Final Report

Survey of GB Blight Populations

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1. SUMMARY

Analysis of samples of late blight collected via the PCL Fight Against Blight (FAB) campaign has shown a dramatic increase in the frequency of a single genotype of the A2 mating type of *Phytophthora infestans.* This project has tracked the change in great detail, assessed the likelihood and risks of oospore formation and the aggressiveness of the new population and informed the industry of the implications of this population shift.

Blight Scouts working for the FAB campaign began monitoring blight activity in 2003 and a change in the pathogen population was noted in 2005 which lead to a more detailed sampling of over 670 GB blight outbreaks over the 2006, 2007 and 2008 seasons. Mating type testing and genetic fingerprinting of almost 4000 isolates has shown the A2 mating type of *P. infestans* in GB has increased from 5% to over 90% since 2003.

Oospores form when isolates of the A1 and A2 mating type interact during infection. The probability of this occurring increase as the mating type ratio reaches 50:50. Over this project both mating types were found in 19% of the 672 blight outbreaks sampled (15% in 2008). Detailed Superscout monitoring of eleven outbreaks in their early stages revealed no evidence of oospores as a source of inoculum. Evidence from the other 661 outbreaks from standard scouting (up to 8 samples per outbreak) supported this finding. Over 90% of the sampled 2008 population was represented by only two genotypes (13_A2 78% and 6_A1 12%) of *P. infestans* and in only eight of sampled outbreaks over the whole project were novel genotypes and both mating types found. The widespread involvement of oospores as a source of primary inoculum would have generated a considerably more diverse pathogen population. Studies on experimentally generated oospores in trial plots of potatoes in polythene tunnels that were cropped twice a year indicated that oospores of GB genotypes survive in the soil for 6 months and can act as a source of primary inoculum. However neither the novel genotypes from the tunnel crops or from the outbreaks from commercial crops mentioned above were found beyond their immediate locality suggesting they were not as aggressive or fit as the clonal genotypes.

The aggressiveness of many GB and foreign *P. infestans* genotypes to foliage and tubers in the laboratory and the field was examined. Lesion size and latent period were measured on leaves of five cultivars inoculated with *P. infestans* isolates and held at either 13°C or 18°C in growth rooms. Isolates of genotype 13_A2 generated larger lesions that sporulated faster than isolates of almost all other genotypes. The result was clearer at the cooler temperature. Tests on whole tubers stored at 15°C did not show 13_A2 to be consistently more aggressive than other genotypes. A field trial clearly demonstrated the increased fitness and aggressiveness of 13_A2 compared to four A1 genotypes.

A comprehensive programme of KT has informed the industry of this more aggressive form of blight that is now common in GB crops. Evidence from other studies has shown 13_A2 to be resistant to phenylamide fungicides and able to overcome some sources of cultivar resistance. In general, an earlier start to the spray programme, shorter spray intervals, a greater focus on primary inoculum sources and care over cultivar and fungicide product selection is now more critical than ever. The threat of oospore inoculum remains; most of the land potentially contaminated with oospores has yet to return to potatoes in the rotation.

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2. INTRODUCTION

Primary inoculum carried over from one season to the next is the source of all late blight outbreaks and growers attempt to avoid such sources of the pathogen (*Phytophthora infestans*) by using healthy seed and eliminating infected outgrade piles and groundkeepers. This vegetative or asexual inoculum is a familiar threat to the industry; a potentially more serious but less familiar threat is, however, the sexual stage of the pathogen (the oospore). Since these thick-walled oospores can survive in the soil for several years their management is more challenging and they are therefore a cause for real concern to the GB potato industry. The implications of oospore production are two-fold. Firstly, soil-borne oospores are an additional and potentially more damaging reservoir of long-lived primary inoculum, increasing the likelihood of crop infection. Oospore-borne infections have been reported to occur earlier in the season (Andersson et al 1998, Hannukkala et al., 2002) and result in infections in the lower part of the canopy that are more difficult to identify early and treat with fungicides. Secondly, oospores are the result of sexual recombination that generates new variation in the pathogen population that allows the pathogen to adapt more rapidly to management tools such as fungicides or resistant cultivars.

Oospores are formed when the two mating types of the pathogen, termed A1 and A2, meet during plant infection. Prior to the 1980s, the A2 mating type was not found in Europe and despite its presence in GB for around 20 years it has remained at very low levels (e.g. Cooke *et al*., 2003; Day *et al*., 2004). BPC-funded reporting of blight outbreaks in 2005 revealed an increase in the proportion of A2 isolates (Shaw *et al*., 2006). Further examination of these A2 mating type isolates using the restriction fragment length polymorphism (RFLP) analysis with the RG57 probe indicated the presence of several RG57 types not recorded in the 1995-1998 GB survey (Day *et al*., 2004). The three most common A2 types were labelled blue (7 isolates), green (8 isolates) and yellow (3 isolates) (Shaw *et al*., 2006). It appeared that the GB *P. infestans* population was changing; hence the concern over the impact oospores may have on the ability of the GB industry to control blight. Scouting by the BPC-funded Fight Against Blight campaign (FAB) aids disease management by documenting late blight outbreaks across the country but, prior to this project, only required a single sample per outbreak/site to confirm the presence of blight. Such sampling offered little resolution and to provide a better appreciation of any *P. infestans* population change it was recognised that more samples per outbreak were needed to investigate the overall frequency of the A2 mating type and the breakdown of mating types within individual crops. In this way an assessment of the likelihood of new oospore formation and whether the disease outbreak was itself derived from soil-borne oospores can be made. Furthermore, genetic analysis tools are now available to enable a low-cost but high-throughput and objective view of the genetic structure of the GB population (Cooke & Lees, 2004; Lees *et al*., 2006). In parallel to the above surveys, a need was seen to collate data on other groups' experiences of managing *P. infestans* oospores and commence research to investigate the risks and implications of oospore formation and survival under GB conditions. For example, most previous studies on oospore survival and viability have not investigated natural soil infestations. For how many years do oospores produced in a GB potato crop remain viable and a threat to future potato crops? Similarly it is important to review the disease control options to ensure the GB industry is best placed to respond to any threat of oospores appropriately.

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2.1. Objectives as detailed in project proposal

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)

2.2. Aggressiveness studies

The Potato Council recognised the need for data on the implications of the changing *P. infestans* population on disease management and funded SCRI for an additional series of experiments to examine aggressiveness of GB and other genotypes as an extension of project R274. Background information on aggressiveness can be found in the review on the Potato Council website (Cooke *et al.*, 2007). The drivers of the population change are unknown but it is implicit that the single dominant A2 type (genotype 13_A2) is fitter than other strains. Fitness is associated with aggressiveness (Cooke *et al.*, 2007) and an increase in the ability of a pathogen to cause disease is almost certain to make disease control more difficult. The rapid spread of the A2 type across the country indicates its success as a pathogen. The aggressiveness and fitness of a particular strain of *P. infestans* needs to be seen in the context of the environment and variety. The foliar and tuber phases of infection must also be considered as this will affect both the rapid spread during a season and the capacity to survive in seed tubers and cause disease in the next season. There was however, no objective data on the aggressiveness of these new populations and thus uncertainty in the industry over what these changes meant in terms of disease control.

2.2.1. Objectives as detailed in project proposal

6. Complete study of foliar aggressiveness of GB and European *P. infestans* isolates against a selection of potato cultivars in growth cabinets under ‘cool’ and ‘optimal’ infection conditions.
7. Complete assessment of tuber aggressiveness of GB and European *P. infestans* isolates against a selection of potato cultivars.
8. Establish, infect and monitor fitness and aggressiveness of *P. infestans* isolates in a field trial against a selection of potato cultivars.

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3. MATERIALS AND METHODS

3.1. Objective 1. Determine levels of A2 mating type of *P. infestans* in 2006-8 GB potato crops

3.1.1. Outbreak sampling

Potato Council's 'Fight Against Blight' (PCL FAB) scouts increased their sampling, collecting up to 8 lesions per crop. As in previous years, the samples were sent to Central Science Laboratory (now Fera but referred to as CSL in this report) and, if confirmed as blight, the FAB map was updated, generating a red spot for each outbreak. Positive samples were placed within small potato tubers and sent in batches to SCRI (Dundee) for further testing. In addition to these (PCL FAB) scouts, sampling packs were posted to Superscouts and handed out to the SASA seed potato inspectors during their training events in Edinburgh (June 2006-8).

3.1.2. Sample processing

Upon arrival at SCRI, a slice of tuber ca. 5mm thick was taken from the zone in contact with the blighted plant material and laid in a Petri dish with the newly cut surface uppermost. After 1-4 days incubation at room temperature (ca. 19°C) tuber tissue sporulating with *P. infestans* was plated onto a primary isolation plate of Rye A agar with antibiotics. After further culturing (ca. 19°C) on a secondary isolation plate, the culture was plated onto a series of media as follows; a pea broth plate to yield mycelium for subsequent DNA extraction, two plates each pre-inoculated with either the A1 or A2 tester strain and finally a Rye A agar screw-cap slope for longer-term storage. Each of these plates was further incubated at ca. 19°C. After ca. 7 days the pea broth cultures were rinsed in sterile distilled water, the agar plug removed and the mycelium was freeze-dried and stored. Once the tester and unknown isolate colonies had grown together for several days, the central zone of the agar plate was examined under the microscope for the presence of abundant oospores at the interface of the two colonies that would indicate that the unknown isolate is the opposite mating type to the tester strain. Other regions of the colony of each unknown isolate were also screened for the presence of oospores that may indicate the presence of a mixed culture or a self-fertile isolate.

3.2. Objective 2. Examine specific early outbreaks to determine the likely source of inoculum

A detailed sampling protocol developed by Ruairidh Bain and Nick Bradshaw was trialled in 2005 and a simplified version used in the 2006-2008 seasons. Sampling packs were assembled at SCRI and posted to the 15-21 Superscouts with instructions to sample up to 32 lesions from blight outbreaks at a very early stage of development and to provide a detailed account of the outbreak. In this way we could maximise the likelihood of identifying the primary inoculum source via the detailed outbreak and genetic fingerprint data. Samples were returned directly to SCRI and entered onto the FAB outbreak map via the web-based interface with the database.

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3.3. Objective 3. Determine genetic diversity of GB *P. infestans* populations

A small 2mm^3 fragment of freeze-dried mycelium was subject to DNA extraction using a 'Quick and Easy' protocol (www.eucablight.org) modified from Wang and Cutler (1993). The DNA (1 µl) was subsequently used for SSR analysis with the previously published primers (Lees *et al*., 2006) using methods optimised for the ABI3730 as per the protocol available at www.eucablight.org. The SSR data was manually checked and peaks scored prior to export to XL spreadsheets for further analysis. Data were also entered into the Eucablight database.

3.4. Objective 4. Analysis of oospore production and survival under GB conditions.

Four semi-permanent potato plots were established in separate polythene tunnels (20 x 5.5 m) at Henfaes Research Centre (Sárvári Research Trust, SRT) on the North Wales coast. Tunnels were used to allow two crops per year, to give better control of the epidemics and to minimise spore contamination amongst and away from the tunnels. Crops of Bintje and Maris Piper were established in cycle 1, planted Feb 2007 (Table 1). Standard-sized, 3-row beds were constructed in each tunnel with the rows 70cm apart and the tubers spaced at 25cm within a row. Each tunnel was laid out in an identical manner with a total of 480 plants; 60 per "plot" on each side of a central pathway (Fig. 1). Bintje seed was sourced from Danespo (Denmark) and Maris Piper from SE1 grade seed (Black Isle, Scotland). Crops in the first three tunnels (T1-T3) were inoculated with a different pair of A1 and A2 isolates (Fig. 2) while the crop in T4 was inoculated with all six isolates.

Crop cycle	Planting date	Infection period	Sample no.
1	Feb 2007	May 2007	n/a
2	Oct 2007	Nov 2007	270
3	Feb 2008	April-May 2008	350
4	Oct 2008	Nov-Dec 2008	26
5	Feb 2009	March-May 2009	129

TABLE 1. SUMMARY OF PLANTING DATES AND EPIDEMIC DATES IN THE OOSPORE BIOLOGY EXPERIMENTS IN THE POLYTHENE TUNNELS AT SRT.

Prior to planting of each cycle, 25 tubers of the Maris Piper and Bintje seed batches were selected at random, incubated in the dark at room temperature in moist peat for 1 month and examined for internal and external blight symptoms. Seed tubers for the subsequent cycles were planted as detailed in Table 1. From cycle 2 onwards no inoculum was applied and daily crop inspections were carried out from emergence. Tunnels were misted at least once a day to keep beds wet. Blight infection was noted as early as 2.5 weeks after planting (cycle 3) and as late as 7 weeks after planting (cycle 4) and lesions were sampled from throughout the epidemic with either isolates or FTA cards sent to SCRI for SSR analysis.

A 300 ml soil sample (4 sub-samples per bed from top 5cm) was taken from each plot after cycles 1 and 2 and stored for subsequent use. Air and soil temperature were also recorded for each tunnel.

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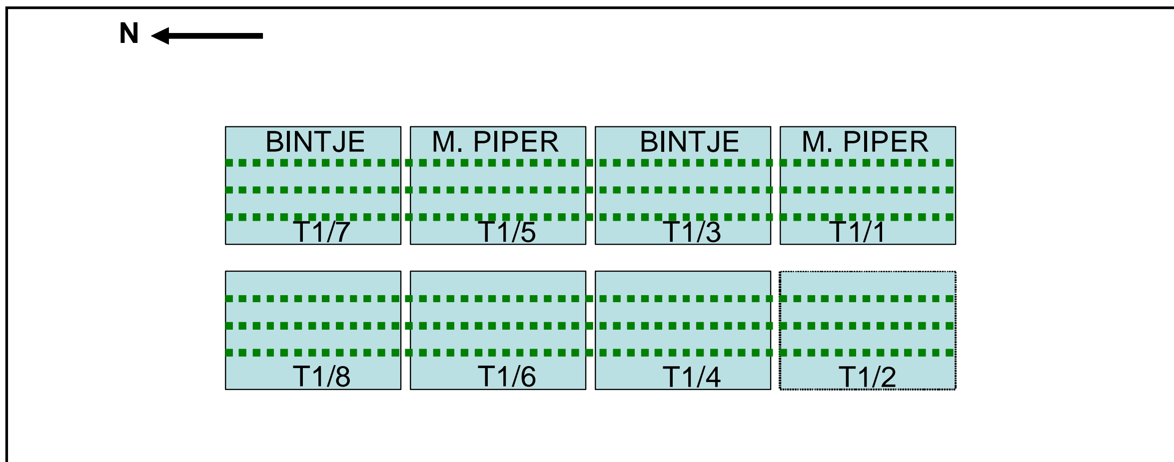

FIGURE 1. LAYOUT OF THE FIRST OF THE FOUR TUNNELS, (T1), WITH PLOTS T1/1 AND T1/2 PLANTED WITH MARIS PIPER, T1/3 AND T1/4 WITH BINTJE ETC. EACH PLOT COMPRISES 3X20 TUBERS.

Postcd	MT	Number of isolates found in 2006 (%)	Cv	Sample Name	genotype	Pi02	Pi02	D13	D13	Pi33	Pi33	Pi04	Pi04	Pi4B	Pi4B	Pi16	Pi16	G11	G11	Pi56	Pi56	Pi63	Pi63	Pi70	Pi70	Pi89	Pi89	Pi89
CB7	A1	96 (11.2)	Maris Piper	2006_3984C	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
CT7	A2	316 (36.8)	King Edward	2006_3928A	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
IP10	A1	56 (6.5)	Charlotte	2006_4168B	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
TR12	A2	32 (3.7)	Charlotte	2006_4012F	3_A2	162	162	118	136	203	203	166	170	213	217	176	178	154	160	176	176	148	157	192	195	179	179	0
SA62	A1	48 (5.6)	Marfona	2006_4232E	8_2a_A1	162	162	118	136	203	206	166	170	205	217	176	178	166	166	176	176	151	157	192	192	179	181	0
AB41	A2	8(0.9)	Other Maincrop	2006_4388D	17_A2	160	162	118	118	203	203	0	0	213	217	176	178	160	162	176	176	148	148	192	192	179	179	

FIGURE 2. CHARACTERISTICS OF THE THREE PAIRS OF ISOLATES SELECTED TO INOCULATE CROPS FOR THE OOSPORE BIOLOGY STUDIES IN THE POLYTUNNELS AT SRT. THE POSTCODE, MATING TYPE, FREQUENCY IN THE 2006 GB POPULATION, GENOTYPE, VARIETY, ISOLATE NAME, AND SSR GENOTYPE ARE SHOWN. THE UPPER TO LOWER PAIRS WERE USED TO INOCULATE T1 TO T3 RESPECTIVELY AND ALL SIX ISOLATES WERE USED IN T4.

3.5. Aggressiveness studies

3.5.1. Objective 6. Foliar assay on detached leaves under controlled conditions in growth rooms

Twenty six isolates of *P. infestans* were selected as follows; four isolates of genotype 13_A2, pairs of isolates representing other dominant genotypes from the 2006 GB population, additional isolates from other parts of Europe and two reference isolates from Scotland in the 1990s (Table 2). It was noted that the isolates from the Netherlands were also genotype 13_A2 whereas those from Poland and Sweden did not match dominant GB genotypes. Over time, the aggressiveness of isolates reduces in culture so prior to the experiment, all isolates were inoculated onto detached leaves of glasshouse-grown potato cultivar Craigs Royal. Sporangia were harvested after one week and used to re-inoculate more leaves of the same cultivar. Each isolate was passaged by this method at least three times prior to their use in the experiments.

Five commonly grown potato varieties (Table 3) were selected with a range of blight resistance and grown, 3 plants per pot, in 30cm pots in the glasshouse for 5-6 weeks. For each variety, leaves of a similar age and size were harvested and placed in clear plastic boxes (26 leaves per box) lined with moist tissue paper (Fig. 3). Sporangial inoculum of each isolate was washed off the infected Craigs Royal leaves and

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adjusted to 14,000 sporangia per ml before cooling at 4°C to encourage zoospore release. A 30µL droplet of inoculum from each isolate (approx 420 sporangia) was applied to each leaf and the boxes placed in illuminated walk-in growth rooms at 18 or 13°C. A total of 60 boxes of leaves were inoculated and 30 placed in each growth room in a randomised block design.

The 1560 leaves were scored daily for appearance of symptoms (i.e. Infection Period, IP), appearance of sporulation (i.e. Latent Period, LP) and at six days post-inoculation, lesion size was measured in two orientations using electronic callipers connected to a laptop computer. The data was analysed using GenStat.

Isolate name	Year	Country	County	Mating type	Cultivar	Genotype	Test used
2006_3888A	2006	GB	Cheshire	A1	Lady Rosetta	2_A1	L, T, F
2006_4068B	2006	GB	Dyfed	A1	Charlotte	2_A1	L
2006_4100A	2006	GB	Essex	A1	Marfona	6_A1	L, T, F
2006_3920A	2006	GB	Norfolk	A1	Estima	6_A1	L
2006_4168B	2006	GB	Suffolk	A1	Charlotte	7_A1	L, T, F
2006_4168C	2006	GB	Suffolk	A1	Charlotte	7_A1	L
2006_4232E	2006	GB	Dyfed	A1	Marfona	8_2a_A1	L
2006_4256B	2006	GB	Shropshire	A1	Marfona	8a_A1	L, T, F
2006_3928A	2006	GB	Kent	A2	King Edward	13_A2	L, T, F
2006_4132B	2006	GB	Somerset	A2	Estima	13_A2	L, T
2006_3964A	2006	GB	Suffolk	A2	King Edward	13_A2	L, T
2006_3884B	2006	GB	Lincolnshire	A2	Maris Piper	13_A2	L, T
2006_3936C2	2006	GB	Cornwall	A2	Unknown	10_A2	L
2006_4440C	2006	GB	Staffordshire	A2	Maris Piper	10_A2	L, T
2006_4012F	2006	GB	Cornwall	A2	Charlotte	3_A2	L, T
2006_4244E	2006	GB	Dyfed	A2	Other Maincrop	3b_A2	L, T
2006_4388D	2006	GB	Aberdeenshire	A2	Other Maincrop	17_A2	L, T
C2-95.17.3.2	1995	GB	Edinburgh	A1	Unknown	8a_A1	L
C4-96.9.5.1	1996	GB	Angus	A1	Unknown	5_A1	L
NL06269	2006	NL	Unknown	A2	Unknown	13_A2	L, T
NL04246	2004	NL	Unknown	A2	Unknown	13_A2	L
SE03058	2003	SE	Unknown	Unknown	Unknown	unknown	L, T
SE03087	2003	SE	Unknown	Unknown	Unknown	unknown	L
MP618	2005	PL	Unknown	Unknown	Unknown	unknown	L
MP622	2005	PL	Unknown	Unknown	Unknown	unknown	L, T
LD151 D	n/a	Ireland	Unknown	A1	Unknown	2_A1	L

TABLE 2. DETAILS OF THE 26 ISOLATES USED FOR THE AGGRESSIVENESS STUDIES. THE LAST COLUMN IDENTIFIES ISOLATES USED FOR THE LEAF, TUBER AND FIELD STUDIES (L, T, AND F RESPECTIVELY).

Variety	Foliar blight resistance	Tuber blight resistance
Lady Balfour	8	7
Cara	7	7
Estima [1]	4	5
Maris Piper	4	5
King Edward	3	4

TABLE 3. VARIETIES SELECTED FOR TESTING THE AGGRESSIVENESS OF *P. INFESTANS* GENOTYPES AND THEIR FOLIAR AND TUBER BLIGHT RESISTANCE RATINGS FROM THE POTATO COUNCIL VARIETY DATABASE, JULY 2008.
[1] ESTIMA NOT USED FOR TUBER AGGRESSIVENESS TESTING.

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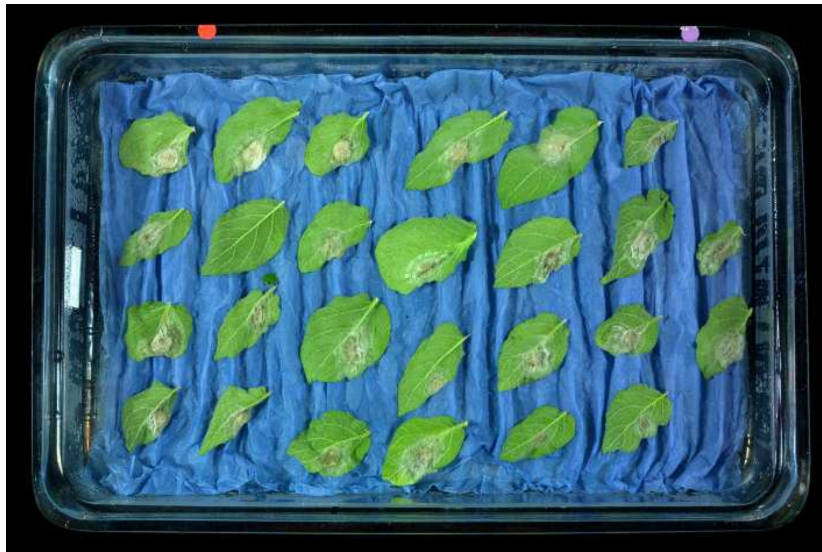

FIGURE 3. EXAMPLE OF A SINGLE BOX OF INFECTED LEAVES SHOWING THE EXPERIMENTAL SET UP AND THE BLIGHT LESIONS FORMED SIX DAYS AFTER INOCULATION.

3.5.2. Objective 7. Assessment of tuber aggressiveness of GB and European *P. infestans* isolates

Testing aggressiveness on field-grown tubers is more time consuming than the above test of foliar symptoms, thus only a subset of the isolates (15; Table 2) and varieties (4; Table 3) used above were examined. Eight plots of each variety were planted in 2 blocks in the field on 20 April and tubers carefully harvested by hand on the 14th August 2007. The tubers were gently washed under running water and eight tubers of each of two varieties were placed rose-end upwards in each plastic box (Fig. 4). As above, sporangial inoculum of each of the 15 isolates was washed off the Craigs Royal leaves and adjusted to 14,000 sporangia per ml before cooling at 4°C to encourage zoospore release. Each group of eight boxes was inoculated with a single isolate of *P. infestans* using a hand-held garden sprayer until run-off (approx 250mls) and all 120 boxes were arranged in a randomised block design in a dark store room at 15°C for 15 days. The percentage area affected by tuber blight was scored on each of the 1920 tubers 15 days after inoculation.

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FIGURE 4. EXPERIMENTAL SET UP FOR THE TUBER BLIGHT AGGRESSIVENESS EXPERIMENT.

3.5.3. Objective 8. Field study: Isolate fitness and aggressiveness monitoring over the course of an epidemic

A randomised complete block (4 blocks) field trial comprising four 25-plant plots of each of five varieties (Table 3) was sown on 9 May 2007. Each plot was laid out as a 5 x 5 plant grid (Fig. 5) with a surrounding guard row of variety Stirling which, at the time, was reported to have a foliage blight resistance rating of 8. Once the plants had met in the rows the trial was sprayed weekly with Bravo (Chlorothalonil) until 10 July to minimise the risk of blight entering the trial from other crops. On the 18th July, inoculum of a genotype 13_A2 isolate and 4 other A1 isolates (Table 2) was prepared as described above. The suspension of all five isolates was then mixed and approximately 50mls used to spray-inoculate the lower leaves of the central plant (plant 13) of each plot. Inoculations took place in late afternoon when rain was forecast. From the date on inoculation onwards, thirty minutes of mist irrigation was used morning and afternoon in each plot to encourage infection and disease spread. The plots were inoculated again in the same manner six days later to ensure the infection was successful. By 24th July blight symptoms were observed in the central plant (13) of most plots and by the 30th July this had spread to the immediately surrounding plants and the sampling of leaves with clear single blight lesions began. Lesions were never taken from plant 13 as the sampling was intended to examine secondary spread of blight. At the early sampling dates, lesion samples were collected from plants adjacent to plant 13 moving out to other plants within the plot (shown in green in Fig. 5) as the epidemic spread. Sample numbers varied according to the stage of the epidemic but the objective was to sample 10 blight infected leaves per plot on each of four dates (n= 800 lesions). As far as possible, leaves with single lesions were selected and placed in individual plastic bags. Once back in the laboratory the lesion margin from each leaf was pressed onto an FTA card (Whatman, UK) using a pair of pliers. Once dry the cards were stored for genotyping at a later date.

Small disks were cut out from the cards and processed according to the manufacturer's instructions (Whatman FTA plant Protocol BD05) prior to dropping into SSR genotyping PCR mixes (see protocol on www.eucablight.org). The SSR data was scored and the fingerprints compared to those of the isolates initially released into the trial.

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G	G	G	G	G	G	G
G	01	06	11	16	21	G
G	02	07	12	17	22	G
G	03	08	13	18	23	G
G	04	09	14	19	24	G
G	05	10	15	20	25	G
G	G	G	G	G	G	G

FIGURE 5. LAYOUT OF EACH OF THE 20 INDIVIDUAL PLOTS IN THE FIELD TRIAL TO MONITOR FITNESS AND AGGRESSIVENESS OF *P. INFESTANS* GENOTYPES IN 2007. G = GUARD PLANT CV STIRLING, PLANT 13 WAS INOCULATED.

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4. Results

4.1. Objective 1. Determine levels of A2 mating type of *P. infestans* in 2008 potato crops in GB

Over the course of the 2008 season, blight isolates were recovered from 202 FAB blight outbreaks and five outbreaks from the SASA seed inspectors. No samples were provided by the Superscouts in 2008. From these samples a total of 1640 isolations were attempted at SCRI yielding 1018 isolates from 207 blight outbreaks. Combined with the equivalent surveys in 2006 and 2007 this project processed data from 672 blight outbreaks and yielded 3649 isolates that were mating type tested. The geographical range of the standard and superscout samples and the regions used to define population structure are shown (Fig. 9). The samples provided by SASA seed inspectors are not shown on the maps but came from Angus, Aberdeenshire, Perth and Kinross, Fife and the Highlands.

The summary data on FAB samples over the six years (2003 to 2008) shows some general trends. Broadly similar proportions of samples were recovered from outbreaks of different severities each year indicating that most samples came from fields with scattered outbreaks (Fig. 6). The majority (70-90%) of sampled outbreaks were in commercial crops (organic or conventional) with other sources such as discard piles, volunteers, trials and private gardens and allotments remaining below 10% each year (Fig 7). The dates of sample arrival provide an rough indication of the timing of the epidemic in each season. The latest season was 2004 and the earliest peak was coincided with the severe weather in the southeast in 2006. The extremely wet 2007 however had the steepest curve which reflected the severe blight problems experienced (Fig 8).

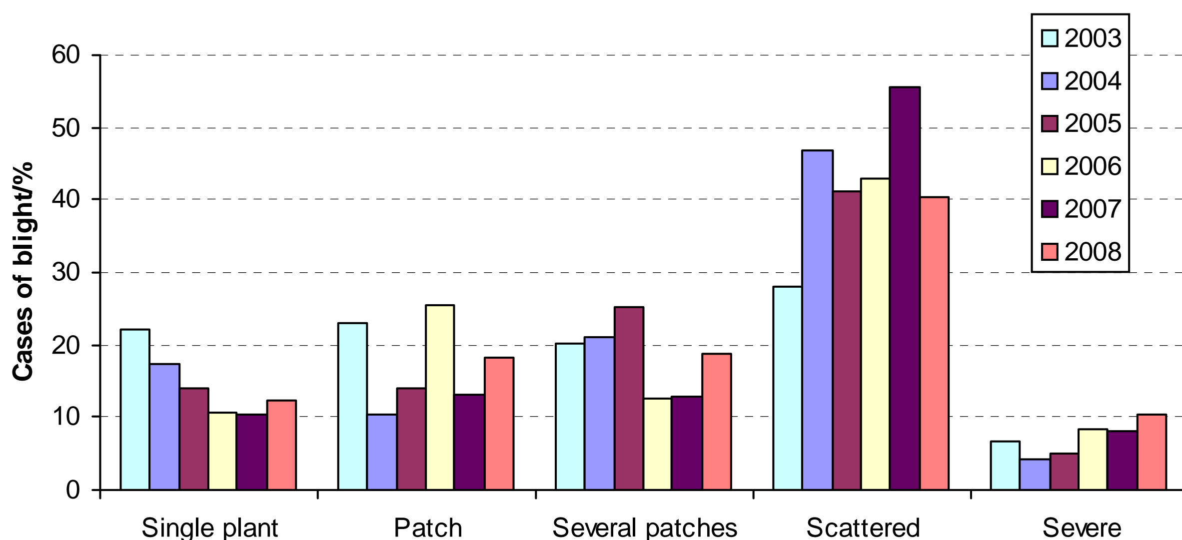

Figure 6. Severity of blight infection recorded in the outbreaks sampled by FAB Scouts during 2003-2008.

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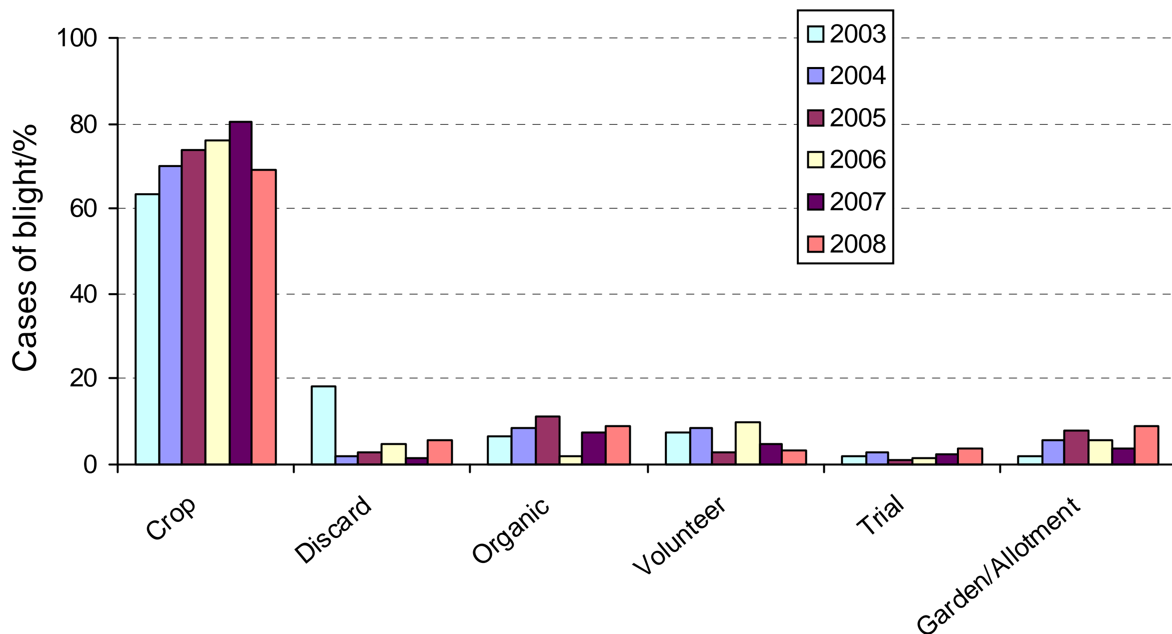

FIGURE 7. SOURCE OF FAB LATE BLIGHT SAMPLES 2003 - 2008

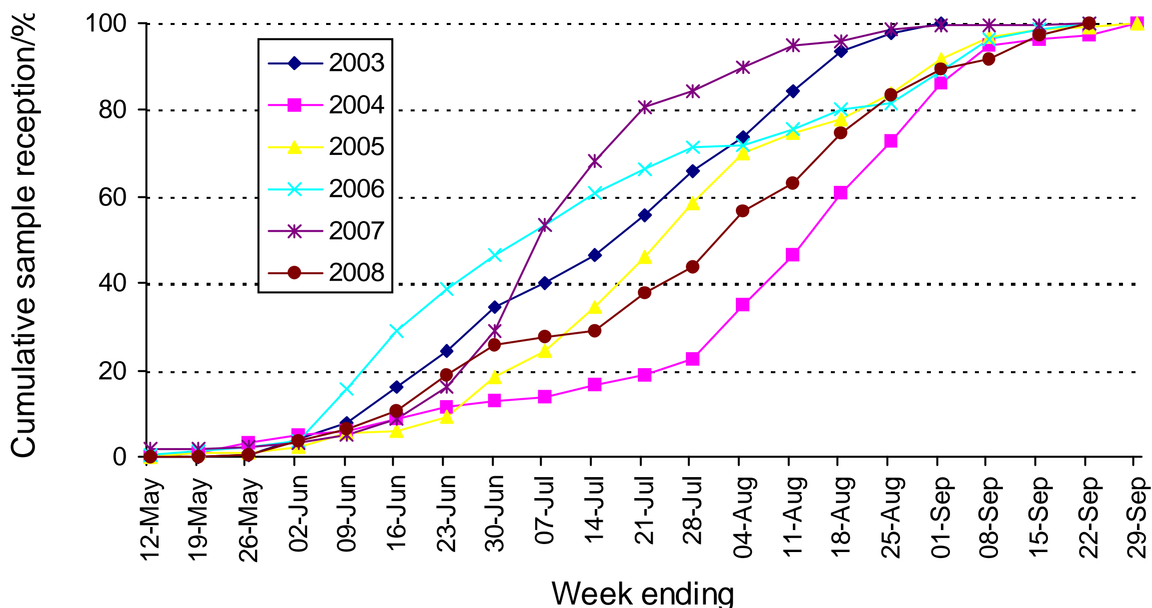

FIGURE 8. WEEKLY CUMULATIVE FAB BLIGHT SAMPLE RECEPTION 2003-2008

The mating type data can either be presented as the percentage of the total number of isolates or broken down and examined per disease outbreak. Since a major objective of this project was to assess the risks of oospore formation that would occur when both mating types are present in the crop, this report focuses on the data per outbreak. There were four sources of sampled outbreaks; FAB standard and Superscouts, SASA seed inspectors and miscellaneous home & garden samples. All samples are grouped and the raw mating type data (Table 4) and breakdown according to each disease outbreak (Table 5) presented.

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The proportion of tested isolates of the A2 mating type has increased from 54 in 2006 to 80% in 2008. When broken down by disease outbreak a similar picture is seen with the A2 mating type being found at 65% of outbreaks in 2006 and 91% in 2008 (Table 5). The proportion of outbreaks in which both mating types were found has declined from 22 to 15% over this period.

Mating type of isolates	2006	2007	2008	Total
A1	464	411	204	**1079**
A2	550	1190	830	**2570**
Total	1014	1601	1034	**3649**
%A1	45.8	25.7	19.7	**30.4**
%A2	54.2	74.3	80.3	**69.6**

TABLE 4. THE NUMBERS AND PERCENTAGES OF *P. INFESTANS* ISOLATES OF EACH MATING TYPE COLLECTED DURING OVER THE 2006-8 SEASONS.

Outbreak type	2006	2007	2008	Total
A2 only	72	188	157	**417**
A1 only	57	55	19	**131**
Mixed	36	57	31	**124**
Total outbreaks	165	300	207	**672**
Total with A2 present	108	245	188	**541**
% outbreaks with A2	65.5	81.7	90.8	**79.3**
% mixed outbreaks	21.8	19.0	15.0	**18.6**

TABLE 5. THE NUMBER AND PERCENTAGE OF BLIGHT OUTBREAKS SAMPLED OVER THE 2006-8 SEASONS CATEGORISED ACCORDING TO THE *P. INFESTANS* MATING TYPES PRESENT.

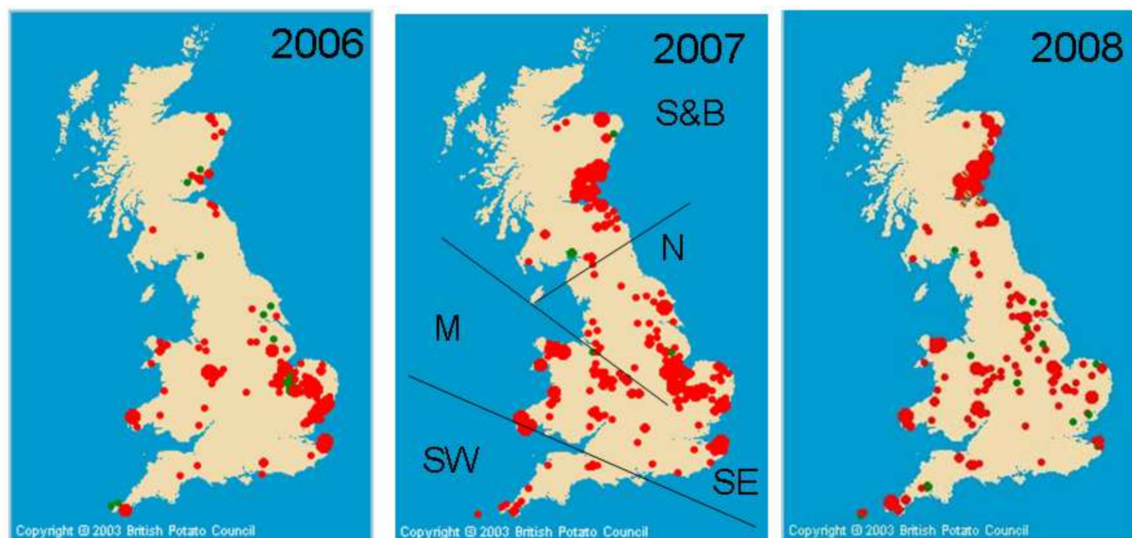

FIGURE 9. LOCATIONS OF THE 672 LATE BLIGHT OUTBREAKS REPORTED DURING THE 2006, 2007 AND 2008 FAB CAMPAIGNS. THE BLACK LINES ON THE 2007 MAP INDICATE THE REGIONS (SOUTH EAST, MIDLANDS, SOUTH WEST, NORTH AND SCOTLAND AND THE BORDERS) USED TO DESCRIBE THE REGIONAL BREAKDOWN OF THE *P. INFESTANS* POPULATIONS.

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4.2. Objective 2. Examine specific early outbreaks to determine the likely source of inoculum

The superscouts specifically sought outbreaks in their early stages of infection in which the primary focus was apparent. Eleven samples were sent to SCRI over the three seasons with eight in 2006, three in 2007 and none in 2008 (Tables 6 and 7). Of the eleven samples, three were of mixed mating type. A detailed examination of the data provided by the scouts on the extent of the outbreak, position of lesions in the canopy, evidence of seed tuber infection and other factors was completed. This data was combined with evidence from the SSR genotyping and the results for each outbreak summarised and reported back to the scouts. Oospores were not implicated in any of the outbreaks. The signature of an oospore-derived outbreak would be severe infection in the lower part of the canopy consistent with a below-ground source combined with a mixture of A1 and A2 mating types and unique SSR genotypes found within the outbreak. The right-hand columns of Table 6 and 7 indicate that, in each case, the isolates recovered were clonal genotypes of *P. infestans* also found in previous seasons. The structured sampling was however informative; within the two mixed mating type sites one could note that specific mating types or genotypes were confined to individual plants or foci within the crop.

If the outbreaks were not derived from oospores was it possible to determine the inoculum source from the other data? There was no evidence in either year that any of the outbreaks were caused by a below-ground source and, in the seven outbreaks with sufficient data, it was concluded that the inoculum had blown in from outgrade piles, volunteers or neighbouring crops.

Eight lesions were provided from the majority of the outbreaks sampled by standard scouting and this also provided an opportunity to examine signatures of oospore infection. Data for all 124 mixed mating type outbreaks over the three seasons were examined carefully but in the vast majority of cases, comprised common A1 and A2 genotypes. In only eight outbreaks were a combination of A1 and A2 isolates and novel genotypes noted. Such outbreaks were consistent with an origin from germinating oospores but in no case were the resultant genotypes observed again either in the same season or the following one which suggests that these new genotype combinations were not sufficiently fit or aggressive to establish themselves (see comments on miscellaneous genotypes in next section).

SCRI code	BPC no.	Postcode	Type	Variety	Created	No. samples	No. isolates	A1	A2	Geno types	% of 2006 Pop.
06_SS1	3900	PE34	Single Plant	Maris Piper	05 Jun 2006	6	5	5	0	6_A1	7
06_SS2	3896	IP12	Several Patches	Maris Peer	05 Jun 2006	32	28	28	0	7_A1	6
06_SS3	3988	CT7	Several Patches	Desiree	08 Jun 2006	18	15	11	4	A1/13	10/41
06_SS4	3956	TF6	Patch (1m2)	Unknown	07 Jun 2006	24	23	23	0	8_A1	11
06_SS5	4160	NR16	Patch (1m2)	Unknown	27 Jun 2006	14	9	0	8	13_A2	41
06_SS6	4212	NR17	Patch (1m2)	Maris Piper	29 Jun 2006	20	17	0	17	13_A2	41
06_SS7	4336	AB53	Patch (1m2)	Marfona	18 Jul 2006	12	8	8	0	2_A1	6
06_SS8	4332	AB51	Patch (1m2)	Marfona	18 Jul 2006	18	13	13	0	18_A1	1

TABLE 6. DETAILS OF THE EIGHT OUTBREAKS SAMPLED BY THE SUPERSCOUTS IN 2006 SHOWING THE MATING TYPE AND SSR GENOTYPE INFORMATION FOR THE *P. INFESTANS* ISOLATES RECOVERED.

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SCRI code	BPC no.	Postcode	Type	Variety	Created	No. samples	No. isolates	A1	A2	Genotypes	% of 2006 Pop.
07_SS1	4742	SA62	veral Patch	Rocket	01 Jun 2007	32	27	0	27	13_A2	71.5
07_SS2	4758	B78	n/a	Maris Piper	07 Jun 2007	32	28	14	14	6_A1 1_A1 13_A2	10.1 2.8 71.5
07_SS3	4766	NR11	veral Patch	Maris Piper	07 Jun 2007	21	18	12	6	7_A1 13_A2	1.8 71.5

TABLE 7. DETAILS OF THE THREE OUTBREAKS SAMPLED BY THE SUPERSCOUTS IN 2007 SHOWING THE MATING TYPE AND SSR GENOTYPE INFORMATION FOR THE *P. INFESTANS* ISOLATES RECOVERED.

4.3. Objective 3. Determine genetic diversity of GB *P. infestans* populations

Over the course of the project 3754 GB isolates from the 2003 to 2008 seasons were genotyped. Eleven SSR loci were PCR amplified and the allele(s) present were scored. The combination of alleles at the 11 loci observed in a single isolate are used to define its genotype. These are shown as colour coded cells with, in general, two cells at each locus representing the two alleles in the diploid form of *P. infestans* (Appendix 1). If both alleles are identical, the isolate is termed homozygous at that locus and the cells within that markers column are shown in the same colour. Heterozygous loci have two (or sometimes more) different alleles at a single locus and the cells are shown in different colours.

The isolate genotypes were sorted in an Excel spreadsheet and the genotype categories defined and numbered arbitrarily with a number and the mating type (i.e. 1_A1, 2_A1, 3_A2 etc). An additional category of genotype termed 'miscellaneous' was defined for isolates with rare combinations of alleles that were found at a very low frequency and commonly in only a single blight outbreak. The overall numbers and frequencies of all the different genotypes in each of the four seasons are shown in Table 8 and presented graphically (Fig. 11).

A considerable amount of detailed data was generated in this part of the project. This report will, however, focus on the findings that relate directly to the main questions raised in the project; how is the *P. infestans* population (and particularly the A2 mating type) changing and are oospores acting as a source of primary inoculum?

In 2007, the 1452 isolates genotyped indicated that 96% belonged to *P. infestans* lineages (genotypes) found in multiple outbreaks and also found in previous years. This was mirrored in 2008 with 97% of isolates belonging to lineages from previous seasons. In 2007, genotype 13_A2 showed a 30% increase to 71.5% and genotype 6_A1 increased by 3% to 10.1%. In 2008, the same genotypes increased with 13_A2 and 6_A1 increasing by 7% and 2% respectively and representing over 90% of the sampled GB *P. infestans* population. The percentage of isolates in the miscellaneous category was at its lowest for the whole project at in 2008 at 3%. It has ranged from 3 to 5.5% and shown no consistent trend over the course of the project.

The sample distribution in 2008 was similar to that in 2007 with most isolates from crops in Scotland and the Borders, the Midlands and the North and a decreasing proportion coming from the southeast region that dominated the sampling in 2006 (Fig. 10). The genotype frequencies *within* the 2006-8 seasons (Fig. 12) clearly indicates the transition to 13_A2 in 2006 and the prevalence of 13_A2 from the start to the end of the following seasons. The isolates at the start of the 2007 and 2008

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seasons reflected the isolate frequencies at the end of the previous season and did not suggest any differences in overwinter survival rates between the genotypes. Similarly, the date of appearance of the miscellaneous category of isolate showed no specific pattern.

The changes in *P. infestans* population within each region over the three years of the project indicate a transition that broadly matches the national picture (Fig. 13) However, some regional differences were apparent. A clear delay in the arrival of 13_A2 was noted in the Midland, North and Scottish and Borders regions in 2006 compared to that in the Southeast. More recently in 2008 in the Midland region the frequency of 13_A2 was unchanged in 2008 whilst 6_A1 increased slightly and in the Southeast region 13_A2 decreased in 2008 whilst 6_A1 increased. Genotype 6_A1 is the only other genotype that seems to be able to compete with 13_A2 but this type remains at a low frequency in the Southwest and Scotland and the Borders regions.

Genotype	2003	2004	2005	2006	2007	2008	Total
21_A1	0	3	0	0	0	0	3
4_A1	2	10	0	2	3	5	22
12_A1	3	6	5	6	11	1	32
20_A1	0	0	0	6	0	0	6
19_A1	0	0	0	6	0	0	6
5_A1(RF2)	9	5	4	8	8	7	41
18_A1	0	0	0	13	2	0	15
2_A1(RF39)	22	54	13	58	40	3	190
6_A1	0	5	2	63	147	136	353
1_A1	2	10	0	88	40	3	143
7,8_A1(RF6)	28	26	20	159	66	44	343
17_A2	0	0	0	8	0	0	8
16_A2	0	0	0	13	0	0	13
13_A2 blue	0	0	9	370	1038	877	2294
10_A2 yellow	0	4	3	31	9	10	57
15_A2	0	2	0	0	0	0	2
22_A2 (RF40)	1	1	0	0	0	0	2
3_A2 green	4	7	13	34	30	0	88
misc	3	5	4	34	58	32	136
Total	74	138	73	899	1452	1118	3754
Outbreak number	100	120	120	165	300	207	1012

TABLE 8. RESULTS OF SSR GENOTYPING *P. INFESTANS* ISOLATES RECOVERED FROM GB OUTBREAKS OVER EACH OF SIX YEARS. ISOLATE NUMBERS AND THE PERCENTAGE OF EACH YEARS SAMPLE FALLING INTO EACH GENOTYPE CLASS ARE PRESENTED WITH THE A2 AND MISCELLANEOUS CATEGORIES SHOWN IN THE LOWER HALF OF THE TABLE. WHERE POSSIBLE, REFERENCE IS MADE TO GENOTYPES DEFINED BY OTHER MEANS IN OTHER STUDIES ([1]= DAY *ET AL.*, (2004) [2]= SHAW *ET AL.*, (2006)).

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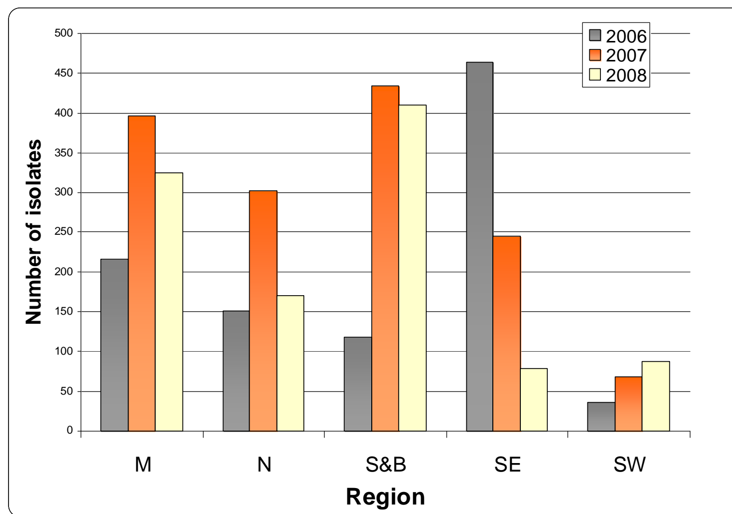

FIGURE 10. REGIONAL BREAKDOWN OF NUMBERS OF *P. INFESTANS* ISOLATES GENOTYPED FROM OUTBREAKS IN DIFFERENT GB REGIONS OVER THE 2006-8 SEASONS.

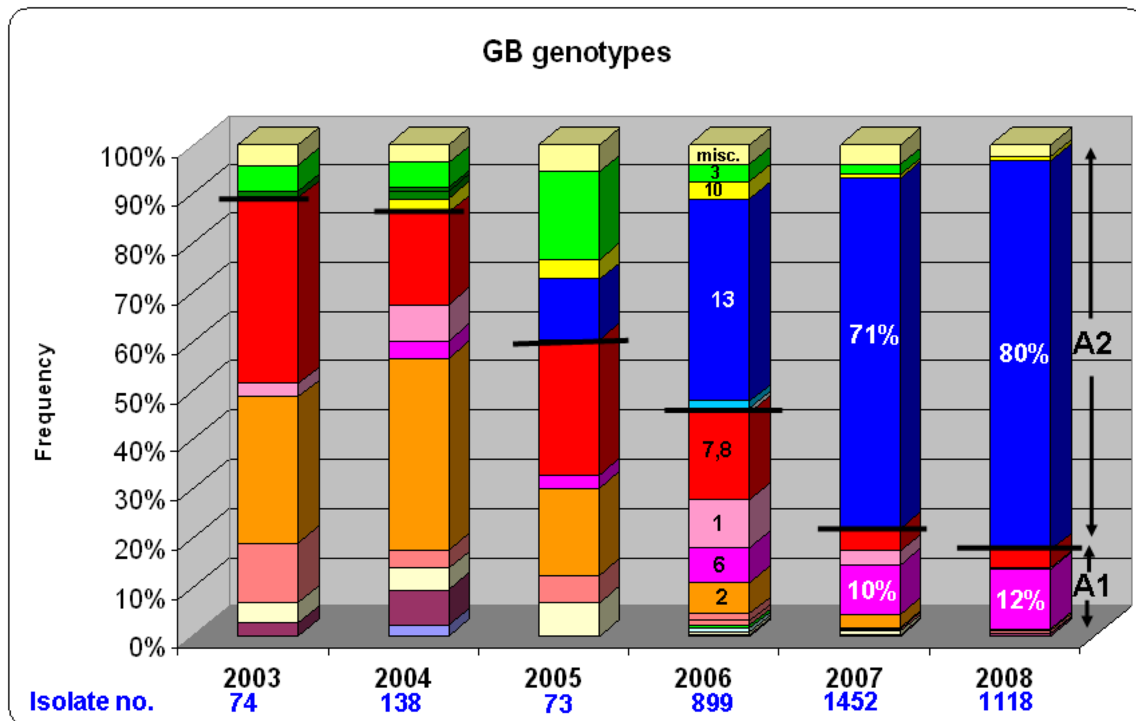

FIGURE 11. BAR CHARTS INDICATING THE FREQUENCY OF *P. INFESTANS* ISOLATES WITHIN EACH SSR GENOTYPE OVER THE COURSE OF SIX SEASONS (2003-08). ALL COLOURS ABOVE THE HEAVY BLACK HORIZONTAL LINE (EXCEPT THE YELLOW MISCELLANEOUS CATEGORY) IN EACH SEASON ARE THE A2 GENOTYPES.

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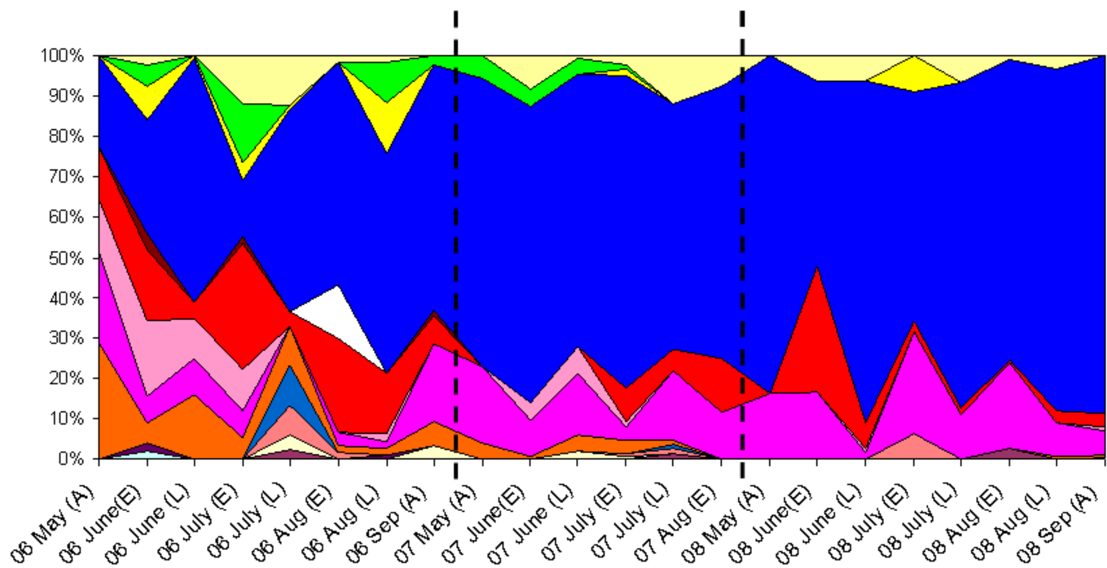

FIGURE 12. THE FREQUENCY OF EACH OF THE *P. INFESTANS* GENOTYPES EXPRESSED EITHER MONTHLY (A = ALL MONTH) OR TWICE-MONTHLY (E = EARLY, L = LATE) WITHIN AND BETWEEN SEASONS OVER THE COURSE OF 2006 TO 2008. THE DOTTED LINES INDICATE THE OVERWINTER BREAKS.

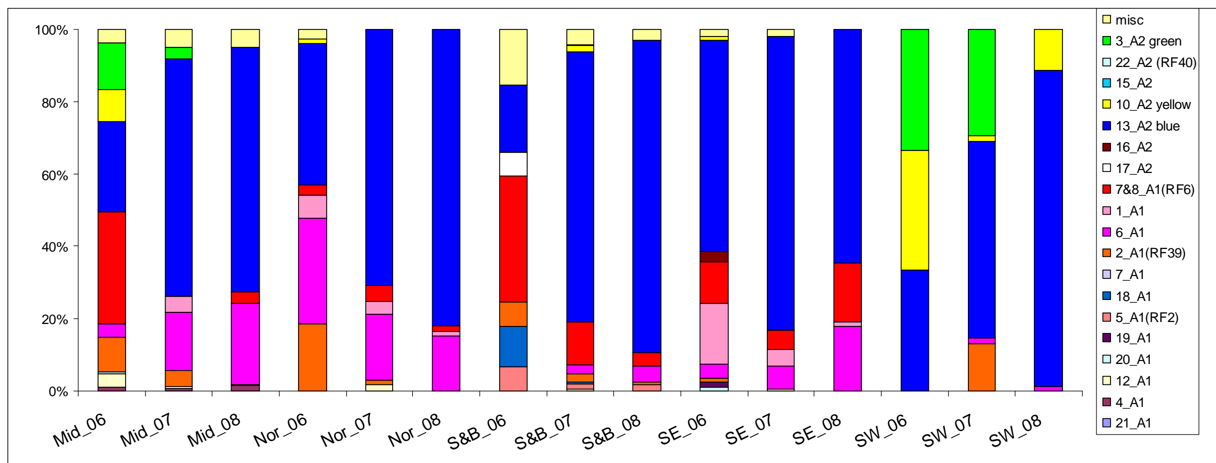

FIGURE 13. BAR CHARTS INDICATING THE CHANGE IN FREQUENCY OF *P. INFESTANS* SSR GENOTYPES IN EACH GB REGION FROM 2006 AND 2008. EACH SET OF THREE BARS REPRESENTS A SPECIFIC GB REGION. COLOURS MATCH THOSE IN FIG. 7.

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4.3.1. Additional analysis

This large dataset of genotyped isolates from blight outbreaks with a range of characteristics can be analysed in many different ways. For example, it would be interesting to note any links between the *P. infestans* genotype causing an outbreak and the potato variety it is infecting. A positive association may indicate susceptibility of a specific variety to a particular genotype. Conversely, a negative association may suggest a variety has resistance to a particular genotype. Over the project, samples were collected from many varieties. An analysis of the ten sampled at the highest frequency was completed but no clear associations between variety and genotype were observed (Fig. 14). Genotype 13_A2 was prevalent on all the varieties. Some differences were noted; genotype 7 and 8 were recovered from all varieties except Estima and genotype 6_A1 was found at 5% or above on all cultivars except Desiree, Maris Peer and Charlotte. In this latter case it may simply be a chance event as the data becomes more sensitive to such patterns as the sample size reduces. The absence of 7_A1 or 8_A1 from Estima was unexpected. However it was not supported by the aggressiveness data on this variety and may be simply a chance event. This data was also examined for each of the individual 2006, 2007 and 2008 seasons and no variety-specific trends over time were noted (data not shown). An analysis of whether *P. infestans* genotype or mating type frequencies were influenced by the type of outbreak was also conducted. Based on these sampled outbreaks, the presence of both A1 and A2 mating types (i.e. mixed mating type outbreaks) was no more or less likely in samples from crops than other types such as outgrade piles, volunteers or gardens and allotments. Similarly no consistent trend in the frequency of the different *P. infestans* genotypes at each type of outbreak was observed (data not shown).

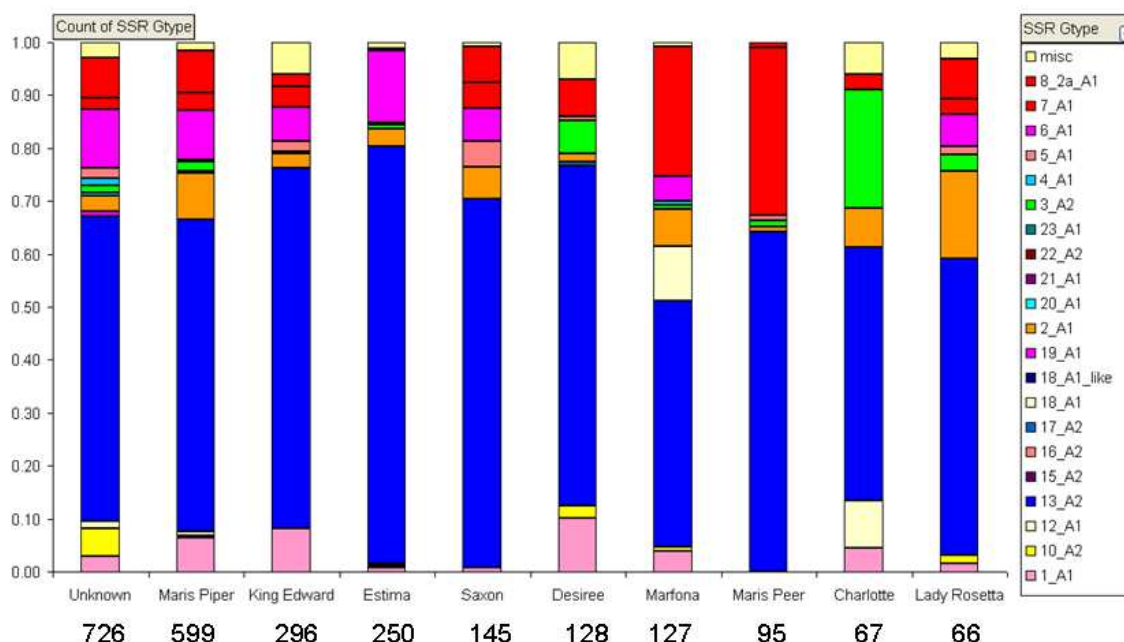

FIGURE 14. EXAMINATION OF THE FREQUENCY OF EACH OF THE *P. INFESTANS* GENOTYPES SAMPLED FROM THE TEN MOST SAMPLED VARIETIES OVER THE SIX SEASONS FROM 2003-2008. THE NUMBERS BENEATH EACH COLUMN REFER TO THE NUMBER OF ISOLATES COLLECTED.

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4.4. Objective 4. Analysis of oospore production and survival under GB conditions.

Soil samples collected from the four tunnels at the end of the first cycle were tested for the presence of oospores using a floating leaflet soil baiting test. The results obtained showed no evidence of recombination, with the few isolates recovered being identical to the parental isolates. At this stage it was decided not to carry out this particular baiting test again as it is time consuming and very few isolates were obtained. This may be due to the relatively small volume of soil which can be sampled compared to planting a new crop which effectively acts as a baiting test for all the soil in the tunnel. The low frequency of primary infection found in all tunnels over cycles 2-5 justifies this decision (see text below).

Tunnel	Cycle	1st infection date (days after planting)	Genotypes found (number of samples)	Mating type
1	2	30	New_1 (18) New_2 (5)	A1 A2
	3	49	New_14 (93)	n/a
	4	52	6_A1 (2)	A1
	5	56	New_17	A2
2	2	31	New_3 (17) New_4 (7)	A1 A2
	3	49	New_17 (10) 13_A2_like (10) New_15 (1) New_4_like (37)	n/a
	4	>52	13_A2 (4) New_21	A2 A1
	5	27	New_18	A2
3	2	33	New_5 (2) New_6 (24)	A2 A2
	3	37	New_14 (4) New_16 (46)	n/a
	4	>52	13_A2 (4)	A2
	5	72	New_18 (4) New_19 (18) New_20 (1)	n/a A2 n/a
4	2	43	New_6 (3) New_7 (7) New_8 (2)	A2 A2 A1
	3	37	New_15 (59)	n/a
	4	>52	6_A1 (2)	A1
	5	54	New_19 (30) New_18 (1)	A2 n/a

TABLE 9. DETAILS ON THE TIMING OF BLIGHT OUTBREAKS IN EACH OF THE FOUR TUNNELS AND THE GENOTYPE AND MATING TYPE OF THE RESULTANT SAMPLES OVER CYCLES 2-5.

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The tunnel crops from each cycle were inspected daily and blight infection occurred from 30 days after planting (cycle 2) up to 72 days after planting. Infection was normally first seen between 30 and 60 days (Table 9). The blight symptoms in each cycle were first noted low down on the stems or on lower leaves. Local foci of infection then developed and, despite the overhead irrigation, in general, spread slowly and infected the whole crop over several weeks. Very few isolates were recovered from cycle 4 due to problems with poor crop emergence.

Over all 4 tunnels and 4 cycles in which oospore-based infection was anticipated, a large range of 22 novel *P. infestans* genotypes were detected. Broadly speaking, specific genotypes were confined to a single tunnel with only trace levels cross infecting from one tunnel to another during cycles 2-5 (Table 9 and Figs. 15–18). However, from a detailed examination of the parental A1 and A2 genotypes in each tunnel and the SSR alleles found in the subsequent crops it was clear that some tunnel-to-tunnel cross infection occurred during cycle 1 when the initial oospore load was created. Despite precautions such as having different sets of boots and overalls for each tunnel, this spread was difficult to avoid given that tunnel doors had to be left open during the day at some stages to prevent the crop overheating. A striking feature of these studies was the absence of carry-over of any genotype from one cycle to the next as asexual clonal inoculum. In the single case (cycle 4) where some isolates of the widespread clonal types, 13_A2 and 6_A1, were recovered these were not found causing infection in the subsequent crop. This absence of clonal GB genotypes indicates that the strategy of growing the crops under plastic and out of the main potato cropping period was successful in minimising outside influences on this trial.

Mating type testing of the isolates from each tunnel indicated that both A1 and A2 type isolates were present in several of the tunnel outbreaks so the possibility of rounds of oospore formation in addition to that in cycle 1 cannot be ruled out. The generally slow spread of specific foci of infection within the tunnels with limited airflow compared to an open field crop may have reduced the mixing of inoculum and thus the likelihood of mixed infections and oospore formation. The examination of leaves with multiple lesions sampled from cycle three supports this as no oospores were observed.

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Isolate	genotype	Pi02	Pi02	Pi02	D13	D13	D13	Pi33	Pi33	Pi33	Pi04	Pi04	Pi04	Pi4B	Pi4B	Pi4B	Pi16	Pi16	Pi16	G11	G11	G11	Pi56	Pi56	Pi63	Pi63	Pi63	Pi70	Pi70	Pi89	Pi89	Pi89	
11	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	
3	1-A1	160	162	0	136	136	0	203	203	0	166	170	0	213	217	0	178	178	0	140	162	0	176	176	148	157	0	192	192	179	195	199	
																																	Frequency
	New_1	160	162	0	118	136	0	203	203	0	170	170	0	205	213	0	176	176	0	140	160	162	176	176	157	157	0	192	192	179	195	199	2
		160	162	0	136	136	0	203	203	0	170	170	0	205	213	0	176	176	0	140	160	162	176	176	157	157	0	192	192	179	195	199	14
		160	162	0	136	136	0	203	203	0	170	170	0	205	213	0	176	176	0	*140*	*162*	0	176	176	157	157	0	192	192	179	195	199	1
		160	162	0	136	136	0	203	203	0	170	170	0	205	213	0	176	176	0	*140*	*160*	0	176	176	157	157	0	192	192	179	179	0	1
Cycle 2																																	
	New_2	162	164	0	118	136	0	203	206	0	166	170	0	205	217	0	176	178	0	162	162	0	174	176	151	157	0	192	192	179	179	0	2
		162	164	0	118	136	0	203	206	0	166	170	0	205	217	0	176	178	0	160	162	0	174	176	151	157	0	192	192	179	179	0	1
		162	164	0	118	136	0	203	206	0	166	170	0	205	217	0	176	178	0	160	166	0	174	176	151	157	0	192	192	179	179	0	1
		162	164	0	118	136	0	203	203	0	166	170	0	205	217	0	176	178	0	0	0	0	174	176	151	157	0	192	192	179	179	0	1
																																	Frequency
	New_14	160	160	0	136	154	0	203	203	0	166	170	0	0	0	0	178	178	0	140	160	162	176	176	157	157	0	192	192	0	0		20
		160	160	0	136	136	0	203	203	0	166	170	0	0	0	0	178	178	0	140	160	162	176	176	157	157	0	192	192	0	0		2
		160	160	0	136	154	0	203	203	0	166	170	0	205	217	0	178	178	0	140	160	162	176	176	157	157	0	192	192	195	195		7
		160	160	0	136	154	0	203	203	0	166	170	0	205	217	0	178	178	0	140	160	162	176	176	?	157	0	192	192	199	199		1
		160	160	0	0	0	0	0	0	0	166	170	0	0	0	0	0	0	0	140	160	162	176	176	0	0	0	192	192	0	0		20
		160	160	0	0	0	0	0	0	0	166	170	0	205	217	0	0	0	0	140	160	162	176	176	0	0	0	192	192	195	195		7
Cycle 3																																	
	New_7?	160	160	0	136	154	0	203	203	0	166	170	0	0	0	0	176	178	0	140	160	162	176	176	0	0	0	192	192	0	0	0	22
		0	0	0	136	136	0	203	203	0	166	170	0	0	0	0	176	178	0	140	160	162	176	?	0	0	0	192	192	0	0	0	1
		0	0	0	136	158	0	203	203	0	166	170	0	0	0	0	176	178	0	140	160	162	176	176	0	0	0	192	192	0	0	0	1
		0	0	0	136	154	0	203	203	0	166	170	0	0	0	0	176	178	0	140	160	166	176	176	0	0	0	192	192	0	0	0	1
																																	Frequency
	New_17	160	160	0	136	154	0	203	203	0	166	170	0	205	213	0	176	178	0	140	160	0	174	176	151	157	0	192	192	179	179	0	13
Cycle 5		160	160	0	136	154	0	203	203	0	166	170	0	205	213	0	176	178	0	140	160	0	174	176	151	157	0	192	192	179	199	0	7

FIGURE 15. SUMMARY OF GENOTYPES IDENTIFIED FROM OUTBREAKS OF BLIGHT IN TUNNEL 1. THE LOCI EXAMINED ARE SHOWN IN THE COLUMNS AND THE ALLELES AT EACH LOCI FOR SPECIFIC ISOLATES IN THE ROWS. THE NUMBER OF TIMES EACH COMBINATION OF ALLELES (GENOTYPE) WAS RECOVERED IS SHOWN IN THE RIGHT HAND COLUMN. THE UPPER TWO ROWS INDICATE THE PARENTAL A1 AND A2 ISOLATES USED TO INFECT THE CROP IN THE FIRST CYCLE.

The SSR fingerprints of *P. infestans* from blight lesions examined in cycles 2-5 in Tunnel 1 (Fig. 15) were consistent with the germination and infection of oospores formed as a result of sexual recombination of the isolates introduced in cycle 1 (parental isolates). The new combination of the 140bp allele with 160bp and 162bp alleles at the G11 locus, in particular, was diagnostic. Similarly, the 195/195bp combination at locus Pi89 has never been amplified in any of the 3754 GB field isolates amplified to date. No isolates with SSR profiles identical to the parental isolates were recovered. In cycle 2 eight isolates with alleles not present in either of the Tunnel 1 parent isolates were noted (cells with green border in Fig. 15). In three of the loci this suggested limited cross-contamination of the pathogen from Tunnels 2 or 3. The 164bp allele at locus Pi02 was not, however, present in the parental isolates used in any tunnel and is found at a very low frequency in all GB isolates. The presence of this allele may represent a rare mutation at this locus. The majority of isolates of a specific named (e.g. New_14) SSR genotype were identical at all SSR loci. For example 14 isolates of genotype New_1 were noted in Cycle 2 and 55 of New_14 in cycle 3. However, some instability amongst the profiles was observed.

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Isolate	genotype	Pi02	Pi02	Pi02	D13	D13	D13	Pi33	Pi33	Pi33	Pi04	Pi04	Pi04	Pi4B	Pi4B	Pi4B	Pi16	Pi16	Pi16	G11	G11	G11	Pi56	Pi56	Pi63	Pi63	Pi63	Pi70	Pi70	Pi89	Pi89	Pi89	Frequency
6	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	
15	3_A2	162	162	0	118	136	0	203	203	0	166	170	0	213	217	0	176	178	0	154	160	0	176	176	148	157	0	192	195	179	179	0	
	New_3	160	162	0	118	136	154	203	206	0	166	170	0	205	205	0	176	178	0	154	160	0	176	176	151	157	0	192	192	179	179	0	1
		160	162	0	118	136	154	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	14
		160	162	0	118	136	140	203	203	0	*166*	*170*	0	205	205	0	176	178	0	*154*	*160*	0	176	176	*151*	*157*	0	*192*	*192*	179	181	0	1
Cycle 2		160	162	0	118	136	0	203	206	0	166	170	0	205	217	0	176	178	0	154	160	0	174	176	151	157	0	192	192	179	181	0	1
	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	5
		160	162	0	118	136	0	203	206	0	166	170	0	205	213	217	176	178	0	154	160	0	176	176	151	157	0	192	192	179	179	0	2
	New_4 Like	160	162	0	118	136	0	203	206	0	166	170	0	205	213	0	176	178	0	154	160	0	176	176	0	0	0	192	192	179	179	0	6
		160	162	0	118	136	0	203	203	0	166	170	0	205	213	217	176	178	0	154	160	0	174	176	0	0	0	192	192	179	181	0	10
		160	162	0	118	136	0	203	203	0	166	170	0	0	0	0	178	178	0	154	160	0	174	176	151	157	0	192	192	179	179	0	15
		160	162	0	118	136	0	203	203	0	166	170	0	0	0	0	178	178	0	154	160	0	174	176	151	157	0	192	192	179	181	0	6
		160	162	0	118	136	0	203	206	0	166	170	0	0	0	0	178	178	0	154	160	0	176	176	157	157	0	192	192	179	181	0	1
	New_16?	0	0	0	118	118	0	203	203	0	166	170	0	0	0	0	176	178	0	154	154	0	176	176	0	0	0	0	0	0	0	0	1
		160	162	0	118	136	0	203	203	0	166	170	0	0	0	0	176	178	0	154	154	0	176	176	0	0	0	192	192	0	0	0	7
		160	162	0	0	0	0	0	0	0	166	170	0	0	0	0	0	0	0	154	154	0	176	176	0	0	0	192	192	179	181	0	1
	13_A2_like	160	162	0	136	154	0	203	203	0	166	170	0	205	213	217	176	178	0	160	160	0	176	176	0	0	0	192	192	179	179	0	2
Cycle 3		160	162	0	136	154	0	203	203	0	166	170	0	205	213	217	178	178	0	160	160	0	176	176	151	157	0	0	0	179	179	0	5
		160	162	0	136	154	0	203	203	0	166	170	0	0	0	0	178	178	0	154	160	0	0	0	151	157	0	0	0	179	179	0	2
		160	162	0	136	154	0	203	203	0	0	0	0	0	0	0	178	178	0	0	0	0	0	0	151	157	0	0	0	179	179	0	1
	New_16	0	0	0	118	136	0	203	203	0	166	170	0	0	0	0	176	178	0	0	0	0	176	176	0	0	0	0	0	0	0	0	1
	New_15	160	162	0	136	154	0	203	203	0	160	168	0	0	0	0	176	178	0	140	160	162	174	176	151	157	0	192	192	179	195	199	1
cycle 5	New_18	160	162	0	136	136	0	203	203	0	166	170	0	205	217	0	176	178	0	154	160	0	176	176	151	157	0	192	192	179	179	0	17

FIGURE 16. SUMMARY OF GENOTYPES IDENTIFIED FROM TUNNEL 2 (LEGEND AS PER FIGURE 15).

The blight outbreaks in Tunnel 2 also comprised novel combinations of SSR alleles that differed from the parental genotypes (Fig. 16). The presence of the 154bp allele at D13 and the 160bp allele at Pi02 are however inconsistent with their origin from the introduced 7_A1 and 3_A2 parent strains. In another case, the 205/205bp combination at Pi4B would not be predicted from the combinations introduced in the parental strains. Collectively this suggests the outbreaks are from recombinant strains but that, during cycle 1, Tunnel two has become contaminated with genotype 13_A2 from tunnel one. The presence of only a single isolate (New_15) with the 140bp allele at G11 in cycle 3 suggests genotype 1_A1 did not infiltrate the tunnel during cycle 1.

As with the other tunnels, the outbreaks in Tunnel 3 cycles 2-5 also comprised novel combinations of SSR alleles different from the parental genotypes (Fig. 17). Again alleles that were not found in either parent suggested some cross-contamination had occurred between tunnels. The 170/170bp combination at locus Pi04 in cycle 2 is rare in the wider GB crop having been found in only two rare genotypes and a very few isolates of the miscellaneous SSR category. This is further evidence that sexual recombination has re-assorted these alleles.

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Isolate	genotype	Pi02	Pi02	Pi02	D13	D13	D13	Pi33	Pi33	Pi33	Pi04	Pi04	Pi04	Pi4B	Pi4B	Pi4B	Pi16	Pi16	Pi16	G11	G11	G11	Pi56	Pi56	Pi63	Pi63	Pi63	Pi70	Pi70	Pi89	Pi89	Pi89	Frequency
8	8_2a_A1	162	162	0	118	136	0	203	206	0	166	170	0	205	217	0	176	178	0	166	166	0	176	176	151	157	0	192	192	179	181	0	
19	17_A2	160	162	0	118	118	0	203	203	0	0	0	0	213	217	0	176	178	0	160	162	0	176	176	148	148	0	192	192	179	179	0	
	New_5	160	162	0	136	154	0	203	203	0	170	170	0	213	217	0	178	178	0	140	160	162	176	176	148	157	0	192	192	179	199		1
		160	162	0	136	154	0	203	203	0	170	170	0	213	217	0	178	178	0	140	160	162	176	176	148	157	0	192	192	179	179		1
	New_6	160	160	0	136	154	0	203	203	0	0	0	0	205	213	217	178	178	0	0	0	0	174	176	0	0	0	0	0	179	179	0	1
		160	162	0	136	154	0	203	203	0	170	170	0	205	213	217	178	178	0	140	160	162	174	176	148	151	157	192	192	179	195	199	15
Cycle 2		160	162	0	136	154	0	203	203	0	170	170	0	205	213	217	178	178	0	0	0	0	174	176	151	157	0	192	192	179	195	199	1
		160	162	0	136	154	0	ff	ff	0	170	170	0	ff	ff	0	178	178	0	140	162	0	174	176	148	151	157	192	192	ff	ff	0	2
		160	162	0	136	136	0	ff	ff	0	170	170	0	ff	ff	0	178	178	0	140	162	0	174	176	148	148	0	192	192	179	179	0	1
		160	162	0	118	136	0	203	203	0	170	170	0	205	213	217	178	178	0	140	160	162	174	176	148	151	157	192	192	179	195	199	1
		160	162	0	118	136	0	203	203	0	170	170	0	205	213	217	178	178	0	140	162	0	174	176	148	151	157	192	192	179	179	0	1
		160	162	0	136	154	0	203	203	0	170	170	0	ff	ff	0	178	178	0	140	160	162	174	176	148	151	157	192	192	179	179	0	1
		160	162	0	132	150	0	0	0	0	170	170	0	205	213	0	176	178	0	140	160	162	174	176	148	151	157	192	192	0	0	0	1
	New_16	0	0	0	0	0	0	0	0	0	166	170	0	0	0	0	0	0	0	140	154	0	174	176	0	0	0	192	192	0	0	0	17
		160	160	0	0	0	0	0	0	0	166	170	0	0	0	0	0	0	0	140	160	162	176	176	0	0	0	192	192	0	0	0	1
		160	160	0	0	0	0	0	0	0	166	170	0	0	0	0	0	0	0	140	154	0	174	176	0	0	0	192	192	0	0	0	7
		160	160	0	0	0	0	0	0	0	170	170	0	0	0	0	0	0	0	140	140	0	174	176	0	0	0	192	192	179	179	0	1
		160	162	0	0	0	0	0	0	0	166	170	0	205	213	0	0	0	0	140	154	0	174	176	0	0	0	192	192	179	179	0	1
		160	162	0	136	136	0	203	203	0	166	170	0	0	0	0	176	178	0	140	154	0	174	176	0	0	0	192	192	0	0	0	23
Cycle 3		160	162	0	136	136	0	203	203	0	166	170	0	0	0	0	176	178	0	140	160	162	174	176	0	0	0	192	192	0	0	0	1
		160	160	0	136	136	0	0	0	0	166	170	0	0	0	0	0	0	0	140	154	160	174	176	0	0	0	192	192	0	0	0	1
		0	0	0	118	136	0	203	203	0	0	0	0	0	0	0	176	178	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
		0	0	0	0	0	0	203	206	0	166	170	0	0	0	0	0	0	0	0	0	0	174	176	0	0	0	0	0	0	0	0	1
	New_14	160	160	0	136	154	0	203	203	0	166	170	0	0	0	0	178	178	0	140	160	162	176	176	0	0	0	192	192	0	0	0	1
	New_18	160	162	0	136	136	0	203	203	0	166	170	0	205	217	0	176	178	0	154	160	0	176	176	151	157	0	192	192	179	179	0	4
	New_19	160	162	0	136	136	0	203	203	0	166	170	0	205	213	0	178	178	0	140	160	162	176	176	157	157	0	192	192	179	179	0	14
Cycle 5		160	162	0	118	136	0	203	203	0	166	170	0	205	213	0	178	178	0	140	160	162	176	176	157	157	0	192	192	179	179	0	4
	New_20	160	162	0	136	136	0	0	0	0	166	170	0	205	217	0	0	0	0	140	160	162	176	176	151	157	0	0	0	179	179	0	1

FIGURE 17. SUMMARY OF GENOTYPES IDENTIFIED FROM TUNNEL 3 (LEGEND AS PER FIGURE 15).

Isolate	genotype	Pi02	Pi02	Pi02	D13	D13	D13	Pi33	Pi33	Pi33	Pi04	Pi04	Pi04	Pi4B	Pi4B	Pi4B	Pi16	Pi16	Pi16	G11	G11	G11	Pi56	Pi56	Pi63	Pi63	Pi63	Pi70	Pi70	Pi89	Pi89	Pi89	Frequency
3	1_A1	160	162	0	136	136	0	203	203	0	166	170	0	213	217	0	178	178	0	140	162	0	176	176	148	157	0	192	192	179	195	199	
11	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	
6	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	
15	3_A2	162	162	0	118	136	0	203	203	0	166	170	0	213	217	0	176	178	0	154	160	0	176	176	148	157	0	192	195	179	179	0	
8	8_2a_A1	162	162	0	118	136	0	203	206	0	166	170	0	205	217	0	176	178	0	166	166	0	176	176	151	157	0	192	192	179	181	0	
19	17_A2	160	162	0	118	118	0	203	203	0	0	0	0	213	217	0	176	178	0	160	162	0	176	176	148	148	0	192	192	179	179		
	New_6	160	162	0	136	154	0	203	203	0	170	170	0	205	213	217	178	178	0	140	160	162	174	176	148	151	157	192	192	179	195	0	2
		160	162	0	136	154	0	203	203	0	170	170	0	205	213	217	178	178	0	140	160	162	174	176	148	151	157	192	192	179	179	0	1
	New_7	160	162	0	136	154	0	203	203	0	166	170	0	205	213	217	178	178	0	140	160	0	176	176	148	157	0	192	192	179	195	0	4
Cycle 2		160	162	0	136	154	0	203	203	0	166	170	0	205	213	217	176	178	0	140	160	0	176	176	148	157	0	192	192	179	195	0	1
		160	162	0	118	136	154	203	203	0	166	170	0	205	213	217	178	178	0	140	160	0	176	176	148	157	0	192	192	179	195	0	1
		160	162	0	122	136	154	0	0	0	166	170	0	205	213	217	178	178	0	140	160	0	176	176	148	157	0	192	192	179	195	0	1
	New_8	160	162	0	136	154	0	203	206	0	168	168	0	213	217	0	176	178	0	154	166	0	174	176	151	157	0	192	192	179	179	0	1
		160	162	0	136	152	0	203	206	0	160	168	0	213	217	0	176	178	0	154	166	0	174	176	151	157	0	192	192	179	179	0	1
	New_15	160	162	0	136	154	0	203	203	0	0	0	0	205	213	217	176	178	0	140	160	162	174	176	151	157	0	0	0	179	195	199	8
		160	160	0	136	154	0	203	203	0	160	168	0	0	0	0	176	178	0	140	160	162	174	176	0	0	0	192	192	0	0	0	3
		160	162	0	136	136	0	203	203	0	0	0	0	0	0	0	176	178	0	0	0	0	174	176	0	0	0	0	0	0	0	0	2
		160	162	0	136	154	0	203	203	0	160	168	0	205	213	217	178	178	0	140	160	162	174	176	151	157	0	192	192	179	195	199	4
		160	162	0	136	154	0	203	203	0	0	0	0	0	0	0	176	178	0	0	0	0	0	0	148	151	157	0	0	179	179	0	1
		160	162	0	136	154	0	203	203	0	160	168	0	205	213	217	176	178	0	140	160	162	174	176	151	157	0	192	192	179	179	0	11
Cycle 3																																	
	New_15?	160	162	0	0	0	0	0	0	0	160	168	0	0	0	0	0	0	0	140	160	162	174	176	0	0	0	192	192	179	179	0	21
		0	0	0	0	0	0	0	0	0	166	170	0	0	0	0	0	0	0	140	160	162	0	0	0	0	0	0	0	0	0	0	1
		160	162	0	0	0	0	0	0	0	160	168	0	0	0	0	0	0	0	140	160	162	176	176	0	0	0	192	192	179	179	0	2
	13_A2_like	160	162	0	136	154	0	203	206	0	166	170	0	0	0	0	178	178	0	160	160	0	174	176	151	157	0	192	192	179	181	0	1
	New_18	160	162	0	136	136	0	203	203	0	166	170	0	205	217	0	176	178	0	154	160	0	176	176	151	157	0	192	192	179	179	0	1
Cycle 5	New_19	160	162	0	136	136	0	203	203	0	166	170	0	205	213	0	178	178	0	140	160	162	176	176	157	157	0	192	192	179	179	0	28
		160	162	0	118	136	0	203	203	0	166	170	0	205	213	0	178	178	0	140	160	162	176	176	157	157	0	192	192	179	179	0	2
		160	162	0	136	136	0	203	203	0	166	170	0	205	213	0	178	178	0	140	160	162	176	176	151	157	0	192	192	179	179	0	2

FIGURE 18. SUMMARY OF GENOTYPES IDENTIFIED FROM TUNNEL 4. (LEGEND AS PER FIGURE 15).

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All six parental isolates were introduced into tunnel 4 during cycle 1 and a greater diversity of SSR genotypes would therefore have been predicted. There were however, no more genotypes present in tunnel 4 than any other (Fig. 18).

In general, multiple replicate lesions of the same genotype were identical in SSR fingerprint but variation amongst the SSR profiles of the some genotypes was evident (Figs. 15-18). There are three possible explanations for this: a) more than one genotype was pressed onto an FTA card and the fingerprint thus represents a mixture of DNA *P. infestans* genotype b) instability in the *P. infestans* genome during recombination or c) technical failure of PCR amplification of low levels of the pathogen DNA captured on FTA cards. The cause of this is not clear but amplification of the variant SSR profile in several samples would suggest that experimental error and artefacts are unlikely (see discussion for further considerations). The FTA results were confirmed in many cases by the parallel fingerprinting of an isolate recovered from a specific lesion that was also processed via FTA card analysis.

4.4.1. Oospore detection

Test crosses of all three parental combinations on agar and on leaf material in the laboratory generated abundant oospores. A sample of leaves from the tunnels in cycle 1 also confirmed that oospores were abundant. Oospores were not detected in leaves from other cycles. Particularly, leaflets with multiple lesions collected in cycle 3 were examined along with experimentally infected leaflets inoculated with A1 and A2 isolates. Only the latter showed the presence of oospores. We conclude that oospores were not abundant in leaflets from cycles 2 and 3 in any tunnel.

The PCR-based detection of *P. infestans* oospore DNA in the tunnel soil samples was not attempted as testing of the assay in soils 'spiked' with large numbers of oospores indicated that the oospores were not being disrupted by even aggressive ball-milling (Retsch mill) that has proved effective for other pathogens. Other methods are now being reviewed and investigated in project R423.

All the data above suggests that oospores are responsible for the blight infection in the tunnel-grown crops. The presence of only 22 genotypes in tunnel- and cycle-specific patterns also suggests that a very small proportion of oospores in each tunnel germinated and infected in each crop (see discussion for more detail).

4.5. Aggressiveness studies

4.5.1. Objective 6. Foliar assay on detached leaves under controlled conditions in growth rooms

The use of six replicates for each treatment proved valuable as analysis of variance (ANOVA) indicated that there were highly significant differences between isolates, genotypes and cultivars in this experiment (example shown in Table 10). There were also significant interactions between variety and *P. infestans* isolates/genotypes.

The mean lesion areas for individual isolates indicate that genotype 13_A2 isolates, on average, caused larger lesions than other genotypes at 13°C (Fig. 19). This result is not so pronounced at 18°C with the genotype 13_A2 isolates falling to the left and centre of the chart (Fig. 20). The mean of the lesion area caused by the two isolates of each genotype (or six isolates in the case of genotype 13_A2) are shown in Fig. 21. In this case, genotype 6_A1 was shown, on average, to cause the largest lesions

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closely followed by genotype 13_A2 and 17_A2. Analysis of variance indicated statistically significant variety by genotype interactions in lesion area. The lesion size caused by each genotype varied according to variety. All genotypes generated the smallest lesions on Cara at 13°C and, somewhat surprisingly the lesion sizes were not always greatest on King Edward, the variety with the lowest foliar resistance score (Fig. 22). An interaction was evident on Lady Balfour with some genotypes causing markedly larger lesions (6_A1 & 13_A2) than others (17_A2, 7_A1 & 10_A2) where the host resistance was more effective and lesions smaller. At 18°C the *P. infestans* genotypes caused more similar sized lesions on all varieties (Fig. 23) and the interactions seen at 13°C were not apparent. The largest lesions were formed by six of the eight genotypes on King Edward, the variety with the lowest resistance rating.

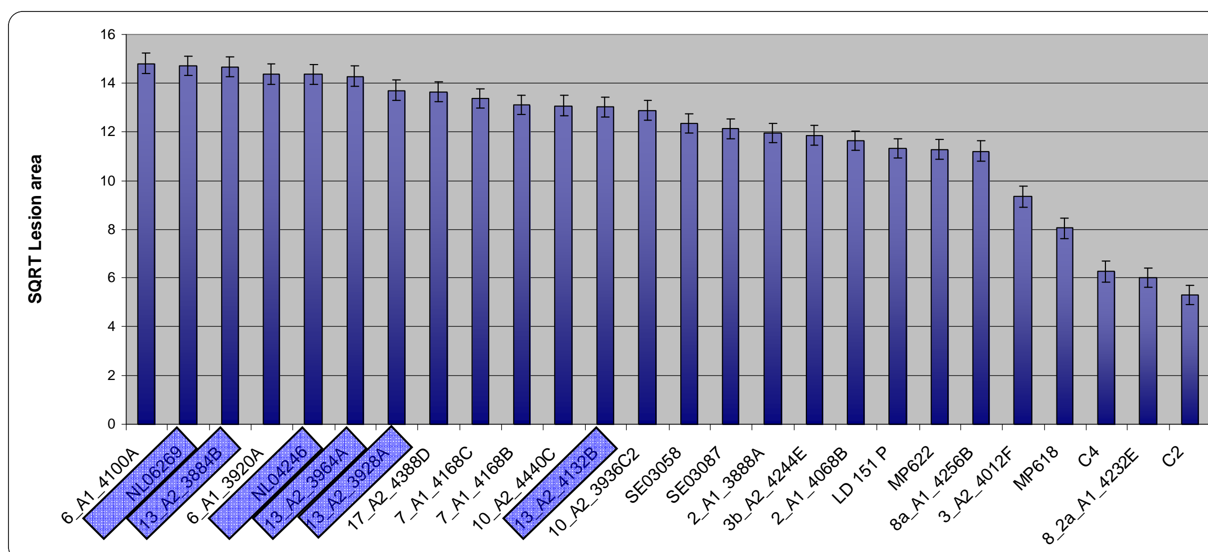

FIGURE 19. MEAN LESION AREA CAUSED BY EACH ISOLATE OF *P. INFESTANS* ON LEAVES INCUBATED AT 13°C. EACH ISOLATE IS LABELLED WITH THE GENOTYPE IDENTIFIER FOLLOWED BY THE ISOLATE NUMBER (SEE TABLE 2). GENOTYPE 13_A2 ISOLATES ARE MARKED IN BLUE. BARS SHOW THE STANDARD ERROR OF THE MEAN = 0.4064 D.F. 624.

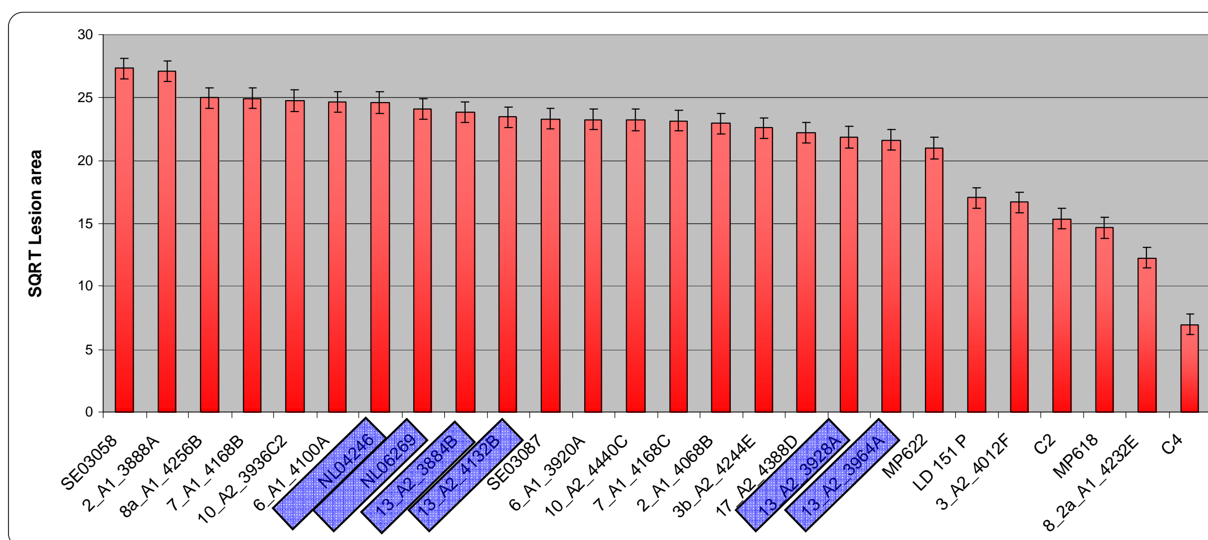

FIGURE 20. MEAN LESION AREA CAUSED BY EACH ISOLATE OF *P. INFESTANS* ON LEAVES INCUBATED AT 18°C. EACH ISOLATE IS LABELLED WITH THE GENOTYPE IDENTIFIER FOLLOWED BY THE ISOLATE NUMBER (SEE TABLE 2). GENOTYPE 13_A2 ISOLATES ARE MARKED IN BLUE. BARS SHOW THE STANDARD ERROR OF THE MEAN = 0.823 D.F. 619.

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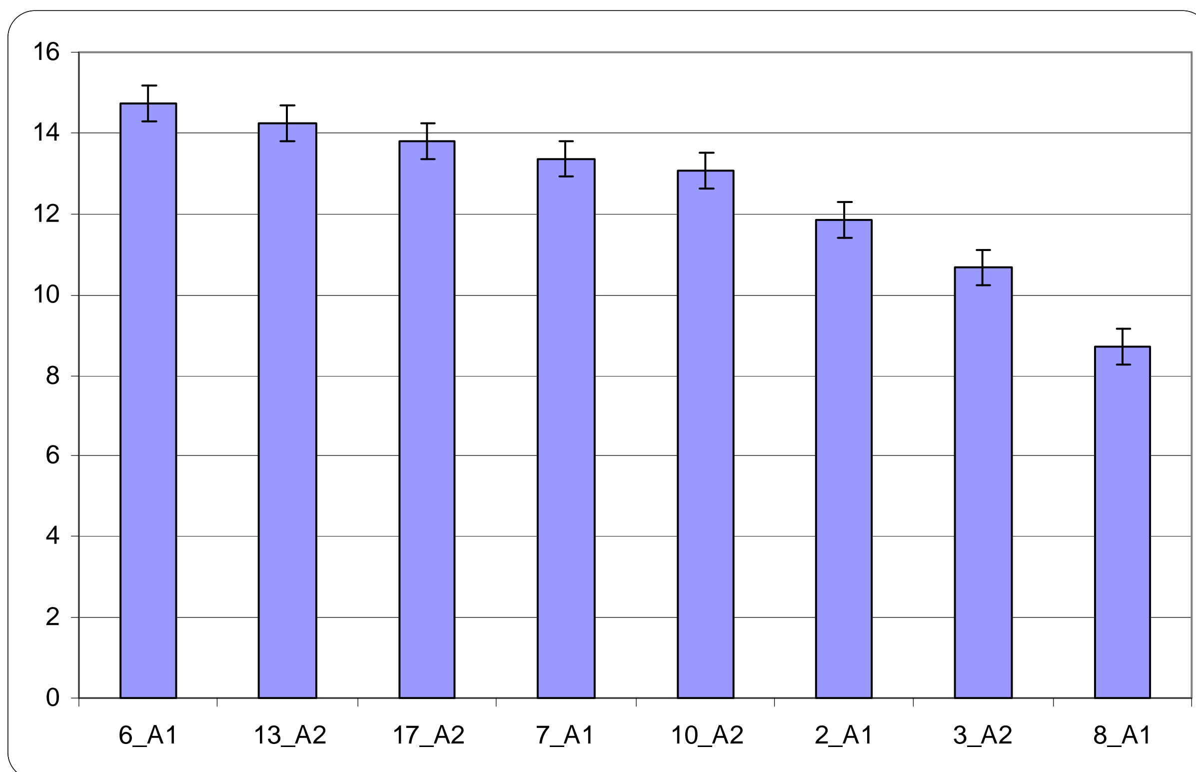

Figure 21. Mean lesion area caused by each of the eight genotypes of *P. infestans* on leaves incubated at 13°C. Each column comprises the mean of all the isolates of that genotype on all varieties. Bars show the standard error of the mean =0.4425 d.f. 175.

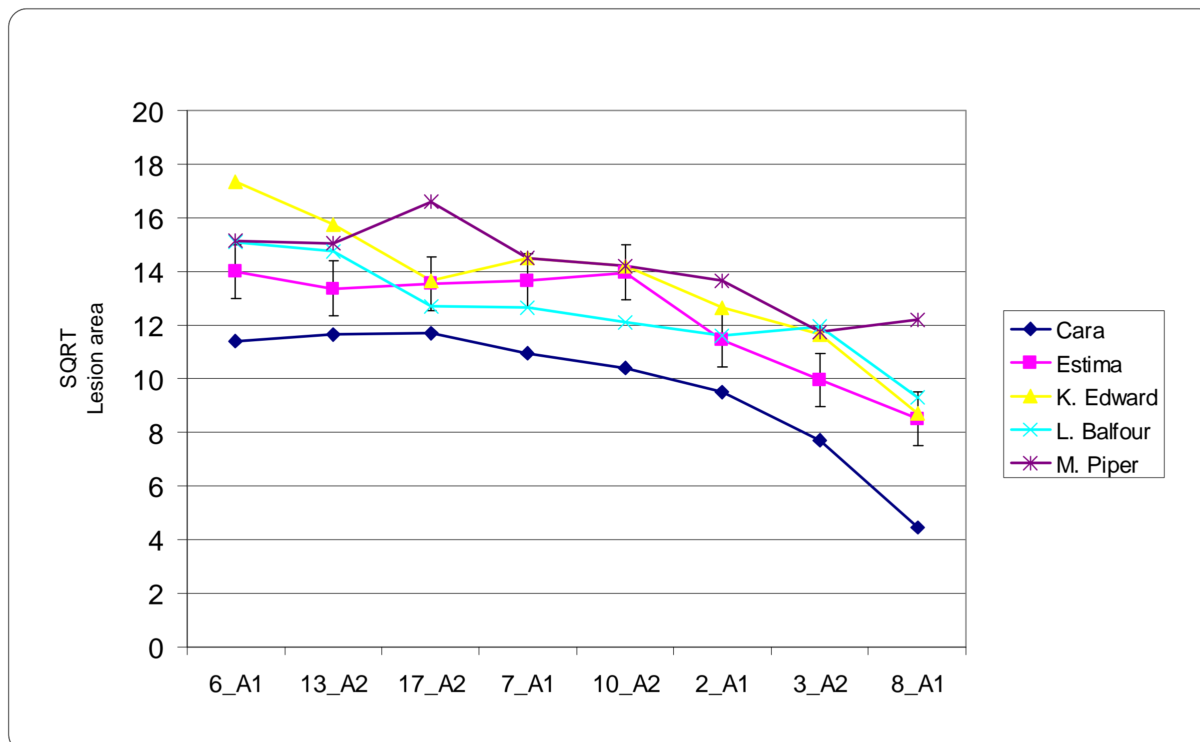

Figure 22. Mean lesion area caused by each of the eight GB genotypes of *P. infestans* on leaves of each of five potato varieties after incubation at 13°C. Each point comprises the mean of all the isolates of that genotype (Table 2). Bars on Estima line show the standard error of the mean for the genotype by variety comparisons: s.e. =1.005; d.f.=195.

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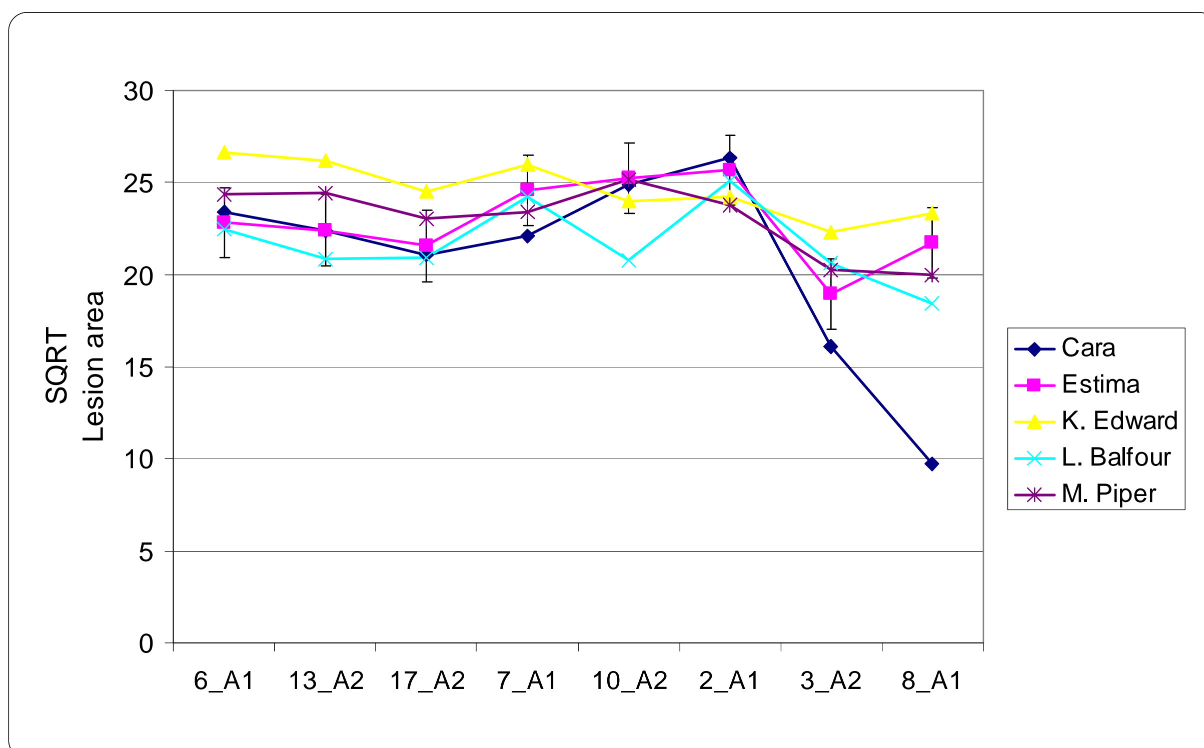

FIGURE 23. MEAN LESION AREA CAUSED BY EACH OF THE EIGHT GB GENOTYPES OF *P. INFESTANS* ON LEAVES OF EACH OF FIVE VARIETIES INCUBATED AT 18°C. EACH POINT COMPRISES THE MEAN OF ALL THE ISOLATES OF THAT GENOTYPE (TABLE 2). BARS ON ESTIMA LINE SHOW THE STANDARD ERROR OF THE MEAN FOR THE GENOTYPE BY VARIETY COMPARISONS: S.E.D.=1.916; D.F.=195.

Variate: av_length					
Source of variation	d.f.(m.v.)	s.s	m.s	v.r	F pr
Rep stratum	5	122.780	24.556	2.16	
Rep.box stratum					
Cv	4	1343.87	335.970	29.62	<.001
Residual	20	226.846	11.342	1.93	
Rep.box.genotype stratum					
Genotype	7	1987.061	283.866	48.32	<.001
Genotype.cv	28	285.260	10.188	1.73	0.018
Residual	175	1028.072	5.875	1.12	
Rep.box.genotype.*Units* stratum					
Genotype.isolate	11	579.890	52.717	10.09	<.001
Genotype.cv.isolate	44	266.661	6.060	1.16	0.238
Residual	274	1431.680	5.225		
TOTAL	568	7266.737			

TABLE 10. EXAMPLE OF ANOVA OUTPUT FROM GENSTAT OF AVERAGE LESION LENGTH CAUSED BY EACH GENOTYPE AFTER INCUBATION AT 13°C INDICATING HIGHLY SIGNIFICANT EFFECTS OF CV (CULTIVAR) AND *P. INFESTANS* GENOTYPE.

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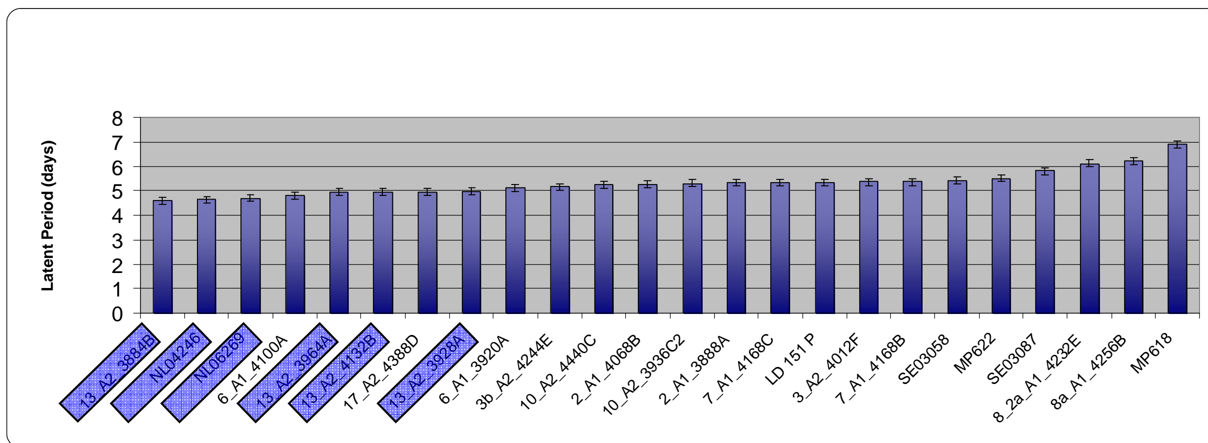

FIGURE 24. MEAN LATENT PERIOD AFTER INOCULATION WITH ISOLATES OF *P. INFESTANS* ON LEAVES INCUBATED AT 13°C. EACH ISOLATE IS LABELLED WITH THE GENOTYPE IDENTIFIER FOLLOWED BY THE ISOLATE NUMBER (SEE TABLE 2). GENOTYPE 13_A2 ISOLATES ARE MARKED IN BLUE. BARS SHOW THE STANDARD ERROR OF THE MEAN = 0.142 D.F. 597.

An assessment of latent period (LP = time in days from inoculation until sporulation was visible to the naked eye) also indicated statistically significant effects of variety, isolates and genotypes. At 13°C genotype 13_A2 isolates had, on average, the lowest LP; i.e. they sporulated sooner than other isolates (Fig. 24). As with lesion length, the results were not so pronounced at 18°C but all six 13_A2 isolates were amongst the 12 isolates with the shortest LP (Fig. 25).

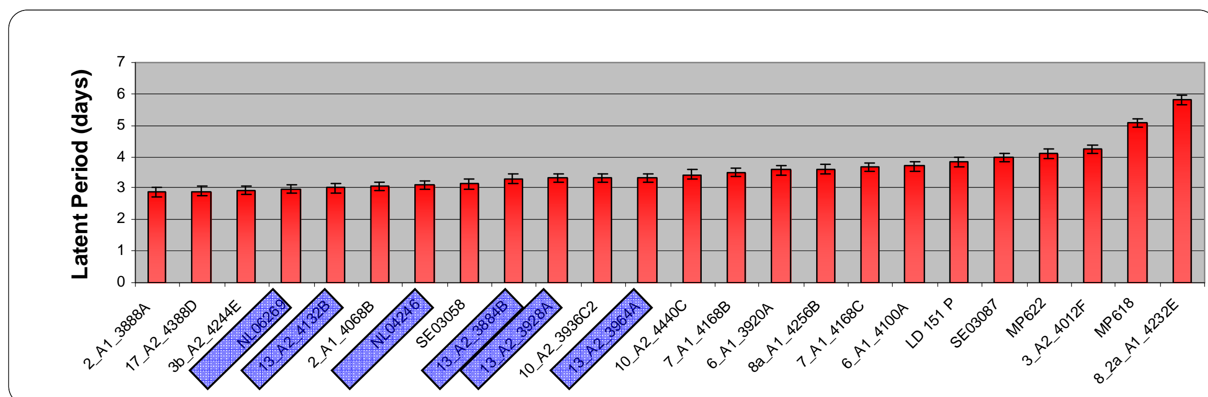

FIGURE 25. MEAN LATENT PERIOD AFTER INOCULATION WITH ISOLATES OF *P. INFESTANS* ON LEAVES INCUBATED AT 18°C. EACH ISOLATE IS LABELLED WITH THE GENOTYPE IDENTIFIER FOLLOWED BY THE ISOLATE NUMBER (SEE TABLE 2). GENOTYPE 13_A2 ISOLATES ARE MARKED IN BLUE. BARS SHOW THE STANDARD ERROR OF THE MEAN = 0.1414 D.F. 595.

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4.5.2. Objective 7. Assessment of tuber aggressiveness of GB and European *P. infestans* isolates

The mean area of the tuber surface that was blighted on each cultivar ranged from approximately 10 to 40% with lower levels on the two cultivars with the higher resistance ratings (Fig. 26; Table 3.). Lady Balfour (resistance scale 7) was more blighted than Cara (7) and unexpectedly, blight was less severe on King Edward (4) than Maris Piper with a higher resistance rating (5). A chart of the mean tuber blight levels across all four varieties indicates that three of the four most aggressive isolates were of genotype 13_A2 causing 30% or more of the tuber surface to be blighted (Fig. 27). Two other genotype 13_A2 isolates, however, caused less tuber blight with one resulting in only 14% blight (isolate 06_3884B). The levels of tuber blight in relation to variety (Fig. 28) indicate several interactions between varietal resistance and *P. infestans* genotype. Seven genotypes, for example, cause 10% or less tuber blight on Lady Balfour but isolates of genotypes 3_A2, 13_A2, 17_A2 and 6_A1 cause more than twice this level. Neither Cara nor the other varieties, however, respond to these genotypes in the same manner. An increase in susceptibility of Maris Piper to genotypes 10_A2, 8_A1 and 2_A1 was also apparent.

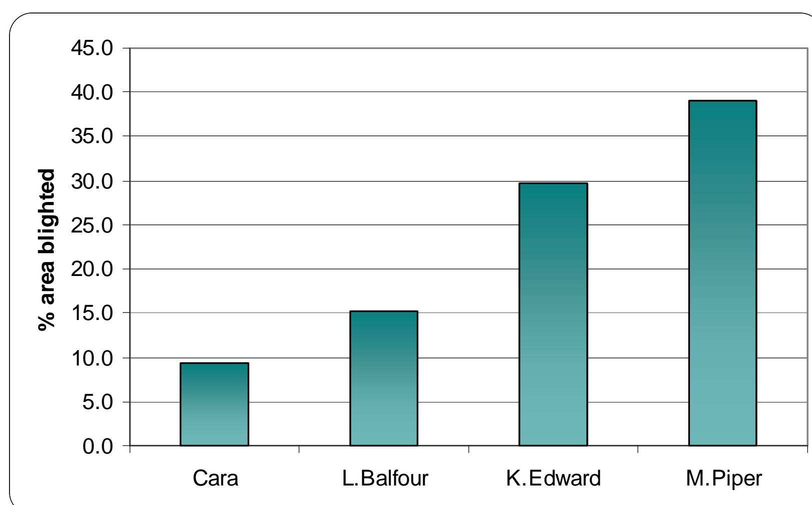

FIGURE 26. THE MEAN PERCENTAGE AREA OF THE TUBER SURFACE THAT WAS BLIGHT INFECTED IN EACH OF THE FOUR CULTIVARS. (S.E. = 1.29 D.F. 135).

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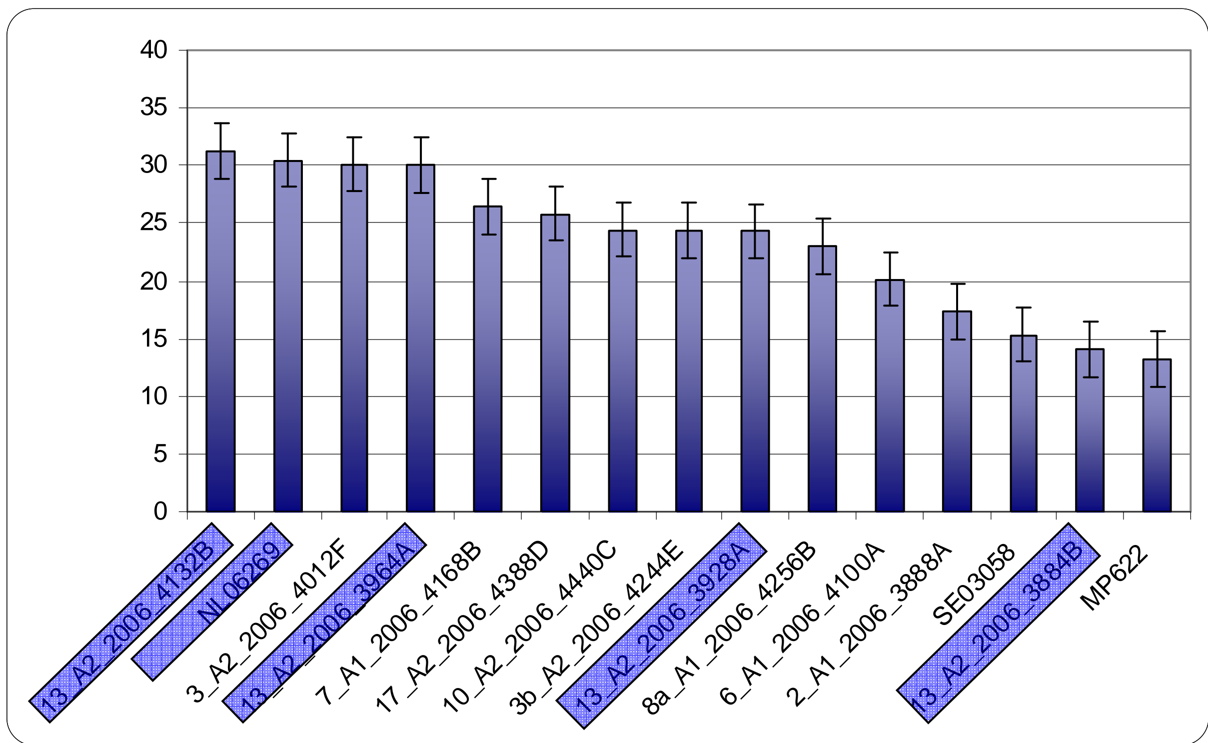

FIGURE 27. MEAN TUBER BLIGHT CAUSED BY EACH OF 15 *P. INFESTANS* ISOLATES. BARS SHOW THE STANDARD ERROR OF THE MEAN: S.E.D. = 2.37 ; D.F.=42.

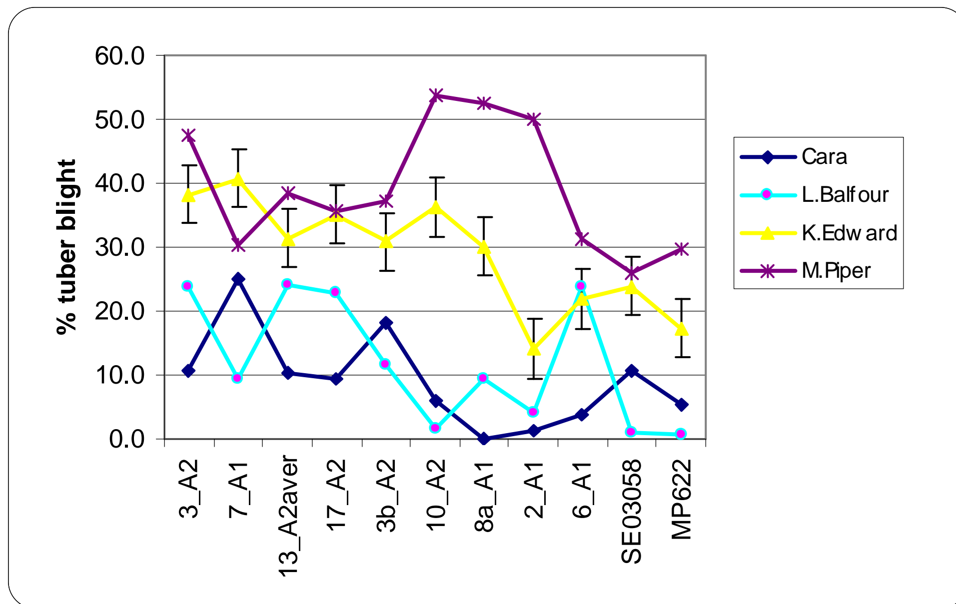

FIGURE 28. MEAN PERCENTAGE TUBER BLIGHT CAUSED BY EACH OF 11 *P. INFESTANS* GENOTYPES ON A RANGE OF FOUR POTATO VARIETIES. BARS REPRESENT STANDARD ERROR FOR GENOTYPE VARIETY INTERACTION = 4.614 DF = 116.

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4.5.3. Objective 8. Field study in which isolate fitness and aggressiveness are monitored over the course of an epidemic

In a laboratory test at the date of inoculation, all isolates caused infection and four of the five genotypes introduced into the trial were re-sampled from the epidemic at least once. In each plot, disease first appeared on the central inoculated plant and was sampled as it spread to neighbouring plants and then across the whole plot. The samples were taken over a three week period on 30 July, 2 Aug, 7 Aug, 13 Aug and 20 Aug 2007. It is clear that genotype 13_A2 has out-competed the other genotypes in the trial as 90% or more of the lesions at each sampling date were caused by genotype 13_A2 (Fig. 29). An additional genotype that was not introduced was also observed. There was no interaction between variety and the *P. infestans* genotypes recovered (data not shown). Despite weekly spraying prior to inoculation, some blight was evident in the guard rows of two plots at around the same stage as the infection first appeared in the inoculated plants (24 July). These lesions were tested and also found to be genotype 13_A2. As the epidemic progressed, blight infection was also noted in the guard rows around each plot which were planted with the blight-resistant variety Stirling that was intended to delay plot-to-plot spread. However, it is now known that Stirling's resistance is broken by genotype 13_A2 and these guard rows may thus have preferentially supported the spread of genotype 13_A2 at the later sample dates. It was, however, clear that genotype 13_A2 also dominated at the first three sample dates when the foci of infection in each plot was clearly in the plants immediately adjacent to the inoculated central plant (Fig. 29).

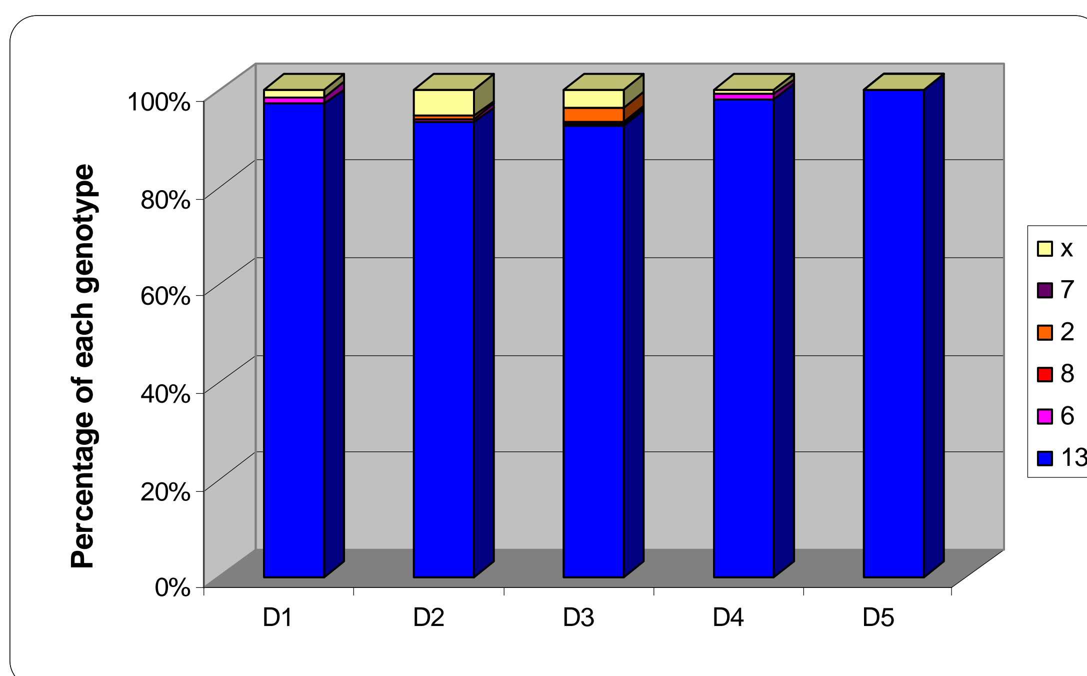

FIGURE 29. RESULTS OF FTA FINGERPRINTING OF BLIGHT LESIONS FROM THE FIELD TRIAL INDICATING THE PROPORTION OF LESIONS CAUSED BY EACH OF THE FIVE INTRODUCED GENOTYPES AT EACH SAMPLING DATE. N = 627 LESIONS WITH 67, 166, 189, 107 & 98 LESIONS SAMPLED AT D1 TO D5 RESPECTIVELY.

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5. Discussion

5.1. Summary

This third and final report summarises the 2008 season and the overall findings of the project. Over the final year of the project considerable progress has been made in our understanding of the changing GB *P. infestans* populations. The frequency of the A2 mating type has continued to increase, being found in 91% of GB outbreaks in 2008. Over 3700 *P. infestans* isolates have been SSR genotyped over the three seasons and this data has proved particularly valuable in understanding the details of the changing population and any role of oospores as primary inoculum. A single A2 genotype (13_A2) has increased in frequency from 12% in 2005 to 78% in 2008 with a single A1 genotype (6_A1) proving the only other form of *P. infestans* able to compete with 13_A2 and a 2008 frequency of 12%. There is no strong evidence from the monitoring of 1012 outbreaks to suggest that oospores are a significant source of primary inoculum in GB crops. However, studies at SRT have indicated that oospores generated from in-crop crosses of GB A1 and A2 *P. infestans* isolates were able to survive within the soil for six months in this intensive rotation of polythene tunnel protected crops and infect the subsequent crop. Such crosses, however, do not appear to be generating genotypes that are more aggressive and can outcompete the clonal genotypes currently dominant in GB field crops. Aggressiveness studies have suggested that the rapid spread of genotype 13_A2 is related to its ability to generate larger lesions that sporulate more rapidly on a range of potato varieties. Interactions were noted with variety, suggesting that genotype 13_A2 has broken down some sources of blight resistance.

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

Although the A2 type has been present in GB since the 1980s, surveys in 1995-1998 indicated a frequency of 3% across GB (Day et al., 2004) and 19% in Scotland (Cooke et al., 2003). In Scotland, subsequent monitoring in 2003-4 showed a drop in A2 to less than 1% (D. Cooke unpublished data). Over the course of the current project, the A2 frequency has continued to rise dramatically with 80.5% of the 1034 isolates collected in 2008 being A2. The A2 mating type was found in 91% of all the 207 sampled outbreaks which is a dramatic rise from 35% in 2005. Similar increases have been reported in the Netherlands and Northern France in recent years (van Raaij *et al.*, 2007; Detourne et al., 2007).

5.1.2. Objective 2. Examine specific early outbreaks to determine the likely source of inoculum

Despite the threats that oospores pose, few conclusive examples of their role in initiating blight have been published. Studies of 20 blight foci in Finnish crops (2000-2002) planted in land with blight infected crops in at least one of the previous four years provided strong circumstantial evidence of oospore inoculum. All primary infections involved lower leaves in contact with the soil, in every case A1 and A2 isolates were isolated from such primary infections and soil bioassays confirmed the presence of overwintering soil-borne inoculum (Lehtinen & Hannukkala, 2004). In Finland, the rotations tend to be short and in the 3 year period leading up to this study

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the frequency of A2 was between 20 and 50% (Lehtinen *et al.*, 2007). Six discrete foci in a blight outbreak in a Swedish field were examined and 68 isolates of *P. infestans* recovered. Analysis of the mating type, mitochondrial DNA and SSR fingerprinting indicated a diverse mix of genotypes of both mating types and strongly suggested that oospores were the source of the infection (Widmark *et al.*, 2007).

A focus of this project was to identify signatures of oospore infection. Training and distribution of sampling packs to Superscouts was a key part of the project. Detailed sampling and records of blight outbreaks in their early stages were provided by the scouts in 2006 (eight outbreaks) and 2007 (three outbreaks). However no samples were provided in 2008. From discussions with a number of the superscouts it seems that two factors contributed to this relatively low level of reporting. In 2007 the blight pressure was extreme which likely meant that scouts had other priorities in managing blight. Also under severe blight pressure background levels of inoculum may have generated low level infections found in every crop that made it difficult to identify the initial focus of infection. Amongst the eleven outbreaks sampled there was no evidence of oospore infection. The sampling strategy, reporting and genetic analysis did, however, prove a very effective means of monitoring the inoculum source in such outbreaks.

Examination of the other 661 outbreaks reported in the standard scouting supports the view that oospores currently play an insignificant role as a source of inoculum in GB blight outbreaks. Between 3 and 5.5% of the isolates were defined in the ‘miscellaneous’ category in each season. Put another way, 94.5 to 97% of isolates were of a clonal genotype that had been present in the previous season. The main means of *P. infestans* overwinter survival is thus still the asexual clones in tubers (either as seed, in outgrade piles or as volunteers). Careful examination of all the ‘miscellaneous’ category genotypes over the three seasons confirmed that each is limited as a source of disease within the local crop only. None were sampled a second time either within the same season or a subsequent one. Eight outbreaks were noted in which both mating types were found alongside novel SSR genotypes. This is the type of signal expected from oospore-borne inoculum but these were standard scouting samples so we are lacking further information needed to judge whether the outbreak was soil-borne. Such outbreaks may also have originated from a nearby outgrade pile in which sexual recombination had occurred. Although the density of volunteer plants is generally low compared to a full crop, the risks they pose should be seriously considered. They represent a very short crop rotation and any oospore inoculum in the soil may cause infection on unprotected volunteer plants from which it may spread to neighbouring crops.

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5.1.3. Objective 3. Determine genetic diversity of GB *P. infestans* populations in 2006, 2007 and 2008 seasons.

A striking change in the population of *P. infestans* was observed over the 2003-2008 seasons. The main feature was an increase in the percentage of genotype 13_A2 from 41 to 78% with a reduction in the number of other A1 and A2 genotypes and the frequency of each. The most diverse and balanced population was observed in 2005 with six genotypes at 5% incidence or higher. This is even more striking considering how few isolates were examined in 2005 compared to 2006 onwards. The only genotype other than 13_A2 to increase in frequency since 2006 was genotype 6_A1 that has moved from 3 to 12% of the population in the past four years. The other A2 genotypes reported in 2005 (10_A2 (yellow) and 3_A2 (green)) have declined across the whole of GB crops. The patterns of change at a regional level clearly indicate a delay in the dominance of 13_A2 in the Scotland and Borders region (and to a lesser extent in the North and Midland regions) indicative of a progression of genotype 13_A2 via crop-to-crop movement of airborne inoculum towards the North and West over this period (Fig. 13). Genotype 13_A2 occurred at a high frequency right from the start of the 2007 and 2008 seasons (Fig. 12) which confirms that it is a fit genotype able to overwinter successfully in tubers; whether they are seed tubers, on outgrade piles or as volunteers.

In other studies at SCRI, a bridge between contemporary and past survey data has been made by examining isolates from previous studies with the new SSR markers (Lees et al., 2006) used in this study. It was thus shown that the A2 genotype most commonly found in 1995-1998 and named RF040 by Day *et al*., (2004) and known in this study as genotype 22_A2, was found only rarely in FAB isolates in 2003 and 2004 and never in 2006-8. This A2 lineage thus no longer plays a significant role in GB blight epidemics and is unrelated to the 13_A2 genotype. Similarly, an A1 genotype (RF039; equivalent to 2_A1 in this study) that comprised 46% of the 1995-8 GB population (Day et al., 2004) has decreased to only 0.3% of the 2008 population.

Genetic fingerprinting at SCRI has now confirmed the widespread occurrence of genotype 13_A2 in GB and other parts of Europe. First reported in the Netherlands in 2004 it has now been confirmed as the main genotype in both Northern France (Detourne *et al*., 2007; D. Cooke unpublished results) and Germany in 2007 (D. Cooke unpublished results).

5.1.4. Objective 4. Analysis of oospore production and survival under GB conditions.

5.1.4.1. Superscouts and SRT studies

Parental A1 and A2 genotypes found in GB crops in 2006 were introduced into the first cycle tunnel crops in April 2007. Since this date, blight epidemics have occurred naturally in the tunnel-grown crops in the subsequent four crops grown in early spring or late autumn. The timing and location of the trials were designed to minimise the chance of inoculum arrival from other sources. Blight was nonetheless frequently detected soon after plants had emerged and, in one case, as early as the third week after planting (cycle 3, tunnel 1). The initial finding of blight in all tunnels was on the lower parts of stems as well as on leaflets which supports the idea of a below-ground inoculum source. Blight was not detected in samples of seed incubated before the planting date at of any cycle and great care was taken to remove any small tubers

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from the previous crop. It was thus concluded that infectious propagules, most probably oospores, were stimulated to germinate in the soil and resulted in this infection. Further support for this came from the genotyping of the *P. infestans* DNA from the sampled lesions. These were either genotyped directly from the pathogen DNA pressed from lesions onto FTA cards or from isolates of *P. infestans* recovered in the standard methods (see above).

Virtually none of the 500+ lesions/isolates recovered from the tunnels were of genotypes that were introduced in cycle one or matched others found in GB potato crops. The genotypes were distinct novel genotypes that, in many cases, were discrete to the particular tunnel in which they were first found. This provides clear evidence that asexual inoculum (sporangia or hyphae) did not survive over the fallow period between crops; even over the short and wet winter fallow. Novel combinations of alleles at specific SSR loci that have never been recovered in thousands of GB crop isolates were found in the tunnels which is strong support for genetic recombination. Isolates with triple alleles at one or more single loci were commonly found amongst the new genotypes detected in all cycles. This is to be expected in cases where parents differ in their chromosome complement (or ploidy). The occurrence of 3-allele genotypes suggests that many of the hybrids were either trisomic (aneuploid) or triploid. These have been detected amongst the progeny established from germinated oospores in the laboratory (Carter *et al.*,1999). Recent evidence from flow cytometry (Masatoki Taga, unpublished) has indicated that genotype 13_A2 is triploid. Such a genotype would be expected to be either sterile or to yield a range of aneuploids in sexual progeny.

Although both mating types were detected in some tunnels in cycles 2 – 5, each mating type tended to be clumped within a tunnel and not mixed with the other. This would suggest that mating of hybrid genotypes was absent or rare in cycles 2 – 5. Microscopic examination of leaflets for oospores, particularly in cycle 3, is consistent with this view and suggests that the majority of the recombination and oospore formation occurred in cycle 1.

Although all the tunnel genotypes were novel the actual genotype number found within each tunnel in each cycle remained low (2-3) compared to the theoretical number of detectable genotypes expected if many (e.g. hundreds) oospores were germinating. This suggests either that germination rates were low or few of the germinated oospores produced progeny that were viable and able to infect the new crop. This is consistent with the observation that oospores produced in laboratory crosses exhibit prolonged dormancy and often have low rates of germination. Such low rates may explain the failure of other studies in which small soil samples are tested for oospores using potato leaf baits. In this experiment we tested 3 different pairwise combinations in single tunnels and nine possible combinations in tunnel 4. Unexpectedly, the latter did not yield more combinations than the former although some isolate movement from tunnel to tunnel may have masked any such effect.

Only in cycle 4 were the common GB *P. infestans* clones 13_A2 and 6_A1 found. However, they did not survive in the soil as asexual inoculum as cycle 5 was again infected with novel genotype combinations.

Careful examination of the SSR data offers some clues as to which parents were most involved in the production of viable oospores. The only source of the 154bp allele at D13 and 174bp allele at Pi56 in this experiment was 13_A2. New genotypes

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containing these alleles were thus extremely likely to have involved 13_A2 as the A2 parent. Such genotypes were common and suggest 13_A2 was able to generate viable oospores. Similarly the 140bp allele at G11 and 195 and 199bp at locus Pi89 were unique to genotype 1_A1. These alleles were also found in many of the new genotypes again providing strong evidence that it acted as an A1 parent. Such alleles were notably absent in tunnel 2 suggesting that the introduced 7_A1 isolate was the A1 parent that generated the oospores in this tunnel. Conversely, according to these criteria, 8_A1 and 3_A2 contributed little to the production of viable oospores under these conditions as their distinct alleles were rare or absent. Other studies have indicated that the success of particular crosses depends on the parental combinations that generate the oospores. However, no specific factors that govern mating success have been identified to date so it is not possible to predict viable combinations of A1 and A2 types. The isolates for this study were selected in 2006 and it was not clear, at this stage, that genotype 6_A1 was going to increase in frequency. With hindsight it would have been interesting to include this now prevalent A1 genotype along with 13_A2 to assess the potential of these two genotypes to generate viable oospores.

5.1.5. Objective 5. Assess the implications of and risks arising from the observed *P. infestans* population structure

The above findings raise many questions in terms of the origins of genotype 13_A2; whether it is harder to control and whether it is combining with A1 isolates to generate sexual oospores.

An equal ratio of A1 and A2 mating type isolates in every crop will theoretically provide the greatest opportunity for oospore formation and the increase in the frequency of the A2 mating type to well above 50% will, in theory, reduce the chances of oospore formation. The proportion of outbreaks in which both mating types were found decreased from approximately 20% in 2006 and 2007 to 15% in 2008. This was expected given the prevalence of the 13_A2 mating type that has out-competed A1 genotypes. Nonetheless, if we presume the outbreaks sampled are representative then oospores are very likely to have formed in 18.6% of blight infected GB crops over the past three seasons. This was particularly likely in the very wet 2007 season when most crops were reported to be infected. It is thus inevitable that oospores have been formed in many GB blight outbreaks and are now present in the soil in these fields. Although there is very little evidence for a large-scale direct involvement of oospores as a source of primary inoculum in GB fields to date it should be remembered that most of the crops sampled in this study were grown on land last cropped between 1999 and 2003 when A2 levels were much lower (e.g. 5% in 2003). Thus we should not be complacent as to the risks of oospores, especially given the evidence that 13_A2 is able to produce viable oospores.

The studies in polytunnels provide strong support for soil-borne oospores acting as a source of inoculum. This experiment was successful in demonstrating the ability of common GB *P. infestans* genotypes to form viable oospores that can survive an approximately six month fallow period between crops. This intense programme of short rotation cropping was required to demonstrate this fact as it would seem that relatively low numbers of oospores were involved in crop infection. Experiments on a wider scale with longer rotations may not easily recover such infection and this may explain the challenges that many international research groups have had in studying oospore biology. It may also explain the low frequency of GB blight outbreaks that are

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attributable to oospores given the long rotation period and larger cropped area in which to identify such infection. Key questions remain; How 'typical' was the behaviour of the specific GB A1 and A2 genotypes used in this study and how can we extrapolate from this study to understand the risks of oospores to the wider GB potato crops? In answer to the first question, it is clear from the literature that different genotypes of *P. infestans* mate more effectively than others. In Nordic countries there is good evidence from a high diversity in genetic fingerprints on a broad (Brurberg *et al.*, 1999) and local (Widmark *et al.*, 2007) scale that suggests that oospores are commonly a source of primary inoculum. Similarly, in Finland and Denmark there is evidence that short rotations increase the risk of earlier blight outbreaks from oospores (Bodker *et al.*, 2006; Hannukkala *et al.*, 2006; Lehtinen & Hannukkala 2004). Oospores have been confirmed as a significant source primary inoculum in some regions of the Netherlands (Evenhuis *et al.*, 2007). However in the GB despite intensive studies in this and other projects (Cooke *et al.*, 2003; Day *et al.*, 2004) such evidence of oospores is missing. It seems probable that the specific *P. infestans* genotypes have a large influence on this pattern but differences in the length of rotation or other agronomic practices cannot be ruled out.

The fit and aggressive 13_A2 genotype has presumably originated from such a recombination event between two fit genotypes and illustrates the potential risks posed by such 're-shuffling of the genetic pack'. This should be recognised in understanding and countering the threat of oospores by extending rotations and keeping good control of likely hotspots of oospore germination such as dumps and volunteers continues to be important.

The seven genotype 13_A2 isolates recovered in 2005 were resistant to the phenylamide fungicide metalaxyl (Shaw *et al.*, 2006) but no independent testing has been conducted since. Prior to this, A2 mating type isolates with metalaxyl resistance have either been absent (Day *et al.*, 2004) or very rare (Cooke *et al.*, 2003). It was furthermore suggested that their sensitivity to this product may have kept their frequency low (Cooke *et al.*, 2003, Day *et al.*, 2004). Syngenta have more recently confirmed that increases in metalaxyl resistance are associated with the increase in prevalence of genotype 13_A2 (Farmers Weekly; Abraham, 2008). In the past, metalaxyl resistance in A1 genotypes has been associated with a reduced rate of overwinter survival (e.g. Gisi and Cohen, 1996) which has meant that early metalaxyl applications have been effective. However, the appearance of this A2 genotype that is both resistant to metalaxyl and dominates the population early in the season has implications for the management of blight using phenylamides. This Potato Council-funded project has supported Syngenta by providing *P. infestans* isolates from early-season blight outbreaks in 2006, 2007 and 2008 for their resistance monitoring programme. The project has played a direct role in updating the industry on genotype 13_A2 frequency and has ensured the industry is well placed to make informed decisions on fungicide product choice.

It is implicit in the dramatic spread of genotype 13_A2 that it has a competitive advantage over other *P. infestans* genotypes (i.e. is a better pathogen). If it is more aggressive and/or fit it is also likely to be more difficult to control. Additional studies on the aggressiveness of GB genotypes against leaves and tubers of a range of potato varieties were conducted at SCRI in 2007 and are reported in the next section.

The results of this project are being updated onto the Eucablight web site to allow comparisons at an international scale. The *P. infestans* population change discovered

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in this project has significant implications for blight management, for example, the effectiveness of Decision Support Systems, cultivar resistance and fungicide choice. The data are being presented and discussed at national and international levels (e.g. Cooke *et al.*, 2007; Cooke *et al.*, 2008a; Cooke *et al.*, 2009).

5.1.6. Objective 6. Complete study of foliar aggressiveness of GB and European *P. infestans isolates*

The aggressiveness of twenty six different isolates against potato varieties of differing blight resistance was examined in a comprehensive experiment on detached leaf assays in growth rooms set at 13 and 18°C. The isolates represented eight of the most commonly found GB genotypes in 2006 and included six foreign and three 'other' isolates. Lesion size and latent period were scored as these factors have been shown to correlate well to other parameters such as sporulation capacity (Carlisle *et al.*, 2002). Highly significant differences were noted in lesion size and latent period between *P. infestans* genotypes and potato varieties with a significant interaction also being observed. At 13°C the main findings were that isolates of two genotypes (13_A2 and 6_A1) were most aggressive. Each caused larger lesions that had a shorter latent period than other genotypes on the five varieties selected. It is significant to note that these two genotypes were also the only ones that have increased in frequency in GB blight outbreaks sampled in recent years. The single isolate of genotype 17_A2 was similarly aggressive but in this case, has only been found at a single site in Aberdeenshire in 2006. It would appear that the increased aggressiveness of genotype 13_A2 at least partly explains its rapid recent spread. In a polycyclic disease such as late blight even what might appear as a slight advantage such as a 12 hour reduction in latent period will allow more generations per season. If the lesions are also 10-15% larger than those caused by other genotypes at a given point after infection then each of the cycles of disease also causes more damage and rapidly generates more spores. The US-8 genotype that displaced the US-1 genotype in the USA was similarly found to be more aggressive (Miller *et al.*, 1998; Young *et al.*, 2005).

Interactions with varietal resistance were also observed at 13°C with unexpectedly large lesions being formed by some genotypes on Lady Balfour which is reported as having a foliar resistance rating of 8, above that of Cara at 7. In this assay, Cara was the most resistant variety against all genotypes but the largest lesions were caused by isolates of genotype 13_A2 and 17_A2. All genotypes caused larger lesions on Lady Balfour than on Cara but an interaction was apparent in the case of 13_A2 and 6_A1 that caused markedly larger lesions on Lady Balfour than other genotypes on this variety. This suggests that the resistance in Lady Balfour has been broken by these *P. infestans* genotypes; this is backed up by other evidence of genotype 13_A2 causing resistance breakdown in variety Stirling and other varieties in both glasshouse tests and in the field (D. Cooke and A. Lees unpublished data). More detailed studies on the varietal resistance were undertaken in the Potato Council-funded Independent Variety Trials (IVT) testing and RERAD-funded work in 2008 and also in the Sustainable Arable LINK project which started in 2009. Reliable and up to date variety resistance ratings are clearly important in planning blight management strategies and particularly any that involves using resistance to replace fungicide inputs. It is thus important to evaluate variety resistance using an isolate (or isolates) that are representative of the current population. The findings of this project are fundamental to the use of resistance as a management tool and invaluable in making a selection of *P. infestans* isolate(s) for resistance screening.

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At 18°C the findings were less clear; genotype 13_A2 isolates did not generate the largest lesions although all six 13_A2 isolates were amongst the 12 isolates with the shortest latent period. The interactions with varietal resistance were also less clear with the genotypes, in general, causing more similar sized lesions on all varieties. The Lady Balfour interaction noted at 13°C was not apparent. It is not clear why the findings at 13°C were not mirrored at 18°C. Perhaps differences in performance of the genotypes are more pronounced at sub-optimal temperatures? It could also be an artefact due to the experimental design. With so many leaves in the experiment there was a practical need to score the lesion size at a single time point when the lesions at both temperatures were at an appropriate size. Six days was selected as a point when the lesions at 13°C had developed sufficiently but at this stage the lesions incubated at 18°C were clearly much larger and nearer to the edges of the leaves. It is possible that more pronounced differences would have been observed at an earlier stage of lesion development in the 18°C treatment.

The evidence of differential performance of *P. infestans* genotypes at different temperatures in this study suggest this should be examined in more detail. In particular, identifying the thresholds of temperature and duration of high humidity of different genotypes will be important in the accurate prediction of blight activity. An answer is needed to the commonly asked question in the industry 'How relevant are Smith periods today?' This is one area of study in the new Potato Council-funded project (R423).

5.1.7. Objective 7. Complete assessment of tuber aggressiveness of GB and European *P. infestans* isolates

The mean percentage tuber blight caused by each isolate varied from 13 to 32% but isolates of genotype 13_A2 were not, in this case, consistently the most aggressive. In fact they ranged from one of the most to one of the least aggressive to tubers. It is difficult to draw conclusions from a single study but it may be that this highlights the trade off between aggressiveness and fitness. Aggressive isolates that cause higher levels of tuber rot are likely to have a lower survival rate (i.e. lower fitness) until the next season. This will be particularly true in the case of volunteer tubers or those on outgrade piles; blight progression on seed tubers will be held in check by low temperature storage. Other studies on aggressiveness to tubers have identified differences between genotypes in Canada (Peters *et al.*, 1999). Interestingly such differences were obscured when the tubers were wounded prior to inoculation. Perhaps that was a factor in this experiment? Although the tubers were hand-dug and washed very carefully, some wounding was inevitable; this should however have applied to all treatments.

The statistical analysis highlights significant interactions between *P. infestans* genotype and potato variety. Such interactions were again pronounced in Lady Balfour with isolates of some genotypes (13_A2, 3_A2, 6_A1 and 17_A2) causing markedly more tuber blight than the other seven isolates. Other interactions were also noted with, for example percentage bight on Maris Piper varying from over 50% blight in the case of 8_A1, 2_A1 and 10_A2 compared with nearer 30% in other genotypes.

Predicting tuber blight losses is difficult and this is borne out by the range of aggressiveness between *P. infestans* genotypes and the interaction with varietal resistance seen in this study. The study aimed to provide a first indication of the

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aggressiveness of the GB population and was conducted under a high inoculum concentration followed by incubation conditions that favoured infection (15°C for 15 days). A RERAD-funded study at SCRI that compared tuber blight damage caused by isolates of four genotypes when stored for 12 weeks at 4°C indicated a greater level of tuber blight caused by 13_A2 isolate compared to the other genotypes (data not shown). An investigation of tuber infection and losses in storage is also underway in the new project (R423).

5.1.8. Objective 8. Establish, infect and monitor fitness and aggressiveness of *P. infestans* isolates

The studies above investigated aggressiveness in a single cycle of infection under laboratory conditions. These tests were necessary to provide objective data but have potential shortcomings. Firstly, they do not account for sporulation and re-infection to give a measure of fitness and secondly an assay under laboratory conditions may not replicate field behaviour. A field trial was thus established at SCRI in which five different *P. infestans* genotypes were used to inoculate a single central plant in each plot and the disease spreading from these plants was monitored over the course of the three week blight epidemic. Single lesions were sampled and squashed onto FTA cards. DNA fingerprinting of the *P. infestans* indicated that genotype 13_A2 dominated the trial from the first sampling date (30 July) until the last (Aug 20) comprising 95.4% of the 627 lesions fingerprinted. Three of the other four genotypes were recovered, albeit rarely and sporadically, which confirms that the inoculum was viable at the outset. The genotypes were tracked on five different varieties but genotype 13_A2 dominated on each of them; i.e. no variety by genotype interaction was observed. A genotype that was not introduced was also found at a frequency of 2.9% which may be expected in the centre of a potato growing region. This experiment was not inoculated until blight had already been reported in surrounding postcode regions.

This experiment supports the laboratory data; genotype 13_A2 clearly has a significant competitive advantage over other *P. infestans* genotypes in the field. This work indicates that genotype 13_A2 is both aggressive and fit on all five varieties in this trial. This work thus supports the observations from the survey and explains how a single genotype has come to dominate the GB population.

5.2. Research Gaps & Questions

The project was very successful in meeting its objectives but questions remain. A series of these are detailed under the following headings: **population change; blight activity and management; primary inoculum; tuber blight prediction; stability of resistance and scope for future approaches to management.** Some of these research gaps are covered in project (R423).

5.2.1. Population change

How stable is the current population?

The recent transition has demonstrated that populations may change rapidly. Given the risks that new blight strains may emerge by mutation, migration or sexual recombination (either within GB or elsewhere) then ongoing monitoring is advisable (at least in the short-term) to understand if the emergence of genotype 13 is the beginning of a trend for more rapidly changing populations or a ‘flip’ to be followed by

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a period of stability. Genotype 6_A1 is increasing in some regions. Any new populations may, like genotype 13, have altered fungicide sensitivity, overcome existing cultivar resistance or may be difficult to predict using current Smith or Plant Plus criteria.

Continued monitoring of *P. infestans* populations (albeit at a lower intensity) is advisable for the following reasons/questions. Growers are still adjusting to new advice on managing genotype 13 and it is not clear how the population will shift in the near future. Has oospore production increased or decreased? Are oospores acting as a source of primary inoculum and, if so, how should this be managed?

5.2.2. Predicting blight activity and management of 13_A2

Is blight now active out of the Smith Period 'box'?
It is currently assumed that activity of the new pathogen population can be predicted using existing Smith criteria. Limited analysis of past meteorological and blight outbreak data by ADAS has highlighted false negatives (i.e. cases where blight is active with no blight warning prompted by a Smith period). Studies at SCRI also suggest that genotype 13 had more of an advantage over other genotypes at 13°C than it did at 18°C. A more detailed investigation is recommended as such uncertainty is not acceptable in planning a blight spray program. If growers are to have confidence in the Blightwatch warnings then the response of genotype 13_A2 to temperature and relative humidity should be examined in more detail. Such data on the response of different *P. infestans* genotypes to temperature and moisture is required to feed into models that can offer more precision in predicting blight activity under changing environmental conditions. A more detailed examination of the historical meteorological and blight outbreak data would be valuable to help understand the blight forecasting failures on a case-by-case basis.

Is current chemistry still providing good control?
It is clear from 2007 data and from recent experience in fungicide trials that the increased aggressiveness of new populations requires a shortening of fungicide application intervals. The 10-day and 7-day intervals for low and high risk are no longer appropriate for current fungicides. However, the potato industry requires specific information on the appropriate intervals to deal effectively with the shorter latent periods and greater sporulation of the new genotypes. Fungicide manufacturers have, and are continuing to, investigate the efficacy of higher individual doses (e.g. the rates approved in continental Europe). Appropriate intervals will clearly be influenced by fungicide dose. With the exception of specific insensitivity of genotype 13_A2 to the phenylamide fungicides there are no reports that the population change has resulted in insensitivity issues for other fungicide groups. In general, insensitivity monitoring is the responsibility of the agrochemical manufacturers. However, the effectiveness of strategies that combine cultivar resistance and reduced fungicide inputs will be influenced by any changes to cultivar resistance ratings as a result of new *P. infestans* populations (see below).

5.2.3. Primary inoculum

Are oospores still a threat?
This project strongly suggests that oospores are a source of primary inoculum but their role has not yet been demonstrated on a wide scale from field sampling in GB crops. The offspring from these oospores are not currently able to out-compete the

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well-adapted parental strains such as genotype 13_A2. However it may also reflect the timing of crop rotations. The A2 genotype only began to increase markedly in 2005 and fields in which high oospore numbers were generated in blight outbreaks have yet to be replanted with potatoes. It is thus unclear whether the reservoir of oospore inoculum in GB soils awaiting a susceptible crop and suitable (warm and wet) environmental conditions is a real concern. Studies to examine the survival of oospores in soil over time and the risks they pose as a source of inoculum are thus required. We do not currently know the inoculum levels that pose a risk, understand how oospore viability declines over time or know how oospores may be managed (for example the impact of in-furrow fungicides). Technical problems in oospore detection and viability are under investigation in project R423.

5.2.4. Predicting the risks of tuber blight in the crop and store

Can we accurately predict the impact of genotype 13 on tuber blight activity?
Predicting tuber blight levels in the growing crop and in store has remained a challenge for growers. It is currently unclear how population change may influence levels of tuber infection. The low levels of tuber blight in 2007 were likely due to the warm, dry conditions towards the end of the growing season and not related to population change. Tuber infection in relation to *P. infestans* genotype is being investigated in project R423. Research that would help growers understand and manage the risk includes: The validation and improvement of sensitive diagnostic tests for latent tuber blight and the use of this test to investigate the timing of tuber infection/contamination and prediction of losses in store as well as an examination of the true incidence of seed infection in GB and imported seed stocks. Such technology can now be combined with genotyping to examine both the presence and genotype of blight present.

5.2.5. Stability of resistance

Can we be confident in current resistance scores as part of blight management decisions?
Sources of resistance previously considered robust (e.g. Stirling, Lady Balfour, Orla) appear to have been broken by genotype 13_A2. A re-examination of varietal resistance ratings by SAC and SCRI as well as additional variety testing by SASA as part of the IVT programme, has confirmed this breakdown. It is important that the ratings are updated to give growers confidence in using resistance as a part of a successful blight management strategy. This episode highlights the need to be aware of population change and its consequences. Such resistance data should be entered into the Eucablight host database as part of the ongoing monitoring of resistance stability on a European scale.

5.2.6. Future approaches (molecular approaches to breeding & pathogen biology)

What about future approaches to blight resistance breeding?
The resistance breakdown discussed above has prompted the re-testing of Commonwealth Potato Collection material (funded by the RERAD workpackage and SSCR Potato Section) to understand which sources of resistance have remained stable and which have been broken by genotype 13. This screen forms the first step for both conventional and molecular breeding strategies. In the latter case, the objective is to isolate the blight resistance genes and their corresponding *P. infestans*

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effectors. Advances in our understanding of both host and pathogen are being pushed by advances in genome sequencing. Fundamental studies on the basis of genotype 13's aggressiveness, fitness and virulence are underway and such approaches represent an ambitious but exciting approach as part of a longer-term strategy towards sustainable blight management.

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6. CONCLUSIONS

This project has documented a significant change in the *P. infestans* population that causes late blight outbreaks in crops from Cornwall to Inverness. The mating type shift first noted in 2005 is now understood to be due to an aggressive A2 mating type lineage that has displaced the previous pathogen population. This new 13_A2 genotype has had a direct impact on the ability of the industry to control blight. The new lineage is more aggressive, causing larger lesions that produce spores sooner than other genotypes. This explains reports that blight has been more active and challenging to control than in the past. This radical shift has had an impact beyond the specific remit of this project. For example, GB and French 13_A2 isolates have been reported to be resistant to phenylamide fungicides. This has reduced the usage of a key early-season systemic product and is prompting the industry to seek alternative strategies to protect this phase of rapid crop growth from blight. The involvement of 13_A2 in the breakdown of sources of blight resistance used in several contemporary varieties has also been confirmed. This has prompted other studies to re-examine the resistance ratings of many current varieties. This data will be crucial in allowing growers to use variety resistance as an effective part of their blight management program. Longer term, it is also influencing decisions on sources of blight resistance in potato breeding programmes.

A key goal of the project was to assess the role of oospores as a source of primary inoculum in GB crops. The shift towards a 50:50 ratio of A1 and A2 mating types in 2005 and 2006 increased the likelihood of both mating types co-infecting the same plant and generating these long-lived propagules. The detailed protocols we developed for investigating the source of primary inoculum proved effective. However, neither the superscout nor the standard scout sampling of over 1000 blight outbreaks from 2003-2008 have indicated that oospores are currently playing a significant role as a source of primary inoculum. Genetic fingerprinting showed that in GB crops, blight outbreaks were initiated by clonal genotypes present in the previous season. This indicates that infection and survival in tubers (as seed, volunteers or outgrade piles) remains a critical phase of the pathogens lifecycle. However, over the three seasons of this project both A1 and A2 mating types have been found in 15-19% of sampled outbreaks. In 2007, for example, a high proportion of GB crops had blight and many oospores will thus have been formed and will now be present in the soil. The likelihood of such inoculum formed by GB A1 and A2 lineages causing blight in subsequent crops was unknown and prompted its study in this project. An objective view of the generation and significance of oospores was provided by a series of crops grown in short rotation in polythene tunnels. Oospores were generated in the first crop by inoculating with parental A1 and A2 mating type *P. infestans* isolates and allowing the crop to rot into the soil. Blight epidemics in the subsequent four crops were monitored and lesions sampled for genetic fingerprinting. The consistent occurrence of blight, normally within a few weeks of emergence, in these isolated crops grown outside of the normal GB potato cropping cycle suggested the presence of soil-borne oospore inoculum. This was confirmed by the genetic analysis that indicated novel genotypes of the pathogen (i.e. not found in GB or European crops) were causing the disease in each cropping cycle. Such results were consistent with oospores as the source of inoculum. We can thus see that short rotations are a risk and the industry should remain vigilant to the threat of oospore primary inoculum. In particular, note should be taken of land cropped over the high risk period of 2006-2008 which comes back into the rotation in 2011-2013. Although the long-term survival and decline rate

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of *P. infestans* oospores has not been studied, clearly, the risks will decline progressively with increasing periods between potato crops in the rotation.

This far-reaching project successfully informed and engaged many different GB partners within the potato industry, each responding to the reported changes to the benefit of blight management in GB crops. The findings have been presented to industry (both nationally and internationally) in almost 70 KT events/articles.

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8. APPENDICES

8.1. Appendix 1: Main SSR genotypes

Main SSR genotypes found within GB *P. infestans* population in 2003-2008. The eleven SSR loci names are shown in the columns and the allele(s) sizes within each shown by the numbers and coloured blocks. The mating type (MT) of the genotypes is shown with A1 in upper section of table and A2 below. In some cases minor variation within a genotype was observed and the most prevalent of the alternative forms are shown. Most of this sub-genotype variation occurs in the hypervariable loci G11 and D13. For example, the seven most common variants of genotype 13_A2 are shown.

SCRI genotype name	Matches to other codes RF=day etal GB	MT	Pi02			D13			Pi33		Pi04		Pi4B		Pi16		G11			Pi56		Pi63			Pi70		Pi89		
1_A1		A1	160	162	0	136	136	0	203	203	166	170	213	217	178	178	140	162	0	176	176	148	157	0	192	192	179	195	199
1b_A1		A1	160	162	0	132	136	0	203	203	166	170	213	217	178	178	140	162	0	176	176	148	157	0	192	192	179	193	201
1c_A1		A1	160	162	0	134	136	0	203	203	166	170	213	217	178	178	140	162	0	176	176	148	157	0	192	192	179	195	201
2_A1	RF039_IE-6	A1	152	162	0	136	136	0	203	203	166	170	217	217	176	178	154	156	206	174	176	148	151	157	192	195	179	179	0
4_A1		A1	152	162	0	136	136	0	203	203	166	170	217	217	176	178	154	162	206	176	176	148	151	157	192	195	179	181	0
5_A1	RF002_NI-2_IE-2	A1	162	162	0	136	136	0	203	203	166	170	205	217	176	178	156	162	0	176	176	157	157	0	192	192	179	181	0
5f_A1	RF002_NI-2_IE-2	A1	160	162	0	136	136	0	203	203	166	170	205	217	176	178	156	162	0	176	176	157	157	0	192	195	179	181	0
5g_A1	RF002_NI-2_IE-2	A1	160	162	0	130	130	0	203	203	166	170	205	217	176	178	156	162	0	176	176	157	157	0	192	195	179	181	0
6_A1		A1	152	160	162	null	null	null	203	203	166	170	213	217	178	178	160	160	0	174	176	151	157	0	192	195	181	197	0
7_A1		A1	162	162	0	118	136	0	203	206	166	170	205	217	176	178	160	160	0	176	176	151	157	0	192	192	179	181	0
8_2a_A1	RF006_NI-1	A1	162	162	0	118	136	0	203	206	166	170	205	217	176	178	166	166	0	176	176	151	157	0	192	192	179	181	0
8_2e_A1	RF006_NI-1	A1	162	164	0	118	136	0	203	206	166	170	205	217	176	178	166	166	0	176	176	151	157	0	192	192	179	181	0
8a_A1	RF006_NI-1	A1	162	162	0	118	136	0	203	206	166	170	205	217	176	178	162	162	0	176	176	151	157	0	192	192	179	181	0
12_A1		A1	162	162	0	118	136	0	203	206	166	170	205	213	178	178	166	166	0	174	176	157	157	0	192	192	179	181	0
12c_A1		A1	162	162	0	118	136	0	203	206	166	170	205	213	178	178	162	162	0	174	176	157	157	0	192	192	179	181	183
18_A1		A1	162	162	0	136	136	0	203	203	170	170	217	217	178	178	206	206	0	176	176	157	157	0	192	192	179	179	0
19_A1		A1	162	162	0	136	150	0	203	203	166	170	213	217	178	178	154	156	0	176	176	148	157	0	192	195	179	199	0
20_A1		A1	162	162	0	118	118	0	203	203	166	170	213	217	176	178	154	156	160	176	176	157	157	0	192	195	179	179	0
21_A1		A1	162	162	0	136	136	0	203	203	166	170	213	217	178	178	162	206	0	174	176	157	157	0	192	192	179	179	0
23_A1		A1	160	162	164	136	210	0	203	206	170	170	213	217	178	178	140	156	0	174	176	148	157	0	192	192	181	181	0
3_A2		A2	162	162	0	118	136	0	203	203	166	170	213	217	176	178	154	160	0	176	176	148	157	0	192	195	179	179	0
10_A2		A2	162	162	0	136	136	0	203	203	166	170	213	217	178	178	162	208	0	176	176	151	157	0	192	192	181	181	0
13_A2_1		A2	160	162	0	136	154	0	203	203	166	170	205	213	176	178	154	160	0	174	176	151	157	0	192	192	179	179	0
13_A2_2		A2	160	162	0	136	140	154	203	203	166	170	205	213	176	178	154	160	0	174	176	151	157	0	192	192	179	179	0
13_A2_3		A2	160	162	0	136	158	0	203	203	166	170	205	213	176	178	154	160	0	174	176	151	157	0	192	192	179	179	0
13_A2_4		A2	160	162	0	136	152	0	203	203	166	170	205	213	176	178	154	160	0	174	176	151	157	0	192	192	179	179	0
13_A2_5		A2	160	162	0	136	154	0	203	203	166	170	205	213	176	178	154	160	164	174	176	151	157	0	192	192	179	179	0
13_A2_6		A2	160	162	0	134	140	154	203	203	166	170	205	213	176	178	154	160	0	174	176	151	157	0	192	192	179	179	0
13_A2_7		A2	160	162	0	136	154	0	203	203	166	170	205	213	176	178	154	160	0	174	176	151	157	0	190	192	179	179	0
15_A2		A2	162	162	0	118	136	0	203	203	160	168	213	217	178	178	154	154	0	176	176	157	157	0	192	195	na	na	na
16_A2		A2	162	162	0	118	136	0	203	203	null	null	213	217	178	178	160	160	0	174	176	148	157	0	192	192	179	179	0
17_A2		A2	160	162	0	118	118	0	203	203	null	null	213	217	176	178	160	162	0	176	176	148	148	0	192	192	179	179	0
22_A2	RF0040	A2	162	162	0	136	136	0	203	203	166	170	213	217	176	178	140	156	162	174	176	151	157	0	192	195	179	181	0

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8.2. Appendix 2: Glossary of technical terms

Glossary of technical terms as applied to *P. infestans* in this report.

Aggressiveness The quantity of disease caused by a particular *P. infestans* isolate (or genotype) on a specific host.
Alleles Variant forms of the DNA sequence at a particular locus. Different alleles of SSR markers have different numbers of the specific repeat motifs.
Clonal A population that survives and reproduces asexually (i.e. without sexual reproduction).
Fitness The expected contribution of a genotype to the next generation. This describes a genotypes ability to spread from plant to plant within a season and/or its ability to survive from one season to the next and may or may not be linked to aggressiveness.
Genotype An individual *P. infestans* isolate that is distinct from others in its SSR profile. Many isolates of a single genotype represent a clonal lineage.
Locus/Loci A fixed position on a chromosome such as the location of an SSR marker.
PCR **P**olymerase **C**hain **R**eaction. Laboratory based method of amplifying a specific fragment of an organisms DNA sequence to a sufficiently high number to allow their detection.
Recombination Process of breaking and re-joining strands of DNA that allows ‘crossing over’ of parts of chromosomes to occur. This process can re-assort alleles at a specific SSR locus but more importantly creates new combinations of genes and is part of the process that generates the genetic variation that fuels evolution.
SSR **S**imple **S**equence **R**epeat. A short repeated motif of specific DNA nucleotides (e.g. dinucleotide - AGAGAGAG or trinucleotide-GCTGCTGCT) that is prone to length variation as a result of errors in DNA replication during cell division. Such ‘slippage’ causes the length of DNA sequence to increase or decrease by units of 2 or 3 DNA base pairs. Such differences can be scored using sensitive machines to measure the length of the PCR products. Such sequences tend to vary at a rate appropriate for examining variation within populations.

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8.3. Appendix 3: Knowledge transfer activities

8.3.1. Presentations/Posters

Year one

1. Cooke DEL and Bain 2006. Superscout training workshops
   11th May, Novotel Hotel, Birmingham International Airport
   12 th May, Great Northern Hotel, Peterborough
   18th May, SCRI, Dundee
2. Cooke DEL, Lees AK, Shaw DS, Taylor M, Bain RA, Bradshaw NJ, 2006. The monitoring of GB blight outbreaks: mating type and genetic fingerprint analysis. Poster for Potatoes in Practice, Dundee. August 2006.
3. Cooke DEL, 2006. Survey of 2006 GB blight populations. GB Blight Forum. Feb 2007 Peterborough
4. Cooke DEL, Shaw DS 2007. Recent changes in the population of *P. infestans* in Great Britain. Euroblight workshop – potato late blight network for Europe. May 2007
5. Clayton R. 2006. Project background and limited examples of data presented at NJF Seminar (Integrated control of potato late blight in the Nordic and Baltic countries.) Helsingor, Denmark December 2006
6. Prentice M. 2006. Feedback letters to standard scouts that included mating type data from this project.
7. Cooke DEL, Bain R, Bradshaw N. 2007. Feedback letters to Superscouts 31 Jan 2007
8. Cooke DEL. 2007. Data submitted to EUCABLIGHT database May 2007 onwards

Year two

9. **June 2007** David Cooke Presentations to SASA seed inspectors on blight status at training event
10. **July 2007** David Cooke 'Tracking P. infestans populations via molecular finger-printing and a comprehensive isolate database' Invited speaker (EAPR pathology section meeting)
11. **Aug 2007** David Cooke Potatoes in Practice, Dundee August 9th. Discussed results with Mistral Group for various press reports.
12. **Nov 2007** David Cooke "An update on the GB blight survey and its impact on disease management" presentation BP2007 event (Harrogate).
13. **Dec 2007** David Cooke "BPC blight survey update: how might population change impact blight management?" Scottish Society for Crop Research, Potato section meeting at SCRI in Dec 2007.
14. **Jan 2008** David Cooke 'Changing blight populations' Presentation at Bayer CropScience Potato technical meeting, Growers and industry advisors. Peterborough.
15. **Jan 2008** David Cooke 'Changes in Scottish *Phytophthora infestans* populations.' Presentation to Scottish Agronomy - grower group. Technology Transfer Event
16. **Jan 2008** David Cooke 'Changes in Scottish *Phytophthora infestans* populations.' Presentation to Scottish Agronomy – advisors group. Technology Transfer Event
17. **Feb 2008** David Cooke 'Update on GB 2007 Blight population survey' GB Blight Forum, Grantham.
18. **Feb 2008** David Cooke 'Changes in aggressiveness of GB blight populations' GB Blight Forum, Grantham.

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19. **Feb 2008** David Shaw ‘Can our population mate?’ GB Blight Forum, Grantham.
20. **Feb 2008** David Cooke ‘The status of GB blight populations and the threat of oospores’ presentation at Proceedings Crop Protection in Northern Britain 2008.
21. **Mar 2008** David Cooke ‘Implications of blight population change for the potato industry’ Presentation and discussion at 'BPC treater group' meeting, March 2008. Representatives from industry meeting to discuss blight control strategies in light of latest results.
22. **March 2008** Finlay Dale “Late Blight in UK – breeding, research and management.” Presentation to Borders Organic Growers in Border Union Agricultural Society's hall at Springwood Park, Kelso (included slides on GB blight populations).
23. **March 2008** Bain RA (2008) Changes to the GB blight population. Part of a presentation to Masstock agronomists. 12 March 2008.
24. **Apr 2008** David Cooke ‘From a European to a global database of *P. infestans* genetic diversity: examining the nature and significance of population change’ The Third International Late Blight Conference, Beijing, China. 03-05 April 2008

Year three

25. **April 2008** Cooke et al., Presentation of findings and discussion of joint work with the Danish experts
26. **May 2008** D. Cooke Presentation at Oomycete molecular genetics network meeting (May 2008, Birnam, UK)
27. **June 2008** D. Cooke & A. Lees Informal presentation to Pseedco growers at SCRI
28. **June 2008** D. Cooke Presentation and handing out of sampling packs to SASA blight scouts
29. **July 2008** R. Bain Presentation of Potato Council and RERAD funded work on population change at three ‘Potatoes in Partnership’ Events at three Scottish partner farms
30. **August 2008** Ian Toth Findings included in presentation at Improving International Potato Production conference in Dundee
31. **August 2008** Cooke et al., Poster at International Congress of Plant Pathology, Turin, August 2008 “Drivers and possible consequences of a changing population of *P. infestans* on the GB potato industry’
32. **Sept 2008** D. Cooke et al Presentation at East Anglia Potato event (4th Sept 2008) sponsored/organised by Potato Council, Syngenta and Greenvale
33. **Oct 2008** D. Cooke. Presentation of Potato Council and RERAD funded work on population change at meetings with Chilean researchers in SAG Santiago and INIAP Osorno. They are interested in monitoring blight populations in Chile using the same approaches.
34. **Oct 2008** A. Lees and D. Cooke Findings presented at Euroblight meeting in Hamar, Norway,
35. **Nov 2008** I. Toth Presentation of Potato Council and RERAD funded work at RERAD Work Package review meeting SCRI.
36. **Dec 2008** D. Cooke Presentation at Global Potato Conference Delhi, India (9-12th Dec 2008) (Cooke DEL, Lees AK, Zhan J, Hansen JG, Lassen P, Birch PRJ (2008) Molecular tools for analysis of populations of Phytophthora infestans at a local and global scale. Proceedings of the Global Potato Conference, 9-12 Dec 2008, New Delhi. P 273-4)
37. **Jan 2009** R. Bain Presentation of work on population change at County Crops agronomists’ meeting in Troon

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38. **Feb 2009** R. Bain Presentation of work on population change at meeting of Masstock agronomists' meeting in York

8.3.2. Publications

Year two

39. **May 2007** Data submitted to EUCABLIGHT database May 2007 onwards
40. **Nov 2007** 'Understanding Blight Populations: Step by Step Guide' BPC Fight Against Blight Growers Advice Sheet.
41. **Dec 2007** Cooke DEL, Lees AK, Shaw DS, Taylor M, Prentice MWC, Bradshaw NJ, Bain RA 2007. Survey of GB Blight Populations. Proceedings of the 10th workshop of an European network for the development of an integrated control strategy for late blight – PPO special report No. 12, 145-152.
42. **Feb 2008** David Cooke 'The status of GB blight populations and the threat of oospores' Proceedings Crop Protection in Northern Britain 2008. 217-222.
43. **May 2008** Bain RA (2008) Changes to the GB blight population. SAC Crop Protection Report 15 May 2008

Year three

44. Fry WF, Grünwald NJ, Cooke DEL, McLeod A, Forbes GA, Cao K, (2009) Population genetics and population diversity of *Phytophthora infestans.* In Oomycete Genetics and Genomics: Diversity, Interactions and Research Tools. Ed. Lamour K and Kamoun S. Wiley Blackwell. pg 139-164.
45. **May 2009** 2008 Data submitted to EUCABLIGHT database

8.3.3. Media reports/press releases related to project activities

Year 1

46. BPC press release post Blight Forum handled by Mistral Group on behalf of BPC
47. Potato Review May/June 2007 issue article

Year 2

48. **May 2007** How does UK Blight effort measure up? Potato Review (May/June 2007).
49. **July 2007** 'Blight population analysis in full swing' BPC Grower Gateway report on blight population
50. **Aug 2007** Press and Journal "Blight fight proves a struggle" update on BPC survey and other issues.
51. **Aug 2007** Dundee Courier "Blight bruises growers' confidence" update on BPC survey and other issues.
52. **Nov 2007** Farmers Guardian Report on D. Cooke presentation at BP07, Harrogate, UK http://www.farmersguardian.com/story.asp?sectioncode=19&storycode=14809
53. **Nov 2007** 'Don't give blight an inch' Potato Council Press release after BP07, Harrogate, UK
54. **Jan 2008** "Blight will come early" Potato Review (Jan 2008)
55. **Mar 2008** 'Potato growers urged to be vigilant in fight against blight' Eastern Daily Press

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56. **Mar 2008** ''Blue 13' blight found to be more aggressive & fitter' Bayer CropScience Four Seasons potatoes newsletter Spring 2008.
57. **Mar 2008** 'A changing blight threat' Potatosafe news (Certis newsletter) Spring 2008.
58. **Mar 2008** 'Keep tight control on blight' BPC Grower Gateway (March 2008)
59. **Apr 2008** David Shaw BBC 'Farming Today' Radio interview in which project results discussed.
60. **Apr 2008** 'Potato Council gears up to battle blight' Potato Council Grower Gateway story on blight project run by SCRI
61. **Jun 2008** 'Tighter spray intervals needed to counter blight' Farmers Weekly 27 June 2008.

Year 3

62. **Sept 2008** 'Wet weather increases risk of potato tuber blight' Farmers Weekly 7 Sept 2009. **http://www.fwi.co.uk/Articles/2008/09/08/112049/wet-weather-increases-risk-of-potato-tuber-blight.html**
63. **April 2009** 'New blight strain demands extra vigilance' Farmers Weekly 5 April 2009. **http://www.fwi.co.uk/Articles/2009/04/05/114942/new-blight-strain-demands-extra-vigilance.html**
64. **May 2009** "Aggressive threat from new strains" Potato Review May/June 2009. Pages 28-9. Mark Sanderson
65. **Jan 09** Scottish Agronomy (Eric Anderson) used slides and data from PCL study at Growers and Advisors Day
66. **Feb 09** Bayer CropScience (Eileen Bardsley) used slides and data from PCL study at Potato Day
67. **Mar 09** Bayer CropScience (Albert Schirring) used slides and data from PCL study at German Potato Day

May 09 Bayer CropScience (Oluf Juhl) used slides and data from PCL study at Swedish Potato Day.

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