Invasion thresholds for fungicide resistance: deterministic and stochastic analyses

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Fungicide resistance is an important practical problem, but one that is poorly understood at the population level. Here we introduce a simple nonlinear model for fungicide resistance in botanical epidemics which includes the dynamics of the chemical control agent and the host population, while also allowing for demographic stochasticity in the host–parasite dynamics. This provides a mathematical framework for analysing the risk of fungicide resistance developing by including the parameters for the amount applied, longevity and application frequency of the fungicide. The model demonstrates the existence of thresholds for the invasion of the resistant strain in the parasite population which depend on two quantities: the relative fitness of the resistant strain and the effectiveness of control. This threshold marks a change from definite elimination of the resistant strain below the threshold to a finite probability of invasion which increases above the threshold. The fungicide decay rate, the amount of fungicide applied and the period between applications affect the effectiveness of control and, consequently, they influence whether or not resistance develops and the time taken to achieve a critical frequency of resistance. All three parameters are amenable to control by the grower or by coordinating the activity of a population of growers. Providing crude estimates of the effectiveness of control and relative fitness are available, the results can be used to predict the consequences of changing these parameters for the risk of invasion and the proportion of sites at which this might be expected to occur. Although motivated for fungicide resistance, the model has broader application to herbicide, antibiotic and antiviral resistance. The modelling approach and results are discussed in the context of resistance to chemical control in general.

Keywords: epidemiology; stochastic model; resistance management; generation time; time-varying selection

1. INTRODUCTION

Resistance to fungicides amongst plant pathogens is an increasingly important problem in the successful control of many crop diseases (Skylakakis 1987; Milgroom *et al.* 1989; de Waard *et al.* 1993). This is particularly critical in the deployment of highly specific fungicides which are targeted at single species with little effect on other organisms. However, the specificity and mode of action of these fungicides imposes strong selection pressures on the parasite population. Because of the clonal reproduction and haploid expression of many fungal parasites, fungicide-resistant mutants are at a strong selective advantage over the sensitive forms which they may rapidly replace. Much experimental work has focused on the molecular and biochemical aspects of fungicide resistance (Dekker 1976; de Waard *et al.* 1993; Koeller 1996; Steffens *et al.* 1996), but relatively little is known about the dynamics of resistance at the population level. Yet the dynamics of competition between fungicide-resistant and fungicide-sensitive strains determines whether or not resistance becomes established in the parasite population, thereby rendering the fungicide ineffective. This can lead

to significant crop losses if epidemics are uncontrolled. Moreover, it may result in the deployment of a broad-spectrum pesticide that affects non-target species or leads to an expensive arms race in which specific fungicides are successively released only to be overcome by the pest.

The risk of fungicide resistance developing can be analysed by examining how the deployment of the fungicide affects the competition between resistant and sensitive strains. Important practical questions (Milgroom *et al.* 1989) are: Will the resistant strain invade the parasite population or will it be eliminated? If it does invade, will it completely replace the fungicide-sensitive strain or can the two strains coexist? How long will it take for a significant proportion of the parasite population to become resistant to the fungicide? To address these questions it is necessary to resolve the interacting dynamics of the parasite, crop and fungicide into a coherent model which allows the definition of the criteria for invasion, elimination or coexistence in terms of biologically meaningful parameters while taking into account stochastic variation. The latter is important when the number of resistant mutants is small or when parasite densities are low during post-epidemic troughs.

Thresholds for the invasion of resistance have recently been derived using deterministic models for both antiviral

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drugs (Bonhoeffer & Nowak 1997; Bonhoeffer *et al.* 1997a) and antibiotics (Massad *et al.* 1993; Austin *et al.* 1997; Bonhoeffer *et al.* 1997b). Surprisingly, however, invasion criteria have been ignored in most models of fungicide resistance and, with few exceptions (for example, Milgroom 1990; Austin *et al.* 1999), stochastic variation is disregarded in models of fungicide, antiviral and antibiotic resistance. Most previous studies of fungicide resistance are based on assumptions of exponential growth (Milgroom *et al.* 1989; Milgroom 1990), or ignore the density dependence imposed by the changing availability of susceptible host tissue (Skylakakis 1980, 1983; Barrett 1983). Largely as a result of these assumptions, they have concluded that the development of resistance is inevitable, which contrasts with field evidence (Wolfe 1985). Consequently, much theoretical work has focused on strategies which increase the time taken for a parasite population to reach a critical frequency of resistance (Skylakakis 1983; Shaw 1989, 1993; Birch & Shaw 1997) rather than avoiding invasion.

By analysing the dynamics of competition between fungicide-resistant and fungicide-sensitive strains, together with the supply of susceptible host tissue and the rate of application and effects of the fungicide, we show that it is possible to identify thresholds for the invasion of fungicide resistance in a parasite population and, hence, to derive criteria to minimize the risk of resistant forms becoming established. First, we present a deterministic formulation of the model as this allows a detailed analysis of any thresholds for fungicide resistance to develop. Because a deterministic description often breaks down when populations are at very low levels (Anderson & May 1991; Renshaw 1991), we also use a discrete, stochastic formulation of the model to examine the dynamics of resistance and, in particular, to obtain the probabilities that a parasite population becomes resistant and to investigate the rate of increase of resistance in the parasite population.

The analyses are applicable to a wide range of fungal pathogens and fungicides. Examples of pathogens that can be incorporated into the generic structure of the models include rusts, mildews, potato blight, *Septoria* spp. (leaf blotch of wheat), *Pseudocercospora herpotrichoides* (cereal eyespot) and *Rhynchosporium secalis* (barley leaf blotch). The models are motivated primarily for systemic fungicides which include benzimidazoles, the demethylase inhibitor (DMI) and morpholine groups of ergosterol biosynthesis inhibitors, hydroxypyrimidines and strobilurins (see, for example, Parry 1990).

2. THE MODEL

The full model for the population dynamics of fungicide resistance has two components, one describing the host-parasite interactions and the second the dynamics of the fungicide. We present both deterministic and stochastic formulations of the host-parasite dynamics, while the effects of the fungicide are treated as a time-dependent input to the host-parasite system.

(a) Host-parasite dynamics

The model describes the dynamics of a host-parasite system in which there are two strains of the parasite, one

sensitive to the fungicide and the other resistant to it. As such it reflects the dynamics of a parasite with clonal reproduction where there is no recombination. This is a reasonable starting point since most cases of evolved resistance have occurred in haploid fungi, where asexual reproduction is dominant in their life histories, in some cases to the exclusion of sexual reproduction (Milgroom *et al.* 1989).

(i) Deterministic formulation

The deterministic formulation of the model is given by the following system of linked differential equations:

$$\begin{aligned}\frac{dH}{dt} &= rH \left(1 - \frac{H}{K}\right) - (e_b(C)b_1P_1 + b_2P_2)H, \\ \frac{dP_1}{dt} &= e_g(C)g_1e_b(C)b_1HP_1 - \frac{\mu_1}{e_\mu(C)}P_1, \\ \frac{dP_2}{dt} &= g_2b_2HP_2 - \mu_2P_2,\end{aligned}\quad (1)$$

where H is the host density, P_1 and P_2 are the densities of the sensitive and resistant strains of the parasite, respectively, and C is the fungicide concentration (see below). Host population growth in the absence of parasitism is described by the logistic equation with rate r and carrying capacity K . The b_i s are the infection rates, the g_i s are the numbers of parasites produced per host infected and the μ_i s are the death rates for each strain. In the model, the parasite generation time ($1/\mu_i$) provides a natural time-scale for the population dynamics. The $e_i(C)$ s represent the proportionate change in the parasite parameter i and can be conveniently summarized using the functional form $e_i(C) = 1/(1 + a_iC)$. The proportionate reduction in each parameter increases from no effect in the absence of control ($e_i(0) = 1$) to complete inhibition of a process at high fungicide concentrations ($e_i(C)$ tends to zero as C becomes large). In addition to examining the changes in the absolute densities of the sensitive and resistant parasite strains, it is often convenient to consider the frequency of resistance in the parasite population, $p(t) = P_2/(P_1 + P_2)$ (Milgroom *et al.* 1989; see also §4). A summary of the variables and parameters used in the model is given in table 1.

(ii) Stochastic formulation and implementation

In the stochastic version of the model, the population sizes H , P_1 and P_2 take integer values and the transitions (for example, births, deaths and infections) are stochastic processes as summarized in table 2. The number of transitions of each type which occurs during a small time-interval δt is drawn from a binomial distribution $B(n, \theta)$ where n is the population size and θ is the probability that an individual is affected by that particular process (the appropriate per capita rate from the system of equations (1) multiplied by δt ; table 2). However, binomial random variables are expensive to simulate and an approximating distribution is used wherever possible. When one of the following three conditions is satisfied, (i) $n\theta(1 - \theta) > 25$, (ii) $n\theta(1 - \theta) > 5$ and $0.1 < \theta < 0.9$, or (iii) $n\theta > 10$ and $n(1 - \theta) > 10$, an approximating normal variate with mean $n\theta$ and variance $n\theta(1 - \theta)$ is used, while if $\theta < 0.1$ and $n\theta < 10$, an approximating Poisson

Table 1. *Summary of variables and parameters*

symbol	description
variables	
H	host density
P_1	density of parasites sensitive to the fungicide (strain 1)
P_2	density of parasites resistant to the fungicide (strain 2)
p	frequency of resistance in the parasite population ($P_2/(P_1 + P_2)$)
C	fungicide concentration
parameters	
r	host growth rate
K	host carrying capacity
b_i	infection rate of hosts by parasite strain i ($i = 1, 2$)
g_i	number of parasites of strain i produced per host infected
μ_i	death rate of parasite strain i
$e_i(C)$	proportionate reduction in parasite parameter i due to the fungicide effects ($i = b, g$ or μ)
E_i	proportionate reduction in parasite parameter i with constant fungicide levels
a_i	measure of how much the fungicide inhibits parasite process i
Γ	amount of fungicide applied per spray
ν	fungicide decay rate
T	time between fungicide applications

variate with mean $n\theta$ is used (Evans *et al.* 1993; Swinton & Gilligan 1999).

(b) *Fungicide dynamics*

The effects of the fungicide are explicitly incorporated in the model for the host–parasite dynamics and are assumed to depend on the fungicide concentration C . Consequently, a model for the dynamics of the fungicide is needed to complete the description of the dynamics of resistance. We consider two regimes of fungicide usage. In the first case, the fungicide levels are treated as constant so that $e_i(C) = E_i$ where the E_i s are constants satisfying $0 < E_i \leq 1$ (cf. the effects of control in Bonhoeffer & Nowak (1997) and Bonhoeffer *et al.* (1997a)). This is the simplest assumption and allows detailed analytical results to be obtained. In reality, however, fungicide is sprayed periodically during a season, in which case the dynamics of the fungicide are described by

$$\frac{dC}{dt} = -\nu C, \quad C(nT^+) = C(nT^-) + \Gamma \quad (2)$$

where the plus and minus superscripts denote the fungicide concentrations before and after the n th application, respectively, T is the time-period between applications, Γ is the amount of fungicide applied and ν is the loss rate due to weathering and general inactivation.

3. POPULATION DYNAMICS IN THE ABSENCE OF CONTROL

In the absence of control ($e_i(C) = 1$) and when only a single strain is present, the parasite population dynamics

are determined by the basic reproductive number for the strain, R_{0i} ($i = 1, 2$), where

$$R_{0i} = \frac{g_i b_i K}{\mu_i}. \quad (3)$$

If $R_{0i} < 1$ the parasite is eliminated and the host population grows to its carrying capacity. Conversely, if $R_{0i} > 1$ the host and parasite populations tend to a globally stable coexistence equilibrium.

When both parasite strains are present, the population dynamics depend on the relative magnitudes of the strain basic reproductive numbers: essentially the strain with the larger $R_{0i} > 1$ eliminates the other strain from the system. In the model, interaction between the strains is restricted to competition for available infection sites. The strain with the larger R_{0i} is able to infect more sites and, consequently, the other strain is unable to produce sufficient new infections to sustain itself. However, competitive exclusion may only take place over a very long time-scale and, moreover, will be affected by factors not included in the model such as spatial heterogeneity and the ability of the strains to persist between seasons.

In the subsequent analysis we assume that, in the absence of control, the basic reproductive number for the sensitive strain ($R_{01} = g_1 b_1 K / \mu_1$) is greater than or equal to that of the resistant strain ($R_{02} = g_2 b_2 K / \mu_2$), so that resistance may carry some fitness cost (see, for example, Georgopoulos 1987). Moreover, we assume that the basic reproductive numbers for both strains exceed unity so that either strain can persist in the absence of the other (i.e. $R_{01} \geq R_{02} > 1$).

4. TIME-VARYING SELECTION PRESSURE

A measure of the selection pressure on the parasite population is obtained by examining the dynamics of the frequency of resistance $p(t)$. In the deterministic formulation the rate of change of the frequency of resistance is given by

$$\frac{dp}{dt} = s(t)p(1-p),$$

where

$$s(t) = \mu_2 \left(R_{02} \frac{H(t)}{K} - 1 \right) - \frac{\mu_1}{e_\mu(C)} \left(e_b(C) e_g(C) e_\mu(C) R_{01} \frac{H(t)}{K} - 1 \right) \quad (4)$$

is the time-varying selection coefficient (Crow & Kimura 1970; Milgroom *et al.* 1989). Importantly, the selection coefficient (equation (4)) can be positive, indicating selection for resistance (i.e. an increase in the frequency of resistance) or negative, indicating selection against resistance (i.e. a decrease in the frequency of resistance).

The dependency of the selection coefficient on the host density H reflects the effects of competition between the sensitive and resistant parasite strains. More important is the dependence of the selection coefficient on the fungicide concentration C . When the fungicide is applied periodically, the selection coefficient fluctuates, with strong selection pressure for resistance (i.e. positive s) when the

Table 2. *Transitions and probabilities for the stochastic model for fungicide resistance*

(The total number of transitions of each type that occur in a small time-interval δt is drawn from a binomial distribution $B(n, \theta)$, where n is the population size and θ is the probability. Under certain conditions, approximating distributions are used for computational efficiency (see §2(a)(ii)).)

description	transition	population size	probability
host birth	$H \rightarrow H + 1$	H	$r(1 - H/K)\delta t$
host infection by parasite strain 1	$H \rightarrow H - 1$	H	$e_b(C)b_1P_1\delta t$
host infection by parasite strain 2	$H \rightarrow H - 1$	H	$b_2P_2\delta t$
parasite birth (strain 1)	$P_1 \rightarrow P_1 + 1$	P_1	$e_g(C)g_1e_b(C)b_1H\delta t$
parasite death (strain 1)	$P_1 \rightarrow P_1 - 1$	P_1	$(\mu_1/e_\mu(C))\delta t$
parasite birth (strain 2)	$P_2 \rightarrow P_2 + 1$	P_2	$g_2b_2H\delta t$
parasite death (strain 2)	$P_2 \rightarrow P_2 - 1$	P_2	$\mu_2\delta t$

Figure 1. Threshold for the development of fungicide resistance for the deterministic model (equation (1)) with constant fungicide levels ($e_i(C) = E_i$). The figure shows the dependence of the threshold (equation (5)) on the effectiveness of control ($\varepsilon = E_bE_gE_\mu$) and the relative fitness of the resistant strain ($\rho = R_{02}/R_{01}$).

fungicide levels are high, and strong selection pressure against resistance (i.e. negative s) when the fungicide levels are low. Consequently, it is possible that a balanced polymorphism could be maintained in the parasite population with both sensitive and resistant strains coexisting (see §5(b); Leonard & Czochor 1980).

5. THRESHOLDS AND THE PROBABILITY OF RESISTANCE DEVELOPING

To address the question of whether or not the frequency of resistance can increase within the parasite population, we consider the ability of a parasite resistant to the fungicide to invade a parasite population wholly sensitive to the fungicide when it is introduced at low densities (for example, through mutation or migration). In the deterministic formulation, this is investigated by calculating the invasion exponent for the resistant parasite strain. This is essentially its intrinsic growth rate when introduced to a wholly sensitive parasite population (Metz *et al.* 1992; Rand *et al.* 1994) and is found by examining the stability of the sensitive parasite equilibrium (that is, where the host and sensitive parasite populations have a positive density).

In the stochastic formulation, the probability of resistance developing was determined by simulating 20 replicates of the model and counting the number that reached a critical frequency of resistance. Twenty replicates were used to show the underlying qualitative dynamics of resistance. Each simulation was run until one of four things happened: (i) the frequency of resistance reached a critical level, (ii) the resistant strain was eliminated from the parasite population, (iii) the entire parasite population died out, or (iv) a certain length of time had elapsed. However, almost all simulations stopped because either case (i) or (ii) occurred. In practice, the probability of reaching a critical frequency of resistance is unchanged once the frequency exceeds a certain level ($p > 0.01$). Accordingly, we arbitrarily set the critical frequency of resistance at 50%. The results would not change qualitatively for other frequencies though the time to achieve the frequency would.

(a) *Constant fungicide levels*

When the fungicide levels are constant, the parasite population always becomes wholly resistant (i.e. the resistant strain eliminates the sensitive one) provided that

$$E_bE_gE_\mu < \frac{R_{02}}{R_{01}}, \quad (5)$$

where the product of the E_i s represents the cumulative effects of the fungicide and the R_{0i} s are the basic reproductive numbers for each strain defined in equation (3) (figure 1) (cf. Austin *et al.* 1997; Bonhoeffer & Nowak 1997; Bonhoeffer *et al.* 1997). If equation (5) does not hold, the fungicide-sensitive strain eliminates the resistant one (figure 1). The left-hand side of equation (5) is a measure of the effectiveness of control ($\varepsilon = E_bE_gE_\mu$) which ranges from $\varepsilon = 0$ representing complete control to $\varepsilon = 1$ representing no control. The right-hand side of equation (5) is the relative fitness of the resistant strain ($\rho = R_{02}/R_{01}$).

Simulations of the stochastic model also show that there is a threshold below which the probability of resistance developing is zero (figure 2). Above the resistance threshold, the probability of resistance developing within a parasite population increases gradually from zero to a maximum of one (figure 2) and is affected by both the initial frequency of resistance (cf. figure 2a,b) and the parasite generation time ($1/\mu_2$; cf. figure 2a,c). Introducing the resistant strain during the transient phase of an epidemic

Figure 2. Probability of half the parasite population developing resistance in the stochastic model for fungicide resistance (table 2) with constant fungicide levels ($e_i(C) = E_i$). Each figure shows the dependence of the probability on the effectiveness of control ($\varepsilon = E_b E_g E_\mu$) and the relative fitness of the resistant strain ($\rho = R_{02}/R_{01}$). The scale bar indicates the probability of resistance developing. (a–c) The resistant strain is introduced to the sensitive parasite population at equilibrium ($H = K/R_{01}$ and $P_1 = (r/b_1)(1 - 1/R_{01})$): (a) Introduction at equilibrium: default parameters with $P_2(0) = 10$. (b) Reduced initial frequency of resistance: default parameters with $P_2(0) = 2$. (c) Increased parasite generation time and reduced sporulation ($\mu_1 = \mu_2 = 0.3$ and $g_1 = 40.0$) with $P_2(0) = 10$. (d) The resistant strain is introduced under non-equilibrium conditions ($H = K/2$, $P_1 = 10\,000$ and $P_2 = 10$): default parameters. The plots are based on the simulation of 20 replicates of the model using a step size of $\delta t = 10^{-4}$. The default host parameters are $r = 1.0$ and $K = 1000$ and the default parasite parameters are $b_1 = b_2 = 0.00006$, $\mu_1 = \mu_2 = 3.0$, $g_1 = 400.0$ and $g_2 = 200.0$. Changes in the relative fitness are incorporated by letting $g_2 = \rho g_1$ where $0 \leq \rho \leq 1$ is the relative fitness (R_{02}/R_{01}). The fungicide parameters are $E_b = 1.0$ and $E_g = E_\mu = \sqrt{\varepsilon}$ where $0 < \varepsilon \leq 1$ is the effectiveness of control ($E_b E_g E_\mu$).

(i.e. under non-equilibrium conditions) does not alter the probability of resistance developing (cf. figure 2a,d).

(b) Periodically applied fungicide

No analytical results have been obtained when fungicide is applied periodically. However, simulations of both the deterministic and stochastic systems show that, for a given level of the effectiveness of control ($\varepsilon = e_b e_g e_\mu$), resistance develops only if the relative fitness of the resistant parasite ($\rho = R_{02}/R_{01}$) is sufficiently high (figures 3a and 4a; cf. figure 1). Moreover, there are threshold values for the fungicide decay rate (ν), the amount of fungicide applied (I) and the period between fungicide applications (T) for resistance to develop (figures 3b–d and 4b–d). In the stochastic model, the probability of resistance developing above the threshold increases rapidly from zero to one (figure 4). Simulations of the deterministic model confirm the prediction based on considering the selection coefficient (equation (4)) that it is possible for the sensitive and resistant strains to coexist (figure 3) and that this polymorphism may be balanced and, hence, persist in the long term (Leonard & Czocho 1980). However, stochastic simulations did not reveal coexistence and, hence, balanced polymorphism even when the number of replicates is increased substantially.

6. RATE OF DEVELOPMENT OF RESISTANCE

For both constant fungicide levels and periodically applied fungicide, the rate of development of fungicide resistance follows a similar pattern (figure 5). Close to the resistance threshold (i.e. where the probability of resistance developing is zero; figures 2 and 4), resistance develops at a relatively slow rate, taking, on average, many generations for the parasite population to reach the critical frequency of resistance (for example, 20–80 generations for $\rho = 0.8$ and $\varepsilon > 0.6$; figure 5). In practice, the frequency of resistance is unlikely to reach the critical level for many seasons. Beyond the threshold, the rate of development of resistance increases with, on average, fewer generations taken to reach the critical frequency (for example, zero to ten generations for $\rho = 0.8$ and $\varepsilon < 0.6$; figure 5). The variance in the time taken to reach the critical frequency of resistance is relatively small, but increases close to the resistance threshold (figure 5). Increasing the parasite generation time has little effect on the number of generations taken to reach the critical frequency of resistance (cf. figure 5a,b), though the absolute time taken to reach the critical frequency is longer. When the fungicide is applied periodically, the critical frequency of resistance

Figure 3. Thresholds for the development of fungicide resistance for the deterministic model (equation (1)) with periodically applied fungicide (described by equation (2) with $e_i(C) = 1/(1 + a_i C)$). (a) Dependence on the effectiveness of control at the time-averaged fungicide level ($\varepsilon = e_b(C_{\text{avg}})e_g(C_{\text{avg}})e_\mu(C_{\text{avg}})$ where $C_{\text{avg}} = \Gamma/\nu T$) and the relative fitness of the resistant strain ($\rho = R_{02}/R_{01}$). No results were obtained to the left of the dotted line due to problems with the simulations. (b) Dependence on the fungicide decay rate (ν) and the amount of fungicide applied (Γ). (c) Dependence on the period between applications (T) and the amount of fungicide applied (Γ). (d) Dependence on the fungicide decay rate (ν) and the period between applications (T). Each figure was calculated numerically by simulating the model for 250 time units. The default host and parasite parameters (including the relative fitness) are as given in figure 2. The default fungicide parameters are $\Gamma = 3.0$, $\nu = 0.5$, $T = 10.0$, $a_b = 0.0$, $a_g = 0.25$ and $a_\mu = 1.0$. Changes in the effectiveness of control are incorporated by letting $a_g = a_\mu = (wT/\Gamma)(1/\sqrt{\varepsilon} - 1)$ where $0 < \varepsilon \leq 1$ is the effectiveness of control ($e_b e_g e_\mu$). The initial populations are $H = K/2$, $P_1 = 10\,000$ and $P_2 = 10$.

is reached more quickly than for constant fungicide levels (cf. figure 5a,c).

For constant fungicide levels the number of parasite generations taken to reach the critical frequency is approximately constant and varies little with the parasite generation time ($1/\mu_2$) (figure 6a). For periodically applied fungicide, the relationship between the generation time and the number of parasite generations taken to become resistant is more complex. For short generation times, resistance develops relatively quickly (in one or two generations; figure 6b). However, as the generation time increases there is a marked transition from a rapid to a more gradual development of resistance (figure 6b).

7. DISCUSSION

In this paper, we have introduced a simple nonlinear model for fungicide resistance in botanical epidemics

which demonstrates the existence of thresholds for the invasion or elimination of resistant strains in the parasite population. By including parameters for the amount applied, longevity and application frequency of the fungicide, these elaborations provide a mathematical framework for analysing the risk of fungicide resistance developing.

(a) Resistance to chemical control in general

Resistance to chemical control is a widespread problem in many disciplines, including antibiotics (Cohen 1992; Bonhoeffer *et al.* 1997b), antiviral drugs (Bonhoeffer *et al.* 1997a), fungicides (de Waard *et al.* 1993) and herbicides (Warwick 1991). Our model shares a common generic structure with models for antiviral drug resistance (Bonhoeffer & Nowak 1997; Bonhoeffer *et al.* 1997a) and antibiotic resistance (Massad *et al.* 1993; Austin *et al.* 1997; Bonhoeffer *et al.* 1997b) and, consequently some of the

Figure 4. Probability of half the parasite population becoming resistant in the stochastic model for fungicide resistance (table 2) with periodically applied fungicide (described by equation (2) with $e_i(C) = 1/(1 + a_i C)$). The scale bar indicates the probability of resistance developing. (a) Dependence on the effectiveness of control at the time-averaged fungicide level ($\varepsilon = e_b(C_{\text{avg}})e_g(C_{\text{avg}})e_\mu(C_{\text{avg}})$ where $C_{\text{avg}} = \Gamma/\nu T$) and the relative fitness of the resistant strain ($\rho = R_{02}/R_{01}$). (b) Dependence on the fungicide decay rate (ν) and the amount of fungicide applied (Γ). (c) Dependence on the period between applications (T) and the amount of fungicide applied (Γ). (d) Dependence on the fungicide decay rate (ν) and the period between applications (T). The plots are based on the simulation of 20 replicates of the model using a step size of $\delta t = 10^{-4}$. The default host and parasite parameters (including the relative fitness) are as given in figure 2 and the default fungicide parameters and initial populations are as given in figure 3.

results are of broad applicability. Indeed, threshold conditions similar to that given in equation (5) have been derived for models of antibiotic (Austin *et al.* 1997) and antiviral drug (Bonhoeffer & Nowak 1997; Bonhoeffer *et al.* 1997a) resistance. However, the present work extends these models in a number of ways. First, it takes explicit account of the dynamics of the chemical control agent and the host population. These features of the model are transferable to herbicide resistance and to consideration of the within-host dynamics of resistance to antibiotics or antiviral drugs. Second and most importantly, the model allows for demographic stochasticity in the population dynamics. This has been largely overlooked in models of resistance, but our results imply stochasticity can be of fundamental importance in determining whether or not resistance develops.

(b) *Thresholds and the probability of resistance developing*

Most previous models of fungicide resistance have predicted that the invasion of resistance, once it arises, is inevitable (Skylakakis 1983; Milgroom *et al.* 1989; Milgroom 1990). In contrast, both the deterministic and stochastic formulations of our model show that there is a threshold below which resistance cannot develop within the parasite population. This threshold depends essen-

tially on two quantities: the relative fitness of the resistant strain ($\rho = R_{02}/R_{01}$) and the effectiveness of control ($\varepsilon = e_b e_g e_\mu$) (figures 1, 2, 3a and 4a). The threshold reflects the inherent nonlinearity of the host–parasite interactions as the two parasite strains compete for available infection sites within a changing supply of host tissue. They are absent in conventional models which only focus on the parasite dynamics and essentially ignore the epidemiological framework imposed by the host dynamics. Skylakakis (1983) and others (Barrett 1983; Milgroom *et al.* 1989) introduced Lotka–Volterra models for competition between the parasite strains. However, these models do not reflect the dynamic constraints imposed by host growth and, therefore, do not yield the thresholds that emerge in the hybrid model linking the epidemiology and population dynamics of the parasite strains.

Important differences emerge between the deterministic and stochastic versions of the models in the behaviour around the thresholds. In the deterministic formulation, the resistance threshold marks a sharp transition from failure of the resistant strain to invade to the parasite population becoming wholly resistant (figures 1 and 3). In the stochastic model, it marks a change from definite elimination of the resistant strain below the threshold to a finite probability of invasion which increases above the threshold (figures 2 and 4). This

Figure 5. Number of parasite generations taken for half the parasite population to become resistant to the fungicide in the stochastic model for fungicide resistance (table 2). (i) Density plots of the mean number of generations to 50% resistance and its dependence on the effectiveness of control ($\varepsilon = e_b e_g e_\mu$) and the relative fitness of the resistant strain ($\rho = R_{02}/R_{01}$). (ii) Cross-sections of the density plots (including standard errors) when the relative fitness is $\rho = 0.8$. (a, b) Constant fungicide levels ($e_i(C) = E_i$). (a) Default parameters and (b) increased generation time and reduced sporulation ($\mu_1 = \mu_2 = 0.3$ and $g_1 = 40.0$). (c) Periodically applied fungicide (described by equation (2) with $e_i(C) = 1/(1 + a_i C)$); default parameters. The plots are based on the simulation of 20 replicates of the model using a step size of $\delta t = 10^{-4}$. The scale bar indicates the mean number of parasite generations taken to reach the critical frequency of resistance, but in each figure white indicates that no replicates developed resistance. The default parameters (including relative fitness) and initial conditions are as given in figure 2d (constant fungicide levels) and figure 3 (periodically applied fungicide).

difference arises because there is always a risk of elimination of the resistant strain independent of its fitness when it is rare in the parasite population.

(c) *Time-varying selection pressure*

The selection pressure on the resistant strain varies over time (see equation (4)). This reflects the effects of competition between the parasite strains where changes in the selection pressure occur due to changes in the host density. More importantly, the selection pressure also varies because of changes in the selection pressure due to periodic applications of the fungicide. The strong selection pressure for resistance at high fungicide concen-

trations and the strong selection pressure against resistance at low fungicide concentrations imply that a balanced polymorphism could be maintained in the parasite population. Although there is evidence from the deterministic model for coexistence of the parasite strains (figure 3), this is not supported by the stochastic model. Increasing the numbers of replicate simulations did not reveal coexistence, with one or other strain being eliminated at low population densities or frequencies.

Much earlier work on time-varying selection has focused on deterministic models of the population genetics of diploid organisms with discrete generations (Crow & Kimura 1970). In this case, polymorphism can

Figure 6. Effect of the generation time on the number of parasite generations taken for half the parasite population to become resistant to the fungicide in both the deterministic (solid line) and stochastic (open circles with standard error bars) formulations. (i) Dependence of the number of parasite generations to 50% resistance on the parasite generation time. (ii) Dependence of the total time to 50% resistance on the parasite generation time. The absolute parasite fitness (R_{0i}) is kept constant by varying the sporulation rate g_i . (a) Constant fungicide levels ($e_i(C) = E_i$). (b) Periodically applied fungicide (described by equation (2) with $e_i(C) = 1/(1 + a_i C)$). The plots are based on the simulation of 20 replicates of the model using a step size of $\delta t = 10^{-4}$. The effectiveness of control ($e_i e_g e_\mu$) is set to $\varepsilon = 0.3$ and the relative fitness (R_{02}/R_{01}) is set to $\rho = 0.8$. The default parameters (including the relative fitness) and initial conditions are as given in figure 2d (constant fungicide levels) and figure 3 (periodically applied fungicide).

be maintained in the population provided the arithmetic mean of the relative fitness is greater than one and the geometric mean of the relative fitness is less than one. However, this result applies only to discrete generations and, more importantly, ignores the possibility of the elimination of an allele due to chance events when it is rare, as occurs in the fungicide resistance model.

(d) Generation time and the rate of development of resistance

Plant parasites vary considerably in their generation times, from rusts which can pass through 30 generations within a season to smuts which have only one. Consequently, it is important to analyse the influence of the generation time on the rate of development of resistance. For constant fungicide levels, the number of parasite generations taken for resistance to develop varies little with the generation time (figure 6a). This arises largely because each parasite generation experiences a constant

selection pressure and, hence, the population takes the same number of generations to develop resistance. However, when fungicide is applied periodically the selection pressure on the parasite population varies with time (see §4) and an interaction is evident between the generation time ($1/\mu_2$) and the period of application (T) (figure 6b), though detailed analysis did not reveal a simple relationship between them. However, there is a marked change in the number of generations needed to achieve 50% resistance between short and longer generation times (figure 6b). Both the mean number of generations needed to achieve 50% resistance and the variance associated with the stochastic realizations were surprisingly small. Studies of insecticide resistance have also examined the effect of the generation time on the rate of development of pesticide resistance (see, for example, Anderson & May 1991; Rosenheim & Tabashnik 1991), but much of this work is for discrete, non-overlapping generations or for diploid organisms with intermediate expression of

resistance in heterozygotes and the results are not comparable with the analysis here.

(e) *Resistance management*

The fungicide decay rate (ν), the amount of fungicide applied (I) and the period between applications (T) affect the effectiveness of control (ε). Consequently, they influence whether or not the frequency of resistance increases (figures 3*b–d* and 4*b–d*) and the time taken to achieve a critical frequency of resistance (figure 5). The effect of the application rate on the development of resistance is of particular interest where there is controversy over whether reducing the amount of fungicide applied will delay or accelerate the development of resistance (Birch & Shaw 1997). Our model implies that reducing the amount of fungicide applied decreases the probability of resistance developing (figure 4*b,c*) and delays the development of resistance should it occur (cf. figure 5). Deployment of this strategy would have to be discounted against the risk of reduced yield and ultimately demands a formal optimization of the long-term risk of fungicide resistance with the short-term risk of yield loss due to incomplete control (Munro 1997).

All three fungicide parameters (T , I and ν) are amenable to control by the grower or by coordinating the activity of a population of growers. Providing crude estimates of the effectiveness of control (ε) and relative fitness (R_{02}/R_{01}) are available, the results can be used to predict the consequences of changing the fungicide decay rate (ν), the period between applications (T) and the amount applied (I) on invasion. We envisage that crude relative estimates for the parameters for the transmission (b_i), production (g_i) and death (μ_i) of inoculum may be available from laboratory and small plot experiments which will allow computation of the relative fitness R_{02}/R_{01} . Some evidence that resistance to a fungicide imposes a fitness cost on a pathogen is collated in the review by Georgopoulos (1987). More recently, evidence of a fitness cost due to resistance has been reported for dicarboximide–benzimidazole on *Botrytis* spp. (Hsiang & Chastagner 1991), metalaxyl on *Phytophthora infestans* (Kadish & Cohen 1992), propiconazole on *Septoria nodorum* (Schmidt 1995) and *Sclerotinia homeocarpa* (Hsiang *et al.* 1998), and imazalil on *Penicillium digitatum* (Holmes & Eckert 1995). Similarly, laboratory and plot experiments may allow computation of the effectiveness of control so that managers can estimate the risk of invasion and the proportion of sites at which this might be expected to occur.

The models introduced and analysed in this paper essentially describes the population dynamics of fungicide resistance within a single season in the absence of any population genetic effects (i.e. mutation and recombination) and at a spatial scale at which homogeneous mixing is an appropriate description of the transmission dynamics. Greater biological realism can be achieved by extending the models to account for some or all of these features. Each of the above extensions will form the basis for future work aiming to provide a better understanding of the population-level mechanisms which influence the development of fungicide resistance.

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