Final Report

A Decision Aid to Support the use of Curative Late Blight Fungicides

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1. SUMMARY

- Methods were developed to quantify curative activity more completely than have previously been reported.
- Taking account of post-infection temperature and also modelling pathogen development provided a better prediction of curative control than considering only the chronological time between infection and treatment.
- Field experiments in which natural infection was simulated demonstrated that elevated cultivar resistance increased the magnitude of the curative effect and/or extended the curative window.
- A strong relationship between documented 1 to 9 resistance ratings for cultivars and the rates at which *P. infestans* colonises leaf tissue was demonstrated across 16 cultivars.
- A simple decision aid for growers and agronomists, which can be used as a guide as to whether or not a curative a.i. is required for any **scheduled** fungicide application, was developed. Data for the most aggressive 13_A2 isolate were used as the basis of the decision aid.
- The decision aid indicated that the impact of post-infection temperature on curative efficacy was considerably greater over the temperature range 6 to 18 ^{o}C than 18 to 26 ^{o}C.
- Very limited validation of the decision aid using field data provided mostly good predictions, but with some biases. Much greater validation is suggested, most probably leading to model refinements.
- The decision aid has the potential to be a very useful IPM tool for blight control, in addition to reducing fungicide input costs.
- Potential users should be consulted over the exact form of the output from the decision aid so that it matches their requirements.

2. INTRODUCTION

Potato late blight is caused by the pathogenic oomycete *Phytophthora infestans*, and is one of the most damaging potato pathogens (Kromann *et al.*, 2014). The disease has several life-cycle characteristics which make it particularly problematic. Its main method of dispersal in regions such as the United Kingdom is via air-borne spores from infected crops, volunteers or plants in outgrade piles, allotments or gardens, although infected seed can also cause outbreaks (Schepers *et al.,* 2017). The pathogen can grow at a very rapid rate after infection, and visible symptoms can appear in a matter of days. The gap between infection and the appearance of these symptoms is known as the incubation period (Leclerc *et al.*, 2014) and there is some evidence that contemporary strains of the pathogen may have shorter incubation periods than those present previously (Cooke *et al.*, 2012) which has the potential to make the disease more damaging and difficult to control.

Good control of *P. infestans* is possible but is heavily reliant on the use of fungicide treatments at relatively tight time intervals (7–10 days) which can generate potential economic and environmental burdens (Small *et al.*, 2015) and encourage the development of reduced sensitivity in the pathogen to some active ingredients.

Potato growers have access to many fungicides with chemically diverse active ingredients (a.i.s). This is crucial for successful late blight management as plant protection products can be selected to match the growth stage of the crop, or the local disease pressure. The availability of many late blight active ingredients with different modes of action (FRAC, 2018) is also key to sustainable control by helping to minimise the risk of the development of insensitivity. To assist in achieving this there are also some regulatory restrictions on the frequency of applications as well as the quantity which can be used per crop. All the fungicides which are used against late blight are applied as prophylactics (Bain, 2016), but several have some degree of mobility within the growing plant; either systemic or translaminar. This opens the possibility that they will encounter the pathogen at early infection stages and arrest its growth. This form of control is referred to as curative and is usually defined as acting after infection but before the point at which disease symptoms are visible (Ivic, 2010). It would be an unwise strategy to rely solely on curative fungicide treatments, but they form an increasingly

important component of current disease control programmes (Figure 1). Growers may know that high infection risk conditions have occurred but will not know with precision when the infections themselves have occurred. It is also a common occurrence that fungicide sprays are delayed by adverse weather conditions, meaning that the optimum time for protective treatments may be missed. Ratings for the curative efficacy of late blight fungicides are available from the EuroBlight fungicide table[1], and although these are very useful they are expressed as a categorical system and are based on expert consensus rather than a more objective trial result (using a harmonised protocol system).

An important theme in contemporary crop protection is the development of strategies and tools to achieve Integrated Crop Management (ICM). ICM involves consideration and integration of all available interventions with the aim of maximizing agricultural production, whist minimizing detrimental effects on the wider environment or on human health (Knight, 1997). The potato-late blight pathosystem is a challenging case for the application of ICM, chiefly because of the potential destructiveness of the pathogen. A control failure could have serious economic consequences, e.g. under some circumstances a little foliar blight can result in extensive tuber blight and the concomitant bacterial soft rot, and growers are therefore justifiably risk-adverse. An additional substantial barrier to the uptake of ICM for blight control is the potential management complexity of the greater diversity of fungicide inputs and timings associated with the elevated resistance of some cultivars and/or application timings dictated by high-risk weather periods.

[1] The EuroBlight Fungicide Table can be found at: http://agro.au.dk/forskning/internationale-platforme/euroblight/control-strategies/late-blight-fungicide-table/ (accessed 2019)

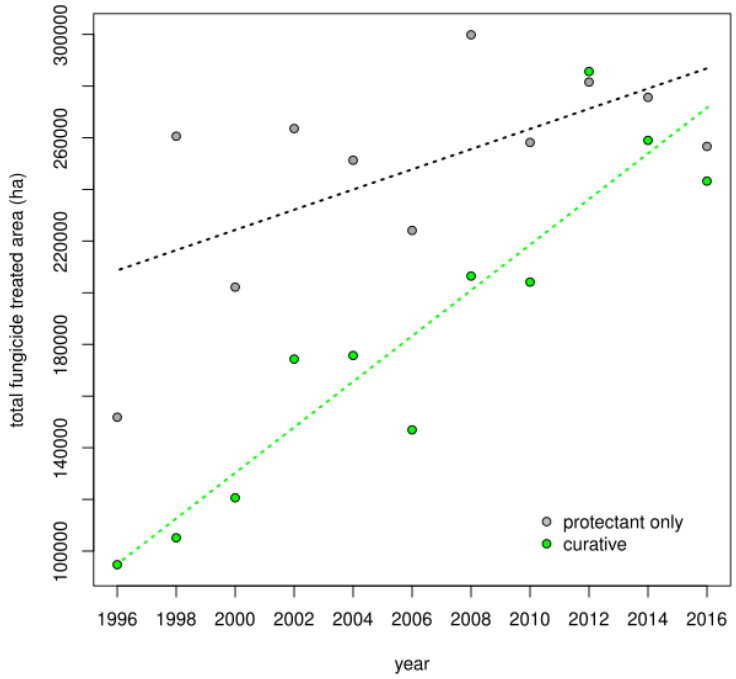

Figure 1. Use of protective and curative fungicide a.i.s on potato crops in Scotland. Data were obtained from SASA Pesticide Usage Survey Reports.

There is a need for tools to better integrate the use of curative fungicides within late blight spray programmes. Growers' comments suggest that it is perhaps not realistic to expect growers to alter the timings of their treatments, as the window for curative control is likely to be very short (see below), but it would be useful for growers to know the likely impact of a curative spray and if the use of a curative a.i. is justified. The goal of this project was to produce a simple decision aid which will help inform growers and agronomists who are considering the use of a curative spray. To achieve this goal it was necessary to gather more detailed characterisations of the curative effect than have been provided previously, and to explore how this control declines with time.

A summary of the key results from the project are detailed within this report. A more complete account, and additional data can be found in the thesis arising from it (Maloney, 2019).

3. METHODS AND KEY RESULTS

3.1 Physiological Time Developmental Model

Consideration of the environmental temperature under which a pathogen develops is an important aspect of plant disease forecasting and modelling. Plant pathogens develop at radically different rates depending on the temperatures which they experience. Previous studies have explored the epidemiological significance of temperature to *P. infestans*, but many of these have focused on infection itself rather than post-infection growth rates (Maziero *et al.*, 2009). It is well established that mode of sporangial germination is sensitive to temperature: cooler temperatures favour indirect germination, i.e. the release of motile zoospores, whilst warmer temperatures favour direct germination, i.e. the development of a germ tube (Sato, 1994). There are also some studies which consider the effect of temperature on the duration of the incubation period or on the rate at which visible symptoms develop (Cooke *et al.*, 2014). However, to our knowledge only a single study (Harrison *et al.* 1994) has made estimates of *P. infestans* development through direct observation within the incubation period (using ELISA), at two different temperatures (10 and 20 ^{o}C).

A central hypothesis of this project is that the efficacy of a curative treatment will decline as the pathogen increases its biomass (see Section 3.2). Estimates of curative control based on physical time alone may be misleading, as the pathogen's development will progress at different rates in response to different environmental temperatures. There are several different approaches to modelling the temperature sensitivity of biological growth and a number of mathematical models, some of which have been used for plant pathogens, are available in the literature (Rebaudo *et al.*, 2017). The first part of this project aimed at collecting growth data for a range of contemporary *P. infestans* isolates from Great Britain, surveying the literature for candidate equations, and establishing which provided the best description of the temperature-growth relationship.

P. infestans growth data were obtained from a sequence of experiments in 2017 using inoculated detached leaves incubated at different temperatures. A single growth cabinet was used for all runs of the experiment. Detached leaves from 6-week-old King Edward plants, a variety which has foliage susceptible to late blight infection, were used in all experimental runs. Leaflets were detached and placed within transparent plastic trays

lined with damp tissue paper. Two distinct tray categories were prepared: ‘symptom measurement’ and ‘biomass estimation’. Four *P. infestans* isolates were assessed separately within this experiment. Two were older genotypes (7_A1 and 8_A1) and the others representative of the newer, more aggressive genotypes prevalent at the time (13_A2 and 6_A1). A sporangial suspension of each was prepared by washing sporangia from King Edward leaf lesions and adjusting the concentration to 10^5 sporangia ml^{-1} after a haemocytometer count. Fresh, healthy individual leaflets were then inoculated with a single 20 µl droplet on their upper surface, avoiding main veins.

Trays were placed within a growth cabinet, set at a different temperature for each run (6, 10, 14, 18, 22, 26 or 30 ^oC). Leaflets were removed randomly from the biomass estimation trays every 12 hours. These were then frozen at -20 ^oC and later qPCR was used to quantify the amount of *P. infestans* DNA present. Leaflets from the symptom measurement trays were photographed 120, 144 and 168 hours after inoculation. The size of lesions present was measured digitally using the programme ImageJ (Rueden et al., 2017), and from this, growth rates could be determined by linear regression (Visker *et al.*, 2004).

From these data, growth of the pathogen both before and after symptoms were present could be estimated. For lesion growth rates (shown in Figure 2), all four of the isolates tested showed similar responses to temperature: no, or very little, lesion growth at the two extreme temperatures (6 and 30 ^oC) and the highest growth rates at 18 or 22 ^oC. The rank order of isolates varied at different temperatures with the 6_A1 isolate the most rapid at 6 – 18 ^oC, but this was not the case at higher temperatures.

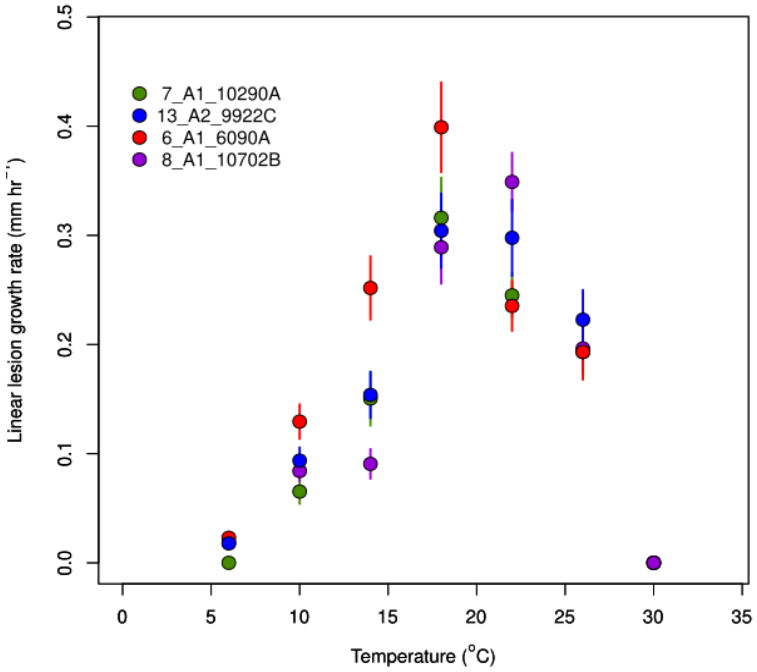

Figure 2. Visible lesion growth rates for four *P. infestans* isolates on detached King Edward leaflets, incubated at different temperatures

P. infestans DNA was detected in all samples from the biomass estimation leaflets (data not shown), but for some isolate-temperature combinations there was not a statistically significant increase over time. The majority of these cases were at the temperature extremes or for isolates which were observed to be less aggressive in the symptom measurement leaflets. Significant increases in pathogen DNA with increasing development time were obtained for the 13_A2 isolate within the temperature range 6 – 26 ^{o}C, with the most rapid accumulation at 26 ^{o}C.

A literature review of temperature-development models was conducted, and fifteen candidate models were selected. These spanned a range of complexity, and preference was given to equations which have been used to describe plant pathogen growth in previous studies. The models were assessed for how well they described the empirical temperature-growth relationship for both visible symptoms and biomass accumulation. A single isolate, belonging to the 13_A2 genotype was used to fit the parameters of the various models, as this isolate showed significant biomass accumulation at more tested temperatures than the other isolates. Generally, very simple models that assumed only a linear relationship or included temperature 'cut-off' values did not provide good

descriptions of the data either for visible symptoms (R^2 = 0.05 to 0.35) or for biomass accumulation (R^2 = 0.01 to 0.58). Quadratic equations provided good descriptions (R^2 = 0.83 for lesion growth, and R^2 = 0.73 for biomass accumulation), but did not capture the asymmetry of the relationship. The other models tested gave solid to excellent characterisations of the relationship (R^2 = 0.83 – 0.99).

The final model needed to be flexible enough to accurately describe the growth of *P. infestans* in both the incubation period and once symptoms become visible, in addition to including as few parameters as possible (too many may make implementation a more complex process). The model which best met these trade-offs was the Analytis function (Analytis, 1977), fitted curves of which are shown in Figure 3.

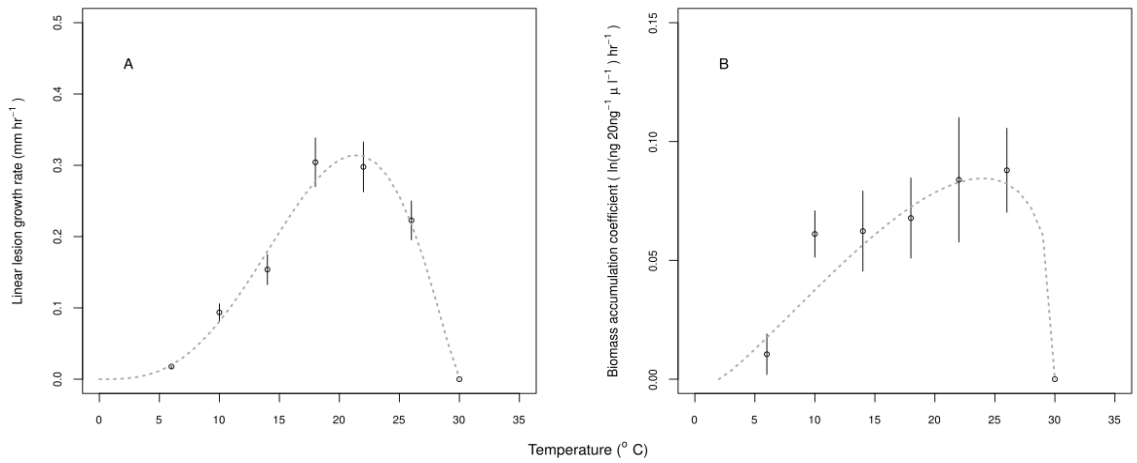

Figure 3. The final temperature-growth model fitted to (A) visible symptom development and (B) a measure of the rate of DNA accumulation during the incubation period

3.2 Curative Control Characterisation

There is some available information on the duration of the curative effect for several different a.i.s, but many of the experiments conducted previously have used long time intervals (for example 24, 48 and 72 hours disease development time). It is also rare for more than a single isolate to be assessed (Bugiani *et al.*, 2010). A series of experiments were conducted within this project to better characterise the curative effect, and how it declines with increasing disease development time. To ensure consistency, a single

fungicide, Infinito (a.i.s: propamocarb-HCl + fluopicolide) was used in the majority of experiments. This fungicide has a curative rating of ++ (good effect) in the EuroBlight fungicide table and represents the middle ranking of fungicides with curative activity.

The first set of experiments exploring curative activity involved a detached leaflet bioassay and was designed to generate a time series of curative control against disease development time. Three runs of this experiment were conducted, with a different isolate each time. Leaflets on whole potato plants (King Edward, approximately 6 weeks old) were inoculated with a 20 μl droplet of inoculum containing 10^5 sporangia ml^{-1}. The plants were then watered and sealed within large plastic bags to ensure high humidity, so that the probability of infection was maximized. Two inoculation timings took place, staggered by 12 hours. After the inoculations were complete, plants were placed in a growth room at 20 ^oC. At 3-hour intervals, between 24 and 72 hours post inoculation, 15 inoculated leaflets were detached from the plants. These were then treated with fungicide at 1.6 l ha^{-1} in 200 l water applied via an AZO compressed air sprayer. The leaflets were then sealed within Petri dishes lined with damp tissue paper, and incubated at 20 ^oC with 16 hours of light per day. After 7 days the leaflets were scored for the presence or absence of late blight lesions. Inoculated but untreated, and 'water-inoculated' leaflets were also included as controls.

The infection rate was high, i.e. between 57% and 87%, but not uniform across experiments and inoculation times so results were expressed as corrected proportions in Figure 4. To enable comparisons between different experiments the results were expressed in thermal time (a function of physical time and temperature). In general, the proportion of leaflets which went on to develop lesions increased with increasing disease development time. For the isolate of genotype 8_A1 and for one of the 13_A2 isolates (13_A2.2), early time points (up to ~ 645 accumulated degree hours) had few lesions developing (0 to 20%) indicating good disease control. However, after 700 accumulated degree hours the proportion of infected leaflets rose to 0.4 to 1.1 for the 13_A2.2 isolate and 0.6 to 1.2 for the 8_A1 isolate. In both cases the relationship between disease development time and disease incidence was significant and is best described by quadratic equations ($y = 0.63 + 0.34x – 0.57x^2$ for the 13_A2 isolate, and $y = 0.68 + 1.33x – 0.43x^2$ for the 8_A1 isolate). In contrast, the other 13_A2 isolate

(13_A2.1) tested never showed less than 50% infection at any of the time points tested, and no statistically significant relationship between development time and disease incidence could be established.

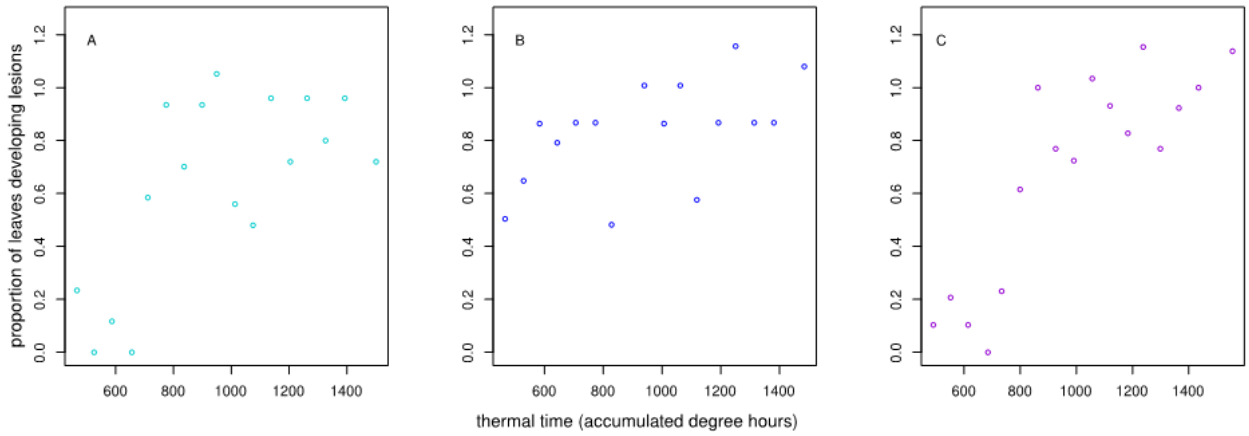

Figure 4. Proportion of leaves with blight lesions in relation to thermal time between inoculation and curative fungicide treatment. Each letter represents the results of one experimental run of the detached leaflet bioassay with a different isolate: (A) 13_A2.2, (B) 13_A2.1, and (C) 8_A1.1.

It was judged that better results than those obtained from the detached leaflet bioassay could be produced for use in the final decision aid. Infection rates were more variable than expected and the relationships which were obtained suggested that data from earlier time points were required for a full characterisation of the curative window. To address these issues an alternative methodology using leaf discs was developed. This allowed an increased sample size (64 compared with 15. Plastic assay frames were constructed which consisted of acrylic tiles (170 mm x 170 mm) with drilled 12 mm-diameter holes in an 8 by 8 grid (= 64 holes), as shown in Figure 5. Each hole within a frame held a single leaf disc, which was cut using a cork borer from 6-week old potato plants. Disc edges were covered using paraffin film so that an area of 100 mm^2 was exposed. Frames were loaded with leaf discs, and each disc was inoculated with a 20 µl droplet of *P. infestans* sporangial suspension (10^5 sporangia ml^{-1}).

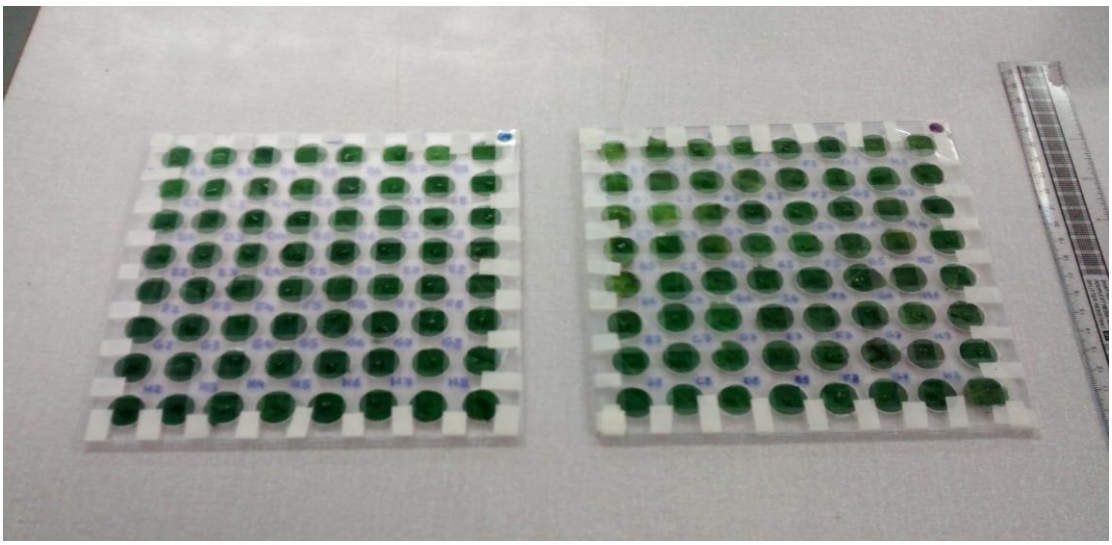

Figure 5. Potato leaf discs within the plastic assay frames.

A full experimental run generated disease development times at 4-hour intervals between 8 and 72 hours, with two staggered inoculation timings to achieve all the times. One untreated control frame, and one non-inoculated control frame for the two staggered inoculations were included in each run, as were non-inoculated control frames. Discs were treated curatively with fungicide as in the detached leaf bioassay detailed above. At each treatment time an additional six discs were frozen for *P. infestans* biomass estimation later (see below). After fungicide treatment, trays were sealed within transparent boxes lined with damp tissue paper and placed in a growth cabinet set at a constant 18 ^{o}C with a 16-hour photoperiod. After 7 days the discs were assessed for the presence of late blight lesions. Those lesions which were fully necrotic or showed abundant sporulation were classified as infected, whereas discs which displayed no symptoms or only small arrested lesions were classified as successful control.

Genomic DNA was extracted from the frozen leaflets and diluted to 20 ng μl^{-1}. The proportion of each sample which was attributable to *P. infestans* DNA was then quantified via qPCR. The assay used primers and followed methods developed by Cullen *et al.* (2001) and Lees *et al.* (2012). A serial dilution of *P. infestans* DNA standards was included, with concentrations between 20 and 2 x 10^{-5} ng $\mu l^{-1.}$ In all samples where *P. infestans* DNA was detected the quantity fell within this range.

Infection rates amongst the untreated controls were much higher in the leaf disc assay, in all but one case exceeding 84%, than the detached leaflet assay. Two isolates were tested in three runs each of the bioassay: an aggressive 13_A2 isolate and a less aggressive 7_A1 isolate, data are summarised in Figure 6. For both isolates the efficacy of curative treatments declined with increasing disease development time (Figure 6). For the 7_A1 isolate, the proportion of leaf discs infected was low, not exceeding 0.3 before 600 accumulated degree hours, after which control declined. In contrast control was less strong for the 13_A2 isolate at early time points, with the proportion of infected leaf discs ranging between 0 and 0.6 when accumulated degree hours were less than 600. The relationship between infection rates and disease development time was integral to the final model and is discussed below.

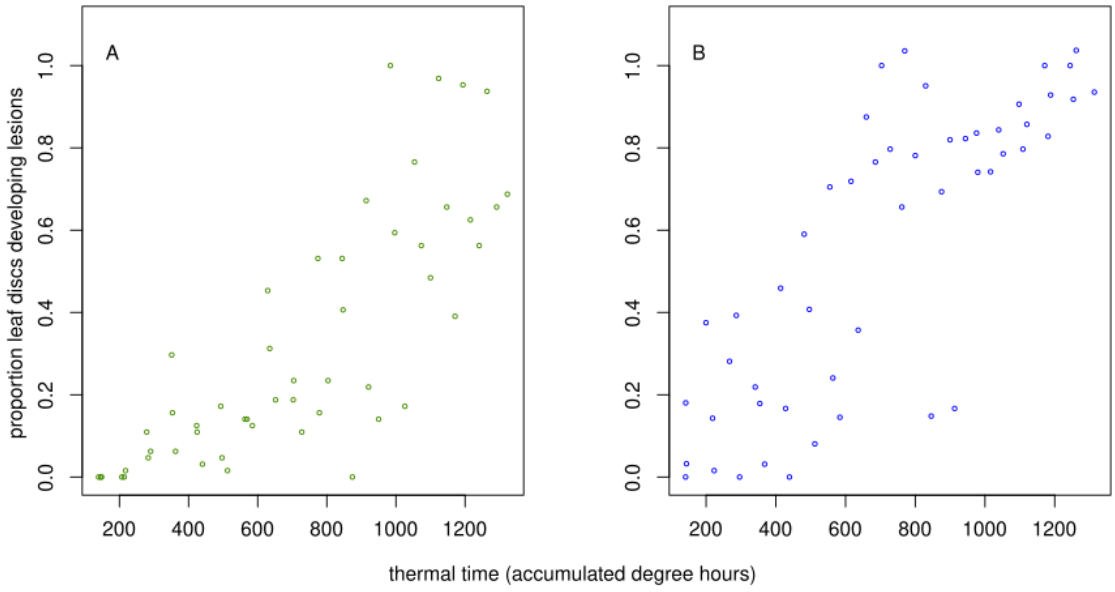

Figure 6. Proportion of leaf discs with blight lesions in relation to thermal time between inoculation and curative fungicide treatment. (A) shows three runs of the experiment with a 7_A1 isolate, and (B) shows three runs of the experiment with isolate 13_A2.1.

Target *P. infestans* DNA was detected in all samples which had been inoculated, mean values are shown in Figure 7. For both isolates there was an initial lag phase followed by an increase in the proportion of *P. infestans* DNA present with increasing thermal time. For both isolates the relationship was exponential, but DNA levels were higher at

later time points for the 13_A2 isolate. The fitted relationships, after natural log transformation were: ln(y) = 0.00244 · 10x – 4.38 ($p < 0.01$) for the 13_A2 isolate and ln(y) = 0.00172 · 10x – 5.40 ($p < 0.01$) for the 7_A1 isolate.

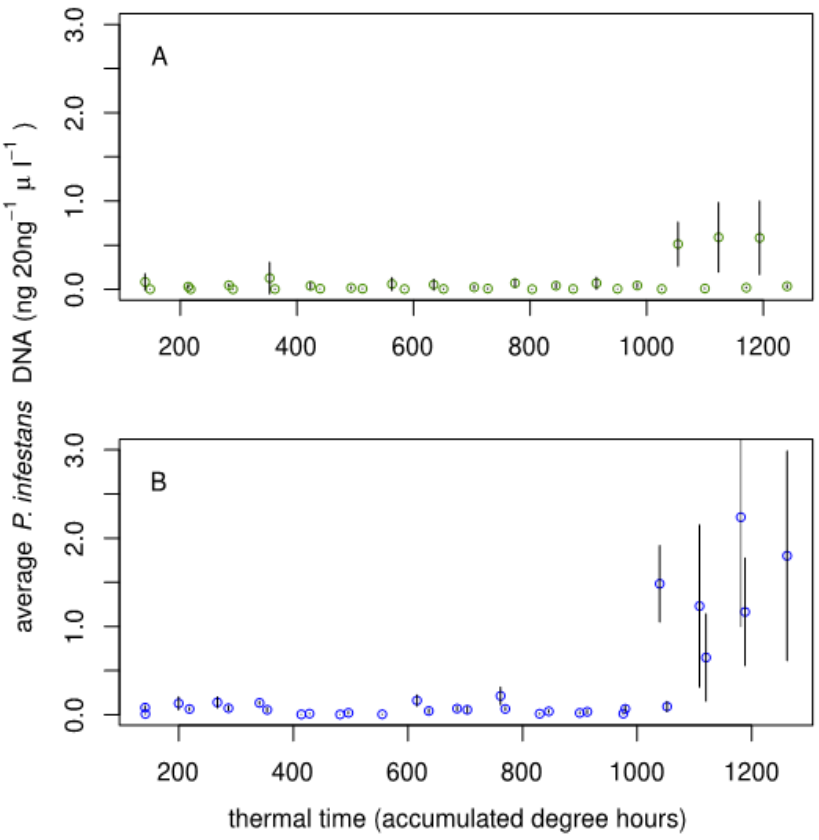

Figure 7. Mean *P. infestans* DNA levels as a proportion of total DNA extracted from leaf discs at the different incubation times (leaf disc bioassay).

A trial was also conducted to assess if a similar pattern of decreasing control with increasing disease development time was evident in a field situation. The trial was in Ayrshire during the summer of 2016 and consisted of 28 plots (4.95 m x 1.7 m) of King Edward potatoes, each plot contained two planted ridges. Plots were inoculated along one ridge in four separate areas with *P. infestans* inoculum (3.8 x 10^4 sporangia per ml) delivered via a spray bottle so that several possible infection sites were present. For logistical reasons the genotype of the isolate was unknown, but was very likely 6_A1 (C. Kennedy, personal communication). Inoculation sites were sealed using transparent

plastic bags to increase humidity and maximize the probability of infection; these were removed after 12 hours. This procedure was repeated 8 hours later along each plot's second ridge. Plots then received one of four treatment categories: curative fungicide applied 1, 2 or 3 days after inoculation, or untreated. The fungicide was propamocarb-HCl + fluopicolide at 1.6 l ha^{-1} in a 200 l water volume from a backpack AZO sprayer. There were 6 replicate plots per treatment category, two types of control plot were used: inoculated but untreated and non-inoculated (to assess background infection levels). After 7 days the number of late blight lesions per compound leaf at each of the inoculation sites was assessed.

Data from the field trial are shown in Figure 8. Lesions were present at all inoculation sites, indicating that curative treatment did not completely contain infection, even at the shortest disease development time. However, there was a statistically significant relationship ($p < 0.01$; $\ln(y + 1) = 1.06 \times 10^{-3} x + 0.06$) between mean lesion counts per leaf and disease development time.

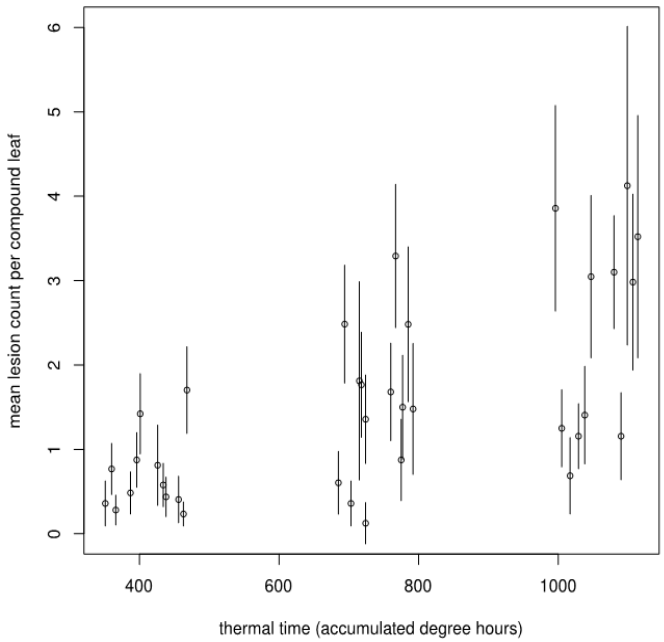

Figure 8. Mean lesion count in relation to development time between inoculation and curative treatment (field trial). The timing of inoculation and fungicide treatment was recorded at the plot level, and it was therefore possible to assign a thermal time value for development to each plot, the three clusters seen above correspond to the three possible curative treatment times (days 1, 2, or 3).

The results from both the laboratory-based assays and the field trial indicated that curative control declines with increasing disease development time, and that there is a ‘critical time window’ for this control after which there is little impact from a curative treatment. Further field trials using very similar methods were conducted as validation of the decision aid and are detailed in Section 3.4.

The leaf disc bioassay results provided a more detailed description of the relationship between disease control and thermal time than is usually reported for experiments of this nature and therefore were selected as the basis for modelling the breakdown in curative control with increasing time. Although there were differences between the two isolates tested, the more aggressive 13_A2 isolate was used as the basis for the decision aid as this probably represents a ‘worst case scenario’ for the duration of the curative window. The relationship between curative control and disease development time is analogous to a dose-response relationship. These relationships are often modelled using sigmoid curves, and a logistic function provided a very good fit to the data from the leaf disc bioassay. This formed the basis of the decision aid and is discussed in further detail in Section 3.4.

3.3 Modifying Factors

To date, very few studies have assessed factors that might modify the duration of the curative control window. It is very likely that the decline in curative control, as the hours pass after infection, is linked to increased pathogen biomass at sites of infection, and so any factor that alters the rate at which *P. infestans* grows and expands into new plant tissue will have an impact on the likely level of curative control. There are many such factors, but the most important are likely to be the post-infection temperature, the aggressiveness of the infecting strain, and the resistance of the cultivar to the progression of infection. There are studies (Bødker and Nielsen, 2000; Genet *et al.*, 2001) which link optimal temperatures for pathogen development with shorter curative control windows, but no other factors have been systematically assessed.

Temperature was included in the decision aid model using the relationship established in Section 3.1. Although experiments conducted in this project (Section 3.2 and

additional experiments not shown) indicated that some isolates responded differently to curative treatment at the same disease development times, only data for the most aggressive isolate tested was carried over to the final decision aid.

Varietal resistance to *P. infestans*, i.e. resistance in terms of rate of leaf tissue colonisation, was considered a key modifying factor in need of characterisation. Plant cultivars differ in their susceptibility to specific pests and pathogens, and this is governed by a number of mechanisms, not all of which are well understood. In potato, late blight incidence and severity can differ between varieties, although the most resistant varieties are often not widely grown as they lack other desirable agronomic traits. Blight resistance ratings from variety trials are available, and whilst these are very useful it is not known if they directly relate to the speed of leaf tissue colonization, or if this varietal attribute can modify the curative activity of fungicides.

A set of experiments was conducted to establish if cultivars with different resistance ratings to leaf blight differed in both the size and duration of the curative effect under field conditions. The experiments were designed to mimic natural infection and took advantage of the late blight field trials conducted annually at SRUC's Auchincruive site. These trials involve medium-sized potato plots (typically 3.4 x 8.0 m) in which epidemics are allowed to develop. As a result, high inoculum pressure can be found within the immediate trial area once the season is advanced.

Small potato plants were propagated from classified seed tubers in small pots (5 cm diameter) kept in a polytunnel for approximately 7 weeks. The experiment was run several times, and due to logistical reasons, each run included slightly different combinations of treatment timings and cultivars, but all followed a common protocol. All experiments included plants of the cultivars King Edward and Cara (with foliar resistance ratings of 3 and 5 respectively), but some also included the more resistant Sarpo Mira (7). Weather conditions were monitored until a Smith Period (the Hutton Criteria were not available when the experiments were conducted) or a 'near miss' was recorded. At this time the small plants were then transported to the field site and left exposed to airborne inoculum for approximately 2 hours. After this exposure time the plants were immediately sealed within transparent plastic sheeting to increase the

humidity and maximize the probability of infection. They were then incubated within a growth room set for a 16-hour photoperiod and at a constant 18 ^{o}C. All experimental runs included control plants which were not exposed to inoculum, to check for background contamination. Selected plants were removed from the growth room during the *P. infestans'* incubation period and treated curatively with propamocarb-HCl + fluopicolide at 1.6 l in a 200 litre water volume per ha. In the first two runs of the experiment a single timing was used (53 hours after exposure in the first run, and 43 hours after in the second) but in the third run three timings were used on separate batches of plants. After fungicide treatment plants were returned to the growth room and after 7 days the number of late blight lesions which had developed per plant was assessed.

Mean lesion count data are shown in Figures 9 – 11. In the first run there were statistically significant differences between the treated and the untreated plants ($p < 0.01$), and between Cara and King Edward plants ($p < 0.01$). Fungicide treatment resulted in an average reduction in lesion number of 44% for King Edward plants and 53% for Cara plants; both reductions were statistically significant. In the second run of the experiment infection rates were lower, but significant differences between the cultivars were still present. Statistical analysis using anova indicated that both fungicide treatment ($p < 0.01$) and cultivar ($p < 0.01$) were important sources of variation. The number of lesions developing on the untreated control plants followed the rank order of resistance ratings (most on King Edward, fewer on Cara, fewest on Sarpo Mira). Interestingly, curative fungicide treatment decreased the number of lesions developing for both Cara ($p < 0.01$) and Sarpo Mira ($p = 0.03$), the two more resistant cultivars, but not for King Edward plants ($p = 0.99$). The final run of the experiment included three separate fungicide treatment times and two cultivars (Cara and King Edward). For the susceptible King Edward plants only the earliest treatment time resulted in a significantly lower number of lesions developing compared with untreated ($p = 0.01$), but for the Cara plants both the first and second treatment timings were significantly lower than the corresponding untreated plants ($p < 0.01$ and $p = 0.04$ respectively).

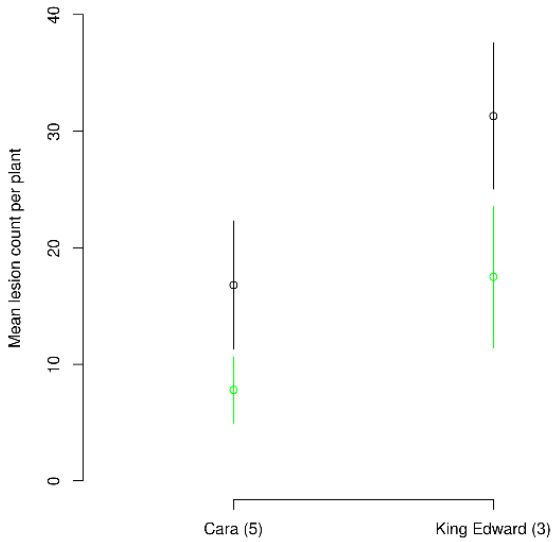

Figure 9. Natural inoculation experiment, 1st run. Mean lesion count on small plants in relation to cultivar resistance level and Infinito treatment 53 hours after inoculation (1008 degree hours). Green represents treated and black untreated plants.

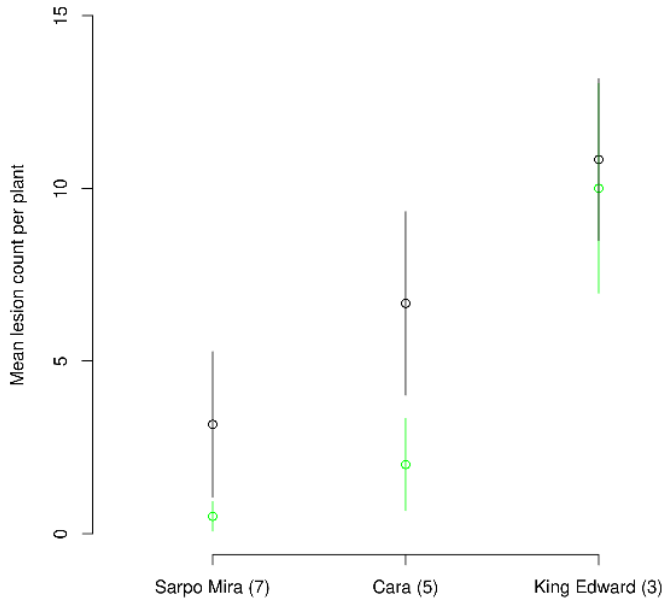

Figure 10. Natural inoculation experiment, 2nd run. Mean lesion count on small plants in relation to cultivar resistance level and Infinito treatment 43 hours after inoculation (846 degree hours). Green represents treated and black untreated plants.

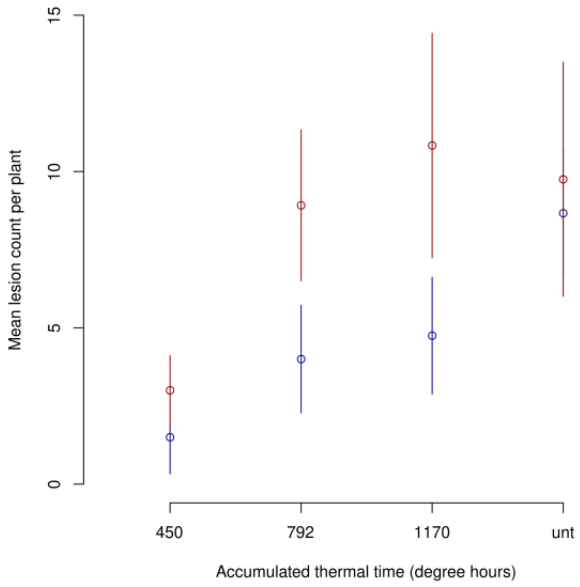

Figure 11. Natural inoculation experiment, 3rd run. Mean lesion count on small plants in relation to cultivar resistance level and Infinito treatment at three timings after inoculation: 25 hours (450 degree hours), 44 hours (792 degree hours) and 65 hours (1170 degree hours). Blue represents Cara, red King Edward. 'unt' denotes untreated plants.

If the 1 to 9 resistance ratings, calculated from independent variety trials[2], are a good guide to the rates at which *P. infestans* colonizes host tissue, they could be a powerful and useful predictor of how much the curative effect is modified by the level of host resistance. However, it is possible that other resistance mechanisms are involved. A selection of cultivars with different late blight resistance ratings were evaluated in the laboratory for both the rates of visible symptom development and the build-up of *P. infestans*-specific DNA within the incubation period. It was not possible to test a large number of cultivars on a single occasion but instead a sequence of experiments was conducted with three cultivars assessed each time. A reference cultivar, King Edward,

[2] These can be found at: http://varieties.ahdb.org.uk/ (accessed 2019)

was included as a standard in each run. Two sequences were conducted over consecutive years, with more detailed measurements taken in the second year.

Plants of the selected cultivars were grown for 6 weeks in a glasshouse. Leaflets were harvested from the plants and labelled with small stickers attached to their petioles. The leaflets were then distributed between transparent plastic trays lined with damp tissue paper. Leaflets were then inoculated with a 20 µl droplet of *P. infestans* inoculum containing a sporangial suspension of 10^5 sporangia per ml. The most aggressive isolate (13_A2.1) from previous experiments within the project was used for all experiments. Once all leaflets within a tray had been inoculated, the tray was sealed inside a transparent plastic bag and placed in a growth cabinet set at a constant 18 ^oC with a photoperiod of 16 hours. For the experiments completed in the first year, trays were opened at 144 hours post inoculation and the size of lesions present was determined using the digital photograph technique. In the second year, the trays were opened at 120, 144 and 168 hours post inoculation and the sizes of lesions present were measured in the same way. In total 32 leaflets were assessed for symptoms from each cultivar. The three timings in the second year experiment allowed growth rates to be determined by simple linear regression. Additionally, in the second year some leaflets (six per timing) of each cultivar were frozen at time points during the incubation period (12 – 72 hours) and qPCR was later used to measure pathogen growth before visible symptoms became visible.

In order to compare pathogen growth across the different experiments, a linear mixed model was used with cultivar as a fixed effect. Estimated means are summarised in Figure 12 for 2016, and in Figure 13 for 2017. For the first year's set of experiments there was a statistically significant effect of cultivar and the rank order of cultivars for late blight lesion size was as expected from their resistance ratings. A larger number of cultivars were tested the following year, and again there was a large and significant effect ($p < 0.01$) from cultivar on lesion growth rates. However, some of the tested cultivars did not meet the rank order expected: the mean growth rate for Charlotte was lower than expected, and the growth rate for Inca Bella significantly higher. The correlation between observed growth rates and foliar late blight resistance rating was strong ($R^2 = 0.57$). However, it may be worth revaluating the ratings for the cultivars

which did not conform to the trend, to determine if their ratings have remained stable in the face of late blight population changes.

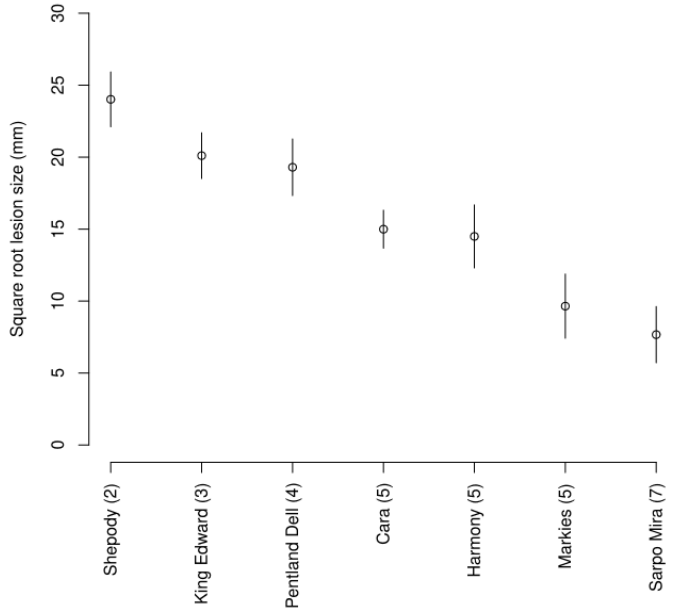

Figure 12. Late blight lesion size 144 hours after inoculation of detached leaflets in relation to documented cultivar resistance ratings (first year experiment)

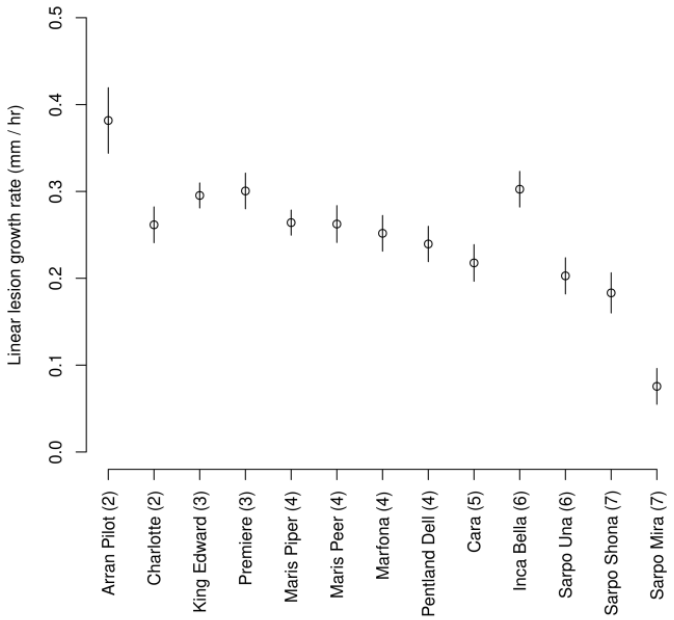

Figure 13. Late blight lesion growth rates on detached leaflets in relation to documented cultivar resistance levels (second year experiment).

In all samples taken within the incubation period, *P. infestans*-specific DNA could be detected. Higher levels of pathogen DNA were associated with later time points, and a growth rate could be determined by simple linear regression of transformed DNA levels on time, as shown in Figure 14. The growth rates obtained were not as different as expected from the published foliar resistance ratings, nor did they correlate well the visible symptom growth rate.

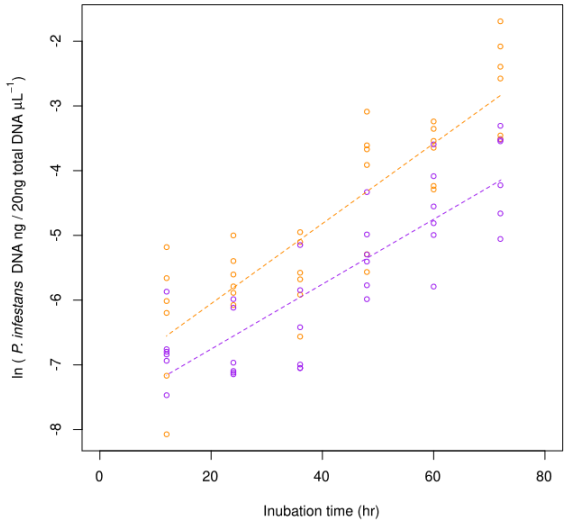

Figure 14. Increase in *P. infestans* DNA with time after infection in the cultivars Sarpo Mira (purple)(resistance rating 7) and Arran Pilot (orange)(resistance rating 2).

3.4 Formulation and Validation of the Decision Aid

The experiments detailed above were used as the basis of the final decision aid, both to decide which factors were of the greatest importance and also to establish appropriate values for its parameters. The final decision aid should inform growers and agronomists, when they are considering a scheduled fungicide treatment, if the use of a product with curative activity is appropriate. It is hoped that the decision aid will complement existing decision support systems and weather-based warnings. A schematic of the decision aid is shown in Figure 15. It consists of two linked models: (i) a pathogen development model and (ii) a curative activity model. The decision aid works by assuming that at the end of a high-risk weather period (i.e. a Hutton Period) late blight infections begin developing within potato foliage, and that the rate at which these

progress depends on the temperature. A provisional term, for the effect of cultivar resistance was also included, and this is discussed further below.

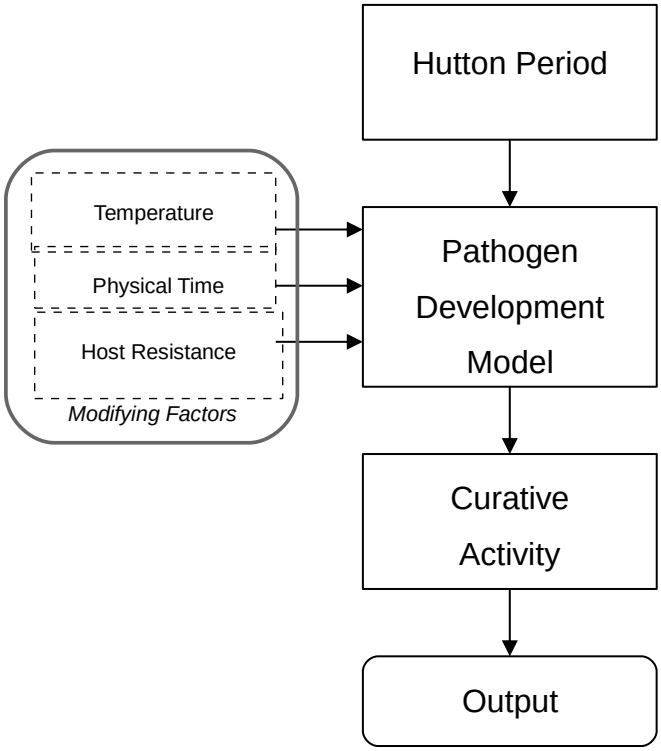

Figure 15. Overview of the decision aid.

The first part of the decision aid is the pathogen development model. This is based on the relationship between temperature and development in the leaf disc bioassays. The decision aid is designed to be conservative, as the costs of failing to control late blight infection can be very high. With this in mind, results for the most aggressive isolate assessed (of 13_A2 genotype) were used to set the model parameters. The model is the Analytis function with parameters derived from the temperature experiments described in Section 3.1. As input it requires a time step (e.g. an hour) and the mean temperature during this time. These data could come from a local weather station or from in-field data loggers. The model outputs a value for expected development within the interval. An additional term was included to adjust for cultivar resistance.

Once the pathogen development model has been provided with the temperature profile, the expected development from each time interval can be summed thereby generating

a projection of the development of infections. This can be thought of as the expected amount of *P. infestans* biomass present at an infection site.

The second model in the decision aid predicts the likely impact of a curative treatment at a specified time. It requires only the output of the pathogen development model as input. The model is based on the relationship observed between curative control and disease development from the leaf disc assay, and thus a value between 0 and 1 is produced as output. This can be thought of as the probability that the curative treatment fails to control the infection. Some example projections at different fixed temperatures, i.e. 6, 10, 14, 18, 22, 26 and 30 ºC, are shown in Figure 16.

It is not appropriate to use the raw output of the curative activity model as the advice to growers, as it is not likely that curative control can be predicted this specifically. The exact form of the output should be tailored to the needs of end users, but for illustrative purposes an example categorical scale is used here. This scale involves five categories which are easy to interpret, describe the probable effect of curative treatment and range from ‘very likely effective’ through to ‘very unlikely effective’ (Figure 17).

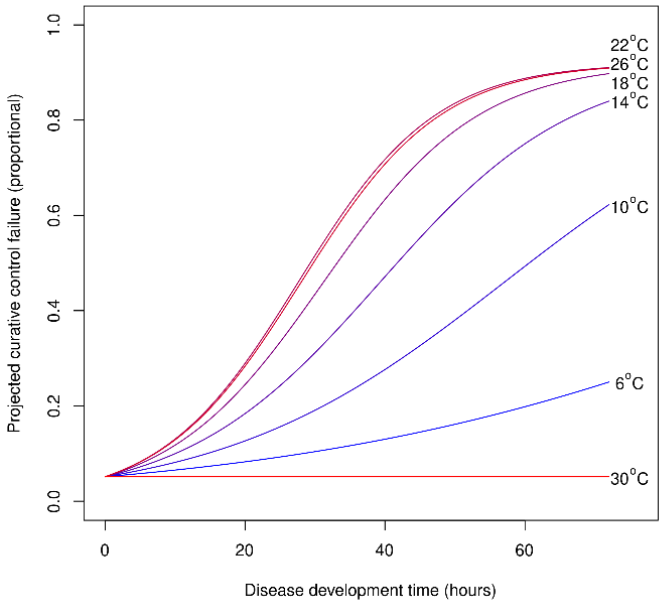

Figure 16. Projected curative control failure in relation to fixed temperature as predicted by the decision aid. Temperatures ranged from 6 to 30 ºC. Blue to red scale indicates low to high temperature. Note that the model predicts no growth at 30ºC, which corresponds to the horizontal red line.

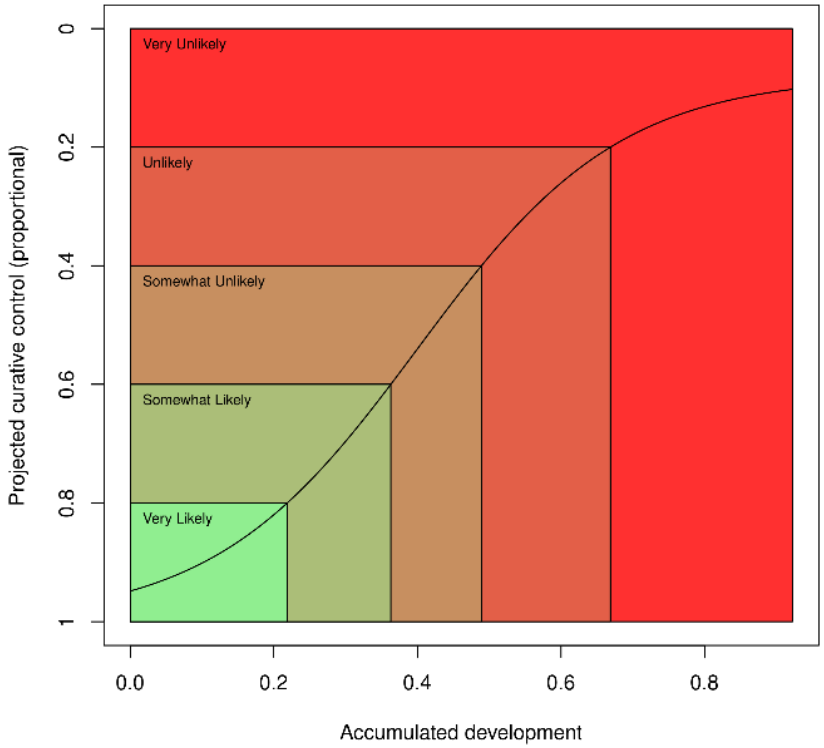

Figure 17. Example output categories for the decision aid. The x-axis represents projected biomass accumulation and is in the same units as the DNA quanity estimates from the qPCR assay (ln(ng 20 ng-1 μl^{-1}). The categories ('very likely', through to 'very unlikely') represent how effective a curative treatment will be in controlling infections within the incubation period.

The parameters used in the decision aid came predominantly from experiments with controlled conditions, so it was necessary to validate the aid under field conditions similar to those of its intended final use. To accomplish this, field trials using similar methods to those detailed in Section 3.2, were conducted at two geographically separate sites 80 miles apart, in Midlothian and South Ayrshire.

The structure of the trial was very similar at each of the two sites. Each trial consisted of 40 plots in four blocks. As before, control plots consisted of inoculated but untreated, and non-inoculated plots. Plots were of one of two varieties with contrasting foliar resistances: Cara (5) or King Edward (3). Artificial inoculation and staggered fungicide

treatments at 1, 2, or 3 days post inoculation took place using the same methodology as in Section 3.2 (product, dose rates, etc.), but in this case the genotype of the isolate was known: the aggressive 13_A2 isolate referred to in previous sections. The staggering of inoculations and fungicide treatment times generated a range of possible disease development times within the pathogen's incubation period at the time of curative treatment. The method of inoculation was as before: 2.6 ml of sporangial inoculum (3.85×10^5 sporangia ml^{-1}) delivered from a spray bottle across the foliage of two bunched stems at 5 sites per plot, generating a scatter of possible infection sites within the canopy. After 7 days the number of lesions which developed per compound leaf was assessed.

Temperature data from local Met Office stations at the two sites were used in conjunction with the recorded inoculation and treatment times to generate a post-inoculation temperature schedule for each plot. This was then used as raw input for the decision aid, allowing the assignment of a categorical prediction for curative control to each plot.

At both sites there were large and statistically significant differences between the number of lesions developing in untreated control plots at the two inoculation times (am and pm) and between the different cultivars (Cara and King Edward). Each of the two inoculation batches was treated as a separate event for later analysis. At one of the sites (Midlothian, shown in Figure 18) there was a strong relationship between disease development time before curative treatment and the number of lesions which developed at both inoculation times ($R^2 = 0.87$ and 0.69, AM and PM respectively) with lesion count increasing with longer development time. There was not a statistically significant relationship at the other site (data not shown).

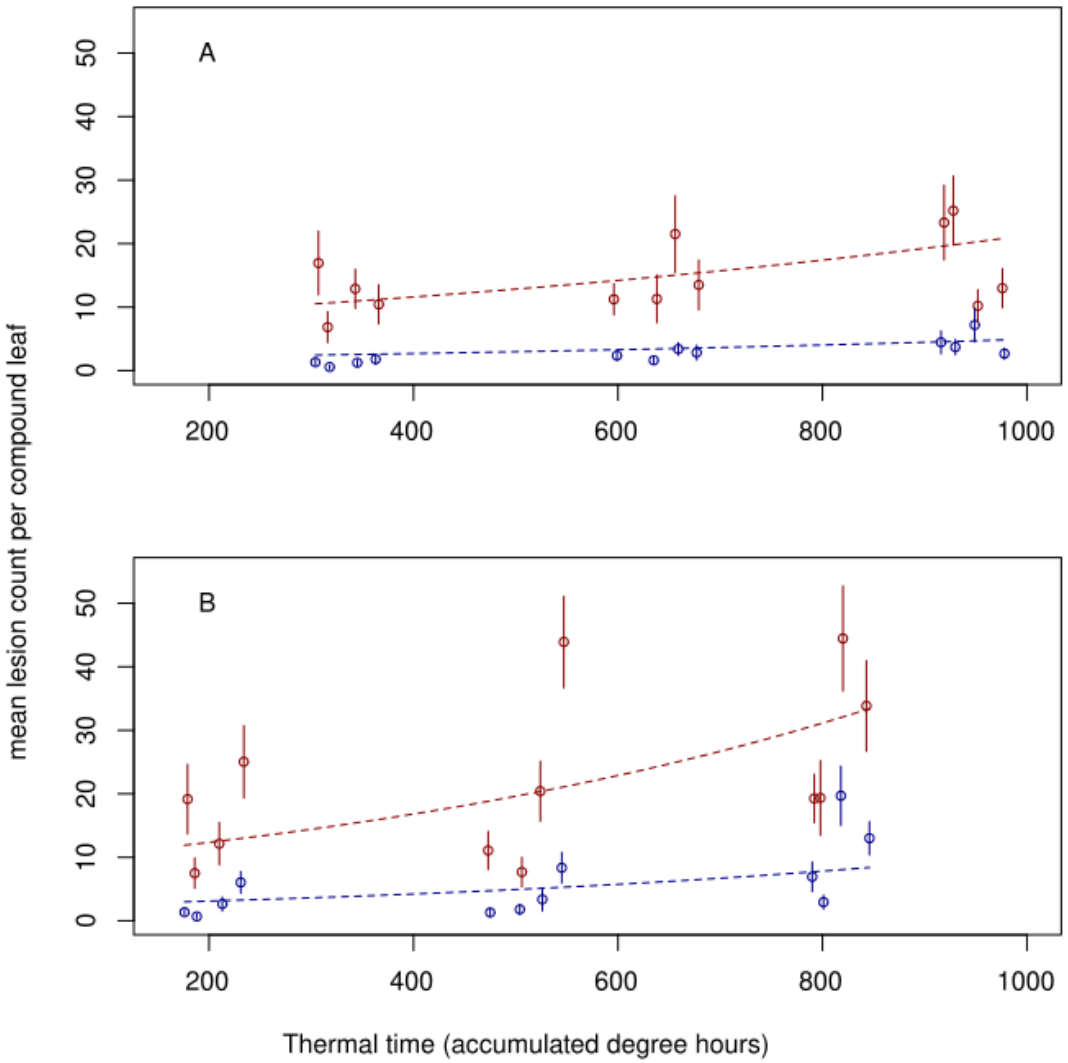

Figure 18. Mean lesion count in relation to Infinito treatment at three timings after inoculation and cultivar resistance level (Validation field trial in Mid Lothian). Red denotes King Edward (resistance rating 3) plants, and blue Cara (5). A and B are separate inoculum batches. All plots represented in the figure were treated with Infinito.

In order to test the decision aid a value of 'observed control' was obtained for each of the treated plots by calculating the ratio of mean lesion count in that plot and the mean lesion count in the untreated control plots. This was then compared with the predicted control proportion generated by the decision aid; see Figure 19 for a summary. For both cultivars at the Midlothian site there was a significant relationship between predicted and observed values ($p = 0.03$ for King Edward, and $p < 0.01$ for Cara).

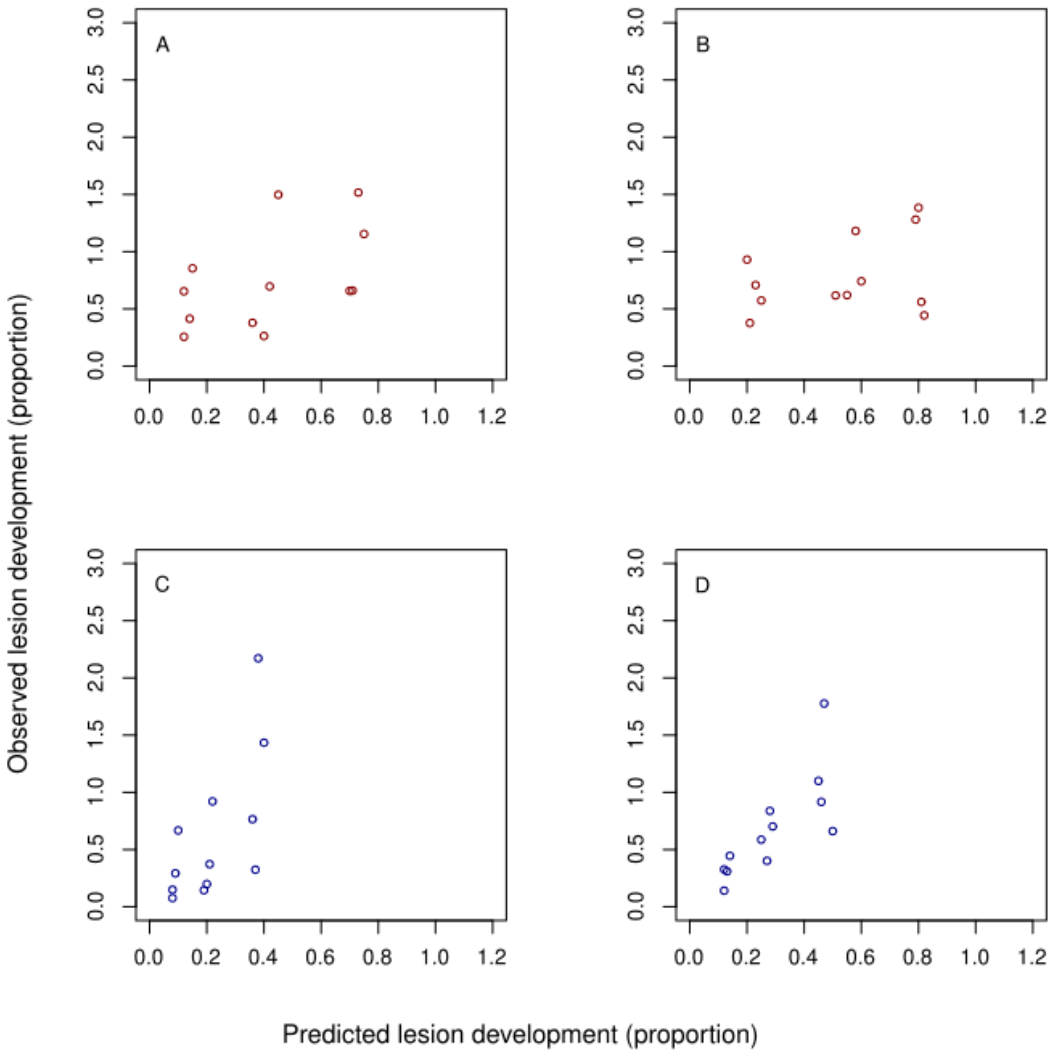

Figure 19. Correlations between observed and predicted infection in relation to timing of curative fungicide application in the validation field trial (Mid Lothian). Red denotes King Edward plants, and blue Cara. A & C were afternoon-inoculated, and B & D morning-inoculated.

The accuracy of the decision aid is summarised in Table 1, which compares the predicted categorical call of the decision aid with the level of curative control that was observed. The categories, which ran from 'very likely effective' through to 'very unlikely effective', were numbered 1 through 5, and the difference between observed and predicted was used as an indication of the 'error' of the decision aid. For example, if the aid predicted a control level of between 100 – 80% ('very likely effective') but the observed control level was between 60 – 79% an error of '+1' would be assigned. If both validation trials are included the decision aid predicted the correct category, or was within one category of the correct value, in 57% of cases. However, the data from the

Auchincruive validation trial were not really suitable because overall there was not a statistically significant relationship between disease development time before curative treatment and the number of lesions which developed. Furthermore, lesion count did not increase with longer development time. Lesion count was much lower in all plots at the Auchincruive site, and this may have been a factor in the lack of a relationship. Using only the Midlothian data, the percentage successful prediction increased from 57% to 69% (range 58 to 92%). At this site the best performance was for Cara plants inoculated in the morning, with 92% of cases being correct or within one category. There was a consistent bias for over-estimating curative control (+1 or + 2 error), indicating that the aid may need further refinement. It should be noted that the categories and their boundaries were chosen for illustrative purposes only and that using fewer categories would most likely improve the aid's accuracy.

Table 1. Accuracy of the decision aid in the validation trials.

			Decision aid error (observed call – predicted call)								
			-4	-3	-2	-1	0	+1	+2	+3	+4
Location	Cultivar	Inoc. Cat.	Proportion of cases								
AU	KE	Morn.	0.00	0.08	0.00	0.08	0.5	0.00	0.25	0.08	0.00
		Even.	0.00	0.08	0.08	0.08	0.33	0.08	0.08	0.16	0.08
	Ca	Morn.	0.00	0.00	0.00	0.25	0.33	0.17	0.25	0.00	0.00
		Even.	0.00	0.00	0.00	0.00	0.25	0.00	0.33	0.08	0.08
ML	KE	Morn.	0.00	0.00	0.17	0.00	0.25	0.33	0.17	0.08	0.00
		Even.	0.00	0.00	0.00	0.08	0.25	0.33	0.17	0.08	0.08
	Ca	Morn.	0.00	0.00	0.00	0.17	0.58	0.17	0.08	0.00	0.00
		Even.	0.00	0.00	0.17	0.17	0.25	0.17	0.17	0.08	0.00
		Mean	0.00	0.02	0.03	0.10	0.30	0.17	0.24	0.10	0.03

5. CONCLUSIONS

Methods have been developed to quantify curative activity more comprehensively than have previously been reported.

A more accurate prediction of the curative control efficacy of Infinito was obtained when it was based on a model of pathogen development post-infection (incorporating air temperature), compared with only considering chronological time between infection and fungicide treatment.

In lab and field experiments, curative activity for Infinito (rated ++ for curative activity on a 0 to +++ scale) declined rapidly with increased pathogen development time post-infection.

The decision aid indicated that the impact of post-infection temperature on curative efficacy was considerably greater over the temperature range 6 to 18 ^{o}C than 18 to 26 ^{o}C.

Factors such as varietal resistance can be modifiers of curative activity. Field experiments, in which natural infection was simulated, demonstrated that elevated cultivar resistance increased the magnitude of the curative effect and/or extended the curative window. More research is needed to develop complete characterisations, but factors which slow the rate of tissue colonization appear to extend the curative control window.

A strong relationship between published varietal foliar blight resistance ratings (AHDB Potato Variety Database) and the rates at which *P. infestans* colonised leaf tissue was demonstrated across 16 cultivars. A few cultivars fell outside the expected rank order. The relationship was less strong for pre-symptomatic growth of the pathogen.

A simple decision aid for growers and agronomists, which can be used as a guide as to whether or not a curative a.i. is required for any **scheduled** fungicide application, was developed. Data for the most aggressive 13_A2 isolate were used as the basis of the decision aid.

The accuracy of the decision aid was only tested in two validation trials and the dataset for the Auchincruive trial was really not suitable for validation. For the Midlothian trial the decision aid predicted the correct category, or was within one category of the correct value, in between 58% and 92% of cases depending on cultivar and the time of day inoculated. This variation, and the weak dataset from the Auchincruive validation trial, identifies the need for greater validation under field conditions. SRUC already has some datasets from commercially funded investigations of curative activity in the field that, with the permission of sponsors, could be used to validate the decision aid further.

The decision aid has the potential to be a very useful IPM add-on tool to existing blight control systems, in addition to reducing fungicide input costs. However, there was a consistent bias for over-estimating curative control, indicating that the aid may need further refinement.

6. DISCUSSION

This report presents valuable new data on curative fungicide performance and factors that can influence it. Firstly, the characterisation of the curative effect used shorter time steps and larger sample sizes than are typically used. Often, studies that model curative activity use very simple rules, such as a single threshold with full control before and no control after. The logistic relationship present here may be useful to those wishing to study curative activity with other fungicides or pathogens. Also, modifying factors of curative activity have not received extensive attention to date. This project provides evidence that both pathogen isolate lineage and cultivar resistance can act as modifying factors. This project focused on the impact of cultivar resistance because the leaf blight resistance rating of the crop is known by the user of the decision aid whereas real-time information on the aggressiveness of the local *P. infestans* population is difficult to ascertain quickly enough with current technology and practices. It is possible that other modifying factors studied in even less detail here, such as the level of disease pressure or inoculum density, also play an important role.

The use of qPCR to monitor *P. infestans* growth within the incubation period has been performed before but previous studies have used fewer sampling times (see for

example Halim *et al*., 2007). Additionally, the observations of the growth of different isolates at different temperatures have been made for visible symptoms, but not for a measurement of growth before symptoms have become visible. The data obtained for *P. infestans* DNA accumulation in different potato cultivars included a much larger range of cultivars than previous studies. The incubation period is a critical growth phase, which clearly has relevance to the efficiency of curative fungicides but may also be of more general interest to other researchers.

Many of the 15 pathogen growth models that were evaluated performed very well at describing the temperature-growth relationship in *P. infestans*. It is interesting that there were slight differences between visible lesion growth, and biomass accumulation within the incubation period. These results should be interpreted cautiously as a relatively small number of isolates were tested. However, previous studies have suggested that 'internal' and 'external' life history stages of late blight have different optimum temperatures.

Fungicides with curative activity are widely used against late blight in the United Kingdom, and they can be an important component of treatment programmes (Figure 1). The decision support systems that include curative activity generally do so as a simple fixed parameter (Hadders, 1997). The decision aid specified by this project provides a useful guide to the use of curative fungicides, particularly as it is parametrized from empirical data derived from contemporary *P. infestans* populations.

The decision aid consists of two linked models: (i) a pathogen development model and (ii) a curative activity model. The decision aid works by assuming that at the end of a high-risk weather period (i.e. a Hutton Period) late blight infections begin developing within potato foliage, and that the rate at which these progress depends on the temperature. A provisional term, for the modifying effect of cultivar resistance was also included. The decision aid inputs are 1) the time at which Hutton Criteria are met, 2) hourly air temperature after this time and 3) the foliar resistance rating of the cultivar being grown.

The decision aid requires periodic checks that foliar resistance ratings are accurate and that the aggressiveness of the *P. infestans* strain used to parameterize the model remains representative of the GB population.

7. PRACTICAL RECOMMENDATIONS

More validation field experiments should be conducted, and/or other existing datasets used, before the decision aid is put into widespread use. Such validation is likely to lead to model refinements.

Potential users should be consulted over the exact form of the output from the decision aid so that it matches their requirements.

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