Fungicide Resistance: Past Experience with Benomyl and Dodine and Future Concerns with Sterol Inhibitors

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ABSTRACT

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The need for a careful introduction of sterol-inhibiting fungicides into apple disease control programs is discussed. Because of the propensity of *Venturia inaequalis* for developing resistance to benomyl and dodine, populations of this pathogen should be monitored closely for changes in their sensitivity to sterol-inhibiting fungicides. In developing strategies for avoiding resistance, cooperation between the various manufacturers of sterol inhibitors will be important.

A large number of fungicides with ergosterol biosynthesis inhibition as their mode of action have been tested on apples in the United States. These include: fenarimol (Eli Lilly EL 222, or Rubigan); triforine (Celamerck Cela W-524, or Saprol); triadimefon (Bayer MEB 6447, or Bayleton); bitertanol (Bayer KWG 0599, or Baycor); 1-[[2-(2,4-dichlorophenyl)-4-ethyl-1,3-dioxolan-2-yl] methyl]-1*H*-1,2,4-triazole (Ciba Geigy CGA 64251); prochloraz (Boots BFN 8206); and fenapanil (Rohm and Haas RH 2161, or Sisthane). Two other pyrimidine compounds, triarimol and nuarimol, were tested on apples before fenarimol was selected for development. In addition, imazalil has been tested for the control of postharvest disease problems.

Sterol-inhibiting fungicides offer several advantages for the control of apple diseases. Most have protective, eradicative, and curative activity against the key, early-season diseases found in apple orchards east of the Rocky Mountains, such as apple scab, caused by *Venturia inaequalis* (Cke.) Wint.; powdery mildew, caused by *Podosphaera leucotricha* (Ell. & Everh.) Salm.; and cedar-apple rust, caused by *Gymnosporangium juniperi-virginianae* Schw. Because of their broad-spectrum activity, these fungicides are likely to be used intensively early in the growing season and, where powdery mildew is a problem, intensive and exclusive use may extend throughout the growing season. Because of their curative activity, they could become the fungicides of choice in emergency disease control situations.

Several of these fungicides will probably be registered for use on apples. Registration will intensify their use and place extreme selection pressure on pathogens like *V. inaequalis*, which have shown a propensity for development of fungicide-resistant strains.

In view of the possible widespread and intensive use of these fungicides on apples, we need to examine the likelihood that pathogens will develop resistance and discuss strategies for using sterol-inhibiting fungicides in ways that will reduce the resistance problem.

CURRENT PROBLEMS OF FUNGICIDE RESISTANCE

Although several fungal pathogens of apple have been subjected to intensive fungicide use for many years, problems with fungicide resistance have only been encountered in practice in *V. inaequalis* and *Penicillium expansum* Lk. ex Thom. Resistance has developed to two fungicides, dodine and benomyl, particularly in the apple production areas of the northeastern United States and eastern Canada. Dodine-resistant strains of *V. inaequalis* were detected in New York State in 1969 (17). They were later detected in Michigan (12,20) and in Nova Scotia (14) and Ontario (13), Canada. Resistance developed where dodine was used in exclusive programs for 10 yr or more. In problem orchards, dodine failed to give the level of control obtained in previous years, and control failures could not be explained by improper spraying or insufficient rates of fungicide.

Following detection in Australia (19), benomyl-resistant strains of the apple scab fungus were detected in Michigan (11,12). Resistant strains are now widely distributed in that state (Fig. 1) and have been detected in Maine, North Carolina, New York, Minnesota, Virginia, Indiana, and in Ontario, Canada. Resistance in North Carolina was widespread (15). In 1978, strains of *V. inaequalis* resistant to benomyl and dodine were detected in an orchard where these fungicides were used in combination following many years of exclusive dodine use (Jones and Ehret, *unpublished*).

Benomyl resistance in Michigan developed rapidly following 2 yr of severe scab pressure. In 1973, the first year benomyl was commercially available for use on apples, scab was widespread and unusually severe, and growers switched to benomyl to control established infections. In 1974 the carry-over of inoculum from the previous season was heavy, and benomyl use increased. Because benomyl controlled all the major diseases of apple in Michigan, it was used exclusively through the growing season by most growers. Resistance was detected in 1975 but was present the previous year because ascospores from leaves infected in the 1974 growing season were resistant to benomyl.

POTENTIAL FOR RESISTANCE TO STEROL INHIBITORS

Many factors must be considered before it can be determined whether a fungus will develop resistance to a fungicide. The characteristics of resistant mutants, the biology of the pathogen, and the selection pressure exerted by the fungicide program all influence the

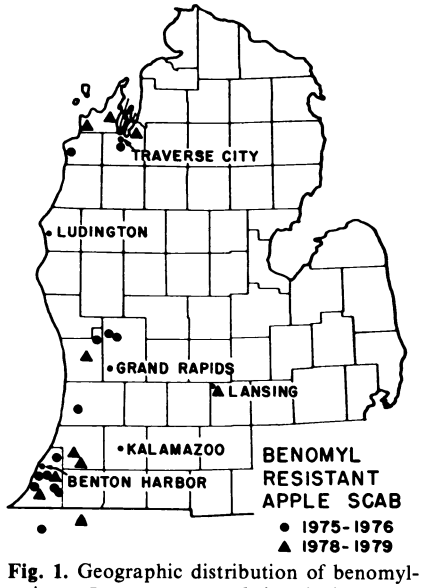

Fig. 1. Geographic distribution of benomyl-resistant *Venturia inaequalis* in Michigan from 1975 to 1979.

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possibility for resistance developing in the field (4).

Under laboratory conditions, mutants resistant to fungicides that inhibit ergosterol biosynthesis are readily induced, and these mutants are frequently cross-resistant to other fungicides that inhibit ergosterol biosynthesis (7). However, Fuchs and co-workers (8,9) have predicted that the development of resistance to fungicides that inhibit sterol synthesis is rather unlikely. Their conclusion is based on several considerations: The level of chemical tolerated by resistant strains is low, resistant mutants have decreased fitness in vitro, and pathogenicity of resistant strains is reduced. Also, mutants resistant in vitro have failed to infect plants treated with fungicide while unsprayed plants were infected (3). It thus appears that the development of resistance to sterol inhibitors in practice depends on the stability of the link between resistance and reduced fitness in the various pathogenic fungi.

In practice, decreased fitness of resistant strains has not always prevented the emergence of resistance under heavy selection pressure. In the Netherlands, resistance to pyrazophos (Curamil) developed in cucumber powdery mildew (*Sphaerotheca fuliginea*) (Schlecht. ex Fr.) Poll. after 4–5 yr use in cucumber and gherkin greenhouses (4). In western Europe, resistance to dimethirimol and ethirimol developed in cucumber mildew and barley mildew (*Erysiphe graminis* DC. f. sp. *hordei* Em. Marchal), respectively (1,10). Strains of *Botrytis cinerea* Pers. ex Fr. resistant to high concentrations of iprodione and vinclozolin were easily selected in the laboratory, and although many strains exhibited poor fitness, field resistance has developed in *Botrytis* spp. on strawberries in England (6). However, experience with pyrazophos and dimetherimol suggests that where resistant populations have reduced fitness and are detected early, the pathogen population may revert to the wild type after fungicide use is stopped.

Because the biology and control strategies for the three major pathogens of apple controlled by sterol-inhibiting fungicides are quite different, the potential for resistance needs to be evaluated for each pathogen.

Apple scab. Pseudothecia and ascospores of *V. inaequalis* are abundant in orchards where scab existed the previous season. The pseudothecia are important as a source of new mutants and for genetic recombination among strains. In designing strategies for resistant strains, it must be remembered that not all ascospores are released from the pseudothecia during any one wetting period (16). Therefore, resistant ascospores from a natural cross between sensitive and resistant strains or two resistant strains must be controlled throughout the ascospore discharge period if their establishment in the orchard is to be prevented. Heterokaryosis is probably of minor importance in resistance because attempts to form heterokaryons with biochemical mutants have not been successful (2).

The most intensive use of fungicides for apple scab control occurs during the period of ascospore discharge, which lasts 6–8 wk in areas east of the Rocky Mountains. Strategies for avoiding resistance by alternating chemical treatments during the primary cycle do not prevent resistant strains from becoming established when the fungicide with a potential for resistance is used. Application of one kind of fungicide during the primary scab period and a second kind for secondary scab would put more control pressure on resistant strains. However, care must be taken in selecting fungicides to use in alternation, because if two compounds with potential for resistance are alternated, resistance to both may develop.

In the apple scab fungus, the possibility is high that fitness of resistant strains can be increased through recombination. Not only fitness, but also resistance may be increased by additive effects of mutation in different genes. This latter situation was demonstrated by van Tuyl (18), who obtained much higher levels of resistance to imazalil in crosses between strains of *Aspergillus nidulans* (Eidam) Winter with different genes for resistance.

Powdery mildew. Control of apple powdery mildew requires frequent applications of fungicides, often weekly, for periods up to 12 wk. Although benomyl has been used intensively to control both scab and mildew, there have not been documented failures of benomyl to control *Podosphaera leucotricha*. Only a few cases of benomyl resistance in other powdery mildews have been reported. Therefore, it appears that where scab and mildew are controlled with the same fungicide, resistance will develop first in the scab fungus. If resistance develops, it would probably arise from mutations in conidia because each cleistothecium has only one ascus and heterokaryosis has yet to be demonstrated in the mildews.

Cedar-apple rust. Only a brief part of the life cycle of the cedar-apple rust pathogen is spent on apple. For 19 mo, and often longer, the pathogen develops on red cedar (*Juniperus virginiana* L.), with infection of apple occurring between the pink and first cover stages of flower bud development. Thus, contact between fungicide and fungus is relatively short. If resistant strains develop through mutation, they must become established on cedar trees and survive in the absence of fungicide pressure. Survival of resistant strains would be reduced where they lack competitiveness or are low in number. Thus resistance would develop more readily in apple scab and powdery mildew than in rust.

PROPOSAL FOR INTRODUCING STEROL-INHIBITING FUNGICIDES

Recently, Delp (5) wrote: "When a chemical with a propensity for resistance is being used, the program must be planned from the beginning to prevent resistance." At least two courses of action should be considered in regard to the introduction of sterol-inhibiting fungicides on apples.

One course would be to incorporate sterol-inhibiting fungicides into present disease control programs. This has been done in the past by government, industry, and university personnel when a new fungicide was registered. It would provide for alternative fungicides where benomyl and dodine resistance is a problem and when public agencies change the registration of existing fungicides. This course assumes that if resistance develops, it will be dealt with at that time.

The second course of action would be to formulate sterol-inhibiting fungicides in mixtures with unrelated fungicides or to restrict their use to certain parts of the growing season to reduce selection pressure on the pathogens. Although this action might delay the development of resistance, product registration, particularly of mixtures, could be delayed for several years and thus deny use of these effective chemicals. However, had such a strategy been followed when benomyl was first introduced, resistance would probably be less of a problem today. Cooperation would be required between the different manufacturers of sterol-inhibiting fungicides because efforts by one manufacturer to avoid resistance could be rendered ineffective by the failure of other manufacturers to follow similar programs.

Regardless of the course of action, the long-term effects of these fungicides on field populations on the scab fungus should be evaluated. Before these compounds are used commercially, samples of *V. inaequalis* should be collected and tested to establish the limits of variation possible within natural populations. Following the use of the compounds, *V. inaequalis* from treated orchards should be tested for possible shifts in sensitivity. These activities need not delay the normal registration process, but the effective life of these compounds may be lengthened by such an approach. Judicious use of fungicides is especially critical when the number of new pesticides introduced each year is declining.

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