Letter to the Editor

Effects of Alternating and Mixing Pesticides on the Buildup of Fungal Resistance

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Systemic fungicide application has increased spectacularly in the last decade. In a recent review, Edgington et al (2) report that one third of the fungicides currently used worldwide are classed as systemics. Early during that period, however, it was recognized that the considerable advantages of systemics often were counteracted by the development of specific resistance (1,3,4). The extent of this risk is well illustrated in, among others, Fehrmann's 1976 review (3), in which a total of 55 fungal species or *formae speciales* are listed as having shown resistance.

It has been suggested that the use of fungicides alternately or in mixtures can delay the buildup of resistance (2), but no direct experimental or other evidence has been provided to support this view. Recently, Kable and Jeffery (6) presented a theoretical model to deal with the problem. After a large number of computer simulations, they concluded: that when spray coverage is complete, use of fungicide mixtures does not delay the buildup of resistance while alternation does; and that when spray coverage is less than complete, the efficacy of mixtures in delaying resistance buildup increases much faster than the efficacy of alternation and (within the range of numerical values used) mixtures are always preferable when coverage is $<90\%$ (their Table 3).

In this paper an attempt is made to assess the effect of fungicide mixtures or alternation by utilizing the relation between relative parasitic fitness and apparent infection rate as developed by MacKenzie (7) and further clarified by Groth and Barrett (5) and Skylakakis (8).

Fungicide mixtures—The model

Let r_1, R_1 and r_2, R_2 represent the apparent infection rate and basic infection rate sensu Vanderplank (9) of the sensitive and resistant populations, respectively, in the presence of the systemic fungicide. Let r'_1, R'_1, r'_2, and R'_2 retain the above meaning in the presence of a mixture of the systemic fungicide with another fungicide which has equal activity on both populations. The activity of the second fungicide is assumed additive to that of the systemic. Then, in the presence of the systemic fungicide (5,7,8):

$$y_t/x_t = (y_0/x_0)\, e^{(r_2 - r_1)t} \tag{1}$$

and in the presence of the mixture:

$$y'_t/x'_t = (y'_0/x'_0)\, e^{(r'_2 - r'_1)t} \tag{2}$$

in which y represents the proportion or amount of resistant and x the proportion or amount of sensitive population. Equations 1 and 2 are valid in the logarithmic stage of an epidemic; ie, when there is no competition for susceptible host sites between the two populations. From Eq. 1 and 2 it is obvious that the use of the second fungicide will delay the buildup of resistance if

$$r_2 - r_1 > r'_2 - r'_1.$$

Since the activity of the second fungicide has been assumed equal on both resistant and sensitive populations and *additive* (neither synergistic nor antagonistic) to that of the systemic, it follows that

$$R_1/R'_1 = R_2/R'_2 \text{ or } R_2/R_1 = R'_2/R'_1. \tag{3}$$

According to Vanderplank (9) the relation between apparent and basic infection rates during the logarithmic stage (ignoring removals) is

$$R = re^{pr}$$

in which p is the latent period. If this is substituted and natural logarithms are taken, Eq. 3 becomes

$$\ln(r_2/r_1) + p(r_2 - r_1) = \ln(r'_2/r'_1) + p(r'_2 - r'_1). \tag{4}$$

Since by definition $R_2 > R'_2$, $R_1 > R'_1$, $r_2 > r'_2$, $r_1 > r'_1$, $r_2 > r_1$, and $r'_2 > r'_1$, Eq. 4 can only hold if $r_2/r_1 < r'_2/r'_1$ for if $r_2/r_1 \geqslant r'_2/r'_1$ then if one sets $r_2/r_1 = K$ it would follow that $r_2 - r_1 = (K-1)r_1$ and $r'_2/r'_1 = K'$ it would follow that $r'_2 - r'_1 = (K'-1)r'_1$ but then $K \geqslant K' \geqslant 1$ and $(K-1) \geqslant (K'-1) \geqslant 0$ at the same time $r_1 > r'_1 > 0$. It then follows that $(K-1)r_1 > (K'-1)r'_1$ which means that $r_2 - r_1 > r'_2 - r'_1$. Therefore, since $p > 0$, we would have $p(r_2 - r_1) > p(r'_2 - r'_1)$ and $\ln(r_2/r_1) > \ln(r'_2/r'_1)$. This is clearly incompatible with Eq. 4 which requires that $p(r_2 - r_1) < p(r'_2 - r'_1)$ if $\ln(r_2/r_1 > \ln(r'_2/r'_1)$. Then, since r_2/r_1 can only be smaller than r'_2/r'_1 it follows from Eq. 4 that $p(r_2 - r_1) > p(r'_2 - r'_1)$; ie, that the intensity of selection pressure in favor of the resistant population is smaller when a second fungicide is used in a mixture.

Fungicide alternation—The model

Let r_1 and r_2 be the apparent infection rates of the sensitive and resistant populations, respectively, in the presence of the systemic fungicide.

Let r'_1 and r'_2 be the apparent infection rates as above in the presence of the alternative fungicide, which is assumed of equal efficacy to both the sensitive and resistant populations. Finally, let r_a and r_b be the average apparent infection rates for the sensitive and resistant populations, respectively, when an alternation of the two above fungicides is used. Then

$$r_a = (t_1 r_1 + t_2 r'_1)/t_1 + t_2$$

and

$$r_b = (t_1 r'_2 + t_2 r'_2)/t_1 + t_2$$

in which t_1 = total duration of effect of systemic fungicide, t_2 = total duration of effect of alternative fungicide, and $t = t_1 + t_2$ = total duration of effect of sequence of alternative sprays.

It then follows that the intensity of selection pressure that dictates the rate of resistance buildup will be:

$$r_b - r_a = [t_1(r_2 - r_1) + t_2(r'_2 - r'_1)]/t_2 + t_1,$$

whatever the values of t_1 and t_2 may be, since according to the assumptions $r'_2 - r'_1 < r_2 - r_1$, it follows that always

$$r_b - r_a < r_2 - r_1$$

which means that alternation will always delay resistance buildup.

In the simplest case, when $t_1 = t_2 = t/2$ and $r'_2 - r'_1 = 0$,

$$r_b - r_a = (r_2 - r_1)/2.$$

In such a case, the use of alternative sprays will double the time needed for the resistant population to increase to a given level.

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Application of the models

Many factors can affect the intensity of selection pressure. Such factors are the degree of resistance to the systemic (expressed by the ratio R_2/R_1), the efficacy of the alternative fungicide (expressed by the ratio $R_2/R'_2 = R_1/R'_1$), the apparent infection rate as it reflects either the inherent properties of the causing organism or the effect of the environment or both, and finally the latent period, p, sensu Vanderplank (9).

In order to study the effects of the factors listed above we need to define a suitable quantitative measure of the intensity of selection pressure. It is proposed to use the "standard selection time" for this purpose. The standard selection time (t_s) is defined here as the time necessary for the proportion of the resistant population (y/x) to increase by e times. If this concept is applied to Eq. 1 we have

$$y_t/x_t = e \cdot (y_0/x_0) = (y_0/x_0)\, e^{(r_2 - r_1)t_s}$$

and if we take logarithms

$$\ln(y_t/x_t) = 1 + \ln(y_0/x_0) = \ln(y_0/x_0) + (r_2 - r_1)t_s$$

and $t_s = 1/(r_2 - r_1)$, expressed in the same time units as the apparent infection rate.

In Table 1, the effects of variation in r_2 and p on t_s have been computed. The data show that: The delaying effect of a mixture increases as the apparent infection rate of the resistant population in the presence of the systemic decreases. Other things being equal, the shorter the latent period the greater the increase in the standard selection time caused by the mixture. Depending on r_2 and p, mixtures can be more or less efficient than alternative sprays in delaying resistance buildup. Use of mixtures is at its optimum when the apparent infection rate of the resistant population in the presence of the systemic is relatively low and the latent period is short.

In Table 2 the effects of the degree of resistance (R_2/R_1) and the efficacy of the second fungicide ($R_2/R'_2 = R_1/R'_1$) have been quantified. The data show that the higher the efficacy of the systemic and the degree of resistance to it, the faster the resistance buildup, and that the higher the efficacy of the second fungicide, the greater the delaying effect of the mixture. It is also interesting to observe that, as the degree of resistance increases, the relative delaying effect of the mixture, measured by the percent increase in standard selection time, also increases.

Discussion

The model presented in this paper and that presented by Kable and Jeffery (6) agree in their basic conclusions. They both indicate: that mixtures and alternation of fungicides delay resistance buildup; that the rate of resistance buildup increases as the efficacy of the systemic and the degree of resistance to it increase; and that the delaying effect of the mixture increases as the efficacy of the second fungicide increases.

One major discrepancy between the two papers is observed in the case of complete coverage when the Kable and Jeffery model forecasts (in contrast to my model presented in this paper) no delaying effect for fungicide mixtures. Because this may have practical implications in some cases, such as fruit or seed dips for instance, it warrants further discussion. This discrepancy is due to the fact that Kable and Jeffery calculate with their model proportions of survivors rather than proportions of populations that increase with time. If one slightly changes their symbols and defines S_2, S_1 as the survivors of the resistant and sensitive populations after one application of the systemic and S'_2, S'_1 as the survivors after one application of the mixture, then according to their model

$$S_2/S_1 = n_2(1 - f_{2A})/n_1(1 - f_{1A})$$
$$S'_2/S'_1 = n_2(1 - f_{2A})(1 - f_B)/n_1(1 - f_{1A})(1 - f_B)$$

in which n_1, n_2 equal the numbers of sensitive and resistant populations, prior to the application of the fungicides f_{1A} = the efficacy of the systemic toward the sensitive population, f_{2A} = efficacy systemic toward the resistant population, and f_B = efficacy of the second fungicide towards both populations.

The above relations mean that by the definition of their model, $S_2/S_1 = S'_2/S'_1$; ie, the second fungicide does not affect resistance buildup. In the terminology of this paper the same relations mean that the effect of the second fungicide is additive to that of the first and thus, it affects the basic infection rates of both populations proportionally ($R_2/R_1 = R'_2/R'_1$). This, by the way, is totally consistent with the postulated effect of protective fungicides on the basic infection rate (9). In spite of a common underlying assumption, the difference in my conclusions and theirs arises because, due to the effect of the latent period p in the relation between apparent and basic infection rates, when $R_2/R_1 = R'_2/R'_1$, $r_2/r_1 < r'_2/r'_1$ and $r_2 - r_1 > r'_2 - r'_1$ and thus, resistance buildup is delayed by the mixture as both populations increase with time.

There are certain uniquely interesting features of the model presented in this paper that possibly make it a closer approximation to reality as well as easier to use. First, it allows for the increase of both the resistant and the sensitive population during the period of their exposure to the effect of the fungicides. Second, it takes into consideration not only the varying efficacies of both the systemic and the alternative fungicide, but also the inherent epidemiological properties of the resistant and sensitive

TABLE 1. Effects of variation of the apparent infection rate for the resistant population in presence of the systemic fungicide (r_2), and the latent period (p), on the standard selection time (t_s)[a]

Apparent infection rate (r_2)	Latent period (p)	Standard selection time (t_s) Systemic only	Mixture	Alternation[b]
0.5	20	8.3	8.7	16.6
	10	5.4	6.1	10.8
	5	3.5	5.4	7.0
0.25	20	10.9	12.1	21.8
	10	6.9	10.7	13.8
	5	5.4	14.1	10.8
0.125	20	13.9	21.5	27.8
	10	10.7	28.2	21.4
	5	9.6	39.0	19.2
0.0625	20	21.5	56.5	43.0
	10	19.3	78.1	38.6
	5	18.4	97.0	36.8

[a]Degree of resistance to systemic $R_2/R_1 = 10$. Efficacy of alternative fungicide $R_2/R'_2 = R_1/R'_1 = 7$.
[b]Calculated for $r_b - r_a = (r_2 - r_1)/2$, when $r'_2 - r'_1 = 0$ and $t_1 = t_2 = t/2$.

TABLE 2. Effects of degree of resistance to the systemic fungicide (R_2/R_1) and efficacy of the alternative fungicide ($R_2/R'_2 = R_1/R'_1$) on the standard selection time (t_s)[a]

Degree of resistance to systemic fungicide R_2/R_1	Efficacy of alternative fungicide $R_2/R'_2 = R_1/R'_1$	Standard selection time (t_s) Systemic only	Mixture	Alternation[b]	Percent increase in t_s caused by mixture[c]
10	9	6.9	11.7	13.8	69.6
	7	6.9	10.7	13.8	55.1
	5	6.9	9.7	13.8	40.6
8	9	7.5	12.5	15.0	66.7
	7	7.5	11.4	15.0	52.0
	5	7.5	10.3	15.0	37.3
5	9	9.4	14.8	18.8	57.4
	7	9.4	13.7	18.8	45.7
	5	9.4	12.5	18.8	33.0

[a]Apparent infection rate of the resistant population in the presence of the systemic fungicide $r_2 = 0.25$. Latent period, $p = 10$.
[b]Calculated for $r_b/r_a = (r_2 - r_1)/2$.
[c]$[(t_s \text{ mixture} - t_s \text{ systemic})/t_s \text{ systemic}]100$.

populations. Third, the variables used (apparent infection rate, latent period, and basic infection rate) have been and can be experimentally measured. Thus, substantial data already exist in the literature and can be utilized in predictions for specific pathogens. Finally, a parameter, standard selection time, is proposed that provides a simple measure for both the rate of resistance buildup and the effect of delaying strategies.

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