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Fungal Resistance to Sterol Biosynthesis Inhibitors: A New Challenge

Yield losses caused by plant pathogens have threatened the security and efficiency of crop production since agriculture became the main source of the human food supply. Fortunately, agriculture has made tremendous progress during the last century, and part of this progress has been the development of modern means of plant disease control. In particular, the introduction of chemical disease control agents has contributed to a substantial increase in crop production, to a smoothing of annual undulations in crop yields, and, ultimately, to today's high level of food security.

The first milestone in fungicide development was the introduction of inorganic fungicides such as sulfur, copper, or mercury compounds, followed by the development of organic fungicides such as dithiocarbamates (e.g., maneb) and phthalimides (e.g., captan). These two classes of protective compounds have been used extensively for decades without development of field resistance. During the same period, organic insecticides had already encountered cumbersome drawbacks. Gordon (16) introduced his 1961 review on insecticide resistance with a clear statement: "The number of insect species or populations resistant to one or more of the synthetic organic insecticides has increased every year since 1947, and there is yet no indication that this trend can be halted or reversed." The conclusion on fungicide resistance drawn 6 years later by Georgopoulos and Zaracovitis (14) was clearly different: "Tolerance to organic fungicides used in the control of fungal diseases of plants or storage rot has created practical difficulties in only a few instances." The future prospects, however, sounded less optimistic, and the authors must have seen the dawn of a major change: "If future fungicides must be selective, interfering with the metabolism of the pathogen and not the plant, the emergence of forms refractory by virtue of acquired resistance will probably be as common as it has been with many human and animal pathogens or with insects."

Such highly desirable compounds with a specific mode of action and a systemic mobility within the plant were discovered during the 1960s. This new class of fungicides offered curative and sometimes eradicative means of plant disease control, along with additional advantages such as lower application rates and longer lasting protection. The benzimidazoles, in particular, were welcomed enthusiastically by plant pathologists and farmers. The initial enthusiasm, however, was soon quelled when the first cases of crop losses owing to rapid development of field resistance were reported. Plant pathologists, like entomologists before them, had to cope with the serious problem of resistance. In spite of this drawback, many new groups of systemic and specific fungicides were developed (6), and most have proved to be valuable tools in plant disease management. Nevertheless, the problem of resistance to fungicides had emerged, and countermeasures had to be developed. The search for reliable antiresistance strategies was of common interest to the farmers, who could suffer from unexpected crop losses, and the manufacturers, who could lose a compound developed at increasingly higher costs.

Although all parties involved agree on the resistance problem, discussions have not been entirely free from tensions and misunderstandings. Even the definitions of terms were, for a long time, a matter of some confusion. A guideline of terminology was proposed in 1985 (11). According to this proposal, "resistance" should be used only to define a stable and heritable adjustment by a fungus to a fungicide, resulting in a considerably reduced sensitivity to the inhibitor. The difference in sensitivity can be defined by the resistance factor, expressed as the ratio EC 50 (resistant)/EC 50 (sensitive). Resistance should be distinguished clearly from a momentary adaptation of a fungal pathogen to a fungicide. Adaptations are neither heritable nor stable and are not expected to cause severe problems. Furthermore, insufficient field performance of a fungicide is not necessarily related to the presence of resistant strains in a field. Poor disease control might be caused by improper application, extremely high infection pressure, or other factors not related to resistance. Thus, the term "field resistance" should be used only when decreased fungicide efficacy is correlated with increased frequency of resistant strains. Unfortunately, this correlation is not always easy to prove or disprove, and appropriate and approved test methods to assess this correlation are urgently needed.

Despite some problems and uncertainties, verified cases of fungicide resistance in the field have become numerous (24), and strategies to continue the beneficial use of these fungicides have had to be developed. The first goal was the search for ways to continue disease control with a particular fungicide even after a substantial level of resistance was established in the field. The ultimate goal, however, has always been a strategy to prevent the buildup of resistance before a new fungicide group is introduced to the market. Guidelines for antiresistance strategies emerged, and their general principles are still entirely valid (10,34):

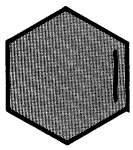
- The total risk of resistance is influenced by management factors, such as conditions of fungicide usage, environmental conditions, or agricultural management methods, and by inherent factors, such as the biology and epidemiology of the pathogen (34). One of the inherent risk factors is the chemistry and biochemistry of the fungicide, and this risk must be evaluated.
- High inherent risk requires more stringent control of risk factors relating to management in order to limit the total risk of resistance. In general, "at-risk"

compounds should not be used alone over long periods of time but, rather, should be used in mixtures or in alternation with second fungicides that lack cross-resistance.

The phenomenon of cross-resistance was soon recognized to be of utmost importance. Switching to a second compound with cross-resistance to the initial one would be useless because any selection of resistant strains would proceed at the same pace. Fungicides with cross-resistance to each other, however, are usually distributed by different companies, and a resistance strategy of one company might be hampered or even counteracted by the marketing strategy of a second company. Intercompany cooperation was deemed necessary. In order to establish this cooperation, the Fungicide Resistance Action Committee (FRAC) was founded in 1981. FRAC brings producers of related fungicides together to coordinate research and develop strategies related to resistance (38). Although the basic outlines of resistance strategies seemed to offer a sound guideline for cooperation, specific cases have been more complex and complicated than originally envisioned. Two main questions must be answered as the basis of decisions on practical action:

- How is the resistance risk assessed and defined before any development of field resistance?
- What are the criteria for a second compound to be used with a fungicide under risk?

We will discuss these questions in light of more recent findings and trends, emphasizing the group of fungicides collectively called sterol biosynthesis inhibitors (SBIs).



Mode of Action and Resistance

Experience with the first systemics such as benomyl was clearly different from that with conventional protective fungicides. Despite extensive use for decades, conventional fungicides have caused few problems, whereas field resistance sometimes developed rapidly shortly after widespread and exclusive use of the new group of systemic compounds. The most popular explanation for this difference came from biochemical discoveries. Benzimidazoles were shown to be inhibitors of tubulin polymerization and, consequently, microtubule assembly (5). This single-site mode of action was clearly different from the multisite modes of action of conventional protective fungicides. A second difference, again biochemical in nature, was the molecular mechanism of resistance. A substantially decreased binding affinity of the benzimidazole to the target tubulin was shown to be responsible for the resistance of *Aspergillus nidulans* (5). Mutation of a single gene was responsible for the structural change of tubulin leading to this decreased binding affinity. Similar biochemical properties—a single-site mode of action and a target mutation leading to resistance—were also reported for the group of carboxamides (6), and the mode of action appeared to offer a rational explanation for the striking differences between conventional and new highly active compounds with a single-site mode of action that were not necessarily of systemic nature. Fungicide biochemistry became the basis of a popular concept (e.g., 10): Conventional multisite inhibitors that interfere with numerous vital metabolic processes of the pathogen allow little chance for resistance because multiple modifications in the pathogen's genome are required to circumvent the action. Specific-site inhibitors, on the other hand, act on only one metabolic site. Resistance is more common because mutations of only one fungal gene might be sufficient to induce a change at the target site leading to decreased binding affinity of the inhibitor.

This statement was highly attractive because it offered both an explanation for the new experience with single-site compounds and a rational foundation for ways to predict the risk of resistance and to counteract or prevent its buildup in the field. The term "mode of action" became commonplace among phytopathologists and influenced our current antiresistance strategies. Simple concepts emerged, for example, the strategy emphasized by Gindrat and Forrer (15): Fungicides with a single-site mode of action are under the greatest risk of resistance development. Therefore, multisite fungicides should be preferred when possible. Single-site compounds should be used only in minimal numbers of applications and, furthermore, only in rotation with a fungicide that differs in mode of action and therefore lacks cross-resistance. An alternative would be a mixture of single-site inhibitors with multisite compounds. To enable and encourage farmers to follow this strategy, a labeling system based exclusively on the mode of action has been suggested.

Biochemistry had found its way to practical advice, and the mode of action became one of the most important characteristics of a new fungicide. When Delp (10) wrote his feature article on fungicide resistance in 1980, two new groups of systemics with a single-site mode of action, the phenylamides and the SBIs, had gained in importance, and problems with resistance development after widespread use were predicted for both groups. Outbreaks of resistance

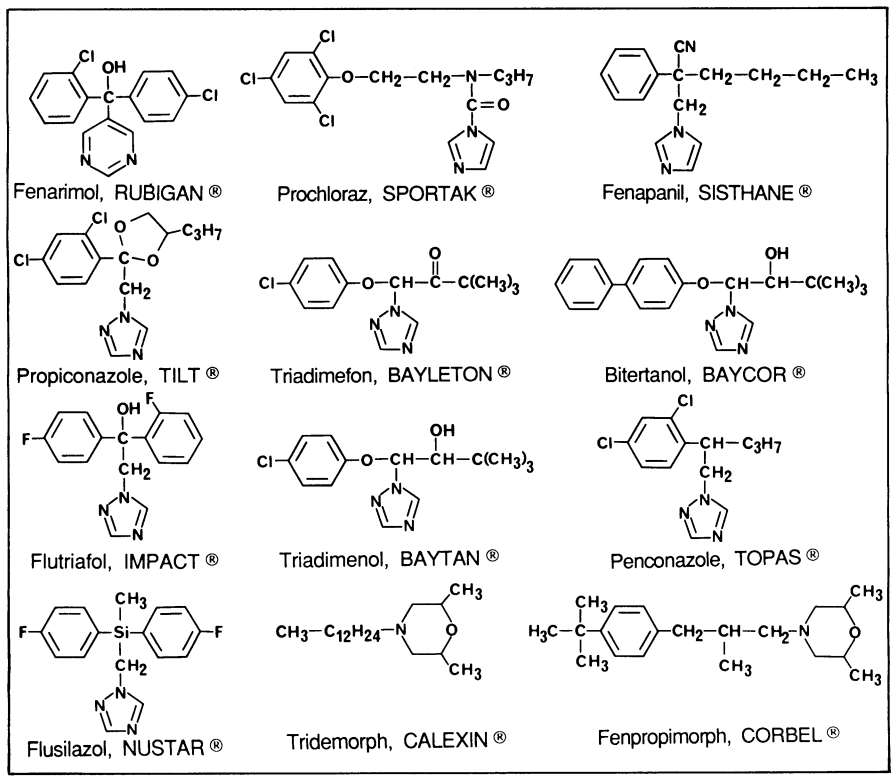


Fig. 1. Representative examples of sterol biosynthesis inhibitors.

followed, as predicted, shortly after metalaxyl, the first phenylamide, was introduced to the market (9), and a resistance strategy based on a mixture with a conventional fungicide was recommended (36). The experience with SBIs has been promisingly different.

SBIs constitute a rather diverse class of modern systemic fungicides. Some of the more important representatives are shown in Figure 1; a complete list has been published in recent reviews (23,26). The mode of action of SBIs has been extensively investigated during the last decade (20). The pyrimidines together with azoles are inhibitors of the C-14 demethylation of lanosterol or 24-methylenedihydrolanosterol, a biosynthetic step that occurs during the conversion of lanosterol to ergosterol, the final product of fungal sterol synthesis (Fig. 2). The mode of action of morpholines is not so well understood. Although the inhibition of sterol biosynthesis seems to be established, uncertainties about the precise site of inhibition still exist (Fig. 3). The most recent report suggests that tridemorph and fenpropimorph inhibit at two different sites to different degrees. The sites of action seem, furthermore, to be somewhat dependent on the fungal species (1). This poses an interesting question with practical relevance: Do all morpholines exhibit the same mode of action? The morpholines are a good example of the difficulties sometimes involved in the elucidation of exact inhibitor sites. This example raises, in addition, a more general question with regard to resistance strategies based on modes of action. It is not certain at what stage of research the mode of action of a fungicide becomes the criterion for practical advice. The appreciation of this problem is especially important for fungicides in an introductory stage and, thus, for the development of strategies aimed at the prevention of resistance.

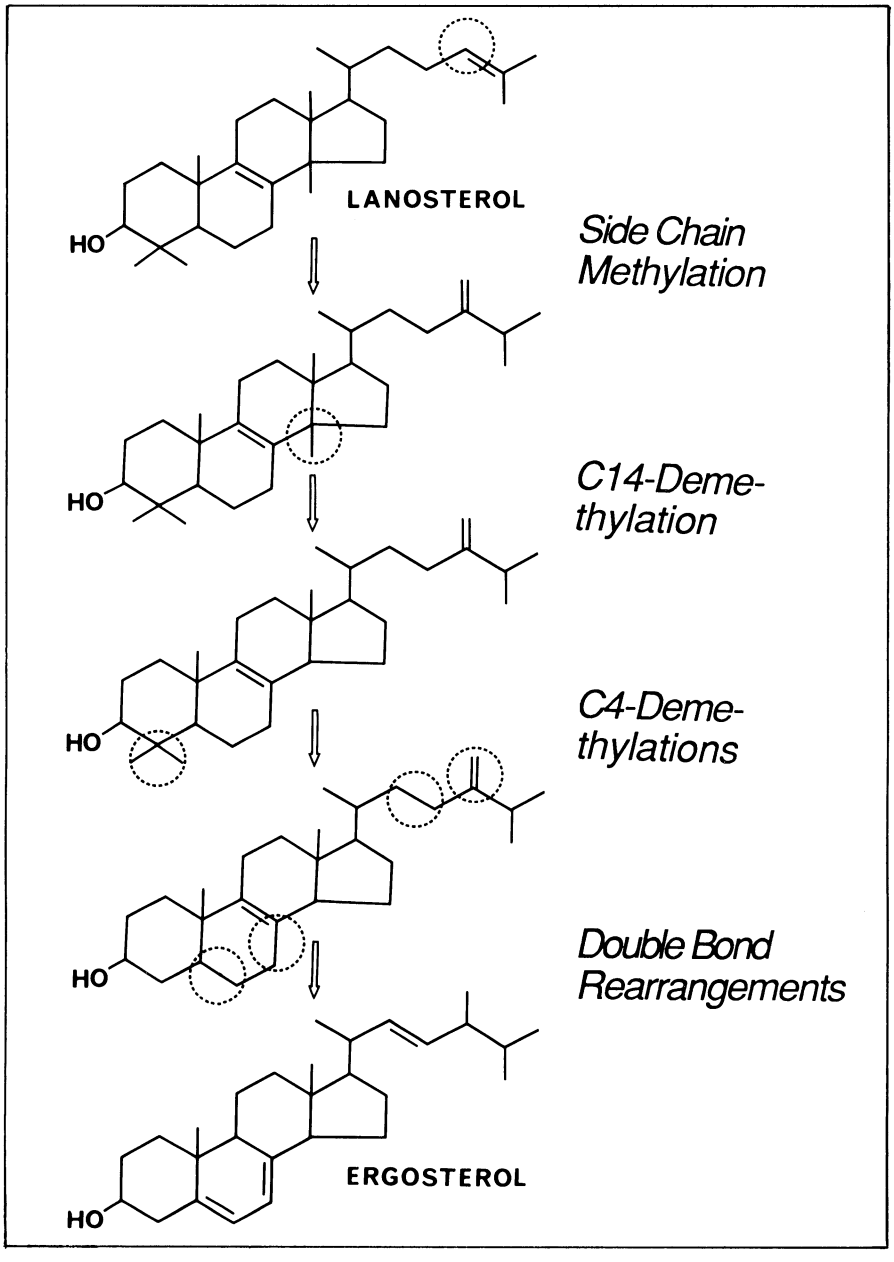


Fig. 2. Simplified scheme of sterol biosynthesis in fungi. The sites of biosynthetic modifications are circled.

Despite some uncertainties with morpholines, the single-site mode of action of most of the SBIs has been established, and this mode of action supposedly indicates a high risk of resistance. It has been frequently pointed out, however, that the mode of action alone is not sufficient for assessing and predicting the risk of resistance development under field conditions, and a second approach to assessments of inherent risk factors has been suggested. The ease of obtaining resistant mutants in the laboratory should be a valuable additional indication. Resistant mutants are not to be expected in the field when resistant strains cannot be obtained in the laboratory with the aid of mutagenic treatment. Conversely, the ready induction of resistant strains in the laboratory suggests a high potential for developing resistance in the field (8).

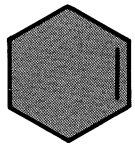
This approach, again, seemed to predict a high risk for the SBIs, especially for pyrimidines and azoles. Resistant laboratory mutants were easily obtained, and reports describing these mutants are numerous (9). All parameters indicated the risk of rapid resistance development after the onset of widespread use. Widespread use is a reality today, and the market share of the SBIs is expected to increase even more in the future (23,26). Among the reasons for the tremendous commercial acceptance of these compounds is their broad spectrum of activity, which provides control of many leaf and seedling diseases of important crops (Table 1). All parameters, taken together, would appear to call for extreme alertness:

- SBIs exhibit a single-site mode of action.
- Resistant laboratory mutants are easily obtained.
- SBIs are used extensively as broad-spectrum fungicides.

The apparent high risk, however, has not been reflected in our current field experience. A rapid outbreak of field resistance has not been observed following widespread use of SBIs, and no case of complete disease control failure has been proved so far. The SBIs appear to be different from benzimidazoles or phenylamides and have been classified as fungicides with a low to moderate risk of resistance (9,22). To explain this difference, a third parameter of risk assessment had to be considered: the pathogenic fitness of resistant strains. Experimental evidence suggested that azole-resistant mutants were less fit than azole-sensitive strains (8,9). Although likely to be selected in the field, resistant genotypes were unlikely to be vigorous

enough to compete successfully and increase in frequency. Reduced fitness offered an explanation but raised a new question as well. Could resistant strains gain in fitness over the time of continuous fungicide stress while retaining their degree of resistance? At least theoretically, a recovery to full fitness and pathogenicity could not be excluded, and careful fungicide management has been advised regardless of a well-recognized lower risk (7). Careful management has become particularly important after a decreased performance of pyrimidines and azoles was confirmed for powdery mildew control on cucumbers and cereal crops (3,30,37,41). All reported cases of resistance are, interestingly enough, restricted to some but not all powdery mildews controlled with pyrimidines or azoles. The practical impact of rare field isolates of *Venturia inaequalis* that show reduced sensitivity has not been evaluated and must remain open (33,35). Decreased efficacy in apple scab control as a consequence of prolonged use of azole fungicides has not yet been observed in the field. Furthermore, changes in sensitivity of field populations have not yet been reported for various other plant pathogens, including rusts, *Septoria, Typhula, Pseudocercosporella*, and *Ustilago* spp. in cereal diseases and powdery mildews and *Monilinia* spp. in tree fruit diseases.

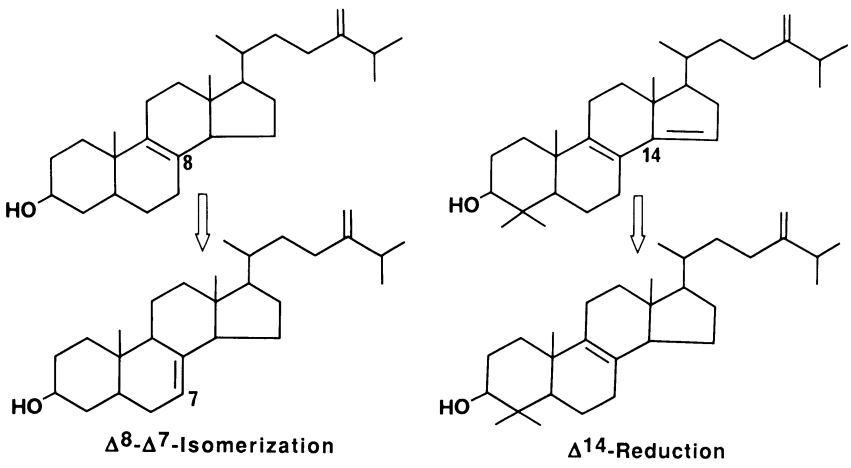
Shifts in powdery mildew populations toward field-resistance have occurred, but development of resistance has not led to a complete loss of disease control. In many cases, the field performance has remained satisfactory. Many phytopathologists became interested in the population dynamics leading to shifts in fungicide sensitivity, and studies related to these shifts gave new insights into the underlying principles of SBI resistance.



Selection Type and Mechanism of Resistance

The entire population of a plant pathogen will respond to any change in its environment. These changes might be affected by changes in agricultural practice such as the introduction of new crop cultivars and the extent to which they are grown. The genotype pattern of a given pathogen population will respond and change until a stabilizing selection results in a new equilibrium. Population dynamics are especially pronounced with airborne diseases such as powdery mildew on cereal crops (40).

Introduction of a new fungicide is an environmental change leading to a population response. There seems to be general agreement that low numbers of fungicide-resistant strains exist in the field before the pathogen population is confronted with the destabilizing action of a new fungicide. The frequency of resistant genotypes, very low but finite under stable conditions, will increase under the selection pressure of the fungicide, and the entire population will shift toward a new equilibrium. This new



Year of Publication	Mode of Action: Tridemorph	Mode of Action: Fenpropimorph
1974	Protein Synthesis	
1975	Respiration	
1978	Sterol Synthesis	
1980	Δ^8-Δ^7-Isomerization	
1981	Δ^{14}-Reduction	
1983		Δ^8-Δ^7-Isomerization
1984	Δ^8-Δ^7-Isomerization	Δ^{14}-Reduction
1984	Δ^8-Δ^7-Isomerization > Δ^{14}-Reduction	Δ^{14}-Reduction > Δ^8-Δ^7-Isomerization

Fig. 3. Modes of action reported for morpholine sterol biosynthesis inhibitors.

Table 1. Activity spectrum of sterol biosynthesis inhibitors for pathogens of major crops

Crop	Pathogens
Cereals	
Stems, leaves	*Erysiphe graminis*
	Puccinia spp.
	Rhynchosporium secalis
	Septoria spp.
	Pyrenophora teres
	P. tritici-repentis
	Typhula incarnata
	Pseudocercosporella spp.
Seed	*Ustilago* spp.
	Tilletia spp.
	Gerlachia nivalis
	Pyrenophora teres
	Septoria spp.
Apples, pears, stone fruits	*Venturia inaequalis*
	V. pirina
	Podosphaera leucotricha
	Gymnosporangium spp.
	Monilinia spp.
	Taphrina deformans
Grapes	*Uncinula necator*
	Guignardia bidwellii
Bananas	*Mycosphaerella* spp.
	Guignardia musae
Peanuts	*Mycosphaerella* spp.
	Puccinia arachidis
Coffee	*Hemileia vastatrix*
Tea	*Exobasidium vexans*

equilibrium might be reached through either a disruptive or a directional selection (32,39). A disruptive response will occur whenever the initial population consists of at least two distinct subpopulations centered around widely different fungicide sensitivities (Fig. 4). The initial exposure of these subpopulations to a new fungicide will lead to a proportional change of both populations, with a decreasing frequency of sensitive propagules and an increasing frequency of resistant propagules. Because the fungicide is almost inactive on the resistant subpopulation, the development of resistance might proceed fast and might result in a sudden loss of disease control. By contrast, a directional selection toward resistance will occur when the initial population consists of one unimodal sensitivity distribution (Fig. 5). Fungicide application at a given rate will initially control the entire population. Dilution of the fungicide within the growing plant, however, will lead to a slowly decreasing concentration, and strains that belong to the less sensitive part of the normally distributed population will propagate first reinfections. As the frequency of these strains gradually increases, the entire pathogen population will eventually shift toward lower sensitivity. Disease control owing to residual fungicide activity might decrease more rapidly between sprays and might result in decreasing safety margins, but population shifts will be gradual, with a relatively low risk of sudden and complete loss of control.

There is increasing evidence to suggest that the different experiences with fungicides such as the benzimidazoles and phenylamides on the one hand and the SBIs on the other might be explained by different selection types. The means by which resistant subpopulations are selected seems to be an additional, and perhaps the most important, factor determining the risk of field resistance. The development of resistance to benzimidazoles and phenylamides proceeded, most likely, through a disruptive selection of a highly resistant subpopulation, whereas any resistance development to SBIs is best described by a directional selection (32). Unfortunately, this latter type of selection, apparently distinguished by a lower resistance risk, is only poorly understood. The sensitivity differences, and thus the resistant factors, are usually very high for a disruptive selection, and population shifts are easily assessed in the laboratory. Growth of field isolates at only one intermediate fungicide concentration is sufficient to classify sensitive or resistant genotypes, and statistical evaluations necessary to prove population shifts are relatively easy to accomplish. The situation with a directional selection is far more complicated and complex, especially with airborne and obligate parasites. Monitoring of population shifts must be based on rather precise sensitivity values, and the sensitive reference strains must reflect the highest frequency before the first widespread use of the fungicide. Any sensitivity shifts must be based again on the highest frequency of sensitivities after the fungicide has been used for a certain period of time. This monitoring is extremely laborious.

The urgent need for simple but appropriate monitoring methods became apparent with the first rumors from Scotland in the early 1980s indicating a declining efficacy of triadimefon in control of barley mildew. Some scientists explained this occasionally unsatisfactory efficacy by the existence of a few field isolates less sensitive than others. However, the results of the first systematic field trials to examine this phenomenon suggested the possibility of a "false alarm." Resistant powdery mildew strains were frequently isolated from fields with excellent mildew control, and isolates from fields with less satisfactory fungicide performance turned out to be highly sensitive in the greenhouse. The development of appropriate test procedures was a necessity (28). One procedure for monitoring fungicide sensitivities of powdery mildew isolates derived from wheat is depicted in Figure 6 (27).

Yet, the results of more recent field trials, employing special experimental techniques for determining directional selection, still remained puzzling. The sensitivity distribution of a powdery mildew population on wheat clearly shifted toward reduced sensitivity to triadimenol. This shift, however, was determined primarily by the date of sampling rather than by the fungicide. The shift observed within a sizable plot extensively treated with triadimenol during the entire season was barely different from the shift within the untreated control. Only the frequencies of highly sensitive and more resistant isolates—and not the maxima of the entire sensitivity distribution—were found to be different in the treated plot (27,29). The reason for the observed seasonal shift remains to be explained, but this example clearly demonstrates the problems encountered in attempts to evaluate such monitoring data. Despite many uncertainties and unresolved questions, recent results of monitoring efforts (3,30,37,41), combined with circumstantial evidence from field experience, strongly suggest that the sensitivity shifts observed for powdery mildews in Europe are following the pattern of directional selection. The evidence is that loss in fungicide efficacy has been gradual and never sudden or complete and that the resistance factors observed with field isolates were comparatively small (< 100). Resistance factors $> 1{,}000$, as frequently described for benzimidazoles or phenylamides, have not been reported for SBIs.

The present experience with major groups of site-specific fungicides indicates that the inherent resistance risk relating to the chemistry of a fungicide is determined not only by the mode of action but, more importantly, also by the type of selection of resistant subpopulations. Genetic analyses and, ultimately, the molecular mechanism of resistance appear to offer an explanation for why the type of selection might be different (13). A disruptive selection is likely to occur whenever a mutation of a single gene leads to a one-step change in fungicide sensitivity. Mutations resulting in a decreased binding affinity of the inhibitor to the corresponding target are single-gene mutations, and these target mutations are apparently responsible for the resistance to benzimidazoles (5) or phenylamides (9). The genetics underlying the directional type of selection are more

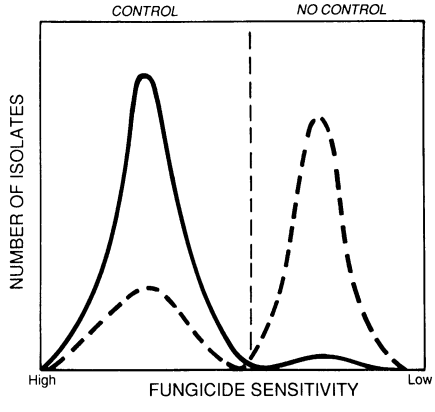


Fig. 4. Scheme of a disruptive selection of a resistant pathogen subpopulation. The initial population (solid line) contains a resistant subpopulation that is completely separated and initially small. The frequency increases under the selection pressure of a fungicide (broken line) and might lead to complete loss of disease control.

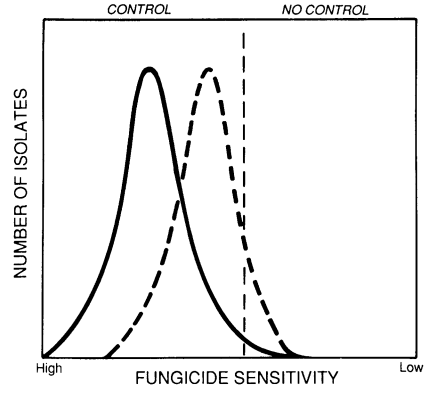


Fig. 5. Scheme of a directional selection of a resistant pathogen subpopulation. The frequency distribution of fungal isolates is unimodal (solid line). The initial population shifts under the selective fungicide pressure toward lowered fungicide sensitivity (broken line). This shift might lead to gradual loss of disease control.

complex and less well understood. Some evidence exists that directional selection might require a positive interaction among several genes other than the gene coding for the target site and that resistance development might proceed in a multistep pattern (13). Mechanisms of resistance different from the mutational change of the target, however, might well be based on the mutation of only one gene. Copper resistance of *Saccharomyces cerevisiae* serves as a good example. This resistance to copper, best described as a detoxification, is based on the amplification of a single gene, and the degree of resistance correlates with the number of gene copies (12). Unfortunately, a similar study with a plant pathogen is not available, and the important question of how the number of gene copies and, thus, the resistance factor might influence the pathogenic fitness must remain open. This example, however, illustrates that the presence of a single-gene mutation is not necessarily an indication for a target mutation and for a high resistance risk, as recently discussed for azole-resistant isolates of *V. inaequalis* (33). The small degree of resistance described for these particular genotypes might well be explained by a mechanism other than a decreased affinity of the fungicide to the target. Genetic analysis alone is obviously not sufficient to indicate a high risk of resistance development. In addition, the molecular mechanism of resistance appears to be among the more important inherent factors for risk assessments.

What is known about the mechanism of resistance to SBIs? Unfortunately, our current knowledge is limited and restricted to pyrimidines and azoles. The morpholines have not been investigated so far, most likely because of the lack of suitable resistant isolates. The widely accepted mechanism of resistance to inhibitors of lanosterol demethylation is based on studies with laboratory mutants of *A. nidulans* and *Penicillium italicum* (6). This model describes resistance as an energy-dependent efflux of the fungicide. In sensitive strains, the corresponding efflux system is induced by the fungicide, which initially accumulates to high intracellular concentrations. In resistant strains, on the other hand, efflux is constitutive and fully operative from the beginning; the fungicide never accumulates to concentrations high enough to saturate the target site (Fig. 7). The mechanism of resistance is best described by a reduced uptake of the fungicide, and a target mutation is obviously not required, although not entirely excluded. A similar reduced uptake, investigated with a different experimental approach, has not been confirmed for azole-resistant laboratory mutants of *Ustilago avenae* (W. Köller, *unpublished*). Preliminary results indicate that the time course of target saturation (rather than uptake) was almost the same for both the sensitive and the resistant strain and that the response of the target enzyme to initial inhibitor binding was not impaired in the resistant strain. Resistance was most likely not caused by a target mutation, although the exact mechanism remains unknown. The absence of a target mutation has been demonstrated by direct means for an azole-resistant isolate of *Candida albicans* (25). Our limited knowledge indicates that mechanisms other than a target mutation are responsible for resistance to SBIs and also that development of field resistance proceeds through a directional type of selection. Both observations are in clear contrast with benzimidazoles and phenylamides, fungicide groups that encountered sometimes rapid resistance development. Thus, the mechanism of resistance, and not the mode of action, appears to be one of the primary determinants of resistance risk.

Parameters that influence resistance risk relating to the chemistry and biochemistry of a fungicide are summarized in Table 2. For the sake of clarity, only benzimidazoles and azoles are compared. Note the departure from previous resistance concepts that emphasize the single-site mode of action as the major determinant of high risk of resistance. The results of recent research combined with circumstantial evidence from practical experience indicate that this concept is an oversimplification. Additional parameters such as the biochemistry and genetics of resistance and the nature of population shifts leading to the selection of resistant genotypes are important factors of inherent resistant risk. It should be clearly pointed out, however, that even the statements summarized in Table 2

Fig. 6. Sensitivity testing of powdery mildew on wheat (27). Detached wheat leaves are placed in agar containing 10 mg/L of benzimidazole as antisenescence compound. The leaves are sprayed with solutions containing various concentrations of triadimefon, then inoculated with conidia of powdery mildew. EC 50 values are determined after 1 week of incubation. Inoculation material is derived by cutting single powdery mildew pustules from leaves collected randomly from a field plot. The pustules are placed in closed tubes containing detached leaves, and infection is initiated by shaking the tubes repeatedly. The leaves are incubated for 1–2 days, then transferred to the agar. Conidia derived from this single transfer are used in fungicide sensitivity tests. Repeated transfer of single-pustule isolates might result in drastic variations of sensitivities.

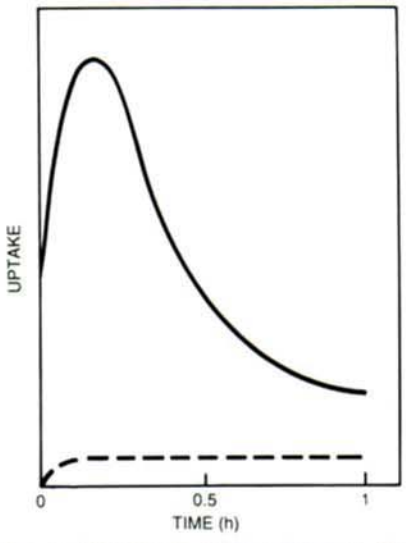


Fig. 7. Schematic time course of the uptake of sterol demethylation inhibitors by sensitive (solid line) and resistant (broken line) isolates of *Aspergillus nidulans* and *Penicillium italicum*. The measure of uptake is the residual fungicide content found in fungal cells after incubation with radioactive fungicide (3).

Table 2. Inherent factors of resistance risk relating to chemistry of site-specific fungicides

Parameters	Benzimidazoles	Azoles
Example	Benomyl	Triadimenol
Mode of action	Tubulin polymerization	Sterol demethylation
Single-site inhibitor	Yes	Yes
Laboratory resistance	Easy	Easy
Genetics	One or two genes	One or several genes
Target mutation	Yes	No
Selection type	Disruptive	Directional
Resistance risk	High	Low to moderate

must be considered tentative. Science is not yet able to assign precise resistance risk values to fungicides. Even the supposedly clear picture presented for the benzimidazoles might be a simplification, and many new questions have arisen in light of more recent findings (5). The tentative character is especially true for the SBIs. The final practical impact of directional selection is only poorly understood, and research efforts aimed at these particular population shifts have just begun. Many questions of great practical importance remain to be investigated: How far might a shifting of the sensitivity distribution toward resistance proceed? Are there potential limits set by the reduced pathogenic fitness of the resistant genotypes, and are these limits different for different pathogens? Will a redistribution of a pathogen population toward sensitivity occur after the use of a fungicide has been discontinued, and how long will it take to reach the stage of a reestablished sensitivity? What is the molecular basis of positive interactions among several resistance genes or a stepwise pattern of resistance development? Is the number of interacting genes limited or even different for different diseases? How many different mechanisms of resistance are possible? How is the mechanism of resistance interrelated with reduced pathogenic fitness? Future research will undoubtedly resolve many of these open questions, but the emergence of field resistance will not wait until then.

Basis for Resistance Strategies

The basic objectives of resistance strategies are to prevent unexpected crop losses and to prolong the effective lifetime of a fungicide. But predicting an unexpected crop loss caused by the development of field resistance is difficult. Experience with benzimidazoles and SBIs clearly demonstrates that resistance development can be different among site-specific fungicides. This difference is pronounced enough to be considered in antiresistance strategies, but many current strategies do not reflect this (e.g., 15). Fungicides with a single-site mode of action are often treated as a homogeneous group, and the special characteristics of SBIs described above are not always taken into account. The justification for this precaution is highly speculative but hard to disprove. One major argument favoring strong preventive countermeasures is that the occurrence of a risky target mutation could be a theoretical possibility for all the single-site inhibitors, including the SBIs. Genotypes with target mutations might be atypical for SBIs and too rare for detection in field monitorings before widespread and prolonged use of the inhibitors. However, an extremely small number of these genotypes could exist in the field. After prolonged selection, these genotypes might develop to sufficient numbers to cause sudden field resistance and complete loss of activity. On the other hand, a contrary theoretical argument would appear to have similar validity: Mutations of SBI targets might always result in defective enzymes and therefore extremely handicapped mutants with little chance of survival under field conditions. There is some experimental evidence from studies with *S. cerevisiae* and *Ustilago maydis* supporting the latter hypothesis (18,21). Both hypotheses are valid from a mere theoretical point of view, but neither is supported by sufficient experimental evidence. Therefore, it is questionable as to which should serve as justification for practical action and advice.

Whenever action is advised, the countermeasures should be based on flexible strategies. We should come to clear decisions on the basis of all information available at a time. Theoretical considerations have their place but should not be overemphasized. Results from field studies and even circumstantial evidence from practical experience should contribute substantially to our decisions as long as the underlying theoretical principles are largely unexplored. This flexibility is especially advisable for SBIs. Current information indicates that SBIs have a lower risk of field resistance than the benzimidazoles or the phenylamides. The gradual decrease of fungicide performance on powdery mildew and the lasting good performance on various other diseases suggest that preventive resistance countermeasures are not necessary for all diseases controlled with SBIs. Preventive countermeasures have their merits, but we must remember that these precautions entail disadvantages, too. Countermeasures make management of plant diseases more complicated and costly, and some advantages of systemics over conventional compounds, such as lower total rates and longer spray intervals, may be lost, at least partly. The threshold for practical countermeasures with respect to SBIs should be the first proven signs of declining efficacy combined with onset of sensitivity shifts. This approach requires a careful monitoring program, a task highly encouraged by FRAC. However, questions of funding and administrative responsibilities for the research, development, and operation of these resistance management activities are yet to be discussed and resolved (2).

Whenever a sensitivity shift toward resistance demands action, countermeasures should constitute an attempt to combine chemical and nonchemical disease control measures through a flexible but not overly complicated range of tactics. Flexibility should reflect the special demands of a particular host-pathogen system and the arsenal of available fungicides in the country involved. Undoubtedly, a wheat farmer in Germany and an apple grower in New York State must go through a different range of tactics. Static schemes of spray regimes, often suggested in reviews covering fungicide resistance, hardly reflect this flexibility. Therefore, we intentionally will not propose a general "SBI-resistance" spray scheme in this article.

A population shift toward field resistance to pyrimidines and azoles has occasionally occurred with powdery mildew of cucumber and cereal crops in Europe, and the threshold for counteraction has been reached. But we may not be wise to adopt tactics from resistance strategies developed for benzimidazoles or phenylamides. In this high-risk situation, current strategies suggest rotation to different compounds or to mixtures with a second fungicide. This second compound is most often the conventional fungicide formerly used to control the particular disease (10,34). The validity of this approach is based on circumstantial evidence from field experience and computer simulations.

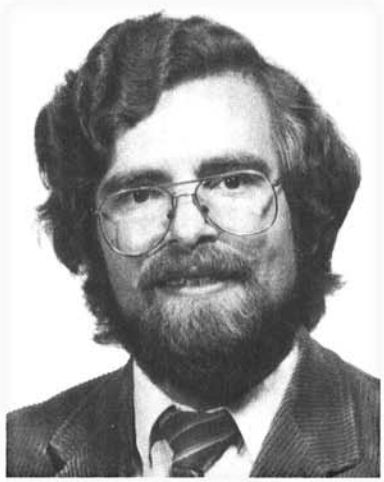
There are questions, however, whether a mixture or a rotation of different compounds should be preferred. An important point of discussion is centered around the spatial separation between a systemic compound under risk and a conventional fungicide restricted to the leaf surface. Those pathogens escaping the protective fungicide layer will exclusively encounter the systemic, and a disruptive selection might still take place. Thus, such parameters as droplet size, leaf coverage, and rate of the protective compounds are important. Unfortunately, experimental evidence from field studies supporting either rotation or mixtures is lacking (31).

The alternative to mix or to rotate two different systemic compounds without cross-resistance to each other is another good choice. Systemic alternatives not affected by field resistance are not always available, however. Fortunately, several systemic fungicides besides azoles and pyrimidines have been developed for the control of powdery mildew on barley or wheat. The morpholines are not cross-resistant to sterol demethylation inhibitors (3), and reports that suggest the possibility of cross-resistance in the field are questionable (18). No resistance development has been reported for the morpholines so far. There is a similar lack of cross-resistance with ethirimol, the second optional fungicide for powdery mildew control, and some evidence that azole fungicides and ethirimol are distinguished by a negatively correlated cross-resistance has been reported (17,19). Thus, antiresistance strategies aimed at azole fungicides can be based on these optional mildew compounds, but should these fungicides be used in a mixture or in rotation? The situation with two systemic fungicides is different from that involving a combination of a systemic and protective compound. Unfortunately, even computer simulations evaluating the combination of two systemics are not available. Nevertheless, preference has been given to alternate application, although the reasons are of theoretical nature (7). Mixtures are considered more likely to select fungal strains with resistance to both inhibitors than is a corresponding fungicide rotation. The results of recent field studies do not support this theory. A mixture of triadimenol and ethirimol effectively prevented any sensitivity shift of powdery mildew in the field (19). Similar results have been obtained with a mixture of triadimenol and tridemorph (K. J. Brent, *personal communication*), results that are supported by studies done in-house by SBI manufacturers. In a recent feature article (34), Staub and Sozzi covered the more general points of discussions concerned with preference of fungicide mixtures vs. rotations. One frequently used argument against prepackaged mixtures is not related to resistance development. Rather, the lack of disease selectivity of these mixtures, which might prevent a flexible response to specific disease control needs, has been discussed as a disadvantage (15). A selective foliar spray applied only when a particular disease has passed a defined threshold level, however, is sometimes rather risky and demands accurate monitoring and observation. An argument in favor of prepackaged mixtures is a greater assurance that resistance strategies will actually be followed by the farmer. Nevertheless, mixtures and rotations of compounds appear to provide equally sound bases for resistance countermeasures aimed at the powdery mildew problem of cereal crops in Europe, and the farmer should decide which to use.

The availability of several unrelated mildew fungicides greatly facilitated the search for sound countermeasures in Europe. The premise for a suitable resistance strategy will, unfortunately, not always be so favorable. But even under less favorable circumstances, sound countermeasures should always be developed on the basis of all information and options available at a certain time and place.

Outlook

Resistance to chemicals, which stands as a reminder of nature's resilience, should be discussed unemotionally and without defensiveness. Resistance to pesticides began with insecticides in the 1940s, extended to fungicides 20 years later, and recently began to affect herbicides. Steady research in the future will continue to reveal the underlying principles and lead to more rational and



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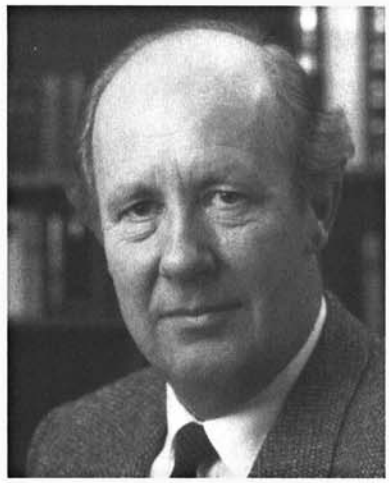

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appropriate solutions. This research should be as multidisciplinary as possible and should combine phytopathology, epidemiology, genetics, physiology, and biochemistry in a joint effort. Cooperation and coordination as emphasized by committees such as FRAC and the Committee on Strategies for the Management of Pesticide Resistant Pest Populations (4) appear to provide a promising start, although scientists from the private and public sectors might not always represent identical points of view.

We will probably never be able to eliminate resistance entirely, but we might learn to manage this phenomenon and greatly restrict its development. SBIs are promisingly different from other site-specific fungicides with respect to the speed and extent of resistance development, and more research aimed at the understanding of this group of fungicides is strongly encouraged. They provide a good opportunity to develop alternative resistance management strategies. Research efforts should also be directed to the development of new systemic and site-specific fungicides or mixtures less threatened by the hazards of resistance. Biotechnology applied to the breeding of disease-resistant crops or the development of more efficient biocontrol agents may make fungicides obsolete, but this goal will not be reached in the near future, if ever. Fungicides will remain valuable and important tools in plant disease management for a long time to come, and innovative progress in this field is still highly desirable and rewarding.

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