Final Report

Strategies for integrated deployment of host resistance and fungicides to sustain effective crop protection.

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Summary

Sustainable crop protection is of key importance to food security. However, pathogens such as *Phytophthora infestans* may evolve insensitivity to fungicides used to control their populations and evolve virulence to overcome cultivar resistance. This may result in a reduction or loss of disease control and associated yield reductions. Classical population genetic theory predicts that integrating chemical and genetic control should delay the evolution of insensitivity and the evolution of virulence, providing more durable effective control.

The project was developed to test three hypotheses:

H1: Deployment of partial cultivar resistance will reduce selection for fungicide insensitivity.

H2: Deployment of fungicides will reduce selection for virulence.

H3: How crop resistance genes and fungicides are integrated is a key determinant of the durability of effective disease control.

To test these hypotheses two approaches were used.
Firstly, three years of field trials were conducted in Wales and Scotland from 2014 to 2016. These compared the selection for either a known fungicide insensitive strain on susceptible and moderately resistant cultivars (Hypothesis 1), or the selection for a known virulent strain at low and high fungicide doses (Hypothesis 2). In each experiment two key variables were recorded, epidemic growth rate and selection ratio. Population genetic theory predicts that higher epidemic growth rates are associated with higher selection ratios.

To measure the selection ratio, leaf samples showing disease lesions were collected from each of the trials at two different sampling times. Genotype analysis of the DNA recovered from these lesions allowed the subsequent quantification of pathogen population composition. The increase or decrease in the frequency of a given strain in the population is the selection ratio. Environmental conditions and naturally occurring inoculum had a large effect on the epidemic timing and growth rate, to the extent that environmental conditions had a larger effect on population growth rates than the treatments. However, the hypothesised positive relationship between epidemic growth rate and selection ratio was observed. This experimentally demonstrates that reduction in population growth rates reduces selection.

Secondly, an epidemiological model was constructed to test the hypotheses. This model described the growth and senescence of a standard UK potato crop, with epidemics of *Phytophthora infestans* being controlled by a combination of cultivar resistance and fungicide application. The model describes the evolution of insensitivity and virulence in *P. infestans*, agreeing with the results of the field trials in the testing of H1 and H2; that reduction in the epidemic growth rates reduces the time taken to evolve insensitivity or virulence. The model was then used to test hypothesis 3, exploring optimum integrated control programmes to maximize the effective life of fungicides and cultivar resistance.

This work demonstrates that the use of cultivar resistance delays the evolution of fungicide insensitivity (hypothesis 1), and the use of fungicide delays the evolution of virulence (hypothesis 2). Integrating these two control methods extends their durability (hypothesis 3). This provides a practical set of tools to manage the evolution of pathogen populations, extending the durability of disease control.

Key messages

The evolution of *Phytophthora infestans* poses risks to effective disease control. Though fungicide insensitivity cases have been rare, when they do occur insensitivity sweeps through the population rapidly and has a large impact.

The challenge of effective and durable disease control contains an apparent conflict. First, in order to ensure effective levels of control, we must use high levels of fungicide. However,

the use of these high levels of control select for pathogen populations which overcome them, resulting in loss of control.
Developing a management programme that effectively controls the pathogen appears to select for insensitivity, and developing a management programme that has low selection for insensitivity appears to compromise control.

This challenge is exacerbated by increased aggressiveness of the strains. Recent evolution of virulence in the populations has mandated increased spray frequency to effectively control them. The evolution of virulence has downgraded cultivar resistance and sprays have to be applied more frequently to compensate. This increased usage of fungicide is expected to further increase the selection pressure for fungicide insensitivity.

A potential solution to this problem is found in the field of integrated disease control. As previously shown, integrated control, when fungicide inputs are adjusted to the level of cultivar resistance, is practical and provides effective levels of disease control.

As shown herein, the use of cultivar resistance delays the evolution of fungicide insensitivity (hypothesis 1), and the use of fungicide delays the evolution of virulence (hypothesis 2). Integrating these two control methods extends their durability (hypothesis 3).

This approach of integrated control is practical and achievable. Even moderate blight resistance is valuable for delaying the evolution of insensitivity, and the levels of cultivar resistance which have a significant effect currently exist within each market sector.

Growers are often justifiably risk adverse and sceptical of lowering fungicide dose (given the high cost of loss of control), however the principle retains flexibility and “room to manoeuvre”. Significant advantages can be found from partial reductions in dose and use of moderately resistant cultivars, and as growers become more familiar with the concept may become more trusting of it.

The principle of integrated control is not necessarily binary. Further mechanisms could be introduced beyond cultivar resistance and fungicide, and several other mechanisms such as accurate blight forecasting can be introduced, all of which could provide significant benefits. This virtuous cycle of control mechanisms results in a system where each mechanism supports the others, resulting in durable and effective disease control.

Introduction

Disease management relies predominantly on the application of fungicides and deployment of crop resistance genes. However, such control measures exert selection pressures on pathogen populations. Maintaining effective control therefore depends on managing two highly dynamic processes: firstly, the introduction of new host resistance genes and their subsequent failure due to pathogen populations overcoming them (the evolution of virulence) and secondly, the introduction of new fungicide modes of action (MOA) and pathogen populations becoming insensitive to those modes of action over time (the evolution of insensitivity).

Integrated control using a range of options which may include host resistance and fungicide application, is widely believed to lead to more durable control than reliance on one control option. There is, however, surprisingly little published evidence and mechanistic understanding of the combined deployment of fungicides and crop resistance genes. The evolution of fungicide insensitivity and the evolution of virulence are virtually always studied in isolation, whereas in practice the processes interact. In this project we studied the integrated use of fungicides and crop resistance genes to exploit evolutionary interactions to maximise the durability of crop protection and apply the knowledge gained to develop strategies for the durable control of late blight, caused by *Phytophthora infestans*, on potato. This was conducted in the context of a changing *P. infestans* population in the UK in which increased aggressiveness, fungicide resistance and changes in virulence have been observed (Cooke *et a*l, 2012; Gilroy *et al*., 2011; Lees *et al* 2012).

The hypotheses concerning the integrated use of fungicides and cultivar resistance tested in this project were based on population genetic theory of clonal organisms. Consider a case where populations of a fungicide-sensitive and a fungicide-insensitive strain are growing exponentially on a cultivar, in the presence of fungicide. The population is predominantly sensitive, as insensitive strains tend to be rare. However, the growth rate of the insensitive strain, r_R, is larger than the growth rate of the sensitive strain r_S, due to the fungicide. Over time this results in an increase of the frequency of the insensitive strain in the pathogen population (this is selection for fungicide insensitivity).

The difference between the rate of increase of the insensitive strain, r_R, and that of the sensitive strain, r_S, is a measure of the rate of selection for fungicide insensitivity, s_F (Crow

& Kimura 1970). This is stated explicitly in Equation 1 (Bosch, Oliver, Berg, & Paveley 2014), and schematically in Figure 1.

$$s_F = (r_R - r_S)T \qquad (1)$$

In Equation 1, s_F is the selection for virulence in a given period of time, and T is the duration of the selection pressure under consideration. If we aim to reduce s_F (which should delay the evolution of fungicide insensitivity) then we can either reduce the duration of exposure, T, or we can reduce the growth rate of the insensitive strain r_R. A third option, which is the basis for this project, is to simultaneously reduce both r_R and r_S, thereby reducing selection for insensitivity (Milgroom & Fry 1988, Bosch, Oliver, Berg, & Paveley 2014).
For example, the selection for insensitivity against fungicide A is reduced by the addition of fungicide which has a different mode of action. This reduction in selection will happen even without any change in the dose of fungicide A. This is because fungicide B reduces the growth rates of the A-insensitive and A-sensitive strains simultaneously. A considerable body of published experimental and modelling evidence corroborates this (Hobbelen, Paveley, Oliver, & Bosch 2013, Bosch, Oliver, Berg, & Paveley 2014).

Equation 1 implies that not only a fungicide mixing partner, but also any method that reduces the growth rate of both strains, will reduce the selection for fungicide resistance. We thus postulate that using a partially resistant cultivar will reduce the selection for fungicide resistance. Equation 1 also applies to the selection for virulence. Replace the selection rate s_F with s_V (selection for virulence) and r_S with r_A, the growth rate of the avirulent strain and r_R with r_V, the growth rate of the virulent strain. Following the same reasoning as for fungicide insensitivity we can postulate that the use of an effective fungicide treatment program will reduce the rate of selection for virulence. This relationship between epidemic growth rate and selection ratio is summarized in Figure 1 right panel.

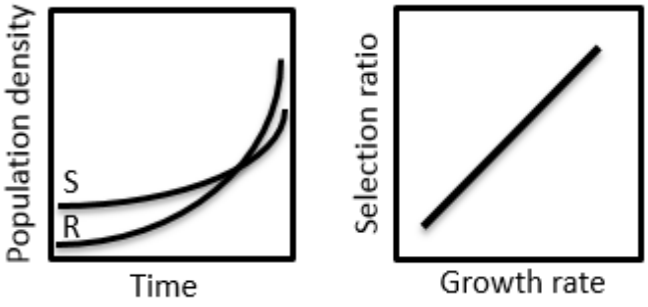

Figure 1 left panel: The dynamics of a fungicide insensitive strain in an existing population of the sensitive strain. The growth rate of the insensitive strain, r_R, is higher than the growth rate of the sensitive strain, r_S, in the

presence of fungicide. The difference in these growth rates is selection for insensitivity. Simultaneous reduction of the growth rates, by use of a fungicide mixing partner or another means, will reduce selection for insensitivity and delay the evolution of insensitivity in the population.

Figure 1 right panel: Relationship between epidemic growth rate (sum of the growth rates of the sensitive and insensitive strains from left pangel 1) and the selection ratio for insensitivity. As a general principle, Equation 1 predicts that simultaneous reduction in the growth rates of both strains (reduction in growth rate) will reduce selection. For the problem of fungicide insensitivity, this simultaneous reduction should be achievable with cultivar resistance. For the problem of virulence, this simultaneous reduction should be achievable with fungicide dose.

This reasoning leads to the following set of hypotheses that form the basis of the project:

H1: Deployment of partial cultivar resistance will reduce selection for fungicide insensitivity.

H2: Deployment of fungicides will reduce selection for virulence.

H3: How crop resistance genes and fungicides are integrated is a key determinant of the durability of effective disease control.

Hypotheses we tested using late blight (causal organism *Phytophthora infestans*) on potato. Late blight is managed with intensive fungicide programmes, complemented with host resistance (conferred predominately by quantitative resistance) in some potato cultivars. Recent changes in the pathogen population raises concerns in the industry and provide a system to critically test the effects of integrated control:

the dominant clonal strains of *P. infestans* in the UK, 13_A2 and 6_A1, are metalaxyl resistant and sensitive respectively, so these can be inoculated at specific ratios in field experimentation;

new races resulting from changes in the highly diverse effector repertoire in *Phytophthora infestans* have evolved, resulting in downgrading of many cultivar resistance ratings.

These hypotheses were tested in field experiments and with a modelling approach. Hypothesis 1 was tested using a susceptible potato cultivar (King Edward) and a partially resistant cultivar (Cara) and selection for insensitivity to phenylamide fungicides was measured. Strains of *P. infestans* which differ in virulence profile due to well characterised mutations in their RXLR effector genes have been isolated (e.g. Gilroy *et al*. 2011). This allowed the effect of the presence or absence of fungicide treatment on virulence selection to be measured for the first time to test hypothesis 2.

A model for the late blight system was developed and used to test both hypothesis 1 and 2, and compare the results with the experiments. Hypothesis 3 could only be tested using the model as multi-year experiments on selection are not feasible.

Methods

1 Experimental field methods

There were several small differences in methodology between sites. This was a constraint of the different locations. The methodologies used are detailed below.

1 A – SRUC Methodology

Planting and design: To minimise the risk of infection by inoculum other than the test isolates the aim was to plant the experiments earlier than standard blight fungicide trials (Table 1). Seed was also physiologically aged between receipt and planting if possible. Trial design: randomised complete block with six replicates (2014) or four replicates (2015 & 2016).

2014: Plot 3.4 m (4 rows) x 8.1 m with 30 cm seed spacing. Plots separated by 2.1 and 2.6 metres of bare earth. 2015 & 2016: Plot 5.7 m (6 rows) x 5.06 m with 23 cm seed spacing. Plots separated by 2.76 and 2.6 metres of bare earth.

Table 1. SRUC Planting dates

Experiment	2014	2015	2016
1	16 May	22 April	-
2	16 May	24 April	30 April
3	29 April	23 April	06 May
4	30 April	23 April	10 May

Inoculum production: The *P. infestans* isolates selected for their appropriate traits and aggressiveness were provided each year as cultures and leaf inoculum by the James Hutton Institute (JHI). As a control measure to confirm that the inoculum was of the correct genotypic profile at key experimental stages, samples of isolates were collected whilst being sub-cultured at Scotland's Rural Collage (SRUC) and from infector plant lesions. The samples were submitted to JHI for genotyping.

Inoculation procedure in 2014: Five isolates of *P. infestans* were grown on Rye B agar for 2 weeks at 16˚C. In all four field experiments eight plants (four adjacent plants in each centre row) in the centre of each plot were identified and marked with canes. Four of the eight central plants were inoculated. The inoculum used had been subbed repeatedly on

detached King Edward leaves. The isolate mixtures are listed in Tables 2 and 3. Each of the four field plants per plot was inoculated with 1 ml of sporangial suspension using a hand-held spray bottle. Each plant was then covered and sealed using a clear polythene bag. Bags were removed the following morning. In 2015 and 2016 infector plants (grown in 8 x 8 cm Jiffy pots) were used to inoculate plots.

Inoculation Procedure in 2015: For each HAPI experiment 48 infector plants (cv. King Edward, grown in a polytunnel) were inoculated with 1 ml of sporangial suspension (The isolate mixtures are listed in Tables 2 and 4) and incubated to promote infection. Plants inoculated with the same isolate combination were kept separate from others (in separate growth rooms) to prevent cross contamination. Two infector plants were placed in the centre of each plot, between rows 3 and 4, after lesions were visible. HAPI 4 was re-inoculated because the epidemic was the slowest of the four trials. Leaf material provided by JHI was used. Plots were re-inoculated using the 2014 method.

Inoculation Procedure in 2016: At the time of machine planting the trials, row 4 of each plot was hand planted with a double-spaced five-plant infector strip. HAPI 2 trial plots were planted with the appropriate variety, i.e. King Edward seed in the King Edward plots and Cara seed in the Cara plots. For the HAPI 3 and 4 trials the double-spaced infector strips of five tubers were Shepody. For each HAPI experiment 96 infector plants (cv. Shepody) in Jiffy pots were inoculated with 1 ml of sporangial suspension (The isolate mixtures are listed in Tables 2 and 5). Approximately 6 days after inoculation four infector plants in Jiffy pots were transplanted into each infector strip. On 8 July for two infector plants per strip in the HAPI 3 & 4 trials, one inoculated infector was bagged with one healthy Shepody infector. This was necessary because the small infector plants were rapidly senescing and rapid spread of the introduced inoculum was required. A small hole was made in each bag to prevent the temperature being raised. The bags were removed on 10 July.

Table 2. Final inoculum concentrations (6_A1 + 13_A2)

Experiment	2014	2015	2016
1	2.5 x 10^5 per ml	7.5 x 10^4 per ml	-
2	2.5 x 10^5 per ml	1 x 10^5 per ml	6.75 x 10^4 per ml
3	2.5 x 10^5 per ml	5 x 10^4 per ml	1 x 10^5 per ml
4	1 x 10^5 per ml	1 x 10^5 per ml*	6.75 x 10^4 per ml

Table 3. Summary of HAPI inoculation and leaf sampling for all trials 2014

	13_A2 isolate	6_A1 isolate	Inoculated*	Sample (T1)	FTA Cards (T1)	Fungicide application	Sample (T2)	FTA Cards (T2)
HAPI 1	H4 (5%)	H5 (95%)	29-Jul	18-Aug	18 & 19 Aug	19-Aug	28-Aug	28 & 29 Aug
HAPI 2	H2 (5%)	H7 (95%)	17-Jul	04-Aug	04 & 05Aug	06-Aug	13-Aug	13 & 14 Aug
HAPI 3	H4 (5%)	H5 (95%)	29-Jul	08-Sep	09-Sep	09-Sep	15-Sep	15 & 16 Sep
HAPI 4	H3 (5%)	H7 (95%)	17-Jul	20-Aug	21-Aug	21-Aug	01-Sep	02-Sep

*Four plants inoculated per plot

Table 4. Summary of HAPI inoculation and leaf sampling for all trials 2015

Experiment	13_A2 isolate	6_A1 isolate	Inoculated	Infector plants placed in plots	Infector FTA	Sample (T1)	FTA Cards (T1)	Fungicide application	Sample (T2)	FTA Cards (T2)
1	H4 (5%)	H5 (95%)	26 Jun	29-Jun	01-Jul	25-Aug	26-Aug	25-Aug	03-Sep	04-Sep
				(68 dap)					(9 days)	
2	H2 (5%)	H7 (95%)	29 Jun	02-Jul	07-Jul	01-Sep	02-Sep	02-Sep	09-Sep	10-Sep
				(69 dap)					(7 days)	
3	H4 (5%)	H5 (95%)	3 Jul	07-Jul	09-Jul	31-Aug	01-Sep	31-Aug**	08-Sep	09-Sep
				(75 dap)					(8 days)	
4	H3 (5%)	H7 (95%)	03-Jul	08-Jul	09-Jul	25-Aug	27-Aug	25-Aug**	02-Sep	03-Sep
				(76 dap)					(8 days)	
4*	H3 (5%)	H7 (95%)	05-Aug	n/a						

Two inoculated Jiffy pot plants were placed in each plot
*Re-inoculation of HAPI 4 - two plants per plot were tagged & inoculated using spray bottle method
** early application of Consento on 02 July

Table 5. Summary of HAPI inoculation and leaf sampling for all trials 2016

	13_A2 isolate	6_A1 isolates	Inoculated	Infector plants placed in plots	Infector FTA Sample	Sample (T1)	FTA Cards (T1)	Fungicide application	Sample (T2)	FTA Cards (T2)
HAPI 2	H3 (5%)	H5 + H7 (47.5 + 47.5%)	29-Jun	05-Jul	06-Jul	02-Aug	05-Aug	04-Aug	15-Aug	17-Aug
				(66 dap)					(13 days)	
HAPI 3	H2 (5%)	H5 + H7 (47.5 + 47.5%)	30-Jun	05-Jul	07-Jul	29-Jul	01-Aug	30-Jul	09-Aug	11-Aug
				(65 dap)					(11 days)	
HAPI 4	H3 (5%)	H5 + H7 (47.5 + 47.5%)	29-Jun	05-Jul	07-Jul	02-Aug	04-Aug	02-Aug	10-Aug	12-Aug
				(65 dap)					(8 days)	

Four inoculated Jiffy pot plants were placed in each plot

Fungicide treatments: In 2014 and 2015 experiments were blanket sprayed at c. 7-day intervals with a range of fungicides to prevent ingress of naturally occurring late blight inoculum prior to test treatments being applied. These blanket sprays were stopped at least 14 days prior to the inoculation date. Treatment fungicides were applied on the dates specified (Tables 6 and 7). To test the selection for fungicide resistance and the modifying effect of cultivar resistance (experiments 1 and 2), metalaxyl-M treatments were applied as outlined in Table 6. The dose of Ridomil Gold 480 SL (480 g/L metalaxyl-M) to apply was calculated using the maximum recommended rate for metalaxyl-M in Fubol Gold as the full recommended rate (or 1.0 dose). To test the effect of fungicide application on the selection

for virulence (experiments 3 and 4), different doses of Consento were applied as outlined in Table 7.

Table 6. Treatments applied to experiments 1 and 2 (hypothesis 1)

			Main application 2014_1	Main application	Main application 2014_2	Main application	Main application 2015_1 & 2 2016_2	Main application
Treatment	Product	Variety (foliar blight resistance rating)	Product L/ha	Proportion of full label dose	Product L/ha	Proportion of full label dose	Product L/ha	Proportion of full label dose
1	Untreated	King Edward (3)	0	0	0	0	0	0
2	Untreated	Cara (5)	0	0	0	0	0	0
3	Ridomil Gold 480 SL	King Edward (3)	0.0195	0.125	0.039	0.25	0.051	0.33
4	Ridomil Gold 480 SL	King Edward (3)	0.039	0.25	0.077	0.50	0.103	0.67
5	Ridomil Gold 480 SL	Cara (5)	0.0195	0.125	0.039	0.25	0.051	0.33
6	Ridomil Gold 480 SL	Cara (5)	0.039	0.25	0.077	0.50	0.103	0.67

Table 7. Treatments applied to experiments 3 and 4 (hypothesis 2)

Treatment	Product	Variety (foliar blight resistance rating)	Early application 2015_3, 4	Early application	Main application 2014_3, 4	Main application	Main application 2015_3, 4 2016_3, 4	Main application
			Product L/ha	Proportion of full label dose	Product L/ha	Proportion of full label dose	Product L/ha	Proportion of full label dose
1	Untreated	King Edward (3)	0	0	0	0	0	0
2	Untreated	Pentland Dell (4)	0	0	0	0	0	0
3	Consento	King Edward (3)	0.5	0.25	0.5	0.25	1.0	0.5
4	Consento	King Edward (3)	1.0	0.5	1.0	0.5	2.0	1.0
5	Consento	Pentland Dell (4)	0.5	0.25	0.5	0.25	1.0	0.5
6	Consento	Pentland Dell (4)	1.0	0.5	1.0	0.5	2.0	1.0

Sampling of lesions from field plots: The ratio of metalaxyl-M-sensitive and –insensitive *P. infestans* strains (experiments 1 and 2) and avirulent and virulent *P. infestans* strains (experiments 3 & 4) just prior to the application of the test fungicides and 7 to 10 days later was determined by intensive sampling of new lesions at the appropriate timings (Tables 3 to 5). All possible steps were taken during sampling and lesion handling to prevent cross contamination, e.g. disposable coveralls and gloves were changed between treatments and Wellington boots washed.

In 2014, sterile Petri dishes lined with damp filter papers were used to detach 16 randomly selected leaflets, each with a single sporulating lesion, from the central rows of each plot. Plants that had been inoculated were avoided. Contact with lesions was circumvented to prevent cross contamination. The lesions were incubated at 16 ^{o}C to promote sporulation. The duration of incubation was generally 24 to 48 hours. In 2015, the same method was used except 24 lesions were collected. In 2016, clear polythene Ziploc bags containing

damp tissue were used to collect lesion samples. In all years the dishes or bags were labelled with experiment number, plot reference and sample (first or second). Incubated lesions were pressed onto FTA cards using the Euroblight protocol method. Once dry, individual FTA cards were placed in separate small Ziploc plastic bags for transport to JHI for genotyping.

1 B – ADAS Methodology

Experimental design: Each experiment was laid out as a fully randomised design with four replicates (six replicates in 2014) per treatment. Seed was hand planted at 30cm spacing. Plots were four rows wide (each row = 0.75m wide) by 8m long (6 replicates) in 2014 and six rows wide by 6m long (4 replicates) in 2015 & 2016. Experiments were planted on 7 and 9 May in 2014 (near Lampeter), 18 and 19 April in 2015 (Lampeter and Aberystwyth), 4 and 5 May in 2016 (Lampeter and Aberystwyth). The field trial site in 2015 is shown in Figure 2.

Figure 2. Field trial site near Aberystwyth at inoculation on 13 July 2015.

Production of inoculum for field experiments: Five isolates of *P. infestans* (supplied by JHI) were grown on potato dextrose agar (PDA) for 3 weeks at 16˚C. Plants (cv. King Edward) were grown in a polytunnel for a minimum of 6 weeks. The leaves were detached and placed abaxial side up in to a box lined with damp paper towel. Boxes were inoculated using a handheld sprayer containing a spore suspension for individual isolates of between 5 x 10^4 sporangia/ml and 5 x 10^5 sporangia/ml. Boxes were sealed and placed into a growth cabinet at 16˚C and relative humidity maintained at 90% until sporulation was visible.

In 2014 and 2015, spore suspensions were produced from these leaves and used to inoculate individual plants in the field plots. The isolates used and the ratios are shown in Table 8. Four plants along the centre two rows of each plot were inoculated with 4ml of 1 x 10^5 sporangia/ml spore suspension and polythene bags placed over the top. The bags were removed after 24 hours. In 2016, infector plants were produced and these were placed in to the field. To produce these plants, individual isolates were grown on leaves as previously described. Polytunnel grown plants (cv. King Edward) were placed into large ziploc bags and inoculated with 5ml of a 1 x 10^5 sporangia/ml suspension of the appropriate isolate ratios (Table 8) using a handheld sprayer. The bags were sealed immediately and plants placed in a growth cabinet at 16˚C maintained at 90% relative humidity. After 48 hours the plants were removed and placed into a polytunnel for a further 5 days until the first "peppering" symptoms were observed. They were transported to the trial sites and two holes (same size as infector plant pots) were dug mid-way along the centre two rows of the plots. A single pot was placed in to each hole and thoroughly watered. Sites were inoculated on 18 July in 2014 and 13 July in 2015, and infector plants placed out in to the field on 5 July 2016.

Table 8. Ratios of isolates used to inoculate the field experiments 2014 to 2016.

Experiment	Year and proportion of each isolate applied to each experiment*					
	2014		2015		2016	
	95%	5%	95%	5%	95%	5%
1	H4	H5	H4	H5	H5 + H7	H2
2	H2	H7	H2	H7	H5 + H7	H3
3	H4	H5	H4	H5	H5 + H7	H2
4	H3	H7	H3	H7	H5 + H7	H3

*where two isolates are stated, these were included in equal proportions.

Experimental treatments – cultivars and fungicides: Experiments were oversprayed at 7 day intervals with a range of fungicides to prevent ingress of naturally occurring late blight inoculum prior to test treatments being applied. If fungicides were applied after plots were inoculated, the inoculated or infector plants were covered prior to fungicide sprays. Oversprays were stopped at least 7 days prior to the inoculation date. To test the selection for fungicide resistance on different cultivars (Hypothesis 1: Experiments 1 and 2), treatments were applied as outlined in Table 9 in 2015 and 2016. The full dose (1.0 dose) of Ridomil Gold 480 SL (480 g/L metalaxyl-M: Syngenta Crop Protection Ltd) to apply was calculated as 0.155 L/ha. This was taken from the maximum recommended rate for

metalaxyl-M in Fubol Gold (64% w/w mancozeb + 3.88% metalaxyl-M: Syngenta Crop Protection Ltd).

Table 9. Treatments applied to experiments 1 and 2 (Hypothesis 1).

Trt No	Product	Variety (foliar blight resistance rating)	Product L/ha	Proportion of full label dose
1	Untreated	King Edward (3)	0	0
2	Untreated	Cara (5)	0	0
3	Ridomil Gold 480 SL	King Edward (3)	0.051	0.33[a]
4	Ridomil Gold 480 SL	King Edward (3)	0.103	0.67[b]
5	Ridomil Gold 480 SL	Cara (5)	0.051	0.33[a]
6	Ridomil Gold 480 SL	Cara (5)	0.103	0.67[b]

[a]proportion of the recommended label dose was 0.25 (0.039 L/ha) in 2014.
[b]proportion of the recommended label dose was 0.50 (0.077 L/ha) in 2014.

To test the selection for virulence on different cultivars (Hypothesis 2: Experiments 3 and 4), fungicides were applied as outlined in Table 10. Consento (75 g/L fenamidone + 375 g/L propamocarb-hydrochloride: Bayer CropScience) was used as the test fungicide. Fungicide doses and number of applications varied over across the three years of experiments.

Table 10. Treatments applied to experiments 3 and 4 (Hypothesis 2).

Trt No	Product	Variety (foliar blight resistance rating)	Prior to visible lesions in the trial area	When lesions are visible in plots after inoculation*
1	Untreated	King Edward (3)	0	0
2	Untreated	Pentland Dell (4)	0	0
3	Consento	King Edward (3)	0.25 L/ha[a]	0.5 L/ha[c]
4	Consento	King Edward (3)	0.5 L/ha[b]	1.0 L/ha[d]
5	Consento	Pentland Dell (4)	0.25 L/ha[a]	0.5 L/ha[c]
6	Consento	Pentland Dell (4)	0.5 L/ha[b]	1.0 L/ha[d]

*not applied in 2014. [a]0.5 L/ha applied in 2015. [b]1.0 L/ha applied in 2015. [c]1.0 L/ha applied in 2015. [d]2.0 L/ha applied in 2015.

Dates of the fungicide applications are shown in Table 11. All experiments were sampled twice during the season. The first sample was taken from experiments 1 and 2 prior to fungicides being applied and the second sample when new lesions were visible after the fungicide application.

Table 11. Fungicide application dates

Experiment	Fungicide application date(s)*		
	2014	2015	2016
1	8 August	6 August	30 July
2	8 August	6 August	30 July
3	8 August	A: 22 July B: 30 July	A: 13 July B: 30 July
4	8 August	A: 22 July B: 30 July	A: 13 July B: 30 July

*for two applications, the first was applied on date A and the second on date B.

Leaf sampling for genotyping: Leaf samples for genotyping were taken when lesions were visible on plants (excluding the infector plants) prior to fungicides being applied in experiments 1 and 2 in all years. In experiments 3 and 4, leaf samples were taken prior to the first fungicide application in 2014 and after the first fungicide application and prior to the second fungicide application in 2015 and 2016. Twenty-four leaves with sporulating lesions were randomly selected from the central two rows. Contact with lesions was avoided to prevent cross contamination. In 2014, leaves were pressed on to FTA cards in the field using the Euroblight protocol. In 2015 and 2016, leaves were removed from the field and placed into Petri dishes containing moist paper tissue. The leaves were incubated at 15 to 20˚C overnight. The following day, the lesions were pressed onto FTA cards using the Euroblight protocol. Once dry, individual FTA cards were placed in separate small Ziploc plastic bags before shipping to JHI. Dates are in Table 12.

Table 12. Dates the trial plots were sampled.

Experiment	First (A) and second (B) sampling date		
	2014	2015	2016
1	A: 8 August B: 18 August	A: 4 August B: 18 August	A: 28 July B: 8 August
2	A: 8 August B: 18 August	A: 4 August B: 18 August	A: 28 July B: 8 August
3	A: 8 August B: 18 August	A: 29 July B: 10 August	A: 28 July B: 8 August
4	A: 8 August B: 18 August	A: 29 July B: 10 August	A: 28 July B: 8 August

2 Genotyping methods

Use of FTA cards for SSR fingerprinting: Sets of FTA cards were provided by ADAS and SRUC at the end of the 2014, 2015 and 2016 field seasons. A total of 25,276 lesions were pressed onto these cards over the three seasons (Table 13). Each FTA card had space to imprint 4 lesions onto specific zones of the absorbent paper matrix. In some cases, particularly in the early stages of the epidemic on more resistance hosts, there were insufficient lesions to sample the full complement per plot. Pathogen DNA was extracted from these cards via 3mm disks cut from the inner edge of the green chlorophyll zone (Figure

3) using the proprietary Whatman® FTA® purification reagent. The cut and eluted disks were subsequently used for SSR analysis with a 12-plex marker set (Li et al., 2012). The SSR allele peaks were checked manually and scored against those of reference isolates prior to export to excel spreadsheets for further analysis.

Figure 3. An example of an FTA card showing the micro-punch and the location of the disks cut from each lesion imprint.

Table 13. Numbers of late blight lesions supplied to The James Hutton Institute pressed onto FTA cards each season from the 4 field trials run by ADAS and SRUC.

		2014	2015	2016	Total
ADAS	Exp1	915	1112	1114	3141
	Exp2	1152	1104	1146	3402
	Exp3	1148	1150	1152	3450
	Exp4	940	1149	977	3066
SRUC	Exp1	1144	1125	na	2269
	Exp2	1124	1152	826	3102
	Exp3	1151	1150	1100	3401
	Exp4	1152	1152	1141	3445
Total		8726	9094	7456	25276

3 Modelling methods

The model: Crop and pathogen biology were incorporated in the model equations. Host growth was described by a pair of logistic growth curves to describe the growth and senescence of the green canopy. This host growth model was parameterized to describe the growth and senescence of a standard UK main crop of potato; emerging at the end of April, reaching full canopy of Leaf Area Index (LAI) 6 in early June, and with senescence beginning just before haulm destruction and harvest in September (Figure 4).

An epidemiological SIR class model was developed to describe epidemics of *P. infestans*. The initial release of spores forming the primary inoculum was described as a truncated normal distribution. This curve was parameterized to cause the epidemics to start the in early summer, the average time for late blight epidemics to emerge (Dowley, Grant, & Griffin, 2008) (Figure 4). The composition of the primary inoculum in the first year was set to be composed entirely of the avirulent strain. New strains emerge by mutation, and change in frequency in the population according to selection. The primary inoculum in subsequent years reflects the strain composition of the pathogen population the previous growing season.

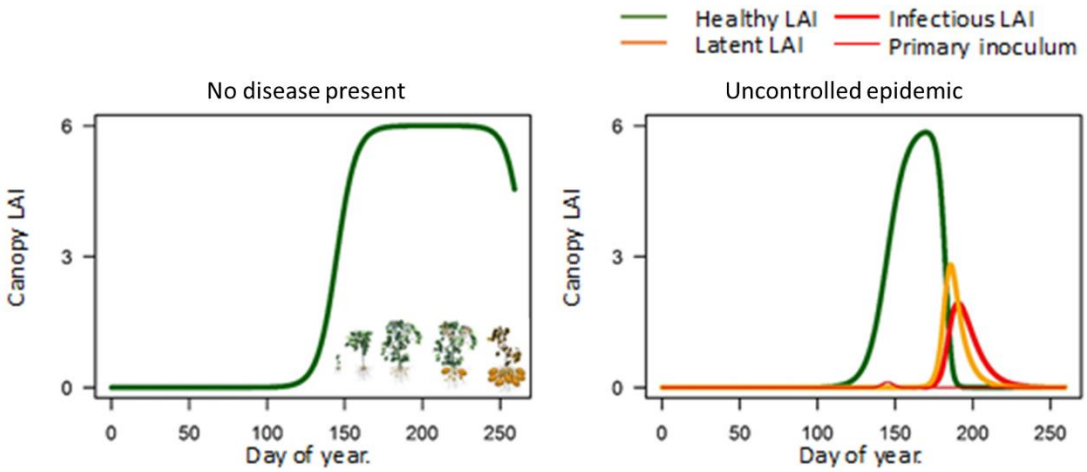

Figure 4. The progress of the host growth model in the case of effective and ineffective disease control.

P. infestans is predominantly tripoid, and, in the UK, asexual. Assuming each QTL has two alleles, avirulent and virulent, the organism can be homozygote avirulent, homozygote virulent and heterozygote (or sensitive and insensitive, for testing the complementary hypothesis). The model can describe any number of QTL and allows mutation at each of the loci to generate new strains. All strains also carry a QTL conferring fungicide sensitivity, which is immutable (or an immutable allele conferring avirulence, for the complementary hypothesis). The main source of new genotypes will be in the formation of spores, not from mutations in a single cell of a fungal colony/lesion. Therefore, we model mutation as the production of spores; infectious tissue of the i^{th} type generates spores a given sporulation rate; and of these a fraction, determined by the mutation rate, are of the j^{th} type. In turn, the j^{th} type sporulates and generates a small amount of i^{th} type spores. The total number of genotypes in a simulation is 3 raised to the power of the number of QTL. Mutation rates are assumed to be constant, and are not affected by the fungicide.

The amount of healthy area, generated by host growth, is reduced through infection by *P. infestans*. A spore from the primary inoculum which lands on a healthy part of a potato leaf causes an infection with a given probability; the infection efficiency. At infection, healthy area becomes latently infected area (non-sporulating) and the lesion grows for a period before becoming infectious and generating new spores; the latent period. After the latent period the latent tissue becomes infectious, generating new spores at a sporulation rate (Figure 5).

These three life cycle parameters are the subject of selection and vary between each strain in the simulation according to the genotype specific parameter values of the strain and the environment (cultivar and fungicide) it is present in. The life cycle parameters are altered in three ways.

Firstly, the three life cycle parameters are altered by the level of cultivar resistance. Cultivar resistance is described as a fractional reduction (or extension, for latent period) in the life cycle parameters of an avirulent strain. The amount of change in these parameters depends on the number and effectiveness of avirulence QTL, and allelic dominance. A range of resistance values are explored, to replicate observed resistance levels in commercial cultivars (Figure 6).

The presence of a virulence allele in the organism carries a cost. This cost of virulence can result in a reduction of the sporulation rate and infection efficiency, and an extension of the latent period. The amount of change in these parameters depends on the magnitude of the cost, the number of virulence QTL, and allelic dominance. The cost of virulence is parameterized to be small, following (Montarry, Hamelin, Glais, Corbi, & Andrivon, 2010).

The application of fungicide can reduce the sporulation rate and infection efficiency and extend the latent period. This depends on the dose and efficacy of the fungicide.

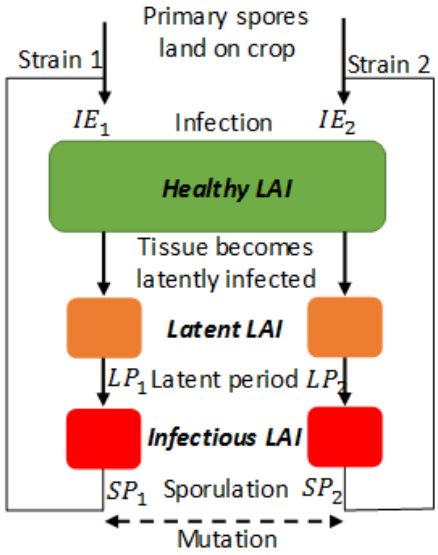

Figure 5. A schematic diagram of the model. Within a given epidemic, spores of the initial inoculum fall on the healthy tissue. The initial inoculum may be composed entirely of a single strain, or may contain a variety of strains. These spores infect at a given infection efficiency (IE), and are latent for a period of time (LP), before becoming infectious and sporulating (SP). These three terms change for different strains, depending on their genetics and environment. For example, carrying fungicide insensitivity genes often reduces the fitness of the strain in the absence of the fungicide (a cost to

insensitivity). This results in differences in the growth rate of the strains. Mutation generates new strains. Strain 1 produces spores, a small fraction of the spores produced are mutants, members of Strain 2.

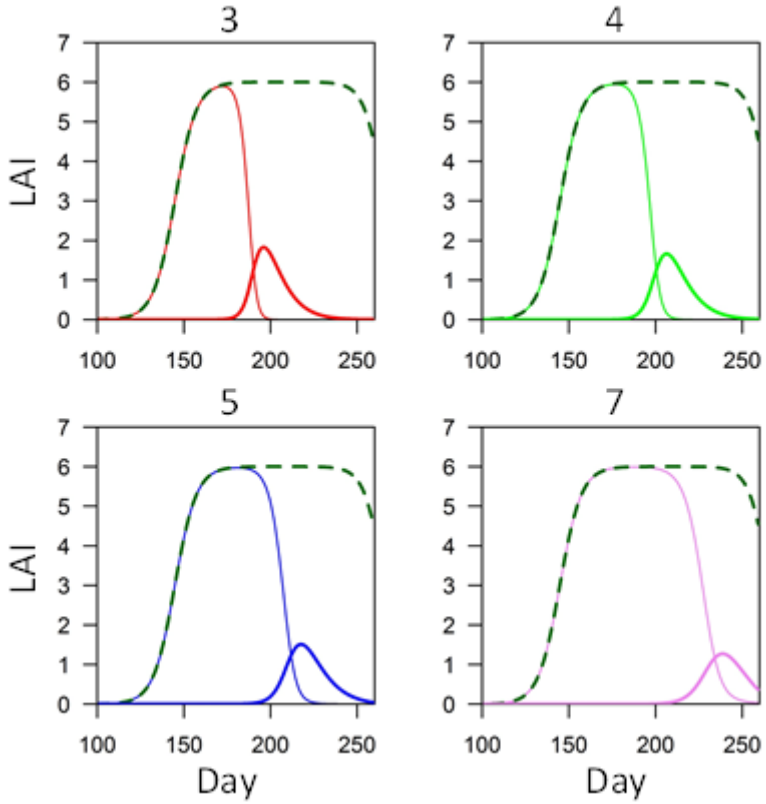

Figure 6. Healthy canopy and disease dynamics of potato crops with different resistance ratings (3,4,5,7 on a 1-9 scale of increasing resistance). This figure is intended as a guide to the term resistance rating, which is more commonly used by growers. The dashed dark green line is healthy area of the crop in absence of disease, senescing just before harvest. The thin coloured line is the healthy area of the crop for an epidemic of the avirulent pathogen with no fungicide, the thick coloured line is the infectious (sporulating) tissue in that epidemic. This rapid loss of the entire canopy is a common feature of potato late blight. The ratings 3-5 and 7 were chosen to represent commercially relevant cultivars.

The epidemic is initially composed entirely of sensitive homozygotes, new strains are generated by mutation and changes in frequency occur over time due to differences in growth rates. Differences in growth rates between strains are a result of differences in the infection efficiency, latent period and sporulation rate. For sensitive homozygotes these are altered by the fungicide dose and efficacy, and for the insensitive homozygotes they are altered by the cost to insensitivity. They are reduced for all strains by cultivar resistance. Each year primary inoculum begins the epidemic, and from one season to the next the composition of the primary inoculum changes according to the composition of the epidemic in the previous year, so insensitivity evolves over multiple years.

Model parameterisation: *P. infestans* has been the subject of many studies, allowing the model to draw upon extensive published and unpublished data for parameterization.

Specific attention is paid to cultivar resistance and fungicide dose response as they are the two key variables in this study.

Fungicide dose response curve: A dose response curve (Figure 7) for the fungicide Infinito (active ingredients propamocarb-hydrochloride and fluopicolide) was established on a sensitive cultivar (King Edward) in field trials (BBSRC project BB/K020447/1). As we will discuss below the results are not qualitatively dependent upon a particular fungicide or mode of action, but the use of realistic dose response curves allows for the efficient translation from theory to application. Dose is reported as the fraction of a full dose, where full dose is defined as the maximum dose that can be applied at a single application timing on the product label. The critical feature for our purposes is the ability of the applied dose to reduce the epidemic growth rate. An advantage of using a model system is that we are able to explore the effect of using doses above the legally permissible dose of 1; these are presented strictly for comparison and clarity; we do not necessarily advocate application of such doses.

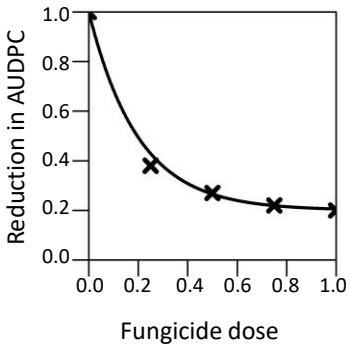

Figure 7. The dose response curve of an epidemic of *P. infestans* treated with the fungicide Infinito on the susceptible cultivar King Edward. Crosses are data generated by BBSRC project BB/K020447/1.

Cultivar resistance: In the UK potato industry, cultivar resistance is expressed as a resistance rating (1 = most susceptible; 9 = most resistant). This rating, is based on disease assessments from experimental plots containing cultivars that have not been treated with fungicide. The ratings are published in the AHDB Potato Variety Database (AHDB, 2016). In a set of experiments exploring the evolution of virulence in the UK population of *P. infestans* the relationship between resistance rating and the area under the disease progress curve (AUDPC) was described (Bain, Bradshaw, & Ritchie, 2009). We have used this AUDPC to parameterise the model to reflect the commercially relevant range of cultivar resistance in the UK. Figure 6 is provided as a guide to show the effect of resistance rating on the healthy area curve as well as on the epidemic development.

Quantifying the evolution of insensitivity: Insensitivity is quantified as T50; the time from the introduction of the fungicide mode of action until the sensitive homozygote has declined to 50% of the population. The remaining 50% is heterozygote and insensitive homozygote. If selection for insensitivity is large we find a small T50 (insensitivity evolves rapidly), if selection is low the T50 is longer (insensitivity takes more time to evolve).

Quantifying the evolution of virulence: Virulence is quantified as T95; the time from the introduction of the cultivar until the avirulent homozygote has declined to 95% of the population. The remaining 5% is heterozygote and virulent homozygote. If selection for virulence is large we find a small T95 (virulence evolves rapidly), if selection is low the T95 is longer (virulence takes more time to evolve). This was used rather than T50 simply to visually clarify the effect.

Results

Field experiment disease progress curves to identify epidemic growth rates

It should be noted when interpreting these results that fungicide treatments consisted of one or two fungicide applications for the purpose of the experimental methods required to test the hypotheses, whereas a standard programme would require applications at 7 day intervals to provide robust control for the duration of the cropping season.

The foliar late blight epidemics progressed differently in all three years in both sets of experiments (Figures 8 to 11). In 2014, foliar late blight was slow to develop as weather was not favourable. In experiments 1 and 2, there were clear differences between the varieties, with a maximum of 9% foliar late blight reported on Cara and 58% on King Edward in experiment 1 by 26 August. Disease pressure was higher in experiment 2, with 78% leaf area affected by foliar blight on King Edward and 38.3% on Cara by 2 September despite the epidemic starting 10 days later than experiment 1. In experiments 3 and 4, there was a similar pattern, with the greatest difference between variety rather than fungicide treatment. In experiment 4, foliar blight levels were similar regardless of fungicide dose and variety.

In 2015, all experiments were inoculated on 13 July. First foliar blight lesions were reported on 4 August on King Edward. The epidemic progressed slowly over the next 10 days, and subsequent blight favourable weather resulted in 100% foliar blight on King Edward and 88% foliar blight on Cara by 1 September. There was a similar pattern of foliar blight development in experiment 2. First blight lesions were recorded in experiments 3 and 4 on 29 July. By 6 August, foliar was 28% on untreated King Edward and 5% on untreated Pentland Dell in experiment 3. By 15 August, foliar blight was over 90% on both King Edward and Cara on both experiments 3 and 4.

In 2016, infector plants were placed in to the plots on 5 July. The first foliar blight lesions were found on 26 July in experiment 1 and 21 July in experiment 2. There were clear differences between the varieties in both experiments. In experiment 1, foliar blight on King Edward reached 100% on 8 August and reached 94% on Cara by 27 August. In experiment 2, foliar blight on King Edward was 100% on 13 August, whereas foliar blight on Cara reached 94% by 27 August. In experiment 3, first foliar blight lesions were observed on 21

July, however, the epidemic did not progress substantially until after 29 July, when weather was more favourable. Experiment 4 was located separately to experiments 1 to 3 in 2016, therefore epidemic progress was slightly different. First lesions were also observed on 21 July, however, no substantial increase in the amount of foliar blight on the most susceptible variety, King Edward, was observed until after 4 August. There were clearly lower levels of foliar blight on Pentland Dell compared with King Edward in experiment 4 compared with experiment 3.

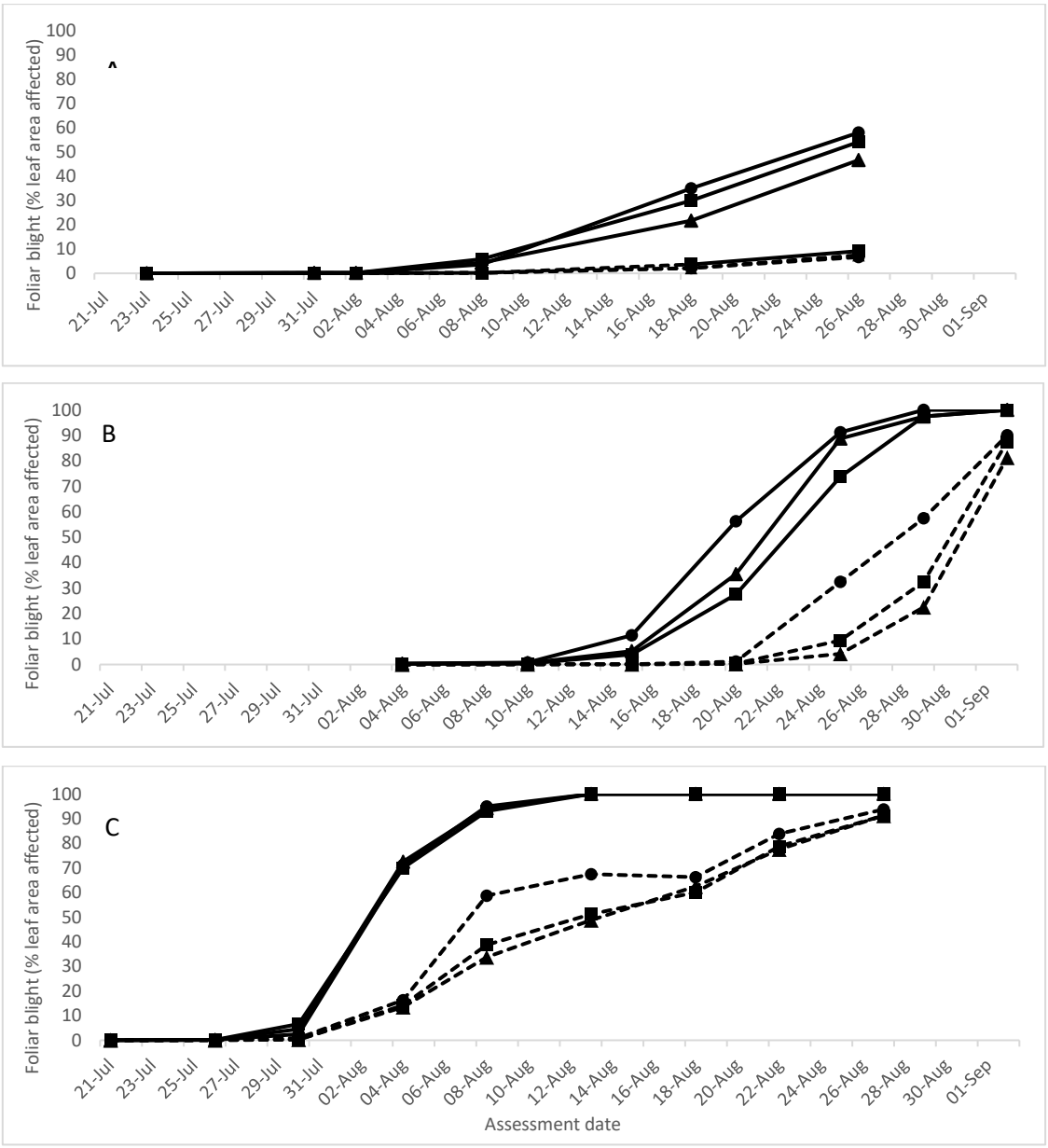

Figure 8. Foliar late blight progress in experiment 1 to test the effect of host resistance on selection for fungicide insensitivity in years 2014 (A), 2015 (B) and 2016 (C).

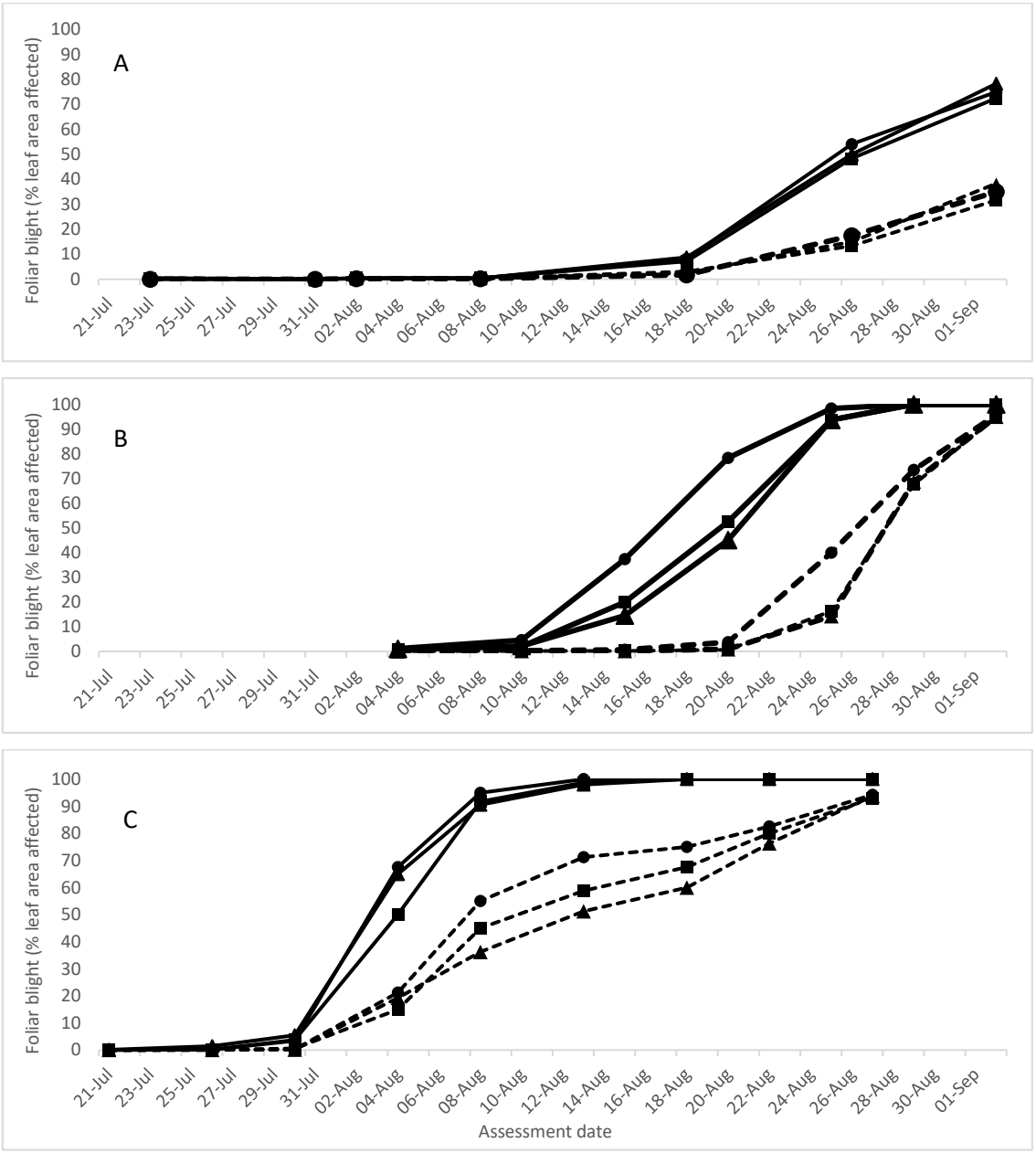

Figure 9. Foliar late blight progress in experiment 2 to test the effect of host resistance on selection for fungicide insensitivity in years 2014 (A), 2015 (B) and 2016 (C).

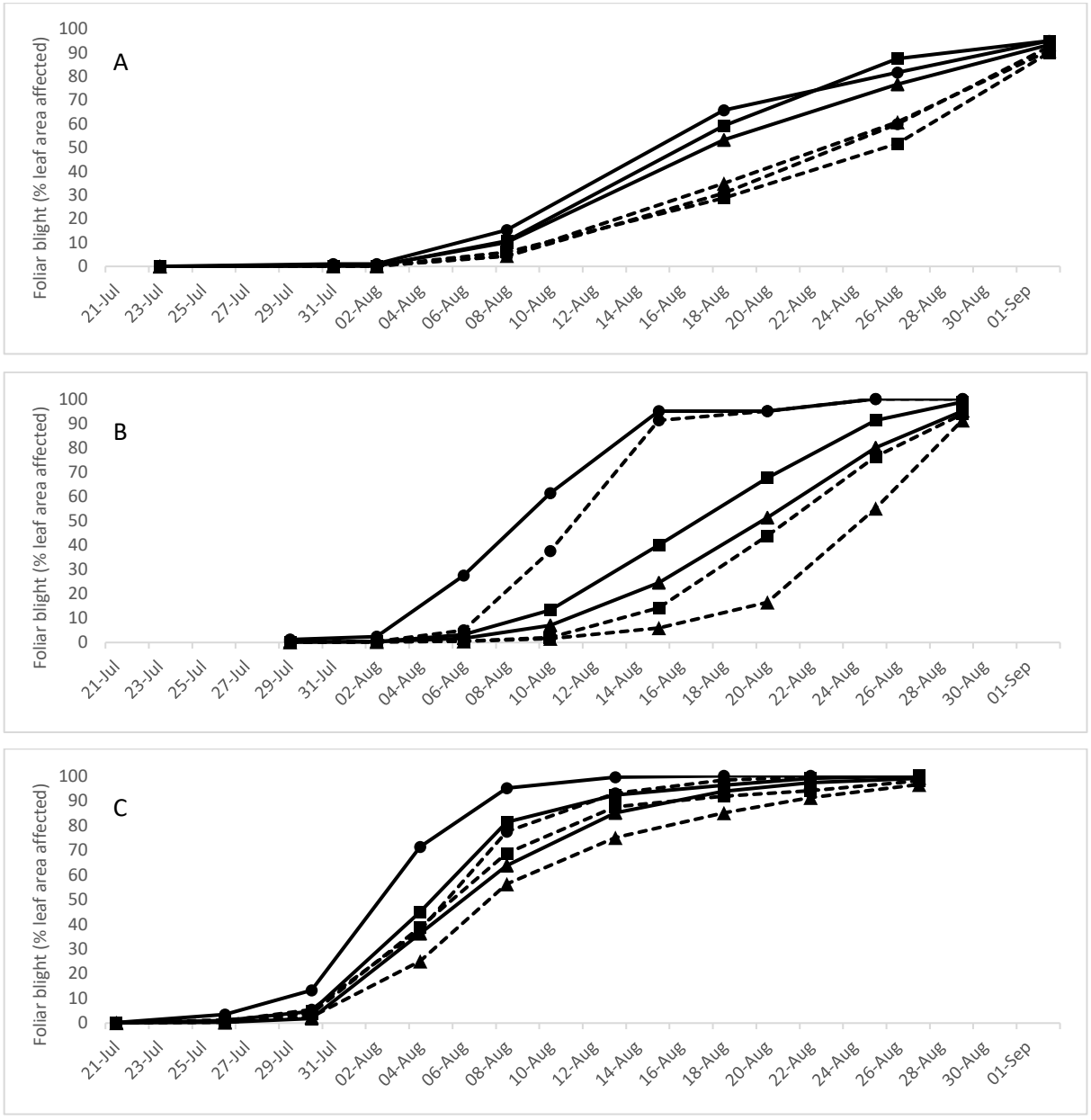

Figure 10. Foliar late blight progress in experiment 3 to test the effect of fungicide treatments on selection for virulence in years 2014 (A), 2015 (B) and 2016 (C).

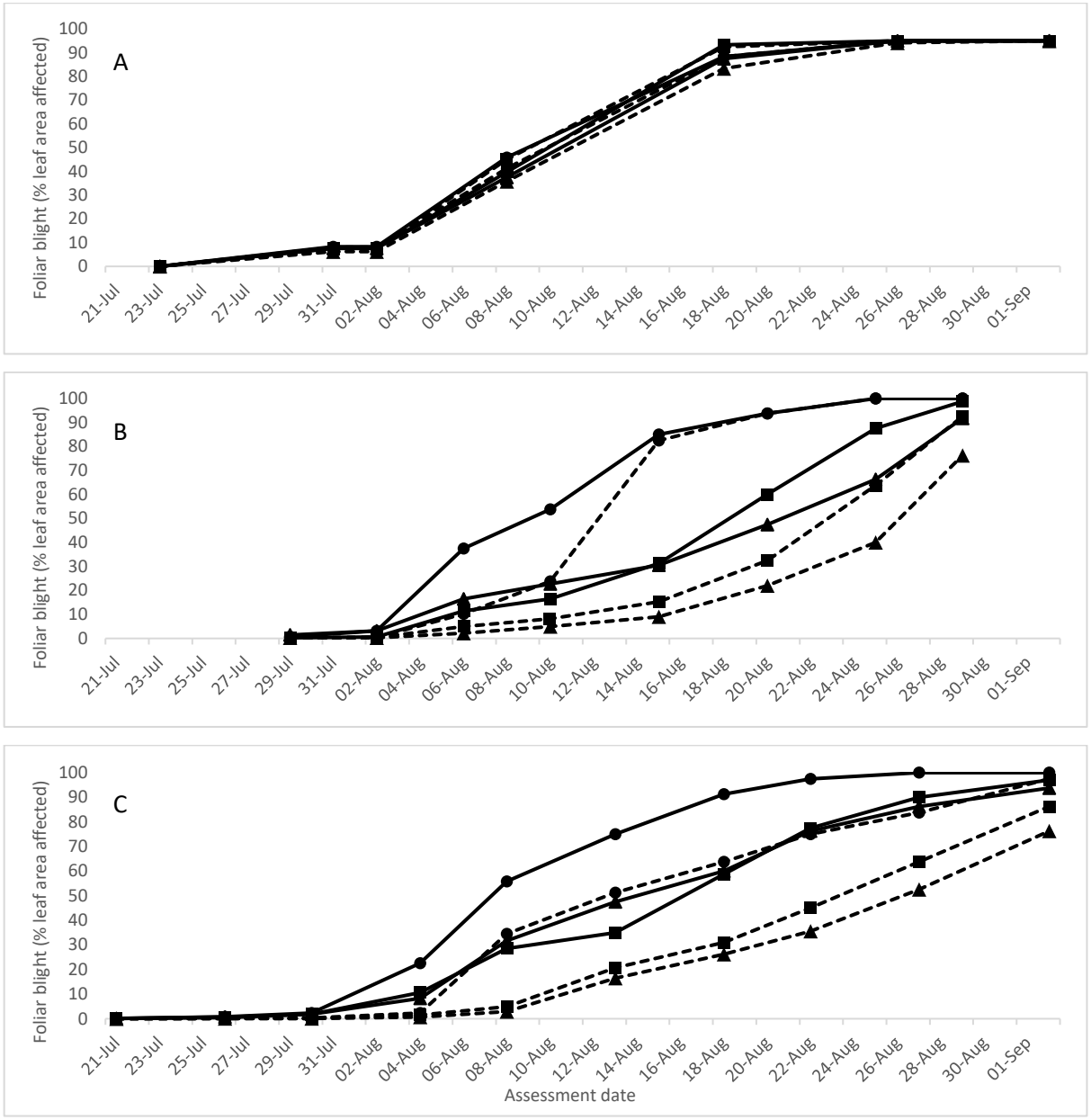

Figure 11. Foliar late blight progress in experiment 4 to test the effect of fungicide treatments on selection for virulence in years 2014 (A), 2015 (B) and 2016 (C).

2. Genotyping results to identify selection ratio in populations.

A total of 16,854 lesions pressed onto FTA cards were scored from the 24 field trials run over the three years of the project (Table 14). The majority of these lesions yielded unambiguous SSR allele peaks from which the genotype could be determined. Despite the care taken to select single distinct lesions a proportion of lesions from each trial, yielded a mix of more than one set of peaks indicating lesions caused by two distinct genotypes had been pressed. Other lesions yielded no fingerprint which may have been due to the lesion drying out or an uninfected section of leaf being punched out (Figure 3). A change to the

sampling method involving an overnight incubation of the lesion in a petri dish improved the success of the genotyping compared to lesions pressed immediately after field collection. All genotyping and scoring was run 'blind' as neither the allocation of treatments or the layout of the field trials was provided to the genotyping team until the end of each season.

Table 14. Numbers of late blight lesions on FTA cards processed to determine the *P. infestans* SSR genotype each season from the 4 field trials run by ADAS and SRUC.

		2014	2015	2016	Total
ADAS	Exp1	909	536	1112	2557
	Exp2	1148	503	1145	2796
	Exp3	1146	539	1150	2835
	Exp4	937	525	977	2439
SRUC	Exp1	288	1120	na	1408
	Exp2	0	532	503	1035
	Exp3	192	1147	574	1913
	Exp4	192	1107	572	1871
Total		4812	6009	6033	16854

The genotype of the *P. infestans* isolate causing the lesions sampled from the trials was determined according to the reference genotypes of the supplied isolates. Despite fungicide treatment prior to inoculation with the specified isolates, ingress of other genotypes in some trials was observed. In some cases the 'contamination' was limited and did not prevent downstream analysis. In other cases it was clear from an initial screen of several hundred samples from the first sample that the ratio of the two inoculated genotypes was severely skewed. For example, across three of the 2014 SRUC trials 672 samples from date 1 were genotyped but all were of the same 13_A2 lineage with no lesions caused by the 6_A1 inoculum (data not shown). In such cases it was not worth the time and expense of genotyping the remaining lesions and resources were instead allocated to the trials in which both genotypes were spreading throughout the trial allowing downstream hypothesis testing.

The results shown below (Figures 12-23) reflect the trials in which substantial genotyping was conducted. Within these trials it is apparent that genotype 8_A1 entered the King Edward plots early in the ADAS 2014 trials 1 and 2 (Figures 12-13) and that the 6_A1 inoculum did not 'take' in SRUC Experiment 2 in 2015 (Figure 17). In addition, a difference in the success of the 13_A2 inoculation was apparent when comparing ingress in ADAS Experiment 3 and 4 in 2016 (Figures 22-23).

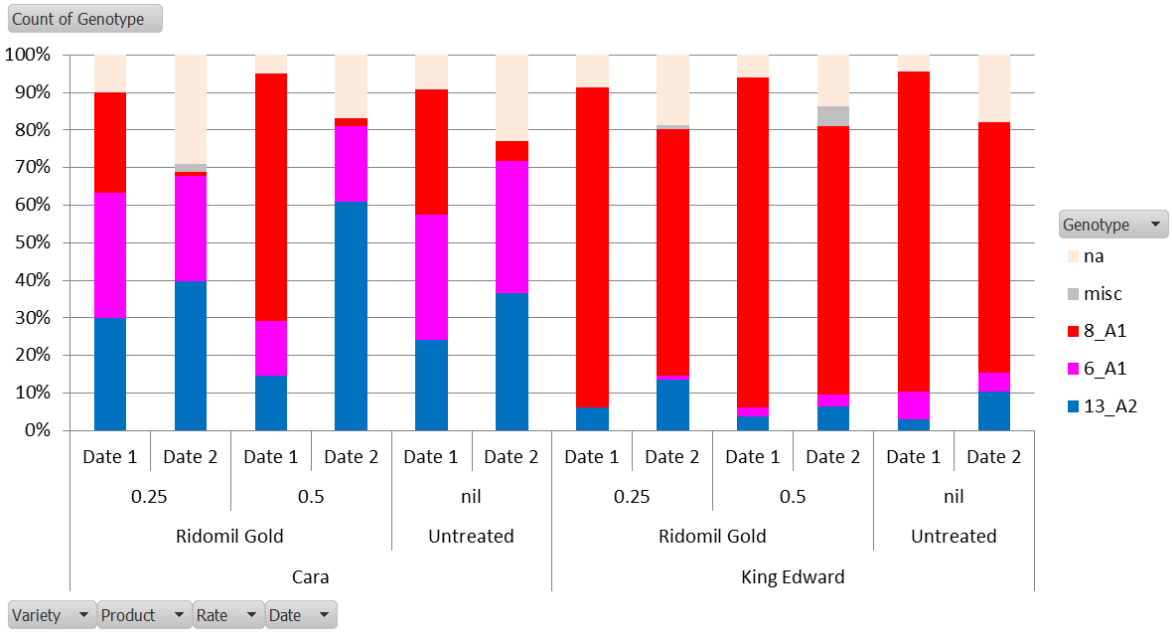

Figure 12. Proportion of each *P. infestans* genotype derived from SSR typing of lesions sampled from ADAS Experiment 1 in 2014. Total lesions processed *n*=909 with 135 that did not generate a clear fingerprint (na).

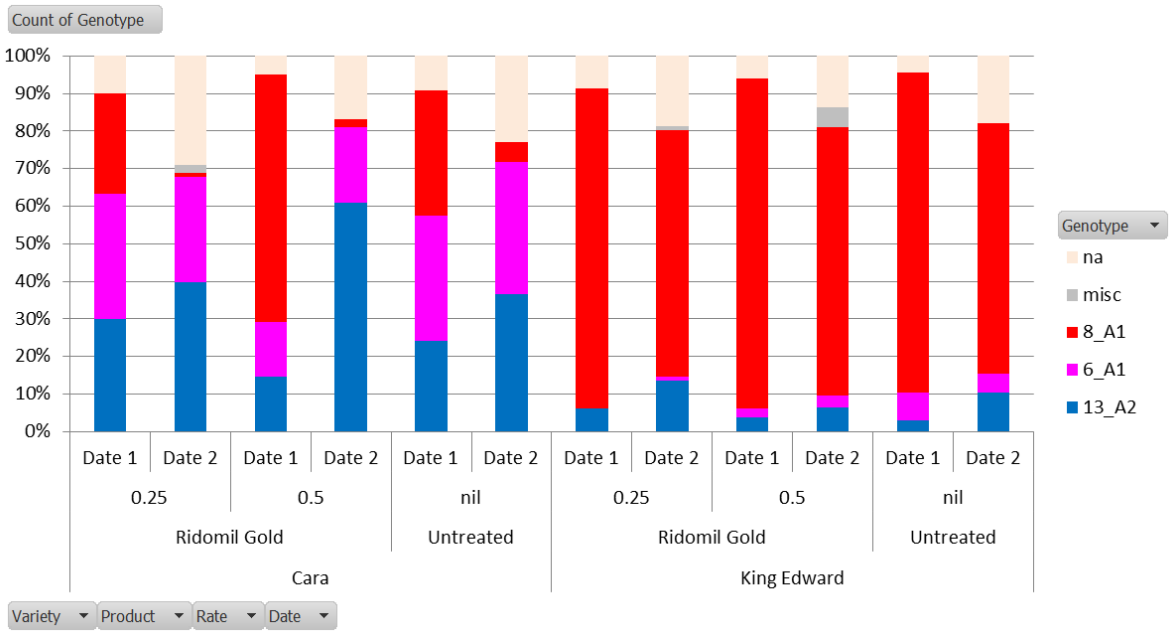

Figure 13. Proportion of each *P. infestans* genotype derived from SSR typing of lesions sampled from ADAS Experiment 2 in 2014. Total lesions processed *n*=1148 with 297 that did not generate a clear fingerprint (na).

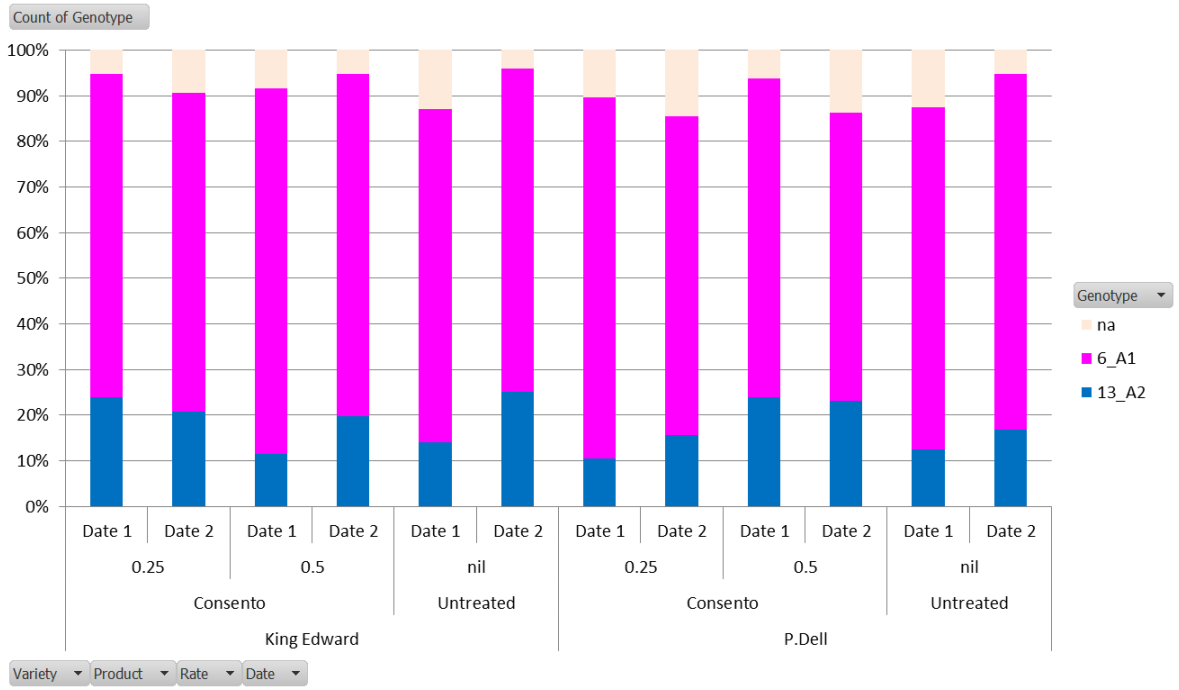

Figure 14. Proportion of each *P. infestans* genotype derived from SSR typing of lesions sampled from ADAS Experiment 3 in 2014. Total lesions processed *n*=1146 with 103 that did not generate a clear fingerprint (na).

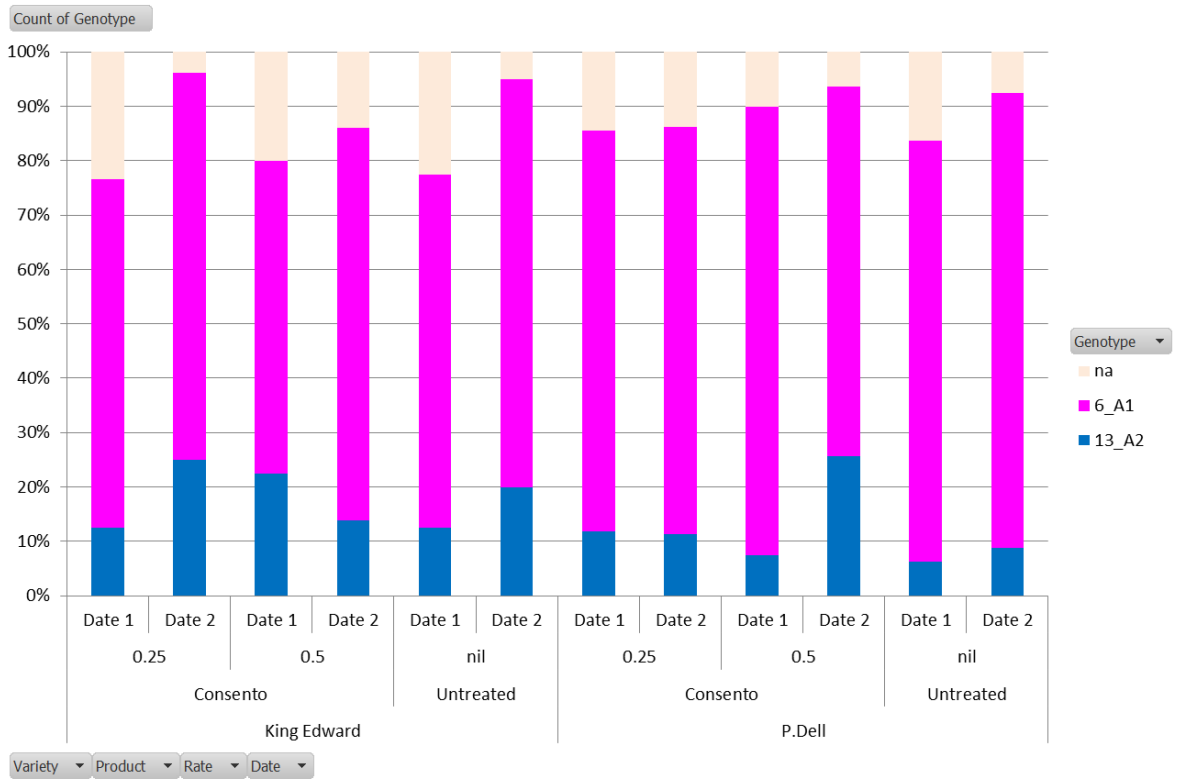

Figure 15. Proportion of each *P. infestans* genotype derived from SSR typing of lesions sampled from ADAS Experiment 4 in 2014. Total lesions processed *n*=937 with 121 that did not generate a clear fingerprint (na).

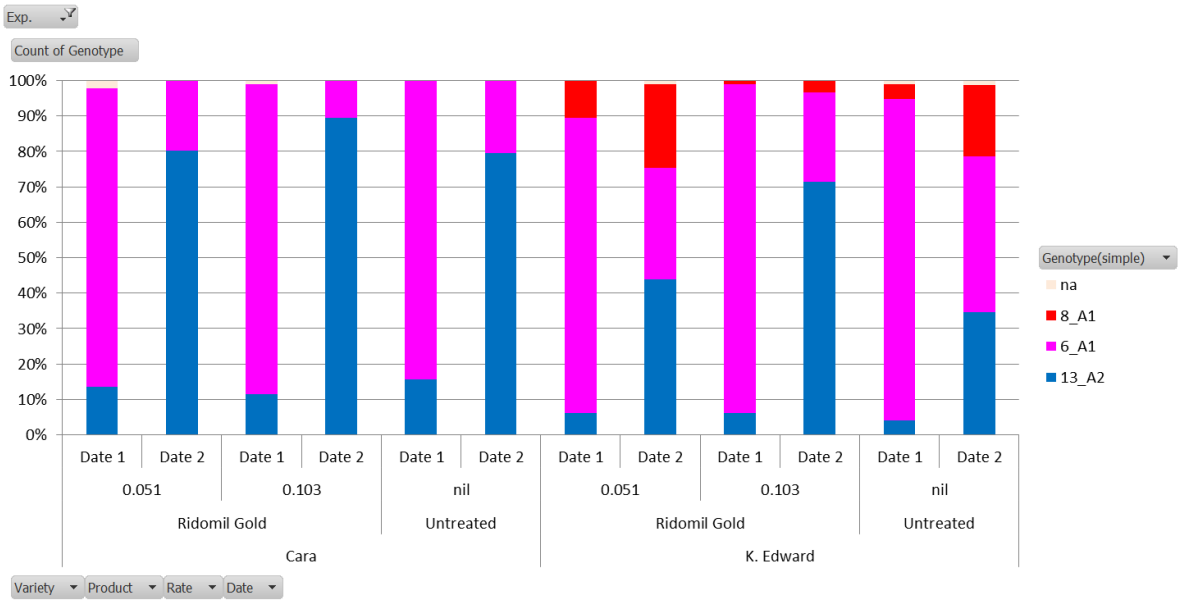

Figure 16. Proportion of each *P. infestans* genotype derived from SSR typing of lesions sampled from SRUC Experiment 1 in 2015. Total lesions processed *n*=1120 with 6 that did not generate a clear fingerprint (na).

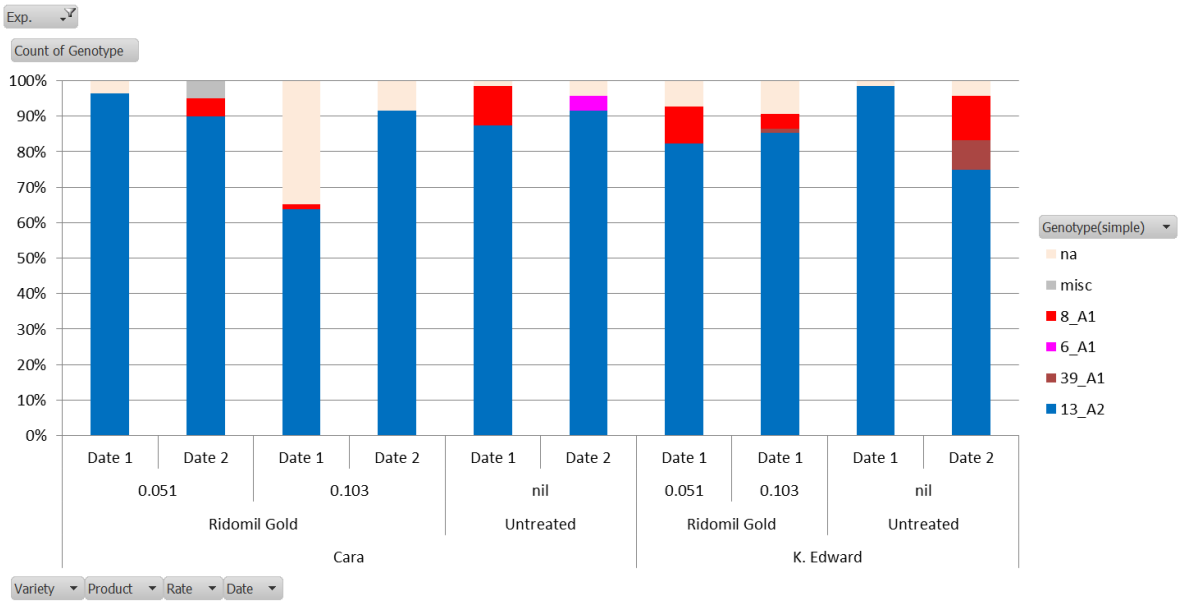

Figure 17. Proportion of each *P. infestans* genotype derived from SSR typing of lesions sampled from SRUC Experiment 2 in 2015. Total lesions processed *n*=532 with 50 that did not generate a clear fingerprint (na). Not all samples genotyped due to a lack of 6_A1 in those typed.

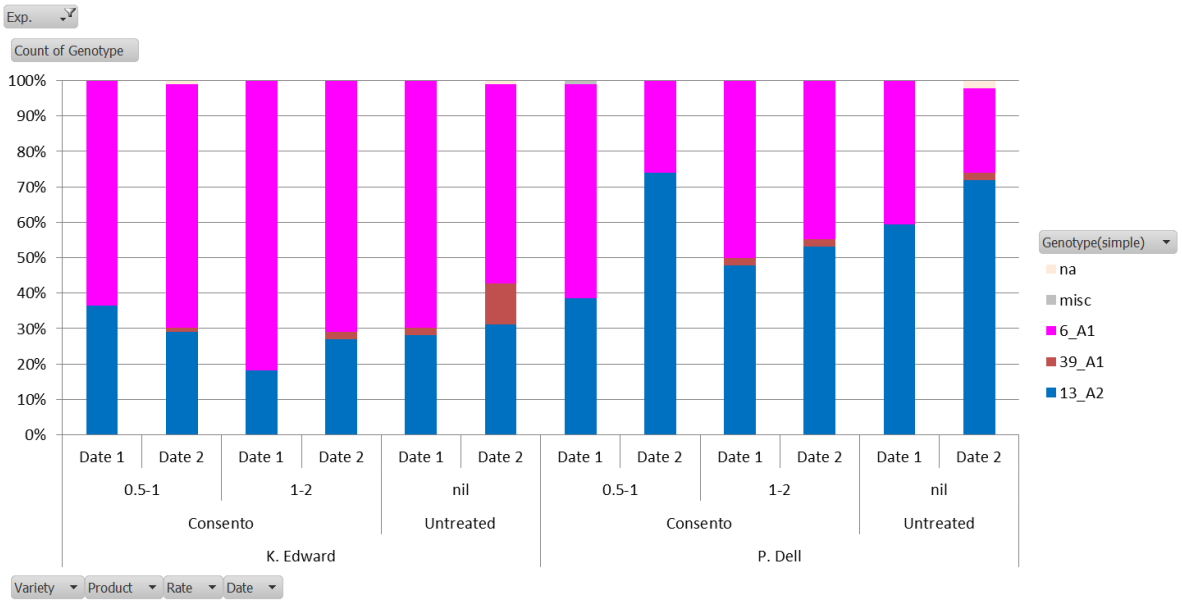

Figure 18. Proportion of each *P. infestans* genotype derived from SSR typing of lesions sampled from SRUC Experiment 3 in 2015. Total lesions processed *n*=1147 with 4 that did not generate a clear fingerprint (na).

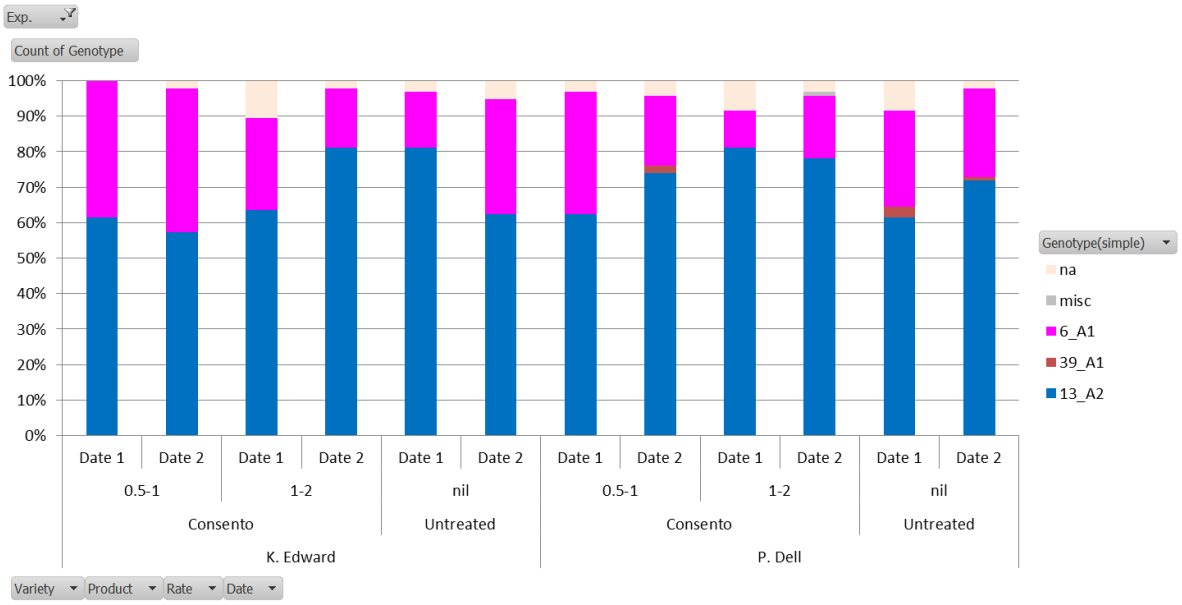

Figure 19. Proportion of each *P. infestans* genotype derived from SSR typing of lesions sampled from SRUC Experiment 4 in 2015. Total lesions processed *n*=1107 with 50 that did not generate a clear fingerprint (na).

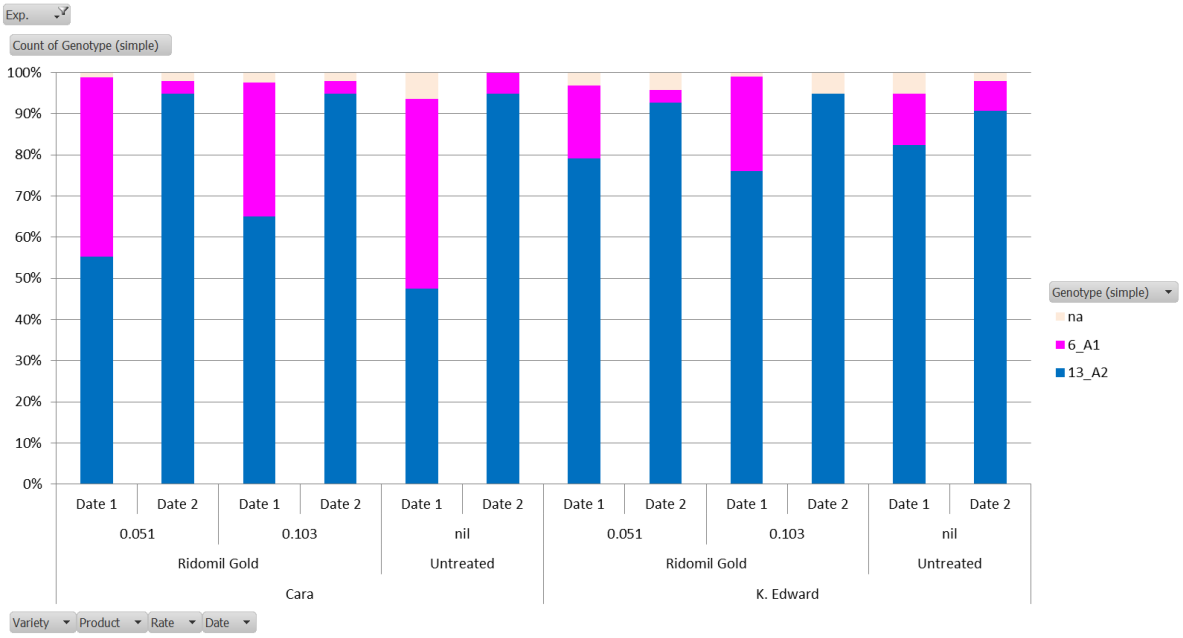

Figure 20. Proportion of each *P. infestans* genotype derived from SSR typing of lesions sampled from ADAS Experiment 1 in 2016. Total lesions processed *n*=1112 with 32 that did not generate a clear fingerprint (na).

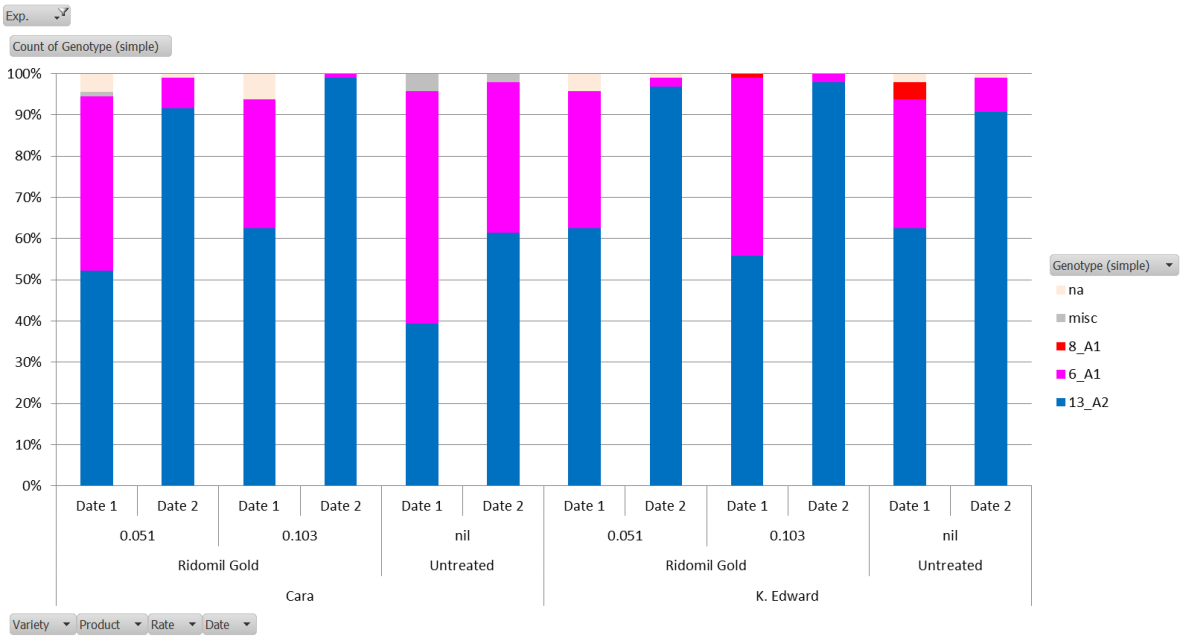

Figure 21. Proportion of each *P. infestans* genotype derived from SSR typing of lesions sampled from ADAS Experiment 2 in 2016. Total lesions processed *n*=1145 with 19 that did not generate a clear fingerprint (na).

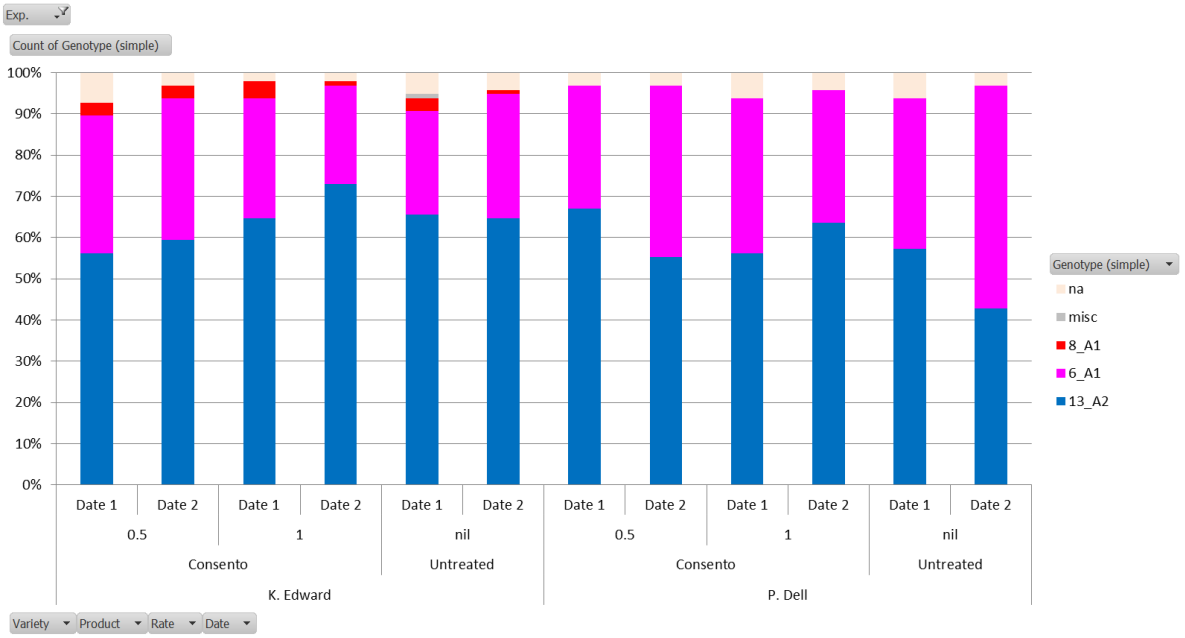

Figure 22. Proportion of each *P. infestans* genotype derived from SSR typing of lesions sampled from ADAS Experiment 3 in 2016. Total lesions processed *n*=1150 with 48 that did not generate a clear fingerprint (na).

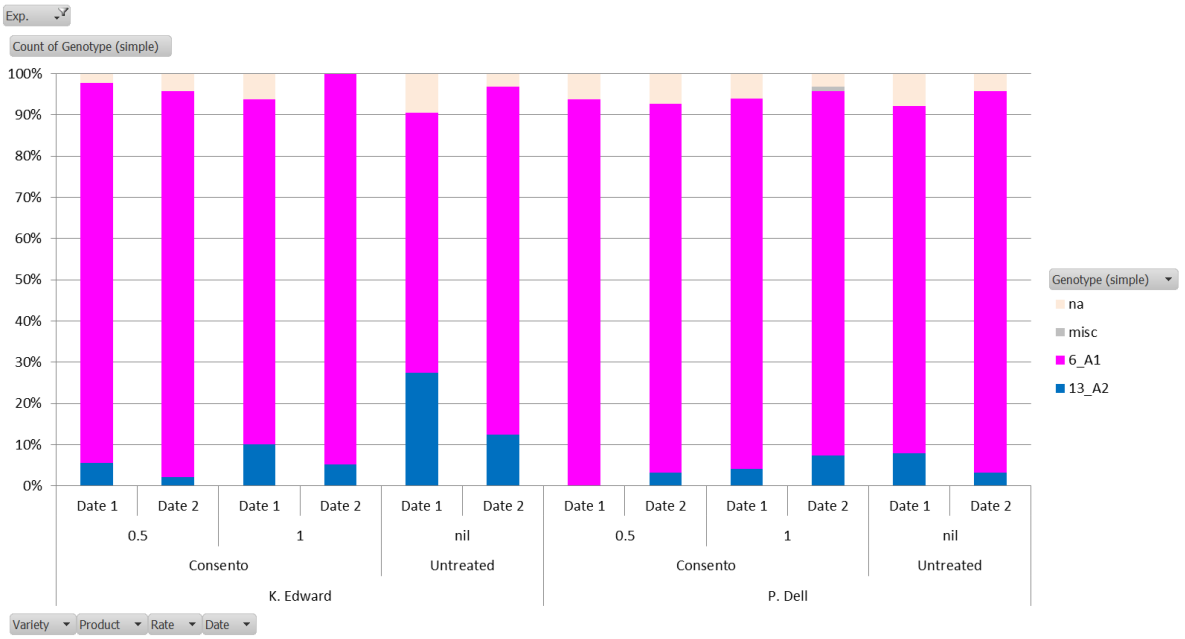

Figure 23. Proportion of each *P. infestans* genotype derived from SSR typing of lesions sampled from ADAS Experiment 4 in 2016. Total lesions processed *n*=977 with 45 that did not generate a clear fingerprint (na).

NOTE: These figures don't contain all the site * years. Eg SRUC 2014 is absent entirely, due to natural inoculum swamping the trial.

3. Result of testing the Hypotheses with field trials

Not all experiments were successful in testing hypotheses 1 and 2 every year, predominantly due to the ingress of naturally occurring *P. infestans* inoculum. Only data from successful experiments (experiment 1 from the SRUC site in 2015 and experiments 1, 2 and 3 from the ADAS site in 2016) were considered in the analysis.

Selection ratios were generated from the genotyping results, showing the change in the population over time. Selection ratio was calculated as the fraction of 13_A2 in the population at date 2, divided by the fraction of 13_A2 in the population at date 1. This gives the fold increase in 13_A2 in the population, as shown in Equation 2.

$$S = \frac{13\_A2_{D2}}{13\_A2_{D1}} \qquad (2)$$

Where S is the selection ratio, 13_A2_{D2} is the fraction of the population composed of the virulent / insensitive 13_A2 strain on Date 2, and 13_A2_{D1} is the fraction of the population composed of 13_A2 on Date 1.

Taking the disease progress curves we generate growth rates, showing epidemic progression. The disease progress curves are used, and during the phase of the epidemic showing exponential growth phase of symptomatic tissue an exponential model is fitted, as in Equation 3.

$$sym = exp^{rt} \qquad (3)$$

Where sym is the symptomatic tissue in the crop (eg Figure 8), t is the amount of time that has passed and r is the growth rate of the epidemic. Based on Equation 1, we expect a positive relationship between epidemic growth rate (r) and selection ratio (S).

Testing hypothesis 1: According to Hypothesis 1, use of cultivar resistance ought to decrease epidemic growth rate resulting in a decrease in selection for fungicide insensitivity. The results are shown in Figure 24.

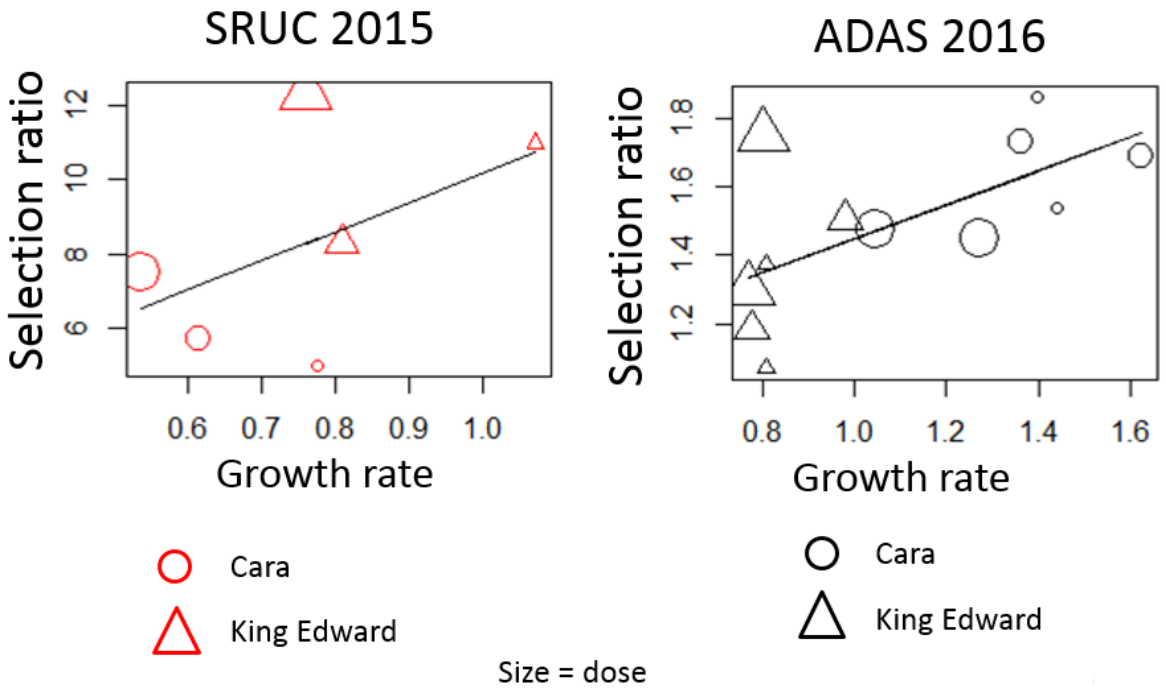

Figure 24. Equation 1 predicts a positive relationship between growth rate of the epidemic and selection ratio for the insensitive 13_A2. Size is dose.

The expected relationship between epidemic growth rate and selection ratio was observed, confirming the hypothesis as true for this set of experiments. Complications were found for the 2016 experiment, where the growth rate of the epidemic was apparently faster on the resistant cultivar Cara. This was due to variation in plot position and climate. However, the general trend for a correlation between epidemic growth rate and selection held. Reductions in the epidemic growth rate led to a reduction in selection ratio.

Testing hypothesis 2: According to Hypothesis 2, use of fungicide ought to decrease epidemic growth rate with a subsequent, decrease in selection for virulence. The results are shown in Figure 25.

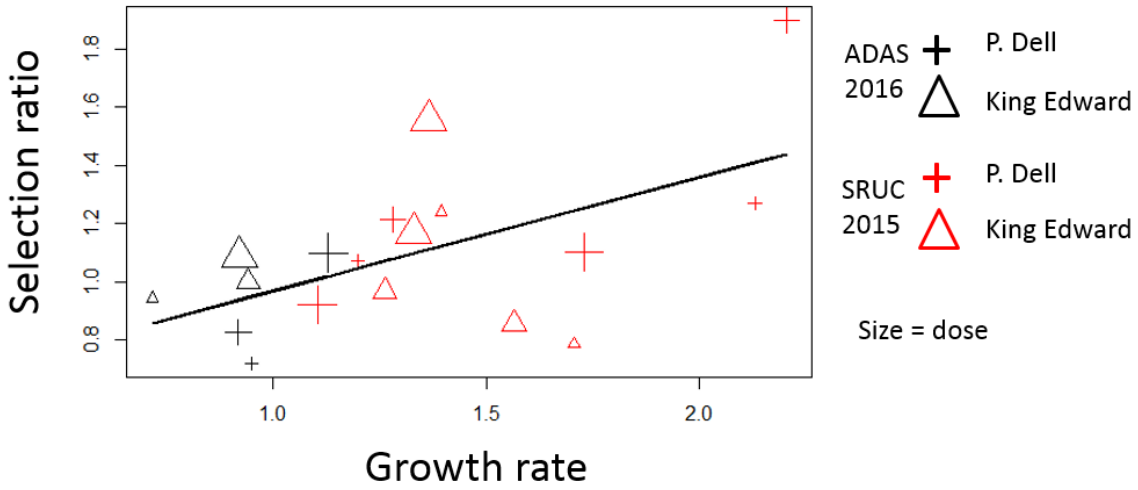

Figure 25. Equation 1 predicts a positive relationship between growth rate of the epidemic and selection ratio for the virulent 13_A2.

The expected relationship between growth rate and selection ratio was observed, confirming the hypothesis as true. Complications were found in that the effect of dose on epidemic growth rate (or cultivar resistance on selection for virulence) was not always in the manner expected. Selection would be expected to be greater for the resistant cultivar and lower at higher fungicide doses, however occasionally was not. Potentially, this discrepancy was due to variation in micro climate between plots (e.g. edge effects). However, the general trend for a correlation between epidemic growth rate and selection held.

4.Results of testing the hypotheses with the epidemic model

Testing hypothesis 1: Hypothesis 1 postulates that the use of cultivar resistance reduces epidemic growth rates and delays the evolution of fungicide insensitivity. To test this a series of simulations were run with a range of cultivar resistance ratings and a range of fungicide doses. The pathogen population was set to be avirulent, with no change in avirulence over the time period of interest. The time taken until the insensitive pathogen passed a certain threshold in the population was then recorded.

Figure 26 provides a guide to interpret cultivar resistance. This is presented as disease progress curves showing that increasing cultivar resistance reduces epidemic growth rates. Adjacent to this, Figure 26 also shows the relationship between T50 (time taken for insensitivity to evolve) and cultivar resistance rating.

This positive relationship between cultivar resistance and time taken to evolve insensitivity was expected under the hypothesis, so we accept the hypothesis as true. If the increase in cultivar resistance rating results in a reduced epidemic growth rate, then increasing cultivar resistance will delay the evolution of fungicide insensitivity.

Testing hypothesis 2: Hypothesis 2 states that the use of fungicide reduces epidemic growth rates and delays the evolution of virulence. To test this, a series of simulations with a range of cultivar resistance ratings and a range of fungicide doses was run. The pathogen population was set to be fungicide sensitive with no change in sensitivity over the time period of interest. The time taken until the virulent pathogen passed a certain threshold in the population was then recorded.

Figure 27 shows the effect of fungicide dose on the time taken to evolve virulence (T95). This is presented for four different resistance ratings. Breeders can generate a resistance rating in different ways. For example; with a single strong resistance QTL, or 2 weaker QTL which together have the same effect, or perhaps with 3 weaker QTL, which together have the same effect as the single strong QTL.

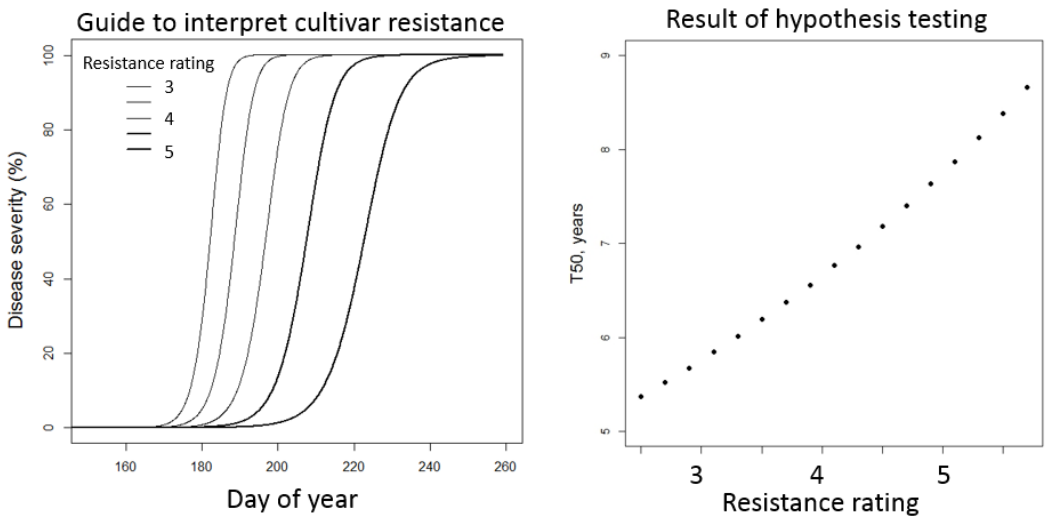

Figure 26. As cultivar resistance rating increases, the epidemic growth rate is reduced, and the time taken to evolve insensitivity (T50) increases.

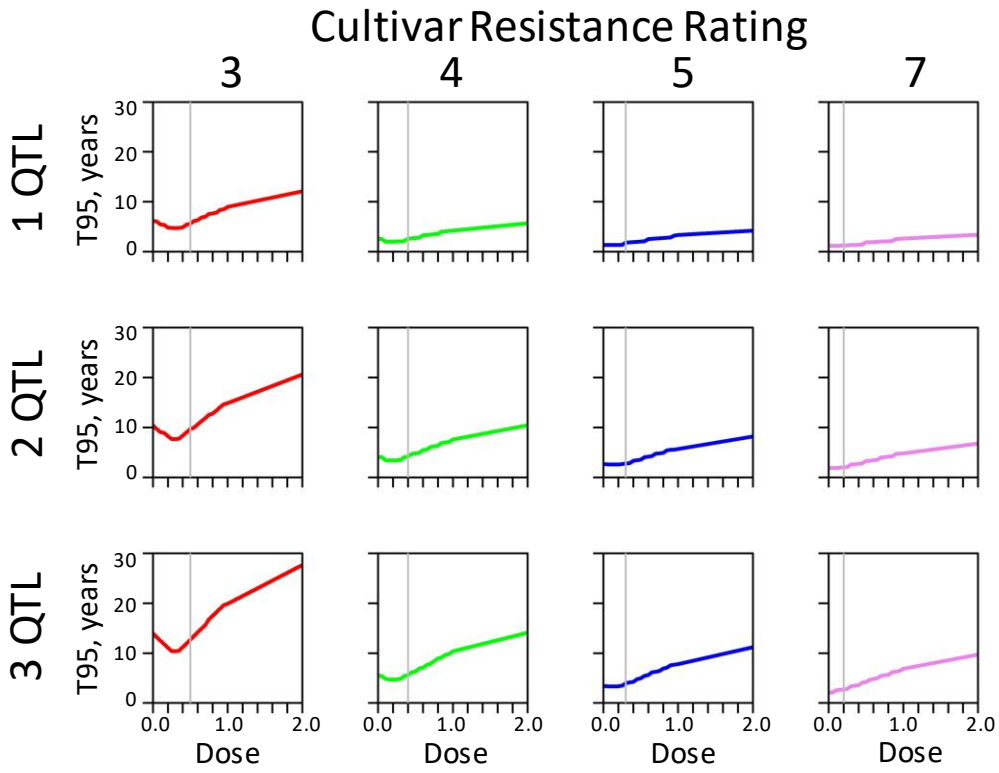

Figure 27. The effect of fungicide dose on the evolution of virulence, as measured by T95. This is the time from the introduction of the cultivar, when the pathogen population is set at 100% avirulent, until the moment the pathogen population is 95% avirulent (the remainder being heterozygotes and the virulent homozygote). The vertical grey line indicates the minimum dose which would in principle be needed for commercially acceptable control of potato blight, lower doses are required for more resistant cultivars.

In the results for testing Hypothesis 2 we find three results in particular to comment on:

There is a positive relationship between dose and time taken to evolve virulence.

This is seen as the increasing positive trend in Figure 27. As the dose increases, the epidemic growth rate is reduced and the difference between the strains decreases (Equation 1), reducing selection and extending time taken to evolve virulence. The hypothesis is accepted as true.

Under certain conditions, there is a negative relationship between dose and time taken to evolve virulence.

For a narrow range of conditions (for low resistance ratings and moving from a zero to a very low dose) a negative relationship is observed. This is due to the increased fungicide dose increasing healthy area available for infection. A small increase in dose will conserve healthy leaf area, which accelerates the epidemic (as infection is dependent upon having tissue to infect). Accelerating the epidemic increases the difference in the growth rates of the strains, increasing selection for virulence (Equation 1). The general principle is accepted as true, and the particular conditions where increasing fungicide dose does not have the expected effect are described. This is annotated in Figure 28, below.

This effect can only occur at doses that are too low to provide commercially acceptable disease control. No grower will willingly accept such a programme, so although the result is interesting, it is not commercially relevant.

Eventually the effect asymptotes, and further increases in fungicide dose do not further extend time taken to evolve virulence.

The effect of increasing dose in delaying the evolution of virulence eventually asymptotes (Figure 27). The point at which the effect asymptotes is a result of the dose response curve (Figure 7). Eventually, no further increases in dose have an effect on the epidemic growth rate – if there are no reductions in epidemic growth rate there will be no effect on selection (Equation 1).

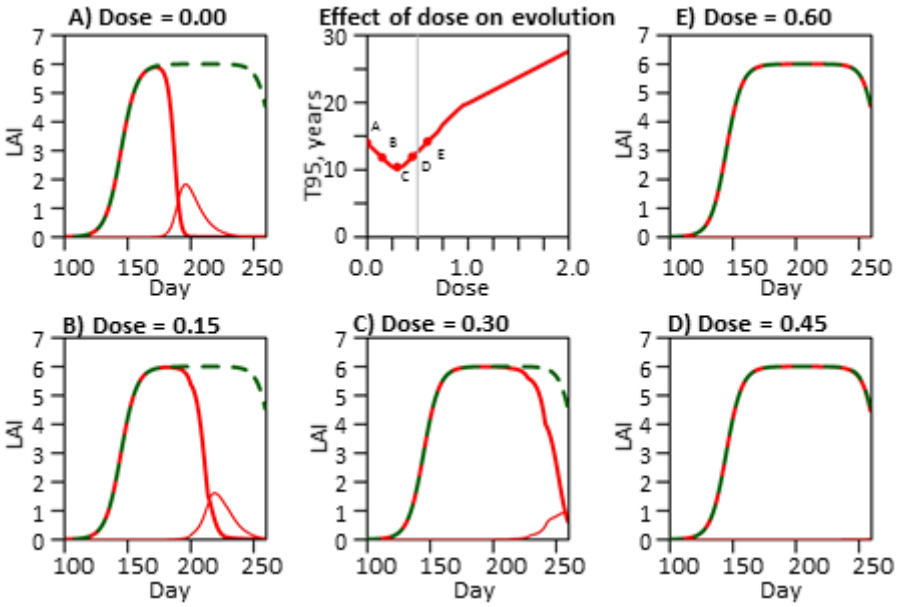

Figure 28. Increase in selection for virulence at low fungicide doses. The cultivar from Figure 27, with 3 QTL and a resistance rating of 3, is presented in the top centre panel. Five fungicide doses are highlighted to demonstrate the effect, and for each of these doses the epidemic progression is presented. As we increase the dosage from 0.00 to 0.30, substantial increases in the canopy area (thick red line) result and a large decrease in infectious tissue area (thin red line). This increase in healthy area from dose 0.00 to 0.30 results in 1) a longer time period for virulence to evolve (increased T, in Equation 1) and 2) a greater selection for virulence as the difference between r_V and r_A increases due to the greater availability of healthy tissue. However, the grey vertical bar indicates the minimum dose for commercially acceptable disease control. All cases where there is an increase in selection for virulence occur below this threshold. As long as the fungicide dose is sufficient to control the disease, increases in fungicide dose that result in reduction in growth rates reduce selection for virulence.

Testing hypothesis 3: Hypothesis 3: integrating disease control methods extends their durability. A series of simulations with a range of cultivar resistance ratings and a range of fungicide doses was run to test this hypothesis. The pathogen population was set to be initially fungicide sensitive and avirulent, allowing both to evolve. The duration of effective disease control, where control is effective if the epidemic is kept below a certain threshold, was then recorded.

There exists an optimum of integrated control, which has a longer effective life than either control method used in isolation (Figure 29). The hypothesis is accepted as true: integration of disease control methods extends their durability.

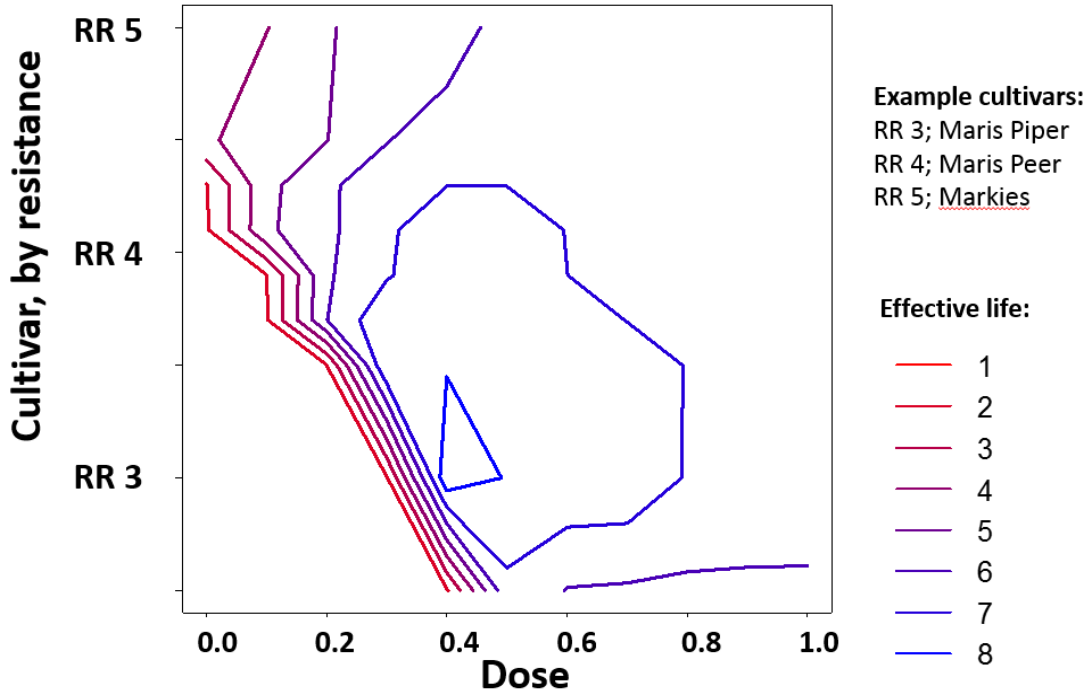

Figure 29. Contour plot showing fungicide dose on the x-axis, and cultivar resistance rating on the y-axis. The number of years of effective disease control in the simulation are shown. High doses select for fungicide insensitivity, resulting in loss of control. High resistance ratings select for virulence, resulting in loss of control. The maximum durability of effective control is found at intermediate doses and intermediate cultivar resistance ratings.

Discussion

1 Field results

The general trend that reducing the epidemic growth rate reduced selection held in both sets of experiments: for the evolution of virulence and the evolution of insensitivity (Figures 18 and 19). This was complicated by external factors having a larger effect on the epidemic growth rate than the doses or cultivars used. However, the qualitative relationship held. This is the first time it has been shown experimentally that a reduction in epidemic growth rate results in a reduction in selection. The use of fungicides generally decreases the epidemic growth rate of the pathogen population, as does the use of resistant cultivars. A range of doses that were much lower than are used for commercially acceptable control were used in order to allow the epidemic to progress; if there was no epidemic it would not have been possible to collect the samples. These low doses mean the effect size of the fungicide is lower, and so more prone to interference from other effects (potentially climate). However, the original hypothesis tested from Equation 1 does not specify that the control method used must be fungicides. Rather, any given technique which slows the development of the epidemic is expected to reduce selection for insensitivity or virulence.

2 Modelling results

The model of the evolution of insensitivity and virulence in P. infestans demonstrated that decreasing epidemic growth rate delayed pathogen evolution, in agreement with the field trials. The model enabled exploration of a wider range of conditions than the field trials, and found that under certain conditions there was a negative relationship with dose, and time taken to evolve virulence (Figure 27). However, the negative relationship between dose and T95 at very low doses was explained under the same paradigm; rather than the fungicide acting to reduce the growth rates of both strains, an increase in healthy area with increasing

dose increased the growth rates of both strains (Figure 28). Fungicide application can thus provide a useful tool to reduce the selection for virulence and cultivar resistance can be a useful tool in managing the evolution of insensitivity. It was observed that the ability of a fungicide to delay the evolution of virulence occurs regardless of whether cultivar resistance is generated by a few genes of large effect or many genes of small effect. The magnitude of the effect changes according to overall level of cultivar resistance and the efficacy and dose of the fungicide. The use of integrated control was demonstrated to have a longer effective life of control than either control method used in isolation (Figure 29).

3 General conclusion

The evolution of agricultural pathogens can result in the breakdown of disease control. Several methods have been suggested to manage evolution. Classical population genetics theory predicted that any additional disease control method that affects both virulent and avirulent strains equally will delay the evolution of virulence, or the evolution of insensitivity if considering sensitive and insensitive strains. In this work classical theory has been applied to demonstrate how control methods implemented by growers can reduce selection for virulence. To test the hypothesis, we considered the case for integrating cultivar resistance and fungicide use. We constructed a model of the evolution of virulence in *P. infestans* on potato crops and found that use of resistant cultivars delays the evolution of insensitivity, and appropriate fungicide application delays the evolution of virulence. Here we have shown the case for fungicides and cultivar resistance in the late blight pathosystem. However, the effect of other disease control methods should be tested both for this system and for other pathogens and other crops. We stress that these two methods, fungicides and resistant cultivars, are predicted to be particular instances of a general principle. If future work explores alternate methods to control disease and finds that the hypothesis holds as expected, we have opened a wide range of possibilities that can contribute to the management of the evolution agricultural pathogens.

Knowledge Exchange

As outlined in the introduction, the predictions made by Equation 1 are expected to be broadly applicable to multiple fungicides, multiple pests and multiple crops. To facilitate the practical application of this principle and the research herein, the results of this research have been presented at a variety of conferences and events, both academic and non-academic (Table 15). The reception has been overwhelmingly positive; agronomists are often attracted by the practical applicability of the technique, industry representatives are excited by the potential to extend the effective life of various products, and academic audiences seek to apply the principle to other systems and other diseases.

Several research papers are in progress and will be submitted after the end of the project (Table 16). Of these papers, three are on the three hypotheses, one is on the results of the field trials, and one is a methods paper to facilitate broader application of this principle to other pathosystems.

Table 15. Knowledge exchange events past and future.

Event	Date	Note
Invited keynote presentation at 10èmes Rencontres de Phytopathologie - Mycologie de la Société Française de Phytopathologie	Jan-14	Academic
Invited keynote presentation at the annual meeting of the Spanish plant pathology society 2014	Jan-14	Academic
Presentation at the Potato Council Winter Forum (West)	Jan-15	Industry
Presentation at the Potato Council Winter Forum (East)	Feb-15	Industry
Presentation at the Potato Council Winter Forum (North)	Feb-15	Industry
Presentation, 4th International Cereal Rusts and Powdery Mildews Conference	Jul-15	Academic and industry

Invited keynote presentation Fungicide Resistance Specialist workshop, University of Melbourne	Nov-15	Academic and Industry
Presentation, Resistance 2015 Conference	Nov-15	Academic and Industry
Presentation at Reinhardsbrunn Symposium	Apr-16	Industry and academics
Presentation at QUB Symposium, Rothamsted	Jul-16	Academics
Poster at EAPR Pests and Pathology section meeting	Aug-16	Industry, academics and agronomists.
Poster at Euroblight workshop	May-17	Industry and academics.
Poster at EAPR Triennial conference 2017	Jul-17	Industry and academics.
Potatoes in Practice	Aug-17	Industry, academics and agronomists.
BP2017	Nov-17	Industry and agronomists.

Table 16. Research papers originating from this project. Titles for un-published papers are subject to change.

Title	**Status**	**Date**	**Note**
Governing principles can guide the development of fungicide resistance management tactics	Published in Annual Review of Phytopathology	2014	This publication was made partly on the basis of the preliminary work for the HAPI project and outlined the theory underpinning the work.

Evidence-based resistance management: a review of existing evidence	Published in Fungicide resistance in plant pathogens	2015	This publication was made partly on basis of the preliminary work for the HAPI project
The use of mathematical models to guide fungicide resistance management decisions	Published in Fungicide resistance in plant pathogens	2015	This publication was made partly on basis of the preliminary work for the HAPI project
Cultivar resistance can help extend the effective life of fungicides	Published in Proceedings of the 18th international Reinhardsbrunn symposium	Apr-17	Preliminary presentation of Hypothesis 1.
Extending the durability of cultivar resistance by limiting epidemic growth rates.	Under review in Proceedings of the Royal Society B	Submitted April 2017	Hypothesis 2.
To delay the evolution of fungicide insensitivity, use resistant cultivars.	Initial draft completed.	Late 2017	Hypothesis 1.
Integration of disease control methods extends their durability	Preliminary work begun.	Mid 2018	Hypothesis 3
Genotype-phenotype mapping for fungicide resistant plant pathogen strains	Preliminary work begun.	2018	Methods paper to facilitate application of this principle to other systems.

Climate limits the evolution of fungicide insensitivity and the evolution of virulence in Phytophthora infestans	Preliminary work begun.	2018	Paper on the results of field trials.

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