

Final Report

Testing fungicide resistance management strategies on the selection for *Phytophthora infestans* on potato

Ref: 11120032

Reporting Period: June 2019 to May 2022

Report Authors: Faye Ritchie (ADAS), Neil Paveley (ADAS), Philip Walker (ADAS), Alison Lees (JHI), James Lynott (JHI) and Ruairidh Bain (SRUC)

Date report submitted: 28 July 2022

© Agriculture and Horticulture Development Board 2021. No part of this publication may be reproduced in any material form (including by photocopy or storage in any medium by electronic means) or any copy or adaptation stored, published or distributed (by physical, electronic or other means) without the prior permission in writing of the Agriculture and Horticulture Development Board, other than by reproduction in an unmodified form for the sole purpose of use as an information resource when the Agriculture and Horticulture Development Board is clearly acknowledged as the source, or in accordance with the provisions of the Copyright, Designs and Patents Act 1988. All rights reserved.

AHDB^{AHDB} is a registered trademark of the Agriculture and Horticulture Development Board.

All other trademarks, logos and brand names contained in this publication are the trademarks of their respective holders. No rights are granted without the prior written permission of the relevant owners.

CONTENTS

1.	SUMMARY	4
2.	Аім	5
3.		6
4. 4.1	MATERIALS AND METHODS	
4.2 4.3	 P. Treatment list Sampling strategy 4.3.1. Sample 1: Disease symptoms visible and prior to test treatments 	8 10 . 10
	 4.3.2. Sample 2: After three test treatment applications have been applied	. 11 11
	 4.4 Determining the effect of fungicide treatments on selection	. 12 . 12
5. 5.1	RESULTS	13 13
	5.1.1. ADAS 5.1.2. SRUC 2. 2020 experiments	. 14
	5.2.1. ADAS 5.2.2. SRUC	. 16 . 19
	 3. 2021 experiments 5.3.1. ADAS 5.3.2. SRUC I. Cross site analyses 	. 21 . 23
6.	Discussion	
7.	CONCLUSIONS AND PRACTICAL RECOMMENDATIONS	30
8.	References	31
9.		33
		34
11.	ACKNOWLEDGEMENTS	.34

1. SUMMARY

Principles governing the selection of fungicide insensitive strains have been derived and tested against available experimental data and have shown that there are generic principles that can be applied to developing fungicide resistance management strategies. Possible resistance management strategies include alternating different modes of action, mixing two modes of action and decreasing the total fungicide dose applied throughout the fungicide programme. The most recent DEFRA Pesticide Usage Survey reports that ware potatoes can receive, on average, 10 fungicide applications in a single season. Given that late blight control requires multiple spray applications there is a need to understand how best to manage resistance selection, particularly where crops receive multiple sprays.

Aim: the aim of this project was to determine effective strategies to slow the selection for strains with decreased sensitivity to fungicides to: 1. determine appropriate strategies to slow selection using alternation, mixtures and multisite fungicides in multi-spray programmes, 2. test the effect on selection of adjusting dose under field conditions (integrated pest management: IPM) and 3. produce a set of evidence-based guidelines outlining best practice and cost effectiveness for the industry. Conclusions and practical recommendations for 3. are included in this report and will be used to update the Fungicide Resistance Action Group (FRAG) and other stakeholders.

Methodology: A method to test the selection for strains with decreased sensitivity to fungicides was developed using the *P. infestans* strain EU_37_A2. This strain is known to have decreased sensitivity to fluazinam and, as a result, experiments could be designed to include fluazinam and test whether the strategies had any effect on the selection for this strain. Experiments were deliberately located in areas where EU_37_A2 would occur naturally and at a low frequency in the population, so an increase in EU_37_A2 during the season could be tracked in field experiments.

Practical recommendations: The repeated and sequential application of a single site mode of action, over multiple sprays, will result in the selection for a fungicide insensitive strain, if such a strain is present in the population. All of the resistance management strategies tested significantly decreased the selection for a fungicide insensitive strain. This diverse range of effective approaches should make it simpler for practical considerations, such as efficacy and cost, and fungicide resistance management, to be implemented across the fungicide programme.

When building a fungicide programme, the following resistance management strategies have been shown experimentally to decrease selection for fungicide insensitive strains and did not differ significantly in their effect on resistance selection:

- 1. Alternation (as both alternating single sprays or blocks of three sprays).
- 2. Multi-site + single-site or single-site + single-site modes of action in tank mixtures.
- 3. Half dose or full dose tank mixtures (this would be applicable for co-formulations)

Recommendations when implementing resistance management strategies:

- 1. Consult up to date FRAG guidelines for potato late blight: <u>https://bit.ly/3J8udYK</u>
- 2. Fully implement all cultural resistance management strategies prior to using fungicides including management of outgrade piles and volunteers, and blight-free seed.
- 3. Use fungicides from different cross-resistance groups for alternation and mixture strategies.
- 4. When using fungicides, do not apply more than three applications in sequence for the same mode of action.
- 5. Balance the use of different modes of action within the fungicide programme.
- 6. Continue to use multisite fungicides in the programme where possible.

2. AIM

The aim of the project was to determine, and rank, effective strategies to slow the selection for strains with decreased sensitivity to fungicides. Experiments were conducted to address the following objectives:

- 1. Determine appropriate strategies to slow selection using alternation, mixtures and multisite fungicides in multi-spray programmes.
- 2. Test the effect on selection of adjusting dose under field conditions (IPM).
- 3. Produce a set of evidence-based guidelines outlining best practice and cost effectiveness for the industry.

3. INTRODUCTION

The control of late blight, caused by *Phytophthora infestans*, requires frequent applications of fungicide every 5 to 7 days depending on the weather risk. The most recent DEFRA Pesticide Usage Survey reports that ware potatoes can receive, on average, 10 fungicide applications in a single season (Garthwaite et al., 2019). This can substantially differ depending on the individual season and late blight weather risk. There is recent evidence that fungicide treatment can shift the sensitivity of *P. infestans* field populations to different modes of action (Andersson et al., 2017). A *P. infestans* strain with decreased sensitivity to fluazinam, EU-37_A2, has been reported in Great Britain (GB) and several European countries (Lees, 2019, EuroBlight, 2020). Given that late blight control requires multiple spray applications there is a need to understand how best to implement fungicide resistance management strategies across the fungicide programme.

The effects of using mixtures to decrease selection for fungicide insensitive strains has been investigated previously for Phytophthora infestans and the active ingredient metalaxyl-M applied as a solo, tank-mixed with mancozeb and with mancozeb+cymoxanil under protected conditions. This method used spore washings and counts from infected leaves to identify whether resistant or sensitive strains were present. Although there was a benefit from using mixtures, the repeated application of the same mixture over 50 days did not prevent the resistant subpopulation from reaching 100% (Cohen and Samoucha, 1989). Since then, scientists have identified a governing principle for fungicide resistance management that exists and holds true across fungal pathosystems and fungicide combinations (van den Bosch et al., 2014). It states that reducing the selection coefficient (the proportion of resistant strains along with or relative to sensitive strains) and reducing the exposure time to particular modes of action will reduce buildup of fungicide resistance. Practical disease management strategies that could theoretically achieve this includes adjusting the fungicide dose, constraining the number of applications, mixing and alternating different modes of action and adjusting the timing of applications. For potato late blight management, a combination of the strategies are already used, however, there is no experimental evidence to demonstrate whether these strategies actually work within a multispray fungicide programme or whether they differ in effectiveness from each other.

There is a need for evidence-based guidance to demonstrate appropriate fungicide resistance management strategies for the industry and identify the tactics that would be most effective at slowing resistance selection. It is important that any strategies deployed must be effective at controlling the disease, be cost effective for growers and have evidence to support their implementation. To do this, an experimental protocol was developed that utilised the presence of both fluazinam insensitive (EU_37_A2) and sensitive (e.g. EU_6_A1/EU_36_A2) to test whether commonly used resistance management strategies for late blight management are effective.

4. MATERIALS AND METHODS

The experiments were designed to test fungicide strategies and their effect on the selection for fungicide insensitive strains. To test this, a protocol that included fluazinam in the fungicide programme, which would select for the *P. infestans* strain with decreased sensitivity to fluazinam, EU_37_A2, was developed. The EU_37_A2 strain was therefore used as a tool to test the effectiveness of different resistance management strategies. The protocol required EU_37_A2 to be present at a low frequency initially, with test treatments applied that may or may not select for EU_37_A2. This way, any increase in the proportion of this strain in the population vs all other strains present could be monitored throughout an experiment and used to calculate whether selection occurred or not, from the start of the fungicide programme, to the end of the programme.

Field experiments were conducted at SRUC in Ayrshire and ADAS in Worcestershire during 2019, 2020 and 2021. One experiment was conducted at each site in each year, giving a total of six experiments carried out during the project. All experiments were laid out in a randomised block design with nine treatments and four replicates. The cultivar King Edward was used in all trials except for the SRUC site in 2021, when c.v. Fontane was used instead, due to a shortage of King Edward seed. The trials were placed in locations where the *P. infestans* strain EU_37_A2 had been reported previously, as the presence of this strain was needed as part of the experimental design. Natural infection was expected to be sufficient, however, isolates of EU_6_A1 and EU_37_A2 (obtained from the James Hutton Institute (JHI)) were bulked up annually on potato leaves and were ready for use to inoculate the trial if late blight did not occur naturally. Pot grown plants or leaves placed in sealed containers were inoculated with an isolate of EU_37_A2 and maintained until required.

At the ADAS site, plot size was $4m \ge 10m$ with two rows of plants surrounding the trial. These were left untreated as spreader rows. A 0.5m gap of untreated plants was left between plots to encourage even disease development. All untreated areas were monitored for signs of *P. infestans* to identify the appropriate time to start the spray programme. The trial programme of fungicide applications began when disease was detected in the spreader rows or after inoculation, if this was necessary. Inoculation with EU_37_A2 was required in 2019 and 2020. The trial area was inoculated evenly at 16 points across outer and inner untreated rows using a concentration of between 5 x 10⁴ sporangia/ml and 5 x 10⁵ sporangia/ml. inoculated plants were sprayed with a minimum of 8ml spore suspension and inoculum applied late afternoon/early evening. Inoculated plants were covered with a plastic bag and secured in place using a tie. Plastic bags remained in place overnight and were carefully removed the following morning.

At the SRUC site, in 2019, plots were 6.8 m (8 rows) x 5 m. The longitudinal gap between plots which became untreated once the oversprays ended was 1.3 m in length. Plots were separated transversely by 6.9 m of bare soil to minimise interplot interference during blightfavourable westerly winds. In these areas were two longitudinal spreader rows of Shepody. There was no artificial inoculation in the first year of the project. Some layout details were changed for 2020 and 2021. In both years plots were 3.4 m (4 rows) x 9 m. The longitudinal gaps between plots which became untreated once the oversprays ended were 1.0 and 1.5 m in length in 2020 and 2021 respectively. Plots were separated transversely by 6.6 m of bare soil in 2020 and 8.6 m in the following year. In each of these bare soil areas there was one longitudinal spreader row (cultivar not recorded). The introduction of genotype 37_A2 was necessary in 2020 because none was detected at the first sampling. The top half of one plant in all infector plots (4 rows x 1.0 m) was inoculated with isolate W1373B on 31 August (1.0 x 10⁵ sporangia per plant) and again on 8 September (7.5 x 10⁴ sporangia per plant). In the 2019 and 2020 SRUC trials genotype 37 A2 was at a lower than ideal frequency; 36 A2 dominated. A similar situation was anticipated in 2021 because at the end of the 2020 season the proportions of genotypes on Auchincruive Estate were 97.3% (36 A2) and 2.7% (37 A2). For this reason genotype 37_A2 was introduced on 30 August (5.2 x 10⁵ sporangia of W1373B per plant), immediately after sample 1 was collected. The methodology was the

similar to that used in 2020. With hindsight 37_A2 did not need to be introduced because for sample 1 the percentage of 37_A2 was 35%.

The experimental protocol, described in more detail in the following sections, consisted of:

- Oversprays during rapid canopy growth to keep the trial site late blight free until stable canopy was reached.
- Nine fungicide strategies to represent typical resistance management strategies, recommended by guiding principles.
- Three samples for genotyping of strains taken from the trial site: the first when late blight was observed to determine the initial ratio of EU_37_A2 to other late blight strains, the second halfway through the spray programme, to confirm the presence of EU_37_A2 in the trial area, and the final sample to determine the effect of the different fungicide strategies on the proportion of EU_37_A2 causing disease symptoms.

4.1. Oversprays

For the ADAS trials, the trial area was oversprayed with alternate applications of Ranman 0.5 L/ha + Sipcam C50 0.24 kg/ha and Infinito 1.6 L/ha, from rosette stage onwards, at 7-day intervals. When late blight was observed in trial plots or in the surrounding untreated areas the oversprays ceased and the experimental treatments began. The number of oversprays was therefore dependent on the time at which late blight was observed in the trial area. After the second overspray was applied, small areas of the experimental site were left untreated, to allow late blight movement around the site to be tracked and for late blight to establish evenly across the site. The number of oversprays applied varied depending on the site and season. Overspray details are given in appendix.

Details of over-sprays for the ADAS and SRUC trials are given in Appendix A1.

4.2. Treatment list

Nine treatments were included in each trial. Each treatment represented a particular strategy that was hypothesised to either select or not select for fungicide insensitivity in a given population. The description and rationale for the testing of the different strategies are shown in Table 1, with the fungicides and doses used in the trial outlined in Table 2.

At the ADAS site, all fungicides were applied at a water volume of 300 L/ha with a medium spray quality using a handheld OPS sprayer. At the SRUC site, fungicides were applied using a tractor-mounted AZO compressed air sprayer, 200 l/ha, medium-fine spray (BCPC classification) at 3.5 bar using Lurmark F03-110 flat fan nozzles.

The target spray interval was 7 days but some flexibility was necessary depending on weather. Actual spray dates for treatments are shown in appendix Table A2. Herbicides, insecticides and nutritional inputs were applied in line with local practice.

Table 1. Treatment descriptions and rationale for inclusion.				
Trt.	Description and rationale	Short description for chart labels		
1	Same mode of action applied repeatedly (no resistance management approach)	Fluazinam (control)		
2	Using a multisite/alternative mode of action only (impact of a multisite or mode of action without any reported insensitivity)	Mancozeb (multisite)		
3	Alternating two modes of action at every application (effectiveness of alternating different single site modes of action)	Alternation (Revus start)		
4	Alternating two modes of action at every application (effectiveness of alternating different single site modes of action)	Alternation (Shirlan start)		
5	Application of two modes of action in 'blocks' of three sequential applications (does blocking, and blocking order, increase the selection for resistance)	Blocks (Shirlan first)		
6	Application of two modes of action in 'blocks' of three sequential applications (does blocking, and blocking order, increase the selection for resistance)	Blocks (Revus first)		
7	Applying two modes of action in mixture at lower doses, that provides effective control (balanced mixtures, containing two contrasting modes of action, are effective resistance management strategies)	Half dose mixture (single site)		
8	Applying two single site modes of action, in mixture, at the full recommended dose (mixtures, with a single site mixture partner, are effective resistance management strategies)	Full dose mixture (single site)		
9	Applying two modes of action, with one multisite acting, in mixture at the full recommended dose (mixtures, with a multisite site mixture partner, are effective resistance management strategies)	Full dose mixture (multisite)		

Table 1. Treatment descriptions and rationale for inclusion.

Table 2. Fungicide products and doses used to test each resistance management strategy.						
Trt	Number and sequence of fungicide application in the spray programme					
m	1	2	3	4	5	6
1	Shirlan 0.4	Shirlan 0.4	Shirlan 0.4	Shirlan 0.4	Shirlan 0.4	Shirlan 0.4
	L/ha	L/ha	L/ha	L/ha	L/ha	L/ha
2	Manzate	Manzate 1.7				
	1.7 kg/ha	kg/ha	kg/ha	kg/ha	kg/ha	kg/ha
3	Revus 0.6	Shirlan 0.4	Revus 0.6	Shirlan 0.4	Revus 0.6	Shirlan 0.4
	L/ha	L/ha	L/ha	L/ha	L/ha	L/ha
4	Shirlan 0.4	Revus 0.6	Shirlan 0.4	Revus 0.6	Shirlan 0.4	Revus 0.6
	L/ha	L/ha	L/ha	L/ha	L/ha	L/ha
5	Shirlan 0.4	Shirlan 0.4	Shirlan 0.4	Revus 0.6	Revus 0.6	Revus 0.6
	L/ha	L/ha	L/ha	L/ha	L/ha	L/ha
6	Revus 0.6	Revus 0.6	Revus 0.6	Shirlan 0.4	Shirlan 0.4	Shirlan 0.4
	L/ha	L/ha	L/ha	L/ha	L/ha	L/ha
7	Shirlan 0.2	Shirlan 0.2	Shirlan 0.2	Shirlan 0.2	Shirlan 0.2	Shirlan 0.2
	L/ha +	L/ha +	L/ha +	L/ha +	L/ha +	L/ha +
	Revus 0.3	Revus 0.3	Revus 0.3	Revus 0.3	Revus 0.3	Revus 0.3
	L/ha	L/ha-	L/ha-	L/ha-	L/ha-	L/ha-
8	Shirlan 0.4	Shirlan 0.4	Shirlan 0.4	Shirlan 0.4	Shirlan 0.4	Shirlan 0.4
	L/ha +	L/ha +	L/ha +	L/ha +	L/ha +	L/ha +
	Revus 0.6	Revus 0.6	Revus 0.6	Revus 0.6	Revus 0.6	Revus 0.6
	L/ha	L/ha	L/ha	L/ha	L/ha	L/ha
9	Shirlan 0.4	Shirlan 0.4	Shirlan 0.4	Shirlan 0.4	Shirlan 0.4	Shirlan 0.4
	L/ha +	L/ha +	L/ha +	L/ha +	L/ha +	L/ha +
	Manzate	Manzate 1.7				
	1.7 kg/ha	kg/ha	kg/ha	kg/ha	kg/ha	kg/ha

4.3. Sampling strategy

Three samples were included as part of the genotyping sampling strategy. The first sample was taken when late blight was first observed in the trial area to establish the baseline frequency of EU_37_A2 compared with other all strains present. The second sample was taken after the third test fungicide application, to confirm that the required isolates were present in the trial area. This was particularly important where EU 37 A2 was not detected during the first sample and the trial was inoculated. The final sample was taken at least 7 days after all treatment fungicides were applied, to confirm the frequency of EU 37 A2 in individual plots compared with other strains after fungicide treatment. The samples were collected on FTA cards following the method outlined in the EuroBlight protocol for sampling leaf lesions: http://euroblight.net/fileadmin/euroblight/Monitoring 2013 project/EuroBlight sampling.pdf. The James Hutton Institute (JHI) supplied barcode labels so that each FTA card had a unique identifier for year, site and trial plot.

4.3.1. Sample 1: Disease symptoms visible and prior to test treatments

One sporulating infected leaf from each of 64 points from the untreated areas of the trial (15 samples from each replicate) was removed as evenly as possible from across the trial area. Each sample was given a code and the location of the sample recorded. Individual infected leaves were placed into Petri dishes lined with moist paper towel, the lid replaced and maintained at 15 to 20°C until sporulation was visible. Approximately 24h was usually found to

be sufficient, however, if there was no sporulation after this time, the samples were left for a further 24 hours. Care was taken to avoid cross contamination between samples during collection and after placing into Petri dishes. Once sporulation was visible, the leaves were pressed onto FTA cards supplied by James Hutton Institute (JHI).

4.3.2. Sample 2: After three test treatment applications have been applied

Sixteen samples were taken from across all four replicates from each of Treatments 1 and 2 separately, immediately prior to the fourth fungicide treatment to determine if the appropriate *P. infestans* strains were in the trial area. This was done by taking four leaves with sporulating lesions for each of the four plots within a treatment. The plot and treatment number for each sample was recorded and leaves were collected from distinct areas of each plot. Multiple samples from individual plants or small areas were avoided. Once collected, the leaves were processed in the same manner as described in section 4.3.1.

4.3.3. Sample 3: After all test treatments have been applied

A minimum of seven days after the final test treatment had been applied (Application 9), plots were inspected for fresh sporulating late blight lesions. Sixteen leaf samples were collected from every plot, taking care to ensure samples were taken from different plants and areas within the plot. Leaves were processed in the same manner as described in section 4.3.1.

4.4. Method for receiving, processing and reporting the samples

A maximum of four individual sporulating lesions were pressed onto individually defined sampling areas on each FTA card. The total number of individual FTA card samples received from each site during each year of trials is shown in Table *3*.

Site	Sample	2019	2020	2021
	Sample 1	64	64	64
ADAS	Sample 2	32	32	32
	Sample 3	576	576	300
	Sample 1	64	64	64
SRUC	Sample 2	32	32	32
	Sample 3	564	570	469

Table 3. Number of FTA card samples received from each site in each trial season.

Late blight lesions pressed onto the FTA cards were used to obtain DNA for SSR analysis with a 12-plex marker set according to the method of Li *et al.*, (2012). To process the samples, 2mm disks were punched from the interface of the green and brown zone of the lesions pressed onto FTA cards, washed with the FTA Purification Reagent (Whatman[™] WB120204) according to the manufacturer's instructions and the disk used as template in the 12-plex PCR assay. The SSR allele peaks were manually checked and scored prior to export to excel spreadsheets for further analysis.

The resultant allelic data for each individual lesion sample was then assigned to a pre-defined genotype based on the allelic profile observed. The data was sorted in an excel database for each trial location and the proportions of genotypes observed in each plot at the different sample dates were calculated.

Results were reported with a quick turnaround, particularly for sample dates 1 and 2, in order to observe the *P. infestans* strain profile within the trials and allow decisions to be made regarding the need for artificial inoculation.

4.4 Determining the effect of fungicide treatments on selection

The effectiveness of strategies to manage resistance was quantified by calculating the selection coefficient for each treatment for individual trials.

The selection coefficient (s) was calculated as follows:

$$s = \left(\frac{1}{t_2 - t_1}\right) ln\left(\frac{d_2(1 - d_1)}{d_1(1 - d_2)}\right)$$

Where $t_2 - t_1$ is the number of days between the first sample inoculation (t_1) and the final sample (t_2), d_1 is the ratio of EU_37_A2 to all other genotypes at the first sampling date and d_2 is the ratio of EU_37_A2 to all other genotypes on the third sampling date. This allowed the effect of the fungicide treatments to be determined using the proportion of EU_37_A2 at the start of the experiment and at the final (after treatment) sample across the time period when test fungicides were applied. The higher the value for the selection coefficient, the less effective that strategy is for reducing the selection for EU_37_A2, therefore strategies with a lower selection coefficient would be considered to be more effective.

4.5 Disease assessments

Late blight was assessed regularly during the season, with the gap between assessments determined by the speed of the epidemic. Disease assessments commenced when late blight symptoms were first seen in the trial. If disease was moving quickly, then the interval between assessments was decreased. The interval between assessments was usually between three and seven days. Assessments continued until at least 7 days after the final sample was taken. Disease assessments were carried out on the same day as the late blight samples were taken. Disease was assessed as described in the EPPO guideline PP 1/002 (5) *Phytophthora infestans* on potato at both sites. The percentage of canopy area affected by late blight, per plot, was recorded at each assessment.

4.6 On-site weather recording and site details

Both sites had weather stations on site or nearby to provide information on late blight risk as well as weather on the days before and after fungicide application. Data collected included maximum, minimum and average air temperature and rainfall. Details about soil type and series, previous cropping (2 years), pre-planting cultivations, planting date and spacing were recorded as well as all pesticides, fertilisers and other treatments applied to the whole crop.

4.5. Statistical analysis

After consultation with an ADAS statistician, all data were analysed using ANOVA followed by Duncan's multiple range test to measure specific differences between pairs of means.

5. RESULTS

5.1. 2019 experiments

5.1.1. ADAS

In 2019, the late blight epidemic occurred relatively late in the season. As a result, two oversprays were applied to the trial area prior to test treatment programmes starting (Table 4).

Table 4. Application dates.		
Application	Date	
Overspray	15 July	
Overspray	24 July	
1 st Application	30 July	
2 nd Application	7 August	
3 rd Application	13 August	
4 th Application	20 August	
5 th Application	27 August	
6 th Application	4 September	
Overspray	11 September	
Overspray	19 September	

No untreated control was included in the trial design as this was not required as part of the protocol, however, disease levels in the untreated areas surrounding the trial (imbricated) were monitored and scored in the same manner as the rest of the trial area. Foliar late blight severity developed rapidly, increasing from 2% to 90% in seven days in the untreated areas (Figure 1).

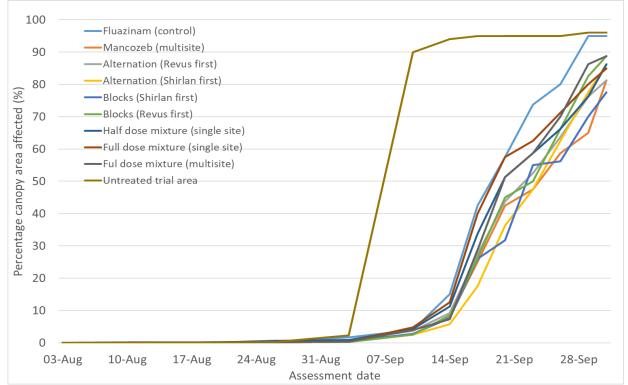


Figure 1. The progress of the late blight epidemic for individual treatments (as the percentage leaf area affected).

In 2019, the first sample, was taken on 30 July, prior to fungicide test treatments being applied. Thirty percent of the samples were EU_37_A2 strain, with the remainder being

EU_6_A1. When the site was sampled midway through the test fungicide applications on 19 August, EU_37_A2 was present in 94% of samples where fluazinam had been applied, compared with 64% of samples were EU_37_A2 where mancozeb had been applied (Figure 2).

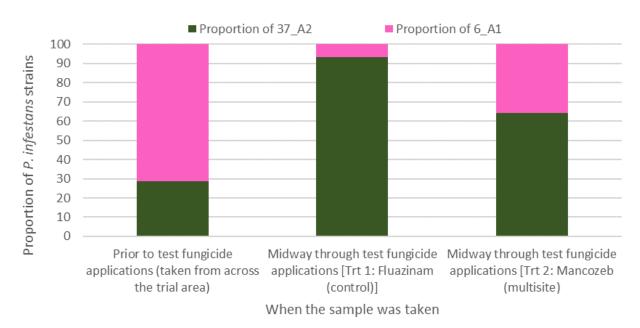


Figure 2. The proportion of EU_37_A2 across the ADAS site prior to fungicide application and the proportion midpoint through the experiment in fluazinam and mancozeb treated plots (Treatments 1 and 2 in Table 1 respectively).

The third and final sample was taken on 10 September (Replicates 1 and 2) and 11 September (Replicates 3 and 4). The fluazinam insensitive strain, EU_37_A2, was most prevalent where fluazinam only (Trt 1) had been applied for the six test treatments. This was observed for both the proportion of EU_37_A2 present relative to other strains and the selection coefficients (Figure 3 and Figure 4). A substantial proportion of the samples taken were 'fails', which was likely due to the leaves not sporulating sufficiently prior to pressing on FTA cards. Interestingly, only EU_37_A2 was detected from the FTA card and not EU_6_A1. Whether this was due to a difference in the rate of sporulation between the genotypes is unknown. This data will therefore not be included in the final cross site analysis or considered to determine the conclusions for this reason so is excluded from the report.

5.1.2. SRUC

In 2019, three oversprays were applied prior to test treatment programmes starting (Table 5).

Application	Date	
Overspray	1 August	
Overspray	8 August	
Overspray	16 August	
1 st Application	22 August	
2 nd Application	28 August	
3 rd Application	5 September	
4 th Application	13 September	
5 th Application	20 September	
6 th Application	27 September	
Overspray	7 October	

Table 5. Application dates.

The epidemic was relatively late in the season, starting in mid-August and reaching around 70% foliar leaf area affected in early September in treated plots (Figure 3). Untreated disease progress was not monitored at this site in 2019.

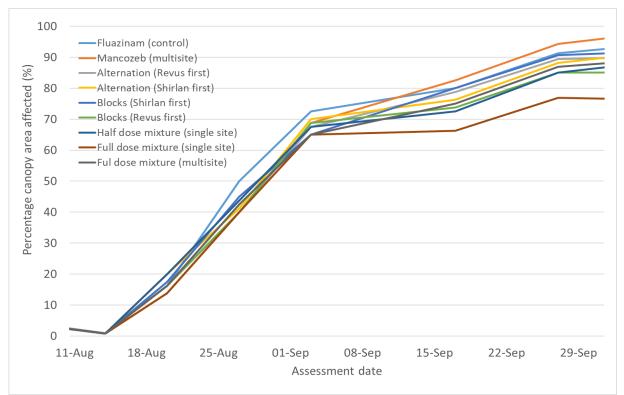


Figure 3. The progress of the late blight epidemic for individual treatments (as the percentage leaf area affected).

The first sample was taken on 14 August, prior to fungicide test treatments being applied. Sixty-nine percent of the samples were 36_A2, with the remainder EU_6_A1 (20%), 13_A2 (9%) and EU_37_A2 (3%). When the site was sampled midway through the test fungicide applications, 98% of samples taken from the fluazinam only treatment were 36_A2 and 2% of samples were EU_37_A2 and 'Other' strains. In the mancozeb only treatment, 92% of samples were 36_A2 with the remainder EU_37_A2 (Figure 4).

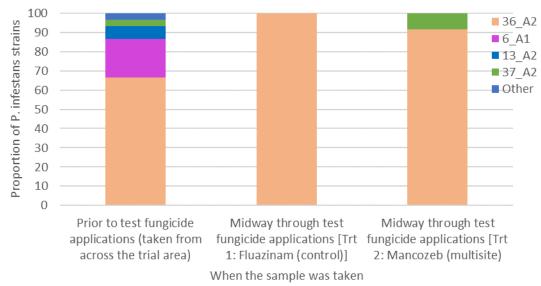


Figure 4. The proportion of EU_37_A2 across the SRUC site prior to fungicide application and the proportion midpoint through the experiment in fluazinam and mancozeb treated plots (Treatments 1 and 2 in Table 1 respectively).

The third and final samples were taken on 30 September (Replicate 1), 1 October (Replicate 2), 2 October (Replicate 3) and 3 October (Replicate 4). The fluazinam insensitive strain, EU_37_A2 , was most prevalent (approximately 10% of the samples taken) where fluazinam only had been applied for the six test treatments. This was demonstrated by both the proportion of EU_37_A2 present relative to other strains, which were predominately 36_A2, and the selection coefficients (Figure 5). There were differences between the selection coefficients, however, these were not statistically significant (*P*=0.467).

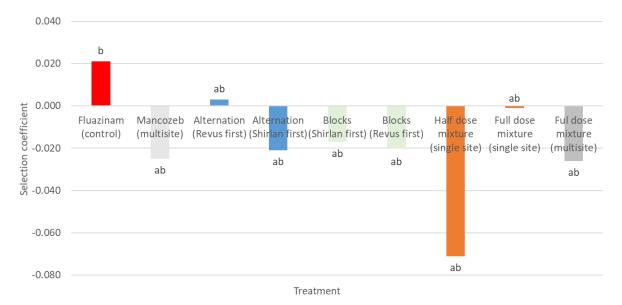


Figure 5. Selection coefficients for the fungicide resistance management strategies tested relative to the fluazinam control. Differences between the selection coefficients were not statistically significant (P=0.409). Letters represent the results from the Duncan's multiple range test.

5.2. 2020 experiments

5.2.1. ADAS

In 2020, two oversprays were applied prior to starting the test treatment programmes (Table 6).

Table 6. Application dates.	
Application	

Application	Date
Overspray	10 August
Overspray	15 August
1 st Application	23 August
2 nd Application	31 August
3 rd Application	7 September
4 th Application	14 September
5 th Application	21 September
6 th Application	28 September
Overspray	8 October
Overspray	14 October

The epidemic was late in the season, starting in mid-August, however, it was relatively slow to develop, with around 10% foliar late blight reported in mid-September and reaching 100% by the end of September (Figure 6).

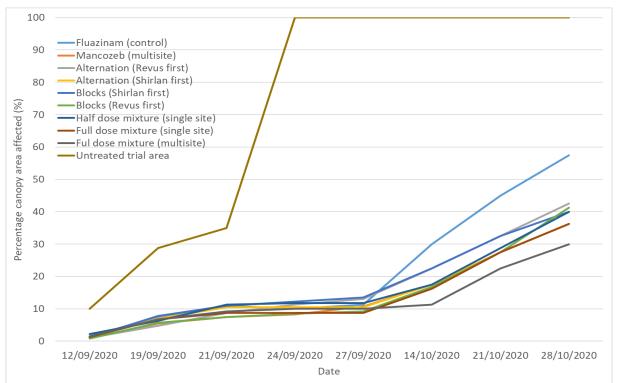


Figure 6. The progress of the late blight epidemic for individual treatments (as the percentage leaf area affected).

In 2020, the first sample was taken on 1 September, prior to fungicide test treatments being applied. Nine percent of the samples were EU_37_A2, with the remainder EU_6_A1. When the site was sampled midway through the test fungicide applications on 14 September, EU_37_A2 was present in 88% of samples where fluazinam had been applied, whereas 19% of samples were EU_37_A2 where mancozeb had been applied (Figure 7).

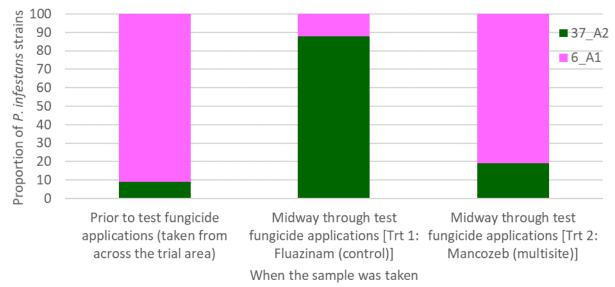


Figure 7. The proportion of EU_37_A2 across the ADAS site prior to fungicide application and the proportion midpoint through the experiment in fluazinam and mancozeb treated plots (Treatments 1 and 2 in Table 1 respectively).

The third and final sample was taken on 5 October. The fluazinam insensitive strain, EU_37_A2, was most prevalent (over 90% of the samples taken) where fluazinam only had been applied for the six test treatments for both the proportion of EU_37_A2 present relative to other strains and the selection coefficients (Figure 12). There were significant differences between the selection coefficients, (P=0.003), with all treatments, from full dose mixture

onwards significantly decreasing the selection coefficient relative to the fluazinam only treatment (Figure 8).

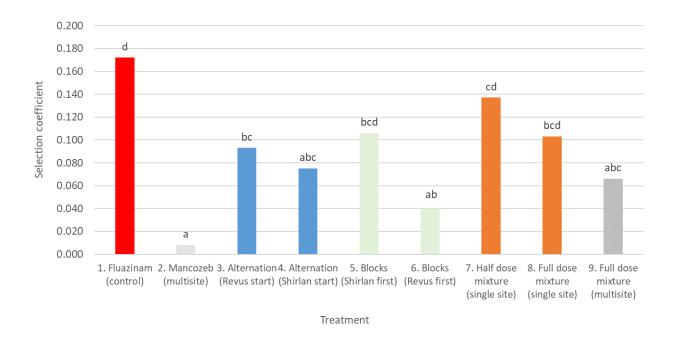


Figure 8. Selection coefficients for the fungicide resistance management strategies tested relative to the fluazinam control (P = 0.003). Letters represent the results from the Duncan's multiple range test.

5.2.2. SRUC

In 2020, two oversprays were applied prior to test treatment programmes starting (Table 7).

Table 7. Application dates.			
Application	Date		
Overspray	6 August		
Overspray	13 August		
1 st Application	19 August		
2 nd Application	26 August		
3 rd Application	2 September		
4 th Application	9 September		
5 th Application	16 September		
6 th Application	23 September		
Overspray	N/A		
Overspray	N/A		

The epidemic was slow and occurred late in the season, with first signs of late blight mid-August, reaching 75% leaf area affected by end of September (Figure 9).

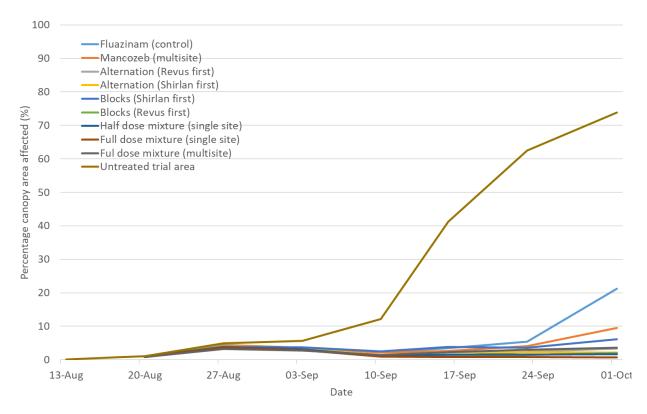


Figure 9. The progress of the late blight epidemic for individual treatments (as the percentage leaf area affected).

In 2020, the first sample was taken on 19 August, prior to fungicide test treatments being applied, and one hundred percent of the samples were 36_A2 (

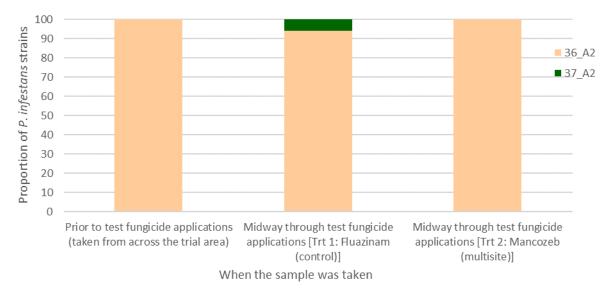


Figure 10). Because EU_37_A2 was not detected, the site was inoculated with EU_37_A2 on 31 August and 8 September. Midway through the treatment fungicides, EU_37_A2 was detected in the fluazinam only plots (6%) but not in the mancozeb only plots. The only other genotype identified was EU_36_A2.

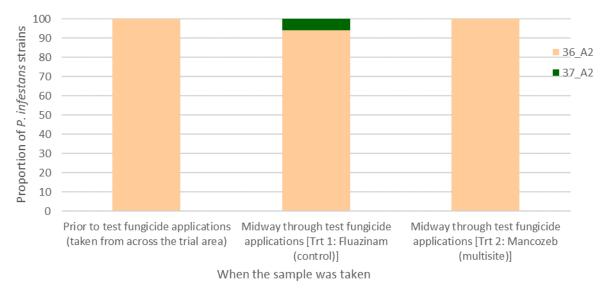
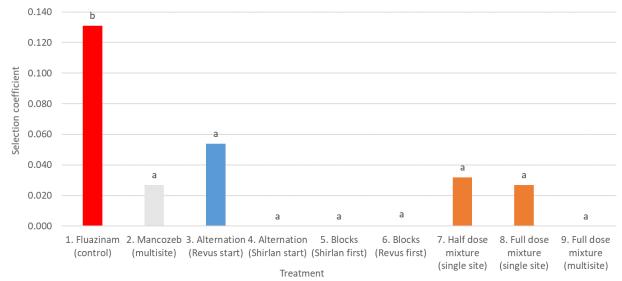


Figure 10. The proportion of EU_37_A2 across the SRUC site prior to fungicide application and the proportion midpoint through the experiment in fluazinam and mancozeb treated plots (Treatments 1 and 2 in Table 1 respectively).

The third and final sample was taken on 1 October (Replicate 1 and 2) and 2 October (Replicate 3 and 4). The fluazinam insensitive strain, EU_37_A2, was most prevalent where fluazinam only had been applied for the six test treatments. This was demonstrated by both



the proportion of EU_37_A2 present relative to other strains and the selection coefficients (

Figure 11Figure 12). There were differences between the selection coefficients and these were statistically significant (P<0.001). All resistance management programmes significantly decreased selection relative to fluazinam applied alone (

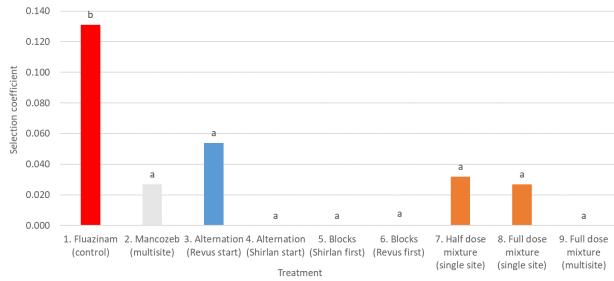


Figure 11).

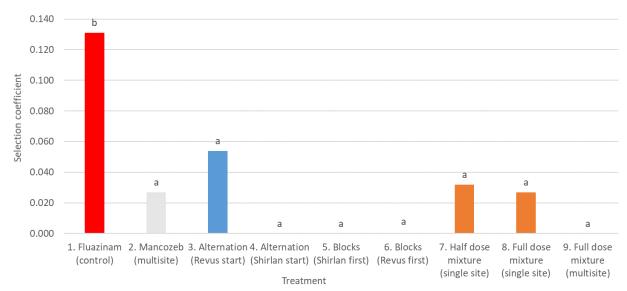


Figure 11. Selection coefficients for the fungicide resistance management strategies tested relative to the fluazinam control (P=0.003). Letters represent the results from the Duncan's multiple range test.

2021 experiments

5.2.3. ADAS

In 2021, five oversprays were applied prior to test treatment programmes starting (Table 8).

Table 8. Application dates.

Application	Date
Overspray	12 July
Overspray	19 July
Overspray	26 July
Overspray	3 August
Overspray	10 August
1 st Application	17 August
2 nd Application	24 August
3 rd Application	1 September
4 th Application	7 September
5 th Application	14 September
6 th Application	22 September
Overspray	N/A

The main part of the epidemic occurred late in the season, despite first signs of late blight in mid-August. Foliar late blight reached 100% in untreated areas at the end of September (Figure 12). Low rainfall was recorded during August and into September, therefore the crop senesced rapidly towards the end of the season and around the time the final samples were being taken.

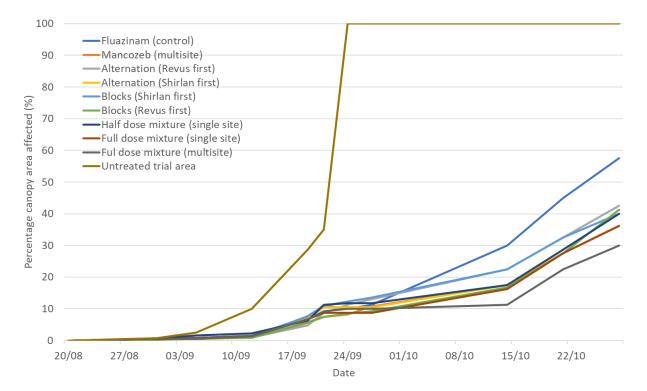


Figure 12. The progress of the late blight epidemic for individual treatments (as the percentage leaf area affected).

In 2021, the first sample was taken on 17 August, prior to fungicide test treatments being applied (Figure 13). A range of *P. infestans* strains were recorded including a high proportion

of EU_6_A1, a rare genotype (EU_44) and three 'other' strains, which are present in such low proportions they have not been assigned a code. The proportion of these strains appeared to increase between the first and second sample (taken on 15 September) where fluazinam had been applied compared with the mancozeb plots, however, it should be noted that the sample size was small (four lesions per plot per replicate). Because EU_37_A2 was not detected, the site was inoculated with EU_37_A2 on 19 August.

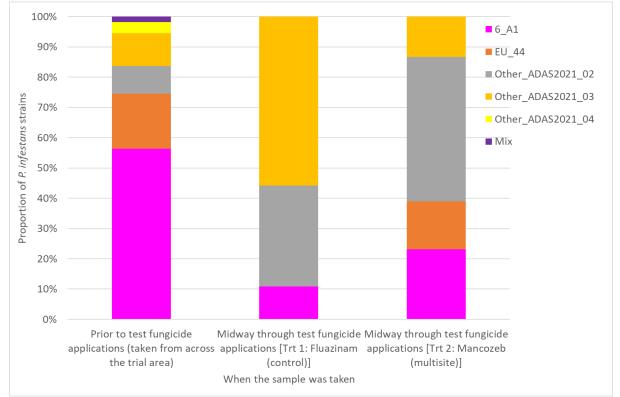


Figure 13. The proportion of different *P. infestans* strains across the ADAS site prior to fungicide application and the proportion midpoint through the experiment in fluazinam and mancozeb treated plots (Treatments 1 and 2 in Table 1 respectively).

The third and final sample had to be taken one week earlier than planned (22 September) due to the crop senescing rapidly. Only trace levels of EU_37_A2 were observed in trial plots in treatments 1 (fluazinam only) and 9 (full dose mixture, multisite). A high proportion of failed samples meant it was not possible to obtain data on selection for EU_37_A2 (Figure 14).

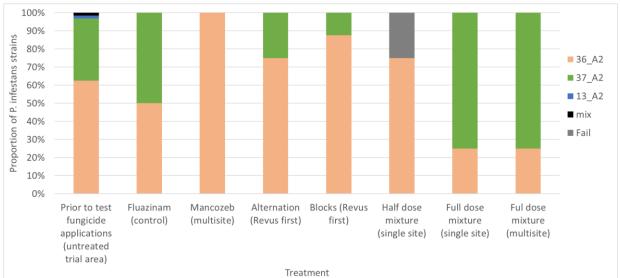


Figure 14. The proportion of strains reported, including failed samples, for the fungicide resistance management strategies tested.

5.2.4. SRUC

In 2021, six oversprays were applied prior to test treatment programmes starting (Table 9).

Table er replication a	
Application	Date
Overspray	6 July
Overspray	13 July
Overspray	20 July
Overspray	27 July
Overspray	3 August
Overspray	10 August
1 st Application	17 August
2 nd Application	24 August
3 rd Application	1 September
4 th Application	7 September
5 th Application	14 September
6 th Application	22 September
Overspray	N/A
3 rd Application 4 th Application 5 th Application 6 th Application	1 September 7 September 14 September 22 September

Table 9. Application dates.

The main part of the epidemic occurred late in the season, despite first signs of late blight in mid-August, similar to the ADAS site. Foliar late blight reached nearly 80% in mancozeb-treated plots by early October (Figure 15). The crop was senescing rapidly during this period, which coincided with the time the final samples were being taken, and it was not possible to complete disease assessments across the entire trial area.

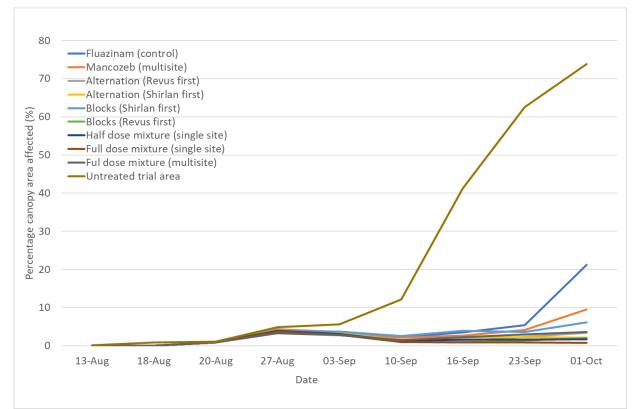


Figure 15. The progress of the late blight epidemic for individual treatments (as the percentage leaf area affected). Only mancozeb and fluazinam plots were assessable after 21 September.

In 2021, the first sample was taken on 30 August, two weeks after test treatments were applied, as this was when sporulating lesions were first observed in trial plots (Figure 16). EU_36_A2 and EU_37_A2 were the predominant strains, with very low levels of EU_13_A2 recorded. The proportion of EU_37_A2 increased only slightly between this first and second sample (taken on 7 September) in fluazinam control plots, however, there was no further selection observed after sample 3. As a result, the data were not analysed further.

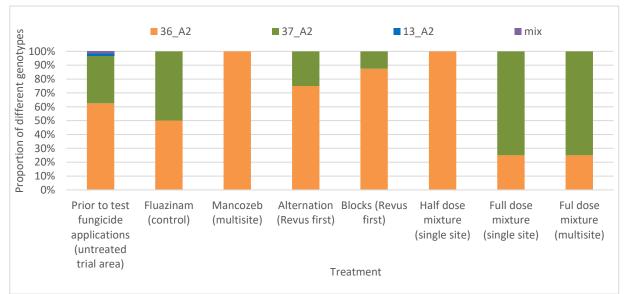


Figure 16. The proportion of different *P. infestans* strains across the ADAS site prior to fungicide application and the proportion at the midpoint in the experiment in fluazinam and mancozeb treated plots.

5.3. Cross site analyses

Given the high number of genotyping fails in the ADAS trials in 2019 and 2021, and the lack of selection in the SRUC trial in 2021, three out of the six sites were included in the final cross site analysis to determine whether strategies differed in their effectiveness to decrease selection. This included the SRUC trial from 2019 and the ADAS and SRUC trials from 2020.

When the datasets from the three successful trials were combined, there was a significant decrease in the selection coefficient for all resistance management strategies tested relative to the fluazinam only control (Figure 17). Although using a tank mixture with a multisite fungicide appeared to reduce the selection coefficient to a greater extent than single site mixture, the difference between these treatments were not statistically significantly different. The order in which fungicide products were applied, in alternation or in blocks of three applications, did not significantly affect selection. It was noted that the selection coefficients were lower when Revus was used to start alternation strategies compared with Shirlan, however the differences were not statistically significantly different. Similarly, there were no differences in selection where a half or full dose mixture was applied.

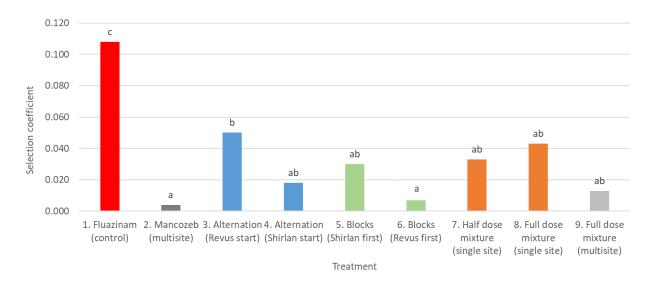


Figure 17. Selection coefficients for the fungicide resistance management strategies tested (P=<0.001). Site.Treatment interaction = P=0.072 (not significant). Letters represent the results from the Duncan's multiple range test.

Given there were no statistically significant differences between the different alternation, block and tank mixture strategies, these were combined (Treatments 3 and 4, 5 and 6, and 7 and 8) and reanalysed to provide a simple summary of the trial results to consider the absence of a resistance management strategy (fluazinam only) and the resistance management strategies (as alternation, blocks and alternation, multisite and single site mixtures) available (Figure 18). It can be seen from the analysis that all strategies significantly decrease the selection coefficient and the proportion of the fungicide insensitive strain in the experiments.

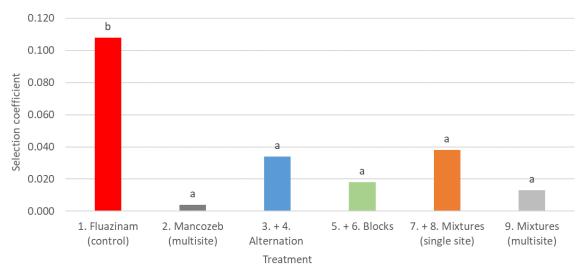


Figure 18. Selection coefficients for the combinations of fungicide resistance management strategies tested (P=<0.001). Site.Treatment interaction = P=0.089 (not significant). Letters represent the results from the Duncan's multiple range test.

6. DISCUSSION

Good late blight epidemics developed at all sites in both 2019 and 2020. All epidemics were relatively late in the season as we were relying on natural epidemics occurring at generally lower risk sites. This was due to the requirement for slower and sustained epidemics against which to test the effects of multiple sprays on selection for fungicide insensitivity; early and rapid epidemics would not provide the opportunity to do this. The option to inoculate the trials as well as rely on natural epidemics was necessary and was successful at the ADAS and SRUC sites in 2020. We developed a protocol that required EU 37 A2 to be present at low levels at the start of the season, providing a base from which to select from and test an experimental hypothesis. As a period of at least 42 days was required to test the resistance management strategies using six consecutive fungicide applications, there was a balance between waiting for a natural epidemic and the crop, particularly foliage, duration. Despite numerous attempts at inoculating the ADAS site in 2021, crop condition towards the end of the season was poor due to weather and no EU 37 A2 was observed. At the SRUC site. despite the presence of EU_37_A2, no selection was observed, perhaps in part due to little or no movement of late blight across the site after the first three fungicide applications due to unfavourable weather. Weather related issues with field trials are difficult to avoid, however, they are noted here in case others wish to conduct similar experiments in future. Taking FTA card samples from 64 untreated locations across the trial area to determine the initial proportions of P. infestans strains prior to applying the resistance management fungicide programmes, rather than taking samples from individual plots as had been tried in similar experiments previously, was reliable and cost effective (Carolan et al., 2018).

It should be noted that the protocol used in this project was designed to test the effectiveness of resistance management strategies by selecting for EU_37_A2, a P. infestans strain with known insensitivity to fluazinam, as an experimental tool. There was a substantial reduction in the use of fluazinam in England and Wales from 2016 (20% of the total fungicide treated area) to 2018 (4%) and 2020 (3%) (Garthwaite 2018, Garthwaite et al., 2019, Ridley et al., 2020) and the proportion of the genotype found in 'Fight Against Blight' samples also dropped from 25% in 2017 to just under 10% in 2021 (Cooke et al., 2022). Using mixtures and alternation that included fluazinam decreased selection for the fungicide insensitive strain EU_37_A2. This has raised questions as to the use of fluazinam in fungicide programmes and there are several considerations to make, most around the efficacy against foliar and tuber late blight. Firstly, it was clear from the experiments that the proportion of EU_37_A2 increased during the season with fluazinam use, regardless of whether it was used in mixtures or alternation with other modes of action. Therefore, increasing the use of fluazinam is likely to still select for EU 37 A2, albeit at a slower rate, regardless of the resistance management strategies implemented, as it is already established in the GB population. This would be expected as fungicide resistance management strategies will slow and not prevent selection for fungicide insensitive strains. Secondly, it is usual for the strains responsible for starting a late blight epidemic to be unknown until a sample is taken for genotyping. In the experiments, we created an environment where EU_37_A2 was at low levels early in the season and we could monitor increases in EU 37 A2 throughout the season. It is possible that, in practice, EU_37_A2 could be the dominant strain at the beginning of the epidemic and strategies such as block alternation would mean late blight was ineffectively controlled for periods of more than 7 days and put the crop at risk from infection. Thirdly, if fluazinam is used and EU 37 A2 is present, there are potential risks from ineffective control of tuber blight to consider e.g. if it was the only tuber blight product included at key points in the fungicide programme.

Phytophthora infestans has proved to be adept at overcoming host resistance genes, reproducing faster and overcoming fungicide active ingredients. Examples include EU_13_A2 (metalaxyl insensitivity/downgrading of variety resistance ratings), EU_33_A2 (fluazinam insensitivity, but with a fitness penalty) and EU_37_A2 (fluazinam insensitivity and competitive with other strains) (Lees *et al.*, 2012, Schepers *et al.*, 2018). The predominant genotypes identified in 'Fight Against Blight' sampling in 2021 were 36_A2 (40%), EU_6_A1 (23%) and EU_37_A2 (10%), EU_8_A1 (6%), with other strains accounting for around 20%. Six out of the 12 modes of action available (Qil, CAA, carbamates, benzamides, OSBPI and

dithiocarbamates) have been screened against 36_A2, EU_37_A2 and EU_6_A1 and although small differences between the strains in their sensitivity were noted in laboratory studies, the degree of those differences were small and not considered to have any impact on field performance (Cooke *et al.*, 2022). Therefore a range of modes of action are available to incorporate into fungicide programmes for resistance management strategies.

The individual experiments and cross site analysis clearly demonstrate that the repeated application of a single site mode of action without the implementation of any resistance management strategy, over six sprays, will result in the selection for fungicide insensitive strains. Interestingly, alternating modes of action, whether as blocks of three sequential fungicide products with the same mode of action or alternating sequential sprays, both appear to reduce the selection for fungicide insensitivity. Mixtures, whether half or full dose, and regardless of whether a single or multisite mixture partner, also appear to be effective. When resistance management strategies were investigated for *Zymoseptoria tritici* on winter wheat, a similar observation was made; alternation is as effective as mixtures at reducing selection (Young *et al.*, 2021). Mixtures have been shown to be more effective for resistance management than alternation in modelling studies that looked to evaluate the strategies, however, these analyses run longer than a single season (Elderfield *et al.*, 2018). The long term impact of resistance management strategies for *P. infestans* on potato remains to be determined.

It is not practical to design a fungicide programme purely for resistance management, as the most effective resistance management strategies (very low doses and infrequent applications) are likely to be ineffective at controlling disease. A fungicide programme should provide effective control of *P. infestans* as well as include the available resistance management guidelines. The data presented here suggests that there is a range of options available for resistance management that could be integrated into a fungicide programme and make resistance management strategies straight forward to implement.

The rationale for the success of the different strategies comes from understanding the principles that govern pathogen evolution (van den Bosch et al., 2014). To reduce selection, the time span over which selection takes place (the exposure time) is reduced and the per capita rate of increase of the resistant strain is reduced for both strains, or the resistant relative to the sensitive strain. In the alternation treatments, whether as blocks of three sprays or alternating single sprays, reducing the number of applications of fluazinam from six (in the control treatment) to three reduced selection for the insensitive strain (EU_37_A2). Similarly, mixtures were also effective in these experiments. The rationale for using mixtures assumes that there is fungicide insensitivity to only one of the two mixture components and that the mixture partner is effective against all strains, reducing the overall population as well as the relative proportion of resistant to sensitive strains (van den Bosch et al., 2014). It should be noted that the total dose of a mode of action applied has been identified as a key driver for resistance selection for Zymoseptoria tritici on winter wheat (Young et al., 2021). This was not investigated in detail in the current study, however, given that this was observed on winter wheat, which receives far fewer fungicide treatments than potato, using a wide range of modes of action within the fungicide programme. limiting the use of individual modes of action and ensuring there is a balance of modes of action used throughout, would be beneficial for resistance management on potato.

In the experiments, there did not appear to be a difference in efficacy or selection for EU_37_A2 between a single site (mandipropamid) or multisite (mancozeb) mode of action. The failure to observe any difference in efficacy may be due to the trials being conducted during stable canopy rather than rapid canopy growth. Therefore the benefits from using an active ingredient, such as mandipropamid, which has greater activity on new growth relative to a protectant active ingredient such as mancozeb, would not be observed. There is no available data on the deployment of resistance management strategies during rapid canopy growth in particular, however, mobility will be a key consideration during this growth period for efficacy. For resistance management, mixture partners with single site and multisite modes of action provided similar reductions in resistance selection. Although it would appear the two are

interchangeable, there are two important differences. Firstly, single site + single site (S + S) provides mutual protection for each individual mode of action, however, the single site mixture also creates a selection pressure against both single site modes of action. With a multisite + single site (M + S) mixture, the multisite fungicide protects the single site fungicide and is at low resistance risk itself. In these trials, we only measured selection against one of the two single site modes of action included in the mixture, and therefore could not demonstrate any potential disadvantage of single site mixtures. There is concern that multisite + single site mixtures (M + S) mixtures may leave the single site mode of action unprotected in curative conditions given the protectant activity of multisite fungicides and, although this is a theoretical risk, there is no evidence for this from this project or elsewhere that this would or does occur.

There is evidence from other pathosystems that concurrent resistance, where resistance exists to two modes of action in the same pathogen strain, is more likely to occur when two or more single site modes of action are being used in the spray programme. For *Zymoseptoria tritici*, the use of chlorothalonil (multisite) + izopyrazam (single site) + prothioconazole (single site) significantly decreased selection for strains with resistance to SDH (izopyrazam) and DMI (prothioconazole) than where only izopyrazam + prothioconazole were applied (Young *et al.*, 2021). Given the relatively low resistance risk for multisites on potato, and the potential risks identified from not including multisites in other pathosystems, the inclusion of mancozeb should still be considered throughout the fungicide programme for resistance management.

It was clear that using half doses of fungicide did not compromise resistance management strategies, however, reducing fungicide doses comes with risks, particularly on the predominately highly late blight susceptible varieties used in GB today. Planting varieties with better resistance and applying lower fungicide doses for late blight control has been demonstrated to be successful in previous European and UK studies (Clayton and Shattock, 1995, Gans *et al.*, 1995, Bain *et al.*, 2008, Ritchie *et al.*, 2018). In an SRUC-led project from 2009 to 2011, it was demonstrated that foliar late blight control was superior with both full and lower doses of fungicides at 7-day intervals where varieties with moderate (5) to resistant (8) foliar late blight resistance ratings were used, when compared to a variety with a foliar resistance and reduced doses of fungicide, can provide good disease and resistance management. There are limitations in practice to using this integrated strategy, as market demands rather than disease resistance will usually dictate the variety grown. However, as pressure to decrease pesticide use increases, it is likely that alternative strategies will need to be deployed in future.

The data presented here supports the guidance currently recommended in the <u>FRAG</u> <u>guidelines</u> for fungicide resistance management in potato late blight, however, there is an opportunity to use the outputs from this project to fine tune general advice. For example, product labels have a range of guidance on application intervals, produce use throughout the season or simply state to use products in blocks and alternate to reduce the resistance risk. Many labels also include the recommendation for products not to be used in curative situations. All fungicide treatments in this experiment were applied in curative conditions, yet selection was still decreased when appropriate resistance management strategies were deployed. The outputs of this project could form part of a future review of resistance management strategies for late blight and contribute to the evidence base, providing new information to update and simplify fungicide resistance guidelines for the management of late blight on potato.

7. CONCLUSIONS AND PRACTICAL RECOMMENDATIONS

The repeated and sequential application of a single site mode of action, over multiple sprays, will result in the selection for a fungicide insensitive strain, if such a strain is present in the population. All of the resistance management strategies tested significantly decreased the selection for a fungicide insensitive strain. This diverse range of effective approaches should make it simpler for practical considerations, such as efficacy and cost, and fungicide resistance management to be implemented across the fungicide programme.

When building a fungicide programme, the following resistance management strategies have been shown experimentally to decrease selection for fungicide insensitive strains and did not differ significantly in their effect on resistance selection:

- 1. Alternation (as both alternating single sprays or blocks of three sprays).
- 2. Multi-site + single-site or single-site + single-site modes of action in tank mixtures.
- 3. Half dose or full dose tank mixtures (this would be applicable for co-formulations)

Recommendations when implementing resistance management strategies:

- 1. Consult up to date FRAG guidelines for potato late blight: <u>https://bit.ly/3J8udYK</u>.
- 2. Fully implement all cultural resistance management strategies prior to using fungicides including management of outgrade piles and volunteers, and blight-free seed.
- 3. Use a range of fungicides from different cross-resistance groups for both alternation and mixture strategies.
- 4. When using fungicides, do not apply more than three applications in sequence for the same mode of action.
- 5. Balance the use of different single site modes of action within the fungicide programme, do not overuse a particular single site mode of action.
- 6. Continue to use multisite fungicides in the programme where possible.

Future research needs

1. Annual collection and screening of new genotypes of *P. infestans* to understand the implications of novel phenotypes e.g. fungicide sensitivity and aggressiveness, risks, such as fungicide insensitivity and aggressiveness for late blight management.

2. Screen the efficacy of different modes of action against novel *P. infestans* genotypes under field conditions to provide agronomists and growers with robust information to make decisions for disease and resistance management.

3. Understand the long-term (more than one season) effect of implementing the recommended resistance management strategies on the selection for fungicide insensitive strains and disease management.

8. REFERENCES

Andersson B, Sjöholm S, Jönsson M, Olsson (2017) Selection for fungicide tolerance in Phytophthora infestans. Euroblight meeting, Aarhus, <u>https://agro.au.dk/fileadmin/4_Bjorn_Andersson.pdf</u>.

Bain RA, Bradshaw NJ, Ritchie F (2008) Matching fungicide inputs to cultivar resistance for the control of *Phytophthora infestans* on potato. In: Shepers HTAM (ed) Proceeding of the eleventh Euroblight workshop. PPO-special report no. 13 pp. 283-289.

Carolan K, Bain R, Lees A, Cooke D, Ritchie F, Paveley N, van den Bosch (2018) Strategies for integrated deployment of host resistance and fungicides to sustain effective crop protection. Report no. 2018/9. AHDB, UK.

Clayton RC, Shattock RC(1995) Reduced fungicide inputs to control *Phytophthora infestans* in potato cultivars with high levels of polygenic resistance. Potato Research 38: 399-405.

Cohen Y, Samoucha Y (1989) Selection for metalaxyl resistance in potato crops infected with *Phytophthora infestans*: effects of fungicides and initial frequency of resistant sporangia. Plant Pathology 38: 382-390.

Cooke D, Lees A, Ritchie F (2022) Population monitoring and fungicide sensitivity testing of *Phytophthora infestans* across GB 2019-21: Final Report P1812278 2019-2021, AHDB, UK.

Gans P, Carson WD, Pearson N, Owen LL (1995) Exploiting cultivar resistance to control potato blight (*Phytophthora infestans*). In: Dowley LJ, Bannon E, Cooke LR, Keane T, O'Sullivan E (eds) *Phytophthora* 150. Boole, Dublin, pp. 345-350.

Garthwaite DG, Barker I, Laybourn A, Huntly G, Parrish P, Hudson S, Thygesen H (2015) Pesticide usage survey report 263: Arable crops in the United Kingdom 2014. FERA, UK

Garthwaite D, Barker I, Ridley L, Mace A, Parrish G, MacArthur R, Lu Y, (2018) Pesticide usage survey report 271: arable crops in the United Kingdom 2016. FERA, UK.

Garthwaite D, Ridley L, Mace A, Parrish G, Barker I, Rainford J, MacArthur R, (2019) Pesticide usage survey report 284: arable crops in the United Kingdom 2018. FERA, UK.

van den Bosch F, Oliver R, van den Berg F, Paveley NP (2014) Governing principles can guide fungicide-resistance management tactics. Annual Review of Phytopathology 52; 175-195.

Elderfield JAD, Lopez-Ruiz FJ, van den Bosch F, Cunniffe NJ (2018) Using epidemiological principles to explain fungicide resistance management tactics: why do mixtures outperform alternations? Phytopathology 108: 803-817.

Lees A (2019) Comparison of sensitivity to a range of fungicides in contemporary genotypes of *Phytophthora infestans*. Report no. 2018/7. AHDB Potatoes, UK.

Lees AK, Stewart JA, Lynott JS, Carnegie SF, Campbell H, Roberts AMI (2012) The effect of a dominant *Phytophthora infestans* genotype (13_A2) in Great Britain on host resistance to foliar late blight in commercial potato cultivars. Potato Research. 55: 125-134.

Ridley L, Mace A, Stroda E, Parish G, Rainford J, MacArthur R, Garthwaite D (2020) Pesticide usage survey report 295: arable crops in the United Kingdom 2020. FERA, UK.

Ritchie F, Bain RA, Lees AK, Boor TRW, Paveley ND (2018) Integrated control of potato late blight: predicting the combined efficacy of host resistance and fungicides. Plant Pathology. 67: 1784-1791.

Schepers HTAM, Kessel GJT, Lucca F, Förch MG, van den Bosch GBM, Topper CG, Evenhuis A (2018) Reduced efficacy of fluazinam against *Phytophthora infestans* in the Netherlands. European Journal of Plant Pathology. 151: 947-960.

Young C, Boor T, Corkley I, Fraaije B, Clark B, Havis N, Kildea S, Paveley N (2022) Managing resistance evolving concurrently against two or more modes of action to extend the effective life of new fungicides. Project Report no. 637. AHDB, UK.

9. APPENDICES

If applicable, include information that supports your analysis, validates your conclusions or pursues a related point.

Site and year	Prior to treatment sprays	After treatment sprays		
ADAS 2019	15 and 24 July 11 and 19 September			
ADAS 2020	21 and 28 July, 3, 10 and 15	8 and 14 October		
	August			
ADAS 2021	12, 19 and 26 July, 3 and 10	none		
	August			
SRUC 2019	1, 8, and 16 August	7 October		
SRUC 2020	6 and 13 August	none		
SRUC 2021	13, 20 and 27 July, 3 and 10	none		
	August			

Appendix A1. Overspray dates for each site and year.

Appendix A2. S	Spray dates for the test fungicide programmes for each site and year.

	Spray number						
Site and year	1	2	3	4	5	6	
						4	
ADAS 2019	30 July	7 August	13 August	20 August	27 August	September	
	23	31	7	14	21	28	
ADAS 2020	August	August	September	September	September	September	
	17	24	1	7	14	22	
ADAS 2021	August	August	September	September	September	September	
	22	28	5	13	20	27	
SRUC 2019	August	August	September	September	September	September	
	19	26	2	9	16	23	
SRUC 2020	August	August	September	September	September	September	
	17	24	1	7	14	22	
SRUC 2021	August	August	September	September	September	September	

10. KNOWLEDGE EXCHANGE ACTIVITIES

An introduction to the project was presented by Cathryn Lambourne from AHDB during SPot farm week (February 2021). Neil Paveley provided an update to the Fungicide Resistance Action Group (FRAG) on the results so far (March 2021). The project was included in an article for Farmers Weekly (9 March 2021) on mancozeb and ways in which the industry could prepare for its loss. It included a short piece on the project to demonstrate how it will test ways to implement resistance management strategies in multispray crops in the absence of multisite fungicides. It was necessary to wait until the end of the project before disseminating data widely, as multiple trials were required to ensure outcomes were representative. Project results were presented at the EuroBlight meeting in May 2022 in Ascona, Switzerland, and will be shared with the Fungicide Resistance Action Group (FRAG) and more widely within the project partner knowledge exchange groups.

11. ACKNOWLEDGEMENTS

We would like to thank the following people for their contribution to the project including providing funding, delivery of the project, and input into the interpretation of results and guidelines: Dennis Churchill and Aldwyn Clarke, ADAS, Mark Hemmant, Agrovista, Sue Cowgill and Rob Clayton, AHDB, Kerry Maguire, Bayer CropScience, Craig Chisholm, Corteva Agriscience, Reuben Morris, Frontier Agriculture, Kuldip Mudhar, Gowan Crop Protection, John Edmonds, Gowan Crop Protection, Mark Britton, Syngenta Crop Protection, Andrew Cunningham, Syngenta Crop Protection, Michael Tait, Syngenta Crop Protection and Pamela Chambers, UPL.