# Competition Between Benomyl-Resistant and Sensitive Strains of *Venturia inaequalis* on Apple Seedlings

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## ABSTRACT

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The fitness of benomyl-resistant and sensitive populations of *Venturia inaequalis* was compared in the absence of benomyl selection pressure by passing mixed (1:1 in initial spore suspension inoculum) populations through several sporulation cycles on apple seedlings under greenhouse conditions. After eight sporulation cycles there was no major change in the original 1:1 ratio of resistant:sensitive components of a population initially composed of 11 isolates of each type taken from the same orchard. Similar results were obtained following four sporulation cycles after inoculation with a 1:1 mixture of spores of 16 sensitive and 16 resistant isolates obtained from different orchards. Mixtures of the same resistant and sensitive populations, at original ratios of 3:1, 1:1 or 1:3, exhibited similar trends during three sporulation cycles. Differences in the fitness of three separate resistant populations were observed when they were separately mixed at a 1:1 ratio with the same sensitive population; in one of the mixtures, a significantly greater decline in the resistant component was observed during five sporulation cycles as compared to the other two. An increase in the benomyl-resistant component of a 1:9 (resistant:sensitive) population occurred over five cycles when it was exposed to sublethal doses of benomyl, benomyl mixed with captan, or benomyl alternated with captan. The original ratio did not change significantly, however, when the population was exposed to captan alone or was not exposed to a fungicide.

*Additional key words:* fungicide tolerance.

The widespread development of benomyl-resistant strains of *Venturia inaequalis* (Cke.) Wint. in apple orchards sprayed exclusively with benomyl (methyl 1-[butylcarbamoyl] benzimidazole-2-yl carbamate) for 2–3 yr (6,8,9,14) has prompted the recommendation that the fungicide be used to control apple scab only in combination with another fungicide with a different mode of action (1). Theoretically this practice should reduce selection pressure on the pathogen population and thus delay the onset of resistance to benomyl. Because no experimental proof for this has been reported, the long-term effectiveness of the strategy is unknown. Information also is lacking regarding the fitness of benomyl-resistant strains of *V. inaequalis* once the use of benomyl is discontinued. It cannot be determined, therefore, whether benomyl can be used in the future in orchards where resistance has developed. Wicks (15) detected benomyl-resistant isolates of *V. inaequalis* in an orchard 3 yr after benomyl applications were stopped, but did not determine any changes in the proportion of the resistant component of the population. McGee (8) showed that benomyl-resistant populations composed of field isolates of *V. inaequalis* had no competitive advantage over corresponding sensitive populations when passed through several sporulation cycles on apple seedlings in the absence of benomyl. Olivier et al (9), however, using similar techniques but comparing single isolates rather than populations, found that the proportions of benomyl-resistant isolates increased.

Generalizations cannot be made regarding the origin and persistence of benomyl resistance for an individual pathogen. There is evidence both for benomyl-resistant strains existing in populations of pathogens that have never been exposed to benomyl (2,5) and for resistant mutants appearing when populations are exposed to benomyl (3). Resistant strains also can either persist or decline after benomyl is withdrawn from use, as shown in studies with *Cercospora beticola* (4,10) and *Sclerotinia homeocarpa* (13), respectively.

This paper substantiates preliminary findings (8) that benomyl-resistant strains of *V. inaequalis* may be as fit as sensitive strains in the absence of benomyl selection pressure and provides experimental data showing the responses of a mixed benomyl-sensitive and resistant population to various fungicide treatments.

## MATERIALS AND METHODS

**Populations of *Venturia inaequalis*.** Isolates of *Venturia inaequalis* were obtained from two apple orchards at Highmoor Farm, Monmouth, ME. In one orchard, which consisted of McIntosh trees on semidwarf Malling rootstocks planted in 1968, benomyl resistance had been detected in 1975 following the exclusive use of benomyl for apple scab control in 1973, 1974, and 1975. The other orchard, which consisted of McIntosh seedling rootstock planted in 1928, had been used for many years to evaluate various fungicides for control of apple scab. There was no evidence of benomyl resistance in this orchard. Apple scab lesions were cut from detached leaves and streaked across the surface of potato-dextrose agar (PDA) plates to deposit conidia of *V. inaequalis*. After 24 hr, small plugs of groups of one, two, or three germinated conidia were transferred to PDA slants. Conidial suspensions of each isolate, prepared on cheesecloth wicks saturated with malt extract broth (12), were streaked sequentially across divided petri plates containing PDA in one half and PDA amended with 10 $\mu$g/ml of benomyl in the other half. After incubation at 20 C for 24 hr, conidia were checked for normal germination on the unamended half of the plate and for benomyl sensitivity or resistance on the amended half. Sensitive isolates were characterized by conidia that produced swollen, aborted germ tubes, no more than twice the length of the conidia, while conidia of benomyl-resistant isolates produced germ tubes at least 10 times the length of the conidia.

Benomyl-sensitive or resistant populations were prepared by mixing equal volumes of conidial suspensions of each component isolate. Mixed populations were made by pooling appropriate volumes of the conidial suspensions of resistant and sensitive populations.

**Sporulation cycles on apple seedlings.** McIntosh apple seedlings, grown for 8 wk in a mixture of field soil, sand, and peat moss (1:1:2, v/v) were used in all experiments. For each sporulation cycle, three seedlings in each of four pots were inoculated by spraying a conidial

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suspension of the test population on the leaves with a DeVilbiss atomizer. Seedlings were placed in a mist chamber, maintained at 20 C for 24 hr with the misting unit on, then kept there for a further 48 hr with the unit off before transfer to the greenhouse. Preliminary histological studies showed that, under these conditions, leaves were covered by sporulating scab lesions originating from infection by numerous conidia. Fourteen days after inoculation, leaf sections, approximately 1 cm × 1 cm, were excised from eight different plants and vigorously agitated in sterile water to dislodge the conidia. The conidial suspension was adjusted to a concentration of 200,000 conidia per milliliter and then used to inoculate another batch of seedlings, thus beginning another sporulation cycle. Before inoculation, however, a sample of the suspension was drawn to determine the percent of resistant conidia present, using divided petri plates, as previously described. The numbers of ungerminated conidia and germinating conidia showing benomyl-sensitivity or resistance were counted for 100 conidia in each of six streaks. The average percentage of benomyl-resistant conidia among the germinated conidia was calculated. The germination count on the unamended half of the plate provided a check on the viability of the conidia. The percentage of nongerminating conidia never was greater than 20%, nor was there any marked difference in this count between the two halves of the plate for any treatment.

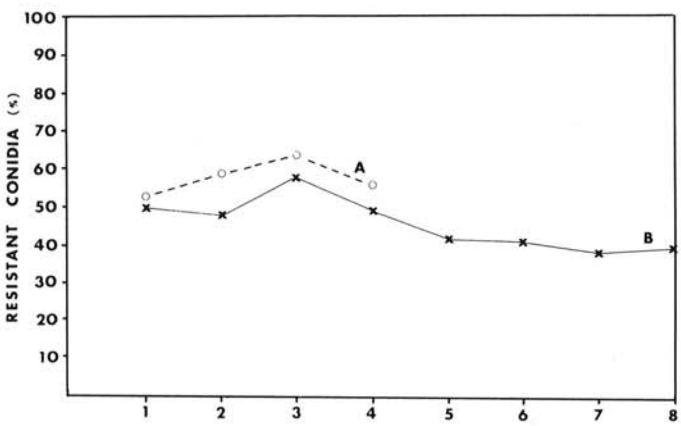


**Fig. 1.** Competition between benomyl-resistant and sensitive components of *Venturia inaequalis* populations originally composed of 1:1 ratios of resistant and sensitive conidia then passed through successive sporulation cycles on apple seedlings in the greenhouse. Population A comprised 16 resistant isolates from one orchard and 16 sensitive isolates from another orchard. Population B comprised 11 isolates of each type taken from the same orchard. Coefficients of variability for all measurements in populations A and B were 9.6 and 9.2%, respectively.

**Competition between benomyl-sensitive and resistant components of populations in the absence of benomyl.** Separate benomyl-resistant and sensitive populations were made from 11 isolates of each type obtained from the orchard in which benomyl resistance was found in 1975. Each population and a 1:1 mixture were passed separately through eight sporulation cycles on apple seedlings. In a similar experiment, a benomyl-resistant population comprising 16 isolates from the orchard with benomyl resistance and a sensitive population comprising 16 isolates from the orchard with no benomyl resistance were passed through four cycles. The fitness of three resistant populations made from sets of 16 different isolates from the orchard with benomyl resistance was compared by separately mixing each population in a 1:1 ratio with a single sensitive population comprising 16 isolates from either orchard and passing the mixtures through five sporulation cycles. Competition between resistant and sensitive populations originally mixed at different ratios was compared by passing 1:0, 3:1, 1:1, 1:3, 0:1 (resistant:sensitive) ratios of the two populations used in the first experiment of this series through three sporulation cycles.

**Effect of fungicides on benomyl-resistant and sensitive populations.** Preliminary experiments determined appropriate doses of the fungicides benomyl and captan (*N*-[[trichloromethyl] thio]-4-cyclohexene-1, 2-dicarboximide) that, when applied to apple leaves, might affect *V. inaequalis* populations inoculated onto leaves but still allow sporulating lesions to develop. A benomyl-resistant population made from 48 isolates and a sensitive population made from 16 isolates were mixed at a 1:9

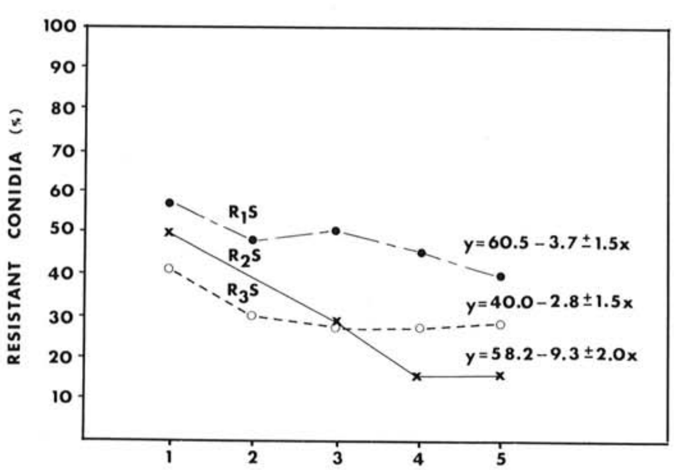


**Fig. 2.** Competition between three separate benomyl-resistant populations ($R_1$, $R_2$, and $R_3$) and one sensitive population (S) of *Venturia inaequalis* originally made from 1:1 ratios of resistant and sensitive conidia then passed through sporulation cycles on apple seedlings in the greenhouse. Each population represents equal numbers of conidia from 16 isolates. The regression slopes include confidence limits, $P = 0.05$. The coefficient of variability for all measurements was 14.5%.

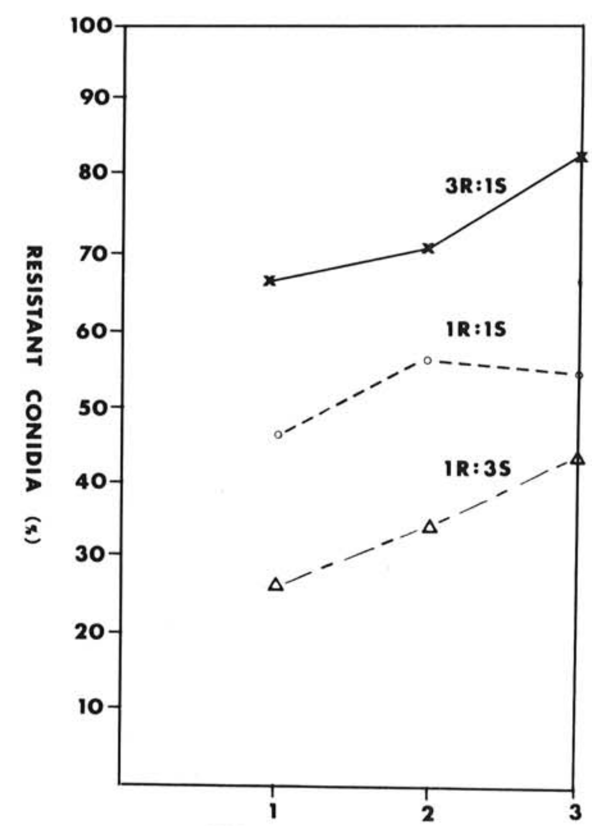


**Fig. 3.** Competition between benomyl-resistant (R) and sensitive (S) components of *Venturia inaequalis* populations originally consisting of three different ratios of conidia of the same resistant and sensitive populations then passed through sporulation cycles on apple seedlings. Each population represents equal numbers of conidia from 11 isolates. The coefficient of variability for all measurements was 8.4%.

(resistant:sensitive) ratio and passed through five sporulation cycles (as previously described) on apple seedlings previously sprayed with benomyl (Benlate 50W [2.0 mg/L]), Captan 80W (2.0 mg/L), Benlate 50W (1 mg/L) and Captan 80W (1 mg/L), Benlate 50W (2 mg/L) alternated with Captan 80W (2 mg/L), or water. Because the percentages of resistant conidia remained unchanged after three cycles, the fungicide doses were doubled for the final two sporulation cycles.

## RESULTS

Very little change occurred after eight sporulation cycles in the original 1:1 ratio of benomyl-sensitive and resistant conidia in a population made from *V. inaequalis* isolates from the same orchard (Fig. 1). A very slight increase occurred after four disease cycles in the resistant component of a mixed population consisting of resistant and sensitive conidia obtained from different orchards. In both experiments, the composition of the sensitive and resistant populations that made up the mixed populations did not change when they were passed separately through all sporulation cycles. When one sensitive population was mixed at a 1:1 ratio with three different resistant populations, the resistant component of each mixture tended to decline after the five sporulation cycles (Fig. 2). The rate of decline as indicated by the slope of the regression line was significantly greater for the population $R_2S$ than for the $R_1S$ and $R_3S$ populations (no data were obtained for the second cycle for $R_2S$).

A gradual increase in the resistant component occurred over three cycles in the populations originally consisting of different ratios of resistant and sensitive conidia (Fig. 3). There were no changes, however, in the 100% resistant or 100% sensitive populations.

Significant increases occurred over five sporulation cycles in the resistant component of a mixed population when treated with benomyl as compared to captan or to no fungicide (Fig. 4). The rate of increase was significantly greater in the benomyl mixed with captan treatment than in the other two benomyl treatments. Captan had no effect on the resistant component compared to the no-fungicide treatment.

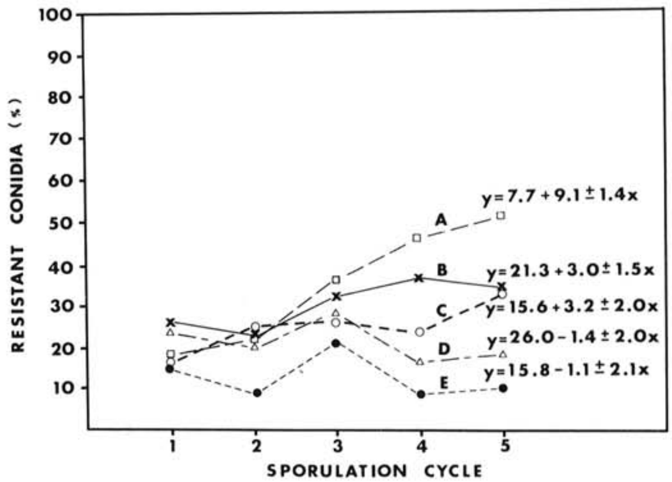


**Fig. 4.** Effect of sublethal doses of fungicides on the ratios of benomyl-resistant and sensitive conidia of a population of *Venturia inaequalis,* originally mixed at the ratio of 1:9 (resistant:sensitive) conidia then passed through five sporulation cycles on apple seedlings in the greenhouse. Fungicide treatments were: **A,** benomyl mixed with captan; **B,** benomyl alternated with captan; **C,** benomyl; **D,** captan; and **E,** no fungicide. The regression slopes include confidence limits at the $P = 0.05$ level. The coefficient of variability for all measurements was 24.8%.

## DISCUSSION

Under the conditions of this study, benomyl-resistant components of mixed populations of *V. inaequalis* seemed to be as competitive as the sensitive components in the absence of benomyl. If benomyl-resistant and sensitive components of wild populations of *V. inaequalis* are equally able to withstand the environmental conditions in the field, it seems likely that benomyl-resistant strains will persist for a prolonged period of time in an orchard after benomyl is withdrawn from use, and therefore, reintroduction of the fungicide would not be advisable in the near future.

The trends for an increase or decrease in the resistant components of the various populations used in this study probably reflect simply the net fitness of the component isolates. The fitness of an individual isolate of *V. inaequalis* will be influenced by characteristics such as virulence, rate of conidial production, etc. Because these characteristics are likely to vary among isolates, fitness also will vary. Comparisons between individual isolates of *V. inaequalis,* as made by Olivier et al (9), therefore, may be of limited value. If, as they suggest, benomyl-resistant strains of *V. inaequalis* have a competitive advantage over sensitive strains, then resistant spores of this airborne pathogen could contaminate other orchards and increase there in the absence of benomyl selection pressure. There are no reports to suggest that this has happened.

While selection pressure for benomyl-resistant strains of *V. inaequalis* obviously has taken place in the field (6,8,9,14) this, seemingly, is the first time that it has been demonstrated experimentally. As might be expected, the resistant components increased when the mixed population was exposed to sublethal doses of benomyl. The increased selection pressure when the population was exposed to benomyl mixed with captan compared to benomyl alone or alternated with captan, however, was unexpected. Possibly, the lower rate of benomyl used in the mixed treatment allowed selection of components of the population with lower resistance that did not survive the higher rate used in the other treatments, thus increasing the overall proportion of benomyl-resistant conidia. A range of benomyl resistance does exist among resistant field isolates of *V. inaequalis* (11). It would be premature to recommend against the use of mixtures, based on the special conditions of this one experiment. These data do, however, indicate potential problems with this approach to controlling pathogen resistance to fungicides. The responses of mixed populations of pathogens to benomyl either mixed or alternated with a fungicide with a different mode of action will depend to a significant degree both on the range of benomyl resistance in the population and on the fungicide application rates. By quantifying the responses of populations to these and to other important variables, such as spray coverage, the management of resistant strains may be possible. Mathematical models such as that designed by Kable and Jeffery (7) are an important step in achieving this goal. Our data agree with the prediction of this model, that when the resistant proportion of a population is greater than 1%, selection of resistant strains by a fungicide mixture will proceed rapidly. Kable and Jeffery (7) postulate, however, that below the 1% level, the use of mixtures may be of value in delaying the onset of resistance. Experimental work to test this claim would be of great value.

## LITERATURE CITED


1. Anonymous. 1980. Benlate fungicide label. Agrichemicals Label Manual/1980. E. I. du Pont de Nemours & Co. Wilmington, DE, p. 9.
2. Bolton, A. T. 1976. Fungicide resistance in *Botrytis cinerea,* the result of selective pressure on resistant strains already present in nature. Can. J. Plant Sci. 56:861-864.
3. Dekker, J. 1976. Prospects for the use of systemic fungicides in view of the resistance problem. Proc. Am. Phytopathol. Soc. 3:60-66.
4. Dovas, C., Skylakakis, G., and Georgopoulos, S. G. 1976. The adaptability of the benomyl-resistant population of *Cercospora beticola* in northern Greece. Phytopathology 66:1452-1456.
5. Fletcher, J. T., and Scholefield, S. M. 1976. Benomyl tolerance in isolates of *Botrytis cinerea* from tomato plants. Ann. Appl. Biol. 82:529-536.
6. Jones, A. L., and Walker, R. J. 1976. Tolerance of *Venturia inaequalis* to dodine and benzimidazole fungicides in Michigan. Plant Dis. Rep. 60:40-44.
7. Kable, P. F., and Jeffery, H. 1980. Selection for tolerance in organisms exposed to sprays of biocide mixtures: A theoretical model.

Phytopathology 70:8-12.
8. McGee, D. C. 1978. Competition between benomyl resistant and sensitive populations of *Venturia inaequalis* in the absence of benomyl. Page 371 in: Abstracts of Papers, 3rd Int. Cong. Plant Pathol., Munich, Germany.
9. Olivier, J. M., Lespinasse, Y., Godicheau, M., Boureau, M., and Martin, D. 1980. Étude de compétitions entre des souches sauvages de *Venturia inaequalis* et des isolats ayant acquis une nouvelle virulence ou une résistance au bénomyl. Pages 145–156 in: Compte reudu du Symposium de la Section Fruits Eucarpia. Station d'Arboriculture Fruitiere Augers 1979.
10. Ruppel, E. G., Jenkins, A. D., and Burtch, L. M. 1980. Persistence of benomyl-tolerant strains of *Cercospora beticola* in the absence of benomyl. Phytopathology 70:25-26.
11. Tate, K. G., and Samuels, G. J. 1976. Benzimidazole tolerance in *Venturia inaequalis* in New Zealand. Plant Dis. Rep. 60:706-710.
12. Tuite, J. F. 1969. Plant Pathological Methods. Burgess Publishing Company, Minneapolis, MN. 161 pp.
13. Warren, C. G., Sanders, P. L., Cole, H., Jr., and Duich, J. M. 1977. Relative fitness of benzimidazole- and cadmium-tolerant populations of *Sclerotinia homeocarpa* in the absence and presence of fungicides. Phytopathology 67:704-708.
14. Wicks, T. 1974. Tolerance of the apple scab fungus to benzimidazole fungicides. Plant Dis. Rep. 58:886-889.
15. Wicks, T. 1976. Persistance of benomyl tolerance in *Venturia inaequalis*. Plant Dis. Rep. 60:818-819.