



An update on the status of genotype 13 in GB

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Scouts
Potato Council FAB
Scottish Government





- Survey results for 2009 season
- Local/regional spread of late blight – SSR tagging
- Oospore experiments
- Conclusions & implications

2009 *P. infestans* Population

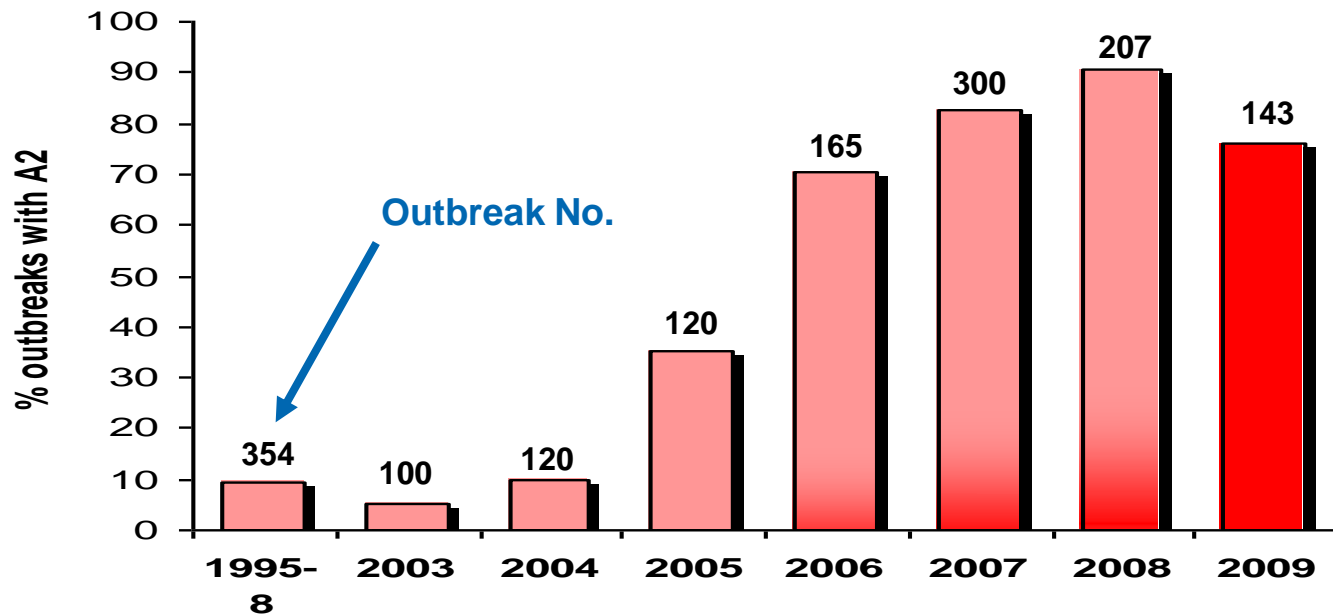


- 4 leaves per outbreak
- 154 positive outbreaks recorded
- 143 outbreaks with isolates recovered

- 681 samples arrived
- 533 isolates recovered
- 533 mating type tested
- **71.5% A2 mating type**



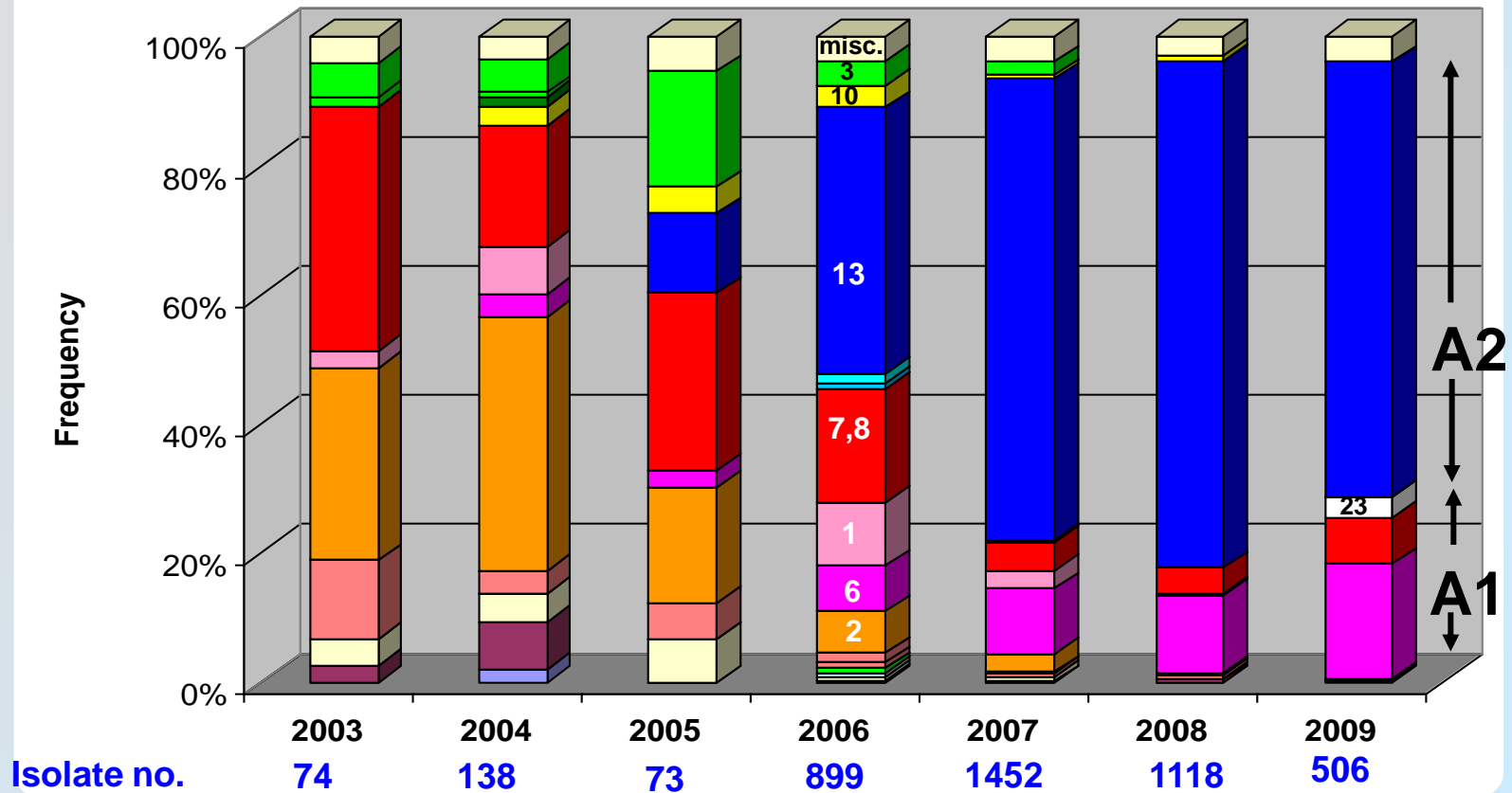
A2 mating type by outbreak



Population diversity 2003-9



GB genotypes

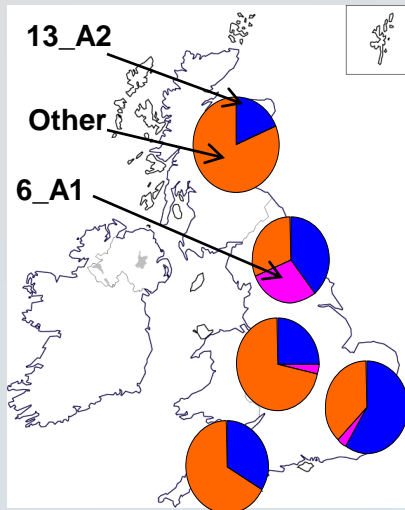


Genotype 23_A1 – tomato, petunia & potato. Increase?
 Misc – no increase

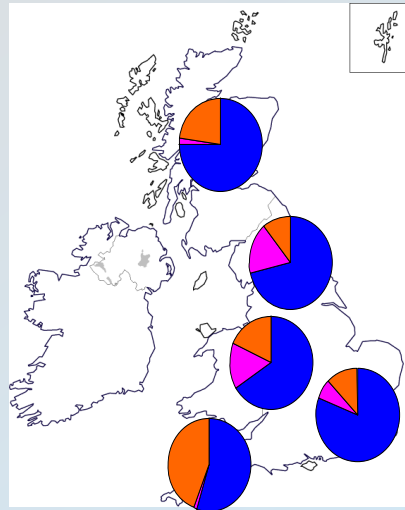
Regional distribution over time



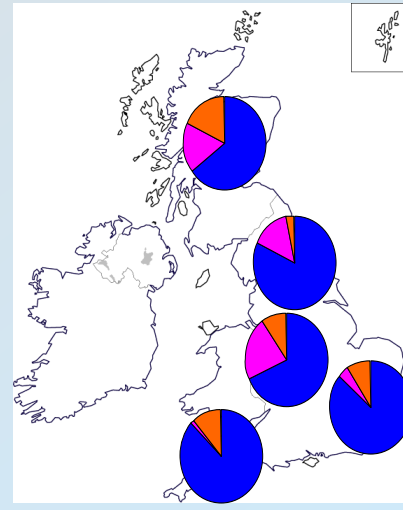
2006



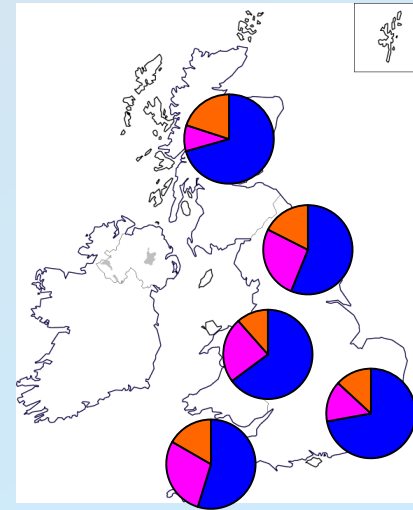
2007



2008



2009



- 13_A2 spread from South East
- 6_A1 spread from North – origin NL?

13_A2 – SSR variation within clonal lineage



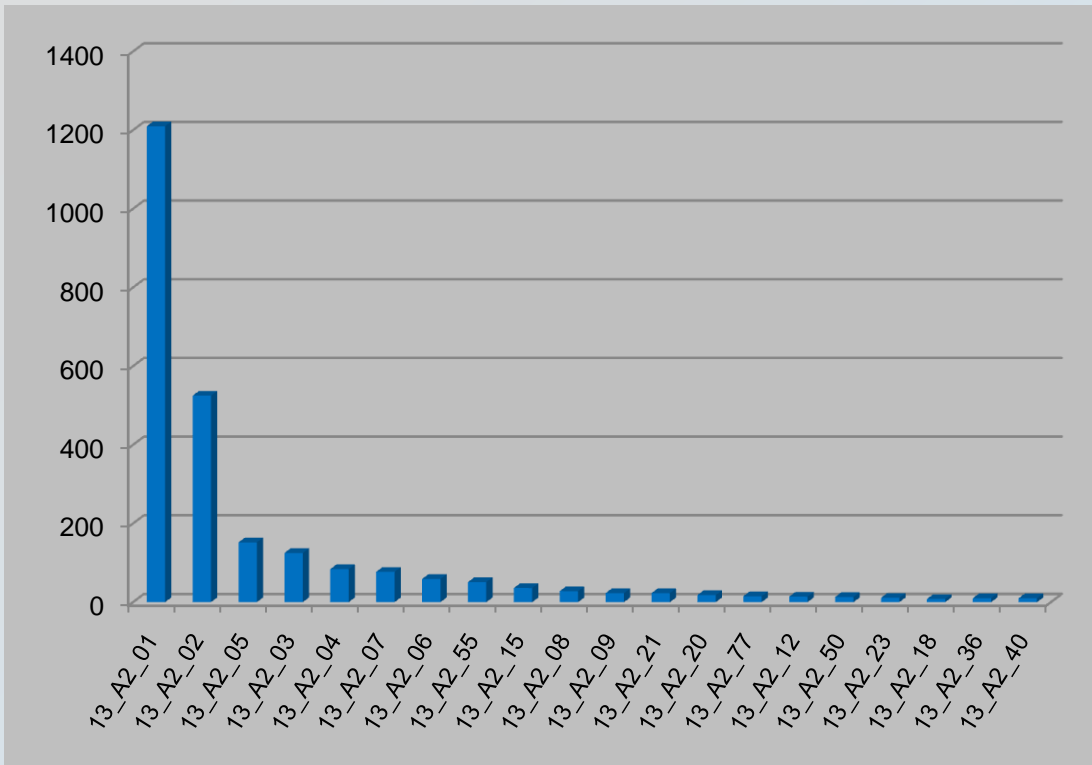
P102	P102	P102	D13	D13	D13	P33	P33	P104	P104	P14B	P14B	P116	P116	G11	G11	G11	P66	P66	P63	P63	P170	P170	P68	P68
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1500
13_A2
isolates
(stable)

13_A2 →

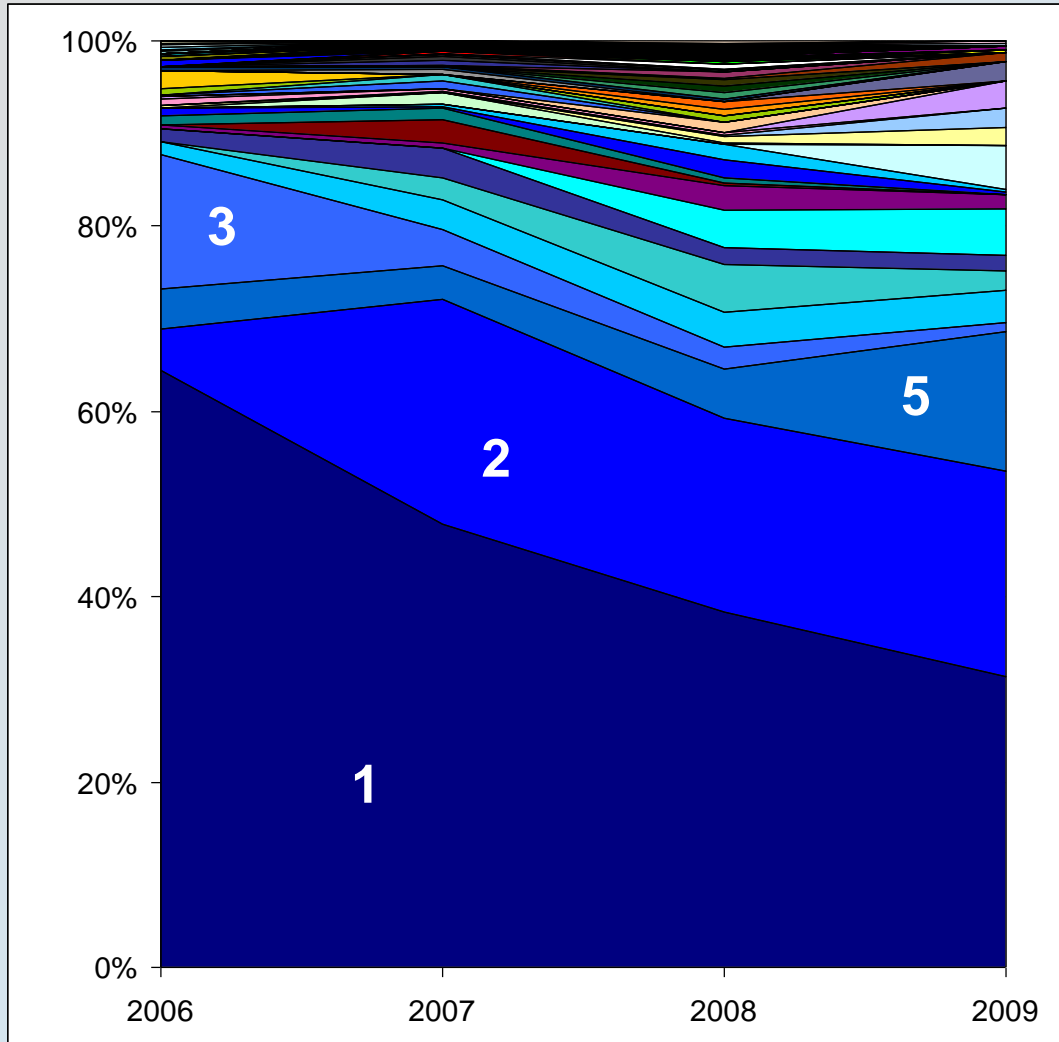
other GB
genotypes

Variation **within** genotype 13_A2



- Minor changes in two markers yields distinct genetic 'signatures' **within** 13_A2 blight.
- One type (13_A2_1) dominates the 2003-2009 population
- Can be used to track inoculum spread

Variation within genotype 13_A2 over time

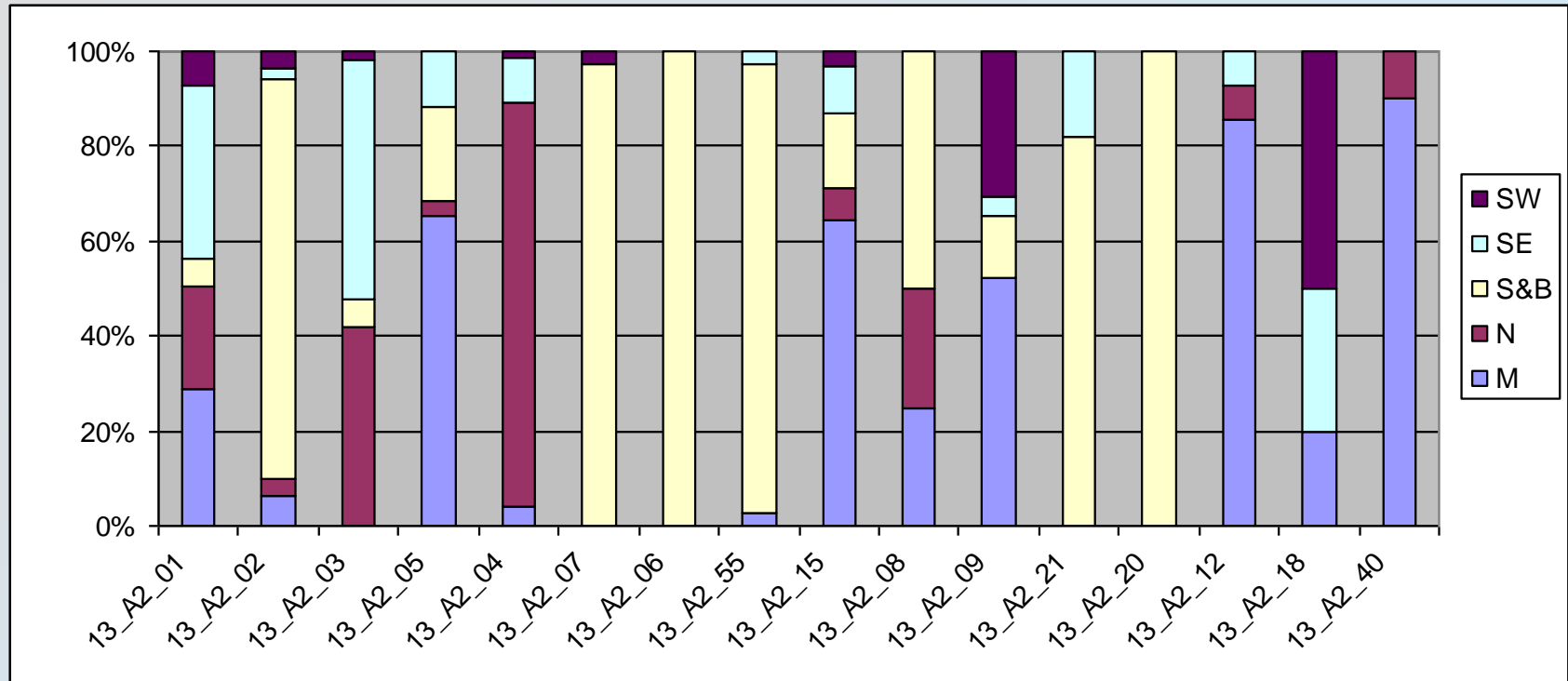


80 minor variants.

Each likely similar properties in field but can be used to track inoculum spread

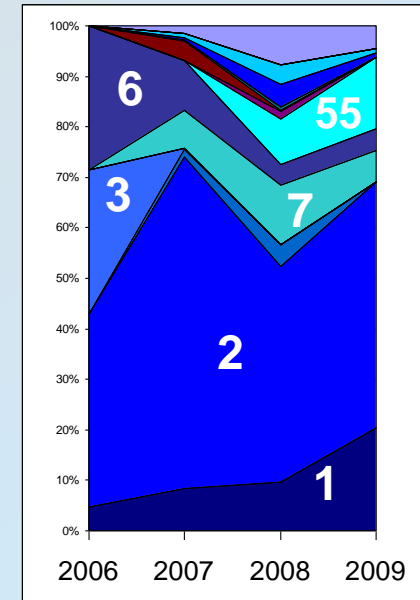
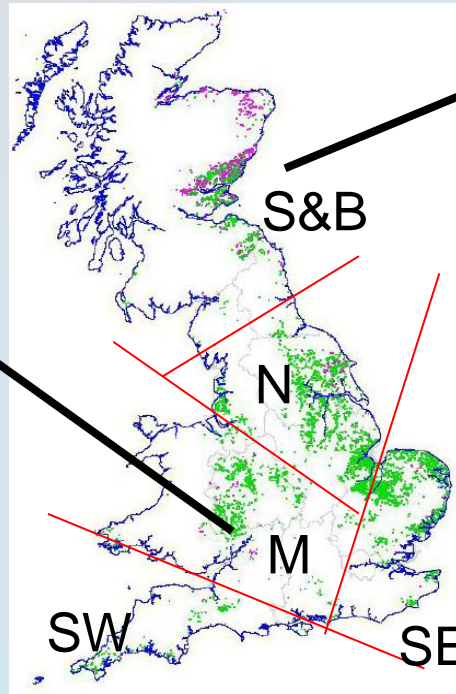
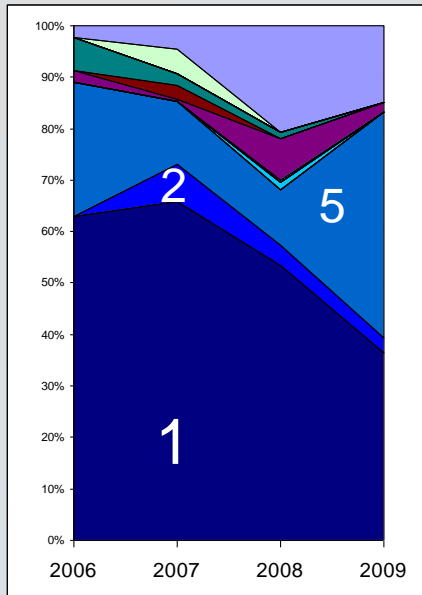
Over whole of GB the original type (1) from 2006 is declining as new forms emerge.

Variation within genotype 13_A2 by region



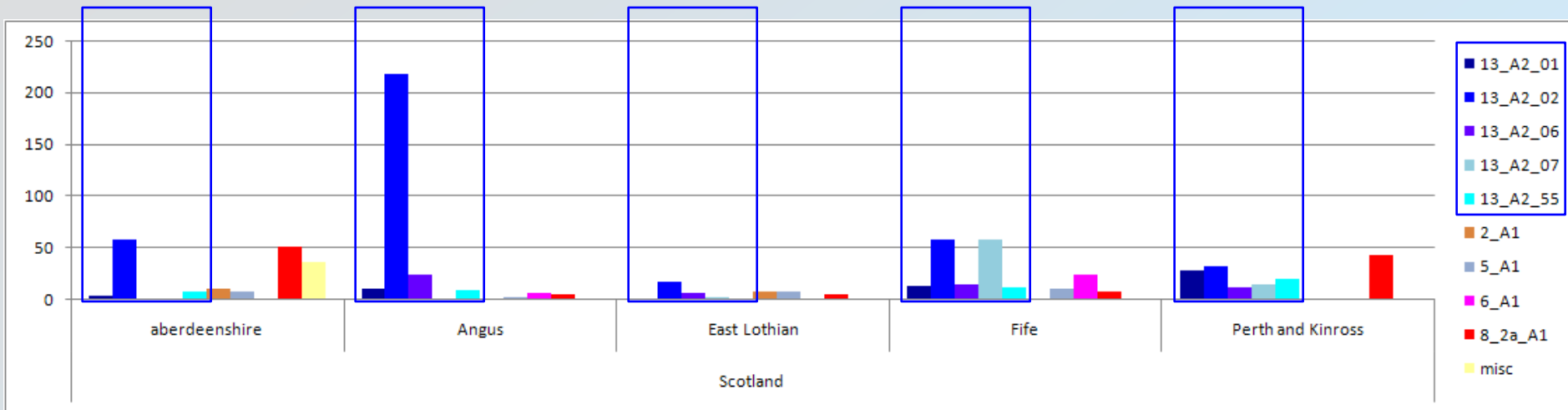
- Regional differences in proportion of the different 13_A2 variants.
- Some common to several regions but several distinct to Scotland & Borders.
- 'founder effect' i.e. local sources of inoculum important in epidemics
- Geographic distance helps isolate populations

Variation within genotype 13_A2 by region and time

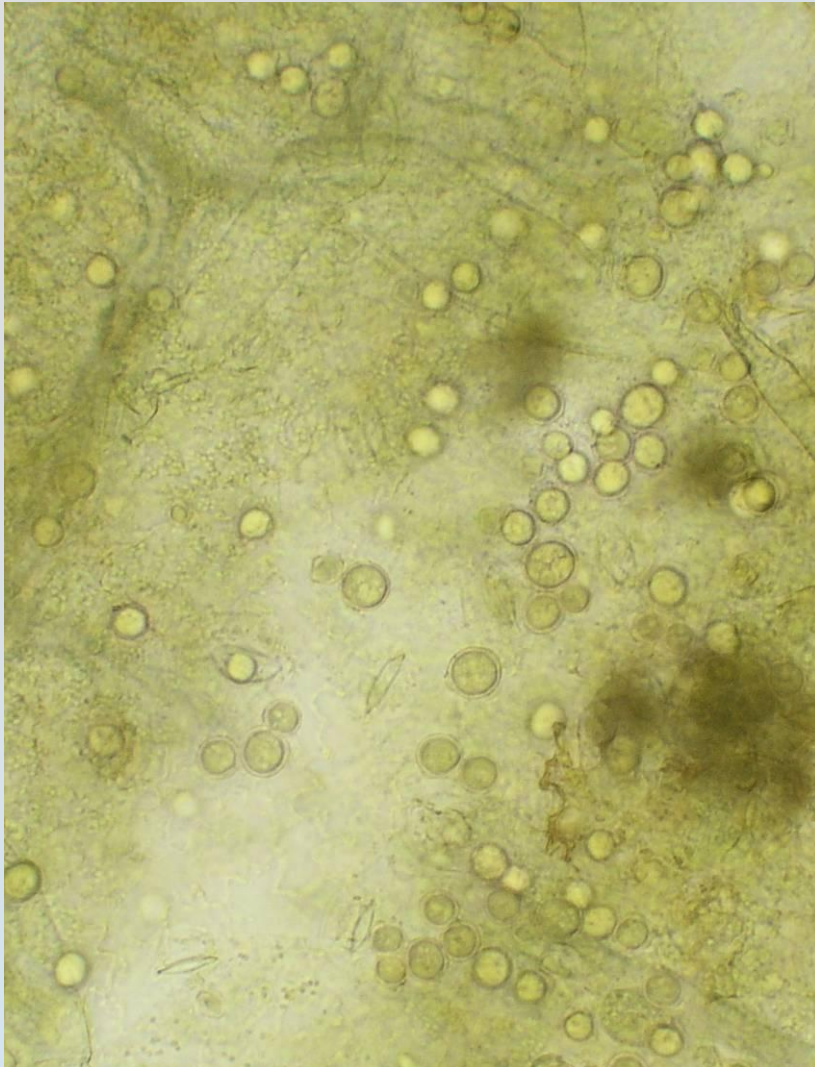


- Type 2 founded Scottish population in 2006 and continues to dominate but rare in Midlands area.
- Type 1 dominates most other regions but is in decline
- Type 5 frequent in Midlands but rare in Scotland

Variation within genotype 13_A2 by Scottish county



Role of oospores?



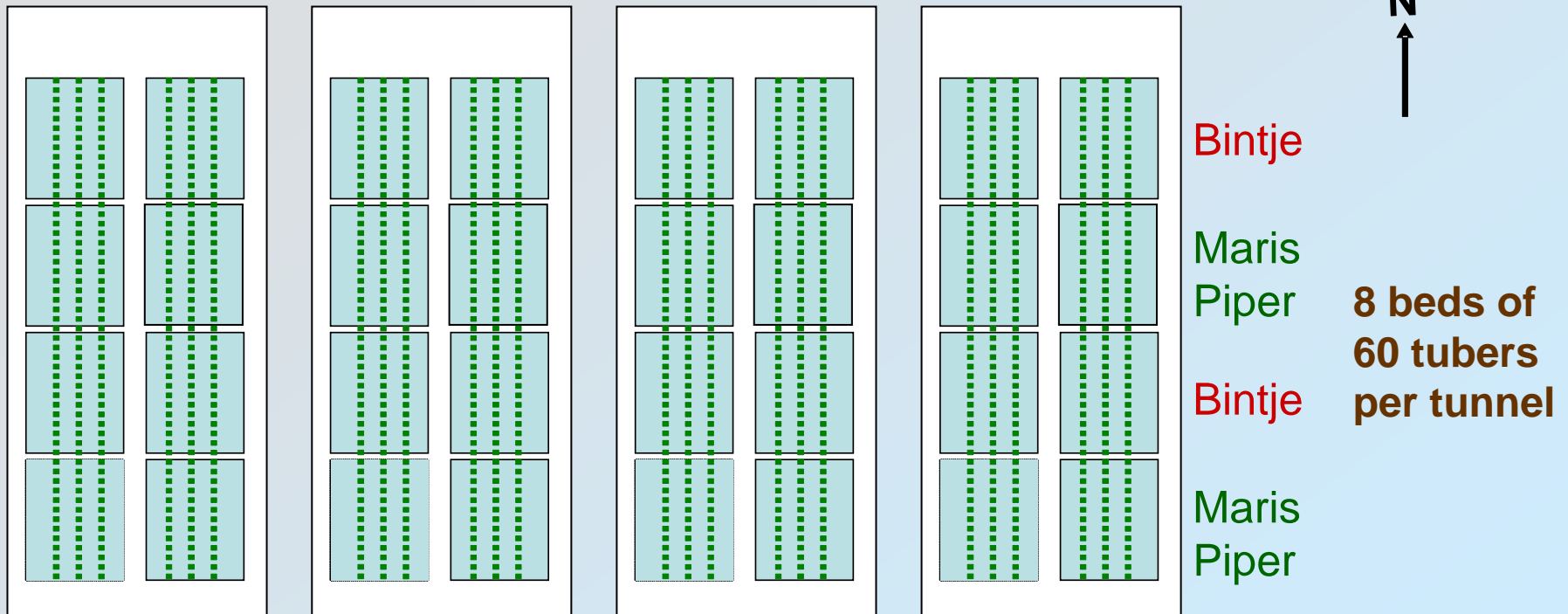
- **2006 to 2009 both mating types have been found in 1/5 to 1/7 of sampled GB outbreaks.**
- **We know GB A1 and A2 strains form oospores in the lab & very likely oospores are in GB fields**
- **Why low frequency of new genotypes in field samples?**
- **Need for good field data on oospore biology**

Main questions



- 1. Do GB *P. infestans* A1 and A2 genotypes form oospores in GB potato crops?**
- 2. Do these oospores survive in the soil between crops?**
- 3. What is the role of the resultant novel isolates on:**
 - a) Disease in current crop**
 - b) Wider population**

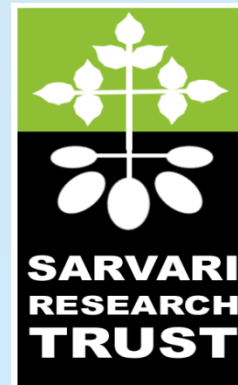
Experimental set up – four tunnels



Why tunnels?

- Increase number of crop cycles
- Contain spread – between tunnels and beyond

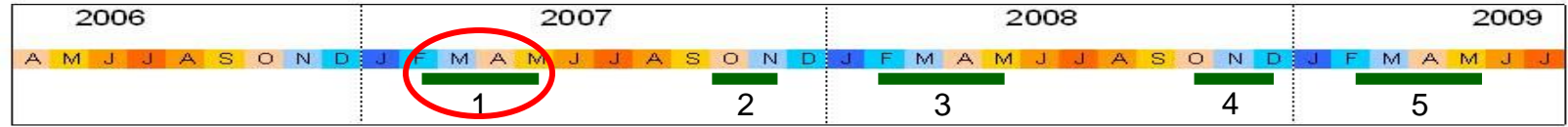
Polytunnels at SRT, Bangor



Dave Shaw
Simon White
Cornelia Bufa
Jonathan Thompson



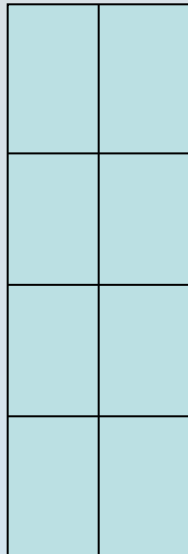
Timeline and first cycle infection



- Different pairs of A1 and A2 used to establish blight in each tunnel
- No further introductions of pathogen in cycles 2-5

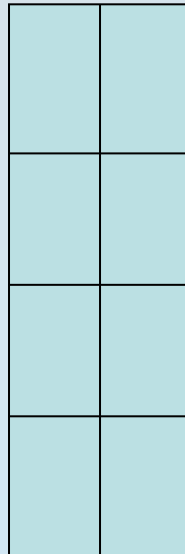
T1

A1 + A2



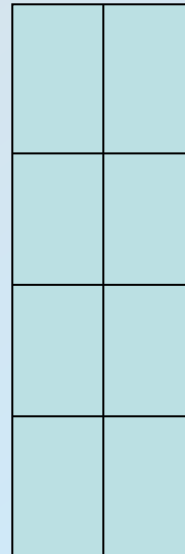
T2

A1 + A2



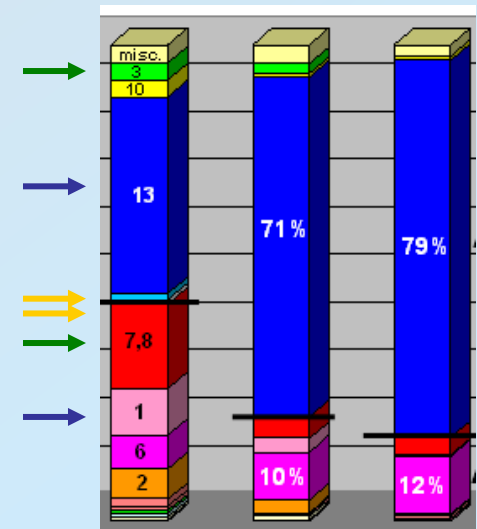
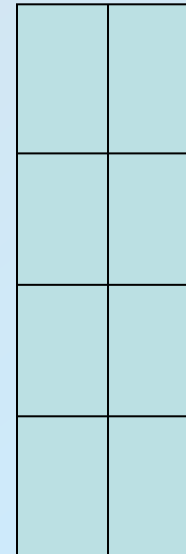
T3

A1 + A2



T4

A1 + A2
A1 + A2
A1 + A2



Cycle 1 infection



- High levels of disease in all tunnels



- Infected leaf samples examined and oospores observed in all tunnels

Parental isolate genotypes



- SSR fingerprints of each isolate distinct
- If sexual recombination occurs we expect to see recombination and re-assortment of the alleles at the markers below
- Some alleles unique to specific isolates

11 markers

	Mating typ	Genotype	Pi02	D13	Pi33	Pi04	Pi4B	Pi16	G11	Pi56	Pi63	Pi70	Pi89
T1	A1	1_A1	160 162	136 136	203 203	166 170	213 217	178 178	140 162	176 176	148 157	192 192	179 195 199
	A2	13_A2	160 162	136 154	203 203	166 170	205 213	176 178	154 160	174 176	151 157	192 192	179 179 0
T2	A1	7_A1	162 162	118 136	203 206	166 170	205 217	176 178	160 160	176 176	151 157	192 192	179 181 0
	A2	3_A2	162 162	118 136	203 203	166 170	213 217	176 178	154 160	176 176	148 157	192 192	179 179 0
T3	A1	8_A1	162 162	118 136	203 206	166 170	205 217	176 178	166 166	176 176	151 157	192 192	179 181 0
	A2	17_A2	160 162	118 118	203 203	0 0	213 217	176 178	160 162	176 176	148 148	192 192	179 179

Observed crops in Cycle 2

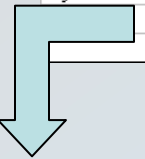
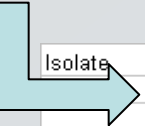


- Dead haulms rotted into soil
- Small tubers possible inoculum source so removed by hand
- Soil kept moist over summer period with misting
- Weeds controlled with herbicide
- A bait-test with soil samples recovered only two isolates and these were of parental type
- Crop planted in October 2007 and inspected daily for blight infection

Genotypes from Tunnel 2 in cycle 2



IN

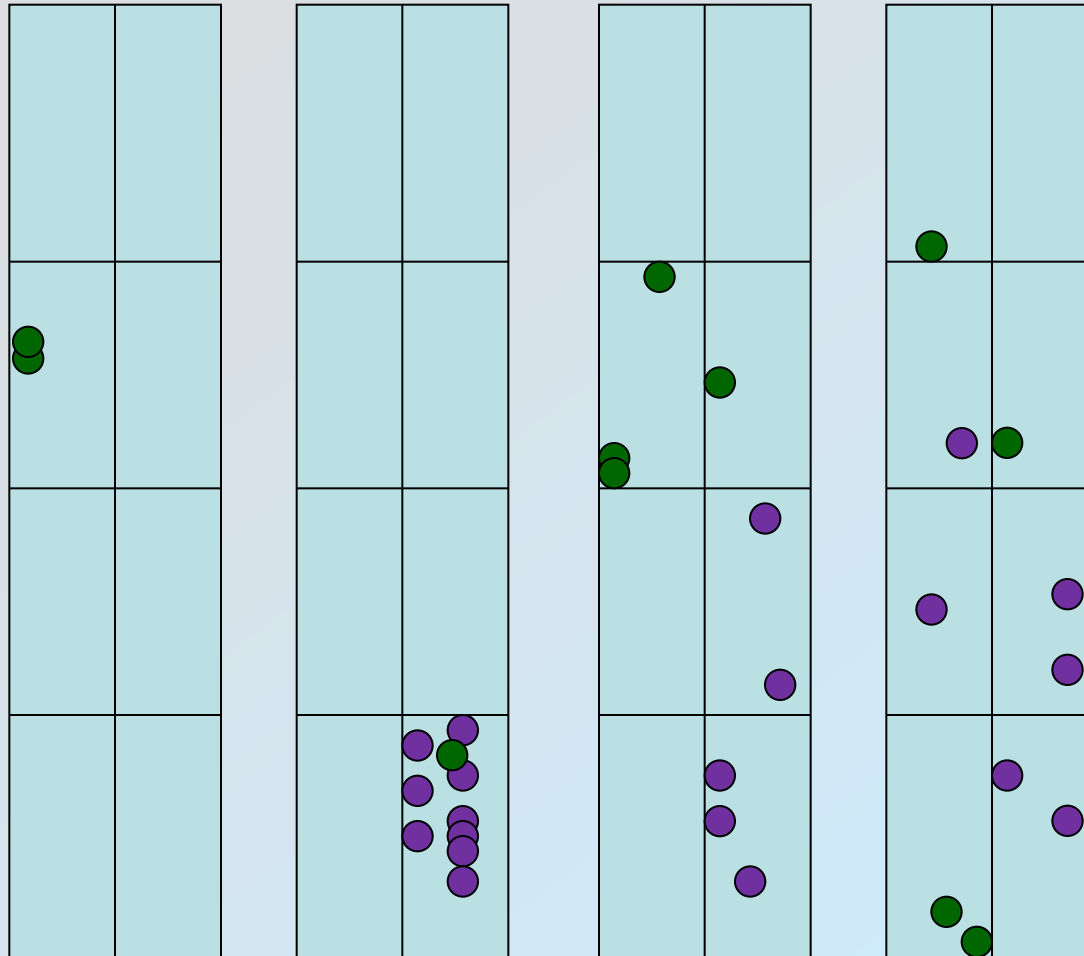


OUT

13-A2	160	162	0	136	154	0	203	203	0	166	170	0	205	213	0	176	178	0	154	160	0	174	176	151	157	0	192	192	179	179	0				
Isolate	genotype		Pi02	D13			Pi33			Pi04			Pi4B			Pi16			G11			Pi56		Pi63			Pi70		Pi89						
7_A1	162	162	0	118	136	0	203	206	0	166	170	0	205	217	0	176	178	0	160	160	0	176	176	151	157	0	192	192	179	181	0				
Cycle 2	New_3		160	162	0	118	136	154	0	203	206	0	166	170	0	205	205	0	176	178	0	154	160	0	176	176	151	157	0	192	192	179	181	0	Frequency
	New_4		160	162	0	118	136	0	203	206	0	166	170	0	205	213	217	178	178	0	154	160	0	176	176	151	157	0	192	192	179	179	0	7	

- Blight seen 31 days after planting
- Novel combinations of markers found consistent with 7_A1 parent but also 13_A2 instead of 3_A2 as partner

Location of infection Tunnel 2 cycle 2



Sampled: 2 Nov.

7 Nov.

13 Nov.

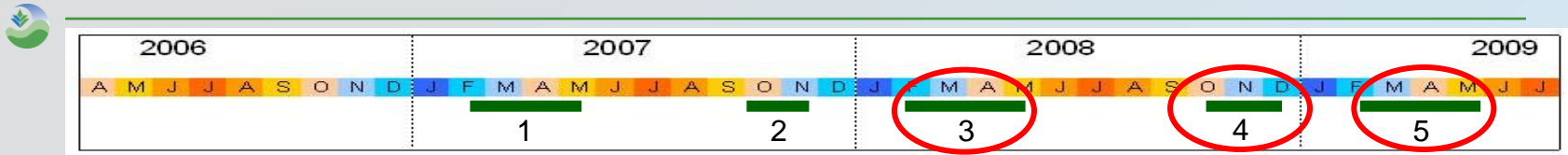
19 Nov.

● new_3

● new_4

- Type 3 more aggressive?

Other cycles - general conclusions



- Blight appeared from 37 to 49 days after planting.
- Further novel tunnel-specific genotypes found. **None matched those in cycle 2.**
- All genotypes absent from the wider GB population.

Oospore conclusions



1. Do GB *P. infestans* A1 and A2 genotypes form oospores in GB potato crops? **YES**
2. Do these oospores survive in the soil between crops? **YES**

Early infections in most crops.

The 500+ isolates recovered from these tunnels over 2 years of 22 genotypes genetically distinct from the parental A1 and A2 isolates and generally specific to a tunnel. This is consistent with germinating oospores.

Many oospores likely generated in cycle 1 but the number that germinated and caused disease pretty low (i.e. 22). Explains poor record of bait testing?

Oospore conclusions



3. What is the role of the resultant novel isolates on:

a. Disease in current crop

Stem and foliar lesions formed but, in general, epidemics spread slowly. Feature of genotypes or environment? Differences in fitness and aggressiveness between types noted. Perhaps new genotypes from these isolates not well adapted.

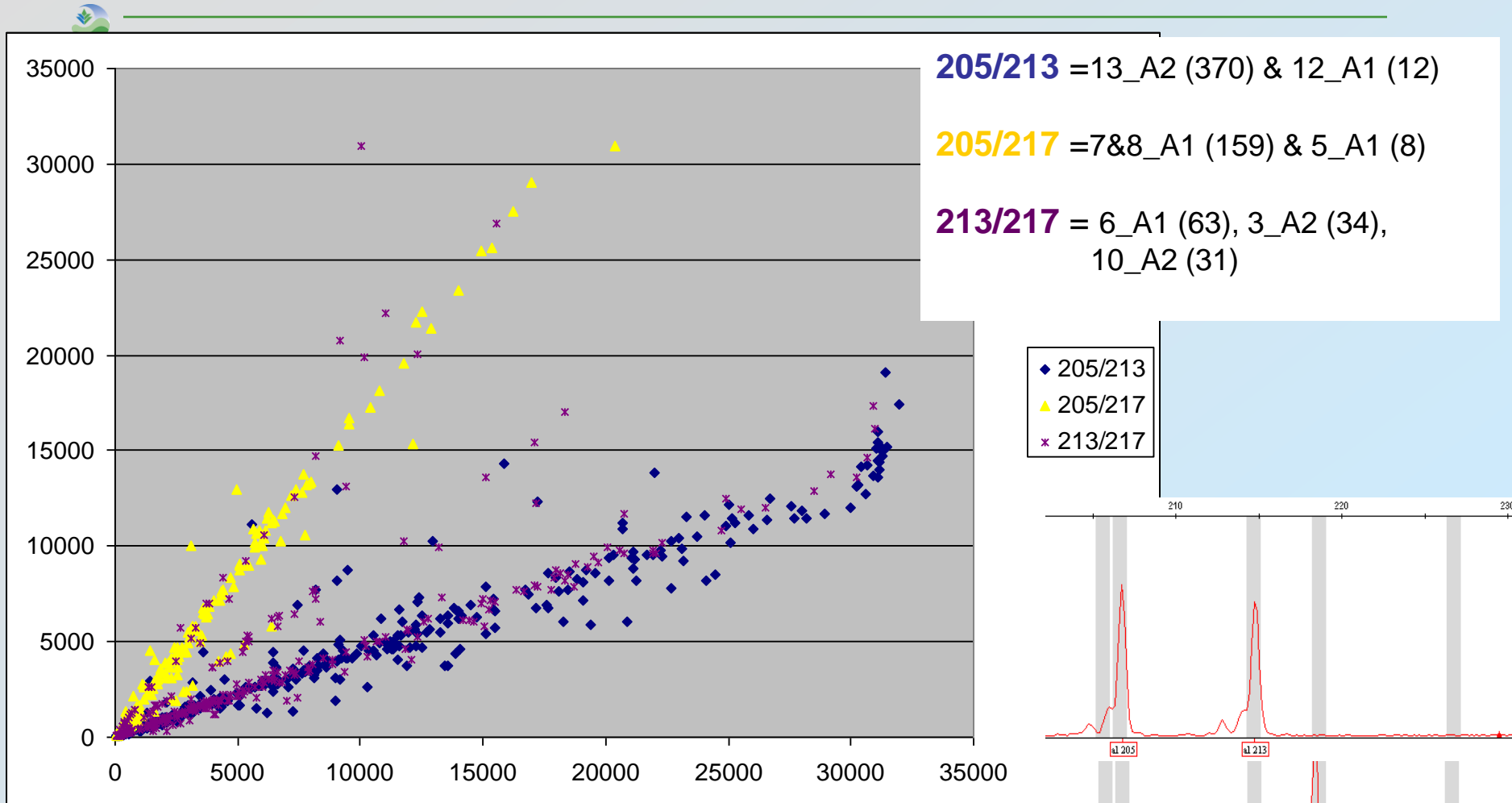
A few A1 and A2 combinations contributed most of novel markers to new population whereas others combinations had very low viability. Variation related to ploidy or other factors?

b. Wider population

Low fitness amongst progeny in this study consistent with low frequency of 'misc' genotypes to date on GB scale. Not the case in other countries.

Threat remains –oospores increase diversity of population and probability of new fitter forms overcoming host or fungicides increased compared to clonal populations. New fit clones generated from such events.

Peak height ratios & ploidy



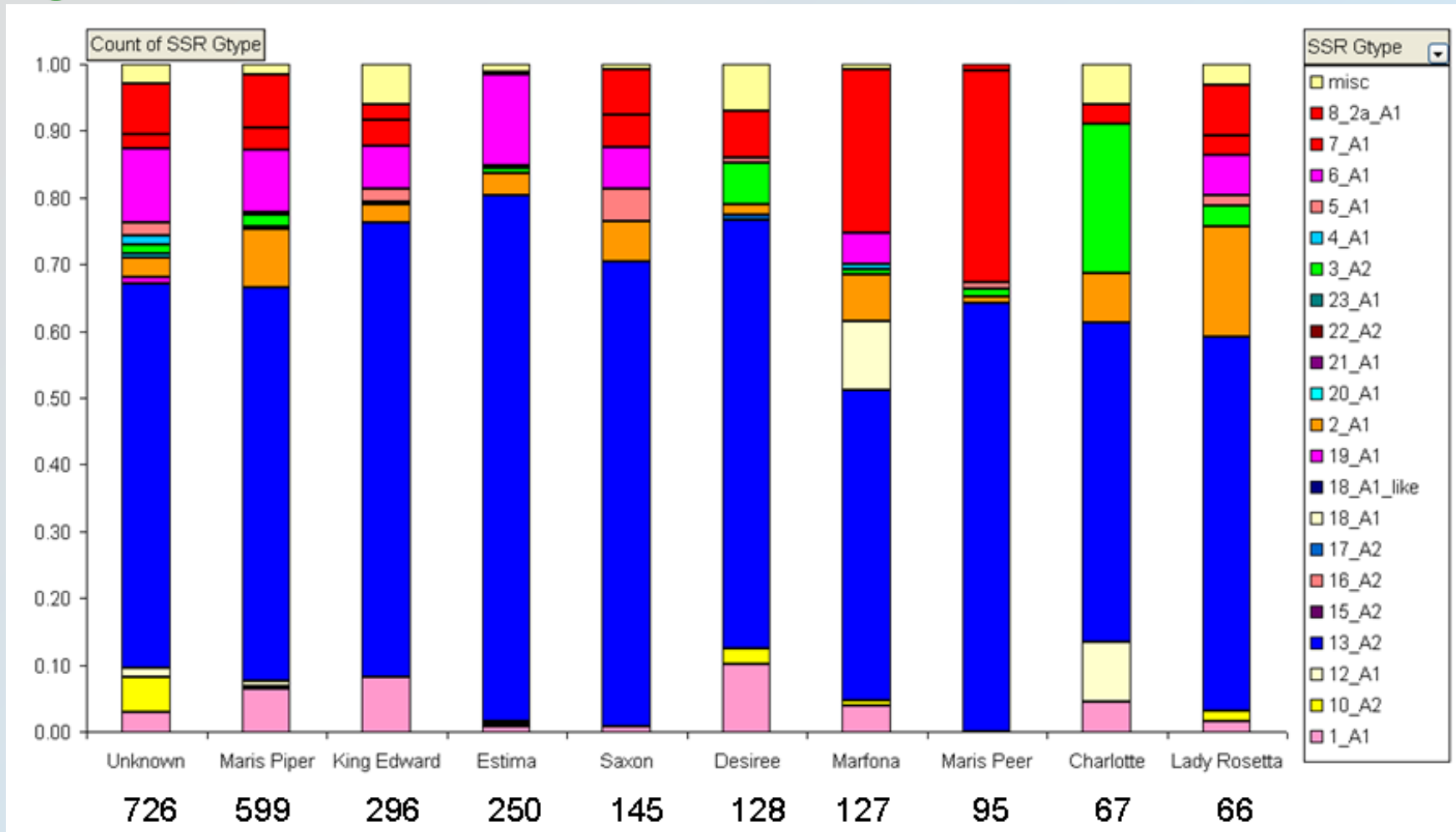
- Pi4B different allele combinations plotted

Conclusions & Implications



- **Mating types:** Plateau of A2 type and genotype 13 in population. Rise of genotype 6 also apparent.
- **Oospores:** Threat of oospore formation remains. Oospores already in soil remain a risk as fields come back into potato. Evidence that oospores will form and infect in short rotations under GB conditions. **Especially relevant as meiosis generates novel combinations of effector genes which may make deployment of any novel resistances challenging.**
- **Other primary inoculum:** local inoculum still an issue – stay alert to seed, volunteers and outgrade piles.
- **Disease management:** Ensure robust protection & consider fungicide products carefully in light of population change. Keep on your toes with spray intervals as blight still more active than in the past
- **Questions remain:** - DSS & Smith periods

Associations between genotype and cultivar 2003-8?



- No clear evidence of association – reflects lack of R?