Final Report

GB Late Blight Populations: Monitoring and Implications of Population Changes

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Reporting Period 2009-2012

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1 SUMMARY

1.1 Aims

The aims of this project were to monitor changes in the population structure of *Phytophthora infestans* in GB between 2009-2011 and to measure, and determine the likely impact of, characteristics of the population that may affect disease incidence, severity and control. This work was to be integrated as far as possible with information resulting from other projects both in the UK and worldwide. In this report aspects of the work relating to the following aims are described:

a) To monitor the GB *P. infestans* population to provide information on the frequency of mating types and genetic diversity of the pathogen

b) To assess the characteristics of the GB *P. infestans* genotypes and the implications for the activity and control of foliar blight and tuber blight infection

c) To study the longevity of oospores under GB conditions and the implications for subsequent potato crops

d) To actively monitor research in other countries and to use this information to inform the research aims and interpretation of results for this project.

1.2 Methodology

The monitoring of the GB *P. infestans* population was continued via the Potato Council 'Fight Against Blight' campaign with volunteer scouts providing samples of blight infected plant material from which the pathogen was isolated, characterised and genetically typed. A series of laboratory and glasshouse assays were conducted on isolates of *P. infestans* representing the GB contemporary lineages. Such tests examined a series of key characteristics of the pathogen population including; aggressiveness, response to different temperature and humidity regimes, type of spore germination and their ability to infect and survive on potato tubers. The survival of *P. infestans* sexual oospores was investigated in a long-term study that involved field burial and recovery of samples that were tested for their viability in several different bioassays. The competitive ability of different pathogen lineages was also examined in a field trial in which a late blight epidemic was initiated and carefully tracked. Lastly, the research team integrated, discussed and compared their findings with international research teams and used a pathogen dispersal model to help inform and interpret the results in a wider context.

1.3 Key findings

The *P. infestans* population in Great Britain has changed dramatically since 2006 and the three years of monitoring (2009-2011) reported here, continue that trend. The predominance of the 13_A2 genotype reduced slightly over the first two years and then markedly in 2011. The cause of the change is complex but is likely to reflect a change in fungicide use, founder

effects due to the late epidemic in 2011 and possible differences in temperature response between lineages. Concerns have been raised about whether this population change affects the reliability of late blight prediction, tuber blight risks and the threat of oospores as a source of inoculum. The Smith Period is commonly used in GB to predict blight risk on the basis of two criteria; on two consecutive days the minimum temperature must be 10°C or above and the relative humidity must be 90% or above for 11 hours on each of the days. In this study we examined both these criteria and showed that infection can occur down to at least 6°C and that 4-6 hours of high humidity are sufficient for infection to occur. With current moves toward precision-agriculture it thus seems sensible to revise the Smith Period criteria to provide more precise information on infection risk. More detailed examination of whether temperature or humidity responses were genotype-specific revealed some trends but the relationship was not clear-cut. In most cases, variation in response amongst isolates of the same clonal genotype masked genotype-specific effects. When four different genotypes were used to inoculate a field trial, disease progress data and genetic fingerprinting of sampled lesions showed clear differences in the aggressiveness and fitness within the blight population. Genotype 6_A1 caused most disease on the plant initially inoculated and caused nearly 40% of lesions across the plots. Isolates of 13_A2, however, spread most effectively across the trial and caused over 60% of the lesions sampled. The other two lineages (7_A1 and 8_A1) were out-competed and caused only low levels of disease.

Despite the increased foliar aggressiveness of the new clonal pathogen population (i.e. genotypes 13_A2 and 6_A1 that have dominated since 2006), anecdotal evidence suggests that the risk of tuber blight severity has declined. A series of experiments examined the effect of this new population on tuber blight infection and tuber survival after infection. There was considerable variation in tuber aggressiveness between isolates of the same genotype. However, isolates of 13_A2 were generally more aggressive (infection and tissue colonisation) on tubers than those of older genotypes (i.e. those established prior to 2006). The generally lower incidence of tuber blight nationally therefore can't be directly related to pathogen aggressiveness on tubers. The severity of secondary bacterial soft rotting was on average significantly greater for tubers infected by 13_A2 compared with older genotypes.

The optimum temperature for the production of motile zoospores from sporangia by new genotypes of *P. infestans* was low, i.e. between 10^{o}C and 4^{o}C, but generally similar to that for isolates of older genotypes. Changes in the ratio of sporangia to zoospore inoculum across the normal temperature range for GB were generally similar for the new and old genotypes tested.

Oospores from crosses of several A1 and A2 lineages were buried at three locations and their viability tested at six-monthly intervals over 36 months in a series of different bioassays. No oospore germination was recorded. This study and the low frequency of recombinant 'misc' progeny in the monitoring work suggest that *P. infestans* oospores are relatively unimportant as a source of inoculum within the GB potato industry. This is probably due to a combination of dominant triploid clonal lineages that do not generate high numbers of viable progeny and long rotations that allow sufficient decline in inoculum viability. Other studies in Europe have found that oospore biology is challenging to study. Oospore germination is difficult to predict which makes the system rather intractable. Nonetheless the significantly greater pathogen

diversity in other regions of Europe indicates that oospores are a prevalent source of primary inoculum and remain a potential threat.

Further dialogue with European research teams allowed a comparison of the Smith Period criteria with other blight prediction models. Sets of GB meteorological data and the Smith criteria were included in a visual tool to compare models developed by Jens Hansen (University of Arhus, Denmark). Using this graphic analysis tool, the merits of different models can be examined. The Smith criteria can be seen to broadly correspond to other models but modifications should be considered to look at the potential benefits of accounting for lower temperatures and shorter humidity periods thresholds. Skills in state-of-the-art disease modelling were sought through collaboration with Dr Geert Kessel (Wageningen University, the Netherlands) and, latterly, via Dr Peter Skelsey (The James Hutton Institute). Changes in the epidemiological parameters of different *P. infestans* genotypes were made and comparisons of their spread in virtual epidemics demonstrated the significance of even minor changes in pathogen performance. This highlighted the value of the models as an experimental platform to predict pathogen infection and disease spread for future studies.

1.4 Practical recommendations

- Knowledge of the contemporary pathogen population remains important in understanding fungicide resistance traits, aggressiveness, host susceptibility and risks of oospore formation to formulate best-practice management approaches.

- Smith criteria remain a useful indication of risk of pathogen infection and disease spread on a regional basis. However, this study has shown that the criteria should be revised and that growers should be aware of the local environmental conditions are very important factors in considering disease risk and that infection can occur in a crop in the absence of a reported Smith period.

- There is no evidence from the tuber blight studies that the current pattern of use of zoospore-active and sporangia-active fungicides needs to be modified to accommodate the population change.

- Progeny tubers blighted by more aggressive strains are more likely to rot away prior to harvest, especially if infection is earlier in the tuber bulking phase of the growing season and conditions favour secondary bacterial activity, i.e. soils are wet and warm.

- If blight infection of tubers is late in the growing season then the risk of secondary bacterial soft rot in store will generally be higher where tubers are infected by more aggressive strains.

- A seed stock infected with a more aggressive blight strain may carry a slightly higher risk of non-emergence (blanking) or earlier expression of blackleg symptoms. The increased risk is likely to be of significance to seed crops entered in classification schemes only, not ware crops.

- Oospore inoculum is important in some regions of Europe but, within the GB industry, the risks remain low. This is, in part, due to the nature of the *P. infestans* population

and, in part, due to long rotations. Growers should remain aware of the threat and keep rotations as long as possible to allow oospore inoculum to degrade.

- Modelling approaches to predicting of early infections and risks of disease spread have great potential to improve decision support and work is ongoing in this area.

- A small associated study, in which two isolates (13_A2 and 8_A1) were studied, has shown that curative fungicide activity is reduced when an isolate (13_A2) with shorter latent and infection periods is compared to a less aggressive genotype (8_A1) [Bain, unpublished- see Appendix 13].

2 Introduction

2.1 P. infestans population

Potato late blight, caused by *Phytophthora infestans*, is a very significant disease in the UK. The pathogen attacks the leaves, stems and tubers of potato crops and, if not adequately controlled, can result in crop failure.

Previous Potato Council-funded research has demonstrated the value of using genotyping methods such as SSR markers (Cooke & Lees, 2004; Lees *et al*., 2006) to monitor genetic diversity in combination with phenotypic characteristics such as mating type and has revealed a significant change in the *P. infestans* population over recent years. Monitoring of approximately 1000 isolates of *P. infestans* per year in 2006, 2007 and 2008 has shown that the proportion of A2 mating type isolates in the population has increased dramatically in comparison with previous years. This increase in A2 mating type isolates has been attributed to an increase in just one genotype of *P. infestans* (the so-called 13_A2 genotype), first noted in the UK in 2005. This genotype represented approximately 70% and 80% of the >1000 isolates collected in 2007 and 2008, respectively. There has also been an increase in a novel A1 genotype termed 6_A1. These genotypes initially dominated in the South East, but spread North in 2007-8 and now dominate in all regions. These differences in distribution were probably due to the physical distances for spore dispersal and differences in seed source between regions. This information has been widely disseminated to the potato industry.

There are two main implications of this change 1) the new population is likely to have traits that differ from the previous population (e.g. aggressiveness, virulence and fungicide resistance) and therefore influence blight management and 2) the risk of both mating types generating long-lived soil-borne inoculum (oospores).

The presence of A2 isolates is not in itself a problem, however, where A1 and A2 mating types are present in the same outbreak there is a risk that oospores can develop. The resulting sexual recombination (each germinating oospore generating a new genotype of *P. infestans* with a new combination of traits) would lead to a dramatic increase in pathogen diversity and a risk of accelerated host resistance breakdown and fungicide resistance.

Currently there is no evidence for increasing levels of recombination in the UK and the *P. infestans* population is made up of relatively few clones. However, populations in other countries such as Norway, where A1 and A2 have been present in an equal ratio for longer, are much more diverse. Increased pathogen diversity may therefore occur in the UK in the

future: oospores can survive in soil, reportedly for up to 48 months and most of the fields in which both mating types have been observed have not yet been replanted with potatoes. There is an additional concern that oospores are a source of primary inoculum that can survive in the soil and this is considered later in this section.

2.2 Sampling and characterising the GB *P. infestans* population

Effective blight control relies on knowledge of the source of inoculum and conditions under which disease occurs, the efficacy of fungicides and host resistance. Given the dramatic changes to the *P. infestans* population and the potential for increasing diversity in the future, management strategies that use host resistance and fungicides must take account of the traits of the contemporary population. It cannot be assumed that the current dominant genotype will remain dominant in the future and, the situation can change radically over a few growing seasons. Therefore the potential for increased population diversity, and its implications for disease control over the coming years, should be monitored.

2.3 Environmental factors affecting blight and its control

Control of blight may become more difficult in the UK in future due to increased virulence, aggressiveness and fungicide resistance in *P. infestans*, proposed EU restrictions on the fungicides approved for blight control which may significantly increase costs and the impact of global warming. A higher frequency of 'blight years', when the disease is inadequately controlled, would have negative consequences for the efficiency of use of nitrogen and irrigation water, and for food prices. It is difficult to predict the impact of climate change on the overall blight risk in the UK (previously reviewed by SCRI in the report to RERAD: 'Impact of Climate Change on Pests and Diseases of Potatoes in Scotland: Risks and Research Recommendations' (2008)). Studies based on simulation models indicate that an increase in mean temperature in southern Finland of 1°C will extend the period when chemical control for late blight is necessary by 10–20 days if soil-borne inoculum is absent (Kaukoranta, 1996).

However, it is known that winters with fewer and less severe frosts allow the overwintering of considerably more blight inoculum which is active earlier. Night air temperatures below 10^{o}C rather than insufficient humidity hours tend to prevent Smith periods occurring at the beginning and end of the growing season. Increases in these night temperatures, earlier planting and the possibility that the current blight population is active at lower temperatures could result in earlier blight epidemics, potentially exacerbated by the increased use of overhead irrigation. Carnegie (2008) noted that the blight epidemic in 2008 in Potato Council-funded Independent Variety Trials (IVT) progressed rapidly under environmental conditions that would not normally be considered optimal for infection. Kirk (2008) reported that in Michigan over 30 years of monitoring weather-based blight risk, the threshold used to trigger the first fungicide application to crops occurred 0.08 to 0.83 days earlier per year over the period.

The characteristics of GB *P. infestans* genotypes that could influence disease epidemics (e.g. activity of foliar blight at a wider range of temperatures; the effect of humidity and temperature in combination; the infection and development of disease in tubers at low temperatures and the reasons for the increased competitiveness and therefore dominance of some strains)

need to be determined and interpreted to determine whether current risk assessment criteria (eg Smith Periods) and control methods are still applicable.

Assuming that good crop management prevents any tuber infection during harvest then the incidence of tuber blight in store is determined by two factors, first how many tubers were infected during the growing season and second how many blighted tubers survive until harvest (Bain, unpublished). In order to determine the effect of *P. infestans* genotypes on tuber blight incidence it is necessary to investigate both factors. Whether sporangia germinate directly, or indirectly, depends on temperature, with zoospores being more likely to infect progeny tubers *in situ*. Information is required on whether the change in the ratio of indirect to direct germination across the temperature range 6^{o}C to 18 ^{o}C varies between *P. infestans* genotypes. The size of the differences between genotypes has implications for the activity of fungicides with strong zoospore activity. The national incidence of tuber blight was low in recent years (2007 to 2011). This coincided with the domination of the GB *P. infestans* population by isolates of the 13_A2 genotype. It isn't clear whether this was simply a coincidence or whether there was a causal relationship between genotype of *P. infestans* and the incidence of tuber blight, with isolates of genotype 13_A2 resulting in low incidences.

Studies in N. Ireland in both the field and laboratory have shown that extreme selection between *P. infestans* genotypes can occur within populations and that this is strongly influenced by the potato cultivars grown (Young *et al.*, 2009). The mechanisms by which some strains of the pathogen preferentially cause infection when present in mixed populations and thus effectively displace other strains are not understood. Differences in aggressiveness alone, as determined for individual isolates, do not appear to account for the observed changes (Young *et al*., 2007). It is proposed to investigate direct competition between well-characterised isolates of *P. infestans* on potato leaf material in the laboratory and to use a range of molecular techniques to identify those which successfully cause infection and to study the processes involved in competition. These competition studies are fundamental to our understanding of the epidemiology of *P. infestans* and, while not having immediate practical applications, will help to identify traits which result in particular genotypes becoming dominant within populations.

2.4 Longevity of oospore survival and implications

In addition to more favourable weather, the shift towards earlier outbreaks of late blight coincides with increasing evidence of oospores as a new primary source of inoculum in some countries. For example in Sweden, suspected oospore-derived symptoms were reported in a field where the previous crop had been heavily infected with blight (Andersson *et al.*, 1998) and in another field which had been in continuous potato production for at least 5 years (Widmark *et al*., 2007); Bødker *et al.* (1998) also reported earlier blight outbreaks in Danish potato fields where potatoes had been grown during one of two previous years in comparison to fields with a break of at least 3 years in potato production and attributed this to the presence of oospores rather than volunteers.

There is convincing evidence that oospores are formed in potato crops throughout Northern Europe, but the relative importance of oospores as a source of primary inoculum is less clear. Oospores have been shown to persist in soil for 3-4 years in the Netherlands (Turkensteen *et*

al., 2000). Work in Finland by Lehtinen & Hannukkala (2004) set out to verify that oospore-derived epidemics were occurring in Finland. It is difficult to prove that an infection is caused by an oospore and so they identified 20 outbreaks where oospores were suspected to be the cause and observed that the following four factors were common to all outbreaks:

1) Early disease foci in fields where blight had been observed in one of the previous 4 years
2) Primary lesions from leaf edges touching soil, or from stems
3) Both mating types present early in the epidemic
4) Soil from the foci able to infect leaves in a bioassay

This work and that of other groups (Hannukkala, Ravnskov, Andersson, pers. comm.) noted the difficulties of using a bioassay to determine infectivity of soil. Results obtained using the commonly used bioassay (Drenth *et al*.,1995) have proved inconsistent. Oospores can also be extracted from soil (Fernández-Pavía *et al*., 2004), but the procedures used are time-consuming and viability must still be determined; techniques such as plasmolysis and vital staining which have been used for viability determination can also be unreliable (Deahl, KL, personal communication).

A real-time PCR assay for *P. infestans* developed at SCRI (Lees *et al*., 2012), which can reliably detect and quantify the pathogen in plant and tuber tissue has been shown not to be reliable for detection of *P. infestans* in soil. Soil sampling and DNA extraction methods found to be effective for a wide range of other potato pathogens (Potato Council- funded projects R249, R253) have been unsuccessfully trialled for *P. infestans*. Without reliable methods for detecting oospores in soil and testing viability it is difficult to examine the longevity of oospores, or the factors affecting viability. By genotyping parental isolates it is possible to infer that isolates showing particular patterns of recombination are the progeny of a particular set of parents and therefore derived from oospore inoculum.

Detailed monitoring of the *P. infestans* population in recent years (Potato Council-funded project R274) indicates that oospore-derived outbreaks are not yet a problem in GB – there is little variation in the population and no confirmed cases in a GB crop where a combination of an early outbreak, mixed mating type isolates and diverse SSR genotypes, which would be characteristic of oospore inoculum, occurs. However, the threat of oospore inoculum remains; most of the land potentially contaminated with oospores has yet to return to potatoes in the rotation. Growers can reduce the risk of an early attack by improving crop rotation where it is inadequate and information on the longevity/viability of oospores in GB soils could help to inform best advice on rotations.

2.5 International Research

In the Netherlands important work on modelling carried out at Wageningen University provides a powerful means of examining the traits of specific *P. infestans* genotypes and predicting their behaviour in an epidemic (i.e. disease spread) with clear implications for effective management. A sophisticated model has recently been developed by Peter Skelsey (Skelsey, 2008) in the laboratory of Geert Kessel. This has been used, for example, to predict the spread of late blight in relation to patterns of resistance deployment. All the parameters

relevant to a potato blight epidemic such as infection frequency, lesion spread and sporulation in response to key criteria such as temperature and humidity are defined in the model. Such parameters were defined from a single 'typical' isolate of *P. infestans* and there has been no attempt, to date, to adjust these parameters according to data for specific *P. infestans* genotypes. The great advantage of such models is that they tightly define the criteria needed to predict disease spread and allow simulations to be run in which the impact of alternative parameters for any particular trait may be examined. For example, the effect of more rapid lesion growth rate, reduced latent period or infection at 8^{o}C (rather than the current minimum of 10^{o}C) can be entered and simulations of the epidemic generated using real meteorological data from previous seasons.

3 Experimental Section

3.1 To monitor the GB P. infestans population to provide information on the frequency of mating types and genetic diversity of the pathogen

Materials and Methods

Outbreak sampling

Following discussion with the Potato Council it was decided that the 400 isolates to be characterised in this study (in collaboration with a DEFRA LINK study) should consist of 4 isolates per outbreak from 100 outbreaks. This is in contrast to the 8 isolates/outbreak sampled in previous years. In this way, the geographical and cultivar distribution of the isolates would be maintained, and although some fine-scale diversity would be lost compared with previous seasons' monitoring, this was considered sufficient to ensure an ongoing monitoring of the GB *P. infestans* population.

Scouts engaged in the Potato Council's Fight Against Blight campaign 2009- 2011 therefore collected up to 4 late blight lesions per crop and the samples were sent to Fera and, if confirmed as blight, the FAB map was updated, generating a red spot for each outbreak (Fig. 1a + 1b). Accompanying information relating to each sample was also recorded (see Appendix 1). Positive samples were placed within small potato tubers and sent in batches to JHI (Dundee) for further testing. Additional samples were requested from any outbreaks with symptoms typical of oospores as a source of inoculum (based on an information sheet included in each scout pack). No such samples were reported or supplied in 2009. In 2010 soils and additional information relating to seed source, geographical proximity of sites etc. were received from sites where an increased number of miscellaneous genotypes were detected.

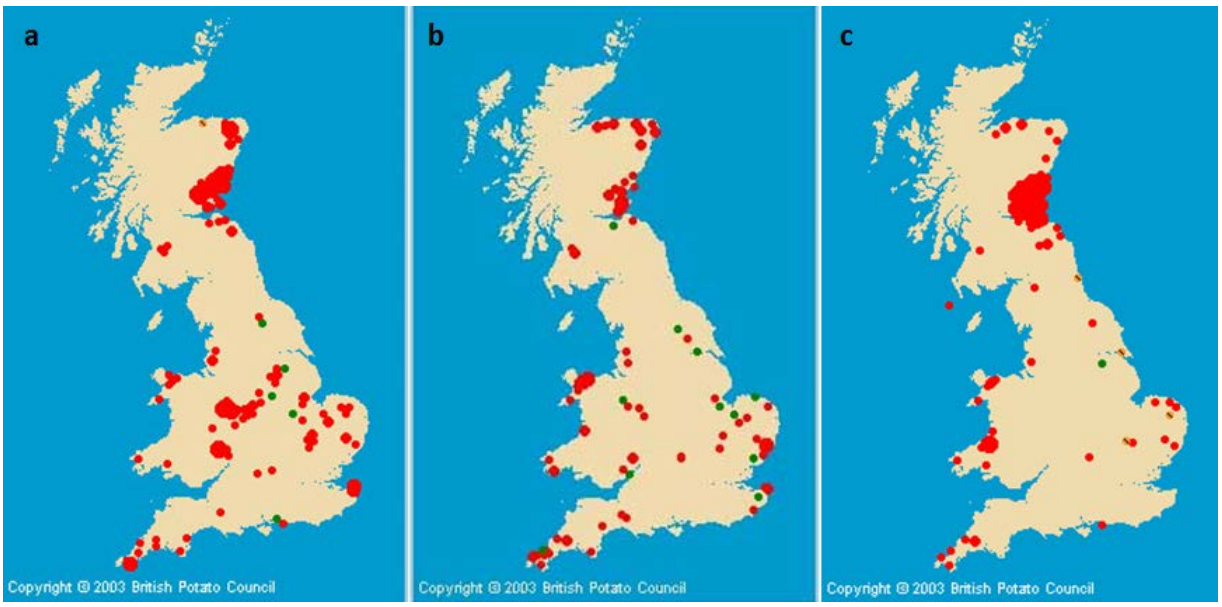

Figure 1. Locations of the late blight outbreaks a) 2009 (154 outbreaks) and b) 2010 (110 outbreaks) and c) 2011 (183 outbreaks) recorded by the FAB campaign.

Sample processing

Upon arrival at JHI, a slice of tuber ca. 5 mm 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^{o}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^{o}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^{o}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 was 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 might indicate the presence of a mixed culture or a self-fertile isolate.

Testing genetic diversity of isolates

A 2 mm^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) and additional 12-plex marker set (Li *et al*., 2012). The SSR data were manually checked and peaks scored prior to export to excel spreadsheets for further analysis. Data were also entered into the Eucablight database.

Results

Sampling

Over the course of the seasons 2009, 2010 and 2011 more than 1800 late blight samples from 304 late blight outbreaks across GB (Fig. 1) were delivered to the James Hutton Institute. All outbreaks were recorded using the FAB postcode-based system. Samples were provided either from Fera or, in the case of many local Scottish samples, direct from the scout. From these samples more than 1200 isolates of *P. infestans* were obtained.

Across GB 2009, 2010 and 2011 were relatively low disease pressure years with blight outbreaks starting later in the year and a higher proportion of samples collected later in the season than the earlier and higher pressure years of 2006 and 2007 (Fig. 2). The geographical spread of outbreaks sampled varied from season to season with 2009 and 2010 having a spread broadly in line with previous years (Fig. 1, Fig. 3) but in 2011 hot dry conditions limited blight activity in England and Wales whereas conducive weather later in the season resulted in high disease pressure and many samples from Scottish crops (Fig. 3).

Pathogen characterisation

Mating type testing of the isolates indicated a slight reduction in the frequency of the A2 mating type from its high of 80% in 2008 to 72% in 2009 and 69% in 2010. This was followed by a marked fall to only 10% in 2011 (Table 1). In line with this, the proportion of sampled

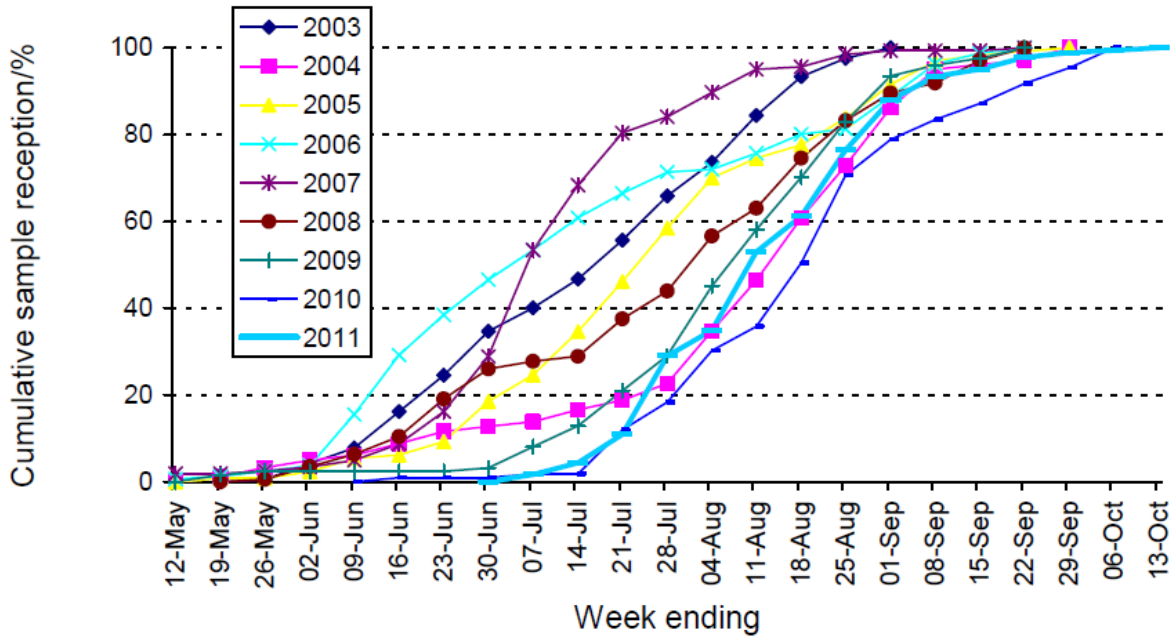

Figure 2. Fight Against Blight sample data for seasons 2003 to 2011. Collated by Moray Taylor (Fera, Sand Hutton, York).

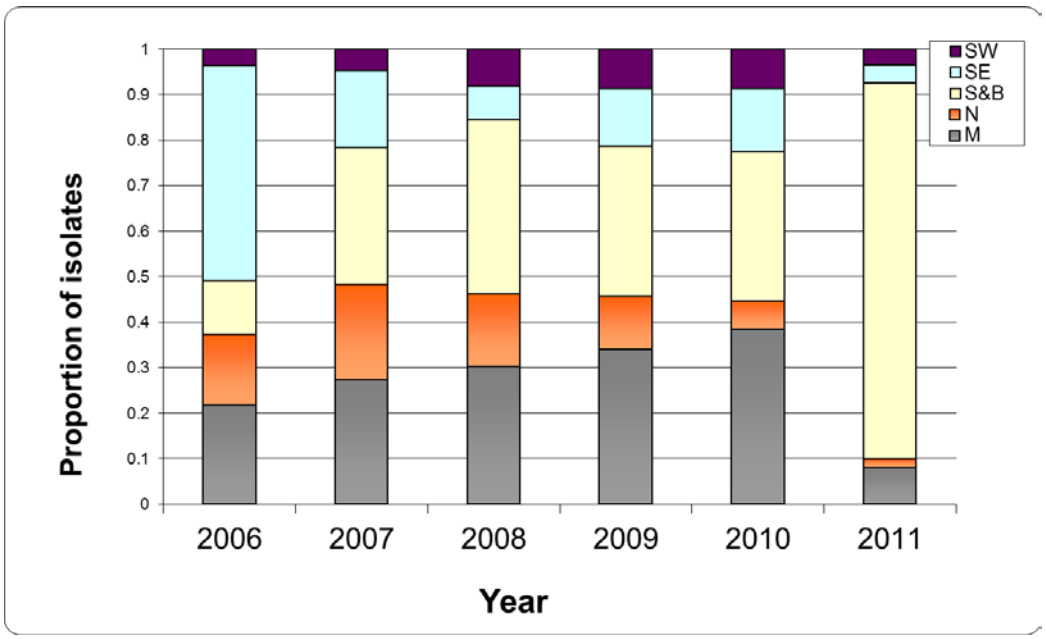

Figure 3. Proportion of isolates originating from regions of GB in from 2006-2011 (SW = South West; SE = South East; S&B = Scotland and Borders; N = North; M = Midlands and Wales)

outbreaks with the A2 mating type present was 77, 72 and 15 % over the three seasons (Fig. 4). The proportion of outbreaks in which both mating types were recovered was 16, 18 and 6% over this period (Table 2). There was thus the potential for oospore formation between A1 and A2 mating type isolates in one in 5 or 6 of sampled crop outbreaks in 2009 and 2010 but this risk fell markedly in 2011.

Table 1. The numbers and percentages of *P. infestans* isolates of each mating type collected during the 2006-11 seasons.

Mating type	2006	2007	2008	2009	2010	2011	Total
A1	464	411	204	152	79	394	1704
A2	550	1190	830	381	177	45	3173
Total	1014	1601	1034	533	256	439	4877
							Mean
%A1	45.8	25.7	19.7	28.5	30.9	89.7	40.0
%A2	54.2	74.3	80.3	71.5	69.1	10.3	60.0

Table 2. The number and percentage of blight outbreaks sampled over the 2006-11 seasons categorised according to the *P. infestans* mating types present. The percentage of outbreaks in which both mating types were recovered is also shown.

Outbreak type	2006	2007	2008	2009	2010	2011	Total
A2 only	72	188	157	89	44	13	**563**
A1 only	57	55	19	33	23	136	**323**
Mixed	36	57	31	23	15	10	**172**
Total outbreaks	165	300	207	143	82	159	**1056**
Total with A2 present	108	245	188	112	59	23	**735**
							Mean
% outbreaks with A2	65.5	81.7	90.8	77.0	72.0	14.5	**66.9**
% mixed outbreaks	21.8	19.0	15.0	16.0	18.3	6.3	**16.1**

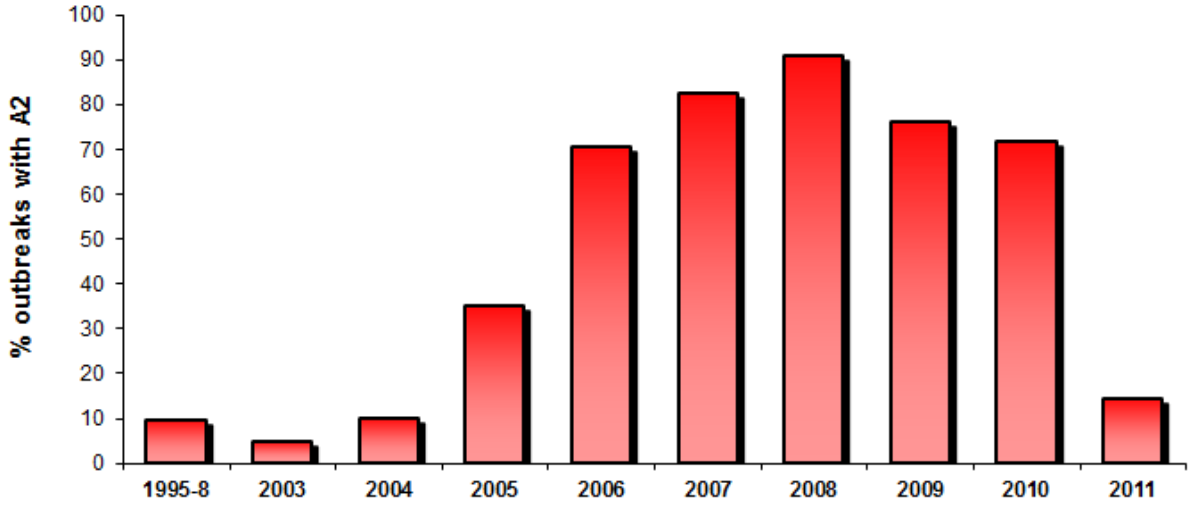

Figure 4. Percentage of all sampled GB blight outbreaks in which A2 isolates of *P. infestans* were found between 1995 and 2011.

Genetic diversity of isolates

In this project, a new efficient system of genotyping in which all twelve simple sequence repeat (SSR) loci were PCR amplified in a single reaction (Li *et al*., 2013) was used. The combination of alleles at the 12 loci observed in a single isolate was used to define its genotype. The genotypes of all isolates were sorted in an excel spreadsheet and combinations present in multiple isolates from many outbreaks are identified by a number and their characteristic mating type (e.g. 1_A1, 2_A1, 3_A2 etc.) as previously defined (Cooke *et al*., 2012, Li *et al*., 2013). An additional category of genotype termed 'miscellaneous', or 'misc', was defined for all isolates with combinations of alleles 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 nine seasons characterised to date are shown (Fig. 5).

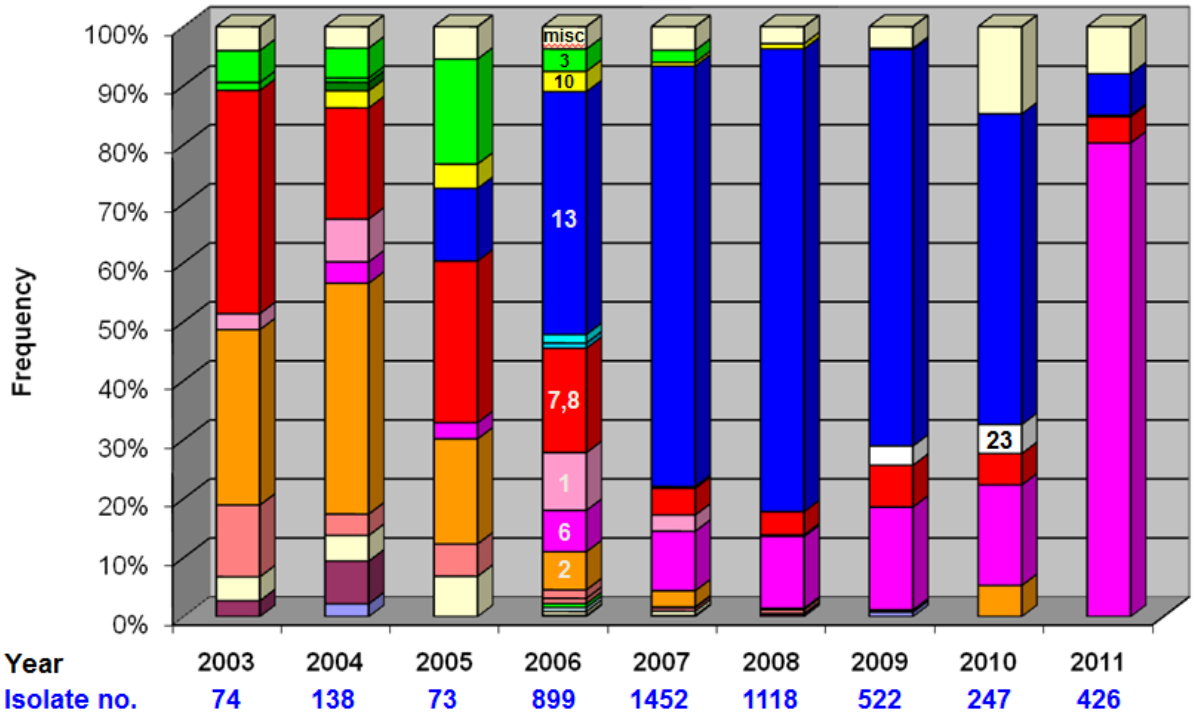

Figure 5. Bar charts indicating the frequency of *P. infestans* isolates of each SSR genotype over the course of nine seasons (2003-11). The main genotypes are labelled with numbers in the 2006 and 2010 columns and represent the following; 3_A2, 10_A2, 13_A2, 7_A1/8_A1 combined, 1_A1, 6_A1, 2_A1, 23_A1 and 'misc'. The number of genotyped isolates per year is also shown.

The results for 2009 were broadly similar to 2008 but with the addition of the genotype 23_A1. This type was first found infecting 1 garden-grown and 2 commercial tomato plants in southern England in 2007 but was not sampled in 2008. In 2009 it was recovered from eight outbreaks in commercial potato crops across England, Wales and Scotland. Such rapid regional spread suggests an additional source of inoculum such as that in the commercial tomato seedling trade as documented in US late blight outbreaks in 2009 (Fry *et al.*, 2013). This lineage was found in tomato and potato crops in Ayrshire, Shropshire and Cambridgeshire in 2010 but is apparently not as fit and aggressive as other lineages under UK conditions as it was sampled only once in 2011. In contrast, genotype 8_A1 and 2_A1 are very persistent, having been in the UK since at least 1995 and 1982 respectively (data not shown). The 13_A2 genotype remained the single most dominant type in 2009 but over these two seasons showed a progressive decline in frequency with corresponding increases in 6_A1 from 12 to 17%. Also in 2010 the frequency of isolates categorised as 'misc' reached its highest value of 15% being found in 17 of the 82 outbreaks sampled (20%). Such isolates are predicted to be the result of the germination of oospores which is indicative of the pathogen having a viable soil-borne sexual stage. In previous years (2003-2009) the frequency of 'misc' isolates has ranged from 2.9 to 5.5% with no observable increasing or decreasing trend. The data for 2010 were thus unexpected and worthy of further scrutiny as to whether they indicate the beginning of a change in the GB *P. infestans* population structure to match that of some other regions of Europe.

Scottish blight outbreaks represented 37% (30 of 82) of the total sampled in 2010 but 71% of the outbreaks with 'misc' isolates (12 of 17) were sampled from Scottish crops. These Scottish crops were from Aberdeenshire (6), Inverness region (5) and Ayrshire (1). The English and Welsh samples were from Hereford and Worcester (1), Devon (1), Gwent (1) and Gwynedd (2). These data suggest a Northern bias to the sampling of 'misc' isolates but it would be unwise to draw any firm conclusions on these data from a single year. There are many factors that need to be considered – for example this may have been a sampling effect as relatively few outbreaks were sampled in England and Wales during the early part of the 2010 season as the dry conditions were not conducive to oospore germination. Warm and wet conditions during planting and emergence have been reported to encourage oospore activity so regional differences in soil conditions during this crop stage need to be considered. Only the first two 'misc' outbreaks sampled on 8th June and 5th July in north-eastern Scotland could be considered unusually early outbreaks within the region they were sampled. Other 'misc' outbreaks were sampled in July (3), August (11) and September (1). There was no clear pattern in the type of crop with the 'misc' samples originating from organic (3), conventional (10), gardens and allotments (3) and one volunteer potato. This increase in 'misc' isolates was not continued in 2011 when the percentage fell back to 7.8%. Nonetheless, of the 11 outbreaks in which 'misc' isolates were found, 8 were in Scotland and predominantly in Aberdeen, Moray and Inverness regions. There is however no clear explanation for this clustering of novel genotype combinations or any indication of significant spread within a season or carry-over of inoculum from one year to the next. This suggests the new genotypes are not particularly fit or aggressive. Soil has been sampled from one outbreak and tested for the presence of viable oospores (see below).

A marked shift in the frequency of genotypes of *P. infestans* was recorded in 2011 (Fig. 5) with an increase of 6_A1 and a decline in 13_A2. Several factors may have led to this change. The start of the 2011 season was unusually warm and dry resulting in low blight pressure for almost all GB potato growing regions. As a result much of the primary inoculum in, for example, discard piles or volunteers will have failed. Unfavourable weather for late blight continued across England and Wales and fewer samples than normal were received from these areas (Fig. 3). The rainfall was significantly higher than average in Eastern Scotland in July and August generating severe disease pressure that resulted in 4.5 times as many samples being received from Scotland than from England and Wales. Such bias in sampling will have affected the mean GB genotype values in Fig 5. A breakdown of the results into those from Scotland versus England and Wales (Fig. 6) shows an even more extreme decline in 13_A2 in Scotland with 6_A1 comprising 88% of the 2011 samples. One explanation for this may be a major 'founder effect' in which 6_A1 inoculum from a severe infection in a Scottish crop in June rapidly spread in Fife, Angus and Perthshire crops. It would appear that this is not the only factor as 6_A1 comprised 55% of isolates in sampled English and Welsh outbreaks which is also markedly higher than the 20% recorded in 2010. In contrast 13_A2 comprised 28% of the samples in England and Wales 2011 compared to 59% in 2010 (Fig. 6).

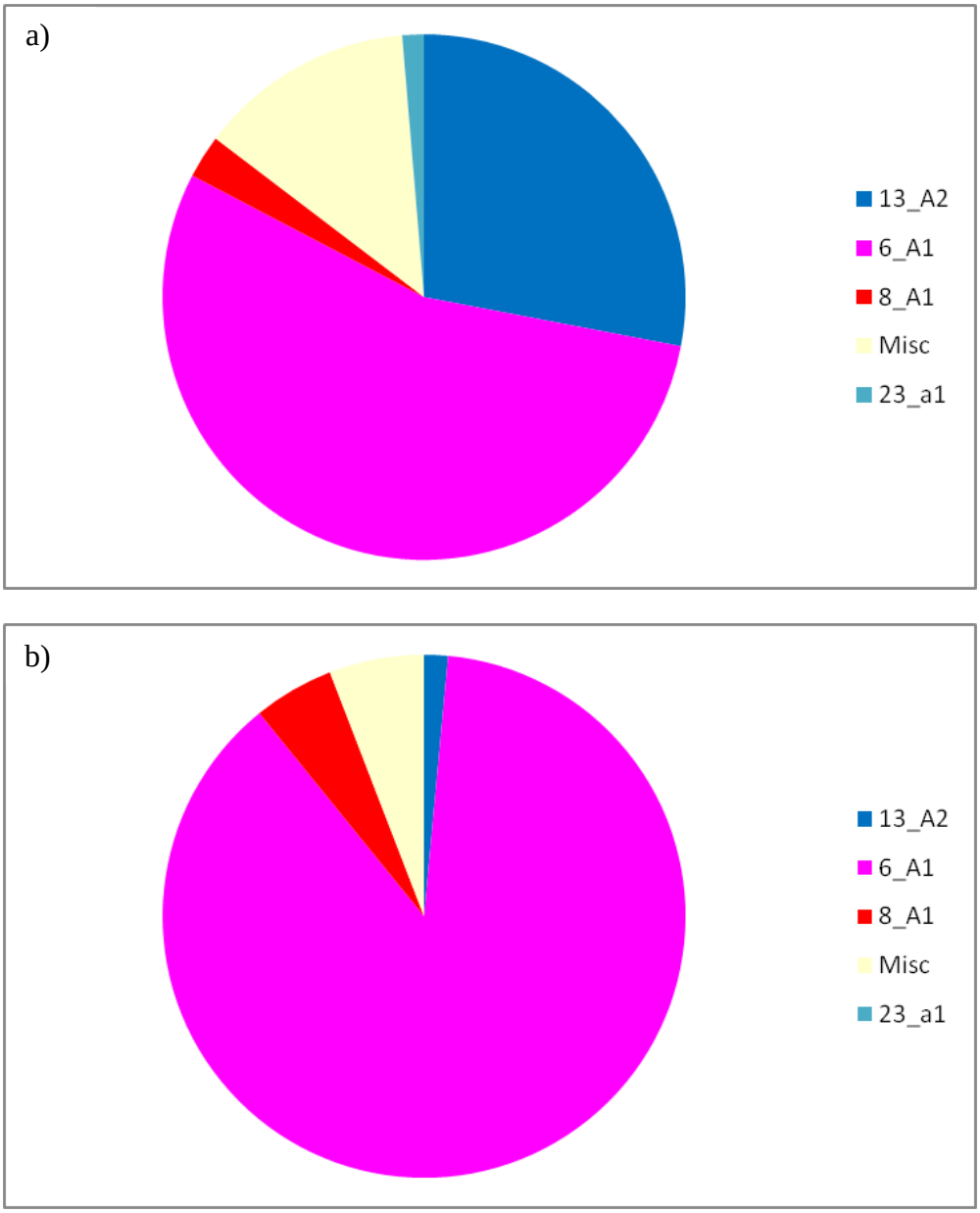

Figure 6. The frequency of *P. infestans* genotypes recovered from outbreaks in the 2011 season from a) England and Wales (*n*=75) and b) Scotland (*n*=341).

Within genotype variation in 13_A2

Each time a *P. infestans* cell divides, minor DNA sequence differences (mutations) will inevitably be introduced into the 250 million or so DNA base pairs in its genome that are replicated. Up to 20,000 sporangia are produced per cm^2 of every late blight lesion each day and clearly therefore, countless billions of *P. infestans* cells are dividing daily. Three of the 12 SSR markers are more prone to mutation than the others which generates minor differences in fingerprint patterns over time. Almost 2900 isolates of the 13_A2 clonal lineage have been fingerprinted and almost 100 minor sub-groups defined (named 13_A2_1, 13_A2_2 etc). Those sub-groups that emerged early had an opportunity to spread and are prevalent in the population but the majority are rare and thus seldom sampled. The rate at which new 13_A2 variants emerge and their stability over time makes them appropriate for tracking inoculum

spread. An analysis of the years 2006-2009 indicates that the frequency of the initial 13_A2_1 type has decreased over time (Fig. 7a) as the population of this clone has splintered into different sub-types. It is also apparent that there are regional differences in the frequency of these variants with for example 13_A2_5 being prevalent in the Midlands region (Fig. 7b) but not in Scotland and the Borders. Conversely 13_A2_7 and 13_A2_55 are found almost exclusively in crops in Scotland and the Borders. A more detailed analysis even showed a strong local pattern amongst Scottish regions (data not shown). This illustrates the marked impact of inoculum generated in last season's crop as a source of local primary inoculum propagating disease in nearby crops the following season.

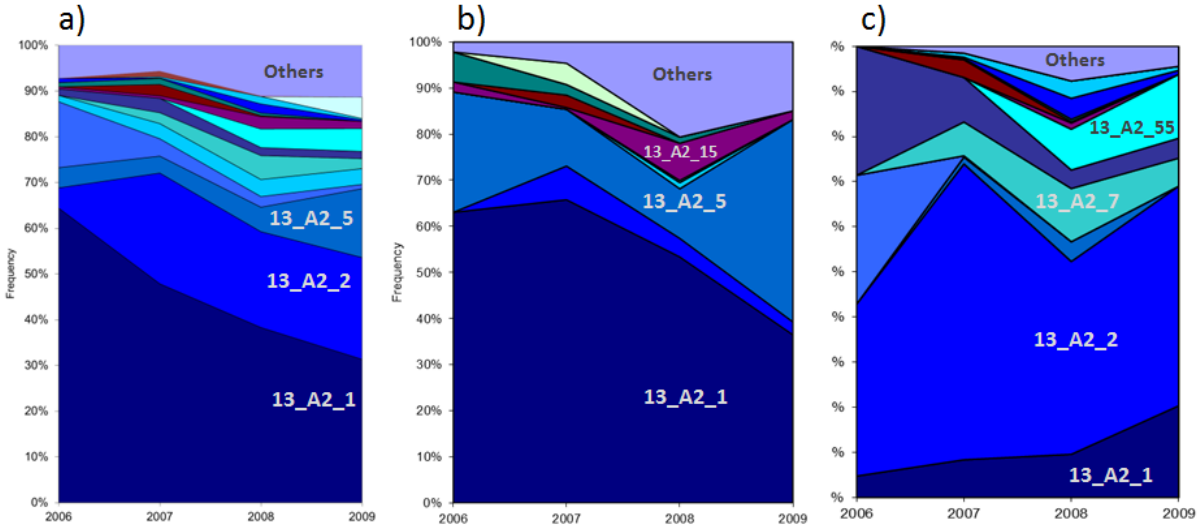

Figure 7. Analysis of the frequency of genetic sub-groups of the 13_A2 lineage a) across the whole of GB (n = 2881) b) in outbreaks in the Midlands and Wales (*n* = 636) and c) in Scotland and the Borders (*n* = 834). Only the 15 most frequently recovered 13_A2 variants are shown with all other minor forms combined in a single category 'others'.

Green33 lineage

A sample of infected volunteer potato collected in Norfolk in October 2011 indicated that another new genotype had migrated into the GB crop. The lineage, Green33, was first identified and studied by Geert Kessel, Bert Evenhuis and Huub Schepers at Wageningen UR (WUR) in The Netherlands. The Green33 lineage was first found in The Netherlands in 2009 and comprised 20% of the *P. infestans* population sampled there in 2009 and 2010. In 2011 a field trial demonstrated that Green33 isolates were not effectively controlled by fluazinam (as Shirlan). Subsequent work by Syngenta and WUR confirmed that some isolates of Green33 were less sensitive to fluazinam. It was also shown that isolates of the Green33 lineage did not appear to be as fit as other lineages such as 13_A2 in the absence of fluazinam treatment (Schepers *et al.*, 2013). The discovery of this lineage in the UK was a cause of concern and was reported at the Potato Council Winter Forum early in 2012.

Discussion

The progressive decline of 13_A2 over the 2009 and 2010 seasons and its marked drop in frequency in 2011 means that, for the second time in 6 years, GB growers are managing a new pathogen population. It is important to understand the causes of the population change

and the implications for blight management in the GB potato industry. Will the decline of the aggressive 13_A2 lineage mean that blight is easier to manage? A study in 2007 indicated that 13_A2 and 6_A1 were the two most aggressive lineages at that time (Cooke *et al*., 2012) and the evidence from this study certainly supports the fact that 6_A1 is an aggressive and fit genotype that will require similar fungicide application schedules to 13_A2. Unlike genotype 13_A2 however, the 6_A1 lineage is known to be sensitive to metalaxyl (Kildea *et al*., 2013). Nonetheless growers, cautious about the proportion of fully resistant lineages within the population, are not reported to be returning to this potentially useful systemic fungicide. Earlier work suggested that 13_A2 had a greater competitive advantage over other lineages at 13^{o}C but less so at 18^{o}C (Cooke *et al*., 2012). In this study (see later in the report) there are indications of 6_A1 causing larger lesions than 13_A2 under warmer conditions in support of this theory and temperature is one possible driver in the changes observed in 2011. If the previous dominance of 13_A2 was in part due to its ability to outcompete other lineages at lower temperatures it may be that the 13_A2 primary inoculum emerged earliest under cooler conditions in 2011 and was severely checked by the subsequent dry conditions. By the time the epidemic began later in the 2011 season the conditions were warmer and may have favoured lineages such as 6_A1 over 13_A2. Another possible cause was a removal of the positive selection pressure imposed on resistant 13_A2s by the use of phenylamide fungicides. The area of UK potatoes treated with metalaxyl-M fell from 82,000 in 2006 to 2600 ha in 2010 (Fera Pesticide Usage Statistics; http://pusstats.csl.gov.uk/index.cfm).

Does the population change influence the likelihood of oospores acting as a source of primary inoculum in GB crop? Both the A1 and A2 mating types are required in the same disease outbreak to induce the formation of oospores and the probability of this occurring has declined with a greater imbalance in the mating type frequencies. Furthermore new evidence indicates that both 6_A1 and 13_A2 are triploid; i.e. have an additional set of chromosomes from the standard diploid complement (Hamed and Gisi, 2012). Sexual recombination between triploid parental strains is possible but often results in anomalies in chromosome segregation which may affect viability and pathogenicity. Hamed and Gisi (2012) reported significantly fewer F1 progeny in a cross between triploid 6_A1 (their isolate 110) and 13_A2 (their isolate 101) isolates than other triploid by diploid or diploid by diploid crosses. This was shown to be the case in the studies in polythene tunnels in a previous Potato Council project (R274 Cooke *et al*., 2010).

Fields planted with a potato crop in 2006, in which both mating types were found in 20% of sampled outbreaks, were likely to have been planted with ware potatoes again in 2011. However, by far the majority of late blight outbreaks in GB potato crops in all seasons are the result of clonal (asexual) inoculum from the previous season. In the rare cases where oospores are likely to be the source of inoculum such pathogen genotypes do not appear to be fit and aggressive as they are rarely found beyond a single crop. This finding that oospores are not a major source of primary inoculum in GB crops is a positive outcome of the practice of maintaining long rotations and is a contrast to some other regions of Europe with evidence of abundant oospores causing disease and generating new damaging genotypes (Nordic regions; Yuen and Andersson, 2012; The Netherlands; Li *et al*., 2012 and Poland; Chmielarz *et al.,* 2013). It is important to be aware of this and maintain a watching brief on the evidence for viable *P. infestans* oospores in GB soils.

Despite the positive news on oospores, the abundant evidence for local sources of clonal inoculum from the previous crops indicates that GB growers could do more to control such sources of the pathogen. Management of inoculum on discard piles and volunteers and using high quality seed remains important.

The presence of the Green33 genotype two years after its first finding in The Netherlands reconfirms the ability of *P. infestans* to spread across national borders. Its prevalence in Dutch crops is a concern but is very probably related to their historically greater reliance on fluazinam with many applications, often in blocks. It is encouraging that Green33 is not as aggressive as 13_A2 which, in the absence of fluazinam application, can outcompete Green33 (Schepers *et al*., 2013). Nonetheless, the emergence of reduced sensitivity against a product that was considered a fungicide with a low risk of resistance development is a warning of the evolutionary potential within *P. infestan*s populations.

3.2 To assess the characteristics of the GB P. infestans genotypes and the implications for control of foliar and tuber blight infection

3.2.1 Characterising the collection for aggressiveness

Materials and Methods

A selection of isolates representing the main genotypes distinguished in previous years was assembled (Appendix 2). The rationale was to select approximately 50 contemporary isolates for aggressiveness testing with several (up to 10) representative isolates per genotype to investigate intra and inter-genotype responses. A greater proportion of 13_A2 and 6_A1 genotype isolates were included to allow within genotype comparison of these dominant lineages. Within the core set, we included; isolates that were to be used in a complementary DEFRA Link project to examine host resistance; reference strains from other studies (e.g. IVT and Potato Council-funded aggressiveness studies; isolates used in previous projects (R274).

The aggressiveness of 49 isolates (representing 11 genotypes) was tested on detached leaves in a fully replicated experiment (using measurements of infection, sporulation and lesion size) on five cultivars (Maris Piper, Cara, King Edward, Lady Balfour, Estima) at 15ºC using a standard Eucablight protocol. Due to the scale of the study, isolates were tested in batches in four separate experiments and, at each date, two control isolates (2006_3928A and 2007_5442F) were included to account for variation between tests. Control isolates were placed in the middle of the box and always in the same place in each box with the other isolates being randomised. The boxes were sealed in plastic bags and transferred to a growth chamber for incubation at 15°C with a light and dark cycle of 16 and 8 hours respectively. A randomised complete block design with six replicate blocks was used. Leaves were examined daily for seven days and the following factors scored: incubation period (IP; days to first symptoms), latent period (LP; days to sporulation) and lesion size (mm) measured on several dates in two directions at 90 degrees to each other with digital callipers. Lesion size data were plotted over time and the curve used to calculate a single value that captured the time series, termed the area under the lesion expansion curve (AULEC).

An ANOVA was conducted on the lesion area (mm^2) data measured for the control isolates in each of the four experiments. Significant differences between tests for both of the reference

isolates (2006_3928A *P*=0.004 and 2007_5442F *P*=<0.001) were observed and this invalidated the plan to combine all the data into a single ANOVA. The results are therefore presented separately for each of the four tests.

Results

Significant differences in IP, LP, lesion size and AULEC were observed between isolates, genotypes and cultivars in all four tests. The time to first appearance of symptoms (IP) did not discriminate between isolates as clearly as the variates LP or AULEC; the range of IP values was from three to almost five days with the mean value of the majority of isolates being around three days (data not shown). The ten isolates of genotype 13_A2 had IP values covering almost the whole observed range. Similarly one isolate of 6_A1 had the shortest IP whilst another had the longest IP. The mean IP of all isolates of the same genotype was calculated and no consistent effect of genotype was observed over the four tests. Given the range of IP amongst isolates of the same genotype, this was to be expected. A similar pattern was observed for LP. The mean time from inoculation to sporulation of individual isolates ranged from 5.5 to 7.7 days (data not shown). The mean LP values of isolates of the same genotype varied from test to test with the 6_A1 isolates in test one having the shortest mean LP but those in test four had one of the longest (data not shown).

Significant differences in lesion size, expressed as AULEC, were observed for individual isolates (Fig. 8) and genotypes (Fig. 9) across all experiments. As for the data for LP and IP, there were significant differences in area under the lesion expansion curve amongst isolates within all experiments (*P*<0.001). Variation in final lesion size between isolates of the same genotype was also observed. In the experiment with most isolates of 13_A2 and 6_A1, the final lesion sizes of 6_A1 isolates were, on average, larger than those of 13_A2 isolates (Figure 8 A). In all four experiments, isolates of genotype 7 and 8_A1 formed some of the smallest lesions whereas those of genotype 2_A1 were, in general, larger.

The effect of cultivar on AULEC was statistically significant in three of the four experiments with the greatest resistance being demonstrated in Cara and the least in King Edward and Maris Piper (Figure 10).

A detailed breakdown of the findings is available in the PhD thesis upon which this report is based (Chapman, 2012). The thesis may be found on the Dundee University research portal (http://discovery.dundee.ac.uk/portal/).

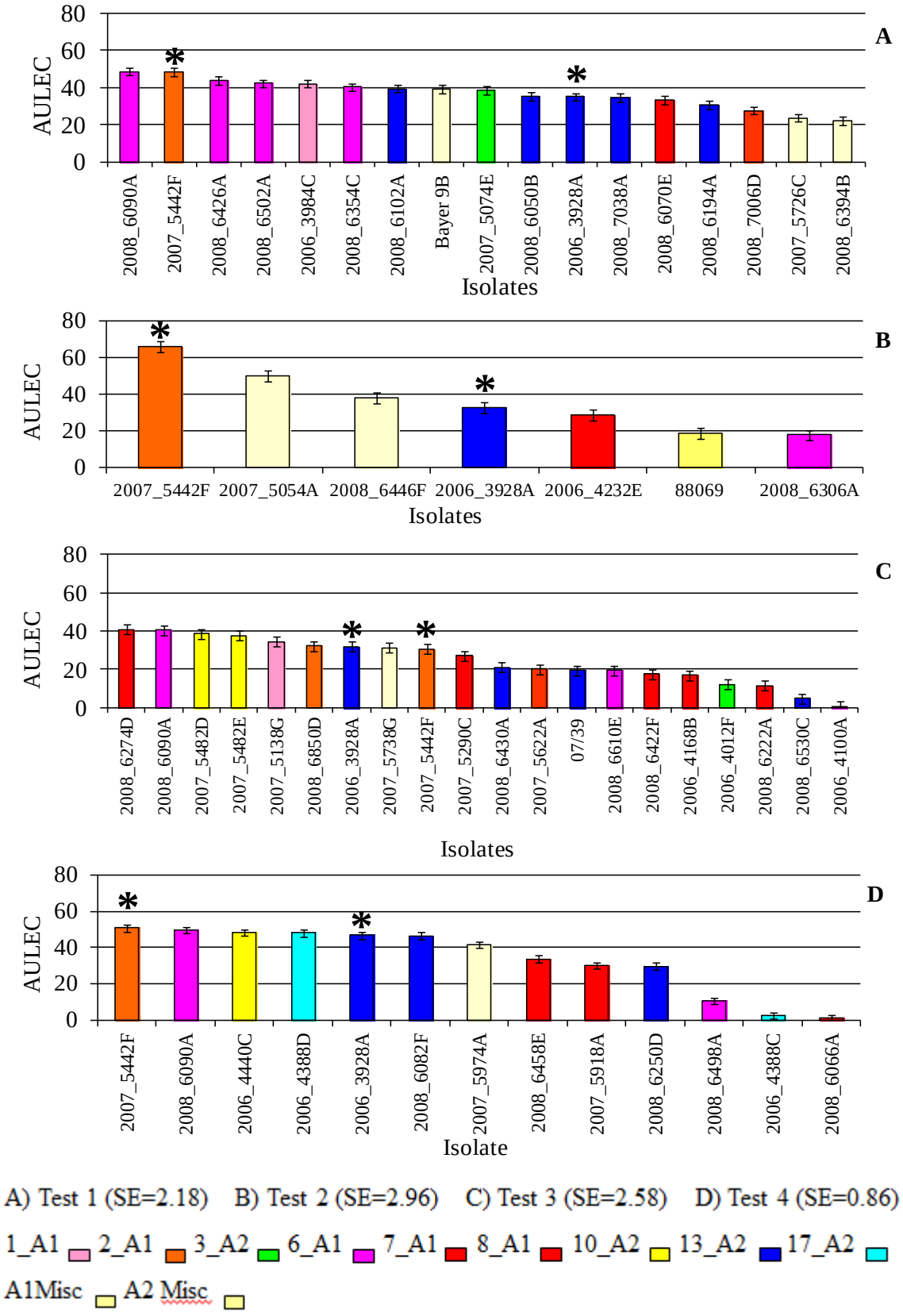

Figure 8 – Mean AULEC values of a range of UK *Phytophthora infestans* isolates on detached potato leaflets (averaged over five cultivars). The error bars represent the standard errors of differences of the means (SE). Bars marked with an asterisk are the reference isolates.

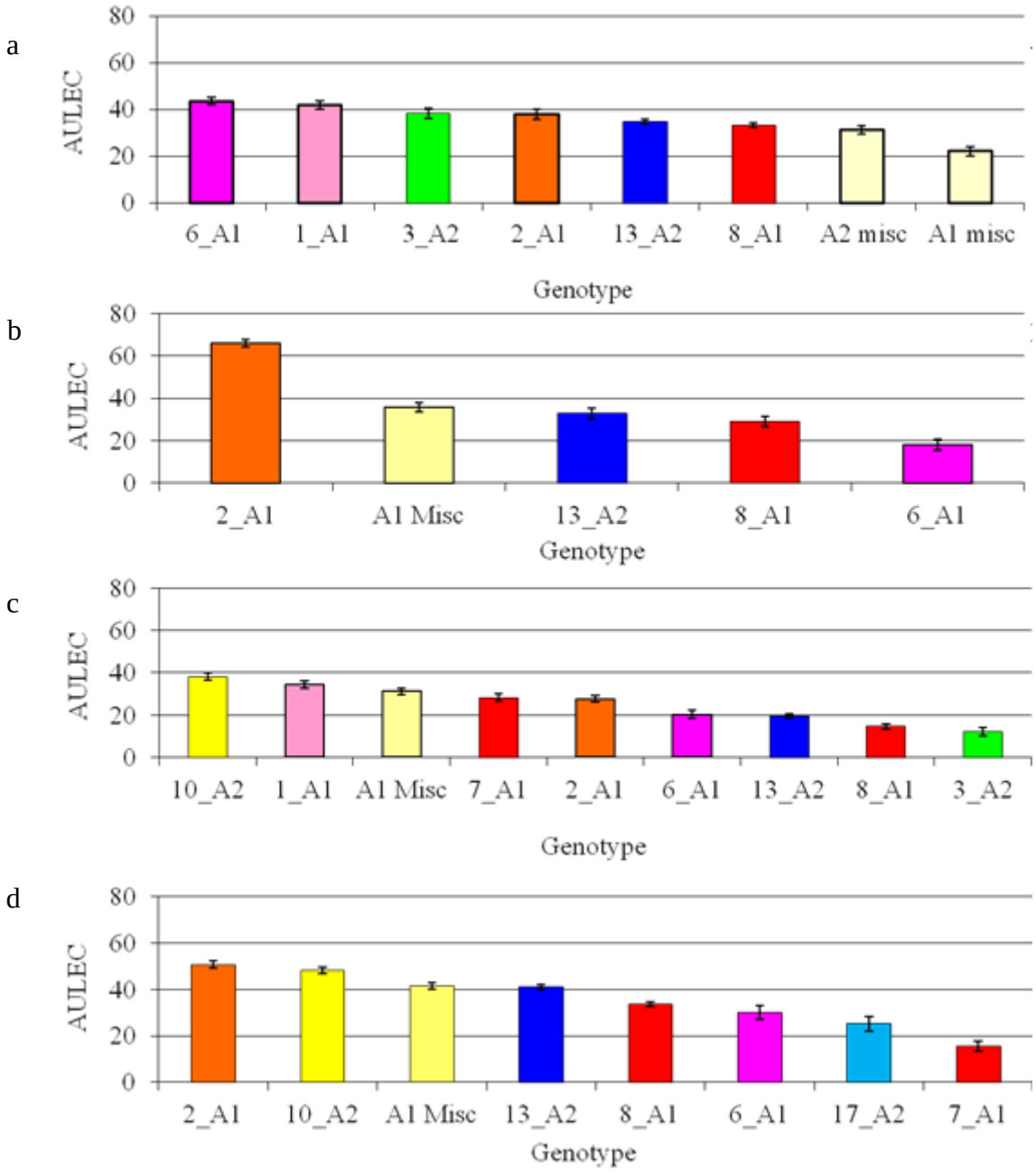

Figure 9 – Mean AULEC values of a range of UK *Phytophthora infestans* genotypes on detached potato leaflets (averaged over five cultivars). The error bars represent the standard errors of differences of the means (SE). Different numbers of isolates were represented in each of the four experiments as detailed; **a)** 6_A1 (n=4), 1_A1 (n=1), 3_A2 (n=1), 2_A1 (n=2), 13_A2 (n=5), 8_A1 (n=1), A2 Misc (n=1) and A1 Misc (n=2) **b)** 2_A1 (n=1), A1 Misc (n=3), 13_A2 (n=1), 8_A1 (n=1) and 6_A1 (n=1) **c)** 10_A2 (n=2), 1_A1 (n=1), A1 Misc (n=1), 7_A1 (n=3), 2_A1 (n=3), 6_A1 (n=3), 13_A2 (n=4), 8_A1 (n=2) 3_A2 (n=1) **d)** 2_A1 (n=1), 10_A2 (n=1), A1 Misc (n=1), 13_A2 (n=3), 8_A1 (n=1), 6_A1 (n=2), 17_A2 (n=2) and 7_A1 (n=2).

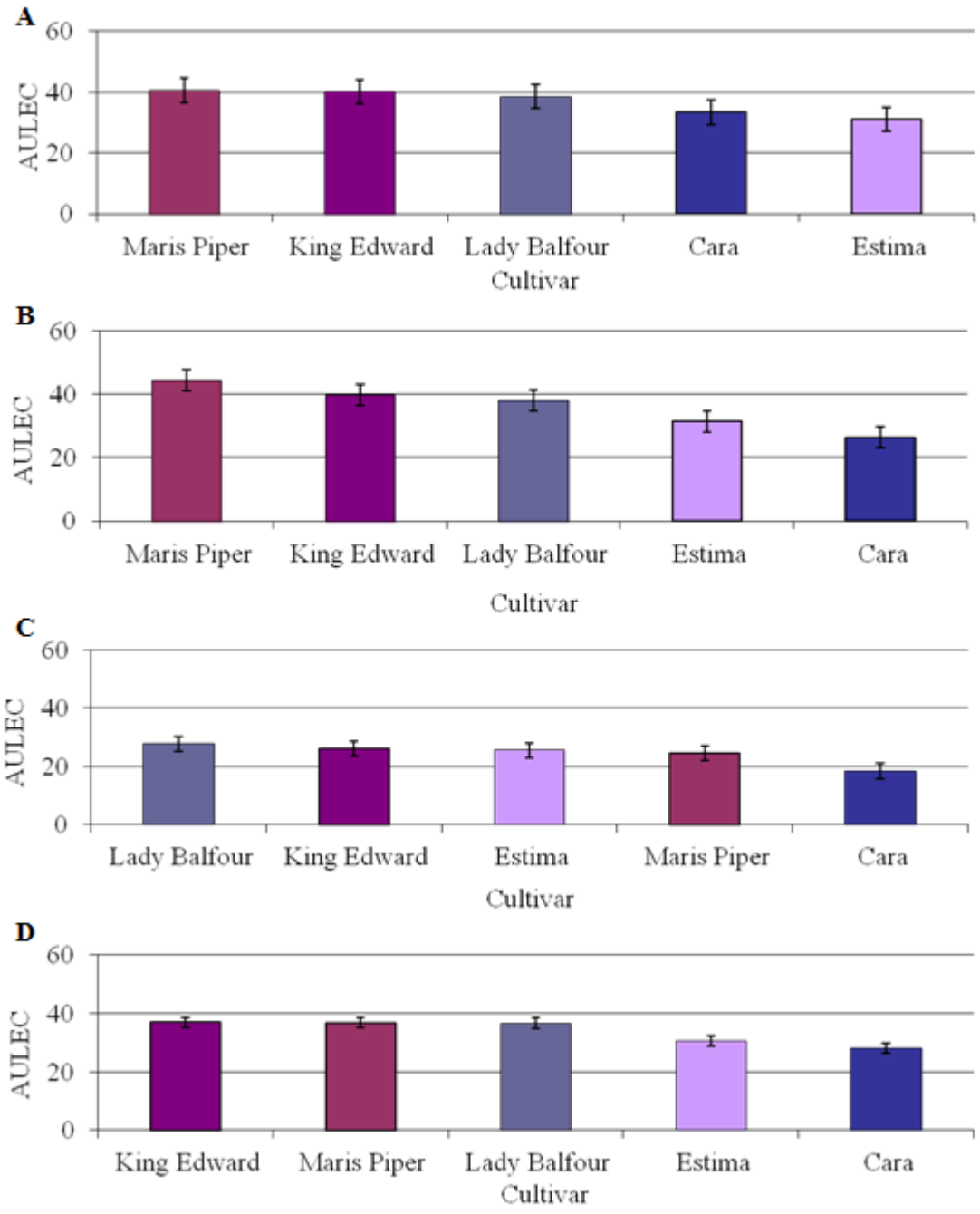

Figure 10 – Mean AULEC values 7 days post inoculation of a range of UK *P. infestans* genotypes on detached leaflets of five cultivars. Error bars represent the standard errors of the differences of the means (SE) A) Test1 (SE=3.954) B) Test 2 (SE=3.318) C) Test 3 (SE=2.56) D) Test 4 (SE=1.793).

Discussion

Significant differences in lesion size, incubation period and latent period between *P. infestans* isolates and genotypes were observed within these experiments. Lesion size and latent period were scored as these variates have been shown to correlate well with other parameters such as sporulation capacity (Carlisle *et al*., 2002). A previous study carried out on 26 isolates at 13°C and 18°C showed that genotype 13_A2 and 6_A1 isolates, on average, caused larger

lesions than other genotypes at 13°C but that this difference was less pronounced at 18°C (Cooke *et al*., 2012, Cooke *et al*., 2010 (R274 report). In this study of a larger sample of 49 isolates we did not, however, find consistent differences in aggressiveness between the genotypes. There are several possible explanations for this observation. Firstly it is possible that genotypes 13_A2 and 6_A1 are better adapted to cooler conditions and the intermediate temperature of 15°C used in this study was sub-optimal for these lineages. A second possibility is experimental variation ('noise') that is inevitable when testing so many isolates in four separate sub-experiments. The previous study on 26 isolates was run as a single study whereas in this study the first test was run in June and the last in October. The small error bars on the figures above indicate the experimental variation between replicates of each treatment was low but the statistically significant differences between the reference isolates in each of the four experiments suggests that variation in factors such as inoculum quality, plant growing conditions and leaf age have contributed to a lack of reproducibility. A third possible factor is variation amongst isolates representing each genotype; in this study isolates from the 2006, 2007 and 2008 seasons were used whereas in the previous study all isolates were from the 2006 season and had thus been in culture for only one year. It is possible that mutations that accumulated over time within these lineages resulted in phenotypic differences in aggressiveness traits. Such challenges in seeking consistent aggressiveness (Lehtinen *et al*., 2009) and virulence (Andrivon *et al*., 2011) tests been observed by other researchers. The variability in this detached leaf study makes it difficult to generalise about the aggressiveness of specific lineages. However, the evidence from the frequency of dominant lineages in GB crops and their competitive ability as demonstrated in the field trial (described in a later section of this report) suggests the differences are genuine but not easily detected in these studies. Despite these between-isolate differences, the differences in cultivar resistance were broadly in line with expectations from their published resistance ratings (Lees *et al*., 2012 and http://varieties.potato.org.uk/).

3.2.1.1 Assessing growth on agar at a range of fixed temperature profiles

Materials and Methods

Fifty-eight isolates of *P. infestans* belonging to the core collection of isolates (Appendix 2) were cultured on Rye A agar at 18°C. Using a 5 mm corer, a plug of each isolate was removed from the edge of the colony and transferred to the centre of a 9 cm Rye A agar plate under sterile conditions. Four replicate plates per isolate were made and each was sealed with Nescofilm. Plates were placed, in a fully randomised design, within an incubator and incubated with 16 hours light and 8 hours dark at the appropriate temperature. The experiment was run at six different temperatures (5, 10, 15, 20, 25 and 30˚C) on six separate dates. The diameter of each growing colony was measured on two axes at regular intervals using digital callipers. Data collection ceased once colonies of the most rapidly growing isolates were within 1cm of the edge of the agar plate. The length of the study varied for different temperatures so the colony size and scoring date figures were used to calculate colony growth rate data (mm per day) to allow meaningful comparisons of growth.

Results

Temperature had a major effect on colony growth rate, with rates of 1 mm per day or less at 5°C and no growth at all at 30°C. The mean optimal temperature for colony growth of all

genotypes was 20°C (Fig. 11). Genotype 3_A2 had the most rapid growth rate at most temperatures and 17_A2 grew most rapidly at 25°C. Amongst the other genotypes however, the differences were less pronounced. At each temperature there were statistically significant differences (P <0.01) between the growth rates of genotypes but the sizes of the differences in growth rate were not very large. There are indications of a group of genotypes that grew more rapidly than the others at 15 and 20°C but, as with the aggressiveness study, the variation amongst isolates of the same genotype (see Appendix 3) makes it difficult to draw any conclusions about genotype-specific patterns of *in vitro* growth. After incubation at 30°C the isolates were returned to room temperature for 7 additional days but no growth was observed which suggests the isolates were killed by exposure to this temperature.

Discussion

One simple explanation for the dominance of the 13_A2 and 6_A1 lineages may be that they have a metabolic advantage that enables them to use nutrients more efficiently and grow faster than other lineages. We thus examined growth on an artificial agar substrate as a logical starting point. A previous study also suggested an advantage for 13_A2 at lower temperatures (Cooke *et al*., 2012) and so this combined analysis of growth rate of different lineages at a range of temperatures was conducted. Previous studies on the response of the pathogen to temperature have been conducted on a single or a few isolates only (e.g. Crosier, 1934). Differences in temperature responses between lineages have been observed (Mizubuti and Fry, 1998). In agreement with this study, Crosier reported 20^{o}C was the optimal temperature for pathogen growth on leaves which supports our finding that 20^{o}C was optimal for growth on agar.

Genotypes of *P. infestans* were also shown to differ in their ability to survive freezing conditions (Kirk, 2003). Isolates of a range of genotypes grown on agar were exposed to a temperature of -3°C for 3-5 days and then incubated for 28 days at 12°C. The isolates that had been exposed to -3°C for up to three days could still grow when subsequently incubated at 12°C, but those exposed to -3°C for 4 and 5 days could not. Isolates of US-8 and US-14 could tolerate lower temperatures than isolates of US-1 which may be part of the explanation for US-8 isolates displacing those of US-1 in North America.

In the present study, differences in colony density were noted in some isolates at lower temperatures such that a difference in growth efficiency may have occurred in two isolates with identically sized colonies. The relationship between radial growth, as measured by image analysis, and weight of mycelium was shown to vary between isolates in another study (Kirk, 2003). An improvement in the assay may be to conduct the experiment in a liquid medium and measure the dry weight of mycelium after a set period.

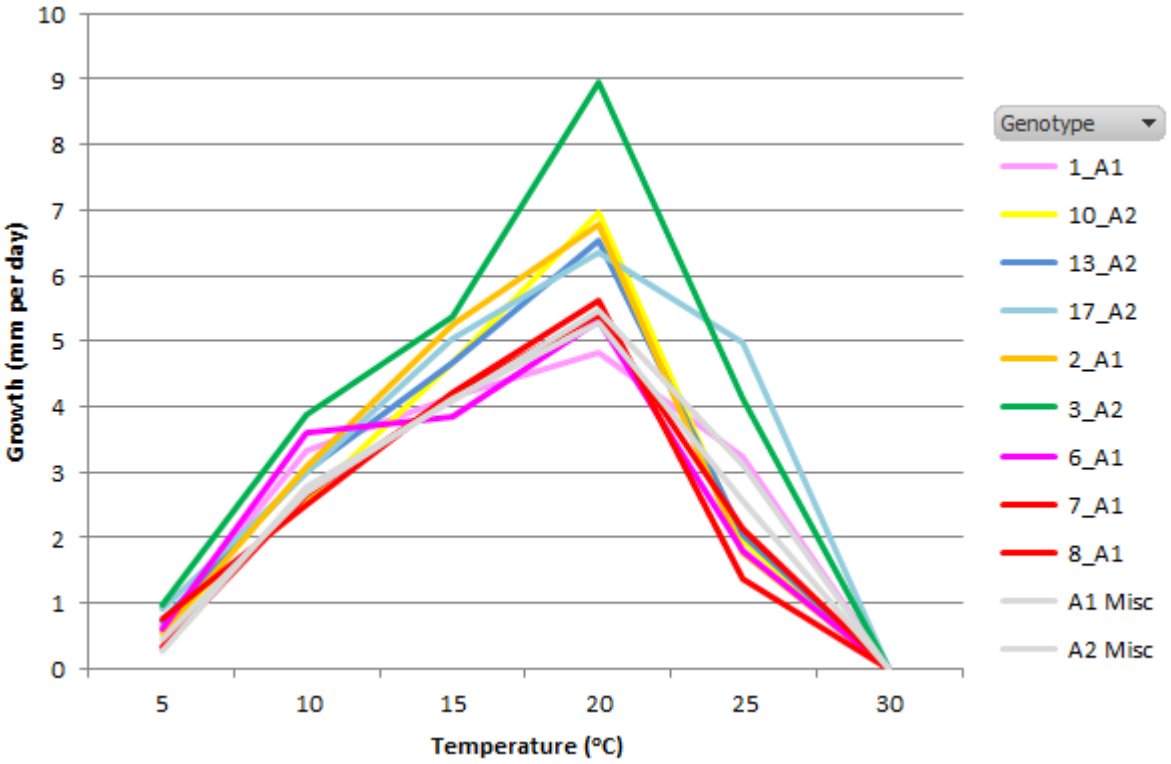

Figure 11. Mean *in vitro* colony growth rate (mm per day) of isolates of a range of *P. infestans* genotypes in response to a range of temperatures.

3.2.1.2 Assessing growth on leaves at a range of fixed temperature profiles

Materials and Methods

In this experiment the influence of temperature on the infection and aggressiveness 47 isolates of *P. infestans* (Appendix 2) was tested on a temperature gradient plate incubator (GRD1, Grant Instruments, Cambridge).

An eight by eight grid of 9 cm Petri dishes lined with damp filter paper were placed on the temperature gradient plate that was set to generate a 6°C to 20°C gradient across the plate with the dishes in a single column incubated at the same temperature (Figure 12). Temperature loggers (DS1921G Thermochron iButton, Maxim) were placed along a central horizontal line in between the Petri dishes. A light and dark cycle of 16 and 8 hours, respectively, was generated by the illuminated lid of the incubator. Two detached Maris Piper leaflets were placed, lower surface uppermost, in each dish. It was not possible to test all isolates in a single run so a series of experiments were completed with seven test isolates and one reference isolate in each. Sporangial inoculum was prepared from isolates previously passaged in leaves of cultivar Craig's Royal and adjusted to a standard concentration (1.4 x 10^4 sporangia/ml) before chilling to promote zoospore release. A single 15 µl droplet was applied to each leaflet with two replicate leaflets inoculated per isolate within each column. Leaflets were monitored daily for signs of infection (infection period), sporulation (latent period) and lesion size was measured before the lesions reached the edge of the leaflet (7 days post inoculation).

Figure 12. Experimental layout on the temperature gradient incubator in which each Column represents one temperature (6°C to 20°C in ca. 2°C increments). Two replicate treatments of each of eight isolates (seven plus a reference isolate) were applied to randomly selected leaflets within each column.

Results

Over 90% of the *P. infestans* isolates tested were able to infect the Maris Piper detached leaflets at temperatures of 10°C and above (Figure 13). At 8°C, 84% of the isolates caused infection and 49% of the isolates (24 out of the 49) showed signs of infection at 6°C. This ability to infect at low temperatures was not genotype-specific (Figure 14) as 10 of the 11 genotypes infected the leaflets at 6°C with only genotype (3_A2) unable to infect. In 14 of the 24 isolates that infected at 6°C, a lesion was formed on only one of the two replicate leaves suggesting that that this is close to the infection threshold. The lesions formed at 6°C were only a few mm across but expanded when the leaflets were subsequently incubated at 15°C. This indicated that once infection had occurred, the pathogen was able to survive in a latent low-temperature phase within the leaf tissue until conditions were more favourable.

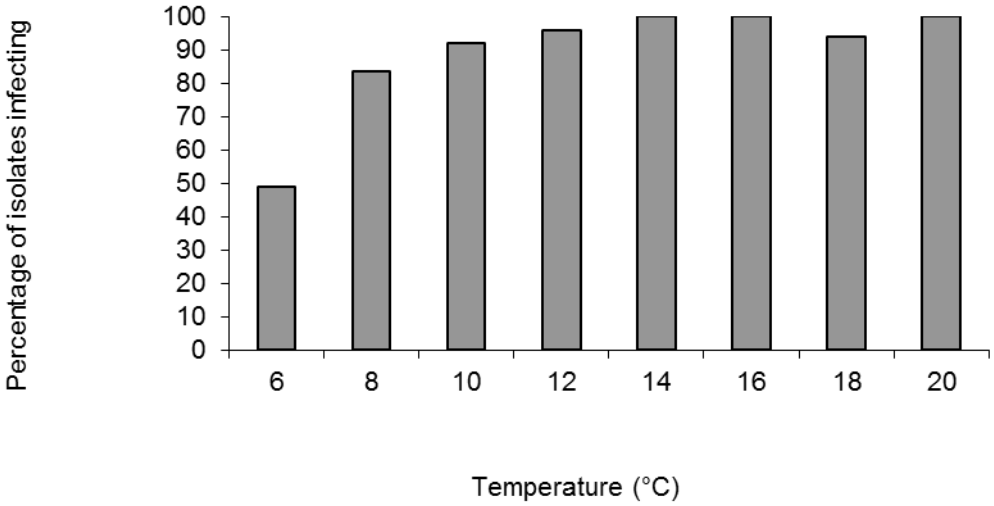

Figure 13. Percentage of isolates showing signs of infection after incubation on leaves of Maris Piper for 7 days at a range of temperatures.

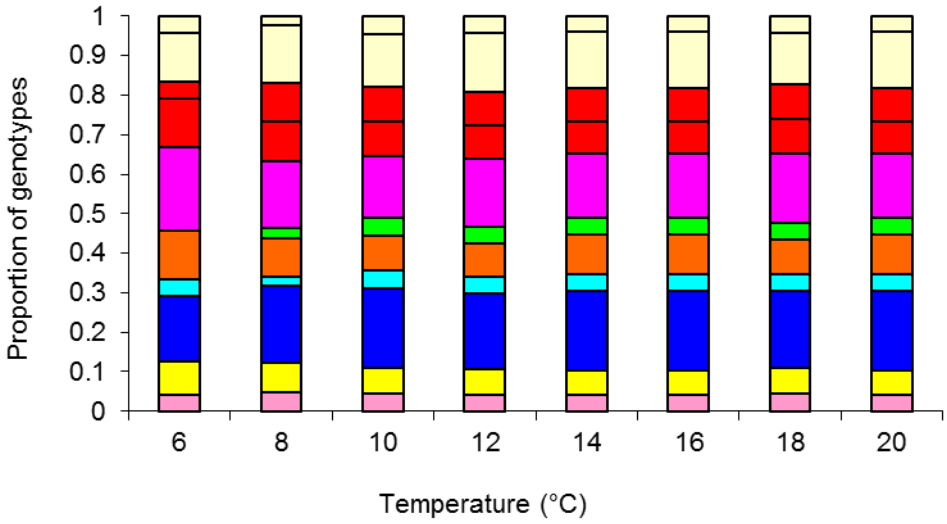

Figure 14. The proportion of isolates of different genotypes infecting potato leaflets at each temperature. Only genotype 3_A2 (green) failed to infect at 6°C.

Temperature had a marked effect on IP (time until disease symptoms were visible) with a range from almost eight days at 6°C to under 3 days at 20°C (Fig. 15). A one to two day range in mean IP values was observed at each temperature but there were, however, few consistent trends in the IP of specific pathogen genotypes. Genotype 3_A2 had a longer IP value than most other genotypes and 13_A2 was amongst the five genotypes with the shortest IP. As in previous studies, a broad range of responses was apparent amongst isolates of the same genotype which masked clear genotype-specific responses (data not shown).

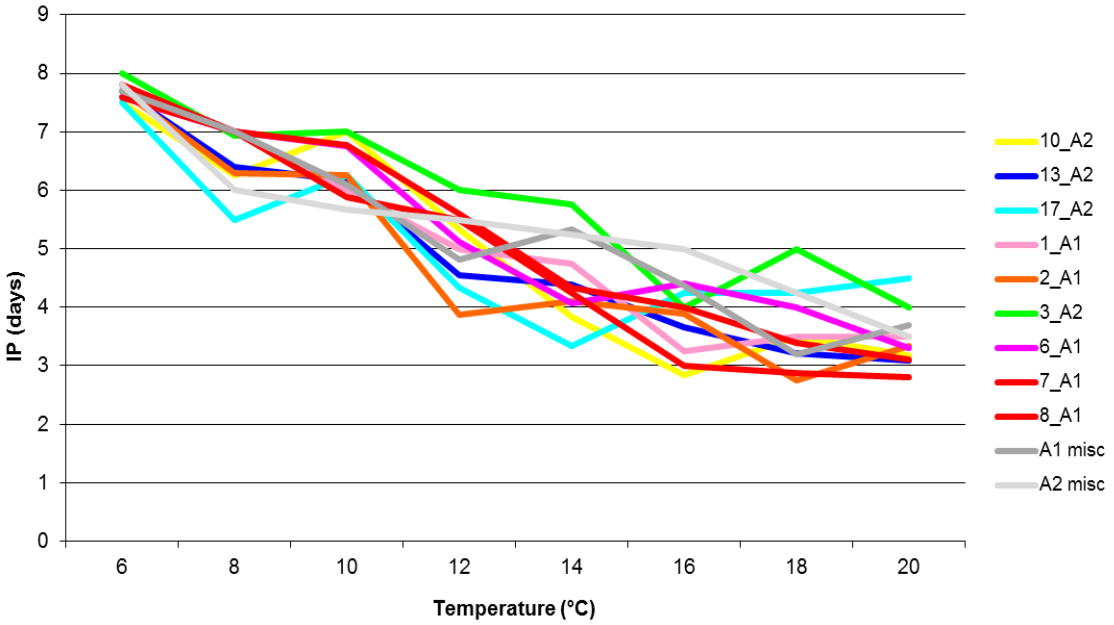

Figure 15. The effect of temperature on the mean infection period (IP) of a range of *P. infestans* genotypes on Maris Piper leaflets.

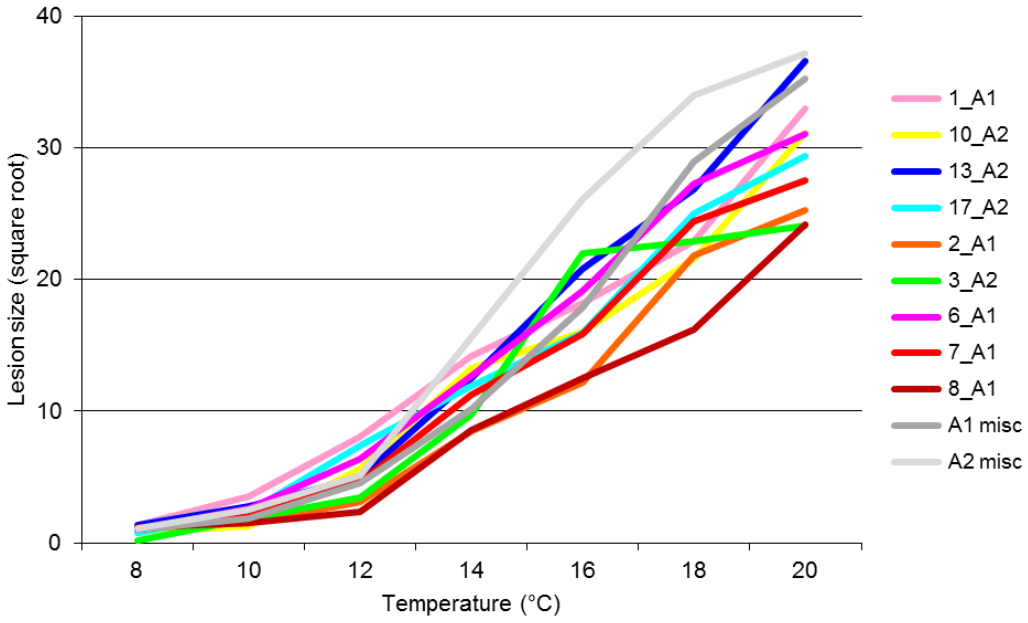

Figure 16. The effect of temperature on mean lesion size caused by a range of *P. infestans* genotypes on Maris Piper leaflets. Data shows the square root of lesion size (mm^2).

Mean lesion size increased over the range of temperatures tested with the largest lesions formed at 20°C. Although significant differences in the lesion sizes caused by individual isolates were observed (data not shown), when the mean size per genotype at different temperatures was examined (Fig. 16) the differences were not pronounced. Some trends

were apparent with genotype 8_A1 forming smaller lesions and genotypes 13_A2 and 6_A1 generating amongst the largest lesions of the 11 groups of isolates.

Discussion

This study confirmed the positive influence of temperature on pathogen growth within leaf tissue with a progressive decrease in time until first symptoms (IP) and an increase in lesion size up until the warmest conditions tested at 20^{o}C. This is in general agreement with other studies that studied single or a few isolates only (Crosier, 1934; Hartill *et al*., 1990). A key finding was that *P. infestans* isolates of all clonal lineages caused infection at temperatures below 10^{o}C which is below the 'Smith Period' threshold for infection and spread of potato blight (Smith, 1956). Disease development at such low temperatures was very slow with the lesions that formed at 6^{o}C being barely visible after seven days. This latent phase is however important as infection provides the pathogen with water and nutrients which prevents death of the germinated spore through desiccation. Infection at low temperatures is particularly important early in the season when cooler wet conditions prevail, in particular during the night. This knowledge suggests that predictions based on the Smith Period alone may underestimate the risk of blight activity. Similarly to the previous tests, significant variation between isolates was noted in this study but the differences in in IP and lesion size did not appear to be genotype specific. Again variation between individual isolates of the same clonal genotype was observed and such differences have been observed in other studies reported here. No clear relationship between pathogen growth on agar and growth on leaves was observed.

3.2.1.3 Assessing growth on leaves at diurnal temperature profiles

Materials and Methods

The incubation temperatures in the above experiments were fixed but in nature the pathogen and host are exposed to a diurnal pattern of changing temperatures. In this study the effect of such diurnal patterns of temperature change were examined. In this case, 49 square Petri dishes (10.5 cm diameter) were lined with damp filter paper and placed in a seven by seven grid (Fig. 17) on the temperature gradient plate incubator (GRD1, Grant Instruments, Cambridge). The gradient plate was again set from 6°C to 20°C but with a 90° shift in the orientation of the gradient for 16 and 8 hour 'day' and 'night' phases over each 24 hour period. The illuminated lid provided light during the 16 hour 'day'. Each dish was thus exposed to a different day/night temperature regime. Four detached leaflets of potato cultivar Maris Piper were placed in each corner of the Petri dishes and a temperature logger (DS1921G Thermochron iButton, Maxim) placed in the centre of each dish (Fig. 18). Due to limited space on the 1 m^{2} grid, four pathogen genotypes were tested with one isolate used to represent each genotype; 13_A2 (2006_3928A), 6_A1 (2008_6090A), 2_A1 (2007_5442F) and 17_A2 (2006_4338D). All isolates were passaged through potato cultivar Craig's Royal (susceptible, lacking R-genes) twice before being used for inoculum. For replication, the experiment was run four times with the isolate placement within each dish rotated by 90^{o}. This was to ensure that data for each isolate were recorded at all four corners to compensate for the small gradients in temperature across each dish. Accumulated day degrees were calculated by multiplying the average 'day' temperature by the number of 'day' hours (16) and adding that to the average night temperature multiplied by the number of night hours (8). Measurements were made of the final lesion size, incubation period and latent period.

Results

All isolates had a similar mean latent period (six days) but the 6_A1 and 2_A1 isolates resulted in the largest lesions with the 17_A2 isolate forming consistently smaller lesions at most temperatures. On average the 13_A2 isolate caused lesions intermediate between the others but some interactions were noted. For example, 13_A2 and 6_A1 isolates caused

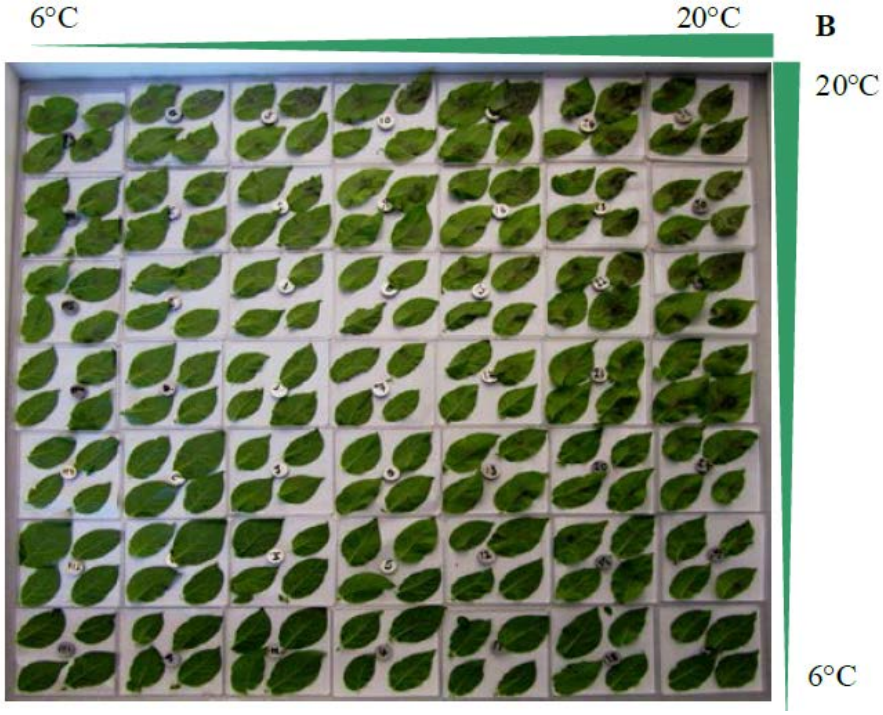

Figure 17. Temperature gradient plate experimental setup; each dish contains four leaves that were inoculated with one of four *P. infestans* isolates. The temperature gradient 'switches' at 90^{o} twice a day, exposing each dish to a different diurnal temperature regime.

Figure 18. Example of lesion development after seven days exposure to a 15 (day) and 13 (night) temperature regime.

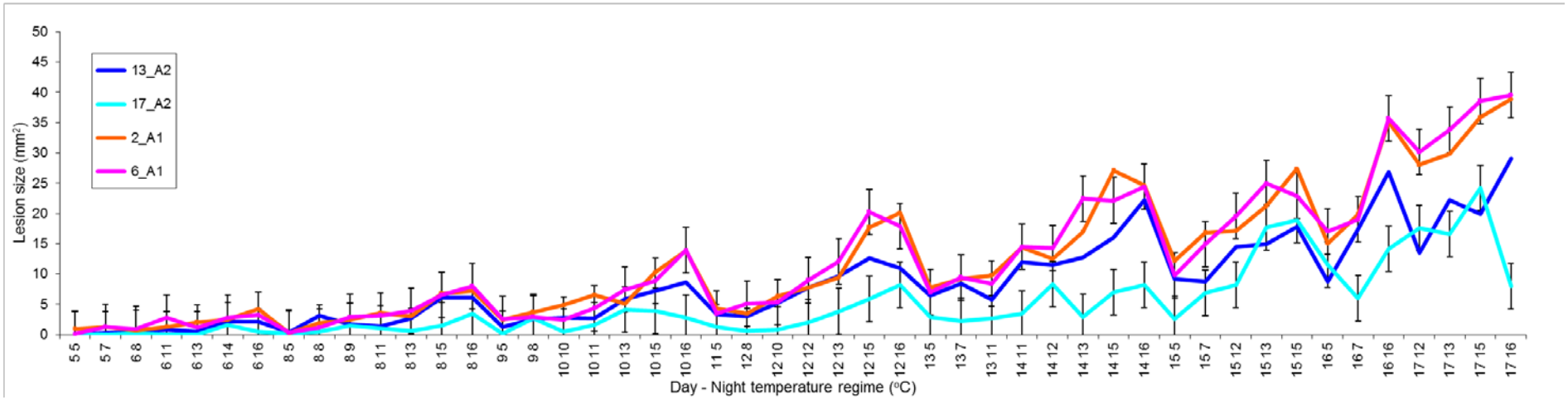

Figure19. Effect of diurnal temperature regime on the mean size of lesions caused by isolates of four different genotypes of *P. infestans*. For clarity error bars shown on two isolates only (S.E. 3.76).

similar sized lesions at cooler day/night combinations such as 13-5 and 13-7 and 15-5 but as the conditions warmed, such as at 12-16, 15-13 and 17-12 day/night combinations, the 6_A1 isolate consistently formed larger lesions than the 13_A2 isolate (Fig. 19). The 2_A1 isolate had an almost identical temperature response curve to that of the 6_A1 isolate. On average, the 17_A2 isolate formed smaller lesions than all the other isolates; but under a few temperature regimes it matched 13_A2. It is interesting to examine whether the infection and growth of the different isolates respond in a direct and linear way to the number of accumulated day degrees (ADD). In this study, for example, there were several ways to reach 296 ADD; day and night at approximately the same temperature (12 & 13, 13 & 11) or greater extremes with a warmer day and a cooler night (15 & 7, 16 & 5). Under both sets of conditions 13_A2 sporulated up to 1 day sooner than 6_A1 (Fig. 20). However, 6_A1 formed larger lesions and, interestingly, its response to the warmer conditions (days at 15 and 16^{o}C) was greater than that of 13_A2 (Fig. 21). This interaction highlights the challenge of identifying the genotype-specific factors that result in a competitive advantage under field conditions.

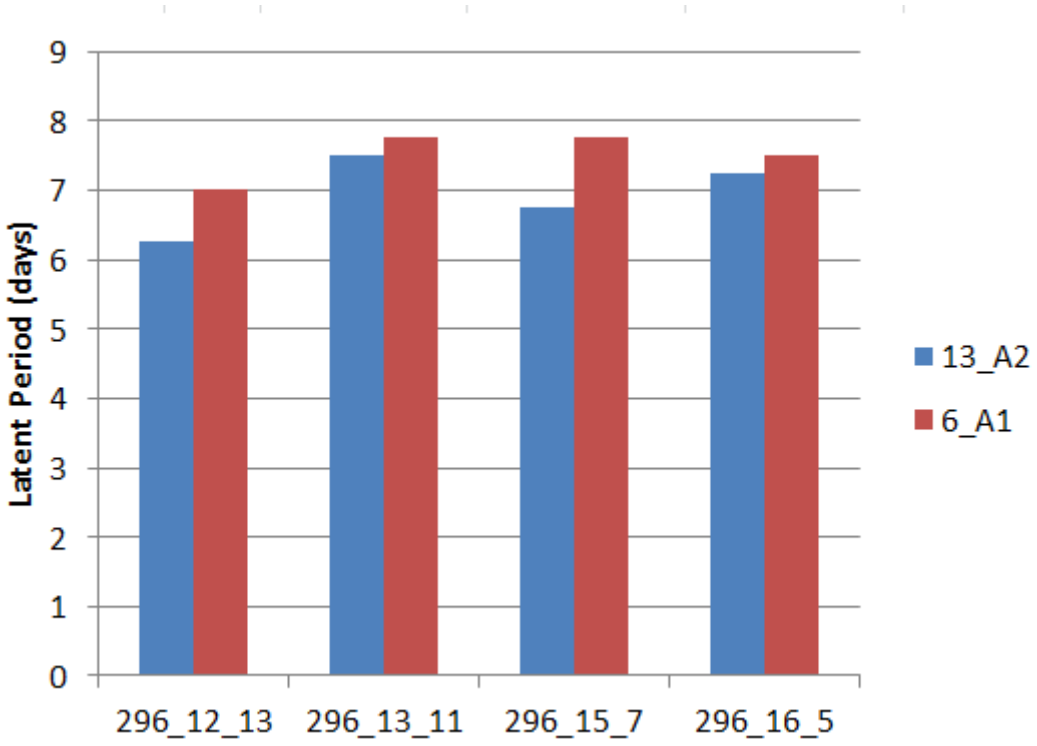

Figure 20. The effect of different diurnal regimes, generating the same ADD, on the latent period of *P. infestans* isolates of genotype 13_A2 and 6_A1.

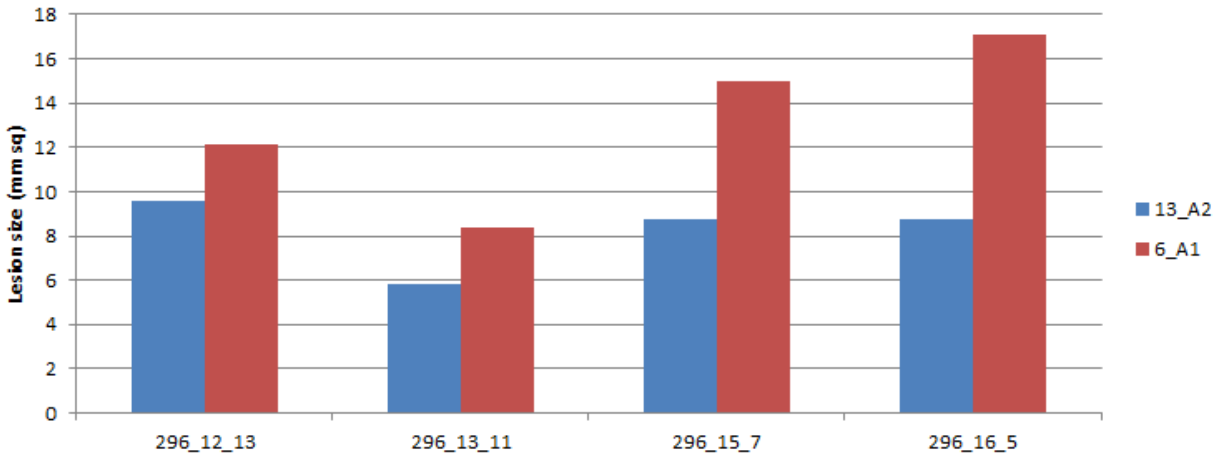

Figure 21. The effect of different diurnal regimes, generating the same ADD, on the lesion size caused by *P. infestans* isolates of genotype 13_A2 and 6_A1.

Discussion

The four replicate experiments and large number of data points reflecting a progressive change in environmental conditions made this a powerful means of studying the relationship between temperature and pathogen growth. As in the previous studies, a clear relationship between temperature and pathogen development was observed. Isolates of the 6_A1 and 2_A1 lineages responded in a very similar manner across the whole range of diurnal temperature regimes. The 13_A2 isolate formed lesions of a similar size to 6_A1 and 2_A1 isolates under cooler conditions but did not, however, respond as favourably to the regimes with warmer conditions (16 to 17^{o}C; Fig. 19). This isolate of 6_A1 thus appears better adapted than 13_A2 to warmer conditions which is also shown in their differential response to matching accumulated day degree profiles (Fig. 21). On average across the whole experiment the mean latent period of all isolates was very similar (data not shown) but under some regimes 13_A2 had a shorter latent period than 6_A1 (Fig. 20) which may outweigh the benefits of larger lesions. Mitzubuti and Fry (1998) commented that the small variations seen in the optimum temperature profiles can have large effects on field epidemics. They, for example observed rather subtle differences in rate of spore germination at different temperatures which may enable a more rapid infection under specific conditions.

3.2.2 Assessing infection at different temperature and humidity regimes

The relative humidity (RH) is critical in the infection process and this is reflected in the Smith criteria which require and RH of over 90% for at least 11 hours on two consecutive days. The aim of this study was to determine how many hours at high humidity are required for isolates of four contemporary *P. infestans* genotypes to infect Maris Piper foliage.

Materials and methods

The growth of *P. infestans* was tested in a whole plant assay to investigate how many hours of high humidity were needed for *P. infestans* to infect and grow. Four isolates were used representing four genotypes; 2006_3928A (genotype 13_A2), 2008_6090A (genotype

6_A1), 2007_5442F (genotype 2_A1) and 2006_4388D (genotype 17_A2). The fifth treatment was a water control. This experiment was conducted at three temperatures; 8°C, 10°C and 15°C. As in other studies, the isolates were passaged through the potato cultivar Craig's Royal (susceptible) twice before being used. Tubers of cv Maris Piper were planted 4 weeks prior to the experiment in a temperature controlled glasshouse which was set at 18°C and had 16 hours of natural light regime supplemented with artificial light.

Fifteen clear plastic boxes (65 x 41 x 59 cm) were lined with damp tissue paper and a beaker of water was placed in the corner of each box to help maintain high humidity throughout the experiment. Humidity loggers (DS1923 Hygrochron iButton, Maxim Direct UK) were placed on top of inverted 100 ml beakers in each box. Ten four-week old, single-stem whole plants were placed in each box and a sealable lid was attached to maintain humidity. Each box was assigned a treatment and the ten single stem whole plants within the box were spray inoculated with the appropriate sporangial suspension (or water). The boxes were covered in black plastic sheeting and incubated in the dark in a walk-in growth chamber at a set temperature. Inoculated plants were exposed to different periods of high humidity; 0, 1, 2, 4, 6, 8, 10, 14, 18, and 24 hours post inoculation. After this incubation phase batches of plants were taken out of the box and placed in a temperature controlled glasshouse set to 15°C for symptom development. Disease development on the single stem plants was visually scored for 10 days and incubation period (days) and percentage foliar blight were scored. The experiment was completed three times with the high humidity incubation temperature set at 8°C, 10°C and finally 15°C.

Results

The 6_A1 isolate used in this study had declined in pathogenicity and failed to cause infection on any of the plants. Data for this isolate were not considered further. The humidity recorded in all boxes across all three studies ranged from 93-100% RH. The water-only control plants showed no sign of infection. The duration of high humidity had a significant effect on both IP and percentage infection. The incubation period shortened with increasing exposure to high RH (Fig. 22). Surprisingly there were some signs of disease at even 1 and 2 hours of exposure to high RH and beyond ten hours there was little decrease in IP. In this study the 2_A1 isolate was the most aggressive causing symptoms sooner than either 13_A2 or 17_A2 after two and four hours of incubation. The effects of temperature were unexpected as they indicated that infection occurred more slowly at 15^{o}C than either 8^{o}C or 10^{o}C (Fig. 23). The amount of blight recorded reflected the IP data with disease level increasing with high RH incubation period. Even exposures of two to four hours were sufficient to cause disease but optimal conditions for pathogen development allowed disease severity to increase through to 18 hours. Differences between the isolates were observed with 2_A1 causing most disease and 17_A2 the least (Fig. 24). Again, the interactions with the temperature were unexpected with less disease development at 15^{o}C than either 8^{o}C or 10^{o}C (Fig. 25).

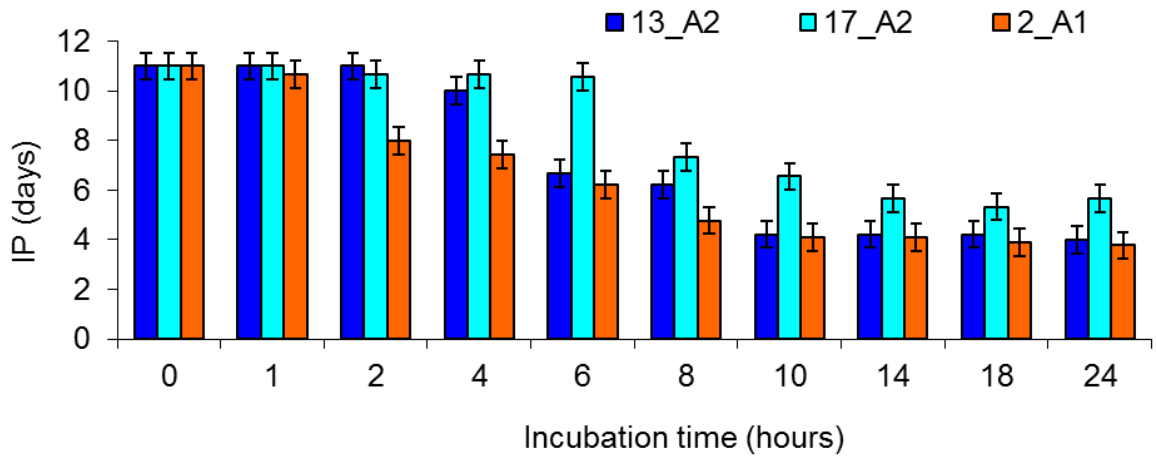

Figure 22. The effect of number of hours at high humidity on the incubation period (IP) of isolates of *P. infestans* genotypes 13_A2, 17_A2 and 2_A1. Eleven days was the length of the experiment and a score of 11 thus indicates that no disease was observed.

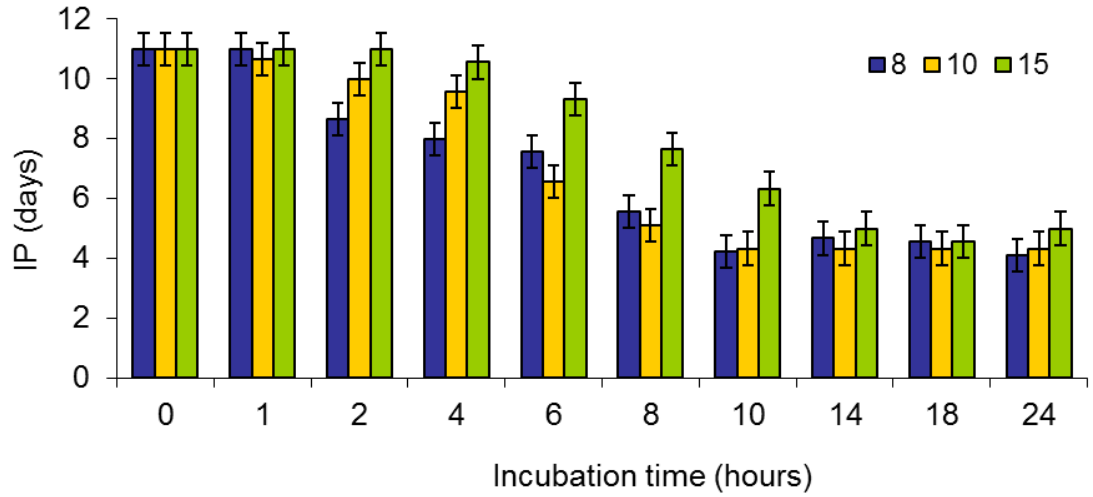

Figure 23. The effect of temperature and number of hours of high humidity on the incubation period (IP) of *P. infestans*. Eleven days was the length of the experiment and a score of 11 thus indicates that no disease was observed.

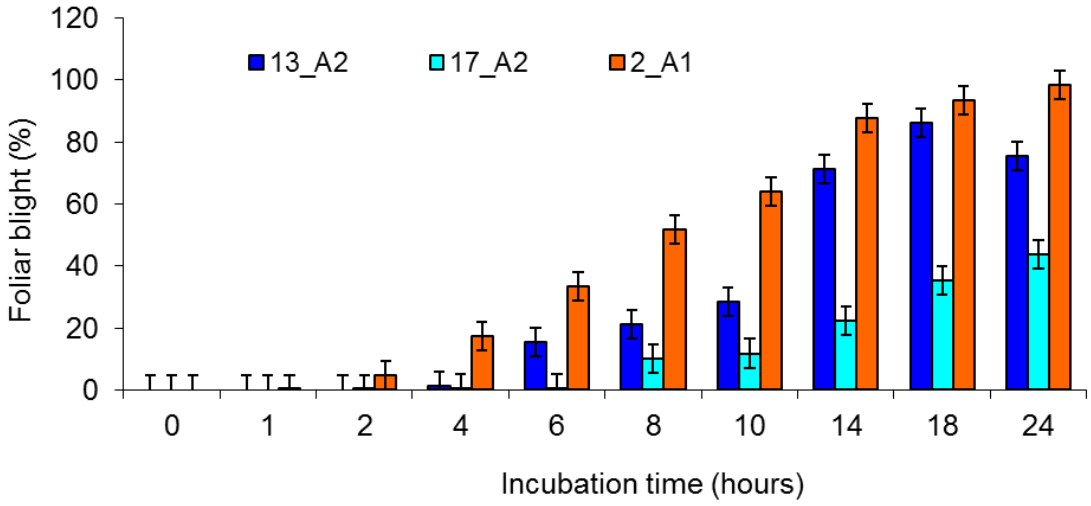

Figure 24. The effect of number of hours at high humidity on the percentage foliar blight caused by isolates of *P. infestans* genotypes 13_A2, 17_A2 and 2_A1.

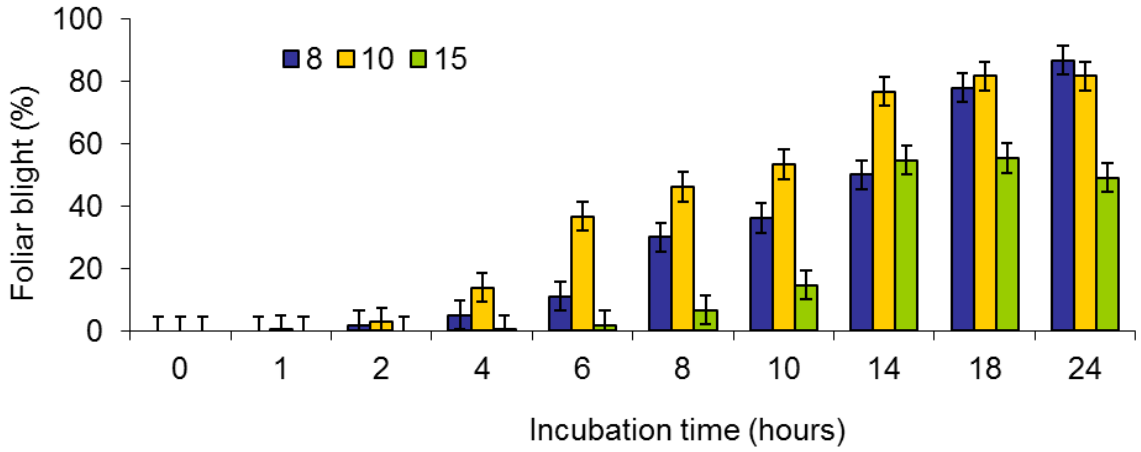

Figure 25. The effect of temperature and number of hours of high humidity on the percentage foliar blight caused by *P. infestans*.

Discussion

In this study the interaction of high humidity duration and temperature on the infection process of three contemporary genotypes of *P. infestans* were examined. Other studies have shown that free moisture or high humidity is critical for infection but, in general, use only single isolates (e.g. Rotem *et al*., 1970). In this study, clear differences were observed

between the three isolates with a minimum of eight hours high RH required for infection of the 17_A2 isolate compared with two to four hours for the other isolates. It is assumed that this reflects increased aggressiveness of the 13_A2 and 2_A1 isolates, able to complete key phases of their life cycle in shorter periods than 17_A2, rather than any intrinsic differences in ability to continue infection under conditions of lower humidity. This study shows the importance of selecting aggressive isolates for such studies. The absence of infection after one hour of high humidity indicates that conditions for symptom expression in the glasshouse were unsuitable for infection.

What are the implications of infection after only two to four hours of high humidity rather than the 11 hours on two consecutive days the Smith criteria suggest for infection and disease spread? In this study the inoculum levels were relatively high and the plants incubated in a closed chamber, but to counter this, the incubation phase was also under cool conditions of 10^{o}C or lower. Such low temperatures will promote zoospore release but are sub-optimal for pathogen growth and infection (Harrison, 1992). It was unexpected that infection levels were reduced at 15^{o}C; perhaps this relates to a decrease in propagules due to more direct rather than indirect sporangial germination under warmer conditions? Indirect germination results in the release of multiple zoospores per sporangium. It is clear that *P. infestans* is able to infect plants during periods of high humidity that are shorter than that defined by the current Smith Period and that a revised set of criteria may provide a more accurate prediction of crop infection. In a field study in Switzerland, Cao *et al*. (1997) indicated that taking six hours of high humidity as a cut-off was a good means of predicting blight activity. In a subsequent field trial the value of three different prediction models were compared and one based on a set of critical weather conditions that include free moisture for 6 hours was considered optimal.

3.2.3 General comment on humidity and temperature studies

This complex series of large-scale experiments were designed to examine the relationship between pathogen genotype and their temperature and humidity requirements for infection and disease development. The experiments were conducted under laboratory conditions and mostly on detached leaves in an attempt to reduce experimental variation. Nonetheless, a clear factor affecting the discriminatory power of these assays was a marked isolate-to-isolate variation in pathogenicity amongst isolates of the same clonal lineage. This tended to mask genotype-specific adaptations observed in previous studies (Cooke *et al.,* 2012). Such variation in virulence (Goodwin *et al*., 1995) and aggressiveness (Carlisle *et al*., 2002) within clonal lineages has been reported previously. It is likely that two factors are important – firstly genuine differences in aggressiveness in a pathogen with a diverse range of mechanisms for generating genetic variation (Cooke and Lees, 2004) and secondly the wide range of possible sources of experimental 'noise' within studies of this nature (e.g. Lehtinen *et al*., 2009). We consider the experiments to have successfully defined the range of environmental criteria over which the isolates performed and these data are proving valuable for incorporating into the disease prediction models.

3.2.4 Assessing the competitive ability of isolates in a field trial

The aim of this study was to investigate the competitive ability of four UK *P. infestans* genotypes on two potato cultivars (Maris Piper and Cara) in the field.

Materials and methods

A field trial comprising 24 16-plant plots of two cultivars (Maris Piper and Cara, resistance ratings for foliage blight of 4 and 5, respectively), arranged as a randomised complete block design, was planted on the 4th May 2011 at the Agri-Food and Biosciences Institute (AFBI), Belfast, Northern Ireland. The treatments comprised two cultivars and three types of inoculum in four replicate blocks. Each plot comprised four rows of four plants with each plot surrounded by a single-plant guard row of the resistant cultivar Sárpo Mira. An unplanted row was left between each plot. Four isolates were chosen to represent each of four genotypes; 13_A2, 6_A1, 7_A1 and 8_A1 (Appendix 2). Isolates were passaged through potato cultivar Up-to-Date before being used to make inoculum (as described previously). Three inoculation treatments were applied; all three included isolates of genotype 13_A2 inoculated onto plant 1 of every plot. The other treatments used isolates of either 6_A1, 7_A1 or 8_A1 to inoculate plant 16 according to the randomised treatment allocation. Genotype 13_A2 and another genotype were thus applied to plants in diagonally opposite corners of the plot. The individual leaflets of two leaves on either plant 1 or plant 16 were inoculated on 29th June and a plastic bag used to maintain the humidity for 24 hours. Meteorological data were collected from a nearby site and irrigation was applied as required. Disease progress was monitored on all plants within each plot and up to four lesions were sampled from each plant as the epidemic progressed. These lesions were pressed onto FTA cards (Whatman, UK) and genotyped using a 12-plex SSR method (Li *et al*., 2013).

Results

For the first four days after inoculation the conditions were cool with an average temperature from 12.4°C to 13.8°C. The conditions over the whole epidemic were also generally cool and wet with a minimum temperature ranging from 6-14^{o}C and the maximum from 14-23^{o}C. Disease symptoms were observed on the inoculated plants 5 days post-inoculation. The percentage disease cover on every plant was scored on nine dates over the 62 day epidemic and, as predicted, showed a lower rate of disease progress and final disease levels on Cara compared to Maris Piper (Fig. 26). Disease levels on individual plants were converted to Area Under the Disease Progress Curve (AUDPC) values which indicated higher mean disease levels on the inoculated plants and progressive spread to the other 14 plants within each plot. The highest disease levels were recorded on plants inoculated with 6_A1 with very little disease induced by inoculation with 7_A1 and 8_A1 (Fig. 27). Disease levels on plant 1 (inoculated with 13_A2) were also high but there was evidence of greater disease spread from these plants than from plant 16s. The genotyping of 994 lesions sampled from across the trial confirmed that all four genotypes were able to cause disease. It also clearly showed that 13_A2 was responsible for

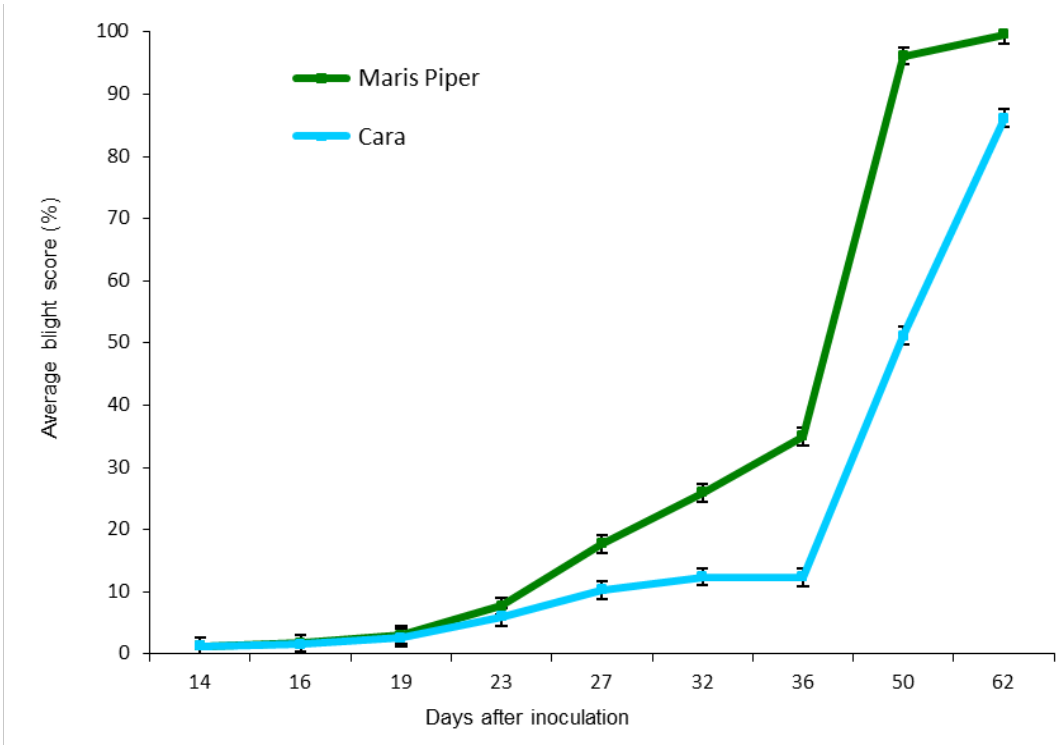

Figure 26. Disease progress curves for cultivars Maris Piper and Cara in the genotype competition trial.

a) 13_A2+6_A1

	1	2	3	4
1	477	224	199	224
2	313	225	226	240
3	238	196	236	262
4	198	179	238	645

b) 13_A2+7_A1

	1	2	3	4
1	377	193	156	166
2	406	203	180	181
3	276	220	171	179
4	210	168	157	322

c) 13_A2+8_A1

	1	2	3	4
1	484	247	158	182
2	287	251	162	170
3	223	201	184	188
4	204	185	161	288

Figure 27. Heat plots showing the mean AUDPC value for the three inoculum treatments (a, b, c) for each plant in the plot (meaned over cultivars).

most of the disease spread (Fig. 28). Of the 825 lesions successfully genotyped, the majority, 625 were 13_A2, 194 were 6_A1, nine were 7_A1 and eight 8_A1. The number of samples confirmed as 13_A2 was divided by three to account for that fact that it was applied to three times as many plots at the time of inoculation (Fig. 28). The spatial pattern of the genotypes recovered indicates the prevalence of isolates within the plots (Fig. 29). In particular the failure

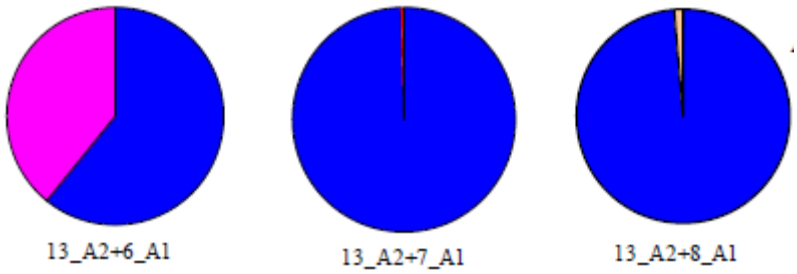

Figure 28 The proportion of each *P. infestans* genotype causing lesions sampled from an epidemic initiated with different combinations of initial inoculum. Very low levels of disease caused by 7_A1 (red) and 8_A1 (pale yellow) were sampled compared to that of 13_A2 (blue) and 6_A1 (pink).

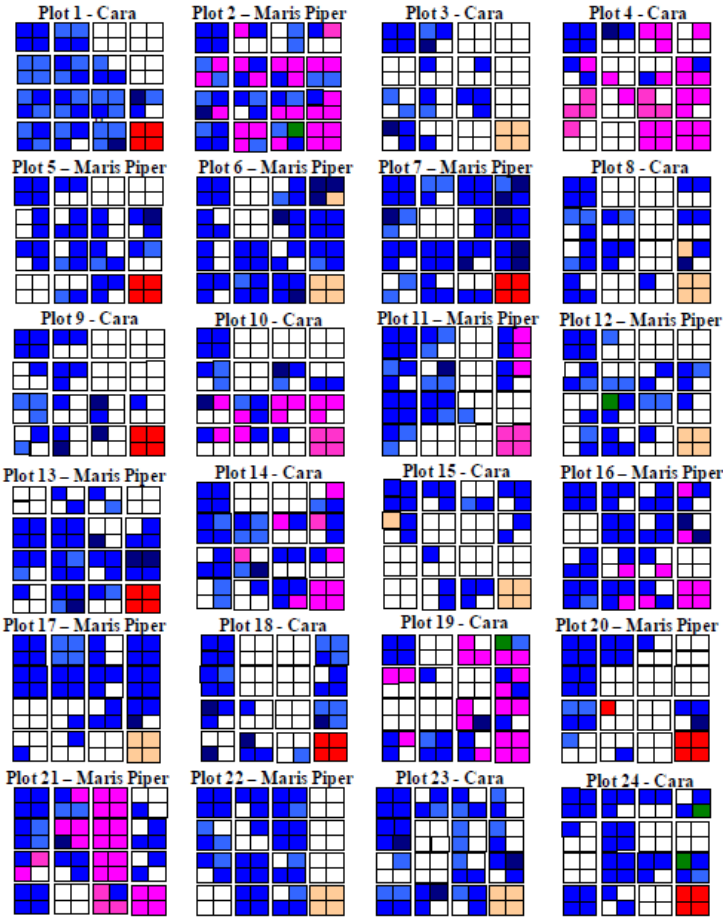

Figure 29. Plan of the field trial indicating the plants (16 per plot) and the sub-plant samples (four per plant) coloured according to the *P. infestans* genotype (see Fig. 28 for key). Genotypes that had spread from one plot to another are not shown (see text). Different shades of blue refer to different sub-genotypes of 13_A2.

of genotypes 7_A1 and 8_A1 to cause disease is apparent. Some plot-to-plot movement of 6_A1 was observed (data not shown) and may relate to the carry-over of inoculum on the assessors' clothing or wind-borne sporangia. The genotyping allowed discrimination within genotype 13_A2 isolates and it was interesting to note that not all variants were recovered in

equal proportions. One isolate of 13_A2_5 (07/39) used to inoculate the trial did not cause any of the sampled lesions, two isolates of 13_A2_1 were used and recovered from 428 lesions compared to 147 lesions for the single isolate of genotype 13_A2_2. There was thus a higher proportion of 13_A2_1 recovered than the 50% initially introduced suggesting that this sub-group may be slightly more aggressive and fit under field conditions than the isolate of genotype 13_A2_2. In agreement with these data, isolate 07/39 did not prove very aggressive in the laboratory study, whereas the other three isolates proved similar in their aggressiveness. Although cultivar Cara was less diseased there was no clear effect of cultivar on the frequency of the genotypes recovered.

Discussion

The experiments reported in previous sections of this report tested the aggressiveness and response to temperature and humidity of *P. infestans* genotypes in a single part of the infection cycle. In this field study, multiple cycles of infection allowed both the aggressiveness and fitness components to be examined. This study proved conclusively that 13_A2 was the fittest and most aggressive of the four *P. infestans* genotypes used to inoculate the field trial. Isolate 6_A1 was, however, also spread although to a lesser extent spread and was the only other genotype to able to compete against 13_A2. The aggressiveness and fitness of 13_A2 isolates was also demonstrated in a previous field study (Cooke *et al*., 2012). Genotype 6_A1 inoculum was also tested in that study but 6_A1 was recovered in a very low proportion of lesions compared to 13_A2. One explanation may be the way the plots were inoculated. Cooke *et al*. (2012) co-inoculated a central plant with a mix of all four isolates and allowed the disease to spread from this plant. In this study, 6_A1 and 13_A2 were inoculated onto separate plants which allowed both to become established. The 6_A1 inoculum used in the current study resulted in disease symptoms on the inoculated plant that were more severe than those caused by the 13_A2 isolates (Fig. 27a). However, disease spread from the initial plant appeared to be more efficient in the 13_A2 inoculated plants which may have accounted for their overall dominance of the trial. It was clear that contemporary isolates of 7_A1 and 8_A1 are able to infect and spread in the field but their rate of spread is insufficient to out-compete the other two genotypes. This evidence corroborates their observed decline in the GB population (Fig. 5). Similarly, the dominance of 6_A1 and 13_A2 as a cause of potato blight in GB potato crops is supported by this field data. It is, however, surprising that the above *in vitro* tests did not indicate any clear competitive advantage that these lineages have over the others. The fact that one of the 13_A2 isolates was not recovered at all in the field trial confirms that within-genotype differences in fitness can occur but this study further highlights the challenge of simulating field conditions in the laboratory. Pathogen infection and disease spread is a complex multifactorial interaction of pathogen, host and environmental factors that can be difficult to dissect. One useful approach to this is the use of pathogen models.

3.2.5 Assessing direct versus indirect germination of sporangia in relation to tuber infection

Materials and methods

Eight genotypes (two isolates per genotype) were examined across eight temperatures (4, 6, 8 to 18°C). Sporangia were produced on tuber tissue.

Suspension preparation

Sporangial suspensions of all 16 isolates of *P. infestans* were prepared using method A. In addition, suspensions for the two 13_A2 isolates were prepared using method B, which is a modified version of that used by Shaw *et al*. (2006). The isolates are listed in Appendix 4.

Method A: Cut tubers

The *P. infestans* isolates were grown on pea agar with vancomycin (Thompson, 2008) for 12 days. Prior to inoculation, tubers were washed, dried and surface sterilised using IMS. Halved tubers were placed in plastic tubs lined with damp tissue (approximately five tuber halves per tub for each isolate). Plugs of 15 mm diameter were cut from the leading edge of the pea agar cultures and placed in the centre of each King Edward tuber half. The tubs were sealed in a clear polythene bag, labelled and incubated for 5-7 days at 15^{o}C in the dark.

Young sporangia were collected from the growing edge of the blight lesion on the tuber surface by removing a core of tissue measuring 25 mm in diameter and 5 mm deep. Cores were taken from the left and right side of the agar plug. The cores were placed in a beaker and the sporangia collected in a mixture of sterile distilled water and potato tuber extract (9:1). The sterile distilled water and potato extract (McKee, 1964) had been pre-incubated at the test temperatures for 24 hours prior to use. The concentration of sporangia was adjusted to c. 5 x 10^4 sporangia per ml. (NB the 10 µl sample for assessment contained c. 500 sporangia).

Method B: Leaf material (Modification of the method used by Shaw et al. *(2006))*

The *P. infestans* isolates were grown on King Edward potato leaves for 7 days at 15°C with a 16-hour day length. Sporangia were then washed off the leaflets and the leaflets dried using clean absorbent paper. The suspensions containing the sporangia were then used to inoculate fresh leaf material for the following week's experiment. The dried leaves were then incubated at 15°C for a further 48 hours to produce a fresh batch of sporangia to be incubated at the test temperatures. The newly formed sporangia were collected in a mixture of sterile distilled water and potato tuber extract (9:1). The sterile distilled water and potato extract had been pre-incubated at the test temperatures for 24 hours prior to use. The concentration of sporangia was adjusted to c. 5 x 10^4 sporangia per ml. (NB the 10 µl sample for assessment contained c. 500 sporangia).

Incubation of suspensions

There were four replicates of each combination of genotype and temperature, i.e. two isolates of each genotype and two suspension samples per combination of temperature and isolate. The test temperatures were 4, 6, 8, 10, 12, 14, 16 and 18°C. Temperatures during the experiment were recorded using i-button thermometers. A pre-prepared glass slide had been divided into two sample grids for assessment. Following thorough mixing of each sporangial suspension a 10 µl droplet was placed on each grid. The slide was then placed in a Petri dish lined with damp tissue, sealed and labelled with isolate reference, test temperature and the time that incubation commenced. The 10 µl suspensions were incubated at the test temperatures for a fixed period of 24 hours. The Petri dishes were randomised within the incubators.

Assessment

Twenty-four hours after incubation commenced the sporangia in the suspension were fixed using a 10 µl droplet of 50% ethanol, i.e. an equal volume to the droplet. Each grid on the glass slide had five clearly marked horizontal lines. The sporangia on these lines were assessed, starting from the top of the slide and working to the bottom, left to right, to ensure the same sporangium was not counted twice. Each grid was assessed for a set time period of 4 minutes (8 minutes per slide), the time required to assess approximately 100 sporangia. Three tally counters were used to record the three possible states of germination which were:

- sporangium with a germ tube longer than half the diameter of the sporangium - directly germinated.
- empty sporangia - indirectly germinated.
- sporangia that are not in either of the above two states - non-germinated.

The controls for each isolate in this experiment were sporangial suspensions assessed prior to incubation, i.e. as soon as possible after preparation of the suspensions. Germination percentages underwent angular transformation prior to analysis of variance.

Results

Eight genotypes (two isolates per genotype) were examined across eight temperatures (4, 6, 8 to 18°C). This was two more genotypes and one more temperature, i.e. 4°C, than originally planned. Sporangia were produced on tuber tissue.

Indirect germination

Temperature, genotype and their interaction all had highly significant effects ($P < 0.001$) on indirect germination (zoospore release from sporangium). The overall relationship between the incidence of indirect germination and temperature was part of a sigmoid curve, i.e. essentially linear between 18 and 8°C with a plateau at 6 and 4°C (Fig. 30). Overall the optimum temperature for indirect germination was 4°C but the values for 4, 6 and 8°C were not significantly different (Fig. 30).

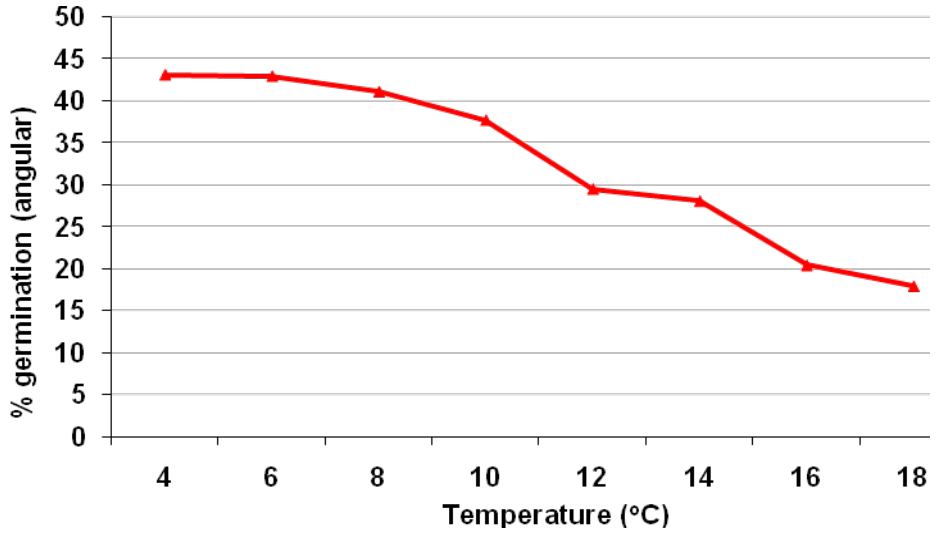

Figure 30. Mean indirect germination of all eight *P. infestans* genotypes in relation to temperature LSD (*P*=0.05) 2.534

In this study the new genotypes were 13_A2 and 6_A1. The old genotypes were 1_A1, 2_A1, 3_A2, 7_A1, 8_A1 and 10_A2. At most temperatures the difference in indirect germination between new and old genotypes was small (Fig. 31, Table 3). However, at 8°C the incidence was significantly greater (*P* <0.001) for the new genotypes. When 13_A2 and 6_A1 were examined separately the difference at 8°C was clearly attributable to the 13_A2 genotype (Fig. 32). The other significant difference between new and old genotypes at 18°C (Table 3) was only marginal.

For some genotypes there was a clear optimum temperature, e.g. for 13_A2 the optimum zoospore production occurred at 8°C (Fig. 33, Table 4). Other genotypes had a wide range as the optimum, e.g. 6_A1 produced most zoospores from 4 to 10°C. All genotypes showed optimum zoospore production within the range 4 to 10°C.

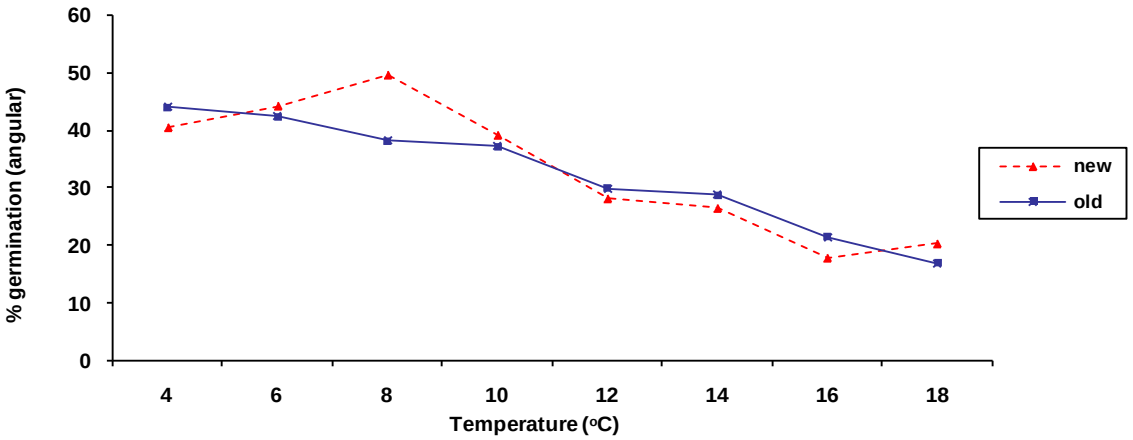

Figure 31. Indirect germination of old and new *P. infestans* genotypes in relation to temperature LSD (*P*=0.05) 7.166

Table 3. Difference in percentage (angular transformed) zoospore release between new and old genotypes

Temperature°C	new	old	F pr.	Difference
4	40.50	44.07	0.298	-3.57
6	44.25	42.43	0.594	1.82
8	49.65	38.33	<0.001	11.32
10	39.17	37.19	0.233	1.99
12	28.11	29.98	0.231	-1.87
14	26.44	28.74	0.178	-2.31
16	17.78	21.42	0.085	-3.64
18	20.26	16.92	0.041	3.33

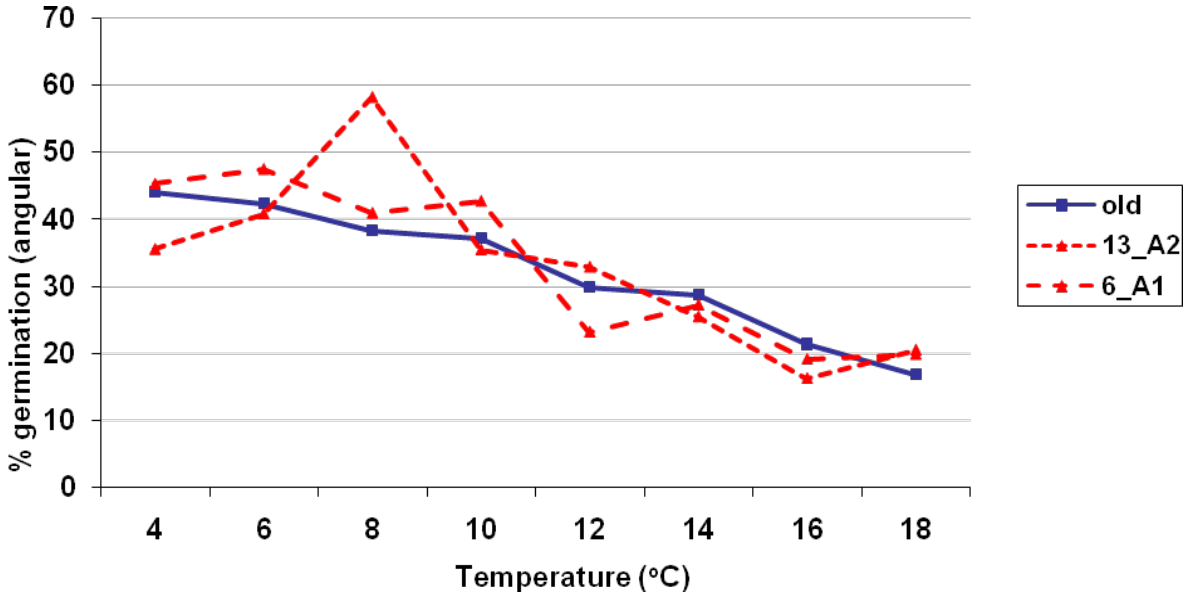

Figure 32. Indirect germination of old and new *P. infestans* genotypes in relation to temperature LSD (*P*=0.05) 7.166

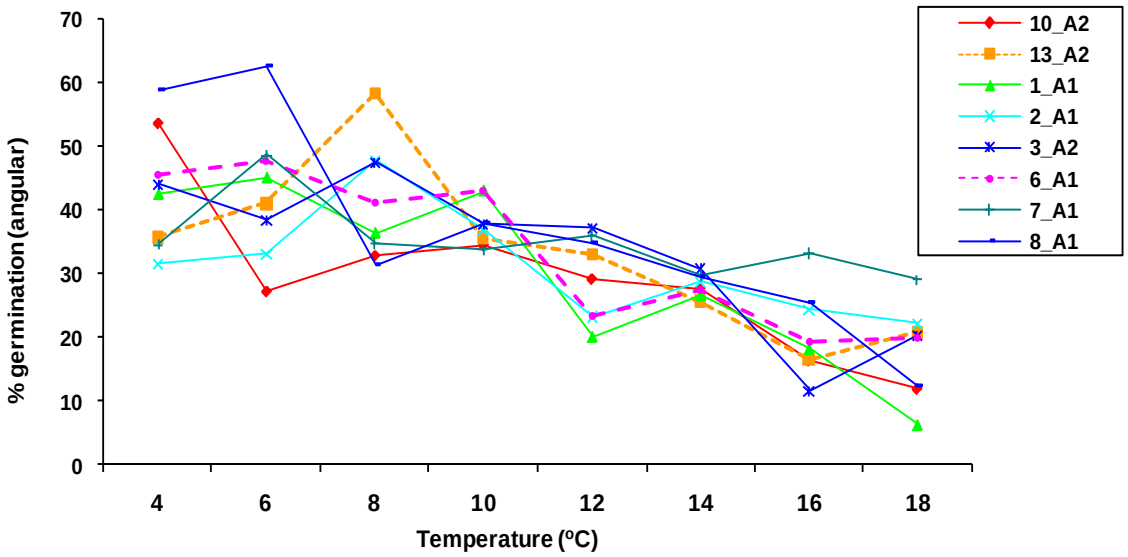

Figure 33. Indirect germination of individual *P. infestans* genotypes in relation to temperature LSD (P=0.05) 7.166. The average indirect germination for the controls was 8.8%.

Table 4. Optimum temperature range[1] for the incidence (%) of indirect germination (angular transformed data)

	Temperature (°C)							
	4	**6**	**8**	**10**	**12**	**14**	**16**	**18**
1_A1	**42.4**	**45.0**		**43.0**				
2_A1			**47.6**					
3_A2	**43.9**		**47.3**					
6_A1	**45.4**	**47.6**	**41.0**	**42.8**				
7_A1		**48.6**						
8_A1	**58.7**	**62.5**						
10_A2	**53.4**							
13_A2			**58.3**					

[1] Values in shaded boxes for the same genotype are not significantly different

In an additional small experiment, indirect germination in relation to temperature was examined using sporangia produced by the two 13_A2 isolates on leaf tissue. The results were compared with those obtained using tuber tissue. For sporangia from both tissue types the incidence of indirect germination by 13_A2 was similar and was significantly higher at 8°C than all other temperatures (Fig. 34). Indirect germination at 16 and 18°C was significantly lower for sporangia produced on leaf tissue but at 4 and 6°C was significantly higher for sporangia originating from this source.

Direct germination

The main purpose of the experiment, part of a tuber blight study, was to establish the optimum temperature for indirect germination by current genotypes. However, the incidence of sporangia producing germ tubes was also recorded therefore the main results for direct germination in relation to temperature are presented.

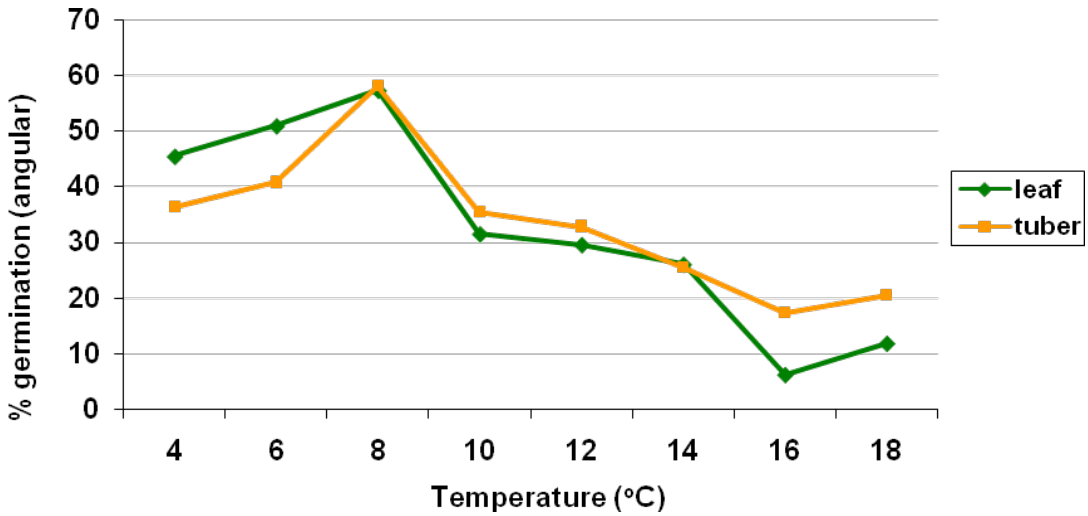

Figure 34. Indirect germination of 13_A2 from leaf and tuber sources of *P. infestans* in relation to temperature. LSD (*P*=0.05) 6.694

Temperature, genotype and their interaction all had highly significant effects (*P*< 0.001) on direct germination. The incidence of direct germination increased with temperature reaching a maximum at 16°C (Fig. 35). The incidence at 18°C was significantly lower than at 16°C. For three of the eight genotypes, 1_A1, 10_A2 and 13_A2, the percentage of sporangia with germ tubes was significantly lower at 18 compared with 16°C. For the other five genotypes there was no significant difference between the two temperatures (Fig. 36).

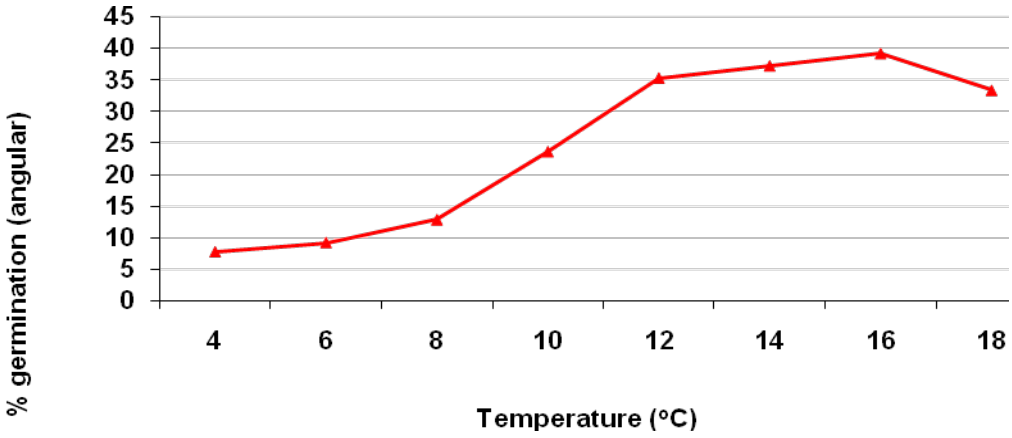

Figure 35. Mean direct germination of all eight genotypes of *P. infestans* in relation to temperature. LSD (*P*=0.05) 2.351

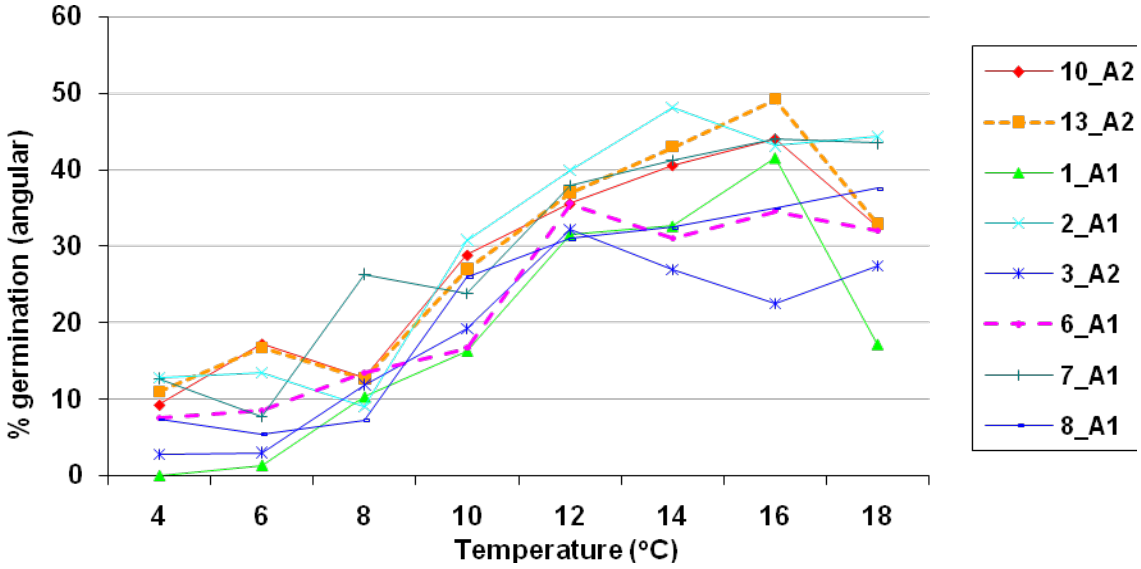

Figure 36. Direct germination of individual genotypes *P. infestans* in relation to temperature LSD (*P*=0.05) 6.65.

Differences between the new genotypes, 13_A2 and 6_A1, and the six older genotypes were generally small (Fig. 37, Table 5). However, at 6 and 16°C the percentage direct germination was significantly higher for the two new genotypes. Examining the results for the two new genotypes separately demonstrated that the higher germination rate was due to 13_A2 only (Fig. 38). Significantly more sporangia of 13_A2 germinated directly compared with genotype 6_A1 at four of the test temperatures, i.e. 6, 10, 14 and 16°C.

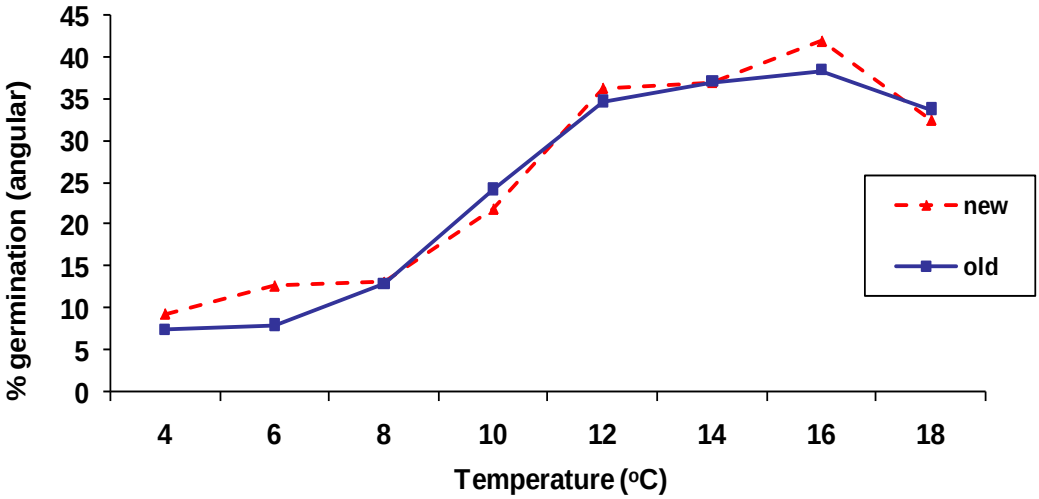

Figure 37. Direct germination of old and new genotypes of *P. infestans* in relation to temperature. LSD (*P*=0.05) 6.650

Table 5. Difference in percentage (angular transformed) direct germination between new and old genotypes

Temperature	new	old	F pr.	difference
4	9.30	7.49	0.430	1.81
6	12.65	8.05	0.027	4.60
8	13.08	12.94	0.942	0.14
10	21.85	24.18	0.361	-2.33
12	36.30	34.73	0.503	1.57
14	37.03	37.03	0.998	0.00
16	41.94	38.42	0.027	3.52
18	32.50	33.80	0.467	-1.30

Only for one genotype was the optimum temperature a single value, i.e. 16°C for 1_A1 (Table 6). Most genotypes had a wide optimum range. All genotypes showed optimum germ tube production between 12 and 18°C. For most genotypes the optimum temperature range for indirect germination was narrower (Tables 4 and 6).

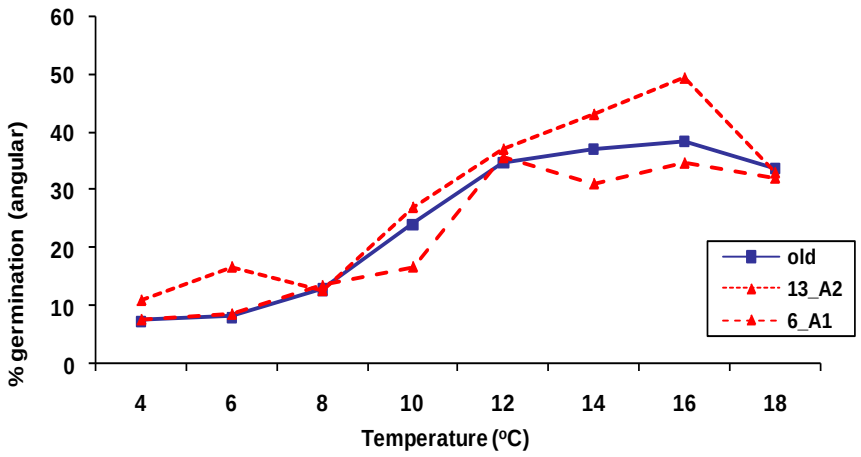

Figure 38. Direct germination of old and new genotypes of *P. infestans* in relation to temperature. LSD (*P*=0.05) 6.650

Table 6. Optimum temperature range[1] for the incidence (%) direct germination (transformed data, angular)

	Temperature (°C)							
	4	6	8	10	12	14	16	18
1 A1							41.5	
2 A1						48.2	43.2	44.5
3 A2					32.2	26.9		27.5
6 A1					35.6	31.1	34.6	32.1
7 A1					39.0	42.4	43.7	42.0
8 A1					31.0	32.5	35.0	37.6
10 A2						40.6	44.1	
13 A2						43.0	48.8	

[1] Values in shaded boxes for the same genotype are not significantly different

Germination across a wide range of temperatures

Genotype 8_A1 had the highest mean germination across the temperature range optimal for indirect germination (4 to 10°C) (Fig. 39). The four genotypes with germination not significantly lower were 6_A1, 13_A2, 3_A2 and 1_A1. The highest mean germination for the temperature range optimal for direct germination (12 to 18°C) was obtained with genotype 2_A1 (Fig. 39). The values for 7_A1, 13_A2 and 10_A2 were not significantly lower. 13_A2 was the only genotype present in both groups. Across the temperature range, the change in the relative frequencies of indirect and direct germination of sporangia was similar for the new and old isolates (Fig. 40).

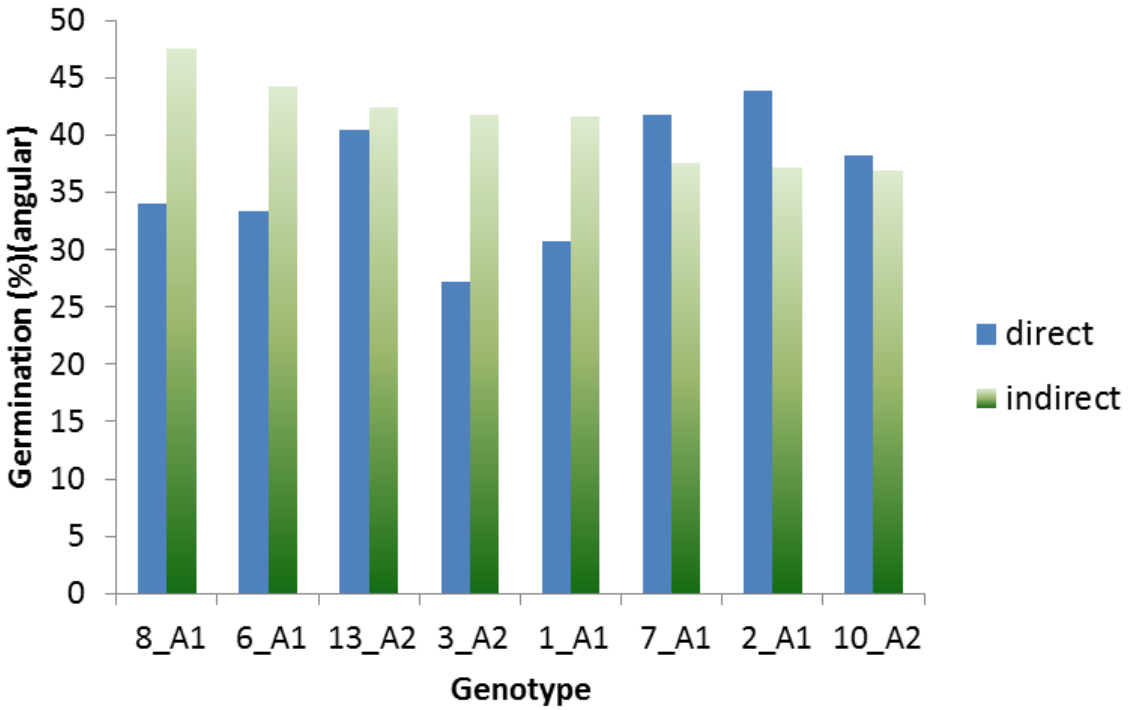

Figure 39. Germination in relation to genotype for low and high temperature optima ranges LSD values (*P*=0.05): direct 6.650, indirect 7.166

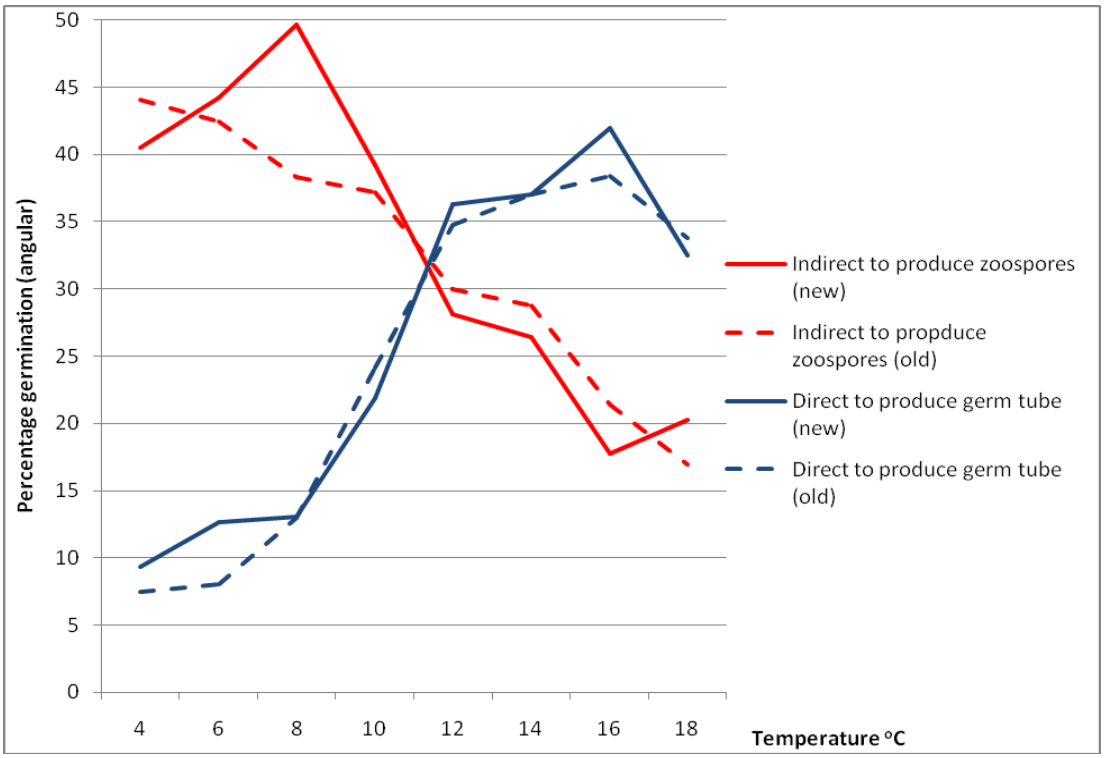

Figure 40. The germination of sporangia to produce zoospores or germ tubes in relation to temperature for two new genotypes (13_A2 and 6_A1) compared with six old (1_A1, 2_A1, 3_A2, 7_A1, 8_A1 and 10_A2)

Discussion

Recent studies (Chapman, 2012) have demonstrated considerable variation in aggressiveness between isolates of the same genotype. Ideally more than two isolates of each genotype should have been tested in the germination experiment but information was sought on eight of the genotypes dominating the GB population. Testing two isolates of eight genotypes allowed four new isolates to be compared with 12 that had been present in the GB population for many years.

Overall, the optimum temperature for indirect germination was 4°C but the values for 4, 6 and 8°C were not significantly different. All eight *P. infestans* genotypes showed optimum zoospore production within the 4-10˚C range. Differences in methodology between studies make direct comparisons of results difficult. It has been demonstrated that indirect germination is affected by the source and age of sporangia. Shaw *et al.* (2006) stated the optima were 9 to 13°C for sporangia from 7-day old rye agar cultures but 5 to 9°C for 1-day old sporangia produced on leaves. In this project (R423), indirect germination in relation to temperature was similar for sporangia from leaf or tuber tissue. Sporangia can lose the ability to produce zoospores as they age (Murphy, 1922). Harrison (1992) noted a marked decline in this ability for 21-day old cultures compared with 10-day old. The optimum range identified by Shaw *et al.* (2006) using young sporangia from leaves was much lower than in earlier work: 12 to 13°C (Melhus, 1915; Crosier, 1934). In 1963 Bohnen obtained an optimum of 12 to 16°C. The sources of sporangia and experimental conditions in these previous studies should be considered. The optimum range identified in the R423 study was very similar to that obtained in experiments using young sporangia from leaf material carried out in 2004 and 2005 (Shaw *et al*., 2006). It should be noted that 13_A2 was not detected in GB until 2005 and in that year did not dominate the population. For some genotypes tested in R423 there was a clear optimum temperature, e.g. for 13_A2 the optimum zoospore production occurred at 8°C. Other genotypes had a wide optimum range, e.g. 6_A1 produced most zoospores from 4 to 10˚C.

It appears that the incubation period for sporangial suspensions affects the incidence of indirect germination. In R423, in which the period of incubation was 24 hours, the overall relationship between indirect germination and temperature was part of a sigmoid curve, i.e. essentially linear between 18 and 8°C with a plateau at 6 and 4°C. In Shaw *et al.*'s work using sporangia from rye agar plates, parabolic curves were consistently obtained. In Shaw *et al.*'s experiment using sporangia from leaves, for seven out of the eleven isolates parabolic curves were obtained but they had asymptotic tails between 13 and 26°C. However, for four other isolates the relationship was linear. The two different types of curve where sporangia were sourced from leaves probably reflect the speed at which zoospores are released by different genotypes of *P. infestans*. Shaw *et al*. measured zoospore release after 2 hours incubation. It is possible that 2 hours at very low temperatures was not long enough for those genotypes that released zoospores more slowly. Yamamoto and Tanino (1961) observed that only 70-80% of the final number of zoospores were released after 2 hours at 12°C. In another study Sato (1979) found that at 16 and 17°C most indirect germination had occurred by 6 hours of incubation. The 24-hour incubation period in R423 was chosen because this period was long enough to allow extensive indirect germination at very low temperatures. In addition this incubation period is reasonable because at lower

temperatures zoospores can remain motile for a long period, e.g. 22 hours at 5 to 6°C (Melhus, 1915) or 24 hours at 1°C (Crosier, 1934). Sato (1979) demonstrated that zoospores were motile for over 8 hours at 10°C.

Infection efficiency is a component of aggressiveness. Dyakov *et al.* (2000) recognised that the proportion of sporangia that germinate will influence infection efficiency. More aggressive genotypes are likely to have high sporangial germination rates across a wide range of temperatures. Such rates were examined for the eight genotypes used in this study. Genotype 8_A1 had the highest mean germination across the temperature range optimal for indirect germination (4 to 10°C). The four genotypes with germination not significantly lower were 6_A1, 13_A2, 3_A2 and 1_A1. The highest mean germination for the temperature range optimal for direct germination (12 to 18°C) was obtained with genotype 2_A1. The values for 7_A1, 13_A2 and 10_A2 were not significantly lower. 13_A2, the most prevalent genotype in the GB population from 2006, was the only genotype present in both groups. It is realised that there are other components that may be of greater importance in determining the relative aggressiveness of genotypes. Further work using a greater number of 13_A2 isolates will be required to confirm this preliminary result.

In R423 the overall optimum temperature for direct germination was 16°C. This is substantially lower than the 24°C optimum observed by Crosier (1934). Crosier found that the number of sporangia germinating directly increased from 6 to 24°C then decreased again up to 30°C.

There are differences between blight fungicide active ingredients in their activities against the two dominant spore types of *P. infestans*. It is reassuring that the change in the relative frequencies of indirect and direct germination of sporangia across the temperature profile was broadly similar for the new and old isolates.

3.3 Determine the influence of P. infestans genotype on the infection and survival of tubers

This study examined the influence of isolates of different genotypes of *P. infestans* on tuber blight using two different direct inoculation methods. The first attempted to simulate the natural infection process in the field. The second was a more artificial point inoculation method. In addition the impact of isolate on the period of survival of blighted tubers was investigated.

Materials and methods

A. Tuber infection in situ *in relation to* P. infestans *genotype (2011)*

Six chitted seed tubers (20-30 mm diam., cv. Rocket) were shallow planted at a depth of 5 cm on 13 April 2011 in 49-litre tubs containing John Innes No 1 compost. Four replicate tubs were planted. Each tub was labelled with replicate and isolate. The tubs were placed in a polytunnel for 15 weeks to encourage rapid tuber initiation and development and to keep the foliage dry. The haulm from each tub was cut c.10 cm above the compost surface to allow the tubs to be stacked in the controlled temperature room. The compost was saturated with the tap water that had been stored at room temperature for 48 hours, to ensure that tuber

lenticels were open prior to inoculation and the tubs incubated on 25 July 2011 for 24 hours at 4 ^{o}C. Tubs were watered from below and overhead so that the compost was wet throughout the complete depth profile.

P. infestans isolates (Appendix 5) were grown on King Edward potato leaves for 10 days at 16^{o}C, 16-hour day length. Sporangial suspensions were prepared by washing leaves with a 9:1 mixture of sterile distilled water and potato tuber extract (McKee, 1964) that had been pre-incubated at 16 ^{o}C for 24 hours. This temperature was to prevent zoospores being produced prior to the time of tuber inoculation. Once the concentration of sporangia for each test isolate was determined, the isolate with the lowest concentration was eliminated and replaced with a *P. infestans*-free control (water only) to check for natural infection. The concentration of sporangia for individual isolates was adjusted to 8.25 x 10^4 sporangia in 100 ml per tub. To assess direct and indirect germination, aliquots of the sporangial suspensions were incubated at 10^{o}C for 24 hours, then fixed and assessed.

All leaf material was removed from the surface of the compost and solid trays were kept in place below the tubs during the inoculation process to prevent cross contamination between tubs. The inoculum suspensions (100 ml) were dribbled evenly onto the surface of the compost using Sarstedt plastic disposable cups with a small hole drilled in the bottom of each cup. A clean cup was used for each isolate. Immediately after inoculum addition, sporangia were watered in using 5.4 litres of tap water per tub. The water had been stored at 10^{o}C for 24 hours. Tubs were stacked two high and arranged as a randomised complete block. Two days after inoculation, trays were drained of any water to alleviate anaerobic conditions, particularly at the base of the tubs. The temperature of the room was increased to 10^{o}C and the tubs were incubated for 21 days. The progeny tubers were harvested, washed, dried and destructively assessed to determine the incidence of blight. Four replicates were assessed, however only the results from two were analysed because of severe bacterial soft rot in tubs of the other two reps. The bacterial soft rot was sufficiently severe to prevent accurate assessment of tuber blight.

B. Tuber infection in situ *in relation to* P. infestans *genotype (2012)*

The experiment was repeated in 2012, with some modifications. Four replicates were planted. The planting date was 25 April 2012. Plants were grown at 15^{o}C in a growth room for 15 weeks. The *P. infestans* isolates (Appendix 6) were grown on Rocket potato leaves for 8 days at 16^{o}C, with a 16-hour day length.

Tuber initiation and bulking were very poor for the Rocket plants in the tubs. There was no alternative supply of immature tubers with open lenticels. As an alternative, ware crop tubers (cv Maris Piper) were washed, dried and surface sterilised using IMS, before being cut in half on 13 August. After removal of the Rocket plants from the tubs 24 tuber halves per tub were laid on to the compost surface, with the cut surface facing upwards. All tuber halves were then covered with c. 5 cm of compost. The growth room was set to 4^{o}C on 13 August for 24 hours. The tubs were moved in on the 13 August and inoculated on 14 August as per the 2011 protocol. Tubs were incubated at 10^{o}C for 9 days before harvesting and assessing the tuber halves. Tuber halves were washed, dried and destructively assessed to determine the incidence of blighted halves.

C. The influence of P. infestans *genotype on the survival of inoculated tubers (2011)*

Tubers (cv. Saxon) were washed, dried and surfaced sterilised. Tubers were placed in rows on trays containing damp tissue. Each tuber was wounded once using a cork borer of 15 mm diameter (sterilised between rows with IMS and flaming), which was pushed into the tuber to a depth of 5 mm. A random sample of intact tubers was tested for contamination by *Pectobacterium atrosepticum* and/or *Pectobacterium carotovorum* subsp. *carotovorum*. A total of eight isolates of *P. infestans* (Appendix 7) and five replicates were used. Four replicates were for tuber burial in the field whilst the fifth replicate was used to determine blight severity in relation to isolate (destructive assessment). In total 100 tubers per isolate were inoculated (20 tubers per replicate).

P. infestans isolates were grown on King Edward potato leaves for 7 days at 16^{o}C, 16-hour day length. Sporangia were washed from the leaflets using sterile distilled water, the concentration was adjusted and 20 µl of sporangial suspension (containing 20 sporangia) were point inoculated into each wound site on 15 September 2011. Individual suspensions were thoroughly mixed between each row of tubers using a Gallenkamp® Spinmix (The Technology Centre, Loughborough, UK). Control tubers were inoculated with sterile distilled water only. Trays containing the inoculated tubers were labelled, placed in large black bags and the bags sealed before being stored at 4^{o}C and high relative humidity for 15 days (Table 7). The black bags were removed after 7 days. Trays were arranged as a randomised complete block (Appendix 8). Following the incubation period, trays were placed in an ambient store for 7 days prior to tuber burial.

Due to heavy and persistent rainfall towards the end of September in 2011, burial was delayed to allow ground conditions at the site to improve. To limit disease progress prior to planting, tuber samples were stored at 4^{o}C for a further 4 days. Tubers from replicate five were transferred to the ambient store 24 hours prior to destructive assessment to encourage disease development. Replicate five was destructively assessed on 12 October 2011. Tubers were cut transversely through the wound/inoculation point and each tuber assessed for blight severity (%).

Table 7 Details of dates and timings (2011 experiment)

Task	Date
Inoculation	15 September
Incubation (4^{o}C)	15 - 30 September
Bags removed	22 September
Incubation (12^{o}C)	30 September – 07 October
Incubation (4^{o}C)	07 – 11 October
Incubation (12^{o}C) (Rep. five)	11 – 12 October
Burial (South Holm)	11 October

Before burial each tuber was individually numbered and weighed. Tubers were transferred to the field in their individual trays and laid out next to the relevant plot. Each plot was 3.4 m (4

rows) x 7.50 m with 2 m spacing between plot ends and 2.6 m between plot sides. Tubers were hand planted in the middle two rows to a depth of 14.5 cm with 20 cm spacing between tuber centres, using a randomised complete block design (Appendix 9). Trowels were thoroughly washed and gloves changed between plots to prevent cross contamination. Extra tubers had been inoculated with isolate 2008_6082F (13_A2) to allow monitoring of tuber rotting throughout the period of burial. All tubers were harvested by hand 6 weeks after burial (21 and 22 November 2011) and individual tubers were washed thoroughly to remove all soft rotted tissue and each tuber re-weighed. Tubers were also assessed for the presence or absence of blight symptoms.

D. The influence of P. infestans *genotype on the survival of inoculated tubers (2012)*

The materials and methods were similar to those used in 2011, with some modifications. Details of dates and timings are given in Table 8. A total of nine isolates (Appendix 10) and five replicates were used. Four replicates were buried whilst the fifth replicate was used for destructive assessment of blight severity. *P. infestans* isolates were grown on King Edward potato leaves for 10 days at 16^{o}C, 16-hour day length. Tubers were point inoculated by placing 20 sporangia in 20 µl sterile distilled water into each wound site on 19 April 2012. Extra tubers were inoculated with isolate 6082F (13_A2) for monitoring tuber rotting throughout the period of burial.

Trays containing the inoculated tuber samples were labelled, placed in black bags and the bags sealed before being stored at 4^{o}C and high relative humidity for 48 days. Trays were arranged as a randomised complete block in the cold store (Appendix 11). The black bags were removed after 7 days. Tubers from replicate five were transferred to the ambient store for 17 days prior to destructive assessment for blight severity on 21 May 2012. Soft rot severity (%) was also recorded during this assessment. The tubers of replicates one to four were buried on 6 June. The experimental layout for the 2012 field trial is detailed in Appendix 12. Tubers were harvested by hand on 29 June 2012, 23 days after burial, and assessed for soft rot severity and tuber blight.

Table 8 Details of dates and timings (2012 experiment)

Task	**Date**
Inoculation	19 April
Incubation (4^{o}C)	19 April – 06 June
Bags removed	26 April
Incubation (12^{o}C) (Rep five only)	04 May
Burial (South Holm)	06 June

Limited irrigation on one occasion, 20 June, using seep hose was required to ensure soil moisture levels were adequate for disease progression.

Results and Discussion

A. Tuber infection in situ *in relation to* P. infestans *genotype (2011)*

In the 2011 experiment, on average 13_A2 isolates caused a significantly higher incidence of tuber blight (cv. Rocket) than representative isolates of the old population (Table 9). However, there was considerable variation between isolates of 13_A2 (Fig. 41). Only one 6_A1 isolate was included in the experiment due to the poor growth of 6_A1 isolates prior to inoculation. The 6_A1 isolate tested gave the lowest incidence of infection. This isolate was grouped with the 13_A2 ones to compare new genotypes with old. The new genotypes resulted in a higher incidence of tuber infection but the difference was just significant (Table 9).

Compared with the old isolates the 13_A2 isolates tested resulted in a significantly higher *in situ* infection (14.7% and 29.2%, respectively) and yet the 13_A2 isolates had significantly lower indirect germination percentages (28.6% and 23.4%, respectively) and direct germination percentages (15.5% and 10.4% respectively).

Table 9 Mean *in situ* infection for isolates of old and new genotypes, cv. Rocket, 2011

	13_A2	**6_A1**	**Old**
	6082F 07/39 7654A	6090A	7006D (2_A1) 7122A (7_A1) 6422F (8_A1)
		New (13_A2 and 6_A1)	
Mean infection (%)	29.2	23.8	14.7
F pr. 13_A2 vs Old	0.008		
F pr. New vs Old	0.046		

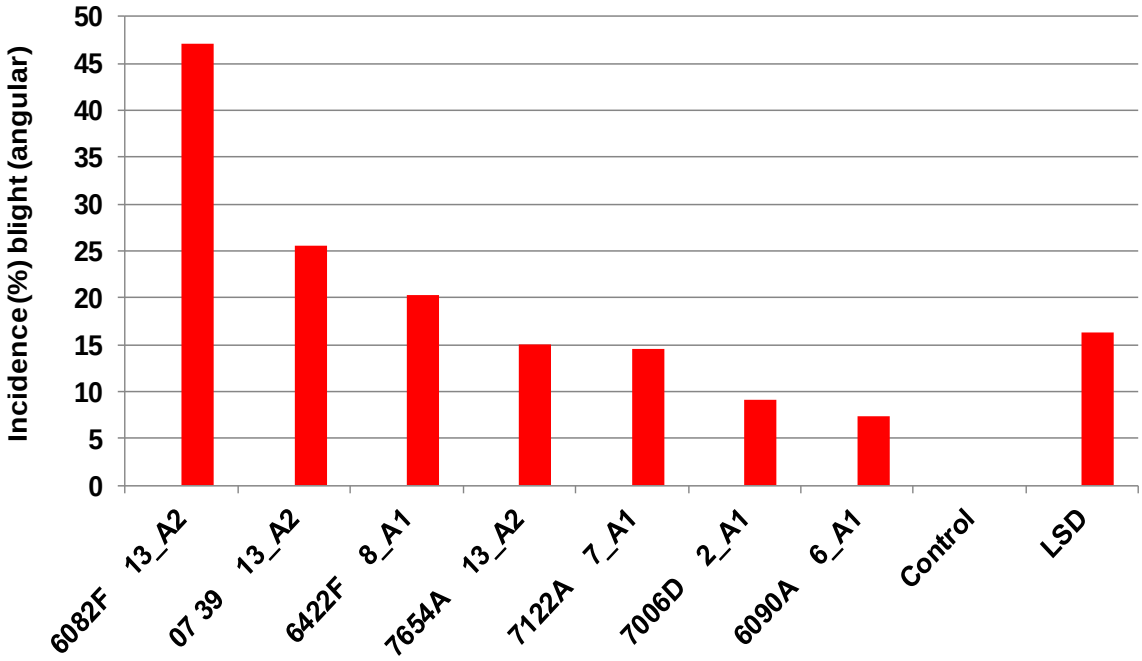

Figure 41. Incidence of *in situ* infection of Rocket tubers by different isolates of *P. infestans*, 2011

B. Tuber infection in situ *in relation to* P. infestans *genotype (2012)*

The mean incidence of infection of tuber halves (cv. Maris Piper) was significantly higher for the two 13_A2 isolates compared with the two isolates of older genotypes (Table 10, Fig. 42).

Table 10. Mean *in situ* infection for isolates of 13_A2 and old genotypes, Maris Piper, 2012

	13_A2	**Old**
	7654A 6102A	7122A 8042B
Mean infection (%)	34.60	25.49
F pr. 13_A2 vs Old	0.002	

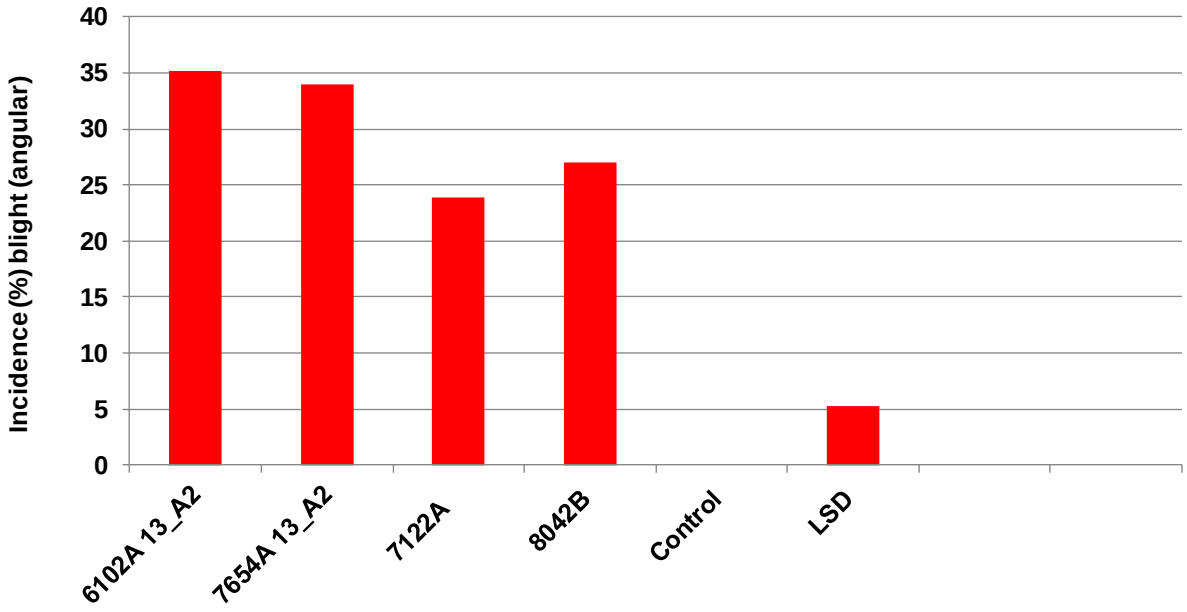

Figure 42. Incidence of *in situ* infection of Maris Piper tuber halves by different isolates of *P. infestans*, 2012

C & D. The influence of P. infestans *genotype on the survival of inoculated tubers (2011 and 2012)*

In both experiments, 2011 and 2012, tubers inoculated with 13_A2 isolates developed significantly more severe tuber blight than those inoculated with older genotypes (Tables 11 and 12). The presence of genotype 13_A2 also accelerated bacterial soft rotting (Tables 11 and 12). This result was anticipated because it is widely known from experience with stored crops that blight infection of tubers predisposes them to secondary rot by bacteria. Sicilia *et al*. (2002) confirmed this scientifically for *Pectobacterium*. However, the absence of a strong relationship between tuber blight severity and soft rot severity was not expected. The weak relationship was partly due to limited soft rot development for some isolates in relation to the tuber blight severity they caused, e.g. isolate 2008_7006D in the 2011 experiment (Fig. 43). Less severe bacterial soft rot than expected was generally limited to isolates of the older genotypes, i.e. 2008_7006D in the 2011 experiment and 2008_7006D, 2010_8042B and 2008_6422F in the follow-up experiment (Fig. 44).

However, although results were consistent in both experiments for isolate 2008_7006D they were not for 2008_6422F. The lack of a consistent result was for soft rot, not blight. This suggests that a factor other than *P. infestans* genotype influenced tuber decay by bacteria. It is assumed that the bacterial soft rot was caused by the *Pectobacterium carotovorum* subsp. *carotovorum* detected in low numbers on non-wounded, non-inoculated tubers. The mean number of bacteria per tuber was 7.0 in the 2011 experiment and 295 in 2012. It is possible that tuber contamination by *Pectobacterium* varied within the tuber stocks used. However, this explanation appears unlikely because the three replicates of each stock tested for

contamination gave a similar result. Also, a large number of tubers were inoculated with each *P. infestans* isolate. The *P. infestans* inoculum for tuber inoculation was prepared from inoculated leaf material. This was a deliberate decision to avoid the issue of axenic culture influencing the aggressiveness of isolates on host tissue. However, the concentration of pectolytic bacteria in the different *P. infestans* suspensions may have been affected by the condition of the leaves used to prepare the inoculum. A higher concentration of bacteria may have been washed from leaf lesions with more advanced blight development. Although it is possible that the loading of pectolytic bacteria was related to isolate aggressiveness on leaves, further experiments, in which bacterial contamination of sporangial suspensions are controlled, are required to re-test the influence of *P. infestans* isolate on the rate of tuber soft rotting.

Many of the isolates of 13_A2 were significantly more aggressive than those of the older population therefore the reported lower incidences of tuber blight in commercial crops cannot be directly related to pathogen aggressiveness on tubers. An alternative explanation is that the more aggressive isolates may cause a higher, or earlier, incidence of tuber blight initially during the growing season but due to more rapid decay of tubers the incidence of tuber blight at harvest may be lower than for less aggressive genotypes. However, the range of experiments that could be carried out in R423 was not sufficient to test this explanation. It is possible that the reduced national prevalence of tuber blight was not related to isolate characteristic but to better crop protection, e.g. shorter fungicide intervals and more frequent use of zoospore-active fungicides.

In the absence of oospores, survival of *P. infestans* between growing seasons is in infected tubers. The more rapid decay of tubers infected by more aggressive strains of *P. infestans* suggests that the survival of blighted buried progeny tubers as groundkeepers or in outgrade piles is less likely if infected by 13_A2 compared with older, less aggressive genotypes. This effect will be more pronounced in milder winters with few penetrating frosts. However, in the one study that specifically examined overwinter survival of inoculated tubers in relation to isolate aggressiveness, survival was unaffected by isolate even although aggressiveness differences were substantial (Montarry *et al*., 2007). Tuber blight development will be limited in a refrigerated store, with little impact of *P*. *infestans* genotype. However, for infected seed brought out of store, blight development after planting will be faster on seed tubers infected with the more aggressive 13_A2 isolates.

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Kelly (unpublished) demonstrated in three field experiments that the incidences of blackleg (*Pectobacterium*) were significantly higher in plots grown from seed tubers inoculated with *P. infestans* compared with non-inoculated tubers of the same stock. The more rapid and more severe bacterial rotting of tubers infected with 13_A2 compared with older genotypes demonstrated in the current study may have implications for non-emergence and seed tuber-borne blackleg. A key early step in blackleg development is rotting of the seed tuber by *Pectobacterium atrosepticum*. If this happens more quickly after planting then either there is a higher incidence of non-emergence (blanking) or, if the plant emerges, blackleg symptoms are likely to develop earlier in the growing season. The impact of seed tuber-borne blight will generally be slight because tuber blight incidences are generally low. However, it is clear that seed stocks infected by more aggressive genotypes such as 13_A2 carry a slightly

higher risk of non-emergence or blackleg. Seed crops entered in classification schemes are more likely to be affected, because of the low tolerances for blackleg, than ware crops.

Table 11 Mean severity of blight and bacterial soft rot on tubers inoculated with different isolates of *P. infestans* and buried in soil, 2011 experiment

Genotype	**13_A2**	**6_A1**	**Old**
Isolates	3928A	6090A	7006D
	6082F	7126A	7122A
	7654A		6422F
Mean blight severity (%)	26.9	15.7	20.3
Mean soft rot severity (%)	35.5	31.6	28.8
	F pr. blight	F pr. soft rot	
13_A2 vs. 6_A1	<0.001	0.004	
13_A2 vs. Old	<0.001	<0.001	
6_A1 vs. Old	0.002	0.039	

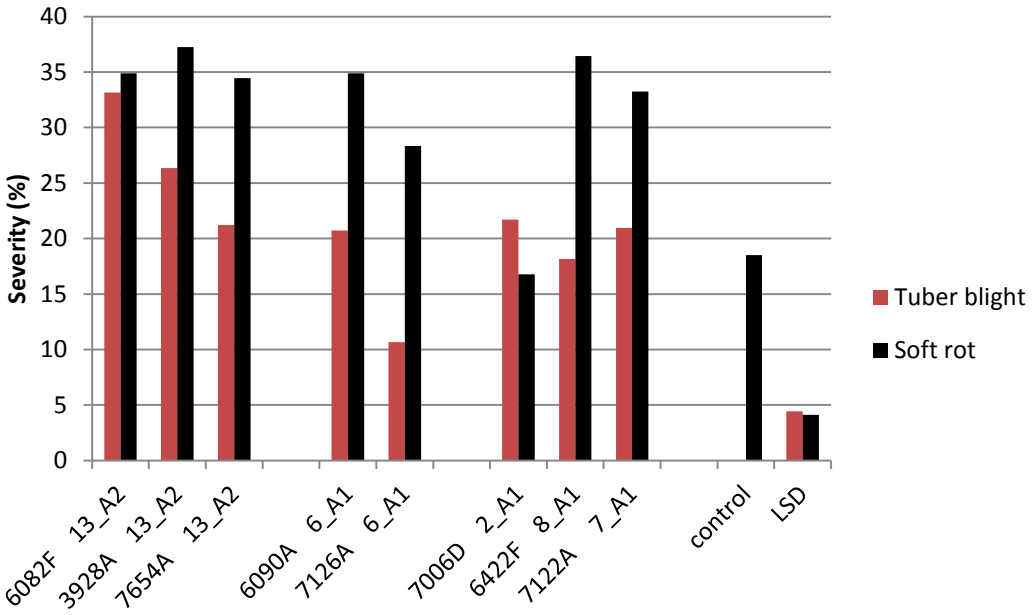

Figure 43. Severity of blight and bacterial soft rot for tubers inoculated with different isolates of *P. infestans* and buried in the field, 2011 experiment.

Table 12 Mean severity of blight and bacterial soft rot on tubers inoculated with different isolates of *P. infestans* and buried in soil, 2012 experiment

Genotype	**13_A2**	**6_A1**	**Old**
Isolates	6082F	6090A	7006D
	07/39	8406A	8042B
	6102A	8986A	6422F
Mean blight severity (%)	31.4	27.5	24.0
Mean soft rot severity (%)	42.8	46.5	11.8
	F pr. blight	F pr. soft rot	
13_A2 vs. 6_A1	0.004	0.007	
13_A2 vs. Old	<0.001	<0.001	
6_A1 vs. Old	0.009	<0.001	

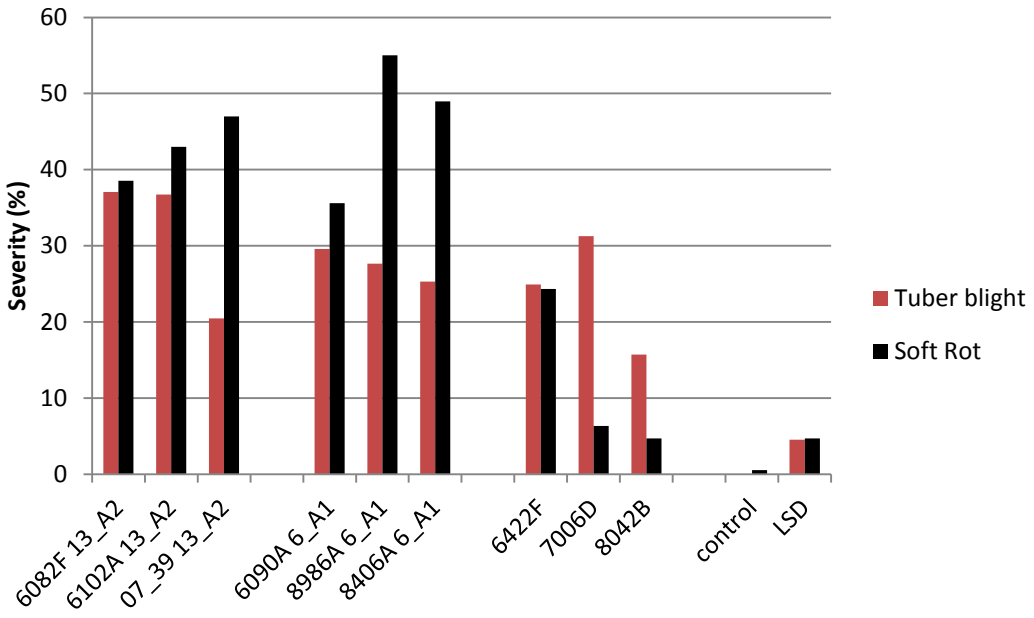

Figure 44. Severity of blight and bacterial soft rot for tubers inoculated with different isolates of *P. infestans* and buried in the field, 2012 experiment.

3.4 To study the longevity of oospores under GB conditions and the implications for subsequent potato crops

Materials and Methods.

Production of oospores

Three A2 mating type isolates and 4 A1 isolates of the most common genotypes found in GB were selected. Each combination of crosses was carried out as follows (Table 13).

Oospores were produced by plating each isolate from the selected cross onto a plate of Rye A agar and incubating at 20°C for several weeks until oospores were formed where the 2 isolates met. Each plate was scored for the presence of oospores and those crosses producing the most oospores were selected. Each site was supplied with different crosses (Table 14).

Table 13 Crosses of *P. infestans* isolates of A1 and A2 mating type for oospore survival experiment.

07/39 (13_A2)	x 2008_6066A (7_A1)
	x 2007_6070E (8_A1)
	x 2008_6090A (6_A1)
	x 2008_6850D (2_A1)
2008_7038A (13_A2)	x 2008_6066A (7_A1)
	x 2007_6070E (8_A1)
	x 2008_6090A (6_A1)
	x 2008_6850D (2_A1)
2007_5482E (10_A2)	x 2008_6066A (7_A1)
	x 2007_6070E (8_A1)
	x 2008_6090A (6_A1)
	x 2008_6850D (2_A1)

Table 14 Crosses of *P. infestans* isolates of A1 and A2 mating type provide to each research site for oospore survival experiment.

AFBI Crosses (T1):
07/39 (13_A2) x 2008_6066A (7_A1)
2008_7038A (13_A2) x 2008_6850D (2_A1)
SRUC Crosses (T1):
07/39 (13_A2) x 2008_6090A (6_A1)
2008_7038A (13_A2) x 2007_6070E (8_A1)
2007_5482E (10_A2) x 2008_6850D (2_A1)
JHI Crosses (T1):
07/39 (13_A2) x 2007_6070E (8_A1)
2008_7038A (13_A2) x 2008_6090A (6_A1)

2007_5482E (10_A2) x 2008_6066A (7_A1)

Half a plate of each cross (split horizontally across plate not vertically along the line of oospores) was mixed with 500 g of soil taken from the site of burial to produce 24 individual samples (6 dates: 6 m (D1), 12 m (D2), 18 m (D3), 24 m (D4), 32 m (D5), 36 m (D6) x 4 replicates) each containing ½ a plate of each of 3 crosses at SCRI and SRUC (2 crosses at AFBI due to fewer genotypes already being present in the NI population). Samples were mixed well and placed into a bag made of permeable nylon mesh. Bags were labelled and tied at the neck and buried individually at a depth of 12 cm from the neck of the bag and at a distance of at least 0.5 m between samples. Samples were randomised and labels were left visible above the ground.

Following discussion at the project meeting, in April 2010 this method was repeated with the same crosses to give an additional 20 samples/site = 4 replicates x 5 dates which would allow longer term sampling of oospore survival beyond the life of the project and the experimental plots were sown with grass seed.

At the James Hutton Institute an additional 48 oospore samples (consisting of 4 replicates x 3 sampling dates (12, 24, 36 months) x 4 treatments) were prepared and buried. Treatments consisted of individual crosses rather than mixed inoculum of 3 crosses as detailed in Table 15.

Table 15 Additional crosses of *P. infestans* isolates of A1 and A2 mating type for burial or soil samples for testing at the James Hutton Institute.

T2: 2007_5482E (10_A2) x 2007_6070E (8_A1)
T3: 07/39 (13_A2) x 2008_6850D (2_A1)
T4: 2008_7038A (13_A2) x 2008_6066A (7_A1)
T5: 07/39 (13_A2) x 2008_6090A (6_A1)
DK1: DK05/3 (misc) x LM2
DK2: DK05/3 (misc) x 203
DK3: DK05/3 (misc) x 106
DK4: DK306 (misc) x LM2
DK5: DK306 (misc) x 203
DK6: DK306 (misc) x 106
GB1: Soil from possible oospore field site

Table 16 Schedule of oospore burial and sampling at the three trial sites.

	James Hutton Institute	SRUC	AFBI	Comments
Buried	26/10/09	27/10/2009	16/12/09	
Sample 1	10/6/2010		30/6/10	
Sample 2	15/11/2010 (T1-T5)	1/9/2010	11/1/11	
Sample 3	17/5/2011	10/3/2011	19/7/11	Additional DK samples at JHI
Sample 4	2/11/2011		24/1/2012	Additional DK samples at JHI
Sample 5	30/4/2012	20/9/2012	26/7/2012	Additional DK samples at JHI
Sample 6	26/11/2012			Additional DK samples at JHI

After recovery of samples, the following steps were made:

1. Each soil sample was weighed and split into six 50 g portions and placed into small sealed plastic bags. The remainder, along with the mesh, was placed into larger plastic bags, sealed and stored at 4°C.
2. At AFBI Positive control inoculum was created by brushing isolate *17/07* sporangia off leaves, chilling the inoculum at 4°C for two hours, then diluting this in 500 ml water.
3. At JHI a positive oospore control was created for inclusion in the third set of samples tested. Isolates were obtained from Sabine Ravnskov, University of Aarhus and crossed to produce oospores. These isolates had previously been shown to produce oospores capable of germinating and producing disease in field trials in Denmark. Oospores were extracted from agar culture and added to soil samples taken from the same source as the original samples and were included in all tests.
4. Additionally, a soil sample obtained from the same source as one of the miscellaneous (potentially oospore-borne) outbreaks discussed above was included in all tests by JHI in 2011.

Tomato leaf, potato leaf and tuber test

For each of the reps (plus negative controls) one 50 g soil portion was mixed with 100 ml water to create a suspension. Droplets of this suspension were placed onto each of 20 detached tomato leaflets (cv. Moneymaker) per rep (contained in 2 humid, perspex boxes – 10 leaflets in each).

One droplet of suspension for each rep and control was also placed onto each of 20 slices of Maris Piper tubers (AFBI = 10 slices of previous season Maris Piper and 10 slices current season Home Guard) . All the inoculated tuber slices were kept in trays lined with damp tissue enclosed in sealed polythene bags, with one tray per rep.

Additionally, droplets of the soil suspension were also pipetted onto detached Maris Piper (AFBI= Up-to-Date) potato leaflets, which were stored on damp tissue in sealed boxes.

'Drenth' baiting test

Two more 50 g portions of the soil sample from each rep and control were each mixed with 100 ml water. Positive controls (oospore-free soil with 100 ml inoculum added) were included in some tests. This mixture was incubated in the blight room at 17°C for two days.

After the incubation period it was noticed that much of the water had evaporated, so an additional 75 ml de-ionised water was added to each soil suspension. Detached Maris Piper leaflets were floated on top of the soil suspension, roughly 3 to 4 large leaflets per box and observed for symptom development. Leaves were replaced at regular intervals (max. of 16 occasions at SRUC) and incubation continued for 4-5 weeks. At SRUC leaves were removed every 48 hours and further incubated in Petri dishes containing damp tissue at 15^{o}C. Similarly, at JHI leaves showing any possible sign of infection were transferred to Petri

dishes for further observation. Water was added to the trays as necessary to replace water lost through evaporation

Tomato plant baiting

The remaining three 50 g soil portions for each rep were used for the tomato plant baiting. Four inch plant pots were three-quarters filled with compost – three pots per rep. One third of the 50 g soil sample was then added to each pot. Two tomato seedlings at the 4 to 5-leaf stage were transplanted into each pot. Each pot was then filled with compost.

At SRUC an extra step was added to this protocol. On nine occasions, starting 1 week after planting , the compost/test soil was saturated with distilled water and detached tomato leaflets from the bait plants placed on the surface of the compost. The temperature of the growth room was reduced to 11°C for 24 hours before being returned to 15°C. The leaflets were left on the compost surface for 48 hours in total and then placed in Petri dishes containing damp tissue and incubated at 15°C. The presence/absence of lesions was assessed after incubation in the Petri dishes for 6 and 11 days. For the second batch of samples, harvested 10 March 2011, King Edward plants and tuber slices were used.

An additional soil sample was provided from a field that, on the basis of genotyping of samples provided by a Fight Against Blight scout, was suspected to be contaminated with oospores. This sample was stored under cool damp conditions for 4 weeks and then included within the baiting regime for the buried oospore samples.

Results

The *in vitro* oospore production was successful in generating from 12 to 38 thousand oospores per ml. After mixing with soil these oospores were buried and tested at regular intervals over a period of three years using a range of tests (Fig. 45 – 47) to study their viability. Despite the multiple screens and lengthy assessments that were completed at the three research centres every sample proved negative. A few positive bait samples at the James Hutton Institute (Fig. 47) were tested by SSR genotyping and proved to be 13_A2 clonal lineages that are likely to have originated from airborne inoculum. In the interests of space, the lengthy details of this testing are not reported here. Additional samples derived from Danish isolates also tested negative after sampling at four dates. The additional soil sample from a GB Fight Against Blight outbreak site also tested negative.

a

b

Figure. 45. Tuber slice assay a) positive control b) negative control

a b

Figure 46. a) tomato plant test positive control b) tomato plant test sample showing no symptoms

a b

Figure 47. a) Drenth bioassay, Maris Piper leaflets b) blight infected leaf from Drenth assay.

Discussion

It was unexpected that all samples in this series of tests proved negative. An obvious question is whether it was the oospores that were not viable or the tests that were insufficiently sensitive to detect oospore germination. Many other studies have successfully germinated oospores that originated from crosses established on agar and there is therefore no reason to suspect that the oospores were not viable. In another study, generating oospores in polythene tunnels it was demonstrated that relatively few of the oospores formed from crosses of GB clonal lineages germinated and resulted in disease in subsequent crops (Cooke *et al*., 2010). It was hypothesised at the time that an abnormal chromosome complement in some dominant lineages may have decreased the viability of oospore progeny. Subsequent to this a laboratory study has confirmed that isolates of 6_A1 and 13_A2 are triploid and crosses that include a triploid parent produced many fewer and less pathogenic progeny than crosses between two parents in their 'normal' diploid state

(Hamed and Gisi, 2013). For this reason isolates were sourced from Denmark that were predicted, on the basis of their SSR fingerprints and evidence of sexual recombination of *P. infestans* in Danish potato crops, to be diploid. Crosses using these mating partners were generated but subsequent burial and testing also proved negative. One possibility is that the frequency of germination is very low and the sample sizes tested here were not large enough to detect the rare events. The high genetic diversity and presumed prevalence of sexual recombination in Nordic *P. infestans* populations (Sjöholm *et al*., 2013) suggests that the pathogen is able to generate viable progeny in these regions and a recent paper reviews the evidence for the role of oospores in Europe (Yuen and Andersson, 2013) . This study and the low frequency of recombinant 'misc' progeny in the monitoring work suggest that, in Great Britain, oospores are relatively unimportant as a source of inoculum. This is probably due to a combination of dominant triploid clonal lineages and long rotations that allow sufficient decline in inoculum viability.

3.5 To actively integrate with research in other countries and to use this information to inform the research aims and interpretation of results for this project.

Parameters used in the Dutch Late Blight models were discussed with Geert Kessel in 2009 and we followed this up by hosting him for a two-day meeting at The James Hutton Institute in March 2011. We identified the data required and how they should be generated to ensure compatibility with those generated for the models produced by Peter Skelsey and colleagues in Wageningen. Protocols were provided and experiments conducted to provide data to update the model. Subsequent to this exchange, Peter Skelsey was appointed to the staff at the James Hutton Institute and this has allowed more detailed testing of the models with parameters determined in this project. An example is presented below.

Updates on the blight forecast sub-models and their comparison were given by Jens Hansen at the Euroblight meeting held in May 2010. Hourly meteorological data from Ayrshire and Dundee for the years 2006-2009 has been supplied to Jens by SRUC and The James Hutton Institute respectively, in order to validate the comparison of these blight forecasting sub-models in GB conditions. An example of such a comparison is shown (Fig. 48) and the graphic analysis tool can be viewed at (http://130.226.173.223/euroblight/EuroBlight.asp). Such a tool allows a visual analysis of the data and it would be good to follow this up with an objective measure of the precision provided by different models in response to a range of real meteorological data.

Little further work on oospore detection had taken place across Europe. Methods for the detection of oospores in soil that were reported by Russian colleagues at the Euroblight meeting in St Petersburg were tested on oospore samples originating in this project (See above).

One of the main outcomes of the Euroblight meeting was the emphasis by participants on the need to link phenotype with genotype in order to inform disease control in the manner of this current project. All presentations and posters from the Euroblight meeting are available at: http://euroblight.net/workshop-proceedings-1996-2013/

Discussions with Sabine Ravnskov (University of Aarhus) in 2010 revealed that they had had some success in generating field epidemics from oospores artificially added to the soil. Results were however variable between years probably due to varying weather conditions. Isolates were obtained from Sabine and crosses made to produce oospores to include in oospore baiting experiments to try to generate a positive control response.

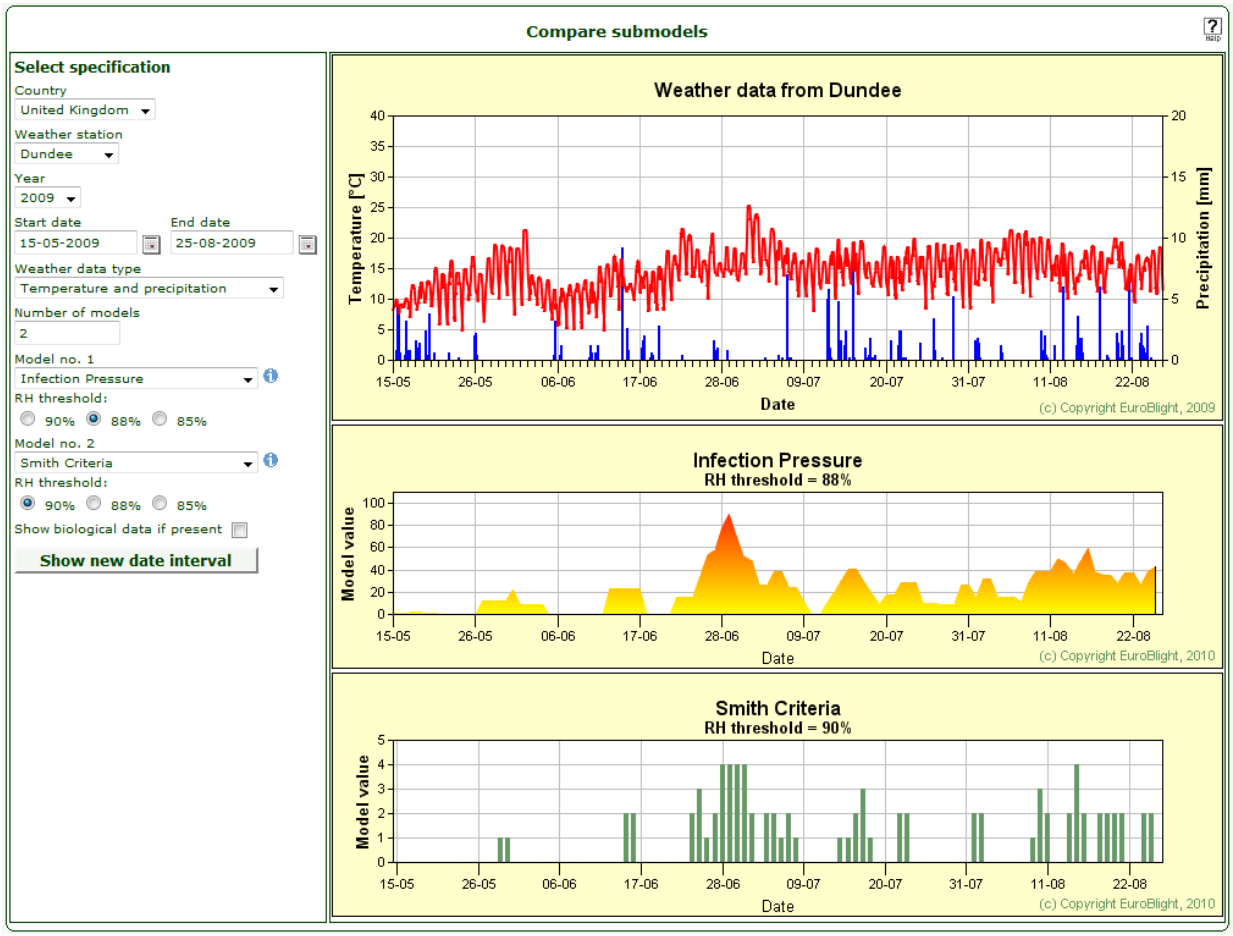

Figure 48. An example of a comparison of Smith criteria and a Danish infection pressure model within the graphic analysis tool developed by Jens Hansen (University of Arhus, Denmark) (http://130.226.173.223/euroblight/EuroBlight.asp).

3.5.1 Example of using a spatiotemporal model of potato late blight spread.

A model that simulates infection and spread of *P. infestans* across potato crops within a landscape has been developed (Skelsey *et al*., 2009). Key to the simulation in this sophisticated model are the epidemiological parameters (e.g. infection frequency, lesion growth rate, response to temperature and humidity) of a representative pathogen lineage as defined during the model development. Towards the end of this project (Nov. 2012) the model was modified by Dr Skelsey to allow two genotypes with slightly modified parameters to co-infect potato crops and 'compete' in a virtual landscape. The model output is the

incidence of potato blight caused by each genotype (Fig. 49). In this particular combination of landscape and weather data, a genotype with a slightly shorter latent period was shown to cause more disease than one with a slight increase in its lesion growth rate. This analysis was replicated over multiple landscapes and years of meteorological data and the mean increase in incidence over all epidemics was plotted (Fig. 50). This simulation shows a clear advantage of earlier sporulation over causing larger lesions and helps explain how a relatively minor change in pathogen biology can affect the pathogen population, disease spread and thus disease management.

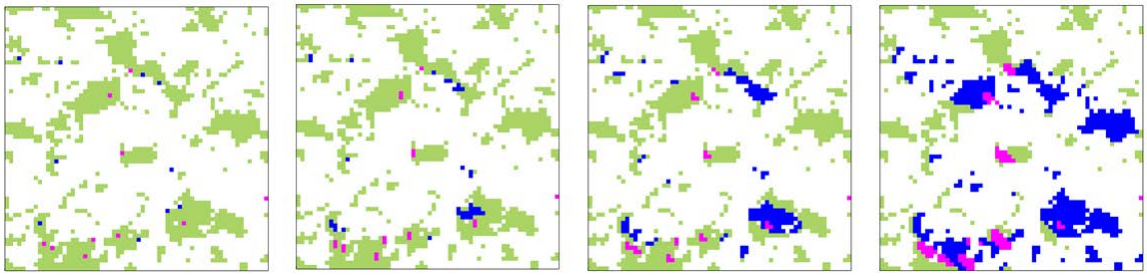

Figure 49 Spatial view of a single late blight epidemic simulation over time (from left to right) where two genotypes, one with a shorter latent period (LP) (13_A2; blue) and the other with a slightly faster lesion growth rate (LGR)(6_A1; magenta) compete to infect potato fields (green cells) in a virtual landscape.

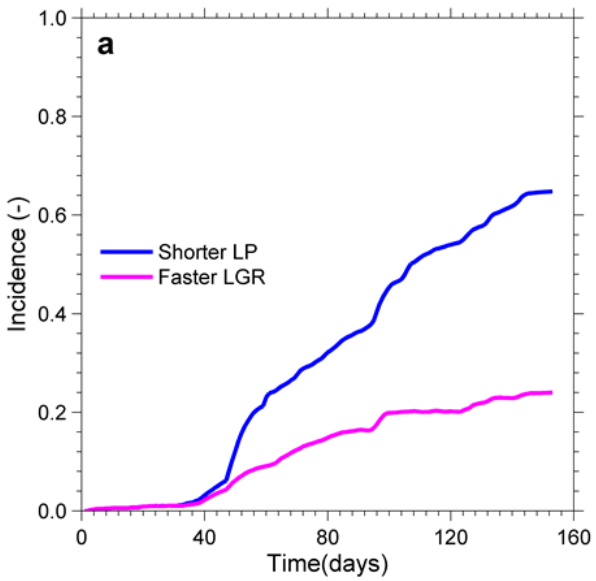

Figure 50. Graphical view of the mean disease incidence within a season of two genotypes with different epidemic parameters (as described in Fig. 49). Times series averaged over 10 years of real meteorological data and 25 random landscapes.

In another simulation, the way lesion growth rate changed in response to temperature was adjusted. Lineages such as 13_A2 with relatively larger lesions and under cooler conditions,

as previously reported for 13_A2 (Cooke *et al*., 2012) were simulated in competition with those forming larger lesions under warmer conditions (see 6_A1 in Fig. 19). Under conditions recorded in the Dundee area in 2009 the simulated incidence of late blight caused by 13_A2 was greater than that caused by 6_A1 at the end of the season (Fig. 51). Both these simulations indicate the value of the models as a virtual experimental platform to aid our understanding of the factors that most strongly influence the spread of disease across a landscape. There are many opportunities for using such detailed spatially explicit models of disease spread to improve disease prediction and management. For example further research is planned in which local disease risk and modifications to the Smith Period criteria can be used to improve the accuracy of disease prediction for GB growers.

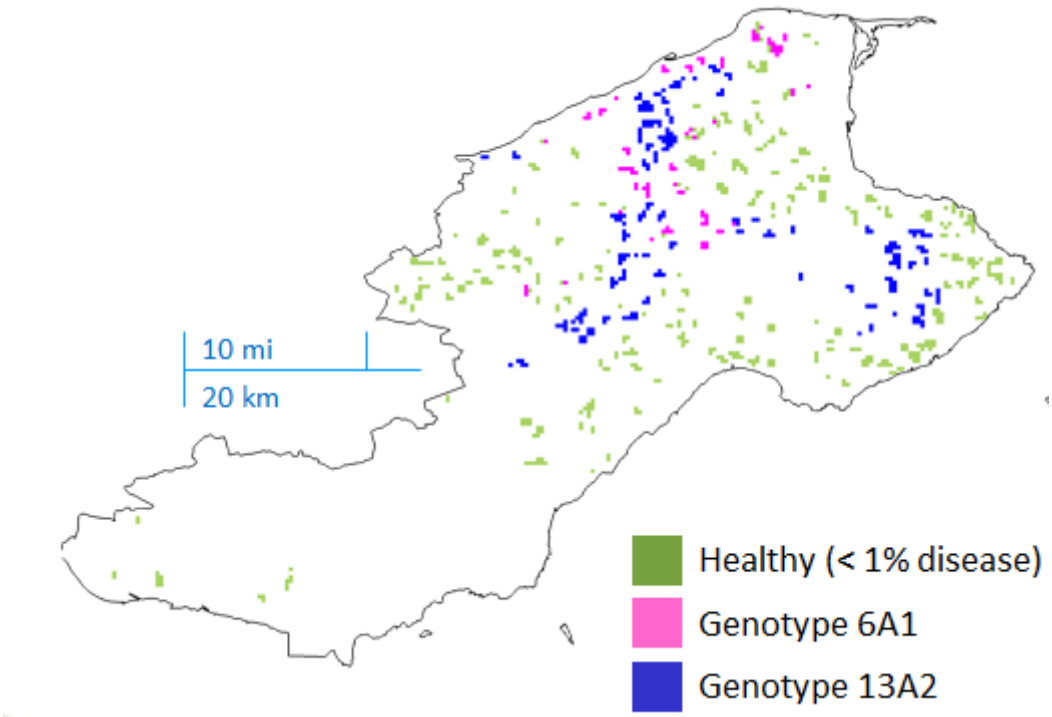

Figure 51. Results of modelling landscape scale spread when the criteria for 13_A2 are more suited to growth under cooler conditions and 6_A1 under warmer conditions. Using meteorological data for 2009 it can be seen that 13_A2 incidence is greater than 6_A1 by the end of the season.

4 Conclusions

The *P. infestans* population in Great Britain has changed dramatically since 2006 and the three years of monitoring (2009-2011) reported here, continue that trend. The predominance of the 13_A2 genotype reduced slightly over the first two years and then markedly in 2011. The cause of the change is complex but is likely to reflect a change in fungicide use, founder effects due to the late epidemic in 2011 and possible differences in temperature response between lineages. Concerns have been raised about whether this population change affects the reliability of late blight prediction, tuber blight risks and the threat of oospores as a source of inoculum. The Smith Period is commonly used in GB to predict blight risk on the basis of two criteria; on two consecutive days the minimum temperature must be 10°C or above and the relative humidity must be 90% or above for 11 hours on each of the days. In this study we examined both these criteria and showed that infection can occur down to at least 6°C and that 4-6 hours of high humidity are sufficient for infection to occur. With current moves toward precision-agriculture it thus seems sensible to revise the Smith Period criteria to provide more precise information on infection risk. More detailed examination of whether temperature or humidity responses were genotype-specific revealed some trends but the relationship was not clear-cut. In most cases, variation in response amongst isolates of the same clonal genotype masked genotype-specific effects. When four different genotypes were used to inoculate a field trial, disease progress data and genetic fingerprinting of sampled lesions showed clear differences in the aggressiveness and fitness within the blight population. Genotype 6_A1 caused most disease on the plant initially inoculated and caused nearly 40% of lesions across the plots. Isolates of 13_A2, however, spread most effectively across the trial and caused over 60% of the lesions sampled. The other two lineages (7_A1 and 8_A1) were out-competed and caused only low levels of disease.

Despite the increased foliar aggressiveness of the new clonal pathogen population (i.e. genotypes 13_A2 and 6_A1 that have dominated since 2006), anecdotal evidence suggests that the risk of tuber blight severity has declined. A series of experiments examined the effect of this new population on tuber blight infection and tuber survival after infection. There was considerable variation in tuber aggressiveness between isolates of the same genotype. However, isolates of 13_A2 were generally more aggressive (infection and tissue colonisation) on tubers than those of older genotypes (i.e. those established prior to 2006). The generally lower incidence of tuber blight nationally therefore can't be directly related to pathogen aggressiveness on tubers. The severity of secondary bacterial soft rotting was on average significantly greater for tubers infected by 13_A2 compared with older genotypes.

The optimum temperature for the production of motile zoospores from sporangia by new genotypes of *P. infestans* was low, i.e. between 10^{o}C and 4^{o}C, but generally similar to that for isolates of older genotypes. Changes in the ratio of sporangia to zoospore inoculum across the normal temperature range for GB were generally similar for the new and old genotypes tested.

Oospores from crosses of several A1 and A2 lineages were buried at three locations and their viability tested at six-monthly intervals over 36 months in a series of different bioassays. No oospore germination was recorded. This study and the low frequency of recombinant 'misc' progeny in the monitoring work suggest that *P. infestans* oospores are relatively

unimportant as a source of inoculum within the GB potato industry. This is probably due to a combination of dominant triploid clonal lineages that do not generate high numbers of viable progeny and long rotations that allow sufficient decline in inoculum viability. Other studies in Europe have found that oospore biology is challenging to study. Oospore germination is difficult to predict which makes the system rather intractable. Nonetheless the significantly greater pathogen diversity in other regions of Europe indicates that oospores are a prevalent source of primary inoculum and remain a potential threat.

Further dialogue with European research teams allowed a comparison of the Smith Period criteria with other blight prediction models. Sets of GB meteorological data and the Smith criteria were included in a visual tool to compare models developed by Jens Hansen (University of Arhus, Denmark). Using this graphic analysis tool, the merits of different models can be examined. The Smith criteria can be seen to broadly correspond to other models but modifications should be considered to look at the potential benefits of accounting for lower temperatures and shorter humidity periods thresholds. Skills in state-of-the-art disease modelling were sought through collaboration with Dr Geert Kessel (Wageningen University, the Netherlands) and, latterly, via Dr Peter Skelsey (now at The James Hutton Institute). Changes in the epidemiological parameters of different *P. infestans* genotypes were made and comparisons of their spread in virtual epidemics demonstrated the significance of even minor changes in pathogen performance. This highlighted the value of the models as an experimental platform to predict pathogen infection and disease spread for future studies.

5 Acknowledgments

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7 Appendices

7.1 Appendix 1

FIGHT AGAINST BLIGHT
Scout response form – 2009

POTATO COUNCIL

Please complete and insert with sample

ID no. *(from SMS confirmation message)* _____

Postcode where sample found ☐☐☐☐ ■■■ (last part not required)

Where was the infection found? *(Please circle)*

Conventional Crop Organic Crop Outgrade pile (dump) Volunteer Other (*please state*) _____

Variety of sample *(optional)* _____

Date sample taken _____

Type of infection *(Please circle)*

Single plant Patch ($1m^2$) Several patches Scattered through field Very severe

☐ Tick the box if you think this might be an **oospore** infection *(see oospore information sheet)*

For suspected oospore infections please take 8 samples (one from each of 8 plants) if possible.

Please describe your sample distribution *(Tick boxes)* * See overleaf for bag labelling guidelines

1 lesion from each of 4 plants* ☐ Were your plants: clustered ☐

2 lesions from each of 2 plants* ☐ scattered through field ☐

lesions from a single plant ☐ Average distance between lesions (in metres)

If your sample distribution did not match any of the above, please describe what you did:

Your name _____ Your mobile phone number _____

Please send me a replacement sampling kit (3 jiffy bags with sample bags) ☐

For laboratory use only

Sample received by _____

Date _____

Confirmed *(Please tick)* ☐ **Negative** ☐

7.2 Appendix 2

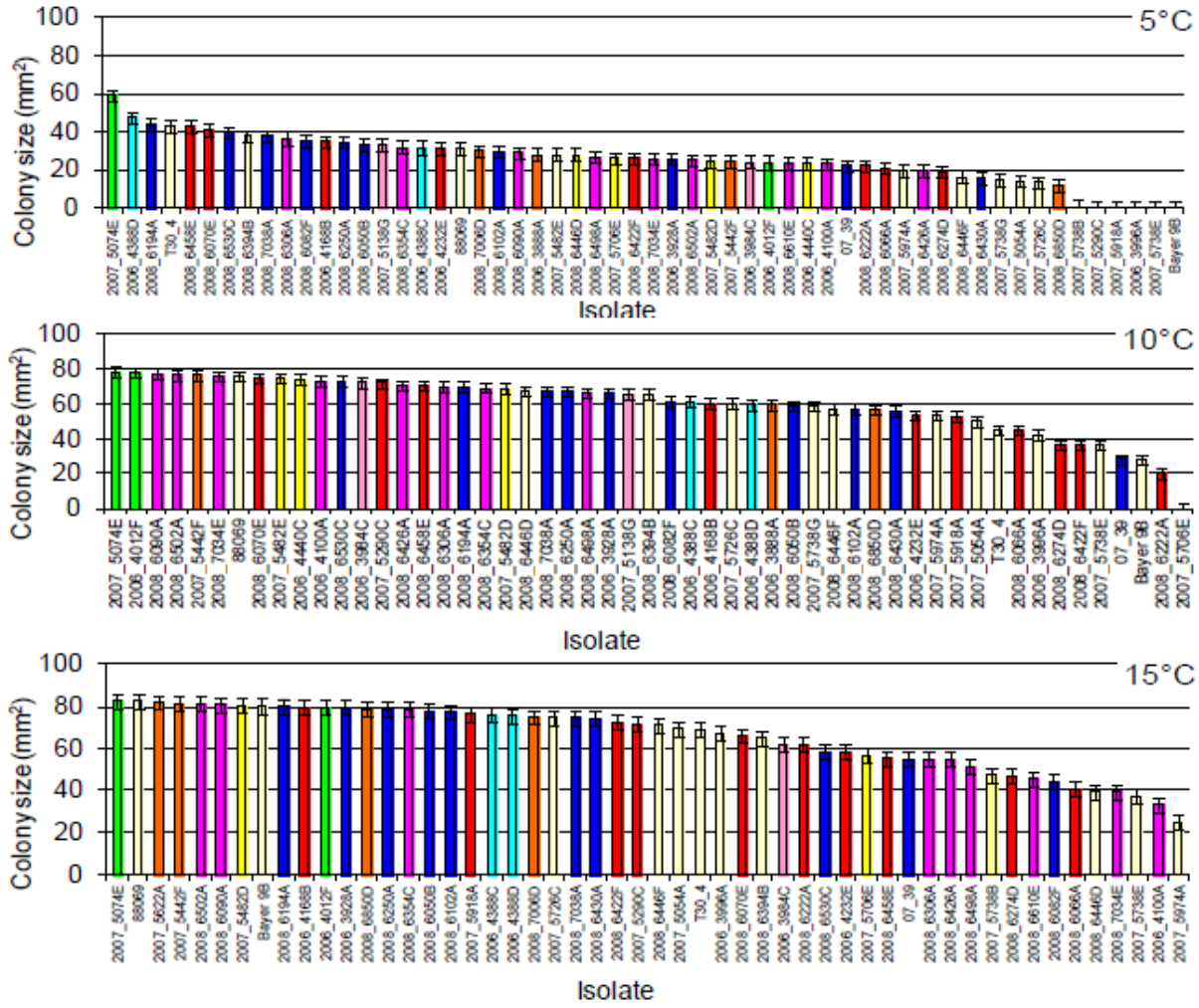
Details of *P. infestans* isolates used in the studies.

Isolate Name	Mating Type	Genotype	County	Region	Cultivar
2006_3984C	A1	1_A1	Cambridgeshire	England	Maris Piper
2007_5138G	A1	1_A1	Kent	England	Maris Piper
2006_4440C	A2	10_A2	Staffordshire	England	Maris Piper
2007_5482D	A2	10_A2	Cumbria	England	Morene
2007_5482E	A2	10_A2	Cumbria	England	Morene
2007_5706E	A2	10_A2	Gwynedd	Wales	Estima
2006_3928A[1]	A2	13_A2_1	Kent	England	King Edward
2008_6194A[1]	A2	13_A2_1	Cornwall	England	Maris Peer
2008_6250A	A2	13_A2_1	Staffordshire	England	Romano
2008_6430A	A2	13_A2_1	North Yorkshire	England	Maris Piper
2008_7038A	A2	13_A2_1	Staffordshire	England	Pentland Dell
2008_6102A[1]	A2	13_A2_2	Somerset	England	Maris Bard
2008_6530C	A2	13_A2_2	Aberdeenshire	Scotland	King Edward
07_39[1]	A2	13_A2_5	Unknown	Unknown	Unknown
2008_6082F	A2	13_A2_5	Suffolk	England	Maris Piper
2006_4388C	A2	17_A2	Aberdeenshire	Scotland	Vivaldi
2006_4388D	A2	17_A2	Aberdeenshire	Scotland	Vivaldi
2006_3888A	A1	2_A1	Cheshire	England	Lady Rosetta
2007_5442F	A1	2_A1	East Lothian	Scotland	Maris Piper
2007_5622A	A1	2_A1	Gloucestershire	England	Pentland Crown
2008_6850D	A1	2_A1	Angus	Scotland	Cultra
2008_7006D	A1	2_A1	East Lothian	Scotland	Unknown
2006_4012F	A2	3_A2	Cornwall	England	Charlotte
2007_5074E	A2	3_A2	Gwynedd	Wales	Bintje
2006_4100A	A1	6_A1	Essex	England	Marfona
2008_6090A[1]	A1	6_A1	Shropshire	England	Maris Piper
2008_6306A[1]	A1	6_A1	Shropshire	England	Vales Emerald
2008_6354C	A1	6_A1	Humberside	England	King Edward
2008_6426A[1]	A1	6_A1	Fife	Scotland	Saxon
2008_6498A	A1	6_A1	Oxfordshire	England	Sante
2008_6502A[1]	A1	6_A1	North Yorkshire	England	Morene
2008_6610E	A1	6_A1	Shropshire	England	Estima
2008_7034E	A1	6_A1	West Midlands	England	Markies
2006_4168B[1]	A1	7_A1	Suffolk	England	Charlotte
2007_5290C[1]	A1	7_A1	Humberside	England	Pentland Dell
2007_5918A[1]	A1	7_A1	Yorkshire	England	Unknown
2008_6066A	A1	7_A1	Warwickshire	England	Markies
2008_6274D[1]	A1	7_A1	North Yorkshire	England	Nicola
2006_4232E[1]	A1	8_A1	Pembrokeshire	Wales	Marfona
2008_6070E	A1	8_A1	Suffolk	England	Shepody
2008_6222A[1]	A1	8_A1	Aberdeenshire	Scotland	Unknown
2008_6422F[1]	A1	8_A1	Herefordshire	England	Russet Burbank
2008_6458A[1]	A1	8_A1	Northumberland	England	Unknown
88069	A1	A1 Misc	Unknown	Netherlands	Tomato
07_SP12_3A	A1	A1 Misc	Highlands	Scotland	Russet Burbank
2006_3996A	A1	A1 Misc	Kent	England	King Edward
2007_5054A	A1	A1 Misc	Kent	England	Maris Piper
2007_5738E	A1	A1 Misc	Aberdeenshire	Scotland	Cara
2007_5738G	A1	A1 Misc	Aberdeenshire	Scotland	Cara
2007_5974A	A1	A1 Misc	Kent	En gland	Tomato
2008_6394B	A1	A1 Misc	Gwynedd	Wales	Arran Victory
2008_6446F	A1	A1 Misc	Dyfed	Wales	Wilja
T30-4	A1	A1 Misc	Unknown	Unknown	Unknown
2007_5726C	A2	A2 Misc	Highlands	Scotland	Unknown
2007_5738B	A2	A2 Misc	Aberdeenshire	Scotland	Cara
Bayer_9B	A2	A2 Misc	Weser-Ems	Germany	Bintje
2008_6446D	A2	A2 Misc	Dyfed	Wales	Wilja

[1] isolates used in field trial (section 2.2.2.6).

7.3 Appendix 3

Sample data from the *in vitro* growth study in which the differences in colony size between different isolates of the same genotype is apparent at each of the three temperatures shown.



7.4 Appendix 4

Details of the GB *P. infestans* isolates used to investigate the ratio of indirect to direct germination of sporangia across temperature

ISOLATE	POST CODE	REGION	CULTIVAR	DATE ISOLATED	MATING TYPE	GENOTYPE
2008_6850D	DD2	Angus	Cultra	29/08/2008	A1	2_A1
2008_7006D	EH39	East Lothian	Unknown	09/09/2008	A1	2_A1
2008_6090A	TF6	Shropshire	Maris Piper	12/06/2008	A1	6_A1
2008_6426A	KY9	Fife	Saxon	08/08/2008	A1	6_A1
2006_3984C					A1	1_A1
2007_5138G	CT3	Kent	Maris Piper	05/07/2007	A1	1_A1
2008_6066A	CV23	Warwickshire	Markies	10/06/2008	A1	7_A1
2008_6274D	DL10	North Yorkshire	Nicola	11/07/2008	A1	7_A1
2008_6070E	IP12	Suffolk	Shepody	04/06/2008	A1	8_2a_A1
2008_6422F	SG8	Hertfordshire	Russet Burbank	31/07/2008	A1	8_A1
07_39					A2	13_A2_1
2008_6102A	TA13	Somerset	Maris Bard	17/06/2008	A2	13_A2_2
2006_4440C					A2	10_A2
2007_5482 E	CA11	Cumbria	Morene	09/07/2007	A2	10_A2
2006_4012F					A2	3_A2
2007_5074 E	LL76	Gwynedd	Bintje	27/06/2007	A2	3_A2

7.5 Appendix 5

Details of the GB *P. infestans* isolates used to investigate the influence of genotype on infection of tuber *in situ* (2011)

ISOLATE	POST CODE	REGION	CV		M TYPE	G'TYPE
2008_7006D	EH39	East Lothian	Unknown	09/09/2008	A1	2_A1
2008_6090A	TF6	Shropshire	Maris Piper	12/06/2008	A1	6_A1
2008_6422F	SG8	Hertfordshire	Russet Burbank	31/07/2008	A1	8_A1
07_39					A2	13_A2_1
2008_6082F		Suffolk	Maris Piper		A2	13_A2_5
2009_7654A					A2	13_A2
2009_7122A					A1	7_A1

7.6 Appendix 6

Details of the GB *P. infestans* isolates used to investigate the influence of genotype on infection of tuber *in situ* (2012)

ISOLATE	POST CODE	REGION	CV		M TYPE	G'TYPE
2008_6102A	TA13	Somerset	Maris Bard	17/06/2008	A2	13_A2
8042B					A1	2_A1
2009_7654A					A2	13_A2
2009_7122A					A1	7_A1

7.7 Appendix 7

Details of the GB *P. infestans* isolates used to determine the influence of genotype on the survival of tubers between infection and harvest 2011

ISOLATE	POSTCODE	REGION	CV		M TYPE	G'TYPE
2008_6082F					A2	13_A2
2009_7654A					A2	13_A2
2006_3928A					A2	13_A2
2008_6090A	TF6	Shropshire	Maris Piper	12/06/2008	A1	6_A1
2008_6422F	SG8	Hertfordshire	Russet Burbank	31/07/2008	A1	8_A1
2009_7122A					A1	7_A1
2008_7006D	EH39	East Lothian	Unknown	09/09/2008	A1	2_A1
2009_7126A					A1	6_A1

7.8 Appendix 8

2011 Tuber survival experiment; randomisation of trays in store (JF Niven Cold Store)
nine stacks, four high

Top	**7006D**
	7122A
	7126A
Bottom	**6082F**

7126A	**CONTROL**
CONTROL	6082F
6422F	7654A
7654A	**3928A**

6090A	**6422F**
7654A	3928A
7006D	6090A
7126A	**7122A**

6082F	**7654A**
7126A	7006D
CONTROL	7122A
7006D	**CONTROL**

3928A	**7122A**
6422F	6090A
6082F	3928A
6090A	**6422F**

door

7.9 Appendix 9

2011 Tuber survival experiment; experimental layout in field (South Holm, Auchincruive Estate)

Plant middle two rows only

Plant at 20 cm spacing within isolate plots (first and last tubers "on the line")

↑ **Oswald Hall**

Block 1	Block 2	Block 3	Block 4			
7006D	7122A	7126A	6082F	1.8	metres	↓
				2	metres	
7126A	CONTROL	6422F	7654A	1.8		**R**
				2		**I**
6090A	7654A	7006D	7126A	1.8		**V**
				2		**E**
6082F	7126A	CONTROL	7006D	1.8		**R**
				2		
3928A	6422F	6082F	6090A	1.8		
				2		**A**
CONTROL	6082F	7654A	3928A	1.8		**Y**
				2		**R**
6422F	3928A	6090A	7122A	1.8		
				2		↓
7654A	7006D	7122A	CONTROL	1.8		
				2		
7122A	6090A	3928A	6422F	1.8		
				32.2		
2.55	4.3	2.55	separation(m)			

7.10 Appendix 10

Details of the GB *P. infestans* isolates used to determine the influence of genotype on the survival of tubers between infection and harvest 2012

ISOLATE	POSTCODE	REGION	CV		M TYPE	G'TYPE
2008_6082F					A2	13_A2
7654A					A2	13_A2
3928A					A2	13_A2
2008_6090A	TF6	Shropshire	Maris Piper	12/06/2008	A1	6_A1
2008_6422F	SG8	Hertfordshire	Russet Burbank	31/07/2008	A1	8_A1
7122A					A1	7_A1
2008_7006D	EH39	East Lothian	Unknown	09/09/2008	A1	2_A1
7126A					A1	6_A1
8042B						
8986A						
8406A						
07/39					A2	13_A2
2008_6102A					A2	13_A2

7.11 Appendix 11

2012 Tuber survival experiment; randomisation of trays in store (JF Niven Cold Store) - 5 stacks, 10 high

Top	Control	8986A
	6422F	**8406A**
	6082F	Control
	8042B	**6422F**
	8986A	8042B
	6090A	**6090A**
	6102A	6082F
	7006D	**07_39**
	8406A	6102A
Bottom	**07_39**	**7006D**
Top	07_39	6422F
	6102A	**8042B**
	8406A	6102A
	6422F	**6082F**
	7006D	Control
	8986A	**07_39**
	Control	8406A
	6090A	**7006D**
	6082F	6090A
Bottom	**8042B**	**8986A**
Top	6422F	
	7006D	
	Control	
	6102A	
	6082F	
	8986A	
	8406A	
	8042B	
Bottom	07_39	
	6090A	

7.12 Appendix 12

2012 Tuber survival experiment; experimental layout in field (South Holm, Auchincruive Estate)

Plant middle two rows only ↑

Plant at 20 cm spacing within isolate plots (first and last tubers "on the line") **Oswald Hall**

Two rows of nine tubers per plot

Block N1	**Block N2**	**Block N3**	**Block N4**			**flow**
6102A	6082F	8042B	7006D	1.6	metres	↓
				2	metres	
6090A	6422F	Control	6090A	1.6		**R**
				2		**I**
7006D	8986A	6422F	6102A	1.6		**V**
				2		**E**
6082F	Control	8406A	8986A	1.6		**R**
				2		
Control	8042B	6102A	07 39	1.6		
				2		**A**
07 39	6090A	7006D	6422F	1.6		**Y**
				2		**R**
8042B	8406A	07 39	Control	1.6		
				2		
6422F	7006D	6082F	8406A	1.6		**flow**
				2		↓
8406A	6102A	8986A	6082F	1.6		
				2		
8986A	07 39	6090A	8042B	1.6		

2.6 3.4 0.6 3.4 2.6 3.4 0.6 3.4 2.6

separation(m)

2.55 4.3 2.55

7.13 Appendix 13

Background

There is concern that the curative efficacy of fungicides may be reduced for more aggressive genotypes/isolates of *P. infestans* but this has not been tested extensively. Two small experiments, carried out by SRUC outside the main project, examined this.

Materials and methods

King Edward (foliar resistance rating 3) leaflets on untreated plants were inoculated with a suspension of sporangia. The plants were then incubated under ideal conditions for infection and development of late blight lesions. At intervals 1, 2 and 3 days after inoculation leaves were either treated with fungicides rated ++ for curative activity or left untreated (control). After further incubation leaves were assessed for sporulating lesions and the mean percentage infection calculated, after angular transformation of the data. The results are presented as percentage infection and also percentage control. In the first experiment the latent periods for the 13_A2 and 8_A1 isolates were 4.5 and 6 days, respectively (Chapman, personal communication).

Results

No general conclusion regarding the relationship between fungicide curative activity and *P. infestans* genotype can be reached from the results of these small preliminary experiments because only one pair of isolates was compared in each experiment. However, in both comparisons fungicide curative activity was greater against the less aggressive isolate (Figs. 1a to 2 b).

Fig. 1a Impact of *P. infestans* isolate on curative activity of fungicides applied 1, 2 or 3 days after inoculation (2010)

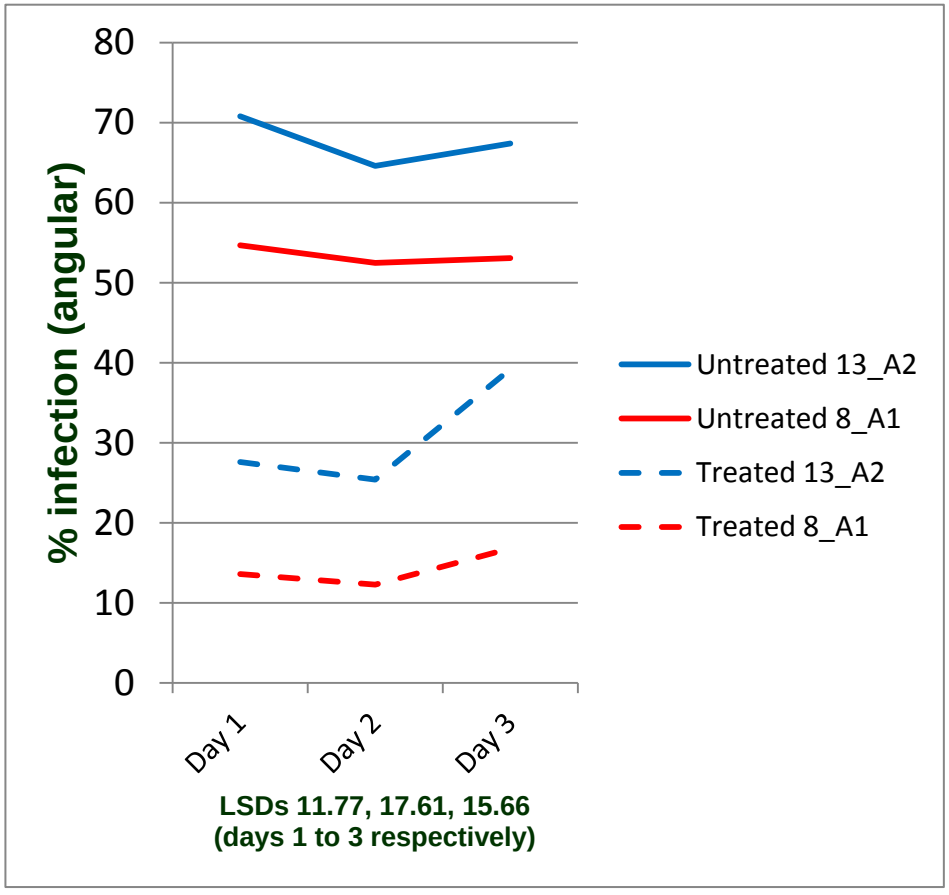
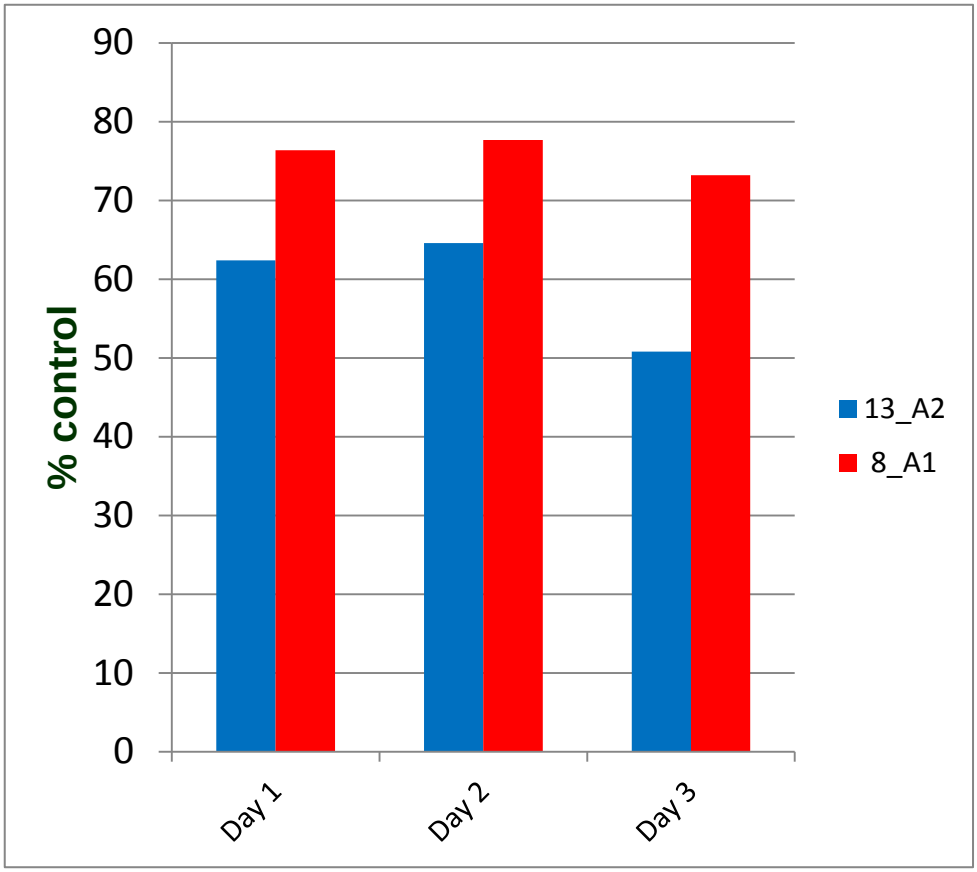


Fig. 1b Impact of *P. infestans* isolate on curative activity of fungicides applied 1, 2 or 3 days after inoculation (2010)



Temperature and isolate aggressiveness promote the speed of development of initial infections, allowing lesions to more rapidly reach the stage of development at which curative fungicides are no longer effective. However, other factors will boost curative activity, e.g. a higher level of cultivar resistance, through delaying the rate of colonisation of leaflets.

Acknowledgements
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