Research Report

Population monitoring and fungicide sensitivity testing of *Phytophthora infestans*

Ref: 11120034

Reporting Period: 2019 and 2020

Report Authors:
David Cooke, Alison Lees The James Hutton Institute, Invergowrie, Dundee, DD2 5DA
Faye Ritchie   RSK ADAS Boxworth, Battlegate Road, Boxworth, CB23 4NN

Contents

1. SUMMARY

Aims

This report presents the outcome of the monitoring of the population structure of *Phytophthora infestans* in GB for the 2019 and 2020 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 north-eastern 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 the pathogen. 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 19 to 17% 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 showed no change in the effectiveness of key fungicide groups.

Data from this study were disseminated to the industry via presentations at AHDB Potatoes events such as AHDB Agronomists' Conference (2019) and the online AHDB Agronomy week (2020) and via press releases and articles in the agricultural press (Crops, Crop Production Magazine, Potato Review, Farmers Weekly, AHDB Grower Gateway etc). Isolates and DNA from isolates were also provided upon request, to the agrochemical industry in support of baseline sensitivity monitoring and other areas of product stewardship.

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 2018-2020 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.

- Both the 2019 and 2020 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. Volunteer and cull pile management is key to reduce 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.

- The use of DNA preserved on FTA cards to complement fresh lesion samples has been successful with industry benefiting from updates on the population within the season to help in decision making. This has been especially important where postal delays have caused the loss of many leaf samples.

- 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

6

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 has not yet been recorded any further west than some crops in Germany (Puidet et al., 2021). 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 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 2020 has been uploaded onto this database and a summary will be presented in this report.

4. EXPERIMENTAL SECTION

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 and 2020. Scouts registered with the AHDB Potatoes FAB campaign thus 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, unlike in previous projects, was sent direct 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 (https://blight.ahdb.org.uk/BlightReport) 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 & 2). Accompanying metadata relating to each sample was also recorded (Appendix 2). Upon receipt in Dundee positive samples were placed 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. 3) 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. 19°C) on a secondary isolation plate, the culture was plated onto a series of media as follows; a pea broth plate to yield mycelium for subsequent DNA extraction, two plates each pre-inoculated with either the A1 or A2 tester strain and finally a Rye A agar screw-cap slope for longer-term storage. 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

9

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. 6), washed with the FTA Purification Reagent (Whatman™ WB120204) according to the manufacturer's instructions and the disk used in the 12-plex PCR. The SSR allele peaks were manually checked and scored prior to export to excel spreadsheets for further analysis. The centroids of each postcode district were converted to latitude and longitude data and the associated outbreak data (cultivar, date and outbreak type) were also entered into the Euroblight database (www.euroblight.net) for further genetic analysis using the R package *poppr* (Kamvar et al., 2015) in addition to more detailed genotype mapping.

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 the first instance, 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 disease samples received through the 2019 Fight Against Blight campaign. Due to the absence of 37_A2 genotypes in isolates sampled at the beginning of the 2019 season, FAB isolates of this genotype were sourced from the 2018 epidemic.

Subsequently, 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 none could be obtained from collaborators.
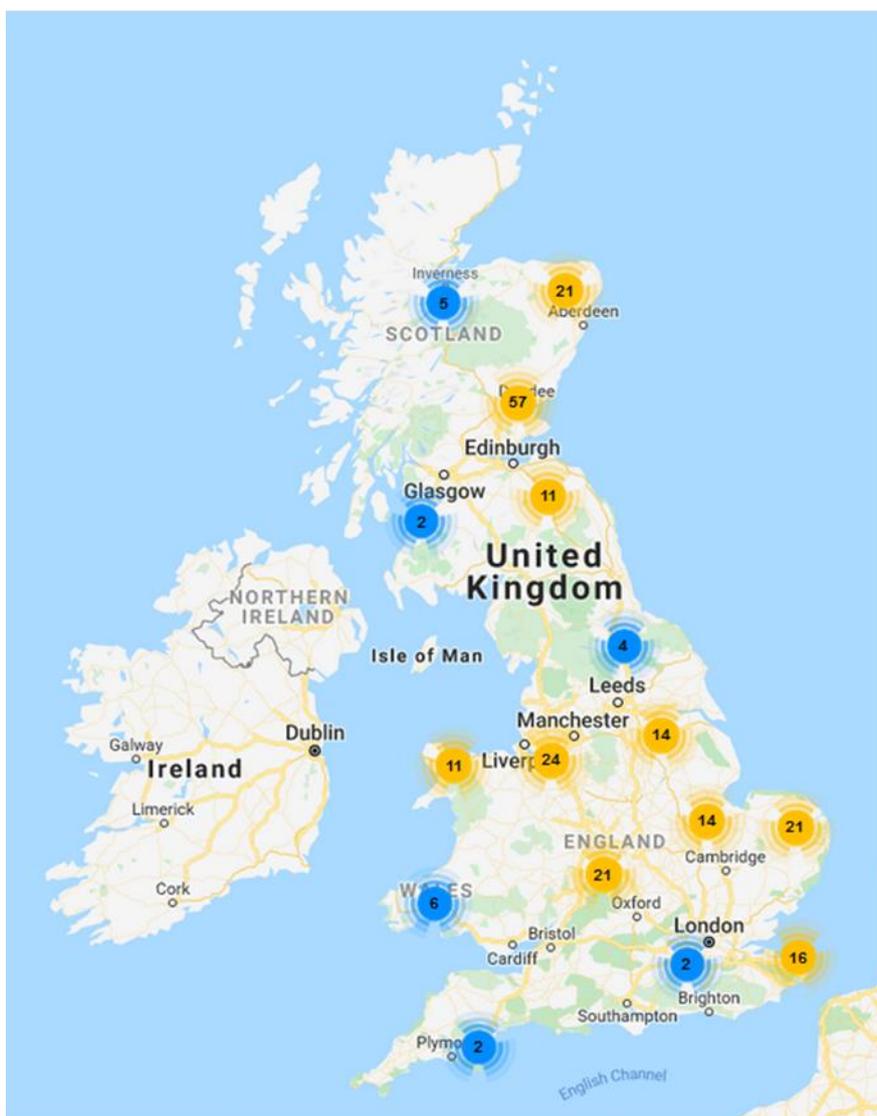
Figure 1. Locations of the late blight outbreaks in 2019 recorded by the FAB campaign. See https://blight.ahdb.org.uk/BlightReport for details.

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).
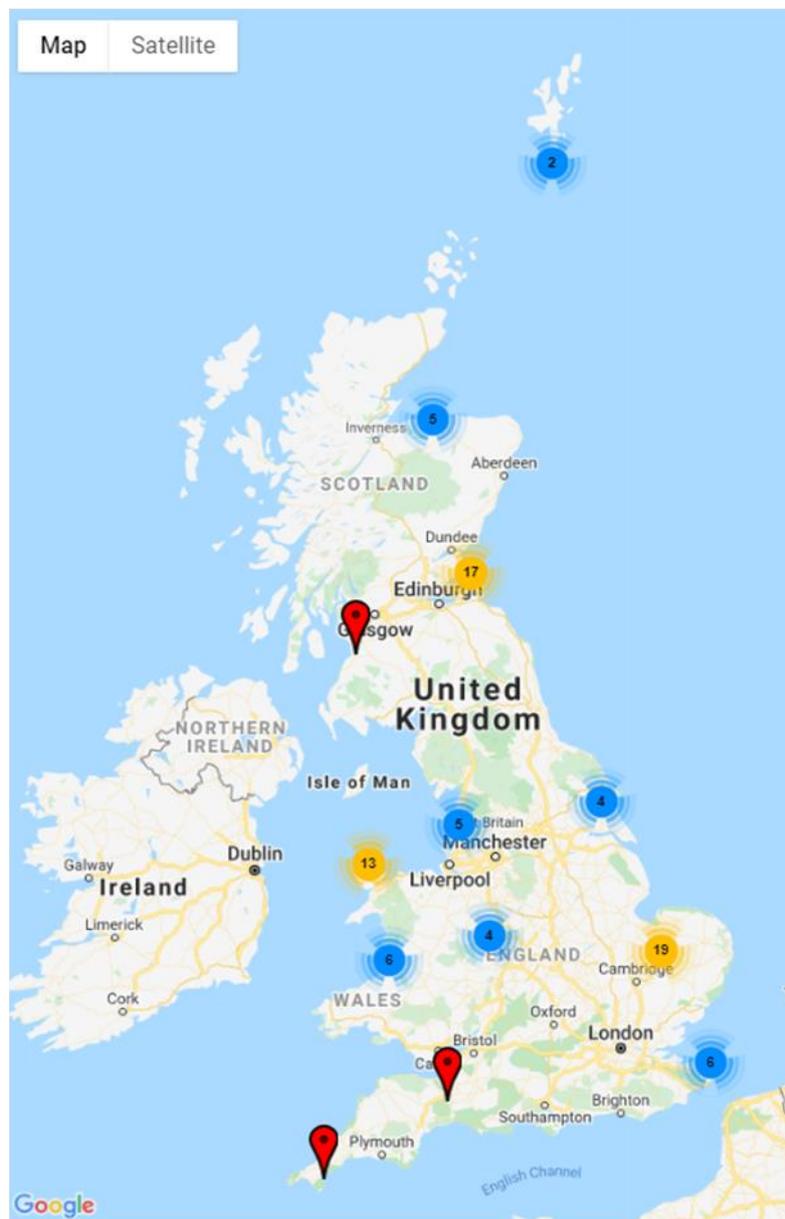
Figure 2. Locations of the late blight outbreaks in 2020 recorded by the FAB campaign. See https://blight.ahdb.org.uk/BlightReport for details.

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.
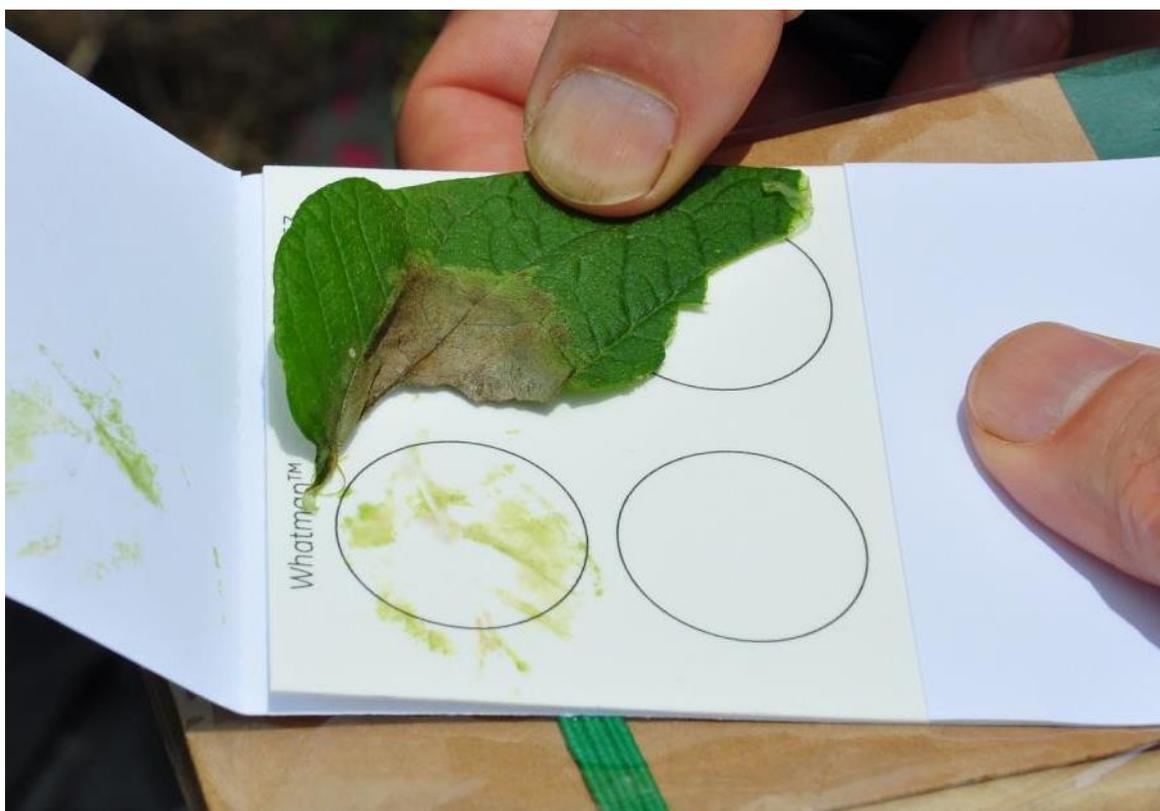
Figure 3. Example of a blight lesion pressed onto an FTA card in the field.

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.

13

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 EC$_{50}$ values in this study. EC$_{50}$ 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).

US Clonal lineage	Mean ± SE EC$_{50}$ (µg ml−1)z 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.

16

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 glasshouse 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

17

samples in the last week of June with a peak of 18 samples in a week (Fig. 4). 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. 6). 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. 5). 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. 6). 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.
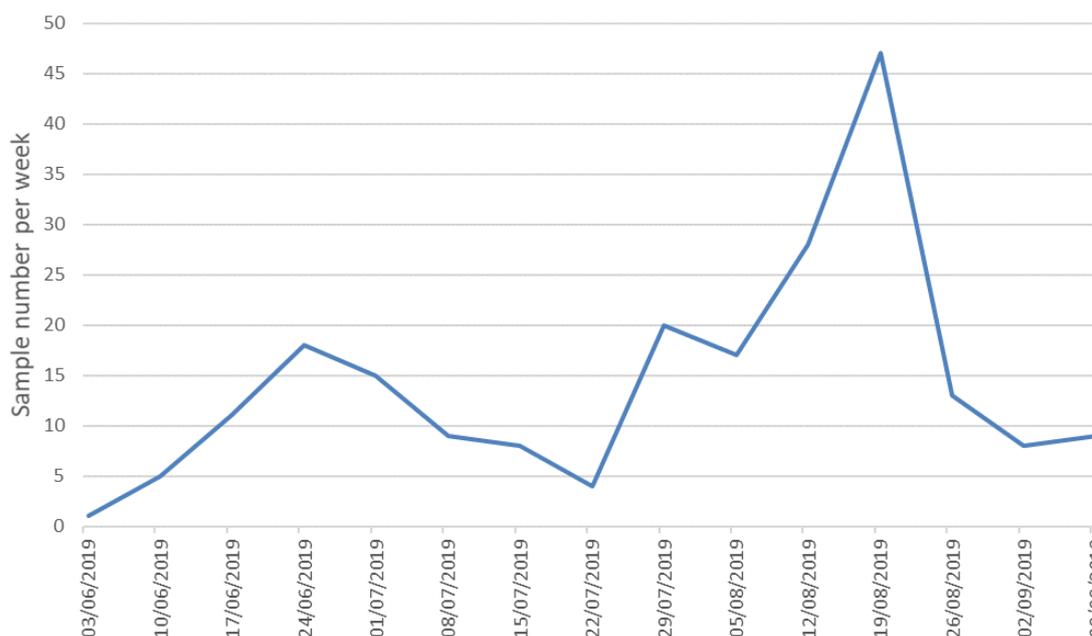
Figure 4. Number of GB late blight outbreaks sampled per week in 2019.
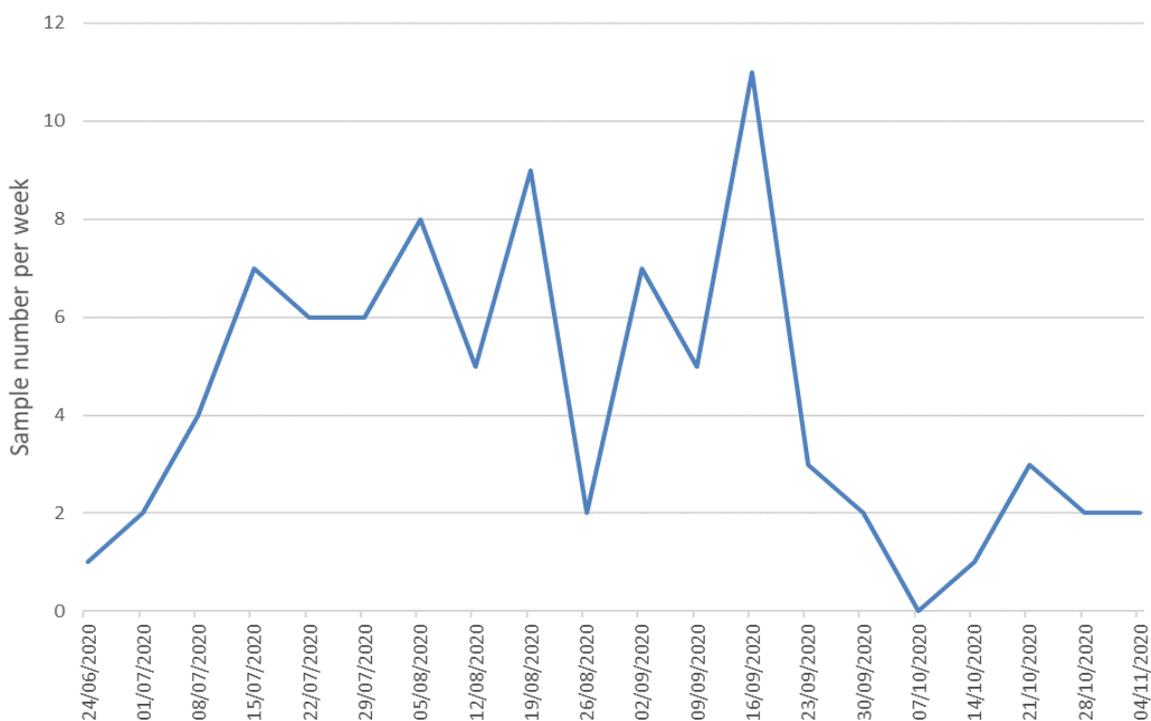
**Figure 5.** Number of GB late blight outbreaks sampled per week in 2020.
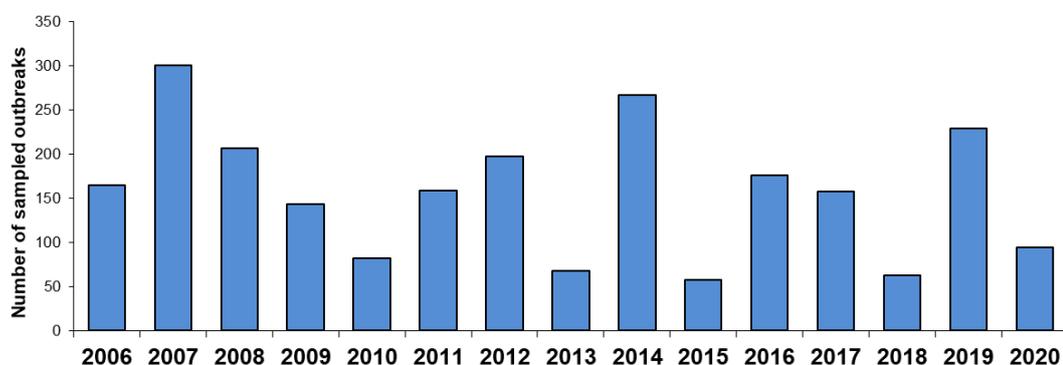
Figure 6. Number of FAB GB late blight outbreaks sampled per year since 2006, providing an approximate guide to late blight disease pressure on a national scale.

Genetic diversity of isolates (2019 to 2020)

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 with an EU_ prefix in the EuroBlight system. An additional 'catch all' category of genotype termed 'Other', was defined for all isolates with novel combinations of alleles found at a very low frequency and commonly in only a single blight outbreak and in a single season. Almost 9500 FAB samples have been genotyped to

19

date; the genotype frequencies and annual total in each of the 18 seasons are presented (Fig. 7).

The genotyping results of almost 1000 samples from 2019 and 432 from 2020 showed that approximately 80% of the samples were of clonal lineages with 6_A1, 37_A2 and 36_A2 dominating. Genotype 6_A1 remained the single most dominant genotype at 36 and 35% of the sampled population over the two most recent seasons. Despite the marked increase in 36_A2 since 2017, it remained stable in 2019 and 2020 at 27 and 29% of the population, respectively. Although genotype 37_A2 increased from 6 to 10% in 2020 this is a marked decline since its high of 24% in 2017. The proportion of 'Other' types was higher than average with 17 and 14% in 2019 and 2020, respectively (Fig. 7). The 13_A2 genotype that had 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 was only sampled in a single outbreak in 2020 and comprised 0.7% of the population. This genotype now appears to be in a continuous decline. An SSR multi-locus genotype (MLG) that was first observed at a low frequency in 2017 but included with the 'Other' category until 2019, has been re-sampled in four consecutive years and was thus formally named as a new genotype, 42_A2, in 2020 (see discussion).
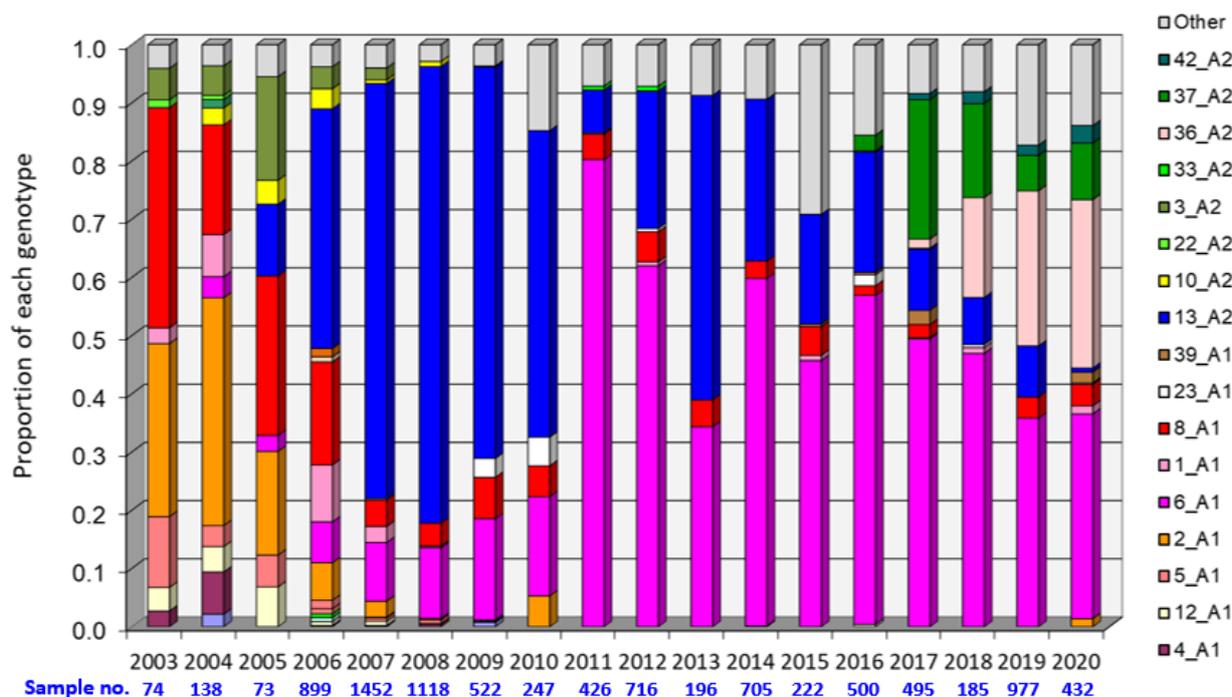
Figure 7. Bar chart indicating the frequency of *P. infestans* isolates of each SSR genotype over the course of 18 seasons (2003-20) and the number of genotyped samples per year.

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. 8). Although 6_A1 predominated in all three countries, its decline in England and replacement by 36_A2 and 37_A2 genotypes is pronounced (Fig. 8). Note that the sampling depth varies from season to season (Figs. 6 & 7) 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 the genotypes 36_A2 and 37_A2 were later to emerge in Wales and Scotland and that the new genotype 42_A2 was sampled predominantly in Wales. The consistently higher frequency of 'Other' genotypes in Scottish crop samples is also evident (Fig. 8).
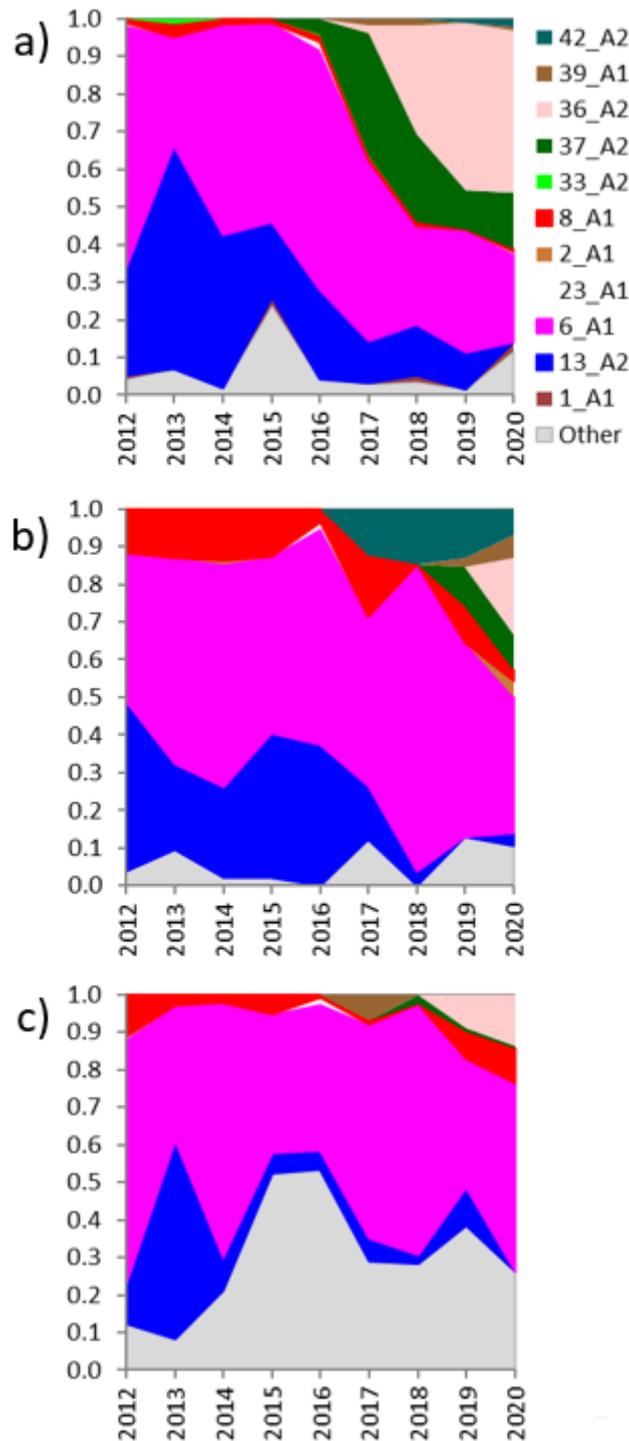
Figure 8. The proportion of different clonal genotypes of *P. infestans* from blight outbreaks sampled over the 2012 to 2020 seasons from a) England (*n*=2579) b) Wales (*n*=548) and c) Scotland (*n*=1353).

Submission of the 2019 and 2020 FAB data to the EuroBlight database allows the outbreaks to be mapped by genotype (Figs. 9 & 10) 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 is shown (Fig. 11). 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. 7) but its range widened into crops in Wales and eastern Scotland (Fig. 11). 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 over 40% of samples from crops in England in 2019 and 2020 (Fig. 8).

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.12). 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.
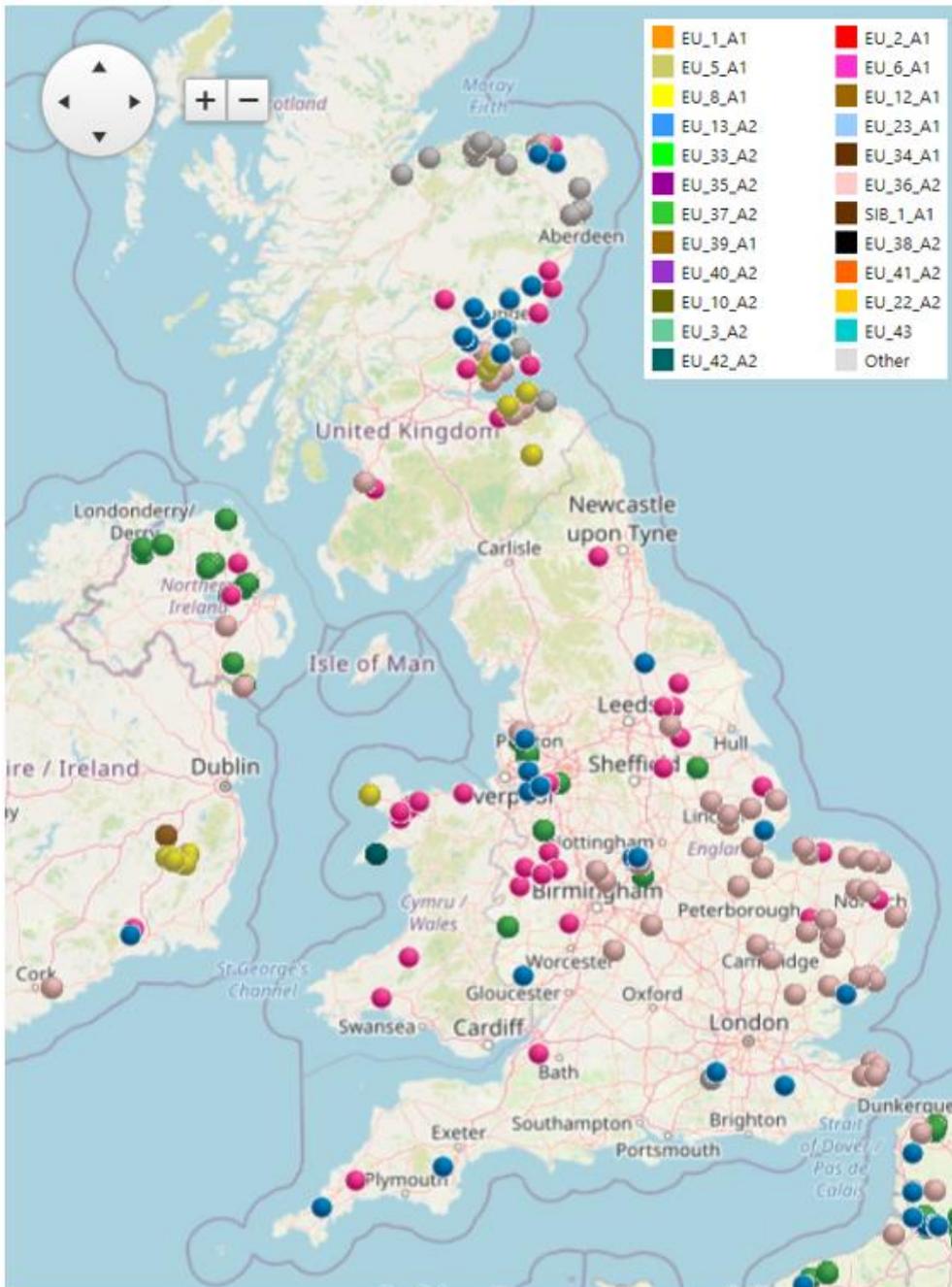
Figure 9. Spatial distribution of all *P. infestans* genotypes collected from 2019 late blight outbreaks submitted to the EuroBlight database (www.euroblight.net).

Figure 10. 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 9.
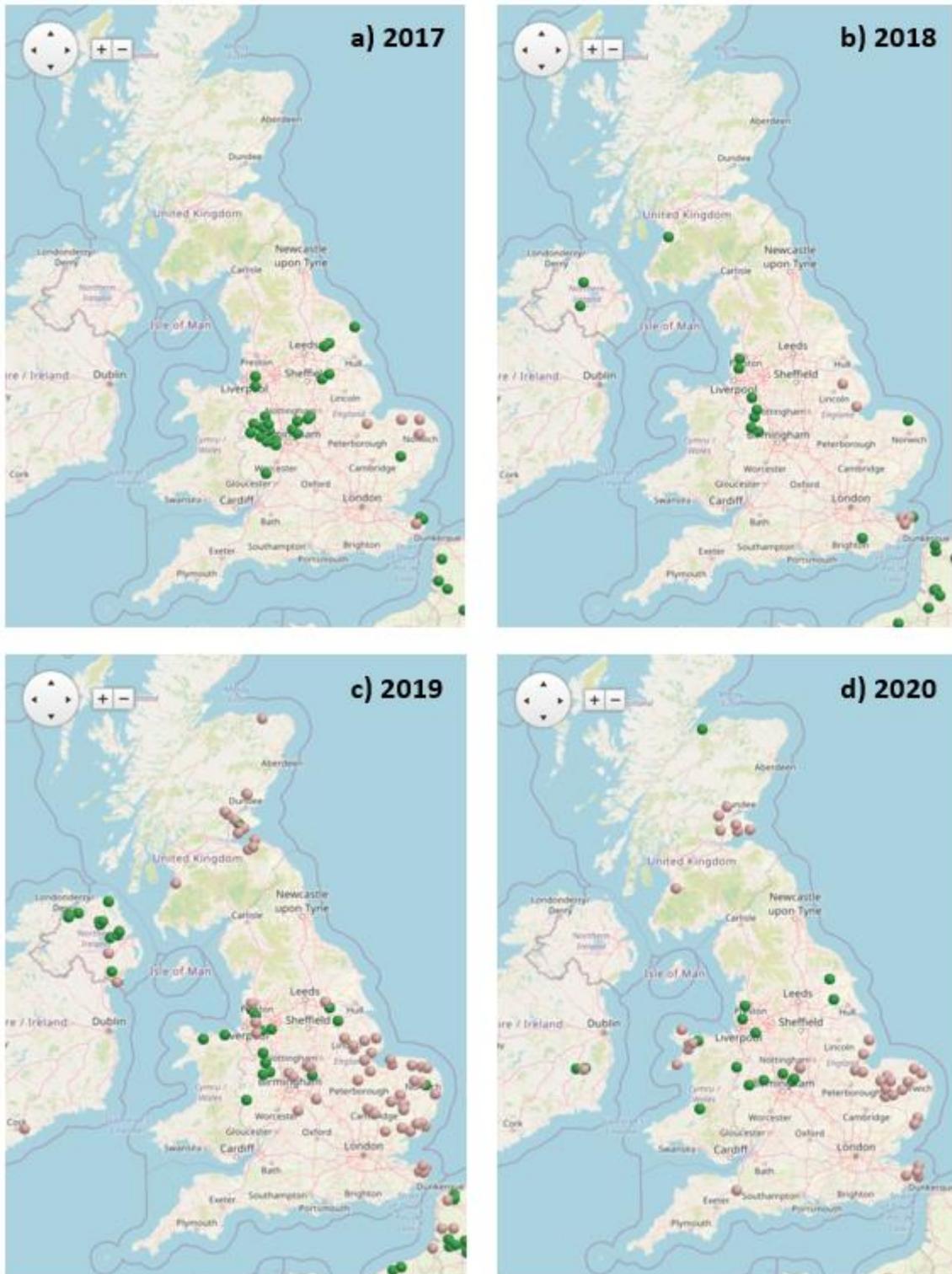
Figure 11. Spatial distribution of *P. infestans* genotype 37_A2 (green) and 36_A2 (pale pink) sampled from a) 2017 b) 2018 c) 2019 and d) 2020 late blight outbreaks in the EuroBlight database (www.euroblight.net).
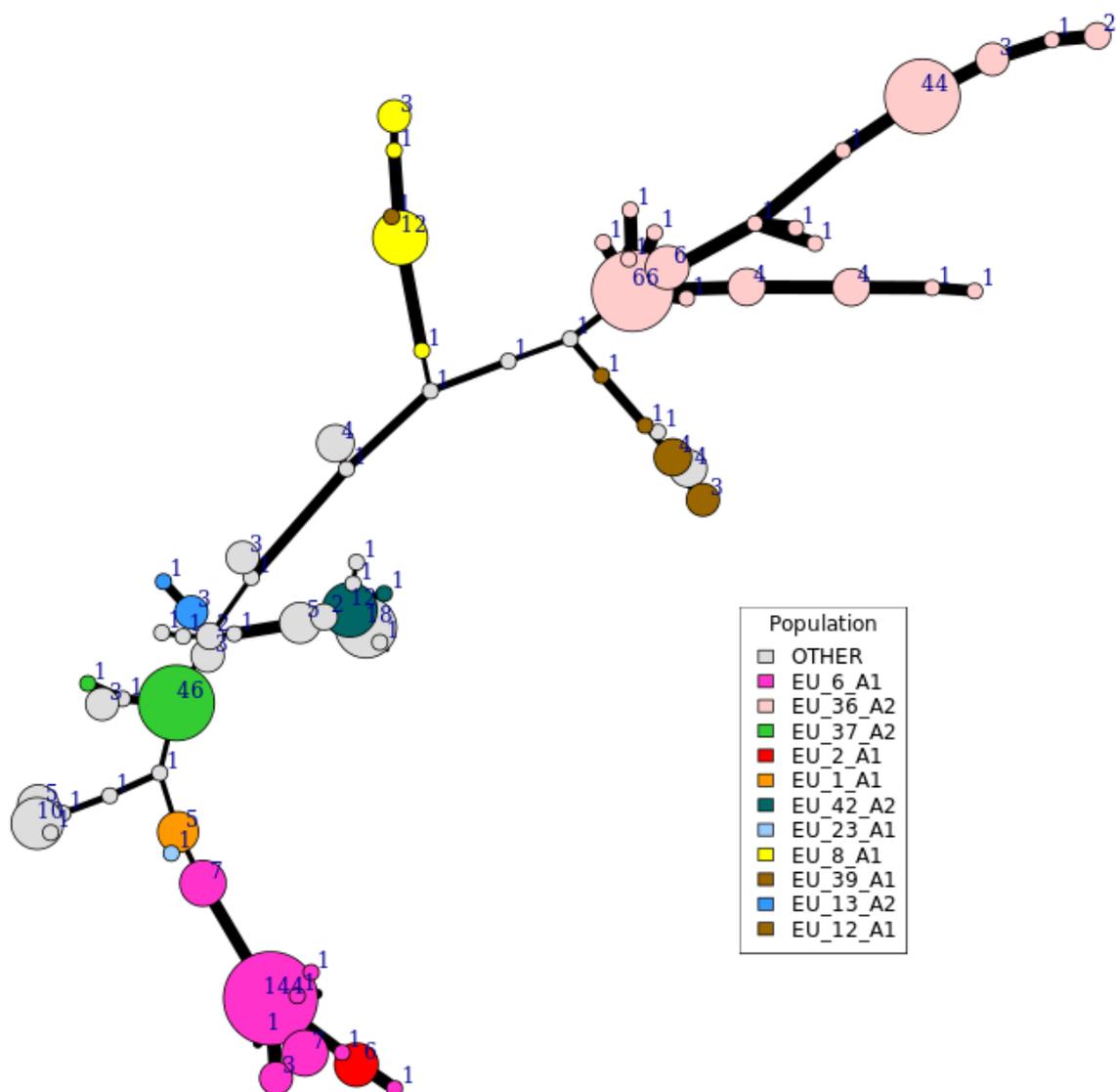
Figure 12. An SSR-based minimum spanning network tree of the 486 genotyped *P. infestans* samples from GB crops in 2020. The data shows the range of diversity within each clone. The figure was generated using *poppr* (Kamvar et al., 2015) via the EuroBlight toolbox.

Fungicide sensitivity testing

General observations

All untreated leaves in all fungicide tests in both 2019 and 2020 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. 13). 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.
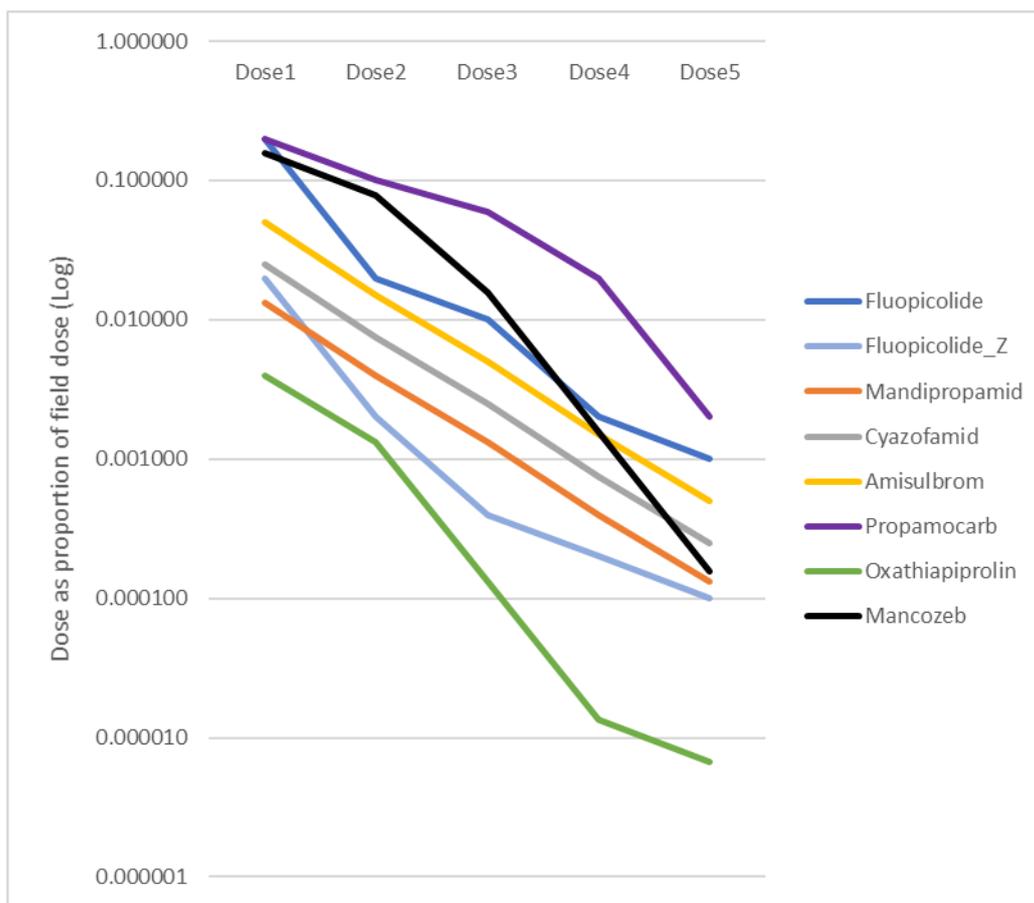
Figure 13. Fungicide dose ranges used to generate sensitivity data against genotypes of *P. infestans* expressed as a proportion of field dose and plotted on a log scale. Fluopicolide_Z is the lower dose range used for the zoospore motility assay.

Fluopicolide 2019

The data for each genotype at different concentrations of fluopicolide shows that at each dose there was a higher incidence of lesions in isolates of 36_A2 than other genotypes (Fig. 14). There was a very low incidence of disease caused by isolates of 37_A2 and 6_A1 at concentrations ≥ 5ppm but a 40% (5ppm) and 17% incidence of lesions caused by 36_A2 at 5 and 10ppm fluopicolide, respectively. No lesions were observed at 100ppm. This indicates that the range of concentrations under test is appropriate. The mean lesion size calculated, for the infected leaves only, decreased with increasing concentration (Fig. 15) and an example of the lesions seen at 10ppm in leaflets infected with isolates of 36_A2 is shown (Fig. 16). 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 (Fig. 17).

EC_{50} values are given in Table 5. There was a statistically significant difference in mean EC_{50} value between genotypes with isolates of 36_A2 showing, on average, a greater EC_{50} value than the other genotypes. 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).
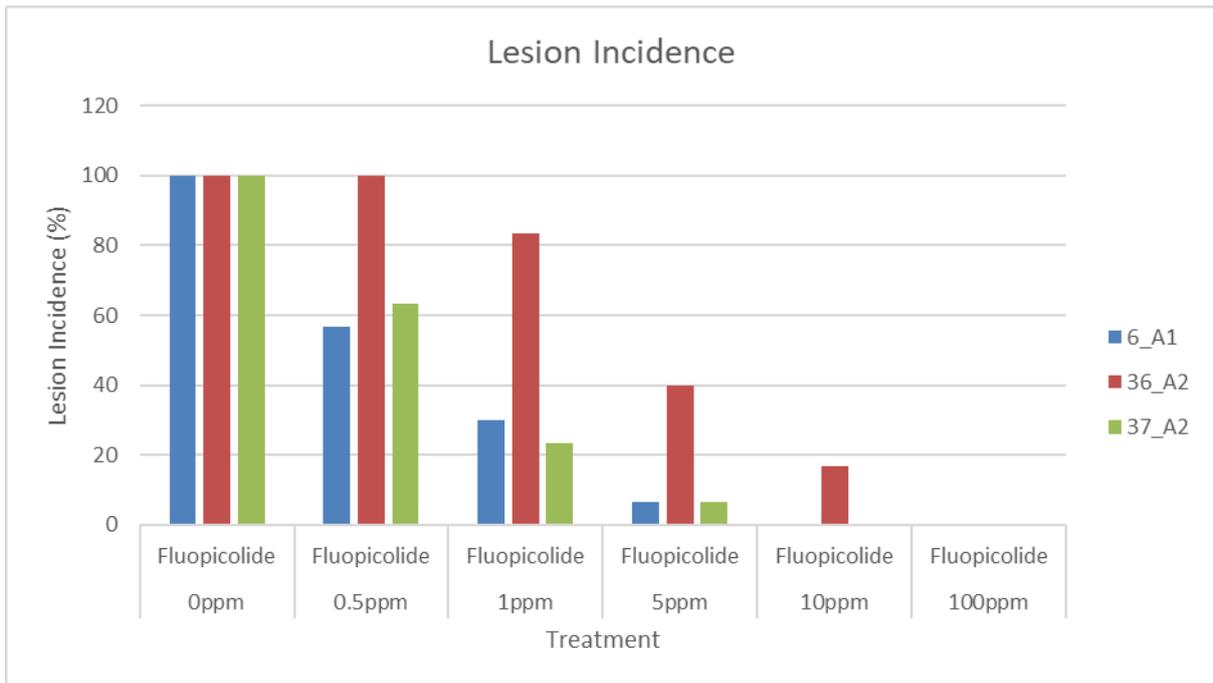
Figure 14. Incidence of lesions (%) caused by each genotype observed at different concentrations of fluopicolide in the 2019 testing.
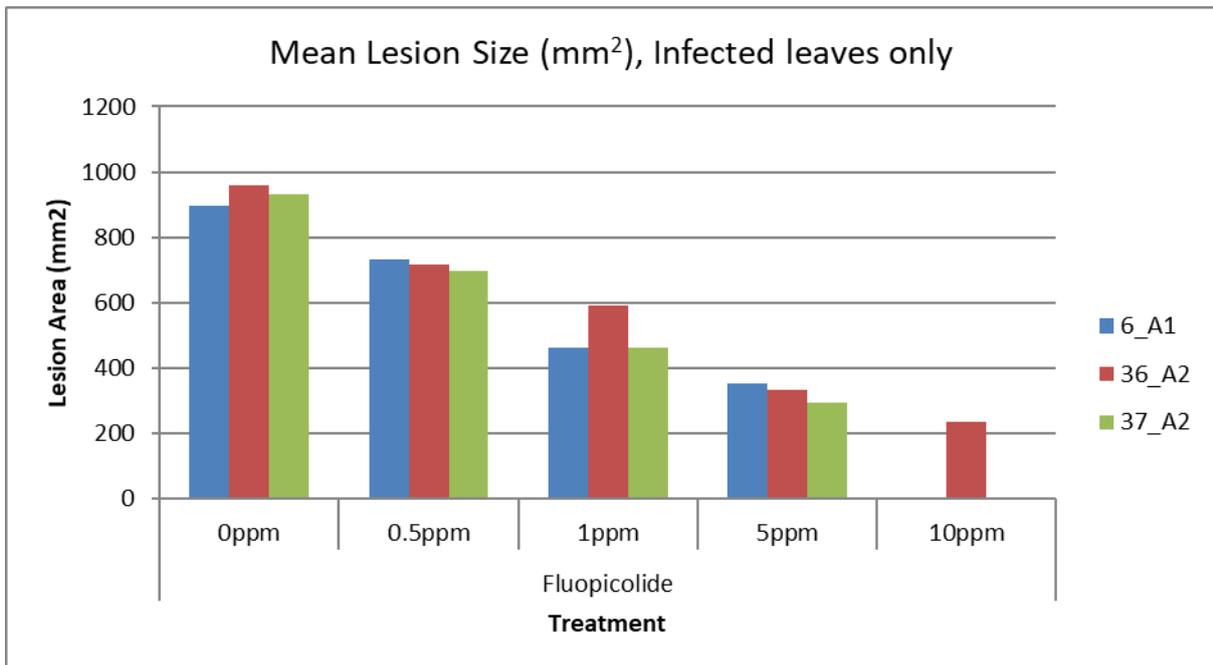

Figure 15. Mean lesion size (mm^2) of genotypes (n= 5 isolates) at different concentrations of fluopicolide (mean of infected leaves only) in the 2019 testing.
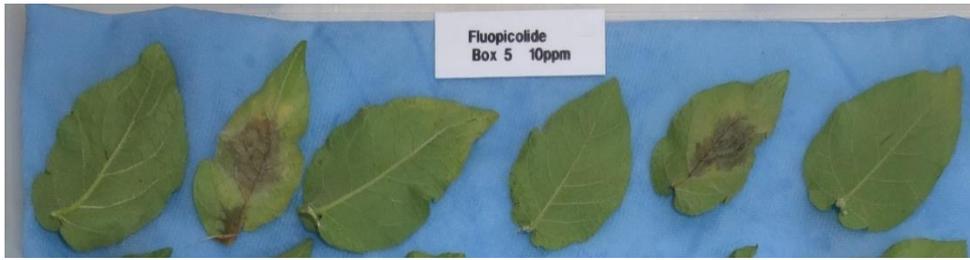
Figure 16. Potato leaflets showing late blight disease symptoms caused by isolates of 36_A2 at 10µg/ml fluopicolide.
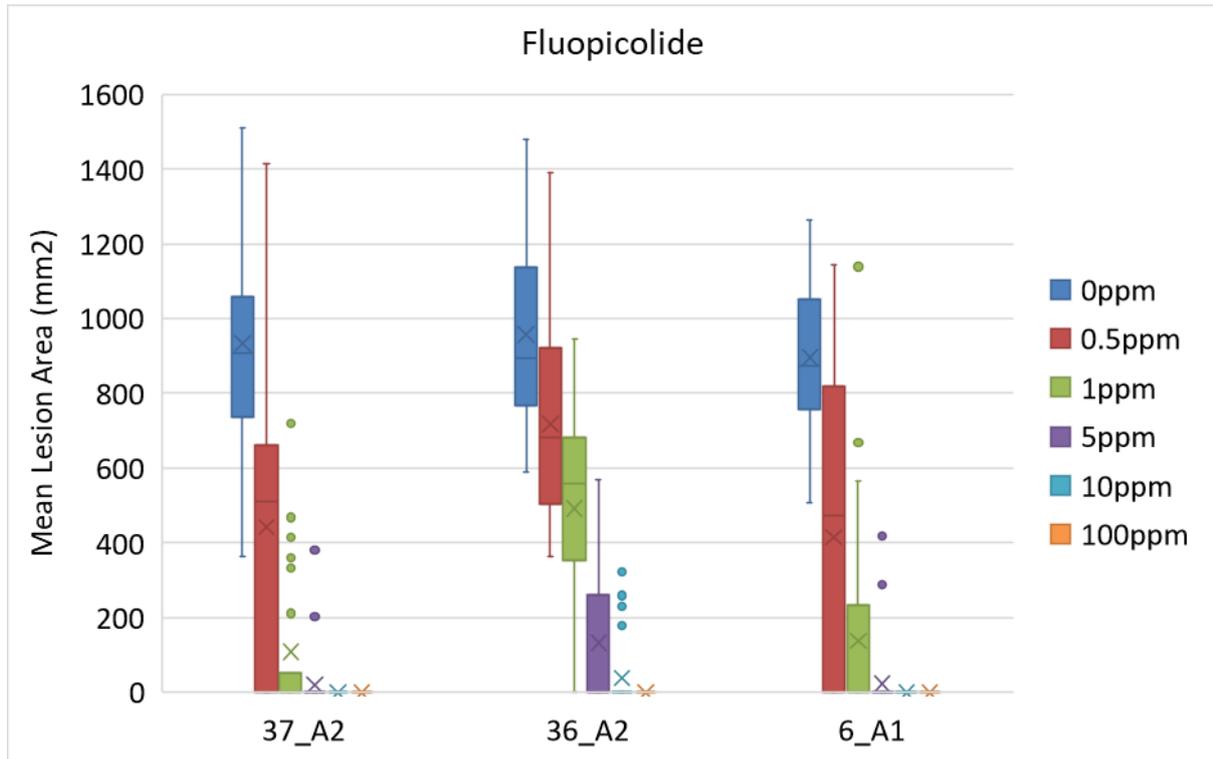

Figure 17. Mean Lesion area (mm^2) of isolates tested in 2019 belonging to 36_A2 and 37_A2 compared with control isolates (6_A1) presented as a box and whisker plot. The maximum field concentration for Fluopicolide (as Infinito) is 500ppm.

The MIC values in this zoospore motility test were also in line with previous baseline sensitivity testing of fluopicolide (Table 6). The mean MIC values for genotype 36_A2 were statistically higher than for other genotypes in 2019 but were still within the expected range.

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. 2018 results are shown in brackets.

Genotype	6_A1	37_A2	36_A2
Number of isolates	5	5	5
Mean EC$_{50}$ (mg/L)	**0.623a** (0.53a)	**0.591a** (1.40a)	**1.561b** (2.57b)
EC$_{50}$ min (mg/L)	0.25 (0.33)	0.25 (0.33)	0.25 (0.33)
EC$_{50}$ max (mg/L)	2.99 (1.49)	2.45 (5.46)	16.40 (24.47)

Table 6. Minimum inhibitory concentration (MIC) of fluopicolide on the motility of zoospores of isolates of *P. infestans* of different clonal lineages tested in 2019. 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. 2018 results are given in brackets but can only be compared with other values in brackets from the same year.

		MIC value (µg/ml)	
Clonal lineage	**Number of Isolates tested**	**Incubation time 1 h**	**Incubation time 2 h**
EU_6_A1	5	**0.1a** (0.075ab)	**0.065a** (0.075b)
EU_36_A2	5	**0.76b** (0.107b)	**0.13b** (0.075b)
EU_37_A2	5	**0.075a** (0.068a)	**0.055a** (0.05a)

Fluopicolide 2020
The results of 2020 testing were broadly similar to that in 2019 with infection and lesion growth inhibited above 5ppm. Unlike the 2019 testing, the 2020 36_A2 isolates did not result in mean incidences or lesion sizes markedly larger than isolates of the other genotypes (Figs. 18, 19 & 20). There were no statistically significant differences in EC$_{50}$ values amongst the genotypes (Table 7) however the higher mean MIC for zoospore activity of 36_A2 isolates after one and two hours was statistically significant (Table 8). Nonetheless, such differences were very small when compared to the field dose of this product.
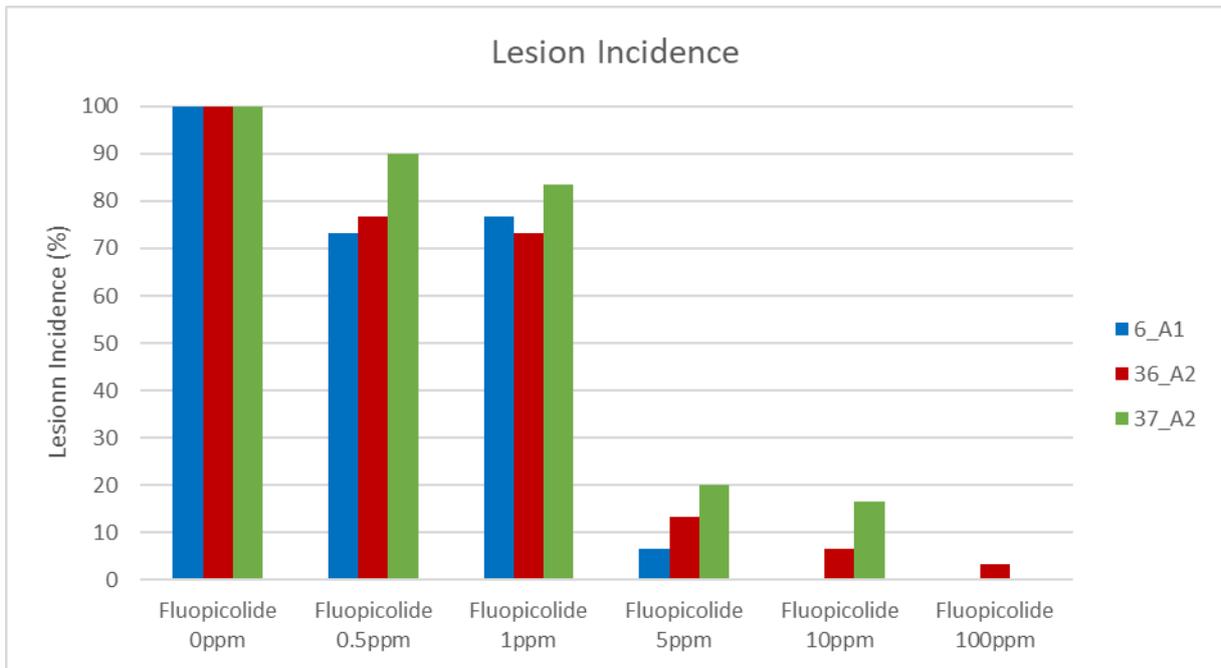
Figure 18. Mean incidence of lesions (%) caused by each genotype observed at different concentrations of fluopicolide in the 2020 testing.
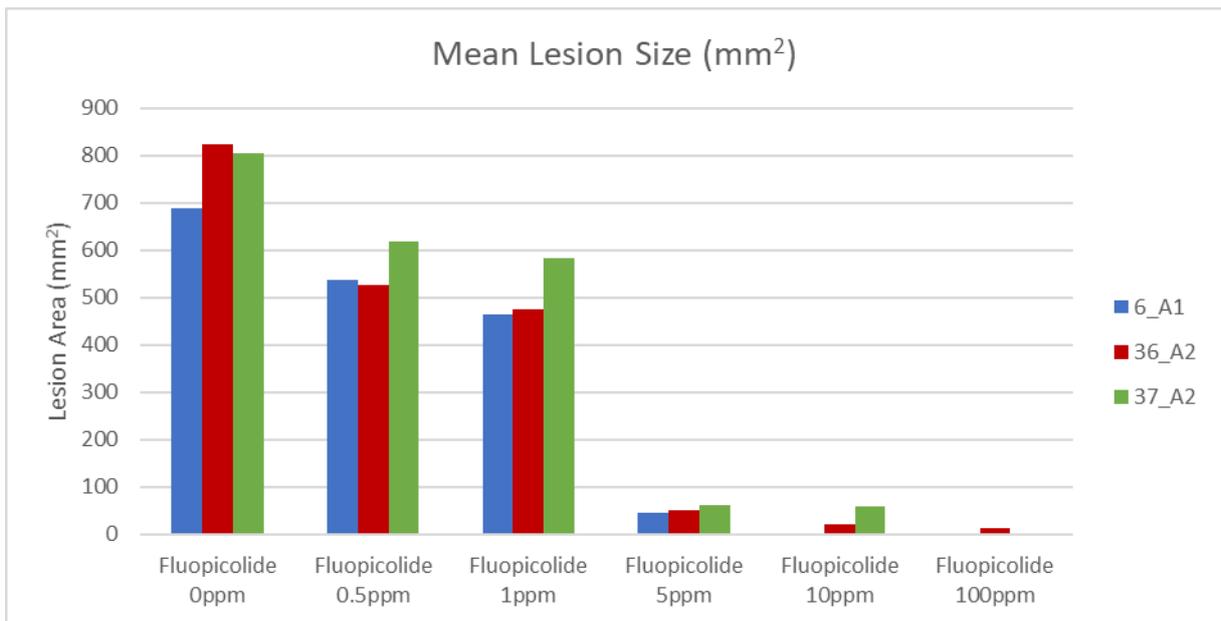

Figure 19. Mean lesion size (mm^2) of genotypes (n= 5 isolates) at different concentrations of fluopicolide (mean of infected leaves only) in the 2020 testing.
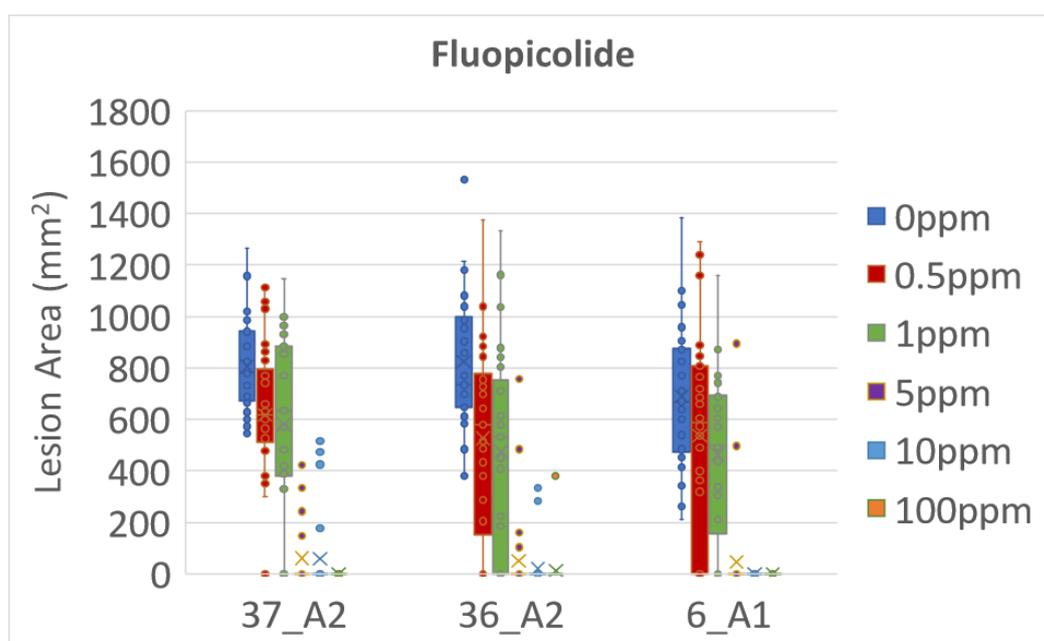
Figure 20. Mean Lesion area (mm^2) of isolates tested in 2020 belonging to 36_A2 and 37_A2 compared with control isolates (6_A1) presented as a box and whisker plot. The maximum field concentration for Fluopicolide (as Infinito) is 500ppm.

Table 7. Mean, maximum and minimum EC50 values for isolates of *P. infestans* of various genotypes tested at a range of concentrations of fluopicolide in 2020. 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)	**1.677a**	**1.81a**	**1.585a**

Table 8. Minimum inhibitory concentration (MIC) of fluopicolide on the motility of zoospores of isolates of *P. infestans* of different clonal lineages tested in 2020. 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. 2019 results are given in brackets but can only be compared with other values in brackets from the same year.

		MIC value (µg/ml)	
Clonal lineage	**Number of Isolates tested**	**Incubation time 1 h**	**Incubation time 2 h**
EU_6_A1	5	**0.125a** (0.100a)	**0.075a** (0.065a)
EU_36_A2	5	**2.72b** (0.76b)	**0.43b** (0.13b)
EU_37_A2	5	**0.100a** (0.075a)	**0.065a** (0.055a)

Mandipropamid 2019

The mean lesion incidence data 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

© Agriculture and Horticulture Development Board 2021

concentrations up to 0.3 ppm of mandipropamid with a lower incidence at 1-10 ppm. The lesion incidence caused by genotype 37_A2 was greater than that of other lineages at four of the five doses (Fig. 21). The mean lesion size, on infected leaves only, is shown in Fig 22. 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 (Fig. 23).
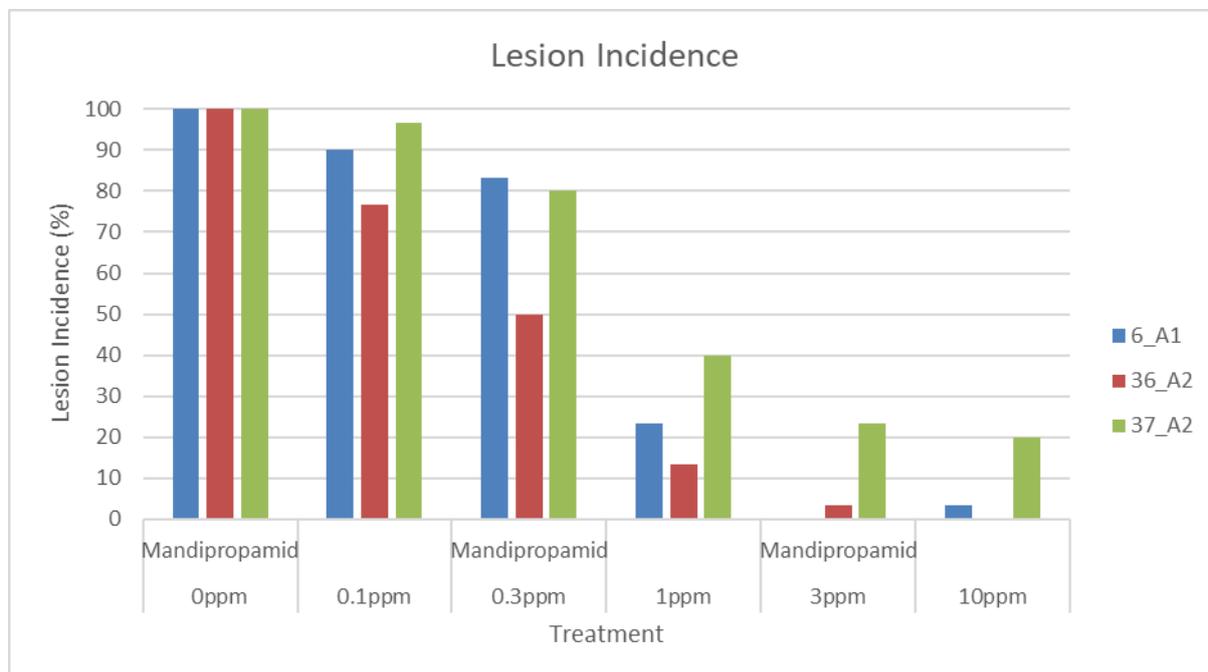
Figure 21. Mean incidence of lesions caused by different *P. infestans* genotypes at a range of concentrations of mandipropamid (0-10 µg/ml) in the 2019 testing.
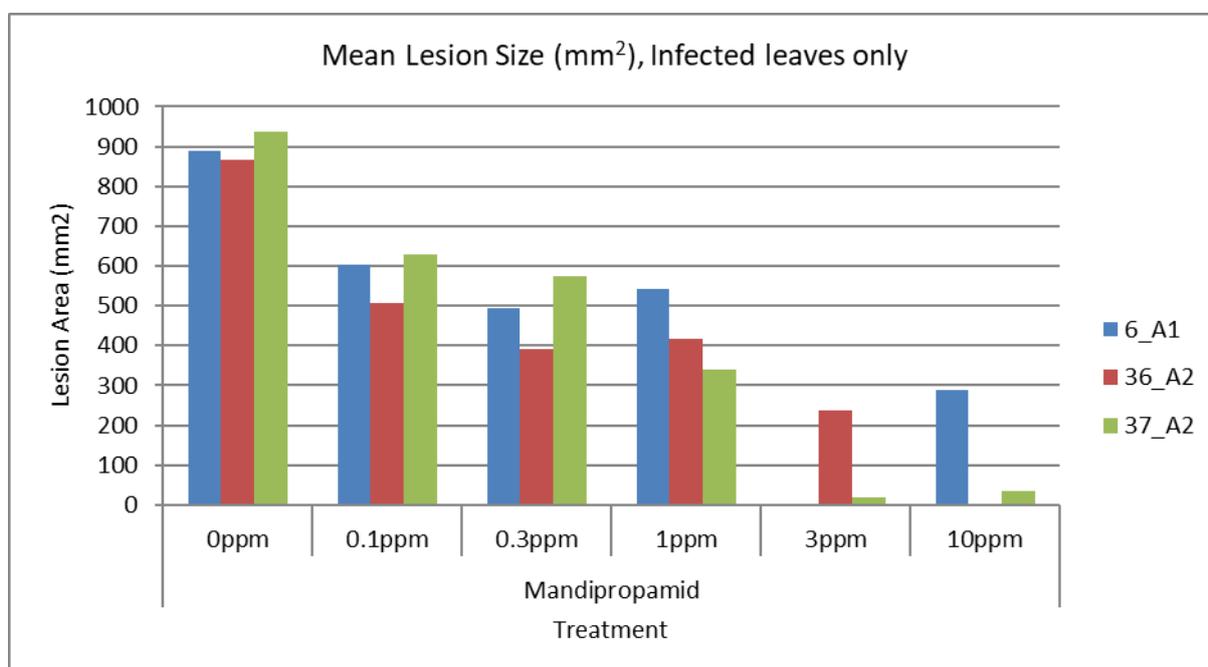

Figure 22. Mean lesion size (mm^2) on infected leaves only after treatment with a range of concentrations of mandipropamid in the 2019 testing. The field rate of mandipropamid is 750ppm.
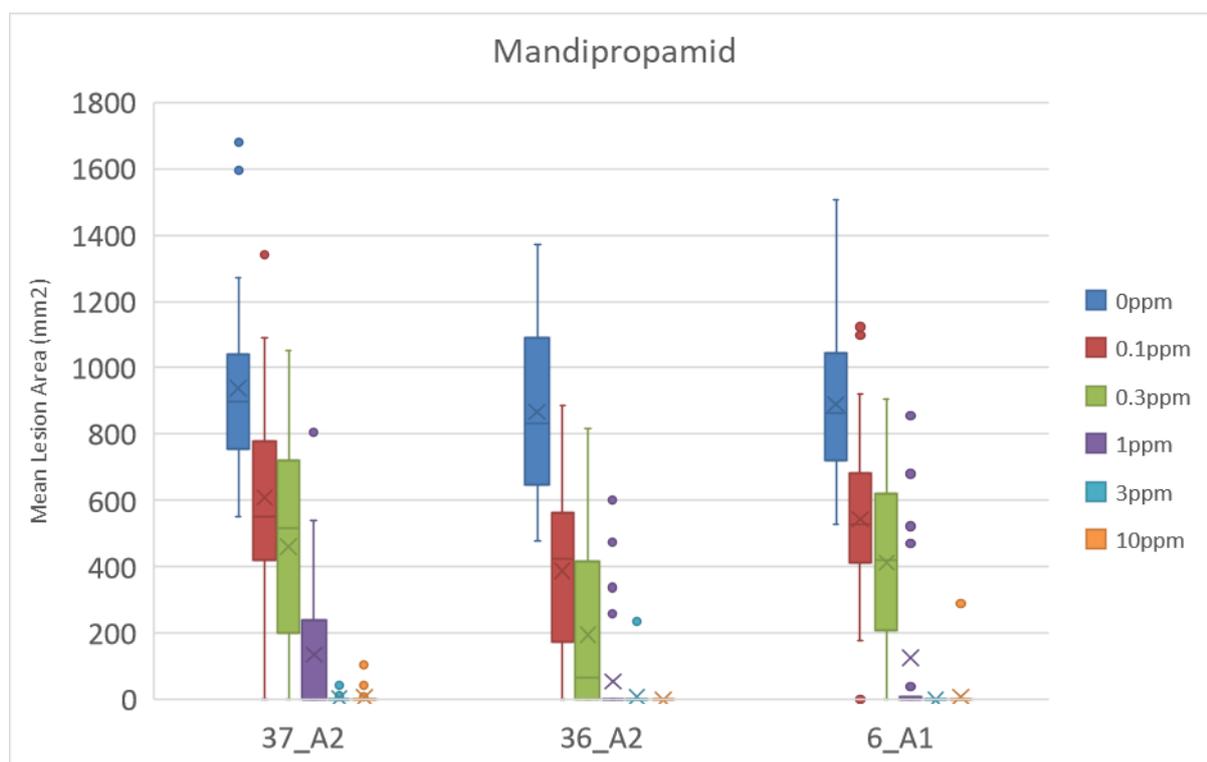
Figure 23. Mean Lesion area (mm2) of isolates tested in 2019 belonging to 36_A2 and 37_A2 compared with control isolates (6_A1) presented as a box and whisker plot. Mandipropamid maximum field concentration = 750ppm

The EC$_{50}$ values in this test were in line with previous sensitivity testing of mandipropamid (Table 9). Mean EC$_{50}$ values for genotype 37_A2 were statistically higher than for 36_A2 but neither of these genotypes was statistically different from the control, 6_A1.

Table 9. 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 are indicated by different letters. 2018 results are given in brackets for comparison but can only be compared with other numbers in brackets.

Genotype	6_A1	37_A2	36_A2
Number of isolates	5	5	5
Mean EC$_{50}$ (mg/L)	**0.444ab** (0.74a)	**0.559b** (0.54a)	**0.289a** (1.26b)
EC$_{50}$ min (mg/L)	0.16 (0.16)	0.16 (0.16)	0.15 (0.27)
EC$_{50}$ max (mg/L)	1.64 (4.94)	1.82 (2.99)	4.47 (5.46)

Mandipropamid 2020
The testing of samples from the 2020 season showed a slightly higher activity of the product than in 2019 with no lesions formed at the 3ppm dose. Mean disease incidence and lesion size was again slightly higher on leaflets inoculated with 37_A2 and was most pronounced at 0.3ppm

(Fig 24, 25 & 26). This was also reflected in a statistically significant increase in the EC_{50} of isolates of 37_A2 compared to other genotypes (Table 10). However, the between genotype differences remained very small in comparison to the 750 ppm field rate of mandipropamid.
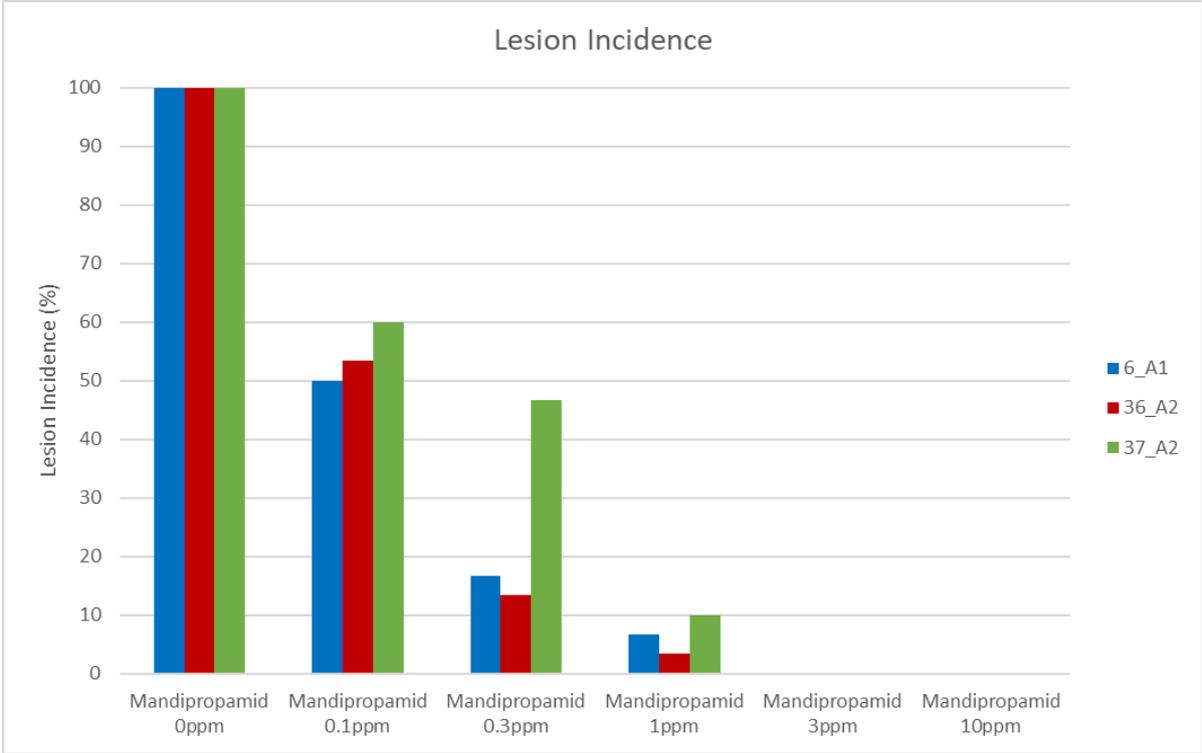

Figure 24. Mean incidence of lesions caused by different *P. infestans* genotypes at a range of concentrations of mandipropamid (0-10 µg/ml) in the 2020 testing.
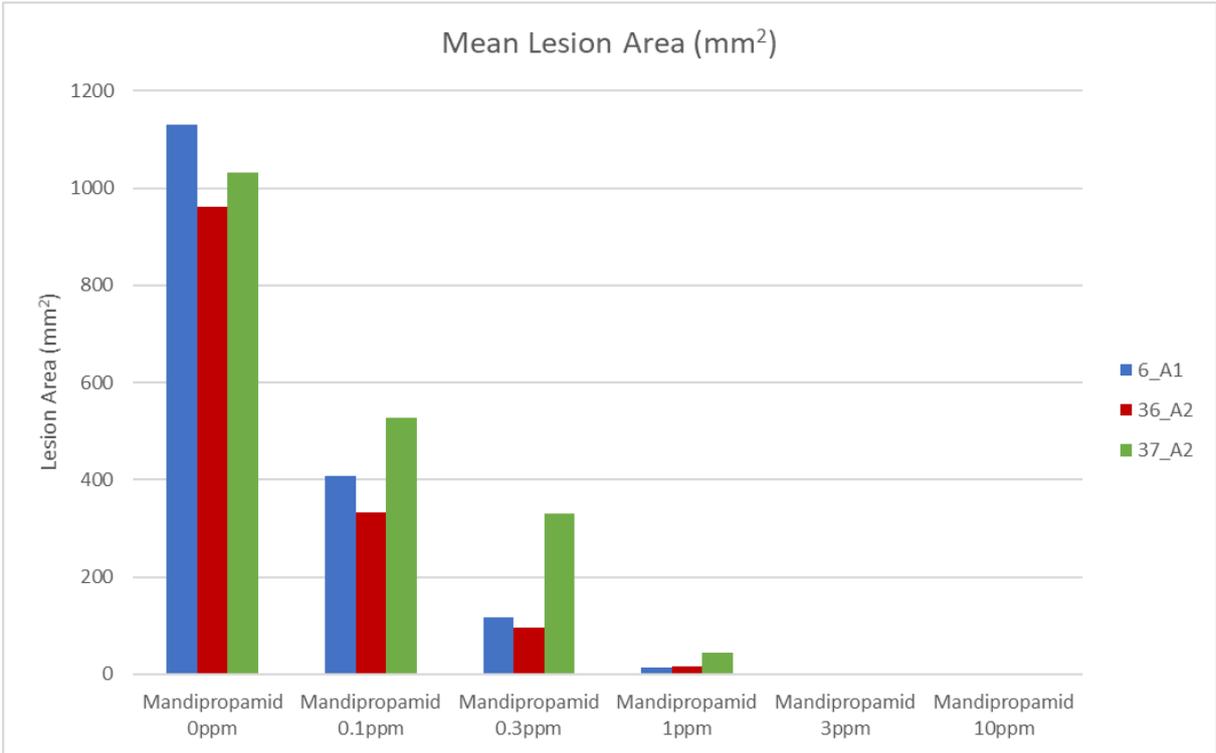
Figure 25. Mean lesion size (mm2) on infected leaves only after treatment with a range of concentrations of mandipropamid in the 2020 testing. The field rate of mandipropamid is 750ppm.
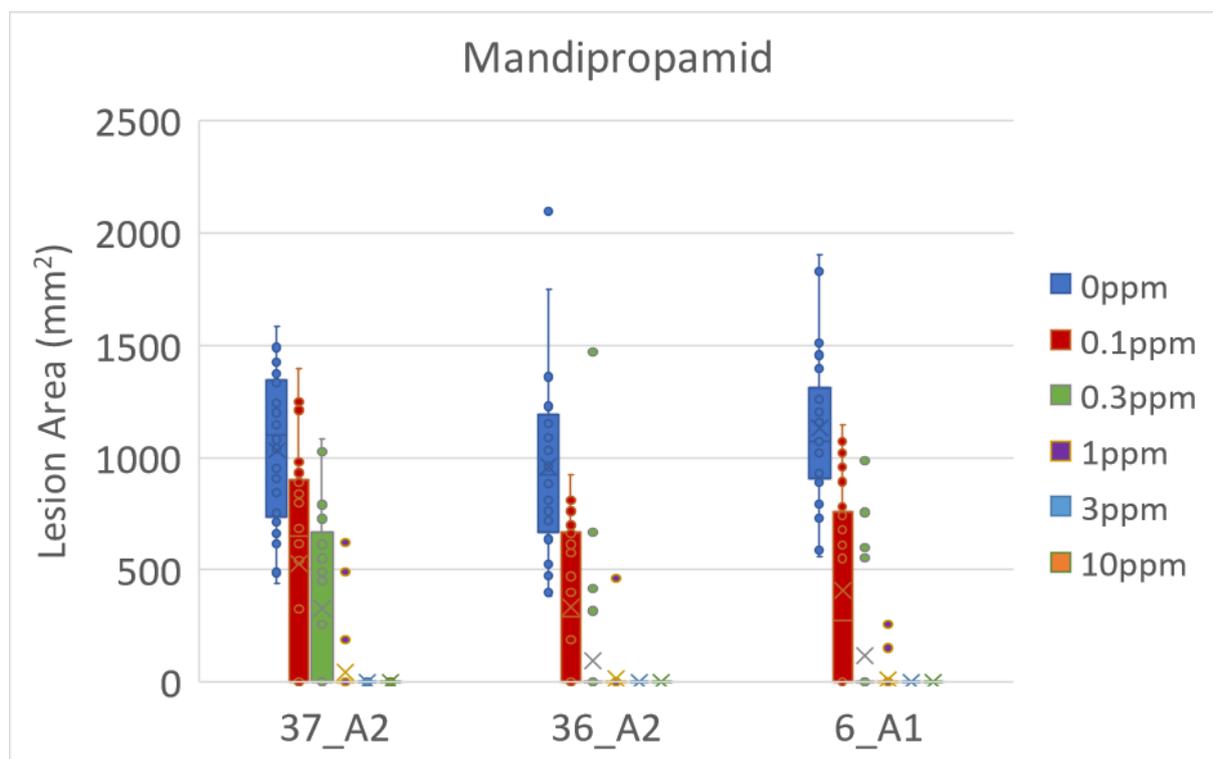

Figure 26. Mean Lesion area (mm2) of isolates tested in 2020 belonging to 36_A2 and 37_A2 compared with control isolates (6_A1) presented as a box and whisker plot. Mandipropamid maximum field concentration = 750ppm

Table 10. Mean EC50 values for isolates of *P. infestans* of various genotypes tested in 2020 at a range of concentrations of mandipropamid (0, 0.1, 0.3, 1.0, 3.0, 10.0µg/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)	0.2257a	0.4122b	0.2516a

Cyazofamid 2019

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 (Fig. 27). A single lesion caused by one isolate of 6_A1 at 10µg/ml cyazofamid (Fig. 28) influenced the mean values (Figs. 27, 28 & 29) but was likely due to experimental error as it was not replicated at lower doses of the same product. The range of concentrations under test (0-10µg/ml) 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 (Fig. 30)

EC_{50} values calculated from test data show there was no statistically significant difference in mean EC_{50} value between genotypes (Table 11). 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. The concentrations of cyazofamid required to control all isolates in this assay are very low when compared with permitted field rates.
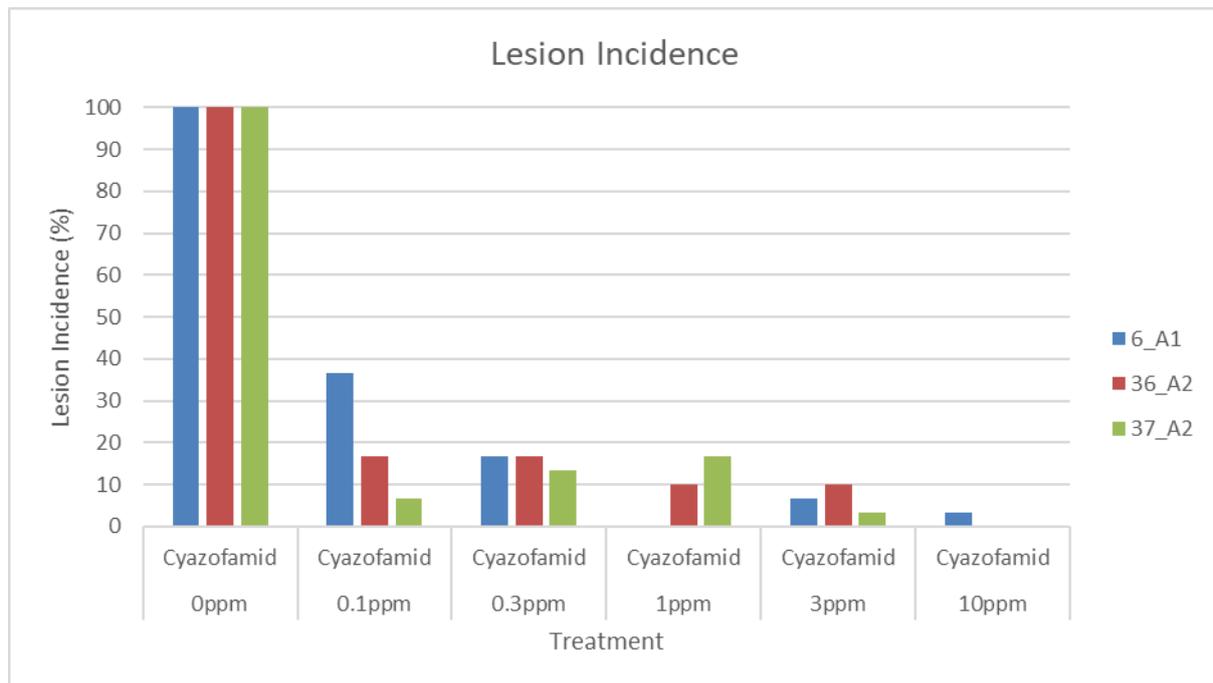

Figure 27. Mean percentage of lesions caused by different genotypes at a range of concentrations of cyazofamid tested in 2019 (0-10 μg/ml).

**Figure 28.** A single lesion caused by an isolate of 6_A1 at 10ppm cyazofamid
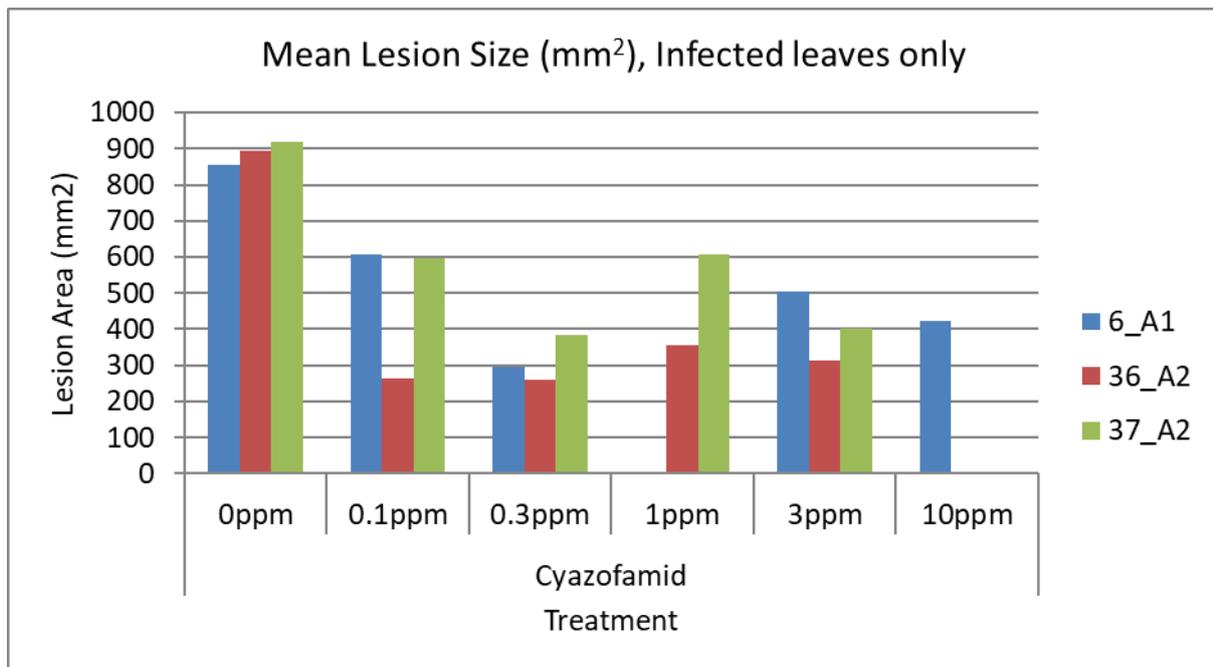
Figure 29. Mean lesion size (mm^2) measured, on infected leaves only, at a range of concentrations of cyazofamid tested in 2019.
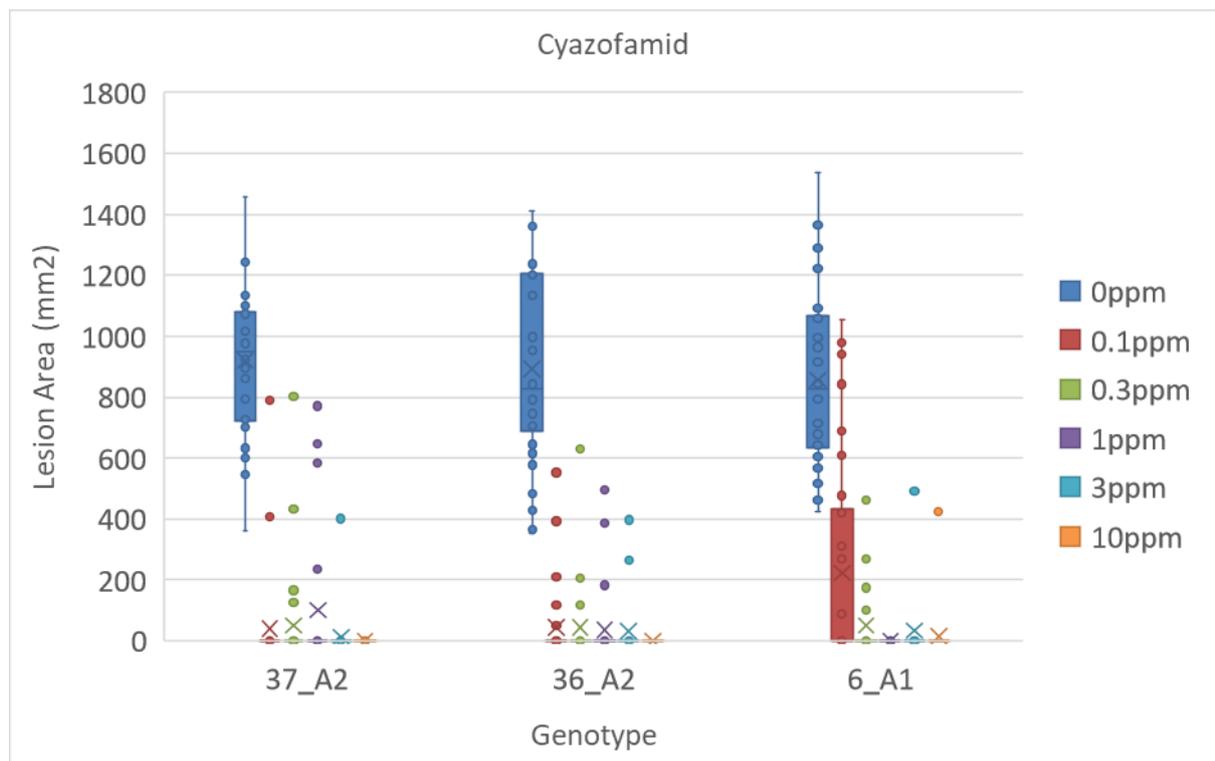

Figure 30. Mean Lesion area (mm2) of isolates belonging to 36_A2 and 37_A2 compared with control isolates (6_A1) tested in 2019 and presented as a box and whisker plot. Cyazofamid Max Field concentration = 400ppm.

Table 11. Mean, maximum and minimum EC$_{50}$ values for isolates of *P. infestans* of various genotypes tested in 2019 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. 2018 values are given in brackets for comparison but can only be compared with other values in brackets

Genotype	6_A1	37_A2	36_A2
Number of isolates	5	5	5
Mean EC$_{50}$ (mg/L)	**0.27a** (0.18a)	**0.25a** (0.19a)	**0.22a** (0.22b)
EC$_{50}$ min (mg/L)	0.15 (0.15)	0.15 (0.15)	0.15 (0.15)
EC$_{50}$ max (mg/L)	2.21 (0.30)	1.49 (0.30)	1.48 (0.55)

Cyazofamid 2020

Testing of samples collected in 2020 indicated a slightly lower activity of the product than the testing in 2019 with a mean of between 50-70 percent of isolates forming lesions on leaflets treated with 0.3 ppm which was consistent with greater lesion numbers at the lower dose of 0.1ppm. This effect was however not related to the isolate genotype (Figs. 31, 32 & 33). The calculated EC$_{50}$ values against the isolates tested in 2020 were similar to 2019 and no significant differences were observed between the genotypes (Table 12).
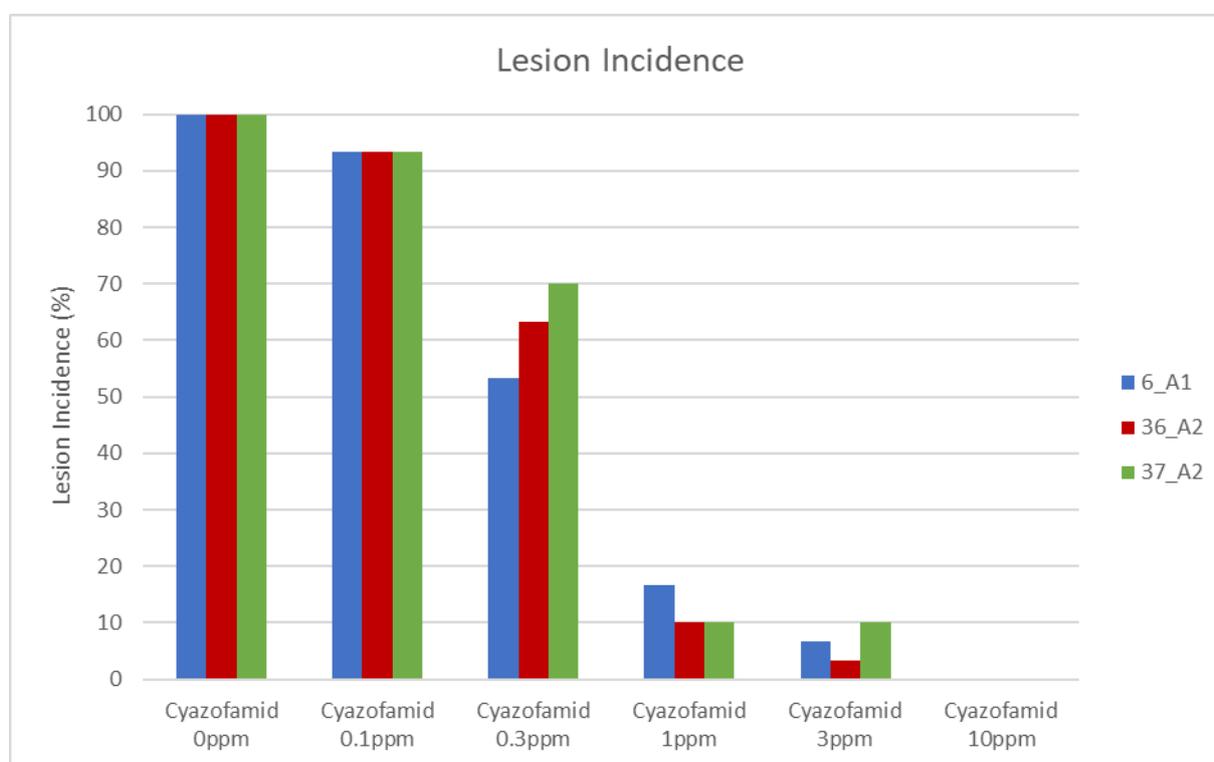

Figure 31. Mean percentage of lesions caused by different genotypes at a range of concentrations of cyazofamid tested in 2020 (0-10 μg/ml).
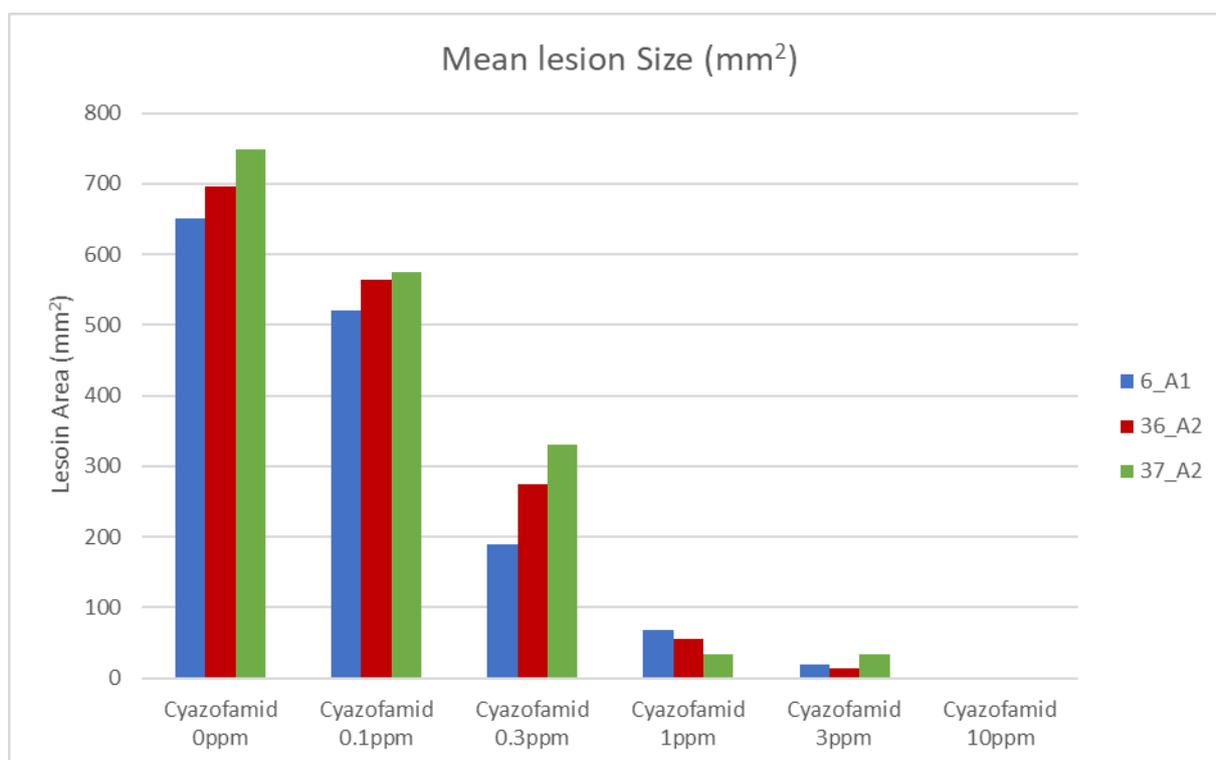
Figure 32. Mean lesion size (mm^2) measured, on infected leaves only, at a range of concentrations of cyazofamid tested in 2020.
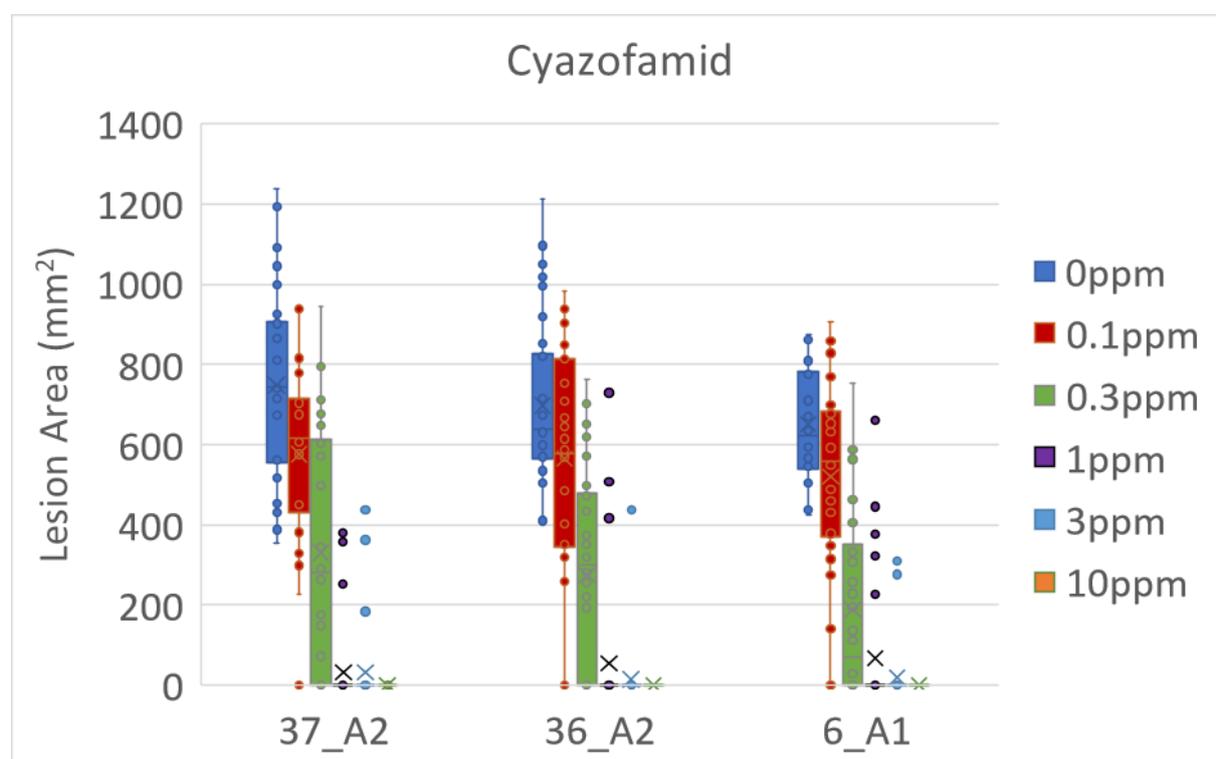

Figure 33. Mean Lesion area (mm2) of isolates belonging to 36_A2 and 37_A2 compared with control isolates (6_A1) tested in 2020 and presented as a box and whisker plot. Cyazofamid Max Field concentration = 400ppm.

Table 12. Mean EC_{50} values for isolates of *P. infestans* of various genotypes tested in 2020 at a range of concentrations of cyazofamid (0, 0.1, 0.3, 1.0, 3.0, 10.0 ppm). Significant differences

between mean values are indicated by different letters. 2018 values are given in brackets for comparison but can only be compared with other values in brackets

Genotype	6_A1	37_A2	36_A2
Number of isolates	5	5	5
Mean EC$_{50}$ (mg/L)	0.4778a	0.398a	0.538a

Amisulbrom 2019

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 (Fig. 34). The mean lesion size, on infected leaves only, is shown as a bar graph (Fig. 35) and a box and whisker plot (Fig. 36). 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$_{50}$. 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 (Table 13).
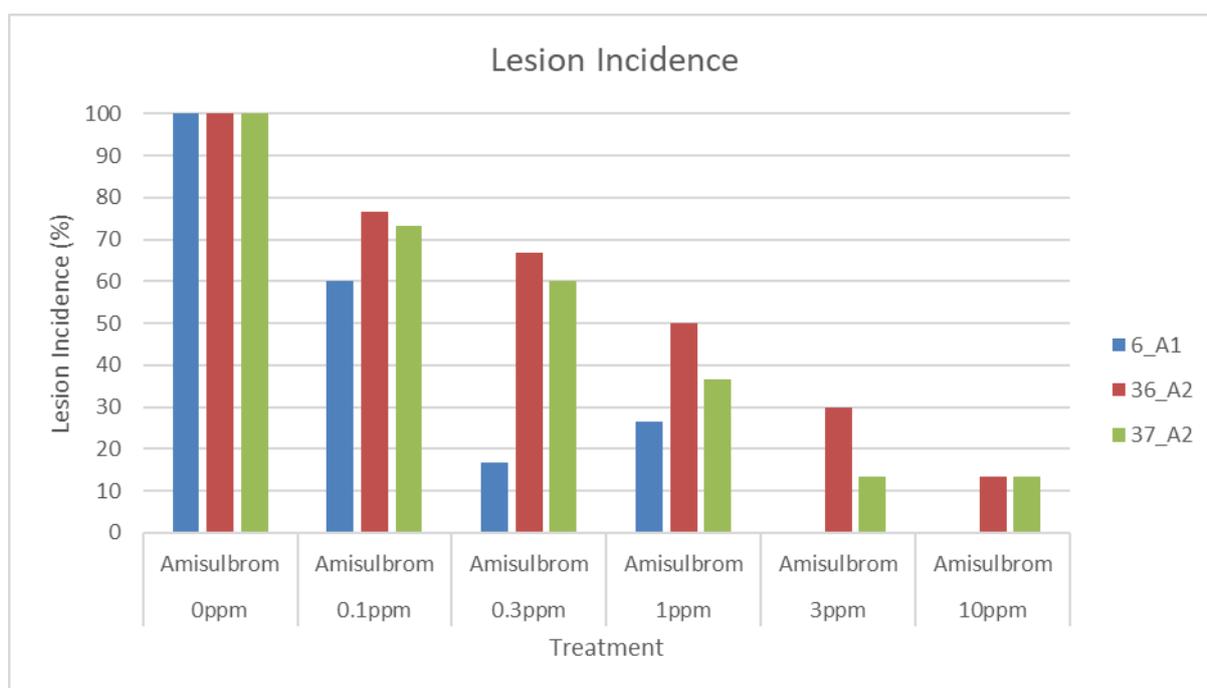

Figure 34. Mean percentage incidence of lesions caused by different genotypes at a range of concentrations of amisulbrom tested in 2019 (0-10 µg/ml).
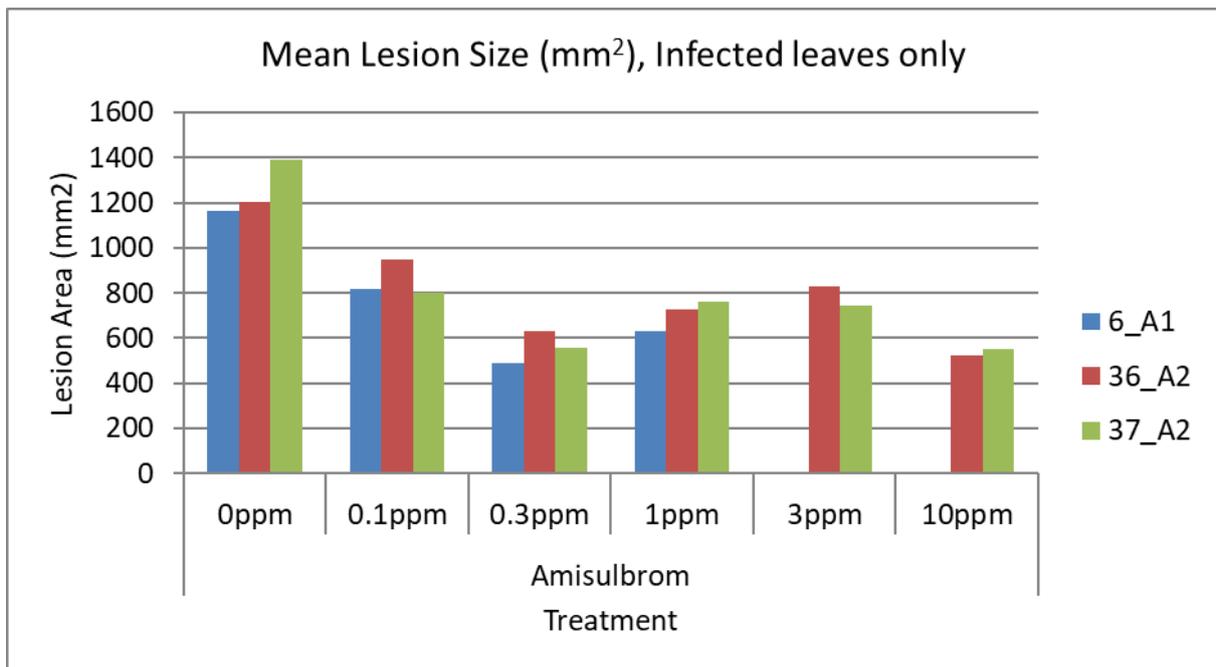
Figure 35. Mean lesion size (mm2) measured on infected leaves only at a range of concentrations of amisulbrom tested in 2019.
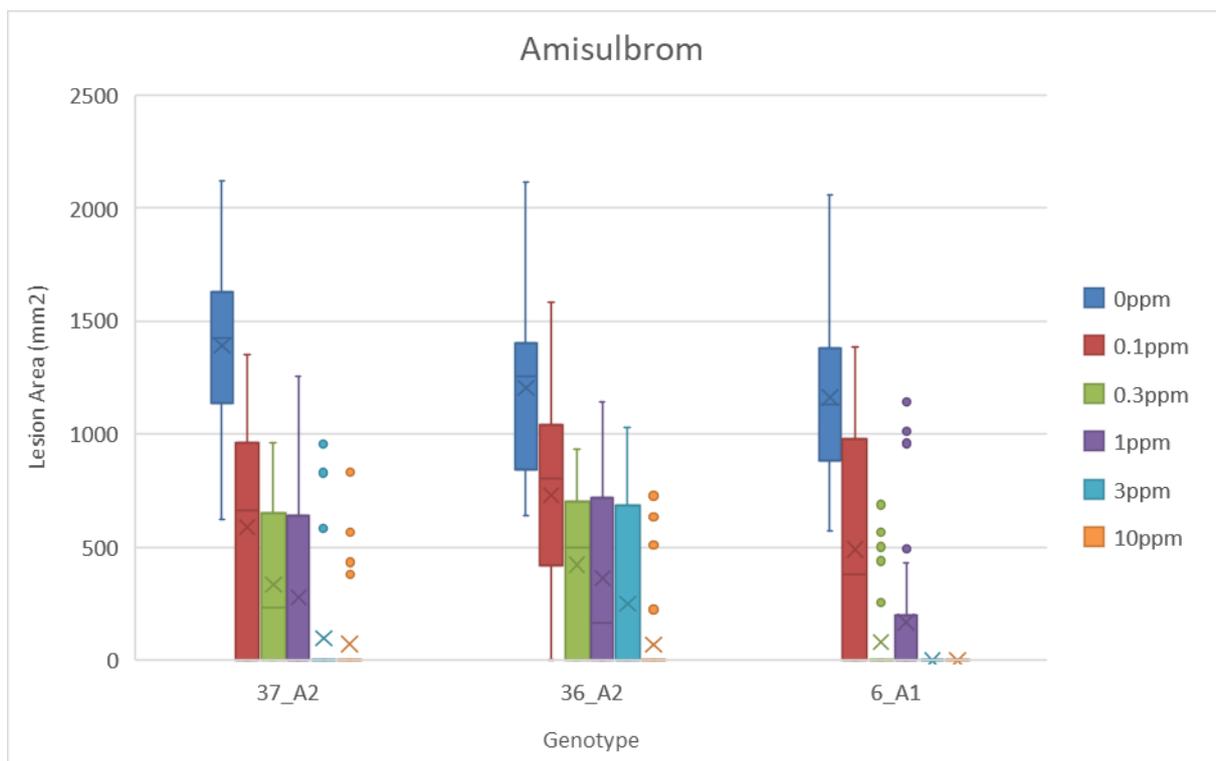

Figure 36. Mean Lesion area (mm2) of isolates belonging to 36_A2 and 37_A2 tested in 2019 compared with control isolates (6_A1) presented as a box and whisker plot. Amisulbrom maximum field concentration = 200ppm.

Table 13. Mean, max and min EC50 values for isolates of *P. infestans* of various genotypes tested in 2019 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)	**0.36a**	**0.36a**	**0.98b**
EC$_{50}$ min (mg/L)	0.15	0.15	0.15
EC$_{50}$ max (mg/L)	2.22	1.64	8.14

Amisulbrom 2020

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 (Figs. 37. 38 & 39). Some very small lesions were observed for all lineages even in the highest dose of 10 ppm but this was only 0.05% of the field rate and is unlikely to be significant in the field. No significant differences in EC$_{50}$ were observed between the clonal lineages of *P. infestans* sampled in 2020 (Table 14).
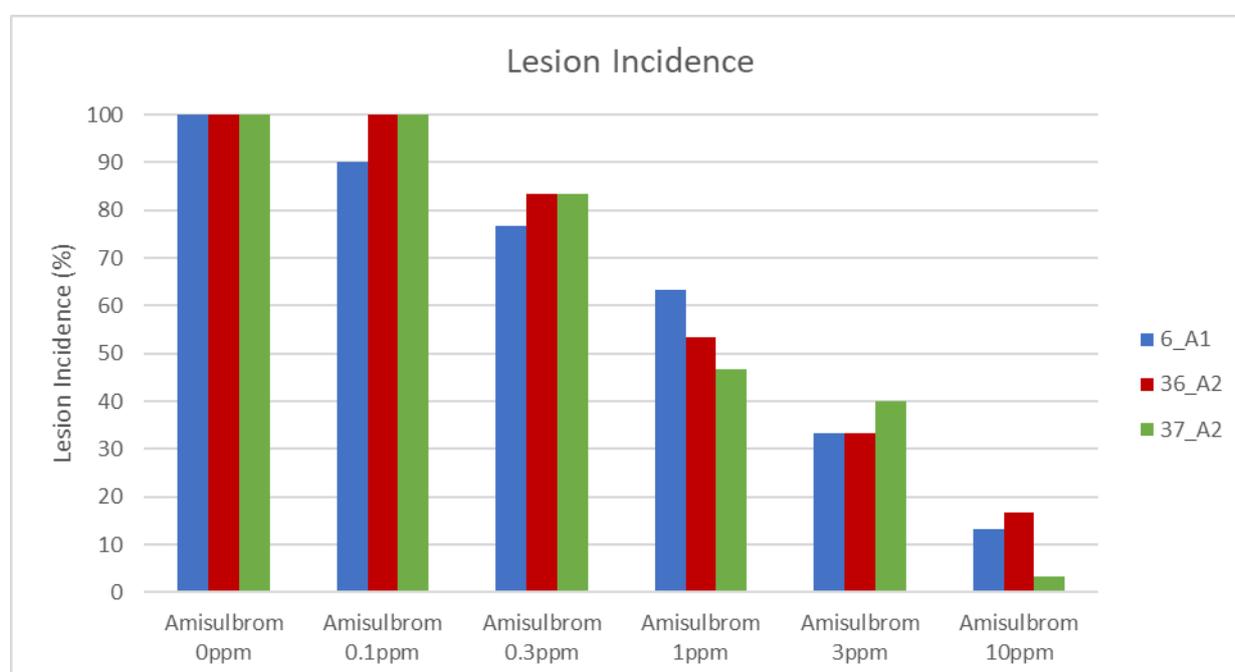

Figure 37. Mean percentage incidence of lesions caused by different genotypes at a range of concentrations of amisulbrom tested in 2020 (0-10 μg/ml).
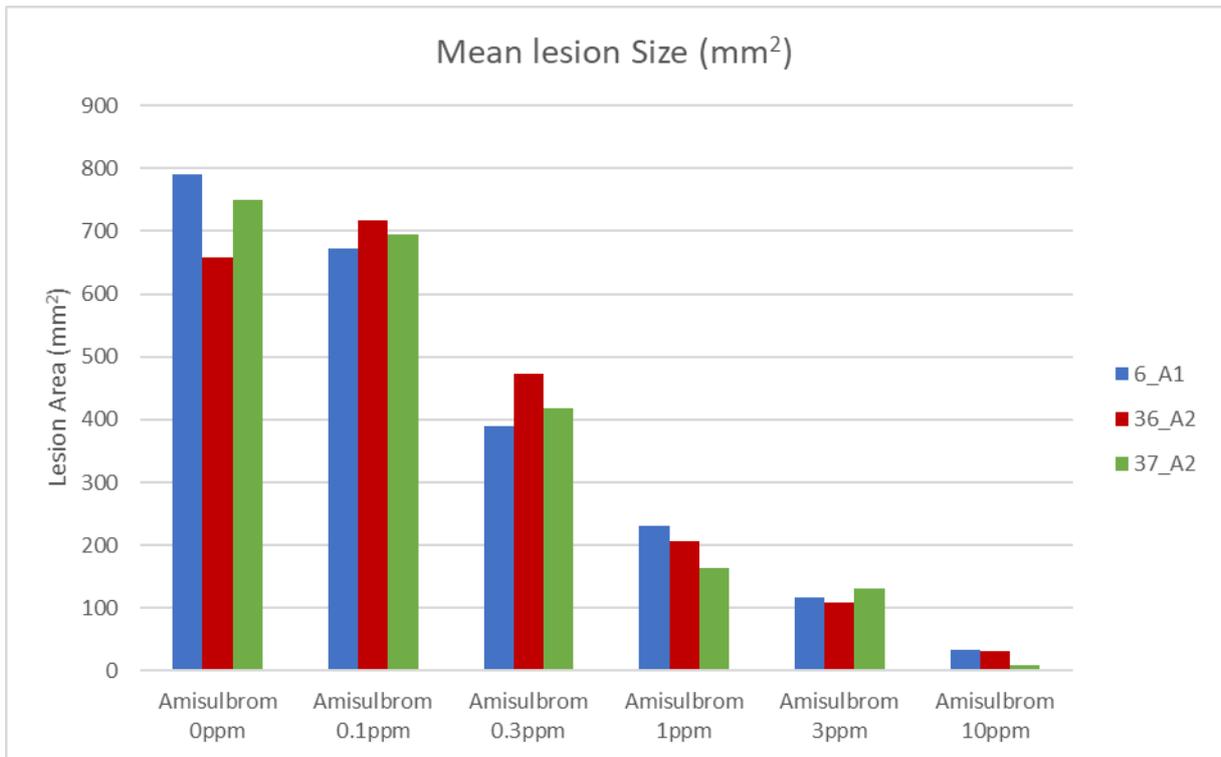
Figure 38. Mean lesion size (mm2) measured on infected leaves only at a range of concentrations of amisulbrom tested in 2020.
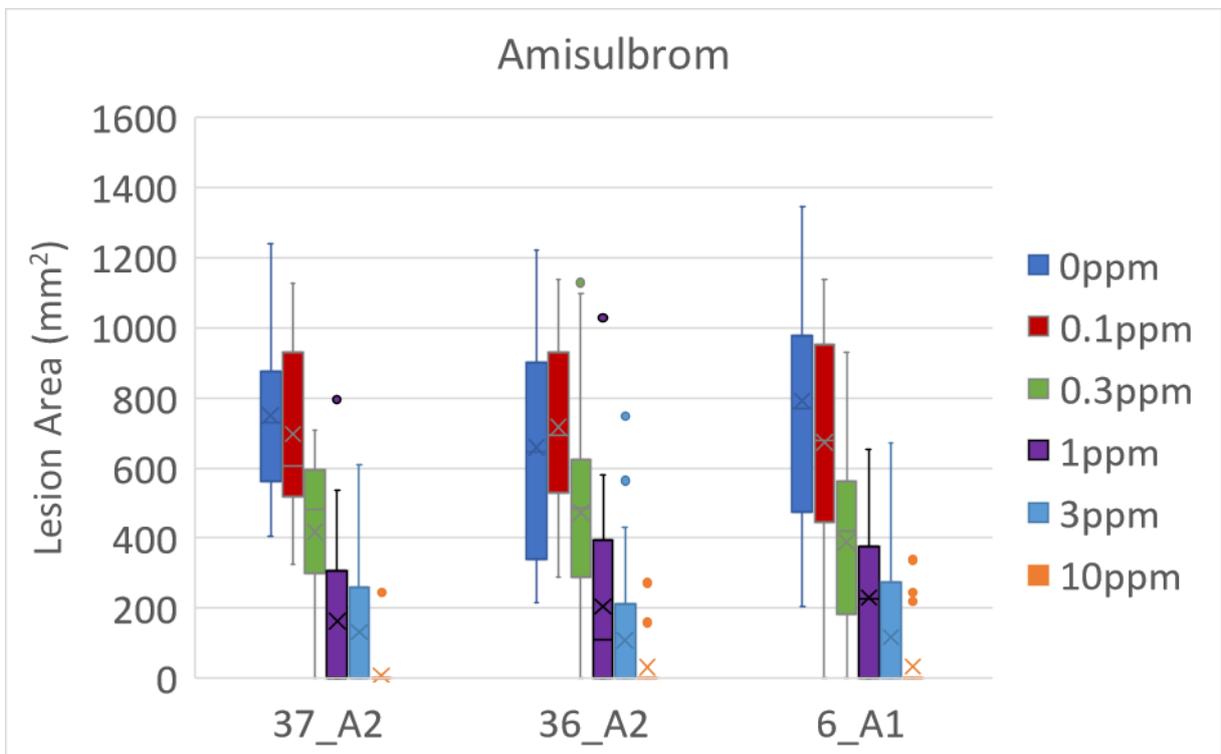

Figure 39. Mean Lesion area (mm2) of isolates belonging to 36_A2 and 37_A2 tested in 2020 compared with control isolates (6_A1) presented as a box and whisker plot. Amisulbrom maximum field concentration = 200ppm.

Table 14. 2020 Mean EC_{50} values for isolates of *P. infestans* of various genotypes tested in 2020 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)	1.073a	0.933a	1.492a

Propamocarb 2019

A high incidence of lesions (25 to 65%) was caused by all genotypes at concentrations up to 300ppm propamocarb (Fig. 40). 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. These lesion incidences with a product at 10% of its field dose are relatively high compared to other active ingredients. Only mancozeb also failed to prevent lesions at 10% of maximum field rate (Fig. 54). No lesions were observed at 1000µg/ml (Fig. 40). Mean lesion size, on infected leaves only, reduced with increasing fungicide concentration (Fig. 41). The box and whisker plot (Fig. 42) shows slightly larger mean lesion sizes caused by isolates of 36_A2 in critical points of the dose curve (10 and 100 ppm) than those of other genotypes. This is reflected in the statistically significant difference in mean EC_{50} value between genotypes, with isolates of 36_A2 having, on average, a higher EC_{50} value than 37_A2 and 6_A1 (Table 15). The EC_{50} values are, however, in line with previous findings.
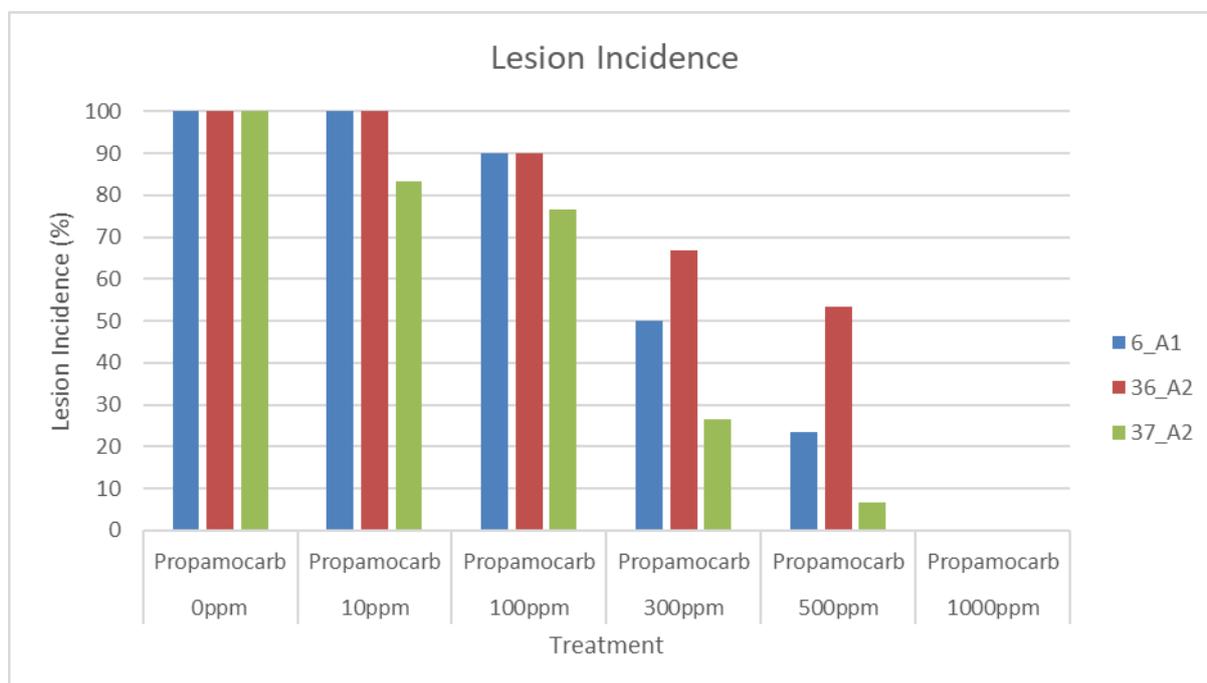
Figure 40. Mean percentage of lesions caused by different genotypes at a range of concentrations of propamocarb tested in 2019 (0-1000 µg/ml).
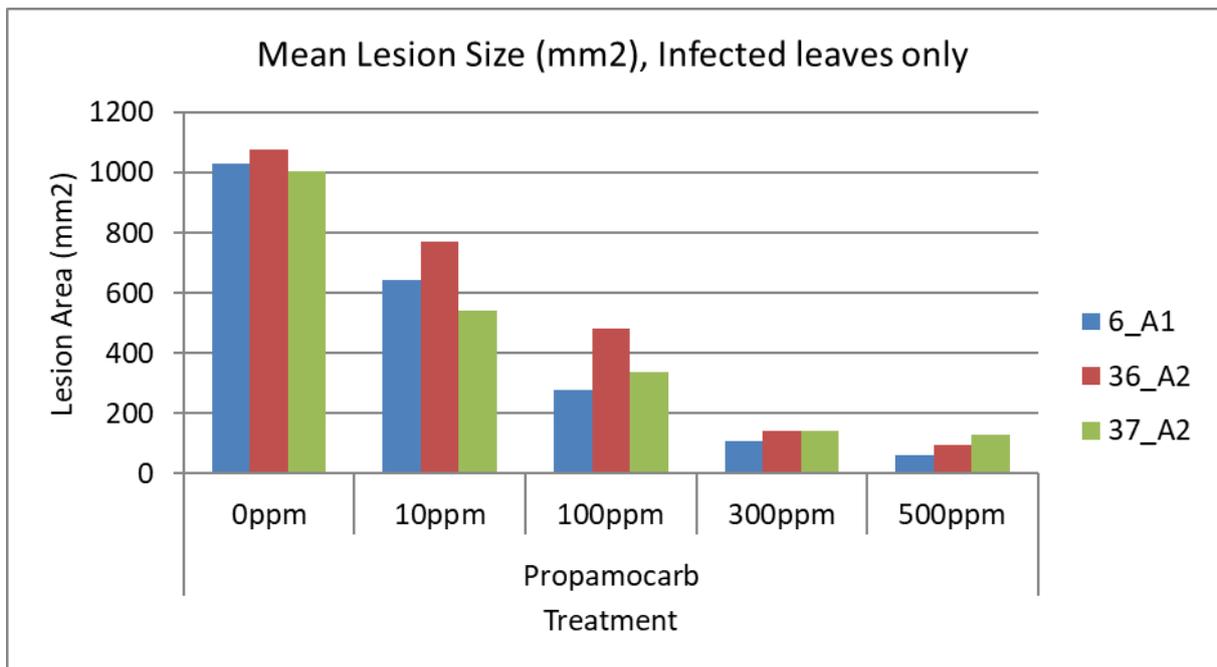
Figure 41. Mean lesion size (mm2) measured on infected leaves only at a range of concentrations of propamocarb tested in 2019.
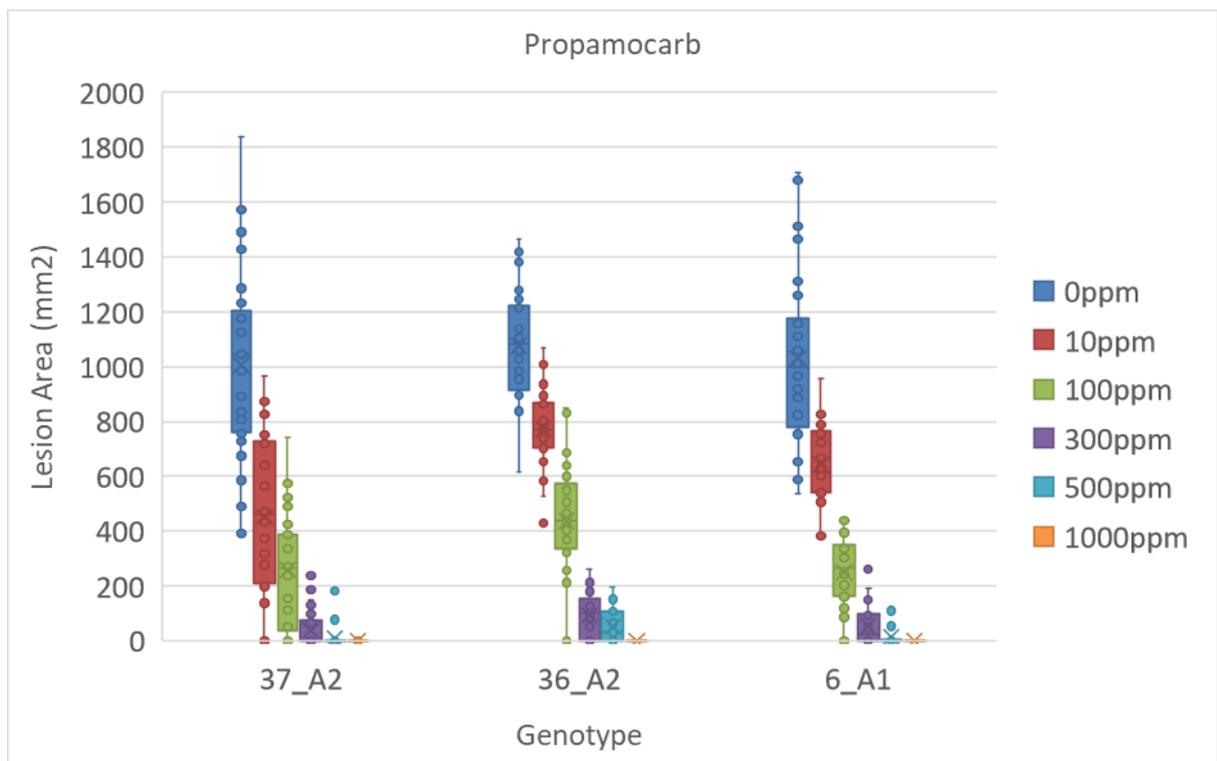
Figure 42. Mean Lesion area (mm^2) of isolates belonging to 36_A2 and 37_A2 compared with control isolates (6_A1) tested in 2019 and presented as a box and whisker plot. Propamocarb Max Field concentration = 5000 ppm.

Table 15. Mean, max and min EC_{50} values for isolates of *P. infestans* of various genotypes tested in 2019 at a range of concentrations of propamocarb (0, 10, 100, 300, 500, 1000 ppm). Significant differences between mean values are indicated by different letters. Numbers in brackets are 2018 data but can only be compared with other numbers in brackets.

Genotype	6_A1	37_A2	36_A2
Number of isolates	5	5	5
Mean EC_{50} (mg/L)	**39.92a** (8.41a)	**38.08a** (21.56a)	**64.67b** (62.03b)
EC_{50} min (mg/L)	2.45 (3.31)	0.99 (3.31)	1.49 (3.31)
EC_{50} max (mg/L)	133.94 (44.58)	244 (133.94)	180.80 (220.83)

Propamocarb 2020

The assay used to test the isolates in 2020 indicated a greater sensitivity than tests in 2019 with no lesions formed by any isolate at either 500 or 300 ppm of propamocarb (Fig. 43 & 44). unlike the data for 2019, the box and whisker plot and calculated EC_{50} values show now significant differences in response of the three genotypes to this active ingredient (Fig. 45; Table 16).
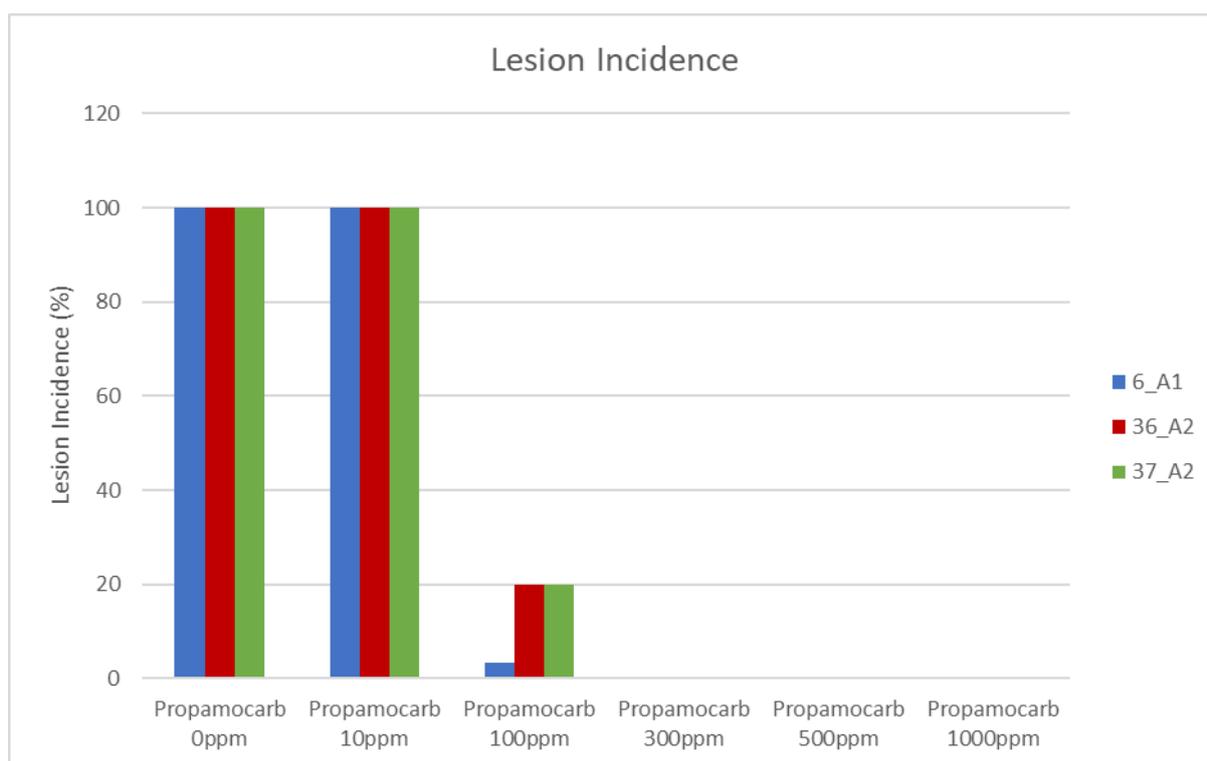

Figure 43. Mean percentage of lesions caused by different genotypes at a range of concentrations of propamocarb tested in 2020 (0-1000 µg/ml).

47
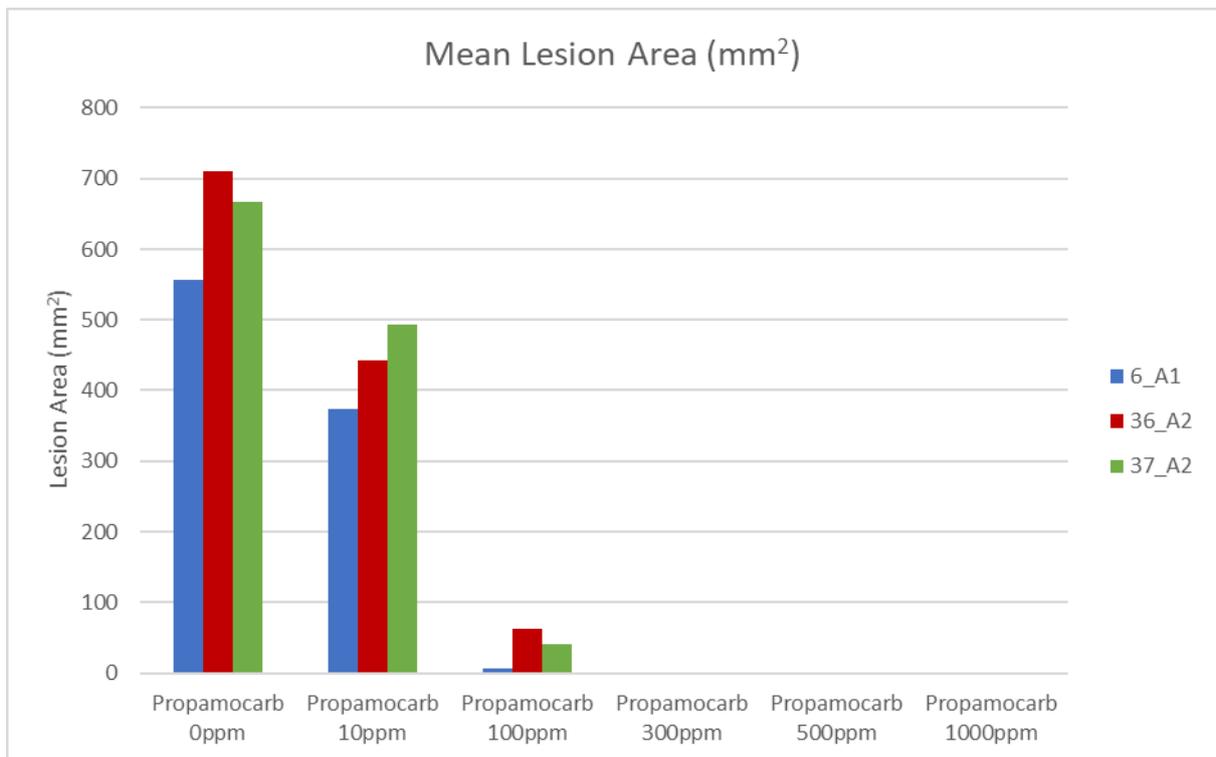
Figure 44. Mean lesion size (mm2) measured on infected leaves only at a range of concentrations of propamocarb tested in 2020.
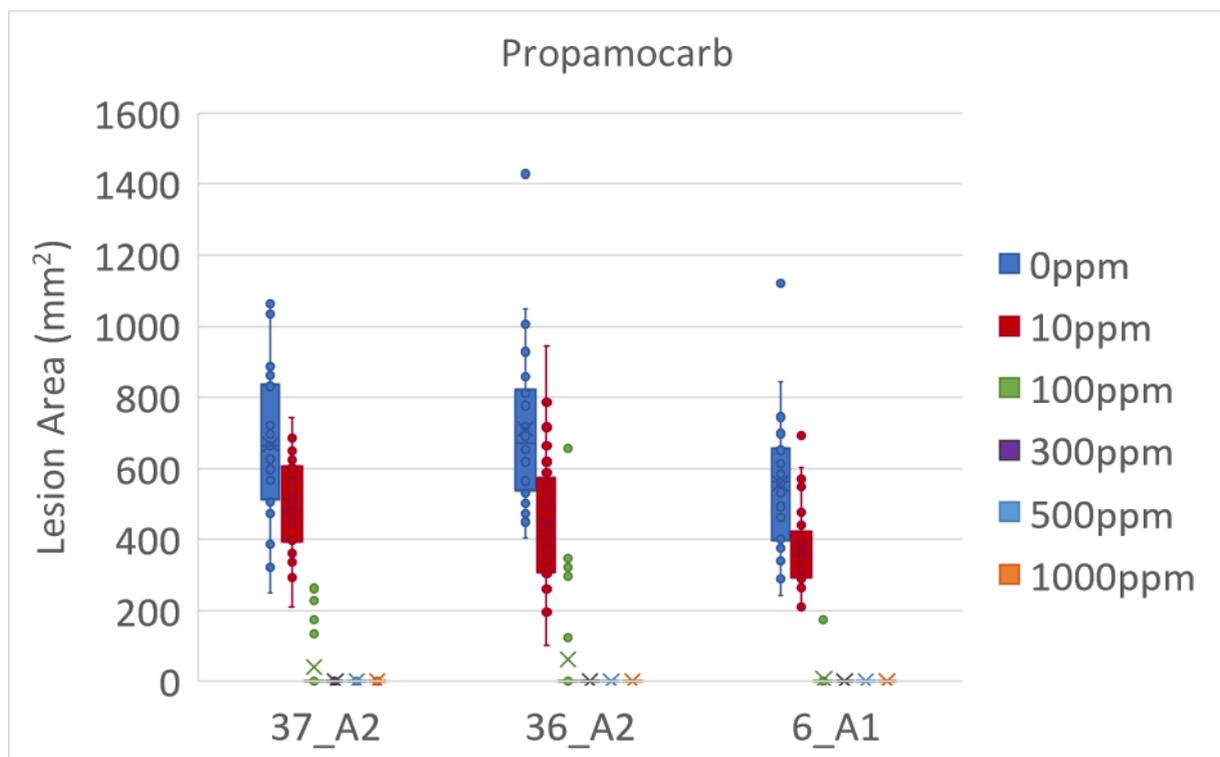

Figure 45. Mean Lesion area (mm^2) of isolates belonging to 36_A2 and 37_A2 compared with control isolates (6_A1) tested in 2020 and presented as a box and whisker plot. Propamocarb Max Field concentration = 5000 ppm.

Table 16. Mean EC_{50} values for isolates of *P. infestans* of various genotypes tested in 2020 at a range of concentrations of propamocarb (0, 10, 100, 300, 500, 1000µg/ml). Significant differences between mean values are indicated by different letters. Numbers in brackets are 2018 data but can only be compared with other numbers in brackets.

Genotype	6_A1	37_A2	36_A2
Number of isolates	5	5	5
Mean EC_{50} (mg/L)	19.91a	26.01a	25.08a

Oxathiapiprolin 2019

A high incidence of lesions was caused by all genotypes at concentrations up to 0.001 ppm oxathiapiprolin (Fig. 46). However, this represents only 0.0013% of the maximum field dose. At 0.01 ppm 40% of isolates of 36_A2 produced lesions compared with 6_A1 (10%) and 37_A2 (27%). No lesions were observed at 0.1ppm. Mean lesion sizes, on infected leaves only, are shown (Fig 47 & 48). The statistical significance, or otherwise, of differences in lesion size is captured in the calculation of EC_{50} and indicated there were no statistically significant difference in mean EC_{50} value between genotypes (Table 17). The EC_{50} values correspond well to those in other studies.
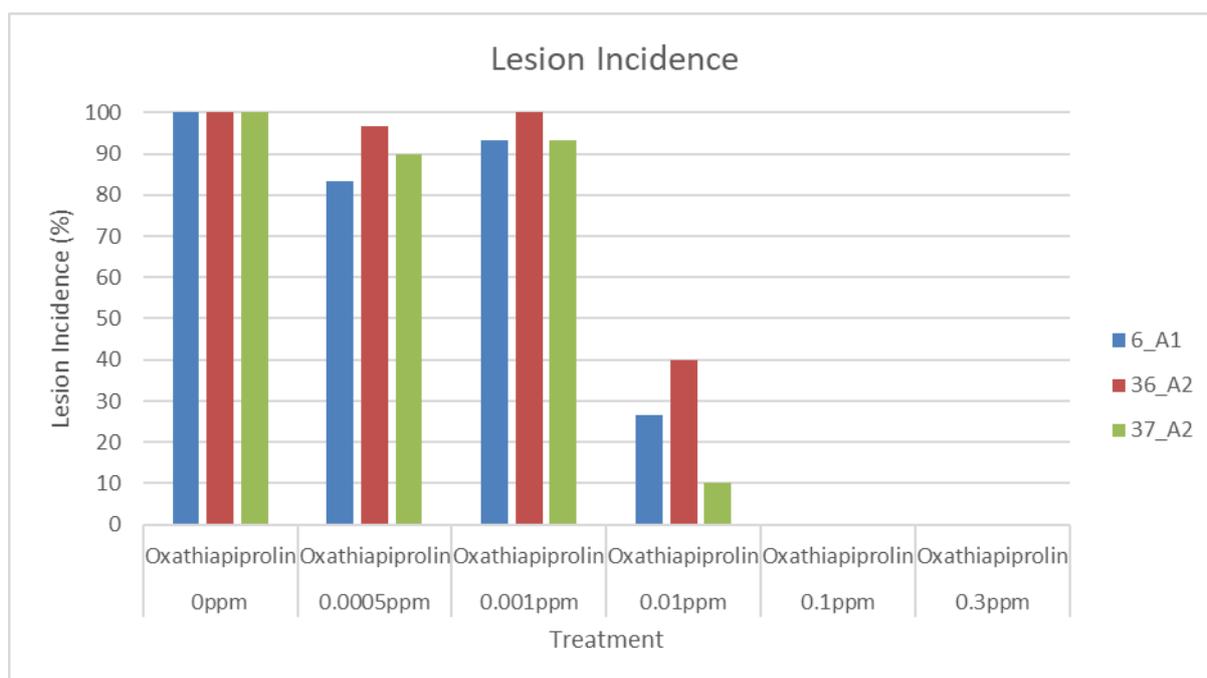

Figure 46. Mean percentage of lesions caused by different genotypes at a range of concentrations of oxathiapiprolin tested in 2019.
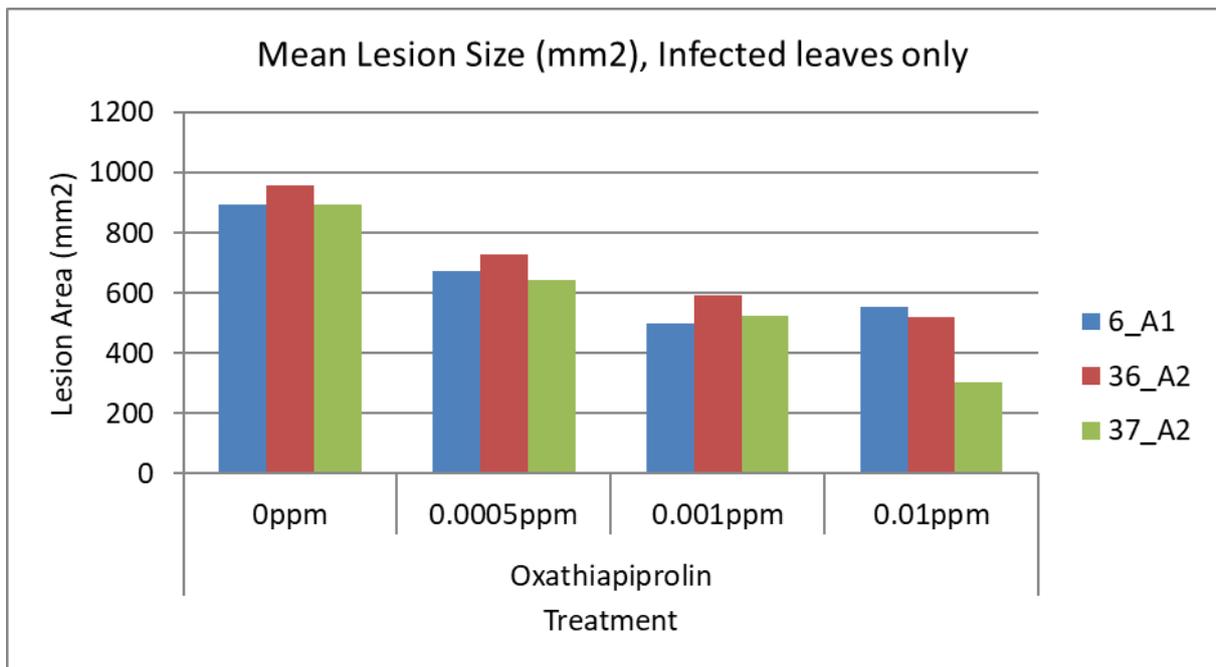
Figure 47. Mean lesion size (mm^2) measured on infected leaves only at a range of concentrations of oxathiapiprolin tested in 2019. No lesions were observed at 0.1 and 0.3ppm.
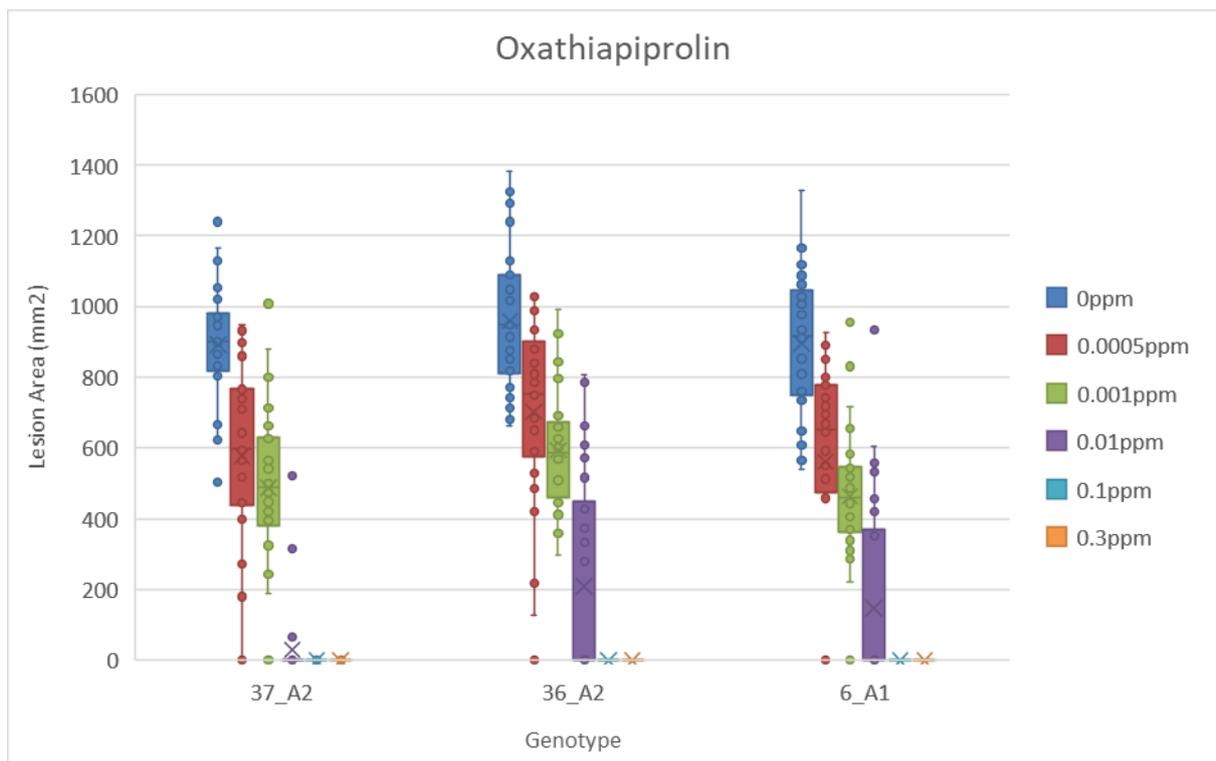
Figure 48. Mean Lesion area (mm2) of isolates tested in 2019 belonging to 36_A2 and 37_A2 compared with control isolates (6_A1) presented as a box and whisker plot. Oxathiapiprolin maximum field concentration = 75 ppm.

Table 17. Mean, max and min EC_{50} values for isolates of *P. infestans* of various genotypes tested in 2019 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)	**0.0053a**	**0.0015a**	**0.0048a**
EC_{50} min (mg/L)	0.00030	0.00037	0.00037
EC_{50} max (mg/L)	0.054	0.0033	0.049

Oxathiapiprolin 2020

Isolates tested in 2020 showed the same response as those in 2019 with no lesions formed when treated with a dose of 0.1 ppm. Incidences of 70-80% at 0.01ppm were marginally higher than in 2019 but, again, there was no difference in response between the three genotypes (Fig. 49 & 50). The box and whisker plot for the 2020 results (Fig. 51) differed from that in 2019 (Fig. 48) in that the lesion sizes of the lowest dose (0.0005 ppm) were not markedly different from the doses either side, in particular for isolates of 36_A2 and 6_A1. These differences at the critical dose of 0.01ppm which is the inflection point between full efficacy, that is zero lesions, at 0.1ppm and much weaker efficacy at 0.001ppm caused problems in fitting a dose response curve and probably explains the markedly higher EC_{50} values in 2020 (mean 0.1322; Table 18) compared to 2019 (mean 0.0039; Table 17). It is likely that the 2020 values are an artefact as the calculated EC_{50} values are very sensitive to such curve fitting anomalies. Nonetheless, no significant differences between the genotypes were observed.
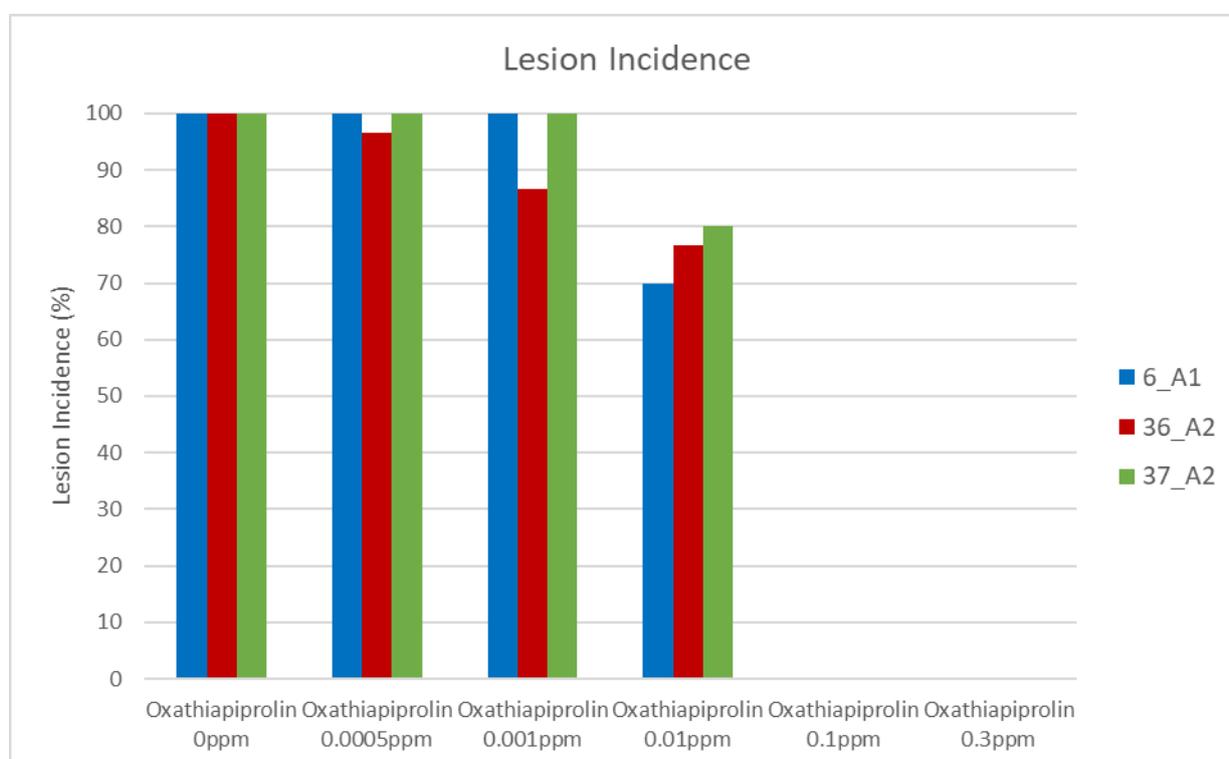

Figure 49. Mean percentage of lesions caused by different genotypes at a range of concentrations of oxathiapiprolin tested in 2020.
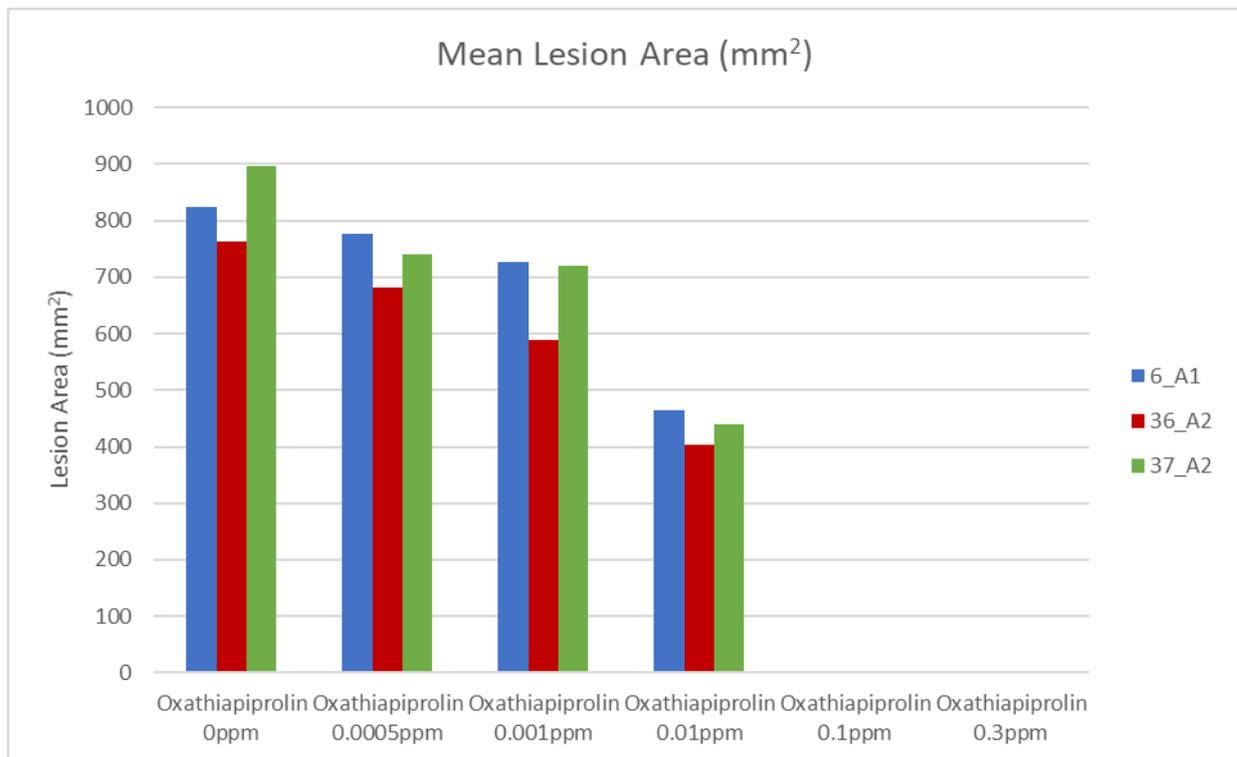
Figure 50. Mean lesion size (mm^2) measured on infected leaves only at a range of concentrations of oxathiapiprolin tested in 2020.
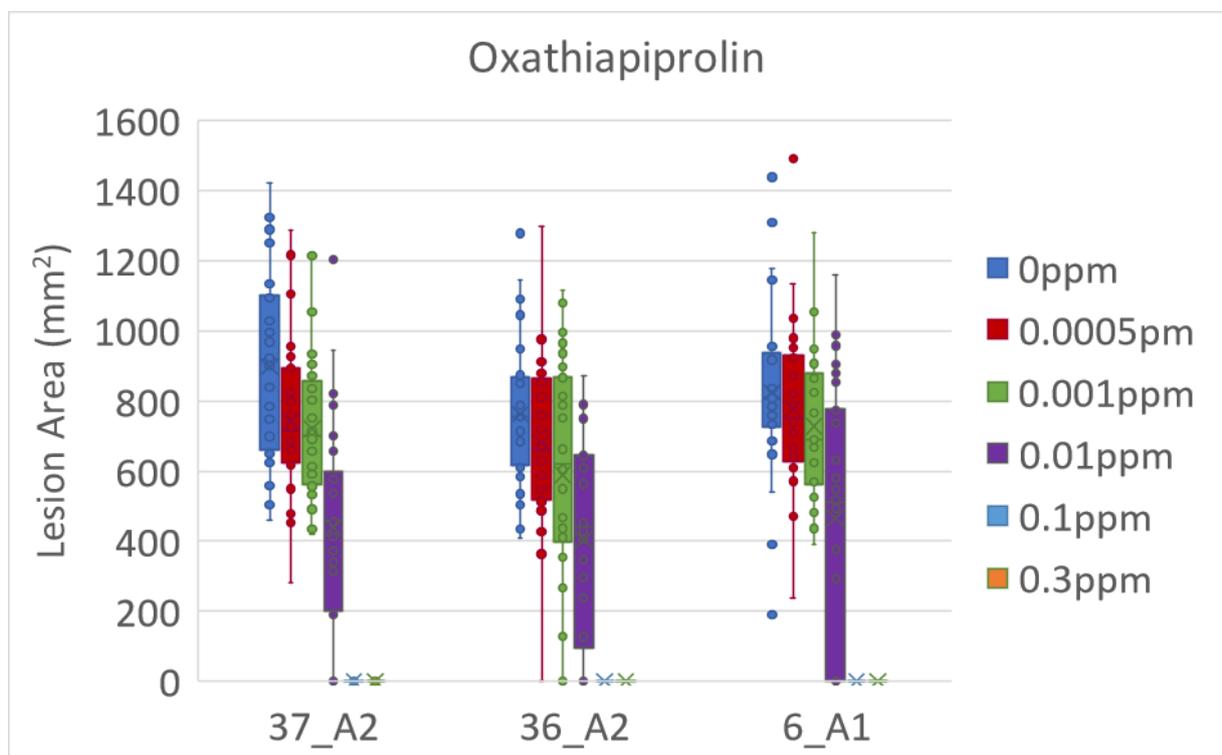
Figure 51. Mean Lesion area (mm2) of isolates tested in 2020 belonging to 36_A2 and 37_A2 compared with control isolates (6_A1) presented as a box and whisker plot. Oxathiapiprolin maximum field concentration = 75 ppm.

Table 18. Mean, max and min EC50 values for isolates of *P. infestans* of various genotypes tested in 2020 at a range of concentrations of oxathiapiprolin. The letters indicate that there were no statistically significant differences between mean values for each genotype.

Genotype	6_A1	37_A2	36_A2
Number of isolates	5	5	5
Mean EC$_{50}$ (mg/L)	0.1321a	0.1351a	0.1294a

Mancozeb 2019

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 and 0% incidence at 1000 ppm (Fig. 52). Mean lesion size, on infected leaves only, reduced with increasing rate (Fig. 53). 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 (Fig. 54). The mean EC$_{50}$ values show there was no statistically significant difference between genotypes (Table 19). The EC$_{50}$ values are higher than those previously reported but were obtained using a different method and are well within the field rate concentration (6375µg/ml).
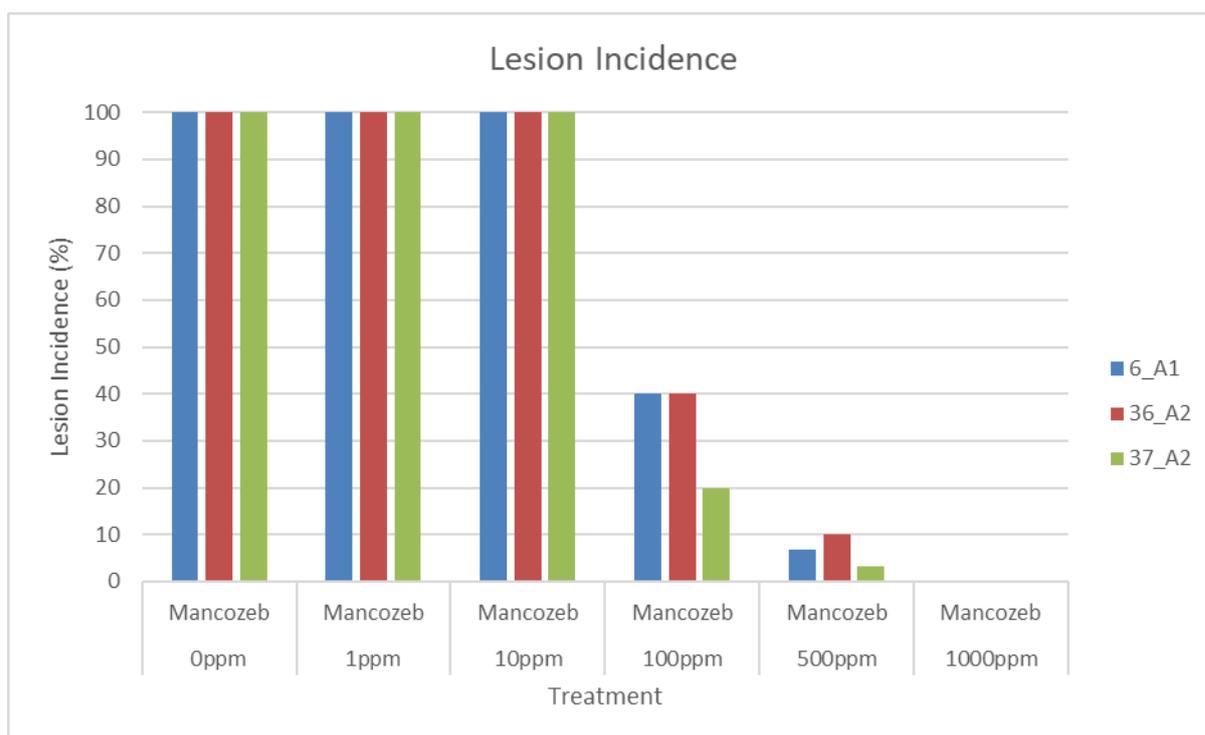
**Figure 52.** Mean percentage of lesions caused by different genotypes at a range of concentrations of mancozeb tested in 2019 (0-1000 µg/ml).
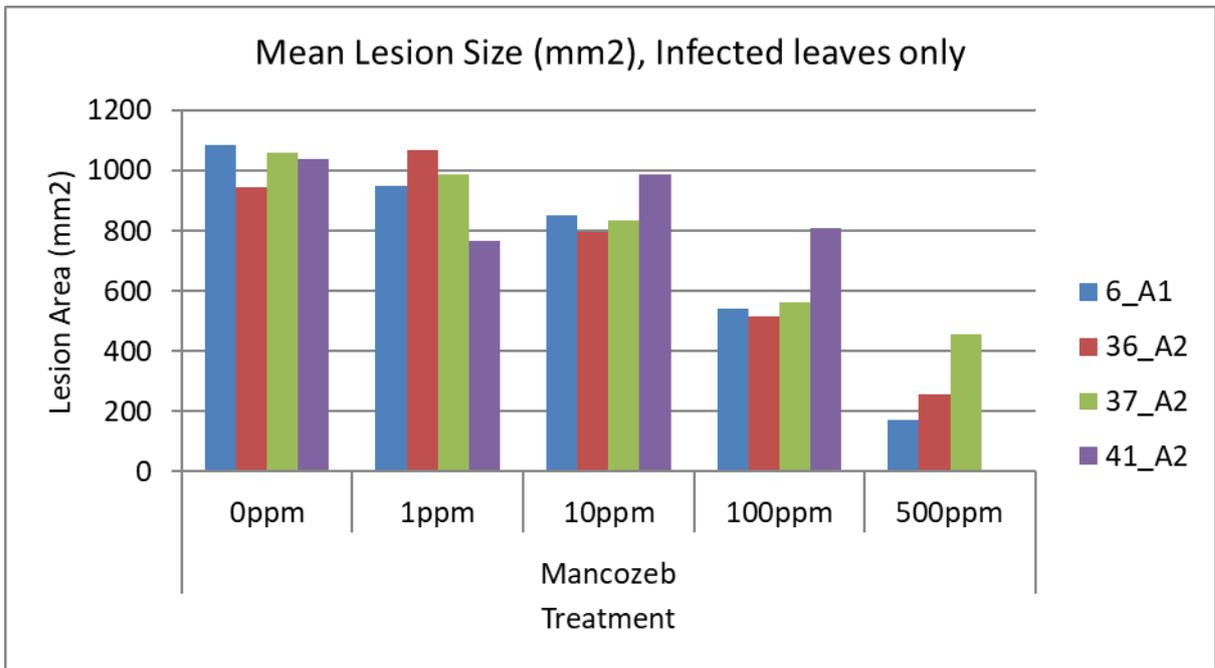
Figure 53. Mean lesion size (mm^2) measured on infected leaves only at a range of concentrations of mancozeb tested in 2019.
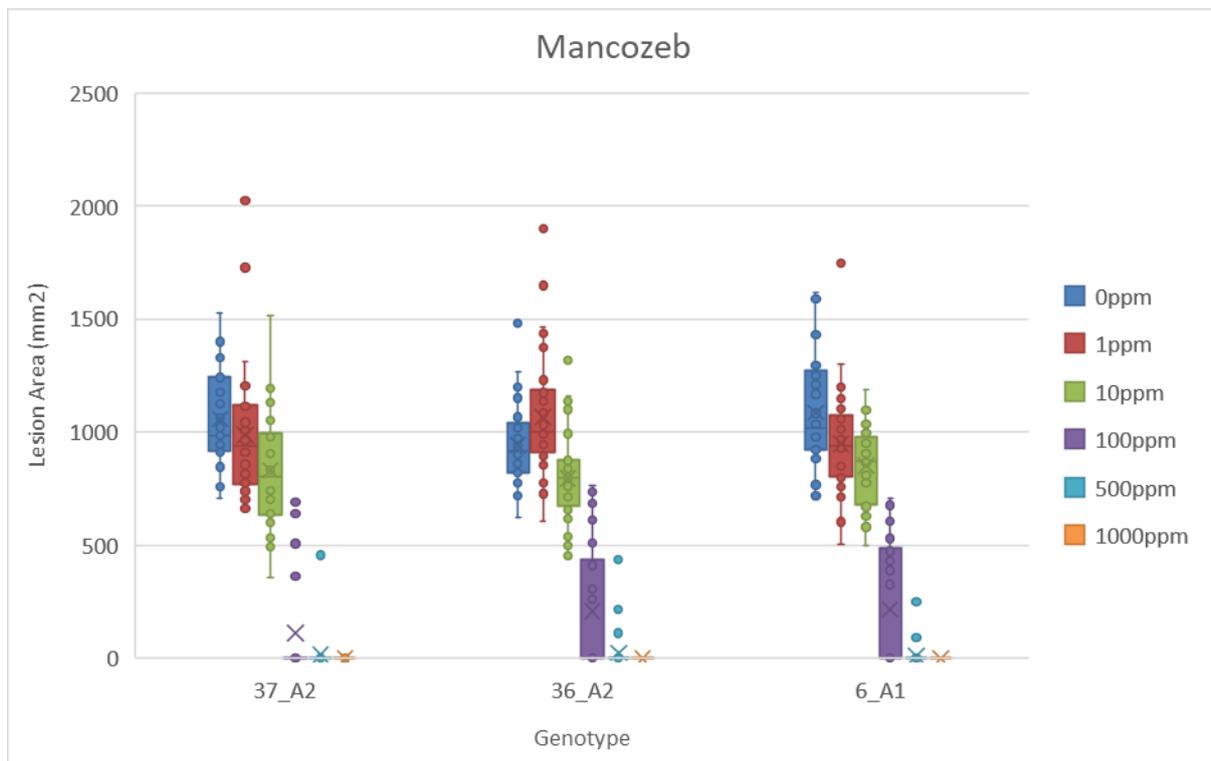

Figure 54. Mean Lesion area (mm^2) of isolates belonging to 36_A2 and 37_A2 tested in 2019 compared with control isolates (6_A1) presented as a box and whisker plot. Mancozeb maximum field concentration = 6375 ppm.

Table 19. Mean, max and min EC_{50} values for isolates of *P. infestans* of various genotypes tested in 2019 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
Mean EC_{50} (mg/L)	**53.05a**	**43.27a**	**66.62a**
EC_{50} min (mg/L)	0.82	7.37	0.90
EC_{50} max (mg/L)	244.06	298.10	329.45

Mancozeb 2020

The efficacy of doses tested against isolates from 2020 was very similar to the testing in 2019 with lesion incidence and size being unaffected by the lowest dose of 1 ppm and only slight reductions at 10 ppm (Fig. 55 & 56). At 100 ppm however lesion incidence fell to a mean around 10% and the lesions were smaller even than those in 2019 (Fig. 53). This generated very steep dose response curves (Fig. 57) used to calculate the EC_{50} values and, like in the 2019 tests, this may explain the relatively high EC_{50} values compared to other studies. Nonetheless, the mean responses of isolates of all three genotypes were similar and no significant differences in EC_{50} were indicated (Table 20).
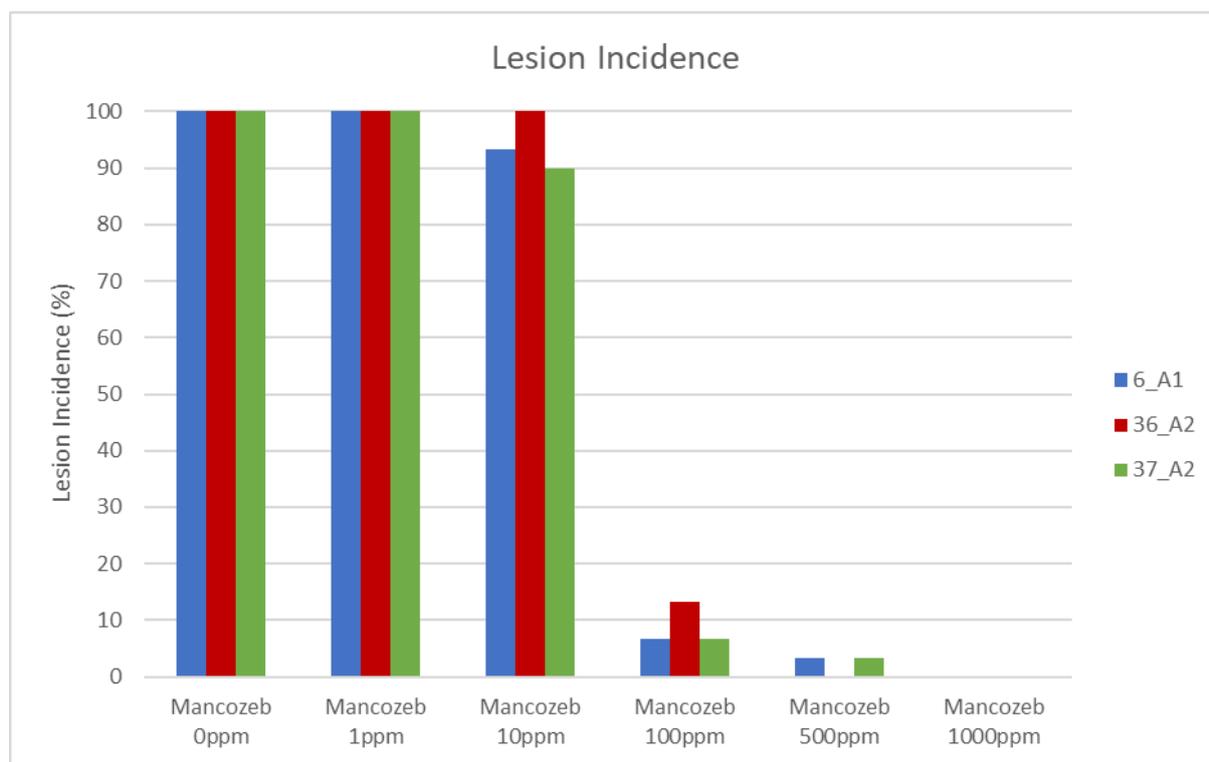

Figure 55. Mean percentage of lesions caused by different genotypes at a range of concentrations of mancozeb tested in 2020 (0-1000 µg/ml).
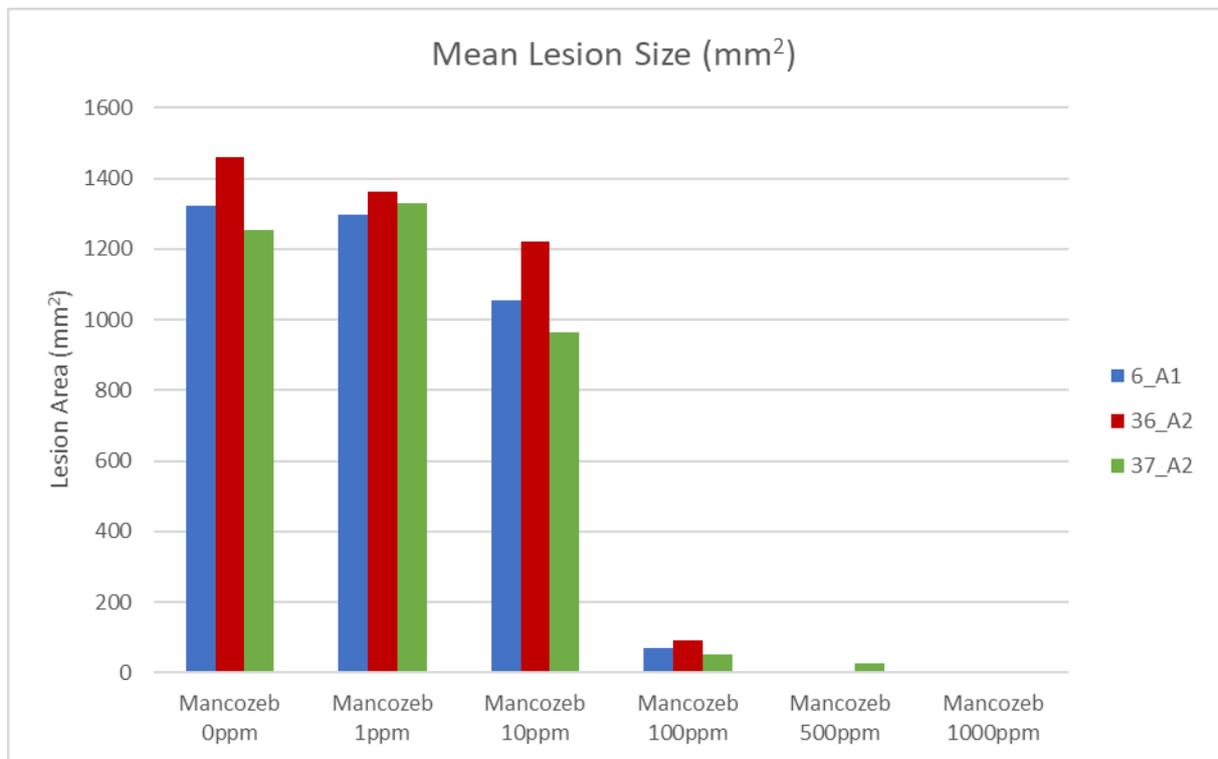
Figure 56. Mean size (mm^2) of lesions, measured on infected leaves only, at a range of concentrations of mancozeb tested in 2020.
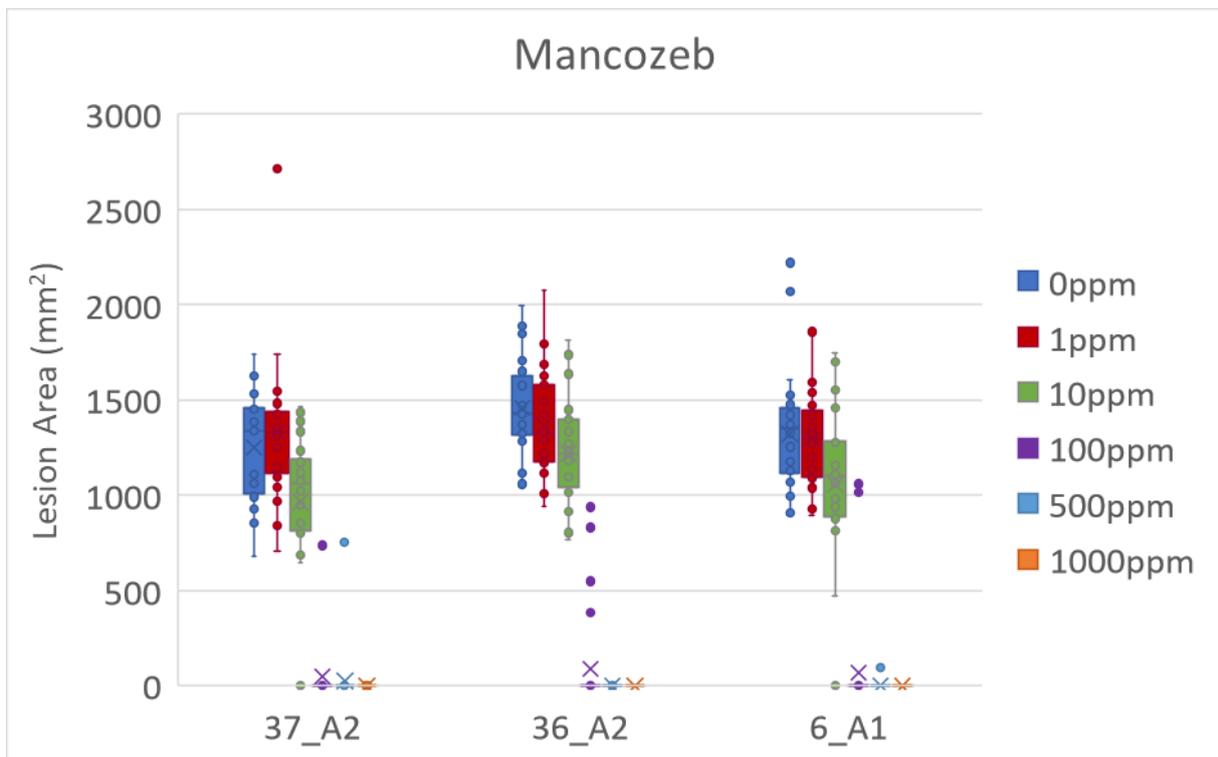

Figure 57. Mean Lesion area (mm^2) of isolates belonging to 36_A2 and 37_A2 tested in 2020 compared with control isolates (6_A1) presented as a box and whisker plot. Mancozeb maximum field concentration = 6375 ppm.

Table 20. Mean, max and min EC_{50} values for isolates of *P. infestans* of various genotypes tested in 2020 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
Mean EC_{50} (mg/L)	37.02a	33.58a	29.51a

Comparisons of EC_{50} values across 2-3 seasons

Having tested batches of isolates collected across two (amisulbrom, oxathiapiprolin, mancozeb) or three (fluopicolide, mandipropamid, cyazofamid, propamocarb) consecutive years a comparison of the EC_{50} values across the time period was possible to examine the data for any annual shifts in sensitivity. However, this comparison did not indicate any consistent shift over time in any product (data not shown). 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.

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. 6). The 2020 season was on average warmer and drier and the lower blight pressure resulted in fewer samples than average. However, the combined sampling of 287 outbreaks and genotyping of over 1400 samples of *P. infestans* provides a good record of the pathogen population (Fig. 7). 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. 8) with an extension of their range into Wales and Scotland (Figure 11). Although not part of this study, an expansion of both 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. 11). 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. 11). In 2020 the proportion increased slightly to nearly 10% across sampled GB blight outbreaks.

An active 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. 58).
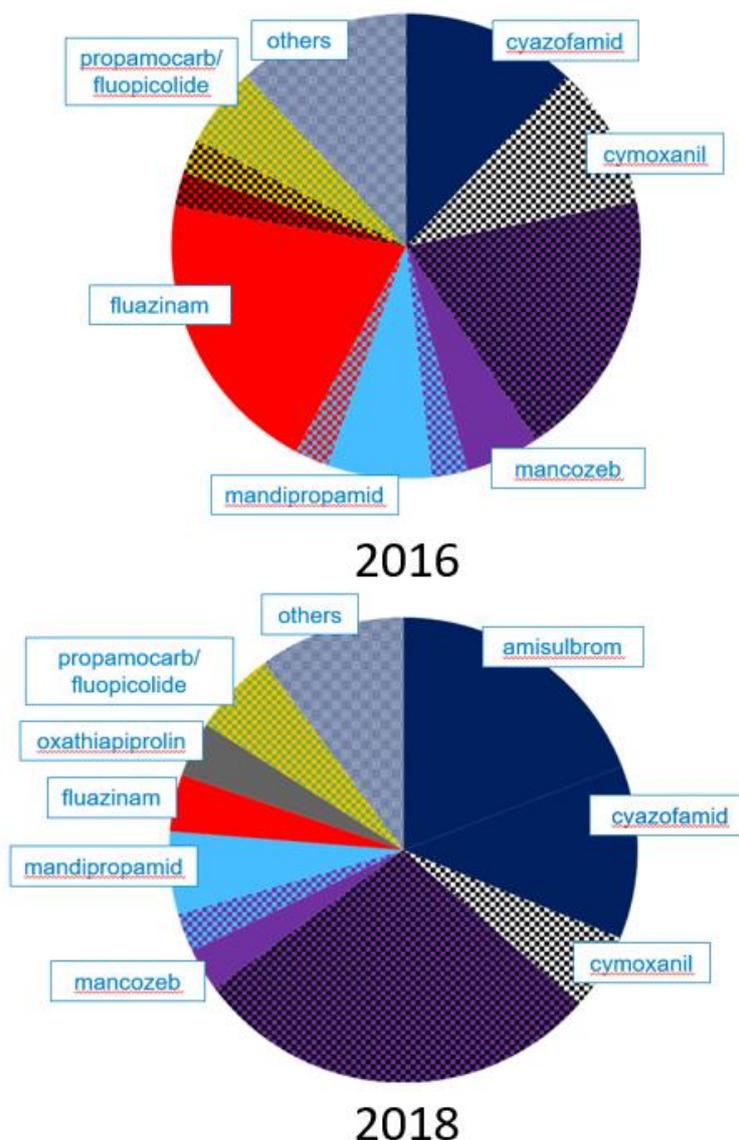
Figure 58. Change in use of fungicide active ingredients on UK potato crops between surveys in 2016 (upper) and 2018 (lower). The proportion of fluazinam applied reduced markedly over this period. Based on data from Garthwaite et al., (2018) and Garthwaite et al., (2019).

This campaign, the related drop in fluazinam usage and the frequency of 37_A2 in the GB pathogen population are correlated and should be seen as a clear success of the FAB campaign. In the absence of this data and publicity it is highly probable that many blight control failures and incidences of tuber blight in GB crops could have occurred.

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 and 2020 seasons 36_A2 increased from 17% of GB samples in 2018 to 27% and 29% in 2019 and 2020, respectively. It has also spread to and become established in Wales and Scotland but remains most prevalent in English crops (Fig. 8). The spread of 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). Despite some evidence of higher lesion incidence at very low doses of fluopicolide and propamocarb in 2019 testing (Figs. 14 & 40) and in 36_A2 and 37_A2 in amisulbrom in 2019 (Fig. 34) this was not supported by identical testing on samples collected in 2020. 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 that 36_A2 has 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 (Figs. 7 & 8) 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 held 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.

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 an absence 2018 it comprised almost 4% of samples in 2019 and 2020 (Fig. 7). It remains more prevalent in crops sampled in Wales and Scotland than in England (Fig. 8). 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 and 39_A1

The genotype 42_A2 was newly defined in 2020 after the first being sampled in 2017 and present in all four consecutive seasons. 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. The 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.

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. 12) 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.12). 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. 12) 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 9.3% with highs of 29% in 2015 a low of 2.9% in 2008 (Fig. 7). This proportion is higher in Scotland ranging from 8.3% 2013 to 53.4% in 2016 (Fig. 8). In 2019 and 2020 the proportion across GB crops was 17.3 and 13.9% respectively.

Genetic analysis of the SSR data from isolates from GB in 2020 highlights the diversity of this group of 'Other' isolates (Fig. 12). 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). Careful analysis of all 'Other' isolates collected from 2003 to 2020 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 outside the country (Fig. 12).

This 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 local 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 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 this region may relate to physical geography and the seed trade. The land suitable for agriculture in this region is constricted to a narrow coastal strip in the area around Stonehaven where upland heath associated with the Cairngorm mountain range meets the coast. 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 6_A1 and 13_A2 lineages and growers should remain alert to the presence of soil-borne oospore inoculum and the threats it poses to genetic diversity and early infection pressure.

Fungicide sensitivity testing
The key main finding from the comprehensive testing of multiple isolates of three clonal lineages examined in 2019 and 2020 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 note in these tests. For example, fluopicolide was less active against 36_A2 with reduced EC_{50} values in 2019 tests (Table 5) and lower zoospore MICs in 2019 and 2020 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). While these differences were statistically significant in individual years, they were generally not supported in a second year of testing and related to low doses that may not reflect field performance. 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, but such reports are important and should be followed up.

The assays 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 of 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 of such exposure that is important. The risk of changes 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 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.

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 and 2020 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. Current research the James Hutton Institute has updated the blight risk conditions which is allowing decision support tools such as AHDB potatoes' BlightSpy (https://ahdb.org.uk/blightspy) to be used alongside the FAB Campaign. 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 2019 and 2020 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 an alternative to this, will remain crucial to the future success of such an approach.

6. ACKNOWLEDGMENTS

7. REFERENCES

Aktaruzzaman M, Lee YG, Lee JE, Choi HY, Park U, Kim BS, 2016. Baseline Sensitivity to Oxathiapiprolin of Potato Late Blight (*Phytophthora infestans*) in Korea. Proceedings of 2016 Annual Meeting *The Korean Society of Pesticide Science* 2016. 180 - 180 (1 pages). http://kspsjournal.or.kr/_common/do.php?a=full&b=12&year=2015

Bain R, Ritchie F, Paveley N 2018. Guidance on how the potato industry should respond to reduced fluazinam sensitivity in late blight populations. AHDB special report. https://potatoes.ahdb.org.uk/sites/default/files/Late%20blight%20fluazinam%20guidance%20doc%20Final%20to%20AHDB%2017%20Apr%2018.pdf

Blum M, Boehler M, Randall E, Young V, Csukai M, Kraus S, Moulin F, Scalliet G, Avrova AO, Whisson SC, Fonne-Pfister R, 2010. Mandipropamid targets the cellulose synthase-like PiCesA3 to inhibit cell wall biosynthesis in the oomycete plant pathogen, *Phytophthora infestans*. *Molecular Plant Pathology* **11**, 227-43.

Bødker L, Pedersen H, Kristensen K, Møller L, Lehtinen A, Hannukkala A, 2006. Influence of crop history of potato on early occurrence and disease severity of potato late blight by *Phytophthora infestans*. In: Westerdijk CE, Schepers HTaM, eds. *Proceedings of the Ninth Workshop of an European network for development of an integrated control strategy of potato late blight.*, *2005*. Tallinn, Estonia, 53-6.

Bosch FVD, Oliver R, Berg FVD, Paveley N, 2014. Governing principles can Guide Fungicide-resistance management tactics. *Annual Review of Phytopathology* 52, 175-95.

Bourke PMA, 1964. Emergence of Potato Blight 1843-46. *Nature* **203**, 805-8.

Brasier CM, 1992. Evolutionary Biology of *Phytophthora. Annual Review of Phytopathology* 30, 153-70.

Brurberg MB, Elameen A, Le VH, Nærstad R, Hermansen A, Lehtinen A, Hannukkala A, Nielsen B, Hansen J, Andersson B, Yuen J, 2011. Genetic analysis of *Phytophthora infestans* populations in the Nordic European countries reveals high genetic variability. *Fungal Biology* **115**, 335-42.

Chapman AC, 2012. *The changing Phytophthora infestans population: implications for late blight epidemics and control*: University of Dundee, PhD.

Chowdappa P, Nirmal Kumar BJ, Madhura S, Mohan Kumar SP, Myers KL, Fry WE, Cooke DEL, 2015. Severe outbreaks of late blight on potato and tomato in South India caused by recent changes in the *Phytophthora infestans* population. *Plant Pathology* **64**, 191-9.

Cohen Y, Rubin E, Hadad T, Gotlieb D, Sierotzki H, Gisi U, 2007. Sensitivity of *Phytophthora infestans* to mandipropamid and the effect of enforced selection pressure in the field. *Plant Pathology* **56**, 836-42.

Cohen Y, Rubin AE, Galperin M, 2018. Oxathiapiprolin-based fungicides provide enhanced control of tomato late blight induced by mefenoxam-insensitive *Phytophthora infestans*. *PLoS One* **13**, e0204523.

Cooke DEL, Andersson B, 2013. *Phytophthora infestans* and potato late blight in Europe. In: Lamour K, ed. *In Phytophthora: A global perspective.* CABI International, 59-67.

Cooke DEL, Cano LM, Raffaele S, Bain RA, Cooke LR, Etherington GJ, Deahl KL, Farrer RA, Gilroy EM, Goss EM, Grunwald NJ, Hein I, Maclean D, Mcnicol JW, Randall E, Oliva RF, Pel MA, Shaw DS, Squires JN, Taylor MC, Vleeshouwers VG, Birch PR, Lees AK, Kamoun S, 2012. Genome analyses of an aggressive and invasive lineage of the Irish potato famine pathogen. *PLoS Pathogens* **8**, e1002940.

Cooke DEL, Kessel GJT, Lassen P, Baby S, Hansen JG, 2015. Update on European *P. infestans* populations; new tools, new insights. In. *15th EuroBlight Workshop.* Brasov, Romania.

Cooke DEL, Lees AK, 2004. Markers, old and new, for examining *Phytophthora infestans* diversity. *Plant Pathology* **53**, 692-704.

Cooke DEL, Lees AK, Chapman AC, Cooke LR, Bain RA, 2014. GB Late Blight Populations: monitoring and implications of population changes. *Potato Council project R423 final report.* Agriculture and Horticulture Development Board. p96. https://projectblue.blob.core.windows.net/media/Default/Research%20Papers/Potatoes/Final%20Report_R423.pdf

Cooke DEL, 2019. GB Late Blight Population Monitoring 2014 to 2018. AHDB Potatoes Report No. 2019/10. Agriculture and Horticulture Development Board. p41. https://projectblue.blob.core.windows.net/media/Default/Research%20Papers/Potatoes/FAB_Final%20Report_2014to2018.pdf

Cooke DEL, Lees AK, Shaw DS, Bain RA, Ritchie F, Taylor MC, 2009. Survey of GB Blight Populations In. *Final report of Potato Council project R274.* AHDB Potatoes web site https://projectblue.blob.core.windows.net/media/Default/Research%20Papers/Potatoes/20106%20Final%20Report%20Blight%20Populations%20R274.pdf

Cooke DEL, Randall E, Sullivan L, Lees AK, 2018. The origins and implications of a novel population of *Phytophthora infestans* on potato crops in Scotland. *Proceedings of the Crop Protection in Northern Britain 2018: The Dundee Conference, Environmental Management and Crop Production*, *2018*. Apex City Quay Hotel, Dundee, pp203-8.

Cooke DEL, Young V, Birch PRJ, Toth R, Gourlay F, Day JP, Carnegie SF, Duncan JM, 2003. Phenotypic and genotypic diversity of *Phytophthora infestans* populations in Scotland (1995-97). *Plant Pathology* **52**, 181-92.

Cooke DEL, Lynott L, Sullivan L, Skelsey P, Torro-Galiana A, Lees AK 2020. Integrated potato late blight management in response to an evolving pathogen population. The Dundee Conference, Crop Production in Northern Britain, Dundee, UK, 25-26 February 2020 p.205-210.

Cooke LR, 2015. The potato blight population in Northern Ireland. In: Schepers HTaM, ed. *Proceedings of the 15th EuroBlight workshop*, *2015*. Brasov, Romania, 31-44.

Cooke LR, Carlisle DJ, Donaghy C, Quinn M, Perez FM, Deahl KL, 2006. The Northern Ireland *Phytophthora infestans* population 1998-2002 characterized by genotypic and phenotypic markers. *Plant Pathology* **55**, 320-30.

Cooke LR, Little G, Wilson DG, 1998. Sensitivity of *Phytophthora infestans* to fluazinam and its use in potato blight control in Northern Ireland. In: *Proceedings Brighton Crop Protection Conference, Pests and Diseases*-1998, 517-522.

Defra 2013. UK National Action Plan for the Sustainable Use of Pesticides (Plant Protection Products) February 2013. www.defra.gov.uk

Day JP, Wattier RaM, Shaw DS, Shattock RC, 2004. Phenotypic and genotypic diversity in *Phytophthora infestans* on potato in Great Britain, 1995–98. *Plant Pathology* **53**, 303-15.

Dey T, Saville A, Myers K, Tewari S, Cooke DEL, Tripathy S, Fry WE, Ristaino JB, Guha Roy S, 2018. Large sub-clonal variation in *Phytophthora infestans* from recent severe late blight epidemics in India. *Scientific Reports* **8**.

Drenth A, Janssen EM, Govers F, 1995. Formation and survival of oospores of *Phytophthora infestans* under natural conditions. *Plant Pathology* **44**, 86-94.

Duvauchelle S, Ruccia D, 2015. Mancozeb: essential tool for sustainable protection of potato against late blight. Proceedings of the Euroblight workshop PPO- Special Report No17, 2015, 109-118.

Förch M, Kessel G, Spits H, Hasunuma N, 2017. Baseline sensitivity of *Phytophthora infestans* lifecycle components to NC–224 20SC (Amisulbrom 200 g/l). Tenth Workshop of an European Network for development of an Integrated Control Strategy of potato late blight Bologna (Italy).

FRAG-UK, 2018. Fungicide resistance management in potato late blight. Report of the Fungicide Resistance Action Group UK, May 2018. p8. https://media.ahdb.org.uk/media/Default/Imported%20Publication%20Docs/AHDB%20Cereals%20&%20Oilseeds/Disease/FRAG%20Potato%20late%20blight%20guidelines%20(May%202018).pdf

Garthwaite D, Barker I, Ridley L, Mace A, Parrish G, MacArthur R, Lu Y, 2018. Pesticide usage survey report 271: arable crops in the United Kingdom 2016. Pesticide Usage Survey Team, Fera UK. https://secure.fera.defra.gov.uk/pusstats/surveys/documents/arable2016-v9.pdf

Garthwaite D, Ridley L, Mace A, Parrish G, Barker I, Rainford J, MacArthur R, 2019. Pesticide usage survey report 284: arable crops in the United Kingdom 2018. Pesticide Usage Survey Team, Fera UK. https://secure.fera.defra.gov.uk/pusstats/surveys/documents/arable2018.pdf

Gisi U, Cohen Y, 1996. Resistance to phenylamide fungicides: a case study with *Phytophthora infestans* involving mating type and race structure. *Annu Rev Phytopathol* **34**, 549-72.

Grünwald NJ, Sturbaum AK, Montes GR, Serrano EG, Lozoya-Saldaña H, Fry WE 2006. Selection for fungicide resistance within a growing season in field populations of *Phytophthora infestans* at the center of origin. *Phytopathology*. **96**, 1397-403.

Guha Roy S, Dey T, Cooke DEL, Cooke LR, 2021. The dynamics of *Phytophthora infestans* populations in the major potato-growing regions of Asia – A review. *Plant Pathology* **70**, 1015-31.

Haas BJ, Kamoun S, Zody MC, Jiang RH, Handsaker RE, Cano LM, Grabherr M, Kodira CD, Raffaele S, Torto-Alalibo T, Bozkurt TO, Ah-Fong AM, Alvarado L, Anderson VL, Armstrong MR, Avrova A, Baxter L, Beynon J, Boevink PC, Bollmann SR, Bos JI, Bulone V, Cai G, Cakir C, Carrington JC, Chawner M, Conti L, Costanzo S, Ewan R, Fahlgren N, Fischbach MA, Fugelstad J, Gilroy EM, Gnerre S, Green PJ, Grenville-Briggs LJ, Griffith J, Grunwald NJ, Horn K, Horner NR, Hu CH, Huitema E, Jeong DH, Jones AM, Jones JD, Jones RW, Karlsson EK, Kunjeti SG, Lamour K, Liu Z, Ma L, Maclean D, Chibucos MC, Mcdonald H, Mcwalters J, Meijer HJ, Morgan W, Morris PF, Munro CA, O'Neill K, Ospina-Giraldo M, Pinzon A, Pritchard L, Ramsahoye B, Ren Q, Restrepo S, Roy S, Sadanandom A, Savidor A, Schornack S, Schwartz DC, Schumann UD, Schwessinger B, Seyer L, Sharpe T, Silvar C, Song J, Studholme DJ, Sykes S, Thines M, Van De Vondervoort PJ, Phuntumart V, Wawra S, Weide R, Win J, Young C, Zhou S, Fry W, Meyers BC, Van West P, Ristaino J, Govers F, Birch PR, Whisson SC, Judelson HS, Nusbaum C, 2009. Genome sequence and analysis of the Irish potato famine pathogen *Phytophthora infestans*. *Nature* **461**, 393-8.

Kato M, Mizubuti ES, Goodwin SB, Fry WE, 1997. Sensitivity to protectant fungicides and pathogenic fitness of clonal lineages of *Phytophthora infestans* in the United States. *Phytopathology*, **87**, 973-978

Kamvar ZN, Brooks JC, Grunwald NJ, 2015. Novel R tools for analysis of genome-wide population genetic data with emphasis on clonality. *Frontiers in Genetics* **6**, 208.

Kessel GJT, Mullins E, Evenhuis A, Stellingwerf J, Cortes VO, Phelan S, Van Den Bosch T, Förch MG, Goedhart P, Van Der Voet H, Lotz LaP, 2018. Development and validation of IPM strategies for the cultivation of cisgenically modified late blight resistant potato. *European Journal of Agronomy* **96**, 146-55.

Kildea S, Quinn L, Mehenni-Ciz J, Cooke DEL, Perez FM, Deahl KL, Griffin D, Cooke LR, 2012. Re-emergence of the Ib mitochondrial haplotype within the British and Irish *Phytophthora infestans* populations. *European Journal of Plant Pathology* **135**, 237-42.

Latorse MP, Kuck KH, 2006. *Phytophthora infestans*: Baseline sensitivity and resistance management for fluopicolide. *Pflanzenschutz-Nachrichten Bayer* 59 2-3 p317-321.

Lees AK, Wattier R, Shaw DS, Sullivan L, Williams NA, Cooke DEL, 2006. Novel microsatellite markers for the analysis of *Phytophthora infestans* populations. *Plant Pathology* **55**, 311-9.

Lees AK, 2018. Comparison of sensitivity to a range of fungicides in contemporary genotypes of *Phytophthora infestans*. AHDB project report Ref: 11120047 https://potatoes.ahdb.org.uk/sites/default/files/publication_upload/11120047%20Fungicide%20Sensitivity%20Testing%202018%20Report_Final.pdf

Lehtinen A, Hannukkala A, 2004. Oospores of *Phytophthora infestans* in soil provide an important new source of primary inoculum in Finland. *Agricultural and Food Science* **13**, 399-410.

Li Y, Cooke DE, Jacobsen E, Van Der Lee T, 2013a. Efficient multiplex simple sequence repeat genotyping of the oomycete plant pathogen *Phytophthora infestans*. *Journal of Microbiological Methods* **92**, 316-22.

Li Y, Van Der Lee T, Zhu JH, Jin GH, Lan CZ, Zhu SX, Zhang RF, Liu BW, Zhao ZJ, Kessel G, Huang SW, Jacobsen E, 2013b. Population structure of *Phytophthora infestan*s in China - geographic clusters and presence of the EU genotype Blue_13. *Plant Pathology* **62**, 932-42.

Li Y, Van Der Lee TA, Evenhuis A, Van Den Bosch GB, Van Bekkum PJ, Forch MG, Van Gent-Pelzer MP, Van Raaij HM, Jacobsen E, Huang SW, Govers F, Vleeshouwers VG, Kessel GJ, 2012. Population dynamics of *Phytophthora infestans* in the Netherlands reveals expansion and spread of dominant clonal lineages and virulence in sexual offspring. *G3 (Bethesda)* **2**, 1529-40.

Mariette N, Mabon R, Corbière R, Boulard F, Glais I, Marquer B, Pasco C, Montarry J, Andrivon D, 2016. Phenotypic and genotypic changes in French populations of *Phytophthora infestans*: are invasive clones the most aggressive? *Plant Pathology* **65**, 577-86.

Martin FN, Zhang Y, Cooke DEL, Coffey MD, Grunwald NJ, Fry WE, 2019. Insights into evolving global populations of *Phytophthora infestans* via new complementary mtDNA haplotype markers and nuclear SSRs. *PLoS One* 14, e0208606.

Miao J, Liu X, Li G, Du X, Liu X, 2020. Multiple point mutations in PsORP1 gene conferring different resistance levels to oxathiapiprolin confirmed using CRISPR-Cas9 in *Phytophthora sojae*. *Pest Manag Sci* **76**, 2434-40.

Mitani et al. 2001. Antifungal activity of the novel fungicide cyazofamid against *Phytophthora infestans* and other plant pathogenic fungi in vitro. *Pesticide Biochemistry and Physiology* 70, 92–99.

Nnadi NE, Datiri AM, Pam DB, Ngene AC, Okonkwo FO, Sullivan L, Cooke DEL, 2019. First report of the EU_33_A2 clonal lineage of *Phytophthora infestans* causing late blight disease of potato in Nigeria. *New Disease Reports* **40**, 20.

Pettitt TR, Keane GJ, John SOL, Cooke DEL, Žerjav M, 2019. Atypical late blight symptoms following first recorded infections by *Phytophthora infestans* genotype EU_39_A1 in UK vine tomatoes. *New Disease Reports* **39**, 16.

Puidet B, Mabon R, Guibert M, Kiiker R, Soonvald L, Le VH, Eikemo H, Dewaegeneire P, Saubeau G, Chatot C, Aurousseau F, Cooke D, Lees AK, Abuley I, Hansen JG, Corbiere R, Leclerc M, Andrivon D, 2021. Examining phenotypic traits contributing to the spread in northern European potato crops of EU_41_A2, a new clonal lineage of *Phytophthora infestans*. *Phytopathology*. 10.1094/PHYTO-12-20-0542-R

Qu T, Shao Y, Csinos AS, Pingsheng J, 2016. Sensitivity of *Phytophthora nicotianae* from tobacco to fluopicolide, mandipropamid, and oxathiapiprolin. *Plant Disease,* **100**, 2119-2125.

Reis A, Ribeiro FHS, Maffia LA, Mizubuti ESG, 2005. Sensitivity of Brazilian isolates of *Phytophthora infestans* to commonly used fungicides in tomato and potato crops. *Plant Disease* **89**, 1279-84.

Saville A, Graham K, Grunwald NJ, Myers K, Fry WE, Ristaino JB, 2015. Fungicide sensitivity of U.S. genotypes of *Phytophthora infestans* to six oomycete-targeted compounds. *Plant Disease* **99**, 659-66.

Schepers HTAM, Kessel GJT, Lucca F, Förch MG, Van Den Bosch GBM, Topper CG, Evenhuis A, 2018. Reduced efficacy of fluazinam against *Phytophthora infestans* in the Netherlands. *European Journal of Plant Pathology* **151**, 947-60.

Schepers HTAM, Strypstein C, Meier-Runge F, Sierotzki H, Scalliet G, Forch M, Van Den Bosch GB, Topper C, Kessel G, Evenhuis A, 2013. Reduced efficacy of fluazinam against some Green 33 isolates of *Phytophthora infestans* in the Netherlands. In. *The 14th EuroBlight workshop* Limassol, Cyprus.

Sjöholm L, Andersson B, Högberg N, Widmark A-K, Yuen J, 2013. Genotypic diversity and migration patterns of *Phytophthora infestans* in the Nordic countries. *Fungal Biology* **117**, 722-30.

Skelsey P, Dancey SR, Preedy K, Lees AK, Cooke DEL, 2018. Forecasting the spread of aerially transmitted crop diseases with a binary classifier for inoculum survival. *Plant Pathology* **67**, 920-8.

Skelsey P, Kessel GJ, Rossing WA, Van Der Werf W, 2009. Parameterization and evaluation of a spatiotemporal model of the potato late blight pathosystem. *Phytopathology* **99**, 290-300.

Gaucher D, Chatot C, Vacher S, Steva H, 2017. Monitoring sensitivity to CAA, QiI and Fluazinam among populations of *Phytophtora infestans* collected from French potato producing areas in 2016. 16th Euroblight Workshop, Aarhus. May 14-17 2017. Presentation https://agro.au.dk/fileadmin/8_STEVA.pdf

Turkensteen LJ, Flier WG, Wanningen R, Mulder A, 2000. Production, survival and infectivity of oospores of *Phytophthora infestans*. *Plant Pathology* **49**, 688-96.

Widmark AK, Andersson B, Cassel-Lundhagen A, Sandström M, Yuen JE, 2007. *Phytophthora infestans* in a single field in southwest Sweden early in spring: symptoms, spatial distribution and genotypic variation. *Plant Pathology* **56**, 573-9.

Widmark AK, Andersson B, Sandström M, Yuen JE, 2011. Tracking *Phytophthora infestans* with SSR markers within and between seasons - a field study in Sweden. *Plant Pathology* **60**, 938-45.

Wynn S, Ritchie F, Foulkes R, Rubinstein O, 2017. Mancozeb: Its Value to the EU Potato Industry. *Journal of Food Science and Engineering* 7. 359-362.

Yuen JE, Andersson B, 2013. What is the evidence for sexual reproduction of *Phytophthora infestans* in Europe? *Plant Pathology* **62**, 485-91.

Zadoks JC, 2008. The Potato Murrain on the European Continent and the Revolutions of 1848. *Potato Research* 51, 5-45.

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 <u>through</u> 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*).

<u>Allow the FTA card to air dry</u> 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.

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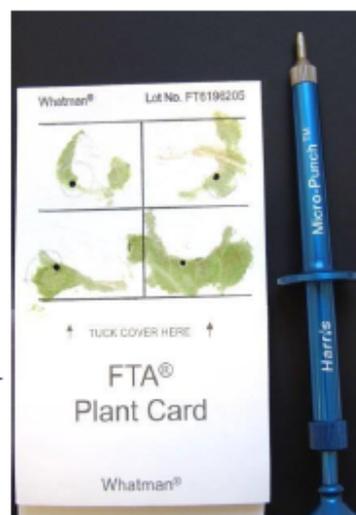
Figure 3: Card after processing in the laboratory (holes punched)

Appendix 2. Blight scout response form

FIGHT AGAINST BLIGHT
Response form - 2020

AHDB

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**