Epidemiological Factors Affecting the Rate of Selection of Biocide-Resistant Genotypes of Plant Pathogenic Fungi

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The resistance problems associated with the use of the site-specific systemic fungicides have been illustrated repeatedly in the last few years. In several extensive reviews, the factors that affect the rate of selection of resistant genotypes have been discussed qualitatively (3,6,8). Three recent publications have dealt quantitatively with the effects of biocide efficacy, biocide mixtures, and biocide alternation on the rate of selection of resistant genotypes (4,10,14).

Delp (4) attempted to calculate the number of lesions per unit of land area covered by the susceptible host after 1 yr or more of fungicide application. With his model, if the discussion is limited to 1 yr (which avoids the need for quantitative assessment of inoculum survival to the next season), the variables necessary for the calculation are: the initial incidence of the resistant population (A), the number of lesions produced by one parent lesion per generation, the percent survival (B) of resistant spores after each exposure to the fungicide ($B = 1$ in the presence of a systemic fungicide), the number of reinfection generations (D) during the season, and a factor ($Y \times Z$) to adjust for partial coverage of the area by the susceptible host population and the proportion of that population treated with the systemic fungicide. According to Delp, the number of resistant lesions A_t at the end of the season will be

$$A_t = A\,[(10 \times B)^D \times (10 \times Y \times Z)].$$

This is a discontinuous compound interest model (16) in which the increase of the resistant population is corrected by $Y \times Z$, as explained above.

Kable and Jeffery (10) attempted direct assessment of the change in the ratio between the resistant and the sensitive populations after repeated challenges by a systemic biocide, by a mixture of biocides, or by alternation with a nonsystemic one. The variables used by Kable and Jeffery were the efficacies of both biocides on both populations as well as the thoroughness of spray coverage that also affects the efficacies of both biocides.

Finally, Skylakakis (14) evaluated the effect of biocide efficacy, mixtures, or alternation on the ratio between the resistant and sensitive populations by utilizing the relation between parasitic fitness and apparent infection rate, which was first suggested by MacKenzie (11). The advantage of this approach is that it relates the problem of selection for fungicide resistance to well-established theory in both population genetics and epidemiology. The ratio of biocide-resistant versus biocide-sensitive genotypes during the logarithmic stage of an epidemic is given by

$$y_t/x_t = (y_0/x_0)\exp(r_2 - r_1)t \qquad (1)$$

in which: x_0, x_t = proportions or amounts of disease caused by the sensitive genotype at times 0 and t, respectively; y_0, y_t = proportions or amounts of disease caused by the resistant genotype at the same time; r_1 = apparent infection rate for x; and r_2 = apparent infection rate for y.

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In equation 1, the ratio of the two genotypes at any time is affected by their initial incidence (y_0/x_0), the duration (t) of the selection pressure, and its intensity ($r_2 - r_1$). It must be assumed that in the absence of the biocide $r_2 - r_1 < 0$; if $r_2 - r_1 \geqslant 0$ the resistant genotype would have been prevalent prior to the introduction of the biocide, the new product would not have provided satisfactory disease control, and it would not have been released for manufacture and use. If this assumption is true, then the initial incidence of the resistant genotype should be defined by its fitness in the absence of the biocide and the mutation rate for the site or sites that generate resistance. The selection pressure duration time (t) is related to the residual activity of the biocide and the pattern of its use. Finally, the intensity of selection pressure ($r_2 - r_1$) is related to the relative fitness of the resistant population in the absence of the biocide, the efficacy of the biocide, and the degree of resistance to it, as well as the epidemiological characteristics of the causal organism.

The objective of this paper is to evaluate how these epidemiological characteristics affect the intensity of selection pressure and thus the rate of selection of biocide-resistant genotypes of plant pathogenic fungi.

THE MODEL

Assume two continuous compound-interest diseases (16) caused by pathogens A and B, respectively. Then, in the absence of the biocide, let: R_A, R_B, r_A, r_B = basic and apparent infection rates of the sensitive populations of pathogens A and B; R_{rA}, R_{rB}, r_{rA}, r_{rB} = basic and apparent infection rates of the resistant populations; and P_A, P_B = latent periods for both resistant and sensitive populations.

Accordingly, in the presence of the biocide, let R_A', R_B', r_A', r_B' = basic and apparent infection rates of the sensitive populations of pathogens A and B; R_{rA}', R_{rB}', r_{rA}', r_{rB}' = basic and apparent infection rates of the resistant populations; and P_A', P_B' = latent periods for both resistant and sensitive populations.

With these symbols, the relative fitness of the resistant populations in absence of the biocide is defined by $r_{rA} - r_A$ and $r_{rB} - r_B$ for pathogens A and B, respectively; the efficacy of the biocide on the sensitive populations is defined by R_A/R_A' and R_B/R_B'; the degree of resistance of the resistant populations is defined by R_{rA}/R_{rA}' and R_{rB}/R_{rB}'; and, finally, the intensity of selective pressure in the presence of the biocide is defined by $r_{rA}' - r_A'$ and $r_{rB}' - r_B'$ for pathogens A and B, respectively.

To isolate the effect of the epidemiological properties of the causal organisms, the following additional assumptions must be made:

1. Equal relative fitnesses of resistant populations in the absence of biocide, therefore:

$$r_{rA} - r_A = r_{rB} - r_B = a \text{ or } r_{rA} = a + r_A \text{ and } r_{rB} = a + r_B.$$

2. Equal biocide efficacy against the sensitive populations of pathogens A and B, therefore:

$$R_A/R_A' = R_B/R_B'.$$

3. Degree of resistance is high and equal for both resistant populations A and B, therefore:

$$R_{rA}/R_{rA}' = R_{rB}/R_{rB}' = 1.$$

4. Latent period is not affected by the presence of the biocide, therefore:

$$P_A = P_A',\ P_B = P_B'.$$

In view of assumptions 3 and 4,

$$r_{rA} = r_{rA}' \text{ and } r_{rB} = r_{rB}'.$$

In this case, taking into consideration assumption 1, the intensity of selective pressure in the presence of the biocide becomes:

$$r_{rA}'-r_A' = r_{rA}-r_A' = (a+r_A) - r_A' \quad (2)$$

and

$$r_{rB}'-r_B' = r_{rB}-r_B' = (a+r_B) - r_B' \quad (3)$$

Obviously, if $r_A-r_A' > r_B-r_B'$, it also follows that $r_{rA}-r_A' > r_{rB}-r_B'$ and $r_{rA}'-r_A' > r_{rB}'-r_B'$. Thus, the intensity of selective pressure in favor of biocide-resistant populations of various pathogens can be compared by using the apparent infection rates of the sensitive populations within the limitations set by assumptions 1 through 4. Since $R = r\exp(pr)$ (16), after taking natural logarithms, assumption 2 can be rewritten as:

$$\ln (r_A/r_A')+P_Ar_A-P_A'r_A' = \ln (r_B/r_B')+P_Br_B-P_B'r_B'$$

or, since $P_A = P_A'$ and $P_B = P_B'$ (assumption 4),

$$\ln(r_A/r_A') + P_A(r_A-r_A') = \ln(r_B/r_B') + P_B(r_B-r_B') \quad (4)$$

The implications of equation 4 are as follows: if $P_A = P_B$ and $r_A > r_B$, equation 4 is possible only if $r_A/r_A' < r_B/r_B'$, for if $r_A/r_A' \geq r_B/r_B'$ then also $r_A-r_A' > r_B-r_B'$, which is clearly incompatible with equation 4. Since $r_A/r_A' < r_B/r_B'$ equation 4 can only hold if $P_A(r_A-r_A') > P_B(r_B-r_B')$; ie, $r_A-r_A' > r_B-r_B'$.

For pathogens with the same latent period, therefore, selection for resistance will proceed more rapidly for the one that spreads faster in unsprayed fields.

TABLE 1. Effects of epidemiological characteristics (apparent infection rate, latent period) of the sensitive population of a fungus on standard selection time[a]

Apparent infection rate (r^b)	Standard selection time (t_s)[b] for latent period (p) equal to:				
	20	10	8	5	3
0.500	No entry[c]	5.4	4.6	3.5	2.8
0.450	No entry	5.7	4.8	3.7	3.1
0.400	No entry	5.8	5.0	3.9	3.3
0.350	No entry	6.0	5.3	4.2	3.7
0.300	10.4	6.4	5.6	4.7	4.2
0.250	10.9	6.9	6.3	5.4	4.9
0.200	11.5	7.9	7.2	6.4	6.0
0.175	12.0	8.5	7.9	7.2	6.8
0.150	12.8	9.4	8.8	8.2	7.8
0.125	13.9	10.7	10.2	9.6	9.3
0.100	15.7	12.8	12.4	11.8	11.5
0.075	18.8	16.3	16.0	15.5	15.2
0.050	25.6	23.6	23.3	22.7	22.6
0.025	47.2	45.5	45.4	45.0	44.8

[a] Efficacy of biocide on sensitive population $R_A/R_A' = R_B/R_B' = 10$. Degree of resistance to biocide high, $R_{rA}/R_{rA}' = R_{rB}/R_{rB}' = 1$.

[b] Expressed in same time units; ie, if $r = 0.5$ per day then t_s and p are also in days.

[c] Because there are no estimates for R when $p = 20$ and $r > 0.3$ in Vanderplank (16).

If $P_A > P_B$ and $r_A = r_B$, equation 4 is possible only if $r_A' > r_B'$ for if $r_A' \leq r_B'$, then $\ln (r_A/r_A') \geq \ln(r_B/r_B')$, $(r_A-r_A') \geq (r_B-r_B')$, $P_A > P_B$, and these relations are incompatible with equation 4. Since $r_A' > r_B'$ and $r_A = r_B$, it follows that $r_A - r_A' < r_B-r_B'$.

For pathogens spreading equally fast in unsprayed fields, therefore, selection for resistance will proceed more rapidly for the one with the shorter latent period. If $r_A > r_B$ and $P_A < P_B$, equation 4 is possible only if $r_A-r_A' > r_B-r_B'$ for if $r_A-r_A' = r_B-r_B'$ then also $r_A/r_A' \leq r_B/r_B'$ and equation 4 becomes impossible.

Thus, selection for resistance will proceed faster for the pathogen that spreads faster in unsprayed fields and has the shorter latent period. Following the same reasoning, if $r_A > r_B$ and $P_A > P_B$, as when $P_A = P_B$, equation 4 is possible only when $r_A/r_A' < r_B/r_B'$ and $P_A (r_A-r_A') > P_B (r_B-r_B')$.

However, since $P_A > P_B$, r_A-r_A' can be smaller, equal or greater than r_B-r_B'. In this case, no overall conclusion can be made on the speed of selection for resistance.

APPLICATION OF THE MODEL

Table 1 includes computed data on the effect of variation of the apparent infection rate of the sensitive population in absence of the biocide and its latent period to the intensity of selection pressure and thus to the rate of buildup of resistance.

As proposed by Skylakakis (14) standard selection time has been used as the quantitative measure of the intensity of selection pressure. It was assumed that in the absence of the biocide the sensitive and the resistant population are almost equally fit ($r_{rA}-r_A = r_{rB} - r_B \leq 0.002$). The computed data show that:

1. The higher the apparent infection rate and the shorter the latent period of the pathogen, the faster the buildup of resistance.
2. The effect of variation of the apparent infection rate to the rate of resistance buildup is greater for slower pathogens (bottom half of Table 1) and for pathogens with relatively short latent periods.
3. The effect of variation of the latent period to the speed of resistance buildup is greater for faster pathogens (top half of the Table) and for pathogens with relatively long latent periods.

PRACTICAL IMPLICATIONS

To what extent can the model account for the observed facts of fungicide resistance outbreaks in the field? In an attempt to provide an answer, a few cases are examined in some detail below.

Cucumber powdery mildew and dimethirimol. According to Bent et al (2) dimethirimol was first widely used in Dutch glasshouses in 1969. It was applied mainly as a soil drench to the base of established plants and provided virtually complete protection to powdery mildew for 6–10 wk. Mildew attacks in January and early February 1970 were still well controlled, however, widespread failures were reported in March-April of the same year. In the absence of more detailed data one has to assume that the breakdown occurred after a minimum of two, and a maximum of four, consecutive applications of the compound; ie, taking as an average a residual effect of 8 wk, after 112–224 days of selection pressure.

In order to proceed with the analysis a few more facts must be established. The latent period of the cucumber powdery mildew under favorable conditions is 5–6 days (13). There are no published estimates of the apparent infection rate, but studies in Dutch glasshouses have given five independent estimates ranging from 0.09083 to 0.26030 with an average of $\simeq$ 0.17189 (*unpublished*). Assuming a latent period of 5 days, an apparent infection rate of 0.17189 and an average of 98% control provided by dimethirimol for 40–45 days on the sensitive population (2), the degree of efficacy of dimethirimol against the sensitive population is approximately 6. $R_A/R_A' = R_B/R_B'$.

Bent et al (2) established a variety of degrees of resistance against dimethirimol. The data in *their* Table 4, although not usable for a direct calculation, seem to support a high degree of resistance (equal to 1) for their more resistant isolates. For the less resistant isolates, we have no data that could lead to an estimate, but we can

TABLE 2. Standard selection time (t_s) and time (both in days) needed for populations of *Erysiphe cichoracearum* (highly or intermediately resistant to dimethirimol) to reach a frequency of (y/x) of 10^{-1} in the total population[a]

Population	Intermediately resistant ($R_{rA}/R_{rA}' = R_{rB}/R_{rB}' = 2$)		Highly resistant ($R_{rA}/R_{rA}' = R_{rB}/R_{rB}' = 1$)	
Initial incidence of resistant population (y_0/x_0)	10^{-6}	10^{-8}	10^{-6}	10^{-8}
Standard selection time t_s (days)	16.5	16.5	8.5	8.5
Time (days) needed for resistant population to reach frequency (y/x) of 10^{-1}	190.0	262.8	97.9	137.0

[a] Apparent infection rate of sensitive population in absence of dimethirimol $r = 0.17189$ (per unit per day). Efficacy of dimethirimol on a sensitive population $R_A/R_A' = R_B/R_B' = 6$. Latent period $p = 5$ days. Selection against the sensitive population in the absence of dimethirimol was very low, $S = 0.01$.

assume it is equal to 2.

Finally, the data of Bent et al (2) provide no indication that resistance was associated with significant reduction in fitness and no estimate of the initial incidence of the resistant populations. For the calculation, we assume fitness of the resistant populations slightly lower than that of the sensitive ones in the absence of dimethirimol and an initial occurrence of 10^{-6} to 10^{-8}.

The theoretical calculations (Table 2) show that we should have expected an outbreak of resistance anywhere between 98–263 days of continuous dimethirimol selection pressure, a rather remarkable agreement with what seems to have happened in practice.

Potato late blight and metalaxyl. The details of this spectacular outbreak are not yet known (1). However, with a latent period of 5 days, an apparent infection rate of 0.45 (16) consistent with the reported severity of the 1980 epidemic (1), and a chemical as efficacious as metalaxyl ($R/R' = 7$–10), the standard selection time t_s will vary between 3.7 and 3.8 days. This means that within 51–70 days of metalaxyl pressure, the frequency of a highly resistant population would increase from an initial level of 10^{-6}–10^{-8} to outnumbering the sensitive population but 10:1 and destroying the potato crop in the process. Again, within the available facts, theoretical prediction and reality show a remarkable fit.

***Cercospora beticola* and benomyl**. The case of sugar beet leaf spot is described in similar terms by Georgopoulos and Dovas (9) for northern Greece and Ruppel and Scott (12) for Texas. By the end of the third year of commercial application, resistance to benomyl had become prevalent. Georgopoulos and Dovas (9) relate that it takes 7–10 benomyl sprays to reach a frequency of 90–100% for the resistant population. Benomyl was used in northern Greece on a 20-day schedule (15), which means that the duration of the selective pressure in the cases reported by Georgopoulos and Dovas (9) ranged 140–200 days. The initial incidence of the resistant population is unknown but the evidence (5) suggests almost equal fitness between sensitive and resistant populations and a high degree of resistance. Finally, prior to the occurrence of resistance, apparent infection rates of 0.10–0.15 for the sensitive population in unsprayed fields, latent periods of 10–15 days, and benomyl efficacy (R_A/R_A') of approximately 10 were obtained by Skylakakis (*unpublished*). The necessary calculations (Table 3) provide an estimate of 130–263 days, again well within reality.

Cereal smut and carboxin. Both Dekker (3) and Georgopoulos (8) comment on the fact that no resistance to carboxin has yet been encountered in practice, although resistant smut mutants have been readily isolated in the laboratory. The situation, however, is totally different from an epidemiological point of view. Loose smut of cereals infects only once per year and infection increases from year to year at most by 20-fold (7). If one applies Vanderplank's (16) approach to such diseases $(1 + R)^t = \exp(r) = 21$ ie, $r = \ln 21 = 3.0445$ per year or 0.00834 per day. Assuming a very high efficacy for carboxin on the sensitive population ($R_A/R_A' = 20$), then in the presence of carboxin $(1 + R')^t = \exp(r) = 2$ ie, $r' = \ln 2 = 0.6931$ per year or 0.00190 per day. Standard selection time t_s is equal to 158.5 days in this case and it would take exactly 1 yr for the frequency of the resistant population to increase 10-fold and 5 to 7 yr of consecutive carboxin applications as well as conditions favoring maximum disease spread, for the frequency of the resistant population to reach 10% starting from an initial incidence of 10^{-6} or 10^{-8}, respectively. Thus, an outbreak of resistance becomes highly improbable indeed.

TABLE 3. Standard selection time and time (both in days) needed for sugar beet leafspot populations of *Cercospora beticola* highly resistant to benomyl to reach a frequency (y/x) of 10^{-1} in total population[a]

Apparent infection rate of sensitive population unsprayed r (per unit per day)	0.10		0.15	
Latent period p (days)	15		10	
Initial incidence of resistant population (y_0/x_0)	10^{-6}	10^{-8}	10^{-6}	10^{-8}
Standard selection time t_s (days)	14.3	14.3	9.5	9.5
Time (days) needed for resistant population to reach frequency (y/x) of 10^{-1}	197.4	263.2	131.3	175.0

[a] Degree of resistance high $R_{rA}/R_{rA}' = R_{rB}/R_{rB}' = 1$. Benomyl efficacy on sensitive population $R_A/R_A' = R_B/R_B' = 10$. Selection against the sensitive population in the absence of benomyl was very low ($S = 0.01$).

Although it should be well understood that the model presented in this paper is only a simplified approximation of reality, it seems to be a useful tool because it provides reasonable quantitative estimates and underlines the kinds of factors that should be experimentally defined to provide even more accurate forecasts on the potential rate of resistance buildup.

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