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Coping with Resistance to Plant Disease

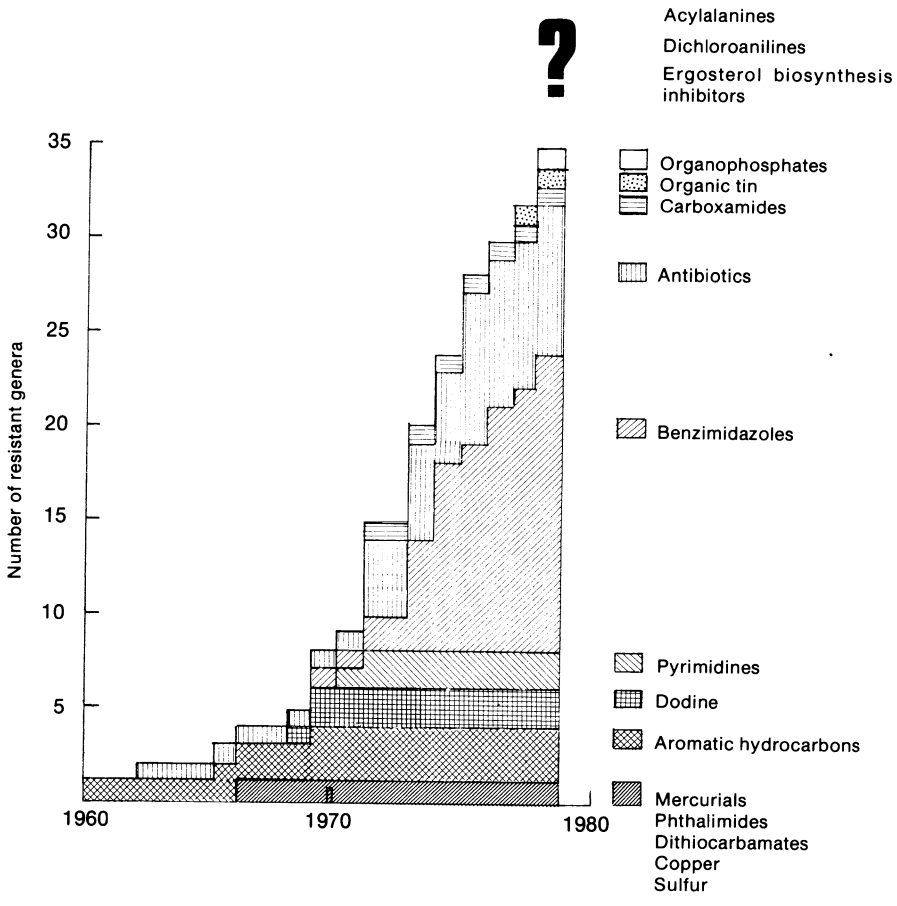

Fig. 1. History of resistance to disease control agents.
(Adapted from Dekker [3], Georgopoulos [9], and Ogawa et al [14]).

A dozen years ago, plant pathologists were not yet concerned with resistance. In fact, in 1967 Georgopoulos and Zaracovitis (10) wrote, "The reported cases of tolerance to agricultural fungicides are very few and the knowledge accumulated hardly justifies a review." The number of practical field problems due to resistance in plant pathogens has increased, however, and recent articles (3,4,7–9,12,14,15,17,18) point to the exclusive and intensive use of potent new disease control agents.

Unfortunately, the scientific breakthroughs that brought powerful chemicals for more effective disease control also triggered the emergence of resistance. This paradox can best be explained in terms of the mode of action and mechanisms of resistance.

Conventional multisite inhibitors interfere with numerous vital metabolic processes of the pathogen and allow little chance for resistance because multiple differences or modifications in the pathogen's genome are required to circumvent the action. Such multisite fungicides as the phthalimides, the dithiocarbamates, the mercurials, sulfur, and the copper compounds have had few practical field resistance problems. Specific-site inhibitors, on the other hand, act at one or two metabolic sites, and resistance is more common because alterations in only one fungal gene are sufficient to induce change at the site of action.

Cross-resistance occurs when resistance to one chemical is conferred to another mediated by the same genetic factor. Resistant strains are frequently cross-

The words *resistance* and *tolerance* are used interchangeably and tolerance is sometimes preferred because of its softer connotation. In 1978, the Food and Agriculture Organization of the United Nations Panel of Experts on Pest Resistance to Pesticides "agreed that resistance should continue to apply to hereditable resistance in fungi or bacteria and recommended that the word tolerance should not be used . . . since it may be ambiguous." (7).

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Control Agents

resistant to structurally related chemicals or to chemicals with similar modes of action. A thorough understanding of cross-resistance among disease control agents is of paramount importance. For instance, for a grower losing disease control because of resistance to one agent to switch to another can be disastrous if there is cross-resistance between the two compounds. Also, combining two agents with cross-resistance is useless. Of course, the lack of cross-resistance between agents does not preclude a strain resistant to one agent from developing resistance to another (multiple resistance). Disease control agents with proven effectiveness are, in some situations, being unnecessarily lost to resistance because of unenlightened use or regulatory restrictions. But there are ways to cope, ways to keep potent agents useful despite their propensity for resistance.

The Emergence of Resistance

The resistance problem is associated with the newer products (Fig. 1). The first fungicide resistance with practical significance was to biphenyl, an aromatic hydrocarbon used primarily for postharvest citrus treatment. Although resistant *Penicillium* strains are common, biphenyl has been used since 1959. Of the other aromatic hydrocarbons, pentachloronitrobenzene (PCNB) became suspect, but substantiated reports of field resistance in *Rhizoctonia* have not resulted in reduced performance (15).

Resistance in the apple scab fungus (*Venturia inaequalis*) emerged only after dodine had been used intensively for about 10 years in certain areas of the northeastern United States. Dodine-resistant strains have become a part of the wild population, but the problem is relatively limited.

The usefulness of the pyrimidines appeared short-lived because of rapid development of resistance in powdery mildews against dimethirimol and ethirimol in western Europe. These products are still in use, however, with restrictions.

The benzimidazoles most dramatically represent the beginning of serious resistance problems. This is true not only because they have been widely and intensively used in crop protection but also because they are specific-site inhibitors and most fungi contain resistant strains in their natural populations. Where problems have occurred, strains resistant to one benzimidazole are cross-resistant to all benzimidazoles but not to unrelated fungicides. Since benzimidazole-resistant fungi survive well, benzimidazoles generally have been ineffective for several seasons after resistant strains dominate large crop production areas. Although resistance to benzimidazoles has presented a variety of problems worldwide, resistance has not become a factor in many cases of long-term use. There is even some evidence that benzimidazoles made ineffective because of resistance may be reintroduced. For example, the powdery mildew fungi were the first to become resistant (16) but in some situations have been the most rapid to revert to sensitive populations.

Benomyl, a benzimidazole, provided excellent control of Cercospora leaf spot of sugar beet, peanut, and celery and was often used exclusively on these crops. Under such conditions, resistance dominated the *Cercospora* populations within 2 to 4 years. In some locations where mixtures of benomyl and unrelated fungicides have been used from the beginning, however, resistance has not become a problem. Similarly, benomyl-resistant *Botrytis* emerged within a few seasons after exclusive use on European grapes and ornamentals. Countless research papers documenting the remarkable effectiveness of the benzimidazoles have been followed during the past few years by reports of loss of effectiveness due to resistance.

Bacterial resistance to streptomycin was predictable from the experience in medicine. Resistance to the agent developed rapidly under intensive use against Xanthomonas bacterial spot of pepper in Florida. Fire blight control, on the other hand, decreased only after extensive, long-term field use. Other antibiotics with limited fungal spectra, such as blasticidin S, kasugamycin, and polyoxin, have also shown a propensity toward resistance problems in *Pyricularia*, *Alternaria*, and *Botrytis*.

Although resistance to carboxamide fungicides is readily demonstrated in the laboratory, the only practical problem has been of *Puccinia* resistance to oxycarboxin on some greenhouse chrysanthemums in Japan. Resistance to triphenyl tin acetate, a nonsystemic fungicide used to control *Cercospora* on sugar beets in Greece since 1960, became a problem in 1976 after many seasons of exclusive use. The organophosphate rice blast fungicides IBP (Kitazin P) and edifenphos have been in use in Japan since 1965; laboratory resistance is easily shown, but field resistance has been reported only recently (17).

At least 15 new compounds (Fig. 2) with a mode of action described as ergosterol biosynthesis inhibition are under development. Many are structurally unrelated, and their biological spectra of activity vary. The common mode of action and cross-resistance in laboratory strains suggests potential resistance problems after widespread use, although none has occurred yet.

Another group of closely related new compounds, the dichloroanilines, presents a similar situation. Resistant *Monilinia, Botrytis, Sclerotinia*, and *Helminthosporium* strains easily develop in vitro and are pathogenic and cross-resistant to all members of the group. Resistant strains do not survive well in the laboratory, but field resistance problems are being reported.

The acylalanines have such unique systemic, curative, and residual properties in the control of Phycomycetes that they will undoubtedly be used exclusively in some situations. This could be risky in view of laboratory and field indications of resistance.

In 1977, a decade after his statements regarding the lack of resistance problems for disease control agents, Georgopoulos (8) wrote, "Plant pathology has witnessed, thus, the collapse of several promising new disease control compounds, and the end is not in sight." We are indeed beginning to see serious problems with some new compounds, but the failure of disease control agents is not inevitable.

Mechanisms of Resistance

Some understanding of the mechanisms of resistance is helpful for a rational approach to the problem. The genetic basis is understood for many cases of resistance, and the mode of action at the biochemical level is being revealed for some.

Nongenetic adaptation is commonly observed in culture (training) and usually is lost after growth on toxin-free media. In most cases adaptation appears to have little practical importance in the field. Evidence suggests, however, that the unstable field resistance in the powdery mildew fungus. *Erysiphe graminis* f. sp. *hordei*, to ethirimol is due, at least in part, to nongenetic adaptation, because resistant strains become sensitive after a few months of nonuse of the fungicide.

Resistance to at least seven unrelated fungicides has been found to depend on changes of chromosomal genes (Table 1). A single gene change confers resistance in some cases, whereas two or more gene modifications are involved in others. Where fungal chromosomes have been mapped sufficiently, as in *Aspergillus nidulans, Ustilago maydis, U. hordei,*

Neurospora crassa, and *Venturia inaequalis,* the exact location of the modified gene has been determined.

Resistance in fungi and bacteria may also be due to extrachromosomal inheritance. For example, the conjugal transfer of plasmid-mediated antibiotic resistance from other bacteria to plant-pathogenic bacteria appears possible in infected plants. To date, field-developed antibiotic resistance in bacteria has not been associated with plasmids.

The biochemical mechanisms that protect resistant plant pathogens are similar to those in insects (Table 2). Although exclusion and detoxification keep the toxicant from the site of action in the fungal cell, modification of sensitive sites is the predominant mechanism of resistance. For example, benzimidazoles appear to inhibit sensitive fungi by binding to protein subunits of the spindle microtubules, thereby disrupting spindle function. Differences in a protein subunit may cause loss of benzimidazole binding and result in resistance (2). Other site-of-action modifications appear to be primary mechanisms of resistance to other agents.

Ways to Cope

Resistance can be mitigated or prevented by early use of spray programs designed to preclude long-term exposure of the pathogen to a single disease control agent. Once resistance is a significant part of a pathogen population, the only choice is to use disease control agents to which there is no cross-resistance—if such agents are available and registered for use. When a chemical with a propensity for resistance is being used, the program must be planned from the beginning to prevent resistance.

Theoretical considerations. Resistant strains may occur naturally in wild populations in very small numbers, probably fewer than 1 propagule in 10^8. Since resistant strains seldom have a fitness advantage over wild strains, they remain at undetectable low levels. When a disease control agent selectively reduces or eliminates the sensitive wild population, resistant strains that are reasonably fit for survival and are pathogenic increase in the population. The most resistant, fit, and pathogenic are further selected by repeated pressure from the agent and environment. Depending on a complex of factors, the pathogen population may

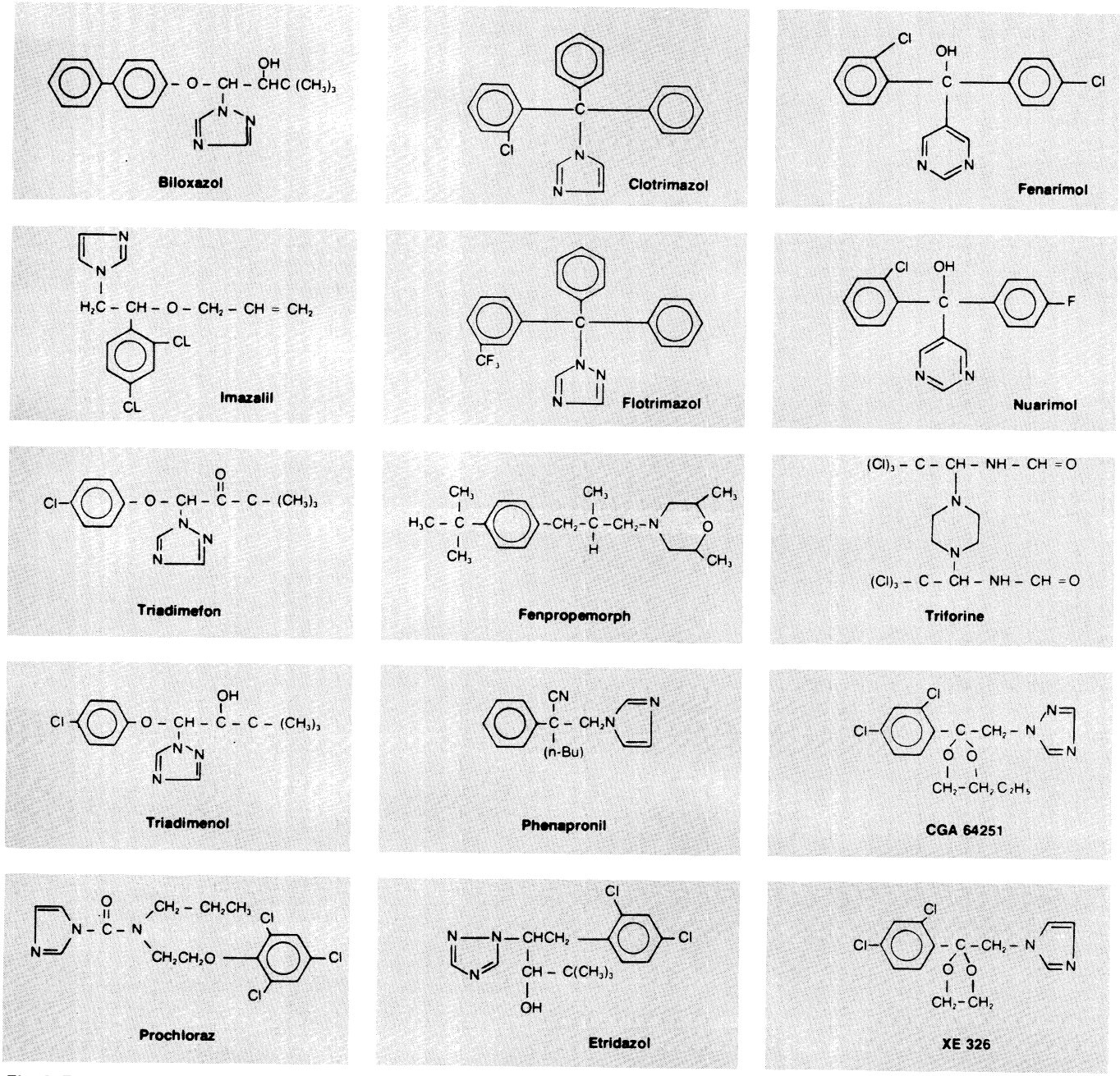

Fig. 2. Ergosterol biosynthesis inhibitors.

change from one with an insignificant level of resistance to one dominated by resistant strains. During this process the disease control agent becomes less effective. Therefore, preventive measures must be initiated before resistant strains can be detected and sole, long-term exposure of the pathogen to the agent must be avoided.

As new disease control agents are discovered, questions arise as to the probability of resistance. The answers seem simple at first because several laboratory tests detect resistance in natural populations and determine if it can be developed with artificial mutagenic treatments. Actually, the answers are far from simple because resistant strains may not survive or be pathogenic in the field. Results are seldom predictable until the new agent is subjected to extensive field use.

Development of resistance in the field has been monitored by various methods (19). The method of sample collection is critical, since small or nonrepresentative samples can distort results. In addition to isolating and growing strains on media amended with normally lethal levels of the agent, inoculating treated plants is needed to confirm pathogenicity. Monitoring studies are valuable first to detect resistance and later to understand the population dynamics under various control regimens.

The use of mixtures. The use of a companion fungicide with a different mode of action may eliminate emerging resistant strains if very few are in the population. Ideally, the rate of the primary agent in the mixture can be reduced. In some crop/disease combinations, however, it is economically and scientifically prudent to use full rates—or even reduced rates—of both agents.

The first guideline toward effective use of mixtures came from English and Van Helsema in 1954 (6). In a report on delaying emergence of resistant laboratory strains of *Xanthomonas* and *Erwinia* with combined streptomycin and terramycin, they predicted resistance and showed the advantage of combinations almost 20 years before the need arose. In 1976 Ogawa et al (15) noted that this information was unfortunately ignored until streptomycin resistance was a widespread problem. This year Kable and Jeffery (13) published a mathematical model demonstrating the advantages of mixtures over exclusive use or alternating schedules. The more the "at risk" fungicide is reduced and the companion disease control agent strengthened in a mixture, the longer resistance problems are delayed. Resistance is also delayed with decreasing spray coverage.

Cercospora arachidicola, a peanut leaf spot pathogen, became highly resistant to benomyl within three to four seasons of exclusive use. This fact was well established in the southeastern United States by 1974. In Texas, where rust is also a serious disease of peanut, benomyl has been used for 9 years in a mixture with a rust fungicide also active against *Cercospora*, and resistance has not become a problem.

Another example is the lack of resistance in *Botrytis* on strawberries in Australia. In several countries where benomyl has been used exclusively for *Botrytis* control, serious resistance problems have developed. Because *Colletotrichum acutatum* (strawberry black spot) is common in Australia and not controlled by benomyl, mixtures of benomyl plus captan are applied. This combination program was started in 1972, the second year after benomyl introduction, and there still is no *Botrytis* resistance.

Mixtures must be included in the program before resistant strains are a significant factor. In some cases where resistance has become a problem, mixture programs were started after a long period of exclusive use. Benomyl-resistant strains that have increased to an easily detected level may dominate the population under selective pressure despite mixture programs.

Rotation of disease control agents. Entomologists prefer alternate use of agents. Clearly, many variations in rotation schedules are possible, but the practice is risky if resistance develops. The alternate use of complementary products increases the spectrum of disease control, a benefit also obtained with mixtures.

The citrus industry combines sanitation methods with monitoring

Table 1. Fungal genes that affect resistance to fungicides[a]

Fungicides	Fungi	Gene changes (no.)
Aromatic hydrocarbons	*Aspergillus nidulans*	2
	Nectria hoematococca	5
Benzimidazoles	*Aspergillus nidulans*	1 or 2
	Ceratocystis ulmi	1
	Neurospora crassa	1
	Ustilago hordei	Poly
	Ustilago maydis	1
	Venturia inaequalis	1
Dodine	*Nectria hoematococca*	4
	Venturia inaequalis	2
Carboxamides	*Aspergillus nidulans*	3
	Ustilago hordei	Poly
	Ustilago maydis	3
Cycloheximide	*Neurospora crassa*	3
	Saccharomyces cerevisiae	8
Imazalil	*Aspergillus nidulans*	8
Kasugamycin	*Pyricularia oryzae*	1 to 3

[a] Adapted from Georgopoulos (9).

Table 2. Biochemical mechanisms of resistance

Reduced permeability (exclusion)
- Benzimidazole in *Sporobolomyces*
- Blasticidin S in *Pyricularia*
- Fenarimol in *Aspergillus*
- Polyoxin D in *Alternaria*

Detoxification (breakdown or binding)
- Benzimidazole in *Verticillium*
- Dodine in *Nectria*
- Organic mercury in *Pyrenophora*
- Organophosphate in *Pyricularia*
- PCNB in *Botrytis*

Decreased conversion into a toxicant
- 6-Azauracil in *Cladosporium*

Modification of sensitive sites
- Decreased affinity for tubulin in *Aspergillus* to benzimidazoles
- Decreased affinity for mitochondrial succinic dehydrogenase complex in *Ustilago* to carboxamides
- Changes in a ribosomal subunit sensitivity in *Saccharomyces* to cycloheximide and in *Pyricularia* to kasugamycin
- Reduced affinity for phosphoramidate enzymes in *Pyricularia* to phosphorothiolates

schemes and rotating fungicides to maintain the efficacy of disease control agents against the constant threat of resistance (5). In Spain, benomyl-resistant powdery mildew of cucurbits forced a switch to unrelated fungicides in 1969. Benomyl was reintroduced in rotation in 1970 and is an integral part of the disease control program. Similarly, dimethirimol rapidly lost effectiveness against powdery mildew of cucurbits in greenhouses in western Europe and was withdrawn; reintroduction in special programs in rotation with unrelated agents has been successful (1).

Limitation of use. Abstinence is a common reaction to anticipated resistance problems. From a business standpoint, a company developing a new agent must consider its potential use and life expectancy; if resistance limits use to an uneconomical level, further development of the agent is not worthwhile. Plant pathologists and growers can preserve disease control agents by limiting use to specific times and areas; this is especially true for agents with unique properties.

In some fruit crops where a number of applications are made, benzimidazoles occasionally are used only once or twice, which helps reduce selective pressure. A similar approach is feasible for other crops when curative effects are needed during critical times, as when disease conditions are severe. Recent results from Greece show that a combination of carbendazim and maneb boosts *Cercospora* control in sugar beet, even though the fungal population is generally resistant to low levels of benzimidazoles. Because restricting applications of the mixture to one or two critical times holds selective pressure to a minimum, the mixtures continue to be beneficial.

In the United Kingdom, *Erysiphe graminis* f. sp. *hordei* appears to temporarily adapt to various levels of ethirimol. To reduce the period of selective pressure, ethirimol is not used on the winter barley crop. Resistance does not build up, and ethirimol controls powdery mildew in the spring barley crop (1,11).

Integrated control strategies. In addition to tactics that optimize the chemical's effect, traditional methods of disease management—cultural practices, resistant cultivars, forecasting, etc.—should be used. Where less than complete disease control is acceptable, leaving refugia of wild, unexposed survivors helps reduce development of resistance. This is an established practice in dealing with insecticide resistance and is a major factor, as demonstrated by the Kable/Jeffery model (13). The integration of all strategies may be necessary to cope with resistance problems.

$$A\left[\left(\left[10xB\right]^{D} x\, C\right) x \left(10\left[YxZ\right]\right)\right]^{E} + \left[\text{Ditto}\right]^{(E-1)} + \left[\text{Ditto}\right]^{(E-2)} \text{ etc.} = \text{resistant lesions/ 30,000 A (74,000 ha)}$$

A = Status of resistant population at the start of the program as a multiple of the natural state. A = 1 if there is 1 resistant lesion in about 1.5×10^{8}.

B = Percent (expressed as a decimal) of the resistant spores surviving the fungicide program (1.0 where benomyl alone is used).

C = Percent (decimal) of the inoculum surviving to infect next season.

D = Number of reinfection generations during the growing season.

Y = Percent (decimal) of the total land area of a minimum statistical compartment (30,000 acres) planted to the susceptible crop.

Z = Percent (decimal) of the susceptible crop acreage treated with benomyl, alone or in mixtures. Assumes that the remaining susceptible crop provides minimal spore load from resistant strains.

E = Number of completed years of continuous use of the particular fungicide program under consideration.

10 = Generation increment based upon a production and release of 1000 viable spores per lesion, 1% of which are successful in starting a new lesion.

Fig. 3. Mathematical model of development of benomyl-resistant fungus strains.

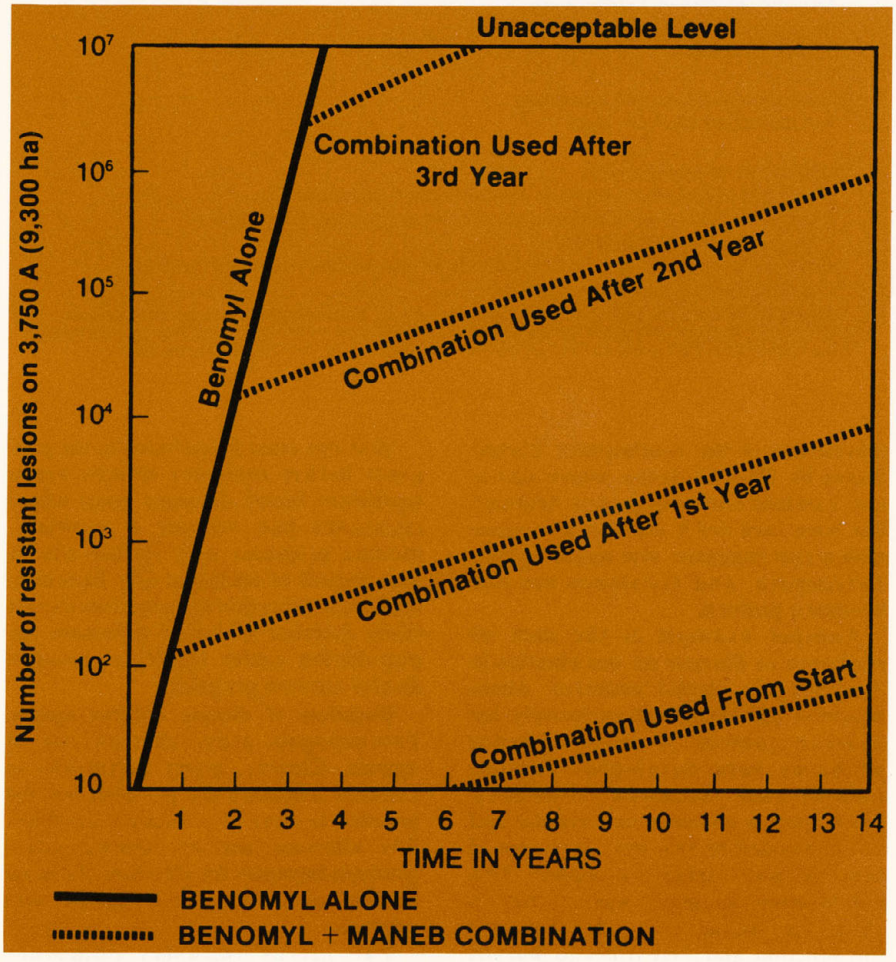

Fig. 4. Development of resistance to benomyl used alone or combined with maneb.

Model for Understanding Resistance Development

R. W. Varner of E. I. du Pont de Nemours & Company (Inc.) devised a theoretical mathematical model to help explain the development of benomyl-resistant fungus strains under various field conditions (Fig. 3). The major factors contributing to selection and development of resistance are as follows:

A. The frequency of resistant spores in nature is estimated at 1.5×10^{-8}. This, of course, varies with the fungus and previous selection pressure but represents the well-established concept that resistant strains are a natural occurrence.

B. The percentage of resistant spores that survive depends on the fitness of the strains and the kinds of control measures used.

C. The amount of inoculum that survives to the next season is also a critical factor in the rate of development.

D. Organisms with only one or two infection cycles per season could be expected to develop resistance much slower than those with more cycles.

Y & Z. The larger the area and the higher the proportion of that area treated, the higher the probability of selection for resistance. A unit of 30,000 A (74,000 ha) of the susceptible crop was chosen for this model. The portion of that area not treated with benomyl provides a minimal spore load of resistant strains.

E. The effect of continuous use of a particular treatment program each year is cumulative.

Because the numerical values assigned to these factors vary widely among particular combinations of hosts, pathogens, and environments, the time and intensity of resistance development also vary.

One of the various situations and experiences that have been computed on this model is presented in Fig. 4. Under conditions conducive for development of Cercospora leaf spot on an annual crop such as peanut, an unacceptable level of resistance is reached after the third year of frequent use of benomyl alone. When a benomyl and maneb combination is used from the start, with maneb providing at least 80% control of the resistant strain, development of significant resistance is delayed indefinitely. Development of resistance is delayed for a lesser but still significant period when the combination is started after the first or second year.

When diseases with few reproductive generations and requiring fewer chemical treatments are computed on the model, resistance develops more slowly.

Although not designed to predict the emergence of resistance in a specific grower's situation, the model is useful in understanding the theory associated with various spray regimens and in giving general trends in population dynamics. It definitely demonstrates the advantages of using mixtures as soon as possible.

Future Prospects

Disease control is now and will continue to be much more complex than when only a few multisite agents were available. But the rewards from greatly improved disease control with the newer, more diverse agents are worth the efforts toward enlightened use. As part of an integrated pest management concept, the most effective use of all chemicals includes ways to cope with resistance. Conventional disease control agents are needed alone and combined with specific new chemicals, including those yet to be discovered. Sufficient information is urgently needed and, when obtained, should be used rationally in registering agents for safe and effective use. Restrictions to control abuse should not rule out necessary flexibility of use. Resistance problems compound the need to keep old agents effective and find new ones. The loss of proven fungicides and delays in availability of new agents because of government regulatory actions clearly complicate production of healthy crops. In a world on the brink of massive food shortages, making the best possible use of old and new disease control agents is imperative. Coping with resistance will require intensive effort, but it can be done.

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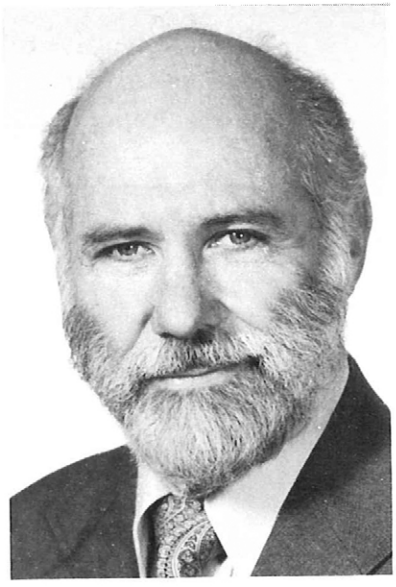
Dr. Delp is an associate plant pathologist with the Du Pont Company, where he serves as an international consultant on plant disease control problems. He was one of the scientists involved in the discovery and development of benomyl (Benlate) fungicide, and he supervised a group conducting research on disease control agents. He earned a B.S. from Colorado State University and a Ph.D. in plant pathology from the University of California at Davis.



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