Population monitoring and fungicide sensitivity testing of *Phytophthora infestans* across GB 2019-21

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1. Summary

Aims

This report presents the outcome of the monitoring of the population structure of *P. infestans* in GB for the 2019, 2020 and 2021 seasons and the results of fungicide sensitivity testing on a sample of the collected isolates from each year. It provides feedback to the industry on the impact of such changes for ongoing 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. A procedure used for the first time in 2019 involved pressing lesions onto FTA cards from which the pathogen DNA could be typed, providing within-season feedback on population change. Rapid population change and concerns about fungicide efficacy also prompted a laboratory-based screen of fungicide sensitivity on detached potato leaves. Lastly, the sample data was integrated into the EuroBlight international late blight database allowing more detailed mapping and genotypic analysis to place the results on GB populations in a wider European context.

Key findings

2019

- The weather in 2019 was relatively warm and blight pressure was variable. Some high regional rainfall such as across much of the potato growing region of England and Wales in June and parts of northeast Scotland in May and August drove locally challenging blight outbreaks. However, intervening spells of warm dry weather checked disease progression and eased the disease pressure in some regions.

- A higher than average 229 outbreaks were reported by 63 blight scouts resulting in 1434 samples. Over 1000 samples were successfully genotyped and showed the GB population of *P. infestans* remained dominated by genotype 6_A1 at 36%. Of the two newer lineages, 36_A2 genotype increased from 17 to 27% while 37_A2 dropped from 16 to 6% of the sampled population. The 13_A2 genotype comprised 9% of the population and the genetically diverse class of pathogen types (termed 'Other') increased from 10 to 19%. Within-season genotyping of samples on FTA cards was successful with some samples processed one day after delivery and most processed within one week.

- A feature of the 2019 season was the spread and establishment of genotype 36_A2. Marked regional differences in the incidence of 36_A2 were apparent; it comprised 44% of the samples in England compared to 29% in 2018. This was driven by high disease pressure and a spike in samples from eastern England in June. It was recorded for the first time from crops in Scotland in 2019 and made up 8% of samples. It was not reported from southwest England or from Wales. The continued displacement of existing populations by this lineage suggests it is aggressive and more challenging to manage than other lineages.

- Outbreaks in northeastern Scotland (Aberdeenshire and Moray) were again dominated by the diverse 'Other' genotypes. This is further evidence that late blight outbreaks are

caused by sexually generated oospores in this region but there is no evidence that these forms of the pathogen are more persistent or damaging than the well-adapted clones.

- The sensitivity of contemporary isolates of new (36_A2 and 37_A2) and older (6_A1) lineages to seven fungicide active ingredients (cyazofamid, propamocarb, mandipropamid, fluopicolide, oxathiapiprolin, amisulbrom and mancozeb) was tested. All isolates were sensitive to even very low doses of the products and no significant changes in sensitivity were observed.

2020

- Following a warm and wet February that caused concerns regarding active overwinter growth of hosts of *P. infestans*, an exceptionally dry and often warm three months suppressed pathogen activity. Blight outbreaks began in late June and early July and disease pressure was generally lower than average.

- A total of 94 outbreaks were reported and of 681 samples, 432 were genotyped. Since the pathogen population was suppressed by the weather conditions, the population remained relatively stable. The frequency of 6_A1 was similar to 2019 at 35% but 13_A2 declined and made up only 1% of samples. The frequency of 36_A2 increased slightly from 27% to 29% of the population. The fluazinam insensitive 37_A2 genotype also increased from 6 to 10%. The proportion of the genetically diverse class of pathogen types (termed 'Other') decreased from 17 to 14% of the population and was again predominantly found in northeast Scotland.

- Data from the 2019 and 2020 AHDB Potatoes-sponsored FAB campaign has 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/).

- Further fungicide sensitivity testing of isolates from the 2020 population against the same active ingredients tested in 2019 showed no change in the effectiveness of these key fungicide groups.

2021

- Warmer and drier weather conditions than average in March and a much colder and drier than average April across much of the GB cropping area severely checked any sources of late blight primary inoculum on dumps and volunteer potatoes. Although, on average, the conditions were less conducive for blight across the country there were localised spells of exceptionally high risk with, for example, 30 consecutive days with Hutton Criteria across parts of the Fens in July. Blight outbreaks in Scotland were later than average.

- A total of 210 positive blight outbreaks were reported from 67 active blight scouts with the earliest in the south on 26th May in Kent and in the north on the 15th July in Angus. The principle change in the population of *P. infestans* was a further dominance of genotype 36_A2 which increased from 29 to 40% of the sampled population across GB crops (64% of samples from crops in England). Genotype 6_A1 reduced from 35 to 24%, 13_A2 remained below 1% and 37_A2 was stable and comprised 9% of samples. The proportion of the genetically diverse class of pathogen types (termed 'Other') increased from 14 to 18% of the population and was again predominantly found in northeast Scotland. Two new genotypes were found in GB crops; EU_41_A2 (first found in Denmark in 2013) was reported in a single crop in Fife, Scotland and a new genotype EU_44_A1 was sampled

in Scotland, Wales and England. FTA cards again proved successful in returning in-season data to growers and advisors to help shape effective late blight fungicide programmes.

- Data from the 2019 and 2020 AHDB Potatoes-sponsored FAB campaign has 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/).

- Further fungicide sensitivity testing of isolates from the 2021 population against the same active ingredients tested in 2019 showed no change in the effectiveness of these key fungicide groups.

Data from this study were disseminated to the industry via presentations at AHDB Potatoes events such as AHDB Agronomists' Conferences (2019 & 2020) and the online AHDB Agronomy week (2020) and via multiple press releases and articles in the agricultural press (Crops, Crop Production Magazine, Potato Review, Farmers Weekly, AHDB Grower Gateway etc). In the absence of a formal AHDB Potatoes knowledge transfer activities in 2021, information was disseminated via key agronomists, a press release and the agricultural press. Isolates and DNA from isolates were also provided upon request, to the agrochemical industry in support of baseline sensitivity monitoring and other areas of product stewardship. At the end of formal AHDB Potatoes activities in 2022 reports on Fight Against Blight were archived on the following web page https://potatoes.ahdb.org.uk/fight-against-blight-monitoring-phytophthora-infestans-populations-in-gb for future access.

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.

- The spread of the fluazinam insensitive clone, 37_A2, has been checked, likely in part by a change in product recommendations that has led to a marked decline in the use of fluazinam over the 2018-2021 seasons. This reduction has prevented disease control failures and incidences of tuber blight caused by 37_A2 that were being reported in 2017. The ability to react within a single season to a significant change in the population that compromises late blight control demonstrates the benefits of FAB monitoring and the importance of sample submission by blight scouts.

- All three 2019-2021 seasons have seen unusually dry conditions during planting and emergence. This has suppressed primary inoculum and reduced disease pressure, but growers need to remain vigilant and be aware of the risks of blight occurring from crop emergence onwards, particularly after mild winters when primary inoculum can survive on host foliage. Most inoculum is of clones surviving overwinter demonstrating that volunteer and cull pile management is vital to reduce this primary inoculum load.

- The continued expanding range of 36_A2 remains a cause for concern. Detached leaf sensitivity testing of isolates of the 'new' 37_A2 and 36_A2 and the older 6_A1 clones against seven key active ingredients showed no significant issues for resistance. No changes to current best practice are required. Nonetheless, there are reports of severe

field infections caused by 36_A2 and some data suggesting it is more aggressive and damaging than other lineages. Tight control of spray intervals and careful use of fungicides following FRAG guidelines remains important for optimal blight management and to protect active ingredients from future fungicide resistance issues. This will be particularly important when mancozeb is withdrawn from use.

- Blight scouts have reported that the use of DNA preserved on FTA cards to complement fresh lesion samples has been successful as they have benefited from updates on the population within the season to help in decision making.

- Although not studied in this project, the loss of diquat for crop burn-down at the end of the season has caused concerns. Low level spread of blight on crop regrowth is a serious threat to tuber health. Growers should be aware of the risk and maintain fungicide applications where regrowth is a problem.

- 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 to allow soil-borne oospore inoculum to degrade. Infection of volunteer potatoes from soil-borne inoculum continues to be a concern. Infected potato volunteer plants in crops and on fallow land remain problematic.

3. Introduction

Potato late blight, caused by *Phytophthora infestans*, remains 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 yield losses and even crop failure. Late blight disease is also problematic on tomato and potato crops in home gardens and can spread from these sources. Active sporulation, in which every square centimetre of lesion can produce 20,000 sporangia per day (Skelsey et al., 2009), can, under optimal conditions, lead to explosive disease epidemics. The population of *P. infestans* has been shown to evolve over time 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's border; lastly, chance events related to the dramatic change in population size between seasons and driven primarily by the weather (i.e. genetic drift and founder effects). The implications of population change are twofold; firstly, new populations 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 management relies on knowledge of the source of inoculum and conditions under which disease occurs, the efficacy of fungicides and host resistance. Given the marked changes to the *P. infestans* population and the potential for increasing diversity in the future, integrated management strategies 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 DNA fingerprinting technology that is similar to that used in criminal forensics. The method examines

genetic variation at twelve locations (loci) within the genome of *P. infestans*. These microsatellite, or simple sequence repeat (SSR), markers are sections of DNA with repeated sequence motifs (e.g. AG-AG-AG-AG or GCA-GCA-GCA) which are prone to expansion and contraction mutations that alter their length. These changes in length of alleles at each locus are detected by running the PCR-amplified fluorescent dye-tagged DNA fragments on a capillary electrophoresis instrument against a size standard. The resultant allelic data for all 12 loci is very powerful as it can discriminate genotypes that represent clonal lineages and also detect minor variation within a lineage (Li et al., 2013a). In addition, this SSR data allows genetically distinct pathogen types that may have arisen via sexual recombination to be detected. When 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, it 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 or Green 33) 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). More recently, the spread of a lineage called 36_A2 has been documented since it was first identified in the potato starch production regions of northern Germany and the Netherlands in 2014. Another recent clone, 41_A2, originated in Denmark in 2013, has since spread to neighbouring countries but until 2021 had not been recorded any further west than some crops in Germany (Puidet et al., 2022). Such changes in European lineages can be tracked via the EuroBlight web pages (www.euroblight.net). This continued spread of *P. infestans* from mainland Europe to British crops has mirrored the situation in 1845 when potato blight first occurred in the 'low countries' of mainland Europe and spread across to Ireland in a single season (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 across all potato growing 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 blight pathogen propagates mostly through the generation of asexual sporangia from sporulating lesions, however sexual oospores can also form an important part of the disease cycle. The risk of oospore formation has increased following the spread of the A2 clonal genotypes such as 13_A2 and 36_A2 amongst prevalent A1 types such as 6_A1 and 8_A1. 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 any co-infection will result in their interaction and subsequent oospore formation. Once the crop rots, such propagules end up in the soil 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 the occurrence 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 (Cooke et al., 2014). A very 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, oospores in soil act as an extra source of long-lived primary inoculum that survives 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.

Successful management of late blight has long been reliant on agrochemical inputs. Since observations in the 19th century that copper sulphate and fumes from zinc factories reduced disease (Zadoks, 2008), the use of chemistry has expanded. Although copper and zinc are still used in some circumstances, the progression to synthetic products with activity against oomycetes has been dramatic and twelve different active ingredient groups are currently listed for late blight control (FRAG-UK, 2018). This range of fungicides enables management strategies that mix or alternate active ingredients across the season to minimise the risks of resistance developing in the population of *P. infestans*. However, the size of the pathogen population and the adaptability of its breeding system (Brasier, 1992) and the large and genetically plastic genome (Haas et al., 2009; Cooke et al., 2012) has led to prior fungicide resistance problems. Until recently the principal example was resistance to a valuable systemic fungicide in the phenylamide group. Problems with resistance to metalaxyl and its more active R-enantiomer known as Metalaxyl-M or mefenoxam, were reported from as early as 1979 (Gisi & Cohen, 1996) but, as detailed above, it was full resistance of isolates of the 13_A2 lineage (also known as Blue-13) that resulted in a marked decline in the use of this active ingredient in the UK. Resistance to fluazinam was considered unlikely but isolates of the 33_A2 and 37_A2 genotypes that emerged in the Netherlands in 2009 and 2013, respectively have proved problematic (Schepers et al., 2018). As part of fungicide product stewardship, agrochemical companies have a duty to investigate and report issues with product efficacy. However, concerns about further emergence and spread of fungicide insensitivity in 2019 led to proposals that AHDB Potatoes conducted further testing.

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 (e.g. 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 with this applied research. The EuroBlight consortium has developed a pathogen population database, hosted at the Aarhus University and managed at the James Hutton Institute, which provides a platform for mapping the data and comparing genetic diversity across different parts of Europe. All AHDB Potatoes FAB data on *P. infestans* from GB crops from 2006 to 2021 has been uploaded onto this database and a summary will be presented in this report.

4. Experimental Section

Population monitoring

Outbreak sampling

As per the previous reporting period (Cooke, 2019), a target of 100 outbreaks each season was set with multiple samples per outbreak to provide the best compromise between breadth and depth of sampling. In response to industry requests for within-season genotyping FTA cards were also distributed with sampling packs in 2019, 2020 and 2021. Scouts registered with the AHDB Potatoes FAB campaign website which provided them with access to their own sample data throughout the season. Each scout collected up to eight late blight lesions per crop; four of which were provided as fresh material with the other four preserved by pressing onto FTA cards (see Appendix 1). Each sample was located by postcode district and was sent to the James Hutton Institute in Dundee within a postage-paid padded envelope. At the point of submission, scouts entered sample details via the FAB web-site which generated a sample ID. Once in the laboratory and confirmed as blight, the FAB database was updated, the scout informed and the map was updated (Figs. 1-3). Accompanying metadata relating to each sample was also recorded (Appendix 2). Upon receipt in Dundee, positive samples were placed within between two halves of a small potato tuber and incubated at room temperature (17-19˚C) for 24 hours. FTA sampling involved pressing sap from the growing edge of actively sporulating late blight lesions (Fig. 4) 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) for at least 12 years.

Sample processing

Slices of tuber ca. 5 mm thick were taken from the zone in contact with the blighted plant material and laid in a Petri dish with the freshly cut surface uppermost. The Petri dishes were stored in a sealed box to prevent them drying out. The tuber slices were inspected daily over a 1 – 4-day incubation period at room temperature on the laboratory bench. Any tuber tissue with white fluffy sporulation of *P. infestans* was plated onto a primary isolation plate of a 50:50 mix of Pea and Rye A agar supplemented with antibiotics (final concentrations Chloramphenicol 34 µg ml^{-1}, Rifampicin 30 µg ml^{-1}, Ampicillin 150 µg ml^{-1}, Pimaricin 10 µg ml^{-1}). An improvement using 'wanding' was used to decrease the risk of bacterial contamination and increase isolation success. This involved cutting a 5 x 5 mm square of isolation media and very gently touching the surface of 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. 19ºC) 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. 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. A small number of tuber samples were also provided direct to the James Hutton Institute. These were washed and cut in the same way as the foliar samples.

Testing genetic diversity of isolates

In most samples, small fragments (ca. 2 mm^3) 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. 4), 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 using the R package *poppr* (Kamvar et al., 2015) in addition to more detailed genotype mapping. Once the genotypes were determined the data was uploaded to the FAB web page which prompted an automated email update to the specific scout who had logged those samples.

Fungicide sensitivity testing

The aim of this testing was to determine the relative sensitivity of isolates of genotypes 36_A2 and 37_A2 compared with control isolates of older lineages to cyazofamid, propamocarb, mandipropamid, fluopicolide, oxathiapiprolin, amisulbrom and mancozeb (Table 1).

Isolates

In each of three years, isolates of 36_A2 (n=5), 37_A2 (n=5) and 6_A1 (n=5) were selected for testing to provide a comparison of fungicide sensitivity between newer (36_A2 and 37_A2) and older (6_A1) genotypes of *P. infestans*. To provide results based on the most contemporary populations, isolates of 36_A2 and 6_A1 were sourced in-season from blight samples received through the 2019-2021 Fight Against Blight campaign. Due to the absence of 37_A2 genotypes in isolates sampled at the beginning of the 2019 season in this year only FAB isolates of this genotype were sourced from the previous years' epidemic.

In one season, four additional isolates of 36_A2 were tested along with 6_A1 controls. These isolates were obtained from fields with late blight control failure as reported by agronomists participating in the FAB campaign. As they were tested independently, the results of this second round of testing are presented separately.

The inclusion of isolates of genotype 41_A2 was not possible in the comparison. This genotype has been emerging in other European countries, but no samples were received in 2019 and insufficient isolates were available in 2020 and 2021 to make a legitimate comparison across genotypes. The Scottish outbreak in 2021 emerged too late for inclusion in the testing.

Production of plant material

All sensitivity tests were carried out using detached leaf protocols and used plant material produced as follows. Plants of Maris Piper (blight susceptible cultivar lacking R genes) grown in pots from seed tubers were maintained under glasshouse conditions. No pesticides were applied. When plants were approximately 5 weeks old, leaflets for inoculation were harvested from plants immediately before use.

Detached leaf treatment and inoculation method

All tests: six leaflets per isolate (2 replicates x 3 leaves) and fungicide concentration were tested (24 leaflets per a.i.). Leaflets were individually dipped in the appropriate fungicide solution and placed abaxial side up in a clean plastic tray lined with damp tissue paper and the lid replaced. Trays were then kept at 18C for 24 hours before inoculation. The range of fungicide

concentrations tested (6 concentrations per active ingredient) was based a) on those specified in the FRAC protocol for testing CAA and other fungicides and b) concentrations tested in similar work carried out in 2018 and known to be appropriate for the calculation of EC_{50} values in each case. The concentrations tested for each active ingredient are listed (Table 2).

Inoculation and incubation

For detached leaf assays, each leaflet was inoculated by depositing one 20µL droplet of the inoculum suspension on the abaxial (lower) side of the leaflet. Inoculated leaflets were incubated for 7 days in a north-facing glasshouse maintained at 16–18°C under natural daylight conditions. The number of sporulating lesions was then counted and lesion size was measured. All treatments were compared with untreated controls.

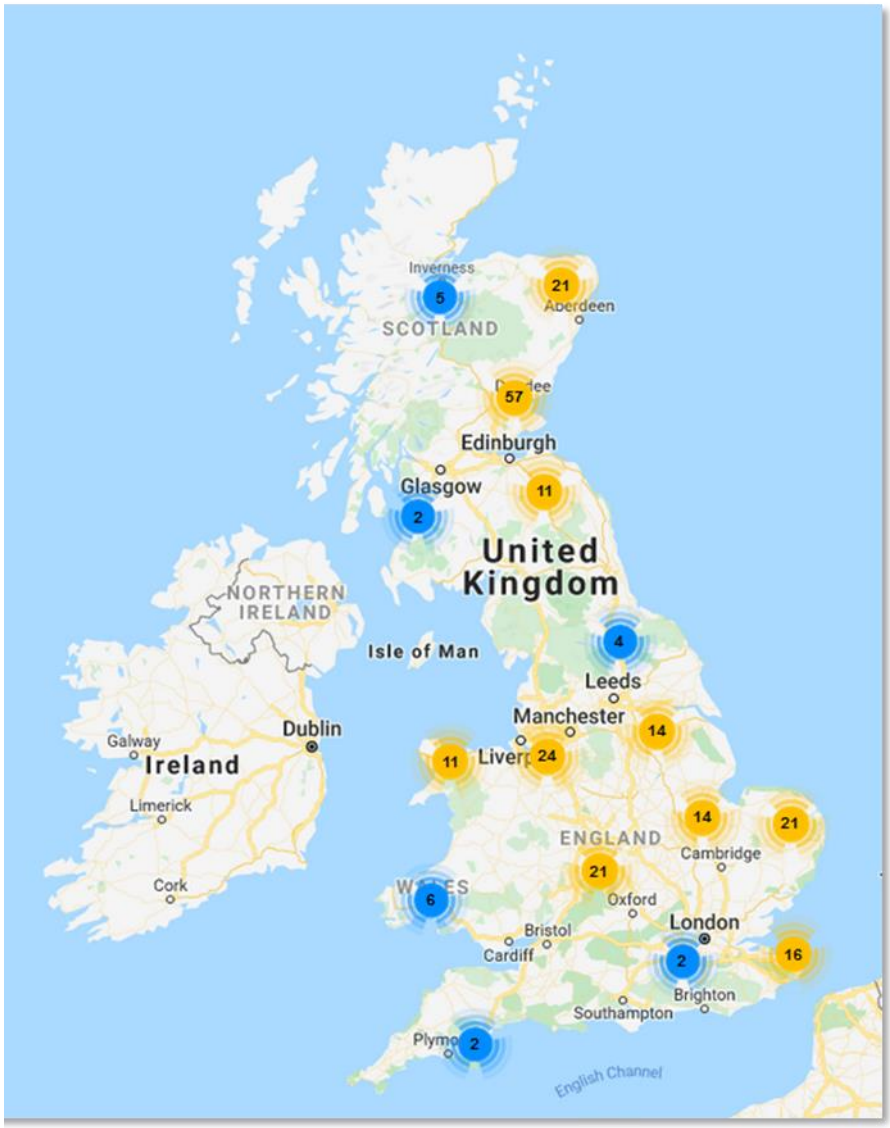

Figure 1. Locations of the late blight outbreaks in 2019 recorded by the FAB campaign.

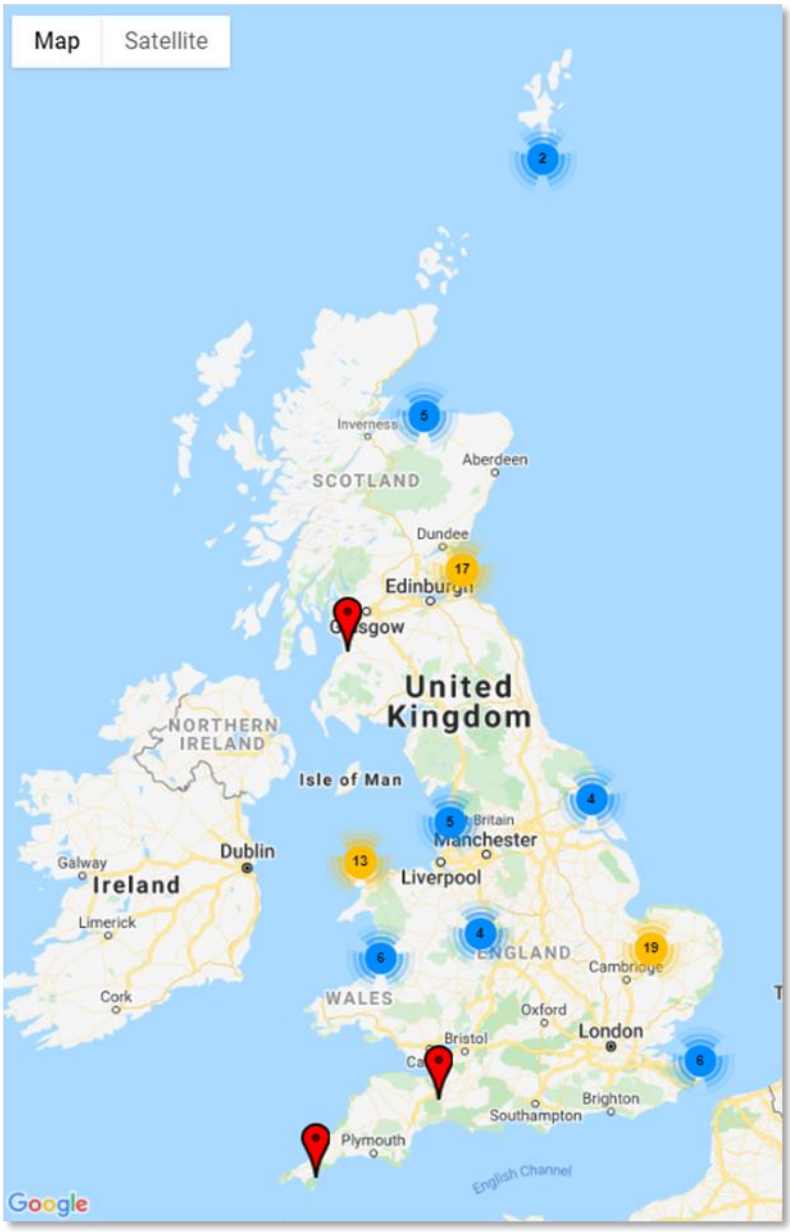

Figure 2. Locations of the late blight outbreaks in 2020 recorded by the FAB campaign.

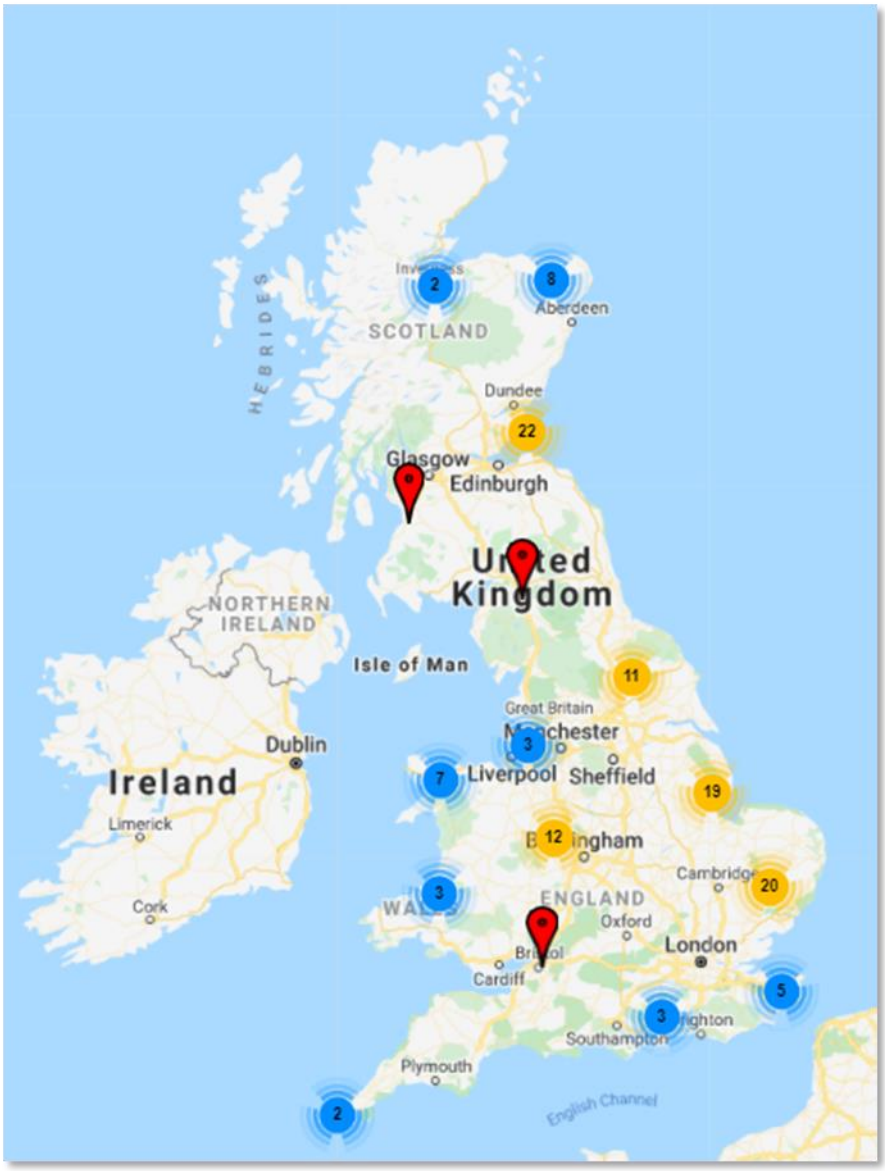

Figure 3. Locations of the late blight outbreaks in 2021 recorded by the FAB campaign.

Figure 4. Example of a blight lesion pressed onto an FTA card in the field.

Table 1. List of tested fungicide active ingredients and their characteristics

Fungicide Group (FRAC Code)	Active Ingredient	Product	Max dose (l/Ha)	Volume (l/Ha)	Max Tank Mix (ppm)
Qil (21)	Cyazofamid 160g/l	Ranman	0.5	200-400	400
Qil (21)	Amisulbrom 200g/l	Shinkon	0.5	200-500	200
CAA (40)	Mandipropamid 250g/l	Revus	0.6	>200	750
Carbamates (28)	Propamocarb 722g/l (625g/l as Infinito)	Promess	1.6	200-400	5000
Benzamides (43)	Fluopicolide 5mg/ml (62.5g/l as Infinito)	Pure active (Sigma Aldrich)	1.6	200-400	500
OSBPI (49)	Oxathiapiprolin 100g/l	Zorvec	0.15	200	75
Dithiocarbamates (M03)	Mancozeb 750g/Kg	Penncozeb	1.7(kg/Ha)	200	6375

1 mg/l = 1 µg/ml = 1 ppm

Table 2. Fungicide dose ranges tested in parts per million of active ingredient with a comparison to the maximum tank mix dose currently approved for field use.

Active ingredients	ppm a.i.						
	level-1	level-2	level-3	level-4	level-5	Level-6	Max. tank mix
Cyazofamid	0	0.1	0.3	1	3	10	400
Amisulbrom	0	0.1	0.3	1	3	10	500
Mandipropamid	0	0.1	0.3	1	3	10	750
propamocarb	0	10	100	300	500	1000	5000
fluopicolide	0	0.5	1	5	10	100	500
oxathiapiprolin	0	0.0005	0.001	0.01	0.1	0.3	75
mancozeb	0	1	10	100	500	1000	6375

1 mg/l = 1 µg/ml = 1 ppm

Calculation of EC_{50} values

According to the FRAC definition, EC_{50} stands for effective control to 50% (i.e. the dose of fungicide that provides 50% inhibition of the isolate as compared to a non-fungicide-amended control). Advice was sought from BioSS regarding the calculation of EC50 values in this study. EC50 for each replicate was estimated by fitting a non-parametric spline to the lesion size data at different concentrations of fungicide. Interpolation was used to obtain the level of fungicide corresponding to the estimate of lesion size at a point midway between the maximum and minimum lesion size values. Differences for EC_{50} between genotypes were then analysed using Fisher's protected least significant difference test at P = 0.05 using Genstat (VSN International).

Lesion Area (mm^2) data is presented as Box & Whisker plots defined as follows: a box and whisker chart shows the distribution of data into quartiles, highlighting the mean and outliers. The boxes may have lines extending vertically called "whiskers". These lines indicate variability outside the upper and lower quartiles, and any point outside those lines or whiskers is considered an outlier.

Fluopicolide

Fluopicolide is usually formulated as a mixture with propamocarb (as Infinito) at a rate of 62.5g/l fluopicolide and 625g/l propamocarb. For the purposes of this test pure active ingredient of fluopicolide (5mg/l) was purchased (Sigma Aldrich) and the technical grade product was first dissolved in acetone to a concentration 100x the final desired concentration. Stock solutions were then diluted in water to final test concentrations (100, 10, 5, 1, 0.5, 0 µg/ml). Detached leaf assays were carried according to a modified version of the method of Latorse & Kuck (2006) using the range of concentrations specified in their original analysis to examine baseline sensitivity changes with isolates from 2001-2006 across Europe. The original assays of Latorse & Kuck (2006) were conducted using a floating leaf disc test and their results presented below (Table 3). This study was carried out using the detached leaf tests, as used for the other fungicides. It should be noted that EC_{50} maximum and minimum values can be affected by use of slightly different tests. However, differences between genotypes should be identifiable.

Table 3. Fluopicolide baseline sensitivity data for *P. infestans* taken from Latorse & Kuck (2006)

Year	2001	2002	2003	2004	2005	2006
Number of isolates	36	75	59	38	33	37
Mean EC_{50} (mg/L)	4.7	4.1	5	4.8	2.7	3.5
EC_{50} min (mg/L)	1.8	0.7	1.6	0.5	1.3	1.5
EC_{50} max (mg/L)	19	16	14.3	11	5.4	8.5

Fluopicolide Zoospore Motility Test

As fluopicolide is known to have activity against zoospores, isolates were also tested for zoospore motility using the method as described as conducted in the studies of Schepers et al (2018) which is a modified version of that used by Cooke et al (1998) for fluazinam.

Sporangial suspensions (105 sporangia/ml) were prepared from infected leaflets (as previously described) and were incubated at 4°C for 3h to stimulate zoospore release. Serial dilutions of fluopicolide were prepared from a 5mg/ml stock and 250 µl aliquots pipetted into each well of 24-well plates (Cellstar, Cat.-No.662 160). Subsequently, 250 µl aliquots of sporangial suspension were added to each well to give final concentrations of 10, 1, 0.2, 0.1 and 0.05 µg fluopicolide/ml (ppm). Two replicate wells per isolate were used for each concentration and water controls were included. The solutions and plates were chilled to 4°C before use to maintain zoospore motility. After 1 and 2 hours of incubation at 4°C, zoospore motility was assessed on a 1-3 scale, where 1 = not motile, 2 = motile, 3 = very motile. Results were expressed in terms of the minimum inhibitory concentration (MIC), defined as the lowest concentration which completely inhibited zoospore motility.

Mandipropamid

Cohen et al (2007) previously tested sensitivity to the carboxylic acid amide (CAA) fungicide mandipropamid in isolates of *Phytophthora infestans* collected between 1989 and 2002 in Israel prior to its commercial use. Leaf disc and detached leaf assays provided baseline sensitivity information for 44 isolates. They further tested isolates from treated (25 isolates) and untreated fields (215 isolates) originating from nine European countries and Israel between 2001 and 2005. All isolates were sensitive to mandipropamid, with EC_{50} values ranging between 0·02 and 2·98µg/mL. Subsequently, a subset of USA dominant lineages (n = 45) collected between 2004 and 2012 was tested in vitro on media amended with a range of concentrations of either azoxystrobin, cyazofamid, cymoxanil, fluopicolide, mandipropamid, or mefenoxam by Saville et al (2015). No insensitivity to azoxystrobin, cyazofamid, cymoxanil, fluopicolide, or mandipropamid was detected within any lineage. EC_{50} values for mandipropamid from this work are presented in Table 4. As described previously a detached leaf test was conducted with isolates: 36_A2 (n=5), 37_A2 (n=5), 6_A1 (n=5) at mandipropamid concentrations of 0, 0.1, 0.3, 1.0, 3.0, 10.0 µg/ml (according to FRAC protocol concentrations).

Table 4. Mean effective concentration at which 50% of growth was suppressed (EC_{50}) values for mandipropamid of US clonal lineages of *Phytophthora infestans* collected from 2004 to 2012 in the US (from Saville et al., 2015).

	Mean ± SE EC_{50} (µg ml−1)z
US Clonal lineage	Mandipropamid
US-8	0.02 ± 0.01 (0.01–0.04) ab
US-11	0.01 ± 0.00 (0.01–0.02) c
US-20	0.03 ± 0.01 (0.02–0.03) a
US-21	0.01 ± 0.00 (0.01–0.01) bc
US-22	0.01 ± 0.00 (0.01–0.02) bc
US-23	0.01 ± 0.00 (0.00–0.02) c
US-24	0.01 ± 0.00 (0.01–0.02) bc

Fungicide EC_{50} values (minimum–maximum) are based on pooled data from two independent trials and three replicates per trial. Mean EC_{50} values followed by the same letters are not significantly different according to Duncan's multiple range test. SE = standard error.

Cyazofamid

In tests conducted on amended media, Saville et al (2015) found that most isolates of US genotypes failed to grow on media amended with cyazofamid, and a sharp decline in growth was observed at all concentrations above 0.1 µg ml^{-1}. The only exception was a single US-8 lineage isolate collected in 2010 (EC_{50} = 0.30). Mitani et al (2001) reported that cyazofamid strongly inhibited all stages in the life cycle of *P. infestans*. Minimum inhibitory concentrations (over 90% inhibition) against indirect germination of zoosporangia (zoospore release), zoospore motility, cystospore germination, and oospore formation were 0.1–0.5, 0.005, 0.05, and 0.01 mg/ml, respectively. Cyazofamid at 0.1 mg/ml exhibited complete fungicidal activity on zoospore release of *P. infestans* 60 min after treatment. Sensitivity tests conducted on French populations of P. infestans unknown genotype in 2016 (Gaucher et al., 2016) using leaf disc assays inoculated with fungicide amended inoculum reported no resistance with all isolates controlled by a concentration of 1mg/l (1µg/ml). As described previously, the detached leaf test was conducted with isolates: 36_A2 (n = 5), 37_A2 (n=5), 6_A1 (n=5) at cyazofamid concentrations of 0, 0.1, 0.3, 1.0, 3.0, 10.0µg/ml (according to FRAC protocol concentrations).

Amisulbrom

Previous work (Förch et al., 2007) was carried out to determine EC_{50} values of NC-224 20SC (active ingredient amisulbrom) for four stages in the life cycle of *P. infestans*. The four stages selected were the release of zoospores, motility of zoospores, germination of cystospores and the formation of oospores in planta. The EC50 of NC-224 20SC for zoospore release, motility of zoospores and germination of cystospores was found to be 0.016 ppm, 0.0002 ppm and 0.061 ppm, respectively. Oospore formation was also sensitive to exposure to NC−224 20SC. Both, the total number of oospores and the number of viable oospores formed were reduced. The EC_{50} value for the fraction of viable oospores formed was determined to be 35% of the recommended dose rate. As described previously, the detached leaf test was conducted with isolates: 36_A2 (n = 5), 37_A2 (n=5), 6_A1 (n=5) at amisulbrom concentrations of 0, 0.1, 0.3, 1.0, 3.0, 10.0µg/ml (according to FRAC protocol concentrations).

Propamocarb

Propamocarb is usually formulated as a mixture with fluopicolide (as Infinito) at a rate of 62.5g/l fluopicolide and 625g/l propamocarb. For the purposes of this test propamocarb was purchased as a single active in the product 'Promess' (722g/l a.i.) and dilutions made accordingly. Grunwald et al., (2006) examined baseline sensitivity of 4-60 isolates of Mexican *P. infestans* isolates using amended media assays and found a range of EC_{50} values from 0.1 to 1000 µg/ml (converted from log values).

As described previously. Detached leaf test conducted with isolates: 36_A2 (n=5), 37_A2 (n=5), 6_A1 (n=5) at propamocarb concentrations of 0, 10, 100, 300, 500, 1000 µg/ml. These were shown to be the best discriminatory doses for calculation of EC_{50} in 2018 based on a combination of FRAC and C-IPM protocols.

Oxathiapiprolin

Cohen et al (2018) tested the preventive and curative (1 day post inoculation) efficacy of oxathiapiprolin against tomato late blight induced by 106 and 90 field isolates of *P. infestans*, respectively. Minimal inhibitory concentration (MIC) values in preventive application ranged between 0.0001 and 0. 1-ppm ai with 17, 51, 35 and 3 isolates fully inhibited at 0.0001, 0.001, 0.01 and 0.1 ppm ai, respectively. Baseline sensitivity testing to oxathiapiprolin carried out in Korea (Aktaruzzaman et al., 2016) on unknown genotypes of *P. infestans* using a leaf disc assay found mean EC_{50} values ranging from 0.00102-0.00120 ppm. Similarly, the EC_{50} value for inhibition of mycelial growth of *P. nicotianae* was shown to be 0.001 ppm a.i. oxathiapiprolin (Qu et al., 2016).

As described previously the detached leaf test were conducted with isolates: 36_A2 (n=5), 37_A2 (n=5), 6_A1 (n=5) at oxathiapiprolin concentrations of 0, 0.0005, 0.001, 0.01, 0.1, 0.3 µg/ml. These low concentrations were chosen based on the previous literature, as cited above, as those most likely to provide robust data for the calculation of EC_{50} for this product.

Mancozeb

Mancozeb is a zinc and manganese-based fungicide that has been registered for more than 60 years. It is a protectant fungicide with multisite inhibitory activity that should result in little or no selection pressure for resistance. Four clonal lineages of *P. infestans* common during the early 1990s in the United States and Canada were evaluated for sensitivity to the protectant fungicides mancozeb and chlorothalonil using amended agar assays for isolates collected from 1990 to 1994 (Kato et al., 1997). No isolate or lineage was resistant and the mean EC_{50} values for mancozeb ranged from 1.61 to 4.22 µg/ml. Similarly, tests on mancozeb amended agar conducted on Brazilian *P. infestans* isolates (Reis et al., 2005) found that the ED_{50} of most isolates (53 of 59) was <1.0 µg/ml. For five isolates, ED_{50} values varied between 1 and 10 µg/ml and, for one isolate, ED_{50} was 25.7 µg/ml. Duvauchelle & Ruccia (2015) presented results of sensitivity testing of mancozeb against 4 genotypes of *P. infestans* (13_A2, 6_A1 and 33_A2) in leaf disk tests and concluded that mancozeb gave effective control against all genotypes but did not state EC_{50} values. There does not appear to be sensitivity data from contemporary European populations.

As described previously, the detached leaf test was conducted with isolates: 36_A2 (n=5), 37_A2 (n=5), 6_A1 (n=5) at mancozeb concentrations of 0, 1, 10, 100, 500, 1000 µg/ml.

Results

2019 Sampling

Late blight outbreaks began very early in 2019 with two outbreaks from dumps in Kent and Suffolk in March although the first reported crop outbreak was not until 9th June in Suffolk. A much wetter than average June over much of England resulted in an early surge in blight samples in the last week of June with a peak of 18 samples in a week (Fig. 5). A second peak of 47 in the week of 19th August comprised samples from both England and Scotland. In total, more than 1400 late blight samples submitted from 229 disease outbreaks across GB (Fig. 1) were delivered to the James Hutton Institute. This was higher than the average of 158 outbreaks sampled per year since 2006 (Fig. 7). From these samples, 519 isolates of *P. infestans* were obtained.

2020 Sampling

After a warm and wet February, very dry warm conditions persisted in March, April and May which acted to suppress the primary inoculum of *P. infestans*. Late blight outbreaks thus began late in 2020 and, unusually, the first positive outbreak was reported in Scotland on 26th June (Highland) and not until the 7th July in England (Shropshire). Sampling intensity was probably impacted by the lockdown conditions required due to the COVID-19 pandemic, but disease pressure was also low. Sample reception was generally low with a peak of only 11 samples in a week in September (Fig. 6). Fight Against Blight scouts frequently sampled late blight outbreaks on volunteer crops later in the season. In total, only 289 late blight samples from 58 disease outbreaks across GB were delivered to The James Hutton Institute. This was lower than the average of 158 outbreaks sampled per year since 2006 (Fig. 7). From these samples, 107 isolates of *P. infestans* were obtained. This isolation rate was impacted by significant delays in mail deliveries, and thus low-quality samples, due to COVID-19 restrictions imposed on the GB postal service.

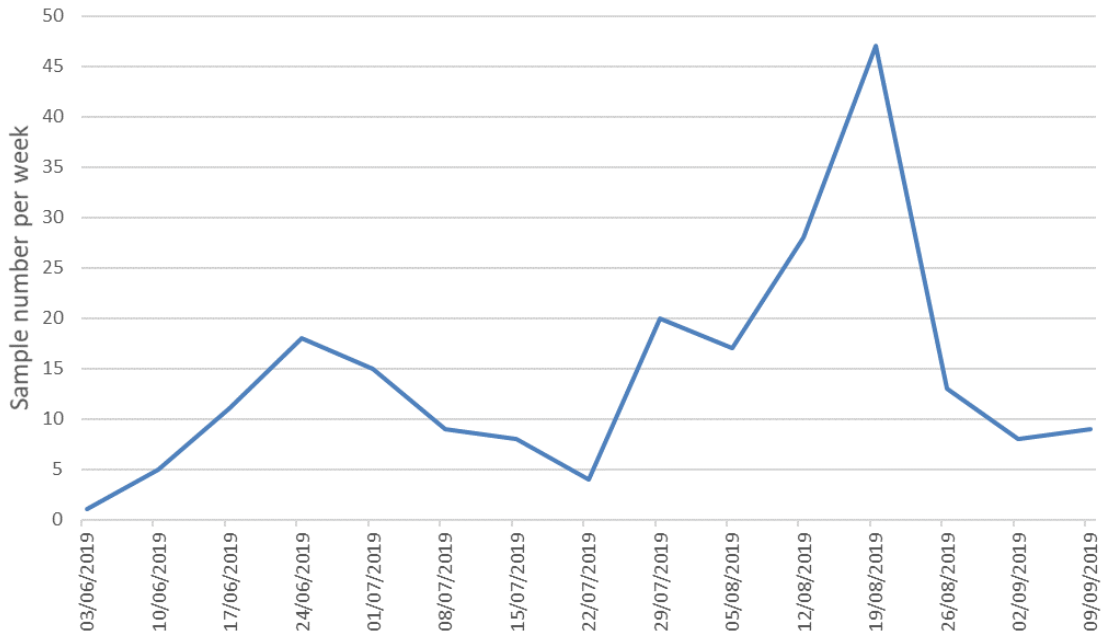

Figure 5. Number of GB late blight outbreaks sampled per week in 2019.

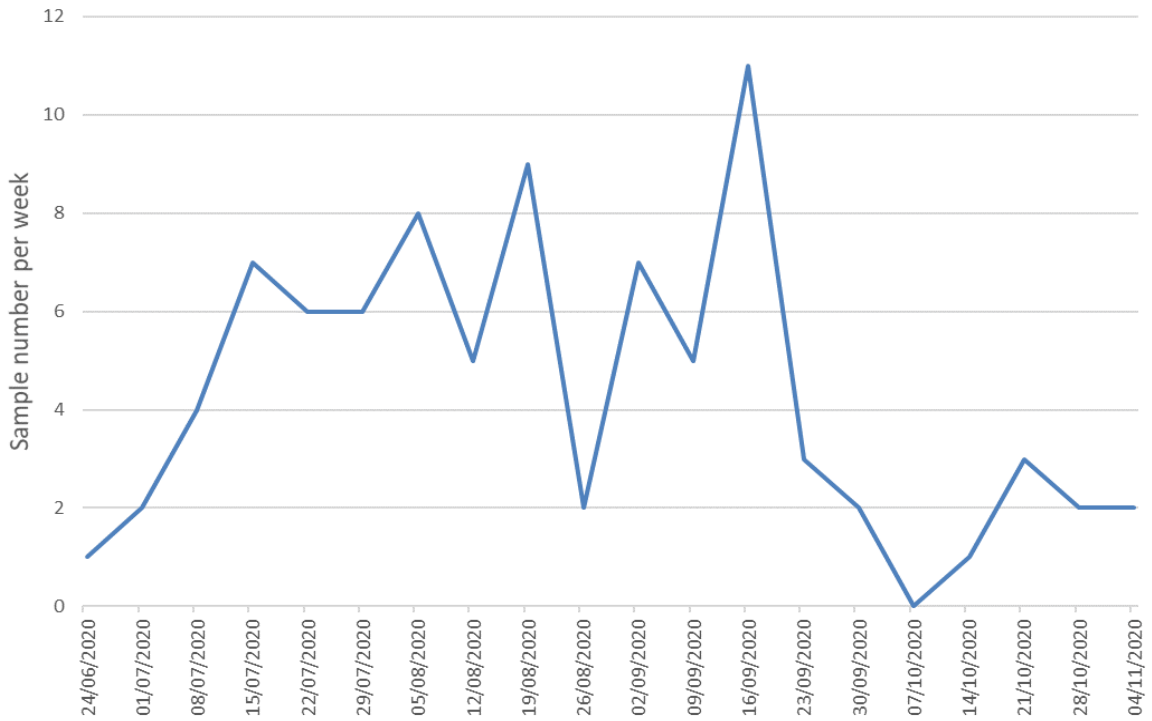

Figure 6. Number of GB late blight outbreaks sampled per week in 2020.

2021 sampling

The blight season again started late due to dry weather in March and April which will have suppressed any emerging overwintering inoculum. Despite the general view that the season was of lower blight risk than average, over 200 outbreaks were reported. This above average number of outbreaks (Fig. 7) was in part due to localised high-risk conditions such as those in eastern England during which Hutton Criteria were met on 30 consecutive days in July 2021 resulting in a spike of 15 to 30 outbreaks per week reported from 19th July to 16th Aug. Blight conditions in Scotland peaked late with 35 outbreaks per week at the end of August (Fig. 8). In total over 1700 late blight samples were submitted to The James Hutton Institute and over 500 isolates of *P. infestans* were cultured.

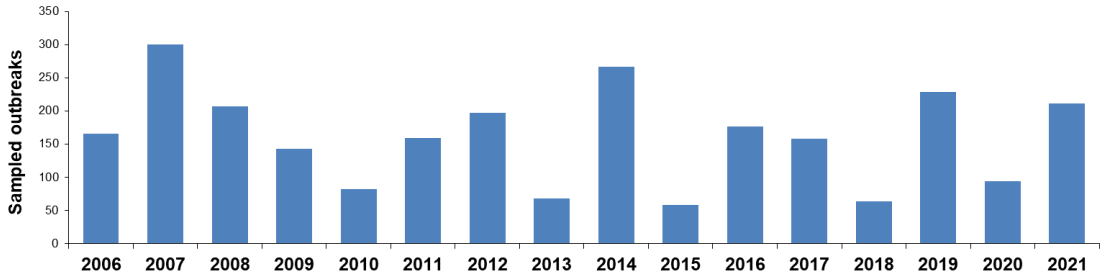

Figure 7. Number of FAB GB late blight outbreaks sampled per year since 2006, providing an approximate guide to late blight disease pressure on a national scale. (n = 2917)

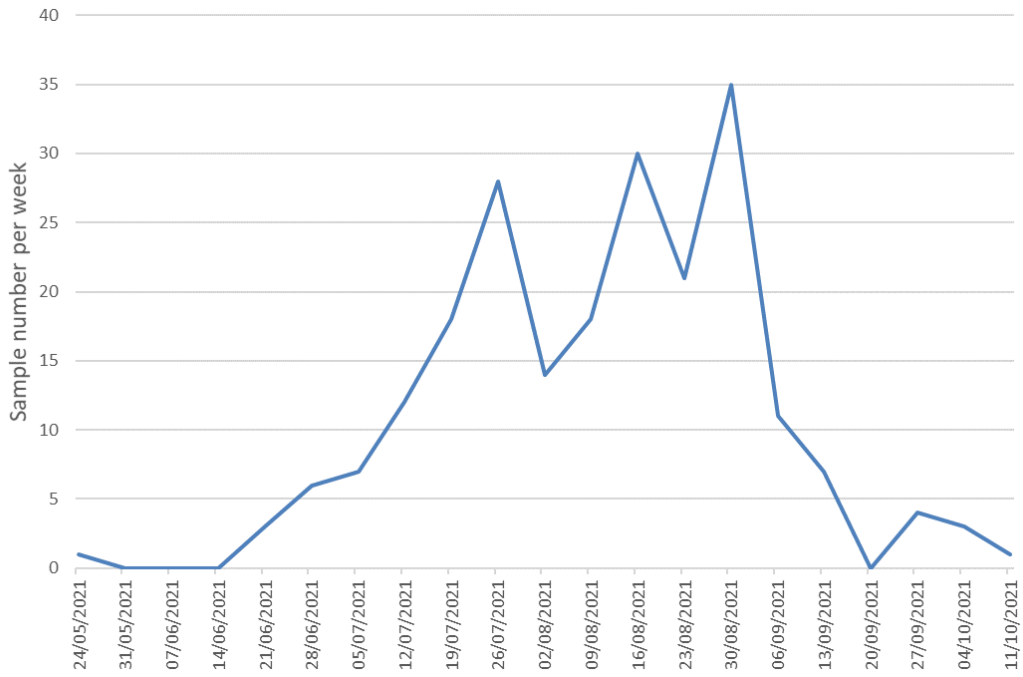

Figure 8. Number of GB late blight outbreaks sampled per week in 2021.

Genetic diversity of isolates (2019 to 2021)

An efficient multiplex genotyping system of 12 SSR loci was used (Li et al., 2013a) with alleles, defined at each locus scored for each *P. infestans* sample. The combinations of alleles for each sample were collated 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, (Cooke et al., 2012)). The system matches that used in the EuroBlight system with the formal name having an EU_ prefix to indicate the region it was first reported. An additional 'catch all' category of genotype termed 'Other', was defined for isolates with novel combinations of alleles found at very low frequency and commonly in only a single blight outbreak and in a single season. A total of 10608 FAB samples from almost 3000 outbreaks have been genotyped to date; the genotype frequencies and annual total in each of the 19 seasons are presented (Fig. 9).

The genotyping results of 977, 432 and 1170 samples from the 2019, 2020 and 2021 seasons, respectively, showed that approximately 80% of the samples were of clonal lineages with 6_A1, 37_A2 and 36_A2 dominating. Genotype 6_A1 was the single most dominant genotype at 36 and 35% of the sampled population in 2019 and 2020 but declined to 24% in 2021 and was displaced by 36_A2 which comprised over 40% of the samples in 2021. This continued the rapid rise in genotype 36_A2 from 1.6% in 2017. Genotype 37_A2 appears stable; although it dropped from 10% to 9% in 2021 this is a marked decline since its high of 24% in 2017. Genotype 8_A1 remains a low but persistent presence at 5% of the 2021 sampled population. The average proportion of 'Other' types over all seasons was 10% so the last 3 years at 17, 14, and 18% are above average (Fig. 9). Genotype 13_A2 is in continuous decline. Having recovered slightly in recent years from a low of 7% in 2011 up to 21% in 2016 and stable at around 10% from 2017-2019 13_A2 comprised less than 1% of the population in 2020 and 2021. An SSR multi-locus genotype (MLG) that was first observed at a low frequency in 2017 but included with the 'Other' category until 2019, was re-sampled in four consecutive years and thus formally named as a new genotype, 42_A2, in 2020. In 2021 this new genotype was not sampled

(see discussion). Two other genotypes are new to the GB potato crop in 2021. EU_41_A2 formerly only sampled in Nordic regions, Poland and Germany was sampled in a single crop in Fife, Scotland in late August 2021. Lastly, a new genotype EU_44_A1 was defined after being sampled in Kent, Ceredigion and three regions of Scotland in addition to other parts of mainland Europe in 2021.

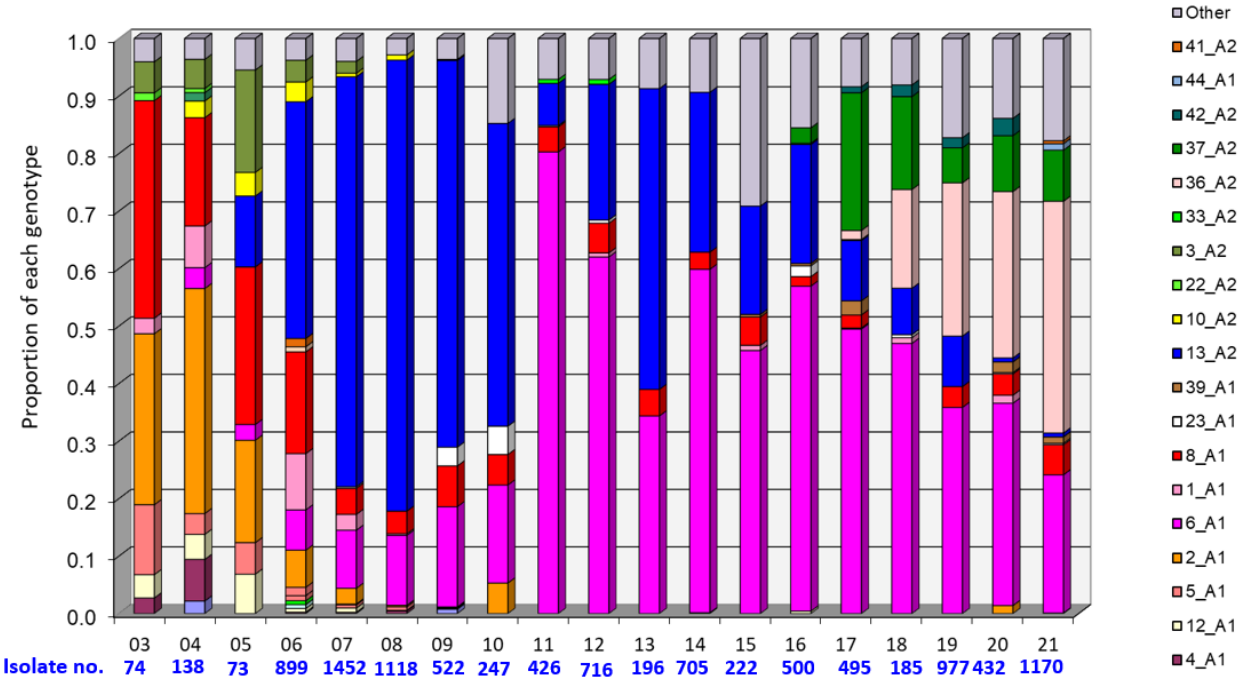

Figure 9. Bar chart indicating the frequency of *P. infestans* isolates of each SSR genotype over the course of 18 seasons (2003-20) and the number of genotyped samples per year.

A breakdown of the population data within GB, indicates marked national differences in the population of *P. infestans* with the samples collected from English crops showing the earliest and most marked shift in population structure (Fig. 10). Although 6_A1 predominated in all three countries, its decline in England and replacement by 36_A2 and 37_A2 genotypes is pronounced. Note that the sampling depth varies from season to season (Figs. 7 and 9) and the relatively low sample numbers in 2013, 2015 and 2018 increases the probability of a skew in datasets coming from relatively few outbreaks. The data plotted by country also reveals that both of the newer genotypes 36_A2 and 37_A2 were later to emerge in Wales and Scotland, that the new genotype 42_A2 and the tomato-specific 39_A1 were sampled predominantly in Wales and that 8_A1 is sampled most often in Scottish crops. The consistently higher frequency of 'Other' genotypes in Scottish crop samples is also evident (Fig. 10).

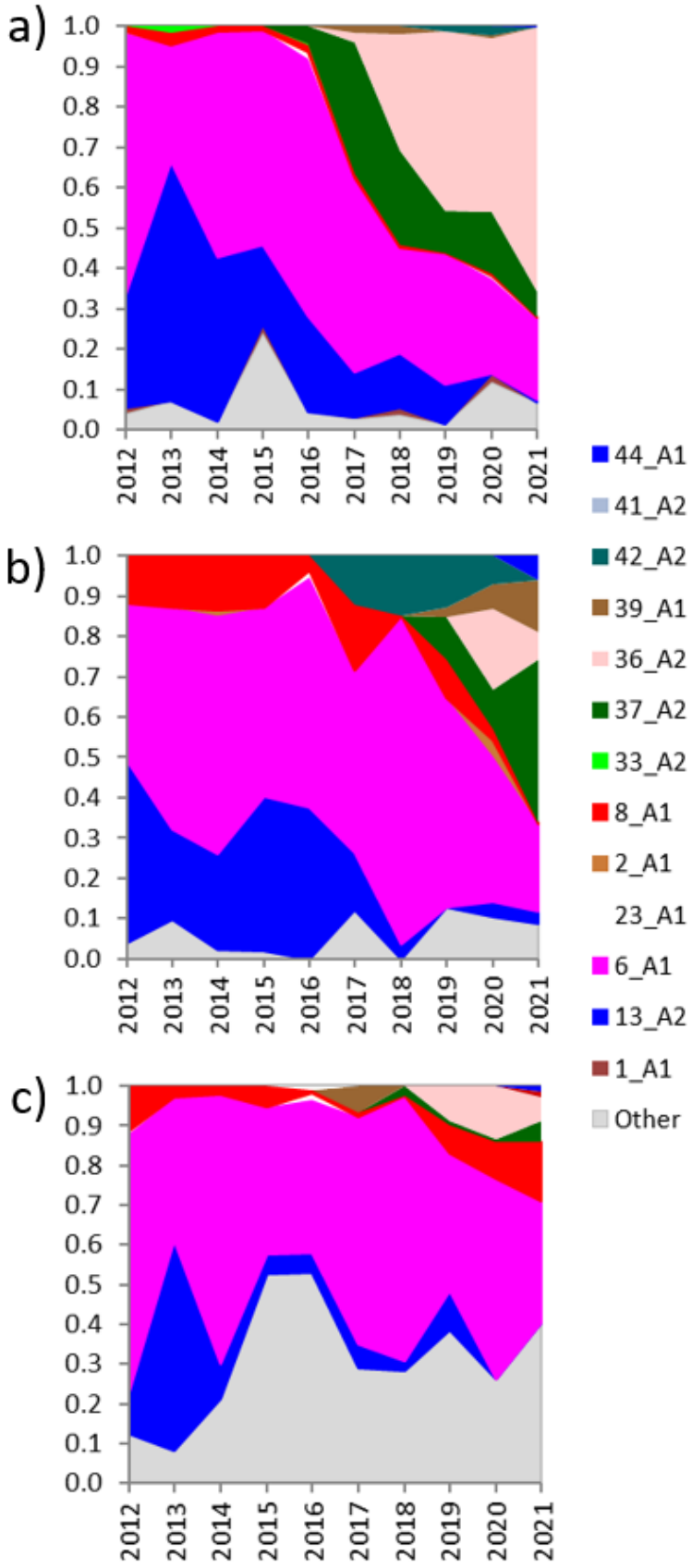

Figure 10. The proportion of each clonal genotype of *P. infestans* from blight outbreaks sampled over the 2012 to 2021 seasons from a) England (*n*=3252) b) Wales (*n*=649) and c) Scotland (*n*=1760).

Submission of the FAB data to the EuroBlight database allows the outbreaks to be mapped by genotype (Figs. 11 - 13) and compared to those from crops in mainland Europe. The mapped outbreaks are open-access and available online https://agro.au.dk/forskning/internationale-platforme/euroblight/pathogen-monitoring/genotype-map/. Note that different geo-located data points from the same location overlay each other which may obscure some of the diversity. In the live mapping tool 'radio buttons' for each genotype may thus be selected to allow specific genotypes to be plotted individually. The spread of genotypes 36_A2 and 37_A2 from 2017 to 2020 (Fig. 14) and in 2021 (Fig. 15) are shown. Genotype 37_A2 was first sampled in the England in an outbreak in the Midlands in late June 2016 with subsequent findings from blight outbreaks in Cheshire, Staffordshire, Nottinghamshire and Cambridgeshire (Cooke, 2019). In the 2017 season it comprised one third of samples in England with outbreaks centred on the Midlands but widening to northeast and southeast England. Even in the dry season of 2018, it spread further to Scotland and Northern Ireland. By 2019 the overall frequency of 37_A2 had begun to decline (Fig. 9) but its range widened into crops in Wales and eastern Scotland (Fig. 14). The expansion of the 36_A2 genotype showed a similar pattern to 37_A2 but it was first sampled one year later in 2017 with the findings in eastern England. It has also become more dominant than 37_A2, comprising 64% of the samples from crops in England in 2021 (Fig. 10).

The genetic markers used in this study also resolve sub-genotype variation which can be used to examine patterns of inoculum evolution and spread. The samples of the 36_A2 lineage from 2020 are, for example, subdivided into 19 sub-clonal forms (Fig.16). These sub-clonal types were sampled at different frequencies with the dominant 'mother type' found 66 times (see number in node) and another type at least three genetic steps away sampled 44 times.

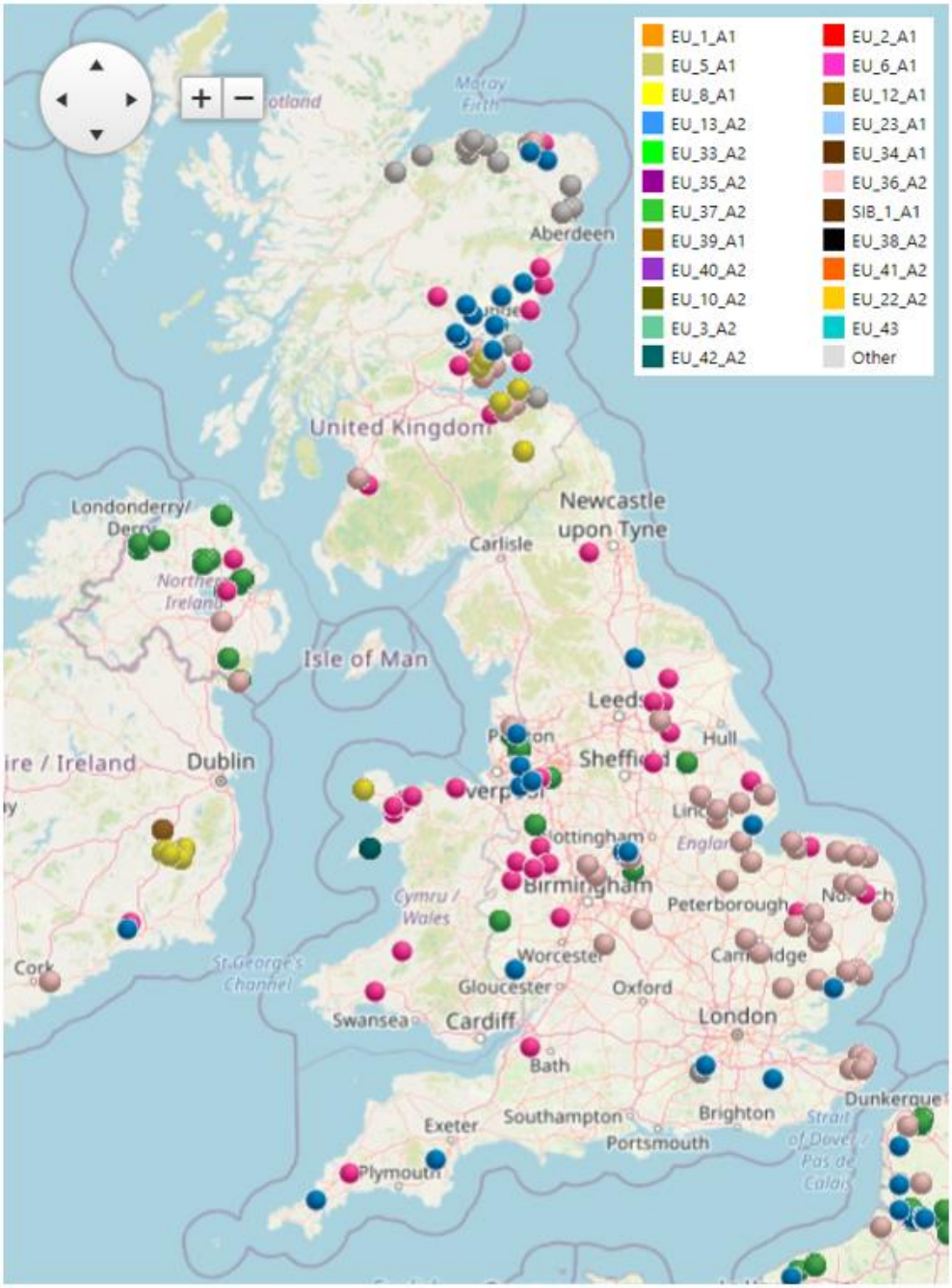

Figure 11. Spatial distribution of all *P. infestans* genotypes collected from 2019 late blight outbreaks submitted to the EuroBlight database (www.euroblight.net).

Figure 12. Spatial distribution of all *P. infestans* genotypes collected from 2020 late blight outbreaks submitted to the EuroBlight database (www.euroblight.net). The legend is the same as for Figure 11.

Figure 13. Spatial distribution of all *P. infestans* genotypes collected from 2021 late blight outbreaks submitted to the EuroBlight database (www.euroblight.net). The legend is the same as for Figure 11.

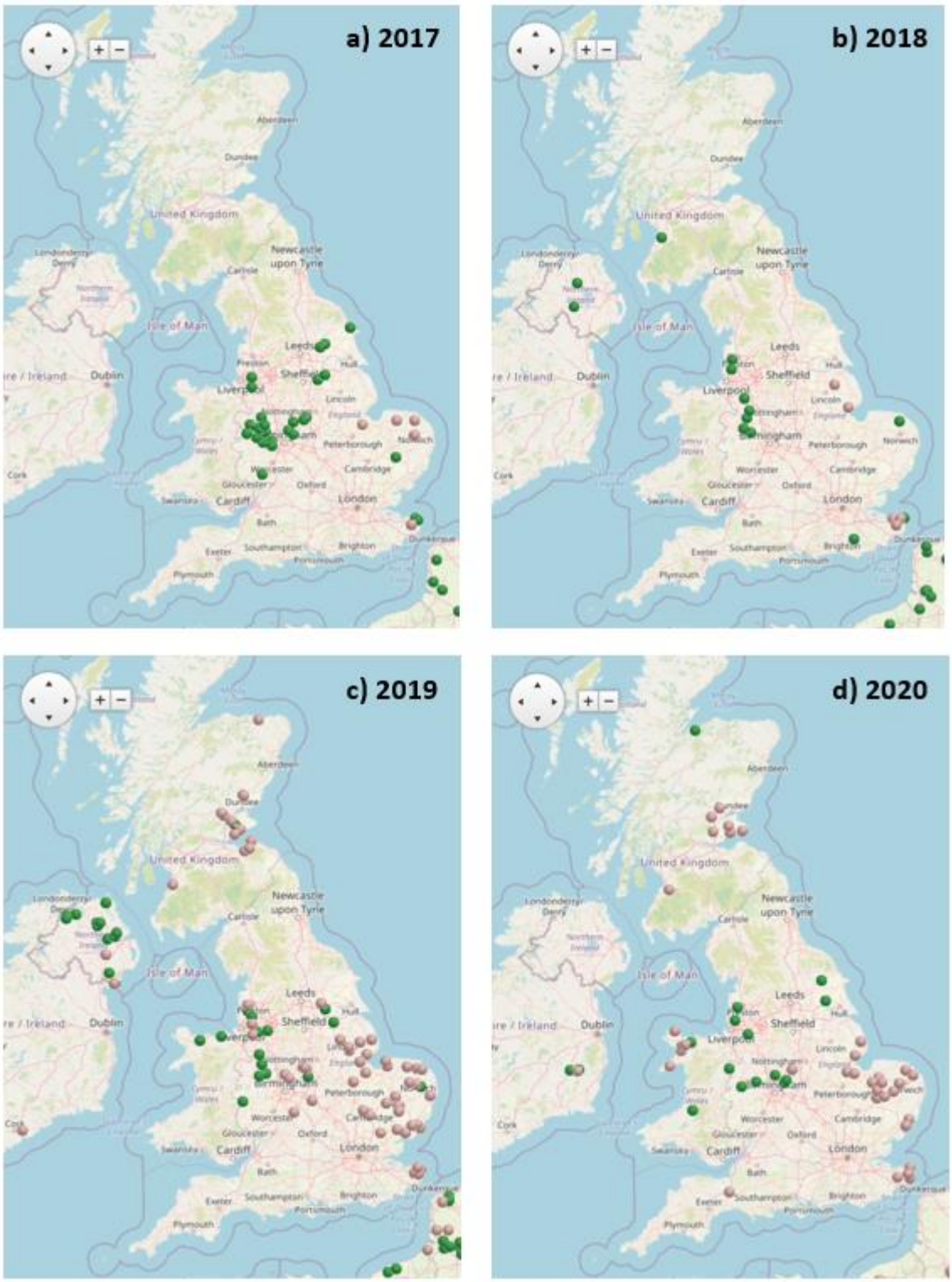

Figure 14. Spatial distribution of *P. infestans* genotype 37_A2 (green) and 36_A2 (pale pink) sampled from a) 2017 b) 2018 c) 2019 and d) 2020 late blight outbreaks in the EuroBlight database (www.euroblight.net).

Figure 15. Spatial distribution of *P. infestans* genotypes 37_A2 (green) and 36_A2 (pale pink) sampled from 2021 late blight outbreaks in the EuroBlight database (www.euroblight.net).

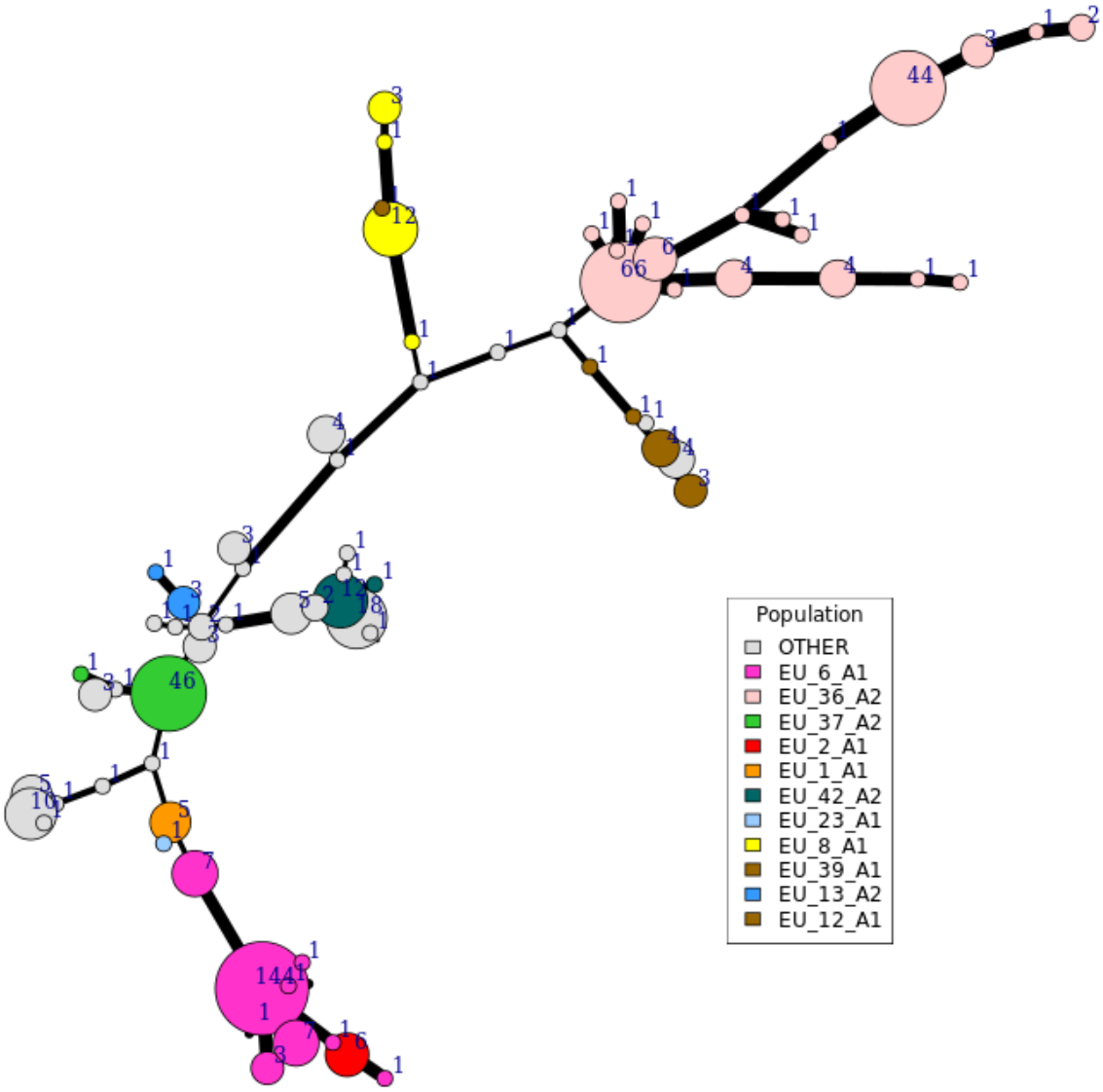

Figure 16. An SSR-based minimum spanning network tree of the 486 genotyped *P. infestans* samples from GB crops in 2020. The data shows the range of diversity within each clone. The figure was generated using *poppr* (Kamvar et al., 2015) via the EuroBlight toolbox. Numbers at each node represent the number of samples.

Fungicide sensitivity testing

General observations

All untreated leaves in all fungicide tests in 2019, 2020 and 2021 testing produced lesions with all test isolates indicating that the test conditions were favourable and the isolates all pathogenic on the test cultivar. Preparative work in other studies and reference to the literature identified a dose range for each product that spans a range of efficacy from 100% effective (no lesions) to a very low efficacy (similar to the control inoculum with no fungicide). This range of doses proved suitable for the calculation of the EC_{50} data. The dose ranges of each product expressed as a percentage of the maximum field dose are presented on a logarithmic scale (Fig.

17). The highest dose of each product ranged from fluopicolide and propamocarb at 20% of their field rate to oxathiapiprolin at 0.4%. The lowest doses of each ranged from propamocarb at 0.2% of field rate to oxathiapiprolin at 0.0007%. Fungicide doses are expressed as parts per million (ppm) of active ingredient with 1 ppm being equivalent to 1 µg ml^{-1}.

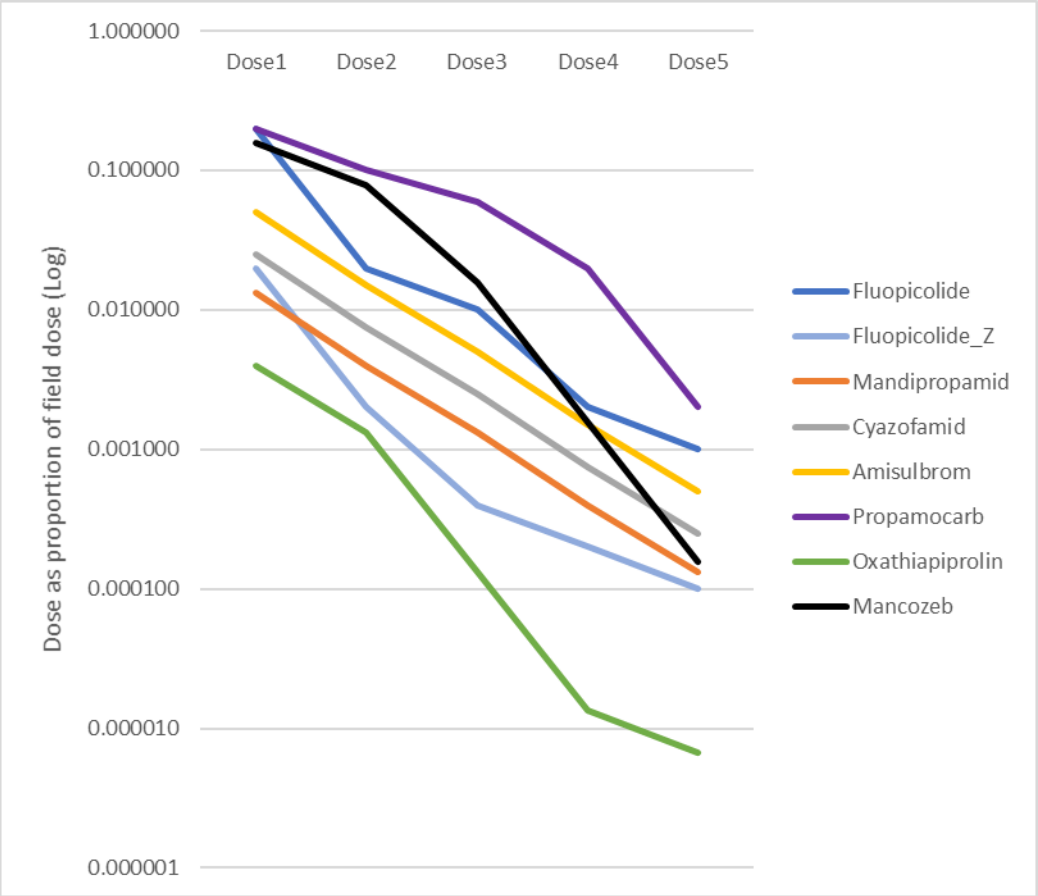

Figure 17. Fungicide dose ranges used to generate sensitivity data against genotypes of *P. infestans* expressed as a proportion of field dose and plotted on a log scale. Fluopicolide_Z is the lower dose range used for the zoospore motility assay.

Fluopicolide 2019-21

Disease Incidence

At every concentration of fluopicolide tested there was a higher incidence of lesions caused by isolates of 36_A2 than other genotypes in 2019 (Fig. 18). This pattern was not consistently repeated in 2020 or 2021 (Fig. 19. Fig. 20). In 2019 there was a very low incidence of disease caused by isolates of 37_A2 and 6_A1 at concentrations ≥ 5ppm but a 40% and 17% incidence of lesions caused by 36_A2 at 5 and 10ppm fluopicolide respectively. No lesions were observed at 100ppm. Results for 2020 (Fig. 19.) were similar, with control achieved at concentrations >5ppm. In 2021 (Fig. 20) there was 100% incidence of lesions at concentrations of fluopicolide up to 1 ppm for all genotypes and 43% (6_A1), 67% (37_A2) and 80% (36_A2) incidence at 5ppm. Fifty percent incidence of disease was observed at 10ppm for isolates of genotypes 36_A2 and 37_A2 only, and at 100ppm good levels of disease control were achieved for all genotypes. Results indicate that the range of concentrations under test is appropriate for calculation of EC50 values.

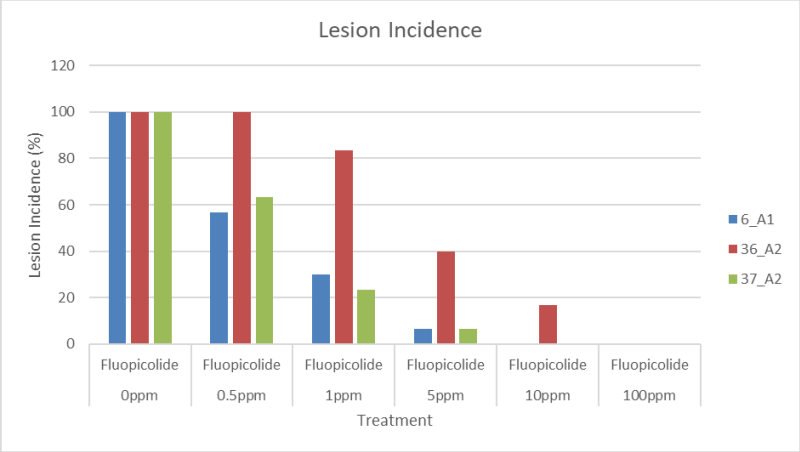

Figure 18. Incidence of lesions (%) caused by each genotype observed at different concentrations of fluopicolide in the 2019 testing.

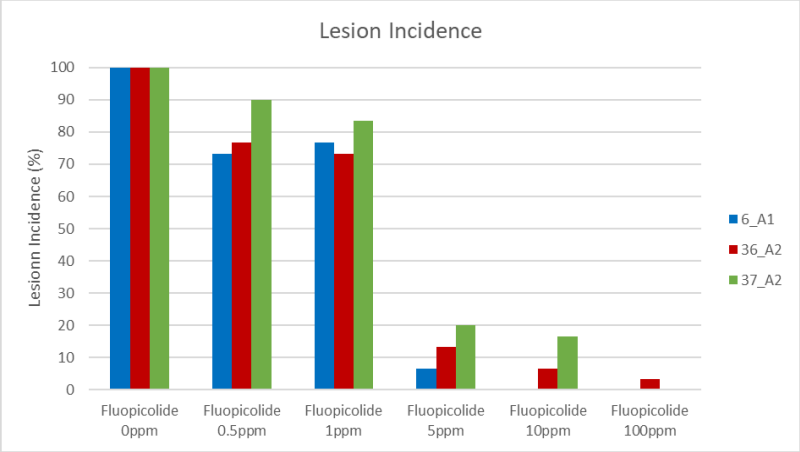

Figure 19. Mean incidence of lesions (%) caused by each genotype observed at different concentrations of fluopicolide in the 2020 testing.

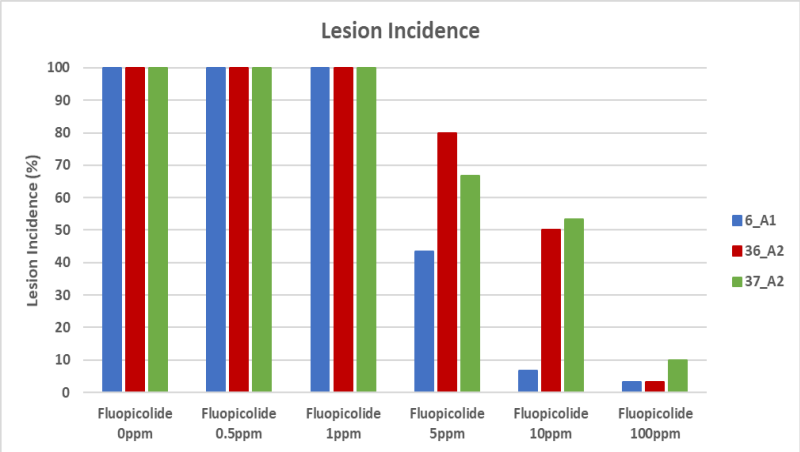

Figure 20. Mean incidence of lesions (%) caused by each genotype observed at different concentrations of fluopicolide in the 2021 testing.

Lesion Size Fluopicolide

Mean lesion size (calculated for infected leaves only) decreased with fluopicolide concentration (Figs. 22-24) with very small lesions observed at higher concentrations in all years. An example of the lesions seen at 10ppm in leaflets infected with isolates of 36_A2 is shown (Fig. 4). The statistical significance, or otherwise, of differences in lesion size is captured in the calculation of EC_{50}. The lesion size data is also represented in a box and whisker plot (Figs. 25-27).

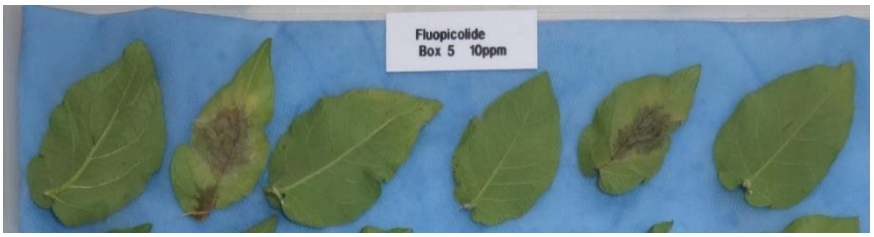

Figure 21. Potato leaflets showing late blight disease symptoms caused by isolates of 36_A2 at 10µg/ml fluopicolide in 2019.

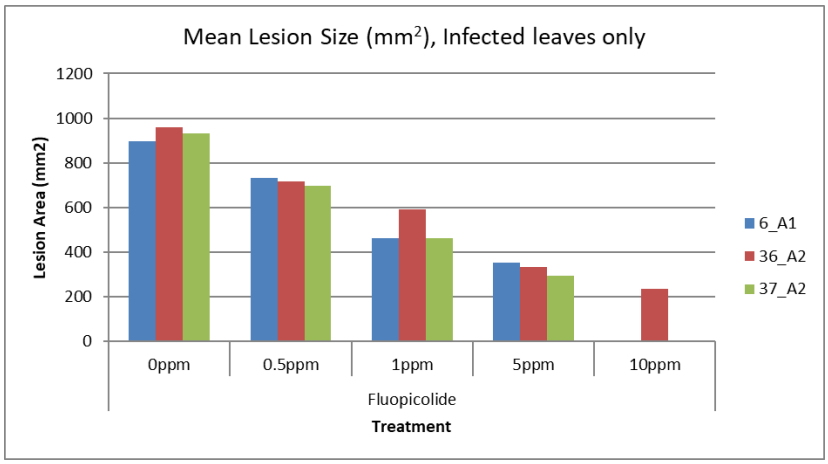

Figure 22. Mean lesion size (mm^2) at different concentrations of fluopicolide (mean of infected leaves only) for each genotype (n= 5 isolates) in the 2019 test.

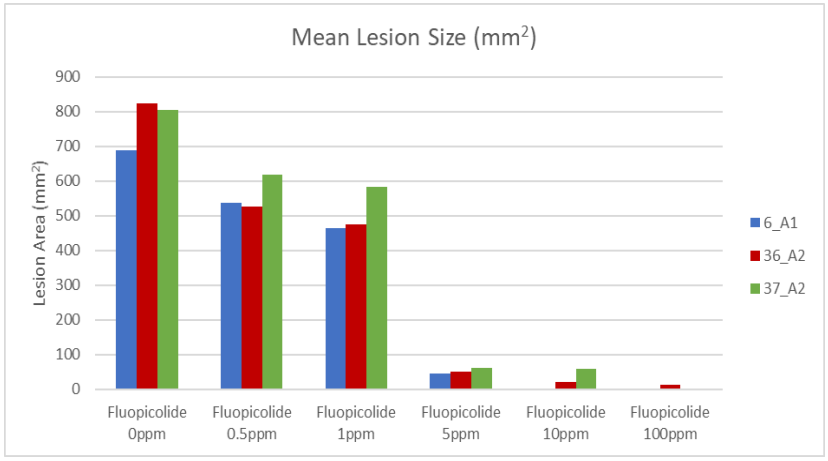

Figure 23. Mean lesion size (mm^2) at different concentrations of fluopicolide (mean of infected leaves only) for each genotype (n= 5 isolates) in the 2020 test.

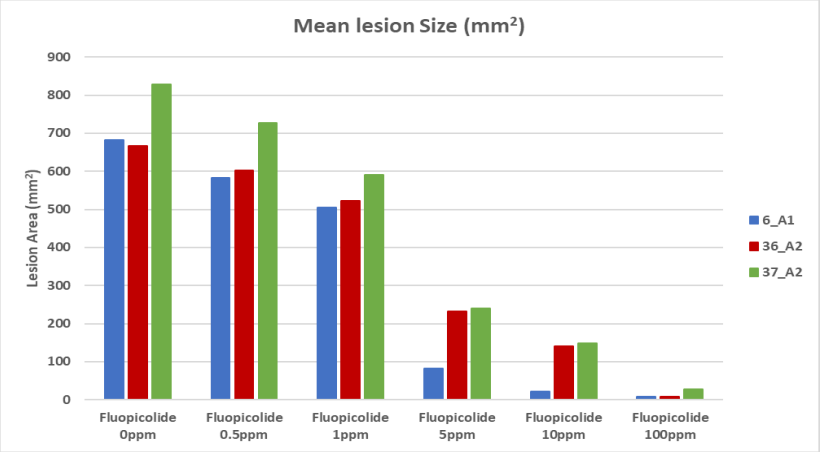

Figure 24. Mean lesion size (mm^2) at different concentrations of fluopicolide (mean of infected leaves only) for each genotype (n= 5 isolates) in the 2021 test.

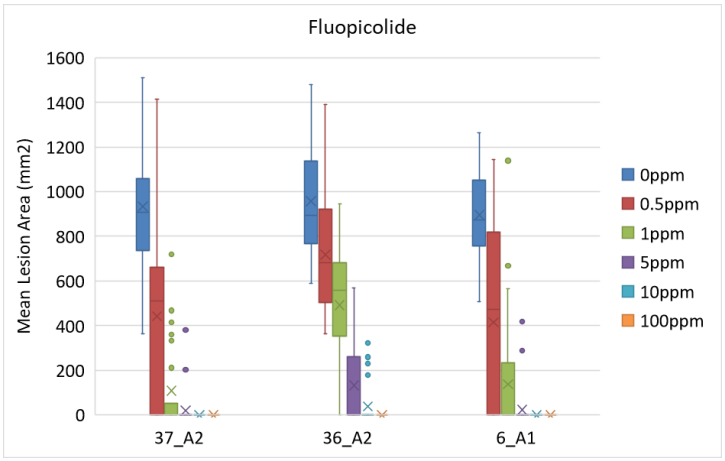

Figure 25. Mean Lesion area (mm^2) of isolates tested in 2019 belonging to 36_A2 and 37_A2 compared with control isolates (6_A1) presented as a box and whisker plot. The maximum field concentration for Fluopicolide (as Infinito) is 500ppm.

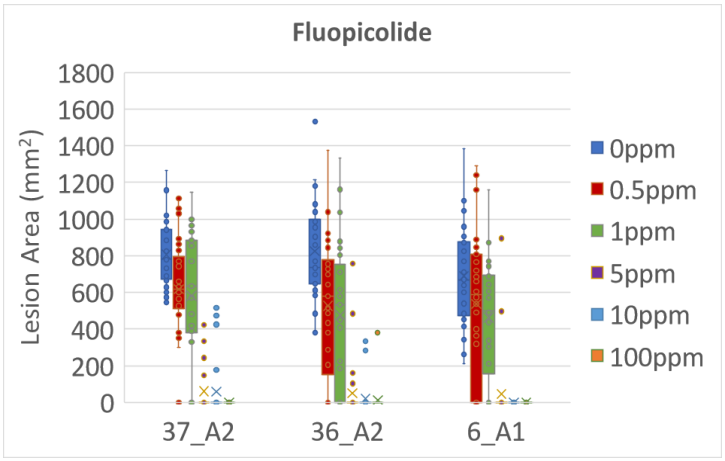

Figure 26. Mean Lesion area (mm^2) of isolates tested in 2020 belonging to 36_A2 and 37_A2 compared with control isolates (6_A1) presented as a box and whisker plot. The maximum field concentration for Fluopicolide (as Infinito) is 500ppm.

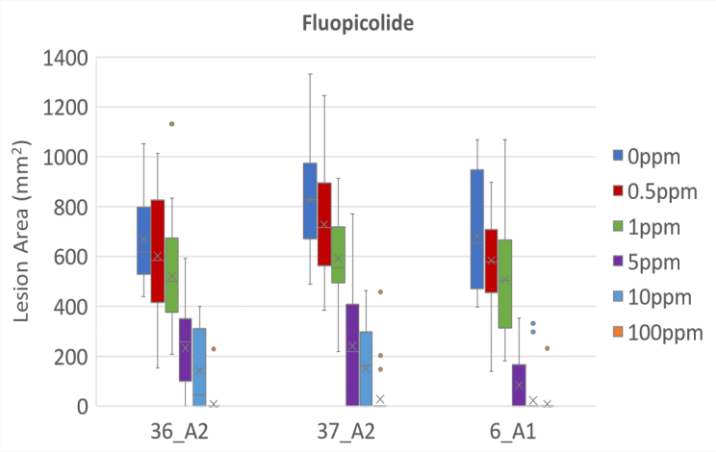

Figure 27. Mean Lesion area (mm^2) of isolates tested in 2021 belonging to 36_A2 and 37_A2 compared with control isolates (6_A1) presented as a box and whisker plot. The maximum field concentration for Fluopicolide (as Infinito) is 500ppm.

EC_{50} Fluopicolide

EC_{50} values are given in Table 5. There was a statistically significant difference in mean EC_{50} value between genotypes in 2019 and 2021, with isolates of 36_A2 showing, on average, a greater EC_{50} value than 6_A1 in both years and greater than 37_A2 in 2019. These differences were not observed in 2020. Values are higher overall in 2021, however the data are collated from different isolates in a biological system and variation is to be expected. However, the mean (and maximum/minimum) EC_{50} values are in line with the original baseline sensitivity data and consistent with those run in 2018 (Lees, 2018).

Table 5. Mean, maximum and minimum EC_{50} values for isolates of *P. infestans* of various genotypes tested at a range of concentrations of fluopicolide in 2019. Statistically significant differences between mean values are indicated by different letters.

Genotype		6_A1	37_A2	36_A2
Number of isolates		5	5	5
Mean EC_{50} (mg/L)	2019	**0.623a**	**0.591a**	**1.561b**
	2020	**1.677a**	**1.81a**	**1.585a**
	2021	**2.200a**	**3.332ab**	**3.695b**
EC_{50} min (mg/L)	2019	0.250	0.250	0.250
	2020	0.246	0.300	0.246
	2021	0.669	0.739	0.367
EC_{50} max (mg/L)	2019	2.990	2.450	16.400
	2020	9.002	4.940	7.370
	2021	4.940	12.151	16.402

The MIC values in this zoospore motility test (results expressed in terms of the minimum inhibitory concentration (MIC), defined as the lowest concentration which completely inhibited zoospore motility) are very consistent across years. The mean MIC values for genotype 36_A2 were

statistically higher than for other genotypes in all years but were still within the expected range. Such differences are very small when compared to the field dose of this product.

Table 6. Minimum inhibitory concentration (MIC) of fluopicolide on the motility of zoospores of different clonal lineages tested in 2019-21. Measurements were taken after 1 and 2 hours of incubation according to the protocol. Within column values followed by the same letter are not significantly different according to Fisher's protected least significant difference test at P = 0.05.

		MIC value (µg/ml) 2019		MIC value (µg/ml) 2020		MIC value (µg/ml) 2021	
Clonal lineage	**Isolates tested**	**1 hour**	**2 hours**	**1 hour**	**2 hours**	**1 hour**	**2 hours**
EU_6_A1	5	0.100a	**0.065a**	0.125a	**0.075a**	0.120a	**0.070a**
EU_37_A2	5	0.075a	**0.055a**	0.100a	**0.065a**	0.105a	**0.055a**
EU_36_A2	5	0.760b	**0.130b**	2.72b	**0.430b**	3.380b	**0.320b**

Mandipropamid 2019-21

Disease Incidence

The mean lesion incidence for each genotype at different concentrations of mandipropamid (0, 0.1, 0.3, 1.0, 3.0 and 10 ppm) shows there was a relatively high incidence of lesions at concentrations up to 0.3 ppm of mandipropamid in all years, with a lower incidence at 1-10 ppm (Figs. 28-30). The lesion incidence caused by genotype 37_A2 was greater than that of other lineages at several doses in 2019 and 2020. The mean lesion size, on infected leaves only, is shown in Figs. 31-33. The range of concentrations under test was appropriate for calculation of EC_{50}. The statistical significance, or otherwise, of differences in lesion size is captured in the calculation of EC_{50}. The lesion size data is also represented in a box and whisker plot (Figs. 34-36).

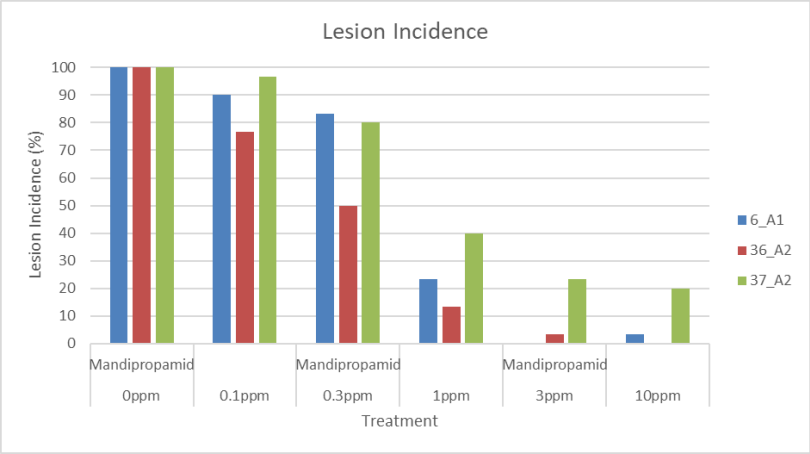

Figure 28. Mean incidence of lesions caused by different *P. infestans* genotypes at a range of concentrations of mandipropamid (0-10 µg/ml) in the 2019 test.

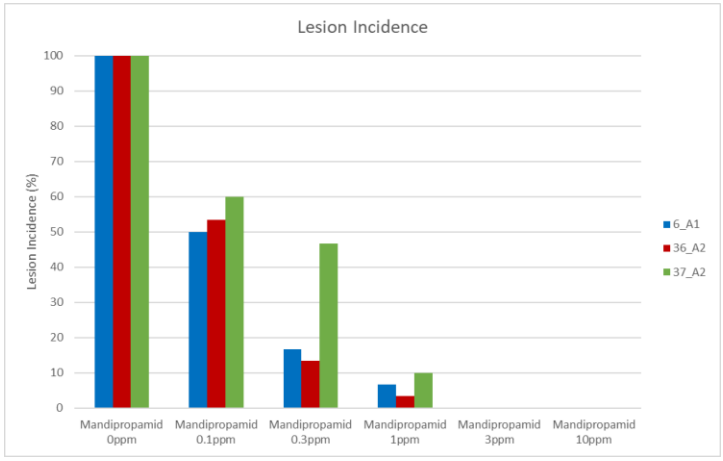

Figure 29. Mean incidence of lesions caused by different *P. infestans* genotypes at a range of concentrations of mandipropamid (0-10 µg/ml) in the 2020 test.

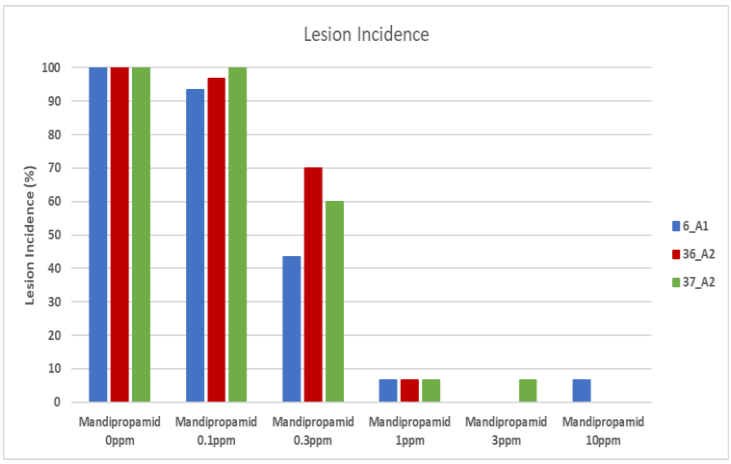

Figure 30. Mean incidence of lesions caused by different *P. infestans* genotypes at a range of concentrations of mandipropamid (0-10 µg/ml) in the 2021 test.

Lesion Size Mandipropamid

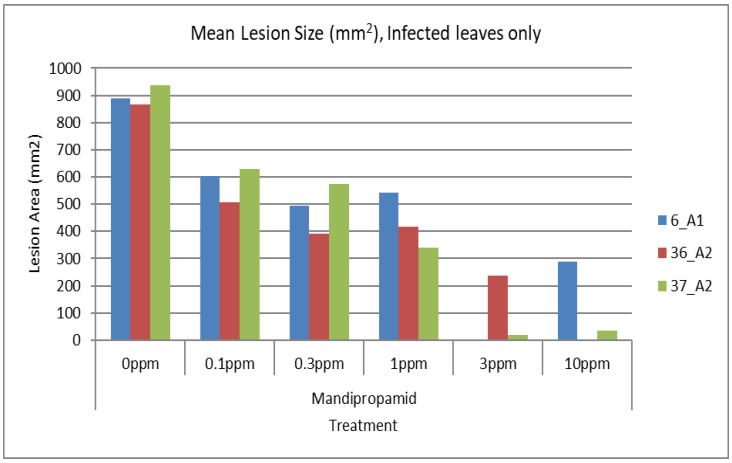

Figure 31. Mean lesion size (mm^2) on infected leaves only after treatment with a range of concentrations of mandipropamid in the 2019 test. The field rate of mandipropamid is 750ppm.

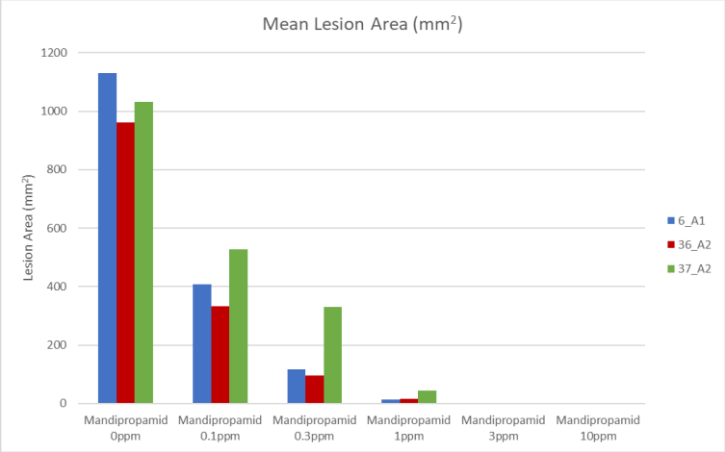

Figure 32. Mean lesion size (mm2) on infected leaves only after treatment with a range of concentrations of mandipropamid in the 2020 test. The field rate of mandipropamid is 750ppm.

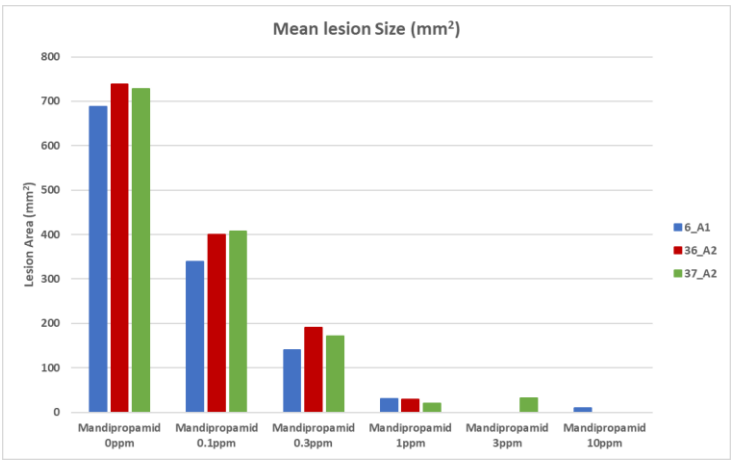

Figure 33. Mean lesion size (mm2) on infected leaves only after treatment with a range of concentrations of mandipropamid in the 2021 test. The field rate of mandipropamid is 750ppm.

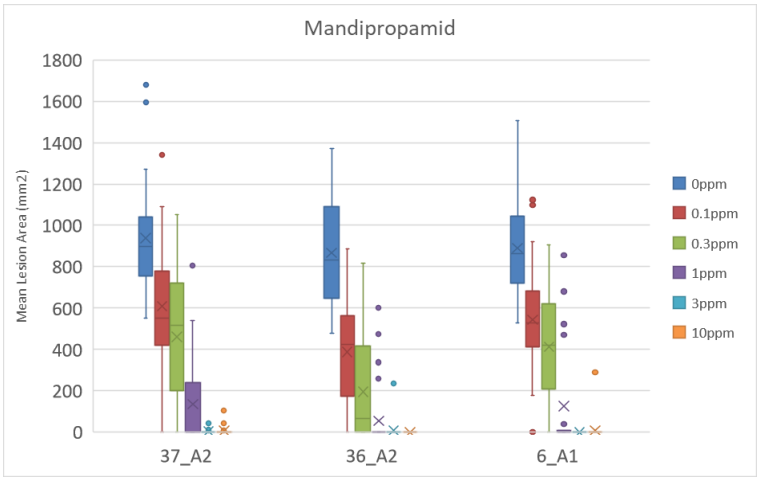

Figure 34. Mean Lesion area (mm2) of isolates tested in 2019 belonging to 36_A2 and 37_A2 compared with control isolates (6_A1) presented as a box and whisker plot. Mandipropamid maximum field concentration = 750ppm

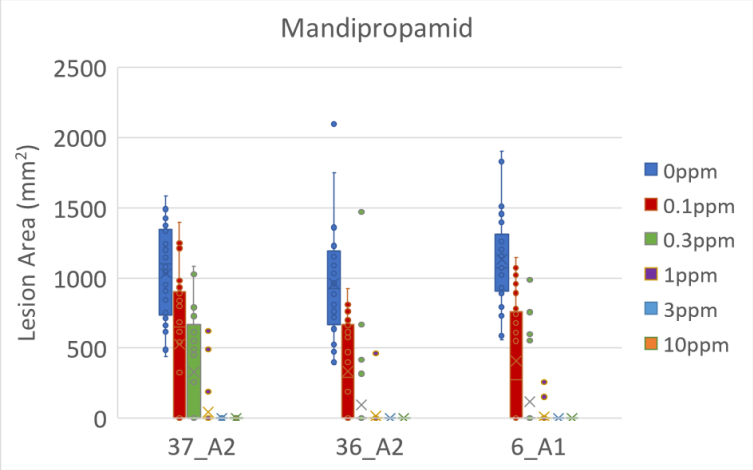

Figure 35. Mean Lesion area (mm2) of isolates tested in 2020 belonging to 36_A2 and 37_A2 compared with control isolates (6_A1) presented as a box and whisker plot. Mandipropamid maximum field concentration = 750ppm.

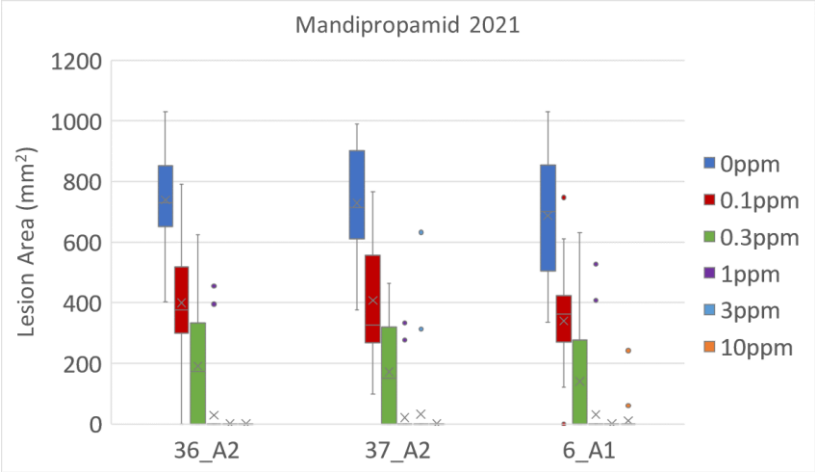

Figure 36. Mean Lesion area (mm2) of isolates tested in 2021 belonging to 36_A2 and 37_A2 compared with control isolates (6_A1) presented as a box and whisker plot. Mandipropamid maximum field concentration = 750ppm.

<u>EC_{50} values Mandipropamid</u>

The EC_{50} values in this test were in line with previous sensitivity testing of mandipropamid (Table 7). There was very little variation for Mean EC_{50} values between years or genotypes.

Table 7. Mean, maximum and minimum EC50 values for isolates of *P. infestans* of various genotypes tested in 2019 at a range of concentrations of mandipropamid (0, 0.1, 0.3, 1.0, 3.0, 10.0µg/ml). Significant differences between mean values (within a year only) are indicated by different letters.

Genotype		6_A1	37_A2	36_A2
Number of isolates		5	5	5
Mean EC_{50} (mg/L)	2019	**0.487a**	**0.559a**	**0.482a**
	2020	**0.226a**	**0.412b**	**0.252a**
	2021	**0.305a**	**0.274a**	**0.296a**
EC_{50} min (mg/L)	2019	0.165	0.165	0.149
	2020	0.149	0.149	0.149
	2021	0.149	0.149	0.165
EC_{50} max (mg/L)	2019	1.644	1.817	4.470
	2020	0.605	2.220	1.488
	2021	1.102	0.605	1.102

Cyazofamid 2019-2021

The mean incidence of lesions for each genotype at different concentrations of cyazofamid (0, 0.1, 0.3, 1.0, 3.0 and 10 ppm) indicates a moderate incidence of lesions at 0.1 ppm cyazofamid with a lower incidence at 0.3 – 3 ppm in 2019 (Fig. 37). More lesions were seen at 0.3ppm in 2020 (50-70%) and 2021 (65-100%), and in 2021 lesions caused by 36_A2 and 37_A2 were observed at all concentrations tested (Figs. 38-39). The range of concentrations tested (0-10µg/ml) was appropriate for calculation of EC_{50}. The statistical significance of differences in lesion size is captured in the calculation of EC_{50}. The lesion size data is represented as bars (Figs. 40-42) and in a box and whisker plot (Figs. 43-45).

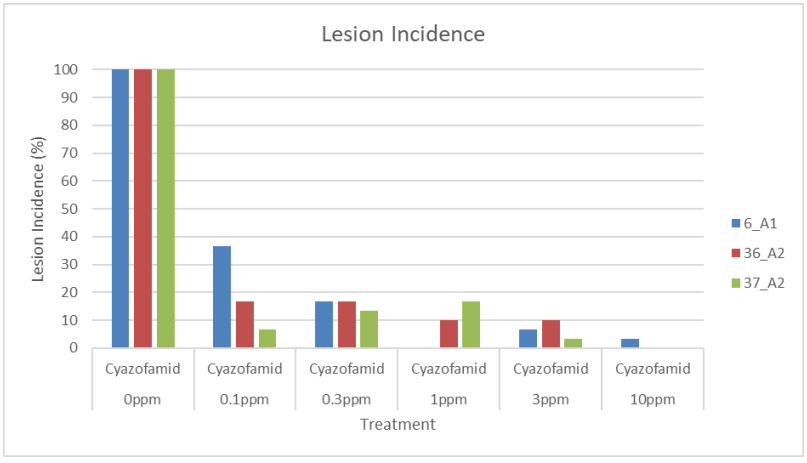

Figure 37. Mean percentage of lesions caused by different genotypes at a range of concentrations of cyazofamid tested in 2019 (0-10 µg/ml).

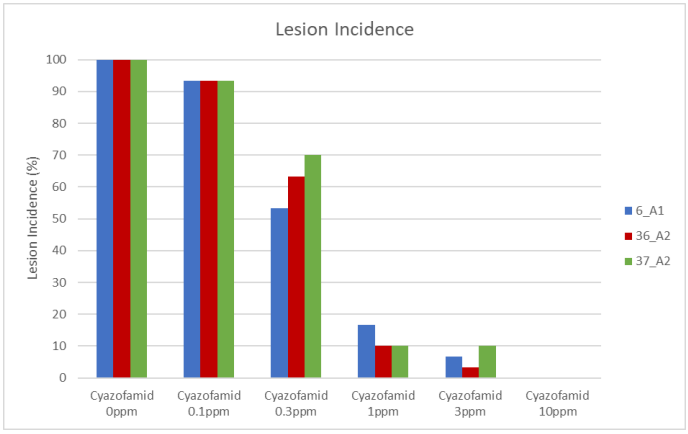

Figure 38. Mean percentage of lesions caused by different genotypes at a range of concentrations of cyazofamid tested in 2020 (0-10 µg/ml).

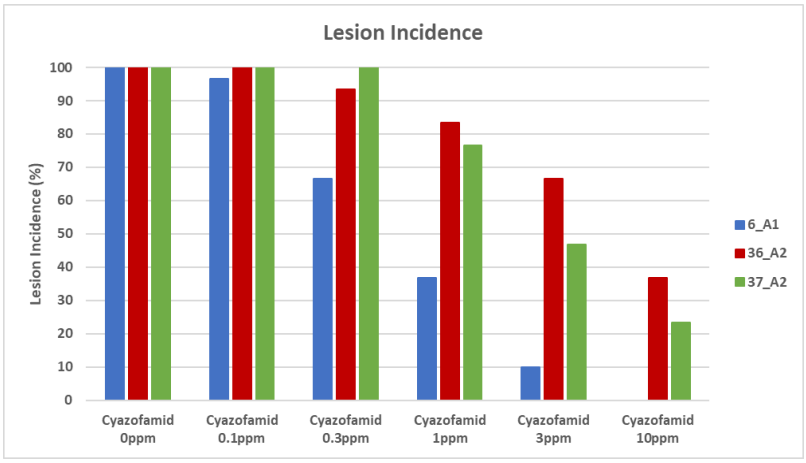

Figure 39. Mean percentage of lesions caused by different genotypes at a range of concentrations of cyazofamid tested in 2021 (0-10 µg/ml).

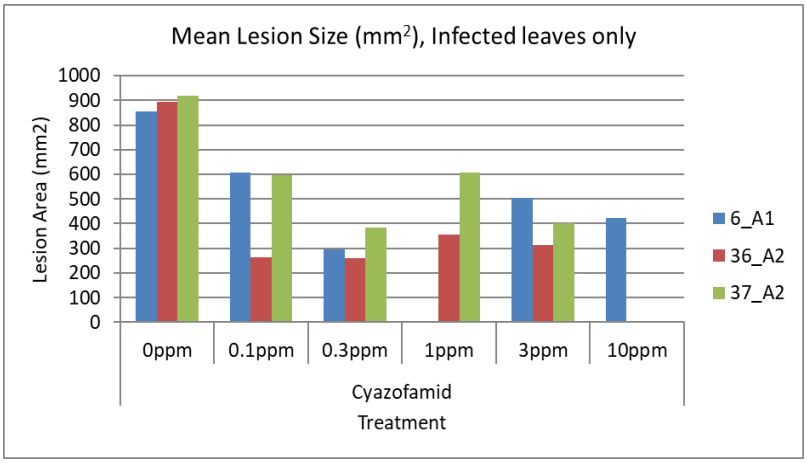

Figure 40. Mean lesion size (mm^2) measured, on infected leaves only, at a range of concentrations of cyazofamid tested in 202019

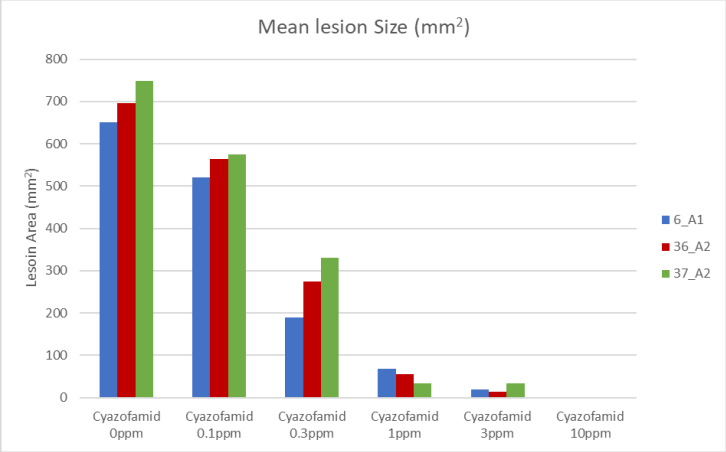

Figure 41. Mean lesion size (mm^2) measured on infected leaves only, at a range of concentrations of cyazofamid tested in 2020.

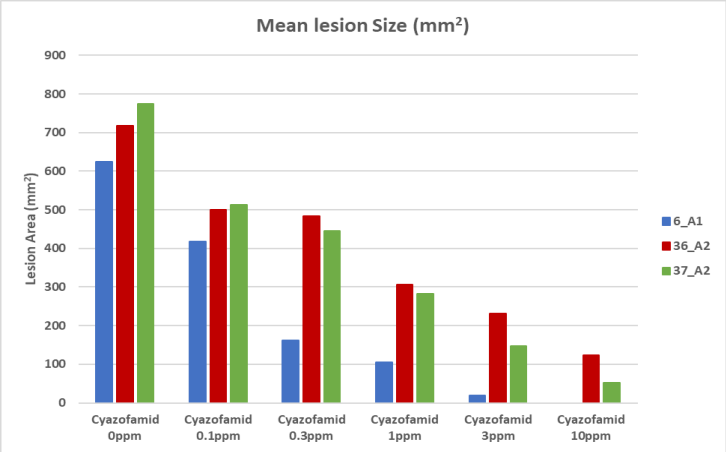

Figure 42. Mean lesion size (mm^2) measured on infected leaves only, at a range of concentrations of cyazofamid tested in 2020.

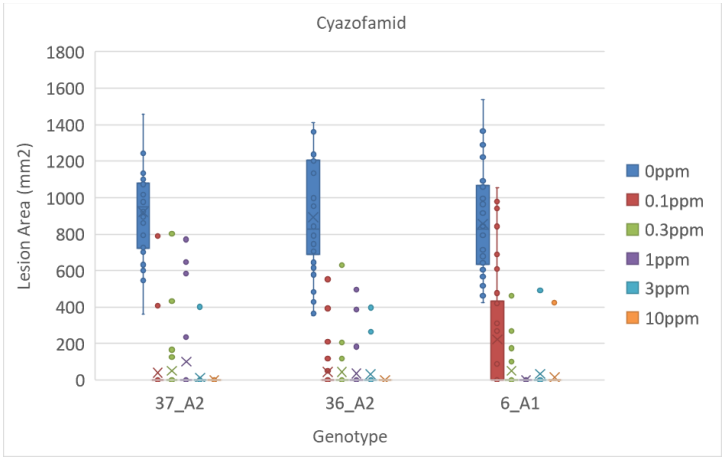

Figure 43. Mean Lesion area (mm2) of isolates belonging to 36_A2 and 37_A2 compared with control isolates (6_A1) tested in 2019 and presented as a box and whisker plot. Cyazofamid Max Field concentration = 400ppm.

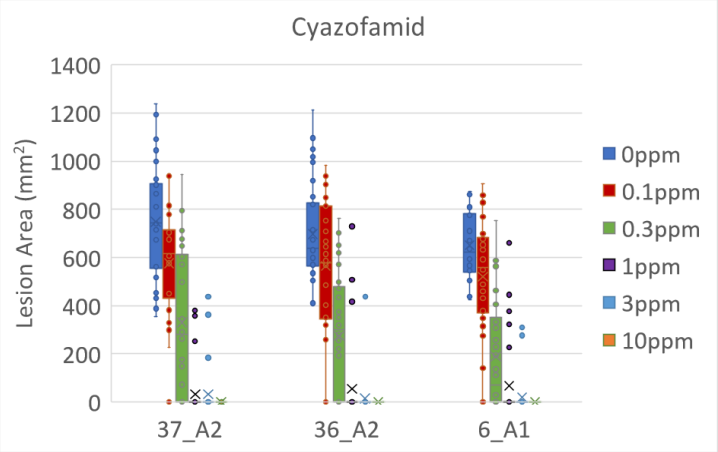

Figure 44. Mean Lesion area (mm2) of isolates belonging to 36_A2 and 37_A2 compared with control isolates (6_A1) tested in 2020 and presented as a box and whisker plot. Cyazofamid Max Field concentration = 400ppm.

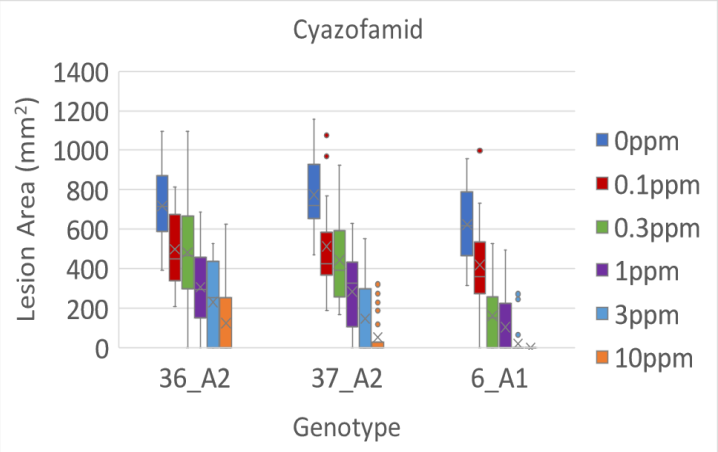

Figure 45. Mean Lesion area (mm2) of isolates belonging to 36_A2 and 37_A2 compared with control isolates (6_A1) tested in 2021 and presented as a box and whisker plot. Cyazofamid Max Field concentration = 400ppm.

EC_{50} values calculated from test data show there was no statistically significant difference in mean EC_{50} value between genotypes in 2019 and 2020 (Table 8). It is difficult to interpret the mean EC50 values in the context the EC_{50} values stated by Mitani et al (2001) as these appear to use incorrect units. Gaucher et al (2007) reported EC_{50} values of between 0.1 – 1.0 ppm cyazofamid when used directly on spore suspensions and these values appear in line with the results reported here using a detached leaf assay in 2019 and 2020. In 2021 the mean EC_{50} values for 36_A2 and 37_A2 were significantly higher than for 6_A1, which remained consistent with previous years. The concentrations of cyazofamid required to control all isolates in this assay are very low when compared with permitted field rates and consistent with values previously reported. We recommend future testing with cyazofamid to determine whether the increased values are real or represent experimental variability as has sometimes been observed in fungicide sensitivity tests.

Table 8. Mean, maximum and minimum EC_{50} values for isolates of *P. infestans* of various genotypes tested in 2019 to 2021 at a range of concentrations of cyazofamid (0, 0.1, 0.3, 1.0, 3.0, 10.0 µg/ml). Significant differences between mean values are indicated by different letters.

Genotype		6_A1	37_A2	36_A2
Number of isolates		5	5	5
Mean EC_{50} (mg/L)	2019 2020 2021	**0.313a** **0.478a** **0.346a**	**0.246a** **0.398a** **1.212b**	**0.285a** **0.538a** **1.936b**
EC_{50} min (mg/L)	2019 2020 2021	0.149 0.149 0.165	0.149 0.149 0.165	0.149 0.149 0.182
EC_{50} max (mg/L)	2019 2020 2021	2.220 2.009 1.102	1.488 1.102 4.940	1.644 4.045 9.948

Amisulbrom 2019-21

The mean incidence of lesions for each genotype at different concentrations of amisulbrom (0, 0.1, 0.3, 1.0, 3.0 and 10 ppm) shows lesions were formed in isolates of 6_A1 in the range 0-1 ppm and for isolates of 36_A2 and 37_A2 in the range 0-10 ppm in 2019 (Fig. 46). The slight reduction in sensitivity of 36_A2 and 37_A2 isolates compared to those of 6_A1 that was observed in 2019 was not replicated in the tests of isolates from 2020 (Fig. 47). In 2021 isolates of 36_A2 developed more lesions on average than those of the other genotypes at 1-10ppm (Fig. 48). This suggests that there is natural variation between years. The mean lesion size, on infected leaves only, is shown as a bar graph (Figs. 49-51) (note larger scale in 2019) and a box and whisker plot (Figs. 52-54). The range of concentrations under test (0-10 ppm) was appropriate for calculation of EC_{50}. The statistical significance, or otherwise, of differences in lesion size is captured in the calculation of EC_{50}.

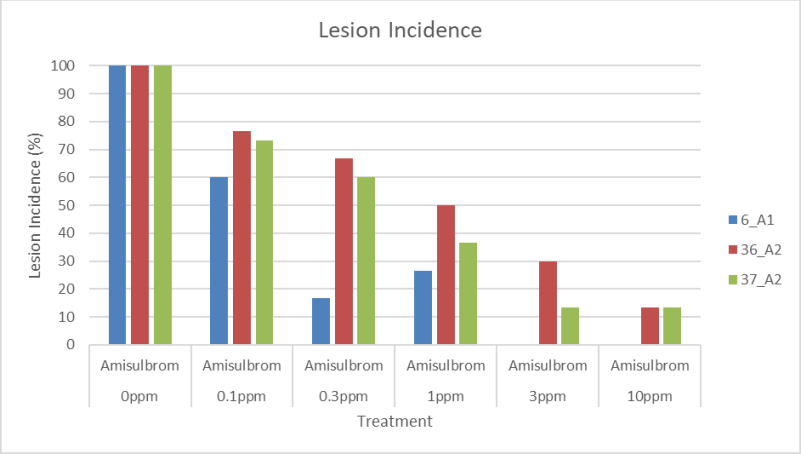

Figure 46. Mean percentage incidence of lesions caused by different genotypes at a range of concentrations of amisulbrom tested in 2019 (0-10 µg/ml).

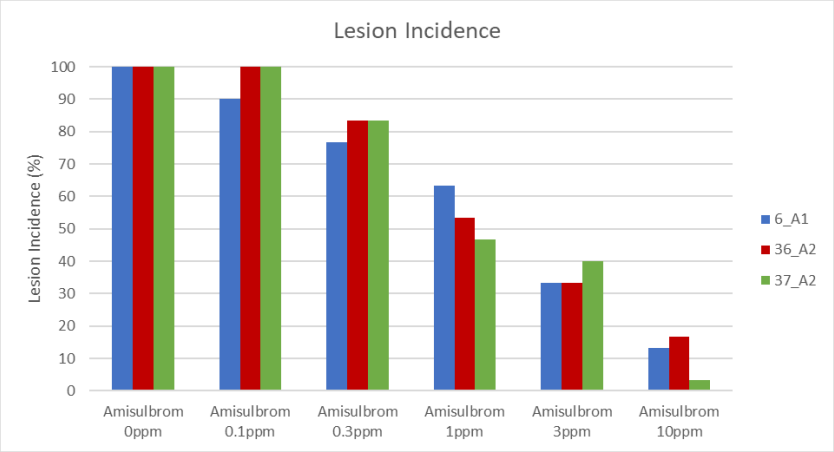

Figure 47. Mean percentage incidence of lesions caused by different genotypes at a range of concentrations of amisulbrom tested in 2020 (0-10 µg/ml).

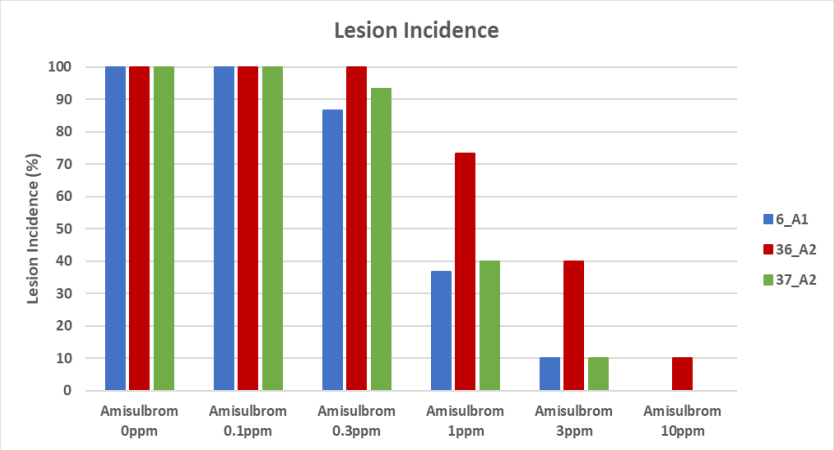

Figure 48. Mean percentage incidence of lesions caused by different genotypes at a range of concentrations of amisulbrom tested in 2021 (0-10 µg/ml).

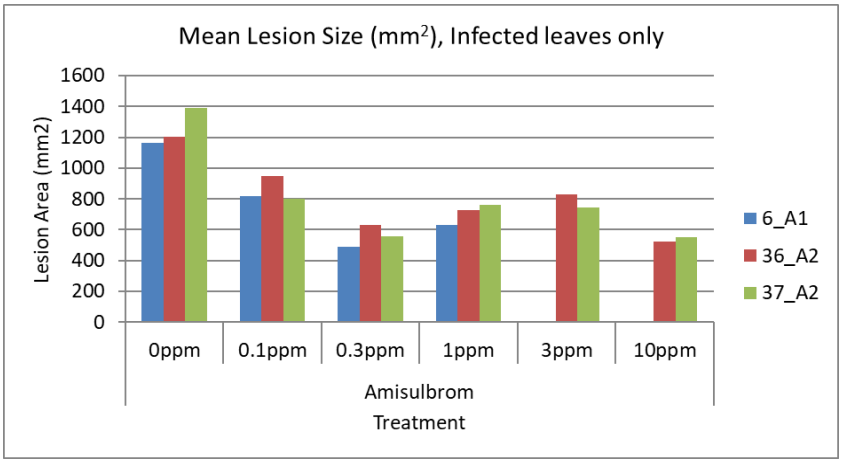

Figure 49. Mean lesion size (mm2) measured on infected leaves only at a range of concentrations of amisulbrom tested in 2019.

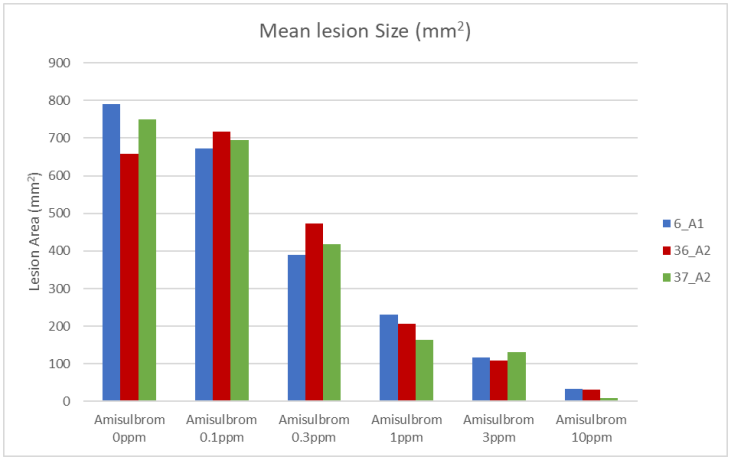

Figure 50. Mean lesion size (mm2) measured on infected leaves only at a range of concentrations of amisulbrom tested in 2020.

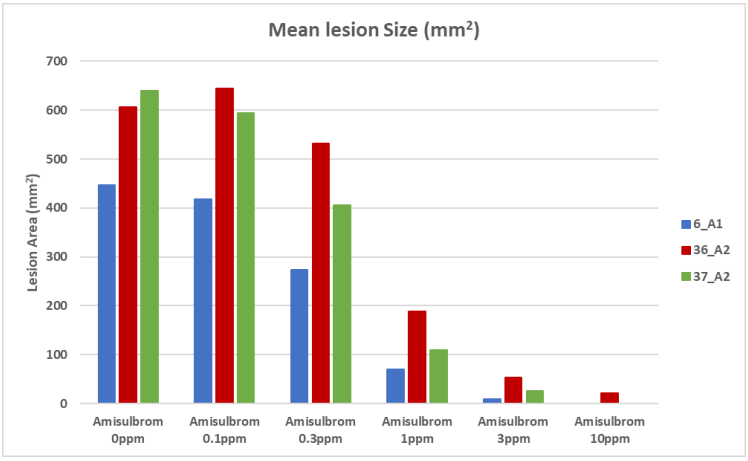

Figure 51. Mean lesion size (mm2) measured on infected leaves only at a range of concentrations of amisulbrom tested in 2021.

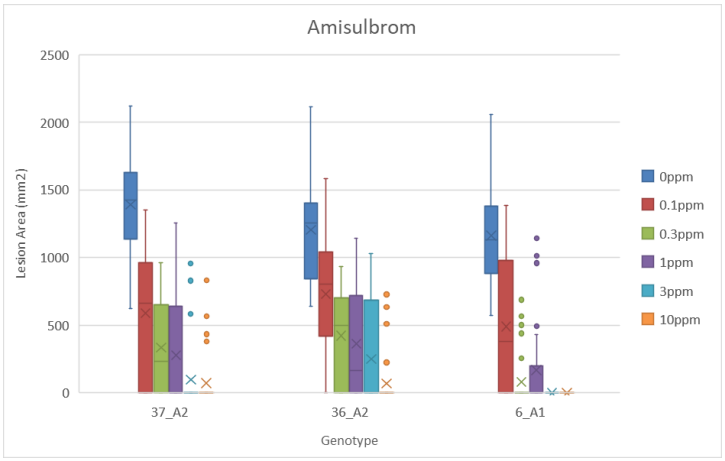

Figure 52. Mean Lesion area (mm2) of isolates belonging to 36_A2 and 37_A2 tested in 2019 compared with control isolates (6_A1) presented as a box and whisker plot. Amisulbrom maximum field concentration = 200ppm.

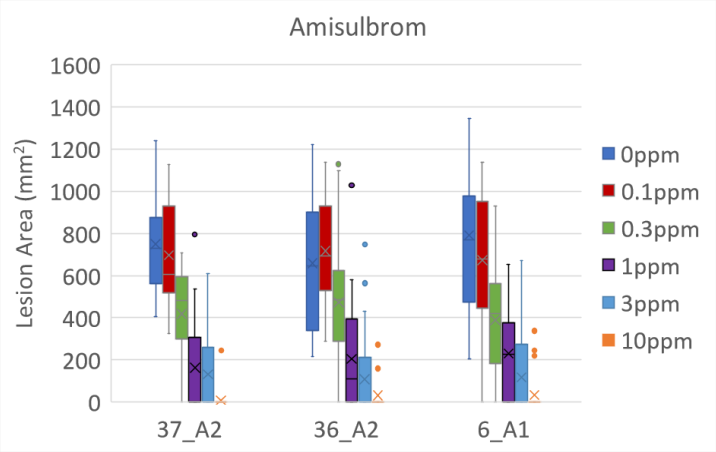

Figure 53. Mean Lesion area (mm2) of isolates belonging to 36_A2 and 37_A2 tested in 2020 compared with control isolates (6_A1) presented as a box and whisker plot. Amisulbrom maximum field concentration = 200ppm.

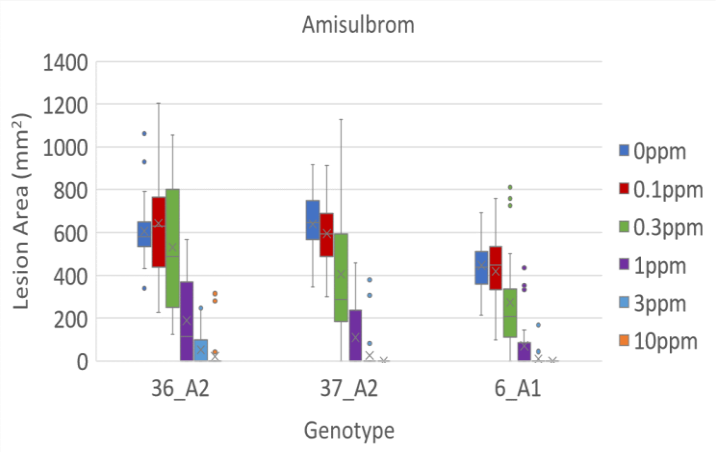

Figure 54. Mean Lesion area (mm2) of isolates belonging to 36_A2 and 37_A2 tested in 2021 compared with control isolates (6_A1) presented as a box and whisker plot. Amisulbrom maximum field concentration = 200ppm.

The EC_{50} values calculated indicate that, on average, isolates of 36_A2 had a significantly greater EC_{50} values than those belonging to genotypes 37_A2 and 6_A1 in 2019 only (Table 9). No other significant differences were observed between genotypes in 2020 and 2021 and results were very consistent.

Table 9. Mean, max and min EC50 values for isolates of *P. infestans* of various genotypes tested in 2019-21 at a range of concentrations of amisulbrom (0, 0.1, 0.3, 1.0, 3.0, 10.0 ppm). Significant differences between mean values are indicated by different letters.

Genotype		6_A1	37_A2	36_A2
Number of isolates		5	5	5
Mean EC_{50} (mg/L)	2019	**0.366a**	**0.363a**	**0.749b**
	2020	**1.073a**	**0.933a**	**1.492a**
	2021	**0.621a**	**0.749a**	**0.842a**
EC_{50} min (mg/L)	2019	0.149	0.149	0.149
	2020	0.149	0.201	0.246
	2021	0.182	0.201	0.201
EC_{50} max (mg/L)	2019	2.220	1.644	8.145
	2020	9.948	4.045	9.948
	2021	2.20	4.045	2.453

Propamocarb 2019-21

A high incidence of lesions (25 to 65%) was caused by all genotypes at concentrations up to 300ppm propamocarb in 2019 (Fig. 55) and a relatively high lesion incidence of 53% of isolates of 36_A2 compared with 6_A1 (23%) and 37_A2 (7%) was noted at 500ppm. No lesions were observed at 1000µg/ml (Fig. 55). The 2019 results are in contrast with 2020 and 2021 (Figs. 56-57) where, in each case, a low number of lesions were observed at 100ppm and good levels of control were observed at concentrations >100ppm. Mean lesion size, on infected leaves only, are shown in Figs. 58-60 and as box and whisker plots (Figs. 61-63).

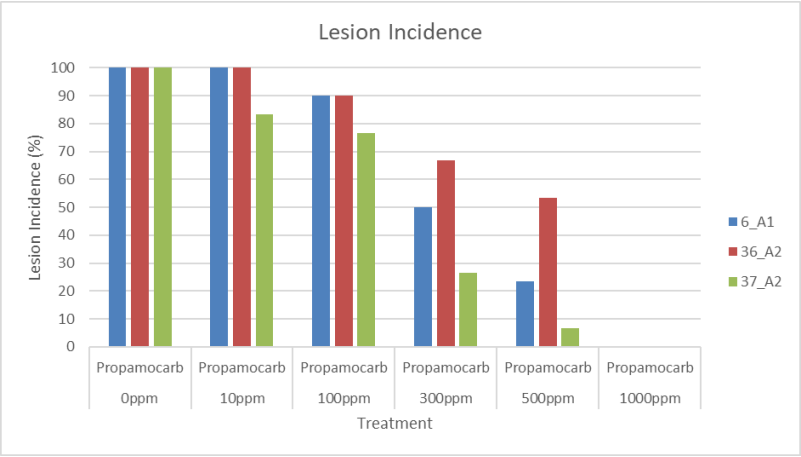

Figure 55. Mean percentage of lesions caused by different genotypes at a range of concentrations of propamocarb tested in 2019 (0-1000 µg/ml).

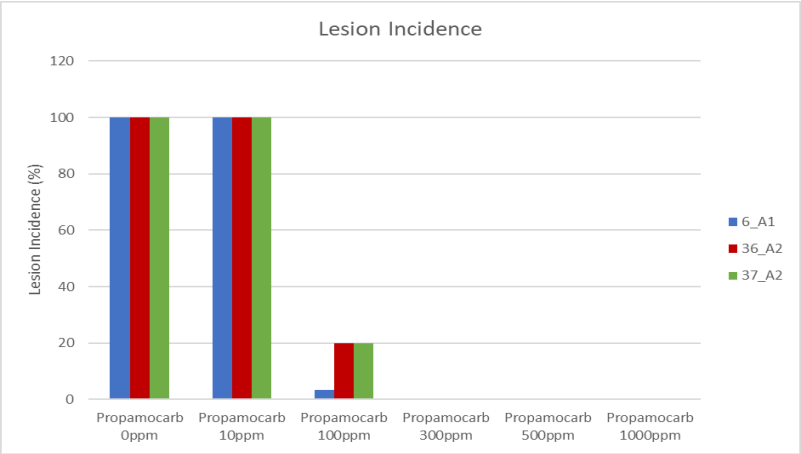

Figure 56. Mean percentage of lesions caused by different genotypes at a range of concentrations of propamocarb tested in 2020 (0-1000 µg/ml).

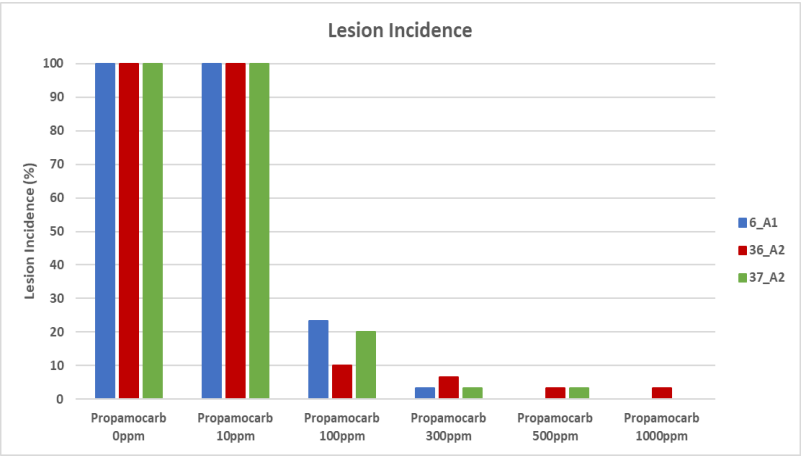

Figure 57. Mean percentage of lesions caused by different genotypes at a range of concentrations of propamocarb tested in 2021 (0-1000 µg/ml).

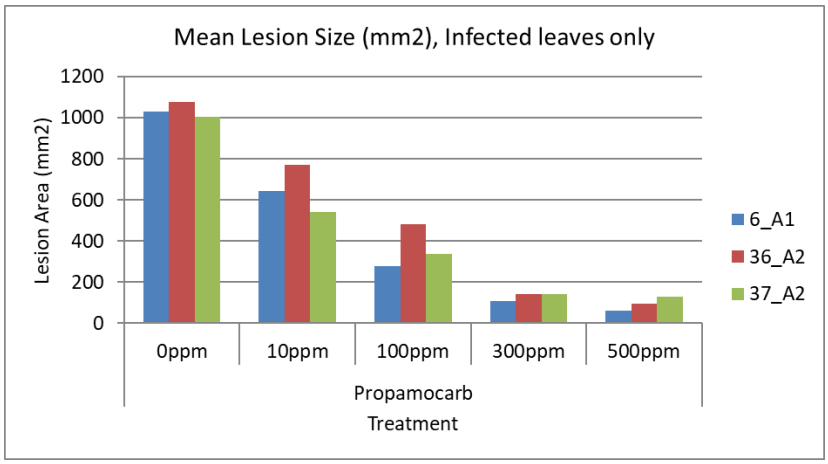

Figure 58. Mean lesion size (mm2) measured on infected leaves only at a range of concentrations of propamocarb tested in 2019.

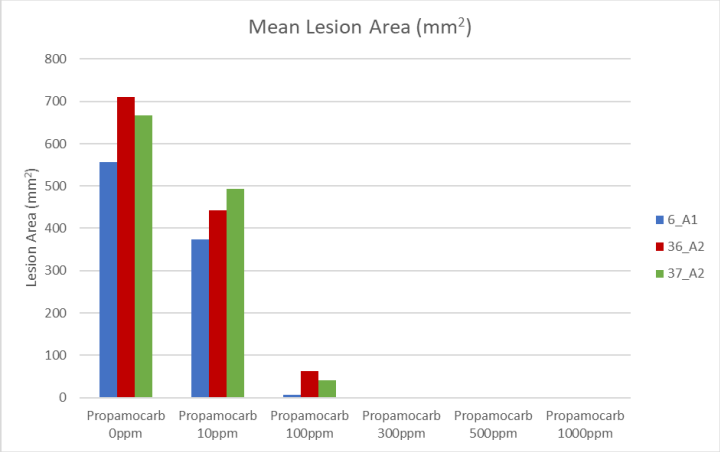

Figure 59. Mean lesion size (mm2) measured on infected leaves only at a range of concentrations of propamocarb tested in 2020.

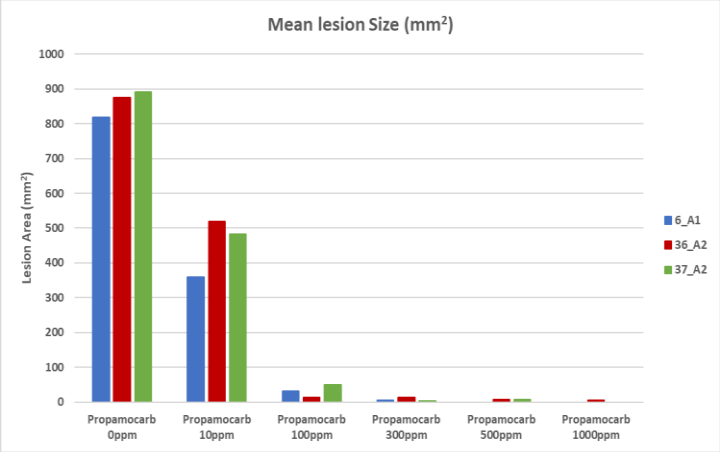

Figure 60. Mean lesion size (mm2) measured on infected leaves only at a range of concentrations of propamocarb tested in 2021.

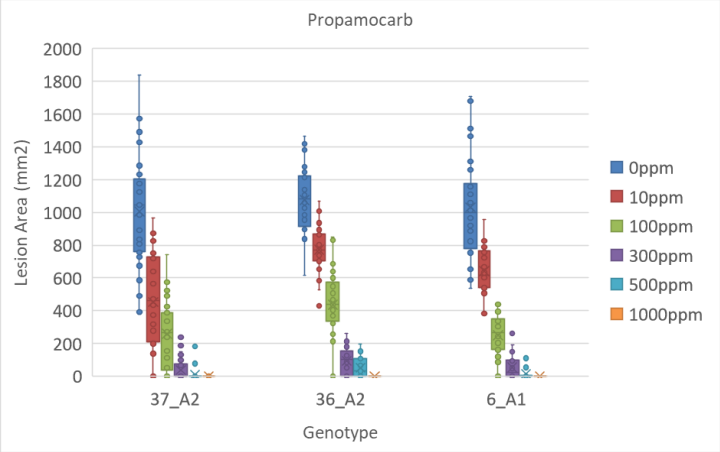

Figure 61. Mean Lesion area (mm^2) of isolates belonging to 36_A2 and 37_A2 compared with control isolates (6_A1) tested in 2019 and presented as a box and whisker plot. Propamocarb Max Field concentration = 5000 ppm.

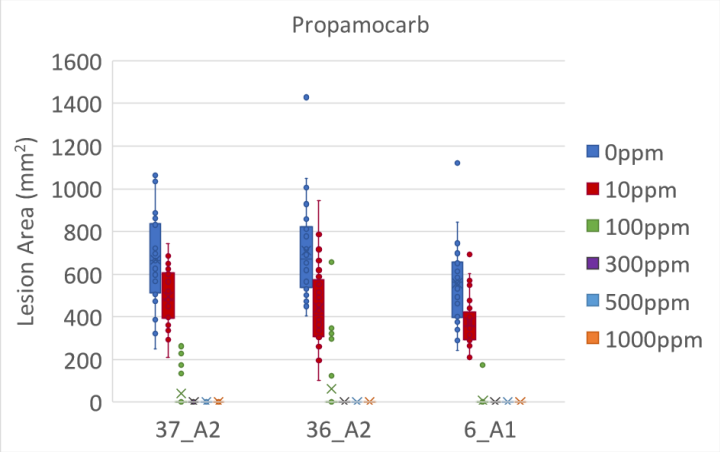

Figure 62. Mean Lesion area (mm^2) of isolates belonging to 36_A2 and 37_A2 compared with control isolates (6_A1) tested in 2020 and presented as a box and whisker plot. Propamocarb Max Field concentration = 5000 ppm.

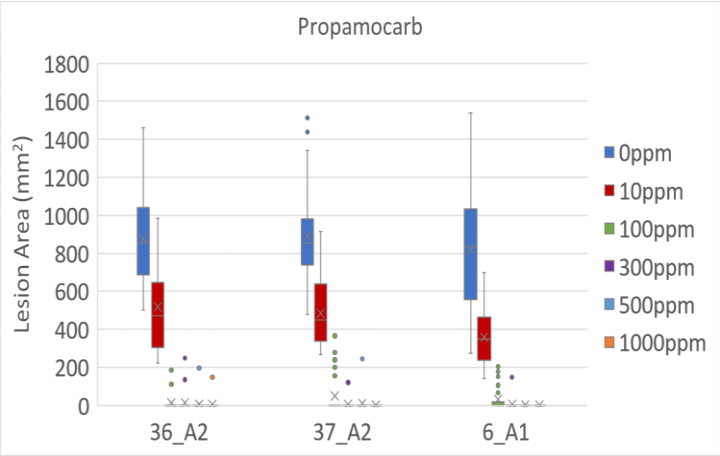

Figure 63. Mean Lesion area (mm^2) of isolates belonging to 36_A2 and 37_A2 compared with control isolates (6_A1) tested in 2021 and presented as a box and whisker plot. Propamocarb Max Field concentration = 5000 ppm.

The EC_{50} values calculated indicate that, on average, isolates of 36_A2 had a significantly greater EC_{50} values than those belonging to genotypes 37_A2 in 2019 only (Table 9).This was a result of slightly larger mean lesion sizes caused by isolates of 36_A2 in critical points of the dose curve (10 and 100 ppm). No other significant differences were observed between genotypes in 2020 and 2021 and results were very consistent over years, with some random variation observed. The EC_{50} values are in line with previous findings.

Table 10. Mean, max and min EC_{50} values for isolates of *P. infestans* of various genotypes tested in 2019 to 2021 at a range of concentrations of propamocarb (0, 10, 100, 300, 500, 1000 ppm). Significant differences between mean values are indicated by different letters.

Genotype		6_A1	37_A2	36_A2
Number of isolates		5	5	5
Mean EC_{50} (mg/L)	2019 2020 2021	**44.40ab** **19.91a** **18.14a**	**38.08a** **26.01a** **17.21a**	**60.87b** **25.08a** **17.1a**
EC_{50} min (mg/L)	2019 2020 2021	2.453 2.711 1.346	0.997 6.034 1.817	4.470 2.009 1.644
EC_{50} max (mg/L)	2019 2020 2021	133.943 44.586 133.943	244.060 109.663 49.275	180.804 148.030 54.457

Oxathiapiprolin 2019-21

A high incidence of lesions was caused by all genotypes at concentrations up to 0.001 ppm oxathiapiprolin in all years (Figs. 64-66). However, this represents only 0.0013% of the maximum field dose. At 0.01 ppm 30-80% incidence of lesions was observed. No lesions were observed at 0.1ppm in 2019 and 2020 (Fig 64-65) and very few in 2021 (Fig. 66). Mean lesion sizes, on infected leaves only, are shown (Figs. 67-69) and represented in box and whisker plots (Figs. 70-72). The statistical significance, or otherwise, of differences in lesion size is captured in the calculation of EC_{50}.

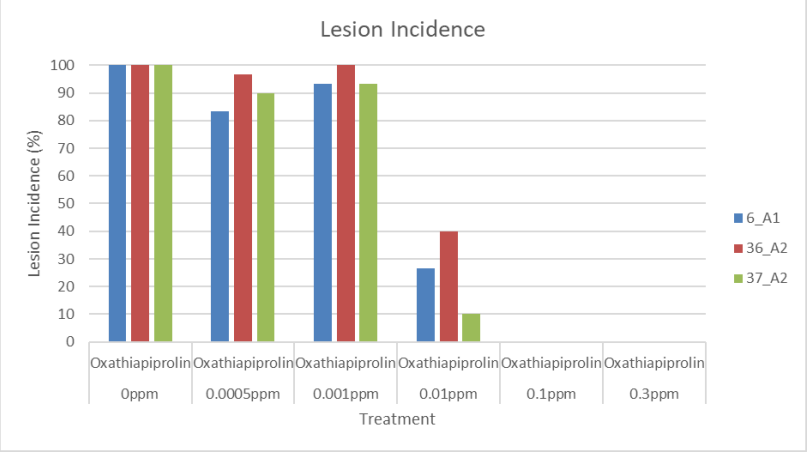

Figure 64. Mean percentage of lesions caused by different genotypes at a range of concentrations of oxathiapiprolin tested in 2019.

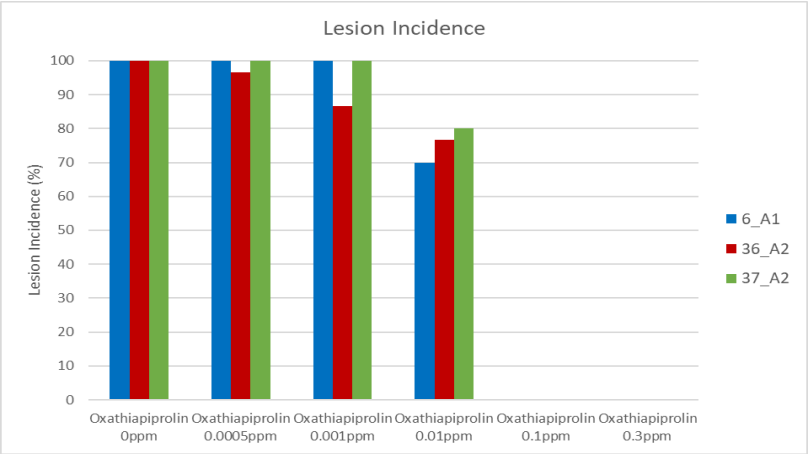

Figure 65. Mean percentage of lesions caused by different genotypes at a range of concentrations of oxathiapiprolin tested in 2020

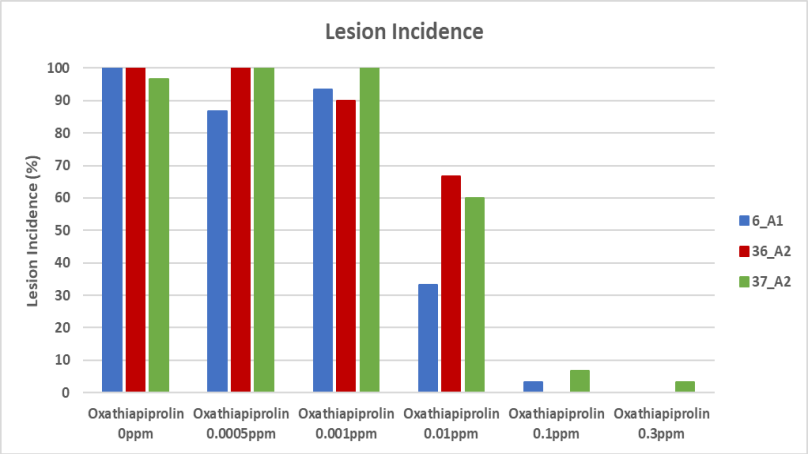

Figure 66. Mean percentage of lesions caused by different genotypes at a range of concentrations of oxathiapiprolin tested in 2021

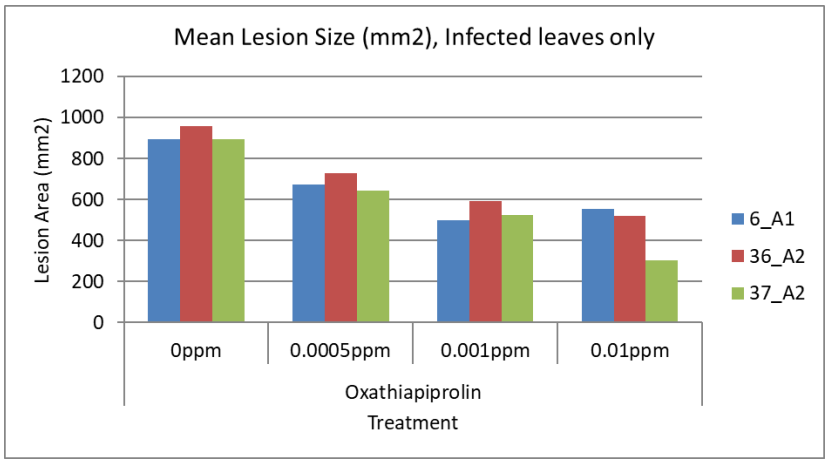

Figure 67. Mean lesion size (mm^2) measured on infected leaves only at a range of concentrations of oxathiapiprolin tested in 2019. No lesions were observed at 0.1 and 0.3ppm.

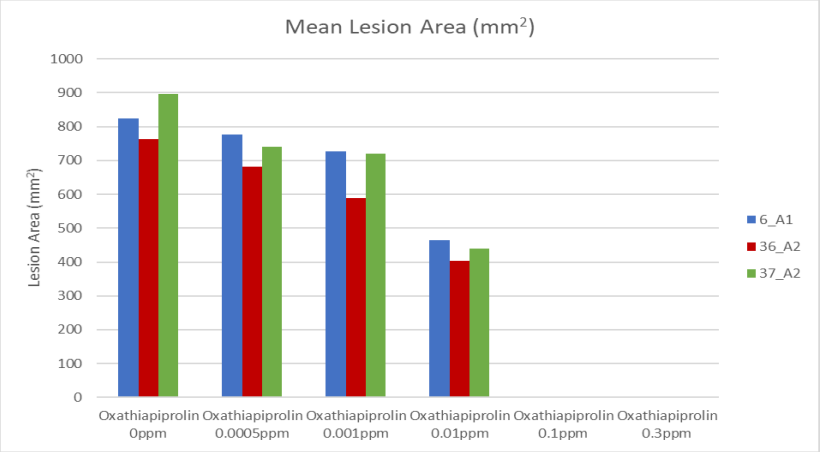

Figure 68. Mean lesion size (mm^2) measured on infected leaves only at a range of concentrations of oxathiapiprolin tested in 2020. No lesions were observed at 0.1 and 0.3ppm.

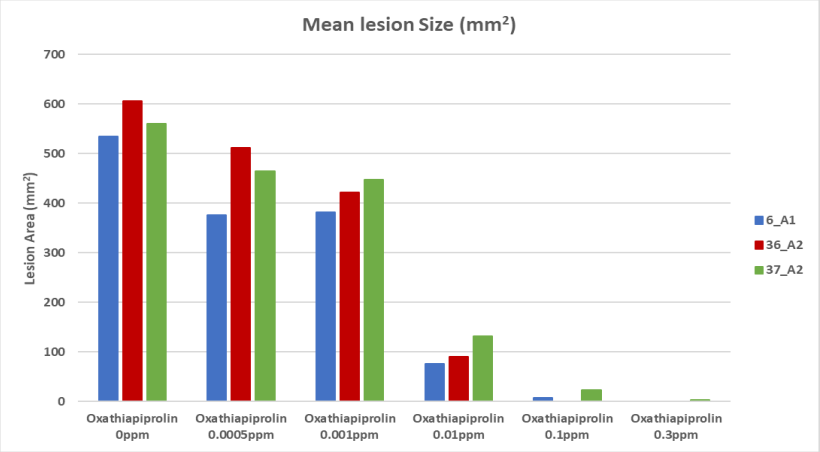

Figure 69. Mean lesion size (mm^2) measured on infected leaves only at a range of concentrations of oxathiapiprolin tested in 2021.

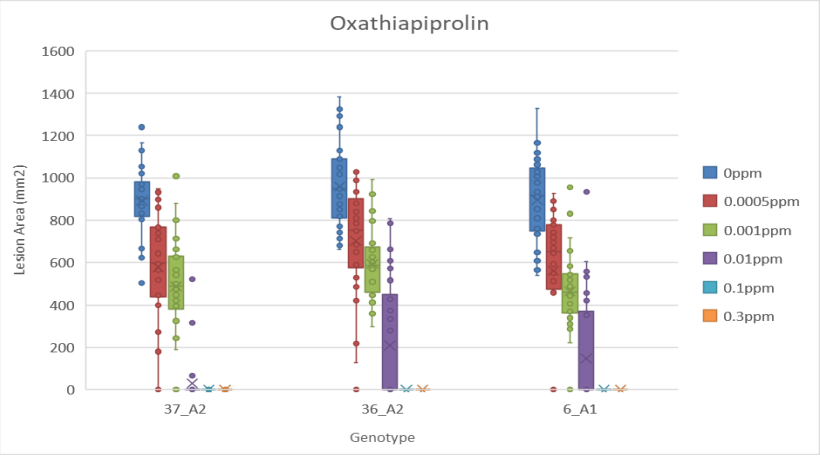

Figure 70. Mean Lesion area (mm2) of isolates tested in 2019 belonging to 36_A2 and 37_A2 compared with control isolates (6_A1) presented as a box and whisker plot. Oxathiapiprolin maximum field concentration = 75 ppm.

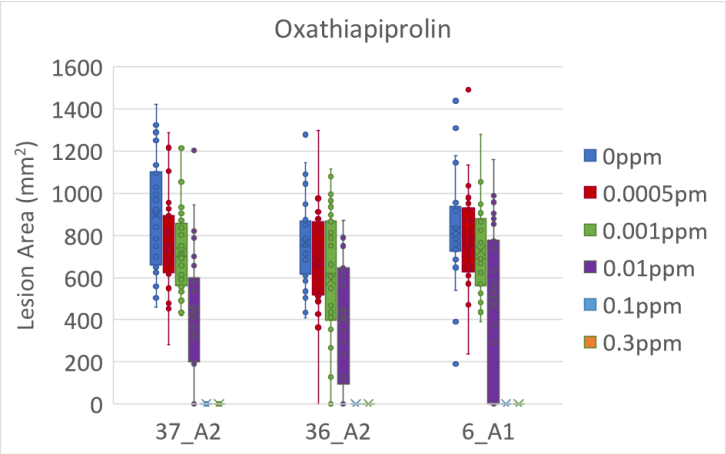

Figure 71. Mean Lesion area (mm2) of isolates tested in 2020 belonging to 36_A2 and 37_A2 compared with control isolates (6_A1) presented as a box and whisker plot. Oxathiapiprolin maximum field concentration = 75 ppm.

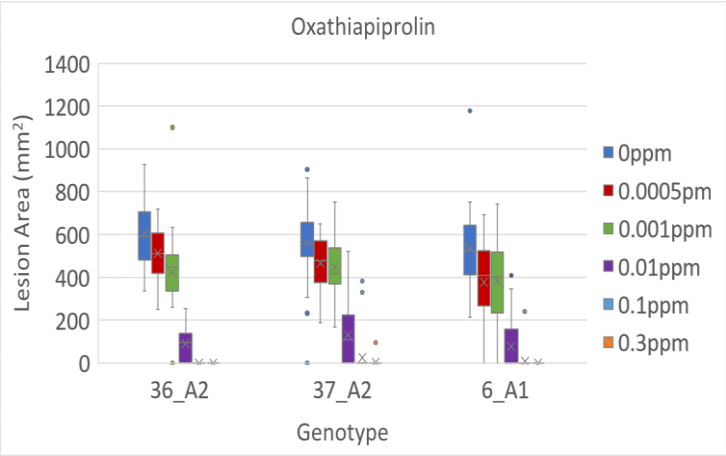

Figure 72. Mean Lesion area (mm2) of isolates tested in 2021 belonging to 36_A2 and 37_A2 compared with control isolates (6_A1) presented as a box and whisker plot. Oxathiapiprolin maximum field concentration = 75 ppm

There were no statistically significant difference in mean EC_{50} value between genotypes (Table 171. The EC_{50} values correspond well to those in other studies.

Table 11. Mean, max and min EC_{50} values for isolates of *P. infestans* of various genotypes tested in 2019-21 at a range of concentrations of oxathiapiprolin. Significant differences between mean values are indicated by different letters.

Genotype		6_A1	37_A2	36_A2
Number of isolates		5	5	5
Mean EC_{50} (mg/L)	**2019**	**0.00761a**	**0.0015a**	**0.0108a**
	2020	**0.1321a**	**0.1351a**	**0.1294a**
	2021	**0.1451a**	**0.1462a**	**0.128a**
EC_{50} min (mg/L)	2019	0.0003	0.0004	0.0004
	2020	0.1000	0.1105	0.1105
	2021	0.1000	0.1000	0.1000
EC_{50} max (mg/L)	2019	0.0545	0.0033	0.0493
	2020	0.2014	0.2014	0.2014
	2021	0.2014	0.3669	0.2014

Mancozeb 2019-21

The mean incidence of lesions for each genotype at different concentrations of mancozeb indicated a 100% incidence of lesions caused by all genotypes at concentrations up to 10 ppm mancozeb (Figs. 73-75). Data for 2021 including the control is slightly variable. This is probably due to the mancozeb test being carried out late in the season when light levels can become limiting and cause increased variation. Disease incidence at 500-1000pm was very low in all cases. Mean lesion size, on infected leaves only, reduced with increasing rate (Figs. 76-78). The statistical significance, or otherwise, of differences in lesion size is captured in the calculation of EC_{50} and the lesion size data is also represented in a box and whisker plot (Figs. 79-81).

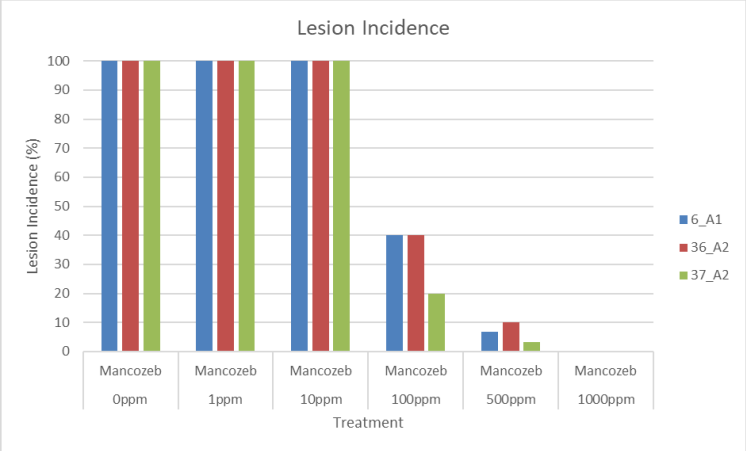

Figure 73. Mean percentage of lesions caused by different genotypes at a range of concentrations of mancozeb tested in 2019 (0-1000 µg/ml).

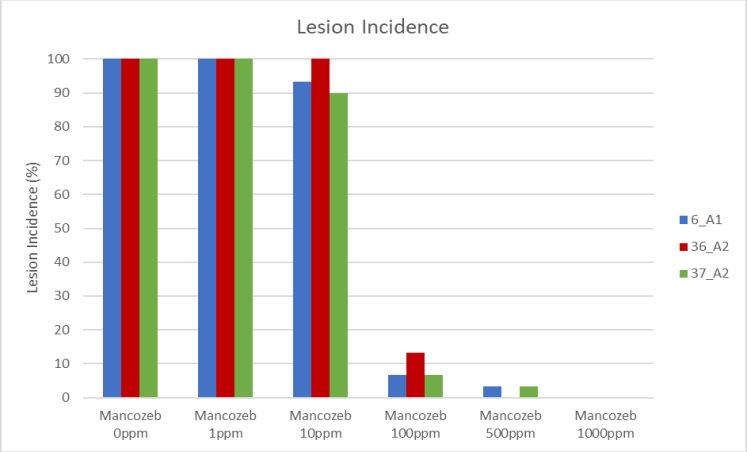

Figure 74. Mean percentage of lesions caused by different genotypes at a range of concentrations of mancozeb tested in 2020 (0-1000 µg/ml).

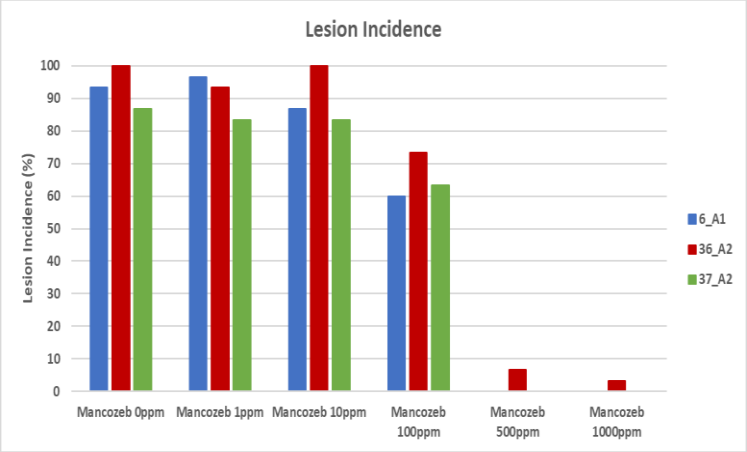

Figure 75. Mean percentage of lesions caused by different genotypes at a range of concentrations of mancozeb tested in 2021 (0-1000 µg/ml).

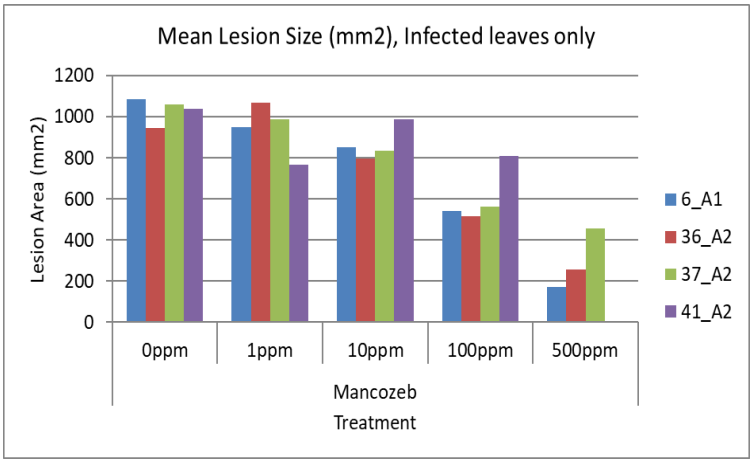

Figure 76. Mean lesion size (mm^2) measured on infected leaves only at a range of concentrations of mancozeb tested in 2019.

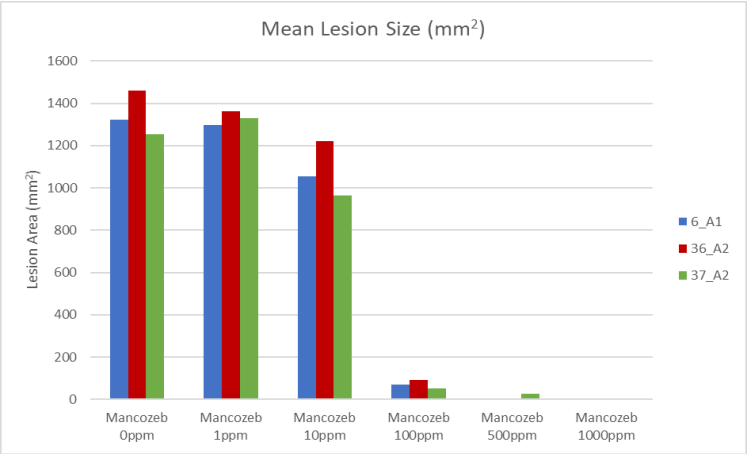

Figure 77. Mean lesion size (mm^2) measured on infected leaves only at a range of concentrations of mancozeb tested in 2020.

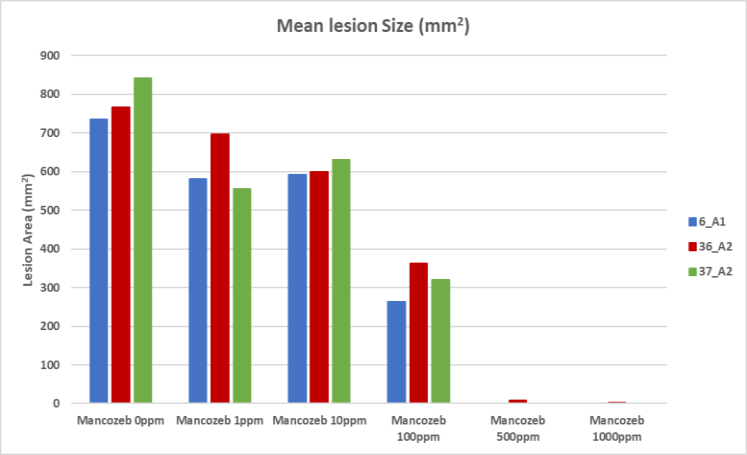

Figure 78. Mean lesion size (mm^2) measured on infected leaves only at a range of concentrations of mancozeb tested in 2021.

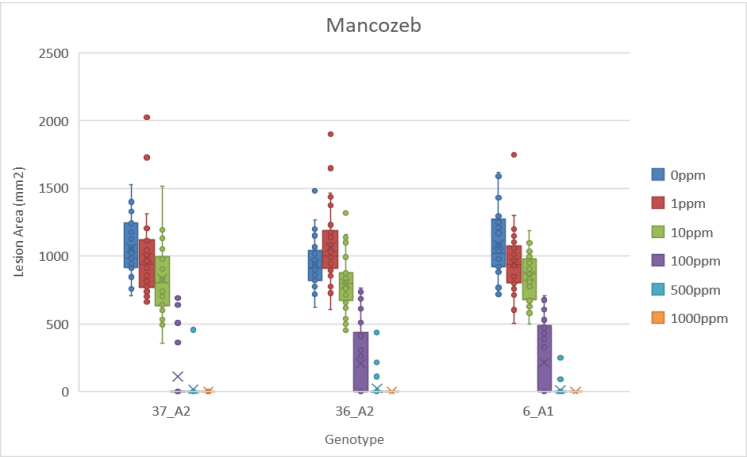

Figure 79. Mean Lesion area (mm^2) of isolates belonging to 36_A2 and 37_A2 tested in 2019 compared with control isolates (6_A1) presented as a box and whisker plot. Mancozeb maximum field concentration = 6375 ppm.

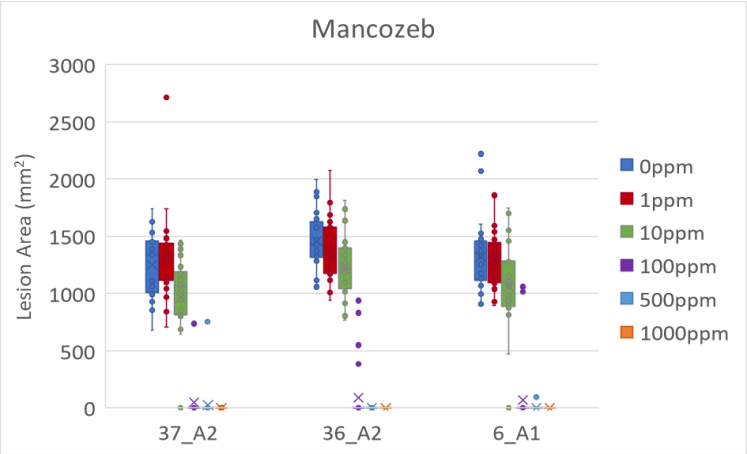

Figure 80. Mean Lesion area (mm^2) of isolates belonging to 36_A2 and 37_A2 tested in 2020 compared with control isolates (6_A1) presented as a box and whisker plot. Mancozeb maximum field concentration = 6375 ppm.

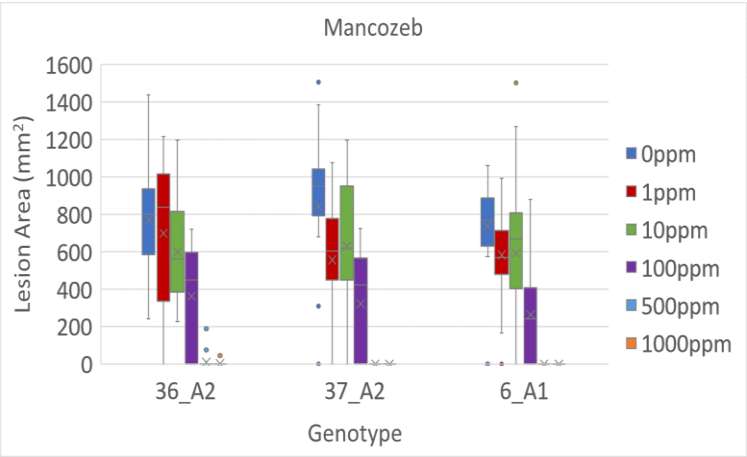

Figure 81. Mean Lesion area (mm^2) of isolates belonging to 36_A2 and 37_A2 tested in 2021 compared with control isolates (6_A1) presented as a box and whisker plot. Mancozeb maximum field concentration = 6375 ppm.

The mean EC_{50} values show there was no statistically significant difference between genotypes (Table 12). The EC_{50} values are higher than those previously reported but were obtained using a different method are very consistent over years and are well within the field rate concentration (6375µg/ml).

Table 12. Mean, max and min EC_{50} values for isolates of *P. infestans* of various genotypes tested in 2019 to 2021 at a range of concentrations of mancozeb. Significant differences between mean values are indicated by different letters.

Genotype		6_A1	37_A2	36_A2
Number of isolates		5	5	5
	2019	**53.13a**	**43.27a**	**45.93a**
	2020	**37.02a**	**33.58a**	**29.51a**
Mean EC_{50} (mg/L)	2021	**95.18a**	**54.96a**	**84.99a**
	2019	0.817	7.370	0.903
	2020	1.102	2.711	9.002
EC_{50} min (mg/L)	2021	0.332	0.100	0.367
	2019	244.060	298.096	329.447
	2020	269.728	133.943	109.663
EC_{50} max (mg/L)	2021	810.308	148.030	298.096

Comparisons of EC_{50} values 2019-21

Having tested batches of isolates collected across three years for sensitivity to seven active ingredients (amisulbron, oxathiapiprolin, mancozeb, fluopicolide, mandipropamid, cyazofamid, propamocarb) it was possible to make a comparison of the EC_{50} values over time to examine the data for any annual shifts in sensitivity. This comparison did not indicate any consistent shift over time in any product (Fig. 82) Minor differences were identified between years but no clear trends and this probably relates to normal biological variation and the fact that different isolates were used each season.

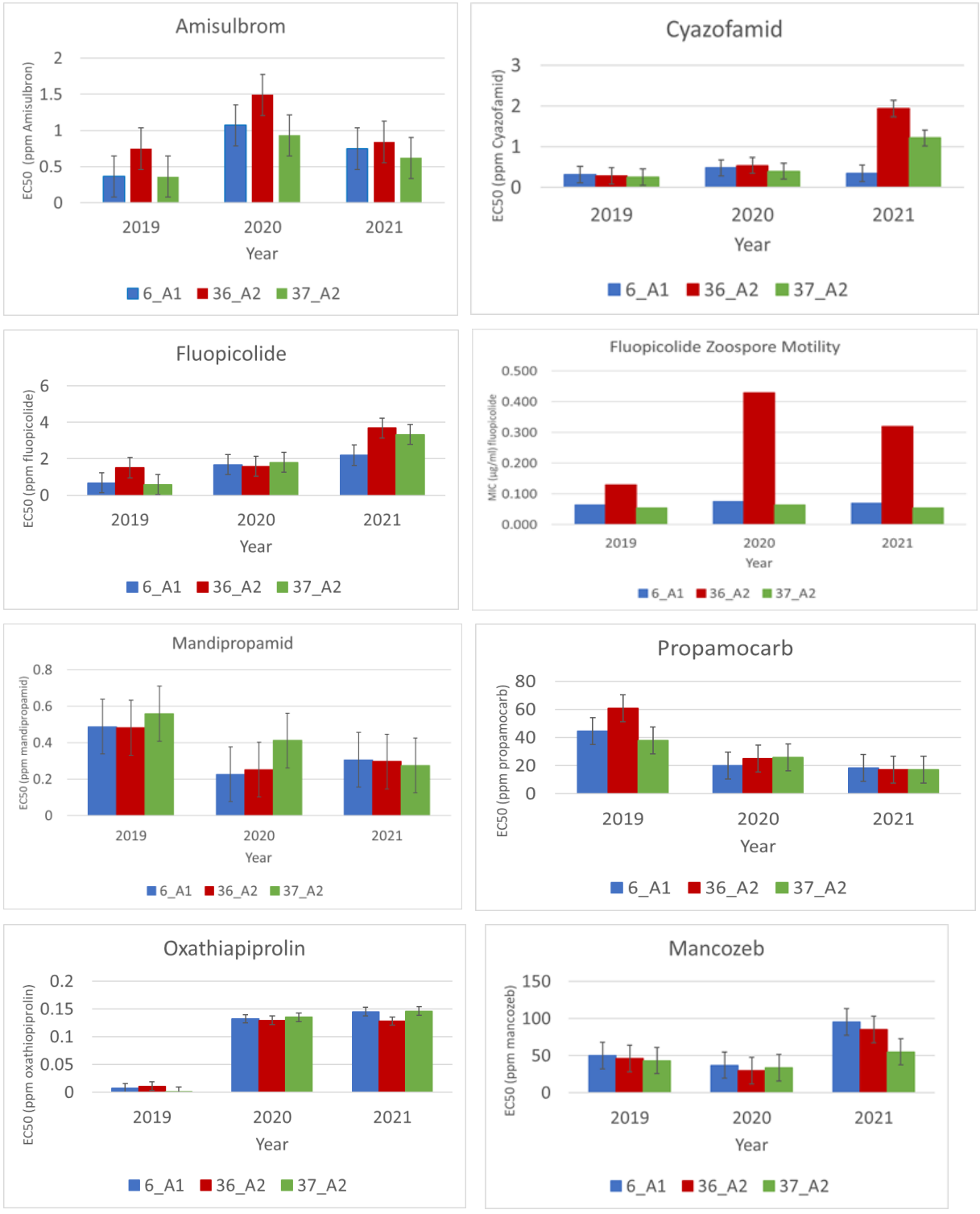

Figure 82. Comparison of mean EC50 values for each fungicide tested across 3 years. Error bars indicate mean sed values.

5. Discussion

Outbreak monitoring and disease risk

The blight sampling in the 2019 season was characterised by two spikes in June and Sept with an above average number of samples by the end of the season (Fig. 7). The 2020 season was on average warmer and drier and the lower blight pressure resulted in fewer samples than average. In 2021, above average number of outbreaks were sampled in a year with localised serious outbreaks. The combined sampling of 534 outbreaks and genotyping of over 2600 samples of *P. infestans* provides a detailed record of the pathogen population (Fig. 9). Compared to recent years the population was relatively stable over the 2019 and 2020 seasons. A consolidation of the new genotypes, 37_A2 and 36_A2 in England was evident (Fig. 9) with an extension of their range into Wales and Scotland (Fig. 10). Under higher disease pressure in 2021 a further expansion of genotype 36_A2 and the first appearance of 41_A2 were key points. Although not part of this study, the expansion of newer clones into Northern Ireland was also observed (Fig. 11). Each of these genotypes is discussed below:

Genotype 37_A2

As a prelude to the challenges of the 37_A2 lineage, issues were first observed in genotype 33_A2 that was detected in the Netherlands in 2009 and demonstrated to have reduced sensitivity to fluazinam (Schepers et al., 2018). Genotype 33_A2 comprised 20% of the sampled population in the Netherlands in 2010 and 2011 (Schepers et al., 2013) and was recently sampled in Nigerian crops (Nnadi et al., 2019). In GB it was sampled in the same location in southeast England in 2011 and 2012 and then not sampled again until 2016 and 2017 (Cooke, 2019). It has not been sampled again since then and this decline is 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 applications (Schepers et al., 2018). The 37_A2 lineage however is both fluazinam insensitive and appears more evolutionarily fit and aggressive than 33_A2. This clone was first observed in the Noordoostpolder region of the Netherlands in 2013 and spread locally and then to Britain over in the following three seasons (Cooke 2019). Within Britain, the infection was first recorded in the Shropshire area in late June 2016 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. 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. 14). It was not reported from Wales, southwest England or Scotland in 2017. Blighted tuber testing from the 2017 season suggested 37_A2 was aggressive and fit on both foliage and tubers. Any spread in 2018 was limited by the weather with only 40 FAB outbreaks sampled across GB. The proportion of 37_A2 declined from 24% in 2017 to 16% of samples in 2018 and by 2019 this had declined further to only 6% of GB samples. Despite the decline, its geographic range had increased into Scotland and Wales (Fig. 14). In 2020 the proportion increased slightly to nearly 10% across sampled GB blight outbreaks and remained stable into the 2021 season (Fig. 9).

An campaign was launched to raise awareness of the need to change the way fluazinam was used in blight control programmes. This involved presentations at AHDB Potatoes' events, Grower Gateway and UK farming press articles and an advisory document on fluazinam use released on the AHDB Potatoes website (Bain et al., 2018). Data from this work was also passed

to the Fungicide Resistance Action Committee UK (FRAG-UK) and the agrochemical industry. The manufacturer's guidelines on the use of fluazinam have been updated (See FRAC website https://www.frac.info/fungicide-resistance-management/by-frac-mode-of-action-group). Unlike past usage, no more than two sequential applications of the solo product are recommended, and growers are encouraged to only use the product in mixtures with other modes of action. The data from UK pesticide use surveys indicate that growers are heeding such advice with a marked reduction in fluazinam use reported between 2016 and 2018 (Fig. 83).

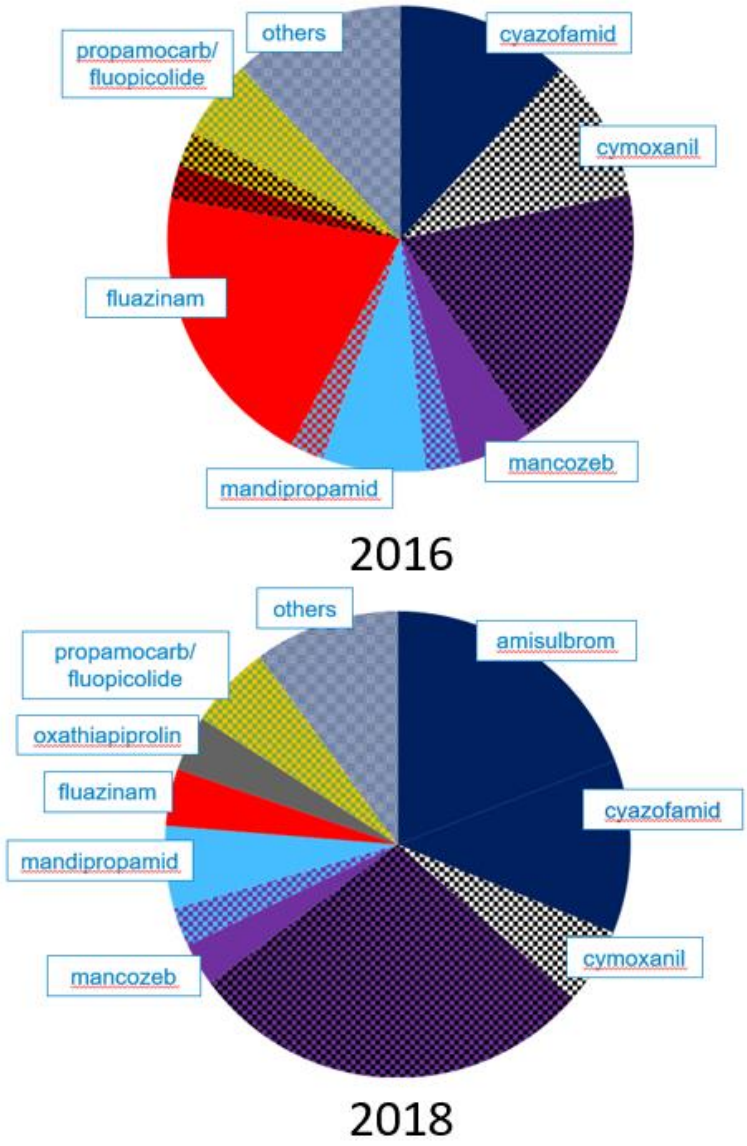

Figure 83. Change in use of fungicide active ingredients on UK potato crops between surveys in 2016 (upper) and 2018 (lower). The proportion of fluazinam applied reduced markedly over this period. Based on data from Garthwaite et al., (2018) and Garthwaite et al., (2019).

This campaign, the related drop in fluazinam usage and the decline and now stable frequency of 37_A2 in the GB pathogen population are correlated and should be seen as a clear success of the FAB campaign. This data and associated publicity have almost certainly prevented many blight control failures and significant incidences of tuber blight in GB crops.

Genotype 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. By 2017 it had spread across the Netherlands into Belgium, the UK, Denmark and Poland and in 2018 was also sampled on crops in Spain, Hungary and Serbia and made up 16% of the EuroBlight samples (up from 10% in 2017). The first reports in British crops were in Kent, Norfolk and Lincolnshire in 2017 and in a similar range but a higher incidence in 2018 where it was reported to cause severe losses in some crops when blight conditions hit later in the summer. Over the course of the 2019 to 2021 seasons 36_A2 increased from 17% of GB samples in 2018 to 27%, 29% and 40% in 2019, 2020 and 2021, respectively. It has also spread to and become established in Wales and Scotland but remains most prevalent in east England crops comprising 64% of these samples in 2021 (Figs. 10 & 15). The spread of the 36_A2 and its ability to displace other genotypes suggests it is fit and aggressive. Fungicide sensitivity testing in laboratories in Wageningen University and The James Hutton Institute indicated 36_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). There was also some evidence of higher 36_A2 lesion incidence at very low doses of fluopicolide and amisulbrom in 2019 and 2021 testing (Figs. 18 & 46) but this was not supported by identical testing on samples collected in 2020 (Fig. 47) which suggests it may be natural experimental variation rather than a shift in sensitivity. A trend for lower suppression of zoospore motility by fluopicolide on 36_A2 isolates relative to other genotypes in all three years of testing may provide indications of a fitness advantage of this lineage. However these values were still within the expected range and the differences very small when compared to the full field dose of this product. The drivers of the population shift for 36_A2 thus remain unclear. However, its ability to displace other lineages and anecdotal reports of control problems supports the hypothesis that is fitter and more aggressive than other lineages and thus more difficult to manage. There are many factors that determine the success of one lineage over another including, overwinter survival rates, infection efficiency, latent period, sporulation capacity, fungicide resistance, virulence and aggressiveness. Each of these may be shaped by a specific set of environmental or crop-specific factors that are challenging to replicate under controlled experimental conditions. Work is underway to identify the specific traits of 36_A2 and how this data can be used to manage it.

Genotype 13_A2

The 13_A2 lineage has been 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, Dey et al., 2018) and other parts of Asia (Guha Roy et al., 2021) 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 gradual displacement by other lineages. If the steep decline in GB populations seen in 2020 and 2021 (Fig. 9) continues growers may once again consider the use of products containing metalaxyl.

Genotype 6_A1

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, still not yet been reported outside of Europe. 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. In 2019 and 2020 it was found in all parts of the GB and at just over one third of all samples remained the single most dominant lineage causing late blight in GB crops. However, by 2021 it dropped to 24% of the population (Fig. 9) and a consistent displacement by 36_A2 is occurring in most regions.

Genotype 8_A1

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% from 2012 to 2015. Despite declines to around 2% in 2016 and 2017 and its absence in 2018 samples it comprised around 4-5% of samples in 2019 to 2021 (Fig. 9). It remains more prevalent in crops sampled in Wales and Scotland than in England (Fig. 10). It appears that 8_A1 had some sort of selective advantage over other lineages but the nature of this advantage remains unclear. Genotype 8_A1 has historically been more dominant in crops on the island of Ireland (Cooke, 2015).

Genotypes 42_A2, 39_A1, 41_A2 and 44_A1

Genotype 42_A2 was newly defined in 2020 after first being sampled in 2017 and present in all four subsequent seasons (Fig. 10). Although it comprised only 1-3% of the population its local spread from the first observations in north Wales and subsequent presence in crops in Cheshire and Lancashire suggest spread on prevailing westerly winds. Despite this persistence and spread there is no current evidence to suggest genotype 42_A2 is particularly difficult to manage or a specific cause for concern but it does represent an unusual example of a named clonal lineage generated within GB crops. It was not sampled in 2021, perhaps because of a lack of fitness but this may also reflect a generally low disease pressure in NW England in 2021.

Genotype 39_A1 represents another lower frequency clone. It first appeared in 2015 and has now been found in several 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 (Pettitt et al., 2019) and it's spread to potato from this source is likely. It was not sampled in 2019 but was recovered 8 times in 2020 on tomato and potato in Wales and once on potato in Kent. In 2021 it was only found in FAB samples from northwest Wales in garden crops.

The arrival of genotype 41_A2 (formally known as EU_41_A2) may be significant. This genotype has been able to displace sexually diverse populations of *P. infestans* in Denmark and other Nordic regions which suggests it is fit and aggressive. In 2020 it comprised 6.3% of European samples in the EuroBlight database (n=1217). It was first sampled in Denmark in 2013 and has slowly spread from there to Sweden, Norway, Finland, Poland and Germany by 2020. The traits of 41_A2 have been examined but no single factor was observed that explains its ability to outcompete other genotypes (Puidet et al., 2022). The five samples with an identical fingerprint to EU_41_A2 from Fife comprised 4 FTA prints and one live isolate. These were from a crop of Maris Piper with seed of GB origin and the outbreak was in a part of the crop that missed a fungicide spray. There is no clear association with imported seed or any other source from mainland Europe so it is unclear how this genotype arrived in Fife. It is 700km across the North Sea from the closest known source of this lineage and the prevailing wind blows westerly rather than from the east. The options are a seed outbreak in the 2020 or 2021 season that remained unsampled until this outbreak or a weather event that carried inoculum eastwards. The latter case is possible but sporangia of *P. infestans* are sensitive to UV light so it would need to occur under overcast conditions.

Genotype EU_44_A1 was named during the analysis of the 2021 GB and EuroBlight datasets. It had not been recorded prior to 2021 and this would normally preclude it from being named until found in a second season. However, its finding simultaneously in several outbreaks in GB and Ireland as well as on mainland Europe prompted the elevation to named clone status.

The phenotype of this genotype has not yet been determined so the risks/consequences are currently unknown.

Within-genotype variation in clones

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 (Haas et al., 2009). Up to 20,000 sporangia are produced per cm^2 of every late blight lesion each day (Skelsey et al., 2009) and therefore, countless billions of cells of *P. infestans* are dividing daily. Genetic analysis based on a population genetics application called *poppr* (Kamvar et al., 2015) offers insights into the data. *Poppr* converts the stepwise variation in SSR data into a matrix of pairwise genetic distances between each isolate. Pairs of isolates with an identical fingerprint will return a value of zero and form a node in the figure (Fig. 16) whereas those that differ by a single step in one marker return a value of around 0.01 (i.e. a 1% difference) and appear as different nodes connected by an edge that is drawn as a thick black line. 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.16). Many thousand isolates of the 13_A2 clonal lineage have been fingerprinted and more than 200 minor sub-groups defined (e.g. Dey et al., 2018). Sub-groups that emerge early have an opportunity to spread and may be prevalent in the population, but the majority are rare and thus seldom sampled. The rate at which new sub-clonal variants emerge and their stability over time makes them appropriate for tracking inoculum movement. For example, genotype 36_A2 isolates form 19 nodes (Fig. 16) and an analysis of this same data based on country of origin (data not shown) shows that two thirds of the largest 66-sample node were found in England with the rest in approximately equal proportions from crops in Scotland and Wales. This node is the original form of 36_A2 which has spread widely. In contrast, the 44-node was found mostly in England with only one fifth sampled from Wales and none from Scotland suggesting it is a more recently evolved variant that was not involved in the migration event to Scotland. It is important to note these are variations in selectively neutral SSR markers and do not necessarily relate directly to differences in the traits of the lineages.

These studies have shown that inoculum generated and surviving locally (as volunteer tubers or in potato dumps) has a marked impact as a source of local primary inoculum propagating disease in nearby crops the following season and stresses the importance of effective management of such local inoculum (Cooke, 2019).

Novel combinations of 'Other' genotypes

A relatively stable proportion of the sampled GB population of *P. infestans* is comprised of samples in a 'catch all' category termed 'Other'. The mean GB proportion since 2003 is 9.7% with highs of 29% in 2015 a low of 2.9% in 2008 (Fig. 9). This proportion is higher in Scotland ranging from 8.3% 2013 to 53.4% in 2016 (Fig. 10). In 2019, 2020 and 2021 the proportion across GB crops was 17.3, 13.9% and 17.9% respectively.

Genetic analysis of the SSR data from isolates from GB in 2020 highlights the diversity of this group of 'Other' isolates (Fig. 16). 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 (e.g. 42_A2 & 44_A1). Careful analysis of all 'Other' isolates collected from 2003 to 2021 has not identified more than a handful of samples with a fingerprint common to more than one outbreak site or season. In 2020 for example, the 28 grey 'Other' nodes were almost exclusively sampled in Scotland and none were sampled from outwith the country (Fig. 16).

The above is strong evidence for local ephemeral populations that are not as fit or aggressive as the clonal genotypes. 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 other sources.

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.

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 northeast Scotland are between 5-7 years; sufficient for significant oospore decay. Samples have been reported from conventional crops but also discard 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 because these disease outbreaks effectively shorten the rotation by spreading inoculum of *P. infestans* to neighbouring ware or seed crops.

The higher frequency of 'Other' types in northeast Scotland 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. 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 limits seed movement 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 is subject of current study (Cooke et al., 2020). Further exploration of the 'recombinants' in this part of Scotland is underway at the James Hutton Institute 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 contemporary clonal 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.

Fungicide sensitivity testing

The key main finding from the comprehensive testing of multiple isolates of three clonal lineages examined in 2019, 2020 and 2021 was that no consistent change in sensitivity was revealed amongst the seven tested fungicide active ingredients. These findings are consistent with other studies (e.g. Saville et al., 2015; Cohen et al., 2007). As described in the introduction to each of the fungicides tested (Section 4), the EC_{50} data generated in this study was broadly in line with other published studies.

A few examples of genotype-specific differences were noted in these tests. For example, fluopicolide was less active against 36_A2 with reduced EC_{50} values in 2019 and 2021 tests (Table 5) and lower zoospore MICs in 2019 to 2021 tests (Tables 6 & 8). Mandipropamid showed lower EC_{50} scores against 36_A2 in 2019 (Table 9) and 37_A2 in 2020 (Table 10). Amisulbrom had lower EC_{50} against 36_A2 in 2019 (Table 13) as did propamocarb in 2019 (Table 15). Lastly, cyazofamid had lower EC_{50} values for 36_A2 in 2021 (Fig. 39) While these differences were statistically significant in individual years, they were generally not supported in a other years of

testing and related to doses well below full field rate. From this we conclude that no consistent shifts in resistance in contemporary lineages were observed and all active ingredients are fully effective at their recommended field rates. Despite these data, there are anecdotal accounts of poor product performance in the field. Further investigation has suggested problems with product timing and high disease pressure. All such reports are important and should be followed up and routine baseline sensitivity testing is important for early identification of any potential insensitivity issues.

The assays in this study were conducted using detached leaves of a single variety with a deliberately low range of doses require to generate a dose response curve. Tests included multiple isolates of each genotype from different parts of UK and six replicates of each treatment. The testing followed FRAG guidelines and examined preventative control in which the product was applied 24 hours before inoculation. Such robust *in vitro* testing has, for example, clearly demonstrated changes in sensitivity to fluazinam (Lees, 2018) that were also apparent in field control failures (Schepers et al., 2018). However, all such *in vitro* tests have limitations as they cannot simulate every possible field scenario. In practice, the pathogen is exposed to fungicide doses lower than full field rates due to factors such as uneven canopy spray penetration, rainfall and the natural decline in active ingredient concentration over time after application. Similarly, despite the advice to use fungicides preventatively, products are inevitably used curatively which generates different selection pressures. It would be interesting to run curative tests in which the products are applied at a range of intervals post-inoculation to investigate whether there are genotype-specific differences in performance.

In this study, testing was conducted against seven principal active ingredients in six FRAG fungicide groups (Table 1). The 2018 FRAG guidelines list 12 groups for control of late blight in the UK. In 2021 twelve groups remain as chlorothalonil use has been banned but the OSBPIs (oxysterol binding protein inhibitors) added. Two of the remaining six groups, phenylamides (e.g. Metalaxyl) and uncouplers of oxidative phosphorylation (fluazinam) have already been tested, leaving four groups for possible future investigation. These are Benzamides (toluamides; zoxamide), Cyanoacetamide-oxime (cymoxanil), QoI fungicides (famoxadone and fenamidone) and the QoSI fungicides (ametoctradin).

To date, the only cases of resistance in the population of *P. infestans* known to reduce fungicide performance in the field have been to metalaxyl (Gisi & Cohen, 1996) and fluazinam (Schepers et al., 2018). Repeated exposure to a single active ingredient is considered a high-risk practice that places a strong positive selection pressure on resistant mutants. However, this depends on the active ingredient and the evidence is mixed. A comparison of resistance amongst isolates from blighted plots untreated or sprayed multiple times with a single fungicide active ingredient (fluazinam, cymoxanil, dimethomorph, metalaxyl, or propamocarb) across a single season in Mexico noted a shift in resistance in only metalaxyl (Grunwald et al., 1996). Similarly, an attempt to force a change in sensitivity to the carboxylic acid amide (CAA) fungicide mandipropamid with repeated sub-lethal doses did not result in any resistant isolates of *P. infestans* in the field (Cohen et al., 2007). It may be the duration and scale of such exposure that is important. The risk of change in sensitivity in fluazinam was considered low (Tucker et al., 1994) and there were no reports of problems between its release in 1992 and when insensitive samples were first collected in 2009 (Schepers et al., 2018). Seventeen years of increasingly intensive use, often in extended blocks across much of the growing season, explain this development. Similarly for other active ingredients, the theoretical risk of field resistance has been demonstrated in the laboratory. Mutagenesis was used to induce resistance in mandipropamid, for example (Blum et al., 2010) and resistance to oxathiapiprolin has been reported in laboratory generated mutants of *Phytophthora capsici* and *Phytophthora* sojae (Miao et al., 2020). This highlights the ongoing risk of mutation and positive selection that can occur if

large populations are subject to prolonged exposure to a single active ingredient. Strategies to minimise the risk include alternating products, mixing active ingredients and limiting the number of applications of a single active in a growing season (Bosch et al., 2014). The pressures on other active ingredients will increase when the approval for use of the commonly used multi-site fungicide mancozeb is withdrawn (Wynn et al., 2017). In the longer term, a strategic approach is needed in which host resistance and fungicide are used in combination to suppress the pathogen population and limit selection (Ritchie et al., 2018). Such strategies are crucial given the continued environmental and political pressure on reducing fungicide usage that is focussing attention on Integrated Pest Management (IPM) systems (Kessel et al., 2018) and working within the UK National Action Plan for the Sustainable Use of Pesticides (Plant Protection Products) (Defra 2013).

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 conditions when the crop is growing rapidly. Over the 2019 to 2021 seasons the risks of primary inoculum build up early in the season have been suppressed by warm dry conditions. However, it remains critical that growers control sources of primary inoculum by management of growth on discard 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.

New genotypes continue to threaten the GB potato crop and the fluazinam insensitive 37_A2 lineage has altered product selection in fungicide programmes in Britain and across Europe. Work is underway to identify the specific traits that have driven the displacement of other lineages by 36_A2 and how this data can be used to best manage it. The use of FTA cards has been valuable in providing the industry in-season feedback in the 2019 - 2021 seasons, allowing growers and advisors flexibility in their fungicide choices. No new sources of insensitivity have been identified. 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, or in the absence of the AHDB Potatoes, an alternative to this, will remain crucial to the future success of such an approach.

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Appendix 1. Sampling instructions

SAMPLING INSTUCTIONS

Sampling:
Experience has shown that **sampling is the most critical process.** Time spent sampling correctly is well spent!

Select:
Select a leaflet with a single fresh, nicely sporulating lesion for each sample. Take samples from different plants if possible – but samples may come from the same plant if necessary. Make a note of how you have sampled. Sampling in the morning tends to be better as the pathogen sporulates overnight. Stem lesions may be sampled if that is the only blight infected material available.

Figure 1: Select a leaflet with a single lesion

Figure 2: Select a fresh sporulating area of the lesion to press onto the FTA card

Avoid:
Dead leaves, old or dry lesions, leaflets with many lesions. Wet, water soaked (bacterially infected) looking leaves.

Protocol for sampling pathogen DNA using FTA cards

Sample onto the card from the area indicated over the page and see YouTube video for further guidance. Search "Blight FTA card" on YouTube for video https://www.youtube.com/watch?v=BQLe0G7vdHY
The method relies on a very sensitive DNA amplification method so please limit cross-contamination between samples and only touch each FTA card sampling area with a single lesion sample.

1. Use **1 card** with 4 sampling areas (circles) **per reported incident.**

2. **Sample 4 lesions per infected field,** 1 lesion for each sample area (E, F, G, H).

3. **Label the FTA card with the same reference number used for leaf sample (obtained from AHDB website).** Don't worry if you haven't been able to get a number but make sure you fill out the Scout response form and write name, date and postcode on card.

4. **Take the sample (instructions below)**
Do not touch the sampling area except with the late blight lesion sample

5. **Air-dry the card,** store and **return card + sampling form** in postage paid envelope.

Place sample on FTA card:
Place the lesion sample (*Figure 2*) inside a clean circular sampling area on the FTA matrix. **Sporulating side facing down.** Cover a single sample area only per lesion.

Replace the cover sheet and press sample:
Apply moderate pounding/pressure to the leaf sample to extract lesion sap through the cover sheet with a round blunt object such as a spoon or a screwdriver handle. Take care not to damage the matrix. Repeat for other three lesions.

When the **green leaf extract is visible on the** FTA matrix the process is complete.

Remove plant residue from card, ensure that no large pieces of plant tissue remain adhered to the FTA card (*Figure 3*).

Allow the FTA card to air dry for a minimum of one hour at room temperature. Store **dry** FTA card in the plastic zip-lock bag.

Return cards and sampling forms in envelope provided.

Figure 3: Card after processing in the laboratory (holes punched)

Materials needed:
Whatman FTA card
Pen/Pencil
Blunt object such as a pliers, marker pen end, small hammer etc.
Zip lock bag to store and return air-dried FTA cards

Appendix 2. Blight scout response form example.

FIGHT AGAINST BLIGHT
Response form - 2020

Please complete and insert with sample

Unique Reference Number from FAB website (used for leaves **and** FTA sample) _____

Postcode where sample found ☐☐☐☐ ☐☐☐ (2nd part optional)

County where sample found:_____

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

Conventional Crop | Volunteer | Outgrade pile (dump) | Garden/Allotment | Other (eg.Trial, Organic Crop) _____

Potato variety _____

Date sample taken _____

Type of infection *(Please circle)*

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

Please describe your sample distribution *(Tick boxes)* * See overleaf

1 lesion from each of 8 plants* ☐ Were your samples: clustered ☐

2 lesions from each of 4 plants* ☐ Scattered through field ☐

Other (please describe) ☐

Any other comments

Your name _____ Your mobile phone number _____

Please send me a replacement sampling kit ☐

For laboratory use only

Sample received by _____

Date _____

Confirmed ☐ **Negative** ☐

IMPORTANT Ensure you are registered on the AHDB website https://blight.ahdb.org.uk/BlightReport

Sampling and Postage Instructions:

Please send us up to 8 lesions per incident (4 fresh leaf lesions **AND** 4 lesions pressed onto an FTA card). *Note: We need live samples for mating type and fungicide sensitivity testing and FTA samples allow us to provide you more rapid feedback.*

Please sample as follows:

Step 1 - Sampling

- Identify an individual blight infected plant.
- Remove an infected leaflet (ideally with a single sporulating lesion) or infected stem piece from each of 8 plants, if available.
- Place **each of four** single leaflet**s** between **the** two pieces of paper towel and into separate plastic sample bags and seal. **NB: please DO NOT add water as this will only encourage rotting of the sample.**
- Press a single lesion from each of the remaining 4 leaflets onto each sample zone of the provided FTA card (labelled E, F, G, H) following the enclosed protocol. Write your name, date and sample postcode on the card.
- Air dry card for minimum of an hour before sealing in plastic bag.

Step 2 - Reporting

- Log onto AHDB FAB page and submit a blight report to generate your unique reference number **https://blight.ahdb.org.uk/BlightReport/Submit** and add the reference number to the form and the FTA card
- *Note If you are unable to generate a number that day or are delayed submitting the report, then please post samples anyway and forward your reference number, when available, to* **fab@hutton.ac.uk**
- Complete the rest of the form overleaf.

Step 3 - Post

- Using the provided pre-paid jiffy bag, post completed forms with the samples, to the **James Hutton Institute, Dundee**.
- Try to ensure that the samples reach the laboratory the next day by posting before the last post, (in some areas this can be as early as 12 noon).
- If the samples are taken on a Friday please store them in your refrigerator and post first thing on Monday

If you are unable to collect lesions in the patterns described above, please just send us what you can.

Thank you for your continued support.

- **Contact:**
- For pack/sampling info: **fab@hutton.ac.uk**
- Any other queries: **https://blight.ahdb.org.uk**