DEVELOPMENT OF A DECISION AID TO SUPPORT THE USE OF CURATIVE LATE BLIGHT FUNGICIDES

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Summary: Curative fungicides, which act within a pathogen's incubation period, are an important component of many late blight control programs. Growers and agronomists would benefit from a simple decision aid to provide justification for the use of curative products. This study aims to ultimately produce such an aid, and interim results which form the basis of its parameters are presented here. Data from glasshouse bioassays with a representative curative fungicide (fluopicolide + propamocarb) and a susceptible potato variety (King Edward) suggest that curative control rapidly declines from 20 hours post inoculation with an aggressive isolate, and from 56 hours with a less aggressive isolate. Further results from bioassays and field trials confirm that post infection temperatures increase the rate of pathogen development up until an optimum, after which it falls, and that varieties with higher resistance ratings increase the time period for curative control.

INTRODUCTION

Late blight (*Phytophthora infestans*) remains a persistent threat to potato crops, particularly in mild, wet climates such as that of northern Britain. Late blight's potential to cause explosive and highly destructive epidemics necessitates the use of routine fungicide applications, usually at 7 to 10 day intervals (Hansen *et al.*, 2016). There is a well developed market for late blight fungicides, and growers and agronomists have access to many active ingredients (a.i.s) with differing modes of action and formulations. A crucial element of a blight spray program is the selection of the most appropriate product based on considerations such as the growth stage of the crop, the local disease pressure, and the prevailing climatic conditions.

Whilst all late blight fungicides are applied with the aim of preventing infection within the crop, several of the a.i.s in widespread use have some mobility *in planta* and have the potential to give control in the pathogen's early developmental stages. This curative activity is commonly referred to as 'kickback' (Genet *et al*., 2001), and can be an important component of a late blight spray program, especially when there is a high probability that infection has taken place due to high risk weather conditions.

The toolkit that can be used to inform the choice of late blight control product is expanding: e.g. fungicide efficacies and characteristics are summarized by the EuroBlight table (Bain, 2016), the most up to date version of which is on the EuroBlight website; weather alerts are issued based on infection cycle criteria (Hutton Criteria); and outbreak warnings are provided by AHDB's 'Fight Against Blight' outbreak mapping service. The aim of this study is to add to this toolkit by producing a simple decision aid for the optimization of curative fungicide use against late blight.

The rapid life cycle of *P. infestans* coupled with the fact that curative fungicides are only effective within the pathogen's incubation period (which in some circumstances can be as little as 3 days) highlights the importance of the fungicide application timings. Treatments outside of a short 'curative window' are unlikely to provide control. Previous studies have explored the decline in the efficacy of curative applications with increasing disease development time (Johnson *et al*., 2000), but there is limited information on how this curative window can be modified by additional factors such as pathogen genotype (Cooke *et al*., 2012) or varietal resistance.

In order for the decision aid to be useful it should be based on empirical data, and to this end a series of bioassays and field trials have been conducted in order to explore the relationship between the duration of disease development and the control given by curative fungicide treatment. Experiments have also been conducted on factors thought likely to modify the curative window, to assess the value of their inclusion within the decision aid. Of particular importance is the developmental temperature because this is known to significantly alter *P. infestans* growth rates (Shakya *et al*., 2015) and it is intended that the temperature profile during the incubation period will be one of the inputs for the decision aid.

MATERIALS AND METHODS

A single representative fungicide product was used in experiments where infected material was treated curatively: Infinito (Bayer CropScience; 62.5 g fluopicolide + 625 g propamocarb l^{-1}). This fungicide is rated by EuroBlight as having 'good (++)' curative activity (Bain, 2016). In all experiments detailed below, a 'curative treatment' refers to a single application of fluopicolide + propamocarb at the manufacturer's recommended label rate, i.e. 1.6 l product in 200 l water ha^{-1}, using an AZO compressed air, precision sprayer.

Curative threshold bioassays

Foliage was collected from 7-week-old, glasshouse-grown King Edward (foliage resistance rating 3) potato plants. Leaf discs (12 mm diameter) were cut from this material using a cork borer. The discs were then placed within a 170 mm x 170 mm Perspex frame, into which holes had been drilled, each frame accommodating 64 discs. Cut edges were covered by Parafilm strips leaving a 1 cm^2 area of disc tissue exposed. Discs were then individually inoculated with a 20 µl droplet of *P. infestans* sporangial suspension (isolate 2012_10290A, genotype 7-A1 for the first run and 2012_9922C, genotype 13-A2 for the second), prepared from 7-day-old infected leaflets and adjusted to 10^5 sporangia ml^{-1}. The two isolates were from Great Britain. Inoculated discs, and non-inoculated controls, were sealed within transparent plastic boxes lined with damp tissue paper. Boxes were in turn placed within a controlled climate chamber (16h / 8h day/night cycle, 18^o C). At timings corresponding to 4-hour intervals between 8 and 72 hours post inoculation, selected frames were removed from the climate chamber and treated curatively – one frame containing 64 discs were treated at each time-point. Two frames of 64 discs were inoculated, but left untreated as controls. Frames were returned to incubation conditions immediately following treatment. Seven days from the initial inoculation discs were assessed for disease development. A disc that was completely necrotic or showed signs of sporulation was classified as a successful infection, whilst one that showed no symptoms or small arrested lesions was categorized as effective control.

Isolate response to temperature

Detached leaves from 7-week-old, glasshouse-grown King Edward potato plants were placed within transparent plastic boxes which had been lined with damp tissue paper. Two boxes were used for each experimental run at each temperature, each containing 8 leaflets. Each

leaflet was then inoculated with a 20 µl droplet of *P. infestans* sporangial suspension (isolate 2012_9922C), adjusted to 10^5 sporangia ml^{-1} as described in the previous section. There were 8 non-inoculated leaflets that served as controls, included in an additional box. The plastic boxes were then sealed and placed within a climate controlled chamber. Conditions in the chamber were set to begin with a 12-hour period at 18 ^oC to maximize the number of leaflets which were successfully infected, followed by 6 days at the experimental temperature (6, 10, 14, 18, 22, 26 or 30^oC). A 16h / 8h day/night cycle was maintained throughout. At 120, 144 and 168 hours post inoculation infected leaflets were removed from their boxes and digital images taken. Lesion size was quantified from these images via the program ImageJ (Schneider *et al*., 2012). Necrotic and/or sporulating tissue was measured using the 'polygon' function. Linear lesion growth rates were then estimated by linear regression of the square roots of these three observations (Visker *et al*., 2003) against time. The experiment was run twice for each experimental temperature.

Varietal resistance field trials

Potato plants of varieties King Edward (foliar resistance rating of 3) and Cara (foliar resistance rating of 5) were grown in small propagation pots within a poly-tunnel for approximately 7 weeks. When high risk weather was forecast (i.e. the Smith criteria, the experiment was conducted before the publication of the Hutton criteria) plants were transported to a trial field where a late blight epidemic was in progress. Plants were placed within open trays on ridges and were left exposed for 2 hours. The plants were then sealed within plastic sheeting and placed within a climate chamber (16h / 8h day/night cycle, 18^oC). After 1, 2 & 3 days, 12 plants per cultivar were treated curatively, or left untreated as controls, and returned to the climate chamber. Plants that had not been exposed in the trial field served as controls. Seven days after exposure to inoculum, the number of late blight lesions per plant was counted.

RESULTS

The time period during which curative fungicide efficacy was high was substantially affected by the aggressiveness of the *P. infestans* isolate, the temperature post infection and also varietal resistance to leaflet colonisation.

Curative threshold bioassays

Figure 1 shows the results of two runs of the leaf disc bioassay with (A) a highly aggressive isolate (2012_9922C) and (B) an isolate that had slower growth rates in other bioassays (2012_10290A). At early time points (approx. 8 – 20 hours) curative sprays generally offered good control on the leaf discs infected with isolate 2012_9922C. This control was then rapidly lost from approx. 20 – 40 hours, and at time points greater than 40 hours curative sprays rarely prevented more than 30% of infection sites from developing into lesions. In contrast for the second run of the bioassay, with isolate 2012_10290A, 40 – 100 % of the leaf discs did not develop expanding lesions after curative sprays in time points 8 – 56 hours.

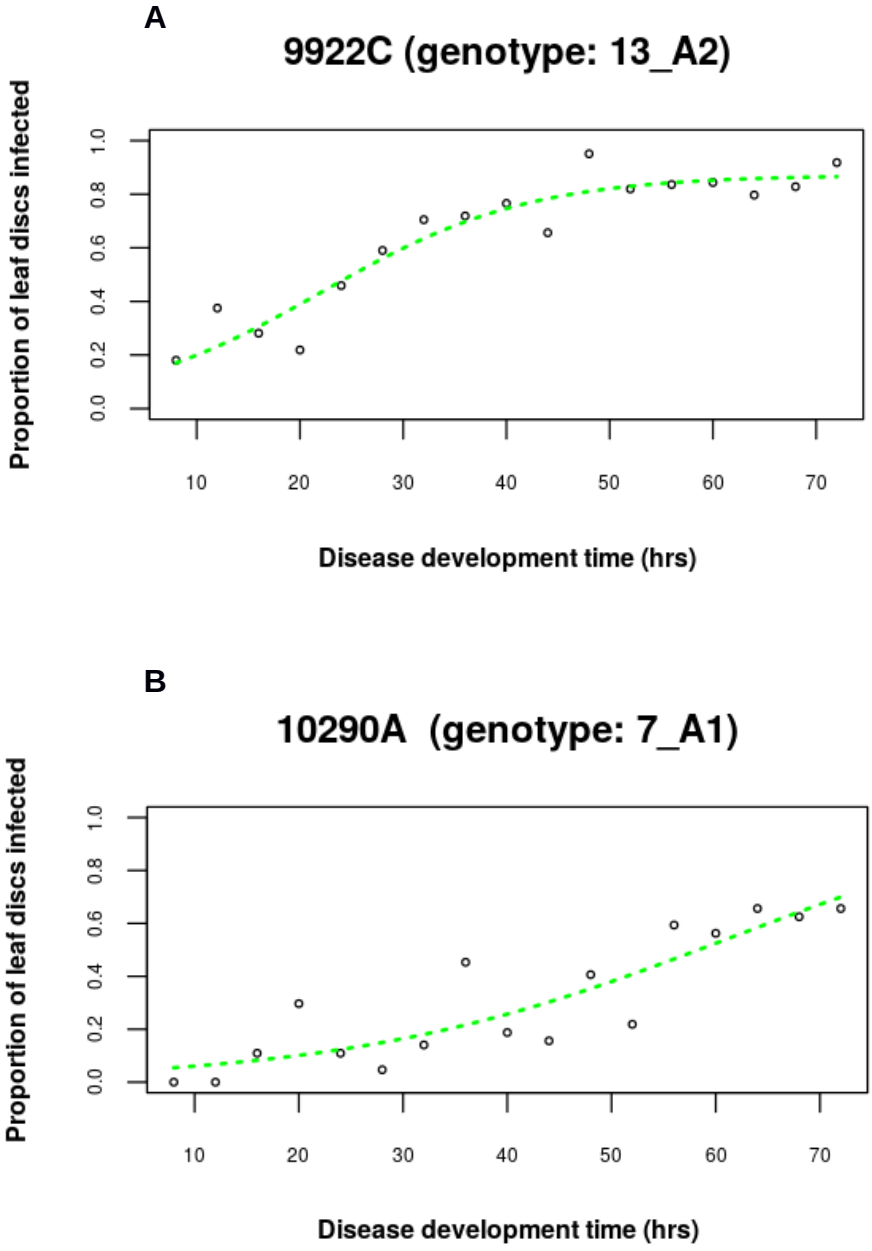

Figure 1. Proportion of leaf disc infection (n=64) in relation to curative fungicide timing (hours at 18 ^{o}C from inoculation to treatment with fluopicolide + propamocarb) for the isolates (A) 2012_9922C and (B) 2012_10290A. Sigmoid curves are fitted to the data (adjusted-R^2 for A = 0.90; adjusted-R^2 for B = 0.78).

Isolate response to temperature

For the lower part of the tested temperature range (6 – 18^{o}C) an increase in temperature was associated with an increase in the linear growth rate of lesions on leaflets. This rate plateaued between 18 and 22^{o}C, and then reduced as temperature increased. No lesion growth was observed at 30^{o}C.

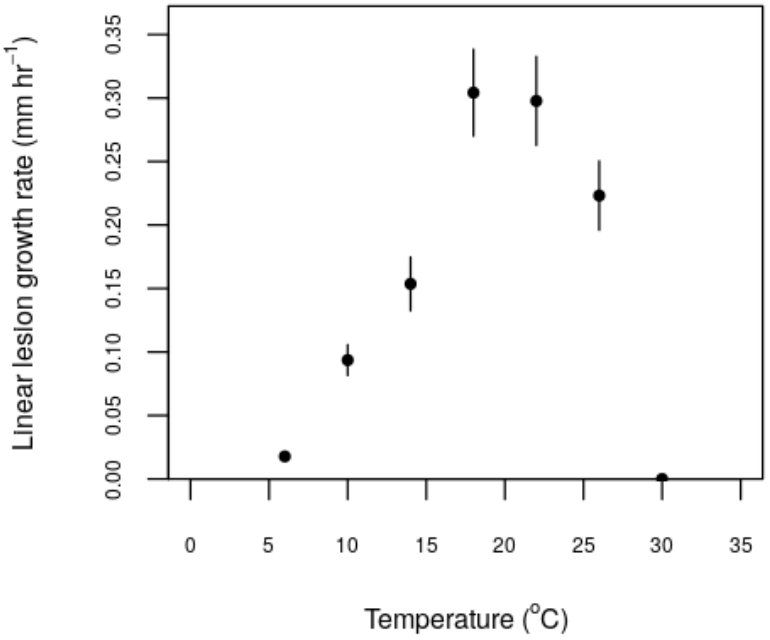

Figure 2. Mean linear lesion growth rates (bars represent 95% confidence intervals) of *P. infestans* (isolate 2012_9922C) on detached leaflets, incubated at 6, 10, 14, 18, 22, 26 or 30 ^{o}C.

Varietal resistance field trials

In this experiment differences between both varietal resistance scores and spray timings were statistically significant ($p<0.01$). On Cara plants lesion numbers were significantly lower for all three curative treatment times compared with untreated control plants. In contrast, only King Edward plants treated after a single day of disease development had significantly lower lesion counts. A full presentation of these results is available (Maloney *et al*., 2018), although they should be interpreted with caution as aggressiveness and genotype data are not available.

DISCUSSION

The curative activity displayed by some fungicides is effective over a relatively short period after infection, after which infections are not well controlled. The results presented here will form the basis of a simple decision aid to assist selection of the most appropriate treatment following a period where infection is suspected. Results from both glasshouse bioassays and field trials suggest that modifying factors can play an important role in altering the duration of the curative window, and these factors should ideally be taken into account when assessing the appropriateness of a curative treatment.

With the susceptible variety King Edward, the reduction in developed lesions of more aggressive isolates was obtained early in both the glasshouse/leaf disc bioassay and the field experiment: less than 20 hours from inoculation in the leaf disc assay, and 1 day from exposure in the field. These times are broadly consistent considering it is possible that the inoculum density and isolate differed between the two experiments. For the more resistant variety Cara, lesion number was reduced for all three fungicide treatment times (1, 2 and 3 days post exposure) in the field trial. The response of the two isolates used in the leaf disc bioassay also differed, with the more aggressive isolate (2012_9922C) reaching a point of limited curative response well before the less aggressive isolate (2012_10290A). This result should not be generalised because these two isolates probably represent extremes in aggressiveness that don't currently exist in the competitive pathogen population. For the

decision aid it is probably best in the short term to take the more risk-averse approach of assuming a crop is under pressure from the most aggressive strain. It may be possible to include genotype aggressiveness as a factor in later versions, once very rapid genotyping of air-borne *P. infestans,* or very early lesions in neighbouring outbreaks, is possible on a large scale. Temperature has a strong influence on the developmental rate of *P. infestans* (Chapman, 2012). The growth rate / temperature profile generated from the detached leaf bioassay (Figure 2) will allow the probable rates of pathogen development to be included in the final decision aid model. This will be crucial because slower *P. infestans* growth at lower temperatures has been shown to improve the performance of curative fungicides (Genet *et al*., 2001). Formulation of the model for the decision aid is ongoing, with the data presented here acting as the basis for parameters and also as justification for which modifying factors to include. Additional results from field trials will be used to assess the predictive power of the aid at different sites and under different conditions.

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