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Research

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Extending the durability of cultivar resistance by limiting epidemic growth rates

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Cultivar resistance is an essential part of disease control programmes in many agricultural systems. The use of resistant cultivars applies a selection pressure on pathogen populations for the evolution of virulence, resulting in loss of disease control. Various techniques for the deployment of host resistance genes have been proposed to reduce the selection for virulence, but these are often difficult to apply in practice. We present a general technique to maintain the effectiveness of cultivar resistance. Derived from classical population genetics theory; any factor that reduces the population growth rates of both the virulent and avirulent strains will reduce selection. We model the specific example of fungicide application to reduce the growth rates of virulent and avirulent strains of a pathogen, demonstrating that appropriate use of fungicides reduces selection for virulence, prolonging cultivar resistance. This specific example of chemical control illustrates a general principle for the development of techniques to manage the evolution of virulence by slowing epidemic growth rates.

1. Introduction

Cultivar resistance is an efficient method of disease control. Pathogen populations can, however, evolve virulence—breaking the resistance of the cultivar. The three main strategies often discussed as methods to delay the evolution of virulence are (i) the deployment of mixtures of cultivars with different host resistance genes (or quantitative trait loci, QTL) conferring resistance, (ii) appropriate deployment of resistant cultivars in time and space, and (iii) combining ('pyramiding') host resistance genes or QTL. Here we present a fourth, complementary, method. We begin with a brief overview of the three widely advocated methods. This is to set the fourth technique in context rather than provide an exhaustive review.

The first advocated method, mixtures of resistant cultivars each with different host resistance genes, has been suggested to reduce selection for virulence, based on the principle that mixtures can reduce total pathogen inoculum or can introduce barrier effects [1,2]. Several papers have demonstrated the efficacy of mixtures for disease control [2–5]; however, empirical evidence for the use of mixtures to reduce the selection of virulent strains is surprisingly sparse. There is at present little uptake of the use of cultivar mixtures by agricultural practice, partly because cultivars have particular agronomic traits which suit them for particular end uses, locations and planting/harvest dates. This limits the range of cultivars that can be mixed and makes growers and food processors reluctant to accept mixtures.

The second method to delay the evolution of virulence is the appropriate deployment of genetic resistance in time and space [6–8]. Evidence for the effectiveness of this method in the management of selection for virulence is lacking, though there are several modelling studies that demonstrate useful effects [8].
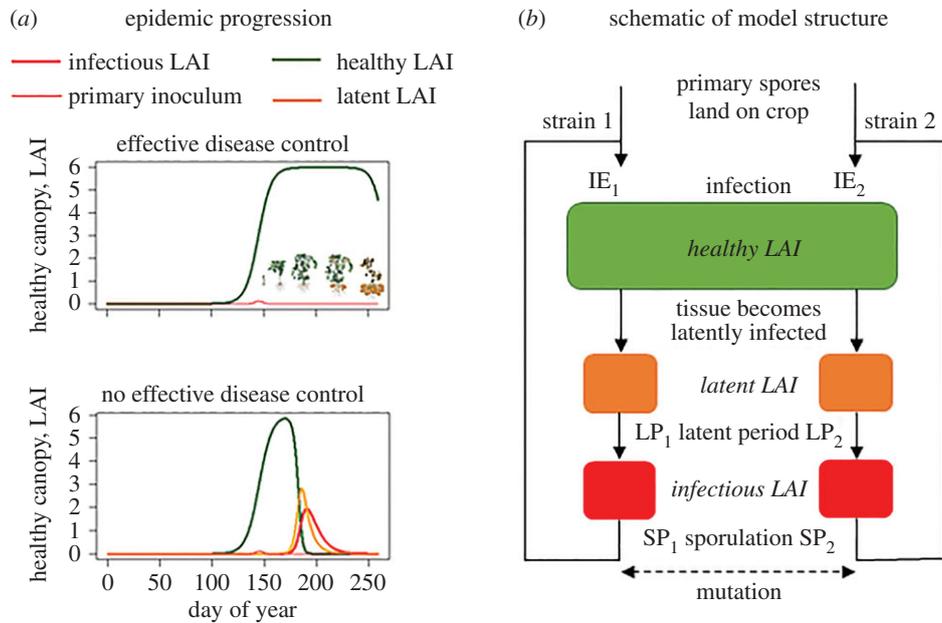
Figure 1. Diagrammatic representation of the epidemiological model of *P. infestans* used in this paper. Panel (*a*) describes crop growth in the presence and absence of effective disease control. Panel (*b*) presents a schematic of the pathogen life cycle for two pathogen strains. LAI is leaf area index, the area of leaf per area of ground. In the model, in the presence of effective disease control, the amount of healthy tissue grows logistically and senesces later in the growing season (*a*). In the absence of effective control, a small amount of primary inoculum (in the figure just before day 150) starts the epidemic, and in cases of incomplete control results in complete crop loss within 4 weeks. Panel (*b*) is a schematic of the model structure (detailed in the electronic supplementary material, appendix). Plant tissue begins as healthy, and spores of the avirulent, A, or virulent, V, are deposited. These spores infect with probability, IE_A and IE_V, respectively. After a latent period, LP_A, and LP_V, they become infectious and generate spores at rates SP_A and SP_V. The values of IE_A, LP_A and SP_A and the values of IE_V, LP_V, SP_V determine the difference in fitness of the avirulent and the virulent strains. Both the virulent and avirulent strains are equally fungicide sensitive. Note that in the model there are several possible genotypes, determined by the number of QTL. In the case, for example, that the cultivar resistance is determined by two resistance QTLs, the model contains two virulence genes in the pathogen, each with a virulent and avirulent allele, leading to $3^2 = 9$ genotypes.

However, in free-market agriculture, there are few mechanisms available to encourage or enforce implementation of controlled large scale spatial/temporal deployment of cultivars.

The third option to delay the evolution of virulence is to pyramid host resistance genes [7]. Deployment of multiple genes in a single cultivar means that a spore virulent against any individual gene will be inhibited by the other genes. Experimental evidence for pyramiding to delay virulence has been put forward [9,10]. Though pyramiding genes into a single cultivar may indeed benefit the breeder by producing a cultivar with a longer effective life, it is an expensive and time-consuming challenge.

Only one of the three methods discussed above, pyramiding resistance genes, is currently widely used (by breeders). At present, no easily applicable method seems to be available to the industry, neither industrial partners nor growers, to contribute to the durability of cultivar resistance. In this paper we introduce an additional general method to delay the evolution of virulence, which supplements current techniques, and is easier for individual growers to apply. The core thesis is derived from classical population genetics theory and can be summarized as follows: selection for virulence can be slowed by introducing additional disease control methods, slowing the growth rate of the entire pathogen population.

Of the various methods that could, in principle, be used to slow the growth rate of the entire population, here we will specifically study the use of chemical control (fungicide). This is a commonly used control method, which is easily applicable. However, we stress that the results are predicted to apply to other disease control methods. There is a range of other methods with the potential to reduce the growth rate of epidemics [11]. For example, biological control organisms of the pathogen (fungi, viruses, virions), changes to soil fertilization levels (which are known to reduce the growth rate of biotrophic plant pathogens) or agronomic measures such as planting date, planting density and intercropping (where other crop species are planted in between the target crop). All these methods have the potential to affect epidemic growth rates; here we have focused on the particular case of fungicides.

For clarity, this principle is not quite identical to integrated pest management (IPM). IPM, combining multiple methods of control, is often advocated for its variety of beneficial and cost-saving effects [12]. For example, by using resistant cultivars and a lower dose of fungicide, growers can save cost compared with sensitive cultivars and a high dose of fungicide. The principle discussed here is that two growers, using the same resistant cultivar, should find there is an optimal fungicide application that delays the evolution of virulence. The optimum for delaying the evolution of virulence may not always be the same as the optimum from the view of IPM.

(a) Population genetics considerations

Suppose populations of an avirulent and a virulent strain are growing exponentially on a partly resistant cultivar. Initially the population is predominantly avirulent; however, as the cultivar is partly resistant, the growth rate of the virulent strain, r_V, is higher than the growth rate of the avirulent strain, r_A. Over time this increases the frequency of the virulent strain in the pathogen population (selection for virulence). The difference between r_V and r_A is a measure of the rate of selection for virulence, s_V [13,14]. This is stated explicitly in equation (1.1) [13].

$$s_V = (r_V - r_A)T, \tag{1.1}$$

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where s_V is the rate of selection for virulence and T is the length of time over which the pathogen is exposed to the cultivar resistance. Equation (1.1) shows that if we introduce an additional disease control measure which reduces the rate of increase of both the avirulent and the virulent strain, this will lead to a decreased rate of selection for virulence [13,15]. For example, introducing a fungicide will reduce both the growth rates of the avirulent and the virulent strains, so equation (1.1) becomes

$$s_V = (f\, r_V - f\, r_A)T = f\, (r_V - r_A)T, \tag{1.2}$$

where f describes the effect of the fungicide on the pathogen population growth rate, ranging between 1 and 0. We thus conclude that, according to this simple consideration, implementing an additional disease control measure should reduce the rate of selection for virulence.

This process of reducing selection has been shown to work for the analogous case of management of fungicide insensitivity. For example, the selection for insensitivity against fungicide A is reduced by the addition of fungicide B with a different mode of action. This reduction in selection will happen even without any change in the dose of fungicide A. This is because fungicide B reduces the growth rates of the A-insensitive ($r_{insensitive}$) and A-sensitive strains ($r_{sensitive}$) simultaneously. A considerable body of published experimental and modelling evidence corroborates this, as is shown in a recent review paper [16] where the existing evidence was analysed and summarized.

Based on the above discussion we postulate that any given disease control method that reduces both r_V and r_A will reduce selection for virulence. A practically relevant and simple example is the use of fungicides. However, the principle described above is generic, and should be applicable to other methods of control.

(b) The pathosystem

There are several features that make the potato late blight pathosystem, caused by *Phytophthora infestans*, an ideal test system. Of the various challenges to potato production, late blight remains the most serious disease in most major potato-producing regions [17]. Late blight control normally requires a combination of appropriate fungicide use and cultivar resistance [18–20]. Highly effective disease control is essential as, compared with other agricultural epidemics, *P. infestans* is particularly destructive—almost the entire canopy of a mature crop can be lost within 20–30 days from the emergence of symptoms [18]. Further, the evolution of virulence in *P. infestans* poses a challenge to the industry at the moment, as evidenced by the downgrading of cultivars historically considered highly resistant to much less resistant or even susceptible [21,22].

2. Methods

Our aim is to demonstrate the concept that additional disease control measures will reduce the rate of selection for pathogen virulence. We therefore describe a model where virulence evolves but the pathogen cannot adapt to the additional control measure, here fungicides. We return to this point in the discussion.

(a) The model

In order to test the hypothesis that additional disease control methods (here the use of fungicide) will delay the evolution of virulence, we constructed and analysed an epidemiological model. A complete mathematical description of the model is provided in electronic supplementary material, appendix S1. Here we summarize the crop and pathogen biology, which is incorporated in the model equations. A host growth model was parameterized to describe the growth and senescence of a standard UK main crop of potato; emerging at the end of April, reaching full canopy with a leaf area index (LAI) of 6 in early June, and with senescence beginning just before haulm destruction and harvest in September (figure 1). This was constructed as a set of logistic growth curves.

An epidemiological healthy–latent–infectious–removed (HLIR) class model was developed to describe epidemics of *P. infestans*. The initial release of spores forming the primary inoculum was described as a truncated normal distribution. This curve was parameterized to cause the epidemics to start in early summer, the average time for late blight epidemics to start [23] (figure 1). The composition of the primary inoculum in the first year is set to be composed entirely of the avirulent strain. Virulence emerges by mutation and changes in frequency according to selection. The primary inoculum in subsequent years then reflects the strain composition of the pathogen population established in the previous growing season.

P. infestans is diploid, and predominantly asexual in the UK [24]. In the model we assume that each host resistance gene or QTL has a paired virulence gene in the pathogen [25]. Each pathogen virulence gene will have one of two alleles: either virulent or avirulent. As a diploid, the pathogen can therefore be homozygote virulent, homozygote avirulent or heterozygote at any particular gene. The model can describe any number of gene-for-gene pairs and allows mutation at each of the loci to generate new pathogen strains. All pathogen strains also carry a gene conferring fungicide sensitivity, which, for the purposes of this study, is assumed to be immutable over the time period of interest.

In the absence of mutation, no new strains are generated. A lesion will produce spores, and when mutation is non-zero a fraction of these are mutant spores—they are a different strain, with a different response to the cultivar than their ancestor. Specifically, infectious tissue of the ith type generates spores at a given sporulation rate; and of these a fraction are of the jth type. In turn, lesions of the jth type sporulate and generate a small amount of ith-type spores. As the pathogen is diploid, the total number of genotypes in a simulation is (3^the number of virulence genes). Mutation rates are assumed to be constant, and not affected by the fungicide.

The amount of healthy leaf area is reduced by infections by *P. infestans*. A spore from the primary inoculum which lands on a healthy part of a potato leaf causes an infection with a given probability: the infection efficiency. At infection, healthy area becomes latently infected area (non-sporulating) and after a period of time, the latent period, it becomes infectious and generates new spores. After the latent period the latent tissue becomes infectious, generating new spores with a sporulation rate (figure 1). These three life cycle parameters, infection efficiency, latent period, and sporulation, vary between each strain according to the interaction between the genotype of the strain and the environment (cultivar and fungicide) the strain is present in. The life cycle parameters are altered in three ways.

(1) The three life cycle parameters are altered by the level of cultivar resistance. Cultivar resistance is described as a fractional reduction (or extension, for latent period) in the life cycle parameters of an avirulent strain. The amount of change in these parameters depends on the number and effectiveness of avirulence QTL and allelic dominance. A range of resistance values are explored, to replicate observed resistance levels in commercially relevant cultivars (figure 3).

(2) The presence of a virulence allele in the organism carries a cost. This cost of virulence can result in a reduction of the
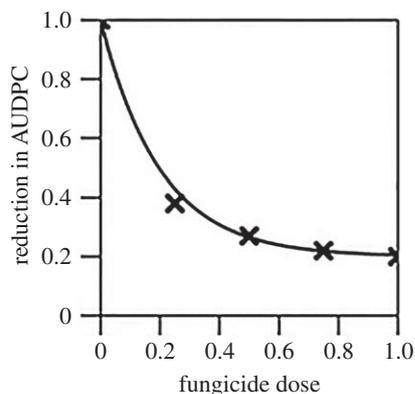
Figure 2. The dose–response curve of an epidemic of *P. infestans* treated with a mixture of the fungicides propamocarb and fluopicolide (as commercial product 'Infinito'; Bayer) on the susceptible cultivar King Edward. AUDPC is area under the disease progress curve which is calculated from a time series of observed disease severity. Dose 1 = the maximum permitted dose. Y-axis is the fractional reduction in AUDPC relative to zero fungicide dose. Crosses are data generated by BBSRC project BB/K020447/1; the curve is fitted to these (electronic supplementary material, appendix S1).

Figure 3. Healthy canopy area and disease dynamics of potato crops with different resistance ratings. LAI is leaf area index. This figure is intended as a guide to the term resistance rating, which is more commonly used by growers. The dashed dark green line is healthy area of the crop in the absence of disease, senescing before harvest. The solid thin coloured line is the healthy area of the crop for an epidemic of the avirulent pathogen with no fungicide, the dotted coloured line is the infectious tissue in that epidemic. This rapid loss of the entire canopy is a common feature of potato late blight. The colour scheme for each level of cultivar resistance is also used in figure 4. The ratings 3–5 and 7 were chosen to represent commercially relevant cultivars.

sporulation rate and infection efficiency, and an extension of the latent period. Again, the amount of change in these parameters depends on the magnitude of the cost, the number of virulence QTL and allelic dominance. The cost of virulence is parameterized to be small [26].

(3) The application of a fungicide reduces the sporulation rate and infection efficiency, and extends the latent period. The extent of this depends on the dose and efficacy of the fungicide. Virulent and avirulent pathogen strains are equally sensitive to the fungicide.

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

(b) Model parameterization

P. infestans has been the subject of many studies, allowing the parameterization to draw upon the extensively published data, and data available to the authors. Individual parameter estimates and a justification are summarized in table S1 of electronic supplementary material, appendix S1. Here we pay specific attention to cultivar resistance and fungicide dose–response as they are the two key variables in our study.

(c) Fungicide dose–response curve

A dose–response curve (figure 2) for a formulated mixture of the active ingredients propamocarb and fluopicolide (as the commercial product 'Infinito', Bayer) was established in field trials (BBSRC project BB/K020447/1). As we will discuss below, model results are not qualitatively dependent upon a particular fungicide or mode of action, but the use of a realistic dose–response curve allows for the efficient translation from theory to practical application. Dose is given here as the fraction of a
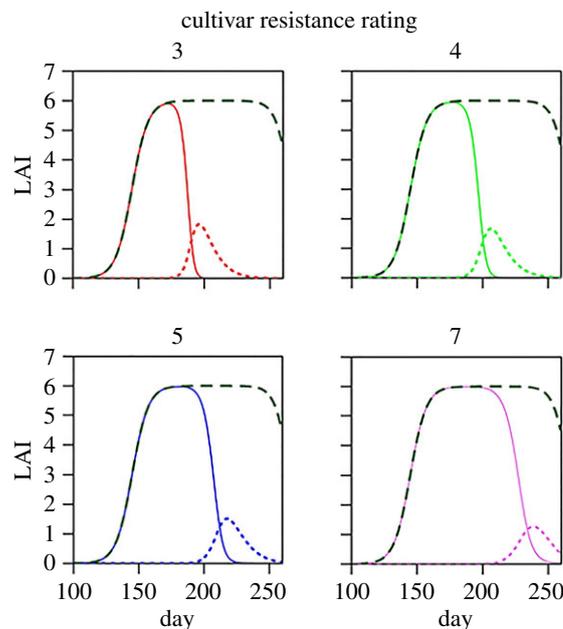
full dose (see note below). The critical feature for our purposes is the ability of that applied fungicide dose to reduce the epidemic growth rate.

Note, a 'full dose' is a concept from crop protection. A 'full dose' is set by the manufacturer in agreement with the pesticide regulator and is an amount of the commercial product applied per hectare such that it provides effective disease control and has environmental impacts below the bounds set by regulation. The number of grams of fungicide active substance applied per hectare in a full dose varies between fungicide products. A grower is allowed to apply one full dose per fungicide application. Application of a higher dose is not permitted. We will thus use the concept of full dose and fractions of a full dose in our analysis.

An advantage of using a model system is that we are able to explore the effect of using doses above the maximum permitted dose of 1; such higher doses are presented strictly for comparison and clarification, but would in practice be illegal.

(d) Cultivar resistance

In the potato industry a cultivar's resistance is expressed as a resistance rating on a 1 to 9 scale. This rating, revised yearly, is based on disease assessments in experimental plots. The ratings are available in [27]. In a set of experiments exploring the evolution of virulence in the UK population of *P. infestans*, the fourth author of the present article described the relationship between resistance rating and the area under the disease progress curve, AUDPC [28]. We have used these AUDPC values to parameterize the model to reflect the commercially relevant range of cultivar resistance in the UK. Figure 3 is a guide to show the effect of resistance rating on the healthy area curve as well as on the epidemic development.
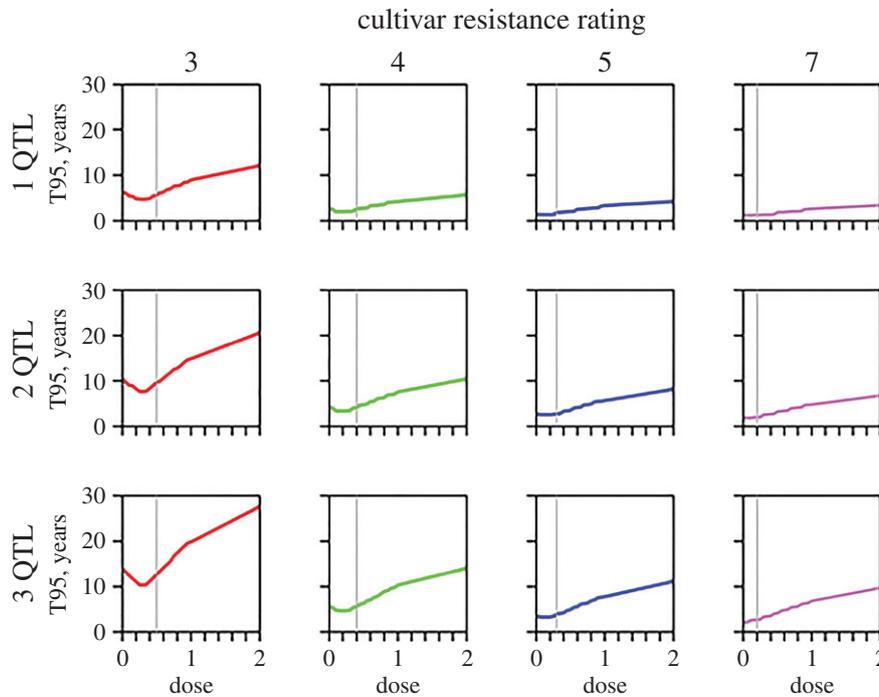
Figure 4. The effect of fungicide dose on the evolution of virulence, as measured by T95. This is the time from the introduction of the cultivar, when the pathogen population is 100% avirulent, until the moment the pathogen population is 95% avirulent (the remainder being heterozygotes and the virulent homozygote). The vertical grey line indicates the minimum dose that would in principle be needed for commercially acceptable control of potato blight; lower doses are required for more resistant cultivars.

(e) A metric for quantifying the evolution of virulence

A metric of speed of virulence evolution is T95, the time from the introduction of the cultivar until the avirulent homozygote has declined from 100% to 95% of the population. The other 5% of the population is heterozygote or virulent homozygote. If selection for virulence is large we find a small T95 (virulence evolves rapidly); if selection is low the T95 is longer (virulence takes more time to evolve). This threshold is chosen as a relevant threshold, beyond which changes to the effectiveness of host resistance might first be noticed in the field. However, the results and conclusions are not dependent upon the 5% threshold. Changing to, for example, T75 would change the results quantitively but not qualitatively: it would take more time to reach a 25% threshold than a 5%.

(f) A metric for quantifying effective disease control

In late blight disease, control is considered to be effective if symptomatic tissue is kept to a virtually zero level. Growers evaluate this threshold visually themselves; we use a criterion to replicate this. If symptomatic tissue (infectious tissue) is more than 1% of the total crop area, disease control is considered to be not effective. That is, if $I/(H + L + I) > 0.01$ then control is lost, where I is LAI of infectious tissue, H is LAI of healthy tissue and L is LAI of latent tissue. This strict criterion is a requirement for blight control, as loss of control can rapidly result in complete crop loss (figure 3). The effective life of the control programme is the number of years of effective control that can be provided by a given combination of fungicide dose and cultivar resistance.

3. Results and discussion

(a) The effect of increasing fungicide dose on selection for virulence; part 1

On the basis of equation (1.1) we predicted that a simultaneous reduction in the growth rate of the virulent and avirulent pathogens via a fungicide application would reduce the selection for virulence. If this hypothesis is true we expect to see a positive relationship between increasing fungicide dose and T95 (time taken to evolve virulence).

As shown in figure 4 such a positive relationship was identified, confirming that increasing fungicide dose can delay the evolution of virulence. The results presented in figure 4 use the dose–response curve in figure 2, and compare four different cultivar resistance ratings (the four cultivars in figure 3). The cultivar resistance rating changes the magnitude of the effect; however, the overall conclusion holds for a commercially relevant range of cultivar resistance ratings: increasing dose can delay the evolution of virulence.

(b) The effect of increasing fungicide dose on selection for virulence; part 2

While figure 4 shows that T95 generally has a positive relationship with fungicide dosage, under certain conditions there can be a negative relationship. At first sight this may seem to contradict our prediction from equation (1.1); however, rather counterintuitively, it is actually a second confirmation of the general principle. The growth rates of the virulent and avirulent strains, r_V and r_A, are influenced by fungicide dose; but also by other features of the system. The rate of new infections on the host tissue depends on the density of healthy host available for infection. Therefore, increasing healthy tissue available to infect will simultaneously increase both r_V and r_A, which should increase the difference between them. Using equation (1.2), the negative trend observed in figure 4 (an increase in selection for virulence) can be explained as a result of an increase in T (a longer epidemic) and a simultaneous increase (rather than decrease) in r_V and r_A as there is more healthy tissue to infect.

In a situation where cultivar resistance is weak and a very low dose of fungicide is used, disease control is insufficient and
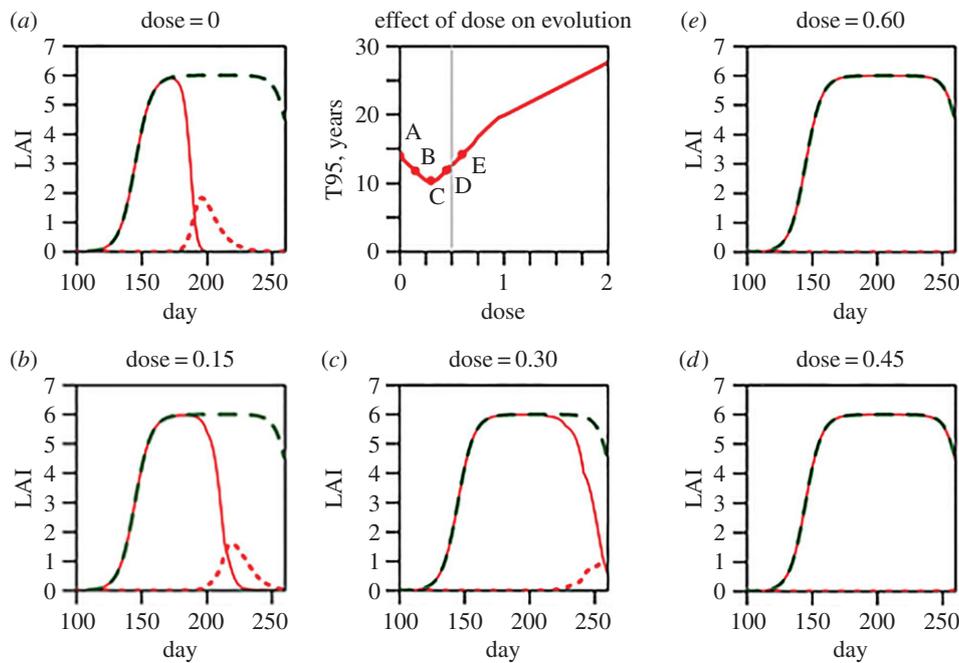
Figure 5. (a–d) Increase in selection for virulence at low doses. The cultivar from figure 4, with 3 QTL and a resistance rating of 3, is presented in the top centre panel. Five doses are highlighted to demonstrate the effect, and for each of these doses the epidemic progression is presented, similar to figure 3. As we increase the dosage from 0 to 0.30, we have substantial increases in the canopy area (thin red line), and a large decrease in infectious tissue area (dotted red line). This increase in healthy area from dose 0.00 to 0.30 results in (i) a longer time period for virulence to evolve (increased T, in equation (1.1)) and (ii) a greater selection for virulence, as the difference between r_V and r_A increases due to the greater availability of healthy tissue. However, the grey vertical bar indicates the minimum dose for commercially acceptable disease control. All cases where there is an increase in selection for virulence occur below this threshold. So long as the fungicide dose is sufficient to control the disease, increases in fungicide dose that result in reduction in growth rates reduce selection for virulence.

the epidemic consumes all the healthy area rapidly. This means that the epidemic is rapid and short. Then, for a narrow range of conditions, an increase in fungicide dose results in a sufficient increase in healthy area to cause both r_V and r_A to increase, increasing selection. At the same time, because at very low doses the epidemic was short because of loss of healthy area, there was less time for selection to proceed. Extending the duration of the epidemic by a small increase in dose extends T, increasing selection.

However, as dose continues to increase, there is little further increase in healthy area or time, and the increased fungicide dose then causes a net decrease in r_V and r_A, delaying the evolution of virulence, which is the effect we expected to see. These effects are shown in detail in figure 5.

Practically speaking, while this additional finding is interesting (and a satisfying confirmation of the general principle that simultaneous reduction reduces selection), it does not affect the management of the evolution of virulence. No grower will willingly accept a control programme (combination of low fungicide dose and low cultivar resistance) that they know in advance results in loss of disease control—and such a programme is the only case where this effect could be observed (figures 4 and 5).

(c) The effect of quantitative trait loci number and strength

Cultivar resistance can be generated in a variety of ways, either by using a few QTL of large effect, or many QTL of smaller effect. The basis of cultivar resistance alters the magnitude of the effect of fungicide use on the rate of virulence evolution.

Reading figure 4 horizontally, QTL number is kept constant and the resistance rating increases. This is a case where we

replace a weak QTL with a more effective QTL, increasing cultivar resistance. This accelerates the rate of virulence evolution (T95 decreases). It is well known that very effective cultivar resistance genes erode faster than less effective (partial) resistance, which is why many breeding programmes breed for partial resistance only. For example, in the 3 QTL case in figure 4, a full dose on a resistance rating 3 cultivar extends the T95 by 1.4-fold over the zero dose case; on a resistance rating 7 cultivar with the same number of QTL, this extension is 3.3-fold. So although virulence evolves faster when the resistance rating is high (as a result of higher selection), the use of an effective fungicide treatment programme delays the evolution of virulence by a proportionally greater amount.

Reading figure 4 vertically, the resistance rating is kept constant and the number of QTL changes. This is the case where we generate the observed level of cultivar resistance either with a single or many QTL. When the resistance rating is fixed and the number of QTL is increased (so that each individual QTL has a smaller effect), T95 increases. This is the same effect as discussed above, where more effective QTL cause higher selection for virulence; the efficacy of each individual QTL decreases, so the selection for virulence against that QTL decreases. Further, the increased number of QTL will, in our model, increase the number of mutations needed to generate the virulent homozygote, and increase the avirulence of each heterozygote towards other resistance QTLs (reducing selection), while the addition of fungicide further reduces selection, extending the time taken to evolve virulence. The causes the magnitude of the effect from equation (1.2) to change according to QTL number: for example, in a cultivar of resistance rating 7 with 1 QTL, a full dose gives a 2.3-fold extension in T95 over zero dose, while increasing QTL to 3 (and decreasing individual efficacy
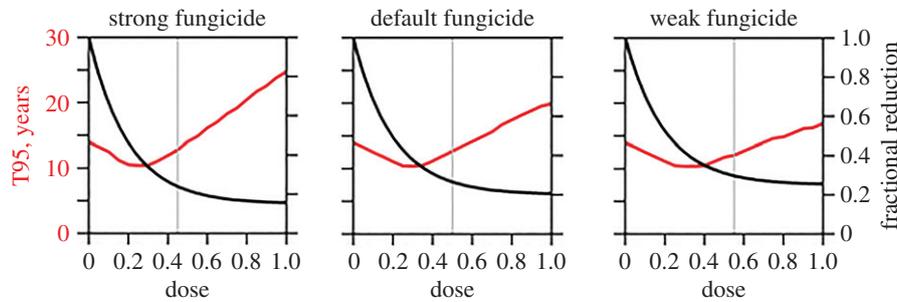
Figure 6. Fungicide dose delays the evolution of virulence according to the efficacy of the fungicide. Figure 4 presented the effect of given fungicide at a range of doses on different cultivars; here we present a single cultivar for a range of different fungicide dose–response curves. On each panel there are two lines: the red is the T95 by dose (the ability of a dose of fungicide to delay the evolution of virulence) and the black is the fractional reduction in life cycle parameters by dose (the fungicide dose–response curve). In all three cases the same cultivar is used: a cultivar of resistance rating 3 with 3 resistance QTL (the lower left cultivar in figure 4). The vertical grey bar is the minimum dose required for at least 1 year of commercially effective disease control. As fungicide efficacy decreases (moving from left to right) the evolution of virulence is delayed by less for a given dose.

so resistance rating is still 7) means the full dose provides a 3.3-fold extension in T95.

(d) The effect of fungicide efficacy

While figure 4 presented the effect of a given fungicide on different cultivars, figure 6 shows the effect of different fungicides on a given cultivar. The figure shows that using a more effective fungicide, here represented as a fungicide with a dose–response curve that is more effective, decreases the selection for virulence further. The effect on the pathogen of the innate toxicity of a fungicide active ingredient, and a fungicide dose, are exactly the same; a low dose of a more toxic fungicide or a high dose of a less toxic fungicide should have the same outcome. The important point is the fractional reduction of the life cycle parameters that the given fungicide causes (figure 1).

(e) The effect of mutation rate

In the absence of relevant published mutation rate data, the effects of a range of mutation rates were explored. Considering the highly resistant cultivar, resistance rating 7 with 3 QTL (figure 4), lowering the mutation rate from 1e-6 to 1e-12 increases T95 at full dose from 6 to 18 years. For the resistance rating 3 cultivar with 1 QTL (figure 4), the same change in mutation rate extended the T95 from 8 to 24 years (data not shown).

4. Conclusion

The evolution of virulence in agricultural pathogens can result in breakdown of disease control. Several methods have been suggested to manage the evolution of virulence. Understandably, all methods have limitations, and there appears to be no 'silver bullet' for this problem. There is currently no commercially acceptable method for growers to contribute to virulence management in their own fields. Classical population genetics theory predicted that any additional disease control method that affects both strains equally will delay the evolution of virulence [1,13–15], and here we have applied classical theory to how control methods implemented by growers can reduce selection for virulence.

To test the hypothesis, we considered the case of fungicide use. We constructed a model of the evolution of virulence in

P. infestans on potato crops and found that appropriate fungicide application does indeed delay the evolution of virulence. Some apparent exceptions were found; however, the negative relationship between dose and T95 at very low doses was explained under the same paradigm: rather than the fungicide acting to reduce the growth rates of both strains, an increase in healthy area with dose increased the growth rates of both strains. Fungicide application can thus provide a useful tool to reduce the selection for virulence. We found that the ability of a fungicide to delay the evolution of virulence occurs regardless of whether cultivar resistance is generated by a few genes of large effect, or many genes of small effect. The magnitude of the effect changes according to overall level of cultivar resistance and efficacy and dose of the fungicide.

Here we have shown the case for fungicides, but the effect of other disease control methods needs to be tested. We stress that fungicides are predicted to be a particular instance of a general principle. If alternative methods of controlling disease are tested and it is found that the hypothesis holds, we have opened a wide range of possibilities that can contribute to the management of the evolution of virulence, extending the effective life of cultivar resistance.

In our model we have assumed that the pathogen does not develop insensitivity to the fungicide. This is justified as we aimed to test the hypothesis that fungicide applications will reduce the rate of selection for virulence. In practical situations, however, pathogens may be evolving and the fungicide then selects for strains insensitive to the fungicide [29–31]. Considering selection for fungicide resistance (and referring again to equation (1.1)), we can also hypothesize that the use of cultivar resistance will reduce the rate of selection for fungicide insensitivity, as growth rate limiting host resistance will slow the growth rate of both sensitive and insensitive strains. The combination of the hypotheses that fungicides reduce the rate of selection of virulence and that cultivar resistance reduces the rate of selection for fungicide insensitivity then leads to the hypothesis that there is an optimum combination of fungicides and cultivar resistance that maximizes the durability of disease control. These two additional hypotheses will be the topic of a further study.

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