



Final Report

GB Late Blight Population Monitoring 2014 to 2018

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1. Summary

Aims

This report combines the results of the monitoring of changes in the population structure of *P. infestans* in GB over the 2014 to 2018 seasons, providing feedback to the industry on the impact of such changes for blight management.

Methodology

Characterisation of the GB *P. infestans* population was continued via the AHDB Potatoes 'Fight Against Blight' campaign with volunteer scouts providing samples of blight infected plant material during each growing season, from which the pathogen was isolated, characterised and genetically typed. In light of an increase in reports of storage problems at the end of the 2016 and 2017 growing season, data was also collected from tuber blight samples and is reported here. Lastly, the data for each year was integrated into the EuroBlight international late blight database allowing more detailed mapping and genotypic analysis to inform and interpret the results on GB populations in a wider European context.

Key findings

2014

- Disease pressure was high from the start of the season with the first of the 267 reported outbreaks on 9th May 2014 in Norfolk; two months earlier than the first in 2013. Sample provision peaked at 40 samples per week on 23rd June, one month earlier than the long-term average.
- A total of 705 samples were genotyped which showed the GB *P. infestans* population to be dominated by the 6_A1 and the 13_A2 clonal lineages at 60 and 28%, respectively. The 8_A1 lineage remained present at a low level (3%) and the genetically diverse class of pathogen types (termed 'Other') comprised 9% of the population.
- An analysis of early and severe blight outbreaks in the Midlands and Lancashire showed that the primary inoculum was of genotypes found in these locations in previous seasons. This suggests that local volunteers or outgrade piles contribute a significant component of early disease risk.
- Outbreaks in eastern Scotland were dominated by a single clone (6_A1) in Angus but a diverse population was found northeast of this in Aberdeenshire and Moray. The latter suggests that some late blight outbreaks are caused by sexually generated oospores in this region but there is no evidence, at this stage, that these forms of the pathogen are more persistent or damaging than the well-adapted clones.

2015

- In contrast to 2014, the 2015 season was not conducive to late blight development and only 58 positive outbreaks were reported. The low disease pressure was due to a generally dry start to the season and below average temperatures when rain did occur later.
- A total of 222 samples were genotyped and a decrease in the frequency of both 6_A1 and 13_A2 at 46 and 19%, respectively was observed. Another clone (8_A1) increased

slightly in frequency to 5% and the proportion of the genetically diverse class of pathogen types (termed 'Other') increased to 29% of the population.

- The increased proportion of genetically diverse novel ('Other') types of *P. infestans* was again focussed in northeast Scotland and suggested oospore infection occurred. There was again no evidence that these genotypes were more aggressive than the dominant clonal lineages. Nonetheless, the presence of this additional source of inoculum is a concern.

2016

- The 2016 season was conducive to late blight development and 176 positive outbreaks were reported with the first in England being at the end of May and the start of July in Scotland. Peak sample reception was in late June indicating that the disease had become established early.
- A total of 500 samples were genotyped with the data showing a decrease in the frequency of both 6_A1 and 13_A2 at 57 and 21%, respectively. A new clone, 37_A2 was sampled for the first time in 2016 and comprised 3% of the population. Tuber blight samples infected with 37_A2 were noted in the Midlands. The proportion of the genetically diverse class of pathogen types (termed 'Other') decreased to 16% of the population.

2017

- The sample receipt over the 2017 season was much later than in previous years due to an unusually dry April that suppressed early sources of primary inoculum. Sample reception peaked at 23 samples per week on 21st August. A wet September in the Midlands resulted in a late and challenging harvest. In total 158 positive outbreaks were reported with more samples from volunteer potatoes than in previous seasons due to the interest in new clonal populations.
- A total of 495 samples were genotyped with the data showing a further decrease in the frequency of both 6_A1 and 13_A2 to 50 and 11%, respectively. The new fluazinam-insensitive clone, 37_A2 increased markedly from 3 to 24% of the sampled population and increased its range from the Midlands to Yorkshire, Kent and Suffolk. Another new lineage, 36_A2, appeared in British crops for the first time. The proportion of the genetically diverse class of pathogen types (termed 'Other') reduced to 10% of the sampled population.

2018

- Blight reporting began early after a slightly warmer and wetter than average April with the first three sampled outbreaks from outgrade piles in Kent from 13-17th April. After a scatter of samples from Wales and the West Country in May, hot and dry conditions prevailed across much of the UK for June and July which suppressed blight development. Sample reception was well below average with only 40 outbreaks reported over the whole season.
- In total 185 samples of blight infected material were genotyped and data followed the trend set in 2017 with a reduction in frequency of the clones 6_A1 from 50 to 46% and 13_A2 from 11 to 6% of the total samples. The fluazinam insensitive clone 37_A2 decreased from 24 to 17% of the sampled population but a new clone, 36_A2 increased from 2 to 18%. With the exception of the finding of 37_A2 in western Scotland, the range of both these clones remained similar to 2018. The proportion of the genetically diverse

class of pathogen types (termed 'Other') remained around the average at 11% of the sampled population.

- In an unusually warm dry season the pathogen distribution was restricted and the sampling frequency below average which may skew the findings compared to previous seasons. Anecdotal reports suggested the 36_A2 clone was a challenge to manage later in the season in some crops. Tuber blight testing was not targeted in 2018 and few samples were submitted.
- Data from the 2012 to 2018 AHDB Potatoes-sponsored FAB campaign has now been uploaded to the EuroBlight database allowing the GB data to be viewed in the context of the mainland European population (see <http://euroblight.net/>).

Data from this study were disseminated to the industry via presentations at AHDB Potatoes events such as the Winter Forums (2014-2015), AHDB Agronomists' Conference (2016-2018), Potatoes in Practice, British Potato and also via press releases and articles in the agricultural press (Crops, Crop Production Magazine, Potato Review, Farmers Weekly, AHDB Grower Gateway etc). Isolates and DNA from isolates were also provided to the agrochemical industry in support of baseline sensitivity monitoring and other product stewardship issues, as required. Fungicide sensitivity testing was also conducted and is reported separately.

2. 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 blight management approaches.
- In 2014 and 2015, the continued dominance of two aggressive and fit genotypes (6_A1 and 13_A2) comprising 76% of the population indicated that existing best practice guidelines should be effective in blight management. However, growers needed to be aware of the risks of blight occurring from crop emergence onwards particularly after mild winters when primary inoculum survived on host foliage. Fungicide application intervals had to be kept short when disease pressure was high.
- The emergence and spread of a new clone, 37_A2, from 2016 to 2017 with its reported insensitivity to fluazinam increased the need to follow FRAG-UK guidelines. Some reports suggest the industry is responding with reduced use of fluazinam in the areas where this genotype is found. The first appearance of 36_A2, in 2017 and its expansion in 2018 is also a cause for concern. Sensitivity testing of isolates of the 37_A2 and 36_A2 clones against fluopicolide, mandipropamid, propamocarb, cyazofamid and fluazinam was completed at the James Hutton Institute in 2018. Apart from confirming the insensitivity of 37_A2 isolates to fluazinam the study did not show any other significant change in efficacy at field rates. However lesions caused by 36_A2 isolates were slightly larger than those caused by other genotypes at very low doses of all five products which may be a factor in its competitive advantage in field populations. All data was uploaded to EuroBlight for mapping and further genetic analysis.
- An increase in reports of tuber blight in 2016 and 2017 raised the possibility that late-season fluazinam use for tuber blight control may be providing a positive selection pressure for the aggressive 37_A2 lineage and resulting in tuber blight control failures.

The 37_A2 clone was widely found in tuber blight samples tested in these seasons. Data from this study was thus provided to the Fungicide Resistance Action Group to update the industry guidelines.

- Continued evidence that early season outbreaks were caused by local inoculum highlights the importance of volunteer and cull pile management in reducing primary inoculum load.
- Oospore inoculum is important in some regions of Europe but, within the GB industry, the risks remain low. Evidence points to oospores infecting crops in some regions and in particular, northeast Scotland. It is important to be aware of the potential threat of this form of inoculum and for scouts, growers and advisors to remain vigilant. Rotations should be kept as long as possible as soil-borne oospore inoculum degrades over time. Infection of volunteer potatoes from soil-borne inoculum continues to be a concern. In particular, infected potato volunteer plants on fallow land produce inoculum as legislation to protect the environment prohibits chemical crop protection on such land.

3. Introduction

Potato late blight, caused by *Phytophthora infestans*, continues to be a significant threat to potato crops in the UK. The pathogen attacks the leaves, stems and tubers and, if not adequately controlled, can result in crop failure. The pathogen population is not however stable and will change in response to several factors, singly or in combination; selection pressure from management practices such as fungicide or host resistance deployment; genetic change due to either mutation or sexual recombination within the existing population or the introduction of new lineages from beyond the UK border; lastly, chance events related to the dramatic change in population size between seasons and driven by the weather (so called genetic drift and founder effects). There are two main implications of population change; the new populations are likely to have traits that differ from the previous population (e.g. aggressiveness, virulence and fungicide resistance) and therefore influence blight management and secondly, the risk that both pathogen mating types interact to form long-lived soil-borne inoculum (oospores). 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 continue to take account of the traits of the contemporary population (Kessel et al., 2018).

Previous research funded by AHDB Potatoes as part of the Fight Against Blight (FAB) campaign has demonstrated the value of genetic fingerprinting (Cooke & Lees, 2004, Lees et al., 2006, Cooke et al., 2012) in tracking pathogen population change. The methods depend on fingerprinting technology similar to that used in criminal forensics that examines genetic diversity of *P. infestans* at twelve locations (loci) within the pathogen genome. These microsatellite, or simple sequence repeat (SSR), markers are sections of DNA with repeated sequence motifs (e.g. AGAGAGAG or GCAGCAGCA) which are prone to expansion and contraction mutations that alter their length. Such variation generates different forms, recognised as alleles at a locus. This length variation is detected by running each PCR-amplified fluorescent dye-tagged DNA fragment in a capillary electrophoresis instrument against a size standard. The resultant allelic data for 12 loci is very powerful as it can discriminate between clonal lineages and detect minor variation within a lineage (Li et al., 2013a). In addition, the genetic diversity data allows genetically

unique pathogen types that may have arisen via sexual recombination to be detected. If the data from such genetic fingerprinting is used in combination with a study of pathogen traits such as aggressiveness, virulence, fungicide resistance, mating type and response to temperature then such knowledge improves decision support systems and effective disease management (Cooke et al., 2014, Chapman, 2012, Cooke et al., 2013). A clear example was the 13_A2 lineage (blue 13) of *P. infestans* that was first detected in Germany and the Netherlands in 2004 (Li et al., 2012, Cooke et al., 2012). Populations in GB (Day et al., 2004, Cooke et al., 2003) and Northern Ireland (Cooke et al., 2006) had been dominated by mating type A1 lineages in the years prior to 2005 but an increase in the A2 type in that year led to more detailed AHDB-funded studies in the 2006-2008 seasons (Cooke et al., 2009). This chronicled the migration and spread of 13_A2 in 2005-2009 (Cooke et al., 2012) and the subsequent emergence of genotype 6_A1 (pink 6) which was first recognised in the Netherlands in 2002 (Li et al., 2012, Kildea et al., 2012). Both lineages were found to be highly aggressive and fit and 13_A2 is resistant to metalaxyl (Cooke et al., 2012). Another new lineage with insensitivity to fluazinam (33_A2) emerged in 2009 in the Netherlands and comprised 22% of Dutch samples in 2011 (Schepers et al., 2018). It was followed by further fluazinam insensitivity with the appearance of 37_A2 in 2013 (Schepers et al., 2018). Another lineage called 36_A2 was first identified in the potato starch production regions of northern Germany and the Netherlands in 2014 and spread in subsequent seasons. This spread of the pathogen from mainland Europe to British crops mirrored the situation in 1845 when potato blight first occurred in Europe (Bourke, 1964). In Britain, genotypes 13_A2 and 6_A1 were initially prevalent in southeast England, but spread north in subsequent years to become dominant in all regions. This pattern of migration probably reflects a mix of local crop-to-crop spore dispersal with occasional longer distance events during windy overcast weather; spores are killed rapidly by UV light (Skelsey et al., 2018). An additional source of longer distance spread is via GB produced or imported seed tubers.

The risk of oospores forming has increased following the spread of the 13_A2 genotype which is of the A2 mating type. The A2 mating type itself is not inherently more damaging than the A1 type but where A1 and A2 mating types are present in the same outbreak they may co-infect a plant, meet and form oospores. Such propagules end up in the soil as the crop rots and can survive for many years in the absence of the host plant. Each germinating oospore generates a new genotype of *P. infestans* with a new combination of traits. It is this sexual recombination that drives increases in pathogen diversity and a risk of accelerated host resistance breakdown and an increased risk of fungicide resistance. In recent years, the majority of late blight samples from British crops have shown the population of *P. infestans* to be dominated by clonal lineages which are, by definition, asexual. A low frequency of novel types of the pathogen have been observed each year which suggests that novel sexually recombinant strains of *P. infestans* do not make a significant contribution to the disease pressure. However, populations in other countries such as Norway, where A1 and A2 have been present in an equal ratio for longer, are more genetically diverse (Brurberg et al., 2011, Sjöholm et al., 2013, Yuen & Andersson, 2013). Similarly, in the Netherlands greater pathogen diversity than in GB crops has been recorded (Li et al., 2012).

In addition to creating genetic diversity, the presence of oospores in soil acts as an extra source of primary inoculum that can survive for several years (Turkensteen et al., 2000) and results in greater and earlier disease pressure, in particular in the early part of the season (Brurberg et al., 2011, Sjöholm et al., 2013, Yuen & Andersson, 2013, Cooke & Andersson, 2013, Bødker et al., 2005, Lehtinen & Hannukkala, 2004, Drenth et al., 1995). It is generally considered

that warm and wet conditions from planting to emergence will increase the risk that oospores will germinate and cause early infection.

As stated above, it is essential to examine the population of *P. infestans* in Britain in the context of that on crops in continental Europe which have proven a source of our recent clonal lineages (Cooke et al., 2012). EuroBlight, a network of European researchers and commercial companies studying pathogen population, breeding for resistance, agrochemical use and decision support systems (www.euroblight.net) provides a good opportunity to integrate applied research. The EuroBlight consortium has developed a pathogen population database hosted at the University of Aarhus which provides a platform for mapping the data and comparing genetic diversity across different parts of Europe. AHDB Potatoes FAB data on *P. infestans* from GB crops from 2014 to 2018 has been uploaded onto this database and will be presented in this report.

4. Experimental Section

Outbreak sampling

Following discussion with AHDB Potatoes it was decided that the target of 400 isolates to be characterised each year would consist of four isolates per outbreak from 100 outbreaks to provide the best compromise between breadth and depth of sampling. Over the 2006-2008 seasons eight samples were provided per outbreak. Scouts engaged in the AHDB Potatoes FAB campaign collected up to 4 late blight lesions per crop which were located by postcode district and sent to Fera Science Ltd for confirmation as blight. Once confirmed, the FAB map was updated, generating a red spot for each outbreak in the 2014, 2015 and 2016 seasons (Figs. 1, 2 & 3). The FAB web page was updated in 2017 and the format of the map changed (Figs. 4 & 5). Accompanying information relating to each sample was also recorded. Positive samples were placed within small potato tubers and sent in batches to The James Hutton Institute (Dundee) for further testing. Many samples from scouts in northeast Scotland were provided direct to James Hutton Institute. Late blight disease pressure was exceptionally high in 2014 and towards the end of season it was clear that many more samples were being provided than it was possible to process due to financial and staff resources. Rather than lose samples, analysis of a subset of samples was thus restricted to DNA analysis only. Lesions from postcode districts that had been sampled previously were stored on FTA cards. This involved pressing sap from the growing edge of actively sporulating late blight lesions (Fig. 6) onto Whatman FTA cards (Whatman™ WB120205) which have been demonstrated to effectively preserve the *P. infestans* DNA for later genetic analysis (Li et al., 2013a). In 2017 and 2018 FTA cards were also tested by some scouts in combination with the standard FAB sampling to shorten the time from sampling to results being available.

Sample processing

Upon arrival at The James Hutton Institute, 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 freshly cut surface uppermost. After 1-4 days incubation at room temperature (ca. 19°C) tuber tissue sporulating with *P. infestans* was plated onto a primary isolation plate of a 50:50 mix of Pea and Rye A agar with antibiotics. An improvement using 'wanding' was used from 2015 onwards to decrease the risk of bacterial contamination and increase isolation success. This involved cutting a 5 x 5 mm square of isolation media and gently touching the sporulating area of the tuber tissue. The agar plug plus sporangia was transferred back to the isolation plate until signs of clean mycelial growth were observed. After further culturing (ca. 19°C) on a secondary isolation plate,

the culture was plated onto a series of media as follows; a pea broth plate to yield mycelium for subsequent DNA extraction, two plates each pre-inoculated with either the A1 or A2 tester strain and finally a Rye A agar screw-cap slope for longer-term storage. Each of these plates was further incubated at ca. 19°C. After ca. 7 days the pea broth cultures were rinsed in sterile distilled water, the agar plug removed and the mycelium was freeze-dried and stored. Once the tester and unknown isolate colonies had grown together for several days, the central zone of the agar plate was examined under the microscope for the presence of abundant oospores at the interface of the two colonies that would indicate that the unknown isolate 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. In 2016 and 2017, tuber samples were also provided direct to the James Hutton Institute. These were washed and cut in the same way as the foliar samples. Isolation and harvesting of small amounts of mycelium for direct DNA extraction were conducted to increase the likelihood of success.

Testing genetic diversity of isolates

In most samples small fragments (ca. 2 mm³) of freeze-dried mycelium were used for DNA extraction using a 'Quick and Easy' protocol modified from Wang and Cutler (1993). The DNA (1 µl) was subsequently used for SSR analysis with a 12-plex marker set (Li et al., 2012). In other cases, 2mm disks were cut from the interface of the green and brown zone of the lesions pressed onto FTA cards (Fig. 6), washed with the FTA Purification Reagent (Whatman™ WB120204) according to the manufacturer's instructions and the disk used in the 12-plex PCR. The SSR allele peaks were manually checked and scored prior to export to excel spreadsheets for further analysis. The centroids of each postcode district were converted to latitude and longitude data and the associated outbreak data (cultivar, date and outbreak type) were also entered into the Euroblight database (www.euroblight.net) for further genetic analysis and genotype mapping.

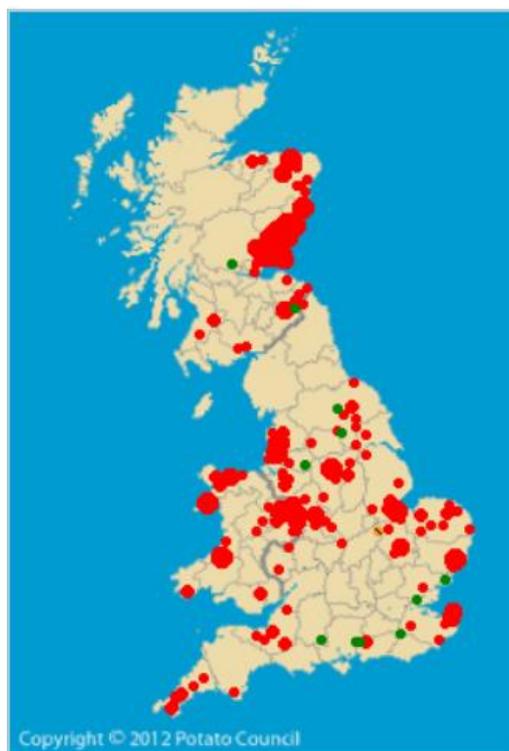


Figure 1. Locations of the late blight outbreaks in 2014 recorded by the FAB campaign. Green indicates reported outbreaks that tested negative for *P. infestans*.

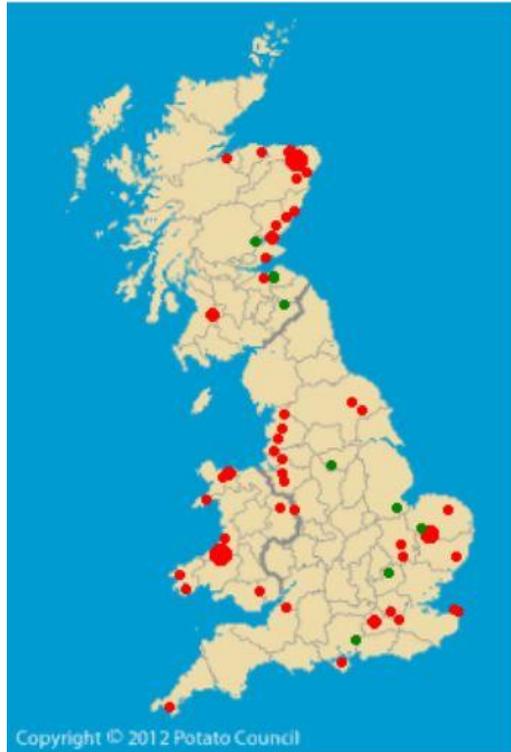


Figure 2. Locations of the late blight outbreaks in 2015 recorded by the FAB campaign. Green indicates reported outbreaks that tested negative for *P. infestans*.



Figure 3. Locations of the late blight outbreaks in 2016 recorded by the FAB campaign. Green indicates reported outbreaks that tested negative for *P. infestans*.

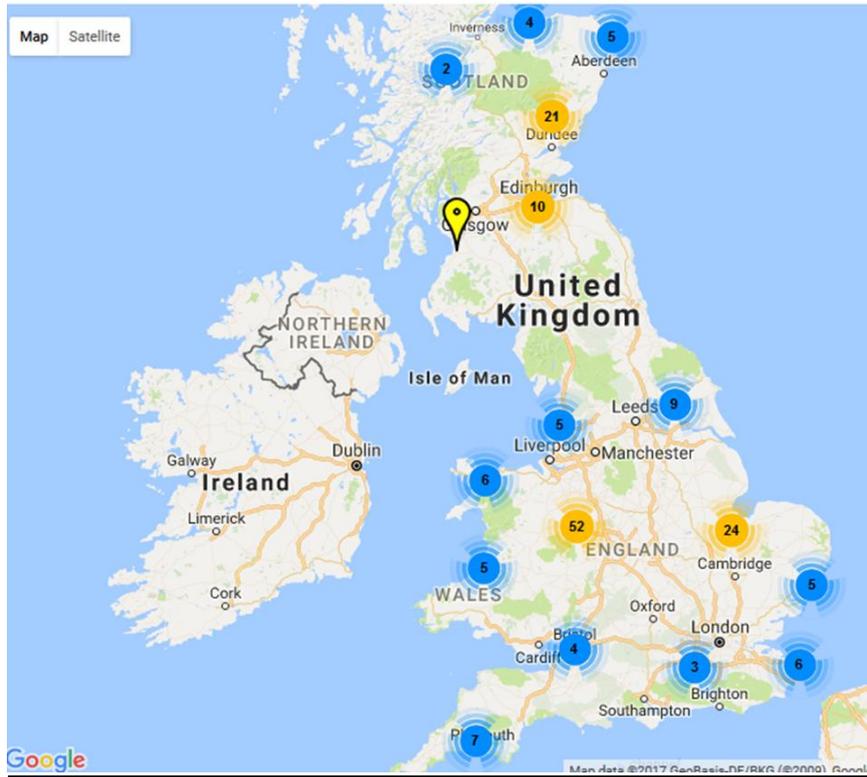


Figure 4. Locations of the late blight outbreaks in 2017 recorded by the FAB campaign.



Figure 5. Locations of the late blight outbreaks in 2018 recorded by the FAB campaign.

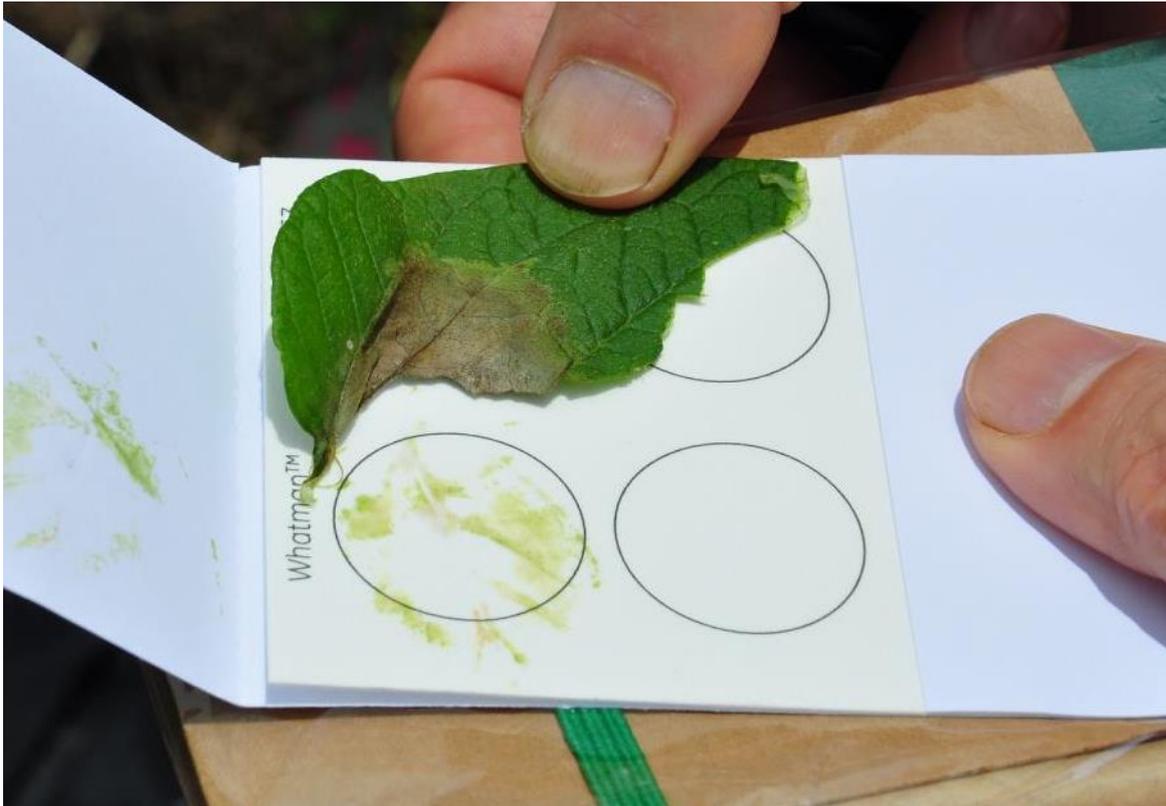


Figure 6. Example of a blight lesion pressed onto an FTA card in the field.

Results

2014 Sampling

Late blight outbreaks began early in 2014 with the first reported outbreak on 9th May in Norfolk. The only earlier sample in the past 12 years was on 4th May in 2007. An early surge in blight samples was seen in June with a peak of 40 samples per week arriving on 23rd June 2014 (Fig. 7). In total, more than 1000 late blight samples from 267 disease outbreaks across GB (Fig. 1) were delivered to the James Hutton Institute. All outbreaks were located by postcode district (e.g. DD2, LL53). From these samples, 600 isolates of *P. infestans* were obtained.

2015 Sampling

Late blight outbreaks began very late in 2015 with the first positive outbreaks reported on 31st June (Kent) and 1st July (Ayrshire). Sample reception showed a different pattern from previous years with a much flatter and later peak of less than 10 samples arriving per week from late July to late August (Fig. 8). In total only 289 late blight samples from 58 disease outbreaks across GB were delivered to the James Hutton Institute. From these samples, 107 isolates of *P. infestans* were obtained.

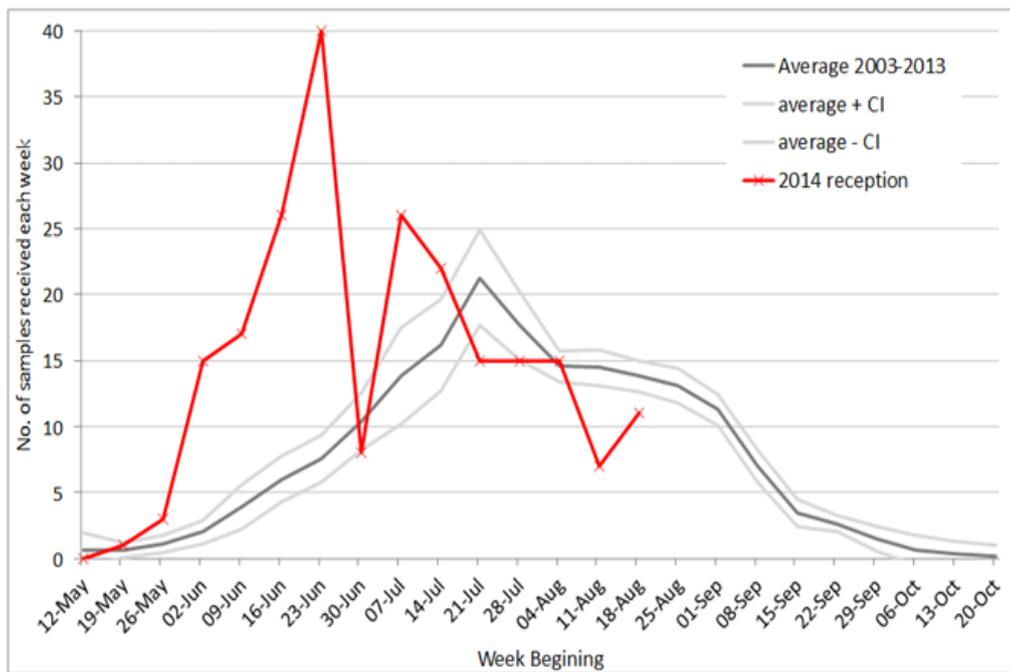


Figure 7. The average number of Fight Against Blight samples received each week from 2003-2013 (grey) compared to that in 2014 (red). Collated by Moray Taylor (Fera, Sand Hutton, York).

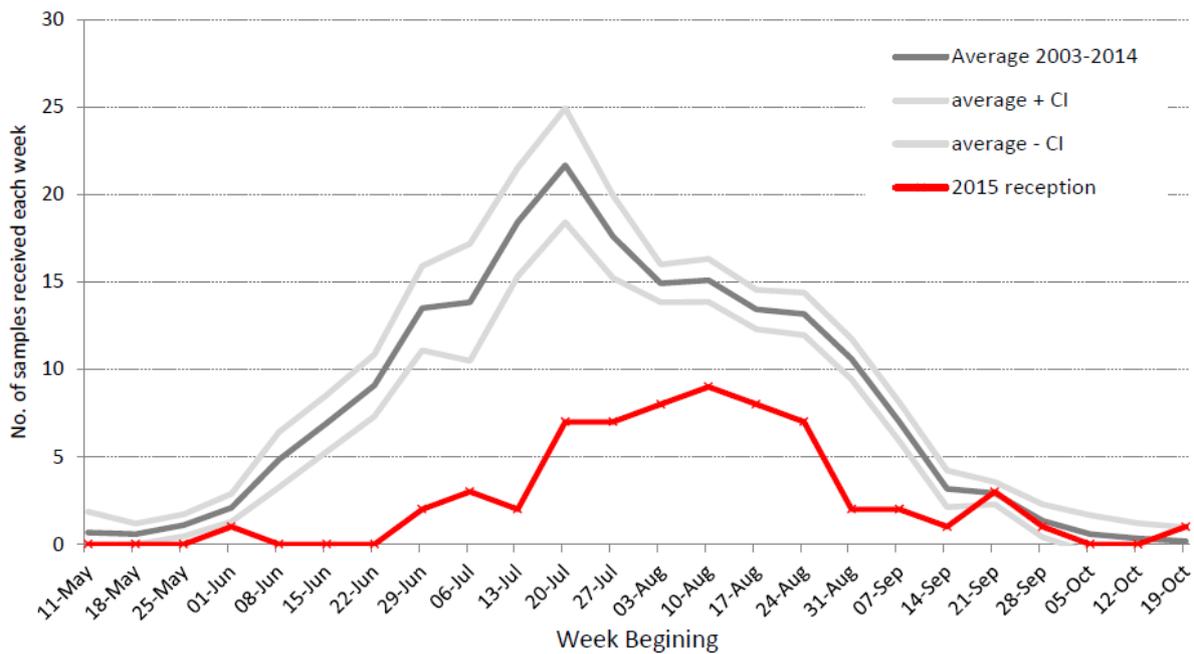


Figure 8. The average number of Fight Against Blight samples received each week from 2003-2014 (grey) compared to that in 2015 (red). Collated by Moray Taylor (Fera, Sand Hutton, York).

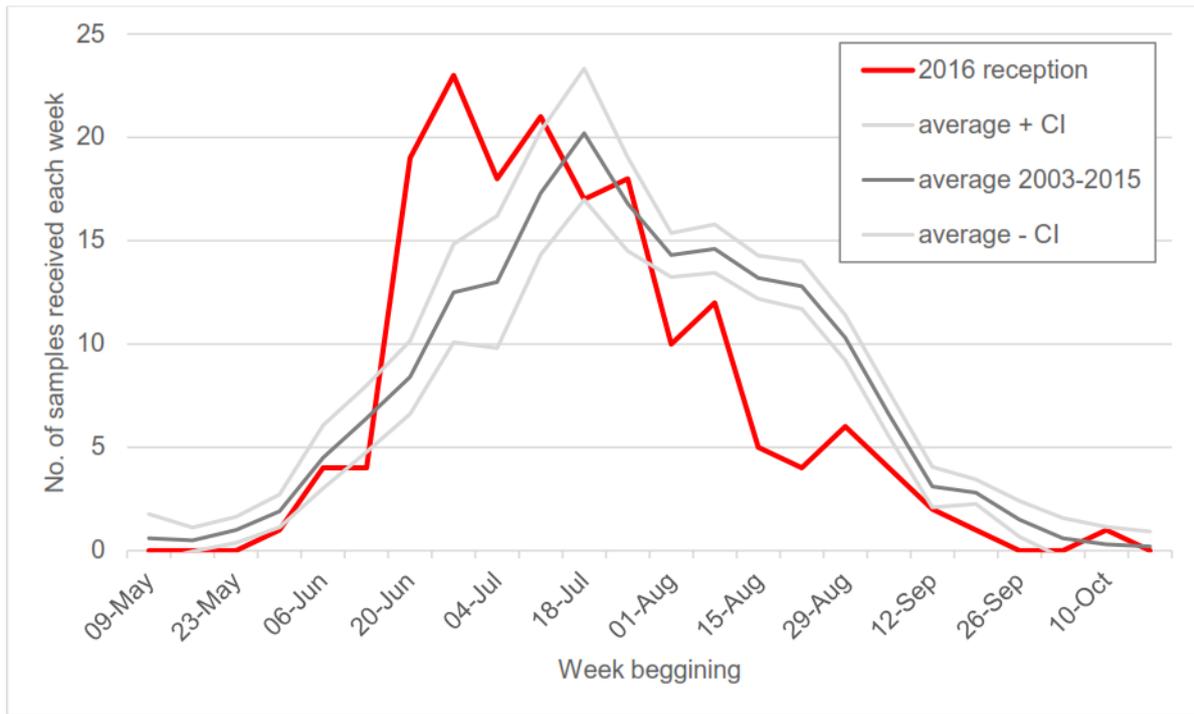


Figure 9. The average number of Fight Against Blight samples received each week from 2003-2015 (grey) compared to that in 2016 (red). Collated by Moray Taylor (Fera, Sand Hutton, York).

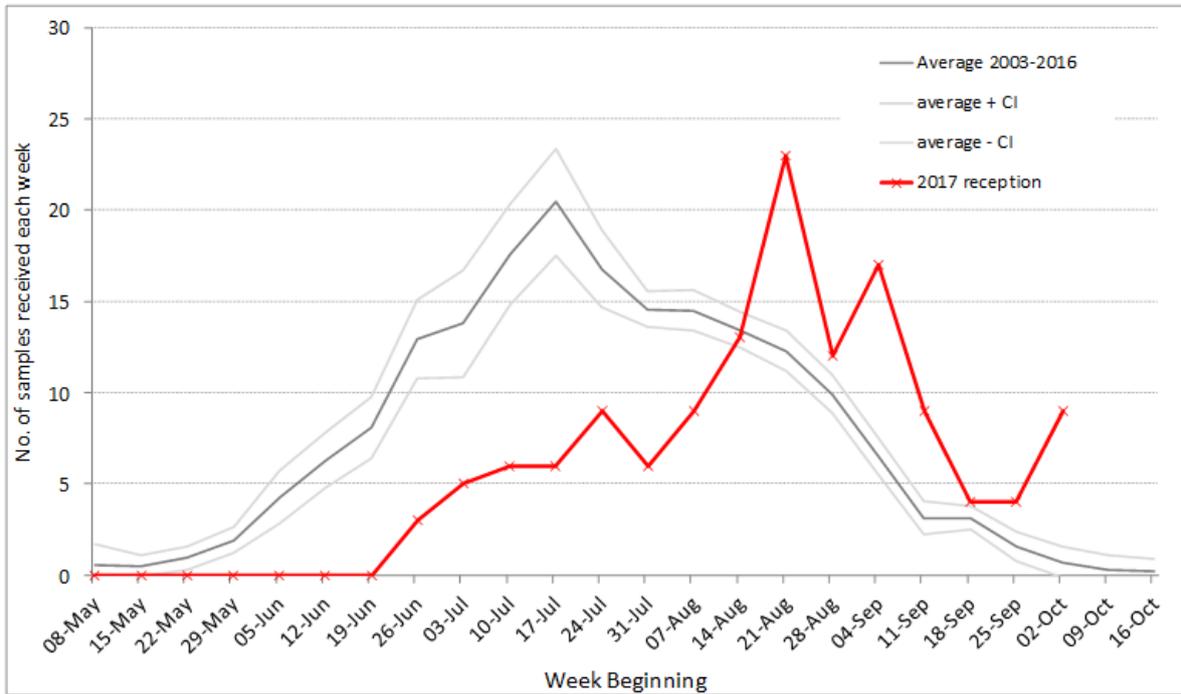


Figure 10. The average number of Fight Against Blight samples received each week from 2003-2016 (grey) compared to that in 2017 (red). Collated by Moray Taylor (Fera, Sand Hutton, York).

2016 Sampling

Late blight outbreaks began at a fairly typical time in 2016 with the first positive outbreaks reported on 17th May (Cornwall) and 16th July (Highland). Sample reception then showed a slightly earlier pattern than in previous years with a typical rate of over 20 samples arriving per week from mid-June to mid-July (Fig. 9). In total, 664 late blight samples from 176 disease outbreaks across GB were delivered to the James Hutton Institute. From these samples 454 isolates of *P. infestans* were obtained. In addition, 68 samples from 14 batches of tubers with suspected tuber blight infection were provided direct to the James Hutton Institute.

2017 Sampling

Although the first blight outbreak reported was from an outgrade pile in Kent on April 4th the blight epidemics were, in general, late in 2017. Eight samples were reported from Scottish crops in June but it was not until after a single positive outbreak reported on 3rd June (Yorkshire) that multiple outbreaks were reported in England in early July. Sample reception showed with a much later peak (Fig. 10) than from previous years. In total 666 late blight samples from 158 disease outbreaks across GB were delivered to the James Hutton Institute. In addition, 110 samples from 31 batches of tubers with suspected tuber blight infection were provided direct to the James Hutton Institute.

2018 Sampling

As in 2017, the first reported outbreak in 2018 was from an outgrade pile in Kent in April. The 2018 season was also late; a slight increase in samples in June was followed by a June and July that were considerably warmer and drier than the 1981-2010 averages and suppressed late blight activity in most areas (Fig 5 & 11). Sample numbers were the lowest in seven years and there was no single peak in sample reception (Fig.11). In total 270 samples were supplied to the James Hutton Institute from 41 outbreaks and this included six samples of tuber blight.

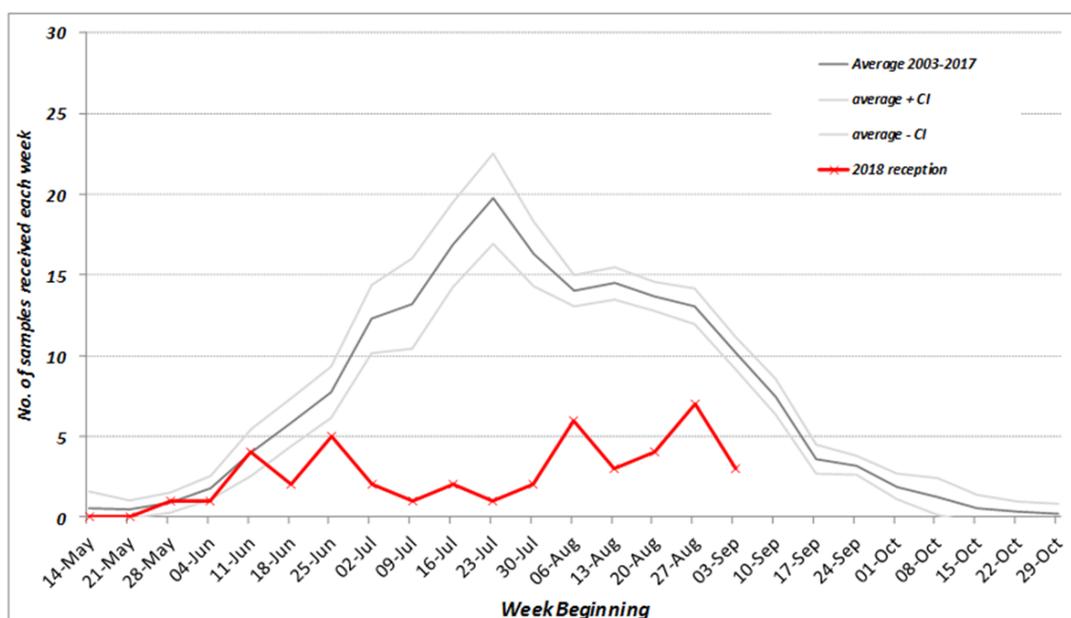


Figure 11. The average number of Fight Against Blight samples received each week from 2003-2017 (grey) compared to that in 2018 (red). Collated by Moray Taylor (Fera, Sand Hutton, York).

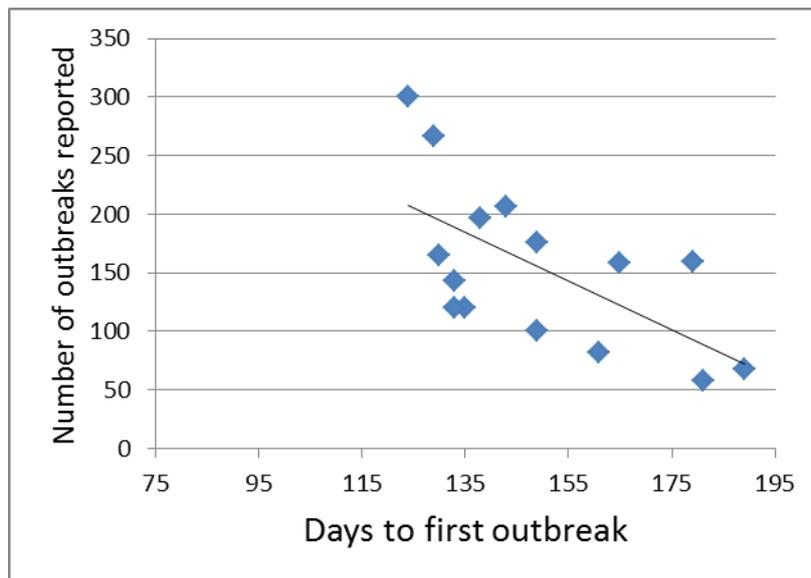


Figure 12. Relationship between the date of the first reported GB late blight outbreak (day of the year) and the total number of samples received in that year. In most years outbreaks began in May (from day 122-152). Data shown are for 15 years from 2003 to 2017.

Data on the date of arrival of the first sample for the 15 years from 2003 to 2017 showed a clear relationship between earliness and the total number of late blight samples received that year (Fig. 12) and that late blight was first reported in the month of May in 10 of the 15 years to date (data not shown). Data for the unusually dry 2018 season was not included in the above analysis.

Genetic diversity of isolates (2014 to 2018)

An efficient genotyping system in which 12 SSR loci (positions in the pathogen genome) were PCR amplified in a single reaction was used (Li et al., 2013a). The alleles, defined as one of a number of alternative forms of the same DNA fragment at the same genetic locus, were scored for each *P. infestans* sample. The combinations of alleles for each isolate were collated in a spreadsheet and those combinations found in multiple isolates from many outbreaks and over more than one season were defined as a clonal genotype. These were named in a series using a number and their characteristic mating type (e.g. 1_A1, 2_A1, 3_A2 etc.; (Cooke et al., 2012). The system matches that used with an EU_ prefix in the EuroBlight system. An additional 'catch all' category of genotype termed 'Other', was defined for all isolates with novel combinations of alleles found at a very low frequency and commonly in only a single blight outbreak and in a single season. The overall numbers and frequencies of all the different genotypes in each of the 16 seasons characterised to date are shown (Fig. 13).

The genotyping results of the 705 isolates from 2014 showed that 90% of the samples were of three clonal lineages (6_A1, 13_A2 and 8_A1) in a ratio like 2012. These lineages had dominated British late blight outbreaks from 2007 to 2016. In 2015, the proportions of these three lineages remained similar but an increase in the 'Other' category from 9.4 to 29.3% was observed (Fig. 13). Genotype 8_A1 remained persistent, having been in the UK since at least 1995 (data not shown). The 13_A2 genotype remained the single most dominant type up until 2010 then declined in the unusual 2011 season when there was little late blight in English and Welsh crops but a local 6_A1-dominated epidemic occurred in eastern Scotland (see previous project report for details; (Cooke et al., 2013)). The 13_A2 genotype has recovered in recent years from a low

of 7% in 2011 to 19-21% in 2015 and 2016 and recently declining again to 11% in 2017 and 6% in 2018.

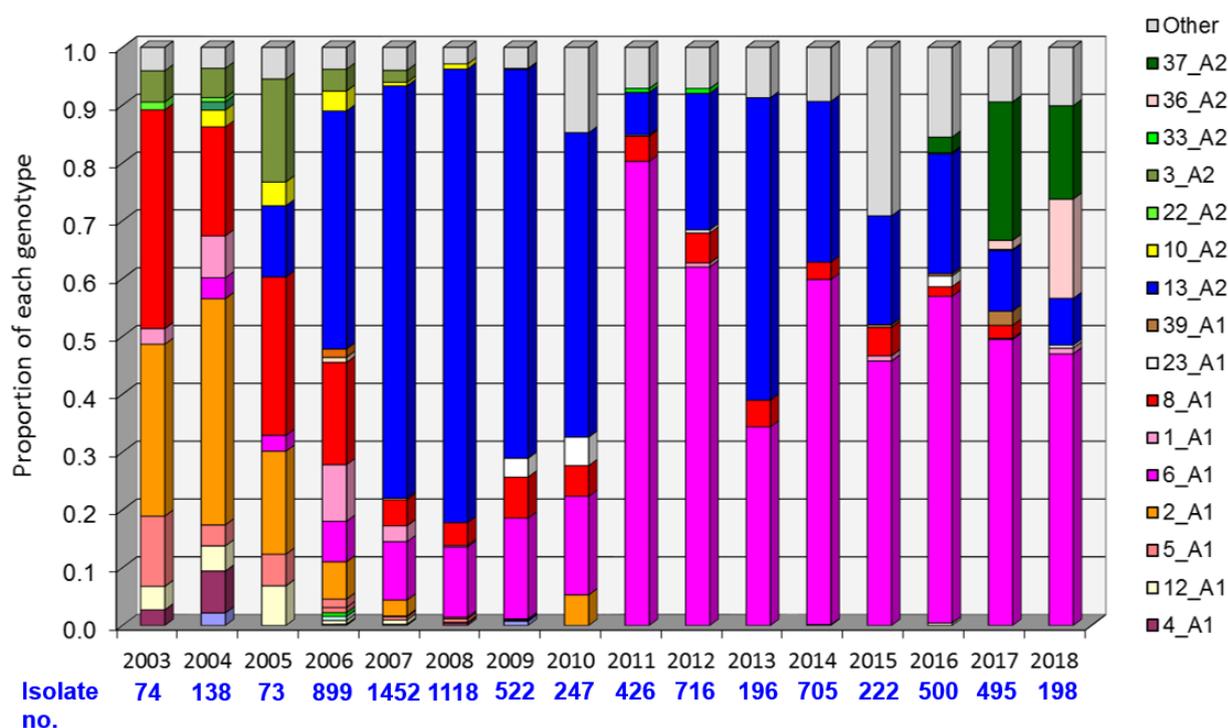


Figure 13. Bar chart indicating the frequency of *P. infestans* isolates of each SSR genotype over the course of 15 seasons (2003-18) and the number of genotyped samples per year.

Despite the 6_A1 lineage being first detected one season before 13_A2, its frequency remained low from 2004 to 2010 but has been dominant since then and comprised 46, 56, 49 and 47% of the sampled population in 2015-2018, respectively. The first fluazinam insensitive lineage named 33_A2 or green 33 had not been sampled in GB since the outbreaks in southeast England (1 late 2011 and 3 in 2012) but was sampled once per year in 2016 and 2017. Two new clones have appeared in the last two to three years; 37_A2, comprised 2.7% of the sampled GB population in 2016 increasing markedly to 24% in 2017 before reducing to 17% in 2018 and 36_A2 which was first sampled in 2017 increased from 1.6 to 18% over the past two seasons. Clone 39_A1 is of note as it has emerged on tomato and potato crops in GB and continued to cause problems in some glasshouse production in 2018.

A breakdown of the population data by country, indicated regional differences with the greatest recent change being in England (Fig. 14). Although 6_A1 predominated in all three countries, the proportion of 13_A2 was markedly lower in crops sampled in Scotland than in those from England or Wales from 2014 onwards. Note that the sampling depth varies from season to season (Fig. 15) and the low sample numbers in 2013, 2015 and 2018 increases the probability of a skew in datasets coming from relatively few outbreaks. This GB data shows the recently emerging clone 37_A2 has, with the exception of a single isolate in Ayrshire, Scotland, been sampled exclusively from England. Clone 36_A2 has not yet been sampled outside of England (Fig. 14) but data from the past two seasons suggests the range of these clones will increase.

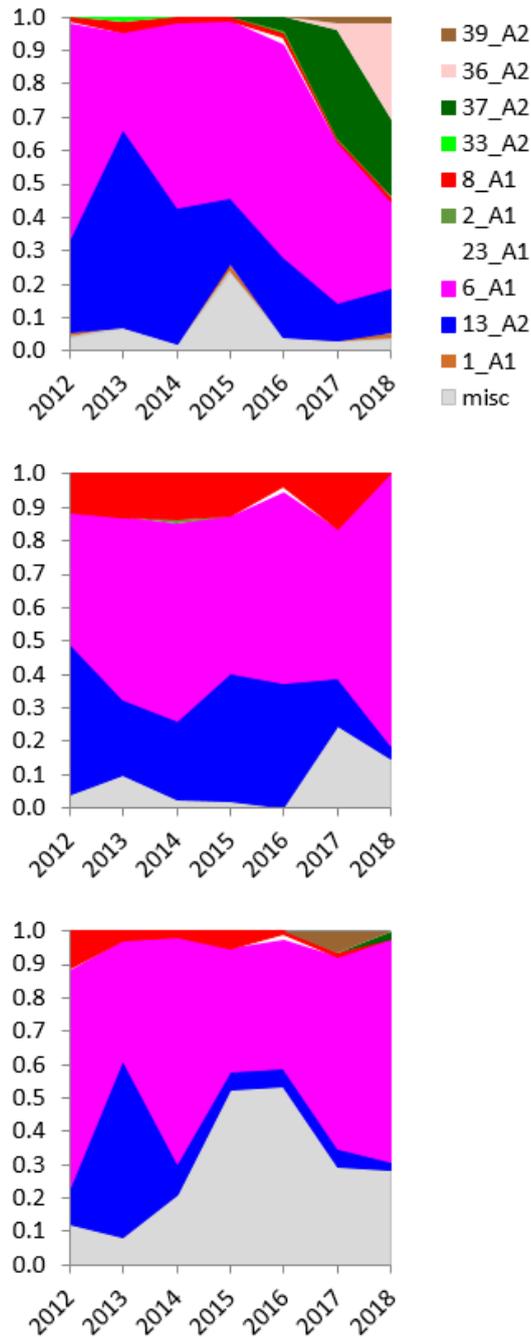


Figure 14. The proportion of different clonal genotypes of *P. infestans* from blight outbreaks sampled over the 2012 to 2018 seasons from top) England ($n=1788$) middle) Wales ($n=344$) and lower) Scotland ($n=771$).

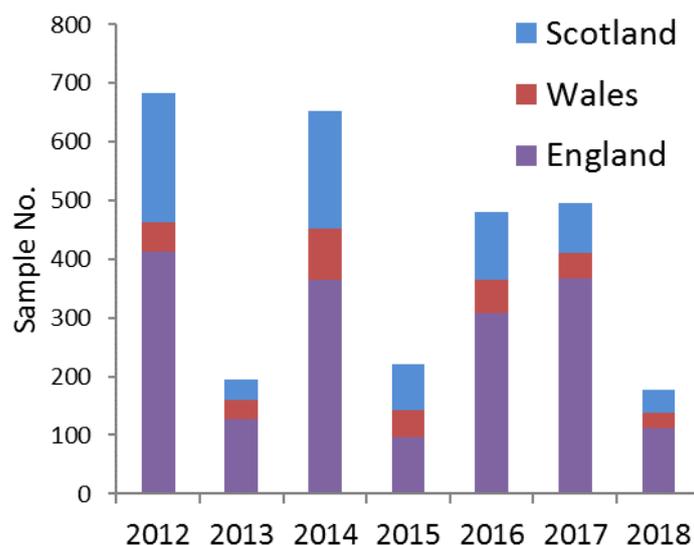


Figure 15. The total number of late blight samples and country of origin for the seasons 2012 to 2018.

The submission of the 2013-2018 FAB data to the EuroBlight database allows the outbreaks to be mapped according to genotype and compared to those from crops in mainland Europe (Figs. 16-23). The mapped outbreaks are open-access and can be viewed online <https://agro.au.dk/forskning/internationale-platforme/euroblight/pathogen-characteristics-and-host-resistance/genotype-map/>. The low number of outbreaks sampled in 2013 compared to 2014 is apparent (Fig. 16 and Fig. 17a). The 2014 data indicates a population in Northern Ireland that was dominated by genotype 8_A1 with an indication of possible spread to western Scotland. A cluster of outbreaks of genotype 1_A1 in northern France and Belgium is also apparent in the 2014-2016 seasons (Fig. 17a, 18 and 19). These maps also demonstrate the clustering of genetically diverse isolates (termed here as ‘Other’) in the northeast parts of the Netherlands (Fig. 17b) and Scotland. Note that different geo-located data points from the same location overlay each other which may obscure some of the diversity. Thus in the live mapping tool ‘radio buttons’ for each genotype may be selected to allow specific genotypes to be plotted individually (Fig. 17b). The recent spread of genotypes 36_A2 and 37_A2 from 2016 to 2018 is shown in Figs 20 and 22. Genotype 37_A2 first emerged in an outbreak in the Midlands in late June 2016 and its geographical spread widened over the subsequent seasons. The appearance of 37_A2 in Northern Ireland and Western Scotland is a significant move north and west in the dry 2018 season (Fig 22 and Fig 23). Genotype 36_A2 was not sampled in 2016 but its incidence in south and eastern England has increased in 2017 and 2018.

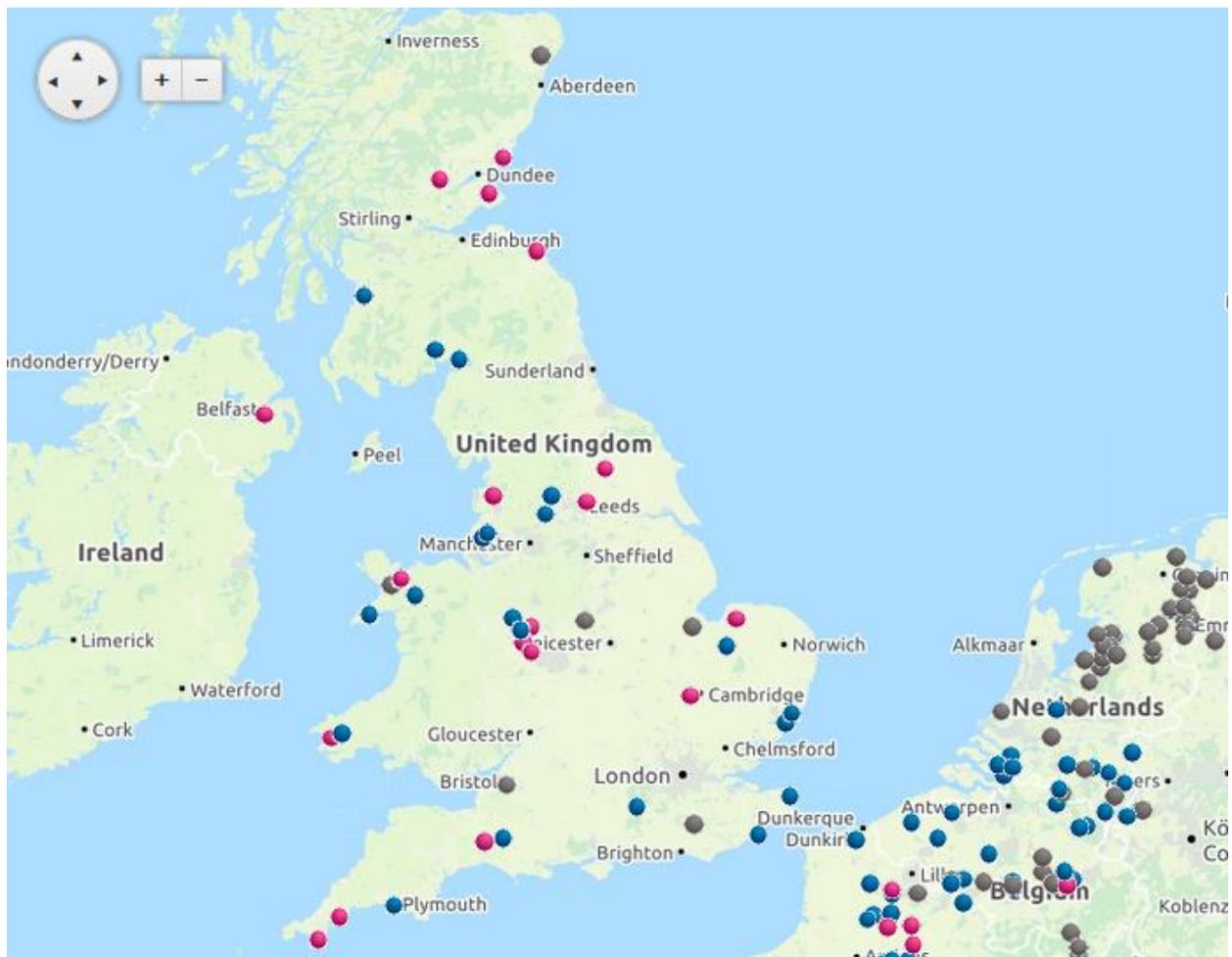


Figure 16. Spatial distribution of *P. infestans* genotypes collected from 2013 late blight outbreaks entered into the Euroblight database (www.euroblight.net). Key as per Fig. 19.

a)



b)



Figure 17. Spatial distribution of *P. infestans* genotypes collected from 2014 late blight outbreaks entered into the Euroblight database (www.euroblight.net). a) All genotypes shown b) the same data with only the 'Other' data shown. Key as per Fig. 19.

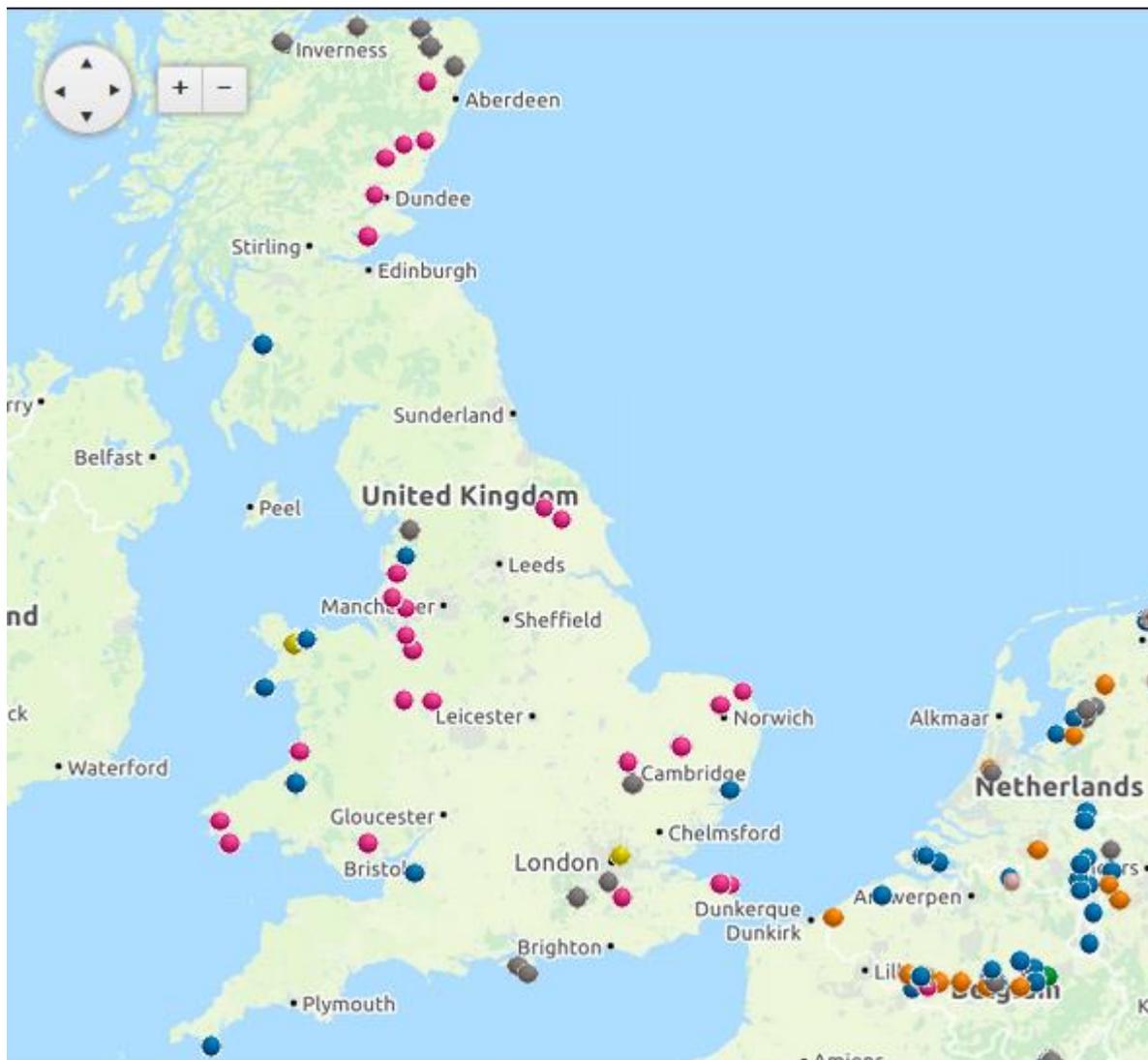


Figure 18. Spatial distribution of *P. infestans* genotypes collected from 2015 late blight outbreaks entered into the Euroblight database (www.euroblight.net). Key as per Fig. 19.

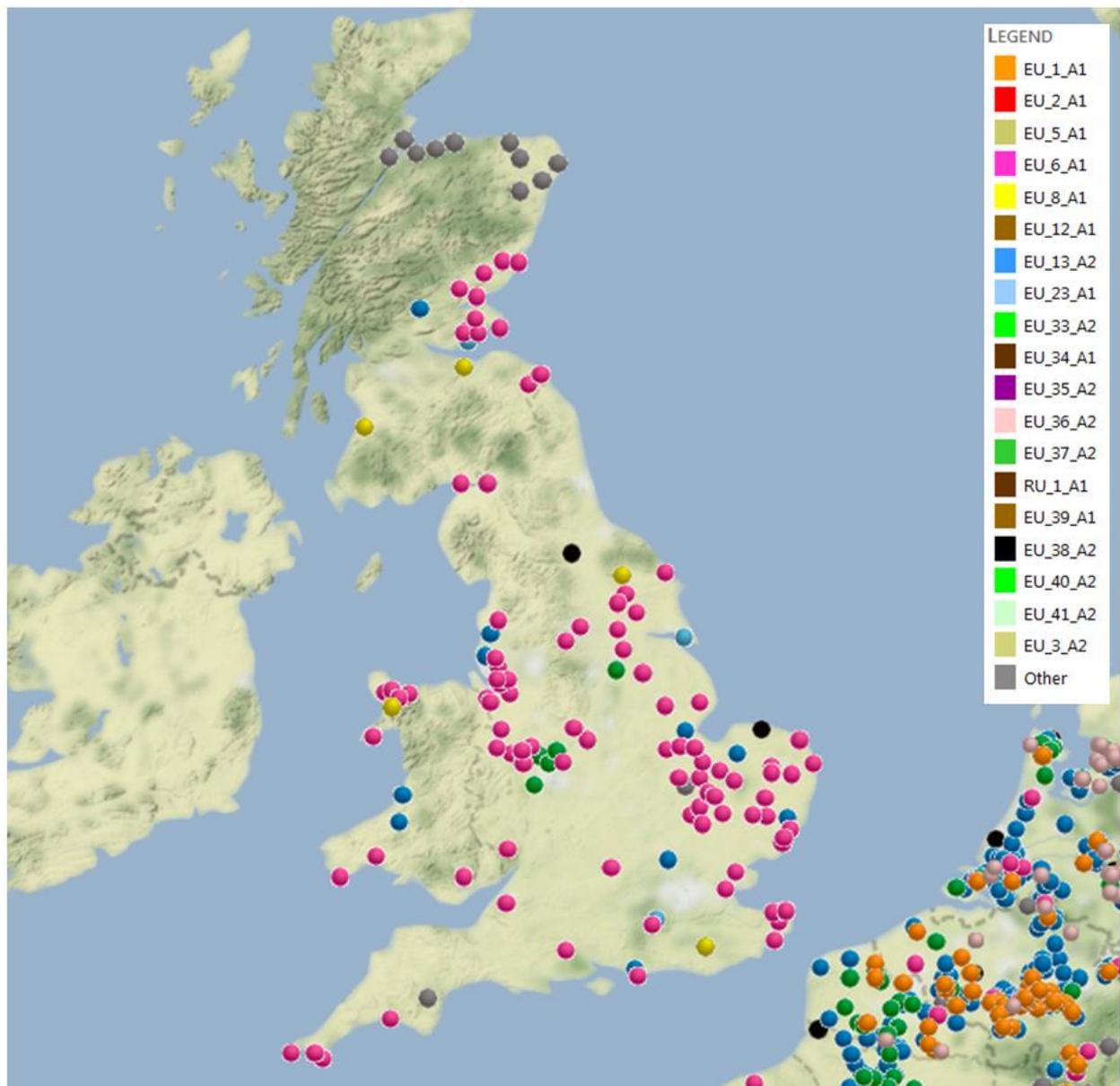


Figure 19. Spatial distribution of *P. infestans* genotypes collected from 2016 late blight outbreaks entered into the EuroBlight database (www.euroblight.net).

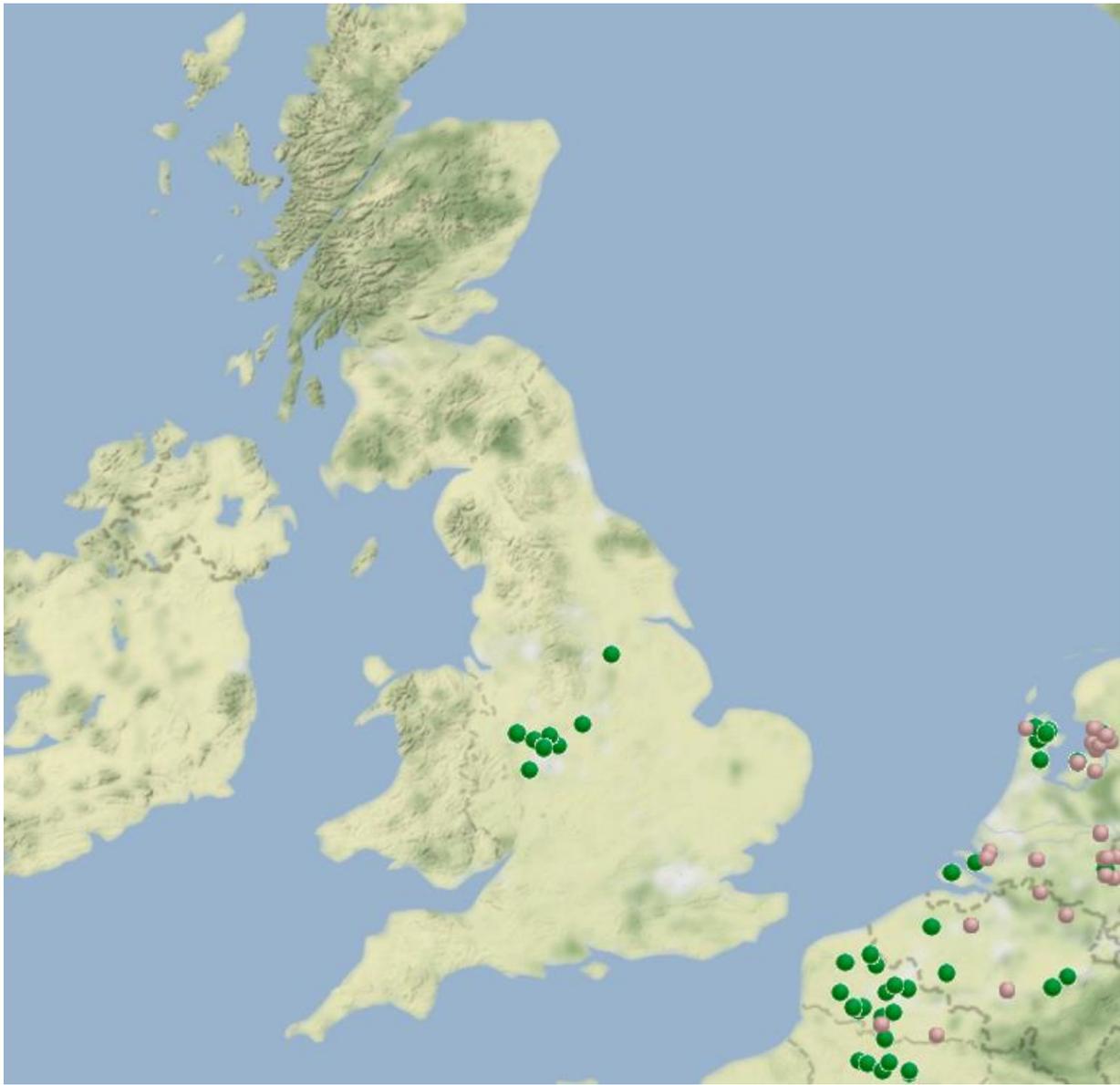


Figure 20. Spatial distribution of *P. infestans* genotype 37_A2 (green) and 36_A2 (pale pink) collected from 2016 late blight outbreaks entered into the EuroBlight database (www.euroblight.net).

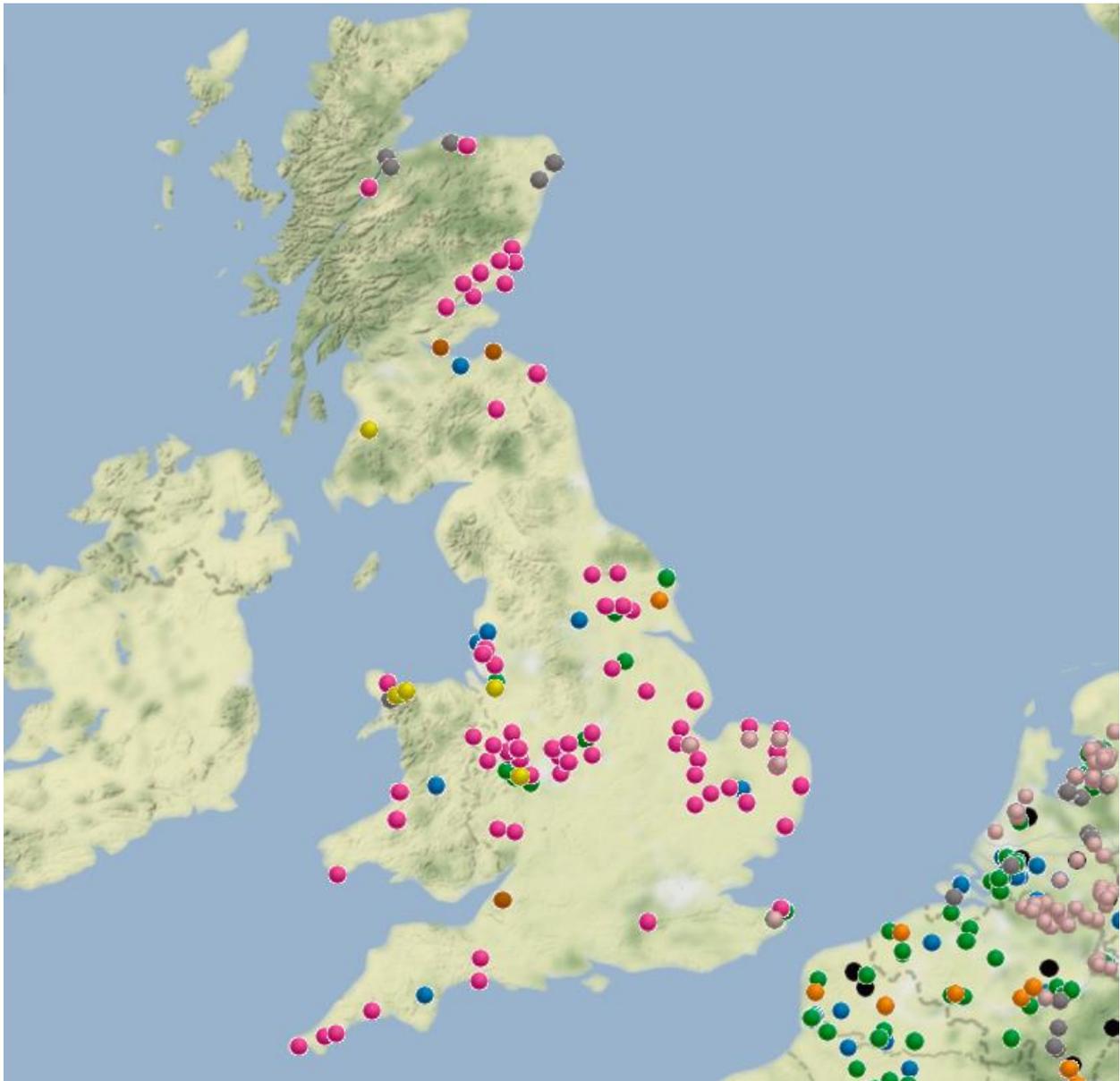


Figure 21. Spatial distribution of *P. infestans* genotypes collected from 2017 late blight outbreaks entered into the EuroBlight database (www.euroblight.net). Key as per Fig. 19.

a) 2017



b) 2018

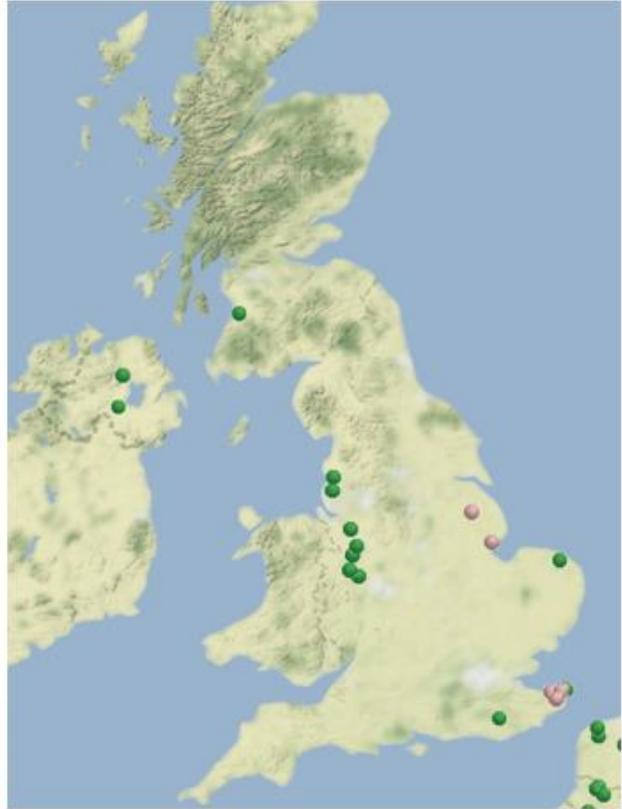


Figure 22. Spatial distribution of *P. infestans* genotype 37_A2 (green) and 36_A2 (pale pink) collected from a) 2017 and b) 2018 late blight outbreaks entered into the EuroBlight database (www.euroblight.net).

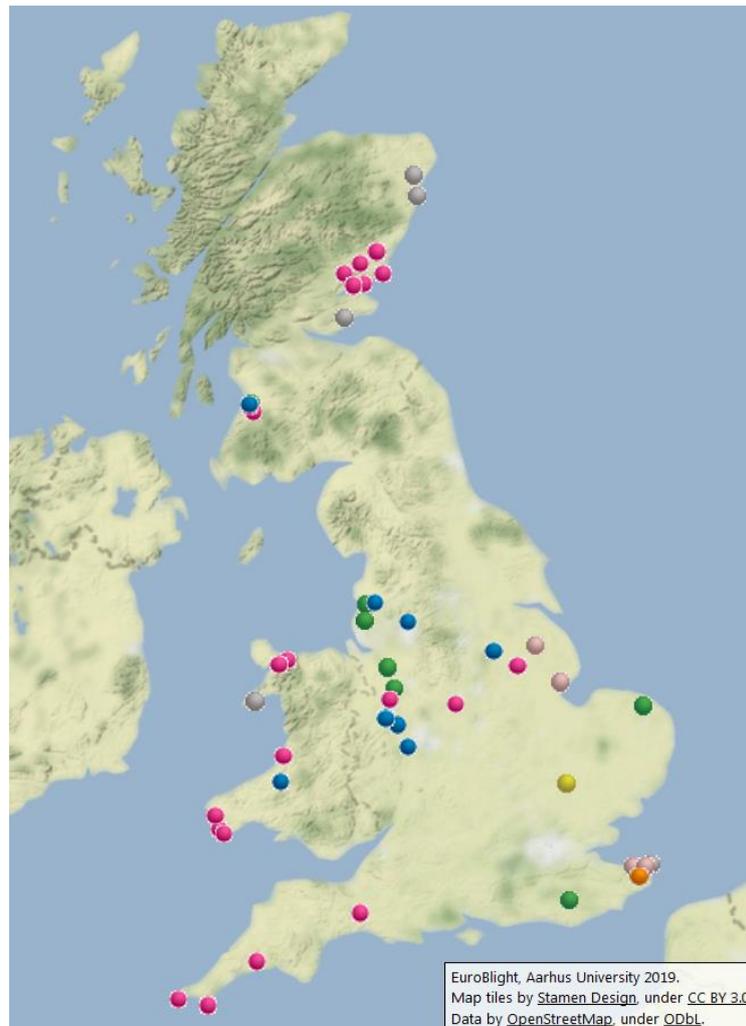


Figure 23. Spatial distribution of *P. infestans* genotypes collected from 2018 late blight outbreaks entered into the EuroBlight database (www.euroblight.net). Key as per Fig. 19.

The genetic markers used in this study also resolve sub-genotype variation and this data has discriminated populations amongst two potato growing regions in England in which the late blight epidemic began early and was problematic in the 2014 crop. Differences were observed between the pathogen genotypes causing blight in Shropshire and the West Midlands compared to those in crop in Lancashire. In both regions, 13_A2 and 6_A1 were responsible for most late blight outbreaks but a distinct form of 13_A2 (termed 13_A2_42) was dominant in the potato growing region in Lancashire but not in the other region (Fig. 24). This genotype was first noted in Lancashire in 2011 and has been locally prevalent in each season since, while uncommon in other potato growing regions of Britain (data not shown).

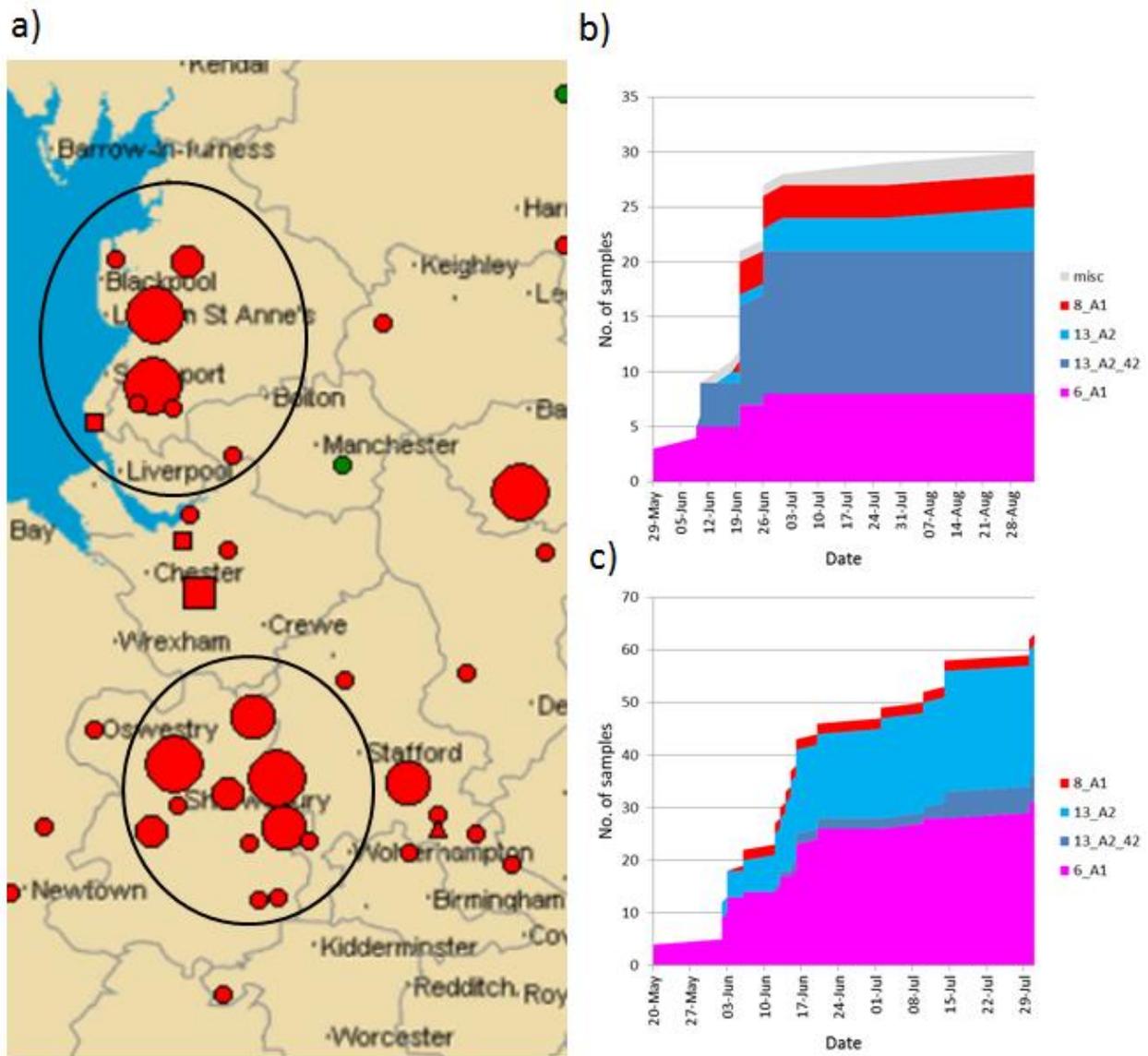


Figure 24. Regional differences in genotypes of *P. infestans* samples collected from outbreaks in northwest England in 2014. a) FAB map outlining the clusters of outbreaks from Lancashire (upper circle) and Shropshire and the midlands (lower circle) postcode districts. Charts indicating the cumulative frequency of different genotypes by arrival date in b) Lancashire outbreaks (31 samples) and c) Shropshire and the midlands postcode districts (65 samples).

Samples categorised as ‘Other’ comprised approximately 5% of the sampled population from 2003-2009, increasing to 15% in 2010 and between 7 and 9% from 2011-2014. In 2015 however, this figure increased to 29% but this may have been as a result of a data skew with the low number of sampled outbreaks (Fig. 15). Detailed examination of the outbreaks from which ‘Other’ genotypes were recovered in 2014 to 2017 also indicated geographical clustering. A striking feature of the many samples provided from Scottish crops in 2014 was the dominance of 6_A1 in DD district postcodes from Northern Fife, Dundee and Angus crops. In the Aberdeenshire, Inverness and Moray postcode regions north of Stonehaven, however, the outbreaks were predominantly a mixture of the many genotypes categorised as ‘Other’ (Fig. 25). The category of outbreak from which the ‘Other’ genotypes was recovered was examined. In 2014, ‘Other’ isolates were found in 21 Scottish outbreaks; 16 in conventional crops, two from gardens, two from organic crops and 1 from volunteer potatoes. In 2015, the 10 ‘Other’ outbreaks

comprised three in conventional crops with the seven others being from gardens, outgrade piles, organic crops and volunteer potato plants. In 2016, 13 outbreaks with 'Other' genotypes comprised ten conventional crop outbreaks and one each from a volunteer, a garden and an organic crop. In 2017, eight outbreaks comprised 'Other' genotypes; three from gardens, three from conventional crops and one each from an outgrade pile and volunteer potatoes.

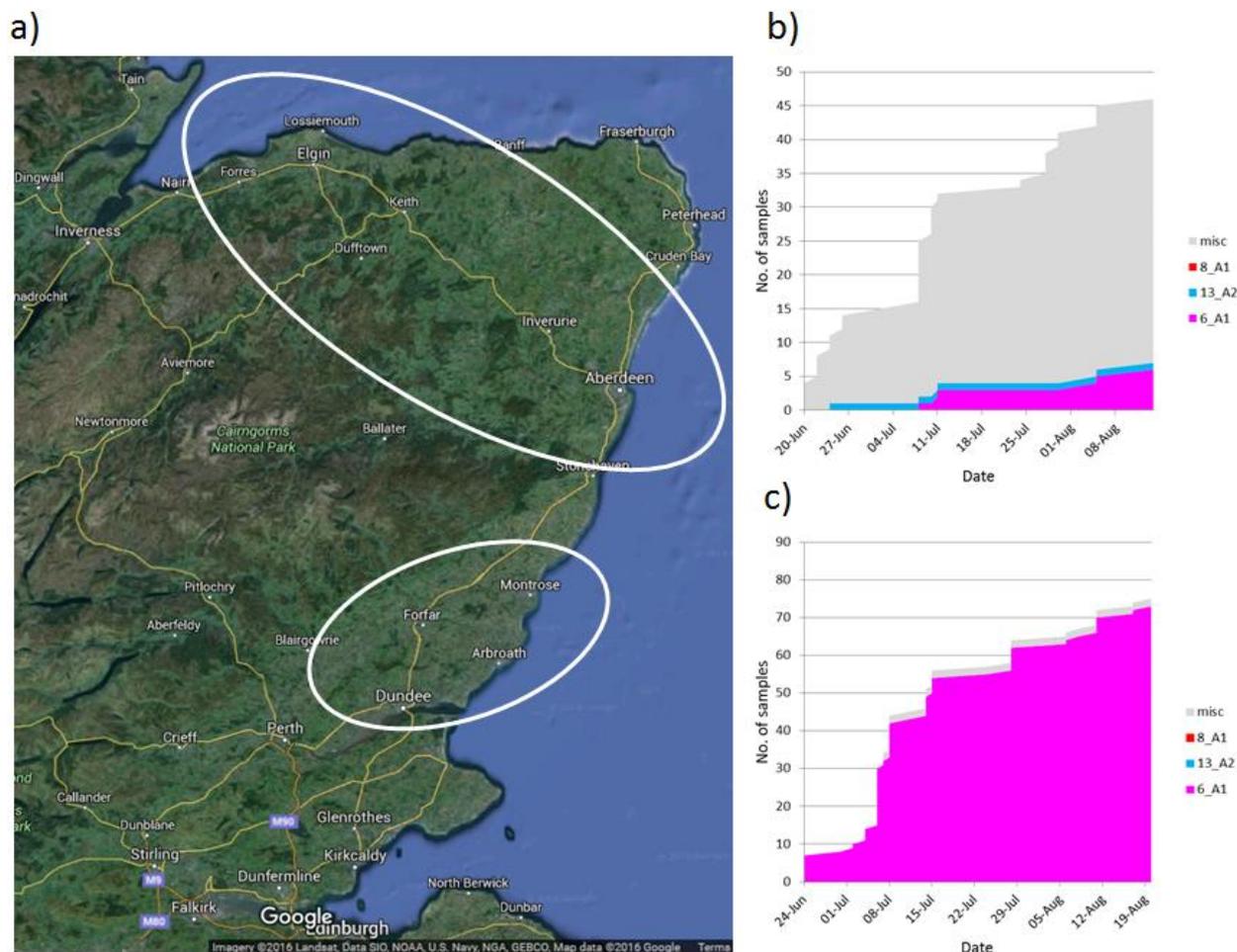


Figure 25. Regional differences in genotypes of *P. infestans* samples collected from outbreaks in northeast Scotland in 2014. a) map outlining the locations sampled from Aberdeen (AB) and Inverness (IV) (upper ellipse) and Dundee (DD) (lower ellipse) postcode districts. Charts indicating the cumulative frequency of different genotypes by arrival date in b) AB and IV postcode districts (46 samples from 20 outbreaks) and c) DD postcode districts (82 samples from 37 outbreaks). The extension of upland heath towards the east coast near Stonehaven (south of Aberdeen just visible beneath the southern boundary of the upper ellipse) is clear on this satellite image map. The category 'misc' is the same as 'Other'.

Tuber Blight testing 2016-2017

In 2016, ten batches of blight infected tubers were supplied to The James Hutton Institute, from Shropshire, Staffordshire, Yorkshire, Nottinghamshire and Cambridgeshire. The previously reported 33_A2 lineage was recovered from Cambridgeshire, genotype 6_A1 from Yorkshire and one sample from Shropshire but six batches were infected with the 37_A2 lineage of *P. infestans*.

Table 1. Tuber blight test data and genotyping results from GB tuber blight samples in 2017.

Sample ID	Postcode	County	Country	Variety	Date	SSR genotype
17_TB02A	TF5	Shropshire	England	Unknown	15-Nov	37_A2
17_TB02B	TF5	Shropshire	England	Unknown	15-Nov	37_A2
17_TB02C	TF5	Shropshire	England	Unknown	15-Nov	37_A2
17_TB04B	DD8	Angus	Scotland	Banba	20-Oct	6_A1
17_TB05B	YO19	North Yorkshire	England	Royal	26-Oct	37_A2
17_TB05C	YO19	North Yorkshire	England	Royal	26-Oct	37_A2
17_TB05D	YO19	North Yorkshire	England	Royal	26-Oct	13_A2
17_TB05E	YO19	North Yorkshire	England	Royal	26-Oct	37_A2
17_TB05G	YO19	North Yorkshire	England	Royal	26-Oct	13_A2
17_TB05H	YO19	North Yorkshire	England	Royal	26-Oct	13_A2
17_TB06A	PL14	Cornwall	England	Unknown	01-Nov	6_A1
17_TB06B	PL14	Cornwall	England	Unknown	01-Nov	6_A1
17_TB07B	NR21	Norfolk	England	Marilyn	14-Nov	36_A2
17_TB07C	NR21	Norfolk	England	Marilyn	14-Nov	36_A2
17_TB07D	NR21	Norfolk	England	Marilyn	14-Nov	36_A2
17_TB08A	NR21	Norfolk	England	Gemson	21-Nov	6_A1
17_TB09A	NR21	Norfolk	England	Charlotte	21-Nov	6_A1
17_TB09B	NR21	Norfolk	England	Charlotte	21-Nov	6_A1
17_TB11A	CT12	Kent	England	King Edward	29-Nov	37_A2
17_TB11D	CT12	Kent	England	King Edward	29-Nov	37_A2
17_TB11E	CT12	Kent	England	King Edward	29-Nov	37_A2
17_TB13A	NR34	Suffolk	England	Soraya	07-Dec	6_A1
17_TB13B	NR34	Suffolk	England	Soraya	07-Dec	6_A1
17_TB13D	NR34	Suffolk	England	Soraya	07-Dec	6_A1
17_TB13E	NR34	Suffolk	England	Soraya	07-Dec	6_A1
17_TB14A	YO26	North Yorkshire	England	Royal	08-Dec	6_A1
17_TB14C	YO26	North Yorkshire	England	Royal	08-Dec	6_A1
17_TB15A	HR8	Herefordshire	England	Russet Burbank	08-Dec	6_A1
17_TB15B	HR8	Herefordshire	England	Russet Burbank	08-Dec	37_A2
17_TB16B	IP31	Suffolk	England	Maris Piper	14-Dec	37_A2
17_TB16C	IP31	Suffolk	England	Maris Piper	14-Dec	37_A2
17_TB16D	IP31	Suffolk	England	Maris Piper	14-Dec	37_A2
17_TB16E	IP31	Suffolk	England	Maris Piper	14-Dec	37_A2
17_TB16F	IP31	Suffolk	England	Maris Piper	14-Dec	37_A2
17_TB16H	IP31	Suffolk	England	Maris Piper	14-Dec	37_A2
17_TB22A	DD9	Angus	Scotland	Shepody	21-Dec	6_A1
17_TB22B	DD9	Angus	Scotland	Shepody	21-Dec	6_A1
17_TB22C	DD9	Angus	Scotland	Shepody	21-Dec	6_A1
17_TB22D	DD9	Angus	Scotland	Shepody	21-Dec	6_A1
17_TB23A	CB7	Cambridgeshire	England	Sagitta	17-Jan	6_A1
17_TB25A	TF6	Shropshire	England	Lady Balfour	01-Feb	6_A1
17_TB30A	DD8	Angus	Scotland	Banba	14-Feb	6_A1
17_TB31D	DN9	South Yorkshire	England	Sagitta	21-Feb	37_A2

With AHDB Potatoes support and funding, 31 batches of blighted tubers were provided from 16 different suppliers in 2017. Batches varied from a single tuber to >20 tubers and were in a range of health conditions. In some cases it was not possible to obtain a *P. infestans* sample due to secondary bacterial infection. In other cases, the tubers were infected with only Fusarium dry rot. A total of 110 samples were processed from 18 different potato varieties. Of the 31 batches, 21 were from crops grown in England, four from Scotland, and six were of non-UK origin. The non-UK samples were of seed destined for UK or non-UK markets. Of the 110 samples, 50% (from 23 tuber batches) yielded genotype data. Genotype 13_A2 was recovered from one, 6_A1 from 11, 37_A2 from eight, 36_A2 from three and a single 41_A2 and an 'Other' genotype from the other two (See Table 1). Genotype 37_A2 was found in six GB batches from the following counties (South Yorkshire, North Yorkshire, Suffolk, Herefordshire, Kent, and Shropshire). This extends the range of this genotype beyond that found on foliar samples during the 2017 season with the finding in Suffolk being of note as the first finding in east England. Genotype 36_A2 was found in a single GB batch from Norfolk. The three Scottish batches comprised only the 6_A1 genotype and eight batches from England were also infected with the 6_A1 genotype. As percentages of the 17 GB batches 37_A2 was found at 35% and 6_A1 at 47% of incidents. Samples of non-UK seed destined for planting in the UK were infected with either the 36_A2, 37_A2 or an unknown 'Other' lineage (data not shown). Tuber blight was not specifically sampled in 2018.

5. Discussion

Outbreak monitoring and disease risk

The seasons 2014 and 2015 contrasted each other with early and severe disease outbreaks in 2014 that were, in some regions, reported to be responding poorly to fungicide application and provoking concern that a new pathogen population was present. Samples from early 2014 outbreaks were thus processed and the genotyping data plotted by reporting date to provide the industry with an in-season update on diversity. It was clearly shown that these early outbreaks were predominantly caused by genotypes 6_A1 and 13_A2 which, after discussions within the industry, suggests that the poor control stemmed from an unusually high disease pressure from crop emergence onwards. A combination of local inoculum and insufficient fungicide protection, either as a result of a lack of appreciation of the high disease pressure or weather conditions being unsuitable for spraying, allowed late blight to take hold in some crops. Management of such established infections proved extremely challenging which highlights the importance of local inoculum sources and the need to be aware of disease risk from the start to the end of the season. In contrast, 2015 was a low disease pressure year with generally below average temperatures and dry conditions in the early part of the season keeping primary inoculum and early infections in check. A comparison of the start dates and final sample numbers over each of 15 seasons confirmed the relationship between an early start and final disease pressure (Fig. 12). It is also clear that the timing of periods of high blight risk with any unfavourable spray conditions is critical; consecutive high risk periods and waterlogged fields pose a greater risk than the same total rainfall spread over a longer period and interspersed with days suitable for spraying.

*Prevalence of the clonal *P. infestans* genotypes*

The pathogen population was relatively stable over the 2014 and 2015 seasons. On a GB scale, the 13_A2 lineage has shown a steady decline from its peak of 78% of the sampled

population in 2008. In recent years it has varied from 24% in 2012 to 52 % in 2013 and down again to 19% in 2015 (Fig. 13). However, note that the apparent spike in the 2013 data may have been biased by the small sample size in this low blight pressure year (Fig. 15). This follows an extreme skew in sampling in Scotland in 2011 which created an impression of a GB-wide decline in 13_A2 but, in fact, represented a late-season epidemic across Scottish crops that was dominated by 6_A1 (Cooke et al., 2013). Plotting the data by country clearly indicates these regional differences (Fig. 14). The mean frequency of 13_A2 across England and Wales combined over the 2012 to 2017 seasons is 29% compared to only 10% in Scotland. The apparently high level of 13_A2 in Scotland in 2013 was based on 19 of 36 isolates, none of which were sampled from the main production areas on the east of the country (data not shown). Genotype 13_A2 has continued to decline to only 6% of the samples collected in 2018. The 6_A1 genotype remained the dominant genotype across British crops over the 2012-2017 seasons with a frequency of 55, 51 and 54% in England, Wales and Scotland, respectively. It has, however, declined slightly in 2018 to 46% of the GB population. The 13_A2 lineage is now reported across France (Mariette et al., 2016), the Netherlands (Li et al., 2012), Northern Ireland (Cooke, 2015), China (Li et al., 2013b), India (Chowdappa et al., 2015) and was recently reported in West Africa. Euroblight data shows it remains widespread in Europe (www.euroblight.net) which supports studies in 2007 showing its aggressiveness (Cooke et al., 2012). However, other studies have not demonstrated a consistently high aggressiveness in isolates of the lineage collected since 2007 (Chapman, 2012, Mariette et al., 2016) and this may partly explain its steady displacement by other lineages.

The 6_A1 lineage was present in GB (Cooke et al., 2012, Cooke et al., 2013, Kildea et al., 2012), Northern Ireland (Cooke, 2015), the Netherlands (Li et al., 2012), France and Belgium (www.euroblight.net) but has, surprisingly, not yet been reported outside of Europe. Recent data shows a 6_A1 cluster in northwest Europe (GB, Belgium and France) but a very low incidence in the Netherlands where it was first reported. Given its aggressiveness (Cooke et al., 2012) and local dominance, it is unclear why the 6_A1 lineage is not more widespread in Europe and globally.

The 8_A1 lineage has been present in Europe since at least 1995 (Cooke et al., 2012) and remained at a frequency of approximately 4% in from 2012 to 2015. However, it declined to around 2% in 2016 and 2017 and was not sampled in 2018. Other lineages at a similar or previously higher frequency (e.g. 23_A1, 3_A2 and 2_A1) have also declined, suggesting their extinction. It appears that 8_A1 had some sort of selective advantage over other lineages but the nature of this advantage remains unclear. In Northern Ireland, genotype 8_A1 has dominated the population in recent years (Cooke, 2015) and its local incidence in 2014 crops can be seen on the EuroBlight map for 2014 (Fig. 17). Regional distribution patterns such as these may be due to a 'founder effect' in which a loss of genetic diversity occurs as a new population expands from a relatively small number of individuals. Under optimal conditions, populations of *P. infestans* are capable of explosive growth from such small founder populations which expand and spread at crop, region and national spatial scales. The prevalence of genotypes 6_A1 in northwest Europe, 8_A1 in Northern Ireland (Fig. 17a), 1_A1 in Belgium (Figs 17a and 18) and 13_A2_42 in Lancashire (Fig. 24) may all be examples of such founder effects. Nonetheless, it is clear that these lineages must each be fit and aggressive to continue to out-compete other lineages after their initial establishment phase. The genotype 39_A1 first appeared in 2015 and has now been found in 3 consecutive years at a low frequency but over a wide geographic range from Slovenia to Scotland. EuroBlight data indicates an association of this genotype with tomato and it's spread to potato from this source is likely (data not shown).

Fluazinam insensitivity; 33_A2 and 37_A2

Genotype 33_A2 with a reduced sensitivity to fluazinam (Schepers et al., 2018) was first detected in the Netherlands in 2009 and comprised 20% of their sampled population in 2010 and 2011 (Schepers et al., 2013). It was found only once per year from British crops in 2016 and 2017 suggesting that revised industry guidelines on the use of fluazinam (limiting application numbers and reduced use of 'blocks' of the same product) had proved successful in reducing the selection pressure and countering the threat this lineage posed to the use of this active ingredient. This decline is however, strongly related to the lack of fitness of 33_A2 isolates compared to other lineages which out-compete it when the selection pressure is not maintained with fluazinam application (Schepers et al., 2018). This is demonstrated by the appearance of the 37_A2 lineage which is both fluazinam insensitive and appears evolutionarily fit and aggressive. This clone was first observed in the Noordoostpolder region of the Netherlands in 2013 and spread locally in the following two seasons. In 2016 however, GB FAB and EuroBlight monitoring revealed its rapid spread to seven other European countries (Fig. 21 & 22). This may have been related to highly conducive weather conditions for late blight in the low countries early in the 2016 growing season. Within Britain, the infection was first recorded in the Shropshire area in late June and further incidents were recorded as far north as Yorkshire as the season progressed. Tuber blight infections were reported at the end of the 2016 season in the west Midlands and many proved to be infected with the 37_A2 genotype. Fluazinam affects zoospore motility and is a key component of the fungicide programme for full-canopy foliar protection and, critically, it also provided tuber blight protection late in the season. Concerns were thus heightened and The James Hutton Institute was requested to provide within-season updates on the incidence of 37_A2 in 2017. From an initial outbreak in Kent on 19 July 2017 it was again documented extensively in Shropshire, Staffordshire and Cheshire but also moved north to Derbyshire, Lancashire and North Yorkshire (Fig. 22). It was also found in one tuber sample at the end of the season in Suffolk but was not been reported from Wales, southwest England or Scotland in 2017. In total, 23 batches of blighted tubers from the 2017 season were tested (Table. 1) and the incidence of 37_A2 in 35% of the batches suggests it is aggressive and fit on both foliage and tubers. Presentations at AHDB Potatoes' events, Grower Gateway articles and attention in the UK farming press made advisors and the wider industry aware of changes to best practice to respond to this threat of fluazinam insensitivity. If patterns of fluazinam application have changed in practice then the incidence of 37_A2 in 2017 confirms its aggressiveness and fitness even in the absence of the product. Data from this work was passed to the Fungicide Resistance Action Committee UK (FRAG-UK) which revised its advice on fluazinam use (https://potatoes.ahdb.org.uk/sites/default/files/FRAG-UK%20Potato%20Late%20Blight%20Guideline%20May%202018_0.pdf). An advisory document on fluazinam use has also been released by AHDB Potatoes (Bain et al., 2018).

Clone 36_A2

Isolates of the clonal lineage 36_A2 were first sampled at low frequencies in the starch potato growing areas in northern Germany and the Netherlands in 2014 and had spread across the Netherlands into Belgium, the UK, Denmark and Poland by 2017. In 2018 it was also sampled on crops in Spain, Hungary and Serbia and made up 16% of the EuroBlight samples (up from 10% in 2017). It was first reported in British crops in Kent, Norfolk and Lincolnshire in 2017 and in a similar range but higher incidence in 2018 where it was reported to caused severe losses in some crops when the weather became more blight favourable later in the summer. The spread of the 36_A2 and 37_A2 lineages and the displacement of other genotypes suggests they are fit and aggressive. Fungicide sensitivity testing in laboratories in Wageningen University and The James Hutton Institute has also shown that EU_36_A2 and EU_37_A2 isolates formed

consistently larger lesions than those of the older lineages on leaves at very low dose rates of four key fungicide active ingredients (Lees, 2018). This supports aggressiveness testing as part of the IPMBlight2.0 project at INRA that revealed that the 36_A2 isolates tested formed large average lesion sizes with abundant sporulation (Roselyne Corbiere, personal communication). Such properties are likely to impact on management practices and may explain the spread of these lineages. Continued environmental and political pressure on reducing fungicide usage is focussing attention on Integrated Pest Management (IPM) systems that combine the use of fungicides, host resistance and decision support tools to increase the sustainability of late blight management (Kessel et al., 2018). The use of AHDB sponsored FAB monitoring data such as this is clearly crucial to the success of this approach both now and in the future.

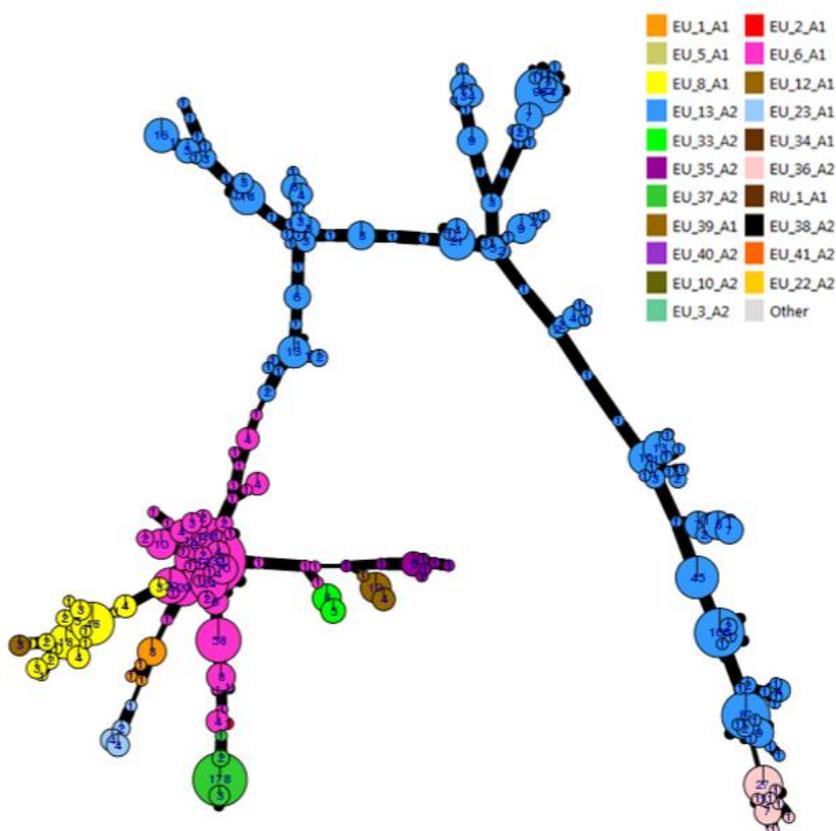


Figure 26. An SSR-based minimum spanning network tree of 2752 genotyped *P. infestans* isolates sampled from GB from 2012-2018. ‘Other’ samples are not shown, only the dominant clones. The data shows the range of diversity within each clone. The figure was generated using *poppr* via the EuroBlight toolbox.

Within genotype variation in 13_A2

Each time a cell of *P. infestans* divides, DNA replication introduces minor DNA sequence differences (mutations) into the approximately 250 million DNA base pairs in its genome. Up to 20,000 sporangia are produced per cm² of every late blight lesion each day (Skelsey et al., 2009) and therefore, countless billions of cells of *P. infestans* are dividing daily. Three of the 12 SSR markers are more prone to mutation than the others and these mutations generate minor differences in fingerprint patterns that can be traced over time (Fig.14, Fig. 26). Almost 6000 isolates of the 13_A2 clonal lineage have been fingerprinted and more than 200 minor sub-groups defined (named 13_A2_1, 13_A2_2 etc). 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 movement. The frequency of the initial 13_A2_1 type has decreased over time (Cooke et al., 2013). Analysis of the many samples from the 2014 outbreaks showed a strong local pattern of 13_A2 variants amongst two potato growing regions of England (Fig. 24). 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 and stresses the importance of effective management of such inoculum.

Novel combinations of 'Other' genotypes

The increase in the frequency of *P. infestans* samples with novel genetic fingerprints to 29% in 2015 is interesting and the causes and possible consequences need to be considered. Detailed genetic analysis of the SSR data from isolates from GB and mainland Europe in the EuroBlight database (Cooke et al., 2015) shows how minor variation within a known clone can be discriminated from the type of genetic differences that are a signature of sexual recombination. The analysis is based on a population genetics application called *poppr* (Kamvar et al., 2015) which converts the stepwise variation in SSR data into a matrix of genetic distances between each isolate. Pairs of isolates with an identical fingerprint will return a value of zero and those that differ by a single step in one marker, a value of around 0.01 (i.e. a 1% difference). In some cases, small step-wise changes in up to 3 of the 12 markers may occur between individuals within a clonal lineage leading to genetic distances of approximately 0.05 (5%). Differences between clones typically involve larger step changes in 8 of the 12 markers translating to genetic distances greater than 0.07 (7%). A matrix of all the pairwise comparisons between each isolate can be processed to view the genetic relationships as a network (Fig. 26), a tree (Fig. 27) or clustered spots in a principal co-ordinate analysis. Using this method and a decade's experience in processing such data has enabled the reliable discrimination of sub-clonal variants and the products of sexual recombination. As described above, the novel types are grouped into a 'catch-all' category called 'Other' and the patterns of such 'Other' isolates are compared both within and between seasons. Any genetic fingerprint common to samples from multiple blight outbreaks and in more than one season would indicate clonal spread and be 'upgraded' to a named clone. Careful analysis of all 'Other' isolates collected from 2003 to 2018 has not identified more than a handful of samples with a fingerprint common to more than one outbreak site or season. This is strong evidence for local ephemeral populations that are not as fit or aggressive as the clonal types such as 6_A1, 13_A2 or 37_A2. In addition, there is no evidence for spread of these types out of Scotland on potato seed, suggesting that seed health status is high and blight dissemination via this pathway does not contribute significantly to primary inoculum compared to local sources (see above). Work within projects such as IPMBlight2.0 (<http://euroblight.net/research-projects/ipmblight20/>) seeks to understand the traits that the 'Other' lineages possess and how their evolution may affect future blight management.

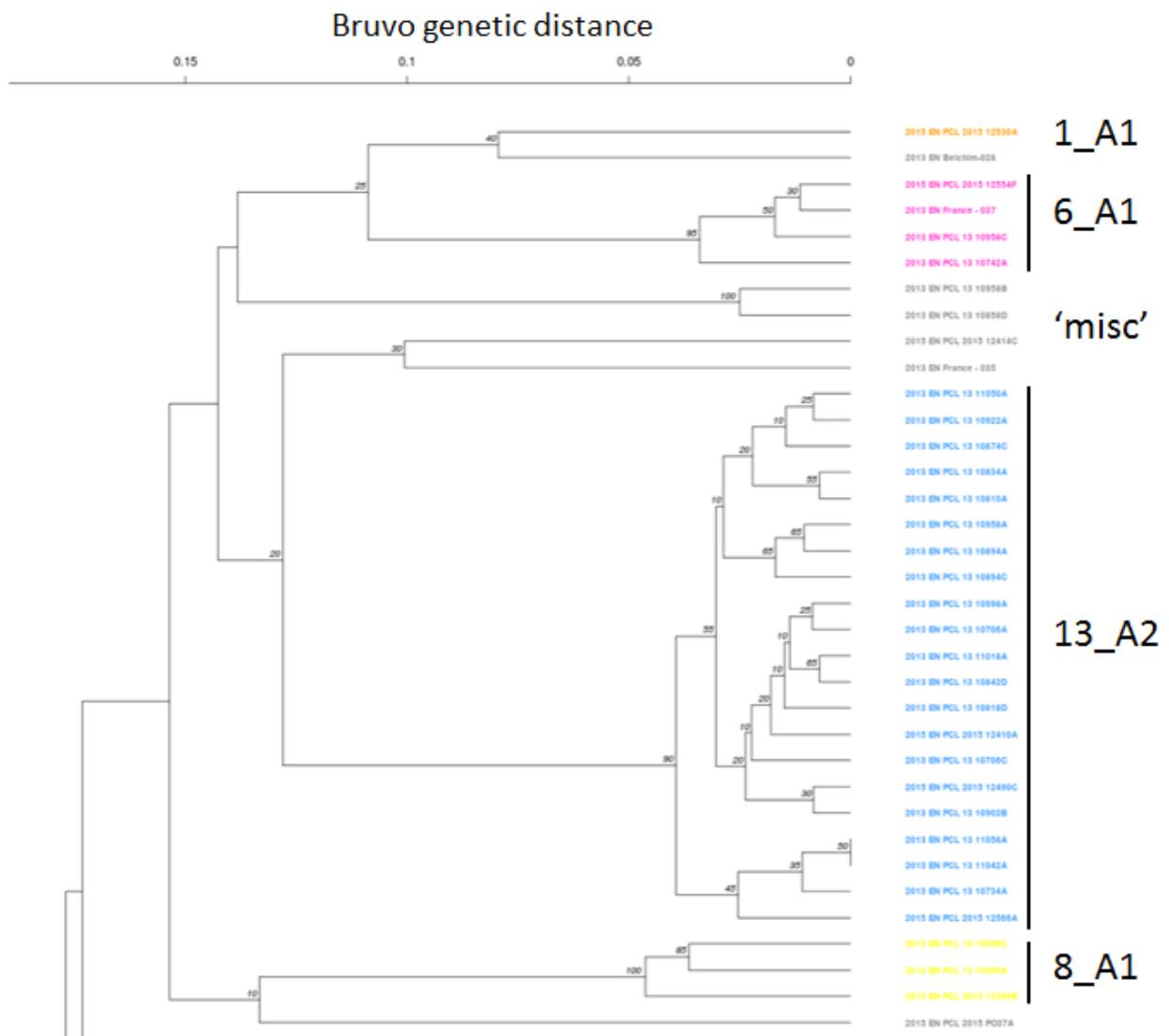


Figure 27. Section of an SSR-based simple neighbour joining tree showing genetic relationships between *P. infestans* isolates sampled from England. Data analysed using a custom Shiny R application based on *poppr* (Kamvar et al., 2015) and implemented on the EuroBlight database. The tree visualises minor variants within a clone at approximately 0 - 0.04 Bruvo genetic distances (see scale bar) compared to differences between clones at distances of approximately 0.07 or greater.

Within the outbreaks having novel 'Other' isolates, some comprise four genetically identical isolates consistent with a single oospore that has germinated to generate a local clonal epidemic. Others comprise several distinct genotypes suggesting multiple oospores germinated to create a mosaic of pathogen genotypes within an outbreak. This is consistent with patterns seen in carefully monitored field outbreaks in Sweden (Widmark et al., 2007, Widmark et al., 2011). This remains indirect evidence and no direct observational data yet exists to validate the hypothesis that oospores are a source of primary inoculum in British crops. Alternative hypotheses are that there is a pool of diverse novel genotypes migrating into the reported outbreaks from other local sources such as gardens, outgrade piles or volunteer potato plants or these diverse types are being imported via wind-borne sporangia or on other sources such as potato seed or whole plants of other solanaceous hosts infected with *P. infestans*. There is some evidence for the latter as the 23_A1 lineage was first found in Britain on tomato plants in 2007 and in 2009 on Petunia

plants from a commercial nursery. British tomato crops in gardens have been reported to contain a diverse population of *P. infestans* (Stroud et al., 2016). Samples of 'Other' isolates from the south of England in 2015 (Fig. 18) were from gardens and tomato production units and clone 39_A1 which similarly, originated on tomatoes in mainland Europe. However, such imports are not consistent with the prevalence of 'Other' combinations in outbreaks reported from northeast Scotland. There are no other clear sources of diversity in *P. infestans* in this region and it is considered highly unlikely that airborne sporangia can migrate to this part of Scotland from Scandinavia. In other parts of Europe, short rotations have been shown to increase the probability of oospore infection in a subsequent crop (Yuen & Andersson, 2013, Bødker et al., 2005, Lehtinen & Hannukkala, 2004) but rotations in seed and ware crops in this region are between 5-7 years; sufficient for oospore decay. Samples have been reported from conventional crops but also outgrade piles, gardens and volunteers and it is possible that these latter outbreak types are sources of novel types of blight. Blight-infected volunteer potato plants in areas of land that cannot be treated due to environmental regulations are a cause of concern as such unchecked disease outbreaks will spread *P. infestans* inoculum to nearby ware or seed crops.

The higher frequency of 'Other' types in this region may relate to physical geography and the seed trade. The land suitable for agriculture in this region is constricted to a narrow coastal strip in the area around Stonehaven where upland heath associated with the Cairngorm mountain range meets the coast (Fig. 25). This, in combination with prevailing westerly winds, creates an effective physical barrier to inoculum spread from crops in Angus to the south. In addition, the area north of Aberdeen is predominantly a seed producing area which restricts the amount of seed, and thus potential sources of *P. infestans* inoculum imported into the region. The absence of competition from the dominant clones may thus allow the 'Other' strains a niche that is seldom available in other parts of Britain. Some genetic diversity in this region was observed using different methods in a survey from 1995-1997 (Cooke et al., 2003) and discussed at a recent meeting (Cooke et al., 2018). Further exploration of the 'recombinants' in this part of Scotland is underway at the Hutton using mitochondrial DNA markers that, in combination with SSRs reveal more about the origins and evolution of these strains (Martin et al., 2019). There is a risk that these sexually reproducing populations can generate new successful clones with traits that allow them to compete with 6_A1 and 13_A2 lineages and growers should remain alert to the presence of soil-borne oospore inoculum and the threats it poses to genetic diversity and early infection pressure.

Conclusions

Although disease pressure varies from season to season, late blight remains a significant threat to the GB crop and can be a difficult disease to manage, especially under warm and wet weather conditions. Warmer winters, such as that in 2015-16 increased the risks of primary inoculum build up early in the season. In this report, we present evidence that local sources of clonal inoculum from previous crops are important and it is critical that growers control sources of primary inoculum by management of growth on outgrade piles, minimising or treating volunteers and continuing to buy high quality seed. They should also be aware of the risks of soil-borne oospores giving rise to patches of severe disease on leaves in contact with the soil early in crop growth. Maintaining long crop rotations is the best way to reduce the risks of oospores. Current research at James Hutton has generated decision support tools that have updated the Smith Period with the Hutton Criteria, providing growers with an improved decision support tool to use alongside the FAB Campaign. New genotypes continue to threaten the GB potato crop and the fluazinam insensitive 37_A2 lineage is altering product selection in fungicide programmes in Britain and across Europe. More work on this and the other rapidly spreading lineage (36_A2) is underway to investigate how 'best practice' should change in the future. FAB

monitoring in 2019 will utilise FTA cards to provide in-season feedback to scouts allowing growers and advisors flexibility in their fungicide choices. Continued environmental and political pressure on reducing fungicide usage is focussing attention on Integrated Pest Management (IPM) systems that combine the use of fungicides, host resistance and decision support tools to increase the sustainability of late blight management. The use of AHDB-sponsored FAB monitoring data such as this is going to remain crucial to the success of such an approach both now and into the future.

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