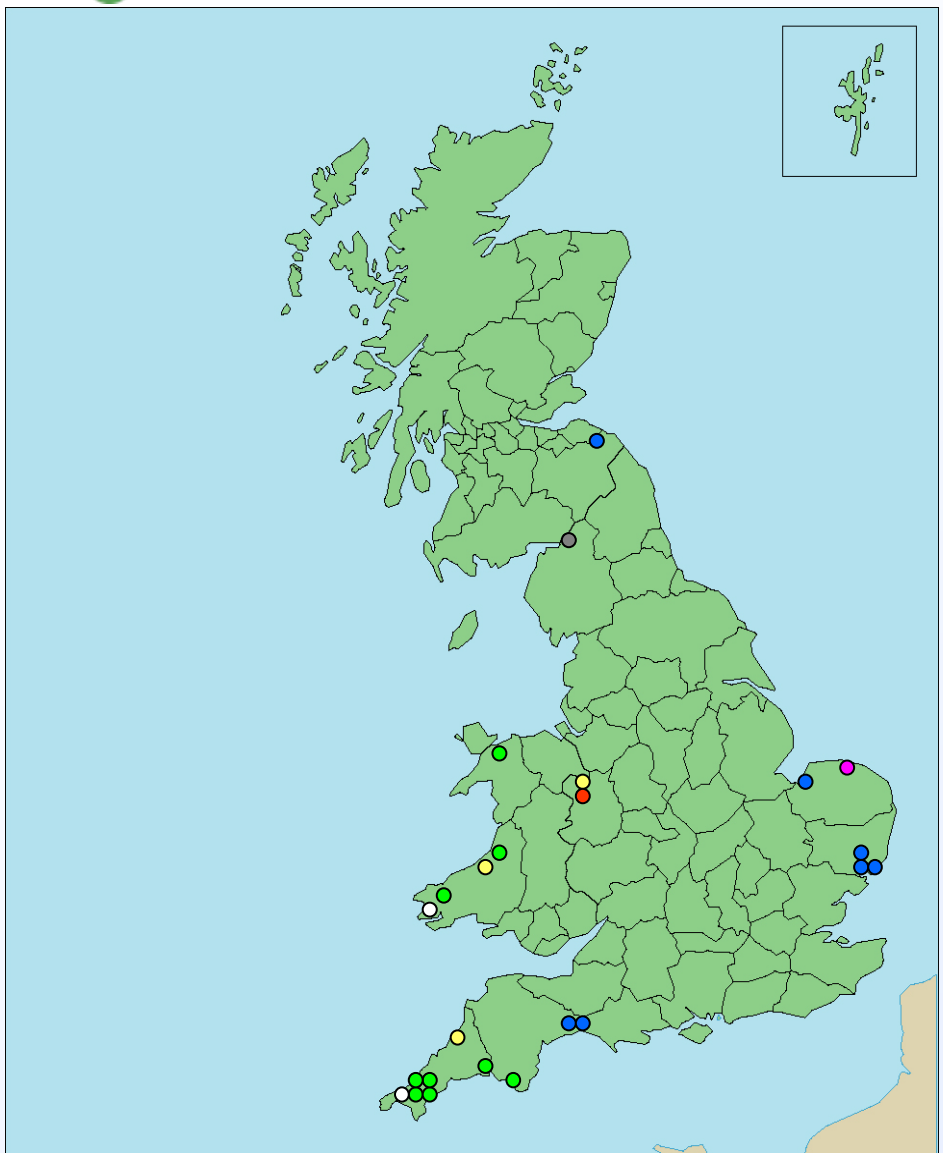
Spatial distribution of A2 types: 2005

Relatively late infection

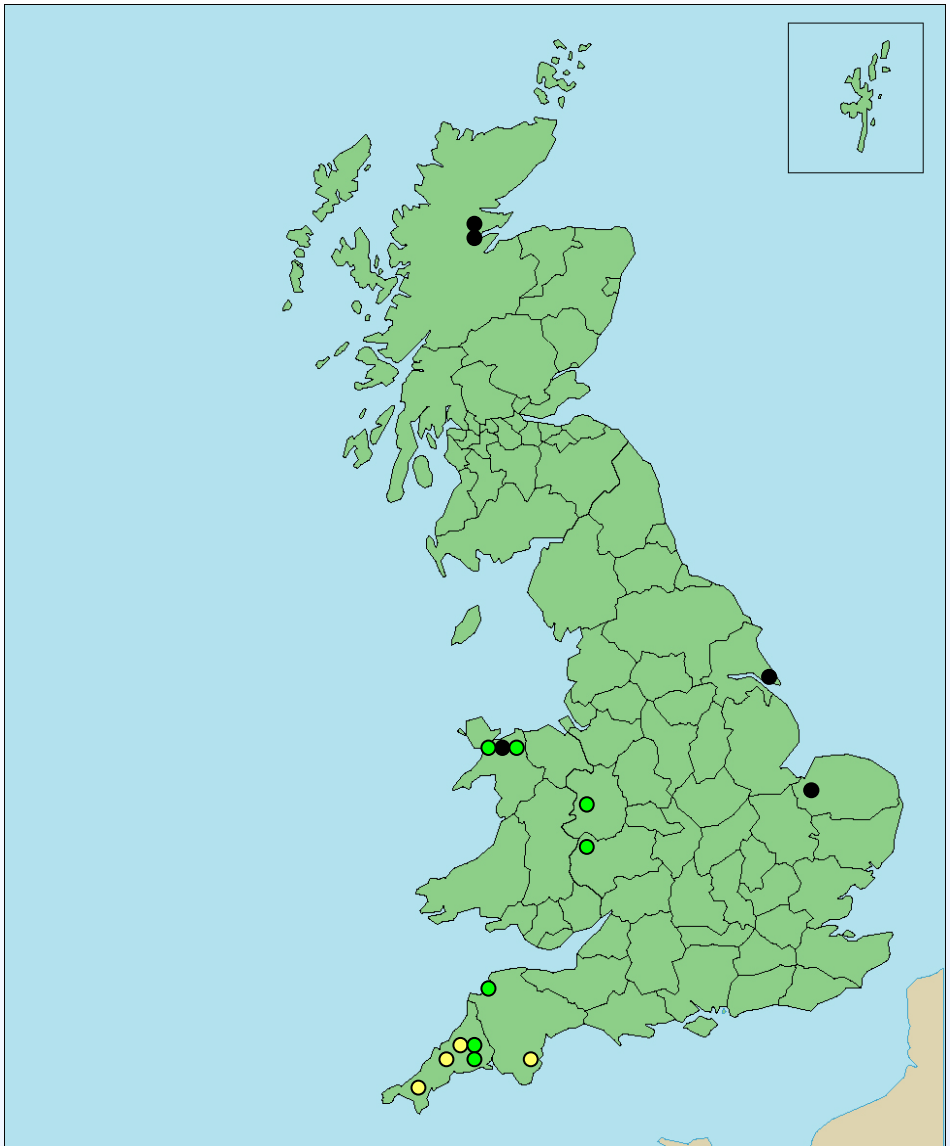


Postcode	County	Date of receipt	mt DNA	
PE31	Norfolk	19/07/2005	Ia	
TD11	ScottishBorders	22/08/2005	Ia	
TA13	Somerset	19/07/2005	Ia	
TA12	Somerset	27/07/2005	Ia	
IP12	Suffolk	18/08/2005	Ia	
IP12	Suffolk	23/08/2005	Ia	
IP12	Suffolk	31/08/2005	Ia	
TR12	Cornwall	23/05/2005	Ia	
TR12	Cornwall	21/06/2005	Ia	
TR2	Cornwall	04/07/2005	Ia	
TR27	Cornwall	07/07/2005	Ia	
PL11	Cornwall	26/08/2005	Ia	
TQ7	Devon	20/07/2005	Ia	
SY23	Ceredigion	12/07/2005	Ia	
SA73	Pembrokeshire	08/06/2005	Ia	
SA48	Ceredigion	21/07/2005	Iia	
PL30	Cornwall	21/07/2005	Iia	
SY4	Shropshire	04/07/2005	Iia	
SY4	Shropshire	04/07/2005	Iia	
NR11	Norfolk	04/07/2005	Iia	
CA6	Cumbria	14/07/2005	Iia	
SA73	Pembrokeshire	22/06/2005	Ia	
AB45	Banffshire	21/09/2005	***	

Seed? Cultivars

M. Piper, M. Peer, Carlingford(x2), Unknown, Estima(x2), Morene

Spatial distribution of A2 types: 2004



Green

HR9	Piper	8 Aug
TF10	Piper	28 Aug
TR14	Marfona	30 Jul
TR4	Caesar	9 Aug
EX39	Wilja	27 Aug
LL61	I. of Skye	12 Aug
LL57	Unkn	16 Aug

Yellow

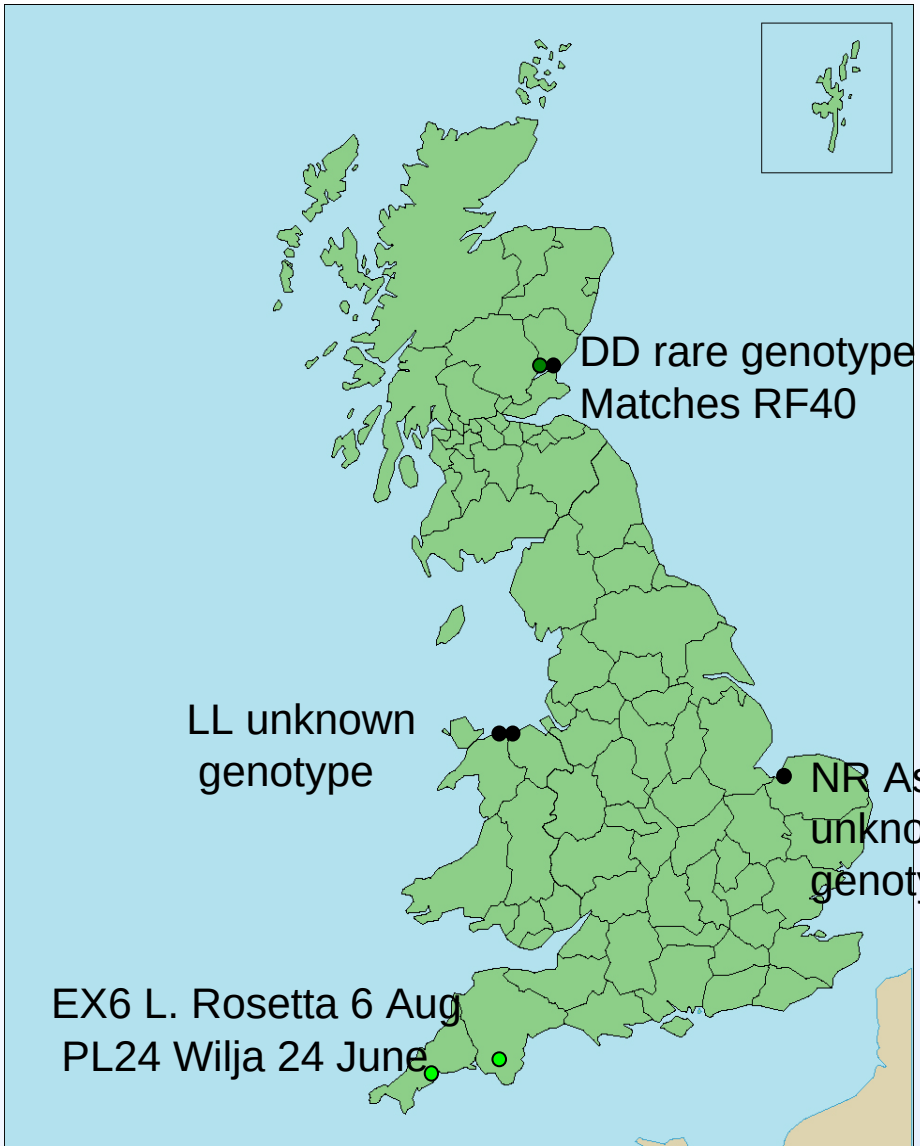
TR14	Marfona	30 Jul
TR11	L. Rosetta	19 Aug
EX7	Cara	3 Sept
TR9	Unkn	13 Sept

Misc

HU12	Dell	31 Aug
IV11	K.Pink	20 Jul
IV8	K. Pink	26 July
LL53	A Blue	30 July
NR11	Piper	5 Aug

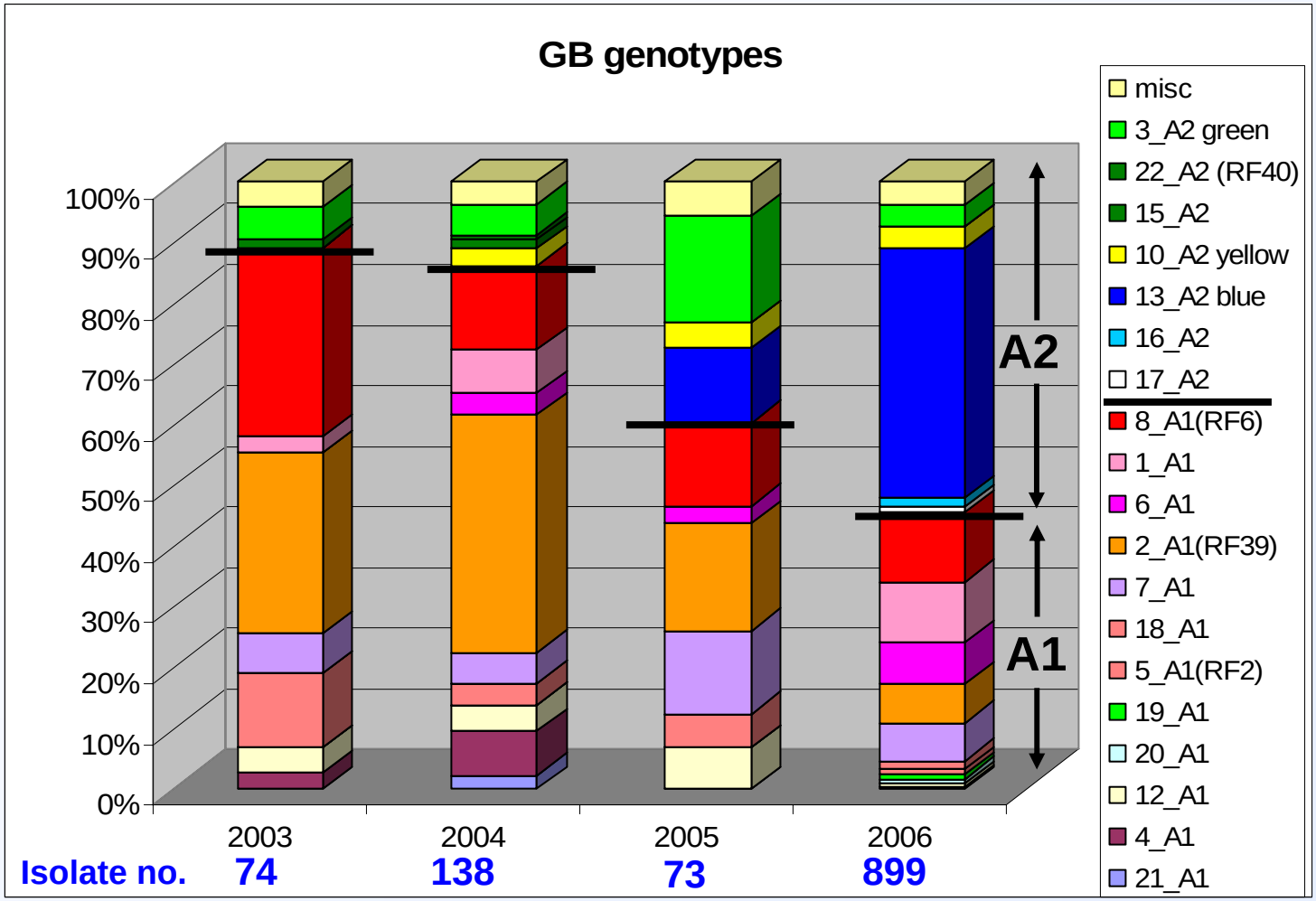
NO BLUE

Spatial distribution of A2 types: 2003



A2 type that was present in 1990s (RF40) has decreased dramatically

Overview of A1 and A2 genotypes (03-06)



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Origins of A2 type?

- Data suggest GB arrival in 2005
- A2 has been increasing across Netherlands and Northern France in recent years
- More detail will come when Eucablight project database is populated with more SSR data

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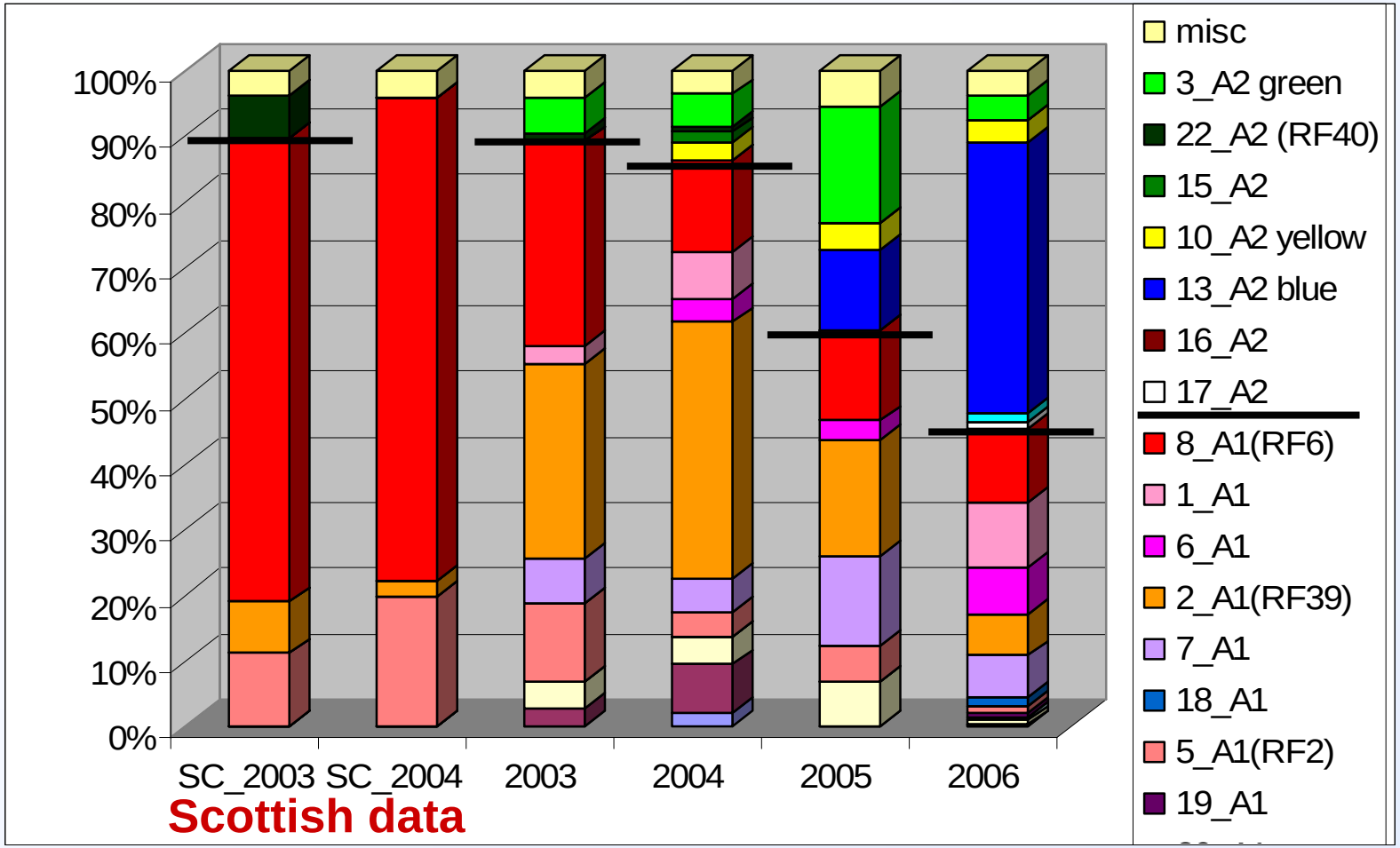
Scottish crops did not contribute new A2s



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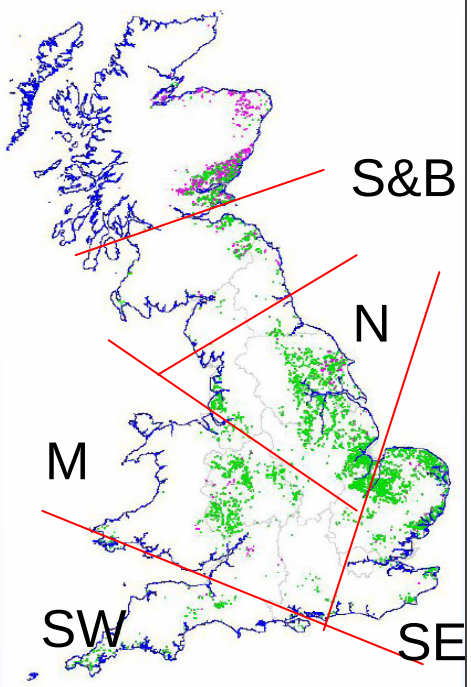
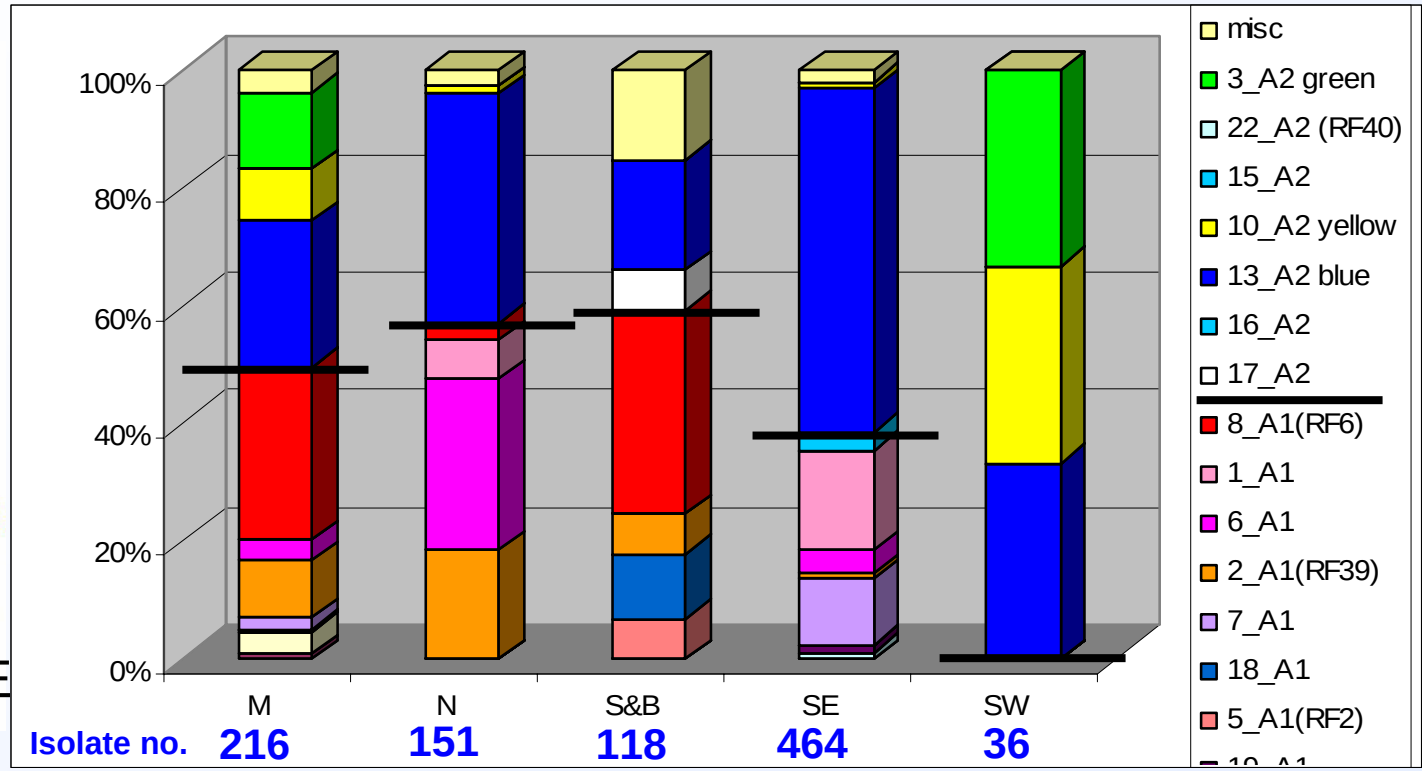
Regional breakdown of 2006 data





S&B blue – all very late (Sept 16th onwards)

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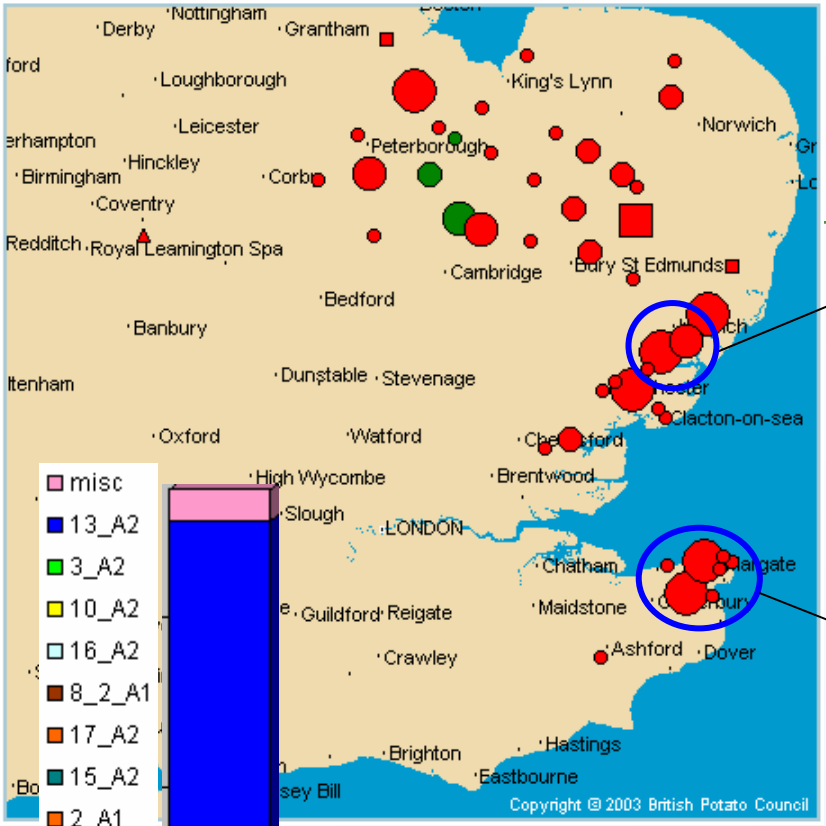

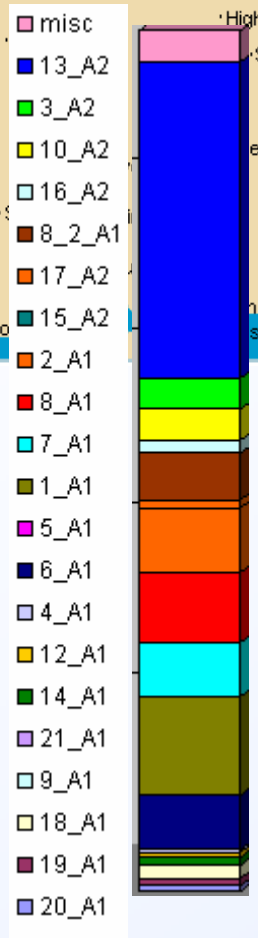

ID	Outbreak size	Variety	Reported	Source	Area	A1	A2
3940	Patch (1m2)	Maris Piper	07-Jun-06	Crop/Other	IP9		
3964	Patch (1m2)	King Edward	07-Jun-06	Crop/Other	IP9		
3952	Scattered through field	Estima	07-Jun-06	Crop/Other	IP10		
4112	Scattered through field	Estima	13-Jun-06	Crop/Other	IP9		
4152	Patch (1m2)	Romano	23-Jun-06	Crop/Other	IP9		
4176	Several Patches	Estima	27-Jun-06	Crop/Other	IP9		
4164	Single Plant	Charlotte	27-Jun-06	Crop/Other	IP10		
4168	Single Plant	Charlotte	27-Jun-06	Crop/Other	IP10		
4204	Scattered through field	Other Maincrop	29-Jun-06	Crop/Other	IP9		

ID	Outbreak size	Variety	Reported	Source	Area	A1	A2
3872	Patch (1m2)	Unknown	10-May-06	Outgrade Pile	CT7		
3876	Several Patches	Unknown	11-May-06	Outgrade Pile	CT7		
3932	Several Patches	Maris Peer	06-Jun-06	Crop/Other	CT12		
3928	Several Patches	King Edward	06-Jun-06	Crop/Other	CT7		
3968	Scattered through field	King Edward	07-Jun-06	Crop/Other	CT7		
3972	Scattered through field	Marfona	07-Jun-06	Crop/Other	CT7		
3992	Scattered through field	Other Maincrop	08-Jun-06	Crop/Other	CT10		
4004	Single Plant	King Edward	08-Jun-06	Crop/Other	CT6		
3988	Several Patches	Desiree	08-Jun-06	Crop/Other	CT7		
3996	Single Plant	King Edward	08-Jun-06	Crop/Other	CT9		
4228	Scattered through field	Other Maincrop	04-Jul-06	Crop/Other	CT3		
4268	Patch (1m2)	Desiree	07-Jul-06	Outgrade Pile	CT3		
4272	Scattered through field	Desiree	07-Jul-06	Crop/Other	CT3		
4276	Scattered through field	King Edward	07-Jul-06	Crop/Other	CT7		
4360	Very Severe	King Edward	21-Jul-06	Crop/Other	CT3		
4368	Scattered through field	Estima	24-Jul-06	Crop/Other	CT3		

SS3

The search for oospores: ‘Superscout’ – objectives and methods

- **Targeted outbreak sampling: early stage of development.**
- **16 Superscouts nominated and ‘trained’**
- **Intensive sampling and recording (up to 32 lesions)**
- **Determine inoculum source from outbreak data**

  Lesion type – stem or leaf and stage of development, mosaic-like symptoms
  Lesion position in canopy- especially lesions at soil level (ASL)
  Nature of outbreak – number of foci, wind direction, plant height

- **Analysis of resultant isolates**

  Mating type
  DNA fingerprinting

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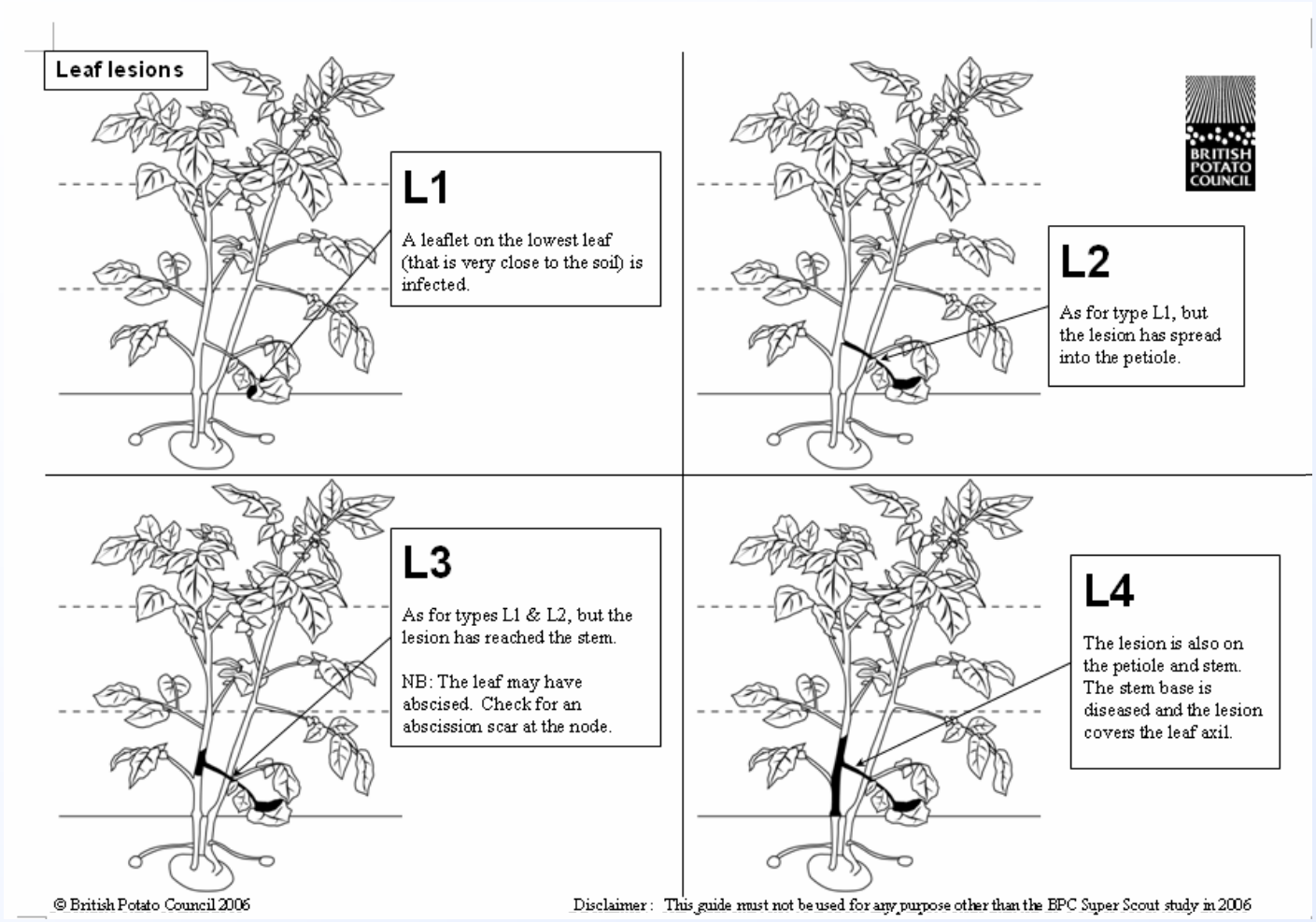
Lesion guide



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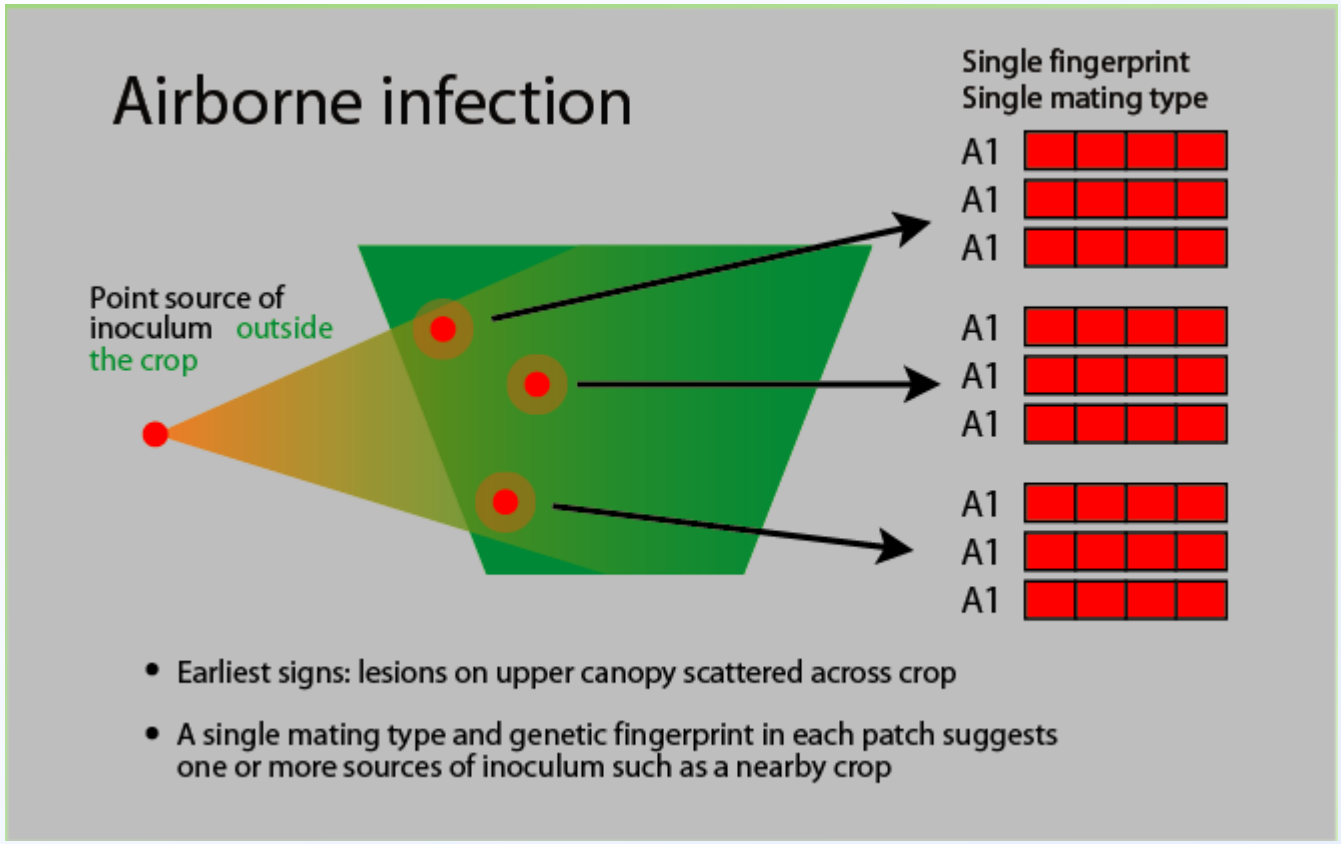
‘Superscout’ – interpretation (1)



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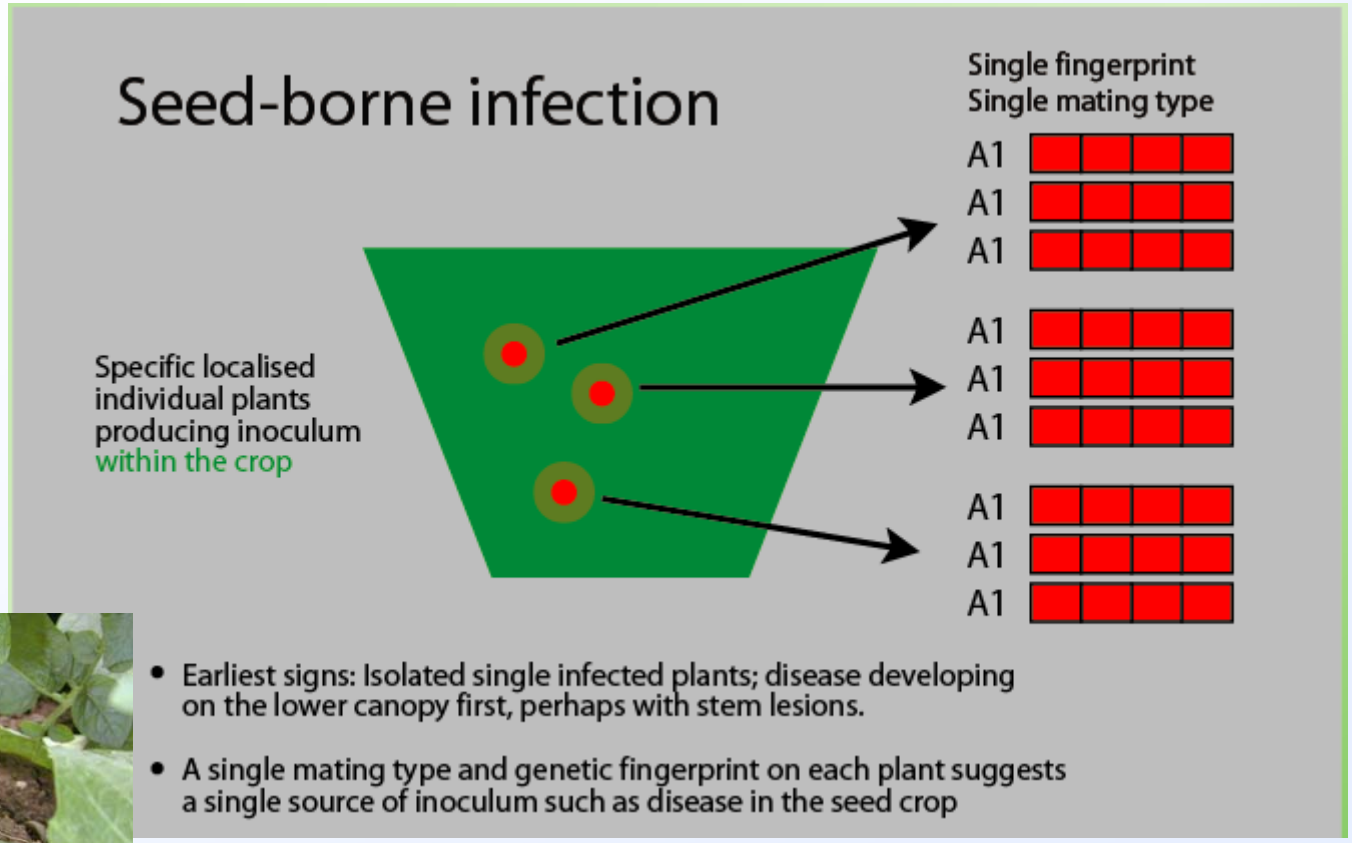
‘Superscout’ – interpretation (2)



Ruairidh Bain

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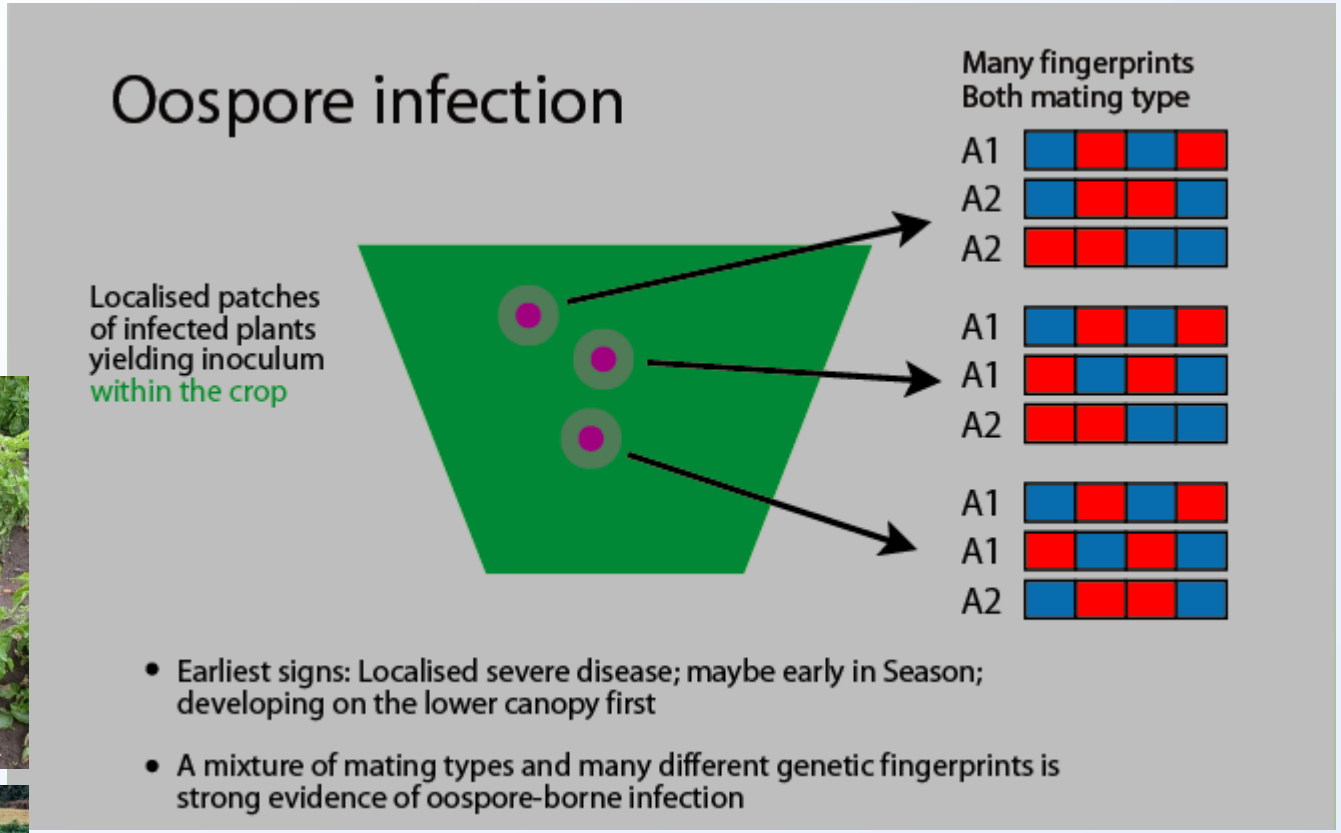
‘Superscout’ – interpretation (3)

Hansen & Bodker

Andersson



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‘Superscout’ – findings

- 8 outbreaks sampled
  5 June to 18 July
  6-32 isolates per outbreak
  Cambs, Kent, Shropshire, Aberdeenshire, Suffolk, Norfolk

- Value of fingerprint data in determining outbreak source

CT7		Dorirea	1	1	A	L1	ASL	06_SS3_01																															
CT7	A1	Dorirea	1	1	B	L1	ASL	06_SS3_02	160	162	0	136	136	0	203	203	0	166	170	0	213	217	0	0	0	140	162	0	176	176	148	157	0	192	192	179	195	199
CT7	A1	Dorirea	1	2	A	L1	ASL	06_SS3_03	160	162	0	136	136	0	203	203	0	166	170	0	213	217	0	178	178	140	162	0	176	176	148	157	0	192	192	181	197	199
CT7	A1	Dorirea	1	2	B	L1	B	06_SS3_04	160	162	0	136	136	0	203	203	0	166	170	0	213	217	0	178	178	140	162	0	176	176	148	157	0	192	192	181	197	199
CT7	A1	Dorirea	1	3	B	L1	B	06_SS3_05	160	162	0	136	136	0	203	203	0	166	170	0	213	217	0	178	178	140	162	0	176	176	148	157	0	192	192	181	197	199
CT7	A1	Dorirea	1	3	A	L1	ASL	06_SS3_06	160	162	0	136	136	0	203	203	0	166	170	0	213	217	0	178	178	140	162	0	176	176	148	157	0	192	192	179	195	199
CT7	A1	Dorirea	2	1	B	L1	ASL	06_SS3_07	160	162	0	136	136	0	203	203	0	166	170	0	213	217	0	178	178	140	162	0	176	176	148	157	0	192	192	181	197	199
CT7	A1	Dorirea	2	1	A	L1	ASL	06_SS3_08	160	162	0	136	136	0	203	203	0	166	170	0	213	217	0	178	178	140	162	0	176	176	148	157	0	192	192	181	197	199
CT7		Dorirea	2	2	B	L1	B	06_SS3_09																															
CT7		Dorirea	2	2	A	L1	ASL	06_SS3_10																															
CT7	A1	Dorirea	3	1	B	L1	ASL	06_SS3_11	160	162	0	136	136	0	203	203	0	166	170	0	213	217	0	178	178	140	162	0	0	0	148	157	0	192	192	179	195	199
CT7	A1	Dorirea	3	1	A	L1	B	06_SS3_12	160	162	0	136	136	0	0	0	0	166	170	0	213	217	0	0	0	140	162	0	176	176	148	157	0	192	192	179	195	199
CT7	A1	Dorirea	3	2	A	L1	B	06_SS3_13	160	162	0	136	136	0	203	203	0	166	170	0	213	217	0	178	178	140	162	0	176	176	148	157	0	192	192	179	195	199
CT7	A1	Dorirea	3	2	B	L1	B	06_SS3_14	160	162	0	136	136	0	203	203	0	166	170	0	213	217	0	178	178	140	162	0	176	176	148	157	0	192	192	179	195	199
CT7	A2	Dorirea	4	1	B	L1	ASL	06_SS3_15	160	162	0	136	154	0	203	203	0	166	170	0	205	213	0	176	178	154	160	0	174	176	151	157	0	192	192	179	179	0
CT7	A2	Dorirea	4	1	A	S4	M	06_SS3_16	160	162	0	136	154	0	203	203	0	166	170	0	205	213	0	176	178	154	160	0	174	176	151	157	0	192	192	179	179	0
CT7	A2	Dorirea	4	2	B	L1	ASL	06_SS3_17	160	162	0	136	154	0	203	203	0	166	170	0	205	213	0	176	178	154	160	0	174	176	151	157	0	192	192	179	179	0
CT7	A2	Dorirea	4	2	A	L1	ASL	06_SS3_18	160	162	0	136	154	0	203	203	0	166	170	0	205	213	0	176	178	154	160	0	174	176	151	157	0	192	192	179	179	0

- Given oospore threat - continuing into 2007

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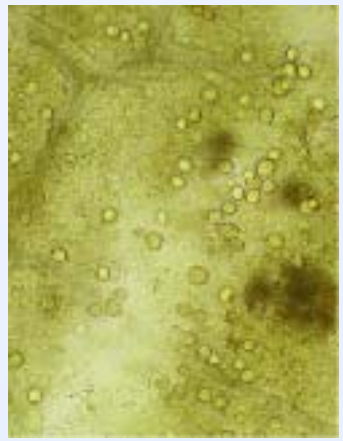
Oospore biology (SRT, Bangor)

- Blight epidemics with dominant A1 and A2 genotypes to be established in polytunnels
- Leaves examined for presence of oospores
- Diseased foliage allowed to rot into soil
- Soil sampled to monitor oospore germination
- Soil stored to monitor oospore longevity
- Outbreaks in subsequent crops sampled
- Samples fingerprinted at SCRI to monitor whether inoculum sexual or asexual



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Conclusions and future work

- **The GB *P. infestans* population is in flux (both A1 and A2)**
- **Predominantly clonal populations to date perhaps due to rotations?**
- **Risk of oospore formation continues to increase along with threat of early epidemics and more rapid pathogen adaptation**
- **Increase in aggressiveness?**
  This is implied but impact on prediction (DSS) & control not known
- **Impact on resistance ratings?**
  IVT & breeders need to be using contemporary isolates
- **Impact on efficacy of current fungicide programmes?**
  fungicide testing needs to be against contemporary isolates

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