Selection for Tolerance in Organisms Exposed to Sprays of Biocide Mixtures: A Theoretical Model

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ABSTRACT

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A mathematical model has been constructed that simulates the process of selection that occurs when populations of organisms with a component tolerant to a particular biocide are repeatedly exposed to that biocide combined with a second biocide to which no tolerance exists. A more general model also was derived which evaluates the selection process when the population is composed of any number of subpopulations of differing sensitivity to a particular biocide. The variables included in the model are: the efficacies of the first biocide against the tolerant subpopulation and against the sensitive subpopulation; the efficacy of the second biocide, which in our evaluation is assumed to be equal for the two subpopulations; and a variable defining the degree of completeness of spray coverage (ie, the proportion of the total population contacted at each spray application). Independent joint action is modeled. To evaluate the model, we commenced with the tolerant subpopulation at a frequency of 1×10^{-9}. Major indications of the model were: That selection proceeds through many spray applications before the level of the tolerant strain builds up to 1%, but once that has happened only a few more sprays are needed for tolerance to dominate; that the variable with the greatest effect on the rate of selection was spray coverage; that when coverage is complete, selection proceeds at the same rate whether the "at-risk" biocide is used on its own or in a mixture; that under these circumstances it is advantageous to use the biocide alternately or in a planned sequence. With less complete spray coverage ($<$ 99%) it is better to use mixtures to slow up the rate of increase of resistant forms; that rates of selection increase with increasing efficacy of the "at risk" biocide to the sensitive subpopulation; that the rate of increase of resistant individuals in a population is reduced with decreasing spray coverage; that when coverage is incomplete, the rate of selection for resistance will be slower with increasing strength of the second biocide; and that the rate of selection for resistance becomes greater as tolerance increases, but the increase is less with decreasing spray coverage.

Additional key words: fungicides, crop disease control.

The problem of fungicide tolerance in plant pathogens has become increasingy important during the past decade (1,6). This has been due largely to the use of new, highly effective fungicides having a specific mode of action involving one or only a few gene sites.

Proven strategies either to prevent or delay the development of tolerance do not exist at present. The factors that influence the development of tolerance and their possible manipulation were discussed by Dekker (5) in a recent review, but experimental data on combating it are virtually nonexistent. There is very little practical field experience from which reasonable inferences can be made. In this informational vacuum, the agrochemical industry and farmers are faced with an immediate need to formulate fungicide-use strategies that will give effective disease control, that will prevent or delay the development of tolerance in target pathogens (thus extending the useful life of the valuable fungicides) and that will provide adequate crop protection should tolerance develop suddenly. It is desirable that there be no failure of control when tolerance arises.

Whether all of these goals are achieveable or even compatible is not clear, but decisions have had to be made on the available evidence. One approach has been to recommend that the "at risk" fungicide be used in a mixture with a second fungicide having a different mode of action. Both fungicides in the mixture are recommended at the same dosage rates that would be used if they were applied singly. It is considered desirable that the second fungicide has a broad-spectrum of activity and that its mode of action involves many gene sites. This policy has been adopted, for example, with benomyl; its use singly is now discouraged by the manufacturers (2).

The present study was prompted by the lack of strong field evidence or other arguments to support the view that mixtures will prevent or delay the appearance of tolerant strains. A simple mathematical model has been constructed to represent the process of selection that occurs when a population of organisms containing two or more components with differing sensitivities to a biocide are sprayed with that biocide in a mixture with a different biocide. The model determines the change in the proportions of the subpopulations with successive spray applications. Although the model was constructed to examine the effect of fungicide mixtures on selection for fungicide tolerance in plant pathogenic fungi, it can equally well be applied to other organism/biocide interactions.

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MATHEMATICAL MODEL OF SELECTION

The model is based on several premises: (i) It assumes the preexistence of subpopulations of the target organism which differ in sensitivity to the "at risk" biocide. In other words, the population of the target organism is sufficiently large in relation to the rate of mutation to tolerance for there to be at least a low frequency of tolerant individuals before exposure to the biocide commences. (ii) It is based on repeated challenges of the organism population by a mixture of biocides. (iii) Efficacies of the biocides against the subpopulations are fixed at the outset and do not change from challenge to challenge. (iv) The proportions of each subpopulation alter with each challenge, but do not change between challenges. (v) Independent joint action of toxicants is modeled. This mode of joint action was chosen because it is most likely to be the operative mode in mixtures of dissimilar biocides such as are being recommended to combat tolerance.

Efficacy (f) of a biocide is defined as a number between 0 and 1. It represents the proportion of individuals killed when contact is made between all individual organisms in the population or subpopulations and the biocidal spray. Specific efficacy values can be considered as points on particular dosage-mortality curves (Fig. 1). Efficacy therefore, reflects two biocide properties. (i) The intrinsic toxicity of the biocide toward the target organism. Efficacy of a biocide, for example, will be lower toward a tolerant, than toward a sensitive subpopulation of a target organism and (ii), the concentration of the biocide—ultimately the amount absorbed by each individual within the population. Thus, efficacy will decline if spray concentration is reduced.

If there are n individuals in a population and a spray of efficacy f is applied, the number of organisms surviving (S) after spraying will be:

$$S = n(1 - f)$$

Incomplete spray coverage is the norm in field spraying, hence its effect on rate of selection for tolerance is examined in the model. The term E defines the proportion of the population escaping contact with the spray due to incomplete coverage. By varying E from 0 to 1, coverage can be represented as complete to none. It is assumed that individuals of both subpopulations are distributed randomly on the crop surface so that E is the same for both subpopulations. When spray coverage is incomplete, the number of individuals surviving after one spraying is given by:

$$\begin{aligned} S &= (\text{Individuals not contacted by biocide + Individuals contacted by biocide, but surviving}). \\ &= En + n(1 - E)(1 - f) \\ &= n(1 + Ef - f) \end{aligned}$$

If biocides A and B with efficacies f_A and f_B, are applied in a mixture to all n organisms in a population and their mode of action is independent, the number surviving will be:

$$S = n(1 - f_A)(1 - f_B)$$

If spray coverage is not complete and a proportion, E, escapes the numbers surviving will be:

$$\begin{aligned} S &= En + n(1 - E)(1 - f_A)(1 - f_B) \\ &= n\{1 + (1 - E)(f_A f_B - f_A - f_B)\} \end{aligned}$$

In the simplest case of biocide tolerance there are two subpopulations of the target organism. If these are defined as subpopulations 1 and 2, the numbers of individuals in them can be represented by n_1 and n_2, respectively.

When a mixture of biocides A and B are applied to such a population having a tolerant component then the efficacy of each biocide on each subpopulation is given by the symbols in the following matrix:

	Biocide A	Biocide B
Subpopulation 1	f_{1A}	f_{1B}
Subpopulation 2	f_{2A}	f_{2B}

If the number of survivors in subpopulations 1 and 2 following m applications of a biocide is defined as S_1^m and S_2^m, then

$$S_1^1 = n_1\{1 + (1 - E)(f_{1A} f_{1B} - f_{1A} - f_{1B})\}$$

and $$S_2^1 = n_2\{1 + (1 - E)(f_{2A} f_{2B} - f_{2A} - f_{2B})\}.$$

Similarly,

$$\begin{aligned} S_1^2 &= S_1^1\{1 + (1 - E)(f_{1A} f_{1B} - f_{1A} - f_{1B})\} \\ &= n_1\{1 + (1 - E)(f_{1A} f_{1B} - f_{1A} - f_{1B})\}^2 \end{aligned}$$

and

$$S_1^m = n_1\{1 + (1 - E)(f_{1A} f_{1B} - f_{1A} - f_{1B})\}^m$$

also $$S_2^m = n_2\{1 + (1 - E)(f_{2A} f_{2B} - f_{2A} - f_{2B})\}^m$$

Consequently, the proportions of subpopulations 1 and 2 individuals after m sprays (P_1^m and P_2^m, respectively) are given by:

$$P_1^m = S_1^m/(S_1^m + S_2^m)$$

and,

$$P_2^m = S_2^m/(S_1^m + S_2^m)$$

These arguments can be extended to k subpopulations of an organism. In that case the number of survivors in subpopulation j after m sprays (S_j^m) is given by

$$S_j^m = n_j\{1 + (1 - E)(f_{jA} f_{jB} - f_{jA} - f_{jB})\}^m$$

and the proportion of subpopulation j organisms after m sprays (P_j^m) is given by

$$P_j^m = S_j^m \Big/ \sum_{i=1}^{k} S_i^m$$

USING THE MODEL

In our simulations to examine the influence of differing biocide efficacies and escape values on the rate of selection for tolerant strains, we have commenced with the initial subpopulation levels differing by a factor of 10^9, the tolerant subpopulation being the lower. This gives an initial size for the tolerant subpopulation approximately the same as that which might be expected if tolerance arose through mutation. Frequencies ranging from about 1×10^{-6} to 1×10^{-9} have been reported (4,7). We have varied the efficacy of one fungicide to simulate differing levels of activity against the subpopulations. These levels reflect differing activity against the sensitive subpopulation due to differences in either intrinsic toxicity or spray concentration, and in the other subpopulation, principally differing levels of tolerance. In each simulation, efficacy of the second biocide has been set at the same value against both subpopulations. This would logically be the case for mixtures having independent joint action, hence, our simulations parallel the usual field situation. The impact on selection rate by increasing or decreasing the efficacy of the second biocide against both subpopulations also has been examined.

Four simulations were carried out. In each simulation, the efficacy of the biocide, A, to which tolerance has developed, was varied against both the tolerant and sensitive subpopulations. The efficacy of the second biocide, B, was constant for both subpopulations in each simulation, but the value differed between simulations. In each simulation, the proportion of the total population escaping contact with the spray was varied between 0 and 50%. Values for the efficacies and levels of escape in each simulation were:

$f_{1A} = 0.50, 0.10, 0.05, 0.01$ (against tolerant subpopulations)
$f_{2A} = 0.99, 0.95, 0.80$ (against sensitive subpopulations)
$E = 0.00, 0.01, 0.05, 0.10, 0.30, 0.50$

The values f_{1B} ($= f_{2B}$) (efficacy of the second biocide) took in the four simulations were 0.95, 0.90, 0.80, 0.00.

The simulations were allowed to proceed with the number of challenges (spray applications) being counted until the tolerant subpopulation became 90% of the total.

The output from each simulation was analyzed as a $4 \times 3 \times 6$ factorial with the three-factor interaction being used as the residual term. To directly compare the F values computed from the four analyses, they have been converted to "relative F values" (Table 1). Relative F is given by:

$$\text{relative F of an effect} = \frac{\text{F value for the effect}}{\Sigma \text{ F values for all effects in the analysis}}$$

Hence, in all analyses the sum of the relative F values total unity, within rounding errors.

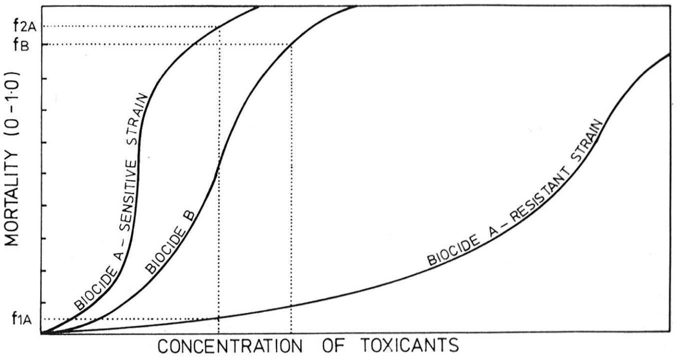

Fig. 1. Schematic representation of the biocide efficacy concept used in the theoretical model. Three dosage-mortality curves are illustrated: the toxicity of one biocide (A) to sensitive and tolerant subpopulations of an organism, and the toxicity of a second biocide (B) which affects the subpopulations equally. Specific efficacy values for particular concentrations of the toxicants are equated with the level of mortality (on a 0 to 1 scale) obtained with those concentrations. For example, in the above figure at the indicated concentration of biocide A, efficacy against the sensitive subpopulation (f_{2A}) will be 0.95 and against the tolerant subpopulation (f_{1A}) it will be 0.05. Biocide B at a different concentration will have an efficacy (f_B) of 0.90 against both subpopulations.

PREDICTIONS OF THE MODEL

The following conclusions can be drawn from our simulations:

(i) When the initial population level of a tolerant strain is low (eg, one individual in a population of 10^9) selection will proceed through many challenges before the level of the tolerant strain builds up to 1% of the total population, ie, many sprays can be applied without tolerance being detected. However, once tolerance reaches a level of about 1%, only a few more sprays are required until almost total tolerance is attained. For example if tolerance exists in a population, arising through mutation at a level of 1×10^{-9}, and fairly strong selection pressure is applied, with model parameter values of $f_{1A} = 0.10$, $f_{2A} = 0.95$, $f_{1B} = f_{2B} = 0.90$ and $E = 0.05$ it takes 18 sprays for tolerance to build up to more than 1% of the total population, yet only a further 6 sprays results in almost three-quarters of the population being tolerant (Table 2).

(ii) Over the range of values chosen, relative F values for the E effect were greater than 0.5 in all simulations (Table 1), thus indicating that level of escape (E) had by far the largest effect on the total number of sprays required for the tolerant subpopulation to become 90% of the total population.

(iii) If complete coverage of the crop is achieved in application of the biocides ($E = 0.00$), then there is no advantage in the use of mixtures (Tables 3 and 4). It can be seen that the efficacy of the second biocide has no effect on the number of spray challenges needed for the population to become 90% tolerant. Even when the second biocide is absent ($f_{1B} = f_{2B} = 0.00$) there is no difference in the rate of selection. When coverage is complete the number of sprays to reach the tolerant situation will depend only upon the efficacy of the "at risk" biocide to both the sensitive and tolerant strains of the pathogen (Table 4). Practical examples of complete

TABLE 1. The relative importance of spray efficacies, spray coverage, and their interactions in determining the number of applications of a spray mixture needed for the population to become 90% tolerant[a]

Variance source[c]	df	Relative F values[b] calculated from model output when the efficacy of the second biocide against both subpopulations ($f_{1B} = f_{2B}$)[c,d] is: 0.95	0.90	0.80	0.00
f_{1A}	3	0.12	0.12	0.12	0.15
f_{2A}	2	0.03	0.04	0.06	0.25
E	5	0.80	0.78	0.76	0.55
$f_{1A}\ f_{2A}$	6	0.01	0.01	0.01	0.02
f_{1A} E	15	0.04	0.04	0.04	0.02
f_{2A} E	10	0.01	0.01	0.01	0.00
Residual	30				

[a]In these simulations the initial frequency of tolerance in the population was set at 1×10^{-9}.
[b]See text for derivation of relative F values.
[c]Variables: f_{1A} = efficacy of the first biocide against the tolerant subpopulation; f_{2A} = efficacy of the first biocide against the sensitive subpopulation; E = spray coverage variable; and variables f_{1B} and f_{2B} are the efficacies (assumed equal) of the second biocide in the mixture against both subpopulations.
[d]Each column represents a separate simulation.

TABLE 2. Proportion of a population tolerant after successive spray applications of a biocide mixture assuming specific model values for efficacies, spray coverage, and initial tolerance level[a]

Second biocide efficacy ($f_{1B} = f_{2B}$)	Proportion tolerant after spray application no. 0	5	10	15	20	25	30	35	40
0.95	.000[b]	.000	.000	.000	.000	.002	.027	.327	.894
0.90	.000	.000	.000	.001	.069	.873	.998	1.000	
0.80	.000	.000	.000	.261	.996	1.000			
0.00	.000	.000	.826	1.000					

[a]The values used in these simulations were: initial frequency of tolerance in the population, 1×10^{-9}; efficacv of the first biocide against the tolerant subpopulation (f_{1A}), 0.10; efficacy of the first biocide against the sensitive subpopulation (f_{2A}), 0.95; efficacy of the second biocide against both subpopulations ($f_{1B} = f_{2B}$), 0.00 to 0.95 as indicated in table; spray coverage variable (E), 0.05.
[b]All values in the table are rounded off to three decimal places.

TABLE 3. The effects of differing biocide efficacies and spray coverage values on the number of sprays needed for the population to become 90% tolerant[a]

Spray efficacy: Second biocide against both subpopulations ($f_{1B} = f_{2B}$)	Spray efficacy: First biocide against the tolerant subpopulation (f_{1A})	No. of sprays before the population becomes 90% tolerant when the proportion escaping biocide contact (E)[b] is: 0.00	0.01	0.05	0.10	0.30	0.50	Mean
0.95	0.50	14[c]	28	78	147	515	1,179	327
	0.10	10	18	44	78	259	584	165
	0.05	10	17	42	73	244	550	156
	0.01	9	17	40	71	233	526	160
0.90	0.50	14	22	48	82	267	598	172
	0.10	10	15	28	45	137	300	89
	0.05	10	14	27	43	129	283	84
	0.01	9	14	26	42	124	270	81
0.80	0.50	14	18	32	49	142	308	94
	0.10	10	13	20	29	75	157	51
	0.05	10	12	19	28	71	148	48
	0.01	9	12	19	27	69	142	46
0.00	0.50	14	15	18	22	42	75	31
	0.10	10	11	13	15	25	42	19
	0.05	10	10	12	14	24	40	19
	0.01	9	10	12	14	24	39	18

[a]In these simulations the initial frequency of tolerance in the population was set at 1×10^{-9}.
[b]These are values of the spray coverage variable. When E = 0.00, no individual within the population escapes contact with the biocide spray, and coverage is complete. When E = 0.01, 1% of the population escapes contact and coverage is 99% complete, etc.
[c]Each of these entries is the mean of three values obtained in simulations varying the efficacy of the first biocide against the sensitive subpopulation (f_{2A}). The values assumed for f_{2A} were 0.99, 0.95, and 0.80.

coverage may include the dipping of seeds, complete plants, plant parts, or animals for disease or pest control, and the treatment of animals for internal parasites.

(iv) The other variables being constant, the greater the efficacy of the "at risk" biocide to the sensitive subpopulation, the fewer will be the number of spray applications needed for the population to become largely tolerant (Table 4).

(v) Poor spraying technique and inadequate coverage with biocide mixtures (high values of E, Tables 3, 4) increase the number of sprays needed for the development of tolerant subpopulations.

(vi) When coverage is incomplete, ($E > 0$) the normal situation in field or orchard spraying, the use of mixtures will retard selection for tolerance. This is shown by comparing the figures in the bottom quarter of Table 3 ($f_{1B} = f_{2B} = 0.00$) which represent use of the "at risk" biocide alone with those in the remainder of the table. In these circumstances selection will be slower with increasing efficacy of the second biocide (Tables 3 and 4).

(vii) The level of tolerance affects the rate of selection. The higher the tolerance the greater is the rate of selection (Table 3). Level of tolerance and spray coverage interact to affect the rate of selection: when coverage is poor, selection for mildly tolerant strains is reduced in comparison to highly tolerant strains. To illustrate this, two examples are taken from Table 3 when $f_{1B} = f_{2B} = 0.95$. Seventeen sprays are required for tolerance to dominate when coverage is good ($E = 0.01$) and the tolerance is high ($f_{1A} = 0.01$), whereas when tolerance is low ($f_{1A} = 0.50$) 28 are needed (an increase of 65% in number of sprays is required). On the other hand when coverage is poor ($E = 0.50$) respective spray numbers are 526 and 1,179 (an increase of 124%).

(viii) The model indicates that the use of different biocides alternately or in a planned sequence may be a preferable strategy instead of the use of full-strength mixtures in some situations. An estimate of the number of sprays needed for tolerance to dominate if the biocides are used alternately may be obtained from the lower quarter of Table 3 ($f_{1B} = f_{2B} = 0.00$). This portion of the table represents the performance of the "at risk" biocide on its own. By definition, alternate applications of the second biocide do not affect the proportions of the sensitive and tolerant subpopulations. Consequently, the estimate of the number of biocide sprays needed with a strategy of alternate spraying can be obtained by doubling the values in the lower quarter of Table 3. Even though the values in Table 3 are means for three simulations in which the efficacy of the "at risk" biocide against the sensitive subpopulation was set at 0.99, 0.95, and 0.80, this principle can be applied to the values obtained in the individual simulations, and similar conclusions will be reached. The effect of spray coverage on the value of alternate spraying is shown in the following example. When spray coverage is complete ($E = 0$), the level of tolerance is high ($f_{1A} = 0.01$), and the second biocide is relatively effective ($f_B = 0.95$), a 90% tolerant population will develop in nine sprays of the mixture. If the same two biocides are used alternately there will be 18 sprays (twice the equivalent value in the lower quarter of Table 3) before the 90% level of tolerance will be reached. The expenditure for spray materials will be the same in both cases. With the alternate use there would be a slight decrease in disease control, but it should still be acceptable. The value of the alternate approach is rapidly lost as spray coverage becomes less complete. With the same efficacy values as the previous example and 99% coverage ($E = 0.01$) a 90% tolerant population will develop in 17 sprays of the mixture, and 20 sprays if the biocides are used alternately. However, when coverage is still less complete ($E = 0.05$) this level of tolerance will develop after 40 applications of the mixture, but after only 24 alternate applications.

INTERPRETING THE MODEL

The model examines the effects of a series of events, but it is not time dependent. Therefore reproduction of the target organism, which would occur in the real world, is not included. Reproduction and population increase are not incompatible with the model, however, provided that the subpopulations reproduce at equal rates; ie, that the proportions of the subpopulations do not alter between biocide challenges. This imposes certain limitations in relating the model to reality. (i) The population being sprayed must almost certainly be a closed system: sensitive or tolerant individuals must not enter the population from outside sources between spray applications in proportions differing from the ratio existing in the population at that point. (ii) Reproduction is asexual: there is no crossing of the two subpopulations. (iii) To maintain the requirement that the proportions do not change between challenges it is necessary to postulate that the action of the biocides must be to kill or otherwise render target individuals reproductively sterile. It is also necessary to assume that tolerant and nontolerant individuals contacted by the biocides, but surviving, reproduce at the same rate. Other types of biocide effect are not compatible with the model. For example, fungicides which differentially affect the latent period or sporulation capacity of sensitive and tolerant strains of a plant pathogen cannot be modeled, because there is no time component.

It should be noted that the use of "rate" or related terms in this paper does not refer to change with time, but to change with number of biocide challenges.

Another basic premise which must be considered when relating the model to the real world is the constancy of the efficacy values from challenge to challenge. In the field, meteorological and biological factors and the residual effects of previous spray applications can cause efficacy to vary. If sprays are applied to

TABLE 4. The effect on selection of the interaction between spray coverage and the efficacy of the "at risk" biocide against the sensitive subpopulation[a]

Efficacy of first biocide: against tolerant subpopulation (f_{1A})	Efficacy of first biocide: against sensitive subpopulation (f_{2A})	No. of sprays before the population becomes 90% tolerant when the efficacy of second biocide against both subpopulations ($f_{1B} = f_{2B}$) is: 0.95, and the proportion escaping biocide contact (E)[b] is: 0	0.95: 0.05	0.80: 0	0.80: 0.05
0.50	0.99	6	61	6	23
	0.95	10	67	10	26
	0.80	26	107	26	46
Mean		14	78	14	32
0.01	0.99	5	36	5	16
	0.95	8	38	8	17
	0.80	15	47	15	24
Mean		9	40	9	19

[a]The means in this table equate with corresponding entries in Table 3.
[b]These are values of the spray coverage variable.

crops at short intervals there can be a contribution to efficacy from previous toxicant deposits. The model, however, requires that the efficacies of the biocides against each subpopulation do not change with successive spray applications. This would be the case, for example, if infection by survivors occurs immediately after each spray application and if the time required for complete degradation of the fungicide deposit was shorter than the latent period of the pathogen, this in turn being shorter than the interval between sprays. A model with capacity to simulte interactions between fungus populations and fungicide deposits of changing efficacy might be more flexible and realistic, however, for the sake of simplicity it was decided to ignore fungicide degradation in the present model. Its representation would require, once again, the introduction of a time component into the model. Also, there is little information on which to base a mathematical representation of fungicide breakdown, residual activity and the additive effects on efficacy which must occur when fungicides are applied to plant surfaces upon which there exist already partially degraded fungicide deposits.

It should be recognized that this model deals with selection, which is only part of the process through which biocide tolerance develops in organism populations. Individuals with tolerant genes must first appear in the population before selection can occur. It is assumed that genes conferring tolerance usually arise through mutation, and that tolerant strains are identical to the sensitive wild-type strain in all aspects other than tolerance.

It may be possible to develop management practices to reduce the number of mutations to tolerance which occur in field populations. For example, it has been recommended that farmers avoid the use of low application rates of "at risk" fungicides in order to prevent the build up of substantial pathogen populations. The rationale for this recommendation was that if resistance arises by chance mutation, the greater the fungus population in the first place, the higher the probability of that mutation, and the sooner a resistant subpopulation would become available for selection (3). However, according to our model this procedure would favor rapid selection for tolerance once it is present. Whether in agricultural practice it is possible to delay the entry of resistant mutants into field populations of plant pathogens by limiting absolute population size is debatable, and obviously depends upon mutation rates, field population sizes, and other factors. Pathogen populations only rarely can be strictly limited over long periods of time: epidemics and consequent high populations occur from time to time due to a variety of causes both climatic and cultural. Such uncontrolled fluctuations must eventually permit the entry of tolerant genes into the population. There are obviously certain organism/biocide combinations for which it would be overly optimistic to expect any delay in the appearance of tolerance by attempting to limit the entry of tolerant mutants (7). In devising strategies to combat tolerance it will be necessary to decide for each pathogen/crop combination whether the greatest delay in the appearance of tolerance can be achieved through limiting effective mutations or by reducing the rate of selection. We are inclined to think that the latter approach will be more generally useful.

In considering the alternate or sequential use of biocides versus the use of mixtures it should be noted that a disadvantage of the alternate and sequential approaches is the lack of protection from the "at risk" biocide which will occur when tolerance reaches the level of economic impact. There could be crop losses from the failure of a single biocide application. On the other hand, it could be argued that with mixtures farmers may never know they have a tolerant form of a pathogen in their crops: the effective second fungicide will mask the failure of the first. They will, therefore, continue to apply a biocide from which no benefit is being derived.

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