Comparison of sensitivity to a range of fungicides in contemporary genotypes of *Phytophthora infestans* in 2019

Aim: Determine the relative sensitivity of isolates of genotypes EU_36_A2 and EU_37_A2 compared with control isolates to cyazofamid, propamocarb, mandipropamid, fluopicolide, oxathiopiprolin, amisulbrom and mancozeb.

Materials and Methods

Fungicides tested are listed below

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 a.i. (Sigma Aldrich)	1.6	200-400	500
OSBPI (49)	Oxathiopiprolin 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

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) *P. infestans* genotypes. In order 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, isolates of this genotype were sourced from archived isolates from the 2018 epidemic.

The inclusion of isolate 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 were obtainable from collaborators.

Inoculum production

Cultures of isolates were established on Rye A agar, transferred onto leaves of cv. Craigs Royal and multiplied for at least two generations, 7–10 days/generation before use in tests. Sporangia were washed off the leaves with sterile water and the concentration of each suspension was adjusted to 5×10^4 sporangia mL^{-1} (unless stated differently in individual methods). Suspensions were chilled for 2h at 4–5°C to release motile zoospores, which was confirmed by microscopic examination before inoculation.

Production of plant material

All sensitivity tests carried out using detached leaf protocols 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 18°C 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 in the table below:

Active ingredients (a.i)	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
oxathiopiprolin	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

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.

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.

Lesion Area (mm^2) data presented as Box & Whisker plots

A box and whisker chart shows 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.

Sensitivity to Fluopicolide

Background

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) 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 and Kuck (2006) using the range of concentration specified in their original analysis to allow changes in baseline sensitivity compared with isolates from across Europe tested from 2001-2006 to potentially be identified – their data is listed below. The original assays of Latorse and Kuck (2006) were conducted using a floating leaf disc test while this assessment 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.

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

Latorse M.P & Kuck K.H. *Phytophthora infestans*: Baseline sensitivity and resistance management for fluopicolide. Pflanzenschutz-Nachrichten Bayer 59 (2006)2-3 p317-321.

Product tested: Fluopicolide technical grade (5mg/ml)

Concentrations tested: 100, 10, 5, 1, 0.5, 0 µg fluopicolide/ml

Isolates tested: 36_A2 (n=5), 37_A2 (n=5) and 6_A1 (n=5)

Results of 2019 sensitivity testing - fluopicolide

All untreated leaves produced lesions with all isolates (Fig 1) indicating good test conditions and suitability of isolates for testing. Figure 1 shows the incidence of lesions for a genotype at different concentrations of fluopicolide. There was a high incidence of lesions at concentrations of fluopicolide ≤ 1ppm, particularly in isolates of 36_A2. 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. Figure 2 shows the mean lesion size calculated for the infected leaves only. 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. 3)

EC_{50} values are given in Table 1. There is 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.

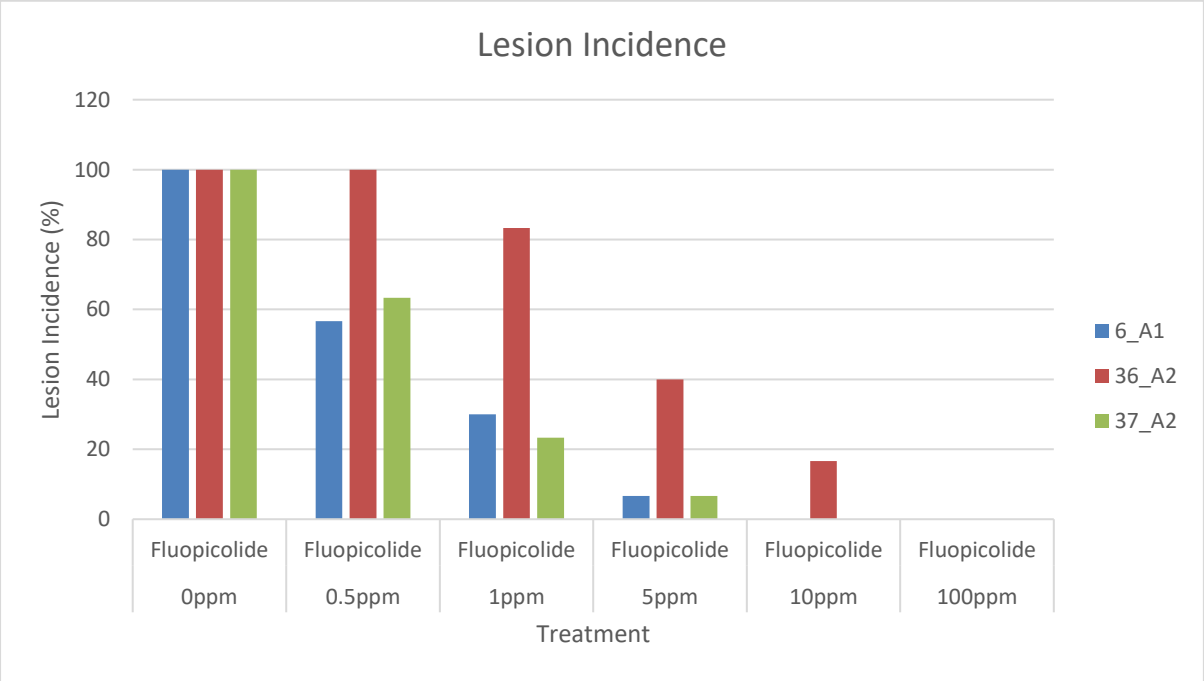


Fig 1. Incidence of lesions (%) caused by each genotype observed at different concentrations of fluopicolide.

Field rate fluopicolide = 500ppm

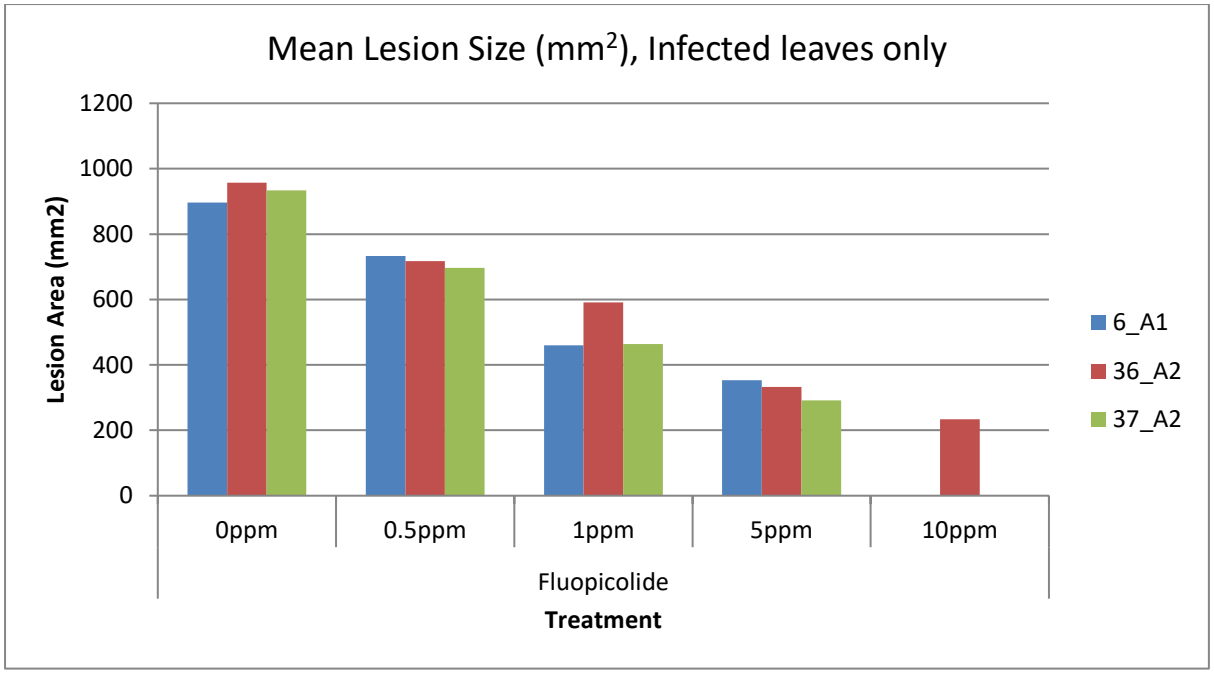
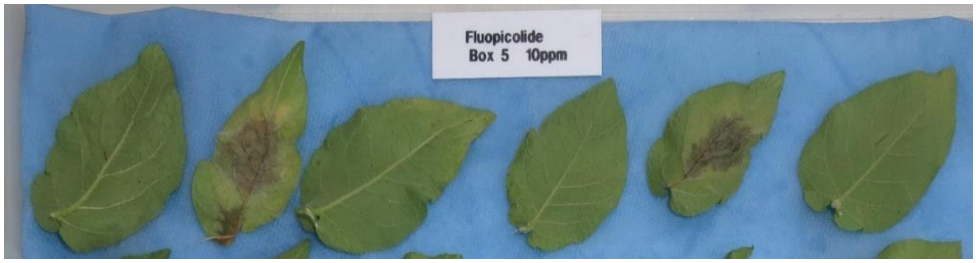


Fig 2. Mean lesion size (mm^2) of genotypes (n= 5 isolates) at different concentrations of fluopicolide (mean of infected leaves only).



Leaves showing disease symptoms caused by isolates of 36_A2 at 10µg/ml fluopicolide.

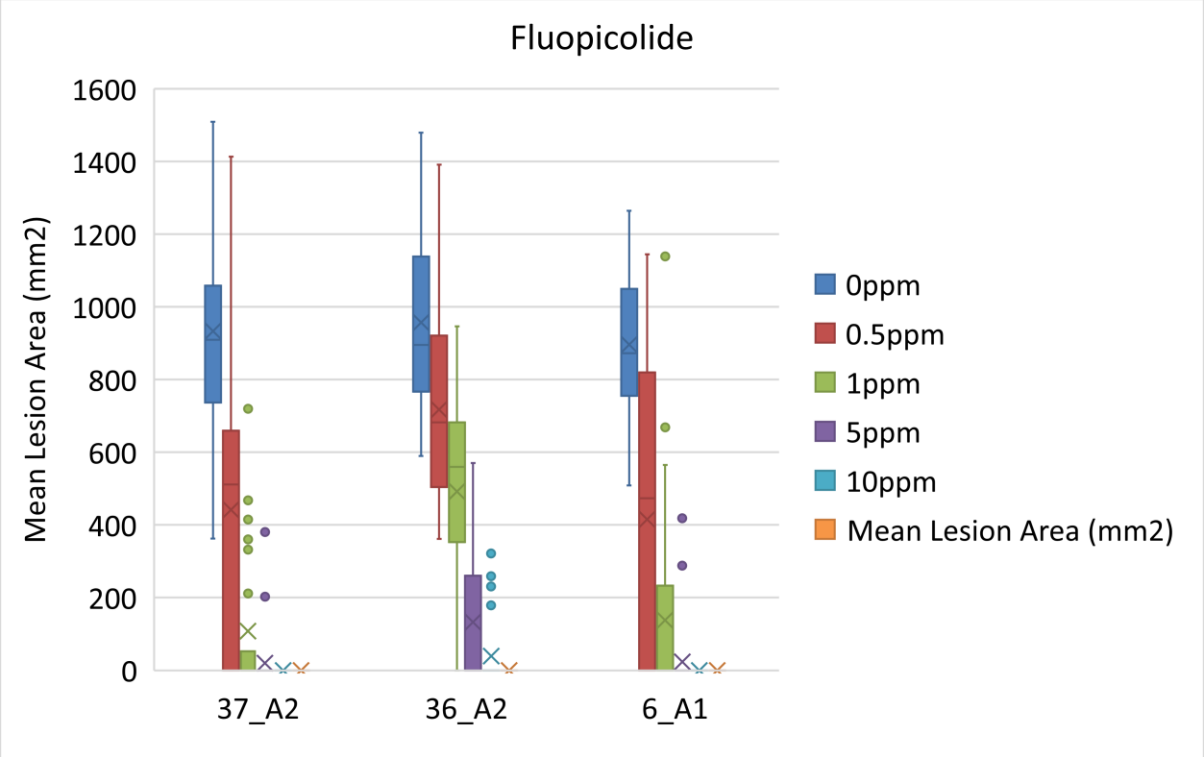


Fig 3. Mean Lesion area (mm2) of isolates belonging to 36_A2 and 37_A2 compared with control isolates (6_A1) presented as a box and whisker plot. Fluopicolide Max Field concentration (as Infinito) = 500ppm

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 1 Mean, max and min EC_{50} values for isolates of *P. infestans* of various genotypes tested at a range of concentrations of fluopicolide. Significant differences between mean values are indicated by different letters. 2018 results are shown in brackets.

Conclusion

EC_{50} values in this test were in line with previous baseline sensitivity testing of fluopicolide. Mean EC_{50} values for genotype 36_A2 were statistically higher than for other genotypes but were still within the expected range. 2019 results are consistent with 2018.

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).

Cooke, L.R., Little, G., & Wilson, D.G. (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.

Schepers, H. T. A. M., et al. (2018). Reduced efficacy of fluazinam against *Phytophthora infestans* in the Netherlands. European Journal of Plant Pathology 151(4): 947-960.
for Fluazinam

Materials and Methods

Sporangial suspensions (10^5 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 scale of 1-3, 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.

Results

		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)

Table 1. 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 and can only be compared with other values in brackets from the same year.

Conclusion

In line with the results of the detached leaf test, the MIC (defined as the lowest concentration of fluopicolide which completely inhibited zoospore motility) was shown to be significantly higher, on

average, for isolates of genotype EU_36_A2 compared with isolates of genotypes EU_6_A1 and EU_37_A2, although the average values appear to be in the same range as observed in 2018.

Mandipropamid

Background

Cohen et al (2007) previously tested sensitivity to the carboxylic acid amide (CAA) fungicide mandipropamid in *Phytophthora infestans* isolates 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). Insensitivity to azoxystrobin, cyazofamid, cymoxanil, fluopicolide, or mandipropamid was not detected within any lineage. EC_{50} values for mandipropamid from this work are shown below.

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

Taken from Saville et al (2015). 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. 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.

Y. Cohen, E. Rubin, T. Hadad,D. Gotlieb,H. Sierotzki, U. Gisi (2007). Sensitivity of *Phytophthora infestans* to mandipropamid and the effect of enforced selection pressure in the field Plant Pathology, 56, 729-910.October 2007

Amanda Saville, Kim Graham, Niklaus J. Grünwald, Kevin Myers, William E. Fry, and Jean Beagle Ristaino (2015). Fungicide Sensitivity of U.S. Genotypes of *Phytophthora infestans* to Six Oomycete-Targeted Compounds. Plant Disease 2015 99:5, 659-666.

Materials and Methods.

As described previously. Detached leaf test 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, 10.0µg/ml (according to FRAC protocol concentrations).

Results

All untreated leaves produced lesions (Fig 4) indicating good test conditions and suitability of isolates for testing. Figure 4 shows the mean incidence of lesions for each genotype at different concentrations of mandipropamid (0, 0.1, 0.3, 1.0, 3.0 and 10µg/ml).

There was a high incidence of lesions at concentrations up to 0.3 µg/ml mandipropamid with a lower incidence at 1-10µg/ml. Mean lesion size on infected leaves only is shown in Fig 5. 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. 6)

EC_{50} values are given in Table 2. EC_{50} values in this test were in line with previous sensitivity testing of mandipropamid. 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.

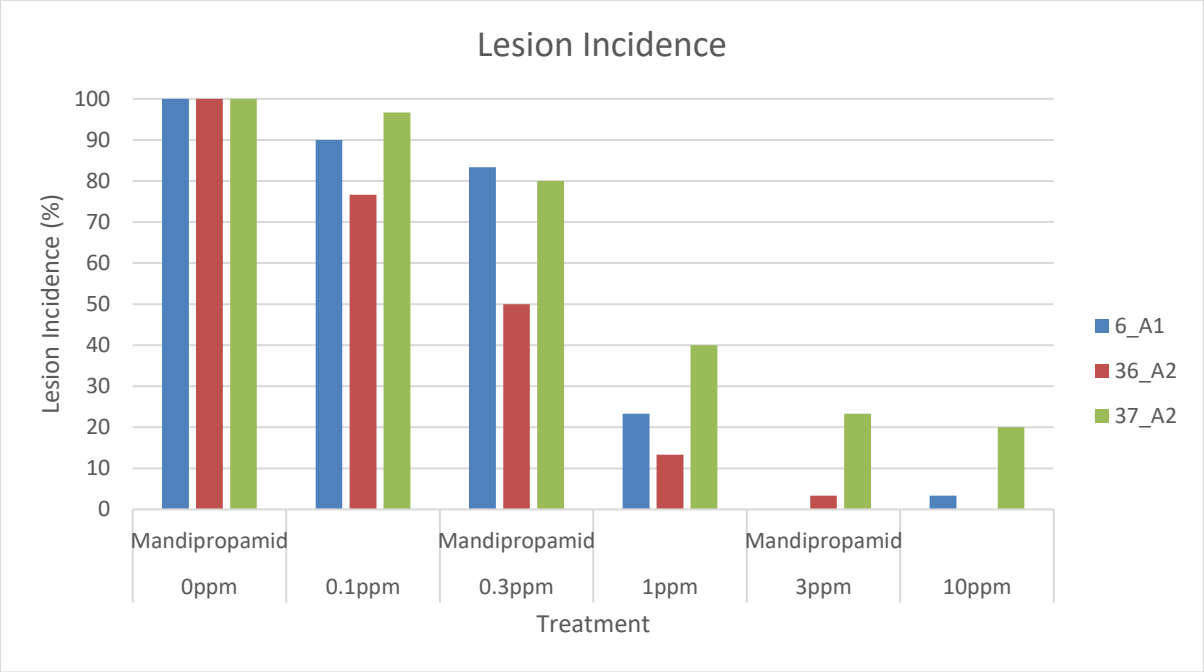


Fig 4. Mean percentage of lesions caused by different *P. infestans* genotypes at a range of concentrations of mandipropamid (0-10 µg/ml).

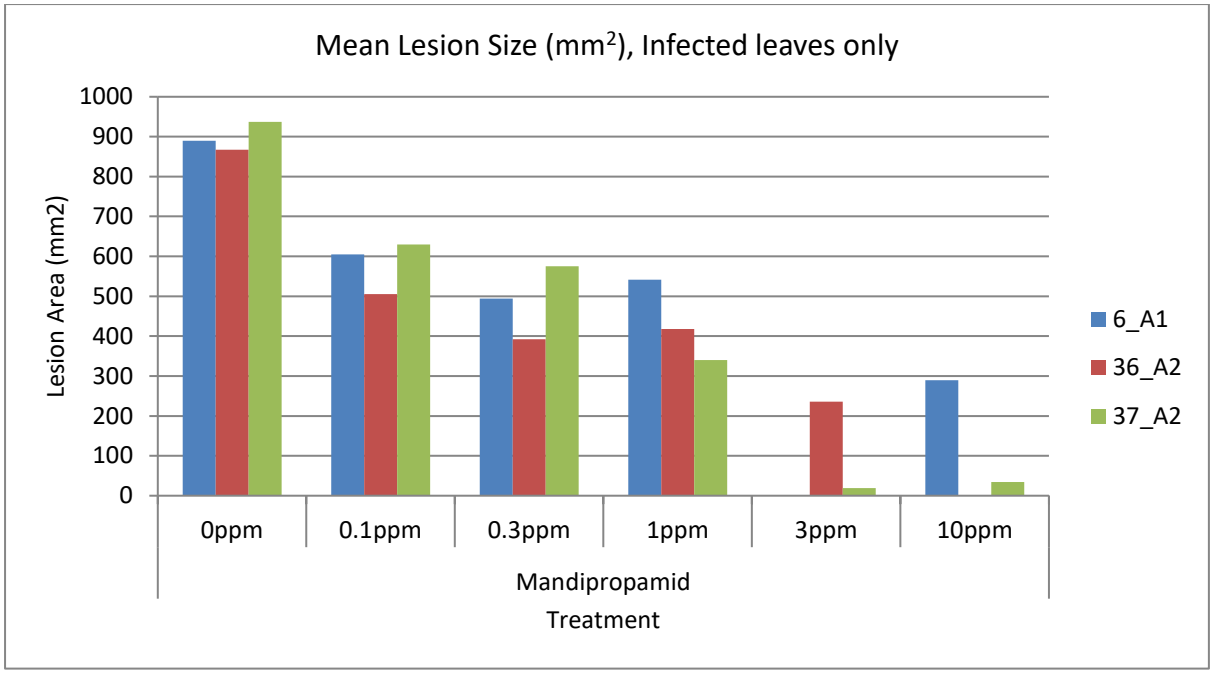


Fig 5. Mean lesion size (mm^2) measured on infected leaves only at a range of concentrations of mandipropamid. Field rate of mandipropamid is 750ppm.

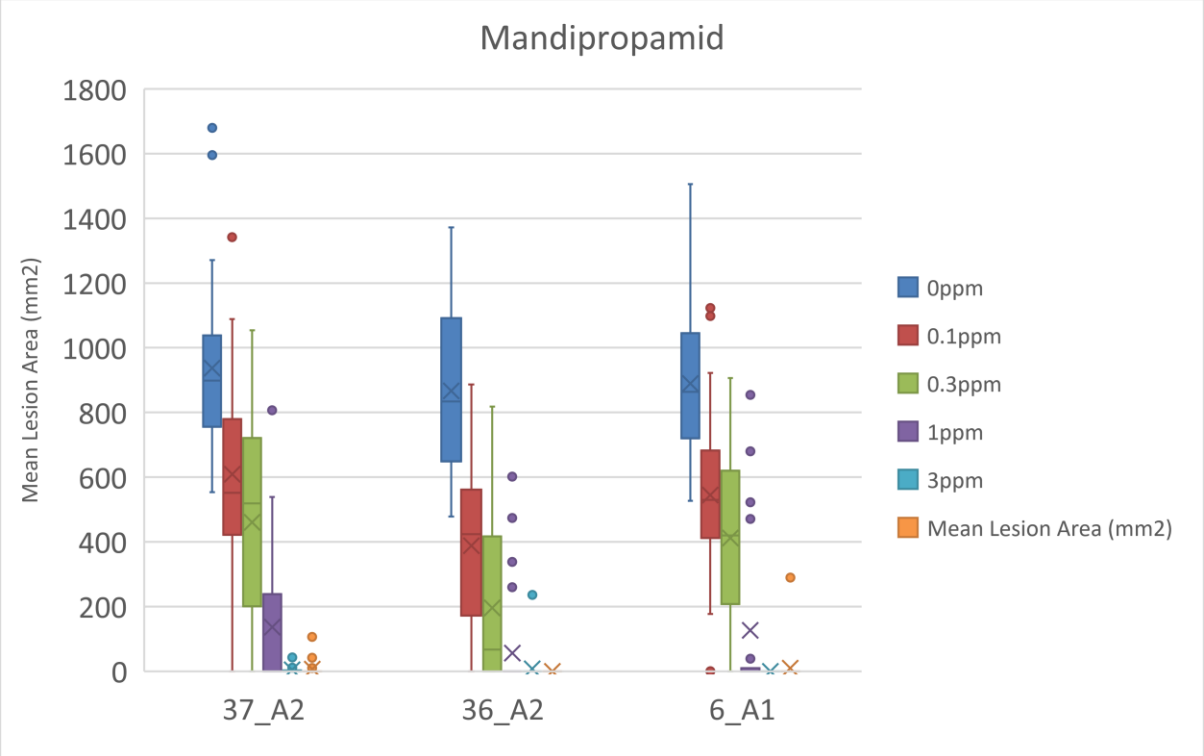


Fig 6. Mean Lesion area (mm2) of isolates belonging to 36_A2 and 37_A2 compared with control isolates (6_A1) presented as a box and whisker plot. Mandipropamid Max Field concentration = **7**50ppm

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)

Table 2. Mean, max and min EC_{50} values for isolates of *P. infestans* of various genotypes tested 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 and can only be compared with other numbers in brackets.

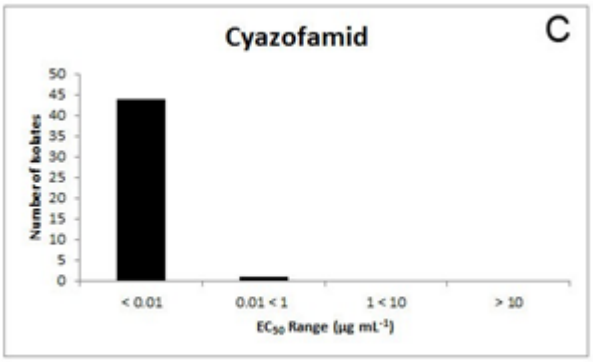
Conclusion

EC_{50} values in this test were in line with previous sensitivity testing of mandipropamid. 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.

Sensitivity to Cyazofamid

Background

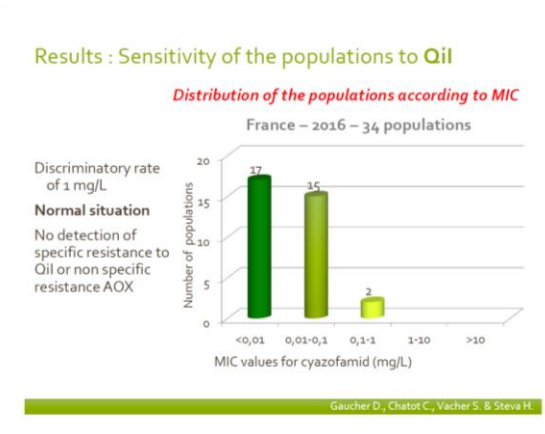
In tests conducted on amended media, Saville et al (2015) found that most isolates of US genotypes tested did not grow on media amended with cyazofamid, and a sharp decline in growth was observed at all concentrations above 0.1 µg ml^{-1}. The Figure below, (taken from Supplementary Fig S1 Saville *et al*., 2015) shows the EC_{50} range of the isolates tested. The only exception was a 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 by *P. infestans* 60 min after treatment.

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 (2001)

Sensitivity tests conducted on French *P. infestans* populations of unknown genotype in 2016 ((http://euroblight.net/fileadmin/euroblight/Presentations/8_STEVA.pdf) using leaf disc assays inoculated with fungicide amended inoculum reported that all isolates were controlled at a maximum concentration of 1mg/l (1µg/ml).



Materials and Methods

As described previously. Detached leaf test 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 concentrations).

Results

All untreated leaves produced lesions (Fig 7) indicating good test conditions and suitability of isolates for testing. Figure 7 shows the mean incidence of lesions for each genotype at different concentrations of cyazofamid (0, 0.1, 0.3, 1.0, 3.0 and 10µg/ml). There was a moderate incidence of lesions at 0.1µg/ml cyazofamid with a lower incidence at 0.3 - 3µg/ml. There was a very low incidence (1 lesion caused by one isolate) at 10µg/ml cyazofamid (shown in Fig.8). This is likely to be due to experimental error. Mean lesion size on infected leaves only is shown in Fig 9. 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. 10)

EC_{50} values calculated from test data are given in Table 3. There is no statistically significant difference in mean EC_{50} value between genotypes. It is difficult to interpret the mean EC_{50} 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 µg/ml cyazofamid when used directly on spore suspensions and these values seem to be in line with the results reported here using a detached leaf assay. Concentrations of cyazofamid required to control all isolates are very low overall compared with permitted field rates.

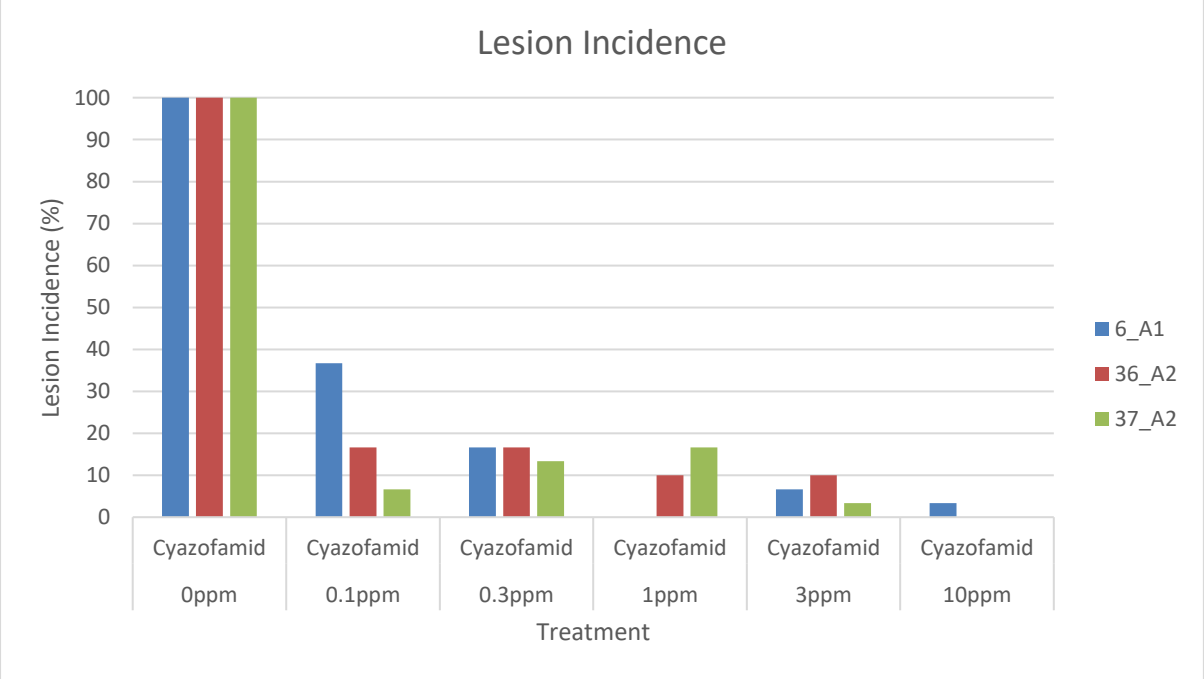


Fig 7. Mean percentage of lesions caused by different genotypes at a range of concentrations of cyazofamid (0-10 µg/ml).

Fig 8. A single lesion caused by an isolate of 6_A1 at 10ppm cyazofamid

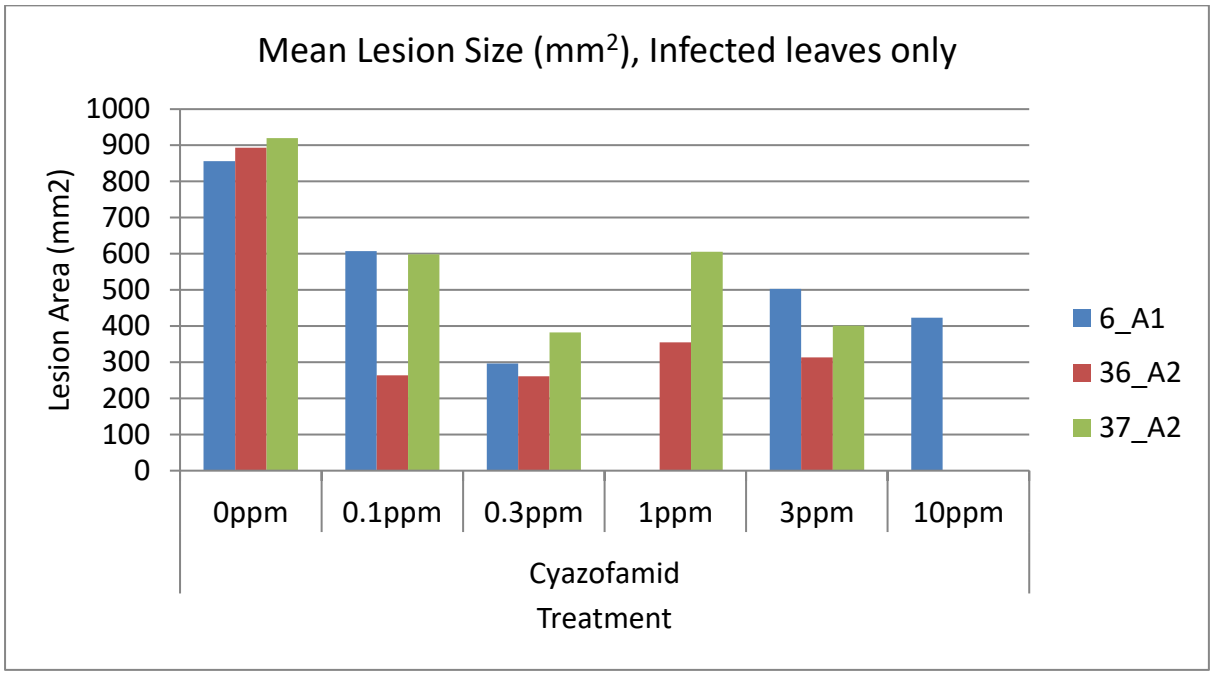


Fig 9. Mean lesion size (mm2) measured on infected leaves only at a range of concentrations of cyazofamid

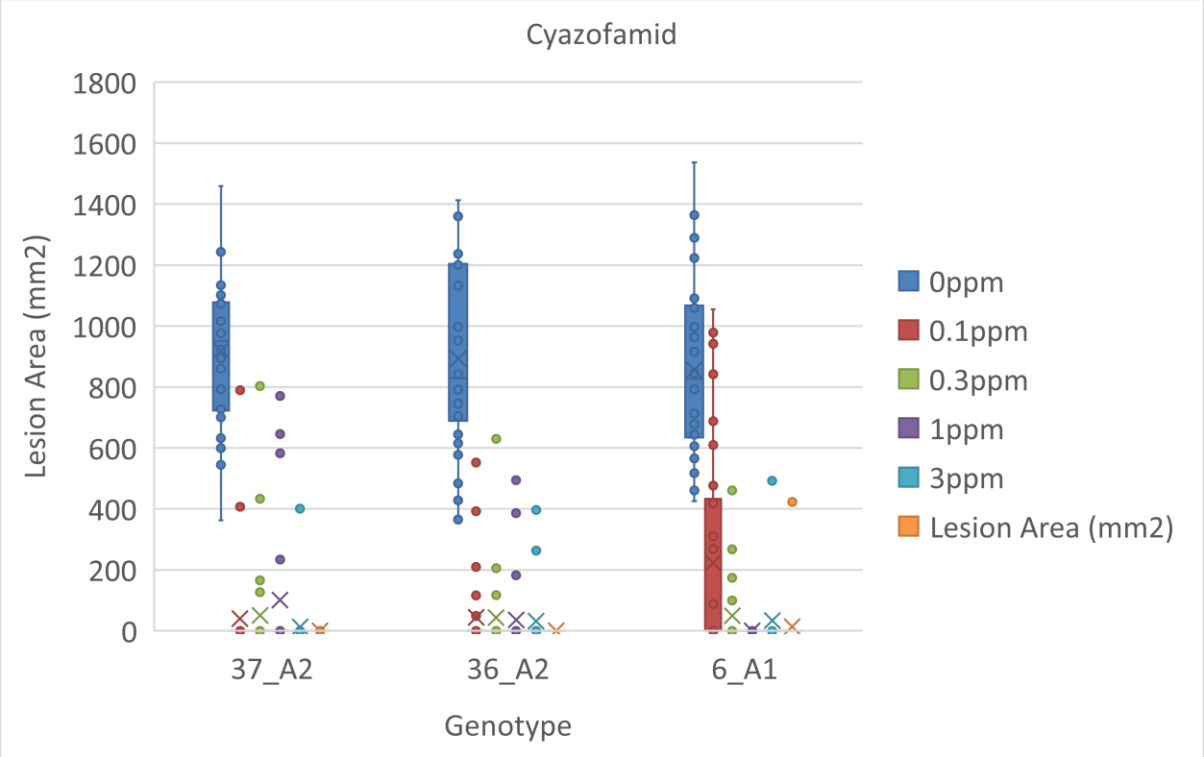


Fig.10 Mean Lesion area (mm2) of isolates belonging to 36_A2 and 37_A2 compared with control isolates (6_A1) presented as a box and whisker plot. Cyazofamid Max Field concentration = 400ppm

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)

Table 3. Mean, max and min EC_{50} values for isolates of *P. infestans* of various genotypes tested at a range of concentrations of cyazofamid (0, 0.1, 0.3, 1.0, 3.0, 10.0µg/ml). Significant differences between mean values are indicated by different letters. 2018 values are given in brackets for comparison and can only be compared with other values in brackets.

Conclusion

EC_{50} values in this test appear to be in line with 2018 data and previous sensitivity testing of cyazofamid conducted in other studies. There was no statistical difference in Mean EC_{50} values between genotypes. In 2018 only very marginal differences were observed and it was noted that additional isolates of all genotypes should be tested to draw any firm conclusion as to differences.

Sensitivity to Amisulbrom

Background

Previous work (Forch 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 *Phytophthora infestans*. The four stages selected were the release of zoospores, motility of zoospores, germination of cystospores and the formation of oospores *in planta*. Th e EC_{50} of NC-224 20 SC for zoospore release, motility of zoospores and germination of cystospores was found to be 0.016 ppm, 0.0002 ppm and 0.061 ppm. Oospore formation also responds 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.

MARIEKE FORCH, GEERT KESSEL, HARRO SPITS AND NAKAKO HASUNUMA. 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), 2007.

Materials and Methods

As described previously. Detached leaf test 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 concentrations).

Results

All untreated leaves produced lesions (Fig 11) indicating good test conditions and suitability of isolates for testing. Figure 11 shows the mean incidence of lesions for each genotype at different concentrations of amisulbrom (0, 0.1, 0.3, 1.0, 3.0 and 10µg/ml). Lesions were formed in isolates of 6_A1 in the range 0-1µg/ml and for isolates of 36_A2 and 37_A2 in the range 0-10µg/ml. Mean lesion size on infected leaves only is shown in Fig 12 and illustrated in a box plot in Fig 13. 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. 13).

EC_{50} values calculated from test data are given in Table 4. On average, isolates of 36_A2 had a significantly greater EC_{50} values than those belonging to genotypes 37_A2 and 6_A1.

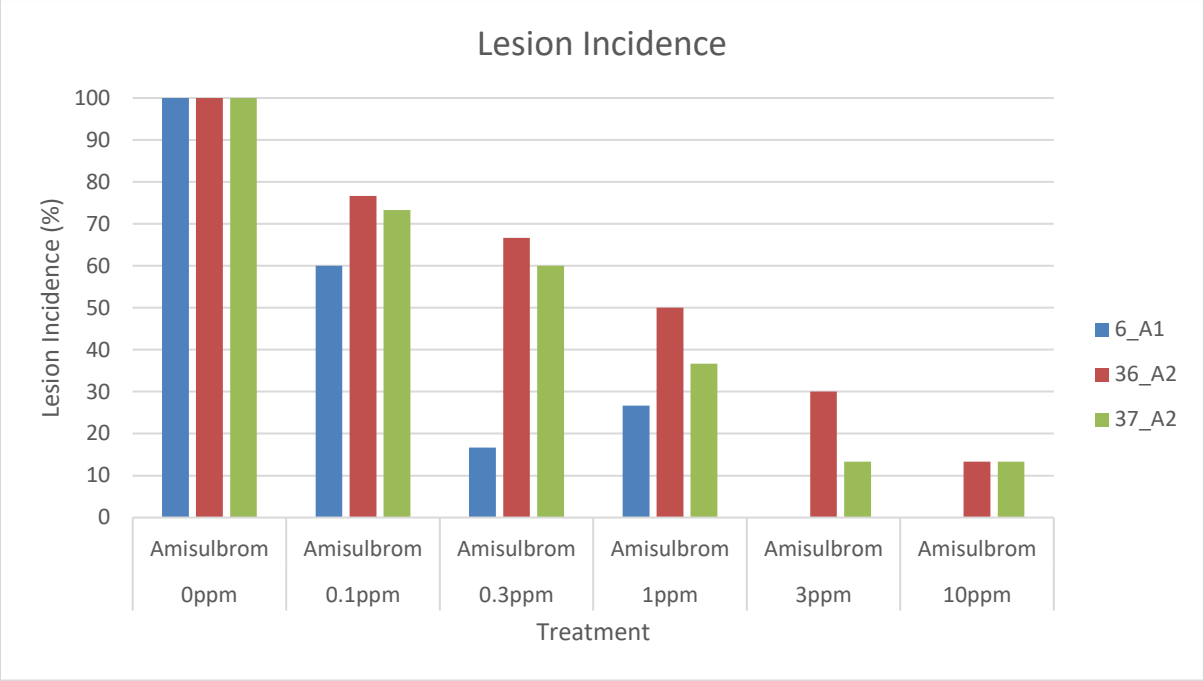


Fig 11. Mean percentage incidence of lesions caused by different genotypes at a range of concentrations of amisulbrom (0-10 µg/ml).

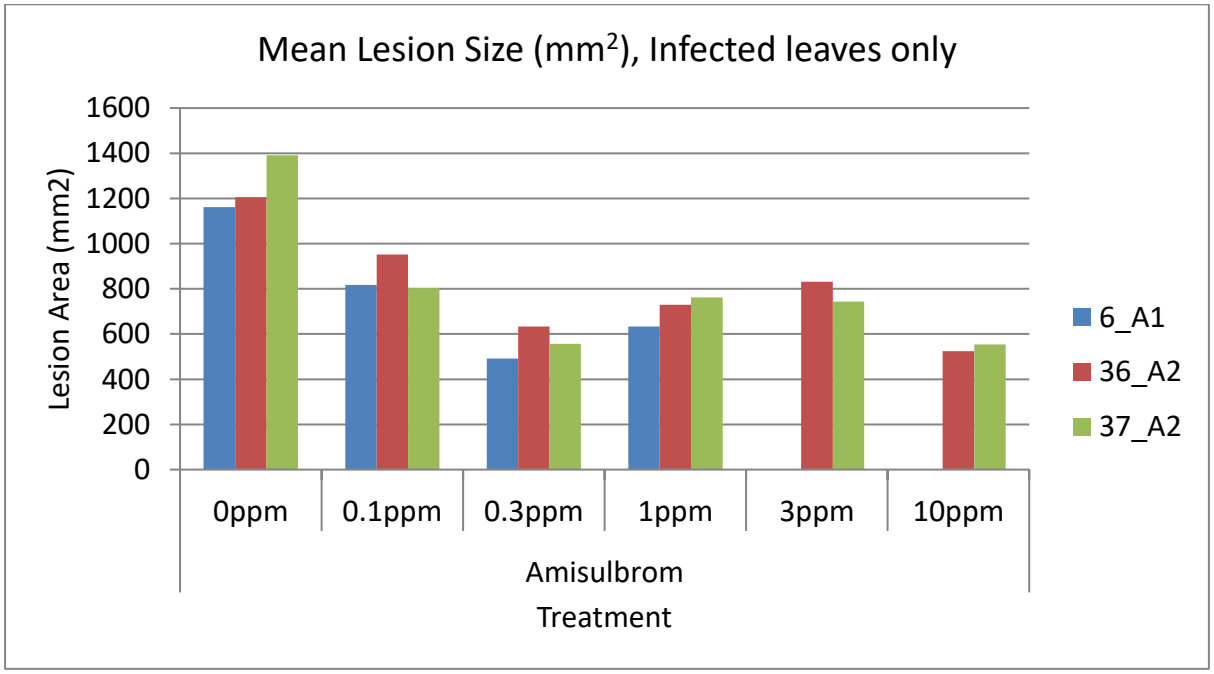


Fig 12. Mean lesion size (mm2) measured on infected leaves only at a range of concentrations of amisulbrom

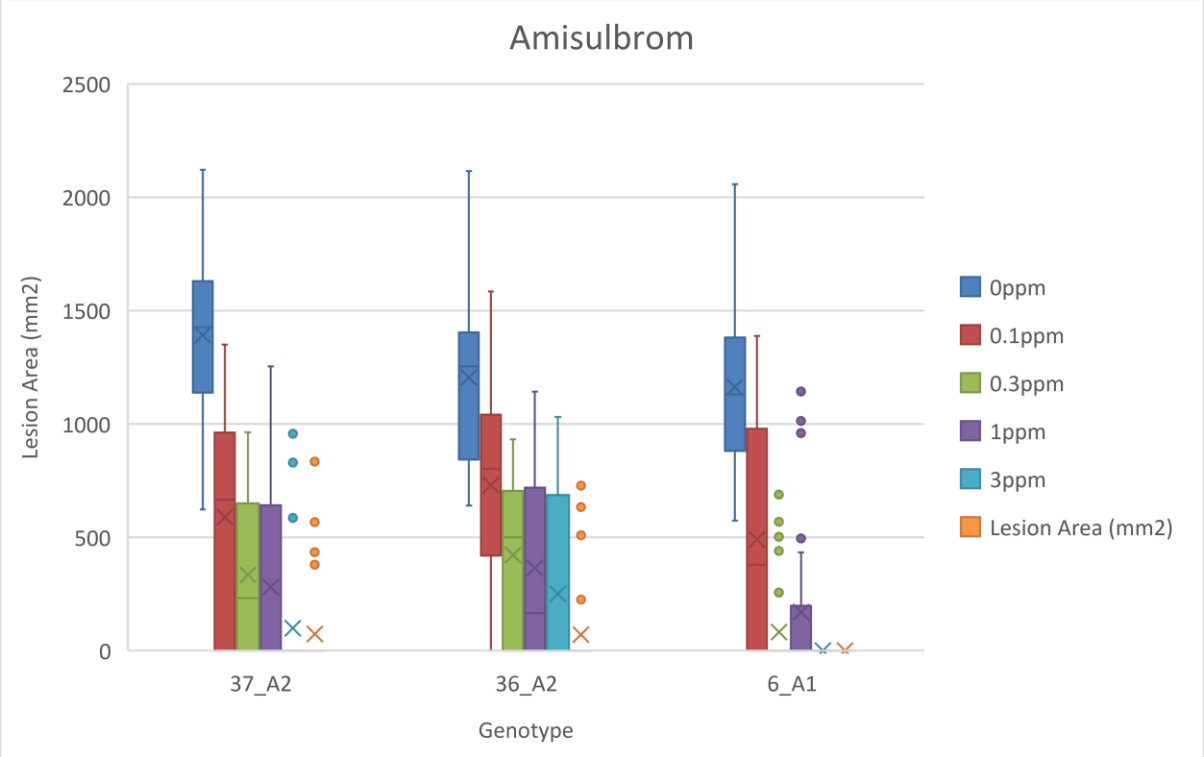


Fig.13 Mean Lesion area (mm2) of isolates belonging to 36_A2 and 37_A2 compared with control isolates (6_A1) presented as a box and whisker plot. Amisulbrom Max Field concentration = **2**00ppm

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

Table 4. Mean, max and min EC_{50} values for isolates of *P. infestans* of various genotypes tested at a range of concentrations of amisulbrom (0, 0.1, 0.3, 1.0, 3.0, 10.0µg/ml). Significant differences between mean values are indicated by different letters.

Conclusion

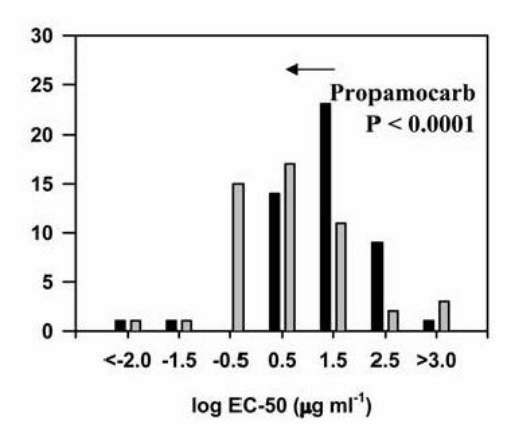
On average, isolates of 36_A2 had a significantly greater EC_{50} values than those belonging to genotypes 37_A2 and 6_A1. Isolates of genotypes 36_A2 and 37_A2 caused lesions at concentrations of 3-10µg/ml amisulbrom whereas isolates of 6_A1 did not.

Sensitivity to Propamocarb

Background

Propamocarb is usually formulated as a mixture with fluopicolide (as Infinito) at a rate of 62.5g/l fluopicolide and 625g/l propamocarb. For the purposes of this test propamocarb was purchased as a single active in the product 'Promess' (722g/l a.i.) and dilutions made accordingly.

Grunwald et al (2006) examined baseline sensitivity of 4-60 isolates of Mexican *P. infestans* isolates using amended media assays and found a range of EC_{50} values from 0.1 to 1000 µg/ml (converted from log values) as shown in the graph below.



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*. 2006 1397-403.

Materials and Methods

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.

Results

All untreated leaves produced lesions (Fig 14) indicating good test conditions and suitability of isolates for testing. Figure 14 shows the mean incidence of lesions for each genotype at different concentrations of propamocarb (0, 10, 100, 300, 500 and 1000µg/ml). There was a high incidence of lesions caused by all genotypes at concentrations up to 300µg/ml propamocarb. At 500µg/ml 53% of isolates of 36_A2 produced lesions compared with 6_A1 (23%) and 37_A2 (7%). No lesions were observed at 1000µg/ml. Mean lesion size on infected leaves only is shown in Fig 15. The range of concentrations under test (0-1000µg/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. 16)

EC_{50} values are given in Table 5. There is a 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. The EC_{50} values in general seem to be in line with previous findings.

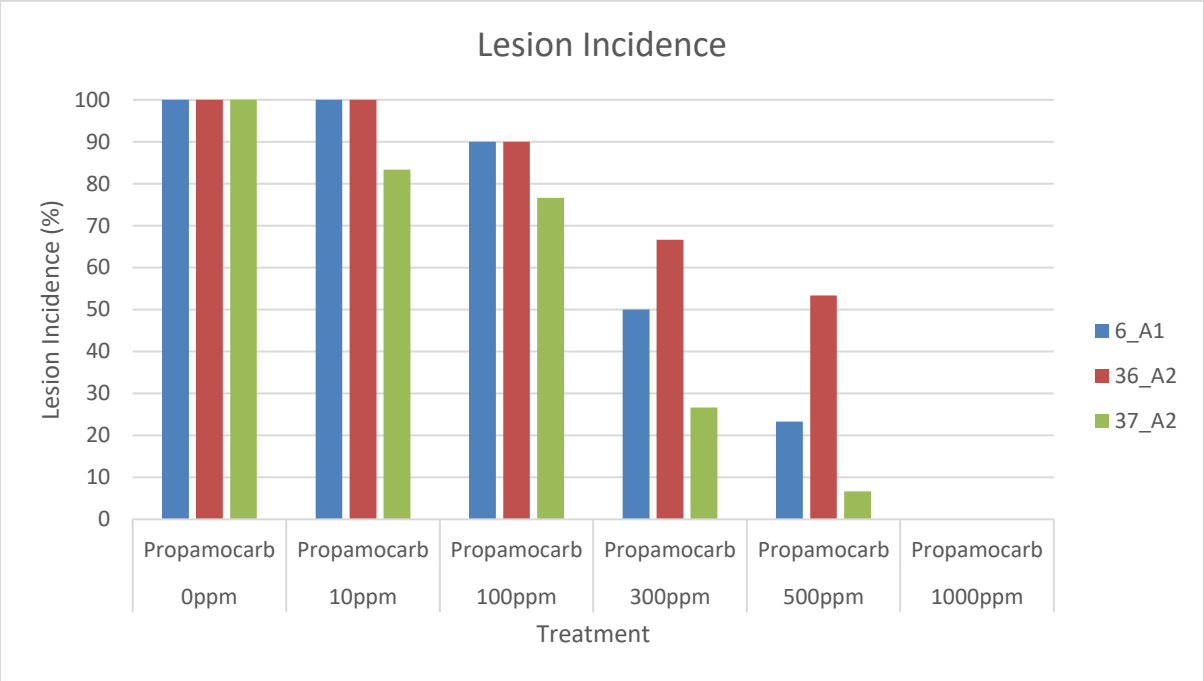


Fig 14. Mean percentage of lesions caused by different genotypes at a range of concentrations of propamocarb (0-1000 µg/ml).

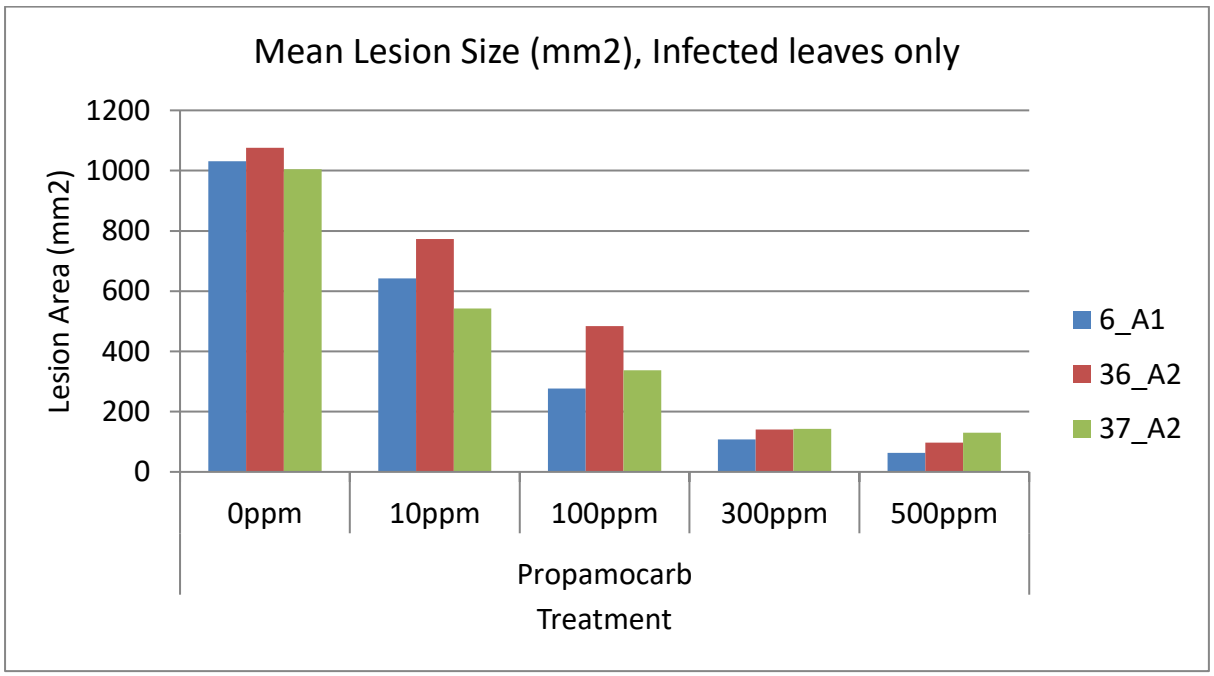


Fig 15. Mean lesion size (mm2) measured on infected leaves only at a range of concentrations of propamocarb

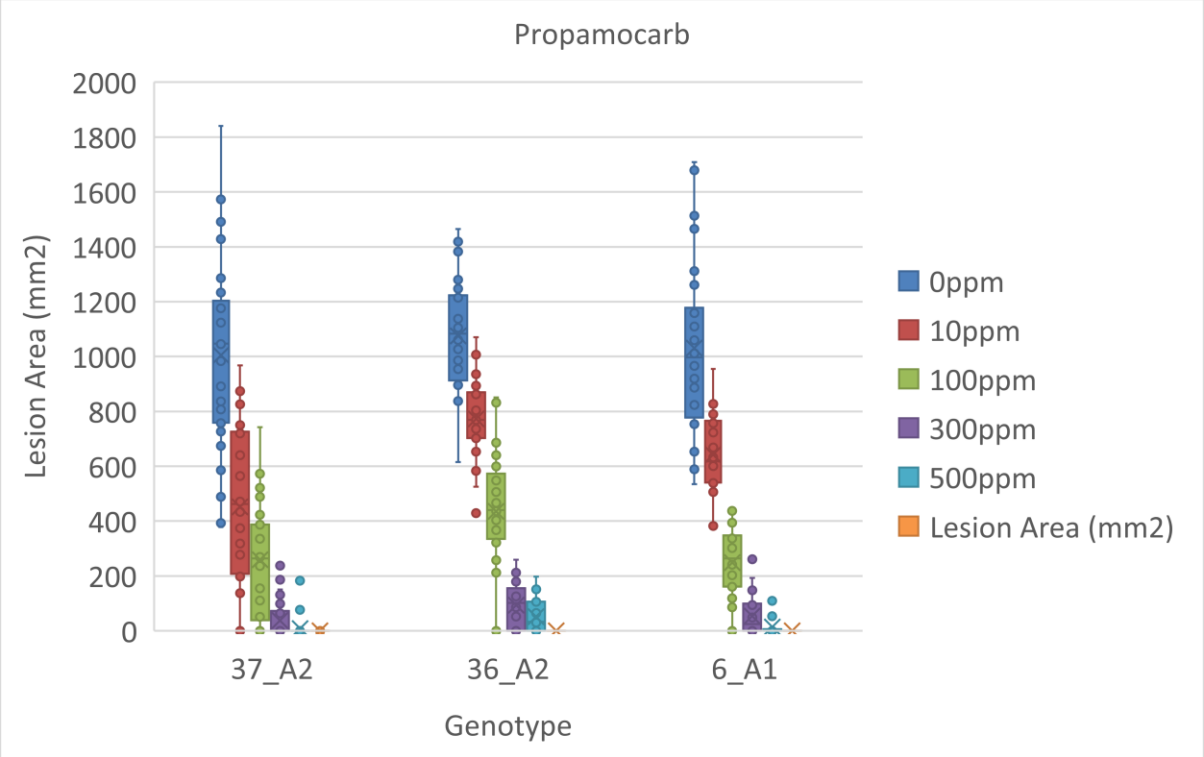


Fig 16. Mean Lesion area (mm^2) of isolates belonging to 36_A2 and 37_A2 compared with control isolates (6_A1) presented as a box and whisker plot. Propamocarb Max Field concentration = 5000 ppm.

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)

Table 5. Mean, max and min EC_{50} values for isolates of *P. infestans* of various genotypes tested 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 and can only be compared with other numbers in brackets.

Conclusion

EC_{50} values in this test appear to be in line with the range found previously for propamocarb. Mean EC_{50} values for genotype 36_A2 were statistically greater than for other genotypes tested in 2018 and 2019.

Sensitivity to Oxathiopiprolin

Background

Cohen et al (2018) tested the preventive and curative (1 dpi) efficacy of oxathiopiprolin against tomato late blight induced by 106 and 90 field isolates of *P. infestans*, respectively. Minimal inhibitory concentration (MIC) values in preventive application ranged between 0.0001 and 0. 1-ppm ai with 17, 51, 35 and 3 isolates fully inhibited at 0.0001, 0.001, 0.01 and 0.1 ppm ai, respectively.

Baseline sensitivity testing to oxathiopiprolin carried out in Korea (Aktaruzzaman *et al.*, 2015, link) 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 0.001µg/ml a.i. oxathiopiprolin (Qu et al, 2016).

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(9): e0204523. https://doi.org/10.1371/journal.pone.0204523

Tianli Qu, Yuanyuan Shao, Alexander S. Csinos, and Pingsheng J. iSensitivity of *Phytophthora nicotianae* From Tobacco to Fluopicolide, Mandipropamid, and Oxathiapiprolin. Plant Disease 2016 100:10, 2119-2125

Materials and Methods

As described previously. Detached leaf test conducted with isolates: 36_A2 (n=5), 37_A2 (n=5), 6_A1 (n=5) at oxathiopiprolin 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}.

Results

All untreated leaves produced lesions (Fig 17) indicating good test conditions and suitability of isolates for testing. Figure 17 shows the mean incidence of lesions for each genotype at different concentrations of oxathiopiprolin (0, 0.0005, 0.001, 0.01, 0.1, 0.3 µg/ml). There a high incidence of lesions caused by all genotypes at concentrations up to 0.001 µg/ml oxathiopiprolin. At 0.01µg/ml 40% of isolates of 36_A2 produced lesions compared with 6_A1 (10%) and 37_A2 (27%). No lesions were observed at 0.1µg/ml. Mean lesion size on infected leaves only is shown in Fig 18. The range of concentrations under test (0-0.3µg/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. 19).

EC_{50} values are given in Table 5. There no statistically significant difference in mean EC_{50} value between genotypes. The EC_{50} values in general seem to be in good correspondence with previous findings in other studies.

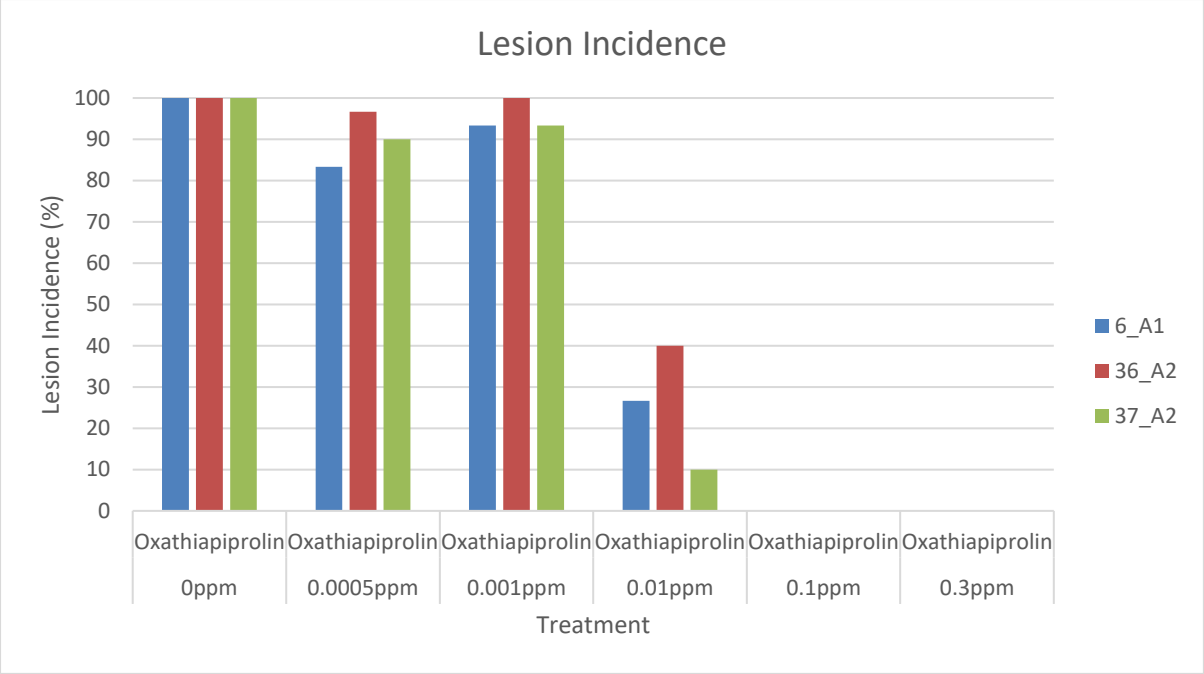


Fig 17. Mean percentage of lesions caused by different genotypes at a range of concentrations of oxathiopiprolin (0-0.3 µg/ml).

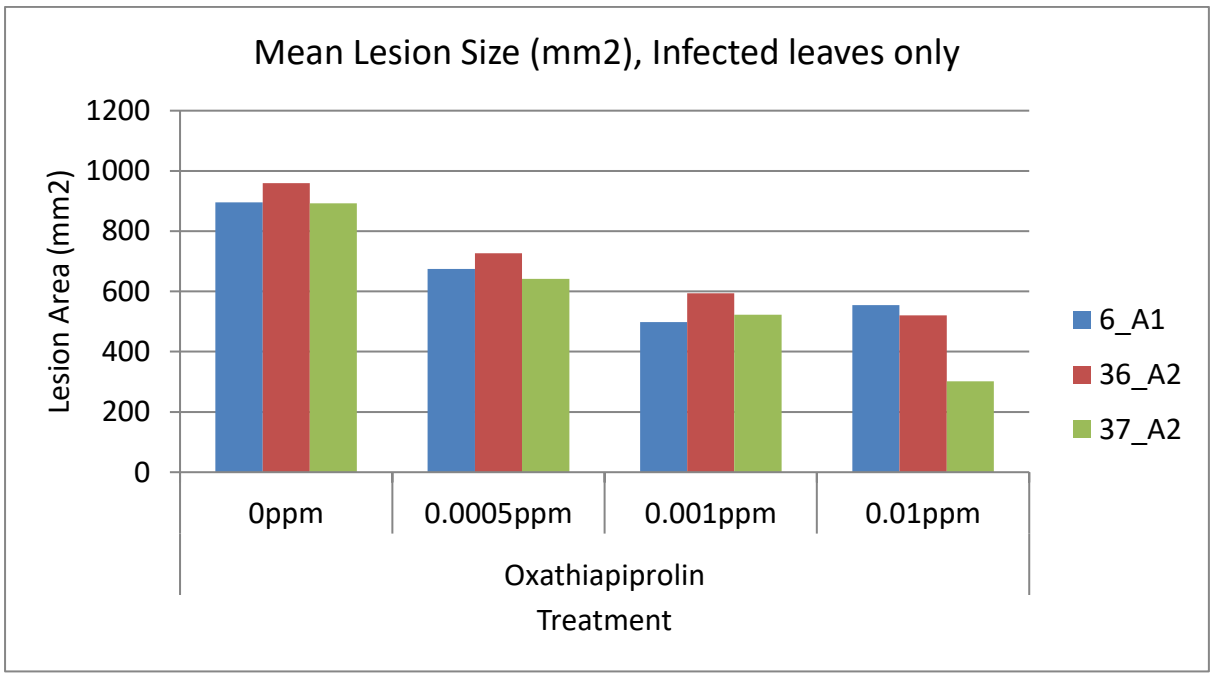


Fig 18. Mean lesion size (mm2) measured on infected leaves only at a range of concentrations of oxathiopiprolin

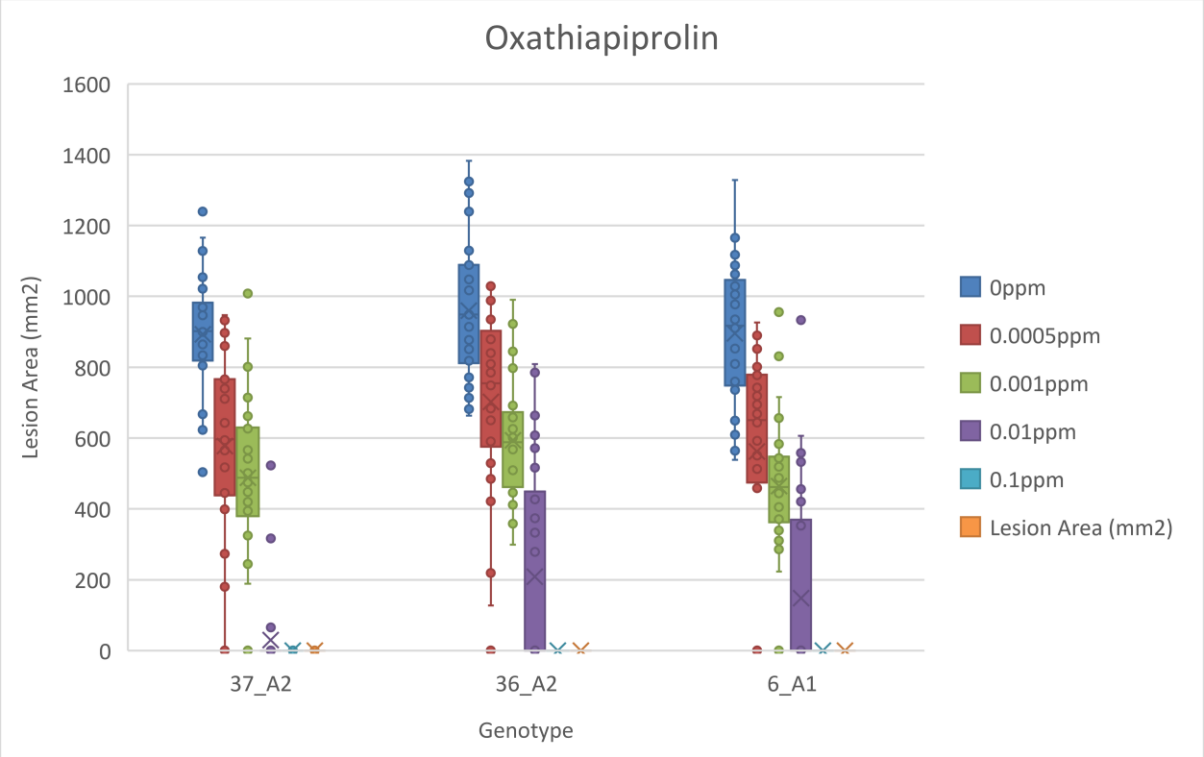


Fig 19. Mean Lesion area (mm^2) of isolates belonging to 36_A2 and 37_A2 compared with control isolates (6_A1) presented as a box and whisker plot. Oxathiopiprolin Max Field concentration = **7**5 ppm.

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

Table 6. Mean, max and min EC_{50} values for isolates of *P. infestans* of various genotypes tested at a range of concentrations of oxathiopiprolin. Significant differences between mean values are indicated by different letters.

Conclusion

EC_{50} values in this test appear to be in line with the range found previously for oxathiopiprolin and no difference between genotypes was observed.

Mancozeb

Background

Mancozeb has been registered for more than 60 years and is a protectant fungicide with multisite inhibitory activity that should result in little or no selection. 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 (Masayasu et al., 1997). No isolate or lineage was resistant and the mean EC_{50}s 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 and Ruccia (2015) presented results of sensitivity testing of mancozeb against 4 genotypes of *P. infestans* (13_A2, 6_A1 and 33_A2) in leaf disc 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.

Masayasu Kato, Eduardo S. Mizubuti, Stephen B. Goodwin, and William E. Fry (1997). Sensitivity to Protectant Fungicides and Pathogenic Fitness of Clonal Lineages of Phytophthora infestans in the United States. Phytopathology 1997 87:9, 973-978

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

Materials and Methods

As described previously. Detached leaf test 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

All untreated leaves produced lesions (Fig 20) indicating good test conditions and suitability of isolates for testing. Figure 20 shows the mean incidence of lesions for each genotype at different concentrations of mancozeb (0, 1, 10, 100, 500, 1000 µg/ml). There was 100% incidence of lesions caused by all genotypes at concentrations up to 10µg/ml mancozeb and 0% incidence at 1000µg/ml. Mean lesion size on infected leaves only is shown in Fig 21. The range of concentrations under test in (0-1000µg/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. 22).

EC_{50} values calculated from test 3 data are given in Table 7. There is no statistically significant difference in mean EC_{50} value between genotypes. The EC_{50} values in general seem to higher than those previously reported but were obtained using a different method and are well within the concentration used as field rate (6375µg/ml).

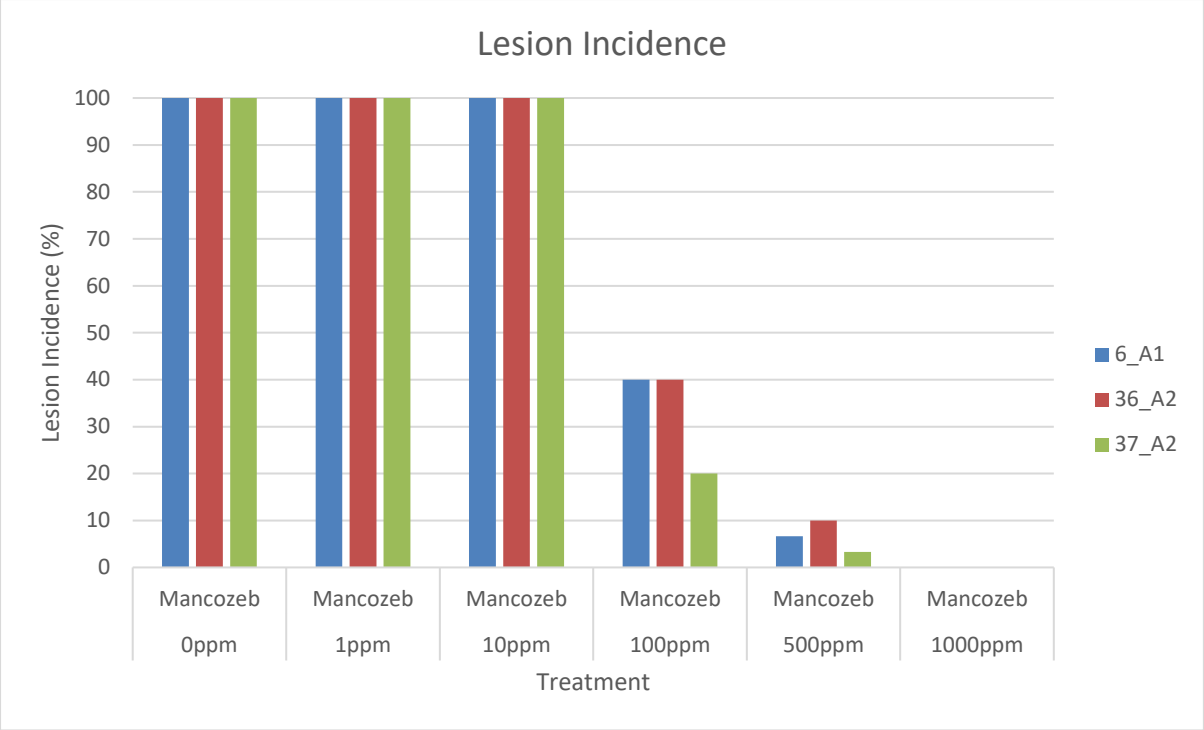


Fig 20. Mean percentage of lesions caused by different genotypes at a range of concentrations of mancozeb (0-1000 µg/ml).

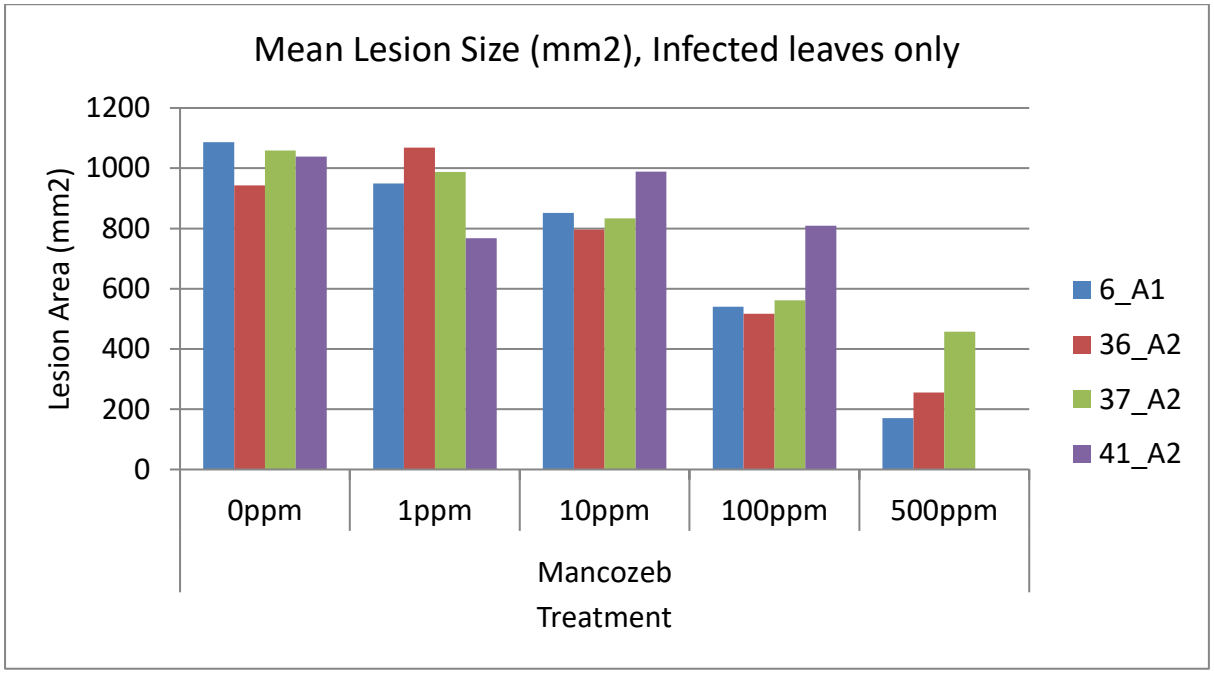


Fig 21. Mean lesion size (mm2) measured on infected leaves only at a range of concentrations of mancozeb

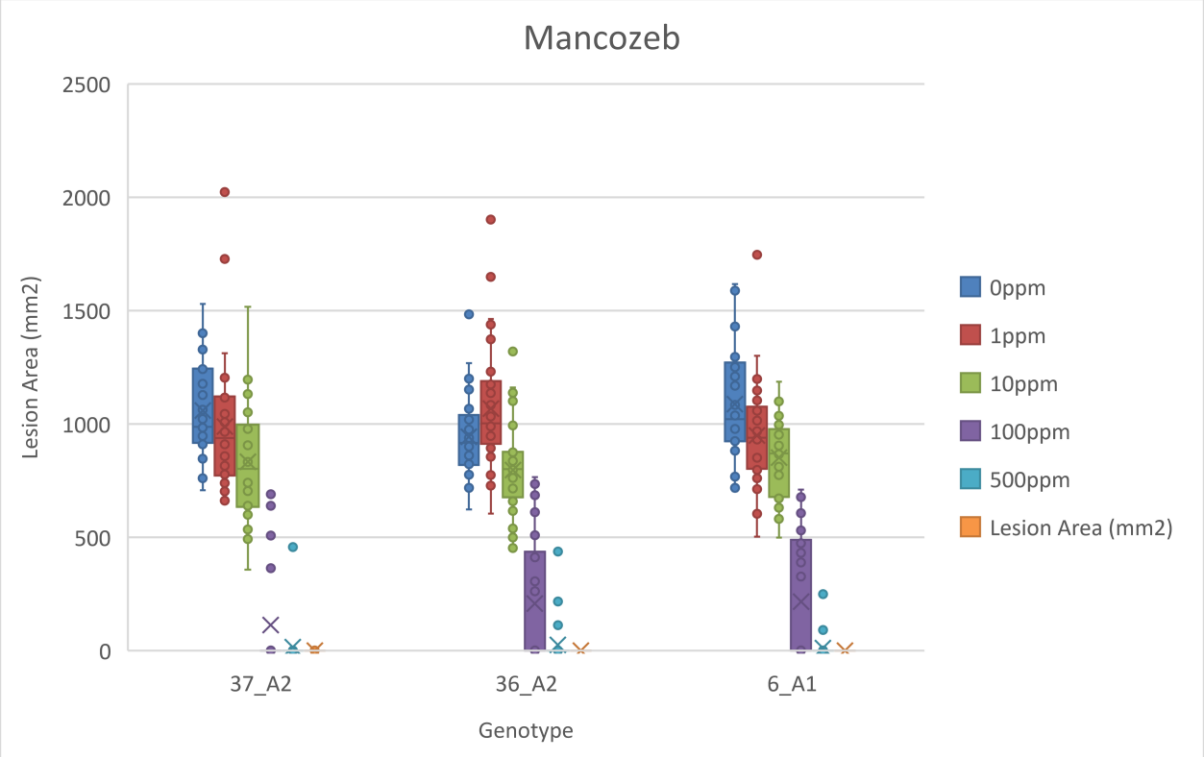


Fig 22. Mean Lesion area (mm^2) of isolates belonging to 36_A2 and 37_A2 compared with control isolates (6_A1) presented as a box and whisker plot. Mancozeb Max Field concentration = **637**5 ppm

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

Table 7. Mean, max and min EC_{50} values for isolates of *P. infestans* of various genotypes tested at a range of concentrations of mancozeb. Significant differences between mean values are indicated by different letters.

Conclusion

No statistical difference in EC_{50} values between genotypes was observed.