FUNGICIDE RESISTANCE: PROBLEM WITH MODERN FUNGICIDES IN JAPAN

Yasuhiko UESUGI*

Abstract

The use of selective fungicides started around 1960 and has become increasingly popular for the purpose of plant disease control in Japan, although the development of resistance to these fungicides by phytopathogenic fungi has caused serious problems since 1970. Characteristics of fungi such as mutability of the gene(s) controlling the site of action of the fungicides and the rate of multiplication may be related to the emergence of mutants resistant to fungicides, and the viability of the resistant mutants may also be an important factor for the development of fungicide resistance in the field. Methods of application and residual effect of fungicides are linked to the efficiency of selection of resistant strains, and the intensive and exclusive use of fungicides belonging to a single group in relation to cross-resistance has often been a cause of fungicide resistance. The best way of solving this problem is to avoid the development of resistance. Recently, some experiments have shown the possibility of controlling resistant strains by using chemicals which exhibit a negatively correlated cross-resistance to the resistant strains or block the biochemical reactions causing the resistance.

Introduction

In the history of the development of fungicides for agricultural use, two distinct periods can be recognized (Table 1). The first period started around the 1940s when organic compounds such as dithiocarbamates, quinones, phenols and organic metal compounds were developed as plant fungicides and replaced inorganic fungicides such as sulfur, lime sulfur and Bordeaux mixture. These organic fungicides were remarkably effective in controlling plant diseases as compared with the inorganic fungicides and therefore they were used widely and abundantly. However they were sometimes toxic to crops, animals and other non target organisms. Around 1960, fungicides endowed with selective toxicity to plant pathogens but not to non target organisms, such as benzimidazole fungicides and antibiotics, were developed. Their selectivity is so strong that even pathogenic fungi on mushroom, namely, fungi on another fungus, may be controlled by some of these fungicides without phytotoxicity to the host fungus. Since the contamination of the environment has been one of the most serious problems since that time, these selective fungicides were immediately used for agriculture to replace the non selective fungicides which may affect the quality of the environment, and these have contributed to a new era in the history of fungicides.

These modern fungicides usually inhibit a single specific site in the fungal cells while the non selective fungicides generally inhibit a number of sites or a site with a specific physiological function which is however common to various organisms, such as the mechanism of oxidative phosphorylation. Although the modern fungicides seem ideal, they have basically an important shortcoming, namely they are characterized by a limited applicability. Most of them have either a therapeutic effect without a preventive one or they display a protective effect but no therapeutic effect, and therefore the timing of application of these modern fungicides is limited. They are also effective in a limited range of plant diseases. This limited applicability is sometimes evident in a species of plant pathogenic fungus. The more resistant strains survive in the presence of the

* Chief, Second Laboratory of Pesticide Chemistry, National Institute of Agricultural Sciences, Yatabe-machi, Tsukuba-gun, Ibaraki 305, Japan.

Table 1 Development of fungicides in relation to chemical composition.

Year	Inorganic fungicides	Organic fungicides	
		Synthetic	Antibiotic
1800			
	Sulfur Lime sulfur Bordeaux mixture		
1940			
		Dithiocarbamates Quinones Phenols Organomercurials Substituted benzenes Halogenoalkylthio-dicarboximides (such as captan)	
1960			
		Benzimidazoles Phosphorothiolates 3,5-Dichlorophenyl-dicarboximides	Blasticidin S Kasugamycin Polyoxins Validamycin A
		etc.	

fungicide and their population increases in the field so long as the fungicide is used.

Factors affecting the development of resistance

The selectivity of the modern fungicides, as stated above, is undoubtedly at the basis of the development of resistance. In fact, the occurrence of resistant fungi in the field was first recognized in 1971, shortly after the development of these selective fungicides, in the case of *Pyricularia oryzae* which became resistant to kasugamycin (Miura *et al.,* 1975) and *Alternaria kikuchiana* to polyoxins (Nishimura *et al.,* 1973) in Japan. A similar situation was also observed nearly at the same time in Europe in the case of *Botrytis cinerea* which developed a resistance to benomyl (Bollen and Scholten, 1971) and in North America in the case of *Venturia inaequalis* which became resistant to dodine (Szkolnik and Gilpatrick, 1969) and of *Sphaerotheca fulginea* resistant to benomyl (Schroeder and Provvidenti, 1969). Since these selective fungicides have been used abundantly and widely, the resistance problem has become very serious for a variety of plant diseases, as shown in Table 2.

Whatever the process of emergence of resistant strains in the field is, strains must meet selection pressure of fungicides under field conditions, and therefore, the behavior of these fungicides on and in the host plants is an important factor for the development of fungicide resistance in the field. In fact, there have been many instances of development of resistance under frequent and/or long-lasting pressures of selection with fungicides. A fungicide having a residual effect or the frequent application of a fungicide results in an efficient selection of strains resistant

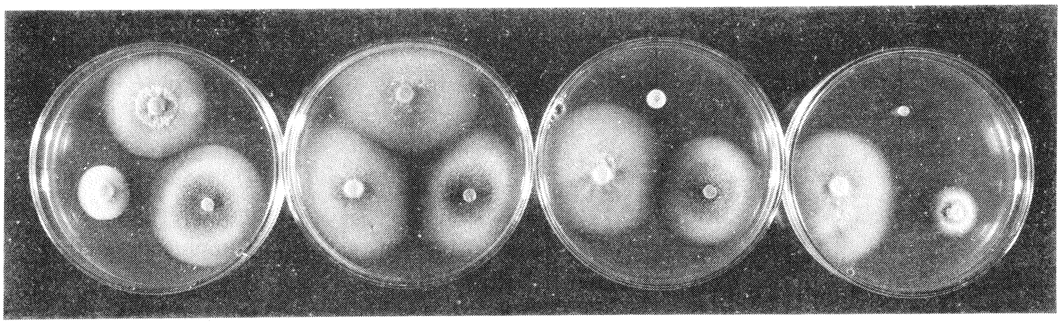
Table 2 Examples of development of resistance of phytopathogenic fungi to fungicides in the field.

Fungus	Crop	Disease	Fungicide
Alternaria kikuchiana	Japanese pear	Black spot	Polyoxins
Alternaria mali	Apple	Alternaria leaf spot	Polyoxins
Botrytis cinerea	Vegetables	Gray mold	Benomyl and Thiophanate-methyl
Botrytis cinerea	Vegetables	Gray mold	Iprodione, Vinclozolin and Procymidone
Cercospora beticola	Beet	Cercospora leaf spot	Benomyl and Thiophanate-methyl
Gibberella fujikuroi	Rice	"Bakanae" disease	Benomyl and Thiophanate-methyl
Mycosphaerella melonis	Watermelon	Black rot	Benomyl and Thiophanate-methyl
Penicillium digitatum	Citrus	Green mold	Benomyl and Thiophanate-methyl
Penicillium italicum	Citrus	Blue mold	Benomyl and Thiophanate-methyl
Puccinia horiana	Chrysanthemum	Rust	Oxycarboxin
Pyricularia oryzae	Rice	Blast	Kasugamycin
Pyricularia oryzae	Rice	Blast	IBP and Edifenphos
Sclerotinia cinerea (=*Monilinia fructicola*)	Cherry	Brown rot	Benomyl and Thiophanate-methyl
Sphaerotheca fulginae	Cucumber and melons	Powdery mildew	Benomyl and Thiophanate-methyl
Sphaerotheca fulginae	Cucumber and melons	Powdery mildew	Dimethirimol
Venturia inaequalis	Apple	Scab	Benomyl and Thiophanate-methyl
Venturia nashicola	Japanese pear	Scab	Benomyl and Thiophanate-methyl

to the fungicide. The method of application may sometimes affect the efficiency of the selection. Application of fungicide granules onto paddy water, submerged application, and soil application have been performed as convenient and labor-saving devices for the control of diseases on growing plants. However, the fungicides applied in this way often remain for a fairly long period of time at certain concentrations in the plants and selection of resistant strains can occur very effectively.

Since fungicide resistance in the field is genetically stable, the resistant strains have presumably emerged by mutation. The probability of emergence of resistant strains may, therefore, depend on the mutability of the related gene(s) of the fungus and also on the rate of multiplication. The mutability may be estimated to some extent by an *in vitro* experiment of selection of fungal cells with the fungicide.

Development of fungicide resistance in the field is, however, not solely controlled by the frequency of mutation. An example was provided by mutants of *P. oryzae* resistant to phosphorothiolate fungicides. The resistant mutants were readily obtained by *in vitro* selection from a large number of conidia on agar medium containing the fungicides without any treatment to induce mutations, and the resistant mutants thus obtained showed a growth rate, sporulation ability and virulence to rice plants similar to those of their mother strains (Uesugi *et al.*, 1969). Recently, development of resistance in the field to phosphorothiolate fungicides by the fungus has been observed in Japan, but most of the resistant field-isolates were definitely different from the resistant mutants obtained in the laboratory by cross-resistance to some phosphoramidates. A negatively correlated cross-resistance was found between phosphorothiolate and phosphoramidate in laboratory-derived resistant mutants and a very few resistant field-isolates, although in most of the resistant field-isolates such correlation was not observed and the latter had a lower resistance to phosphorothiolate than the former (Katagiri *et al.*, 1980) (Plate 1). A type similar



0.1 mM HPA UNTREATED 0.1 mM IBP 0.2 mM IBP

Plate 1 **Growth of a normal type of field-isolate of *Pyricularia oryzae* (top in each petri dish), a popular type of field-isolate moderately resistant to phosphorothiolates (lower right) and a rare type of field-isolate resistant to phosphorothiolates but specifically sensitive to phosphoramidates (lower left) on agar medium containing 0.1 mM HPA (dihexyl *N*-methyl-*N*-phenylphosphoramidate), untreated (control), 0.1 mM IBP and 0.2 mM IBP (left to right).**

to the field-isolates with moderate resistance has not been obtained by *in vitro* selection so far. Thus under field conditions, there may have been some other factors affecting the emergence of resistant strains.

Viability of resistant strains in contrast to normal sensitive ones may be one of the important factors for the development of fungicide resistance in the field. Apparent decreases in growth rate or virulence to the host plants in the resistant strains may be an indication that the development of resistance in the field is unlikely. In addition to this, competition between sensitive and resistant strains must be severe for location, nutrients etc. to survive and grow under field conditions. In this connection, variations in the rate of resistant strains in the total field-isolates is a good index to evaluate variations in fungicide resistance in the field. Development of fungicide resistance in the field has usually resulted from intensive and exclusive use of a single type of fungicide and the rate of the resistant strains tends to increase unless fungicide application is discontinued. There have, however, been various instances where variations in the rate of resistant strains after the cessation of fungicide application have been recorded. In the case of kasugamycin resistance in *P. oryzae*, the rate of resistant strains decreased from about 90% to 20% or less during three years after the repeated use of the antibiotic was discontinued (Miura and Takahashi, 1976). No significant difference was found between resistant and sensitive strains with respect to their growth rate, sporulation ability and virulence to the plants (Miura *et al.*, 1976), but the resistant strains were generally inferior to the sensitive ones on rice leaves inoculated with a mixture of conidia of both strains, presumably because of slower progress of infection in the resistant strains (Ito and Yamaguchi, 1979). On the other hand, the rate of resistance of strains of *Cercospora beticola* to benzimidazole fungicides (Yamaguchi *et al.*, 1976) did not decrease after the application of the fungicides was discontinued. The results obtained in Japan were in accordance with those reported in Greece with the same fungus and fungicides (Dovas *et al.*, 1976).

Prevention of fungicide resistance

The best method to avoid the development of fungicide resistance is to prevent resistant strains from emerging. Among the factors related to the development of resistance in the field, as stated above, factors which can be controlled are those concerning fungicide use. Intensive and exclusive use of a single type of selective fungicides is undoubtedly a cause of emergence of resistant strains, which should be avoided.

Strains resistant to a fungicide are sometimes resistant to another fungicide due to the same biochemical mechanism. This is called cross-resistance and fungicides belonging to a group related to cross-resistance select fungal strains for resistance in common. The use of two or more fungicides belonging to different groups is, therefore, recommendable to avoid the development of resistance. From this standpoint, the development of new types of fungicides is still necessary even when the disease is well controlled by an excellent fungicide. There may be basically two ways of using two or more types of fungicides, i.e. alternate use and use of a mixture of the fungicides. Alternate use may reduce the frequency of application of each of the fungicides and thus decrease or delay the development of resistance. When a mixture of fungicides is used, selection pressure of each of the fungicides may work at the time of each application but may decrease the total amount of remaining resistant strains.

Since the resistant strains are somehow physiologically different from normal sensitive strains, it may be possible to control them by concentrating efforts on the difference. Though there have been no such practical cases for controlling resistant fungi so far, it may be an attractive way to cope with fungicide resistance and the possibility of applying this method has been demonstrated by several experiments. Physiological and biochemical mechanisms of fungicide resistance can be classified into the following three categories: a) modification of site of action of the fungicide to decrease fungicidal action at the site, b) alteration of the metabolism of the fungicide in the fungal cells to increase the detoxification processes or to decrease the activation of the fungicide, and c) decreased permeability of fungal cell membrane to the fungicide.

In the case of resistance by site modification, there are chemicals which are more toxic to the modified site or its related site in the resistant strains than to the corresponding site in the sensitive strains. A negatively correlated cross-resistance should exist between these chemicals and the fungicide. An example was provided by some strains of *Botrytis cinerea* and *Penicillium expansum* resistant to benzimidazole fungicides but specifically sensitive to some *N*-phenylcarbamate herbicides such as barban (Leroux and Gredt, 1979). Both benzimidazole fungicides and *N*-phenylcarbamate herbicides are known as antimitotic compounds which interfere with microtubule formation by interaction with tubulin.

The existence of a negatively correlated cross-resistance seems also possible in the case of resistance due to alteration in the metabolism of the fungicide. Mutants of *P. oryzae* selected for resistance to the phosphorothiolate fungicide IBP were more sensitive to some phosphoramidate compounds than the mother strains (Uesugi *et al.*, 1974). In this type of mutants, metabolic degradation of phosphoramidate as well as of IBP was decreased, and the increase of sensitivity to phosphoramidate could be ascribed to the decreased detoxification of phosphoramidate. However, the development of resistance to IBP has not been elucidated so far (Uesugi and Sisler, 1978). Though the metabolites of IBP were less toxic and the fungal metabolism seemed to be affected by detoxification, the mechanisms involved might include some process of activation or somehow relate to the action of IBP.

Resistance by increased detoxification may be overcome by inhibiting the detoxification process. Mutants of *P. oryzae* selected with phosphorothiolate fungicides are sensitive to some phosphoramidates, while the mother strains are resistant due to higher detoxification, and the detoxification of one of the phosphoramidates in the mother strains was inhibited by IBP and by isoprothiolane and caused a synergistic fungicidal action between the phosphoramidate and IBP or isoprothiolane (Uesugi *et al.*, 1974; Uesugi and Sisler, 1978).

Since the development of fungicide resistance in the field is a comparatively recent problem, investigations should be carried out in the future.

References

1) BOLLEN, G.J. and SCHOLTEN, G. (1971): Acquired resistance to benomyl and some other systemic fungicides in a strain of *Botrytis cinerea* in cyclamen. *Neth. J. Pl. Path.* **77**, 83–70.
2) DOVAS, C., SKYLAKAKIS, G. and GEORGOPOULOS, S.G. (1976): The adaptability of benomyl-resistant population of *Cercospora beticola* in northern Greece. *Phytopathology* **66**, 1452–1456.
3) ITO, I. and YAMAGUCHI, T. (1979): Competition between sensitive and resistant strains of *Pyricularia oryzae* Cav. against kasugamycin, *Ann. Pytopath. Soc. Japan* **45**, 40–46.
4) KATAGIRI, M., UESUGI, Y. and UMEHARA, Y. (1980): Development of resistance to organophosphorus fungicides in *Pyricularia oryzae* in the field. *J. Pesticide Sci.* **5**, 417–421.
5) LEROUX, P. and GREDT, M. (1979): Effect of barban, chlorpropham and propham on strains of *Botrytis cinerea* Pers and *Penicillium expansum* Link resistant to carbendazim and thiophanate-methyl. *C. R. Acad. Sc. Paris* T. **289**, D 691–693.
6) MIURA, H., ITO, H. and TAKAHASHI, S. (1975): Occurrence of resistant strains of *Pyricularia oryzae* to kasugamycin as a cause of the diminished fungicidal activity to rice blast. *Ann. Phytopath. Soc. Japan* **41**, 415–417.
7) ————, KATAGIRI, M., YAMAGUCHI, T., UESUGI, Y. and ITO, H. (1976): Mode of occurrence of kasugamycin-resistant rice blast fungus. *Ann. Phytopath. Soc. Japan* **42**, 117–123.
8) ———— and TAKAHASHI, S. (1976): Transition of effectiveness of kasugamycin and population of strains of rice blast fungus resistant to it. *Ann. Phytopath. Soc. Japan* **42**, 372.
9) NISHIMURA, S., KOHMOTO, K. and UDAGAWA, H. (1973): Field emergence of fungicide-tolerant strains in *Alternaria kikuchiana* Tanaka. *Rept. Tottori Mycol. Inst. (Japan)* **10**, 677–686.
10) SCHROEDER, W.T. and PROVVIDENTI, R. (1969): Resistance to benomyl in powdery mildew of cucurbits. *Pl. Dis. Reptr.* **53**, 271–275.
11) SZKOLNIK, M. and GILPATRICK, J.D. (1969): Apparent resistance of *Venturia inaequalis* to dodine in New York apple orchard. *Pl. Dis. Reptr.* **53**, 861–864.
12) UESUGI, Y., KATAGIRI, M. and FUKUNAGA, K. (1969): Resistance in *Pyricularia oryzae* to antibiotics and organophosphorus fungicides. *Bull. Natl. Inst. Agr. Sci.* C **23**, 93–112.
13) ————, ———— and NODA, O. (1974): Negatively correlated cross-resistance and synergism between phosphorothiolates and phosphoramidates in their fungicidal action on rice blast fungi. *Agr. Biol. Chem.* **38**, 907–912.
14) ———— and SISLER, H.D. (1978): Metabolism of a phosphoramidate by *Pyricularia oryzae* in relation to tolerance and synergism by a phosphorothiolate and isoprothiolane. *Pestic. Biochem. Physiol.* **9**, 247–254.

15) YAMAGUCHI, T., SUGIMOTO, T., HOMMA, Y. and OHATA, K. (1976): Resistance of *Cercospora beticola* to thiophanate-methyl. *Ann. Phytopath. Soc. Japan* **42**, 372–373.

Discussion

Yang, C.Y. (AVRDC): Is it possible to predict the development of fungal resistance to any new selective and systemic fungicide? If so, how long can a new fungicide of this nature be used in the field before we have to look for newer fungicides to encounter possible fungal resistance?

Answer: It is true that the more selective a fungicide is the more likely the development of resistance of fungi to this particular fungicide will occur. However there is an other important factor, namely the viability of the resistant strains in the field, which is difficult to predict.

Leroux, P. (France): Have you conducted experiments to compare the efficacy of mixtures or alternative applications of fungicides with regard to the problem of resistance? In France, we have observed that mixtures are not always effective. For example, the use of mixtures of metalaxyl with conventional fungicides cannot prevent the development of resistance in the case of downy mildew of vine or potato blight.

Answer: In Japan a large number of studies such as those by the Japan Plant Protection Association have been conducted in this regard. I would like to recommend that practical tests be carried out to determine whether it is preferable to use mixtures of fungicides or alternative applications in order to cope with resistance to fungicides.