Effects of Mixtures of Benomyl and Mancozeb on Buildup of Benomyl-Resistant *Venturia inaequalis*

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ABSTRACT

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In 1983 and 1984, a block of 72 Rome Beauty apple trees was inoculated with benomyl-sensitive and benomyl-resistant strains of *Venturia inaequalis*. Fungicide mixtures consisting of different concentrations of benomyl and mancozeb then were applied. In both years, populations of the resistant strains increased rapidly as the concentration of benomyl in the mixture was increased. An increase in the mancozeb concentration tended to delay the buildup of benomyl-resistant strains. In 1983, 98.7% of the initial population was sensitive and the mixture containing the highest benomyl rate (225 mg/L) provided adequate control. In 1984, only 69% of the initial population was sensitive and none of the mixtures gave adequate control. In a second experiment, sprays of benomyl plus mancozeb, each at one-half the recommended rate, were applied to four blocks of 25 trees, each with initial proportions of resistant lesions of 0.006, 0.037, 0.313, and 0.803. The rate of increase in the proportion of resistant lesions was most rapid when neither the sensitive nor the resistant subpopulation dominated in the early stages of selection. The rate was lower when the initial population was composed of mostly sensitive or resistant strains.

The development of highly effective organic fungicides has contributed greatly to the management of fungal pathogens (11). However, in recent years, resistance has developed toward some newer, more site-specific compounds (2). At present, there is concern that resistance may develop against the ergosterol biosynthesis inhibiting fungicides (5).

The use of mixtures or alternation of fungicides with different modes of action has been suggested for reducing selection of resistant pathogens (1,2,4). Several mathematical models have been constructed to simulate the effects of these strategies on the selection process (6,10,15). However, reports of field experimentation specifically designed to examine the effect of these strategies on selection are limited.

The objectives of this study were to determine the relationship between the efficacy of benomyl and mancozeb in mixtures with selection and control of *Venturia inaequalis* (Cke.) Wint. and to examine the effects of the initial proportion of the resistant subpopulation on the selection process. Lower concentrations of benomyl and higher concentrations of mancozeb in mixtures, or lower initial proportions of the resistant strain, were hypothesized to cause a reduction or delay in selection for resistance.

MATERIALS AND METHODS

Evaluation of fungicide mixtures. A block of 72 apple trees (*Malus domestica* Borkh. cv. Rome Beauty) on seedling rootstock and planted on a row spacing of 7 × 10.7 m was used. Because about 15% of the leaves on these trees were infected with scab in the autumn of 1982, elgetol (19% sodium dinitro-*o*-cresylate, 0.5 L/100 L at 4,670 L/ha) was applied to the entire orchard floor before budbreak in 1983 and again in 1984 to reduce ascospore inoculum (8).

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During the tight cluster stage of bud development, 16 vegetative shoots were tagged around the periphery of each tree in each year. Four additional shoots, one in each quadrant, were tagged and sprayed with benomyl at 5 mg/L. A mixture of conidia from benomyl-sensitive isolates was sprayed onto the 16 shoots, and a mixture from benomyl-resistant isolates was sprayed onto the four benomyl-treated shoots during Mills's infection periods (13, Table 1). Each mixture was adjusted to 50,000 spores per milliliter with water. Resistant isolates used in 1983 were supplied by Alice Davis, E. I. du Pont de Nemours & Co., Wilmington, DE, and isolates used in 1984 were from two commercial orchards in Pennsylvania. All resistant isolates grew on media containing 5 mg of benomyl per liter.

The initial proportion of resistant lesions was obtained by dividing the number of lesions on shoots inoculated with the resistant strains by the total number of lesions on all inoculated shoots. After the resistant strains were established, mixtures of benomyl and mancozeb (Benlate 50W and Manzate 200 80W, respectively) were applied to the trees until runoff with a hand-held sprayer at 3,800 kPa pressure. The treatment design in 1983 was a 3 × 2 factorial: benomyl at levels of one-fourth, one-half, and three-fourths the recommended rate were each combined with mancozeb at one-fourth and one-half the recommended rate. In 1984, the treatment design was augmented to a 4 × 3 factorial by incorporating one additional level of benomyl and mancozeb, each at one-eighth the recommended rate. The recommended (label) rates of benomyl and mancozeb were 300 and 1,920 mg/L, respectively. The fungicides were applied once in 1983 and twice in 1984 (Table 1).

The 1983 field design consisted of six blocks of 12 trees each, each block receiving a single fungicide mixture; the blocks were purposely confounded with the treatments to reduce interplot interference. However, to accommodate all 12 treatments in 1984, a randomized complete block design was implemented: the 12 treatments were randomly assigned to each of the 12 trees in each block. Thus, in 1983, there were 12 trees or replicates per treatment, whereas in 1984, there were only six replicates.

Because mancozeb inhibits germination of conidia, the

sensitivity of lesions to benomyl could not be determined until late June or early July, when residues were no longer inhibitory (Table 1). In 1983, 20 lesions per tree (240 lesions per treatment), and in 1984, 30 lesions per tree (180 lesions per treatment) were examined for benomyl sensitivity using a technique previously described (9). Lesions were considered benomyl-sensitive if their conidia produced malformed, nonseptate germ tubes when incubated on water agar containing 5 mg/L benomyl and benomyl-resistant if their conidia produced normal germ tubes with septations on this medium. The lesions were selected from shoots around the periphery of each tree, excluding those 20 shoots that had been inoculated earlier in the season.

An analysis of variance was performed on the estimated proportions of resistant lesions to determine the importance of the benomyl, mancozeb, and interaction factors to the selection process. In addition, the linear form of the monomolecular function (without an intercept) was fit to the data by regression analysis:

$$\ln[K/(K-y)] = r \times *\text{CONC}, \quad (1)$$

where y = proportion of resistant lesions, K = maximum $y + 0.01$, r = estimated intrinsic growth rate, and CONC = mg/L of benomyl. Regression models were derived for each level of mancozeb (full models) and for the combined data set (reduced model). Using the error sums of squares and degrees of freedom from these models, an F-statistic was calculated to determine if mancozeb had a significant effect on the relationship between benomyl concentration and the proportion of resistant lesions (14).

The severity of disease was estimated by selecting 10 shoots around the periphery of each tree and counting the lesions and leaves on each shoot. The average number of lesions per leaf was calculated for each tree by dividing the total number of lesions by the total number of leaves. These disease severity data were subjected to an analysis of variance to examine the relative importance of the benomyl, mancozeb, and interaction factors to disease control. The linear form of the power function was fit to the data using regression analysis:

$$\ln(y) = \ln(a) + b*\ln(\text{CONC}), \quad (2)$$

where y = number of lesions per leaf, $\ln(a)$ = y-intercept, b = slope or rate parameter, and CONC = mg/L of benomyl. To determine the effect of mancozeb on the relationship between benomyl and the severity of disease, an F-statistic was calculated using the same method given above.

The treatment means for each level of mancozeb and benomyl were used in fitting all regression models.

Initial proportion of resistance. In spring of 1983, 2-yr-old Rome Beauty apple trees on M26 rootstock were planted in four blocks of 25 trees each. Each block consisted of a square five trees long by five trees wide with 3 m between trees. The blocks were arranged in a row and spaced 7 m apart. The entire row of blocks was oriented so that it was perpendicular to the prevailing wind. Apple trees had never been planted at the site, and there was separation from the nearest apple orchard upwind by a block of peach trees and downwind by a cornfield. In spring of 1984, each tree was pruned to allow the growth of only five shoots. Conidia from two Pennsylvania isolates, one benomyl-sensitive (BS21) and one benomyl-resistant (SR65), were produced on malt extract wick cultures (17). They were then mixed to yield four suspensions with proportions of resistant conidia of 0.001, 0.01, 0.1, and 0.5, where the proportion = no. resistant conidia/(no. sensitive conidia + no. resistant conidia). The suspensions were adjusted to 50,000 conidia per milliliter and then sprayed onto the four blocks, one suspension per block, during early infection periods (Table 1). All shoots on each tree were inoculated with the suspensions. A fungicide spray, consisting of a mixture of one-half the recommended rates of benomyl and mancozeb, was applied twice during June until runoff, using a hand-held sprayer at 3,800 kPa pressure (Table 1).

Approximate generations of lesions were estimated given a minimum latent period of 8 days, the time of each infection period, and knowledge of which leaves were present during those infections (Table 1). The inoculations in early May 1984 established the first generation. A cold period during the second and third weeks of May lengthened the latent period so that lesions were not visible until late May. Therefore, the three infection periods after inoculation did not contribute to the epidemic.

After estimating the initial proportion of resistant lesions on 31 May, the youngest, most unfolded leaf on each shoot was tagged. An infection period on 7 June allowed spores from the first generation of lesions to infect the younger leaves making up the second section of shoot growth (above the tag): the resulting lesions were labeled as the second generation. Lesions resulting from the 14 June infection period were also labeled as the second generation because fewer than 8 days, the minimum latent period, occurred from 7 to 14 June. Because a minimum of 9 days elapsed between the 7 June infection period and the two periods occurring from 16 to 19 June, the second generation should have contributed spores to infection and, therefore, the third generation was produced (Table 1). The fourth and fifth generations can be similarly deduced.

On 29 June, the second assessment of lesion sensitivity to benomyl was made but only on the younger leaves above the

TABLE 1. Chronology of infection periods and their relation to inoculations, fungicide applications, disease assessments, and generations of scab lesions in 1983 and 1984

Date	Mills's infection period[a]	Event	Experiment[b]	Generation[c]
1983				
2 May	None[d]	Inoculation	1	···
15 May	None[d]	Inoculation	1	···
19 May	Heavy	Inoculation	1	···
21–23 May	Moderate	···	···	···
29 May	Moderate	···	···	···
2 June	···	Assessment	1	···
3 June	···	Fungicide	1	···
4 June	Heavy	···	···	···
18–20 June	Heavy	···	···	···
20–21 June	Heavy	···	···	···
28–29 June	Moderate	···	···	···
8 July	···	Assessment	1	···
1984				
3–4 May	Light	Inoculation	1,2	1
6–8 May	Heavy	Inoculation	1,2	1
21 May	Light[e]	···	···	···
22–23 May	Light[e]	···	···	···
28–29 May	Heavy[e]	···	···	···
31 May	···	Assessment	2	···
1 June	···	Assessment	1	···
4–5 June	···	Fungicide	1,2	···
7 June	Light	···	···	2
14 June	Moderate	···	···	2
15 June	···	Fungicide	1,2	···
16–18 June	Heavy	···	···	3
18–19 June	Moderate	···	···	3
24–25 June	Heavy	···	···	4
25–29 June	···	Assessment	1	···
29 June	···	Assessment	2	···
30 June	Moderate	···	···	4
1 July	Light	···	···	4
5–6 July	Moderate	···	···	5
6–7 July	Moderate	···	···	5
13–14 July	···	Assessment	2	···

[a] Infection periods were based on temperature and duration of leaf wetness (13).

[b] 1 = Fungicide evaluation and 2 = initial proportion of resistance experiment.

[c] A new generation of lesions results from an infection period when more than 8 days (minimum latent period) elapse between that period and the previous period; the inoculations established the first generation.

[d] Duration of wetness was too short for infection to occur.

[e] A cold period extended the latent period of the first generation; lesions were not visible until 31 May, therefore no new generations.

tagged leaf (Table 1). Although the 24–25 June infection period produced a fourth generation, its lesions would not be visible for this second assessment. Therefore, the youngest diseased leaf was tagged during this assessment instead of the youngest leaf. This allowed lesions from this infection period to be part of the third shoot section assessment on 13–14 July. In all assessments, about 10 lesions per tree (250 per treatment) were examined for benomyl sensitivity using the method given for the fungicide evaluation experiment.

The rate of increase in the proportion of resistant lesions was determined for the time intervals between the first and second assessments and between the second and third assessments. The rates were calculated separately for each tree; thus about 25 rates (some trees were missing) or replicates were available for each inoculum treatment. To evaluate treatment effects, the rates within each of the two time intervals were compared using Bonferroni t tests. In addition, the maxima function, $y = axe^{-bx}$, was fit to the rates calculated for the first time interval by using nonlinear regression, where y = proportion of resistant lesions per generation, x = initial proportion of resistant lesions, and $\ln(a)$ and b were the intercept and slope, respectively, of the linear form of the function.

RESULTS

Selection of resistant strains with fungicide mixtures. Although the inoculation procedure was similar in 1983 and 1984, the proportion of resistant lesions observed on the first assessment was 0.013 and 0.31, respectively. Furthermore, no lesions were observed on uninoculated shoots in 1983, whereas some lesions were found on these shoots in 1984. We suggest that the ground spray of elgetol in the spring of 1984 did not significantly reduce the large amount of overwintering inoculum for the experiment in 1983.

Both benomyl and mancozeb had significant effects on the proportion of resistant lesions, as indicated by the relatively low P values in the analysis of variance (Table 2). At the relatively high resistance levels of 1984, the proportion of resistant lesions dramatically increased as the rate of benomyl was increased from one-eighth to one-fourth the recommended rate, then leveled off as the benomyl was increased from one-fourth to one-half the recommended rate (Fig. 1A). The monomolecular function provided an excellent fit to the data, as indicated by the high coefficients of determination and low standard error of the estimates (Table 3). The calculated F-value for comparing the reduced and full models was 5.39, which was significant at $P = 0.03$. Thus, an increase in the mancozeb concentration caused a significant reduction in the proportion of resistant lesions by decreasing the value of the intrinsic growth rate, r. In 1983, the F-value for comparing the reduced and full models was 4.82, which was significant at $P = 0.09$. This lower degree of significance was most likely due to a poor fit by the monomolecular function rather than by the inability of mancozeb to reduce or delay selection. Indeed, a straight line fit the data quite well and the F-value for comparing the reduced and full models was 48.01, which was highly significant at $P = 0.002$.

Scab control with fungicide mixtures. The effect of benomyl concentration on disease severity was significant in both years, as indicated by the analysis of variance (Table 2). In 1984, the power function provided an excellent fit to the data and the F-value for comparing the reduced and full models, 13.88, was significant at $P = 0.003$ (Table 4). This outcome indicated that an increase in the

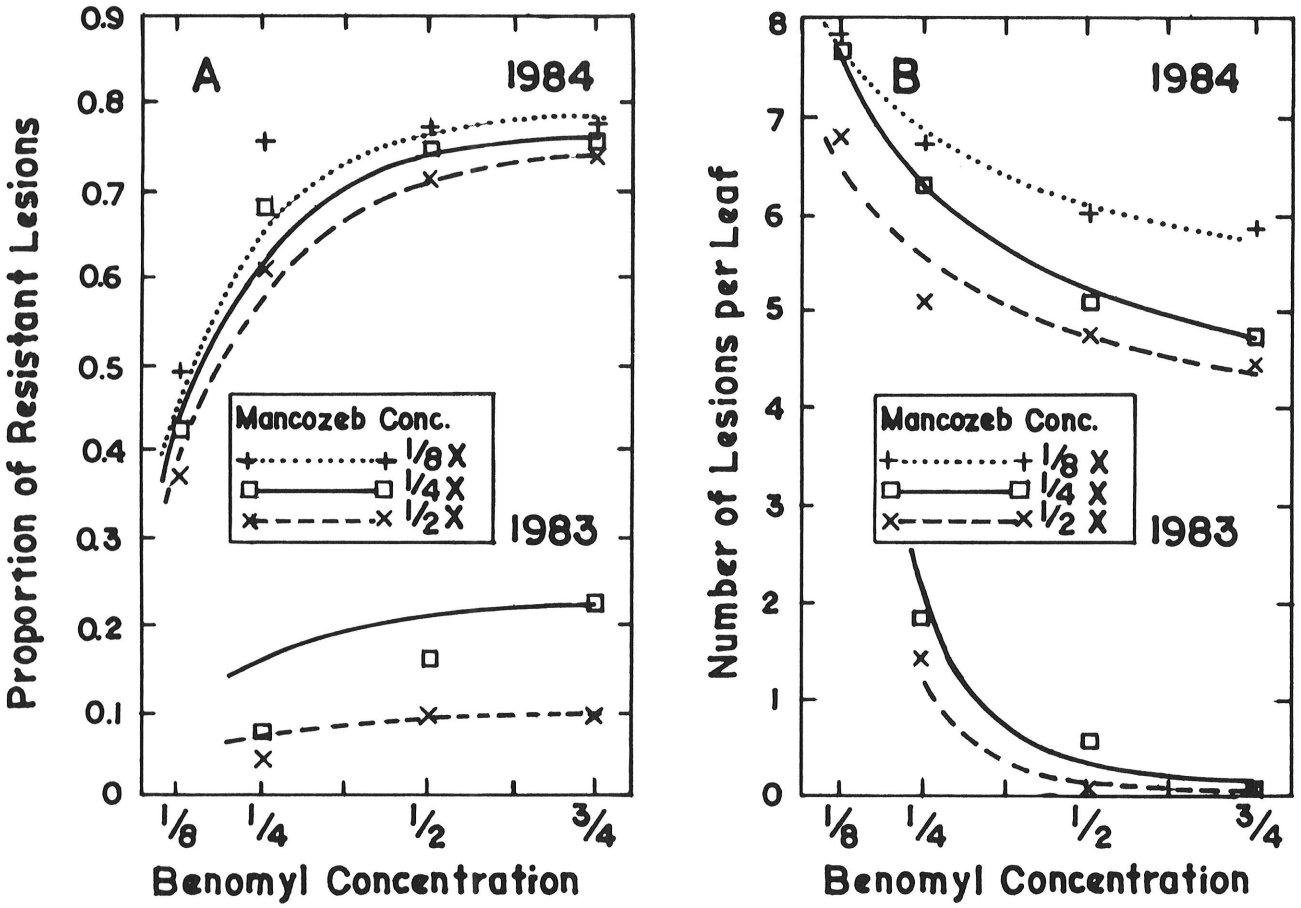

Fig. 1. Relationship **A,** between selection for resistance and benomyl concentration and **B,** between disease control and benomyl concentration for different concentrations of mancozeb in the 1983 and 1984 apple scab epidemics. In A, the curves represent the fit of the monomolecular function (eq. 1) given the parameter estimates in Table 3: each point was an average of 12 trees, 20 lesions per tree, and six trees, 30 lesions per tree in 1983 and 1984, respectively; a single outlier, the results from one tree, was not included in the 1984 analysis. In B, the curves represent the fit of the power function (eq. 2) given the parameter estimates in Table 4: each point was an average of observations from 12 trees, 10 shoots per tree, and from six trees, 10 shoots per tree in 1983 and 1984, respectively. The recommended rates for benomyl and mancozeb used in the spray mixtures were 300 and 1,920 mg/L, respectively.

mancozeb concentration caused a reduction in disease severity by decreasing either or both of the model's parameters. At the lower disease levels of 1983, the F-value for comparing the reduced and full models was 2.14, which was highly insignificant at $P = 0.32$. Although this insignificance may be due to an inadequate fit of the power function, note that the mancozeb factor in the analysis of variance was also less significant (Table 2). The negative exponential function provided a better fit than the power function in only one of the five data sets.

In both years, the benomyl × mancozeb interaction term was not significant in the analysis of variance for either of the dependent variables (Table 2).

Effect of initial proportion of resistance on selection. The proportion of benomyl-resistant lesions in the first generation was consistently greater than the proportion of resistant conidia in the inoculum. Resistant conidia at initial proportions of 0.001, 0.01, 0.1, and 0.5 produced resistant lesions at proportions of 0.006, 0.037, 0.313, and 0.803, respectively. Apparently, the sensitive conidia were either less viable or less capable of inciting disease.

The rate of increase in the proportion of resistant lesions was greater between the first and third generations than between the third and fifth generations (Fig. 2). However, the treatment with the lowest initial resistant proportion of 0.006 had a much slower rate of increase between the first and third than between the third and fifth generations. Furthermore, the rate of increase from the first to the third generation was significantly greater for the 0.037 and 0.313 treatments than for the 0.006 and 0.803 treatments (Fig. 2).

A plot of the rate of increase during this period for each tree against the initial proportion of resistant lesions on that tree indicated that the rate of increase in the proportion of resistance in the population was a function of the initial proportion (Fig. 3). The rate was greatest for the middle proportions of resistance or sensitivity when neither subpopulation was dominant: selection was much slower when either subpopulation dominated. However, the proportional increase in resistance was much greater for lower initial levels of resistance. The proportion of resistant lesions between the first and third generations increased by a factor of 8 or 10 for those trees having the two lowest initial levels of fungicide-resistant strains (Fig. 2). An increase of only 1.8 and 0.2 times the initial amount was recorded on those trees having the two highest initial levels of resistant strains.

The maxima function described 68% of the variation in the rate of increase in the proportion of resistance (Fig. 3).

DISCUSSION

Selection of fungicide-resistant strains is affected by a variety of uncontrollable factors such as the level of resistance in the population, the initial proportion of resistant strains, and the

TABLE 2. The importance of benomyl and mancozeb concentrations in mixtures and their interactive effect on disease severity and selection for resistance in *Venturia inaequalis* as determined by an analysis of variance

Source[a]	df	Sum of squares	F	P value
1983: Number of lesions per leaf[b]				
Benomyl	2	32.083	21.69	<0.001
Mancozeb	1	1.569	2.12	0.150
Benomyl × mancozeb	2	0.596	0.40	0.670
Error	64	47.341		
1983: Proportion of resistant lesions[c]				
Benomyl	2	0.117	4.05	0.022
Mancozeb	1	0.096	6.65	0.012
Benomyl × mancozeb	2	0.030	1.06	0.354
Error	63	0.912		
1984: Number of lesions per leaf[b]				
Blocks	5	74.238	3.99	0.004
Benomyl	3	55.161	4.94	0.004
Mancozeb	2	22.797	3.06	0.055
Benomyl × mancozeb	6	2.618	0.12	0.994
Error	53	197.420		
1984: Proportion of resistant lesions[c]				
Blocks	5	0.127	2.28	0.059
Benomyl	3	1.012	30.36	<0.001
Mancozeb	2	0.179	8.08	0.001
Benomyl × mancozeb	6	0.034	0.52	0.792
Error	53	0.589		

[a] There were 12 and six tress (replicates) for each benomyl-mancozeb treatment combination in 1983 and 1984, respectively. Assessments were made on 8 July 1983 and 25–29 June 1984.

[b] Observed on 10 shoots per tree.

[c] Proportion of resistant lesions = resistant/(resistant + sensitive). A total of 240 and 180 lesions were observed on each tree in 1983 and 1984, respectively.

TABLE 3. Results of regressing the proportion of resistant lesions as a function of the concentration of benomyl for each level of mancozeb by fitting the monomolecular function (eq. 1)

Year	Mancozeb level[a]	df error	F P value	r^2	Estimated r	Standard error
1983	1/4	2	0.17	0.86	0.0159	0.0044
	1/2	2	0.11	0.94	0.0173	0.0029
	Reduced	5	0.06	0.58	0.0094	0.0035
1984	1/8	3	0.02	0.93	0.0233	0.0036
	1/4	3	<0.01	0.99	0.0218	0.0015
	1/2	3	<0.01	0.99	0.0198	0.0006
	Reduced	11	<0.01	0.90	0.0181	0.0018

[a] Proportion of the recommended rate (1,920 mg/L); "reduced" refers to fitting the model to the entire data set, i.e., over all levels of mancozeb.

TABLE 4. Results of regressing the number of lesions per leaf as a function of the concentration of benomyl for each level of mancozeb by fitting the power function (eq. 2)

Year	Mancozeb level[a]	df error	F P value	r^2	Estimates b	Estimates ln(a)	Standard errors b	Standard errors ln(a)
1983	1/4	1	0.16	0.94	−2.56	11.82	0.67	3.32
	1/2	1	0.10	0.98	−2.93	12.89	0.45	2.24
	Reduced	4	<0.01	0.88	−2.74	12.35	0.51	2.51
1984	1/8	2	0.02	0.97	−0.16	2.64	0.02	0.10
	1/4	2	<0.01	0.99	−0.27	3.01	0.02	0.07
	1/2	2	0.05	0.90	−0.22	2.68	0.05	0.24
	Reduced	10	<0.01	0.67	−0.22	2.78	0.05	0.22

[a] Proportion of the recommended rate (1,920 mg/L); "reduced" refers to fitting the model to the entire data set, i.e., over all levels of mancozeb.

biotic and abiotic environment. However, the efficacy of the fungicide used in a mixture is one factor affecting selection that can be controlled through manipulation of the concentration, spray coverage and timing, or choice of compounds. In this study, a decrease in the benomyl concentration or an increase in the mancozeb concentration resulted in a lower proportion of the resistant strain in the population relative to those treatments having higher or lower concentrations of these fungicides, respectively. Because all treatments began with the same initial proportion of resistance, these alterations in efficacy essentially caused a reduction or delay in selection for resistance. This outcome supports the predictions of models by Kable and Jeffrey (6) and by Skylakakis (15). Both models indicated that for incomplete coverage (the usual field situation), the delaying effect of the mixture is augmented by a decrease or increase of the at-risk or nonrisk compounds, respectively. In a recent model by Levy et al (10), the efficacy of the fungicides was continuously decreased over time by including a parameter for fungicide weathering or degradation. Similarly, their model predicted that an increased rate of weathering of the at-risk fungicide in a mixture reduced selection for resistance.

Although an increase in the concentration of mancozeb in this mixture was shown to significantly reduce selection, this reduction was easily offset by increasing the benomyl component. Because mancozeb was used in relatively low concentrations in the mixtures and was most likely subjected to more weathering because of its protectant nature, there may have been periods during which only benomyl was effective. These periods could have reduced the differences between the mancozeb levels. Given this premise, then higher rates of mancozeb in the mixture may have delayed or controlled selection to a greater degree.

The lack of significant interactions between the benomyl and mancozeb for both disease severity and fungicide resistance implies that these two fungicides had additive effects (Table 2). This was particularly evident in the 1984 data, where the P values for the interaction terms were close to unity and yet the separate benomyl and mancozeb P values were relatively low. The parallel nature of the response functions plotted for each level of mancozeb was also evidence of the insignificant interaction. However, in 1983, the fitting of a straight line to the two data sets produced lines with very different slopes, indicating nonadditivity (Fig. 1A). This difference was reflected in the relatively lower interaction P value for that year (Table 2).

The mechanism by which the proportion of resistant strains increased might also be related to the levels of resistance in the inoculum. Although the sensitive isolates did not grow at benomyl concentrations greater than or equal to 5 mg/L, some of these isolates may have been resistant at lower concentrations. Recent evidence indicates that benomyl resistance in *V. inaequalis* occurs at different levels (7). A reduction in the benomyl concentration, either in the mixture or through weathering/degradation, may have resulted in a smaller percentage mortality of the sensitive strains. Hence, the proportion of resistance may be lower not because of less resistant strains but because of more sensitive strains. However, if those "sensitive" strains that survived the lower dosage of benomyl were now to be considered "resistant," then the proportion of resistance may actually increase with decreasing benomyl concentration. In a greenhouse experiment, McGee and Zuck (12) had a greater selection for benomyl-resistant *V. inaequalis* with a mixture of benomyl and captan than with benomyl alone. They concluded that the lower rate of benomyl in the mixture selected for strains with lower resistance that did not survive the full rate of benomyl alone.

Since infection occurs only during discrete wetting periods (13), selection for a greater proportion of resistance in the population is also dependent on the occurrence of those infection periods. Each successive infection period has the capability of producing a new generation of lesions having a different composition of resistant and sensitive strains. In the second experiment, approximate generations of lesions were estimated based on knowledge of infection periods and a minimum latent period of 8 days. Also,

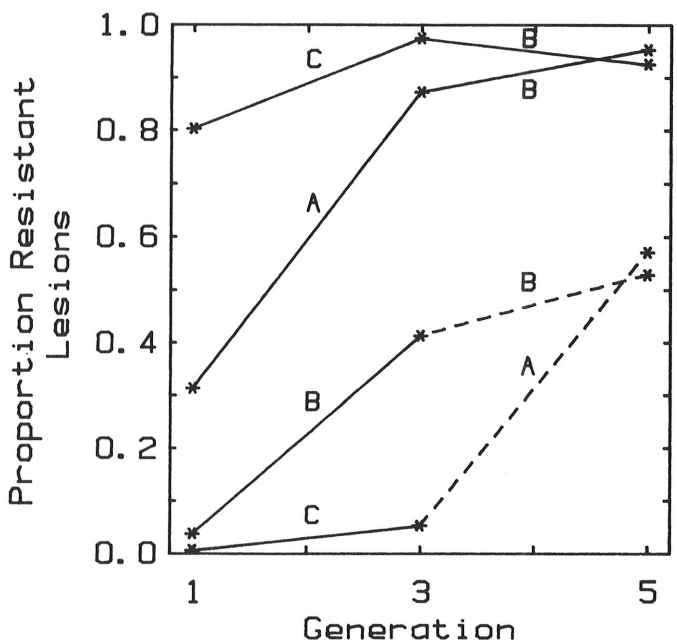

Fig. 2. Effect of the initial proportion of resistance on the selection of benomyl-resistant *Venturia inaequalis* in 1984. Each curve represents the progression of the proportion of resistant lesions as a function of the lesion generation. Generations of lesions were determined from the chronology of infection periods (see Table 1). Between the first and third generations and between the third and fifth generations, any two lines having different letters indicate significantly different rates of increase at $P = 0.05$ using Bonferroni t tests. Two applications of a mixture of benomyl and mancozeb, each at one-half the recommended rate (300 and 1,920 mg/L, respectively) were applied between the first and third generations. Only 14 and 26 lesions were examined during the last assessment of the lowest two initial proportion treatments while a minimum of 172 lesions were observed for all other points. Dashed lines were used to emphasize the relatively small sample size for these points.

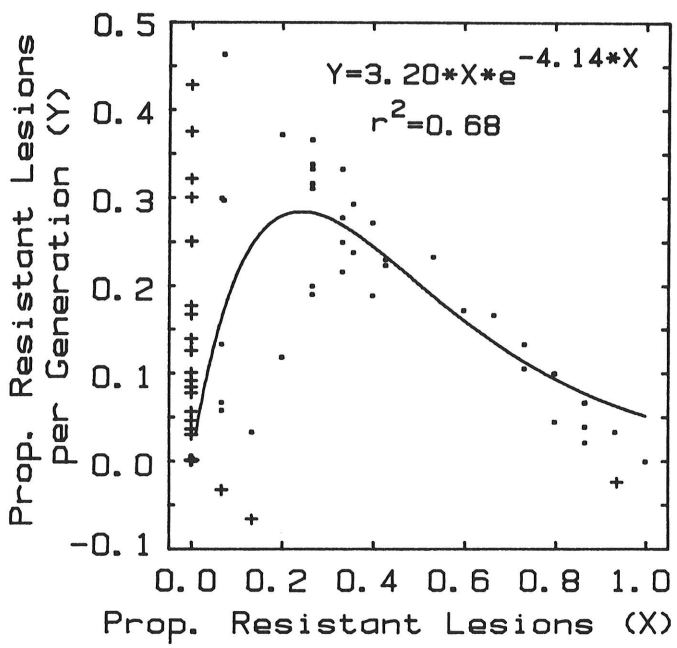

Fig. 3. Rate of increase in the proportion of benomyl-resistant *Venturia inaequalis* lesions between the first and third generations (see Fig. 2) as a function of the initial proportion of resistance in the first generation. Each point was calculated from observations made on a single tree, 10 lesions examined per tree. The maxima function was fit to the data to represent a possible hypothetical relationship. Because of the very low frequency of resistant lesions for the 0.001 treatment, resistant lesions were not detected during the first assessment on some of these trees. The rates for these trees, indicated by a "+," were not used in fitting the model.

lesion assessments were made only on new sections of shoot growth, further separating the older lesion generations from the youngest. Thus, each assessment was only partial in that it sampled only the latest of the generations. This approach allowed greater detection of changes in the population by not allowing older generations to dilute the population sample. Unfortunately, more observations could not be made in June because the presence of the mancozeb residue inhibited spore germination and, hence, determination of lesion sensitivity to benomyl.

The determination of lesion generations assumed that the latent period was constant at 8 days and that each generation was derived only from conidia of the previous generation. Changes in weather conditions and in leaf maturation no doubt altered the latent and sporulation periods, thus leading to generational overlap. Nevertheless, this inaccuracy had no effect on the statistical comparison of rates (Fig. 2) because each rate was calculated over the same period. The abscissa could have been some unit of time instead of generations without causing any change in the results of the analysis. However, lesion generations as the independent variable emphasizes the discrete nature of the selection process.

The rate at which the proportion of the resistant strains increased was a nonlinear function of its initial proportion. Populations with high or low proportions of resistance had lower rates of increase in the proportion of the resistant strains. Conversely, when neither strain dominated, the rate of change to either resistance or sensitivity was greatest. This relationship implies that populations dominated by either strain are much more stable in their composition. A greater intensity or duration of selection pressure would be required to alter these stable populations. These deductions are consistent with the Kable and Jeffrey model (6), which indicated that the rate of selection was more rapid when the proportion of resistance was greater than 0.01. However, if the proposed bell-shaped relationship between the rate of increase and proportion resistance is skewed to the left, then only a small change in the proportion should lead to a dramatic increase in selection.

The initial proportion of resistance was in important factor in disease development as well as selection. In the fungicide evaluation experiment, most of the 1983 population was initially sensitive. The benomyl was highly effective at reducing disease to levels approaching zero lesions per leaf, whereas the low mancozeb rates appeared to add little to disease control. In 1984, however, 31% of the initial population was resistant to benomyl. The mancozeb did not effectively control this subpopulation, allowing disease to increase to a maximum of eight lesions per leaf. The differences between years also could be attributed to differences in the number of infection periods, the number of fungicide applications, or even an alteration in the fitness of the resistant subpopulation due to sexual reproduction. Nevertheless, these results agree with those obtained by Dovas et al (3), who concluded that alternation and mixtures provided satisfactory disease control only if the initial proportion of resistance was low. Similarly, Sutton (16) obtained poor control of *V. inaequalis* with mixtures when applied in the presence of a high initial inoculum level of benomyl-resistant strains.

If selection is truly multidimensional, then fungal strains must undergo selection for fitness in the various epidemiological parameters as well as selection for fungicide resistance. A resistant strain with a low capacity for sporulation, for example, may be less fit than a sensitive strain with a high sporulation capacity, particularly if spray coverage is less than 100%. Because the resistant isolates used in the foregoing experiments were obtained from commercial orchards where they had undergone much selection pressure, they were most likely parasitically fit. Thus, selection for these isolates introduced into the field for experimentation may not be analogous to selection in commercial settings. Assuming that resistant strains must undergo additional selection pressures before becoming dominant, the delay in selection with fungicide mixtures might actually take longer in commercial than experimental situations.

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