A Stochastic Model for the Initial Occurrence and Development of Fungicide Resistance in Plant Pathogen Populations

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ABSTRACT

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A stochastic model was developed to relate pathogen population size, the probability of fungicide-resistant individuals (R) occurring, and the strength of selection for resistance (fungicide efficacy). The model is based on binomial probabilities that R individuals are present in the initial population of size N_0 or occur during an epidemic after selective fungicide is applied. If an R mutant occurs early in the epidemic, the final frequency (q_t) and number of R (N_R) increase to higher levels than if the mutation occurs later. The probability of q_t reaching some given value is always higher when N_0, the pathogen growth rate (r), or mutation rate to resistance (p) is large. The effects of fungicide efficacy ($1 - \alpha$) depend on the other parameters. When N_0 is small, high values of $1 - \alpha$ result in lower probabilities of R occurring. As N_0 increases, this trend is reversed. These results suggest that intensive use of fungicides may be a rational means of resistance management when population sizes are initially small. However, caution must be employed in applying these results to real systems.

Three epidemiological principles that describe the dynamics of resistance buildup in pathogen populations recently have been summarized (15). These principles are that the frequency of resistance will build up more slowly if the initial frequency of resistance is reduced, the apparent infection rates of both fungicide-resistant and fungicide-sensitive genotypes (r_R and r_S, respectively) are reduced, and r_R is reduced relative to r_S. Most discussion of management tactics has focused on reducing apparent infection rates (15). Reduction of initial frequency has received less attention because little can be done to affect the frequency of resistance before a selective fungicide is used. Initial frequency is a function of random effects (9), mutation rates, and the fitness of resistant mutants (16), none of which can be affected directly by management tactics.

However, the interaction of population size and the rare occurrence of resistant mutants offer a management approach that may be useful in some circumstances. One way that initial frequency can be managed to some degree is to keep the population size so small that the probability of a resistant mutant occurring in that population is very low (8). Conceptually, the influence of population size on resistance is similar to the influence of population size on the probability of occurrence of particular pathogenic races (2,9). The size of pathogen populations has been identified as an important factor to consider in designing fungicide resistance management strategies (4,13). The recommendation not to use acylalanine fungicides in a curative manner (20,21) may have been derived from this reasoning; it was after curative use of metalaxyl on large pathogen populations that resistance first appeared (see 20).

If resistance is more likely to occur in a large rather than a small population, a potential strategy for fungicide resistance management is to keep the population size small by using a selective fungicide intensively (and extensively). This strategy has been used for management of resistance to metalaxyl in *Peronospora tabacina* Adam (which causes tobacco blue mold) and *Bremia lactucae* Regel (which causes lettuce downy mildew) (4,5,17). Crute (4) claimed that the appearance of metalaxyl resistance in *Bremia* probably was delayed because of the extensive use of metalaxyl throughout lettuce-growing areas of the United Kingdom. Similarly, there have not been any reports of metalaxyl

resistance in *P. tabacina* in the United States where metalaxyl has been used in this way (17). This approach initially appears contrary to conventional thinking and contrary to strategies that limit the number of fungicide applications (13,15,21). Intensive use of selective fungicide is appropriate only if resistant mutants are not present. Otherwise, intensive use of fungicides will select rapidly for resistance. However, intensive use strategies may be worth considering if the risks of occurrence and buildup of resistance can be evaluated adequately. An evaluation of these risks requires an understanding of the relationships among population size, probability of resistance occurring, and the strength of selection.

Current theory for fungicide resistance dynamics does not accommodate the fact that sometimes no resistant individuals are present in a population. Theoretical models for fungicide resistance (3,6,10–12,15), which are based almost exclusively on selection, will make qualitatively incorrect predictions for small populations in which resistant mutants are not present. All of these models assume that resistance is initially at some low frequency greater than zero (ranging from 10^{-12} to 10^{-5}), and they predict the increase in the frequency of resistance when selective fungicides are applied. If no resistant individuals are present, then the frequency of resistance cannot increase regardless of the potential strength of selection. However, even if there are no resistant individuals initially present, they might arise de novo during an epidemic by mutation as new individuals are added to the population. Again, the probability that a resistant mutant arises increases as the population size increases. To evaluate the importance of population size to the dynamics of fungicide resistance, the probability of resistance occurring, both initially and subsequently by mutation during an epidemic, must be incorporated into selection models.

The objectives of this report are twofold. First, I identify the relationships among population size, the probability of the occurrence of resistance, and selection. Second, I use the theory to evaluate the risks associated with intensive fungicide use as a resistance management strategy. I evaluate this strategy for both single- and multi-season management situations.

MODEL DEVELOPMENT

In this model, "individual" refers to a genetically homogeneous, single lesion. Mutations to resistance are assumed to occur only in the formation of inoculum before infection. For simplicity, there are only two phenotypes (or genotypes): resistant (R) and sensitive (S). Assume that each individual in a population of size N is independent and fungicide resistant with probability p, where p is the mutation rate to resistance. It is reasonable to assume that the number of resistant individuals, N_R, has a binomial distribution with parameters N and p, and, therefore,

$$Pr\{N_R = n\} = \binom{N}{n} p^n (1-p)^{N-n} \qquad \begin{matrix} n = 0, 1, 2,... \\ 0 < p < 1 \end{matrix} \qquad (1)$$

where $Pr\{N_R = n\}$ denotes the probability that the random variable N_R takes the value n. Therefore, the probability that resistant individuals are not present in a population of size N is:

$$Pr\{N_R = 0\} = (1-p)^N \approx e^{-Np} \qquad (2)$$

The approximation, e^{-Np}, is valid when N is "large" and p is "small," as might be expected for plant pathogen populations. The probability that resistant individuals are not present may be high, especially when N and p are both small (8). This model (equation 2) has limited utility: It can only give the probability that n resistant individuals are present.

A more valuable model is one that predicts the probability that the frequency or number of resistant individuals will increase to unacceptable levels due to selection by fungicide use during an epidemic. Such a model must include population growth for the sensitive and resistant types. Suppose the population is growing such that

$$N_t = N_{t-1}(1 + r) \qquad (3)$$

where N_t is the number of individuals at time t, and r is the average number of offspring per individual, per time unit. Equation 3 can be solved recursively to give:

$$N_t = N_0 (1 + r)^t \qquad (4)$$

where N_0 is the initial population size (at time 0). This same growth model applies to the sensitive and resistant types in the absence of selective fungicides. When a selective fungicide is used, assume that r is reduced to αr ($0 < \alpha < 1$) for the sensitive type; $1 - \alpha$ represents a measure of average fungicide efficacy during an epidemic. Fungicide efficacy is assumed to be a function of both dose and number of applications, in addition to the inherent nature of the chemical applied. The parameter α is also a measure of selection; that is, when α is small, sensitive individuals are inhibited more, and, therefore, selection for resistance is stronger (see 15). The growth equations for both types when a selective fungicide is applied are:

$$\begin{aligned} N_{R,t} &= N_{R,0}(1 + r)^t \\ N_{S,t} &= N_{S,0}(1 + \alpha r)^t \end{aligned} \qquad (5)$$

where N_S and N_R are the numbers of S and R individuals, respectively.

From equation 5, we can solve for q_t, the frequency of the resistant type at time t:

$$q_t = \frac{N_{R,t}}{N_{S,t} + N_{R,t}} \qquad (6)$$

However, unless a resistant mutant is present in the initial population, this frequency will equal zero until a mutant occurs sometime during an epidemic. If a single mutation occurs at time x, then selection will operate on the population from time x until some time t. The R subpopulation will increase from $N_{R,x} = 1$ to $N_{R,t} = (1 + r)^{t-x}$ at time t. Substituting for $N_{R,t}$ and $N_{S,t}$ in equation 6 yields:

$$q_t = \begin{cases} 0 & \text{for } t < x \\ \dfrac{(1+r)^{t-x}}{N_0(1+\alpha r)^t + (1+r)^{t-x}} & \text{for } t \geq x \end{cases} \qquad (7)$$

assuming that all individuals in the initial population, N_0, are sensitive. To correct for the loss of a single S individual by mutation to R, $(1 + \alpha r)^{t-x}$ must be subtracted from $N_{S,t}$. The corrected frequency for R is:

$$q_t = \frac{(1+r)^{t-x}}{[N_0(1+\alpha r)^t - (1+\alpha r)^{t-x}] + (1+r)^{t-x}} \quad \text{for } t \geq x \qquad (8)$$

The importance of this correction is greatest when N_0 is small.

To link this population growth and selection model (equations 5 and 8) to the probability of R occurring requires the prediction of the first occurrence of R in a population during an epidemic. This is done with the following reasoning. The probability that no R individuals occur by mutation in any given time interval (from equation 2) is:

$$Pr\{\text{No } R \text{ occurs in } (t-1, t)\} \approx e^{-\Delta N_t p} \text{ for } t = 1,2,... \qquad (9)$$

where $\Delta N_t = N_t - N_{t-1}$, the number of new individuals added to the population in the interval $(t - 1, t)$. It is assumed that mutations to resistance occur only in new individuals added to the population, which are independent and have an equal probability of mutating to resistance (thus satisfying the

requirements for equation 1). Let the random variable X be the time at which a resistant type first occurs in the population. The probability that R first occurs at time x equals the probability that no R occurred before x, multiplied by the probability that R occurred in the interval $(x-1, x)$. Thus,

$$Pr\{X=x\} = e^{-pN_{x-1}}[1-e^{-p(N_x-N_{x-1})}] \quad \text{for } x=1,2,... \quad (10)$$

This is similar to a geometric distribution except that the probability that R occurs changes in each time interval as the population increases. Substituting for N_x and N_{x-1} from equation 5, we obtain:

$$Pr\{X=x\} = e^{-pN_0(1+\alpha r)^{x-1}}[1-e^{-pN_0(1+\alpha r)^{x-1}\alpha r}] \quad (11)$$
$$\text{for } x=1,2,...t$$

$N_{R,t}$ and q_t are functions of the random variable X. Therefore, their expected values are:

$$E[N_{R,t}(X)] = \sum_{x=0}^{t} N_{R,t}(x)\ Pr\{X=x\} \quad (12)$$

$$E[q_t(X)] = \sum_{x=0}^{t} q_t(x)\ Pr\{X=x\} \quad (13)$$

which can be calculated from the definition of $N_{R,t}$ and equations 7 and 11. Expected values are the average outcomes that would be observed if the process were repeated many times.

MODEL RESULTS AND INTERPRETATION

The number and frequency of fungicide-resistant individuals in a population that is under selection depend on the time the first R mutant appears. If there is a single R individual at the start of the epidemic, then at time t, N_R will be $(1+r)^t$ regardless of the initial population size (assuming no density dependence). If R does not arise until some time x after the epidemic begins, N_R will be smaller, that is, $(1+r)^{t-x}$. Similarly, the frequency of R at time t, q_t, will be greater when R occurs early (small x) (Fig. 1). In contrast to N_R, however, q_t depends on the initial population size, N_0. When the same absolute numbers of R individuals are found with larger numbers of S individuals, q_t is smaller (Fig. 1).

This model can be used in at least three ways to understand the dynamics of fungicide resistance. First, it is possible to determine the probability of observing resistance at some given level at the end of an epidemic. Second, the expected outcomes can be found for the number and frequency of resistant individuals. Finally, this model can be used for analyzing multiple-year dynamics in addition to single seasons.

Probability of resistance development. The first application of this model is to find the probability that the frequency of R, q_t, reaches some given level or greater (for example, a detection threshold). Because q_t is a function of the time an R mutant first occurs, X (equation 8), and q_t decreases as X increases (for a given N_0, Fig. 1), then we need to find the probability that X is small enough to result in a value of q_t greater than or equal to the level of interest. More formally, if we want to know $Pr\{q_t \geq q^*\}$, where q^* is some threshold frequency, then we need to find $Pr\{X \leq x^*\}$, where x^* is the time that an R mutant must first occur to result in the frequency q^*. In general,

$$Pr\{q_t \geq q^*\} = Pr\{X \leq x^*\} = 1 - e^{-N_0 p} + \sum_{x=1}^{x^*} Pr\{X=x\} \quad (14)$$

where $1-e^{-N_0 p}$ is $Pr\{X=0\}$ and $Pr\{X=x\}$ is found from equation 11. This cumulative probability is higher for larger values of N_0 (Fig. 2).

To use this probability, consider a simple example. If we want to find the probability that q_t reaches 0.50 or greater, when $N_0 = 10^5$ (with $p = 10^{-6}$, $r = 0.20$, and $\alpha = 0.1$), we see, from Figure 1, that x must be 25 or less. From Figure 2, we can see that $Pr\{X \leq 25\}$ is 0.15 when $N_0 = 10^5$. Therefore, the probability that q_t reaches a frequency of 0.5 or greater is 0.15 for the parameter values in this example. The probability that q_t reaches

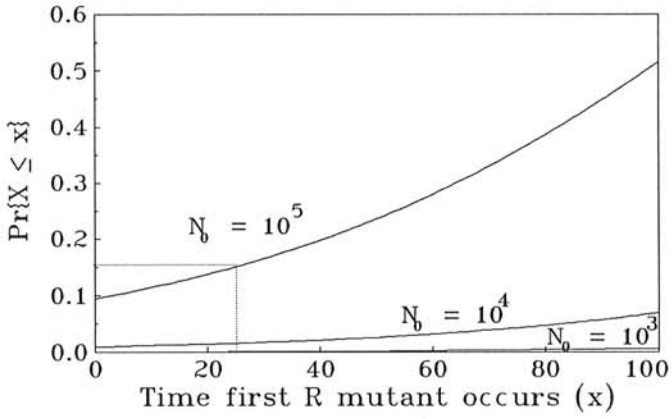

Fig. 2. The cumulative probability that a resistant mutant occurs on or before day x ($Pr\{X \leq x\}$) during a 100-day epidemic, for three initial population sizes (N_0). Parameter values are $p = 10^{-6}$, $r = 0.2$, $1-\alpha = 0.9$. The dotted lines illustrate an example described in the text.

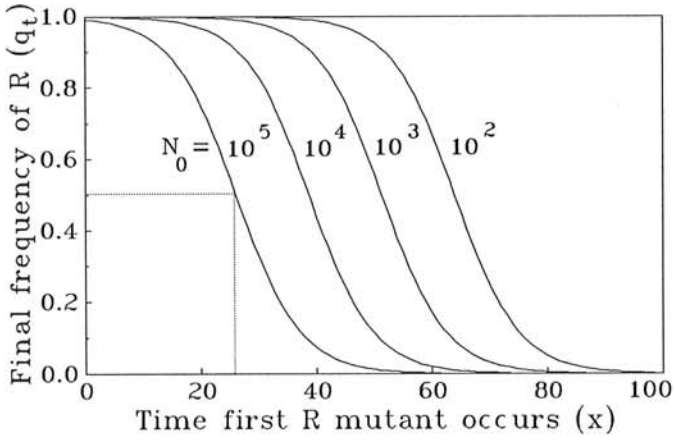

Fig. 1. The final frequency of fungicide resistance (q_t) at the end of a 100-day epidemic as a function of the time (x) the first resistant (R) mutant occurs for four initial population sizes (N_0). These curves were generated using equation 8 (see text). Parameter values for these curves were $p = 10^{-6}$, $r = 0.2$, and $1-\alpha = 0.9$. The dotted lines illustrate an example described in the text.

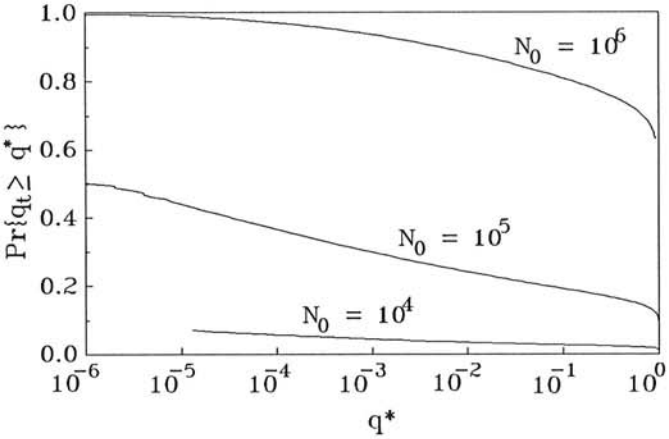

Fig. 3. The probability that the final frequency of fungicide resistance (q_t) reaches some given level (q^*) or greater by the end of a 100-day epidemic ($Pr\{q_t \geq q^*\}$). Probabilities are shown for three initial population sizes (N_0) plotted against q^* on a log scale; the curve for $N_0 = 10^3$ is too close to zero to show on this graph. Parameter values are $p = 10^{-6}$, $r = 0.2$, $1-\alpha = 0.9$.

q^* or greater can be shown directly by plotting $Pr\{X \leq x^*\}$ (which equals $Pr\{q_t \geq q^*\}$, equation 14) against q^* (Fig. 3). The probability that q_t reaches q^* or greater is always lower when N_0 is smaller (Fig. 3). This is because the probability of mutants arising early in an epidemic is much lower in small populations (Fig. 2), and therefore the final frequency is less likely to increase as much.

The effects of fungicide efficacy, $1 - \alpha$ (and, hence, selection), are less intuitive than those for N_0. When $1 - \alpha$ is large (highly effective fungicide on S subpopulation), the population is kept smaller than when $1 - \alpha$ is small; therefore, R mutants are less likely to arise (equation 2). However, if an R mutant arises, large values of $1 - \alpha$ mean that selection is more intense, and the frequency of R will increase more rapidly than if $1 - \alpha$ is small (equation 8). Therefore, the probability that q_t reaches q^* may be lower for large $1 - \alpha$ (high efficacy and selection) in some circumstances, but may be higher in others. This variable effect of efficacy is illustrated in Figure 4. For parameter values used in Figure 4A, the probability that q_t increases to 10^{-3} is higher when $1 - \alpha = 0.50$ (moderate efficacy) than when $1 - \alpha = 0.90$ (high efficacy); the reverse is true for the probability that q_t reaches 10^{-1}. For smaller values of N_0, higher efficacy always is associated with a lower probability of q_t increasing to q^* (except for values of q^* close to 1.0, data not shown). This means that, for small populations, the greater the efficacy of the fungicide (large $1 - \alpha$), the lower the probability that q_t will reach detectable levels (or greater). Conversely, high efficacy has an equal or higher probability of R occurring and increasing to q^* than moderate efficacy when N_0 is larger (Fig. 4B).

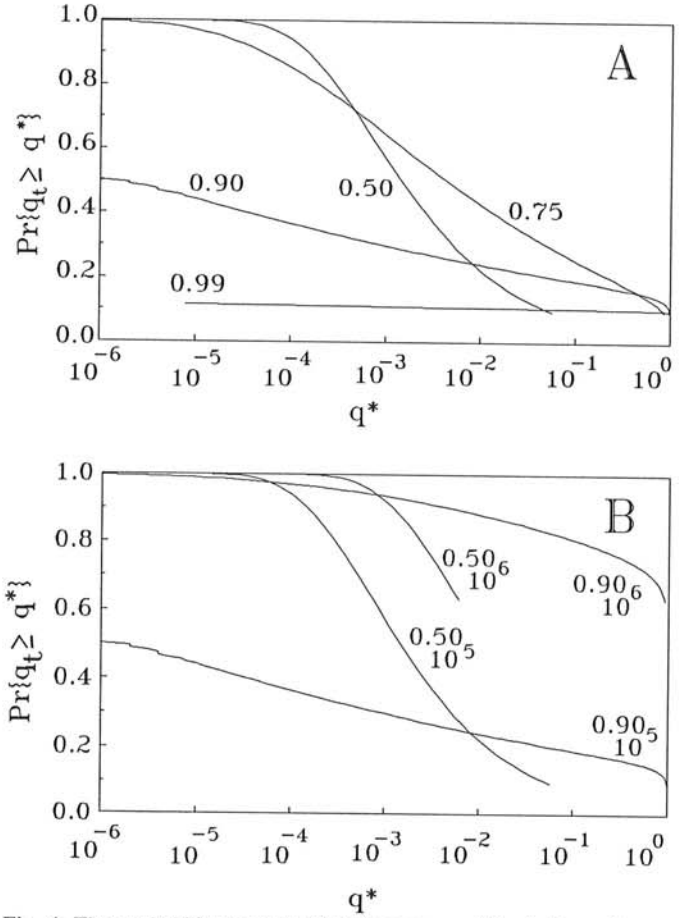

Fig. 4. The probability that the final frequency of fungicide resistance (q_t) reaches some given level (q^*) or greater by the end of a 100-day epidemic ($Pr\{q_t \geq q^*\}$). **A,** Results for four levels of fungicide efficacy ($1 - \alpha$, printed next to each curve) when initial population size (N_0) is 10^5, $p = 10^{-6}$, and $r = 0.2$. **B,** A comparison of the effects of two levels of fungicide efficacy ($1 - \alpha = 0.5$ and 0.9) at two different initial population sizes, 10^5 and 10^6; values for $1 - \alpha$ and N_0 are printed next to each curve. Values for p and r are the same as in A.

The effects of growth rates, r, and mutation rates, p, are similar to those for N_0. For smaller r, population size remains smaller, and, hence, the probability of R occurring stays lower. The same holds for smaller values of p. Lower mutation rates reduce the probability that R mutants occur.

Expected values of $N_{R,t}$ and q_t. The effects of N_0, p, and r on $E[N_{R,t}(X)]$ and $E[q_t(X)]$ are similar to those on the probability of resistance increasing to a given frequency: Larger values for

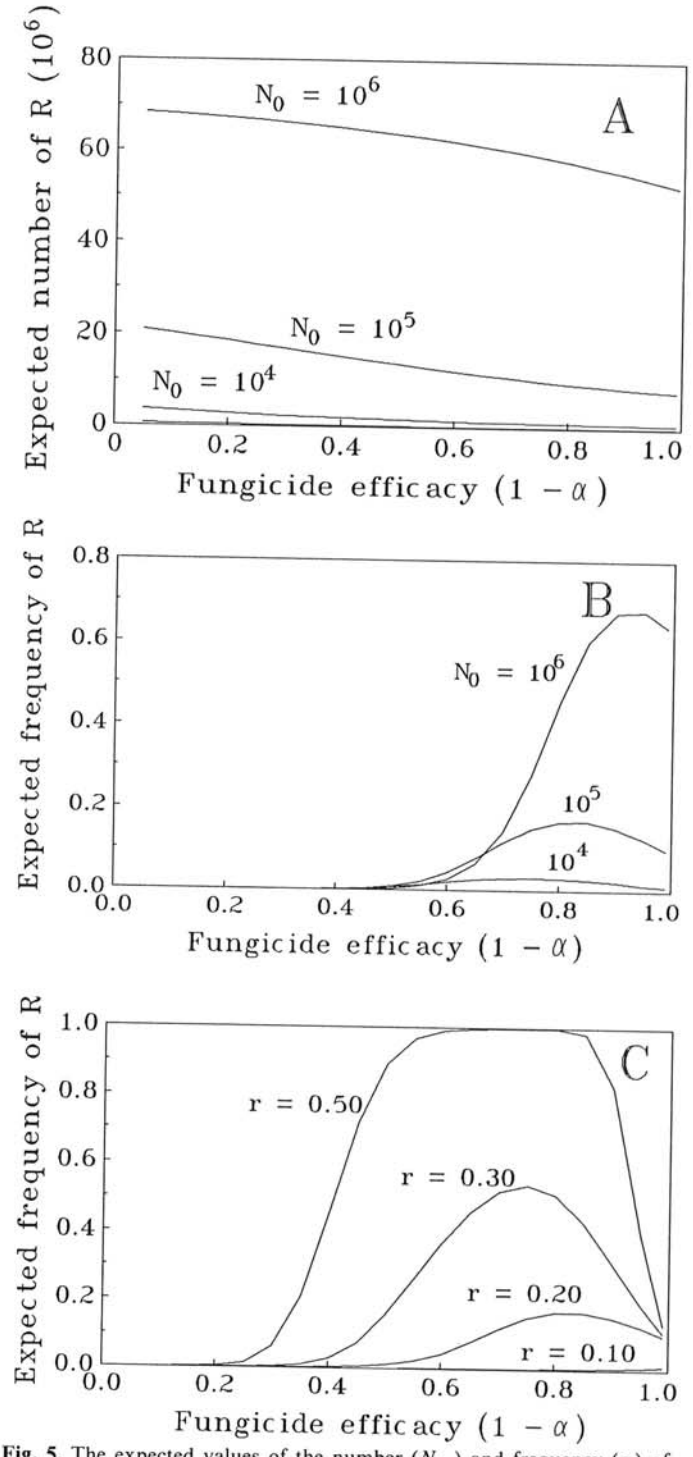

Fig. 5. The expected values of the number ($N_{R,t}$) and frequency (q_t) of fungicide-resistant individuals in 100-day epidemics as functions of fungicide efficacy ($1 - \alpha$). **A,** Expected value of $N_{R,t}$ for three initial population sizes ($N_0 = 10^4$, 10^5, 10^6); the curve for $N_0 = 10^3$ is too close to zero to show on this graph. Values for p and r were 10^{-6} and 0.20, respectively. **B,** Expected values of q_t for the same parameter values as in A. **C,** Expected values of q_t for four values of population growth rates ($r = 0.10$, 0.20, 0.30, 0.50). Initial population size was 10^5 and $p = 10^{-6}$.

these parameters result in larger values of $E[N_{R,t}(X)]$ and $E[q_t(X)]$. For all values of N_0, $E[N_{R,t}(X)]$ decreases as fungicide efficacy increases (Fig. 5A). This occurs because the probability of a resistant mutant arising decreases as fewer new individuals are added to the population when a fungicide suppresses reproduction.

In contrast to $E[N_{R,t}(X)]$, $E[q_t(X)]$ has a maximum value at intermediate values of $1 - \alpha$ (for values of N_0 from 10^3 to 10^6 when $r = 0.20$ and $p = 10^{-6}$). The intermediate maximum is even more evident for values of r greater than 0.20 (Fig. 5C) (when $N_0 = 10^5$ and $p = 10^{-6}$).

Multiple year dynamics. The model results presented thus far pertain to single seasons or epidemics. To evaluate longer term effects of using selective fungicides, I performed Monte Carlo simulations of this model over 10 100-day seasons. The purpose was to discover how often resistance arises and how long it takes the frequency of R to increase to high levels. For these simulations, each season started with the same value for N_0. The number of R individuals present at the beginning of each season was a function of the final frequency of the previous season, q_f. The initial number was determined by randomly sampling from a Poisson(N_0q_f) distribution if N_0q_f was less than 100; otherwise, $N_{R,0} = N_0q_f$, the expected value from the Poisson distribution. The initial number of R individuals for the first season in each 10-yr set was determined as above except that the value for the mutation rate p was used instead of q_f. If there were no R individuals initially present, the first occurrence of R was determined for each season by randomly sampling from the distribution of X (equation 11). The final frequency, q_f, for that season was calculated as a function of the sample value for x using equation 8 with $t = 100$. One thousand 10-season simulations were conducted for each combination of $N_0 = 10^3$, 10^4, 10^5, 10^6 and $1 - \alpha = 0.99, 0.90, 0.75, 0.625, 0.50$. These simulations were conducted 10 times for a total of 10,000 simulations. The parameters r and p were constant for all simulations with values of 0.20 and 10^{-6}, respectively. From each set of 1,000 10-yr simulations, the number of times and the median number of years it took for q_t to reach 0.99 (or greater) were recorded.

The frequency of resistance increased to 0.99 (or greater) more often for larger values of N_0, at the same level for $1 - \alpha$ (Fig. 6A). This is consistent with the single-season dynamics where the probability that q_t reached q^* was higher when N_0 was large (Fig. 3). In almost all cases, R evolved fewer times with higher fungicide efficacy (Fig. 6A). The exception to this was when $N_0 = 10^6$ and R evolved in every 10-yr simulation. The median number of years it took for q_t to reach 0.99 was smaller for larger values of N_0 than smaller ones (Fig. 6B) for a given level of efficacy. The effects of efficacy on the median times were mixed, depending on N_0 (Fig. 6B). At lower values of N_0 (10^3 and 10^4), the longest times for R to build up were when $1 - \alpha = 0.90$. In contrast, the maximum median time was at the highest efficacy value ($1 - \alpha = 0.99$) when $N_0 = 10^5$, and it was at the lowest level ($1 - \alpha = 0.5$) when $N_0 = 10^6$.

DISCUSSION

The model presented above predicts the probability that fungicide resistance reaches a given frequency or absolute population size by the end of an epidemic. These probabilities are a function of the initial population size (N_0), mutation rate (p), pathogen growth rate (r), and fungicide efficacy ($1 - \alpha$). A stochastic model has been developed because in some circumstances, especially when N_0 is small, there is a high probability that the initial frequency R is zero. Selection cannot occur when there is no variability in resistance, and, therefore, the frequency of R will remain zero until an R mutant arises, regardless of how much fungicide is used. In contrast, deterministic selection models that assume initial frequencies greater than zero (3,6,10–12,15) may overestimate the risks of resistance developing when N_0 is small. These models predict that, even if the initial frequency is very low, fungicide use will select for higher frequencies. In the stochastic approach, the number and frequency of resistant individuals will be lower at the end of an epidemic if the first resistant individual does not arise until later in the epidemic. The uncertainty in the stochastic model is the time at which the first R individual occurs in a population.

Many of the model results are intuitively obvious. When N_0 and r are small, the population size remains small and the probability of R occurring remains low. Similarly, if the mutation rate is very low, R is less likely to occur until later in an epidemic when the population reaches a larger size. When the first R mutant occurs late in the epidemic, there is not as much time for selection to act, and, therefore, the frequency of R remains low (Fig. 1).

The implications of this model to fungicide resistance management are simple with respect to N_0 and r: The strategy is to keep N_0 and r small. Not surprisingly, these are the basic strategies previously identified for fungicide resistance management (13,16) and disease control in general (7,22).

The effects of fungicide efficacy on the evolution of resistance are not as intuitive. Deterministic selection models predicted that higher frequencies of resistance result from higher fungicide efficacy (3,10,16,19). However, if a population is kept small by a very effective fungicide (large $1 - \alpha$), then the probability that R occurs is kept low (Fig. 4), and the expected number of R individuals is smaller than for lower values of $1 - \alpha$ (Fig. 5A). Conversely, when $1 - \alpha$ is small, populations may reach larger sizes, and the probability of R occurring early in the epidemic is higher, resulting in larger numbers of R.

The effects of fungicide efficacy depend on initial population size, N_0. When N_0 is large (for example, 10^6), predictions from this stochastic model start to converge with those from deterministic models (10,16,19). This can be seen several ways.

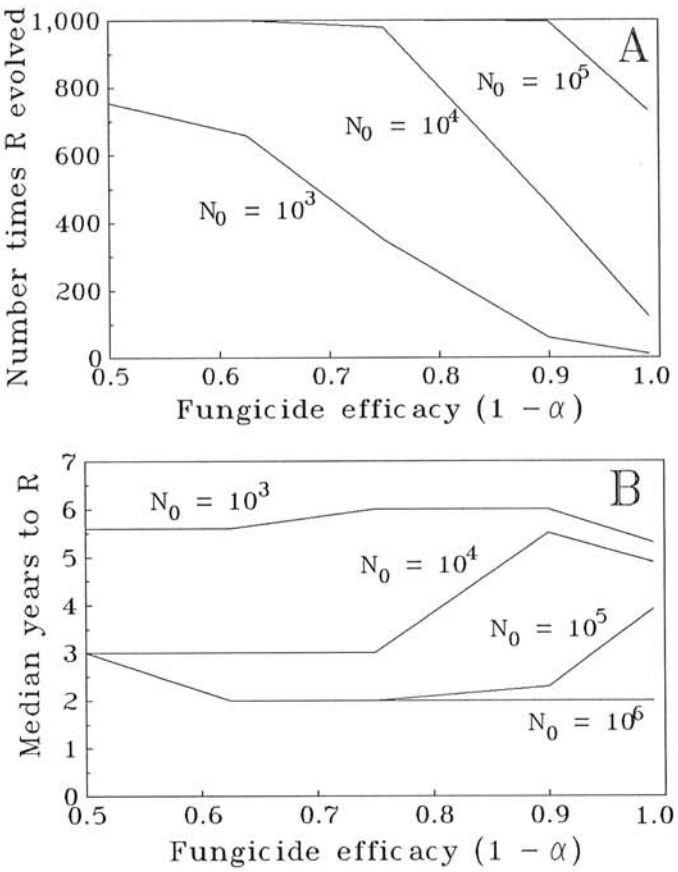

Fig. 6. Mean results of Monte Carlo simulations for 10-yr runs as a function of fungicide efficacy ($1 - \alpha$) and initial population size (N_0) (details of simulations are in the text). One thousand simulations were conducted 10 times for each combination of N_0 and $1 - \alpha$. **A,** Number of times (out of 1,000) that the frequency of fungicide-resistant (R) individuals increased to 0.99 or greater. Resistance evolved in every 10-yr simulation when $N_0 = 10^6$. **B,** The median number of years to reach a frequency of resistance 0.99 or greater, for those simulations in which resistance developed.

In Figure 4B, the curves for the probability of q_t reaching q^* or greater for $1 - \alpha = 0.50$ and 0.90 when $N_0 = 10^6$ are similar until $q^* \approx 10^{-3}$, at which point the probability is much higher for higher efficacy. When $N_0 = 10^5$, the curves are farther apart and do not intersect until $q^* \approx 10^{-2}$. Another way to view the interactions of $1 - \alpha$ and N_0 is in Figure 5B, where the maximum for $E[q_t(X)]$ is at higher level of $1 - \alpha$ as N_0 increases. Finally, it can be seen in Figure 6A that fungicide efficacy has no effect on the number of times resistance evolves to high frequencies when $N_0 = 10^6$ but higher values of $1 - \alpha$ result in fewer occurrences of R when N_0 is less than 10^6.

An evaluation of the strategy of intensive fungicide use for resistance management depends on understanding the effects of fungicide efficacy, $1 - \alpha$. Because α is assumed to be the average reduction in growth rate for an epidemic, fungicide efficacy can be viewed as equivalent to the intensity of fungicide use. As discussed above, the effects of $1 - \alpha$ depend on N_0. Therefore, for some cases when N_0 is small, intensive use of fungicides may be a reasonable strategy for suppressing the evolution of resistance.

An example of a system in which this condition is met is when primary inoculum migrates long distances to areas where inoculum cannot survive over winter, such as blue mold of tobacco caused by *P. tabacina*. Small numbers of sporangia of *P. tabacina* migrate to tobacco-growing areas in Connecticut from areas to the south (1). The probability of any fungicide-resistant types migrating depends on the total number of individuals that successfully migrate (N_0) and the frequency of R in the source population. The fact that relatively few sporangia survive migration, land on susceptible plants, and successfully infect means that N_0 may be extremely small. If the source population had a frequency of R less than a detection threshold (say, 10^{-3}), the probability of R in the initial migrants could be estimated roughly as $1 - e^{-N_0 \cdot 10^{-3}}$ (from equation 2). If this probability is extremely low, then there would be little risk of using fungicides intensively. However, subsequent occurrence and development of R would depend on p, r, and α. Greenhouses are another area for which this model may be applicable because greenhouse populations may be isolated, and intensive sanitation practices could reduce N_0 to very small numbers. The key factors that make intensive fungicide use possible are small initial population size and low (less than detectable) initial frequencies of resistance.

Another system in which initial population sizes are often small is potato late blight caused by *Phytophthora infestans* (Mont.) de Bary. Populations of *P. infestans* in North America undergo drastic reductions in size seasonally because the pathogen only survives between crops in potato tubers. When proper sanitation practices are implemented to minimize inoculum from cull piles and volunteers, seed tubers are the main source of primary inoculum. Furthermore, less than five in 1,000 infected tubers result in primarily infected plants the next season (18; W. E. Fry, *personal communication*). Assuming that seed is produced without any exposure to phenylamide fungicides, then the initial frequency of resistance is approximately p, say 10^{-6}. Also assume that one in 100 seed tubers is infected with *P. infestans* (this is a rather high estimate for seed tubers). Therefore, a 100-ha farm will have approximately (100 ha) $\times$ (48,000 seed tubers ha^{-1}) $\times$ (0.01 infected tubers per seed tuber) = 48,000 infected tubers. Of the 48,000 infected tubers, approximately (48,000)(0.005) = 240 plants will become infected with primary inoculum. Using this estimate of 240 for N_0 and assuming that $1 - \alpha$ is approximately 0.90 (phenylamides are very effective for controlling late blight), then the probability of R reaching detectable frequencies is extremely low during a 60-day epidemic. For this 100-ha potato system (assuming no immigration or inoculum from cull piles, etc.), there is less than a one in 250 chance that the frequency of phenylamide resistance will increase to a level of 10^{-3} under intensive use of phenylamides when r is 0.5. This probability is much lower when r is smaller—for example, when nonselective protectant fungicides also are used or when certified seed with a much lower frequency of infected tubers is used.

It has been suggested that selective fungicides never should be used for potato-seed production because of the risk of disseminating fungicide-resistant inoculum (14). An alternative strategy might be to use fungicides intensively to keep the population so small that the number of infected tubers is minimized. This reduces the probability of R occurring to almost zero because N_0 will be very small. Phenylamide fungicides are used in some potato-seed production with this rationale in mind (S. A. Slack, *personal communication*). These same types of calculations may be applicable to other systems in which inoculum is primarily seedborne and is present in low numbers (for example, certified seed).

To use this model to evaluate the risks in any particular system, one must have reliable estimates of N_0, r, p, and $1 - \alpha$. The model results presented in this paper are for a very limited set of parameter values. It is not possible to make sweeping generalizations that apply to all systems because the four parameters are not independent. It is also difficult to estimate these parameters accurately in many cases. Finally, it is important to know the extent of migration within and between geographic areas. In the late blight example discussed above, I assumed no immigration to simplify the calculations. However, the risk of immigration of R individuals is not considered but obviously could affect the development of resistance. Therefore, caution must be used in applying these model results to any real system.

Some field experiences with resistance may appear at first to contradict model predictions that intensive fungicide use may be an appropriate management strategy under some circumstances. However, rapid development of resistance should prompt an examination of the critical parameters that affect the appearance and evolution of resistance. Was fungicide applied to large populations (large N_0)? Was epidemic development rapid (large r), etc.? In addition, one should ask whether resistance is localized or widespread and, if it is widespread, whether it arose once and migrated throughout an area or arose many times independently. This model predicts probabilities of R occurring in closed populations. It does not predict absolute certainties or anything about movement of resistance once it evolves.

The stochastic model presented here is simplified and, therefore, has obvious limitations. The model assumes geometric growth to simplify the calculations. Logistic or other types of density-dependent population growth generally will tend to slow the evolution of resistance (16). Therefore, the stochastic model, if anything, overestimates the risks of resistance. The assumptions that resistant and sensitive types have equal fitness in the absence of fungicide, and that fungicide has no effect at all on R are other potential sources for overestimating the occurrence of resistance. On the other hand, only single mutations are assumed to occur; mutations to R in multiple individuals are ignored, even though they are likely to occur (especially as N gets large). This simplification is justified, however, because the strength of selection is generally much more important to the evolution of pesticide resistance than are mutations, once resistance is present in a population (16). Other refinements to this model are possible. However, for the purposes of deriving some qualitative generalities, the additional complexity is not necessarily warranted.

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