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

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1. Summary

Five fungicides belonging to a range of FRAC fungicide groups were tested for their ability to inhibit late blight in the laboratory. The active ingredients tested were representatives of Pyridinylmethyl-benzamides (flupicolide), CAA-fungicides (mandipropamid), Carbamates (propamocarb), Qil fungicides (cyazofamid) and Uncouplers of oxidative phosphorylation (fluazinam).

A limited number of isolates of *P. infestans* belonging to lineages known to be either established, or relatively new in GB were tested. Established lineages included 13_A2 and 6_A1. New lineages included 37_A2 and 36_A2. Testing was conducted either as zoospore inhibition tests or detached leaf assays as appropriate and minimum inhibitory concentrations (MICs) or the effective dose for 50% control (EC_{50}) were calculated.

Results of testing genotype 37_A2 provided supporting evidence of its resistance to fluazinam, as previously reported. There were no differences in the MIC of fluazinam required to control the 36_A2 genotype tested.

Results of testing fluopicolide found EC50 values were in line with previous baseline sensitivity testing. The EC_{50} values for genotype 36_A2 were statistically higher than for other genotypes but were within the expected range. Relatively high maximum EC_{50} values in the genotype 36_A2 appear to have been the result of a small number of high readings, not reflected across replicates.

Results of testing mandipropamid found EC_{50} values in this test were in line with previous sensitivity testing. Mean EC_{50} values for genotype 36_A2 were statistically higher than for other genotypes tested.

Results of testing cyazofamid found EC_{50} values to be in line with, or slightly higher, than previous sensitivity testing. EC_{50} values for genotype 36_A2 were statistically higher than for other genotypes. Testing of additional isolates would be needed to draw firm conclusions for this active ingredient.

Results of testing propamocarb found EC_{50} values were in line with previous sensitivity testing. EC_{50} values for genotype 36_A2 were statistically higher than for the other genotypes tested.

These findings do not provide evidence of specific or multiple shifts in resistance in genotype 36_A2 or mean that performance using products at field rates will necessarily be affected. Therefore, recommendations on use for 2018/19 are unchanged. FRAG-UK guidelines on resistance management in potato late blight remain of high importance. It will be important to monitor the situation with regards to the emergence of 36_A2 and other genotypes in the context of their aggressiveness, to test further isolates and to monitor field performance.

2. Materials, Methods and Results

The table below summarises the active ingredients that were studied:

Active Ingredient	Product	Max dose (l/Ha)	Volume (l/Ha)	Max Tank Mix (ppm)
Fluazinam 500g/l	Shirlan	0.4	200-500	1000
Cyazofamid 400g/l	Ranman	0.5	200-400	400
Mandipropamid 250g/l	Revus	0.6	>200	750
Propamocarb 722g/l (625g/l as Infinito)	Promess	1.6	200-400	5000
Fluopicolide 5mg/ml (62.5g/l as Infinito)	Pure a.i. (Sigma Aldrich)	1.6	200-400	500

Isolates

In the first instance, isolates of 36_A2 (n=7), 37_A2 (n=10) and 13_A2/6_A1 (n=2) all isolated in 2017 from the AHDB Fight Against Blight samples were selected for testing to provide a sensitivity test for the newer genotypes (£6_A2 And 37_A2) and to provide some comparison with existing genotypes (13_A2 and 6_A1). Only 7 isolates of 36_A2 were available to test as it was a relatively new occurrence in 2017.

For some tests (Mandipropamid/Cyazofamid/Propamocarb), five additional isolates of 13_A2 were subsequently tested alongside the isolates listed above. These additional isolates have also been tested by Dutch colleagues in 2018 and were included a) to provide a link to similar fungicide testing in the Netherlands should results indicate any shifts in sensitivity and b) to provide a better comparison between the newer genotypes (36_A2 and 37_A2) with the existing population (i.e. 13_A2 and 6_A1). This was carried out at no additional cost.

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, each of 7–10 days 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: This method is in line with the testing being conducted through the EU C-IPM project (IPMBlight2.0) and should allow results to be comparable. Six leaflets per isolate 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.

Mandipropamid/Propamocarb/Cyazofamid: Initial tests were carried out using the detached leaf method as described above and the same isolates (36_A2 (n=7), 37_A2 (n=10) and 13_A2/6_A1 (n=2) all isolated in 2017 from the AHDB Fight Against Blight). The concentrations originally tested were in line with the C-IPM agreed methodology as follows:

Active ingredients (a.i)	ppm a.i. level-1	ppm a.i. level-2	ppm a.i. level-3	ppm a.i. level-4	ppm a.i. Max. tank mix
cyazofamid	0	10	100	400	400
propamocarb	0	100	1000	5000	5000
mandipropamid	0	10	100	750	750

However, after conducting the tests we felt that these concentrations were not entirely appropriate. We therefore re-tested the same isolates in addition to 5 extra isolates of 13_A2 at the concentrations specified in the FRAC protocol for testing CAA and other fungicides. Both sets of results are presented for these 3 fungicides.

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 as illustrated in the example below (Fig1).

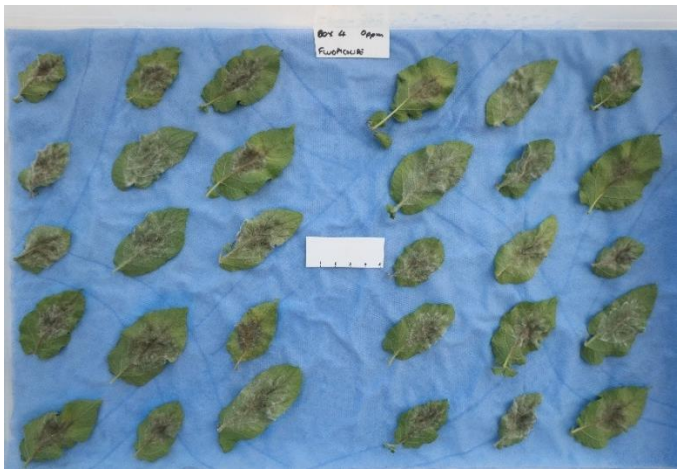


Fig 1. Untreated leaves showing symptoms of late blight 7 days after inoculation.

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 $P = 0.05$.

1 mg/l = 1 µg/ml = 1 ppm

Sensitivity of isolates to Fluazinam

Background

A *P. infestans* genotype (33_A2) with decreased sensitivity to fluazinam was reported in the Netherlands in 2009. In 2011 and 2012, the same strain was reported at low frequency in GB, as part of AHDB's Fight against Blight monitoring, but was rarely detected in subsequent years due to a suspected fitness penalty.

Schepers *et al* (2018) presented the discovery of Dutch *P. infestans* isolates, belonging to the *P. infestans* genotype EU_33_A2, displaying a reduced sensitivity to fluazinam in two field

trials under high disease pressure and in an *in-vitro* fungicide sensitivity assay. They hypothesised that the efficacy of fluazinam to inhibit isolates of *P. infestans* clonal lineages regarding zoospore motility provided a good indication for the efficacy of fluazinam under field conditions.

Additionally, in June 2017 results from Wageningen University & Research showed that all *P. infestans* isolates of genotype EU-37 tested displayed a reduced sensitivity to fluazinam. This finding was supported by tests carried out in the UK. The Dutch researchers stated that there was a strong indication that the rise of EU-37 in Europe was not only caused by its better fitness but also by a selection advantage in situations in which fluazinam is used. The 37_A2 genotype was found in GB as part of AHDB Fight against Blight monitoring in 2016, representing 3% of the samples submitted by blight scouts. However, by 2017, 24% of the samples submitted were 37_A2. Advice concerning the use of fluazinam was then given to agronomists and growers.

In previous surveys in Europe no isolates of *P. infestans* resistant to fluazinam were found prior to the report of Schepers *et al* (2018). Cooke *et al.* (1998) found that Minimum Inhibitory Concentration (MIC) of fluazinam ranged from 0.02 to 0.06 µg/ml in the isolates they tested, which was comparable with the MIC values for the EU_13_A2 isolates reported by Schepers *et al* (2018).

The work described here tests sensitivity to fluazinam in isolates of genotype 36_A2 compared with 37_A2 (which is known to have reduced sensitivity in isolates tested so far (as above)), and isolates of 13_A2 and 6_A1, which have not shown any insensitivity in the isolates tested to date. The method used is the zoospore motility test as conducted in the studies of Schepers *et al* (2018) which is a modified version of that used by Cooke *et al* (1998).

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 fluazinam were prepared from commercial product Shirlan (Syngenta: 500 g/l fluazinam) 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 fluazinam/ml. 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

A collection of 19 *P. infestans* isolates obtained from commercial crops in the UK in 2017 representing 3 clonal lineages, were tested from sensitivity to fluazinam in a replicated in-vitro assay. Analysis of variance of the resulting MIC values (Table 1) demonstrated that isolates of the 37_A2 genotype had on average significantly higher MIC values when compared with isolates of the 36_A2 or 13_A2/6_A1 genotypes. These differences were present after 1 and 2 h of incubation of the zoospores in their respective fluazinam concentrations.

	No. of isolates tested	**MIC value (µg/ml)**		
Clonal lineage		**Incubation time 1 h**	**Incubation time 2 h**	**Combined data**
EU_13_A2 + EU_6_A1	2	0.1a	0.075a	0.088a
EU_36_A2	7	0.246a	0.086a	0.166a
EU_37_A2	10	4.15b	3.2b	3.675b

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

Previous results from Schepers *et al* (2018) are given below for information and comparison – showing significant differences in MIC for genotypes 33_A2 and 37_A2 compared with other genotypes after 1 and 2h of incubation.

	No. of isolates tested	**MIC value (µg/ml)**	
Clonal lineage		**Incubation time 1 h**	**Incubation time 2 h**
EU_13_A2	5	0.2 a	0.2 a
EU_33_A2	5	9.9 b	6.9 c
EU_6_A1	5	0.8 a	0.6 a
EU_37_A2	3	9.0 b	4.0 b
Clone 1	2	1.0 a	1.0 a

Table 2. Sensitivity to fluazinam in the replicated zoospore motility assay for *P. infestans* isolates belonging to five clonal lineages collected in the Netherlands from 2007 to 2014. Taken from Schepers *et al* (2018). Within columns values followed by the same letter are not significantly different according to Fisher's protected least significant difference test at P = 0.05.

Conclusion

The mean Minimum Inhibitory Concentration (MIC) of fluazinam required to inhibit zoospore motility is significantly greater in isolates of genotype 37_A2 than in genotype 36_A2 and other genotypes (concurring with the results of Schepers *et al* 2018). There is no significant difference in the MIC of fluazinam required to control 36_A2 and isolates of 13_A2/6_A1.

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 (40, 10, 1, 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 and this assessment was carried out using the detached leaf tests as used for the other fungicides (apart from fluazinam). 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

Product tested: Fluopicolide technical grade (5mg/ml)
Concentrations tested: 0, 1, 10, 40 µg fluopicolide/ml
Isolates tested: 36_A2 (n=7), 37_A2 (n=10) and 13_A2/6_A1 (n=2)

Results

All untreated leaves produced lesions (Fig 2) indicating good test conditions and suitability of isolates for testing. Figure 2 shows the incidence of lesions for a genotype at different concentrations of fluopicolide. There was a high incidence of lesions at 1 ppm fluopicolide but a very low incidence, confined to genotypes 36_A2 and 37_A2 at 10ppm and 40ppm. This indicates that the range of concentrations under test is appropriate. Figure 3 shows the mean lesion size calculated for the infected leaves only. At all concentrations genotype 36_A2 has the highest mean lesion size compared with the other genotypes. The statistical significance, or otherwise, of this difference is captured in the calculation of $EC_{50.}$

EC_{50} values are given in Table 3. There is a statistically significant difference in mean EC_{50} value between genotypes. However, the mean (and maximum/minimum) EC_{50} values are in line with the original baseline sensitivity data and scrutiny of the raw data shows that for 2 isolates of 36_A2, only one in 6 replicate tests showed growth at 40µg/ml (see Fig 4 for example).

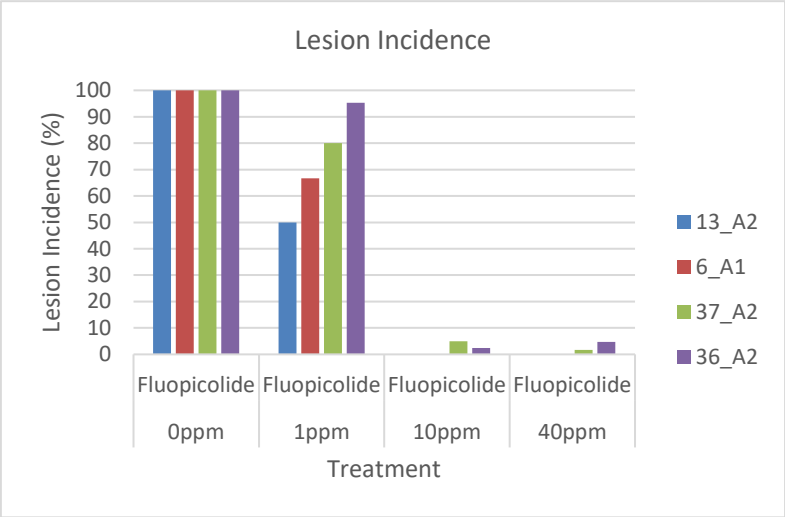


Fig 2. Incidence of lesions (%) caused by each genotype observed at different concentrations of fluopicolide.

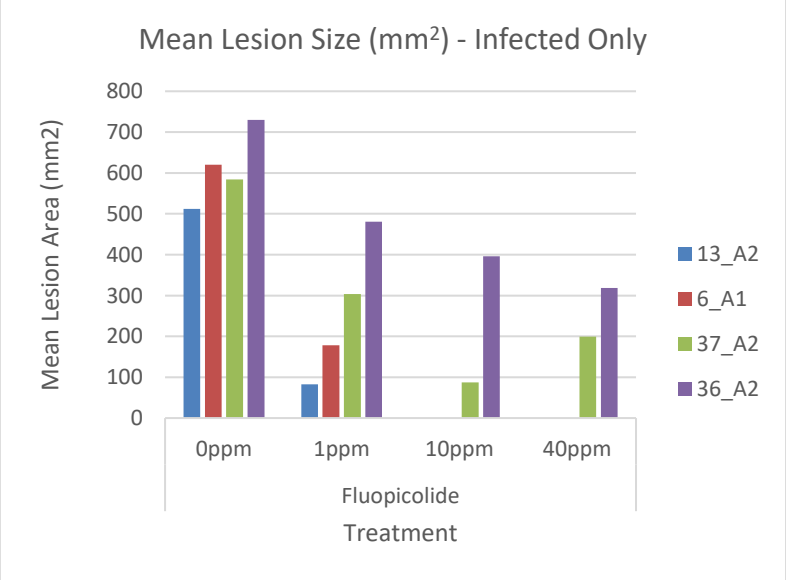


Fig 3. Mean lesion size (mm^2) of genotypes at different concentrations of fluopicolide (mean of infected leaves only).

Fig 4. One replicate leaf of an isolate of 36_A2 showing disease symptoms at 40µg/ml fluopicolide.

Table 3. 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.

Genotype	**13_A2 & 6_A1**	**37_A2**	**36_A2**
Number of isolates	2	10	7
Mean EC_{50} (mg/L)	**0.53a**	**1.40a**	**2.57b**
EC_{50} min (mg/L)	0.33	0.33	0.33
EC_{50} max (mg/L)	1.49	5.46	24.47

As fluopicolide is known to have activity against zoospores, isolates were also tested for zoospore motility using the same method as described for Fluazinam above. Results are given in Table 4 below.

Table 4. Within column values followed by the same letter are not significantly different according to Fisher's protected least significant difference test at P = 0.05

		MIC value (µg/ml)	
Clonal lineage	**Number of Isolates tested**	**Incubation time 1 h**	**Incubation time 2 h**
EU_13_A2 + EU_6_A1	2	0.075ab	0.075b
EU_36_A2	7	0.107b	0.075b
EU_37_A2	10	0.068a	0.05a

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. Relatively high maximum EC_{50} values in genotype 36_A2 appear to have been the result of a small number of high readings, not reflected across replicates.

Sensitivity to 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 EC50 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. EC50 values for mandipropamid from this work are shown below.

Table 5. 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 *P. 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.

	Mean ± SE EC50 (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

The methods are as was described previously.

Test 1 – Detached leaf test conducted with isolates: 36_A2 (n=7), 37_A2 (n=10), 13_A2/6_A1 (n=2) at mandipropamid concentrations of 0, 10, 100, 750 µg/ml (according to C-IPM concentrations)

Test 2 – Detached leaf test conducted with isolates: 36_A2 (n = 7), 37_A2 (n=10), 13_A2/6_A1 (n=7)

at mandipropamid 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 5, 6) indicating good test conditions and suitability of isolates for testing. Figure 5 shows the mean incidence of lesions for each genotype at different concentrations of mandipropamid (0, 10, 100, 750 µg/ml) and Fig 6. for concentrations 0, 0.1, 0.3, 1.0, 3.0 and 10µg/ml. The data is combined in Fig 7 to give an overall picture, but it must be noted that a larger number of isolates were tested at the lower concentrations (Test 2).

There was a high incidence of lesions at concentrations up to 1µg/ml mandipropamid with a lower incidence at 3-10µg/ml and a very low incidence (1 replicate of 1 isolate of 37_A2) at 100ppm. Mean lesion size on infected leaves only is shown in Fig 8. The range of concentrations under test in test 2 (0-10µg/ml) was appropriate for calculation of EC_{50}.

EC_{50} values calculated from test 2 data are given in Table 6. There is a statistically significant difference in mean EC_{50} value between genotypes. Genotypes 13_A2 and 6_A1 have been combined in the analysis due to the low number of 6_A1 isolates tested. This does not alter the result. The mean EC_{50} values appear to be in line with previous data (see background info) although the maximum EC_{50} values are higher than those noted by Cohen *et al* (2007).

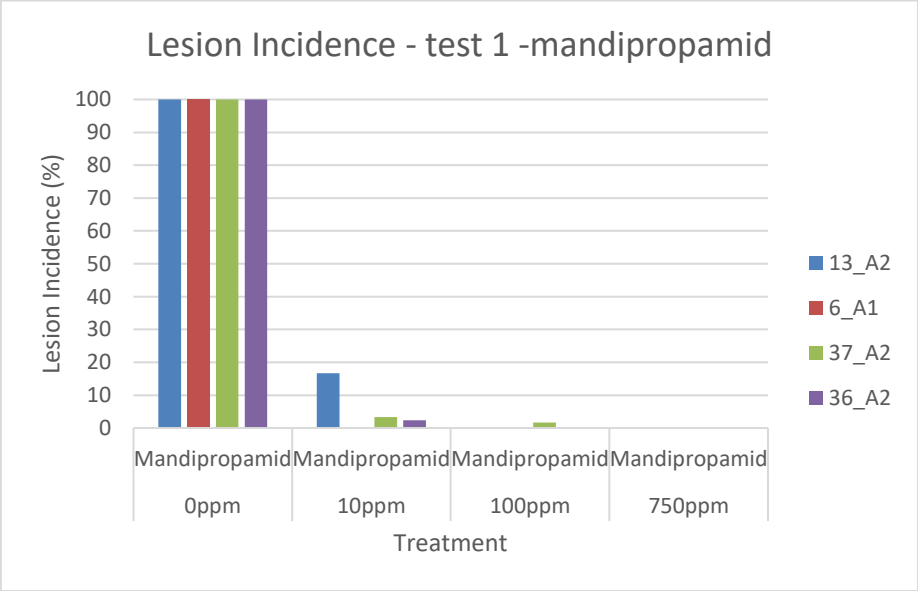


Fig 5. Mean percentage of lesions caused by different genotypes at a range of concentrations of mandipropamid (0-750 µg/ml)

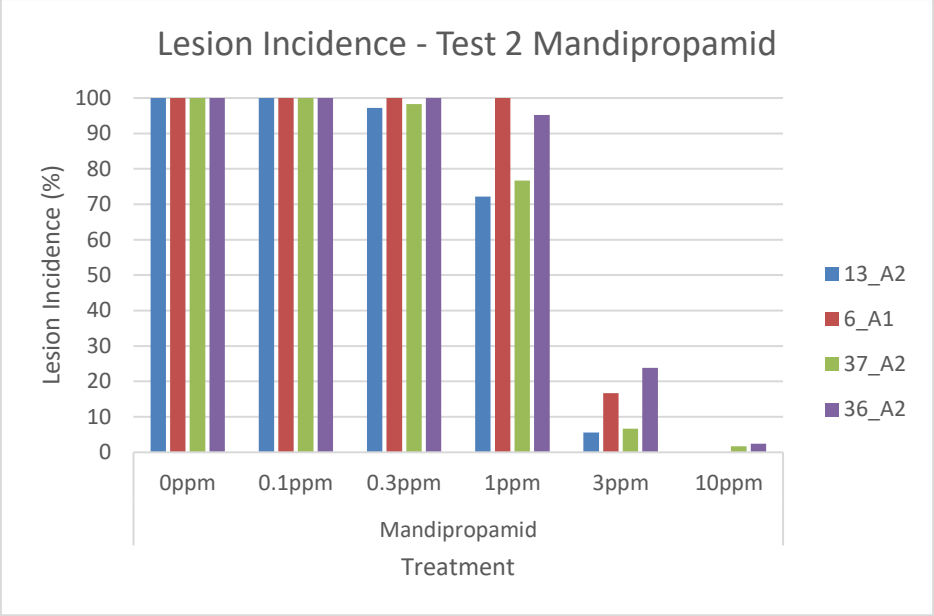


Fig 6. Mean percentage of lesions caused by different genotypes at a range of concentrations of mandipropamid (0-10 µg/ml).

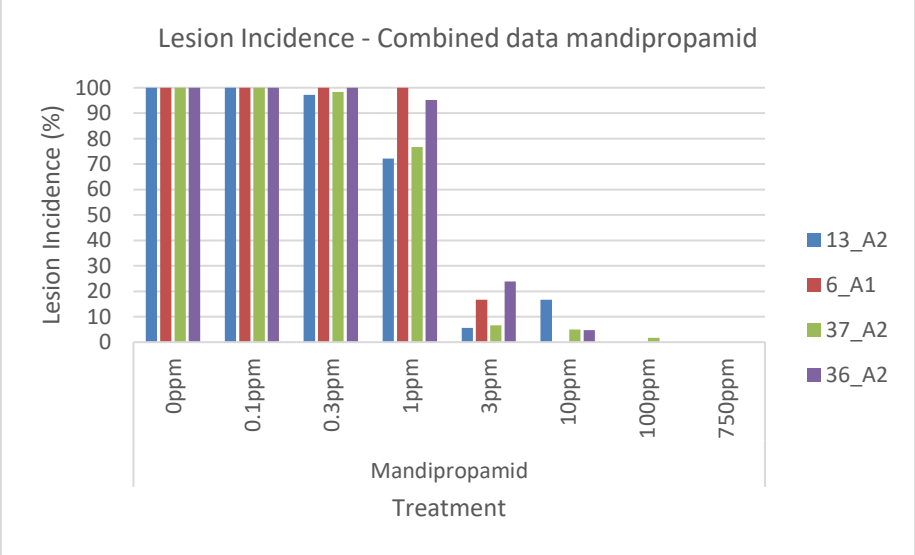


Fig 7. Mean percentage of lesions caused by different genotypes at a range of concentrations of mandipropamid (0-750 µg/ml). This is the combined data of Fig 5 and Fig 6.

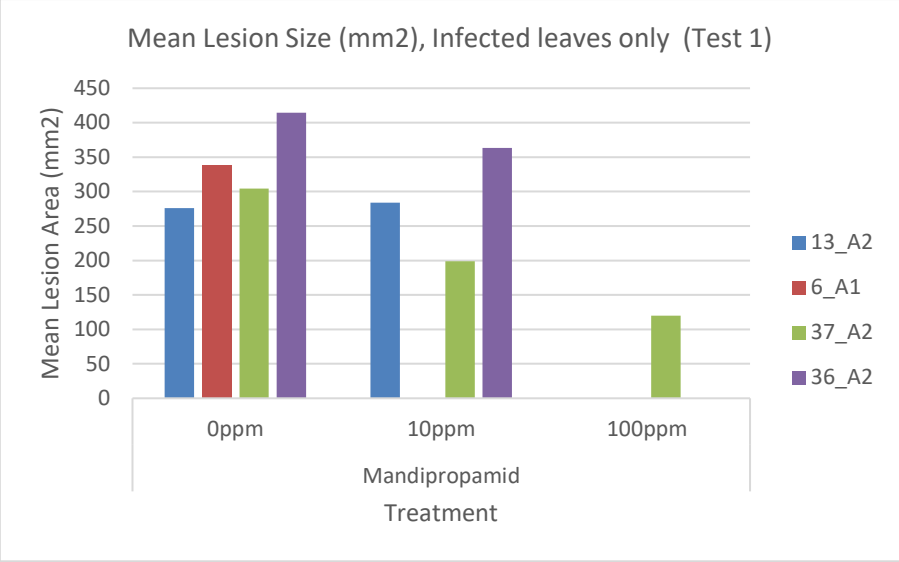


Fig 8. Mean lesion size (mm2) measured on infected leaves only at a range of concentrations of mandipropamid.

Table 6. 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.

Genotype	13_A2 & 6_A1	37_A2	36_A2
Number of isolates	7	10	7
Mean EC_{50} (mg/L)	**0.74a**	**0.54a**	**1.26b**
EC_{50} min (mg/L)	0.16	0.16	0.27
EC_{50} max (mg/L)	4.94	2.99	5.46

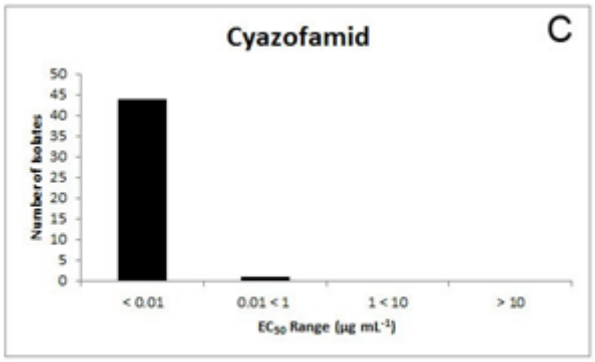
Conclusion

EC_{50} values in this test were in line with previous sensitivity testing of mandipropamid. Mean EC_{50} values for genotype 36_A2 were statistically higher than for other genotypes.

Sensitivity to Cyazofamid

Background

There is little publicly available background information relating to baseline sensitivity of cyazofamid in *P. infestans*. 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.

Inhibition of Mycelial Growth of *P. infestans* by Cyazofamid (data taken from Mitani *et al* 2001). Data is stated in mg/ml.

Isolate	Type	Cyazofamid **(mg/ml)**	
		EC50	MIC (>90% inhibition)
CRI-1	A1	0.008	0.1
TK-918	A2	0.006	0.05
TK-963	A2	0.008	0.01
U-1	A2	0.03	0.5
U-3	A2	0.02	0.1

The methods are as was described previously.

Test 1 – Detached leaf test conducted with isolates: 36_A2 (n=7), 37_A2 (n=10), 13_A2/6_A1 (n=2) at cyazofamid concentrations of 0, 10, 100, 400 µg/ml (according to C-IPM concentrations)

Test 2 – Detached leaf test conducted with isolates: 36_A2 (n = 7), 37_A2 (n=10), 13_A2/6_A1 (n=7)

at cyazofamid concentrations of 0, 0.1, 0.3, 1.0, 3.0, 10.0µg/ml (according to FRAC concentrations).

Results

It was clear that the range of concentrations used in test 1 were not appropriate for calculation of EC_{50} as no lesions were observed. Only the results of test 2 conducted at the lower range of concentrations with the higher number of isolates tested are therefore given here.

All untreated leaves produced lesions (Fig 9) indicating good test conditions and suitability of isolates for testing. Figure 9 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 high incidence of lesions at concentrations up to 0.1µg/ml cyazofamid with a lower incidence at 0.3µg/ml and a very low incidence (1 replicate of each of 3 different isolates of 36_A2) at 1µg/ml. Mean lesion size on infected leaves only is shown in Fig 10. The range of concentrations under test in test 2 (0-10µg/ml) was appropriate for calculation of EC_{50}.

EC_{50} values calculated from test 2 data are given in Table 7. There is a statistically significant difference in mean EC_{50} value between genotypes. Genotypes 13_A2 and 6_A1 have been combined in the analysis due to the low number of 6_A1 isolates tested. This does not alter the result. It is difficult to interpret the mean EC_{50} values in the context of existing data as different tests were used and the EC_{50} values stated by Mitani *et al* (2001) appear to use incorrect units. However, concentrations of cyazofamid required to control all isolates are low overall. The significant difference in EC_{50} values between genotypes is likely to be a result of the 3 isolates of 36_A2 showing lesion development at 3µg/ml. Similarly, this will have influenced the higher maximum EC_{50} values for that genotype.

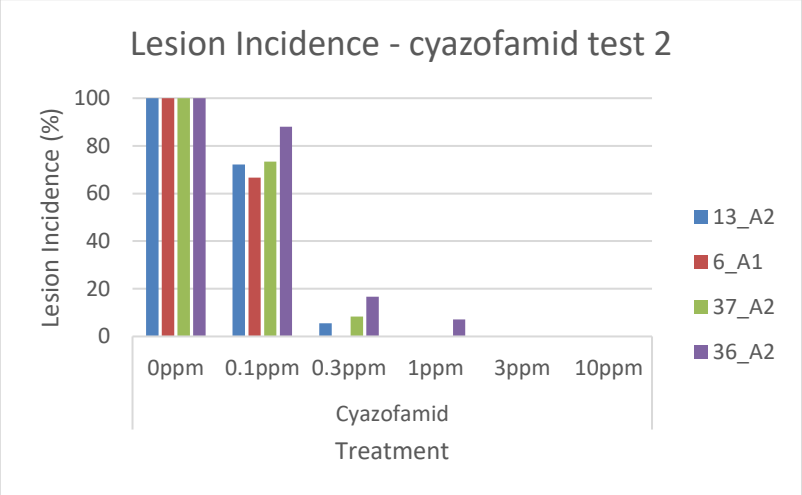


Fig 9. Mean percentage of lesions caused by different genotypes at a range of concentrations of cyazofamid (0-10 µg/ml).

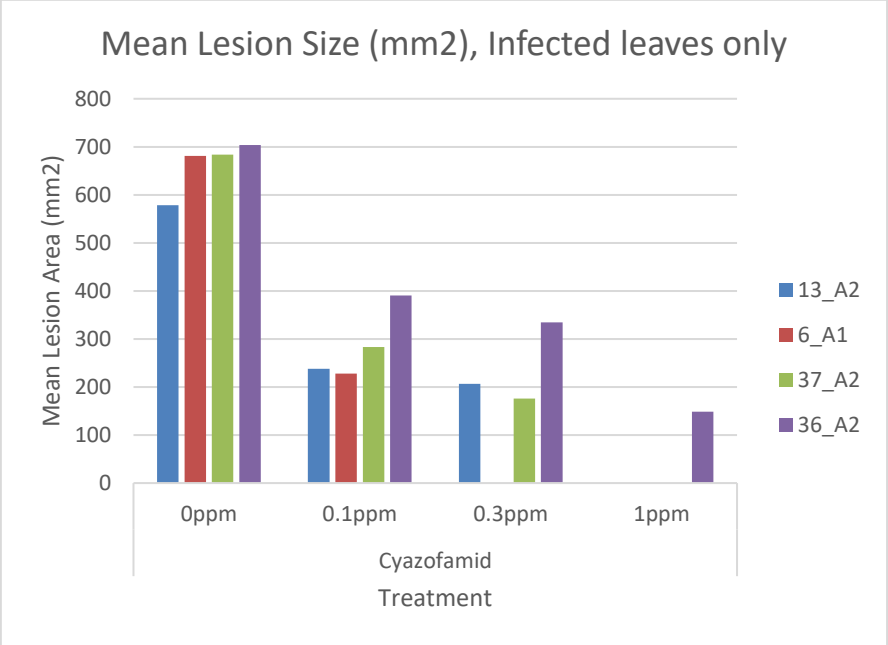


Fig 10. Mean lesion size (mm2) measured on infected leaves only at a range of concentrations of cyazofamid

Table 7. Mean, max and min EC50 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.

Genotype	13_A2 & 6_A1	37_A2	36_A2
Number of isolates	7	10	7
Mean EC_{50} (mg/L)	**0.18a**	**0.19a**	**0.22b**
EC_{50} min (mg/L)	0.15	0.15	0.15
EC_{50} max (mg/L)	0.30	0.30	0.55

Conclusion

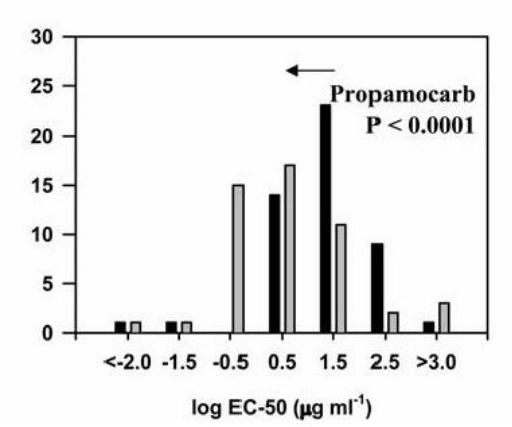
EC_{50} values in this test appear to be in line with, or slightly higher than with previous sensitivity testing of cyazofamid. Mean EC_{50} values for genotype 36_A2 were statistically higher than for other genotypes but this may be marginal. It would probably be necessary to test additional isolates of all genotypes to draw any firm conclusion as to shifts in sensitivity for this active ingredient.

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.



The methods are as was described previously.

Test 1 – Detached leaf test conducted with isolates: 36_A2 (n=7), 37_A2 (n=10), 13_A2/6_A1 (n=2) at propamocarb concentrations of 0, 100, 1000, 5000 µg/ml (according to C-IPM concentrations)

Test 2 – Detached leaf test conducted with isolates: 36_A2 (n = 7), 37_A2 (n=10), 13_A2/6_A1 (n=7)

at propamocarb concentrations of 0, 0.1, 0.3, 1.0, 3.0, 10.0µg/ml (according to FRAC concentrations).

Test 3 - Detached leaf test conducted with isolates: 36_A2 (n = 7), 37_A2 (n=10), 13_A2/6_A1 (n=7)

at propamocarb concentrations of 0, 100, 250, 500, 750, 1000µg/ml (according to FRAC concentrations).

In this case 3 tests were carried out to identify the correct range of concentrations to give the best calculation of EC50 values and discrimination between genotypes. It was clear that the lower range used (test 2) was not appropriate as all leaves infected at these concentrations and EC_{50} could not be calculated. The range of concentrations used in the C-IPM tests (test

1) did show some discrimination but it appeared that the discriminatory range was between 100 and 1000µg/ml.

A third test, including the larger number of isolates (as in test 2) with an intermediate range of concentrations was therefore conducted and EC_{50} values calculated from this data.

Results

It was clear that the range of concentrations used in test 1 and test 2 were not optimal for calculation of EC_{50}. Only the results of test 3 conducted at a mid-range of concentrations (0-1000µg/ml) with the higher number of isolates tested are therefore given here.

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 propamocarb (0, 100, 250, 500, 750 and 10000µg/ml). There was a reasonably high incidence of lesions at concentrations up to 250µg/ml propamocarb with only 1 replicate of 1 isolate of 36_A2 producing a lesion at 750µg/ml. Mean lesion size on infected leaves only is shown in Fig 12. The range of concentrations under test in test32 (0-1000µg/ml) was appropriate for calculation of EC50.

EC_{50} values calculated from test 3 data are given in Table 8. There is a statistically significant difference in mean EC_{50} value between genotypes. Genotypes 13_A2 and 6_A1 have been combined in the analysis due to the low number of 6_A1 isolates tested. This does not alter the result. The EC_{50} values in general seem to be in line with previous findings.

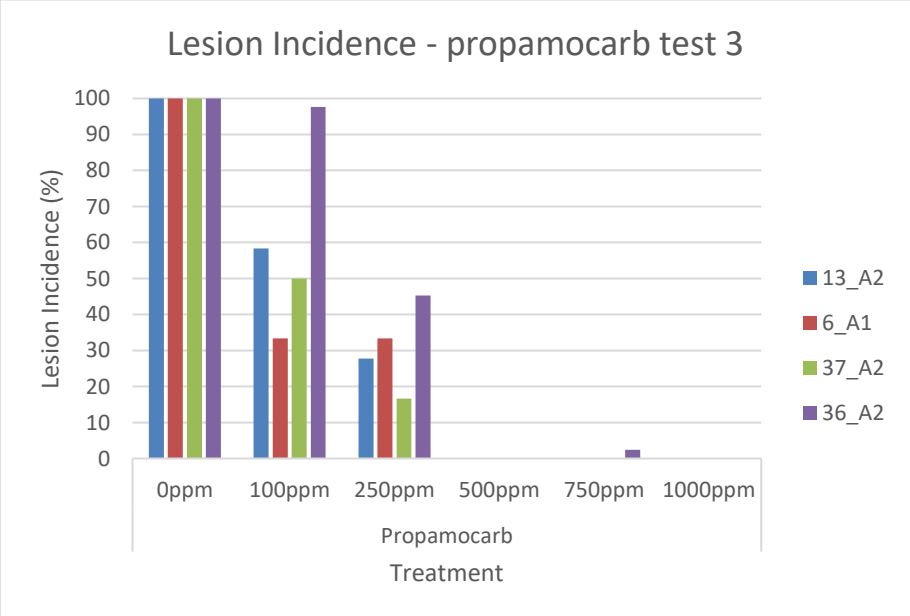


Fig 11. Mean percentage of lesions caused by different genotypes at a range of concentrations of propamocarb (0-1000 µg/ml).

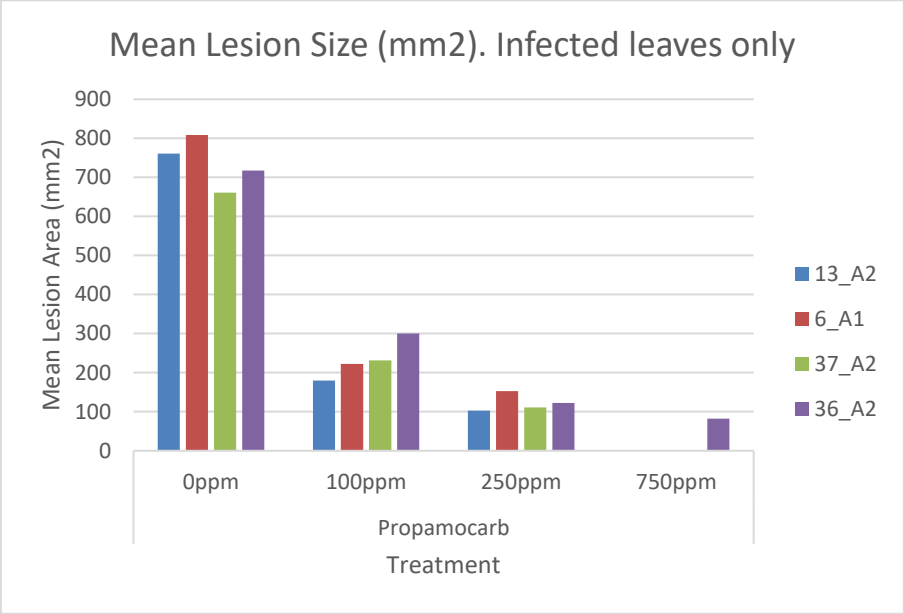


Fig 12. Mean lesion size (mm2) measured on infected leaves only at a range of concentrations of propamocarb

Table 8. Mean, max and min EC50 values for isolates of *P. infestans* of various genotypes tested at a range of concentrations of propamocarb (0, 100, 250, 500, 750, 1000µg/ml). Significant differences between mean values are indicated by different letters.

Genotype	13_A2 & 6_A1	37_A2	36_A2
Number of isolates	7	10	7
Mean EC_{50} (mg/L)	**8.41a**	**21.56a**	**62.03b**
EC_{50} min (mg/L)	3.31	3.31	3.31
EC_{50} max (mg/L)	44.58	133.94	220.83

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 higher than for other genotypes tested.

3. References

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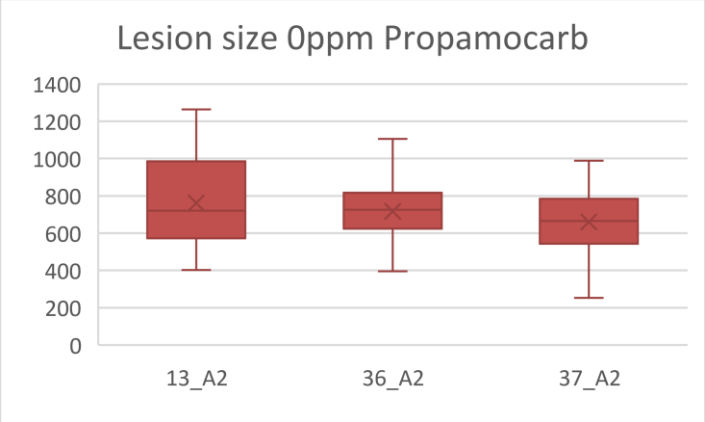
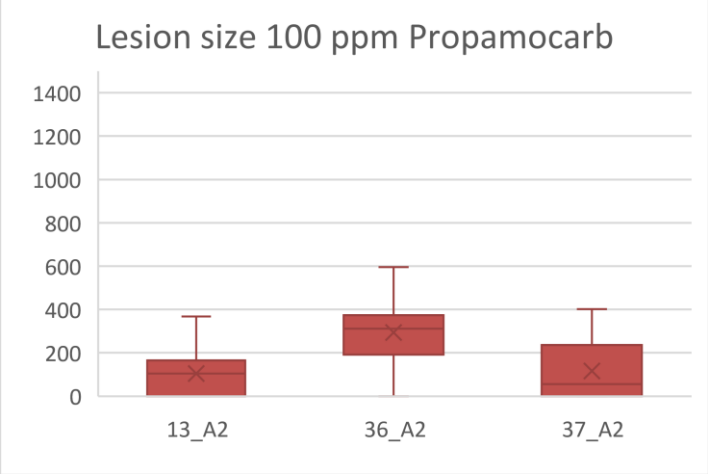
4. Appendix: Lesion Area (mm^2) data presented as Box & Whisker plots:

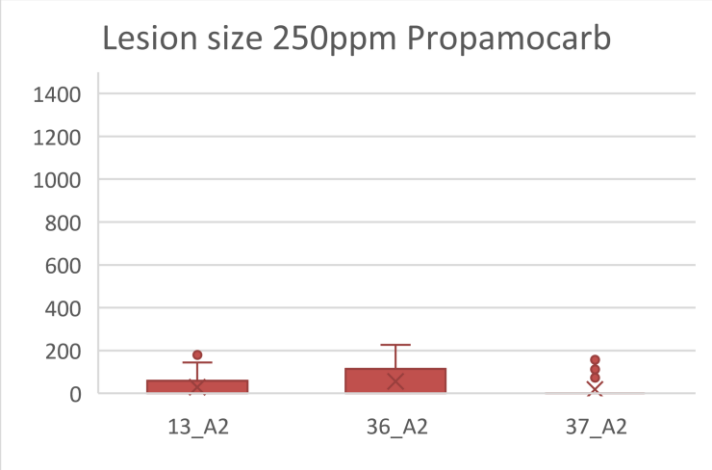
A box and whisker chart shows distribution of data into quartiles, highlighting the mean and outliers. The boxes may have lines extending vertically called “whiskers”. These lines indicate variability outside the upper and lower quartiles, and any point outside those lines or whiskers is considered an outlier.

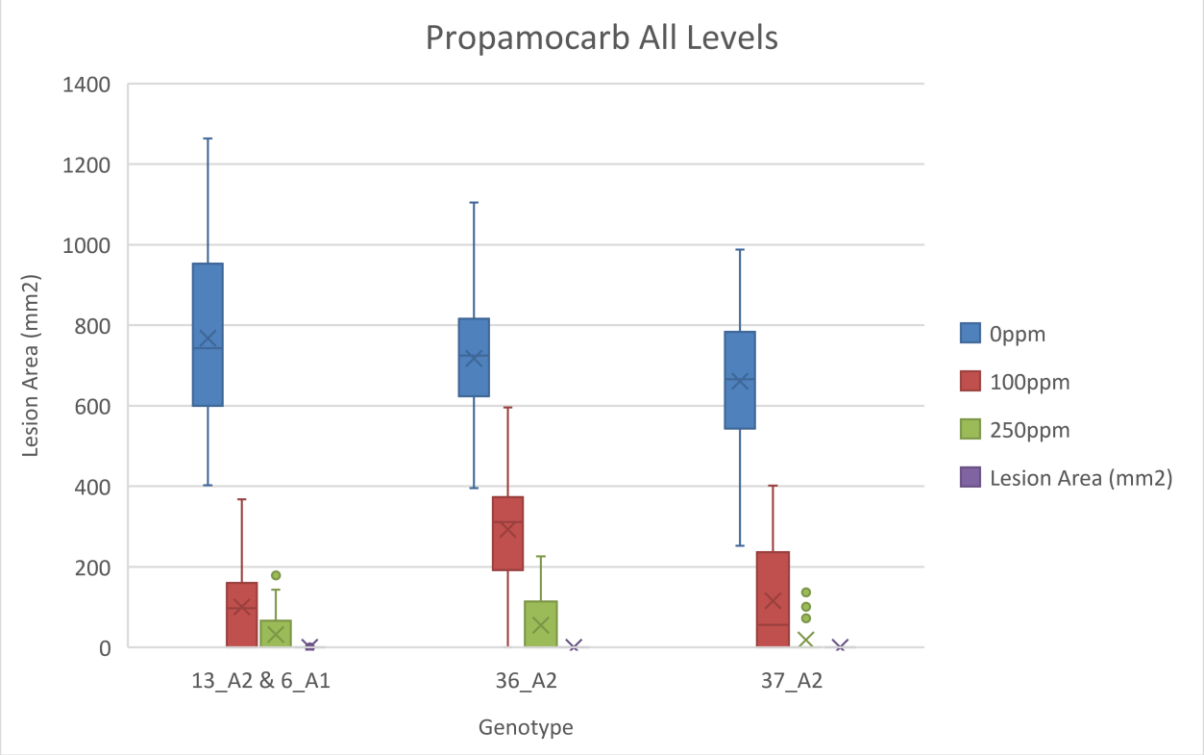
Propamocarb

Max field concentration = 5000ppm

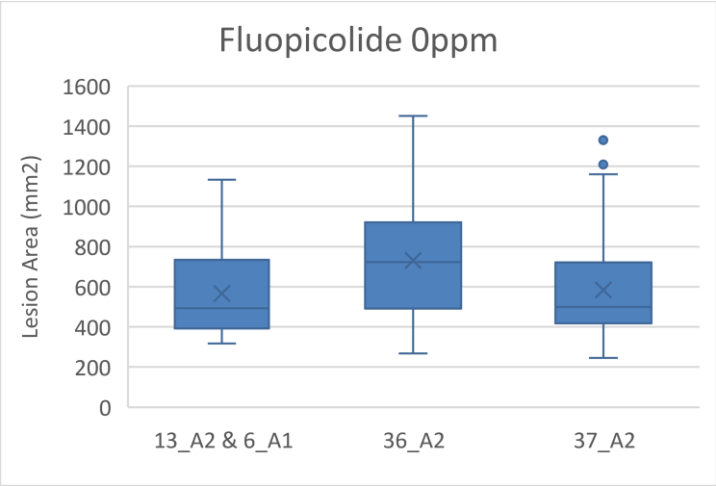


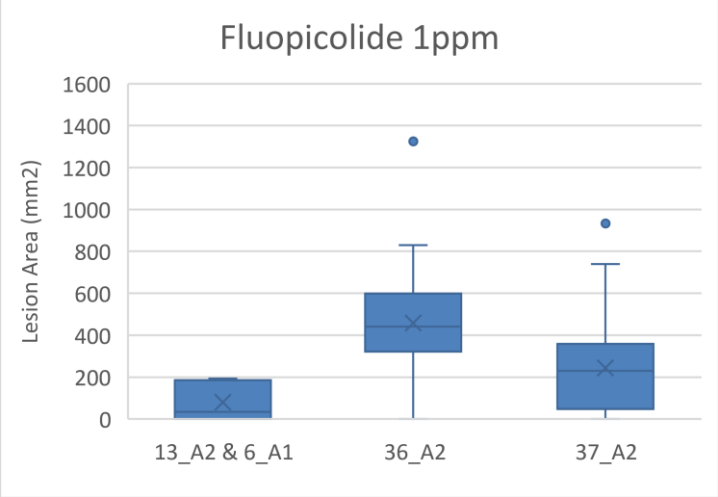


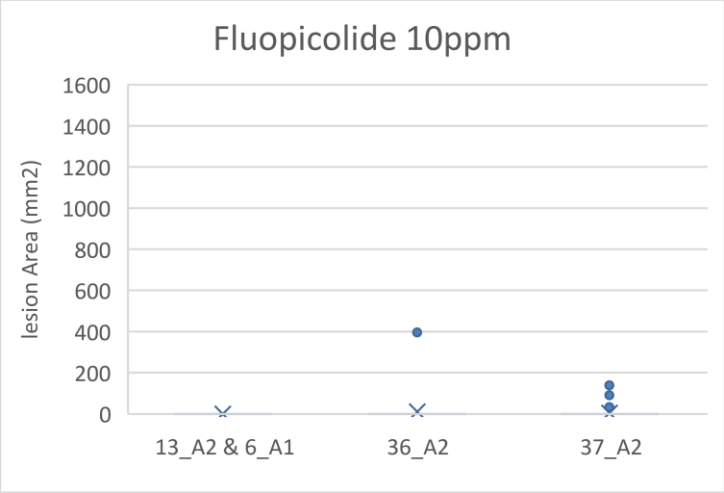


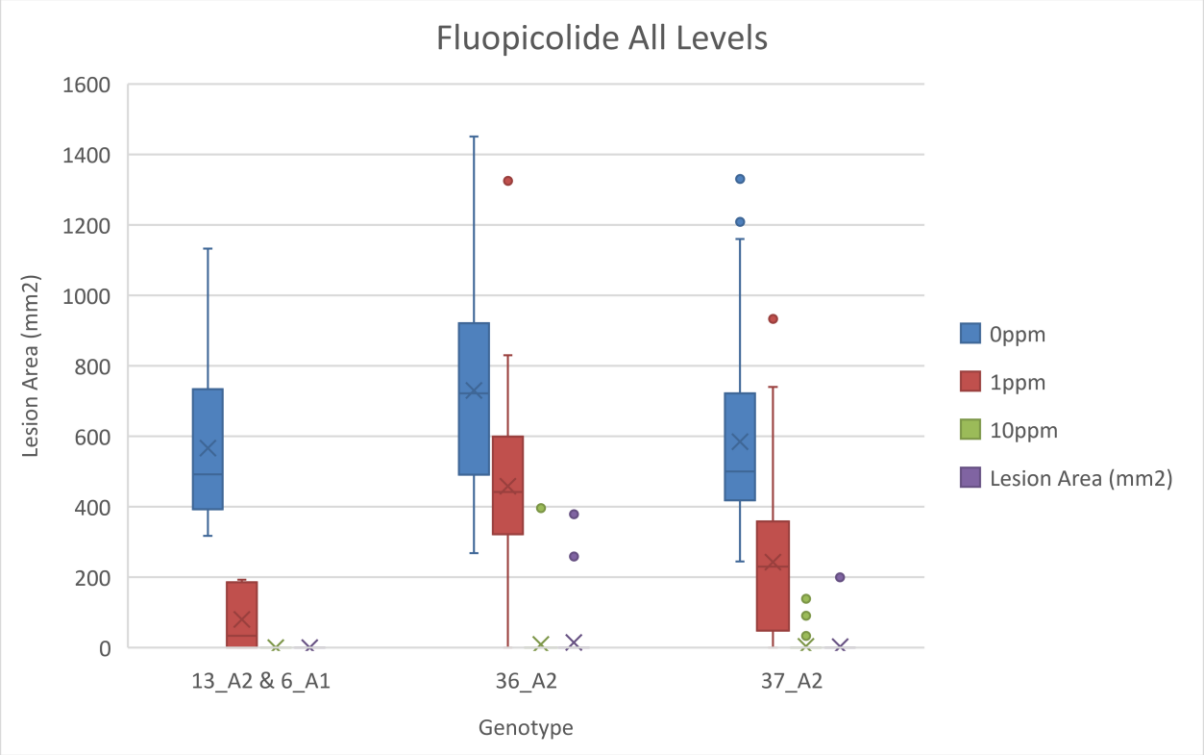


Fluopicolide Max Field concentration (as Infinito) = 500ppm

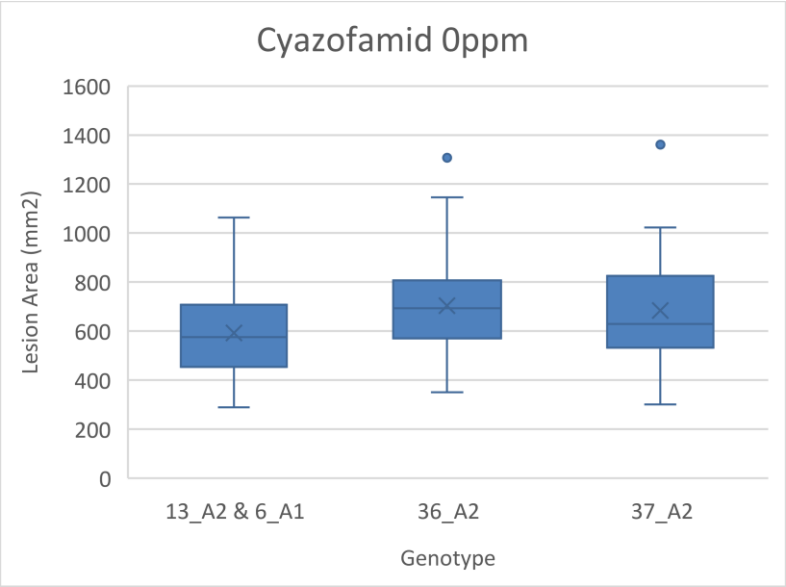


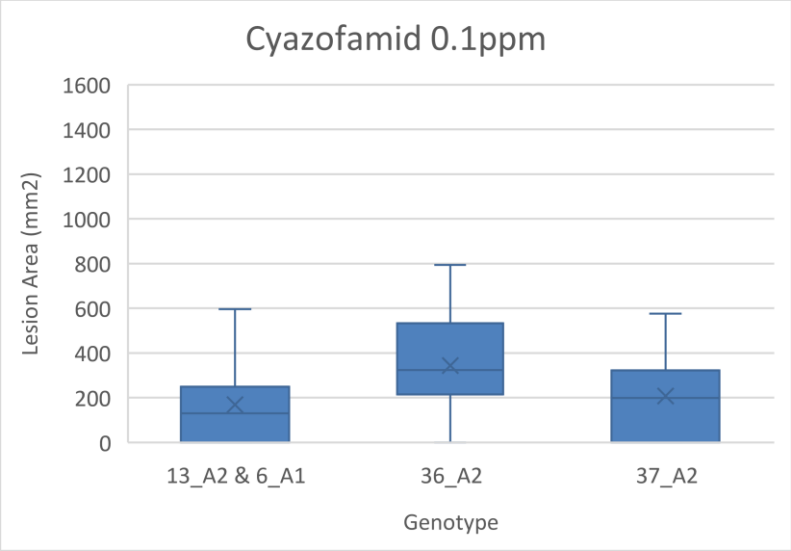


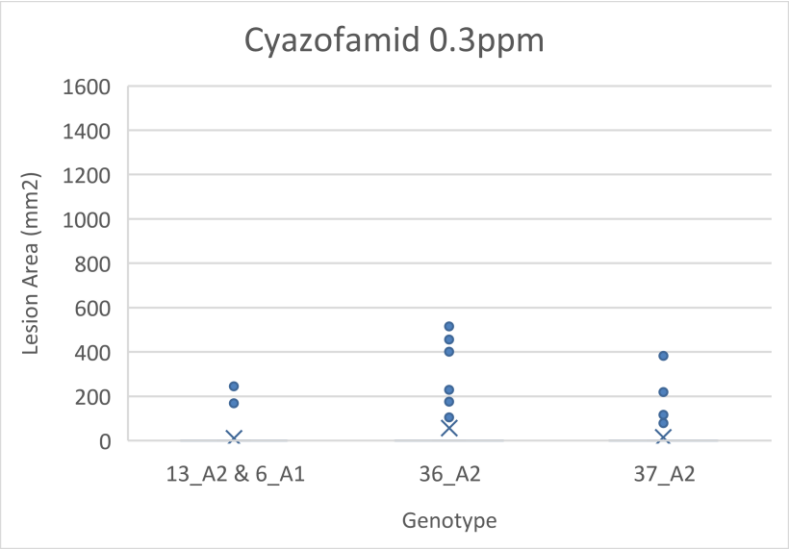


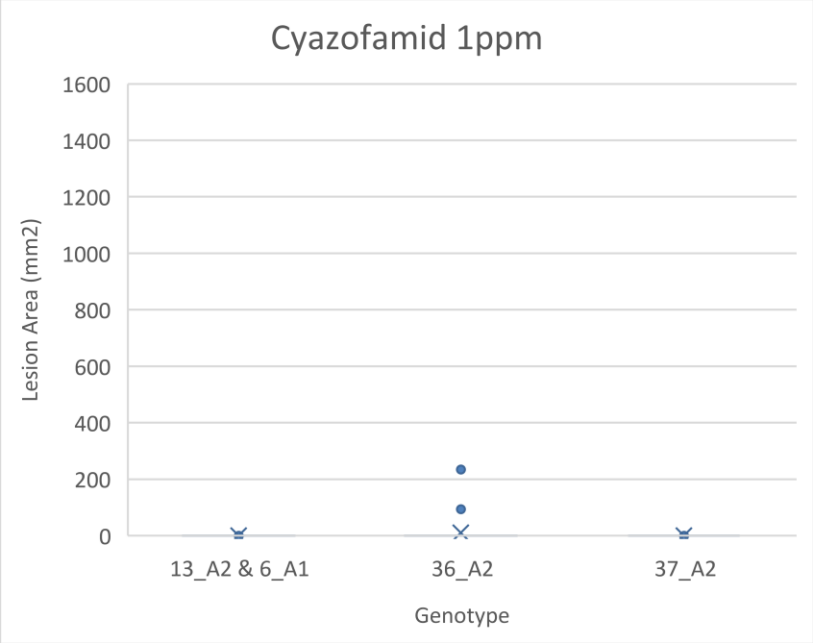


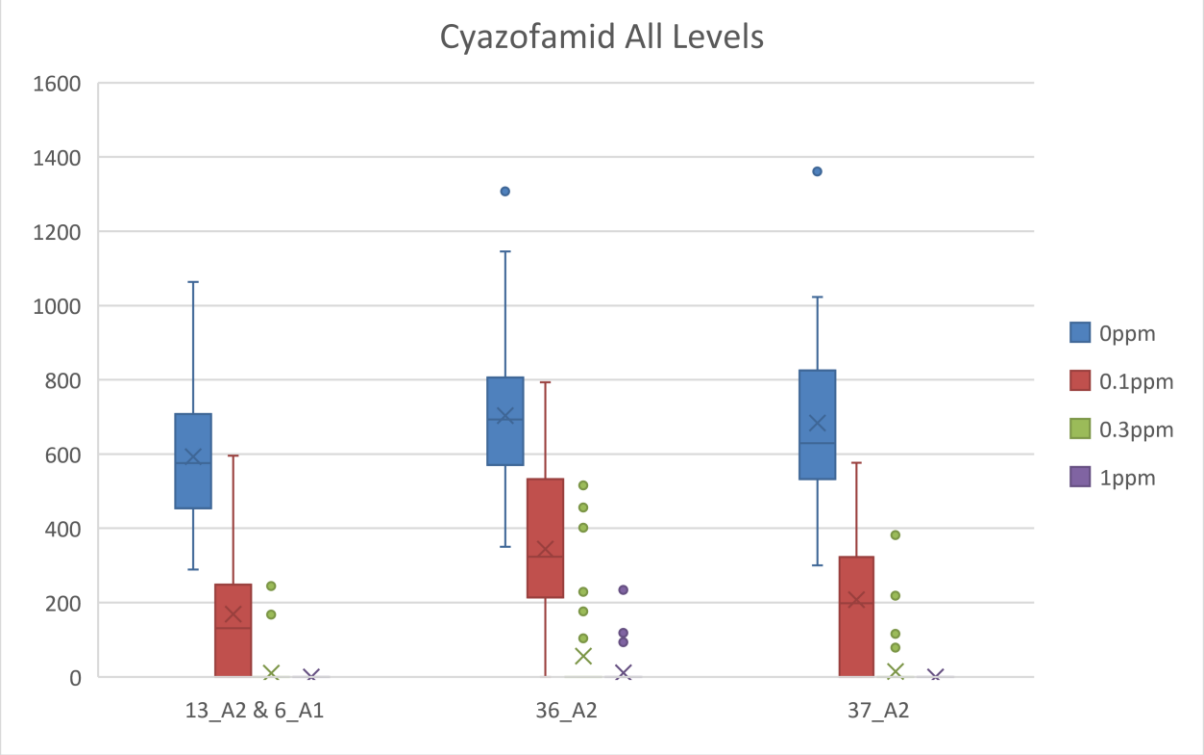
Cyazofamid Max field concentration = 400ppm



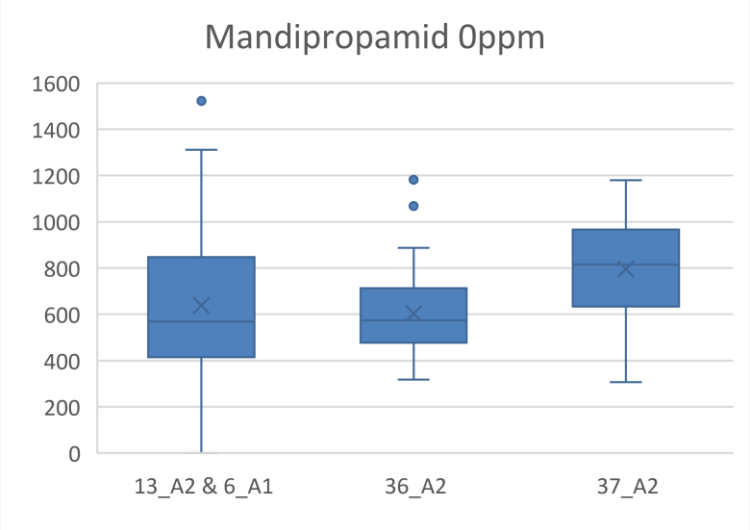


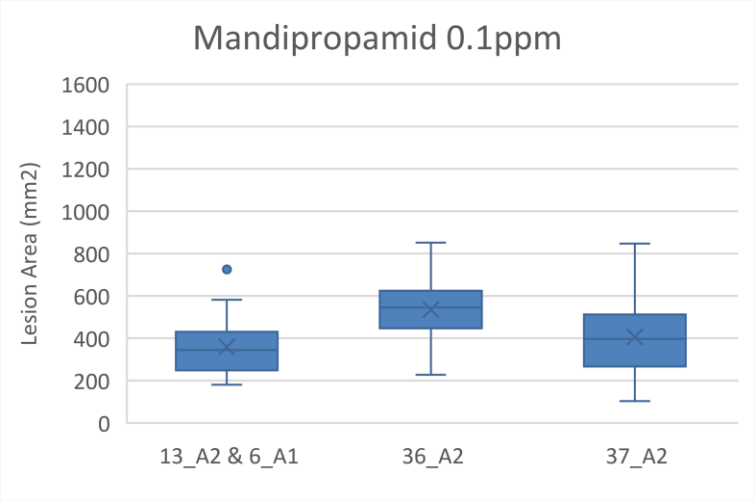


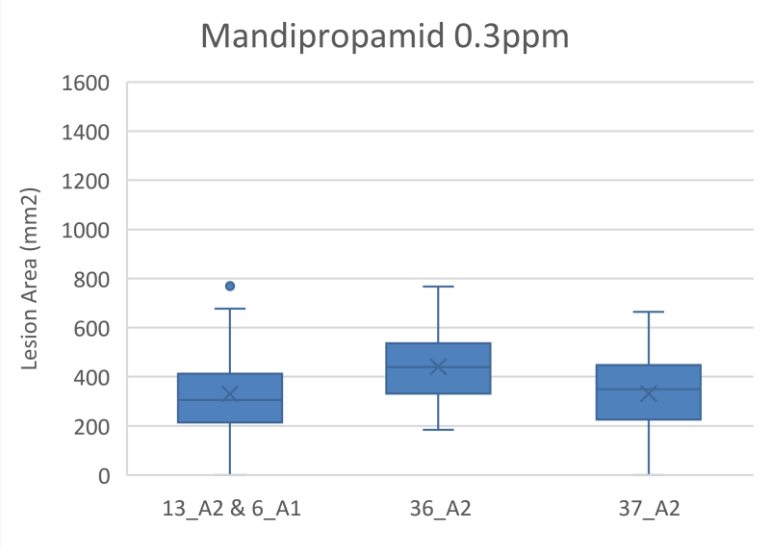


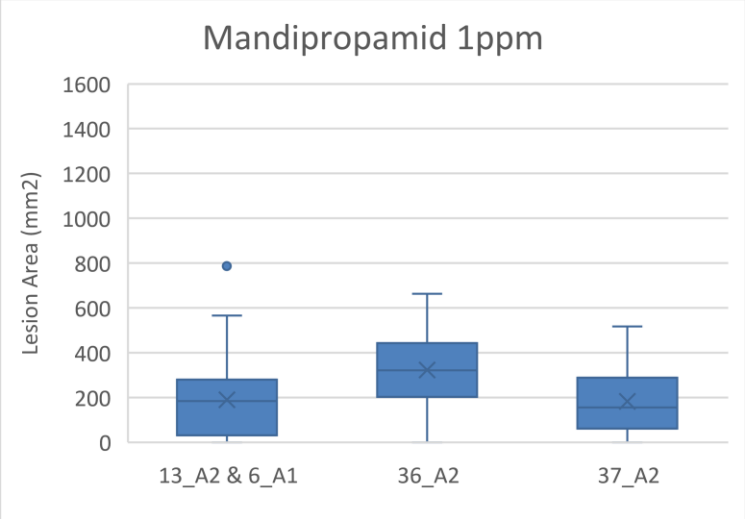


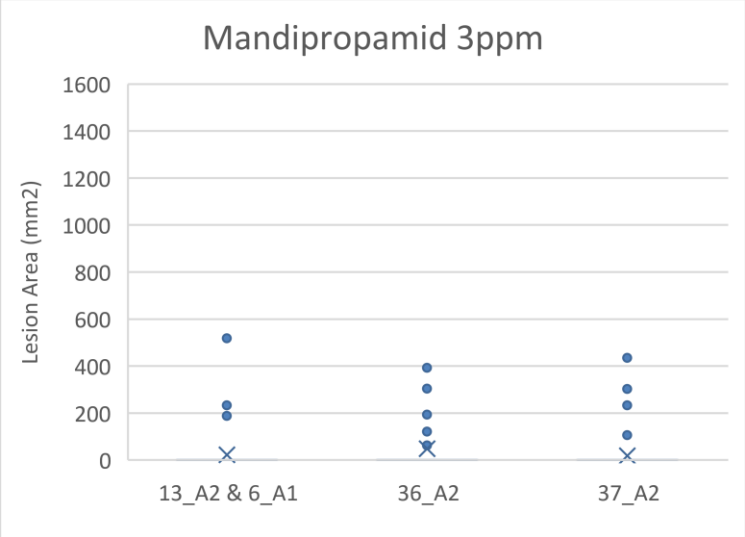
Mandipropamid Max field concentration = 750ppm

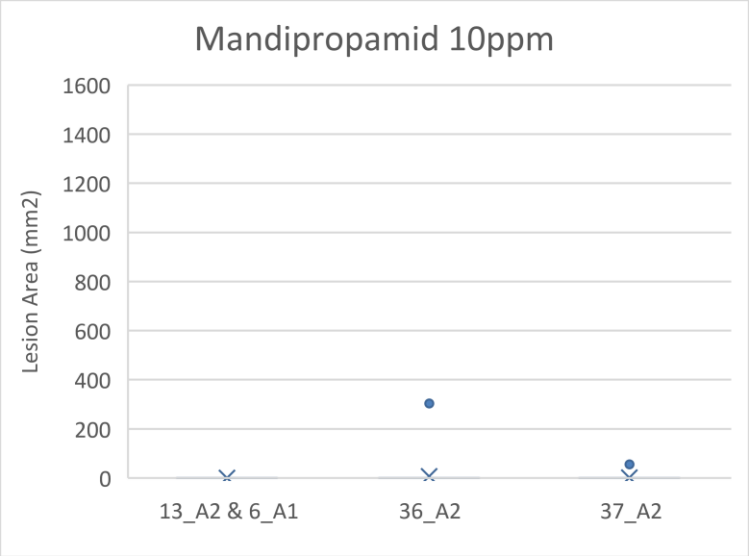


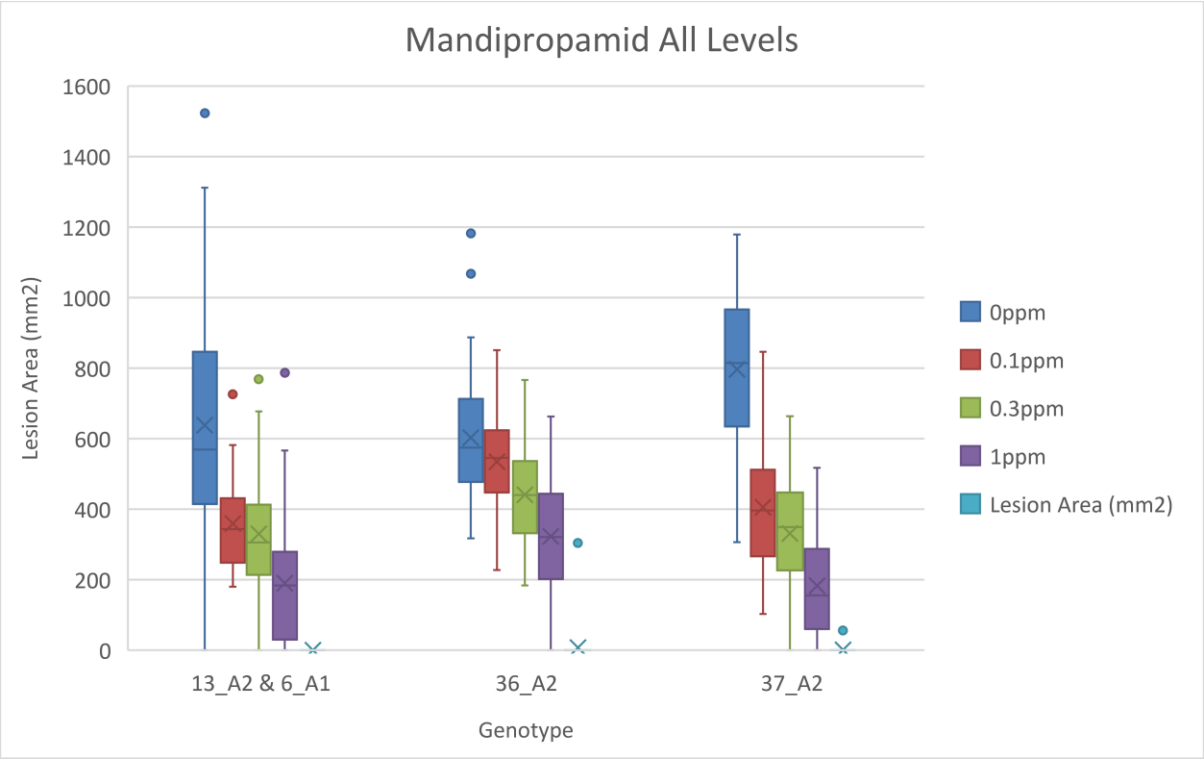












Fluazinam Max field concentration = 1000 ppm