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Cultivar Resistance Can Help Extend the Effective Life of Fungicides

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INTRODUCTION – CURRENT METHODS TO DELAY THE EVOLUTION OF FUNGICIDE INSENSITIVITY

Pathogens can evolve insensitivity to fungicides used to control them. Several methods exist to manage the evolution of fungicide insensitivity, such as fungicide mode of action (MOA) mixtures, and dose alterations (Brent & Hollomon 1998). The efficacy of these methods can be summarized using the following equation, derived from classical population genetics theory.

$$sT = (r_{insen} - r_{sen})T \qquad \text{Equation 1}$$

Where r_{insen} and r_{sen} are the growth rates of the insensitive and sensitive genotype sub-populations. s is the difference in the growth rates of these sub-populations, which is selection for the insensitive strain, and T is the duration over which selection operates.

In order to delay the evolution of insensitivity we aim to reduce sT. To do this we can either (i) reduce T, (ii) reduce r_{insen}, or (iii) reduce both r_{insen} and r_{sen} by the same amount (van den Bosch et *al*. 2014, Milgroom & Fry 1988). All three options reduce selection for insensitivity. For example, the use of mixtures of MOAs has been advocated as a method of delaying the evolution of fungicide insensitivity, supported by a suite of experimental studies (See van den Bosch et *al*. 2014 Table 1 for a review). The ability of a mixing partner to delay the evolution can be described using equation (1). Consider two strains growing in an environment with a given dose of fungicide A. The insensitive strain has a higher growth rate and is selected for. Keeping the dose of fungicide A constant, we add a second fungicide, B, with a different MOA to fungicide A. Because fungicide B has a different MOA it reduces both r_{insen} and r_{sen} by the same amount; the strain insensitive to A is sensitive to B. As a result, the difference in r_{insen} and r_{sen} decreases, and thus selection for resistance to fungicide A decreases.

Equation (1) is not retricted to use of a fungicide mixing partner to slow population growth rates. Any other disease control method that reduces the growth rate of the sensitive and the insensitive strain in the same way should, according to equation (1), reduce the selection for resistance to the fungicide. If both strains are avirulent against a cultivar then the same effect ought to occur, delaying the evolution of fungicide insensitivity in the same manner. This thus

would provide an additional tool to manage the evolution of fungicide insensitivity, based on integrated use of cultivar resistance.

Herein we develop a model of the evolution of fungicide insensitivity in *Phytophthora infestans*, which causes late blight in potato crops, and test the hypothesis that an increase in the resistance rating of a cultivar extends the time taken to evolve insensitivity to the fungicide.

To measure the time taken to evolve insensitivity we use the metric T50. As selection acts the percentage sensitive declines from 100% towards 0%. The point in time when 50% of the population is still sensitive is T50, the rest of the population at that point are heterozygotes or insensitive homozygotes. Longer T50 values indicate the population is changing at a slower rate.

METHODOLOGY

Descriptive model summary

In brief, an SIR model of the epidemiology and evolution of *P. infestans* on potato was developed. A host growth model generates healthy leaf area, spores fall on these leaves and cause infection at a given rate, converting the healthy area to latent lesion area. After a latent period, the tissue becomes infectious, generates spores, and continues the epidemic. From one season to the next the composition of the primary inoculum changes according to the frequency of the strains in the epidemic, and so the pathogen evolves over multiple years.

New genotypes are generated by mutation and their frequency in the population changes according to their growth rate: r in equation (1). This rate is determined by the infection efficiency, latent period, and sporulation of the strain. These lifecycle parameters change according to the strains' susceptibility to fungicide and the level of cultivar resistance.

Fungicide applications reduce the lifecycle parameters of the sensitive strain according to the dose. The fungicide dose decays over time after it is applied. Cultivar resistance is assumed to be constant and reduces the lifecycle parameters according to cultivar resistance level.

Mathematical model summary

The following is a brief summary of the model used, provided to highlight key features of the system. Healthy area grows, senesces and is infected according to

$$\frac{dH}{dt} = g(t)H - s(t)H - y(t)P(t)\sum_{i=1}^{N} \mathrm{IE}_i * \left[W(t)\widetilde{q_i} + \sum_{j=1}^{N} \rho_j \lambda_{ji} I_j(t) \right] \qquad \text{Equation 2}$$

Where g is the growth rate of the healthy area, s is the senescence rate. The term y is the probability of spores landing on crop as opposed to non-crop areas like soil, P is the proportion of leaf area that is healthy tissue, IE_i is the genotype specific infection efficiency,

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W is the amount of primary inoculum, $\widetilde{q_i}$ is the fraction of that primary inoculum that is composed of strain i, ρ is the sporulation rate of the jth strain, λ_{ji} is the rate at which jth strain infectious tissue produces ith strain spores, and I_j is the amount of infectious tissue of strain j. This describes the growth, decay, and infection of healthy tissue.

Latently infected tissue of the ith genotype develops according to

$$\frac{dL_i}{dt} = y(t)P(t)\mathrm{IE}_i\left[W(t)\widetilde{q_i} + \sum_{j=1}^{N}\rho_j\lambda_{ji}I_j(t)\right] - \delta_i L_i(t) - s(t)L_i(t) \qquad \text{Equation 3}$$

Where δ_i is the latent period of the ith genotype. Finally, infectious tissue develops as

$$\frac{dI_i}{dt} = +\delta_i L_i - \omega I_i \qquad \text{Equation 4}$$

where ω is the infectious period. This assumed to be constant for all genotypes. Cultivar resistance and fungicide affect the pathogen through the infection efficiency, latent period and sporulation. Genotype specific infection efficiency, IE_i, is defined as

$$\mathrm{IE}_i = IE_0 * (1 - \theta) * [1 - \pi(1 - e^{-kd})] * \varkappa_i \qquad \text{Equation 5}$$

Where π is the fractional reduction of the infection efficiency at a full label dose of a given fungicide, k is the shape of the dose response curve, and d is the effective dose of the fungicide present on the crop, which is applied and decays. θ is the reduction in infection efficiency caused by the cultivar. Latent period, δ_i and sporulation, ρ_j, are affected in the same way, except that latent period is extended not reduced. Finally, $\varkappa_i$is the fitness cost to the insensitive strain, which causes the fraction of the insensitive strain to reduce in the absence of fungicide.

The key point with this summary is that life cycle parameters for each strain, either sensitive homozygote, heterozygote or insensitive homozygote, are modified both by degree of sensitivity to the fungicide, and the level of cultivar resistance (θ). No strain is virulent. In this way competition between sensitive and insensitive strains (r_{insen} and r_{sen} from equation 1) is influenced by cultivar resistance.

RESULTS

As the level of cultivar resistance increases (the term theta in equation 5) the growth rates of both insensitive and sensitive strains decrease. This results in a slower disease progress curve (Figure 1). The highest level of cultivar resistance simulated delays the epidemic by approximately 40 days.

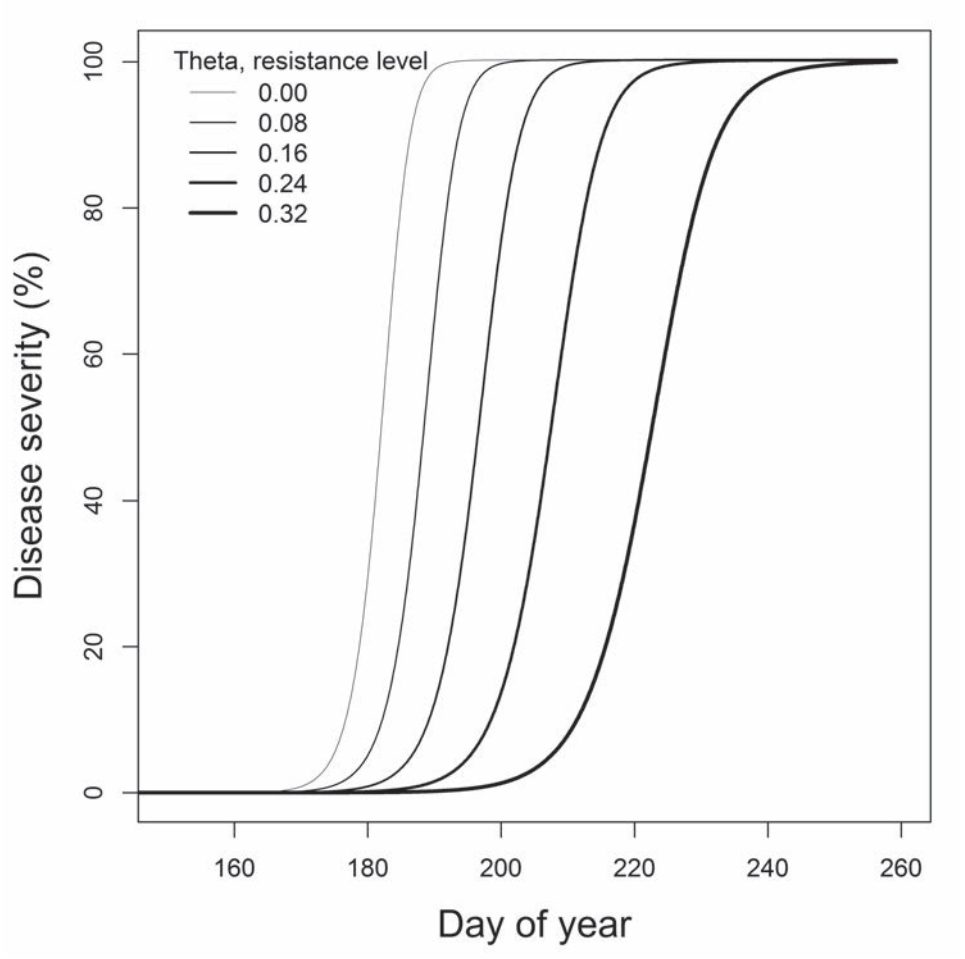

Figure 1 Disease progress curves for the development of late blight on a potato crop, in the absence of fungicide (dose = 0). Cultivar resistance in this pathosystem is not strong enough to provide complete disease control, but slows the epidemic. The model is developed in growing degree days, but presented in Julian days for presentation. Figure 1 serves as a guide to interpret Figure 2, where the x-axis is theta, the cultivar resistance level.

Figure 2 demonstrates the effect this increased cultivar resistance has on the time taken to evolve fungicide insensitivity. Increasing the level of cultivar resistance increases reduces growth rates of both strains, delaying the evolution of fungicide insensitivity, as predicted from equation 1.

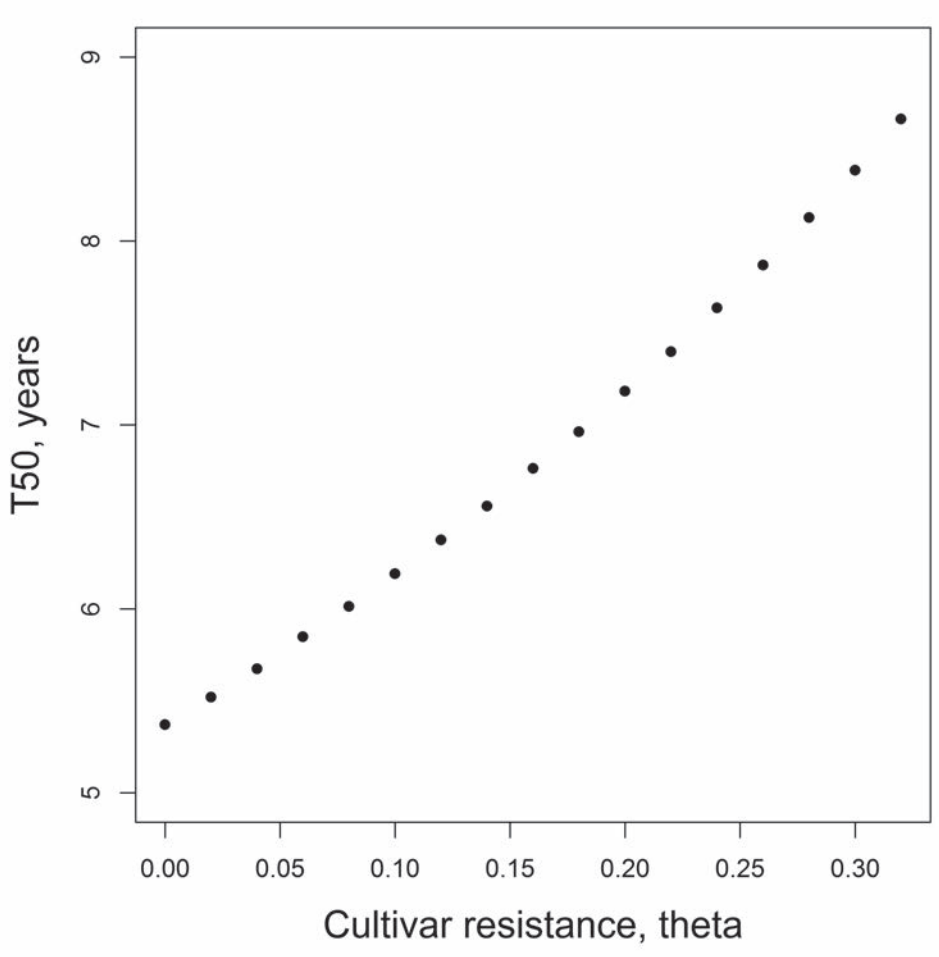

Figure 2 Effect of increasing cultivar resistance on the time taken to evolve insensitivity (T50). As cultivar resistance increases, the time to evolve insensitivity to the fungicide increases. The term theta, used to describe cultivar resistance, is defined in equation (5). Dose is constant at full dose.

Multiple doses, efficacy levels, mutation rates and gene numbers were explored; that an increase in cultivar resistance results in an increase in T50 is consistent, though the magnitude of the effect changes. All increases in cultivar resistance that reduce both r_{insen} and r_{sen} reduce selection and extend the time taken to evolve insensitivity (T50).

DISCUSSION

Increasing cultivar resistance delays the evolution of fungicide insensitivity. Use of cultivar resistance can provide a useful tool to use in managing fungicide resistance, along with, for example, fungicide mixtures, alternation and dose adjustment.

These results are generally applicable to many different types of fungicides. The exact nature of the relationship between T50 and cultivar resistance level changes according to fungicide efficacy, dose and the mutation rate of the pathogen. As in equation 1, the key factor driving the evolution of fungicide insensitivity is the difference in the growth rates of the sensitive and insensitive subpopulations. So long as the growth rates of both populations are affected by cultivar resistance a reduction in selection for insensitivity is predicted to occur, regardless of the particular MOA of the fungicide that the subpopulation is insensitive to.

While cultivar resistance has been presented in this paper as a method by which fungicide resistance could be reduced, cultivar resistance is also eroded by pathogen evolution. High levels of cultivar resistance result in high selection pressure for pathogen populations to evolve virulence. This would lead to a decline in cultivar resistance, and lower levels of cultivar resistance do not delay the evolution of fungicide insensitivity by as much. However, the principles introduced in this paper could be equally applied to the protection of cultivar resistance, and the development of virulence slowed by control factors such as fungicides. How the control of virulence using fungicides and the control of fungicide insensitivity using cultivar resistance interact will be considered in future work.

In summary; effective levels of cultivar resistance can be an additional tool to delay the evolution of fungicide insensitivity, as predicted by classic population genetics theory.

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