KRESOXIM-METHYL

MODIFICATION OF A NATURALLY OCCURRING COMPOUND TO PRODUCE A NEW FUNGICIDE

Historic Perspective

Fungi have been competing with man for crop yields since early crop cultivation. Discovery of the fungicidal effects of lime-sulfur and Bordeaux mixture more than 100 years ago was the precursor of modern fungicide development. Modern methods of fungicide screening rely on random testing of numerous chemical analogs against representative pathogens on their respective host plants to identify lead molecules for fungicide synthesis (18,70). Far less than 0.01% of the chemicals synthesized and randomly screened in this manner reach the market (18). An emerging alternative to random chemical synthesis is the study and exploitation of naturally occurring products with fungicidal properties to identify such lead molecules.

In nature, organisms may possess specialized survival mechanisms, which may be physical, such as spines and thick walls, or chemical, such as toxins or substances with attractive or repellent qualities. Years ago, researchers began to investigate naturally occurring compounds with fungicidal properties. Griseofulvin, isolated from *Penicillium griseofulvum*, showed early promise against several plant pathogens, but its use was discontinued in 1975 due to its high cost for agricultural use (112). During the 1960s and 1970s, Japanese researchers isolated and developed antibiotics (blastidin-S, kasugamycin, polyoxin, and valid-

amycin) from *Streptomyces* spp. for disease control in rice (18,112). Cycloheximide, isolated from *S. griseus*, has fungicidal activity against a wide range of fungi, but it often demonstrated phytotoxicity. Its use was discontinued in 1981 (112).

Phenylpyrroles, used as seed treatments, are a new class of fungicides based on the lead structure pyrrolnitrin, a secondary metabolite of *Pseudomonas pyrocina* and other pseudomonads (70). Several compounds have been isolated from inconspicuous woodland basidiomycetes. The best known examples are oudemansin and strobilurin A, produced by *Oudemansiella mucida* and *Strobilurus tenacellus* (Fig. 1). These compounds, hereafter referred to as strobilurins, presumably inhibit the establishment of competing fungi on substrates utilized by *O. mucida* and *S. tenacellus*. Several agrochemical companies have developed synthetic strobilurins using these natural strobilurins as lead molecules. Some of these synthetic compounds are now marketed as members of a new class of agricultural fungicides. This article focuses on this interesting class of compounds, with primary emphasis on the evolution, characteristics, and potential benefits of kresoxim-methyl, a representative member of the strobilurin fungicides.

Nomenclature

Sauter et al. (77,78) proposed using the name "strobilurins" for all natural and synthetic members of this new class of fungicides based on the most simple, natural lead molecule, strobilurin A, from which the structural variations are derived. The original name for the group was β -methoxyacrylates, or β -MOAs, based on the structure of the toxophore, the active part of the molecule. Other molecules have since been synthesized with the same mode of action and a toxophore structure similar to strobilurin A, but not containing the β -

MOA moiety. Therefore, compounds that will be discussed in later sections, such as those with enol ether ester toxophores (which are β -methoxyacrylates) and those with oxime ether ester toxophores, will all be referred to by the umbrella term "strobilurins."

Fungicide Resistance

The strobilurins and other novel active ingredients play increasingly important roles in managing the development of fungicide resistance. Many fungi have developed resistance to fungicides that once controlled them due to the fact that many fungicides are single-site inhibitors of fungal metabolism (25,31). For instance, this has been confirmed recently for members of the class of demethylation inhibitors (DMI fungicides) in the economically important pathogens *Venturia inaequalis*, which causes apple scab (45,47), and *Uncinula necator*, which causes grape powdery mildew (29,116). Thus, the possibility of fungicide resistance should be considered when a disease management program is set in place. Individuals that

Dr. Ypema's address is: BASF Corporation, 26 Davis Drive, P.O. Box 13528, Research Triangle Park, NC 27709.

SOVRAN and CYGNUS are registered trademarks of BASF Corporation.

"Surface systemic" is a trademark of BASF Corporation.

Publication no. D-1999-1106-01F
© 1999 The American Phytopathological Society

Fig. 1. *Strobilurus tenacellus*, an important basidiomycete from which natural strobilurins were isolated.

express resistance to a particular fungicide are presumed to be present in populations at low frequency prior to introduction of the fungicide. Whether this resistance will ultimately affect fungicide performance depends on many factors, such as the relative fitness of these resistant strains, the reproduction and dispersal rate of the target fungus, the biochemical and biological mode of action of the fungicide, and application regimes (25). Especially when resistance is under polygenic control, application of nonlethal concentrations of fungicides is likely to result in survival of fungal cells that possess some degree of resistance. Repeated fungicide applications on these surviving cells can select further for increased resistance, which ultimately may lead to unsatisfactory control (45,96). The strobilurin kresoxim-methyl offers an effective resistance management tool, because its efficacy against target pathogens is not affected by the occurrence of strains resistant to other fungicides.

Modeled After Nature

Kresoxim-methyl is the culmination of the use of synthetic methods to modify the

chemical structure to improve the fungicidal activity and environmental stability of the naturally occurring strobilurin A. Special physical and chemical properties of kresoxim-methyl result in its novel biochemical and biological modes of action against plant pathogenic fungi, as well as unique uptake and diffusion properties. In laboratory, greenhouse, and field research, kresoxim-methyl has demonstrated protective, postinfection, and antispore activity against economically important fungal diseases. The development of kresoxim-methyl based on natural strobilurins is an excellent example of how natural compounds can be used as models for novel crop protection compounds.

The Strobilurin Story

The strobilurins form a distinct class of fungicidal compounds that can be isolated from several Basidiomycete species that inhabit decaying plant material in woodland soils (4). Original isolations of strobilurins A and B were made from *S. tenacellus*, which occurs naturally in decaying pine cones of *Pinus sylvestris* in Europe (5). Filamentous fungi and yeasts trans-

ferred to agar media containing extracted strobilurins from *S. tenacellus* were inhibited by concentrations of strobilurin A or B as low as 1 mg/liter. The broad-spectrum antifungal activity sparked interest among researchers within the agrochemical industry, and vigorous research programs began, aimed at modifying and enhancing the activity of these naturally occurring compounds. Comparison of the inhibitory actions and binding characteristics of strobilurin A, oudemansin, and related compounds revealed that the enol ether ester toxophore appeared to be required for binding the molecule to the target site and the resulting biological activity (13). These compounds were shown to inhibit mitochondrial respiration by blocking electron transfer in cytochromes b and c_1 (Fig. 2), hereafter referred to as the bc_1 complex. Steglich determined the correct stereochemical structure of strobilurin A (6; Fig. 3). Subsequently, natural and synthetic analogs of strobilurins and oudemansins were compared with regard to differences in molecular structure and concomitant activities (6,7). Synthetic analogs most similar to the natural molecule maintained

Fig. 2. Schematic diagram of the mitochondrial respiratory chain, showing electron transport and the inhibition point of kresoxim-methyl and other strobilurins. The electron flow in the respiratory chain, uninhibited by strobilurins, pumps protons (H^+) across the inner mitochondrial membrane. The proton gradient across the membrane is utilized by ATP-synthetase to produce ATP, thus providing the cell with energy for its metabolism. In some fungi and plants, inhibitors of the respiratory pathway and other stress factors induce the synthesis of an alternative oxidase. This alternative oxidase-mediated respiration pathway diverts electrons at the ubiquinone pool (Q) and confers reduced sensitivity to strobilurin action *in vitro*, but generates much less energy. Induction of alternative oxidase-mediated respiration apparently has no impact on the level of disease control by strobilurins. Q = ubiquinone, Cyt b = cytochrome b, Cyt c_1 = cytochrome c_1 , cyt c = cytochrome c, Cyt a = cytochrome a, Cyt a_3 = cytochrome a_3 . Source: D. Voet and J. G. Voet, 1994. Biochemistry, 2nd ed., John Wiley & Sons, New York; G. C. Vanlerberghe and J. McIntosh, 1997. Annu. Rev. Plant Physiol. Plant Mol. Biol. 48:703-734; J. Smith, 1998. Science 281:58-59.

highest activity, and changes in the toxophore altered respiration inhibition and antifungal activity. The nature of the side chain was significant for biological activity and stability of the strobilurin molecule and also determined critical characteristics that affected how it interacted with the plant and the environment after application. Parallel research performed simultaneously by scientists at BASF and Zeneca (formerly ICI) in the early 1980s resulted in a more stable strobilurin analog, the enol ether stilbene, or β -methoxyacrylate stilbene (22,77,78). This molecule was created by constructing a bridging phenyl ring between the side chain and the enol ether ester toxophore (Fig. 3). These research results were pivotal for further parallel research programs at BASF and Zeneca to synthesize a stable strobilurin fungicide.

The Patent Race

Zeneca began its natural product research program in 1982 with oudemansin A as initial lead molecule before settling on strobilurin A as the best lead molecule

for synthetic analog development, and patents for enol ether ester derivatives were applied for in 1984 (12,20). Several reviews (11,12,22) describe the synthesis steps and rationale behind the development of the final product, ICI A5504 or azoxystrobin (methyl (*E*)-2-[2-[6-(2-cyanophenoxy)pyrimidin-4-yloxy]phenyl]-3-methoxyacrylate). Azoxystrobin retained the enol ether ester toxophore present in strobilurin A, but the side chain was modified to enhance photostability and increase systemicity in the plant (Fig. 3). The presence of a particular combination of heterocyclic and aromatic rings in the side chain provided high activity against fungal plant pathogens and an acceptable balance between phytotoxicity and systemic properties (11).

In 1983, BASF began to evaluate natural products as potential lead molecules for new synthetic fungicides. Sauter et al. (77,78) provided an excellent discussion of the research efforts leading to the selection of BAS 490 F, the internal code name for kresoxim-methyl used in early publications. Strobilurin A was evaluated in

greenhouse tests but was effective only at high concentrations and demonstrated effects on a limited number of fungal pathogens. Modified derivatives of strobilurin A with enol ether ester toxophores were then obtained from Steglich to examine the hypothesis that a more photo-stable molecule would retain higher activity. After subsequent research at BASF, the resulting enol ether stilbene (Fig. 3) exhibited broad-spectrum and strong fungicidal activity in the greenhouse, but remained photolabile in field tests.

Sauter et al. (77,78) discussed the further optimization of strobilurins leading to the development of kresoxim-methyl (methyl-(*E*)-2-methoxyimino-2-[2-*o*-tolxyloxy-methyl]phenyl] acetate). Both the side chain and the toxophore were modified to further enhance the stability and activity of the molecule. The toxophore was changed to the oxime ether ester (77) because the ICI patents mentioned effectively blocked further research of compounds with the enol ether ester toxophore (Fig. 3). BASF filed patents applications on this oxime ether ester chemistry (79,98,99) 2 days before ICI filed applications based on parallel research (9). Research to optimize the side chains revealed increased activity with phenoxymethyl side chains. Large numbers of compounds with these modifications were screened in field tests, with the ultimate selection of BAS 490 F, now referred to by its common name, kresoxim-methyl, as the most promising development product.

In contrast to compounds modeled after the natural strobilurins, Shionogi Co. Ltd. synthesized a number of substituted phenoxyphenyl oxime ether amides as part of its random screening program (65,67). SSF-126 (*E*-2-methoxyimino-*N*-methyl-2-(2-phenoxyphenyl) acetamide) was selected for development because of strong fungicidal activity against rice pathogens and safety to nontarget organisms. The common name of this strobilurin is metominostrobin, and it has the same biochemical mode of action as the other strobilurins.

Target Specificity of Kresoxim-methyl

Like the other compounds in the strobilurin family, kresoxim-methyl specifically inhibits respiration by binding to the mitochondrial bc₁ complex, subsequently blocking electron transfer and ATP synthesis (3,13,77). A model system of submitochondrial particles from several different organisms was used to evaluate target activity of kresoxim-methyl (77). Kresoxim-methyl was equally active in mitochondrial preparations from *Botrytis cinerea*, fly, maize, yeast, and rat, indicating that there was no species selectivity at the mitochondrial target level (75). In contrast to these findings, subsequent studies with intact cells and organisms demonstrated that

Fig. 3. Parallel development at Zeneca and BASF of synthetic strobilurin fungicides. Although Zeneca initiated its research with oudemansin and BASF with strobilurin A, both companies used the enol ether stilbene (or β -methoxyacrylate stilbene) as a lead molecule for synthesis of their fungicide products, azoxystrobin and kresoxim-methyl, respectively. Source: (77).

kresoxim-methyl was specifically active against yeasts and filamentous fungi (77). Fifty percent inhibition of respiration (I_{50}) was achieved with yeast mitochondria and intact *B. cinerea* conidia at 1.4 and 3.3×10^{-8} mol/liter, respectively. In comparison, the I_{50} for intact mouse fibroblasts was 1.9×10^{-5} mol/liter, and the oral LD_{50} in rats exceeded $5,000$ mg/kg.

The biochemical and biological modes of action of kresoxim-methyl have direct bearing on its specific activity against fungi and its low toxicity toward nontarget organisms. Kresoxim-methyl is nontoxic to plants, mammals, birds, insects, and earthworms. The compound is nonirritating to skin and eyes, nonmutagenic, and non-sensitizing. Once kresoxim-methyl is absorbed into cells of nontarget organisms, such as those of wheat, apples, rats, and goats, the methyl ester moiety of its toxophore is rapidly hydrolyzed to its corresponding carboxylic acid, which has no effect on mitochondrial respiration (77,78). Toxicity exists for fish, *Daphnia*, and green algae *in vitro*, but appropriate handling and disposal procedures will prevent exposure of these organisms to kresoxim-methyl. Furthermore, no problems in natural sediment systems were observed due to the rapid degradation of kresoxim-methyl, which has a half-life between several hours and several days in soil (77).

Plant Physiological Effects of Kresoxim-methyl

In addition to affecting fungal metabolism, kresoxim-methyl demonstrated physiological effects on wheat in the absence of disease (34,74). Wheat plants treated with kresoxim-methyl showed increases in grain yields, dry matter, chlorophyll, protein, and delayed senescence (33). Research on the physiological basis for increased yield and dry matter weight increases showed that kresoxim-methyl shifts the CO_2 compensation point (photosynthesis versus respiration) in favor of CO_2 assimilation (74). Carbon dioxide is one of the limiting factors to plant growth, after water and light requirements are met. Bioassays were used in which cell suspensions of maize (*Zea mays*) and algae (*Scenedesmus acutus*), duckweed (*Lemna paucicostata*), seedlings of mustard (*Sinapis alba*) and cress (*Lepidium sativum*), and tobacco stem explants (*Nicotiana tabacum*) were exposed to kresoxim-methyl to generate a physiological response pattern to the compound (34). Growth of maize cells, duckweed, and germinating cress seeds was significantly inhibited, while that of algae and mustard shoots was only slightly inhibited. Duckweed exhibited enhanced chlorophyll pigmentation. Tobacco stem explants on Murashige Shoog medium with amino acids and vitamins differentiated into roots and shoots when kresoxim-methyl was added to the medium. In addition, growth reduction of duckweed by the

cytokinin 6-benzylaminopurine (BAP) was partially reversed by kresoxim-methyl treatments. The latter two responses to kresoxim-methyl were very similar to those observed with indole-3-acetic acid (IAA). To determine whether kresoxim-methyl itself behaved like an auxin or whether it stimulated auxin production, wheat plants were treated and the hormone changes observed. Kresoxim-methyl itself did not have auxin-like properties, nor did it stimulate auxin production, but instead it mimicked the effect of auxin by affecting other metabolic processes in the plant. Following treatment with kresoxim-methyl, the level of 1-aminocyclopropane-1-carboxylic acid (ACC), an ethylene precursor, and ACC synthase activity showed a concentration-dependent decrease and a subsequent reduction in ethylene production. Lower ethylene concentrations have been shown to slow degradation of cytokinins (14), which have been shown to delay senescence (10), and increase chlorophyll production (49,89). The observed increases in growth, yield, and photosynthesis rate in plants treated with kresoxim-methyl appeared to be the result of reduced chlorophyll degradation, greater assimilate concentrations, and delayed senescence. It is clear that kresoxim-methyl has significant positive effects on plant metabolism. However, no phytotoxic or other negative effects on plant growth have been observed in numerous field trials on pome fruits, grapes, pecans, cucurbits, ornamentals, and other crops. The results of these field trials are discussed in a later section.

Physical and Biokinetic Properties of Kresoxim-methyl

Kresoxim-methyl possesses biokinetic and physical properties that build the foundation of its specificity against fungal

pathogens. At room temperature, kresoxim-methyl is a solid white crystal with a low vapor pressure (2.3×10^{-3} mPa) and low solubility in water (2 mg/liter). Due to its lipophilicity ($\log P_{ow} = 3.5$), it has a high affinity for the waxy surface of plants. Upon foliar treatment, kresoxim-methyl forms a particulate deposit on the leaf (Fig. 4). To prove that these deposits were not easily removed by rain, apple seedlings were sprayed with kresoxim-methyl and subjected to 10, 20, 30, and 40 mm of simulated rainfall in the field the next day (23). The seedlings were then returned to the greenhouse and immediately inoculated with *V. inaequalis* and assessed for development of apple scab 20 days later. Kresoxim-methyl maintained effectiveness against scab after 40 mm of rainfall; whereas the standard contact fungicide captan did not sufficiently control the disease after 20 mm of rain or more.

Uptake and movement within the leaf over time was measured by using radioactively labeled kresoxim-methyl. Measurements of radioactivity from the leaf and the epicuticular wax layers revealed that between 5 and 10% of kresoxim-methyl applied to the leaf surface penetrated the leaf within 24 h. After a 4-day period, approximately 60% of the radioactivity was still detected on the surface, and approximately 30% was in the leaf cells (32).

In greenhouse studies, kresoxim-methyl exhibited locally mobile properties as defined by Neumann and Jacob (68), because activity of the product was expressed within the organ of application at points distant from the application site (Fig. 5). To illustrate this, the upper left side of an apple leaf was treated with kresoxim-methyl, and the right side of the leaf was inoculated with *V. inaequalis* conidia. Germination was greatly reduced on the

Fig. 4. One day after application, kresoxim-methyl formed a particulate deposit on this apple leaf surface.

untreated half of the leaf. The level of inhibition was inversely related to the distance from the application site, thereby suggesting translocation of kresoxim-methyl from the treated area (32). Most likely, the fungicidal activity of kresoxim-methyl was expressed at points distant from the application site following diffusion of the compound over and through the waxy cuticular surface of the plant. This type of transport is described by the term "surface systemic," since it mimics systemic transport (68) but occurs exclusively on the plant surface (Fig. 5). Kresoxim-methyl's surface systemicity combined with its later-discussed intercellular and translaminar transport (Fig. 5) have been referred to as quasi-systemic transport (32). The translaminar protective effect of kresoxim-methyl was observed on the underside of a leaf after treatment of the upper leaf surface. The upper surface of the proximal half of bean leaves was sprayed with kresoxim-methyl 1 to 2 days before inoculation of the lower leaf surface with *Uromyces appendiculatus* uredospores. Germination was inhibited on the lower surface directly below the application sites on the upper leaf surface, and more distal areas were protected when higher rates of kresoxim-methyl were used (32). Although

these studies were performed in the greenhouse, the observed properties of kresoxim-methyl appear to be beneficial to its ability to control diseases in the field. When applied on pumpkins to control the powdery mildew fungus *Sphaerotheca fuliginea*, both kresoxim-methyl and the DMI fungicide triflumizole protected both upper and lower leaf surfaces from infection; whereas the contact fungicide chlorothalonil only protected the upper leaf surface (63).

Biological Mode of Action

Kresoxim-methyl showed activity on developmental stages of a wide range of economically important plant pathogenic fungi (Table 1). The protective effects of kresoxim-methyl on germination and sporulation of the rust fungi *Puccinia recondita* and *U. appendiculatus*, and the powdery mildews *Podosphaera leucotricha* on apple, *U. necator* on grape, *S. fuliginea* and *Erysiphe cichoracearum* on cucumber, and *Erysiphe graminis* f. sp. *tritici* and *hordei* on wheat and barley, were investigated on detached leaves of their respective host plants (88). In other studies, inhibition properties of kresoxim-methyl were examined in greenhouse and field studies using four target fungi, *V.*

inaequalis, *E. graminis* f. sp. *tritici*, *P. recondita* (33), and *U. necator* (52). Several other studies have confirmed kresoxim-methyl's efficacy against germination and sporulation of *V. inaequalis* described in this section (23,48). Currently, studies are ongoing to determine the effects of kresoxim-methyl on infection and colonization processes of *Cladosporium caryigenum*, the pathogen causing scab on pecans. *C. caryigenum* infects and colonizes pecan leaves subcuticularly in a manner similar to *V. inaequalis* on apple leaves (50). Kresoxim-methyl may therefore demonstrate inhibition patterns on *C. caryigenum* similar to those described for *V. inaequalis* in this section.

Spore germination. Kresoxim-methyl applied prior to inoculation onto host plants in the greenhouse strongly inhibited spore germination and germ tube growth of all fungi. *V. inaequalis* was the most sensitive pathogen tested: germination was completely inhibited by preinfection applications of 1 mg of kresoxim-methyl per liter (Fig. 6). Germination of *P. leucotricha* and *E. cichoracearum* was completely inhibited at 1.25 mg/liter. Germination of both formae speciales of *E. graminis* was completely inhibited at 2.5 mg/liter, and germination of *U. necator* was completely inhibited at 10 mg/liter. Germination of *S. fuliginea* was not as sensitive as that of the other fungi. Complete inhibition of *S. fuliginea* was not achieved with 100 mg of kresoxim-methyl per liter. Germination of the rust fungi *P. recondita* and *U. appendiculatus* was completely inhibited by kresoxim-methyl concentrations of 5 and 10 mg/liter, respectively.

Mycelial growth. In greenhouse studies, mycelial growth of *E. graminis* f. sp. *tritici* was completely inhibited when 4 mg of kresoxim-methyl per liter was applied to the host plants after inoculation. Under similar conditions, mycelial growth of *U. necator* was greatly reduced by a minimum of 8 mg of kresoxim-methyl per liter. The mycelia of most powdery mildew fungi develop exclusively on the leaf surface. This growth characteristic makes all developmental stages of powdery mildews accessible to kresoxim-methyl.

The rate of subcuticular stroma formation by *V. inaequalis* was reduced when kresoxim-methyl was applied to the host plants after inoculation, but growth was not completely halted (32). However, when kresoxim-methyl was applied after the occurrence of scab infection periods in the orchard, its inhibitory effects on stroma formation and subsequent sporulation appeared to be sufficient to adequately control *V. inaequalis* (19,23).

When kresoxim-methyl was applied after *P. recondita* had penetrated into the leaf tissue, it did not inhibit further mycelial growth in the leaf mesophyll. Due to its biokinetic properties previously discussed, the amount of kresoxim-methyl that actu-

Fig. 5. Schematic diagrams showing surface systemic and translaminar movement of kresoxim-methyl from the site of application (AS). (A) Upon application, kresoxim-methyl diffuses over and through the cuticular wax layers (D). It penetrates the leaf and diffuses in the intercellular spaces translaminarily (TL). (B) Upon reappearance at the other leaf surface after translaminar transport (TL), the compound further diffuses over the lower leaf surface (D). In this manner, kresoxim-methyl can protect plant parts distant from the application site from fungal infection. c = cuticle, e = epidermis, m = mesophyll.

ally penetrated into the leaf mesophyll may have been too low to sufficiently inhibit growth of *P. recondita*.

Sporulation. In the studies on detached leaves in petri dishes, sporulation of all fungi was completely inhibited at kresoxim-methyl concentrations similar to or lower than those needed for complete inhibition of spore germination. Sporulation of *S. fuliginea*, which was least sensitive in the spore germination tests, was almost completely inhibited at 10 mg of kresoxim-methyl per liter. Sporulation of *E. graminis* and *U. necator* was inhibited by postinfection applications of just over 4 or 8 mg/liter, respectively (33,52). Sporulation of *V. inaequalis* and *U. necator* in the greenhouse was reduced by 98% or more when 67 mg of kresoxim-methyl per liter was applied 2 to 4 days after inoculation (Figs. 6 and 7).

In the tests on detached leaves, sporulation of *P. recondita* and *U. appendiculatus* was completely inhibited at 2.5 and 10 mg/liter, respectively, indicating that the preventative application of kresoxim-methyl in this testing system also resulted in a strong reduction in sporulation (88). In greenhouse tests, sporulation of *P. recondita* was not affected by kresoxim-methyl when it was applied after inoculation, most likely because insufficient amounts of kresoxim-methyl had penetrated into the leaf to stop *P. recondita* uredospore initiation and development (33). Probably for the same reason, kresoxim-methyl did not adequately control *P. recondita* and *Septoria tritici* in a field trial on wheat in Missouri (93).

In contrast to DMI fungicides and systemic strobilurin fungicides (11), kresoxim-methyl acts mainly as a protectant on fungi that complete their life cycles deeper in the leaf or plant tissue, such as *P. recondita* and other rusts and downy mildews. Due to its long residual activity and its biokinetic properties, kresoxim-methyl has the greatest activity on stages of fungal development that occur or are initiated on or just below the leaf surface. This includes the processes of germination, penetration, subcuticular growth, and sporulation.

Potential for Development of Strobilurin Resistance

Alternative respiration. The market introduction of strobilurin fungicides, with their single-site mode of action, warrants assessment of the risk of development of fungicide resistance. Some fungi possess an alternative respiration pathway, which confers reduced sensitivity to strobilurins and other bc₁ complex inhibitors (Fig. 2). This alternative pathway branches from the normal cytochrome-mediated pathway at the ubiquinone-pool (Q) and terminates at an alternative oxidase (65), which is encoded by a nuclear gene and subsequently incorporated in the mitochondria (95). Alternative oxidase expression is induced

Table 1. Activity spectrum of kresoxim-methyl

Crop	Disease	Pathogen	Activity ^y
Apple	Scab	<i>Venturia inaequalis</i>	+++
	Powdery mildew	<i>Podosphaera leucotricha</i>	+++
	Sooty blotch	<i>Gloeodes pomigena</i>	+++
	Fly speck	<i>Zygophiala jamaicensis</i>	+++
	Brooks fruit spot	<i>Mycosphaerella pomi</i>	++
	Black rot	<i>Botryosphaeria obtusa</i>	++
	White rot	<i>Botryosphaeria dothidea</i>	++
	Cedar apple rust	<i>Gymnosporangium</i> sp.	P
	Quince rust	<i>Gymnosporangium clavipes</i>	P
	Bitter rot	<i>Colletotrichum</i> sp.	—
Broccoli	Black pox	<i>Helminthosporium papulosum</i>	—
	Downy mildew	<i>Peronospora parasitica</i>	P
Cherry	Powdery mildew	<i>Podosphaera clandestina</i>	+++
Cereals	Powdery mildew	<i>Erysiphe graminis</i>	+++
Cucurbits	Powdery mildew	<i>Sphaerotheca fuliginea</i>	+++
	Gummy stem blight	<i>Didymella bryoniae</i>	+++
	Downy mildew	<i>Pseudoperonospora cubensis</i>	P
Grape	Powdery mildew	<i>Uncinula necator</i>	+++
	Black rot	<i>Guignardia bidwellii</i>	+++
	Phomopsis	<i>Phomopsis viticola</i>	++
	Downy mildew	<i>Plasmopara viticola</i>	P
Lettuce	Powdery mildew	<i>Erysiphe cichoracearum</i>	+++
	Downy mildew	<i>Bremia lactucae</i>	P
Pecan	Pecan scab	<i>Cladosporium caryigenum</i>	+++
Pear	Scab	<i>Venturia pirina</i>	+++
	Powdery mildew	<i>Podosphaera leucotricha</i>	+++ ^z
Rose	Powdery mildew	<i>Sphaerotheca pannosa</i>	+++
	Black spot	<i>Diplocarpon rosae</i>	+++
	Rust	<i>Phragmidium</i> sp.	P

^y +++ = excellent, ++ = good, + = satisfactory, — = unsatisfactory, P = satisfactory when applied preventatively. The activity rating is based on data published in *Fungicide and Nematicide Tests* between 1995 and 1998 and on unpublished data from trials performed by BASF and independent researchers.

^z Efficacy rating derived from greenhouse trial data and kresoxim-methyl's activity on apple powdery mildew, which is caused by the same pathogen.

Fig. 6. Scanning electron microscopy of *Venturia inaequalis*. (A) A conidium germinated and initiated stroma formation under the cuticle of an unprotected apple leaf in less than 24 h. (B) Kresoxim-methyl, applied to leaves at 1 mg/liter 1 day prior to inoculation, caused collapse of conidia and completely inhibited germination and subsequent infection. (C) *Venturia inaequalis* sporulated abundantly on untreated apple leaves 16 days after inoculation. (D) Kresoxim-methyl, applied 2 days after inoculation at 67 mg/liter, provided complete inhibition of sporulation.

by certain stress stimuli and by constrictions in electron flow through the cytochrome-mediated respiration pathway caused by respiration inhibitors (53,86,87,95). Alternative oxidase activity was induced in *Pyricularia oryzae* and *B. cinerea* using metominostrobin (SSF-126) and another strobilurin derivative, respectively (67,97). The subsequent decrease in ATP production resulted in fungistasis, but not death. When these fungi were simultaneously exposed to strobilurins and salicylhydroxamic acid (SHAM), a potent inhibitor of respiration mediated by alternative oxidase, fungal respiration ceased completely (65,97).

Metominostrobin alone never completely suppressed mycelial growth of *P. oryzae* on agar media due to alternative oxidase-mediated respiration (65). However, *P. oryzae* failed to infect rice plants treated with metominostrobin, and mycelial growth of *P. oryzae* was completely halted when the medium on which it grew was amended with flavonoids extracted from rice plants (66,67). The flavonoids did not inhibit alternative oxidase-mediated respiration directly. Presumably, the oxygen radicals resulting from strobilurin inhibition of the cytochrome-mediated respiration were inducers of alternative oxidase-mediated respiration. The flavonoids may have prevented induction of alternative oxidase-mediated respiration by scavenging these oxygen radicals (66,97). Similar findings were obtained with a mutant of *S. tritici* with reduced sensitivity to azoxystrobin (118). In a greenhouse test, the reduced amount of ATP produced in the

mutant via alternative oxidase-mediated respiration was not sufficient to support infection when the plant host had been treated with azoxystrobin. Indications of alternative oxidase-mediated respiration were also observed in *V. inaequalis* conidia and mycelia on agar media amended with kresoxim-methyl alone, SHAM alone, or both compounds combined (71). As in the examples described above, the presumed induction of alternative oxidase-mediated respiration had no impact on the level of scab control in apple seedlings by kresoxim-methyl. In subsequent tests, sensitivity distributions of *V. inaequalis* to kresoxim-methyl were established using both a spore germination test and an apple seedling assay (W. Köller, *personal communication*). The broad sensitivity range obtained with kresoxim-methyl alone in the spore germination tests was in part caused by presumed induction of alternative oxidase-mediated respiration in a fraction of the conidia, and did not correlate to the narrow sensitivity range obtained in the apple seedling assay. This demonstrated again that alternative oxidase-mediated respiration did not enable *V. inaequalis* to infect apple seedlings treated with kresoxim-methyl. Therefore, the host plant may play an essential role in achieving complete inhibition of a strobilurin-exposed pathogen.

Target site mutations. Due to the fact that no strobilurin-resistant plant pathogens have been identified in commercial crop production, strobilurin resistance mechanisms other than alternative oxidase-mediated respiration have been investigated only in the laboratory. These studies have concentrated on the yeast *Saccharomyces cerevisiae* and on organisms that express natural strobilurin resistance, such as the fission yeast *Schizosaccharomyces pombe* and the strobilurin-producing filamentous fungi *S. tenacellus* and *Mycena galopoda*. Several strobilurin-resistant strains of *S. cerevisiae* were generated in the laboratory. Mutations conferring strobilurin resistance in *S. cerevisiae* were associated with single amino acid changes in the cytochrome b

protein (28). Strobilurin resistance in *S. pombe* and the filamentous fungi *S. tenacellus* and *M. galopoda* was also associated with single amino acid changes in the cytochrome b protein when compared with naturally sensitive fungi and yeasts (46).

Recently, the cytochrome b gene was cloned and sequenced from the mitochondrial genome of *V. inaequalis*. Its deduced amino acid sequence showed a high homology to the cytochrome b proteins of other strobilurin-sensitive filamentous fungi (71,117). To what extent the described target site mutations occur in plant pathogens, and to what extent they may result in the appearance of viable, competitive, strobilurin-resistant strains, is currently not clear. Cloning and sequencing of the target site is the first step to addressing this question in the laboratory.

Resistance management. Until resistance occurs in commercial crop production, laboratory data can be used only as indicators for possible resistance. Even if the possibility of reduced strobilurin efficacy due to resistant pathogens in a field situation proves to be remote, responsible handling of strobilurins is important to delay or prevent resistance development. In addition, sensitivity of the target pathogens prior to introduction of strobilurins (baseline sensitivity) should be established, and monitoring programs should be in place upon market introduction. The baseline sensitivity of *V. inaequalis* isolates to kresoxim-methyl has been determined recently and was similar for European and North American scab populations (48; W. Köller, *personal communication*). For strobilurins, the more laborious sensitivity assays on whole or detached plants are preferred over tests on agar plates, due to the discrepancies in strobilurin sensitivity observed in comparative studies (48; S. Heaney and G. Lorenz, *personal communication*; W. Köller, *personal communication*).

Market Introductions and Commercialization

Kresoxim-methyl was introduced to the public at the 1992 Brighton Crop Protec-

Fig. 7. Scanning electron microscopy of *Uncinula necator*. (A) Eight days after inoculation, hyphae grew profusely over the grape leaf surface and conidia formation had been initiated. (B) Kresoxim-methyl at 67 mg/liter, applied 3 days after inoculation, resulted in complete collapse of conidia and hyphae.

Table 2. Countries and crops on which kresoxim-methyl formulations are registered as of 30 September 1998

Countries	Argentina, Austria, Belgium, Brazil, Bulgaria, Chile, Colombia, Croatia, Ecuador, France, Germany, Hungary, Ireland, Israel, Italy, Japan, Kenya, Luxembourg, Moldova, Netherlands, New Zealand, Nicaragua, Norway, Poland, Slovakia, South Africa, Spain, Sweden, Switzerland, Taiwan, Turkey, United Kingdom, United States, Uruguay
Product names ^y	Allegro ^z , Alliage, Brio ^z , Candit, Controlan ^z , Cygnus, Discus, Ensign ^z , Growell ^z , Juwel ^z , Kenbyo, Landmark ^z , Mantra ^z , Mentor ^z , Ogam ^z , Senso ^z , Strobly SC, Strobly WG
Crops	Cereals, citrus, currants, flower bulbs, grapes, mango, ornamentals, pome fruits, stone fruits, strawberries, vegetables

^y All product names are registered trademarks of BASF Aktiengesellschaft or its subsidiaries in some or all of the countries listed above.

^z Combination product.

tion Conference (3). Subsequent research and development efforts resulted in the first crop use registrations for kresoxim-methyl in Europe in 1996 for cereals. In Germany, kresoxim-methyl is sold as a premixed product for use on cereal crops in combination with either fenpropimorph or epoxiconazole to provide broad-spectrum control of the target diseases. Subsequent registrations were obtained for pome fruits, stone fruits, citrus fruits, ornamentals, flower bulbs, grapes, vegetables, strawberries, and other crops (Table 2). On 30 September 1998, the United States Environmental Protection Agency approved kresoxim-methyl's first non-food-use registration on greenhouse ornamentals under the trade name Cygnus. The first food-use registrations of kresoxim-methyl in the United States are expected in 1999 to 2000 and will target apples, pears, and other pome fruits, grapes, pecans, and cucurbits under the trade name Sovran.

Field Performance in the United States

Kresoxim-methyl has been extensively evaluated against the most economically important pathogens in numerous field trials performed by both BASF scientists and independent researchers. These studies focused on performance of kresoxim-methyl alone and in rotations with other fungicides. The rotation studies had the purpose of determining how the particular strengths of kresoxim-methyl could be utilized best in spray programs. This section focuses mainly on performance of kresoxim-methyl on the most important diseases of apples, grapes, pecans, cucurbits, and greenhouse ornamentals, which are the first crops on which kresoxim-methyl will be registered. The efficacy ratings of kresoxim-methyl against all pathogens discussed in this section and against cereal powdery mildew are summarized in Table 1.

Apple diseases. Apple scab (*V. inaequalis*) is economically the most important apple disease in the United States, followed by powdery mildew (*P. leucotricha*). Although both diseases occur nationwide, apple scab is favored in the eastern United States by humid, cool, and wet spring weather; whereas powdery mildew pressure is enhanced by the mild winters and spring conditions in the Pacific Northwest. Both diseases can cause significant reductions in yield and fruit quality. Other apple diseases that are important in the eastern growing areas are cedar apple rust (*Gymnosporangium juniperi-virginianae*), quince rust (*Gymnosporangium clavipes*), and the so-called summer diseases, including fly speck (*Zygophiala jamaicensis*), sooty blotch (*Gloeodes pomigena*), frog-eye leaf spot or black rot (*Botryosphaeria obtusa*), white or bot rot (*Botryosphaeria dothidea*), black pox (*Helminthosporium papulosum*), brooks fruit spot (*Mycosphaerella pomi*), and bitter rot (*Colletotrichum* spp.). The DMI fungicides myclobutanil and fenarimol are the current standards for control of scab, powdery mildew, cedar apple rust, and quince rust. The ethylenebisdithiocarbamates (EBDCs) are used for control of scab and all other major apple diseases except powdery mildew. Captan and benomyl are primarily

used for summer disease control.

Field trials were conducted throughout the United States, Canada, and Europe, and in numerous other countries to investigate the efficacy of kresoxim-methyl against the various apple diseases. In many trials, kresoxim-methyl demonstrated its particular efficacy against apple scab, especially on the fruits (19,21,23,41,80,81,82,100,102; Table 3, Fig. 8). Although BASF recommends protective applications of kresoxim-methyl over postinfection applications, its efficacy against scab was comparable to myclobutanil when applied 96 and 120 h after the occurrence of scab infection periods (19). This illustrates kresoxim-methyl's ability to control *V. inaequalis* after it had penetrated the leaf surface.

In numerous field trials in the United States, kresoxim-methyl generally was as effective against apple powdery mildew as the DMI fungicide standards myclobutanil, fenarimol, or triflumizole (35,39,91,102, 100,114; Table 4). In field trials performed in Israel, its efficacy was comparable to that of several DMI fungicides used alone or mixed with protectant fungicides (80–82). In one field trial, kresoxim-methyl and several DMI fungicides (except triflumizole) failed to control powdery mildew (19). This was mainly due to the fact that a severe powdery mildew epidemic devel-

Table 3. Control of apple scab (*Venturia inaequalis*) by kresoxim-methyl^y

Material (a.i.)	Number of applications ^z	Rate (g a.i./ha)	Scab incidence	
			Terminals (%)	Fruit (%)
Untreated	...	...	91.0	71.6
Kresoxim-methyl	8	84	3.7	0.3
Kresoxim-methyl	8	112	5.8	1.4
Myclobutanil + mancozeb	8	140 + 2,520	10.3	2.3

^y Source: (41).

^z Applications of all fungicides were made on 22 April, 2 May, 8 May, 15 May, 21 May, 30 May, 10 June, and 24 June 1996 at 747 liters/ha. Terminal leaf scab was evaluated on 11 June 1996. Fruit was evaluated for scab incidence on 8 July 1996.

Fig. 8. In apple orchard trials, kresoxim-methyl protected apple fruit against *Venturia inaequalis* (A) compared with untreated fruit (B).

oped in the orchard after the treatments—primarily applied to control scab—had been terminated. Fruit russet caused by *P. leucotricha* was equally well controlled by kresoxim-methyl and DMI fungicides (114).

Kresoxim-methyl reduced incidence of cedar apple rust and quince rust, and its efficacy was comparable to that of myclobutanil when disease pressure was low (76,91). Myclobutanil provided better cedar apple rust control than kresoxim-methyl under heavier infection pressure (39). This can be explained by the fact that kresoxim-methyl has mainly protectant activity against rusts. Alternations of kresoxim-methyl and myclobutanil generally provided better cedar apple rust control than kresoxim-methyl alone (40,90). Subsequently, an apple disease control program, which was based on alternations of kresoxim-methyl with either a standard DMI or EBDC fungicide, demonstrated a broad efficacy spectrum against scab, rust, and mildew (40,42,84,92,101). The spray program developed by BASF for apple scab control focuses on a maximum of three consecutive early-season kresoxim-methyl applications before switching to a DMI fungicide for bloom and petal fall applications. Thereafter, kresoxim-methyl can be applied again for a maximum of three applications before finishing the season with standard applications of captan or captan combined with benomyl. Although the evaluated spray programs with kresoxim-methyl were mainly designed for

apple scab and powdery mildew control, several studies indicated that kresoxim-methyl is efficacious against certain summer diseases, namely brooks fruit spot, sooty blotch, frog-eye leaf spot (black rot), white rot (bot rot), and fly speck (39,76,91,92,113,114). Neither kresoxim-methyl nor myclobutanil appeared to have early season activity against bitter rot and black pox (91,92,114). Ongoing studies with kresoxim-methyl applied later in the growing season will better demonstrate its activity spectrum against summer diseases and the feasibility of late season applications.

Pear diseases. Most pears in the United States are grown in California, Washington, and Oregon. Pear scab (*Venturia pirina*) is economically the most important fungal pear disease in California and Oregon. Heavy crop losses from scab can occur in many commercially important pear cultivars. *P. leucotricha*, causing powdery mildew on both pears and apples, is also important and occurs most frequently in the drier and cooler production areas in Washington. Kresoxim-methyl also showed particular strength in controlling pear scab, especially on the fruits (D. Sugar, *personal communication*; Table 5). No trial results have been published on efficacy of kresoxim-methyl against pear powdery mildew, but greenhouse trials performed by BASF demonstrated efficacy comparable to that demonstrated against apple powdery mildew. Kresoxim-methyl spray sequences on pears recommended by

BASF are similar to those mentioned on apples in the previous section and were tested in California in 1997. Kresoxim-methyl alternated with triflumizole resulted in almost complete pear scab control. The efficacy of this spray sequence was comparable to that of fenarimol mixed with mancozeb, but it did not affect fruit shape (26).

Grape diseases. Approximately 90% of the grapes produced in the United States are cultivated in California. Most of the remaining 10% are in New York, Ohio, Pennsylvania, Michigan, and Washington. Powdery mildew flourishes in the same climatic conditions that favor grape production, and severe epidemics often occur in the western United States. Powdery mildew occurs in the eastern United States as well, but usually develops later in the growing season than in the western United States and is only one of several disease problems. Black rot (*Guignardia bidwellii*) and Phomopsis cane and leaf spot (*Phomopsis viticola*) are typically the first diseases to occur in the east and can cause significant economic damage. Black rot is one of the most economically important diseases of grape in the northeastern United States. Phomopsis is most severe in northeastern grape growing regions, where spring rains are common after bud break. Downy mildew (*Plasmopara viticola*) also primarily affects grape production primarily where it is warm and wet during vegetative growth. Occasionally it is a problem in California when a wet winter is followed by late spring rains or in the event of early fall rains. Myclobutanil, fenarimol, triadimefon, triflumizole, azoxystrobin, and sulfur are the current options for controlling powdery mildew of grapes. Captan, mancozeb, and sulfur are used for Phomopsis control. Myclobutanil, triadimefon, and mancozeb are used to control black rot. Downy mildew is controlled by combinations of metalaxyl and captan, mancozeb, or copper, and more recently by azoxystrobin.

Both kresoxim-methyl and azoxystrobin provided almost complete control of grape powdery mildew under light infection pressure. In many trials, kresoxim-methyl was used at a rate just over half that of azoxystrobin. At these rates, kresoxim-methyl was as effective or more effective than azoxystrobin (83,106,108,109) and showed better residual control in one trial (69). Spray programs with either strobilurin and those with the DMI standard myclobutanil were among the best treatments when infection pressure was moderate or heavy (27,73,105,106,108,109; Table 6, Fig. 9).

Spray programs with kresoxim-methyl and azoxystrobin also effectively controlled black rot (73,103,107,110), and efficacy of both strobilurins was comparable to that of myclobutanil. Both strobilurins also controlled or reduced incidence of Phomopsis comparably (73,111); whereas

Table 4. Control of apple powdery mildew (*Podosphaera leucotricha*) by kresoxim-methyl^y

Material (a.i.)	Number of applications ^z	Rate (g a.i./ha)	Infected terminal leaves (%)
Untreated	...	...	13.9
Kresoxim-methyl	6	140	2.1
Myclobutanil	6	140	1.2
Triflumizole	6	420	0.9

^y Source: J. W. Pscheidt and L. D. Wallace, 1997. Oregon State University, Corvallis. Unpublished data.

^z Applications of all fungicides were made on 19 April, 25 April, 9 May, 20 May, 6 June, and 20 June 1997 at 1,870 liters/ha. Terminal leaves were evaluated for powdery mildew incidence on 25 June 1997.

Table 5. Control of pear scab (*Venturia pirina*) by kresoxim-methyl^x

Material (a.i.)	Number of applications ^y	Rate (g a.i./ha)	Fruit scab incidence (%) ^z
Untreated	...	...	35.2 a
Kresoxim-methyl	4	112	0.0 b
Triflumizole	4	420	3.2 c
Triflumizole + mancozeb	4	280 + 2,688	1.2 c

^x Source: D. Sugar, 1997. Oregon State University, Medford. Unpublished data.

^y Applications of all fungicides were made on 21 March, 20 April, 29 April, and 23 May 1996 at 1,871 liters/ha, following scab infection periods on 18 April, 28 April, 22 May, and 26 May. Fruit was evaluated for scab incidence on 30 June 1996.

^z Means followed by the same letter do not differ significantly (Duncan's NMRT, $P = 0.05$; analysis performed on arcsine square root transformed values).

myclobutanil provided comparable control only when mixed with lime and copper. Kresoxim-methyl reduced incidence of grape downy mildew in one trial, but azoxystrobin combined with a surfactant gave superior control (104). In another trial, both kresoxim-methyl and azoxystrobin provided good control (108), which was equal to that provided by mancozeb alone or alternated with zinc dimethyldithiocarbamate. In the western United States, BASF recommends a kresoxim-methyl spray program in alternation with standard DMI fungicides such as myclobutanil, triflumizole, or fenarimol. This rotation schedule provides efficacy against all major western grape diseases. Kresoxim-methyl mainly provides protectant activity against grape downy mildew and controls the disease well when infection pressure is light and applications are made in a timely manner (108). Incorporation of metalaxyl, copper, or mancozeb in a spray program with kresoxim-methyl or azoxystrobin may provide reliable control and effective management of fungicide resistance (103,104).

Pecan diseases. Most pecans are grown in the southern United States, with major production areas in Texas, Georgia, Oklahoma, Alabama, and New Mexico. Pecan scab (*C. caryigenum*) is the primary disease problem of pecans in the southeastern United States and can result in complete crop losses in susceptible cultivars. A prophylactic spray program starting at bud break to prevent early infection and inoculum buildup can prevent pecan nut scab. Three fungicides currently are used most for pecan scab control: triphenyltin and the DMI fungicides propiconazole and fenbuconazole. Azoxystrobin recently received registration for scab control in pecans. In field trials performed in Georgia (Table 7) and Florida, kresoxim-methyl and azoxystrobin provided pecan scab control similar to that of these standards (1,15,16,30; Fig. 10). In one trial in Florida, programs in which kresoxim-methyl

was alternated with triphenyltin or triphenyltin and fenbuconazole provided numerically better control of leaf scab and nut scab than did programs based on triphenyltin and fenbuconazole (2). In another trial in Georgia, similar spray schedules with kresoxim-methyl and azoxystrobin were among the best treatments for pecan scab control. Their efficacy was comparable to that of the standard DMI fungicides alternated with triphenyltin (17). Current

research focuses on determining the best fungicide alternation programs for pecan scab control.

Cucurbit diseases. All cucurbit species (watermelons, melons, squash, cantaloupes, pumpkins, and cucumbers) are grown in the southern and western states; whereas major pumpkin and cucumber production areas are located in the north-eastern states. Powdery mildew (*S. fuliginea*) and gummy stem blight (*Didymella*

Table 6. Control of grape powdery mildew (*Uncinula necator*) by kresoxim-methyl^y

Material (a.i.)	Number of applications ^z	Rate (g a.i./ha)	Cluster infection	
			Incidence (%)	Severity (%)
Untreated	...	...	100.0	31.0
Kresoxim-methyl	4	112	61.0	2.2
Azoxystrobin	4	196	64.5	2.9
Myclobutanil	4	112	59.5	2.8
Triflumizole	4	140	83.0	3.4
Sulfur	6	3,580	98.5	12.2

^y Source: (106).

^z Applications of all fungicides, except Microthiol, were made on 21 June 1996 at 936 liters/ha, and on 5 July, 17 July, and 31 July at 1,871 liters/ha. Microthiol was applied on the same dates in the same spray volume, and additionally on 23 May and 5 June 1996 at 936 liters/ha. Clusters were evaluated for powdery mildew incidence and severity on 23 August 1996.

Table 7. Control of pecan scab (*Cladosporium caryigenum*) by kresoxim-methyl^x

Material (a.i.)	Number of applications ^y	Rate (g a.i./ha)	Nut scab	
			Incidence (%)	Severity (%)
Untreated	...	...	90	35.1
Kresoxim-methyl	8	168	15	0.3
Azoxystrobin + surfactant ^z	8	168 + 0.13% (vol/vol)	27	0.7
Triphenyltin	8	420	11	0.5
Propiconazole, then triphenyltin	3	189	27	0.7
	5	420		

^x Source: (16).

^y All fungicides were applied on pecan cv. Desirable on 4 April, 18 April, 3 May, 26 May, 15 June, 6 July, and 15 August 1995 at 935 liters/ha. Pecan nuts were evaluated for scab incidence and severity on 29 September 1995.

^z Modified phthalic glycerol alkylid resin (77%).

Fig. 9. In vineyard trials, kresoxim-methyl protected grape clusters against infection by *Uncinula necator* (A), compared with untreated clusters (B).

bryoniae) are major production problems in susceptible cucurbit varieties. Both fungi attack all parts of the plant, including the fruit, and severe infections reduce fruit quality. Other than the use of resistant cucurbit-varieties where available, fungicides are the main component of control programs. To control powdery mildew, good coverage is necessary to reach the undersides of the leaves and the lower canopy to prevent recurring infections on the fruit. Protection of the underside of the leaves can be accomplished also with systemic fungicides, such as the sterol inhibitors and benzimidazoles; but resistance to both

fungicide groups is widespread in commercial production fields and greenhouses in the United States (62,64). Satisfactory chemical control of gummy stem blight is currently obtained by regular application of protectant fungicides and benzimidazoles; although resistance to the latter group of compounds occurred in some production areas recently (44). Cucurbit trials that included kresoxim-methyl have been performed on cantaloupe in Arizona (54,57,60), on pumpkin in New York (63; Table 8), and on watermelon in Indiana (51). Kresoxim-methyl, azoxystrobin only when combined with a surfactant, and

myclobutanil provided the best control of *S. fuliginea* on cantaloupe. Efficacy of kresoxim-methyl and azoxystrobin against cantaloupe powdery mildew was enhanced when the products were tank mixed with chlorothalonil (60). Sulfur, triadimefon, and benomyl were generally not effective (54,57,60), most likely because sulfur acted only as a protectant, and the frequency of strains resistant to the latter two compounds may have been high. On pumpkins, the tested compounds were applied after disease detection in order to establish a threshold for purposes of Integrated Pest Management (IPM). Kresoxim-methyl and triflumizole were both effective against *S. fuliginea* on upper and lower leaf surfaces, but the untreated and triflumizole-treated pumpkins yielded fewer fruit with firm handles than those treated with kresoxim-methyl. Later applications of triadimefon and benomyl lost effectiveness due to the frequency increase of resistant strains, and treatments including chlorothalonil were not as effective as kresoxim-methyl and triflumizole (63). Kresoxim-methyl was not specifically evaluated against downy mildew (*Pseudoperonospora cubensis*) in this trial. However, defoliation due to downy mildew was similar in the untreated plants and in those treated with triflumizole or kresoxim-methyl, and was more severe than defoliation of plants treated with the fungicide programs including chlorothalonil. As mentioned earlier, kresoxim-methyl may act mainly as a protectant against downy mildews and therefore would have to be applied preventively to achieve satisfactory control. In one trial on pumpkins, both kresoxim-methyl and azoxystrobin in alternation with chlorothalonil were comparable and among the best treatments against downy mildew (85). On watermelon, kresoxim-methyl controlled gummy stem blight and was comparable to chlorothalonil in one trial (51). It provided better control than chlorothalonil in another trial (D. Hopkins, *personal communication*; Table 9).

Greenhouse ornamental diseases.

Kresoxim-methyl has been tested against powdery mildews on greenhouse-grown ornamentals in the United States and Europe. Efficacy trial reports are available for control of poinsettia powdery mildew (*Oidium* sp.), and rust (*Phragmidium* sp.) and black spot (*Diplocarpon rosae*) on roses. On poinsettia, kresoxim-methyl provided good control of powdery mildew (24,38). On roses, kresoxim-methyl provided good control of rose powdery mildew comparable to the current standard fungicides (Hausbeck, Cortwright, and Quackenbush, *personal communication*; Table 10). Kresoxim-methyl was also efficacious against black spot and prevented defoliation, similar to the DMI fungicides fenarimol and triforine (72). Kresoxim-methyl and fenarimol reduced rust but

Fig. 10. In pecan orchard trials, kresoxim-methyl protected pecan nuts against pecan scab, caused by *Cladosporium caryigenum* (A), compared with the untreated control (B). Source: T. Brenneman.

were less effective than triforine (72). Rust was already present when the treatments were started, which did not allow for use of kresoxim-methyl as a protectant fungicide.

Other crops. Kresoxim-methyl has been evaluated on pathogens of a number of other crops in the United States. It was evaluated in Arizona on lettuce for efficacy against *E. cichoracearum* and *Bremia lactucae*, which cause powdery and downy mildew, respectively (55,58,61). Both kresoxim-methyl and azoxystrobin provided good and comparable control of lettuce powdery and downy mildew. In one lettuce trial, none of the treatments provided satisfactory powdery mildew control because the disease developed in the extended time between the last application and evaluation (61).

Both strobilurins were also evaluated on broccoli against downy mildew (*Peronospora parasitica*) and provided comparable control equal to or better than the standards (56,59). These results demonstrated again the particular strength of kresoxim-methyl against powdery mildews and showed that kresoxim-methyl, applied as a protectant, has potential to control downy mildews in certain cropping systems.

Kresoxim-methyl was evaluated on sweet cherry in Washington and sour cherry in Michigan for its efficacy against powdery mildew (*Podosphaera clandestina*) and leaf spot (*Blumeriella jaapii*), respectively (36,37,43). In 1996, kresoxim-methyl was among the best treatments against cherry powdery mildew, and it was superior to fenarimol and sulfur. In 1997, both kresoxim-methyl and azoxystrobin were among the best treatments against cherry powdery mildew. Their efficacy was comparable to that of myclobutanil and better than sulfur. Cherry leaf spot infection pressure was light. Both kresoxim-methyl and azoxystrobin almost completely controlled the disease and were comparable to chlorothalonil.

Strobilurins and Disease Management

The strobilurins are an important new class of fungicides with a unique mode of action that targets mitochondrial respiration of fungi. Kresoxim-methyl is effective at low concentrations, and its primary role in disease management is to provide protection against many plant pathogenic fungi. Kresoxim-methyl strongly inhibits spore germination, thereby reducing initial infection and spread of the pathogen. Postinfection applications against powdery mildews and apple scab can control advanced infections, symptom expression, and sporulation, thereby preventing further disease development. These properties make kresoxim-methyl a suitable compound in IPM programs against these pathogens. The new mode of action of strobilurins also may prevent or delay the

occurrence of fungicide-resistant pathogens because there is no cross reactivity with other fungicides currently on the market. The label application requirements for the strobilurins have a direct bearing on their potential to manage fungicide-resistant pathogens. BASF advises strict adherence to resistance management strategies and fungicide guidelines published by the Fungicide Resistance Action Committee (FRAC), which recommend preventive use of strobilurins and limit the number of kresoxim-methyl applications to six per season (8). Kresoxim-methyl should not be used for more than three sequential applications, after which it should be alternated

with at least two applications of an effective fungicide with a different mode of action. Thereafter, kresoxim-methyl can be used again for a maximum of three applications, but it should not be used as the final spray of the season (8). The use of strobilurins in conjunction with other effective fungicides and disease forecasting techniques should serve to obtain effective disease control and to reduce the occurrence of fungicide-resistant pathogens.

Outlook

The development of the strobilurin-fungicides kresoxim-methyl and azoxystrobin by BASF and Zeneca has resulted in the

Table 8. Control of cucurbit powdery mildew (*Sphaerotheca fuliginea*) by kresoxim-methyl^y

Material (a.i.)	Time of application ^z	Rate (g a.i./ha)	Powdery mildew severity	
			Upper leaf surface (%)	Lower leaf surface (%)
Untreated	...	...	0.87	3.57
Kresoxim-methyl	1,3	84	0.00	0.60
Triflumizole	1,3	210	0.03	0.50
Chlorothalonil + triadimefon, alternated with chlorothalonil	1,3 2,4	2,485 + 134 3,026	0.11	3.08

^y Source: (63).

^z Fungicides were applied at 663 liters/ha on pumpkin cv. Wizard on the following dates: 1 = 16 August, 2 = 23 August, 3 = 30 August, 4 = 5 September 1996. Leaf surfaces were evaluated for powdery mildew severity on 11 September 1996.

Table 9. Control of gummy stem blight (*Didymella bryoniae*) on watermelon by kresoxim-methyl^x

Material (a.i.)	Number of applications ^y	Rate (g a.i./ha)	Leaf infection incidence (%)
Untreated	...	...	93 a ^z
Kresoxim-methyl	7	112	10 b
Chlorothalonil	7	2,520	48 c

^x Source: D. Hopkins, 1997. University of Florida, Leesburg. Unpublished data.

^y Applications of all fungicides were made on cv. Crimson Sweets on 8 May, 15 May, 22 May, 29 May, 5 June, 12 June, and 19 June 1996 at 936 liters/ha. Fruit was evaluated for gummy stem blight incidence on 24 June 1996.

^z Means followed by the same letter do not differ significantly (Duncan's NMRT, $P = 0.05$).

Table 10. Control of rose powdery mildew (*Sphaerotheca pannosa*) by kresoxim-methyl^w

Material (a.i.)	Rate ^x (mg a.i./liter)	Powdery mildew incidence	
		Foliage (%)	Flowers (%)
Untreated	...	13.0 a ^y	59.5 a
Kresoxim-methyl + surfactant ^z	120 + 0.016% (vol/vol)	1.0 b	0.0 b
Triadimefon + surfactant ^z	38 + 0.016% (vol/vol)	0.4 b	0.0 b

^w Source: M. K. Hausbeck, B. D. Cortwright, and W. R. Quackenbush, 1997. Unpublished data.

^x Fungicides were applied to runoff on potted miniature roses, *Rosa hybrida* cv. Mini Brite on 28 March, 11 April, and 25 April 1997. Leaves and flowers were evaluated for powdery mildew incidence on 26 April 1997.

^y Means followed by the same letter do not differ significantly (Student-Newman-Keuls, $P = 0.05$).

^z Modified phthalic glycerol alkylid resin (77%).

availability of valuable, novel crop protection compounds with broad activity spectra and many promising uses in agriculture. Both compounds are the first representatives of a new class of fungicides. The great potential of strobilurins in crop protection has been recognized by many other manufacturers, and undoubtedly more strobilurin fungicides will be introduced in the near future (78). Published research data are currently not available on these newer strobilurins, but trifloxystrobin, developed by Novartis, has recently been submitted for registration (94).

The introduction and use of several strobilurins in the same crop-pathogen systems will warrant the need for resistance management strategies for strobilurins in general, in contrast to the current guidelines per separate compound. These guidelines for use of strobilurins should be

agreed upon by all manufacturers involved to preserve the role of strobilurins in crop protection and fungicide resistance management for many years.

The potential of resistance development to strobilurins is not known. Natural strobilurin producers, which are filamentous fungi like many plant pathogens and some other organisms, are resistant to strobilurins by nature, due to single amino acid changes in the target site protein. Although laboratory research was just recently started to address the likelihood of such mutations in plant pathogens (117), the natural strobilurin producers are living proof of the existence of such mutations and resulting adequate fitness under strong selection pressure. Since selection pressure on plant pathogens may increase upon introduction of additional strobilurins, more research is required to study the stro-

bilurin target sites and to investigate the likelihood of target site mutations and the occurrence of strobilurin cross resistance. Both BASF and Zeneca are committing resources to establish strobilurin baseline sensitivities of target plant pathogens and are prepared to investigate instances of unsatisfactory control. These efforts will have to be undertaken by all agrochemical manufacturers to preserve the important role of strobilurins in crop protection for years to come.

The data presented for kresoxim-methyl have shown that strobilurins can have significant effects on plant metabolism. These effects can be beneficial, such as those described for kresoxim-methyl on wheat. Additional studies are needed to acquire a better understanding of the effects of strobilurins on plant metabolism and critical output traits such as yield and nutritional value.

Members of the agrochemical industry will continue their search for molecules that can be developed into environmentally and economically feasible and effective fungicides. The strobilurin case is proof that nature not only afflicts crop plants with pests and diseases, but also provides us with the compounds to combat them. Most likely, biologically active molecules derived from nature will continue to play an important role in the development of crop protection compounds.

Hendrik L. Ypema

Dr. Ypema is an agricultural biologist in fungicide research at BASF Corporation's Agricultural Products Center in North Carolina. He received his B.S. and M.S. degrees in plant pathology from the Agricultural University in Wageningen, The Netherlands, in 1983 and 1986, respectively. From 1986 to 1991, he worked at the Ministry of Agriculture in The Netherlands. He received his Ph.D. in plant pathology from the University of California, Davis, in 1996. His thesis research focused on aspects of fungicide sensitivity of *Uncinula necator*, causing powdery mildew of grapes. He joined BASF Corporation in 1996. His current responsibilities are research on fungicide resistance, resistance management, and product characterization and support.

Randall E. Gold

Dr. Gold is group leader of fungicide research and development at BASF Corporation's Agricultural Products Center in North Carolina. He received his B.S. degree in forestry at the University of Missouri, Columbia, in 1976, and his M.S. in plant pathology at North Dakota State University, Fargo, in 1978. He earned his doctorate in plant pathology from the University of Constance, Germany, in 1983. His dissertation research focused on spore germination and infection processes in rusts. After a postdoctoral position at Cornell University, Ithaca, New York, he joined BASF AG in 1985 in Germany as a laboratory leader in the fungicide discovery department. In 1996, he transferred to BASF's Agricultural Products Division in the United States, and he currently oversees the research and development of fungicides for the NAFTA region.

Literature Cited

1. Aldrich, J. H. 1997. Effect of two experimental fungicides on pecan scab, 1996. *Fungic. Nematicide Tests* 52:88.
2. Aldrich, J. H. 1998. Evaluation of fungicide programs for the control of pecan scab, 1997. *Fungic. Nematicide Tests* 53:111.
3. Ammerman, E., Lorenz, G., Schelberger, K., Wenderoth, B., Sauter, H., and Rentzea, C. 1992. BAS 490F—A broad-spectrum fungicide with a new mode of action. Pages 403-410 in: *Brighton Crop Prot. Conf. Pests Dis.*
4. Anke, T. 1995. The antifungal strobilurins and their possible ecological role. *Can. J. Bot.* 73(Suppl. 1):S940-S945.
5. Anke, T., Oberwinkler, F., Steglich, W., and Schram, G. 1977. The strobilurins—New antifungal antibiotics from the basidiomycete *Strobilurus tenacellus*. *J. Antibiot.* 30:806-810.
6. Anke, T., Schram, G., Schwalbe, B., Steffan, B., and Steglich, W. 1984. Synthese von strobilurin und revision der stereochemie der natürlichen strobilurine. *Liebigs Ann. Chem.* 1984:1616-1625.
7. Anke, T., and Steglich, W. 1989. β -methoxyacrylate antibiotics: From biological activity to synthetic analogues. Pages 9-25 in: *Biologically Active Molecules*. U. P. Schlunegger, ed. Springer-Verlag, Berlin.
8. Anonymous. 1998. Fungicide resistance guidelines (principles for effective resistance management). Global Crop Protection Federation, Brussels, Belgium.
9. Anthony, V. M., Clough, J. M., Godfrey, C. R. A., and Wiggins, T. E. 1986. EP Appl. 254426, ICI, Priority 18.07.1986.
10. Badenoch-Jones, J., Parker, C. W., Letham, D. S., and Singh, S. 1996. Effect of cytokinins supplied via the xylem at multiples of endogenous concentrations on transpiration

- and senescence of derooted seedlings of oat and wheat. *Plant Cell Environ.* 19:504-516.
11. Baldwin, B. C., Clough, J. M., Godfrey, C. R. A., Godwin, J. R., and Wiggins, T. E. 1996. The discovery and mode of action of ICI A5504. Pages 69-77 in: *Modern Fungicides and Antifungal Compounds*. H. Lyr, P. E. Russell and H. D. Sisler, eds. Intercept, Andover, Hants, UK.
 12. Beauteament, K., Clough, J. M., de Fraine, P. J., and Godfrey, C. R. A. 1991. Fungicidal β -Methoxyacrylates: From natural products to novel synthetic agricultural fungicides. *Pestic. Sci.* 31:499-541.
 13. Becker, W. F., von Jagow, G., Anke, T., and Steglich, W. 1981. Oudemansin, strobilurin a, strobilurin b, and myxothiazole: New inhibitors of the BC₁ segment of the respiratory chain with an e- β -methoxyacrylate system as common structural element. *FEBS Lett.* 132:329-333.
 14. Bollmark, M., and Eliasson, L. 1990. Ethylene accelerates the breakdown of cytokinins and thereby stimulates rooting in Norway spruce hypocotyl cuttings. *Physiol. Plant.* 80:534-540.
 15. Brenneman, T. B., and Mixon, J. A. 1995. Evaluation of experimental fungicides for the control of pecan scab, 1994. *Fungic. Nematicide Tests* 50:78-79.
 16. Brenneman, T. B., and Mixon, J. A. 1996. Evaluation of standard and experimental fungicides for the control of pecan scab, 1995. *Fungic. Nematicide Tests* 51:78-80.
 17. Brenneman, T. B., Mixon, J. A., and Sills, P. 1998. Evaluation of standard and experimental fungicides for the control of pecan scab, 1997. *Fungic. Nematicide Tests* 53:112-114.
 18. Brent, K. J. 1996. Pathways to success in fungicide research and technology. Pages 3-15 in: *Modern Fungicides and Antifungal Compounds*. H. Lyr, P. E. Russell, and H. D. Sisler, eds. Intercept, Andover, Hants, UK.
 19. Burr, T. J., Smith, C. A., and Matteson, M. C. 1996. Evaluation of fungicides for control of scab and mildew of apples, 1995. *Fungic. Nematicide Tests* 51:3.
 20. Bushell, M. J., Beauteament, K., Clough, J. M., de Fraine, P., Anthony, V. M., and Godfrey, C. R. A. 1984. EP Appl. 178826, ICI, Priority 19.10.1984.
 21. Cinamowski, J., and Bielenin, A. 1996. Antisporulant activity of new apple powdery mildew fungicides. *J. Fruit Orn. Plant Res.* 4:21-25.
 22. Clough, J. M., de Fraine, P. J., Fraser, T. E. M., and Godfrey, C. R. A. 1992. Fungicidal β -methoxyacrylates: From natural products to novel synthetic agricultural fungicides. Pages 372-381 in: *Synthesis and Chemistry of Agrochemicals III*. D. R. Baker, J. G. Fenyes, and J. J. Steffens, eds. ACS Symposium Series 504.
 23. Creemers, P., De Vleeschauwer, J., Mappes, D., and Defloor, K. 1996. BAS 490 02 F, a new fungicide to control scab and mildew in apple and pear. *Meded. Fac. Landb. Gent* 61 (2A). pp. 431-441.
 24. Daughtrey, M., and Macksel, M. 1998. Evaluation of biological and chemical fungicides for control of powdery mildew in poinsettia and miniature rose. *Phytopathology* 88:S135.
 25. Dekker, J. 1995. Development of resistance to modern fungicides and strategies for its avoidance. Pages 23-38 in: *Modern Selective Fungicides—Properties, Applications, Mechanisms of Action*. H. Lyr, ed. Gustav Fisher Verlag, New York.
 26. Dell, K. J., and Gubler, W. D. 1998. Evaluation of fungicides for control of pear scab, 1997. *Fungic. Nematicide Tests* 53:50.
 27. Dell, K. J., and Gubler, W. D. 1998. Evaluation of fungicides for the control of grape powdery mildew, 1997. *Fungic. Nematicide Tests* 53:91.
 28. Di Rago, J., Coppée, J., and Colson, A. 1989. Molecular basis for resistance to myxothiazol, mucidin (strobilurin A), and stigmatellin. *J. Biol. Chem.* 264:14543-14548.
 29. Erickson, E. O., and Wilcox, W. F. 1997. Distributions of sensitivities to three sterol demethylation inhibitor fungicides among populations of *Uncinula necator* sensitive and resistant to triadimefon. *Phytopathology* 87:784-791.
 30. French, W. J., and Aldrich, J. H. 1996. Evaluation of an experimental fungicide for pecan scab and leaf blotch, 1995. *Fungic. Nematicide Tests* 51:81.
 31. Georgopoulos, S. G. 1995. The genetics of fungicide resistance. Pages 39-52 in: *Modern Selective Fungicides—Properties, Applications, Mechanisms of Action*. H. Lyr, ed. Gustav Fisher Verlag, New York.
 32. Gold, R. E., Ammerman, E., Koehle, H., Leinhos, G. M. E., Lorenz, G., Speakman, J. B., Stark-Urnau, M., and Sauter, H. 1996. The synthetic strobilurin BAS 490F: Profile of a modern fungicide. Pages 79-92 in: *Modern Fungicides and Antifungal Compounds*. H. Lyr, P. E. Russell, and H. D. Sisler, eds. Intercept, Andover, Hants, UK.
 33. Gold, R. E., and Leinhos, G. M. 1995. Fungicidal effects of BAS 490F on the development and fine structure of plant pathogenic fungi. *Pestic. Sci.* 43:250-253.
 34. Grossman, K., and Retzlaff, G. 1997.

An advertisement appears in the printed journal in this space.

- Bioregulatory effects of the fungicidal strobilurin kresoxim-methyl in wheat. *Pestic. Sci.* 50:11-20.
35. Grove, G. G., and Boal, R. J. 1997. Control of apple powdery mildew at Wenatchee, 1996. *Fungic. Nematicide Tests* 52:28.
36. Grove, G. G., and Boal, R. J. 1997. Control of powdery mildew of sweet cherry with oil and soap fungicides, 1996. *Fungic. Nematicide Tests* 52:52.
37. Grove, G. G., and Boal, R. J. 1998. Control of powdery mildew in Washington cherry under extreme disease pressure, 1997. *Fungic. Nematicide Tests* 53:58.
38. Hausbeck, M. K., and Kusnier III, J. J. 1995. Evaluation of fungicides for the control of powdery mildew of poinsettia, 1994. *Fungic. Nematicide Tests* 51:398.
39. Hickey, K. D., May, J., and McGlaughlin, E. 1997. Efficacy of experimental fungicide BAS 490F applied seasonally as dilute sprays for apple disease control, 1996. *Fungic. Nematicide Tests* 52:14.
40. Hickey, K. D., May, J., and McGlaughlin, E. 1998. Efficacy of experimental fungicides applied as dilute sprays for apple disease control, 1997. *Fungic. Nematicide Tests* 53:13-14.
41. Jones, A. L., and Ehret, G. R. 1997. Apple scab control with airblast sprayer, 1996. *Fungic. Nematicide Tests* 52:19.
42. Jones, A. L., and Ehret, G. R. 1998. Apple scab control with SI and strobilurin fungicides, 1997. *Fungic. Nematicide Tests* 53:19.
43. Jones, A. L., and Ehret, G. R. 1998. Disease control on Montmorency cherry with strobilurin fungicides in northwest Michigan, 1997. *Fungic. Nematicide Tests* 53:54.
44. Keinath, A. P., and Zitter, T. A. 1998. Resistance to benomyl and thiophanate-methyl in *Didymella bryoniae* from South Carolina and New York. *Plant Dis.* 82:479-484.
45. Köller, W. 1996. Recent developments in DMI resistance. Pages 301-312 in: *Modern Fungicides and Antifungal Compounds*. H. Lyr, P. E. Russell, and H. D. Sisler, eds. Intercept, Andover, Hants, UK.
46. Kraiczky, P., Haase, U., Gencic, S., Flindt, S., Anke, T., Brandt, U., and Von Jagow, G. 1996. The molecular basis for the natural resistance of the cytochrome bc₁ complex from strobilurin-producing basidiomycetes to center Q_p inhibitors. *Eur. J. Biochem.* 235:54-63.
47. Kunz, S., Deising, H., and Mendgen, K. 1997. Acquisition of resistance to sterol demethylation inhibitors by populations of *Venturia inaequalis*. *Phytopathology* 87:1272-1278.
48. Kunz, S., Lutz, B., Deising, H., and Mendgen, K. 1998. Assessment of sensitivities to anilinoimidazole- and strobilurin-fungicides in populations of the apple scab fungus *Venturia inaequalis*. *J. Phytopathol.* 146:231-238.
49. Kuroda, H., Masuda, T., Ohta, H., Shioi, Y., and Takamiya, K. I. 1996. Effects of light, developmental age and phytohormones on the expression of the gene encoding NADPH-protochlorophyllide oxidoreductase in *Cucumis sativus*. *Plant Physiol. Biochem.* 34:17-22.
50. Latham, A. J., and Rushing, A. E. 1988. Development of *Cladosporium caryigenum* in pecan leaves. *Phytopathology* 78:1104-1108.
51. Latin, R. 1997. Evaluation of fungicides for control of gummy stem blight of watermelon, 1996. *Fungic. Nematicide Tests* 52:196.
52. Leinhos, G. M. E., Gold, R. E., Düggelein, M., and Guggenheim, R. 1997. Development and morphology of *Uromyces necator* following treatment with the fungicides kresoxim-methyl and penconazole. *Mycol. Res.* 101:1033-1046.
53. Lyr, H., and Schewe, T. 1975. On the mechanism of the cyanide-insensitive alternative pathway of respiration in fungi and higher plants and the nature of the alternative terminal oxidase. *Acta Biol. Med. Germ.* 34:1631-1641.
54. Matheron, M. E., and Porchas, M. 1996. Comparison of fungicides for control of powdery mildew of cantaloupe, 1995. *Fungic. Nematicide Tests* 51:103.
55. Matheron, M. E., and Porchas, M. 1996. Effectiveness of fungicides for control of downy and powdery mildew of lettuce, 1995. *Fungic. Nematicide Tests* 51:116.
56. Matheron, M. E., and Porchas, M. 1997. Evaluation of fungicides for management of downy mildew on broccoli, 1996. *Fungic. Nematicide Tests* 52:101.
57. Matheron, M. E., and Porchas, M. 1997. Comparative efficacy of fungicides for management of powdery mildew of cantaloupe, 1996. *Fungic. Nematicide Tests* 52:105.
58. Matheron, M. E., and Porchas, M. 1997. Management of downy and powdery mildew on lettuce with fungicides, 1996. *Fungic. Nematicide Tests* 52:111.
59. Matheron, M. E., and Porchas, M. 1998. Effectiveness of fungicides for management of downy mildew on broccoli, 1997. *Fungic. Nematicide Tests* 53:134.
60. Matheron, M. E., and Porchas, M. 1998. Management of powdery mildew on cantaloupe with fungicides, 1997. *Fungic. Nematicide Tests* 53:135-136.
61. Matheron, M. E., and Porchas, M. 1998. Comparative efficacy of fungicides for management of downy and powdery mildew on lettuce, 1997. *Fungic. Nematicide Tests* 53:153-154.
62. McGrath, M. T. 1996. Increased resistance to triadimefon and to benomyl in *Sphaerotheca fuliginea* populations following fungicide usage over one season. *Plant Dis.* 80:633-639.
63. McGrath, M. T. 1997. Evaluation of fungicide programs initiated after disease detection for managing powdery mildew of pumpkin, 1996. *Fungic. Nematicide Tests* 52:167-168.
64. McGrath, M. T., Stanisewska, H., and Shishkoff, N. 1996. Fungicide sensitivity of *Sphaerotheca fuliginea* populations in the United States. *Plant Dis.* 80:697-703.
65. Mizutani, A., Miki, N., Yukioka, H., and Masuko, M. 1996. Mechanism of action of a novel alkoxyiminoacetamide fungicide SSF-126. Pages 93-99 in: *Modern Fungicides and Antifungal Compounds*. H. Lyr, P. E. Russell, and H. D. Sisler, eds. Intercept, Andover, Hants, UK.
66. Mizutani, A., Miki, N., Yukioka, H., Tamura, H., and Masuko, M. 1996. A possible mechanism of control of rice blast disease by a novel alkoxyiminoacetamide fungicide, SSF126. *Phytopathology* 86:295-300.
67. Mizutani, A., Yukioka, H., Tamura, H., Miki, N., Masuko, M., and Takeda, R. 1995. Respiratory characteristics in *Pyricularia oryzae* exposed to a novel alkoxyiminoacetamide fungicide. *Phytopathology* 85:306-311.
68. Neumann, S., and Jacob, F. 1995. Principles of uptake and systemic transport of fungicides within the plant. Pages 53-73 in: *Modern Selective Fungicides—Properties, Applications, Mechanisms of Action*. H. Lyr, ed. Gustav Fischer Verlag, New York.
69. Northover, J., and Homeyer, C. 1998. Efficacy of fungicides against grapevine powdery mildew, 1997. *Fungic. Nematicide Tests* 53:102.
70. Nyfeler, R., and Ackerman, P. 1992. Phenylpyrroles, a new class of agricultural fungicides related to the natural antibiotic pyrrol-nitrin. Pages 395-404 in: *Synthesis and Chemistry of Agrochemicals III*. D. R. Baker, J. G. Fenyes, and J. J. Steffens, eds. ACS Symposium Series 504.
71. Olaya, G., Zheng, D., and Köller, W. Differential responses of germinating *Venturia inaequalis* conidia to kresoxim-methyl. *Pestic. Sci.* In press.
72. Pscheidt, J. W., and Wittig, H. P. P. 1997. Efficacy of fungicides for the control of black spot and rust on rose, 1996. *Fungic. Nematicide Tests* 52:410.
73. Ramsdell, D. C. 1996. Foliar fungicides to control fruit rots, phomopsis rachis lesions, and downy and powdery mildews, 1995. *Fungic. Nematicide Tests* 68-71.
74. Retzlaff, G. 1995. Untersuchungen zur strobilurinwirkung in Gramineen. *Phytomedizin* 25:45.
75. Rohl, F., and Sauter, H. 1994. Species dependence of mitochondrial respiration inhibition by strobilurin analogues. *Biochem. Soc. Trans.* 22:63S.
76. Rosenberger, D. A., Meyer, F. W., and Engle, C. A. 1998. Evaluation of fungicides for apples, 1997. *Fungic. Nematicide Tests* 53:23-24.
77. Sauter, H., Ammermann, E., Benoit, R., Brand, S., Gold, R. E., Grammenos, W., Koehle, H., Lorenz, G., Muller, B., Rohl, F., Schirmer, U., Speakman, J. B., Wenderoth, B., and Wiggert, H. 1995. Mitochondrial respiration as a target for antifungals: Lessons learned from research on strobilurins. Pages 173-191 in: *Antifungal Agents—Discovery and Mode of Action*. G. K. Dixon, L. G. Copping, and D. W. Hollomon, eds. BIOS, Oxford.
78. Sauter, H., Ammermann, E., and Roehl, F. 1996. Strobilurins—From natural products to a new class of fungicides. Pages 50-81 in: *Crop Protection Agents from Nature: Natural Products and Analogues*. L. G. Copping, ed. The Royal Society of Chemistry Information Services, Essex, UK.
79. Schirmer, U., Karbach, S., Pommer, E. H., Ammermann, E., Steglich, W., Schwalge, B. A. M., and Anke, T. 1985. EP Applications: 203606, BASF, Priority 30.05.1985; 203608, BASF, Priority 30.05.1985; 226917, BASF, Priority 20.12.1985; 226974, BASF, Priority 20.12.1985.
80. Shabi, E., and Elisha, S. 1994. Evaluation of DMI fungicides and a novel compound, BAS-490F, for the control of apple scab and powdery mildew, 1994. *Fungic. Nematicide Tests* 50:31.
81. Shabi, E., and Elisha, S. 1996. Strobilurins and DMI fungicides against scab and powdery mildew (PM), 1995. *Fungic. Nematicide Tests* 51:25.
82. Shabi, E., and Elisha, S. 1996. Apple scab and powdery mildew control in cv. Anna, 1995. *Fungic. Nematicide Tests* 51:26.
83. Shabi, E., and Elisha, S. 1996. Evaluations of strobilurin, dinocap and SBI fungicides, sulfur, potassium and oils for the control of grape powdery mildew (PM), 1995. *Fungic. Nematicide Tests* 51:72.
84. Shabi, E., and Elisha, S. 1997. Apple scab and powdery mildew evaluations in cv. Anna, 1996. *Fungic. Nematicide Tests* 52:30.
85. Shamiyeh, N. B., Straw, R. A., and Mullins, C. A. 1998. Foliar fungicides for control of mildews in pumpkins, 1997. *Fungic. Nematicide Tests* 53:229-230.
86. Sherald, J. L., and Sisler, H. D. 1970. Antimycin A-resistant respiratory pathway in *Ustilago maydis* and *Neurospora sitophila*. *Plant Physiol.* 46:180-182.
87. Sherald, J. L., and Sisler, H. D. 1972. Selective inhibition of Antimycin A—Insensitive respiration in *Ustilago maydis* and *Ceratomyces ulmi*. *Plant Cell Physiol.* 13:1039-1052.
88. Stark-Urnau, M., Gold, R., Guggenheim, R., and Düggelein, M. 1997. Sensitivity of different mildew and rust fungi against kresoxim-methyl. Pages 268-271 in: *Proc. Eur. Medi-*

- terr. Cereal Rusts Powdery Mildews Conf., 9th, Lunteren, The Netherlands.
89. Sundqvist, C., Bjorn, L. O., and Virgin, H. I. 1980. Factors in chloroplast differentiation. Pages 201-224 in: Chloroplasts. J. Reinert, ed. Springer-Verlag, Berlin.
90. Sutton, T. B., and Anas, O. 1998. Cedar apple rust control with Sovran 50W, 1997. Fungic. Nematicide Tests 53:34.
91. Sutton, T. B., and Brown, E. M. 1997. Disease control in Rome Beauty, 1996. Fungic. Nematicide Tests 52:34-35.
92. Sutton, T. B., Brown, E. M., and Anas, O. 1998. Disease control on Golden Delicious, 1997. Fungic. Nematicide Tests 53:39.
93. Sweets, L. E., and Kephart, K. D. 1998. Evaluation of foliar fungicides on winter wheat in Columbia, MO, 1997. Fungic. Nematicide Tests 53:322.
94. Tally, A., Laird, D., Margot, P., and Weiss, B. 1998. CGA-279202—A New Strobilurin Fungicide from Novartis Crop Protection. (Abstr.) Phytopathology 88:S88.
95. Vanlerberghe, G. C., and McIntosh, L. 1997. Alternative oxidase: From gene to function. Annu. Rev. Plant Physiol. Plant Mol. Biol. 48:703-734.
96. Veverka, K. 1996. General concept of fungicide resistance. Pages 283-292 in: Modern Fungicides and Antifungal Compounds. H. Lyr, P. E. Russell, and H. D. Sisler, eds. Intercept, Andover, Hants, UK.
97. Watanabe, M., Hayashi, K., Tanaka, T., and Uesugi, Y. 1996. Cyanide-resistant alternative respiration in *Botrytis cinerea*. Pages 111-115 in: Modern Fungicides and Antifungal Compounds. H. Lyr, P. E. Russell, and H. D. Sisler, eds. Intercept, Andover, Hants, UK.
98. Wenderoth, B., Anke, T., Rentzea, C., Ammermann, E., Pommer, E. H., and Steglich, W. 1989. United States Patent no. 4829085. May 9, 1989.
99. Wenderoth, B., Rentzea, C., Ammermann, E., Pommer, E. H., Steglich, W., and Anke, T. 1986. EP Applic. 253213, BASF, Priority 16.07.1986.
100. Wilcox, W. F., Burr, J. A., Heidenreich, G., Smith, C., and Gadoury, D. M. 1998. Evaluation of solo fungicide products and nontraditional alternatives for control of scab and powdery mildew of apples, 1997. Fungic. Nematicide Tests 53:35-36.
101. Wilcox, W. F., Burr, J. A., Heidenreich, G., Smith, C., and Gadoury, D. M. 1998. Evaluation of fungicide programs for control of scab and powdery mildew of apples, 1997. Fungic. Nematicide Tests 53:37-38.
102. Wilcox, W. F., Burr, J. A., Smith, C. A., and Gadoury, D. M. 1997. Evaluation of fungicide programs for control of scab and powdery mildew of apples, 1996. Fungic. Nematicide Tests 52:38-39.
103. Wilcox, W. F., and Riegel, D. G. 1996. Evaluation of fungicides and spray timings for control of black rot and downy mildew of grapes, 1995. Fungic. Nematicide Tests 51:74.
104. Wilcox, W. F., and Riegel, D. G. 1996. Evaluation of fungicides and spray timings for control of downy mildew and powdery mildew of grapes, 1995. Fungic. Nematicide Tests 51:75.
105. Wilcox, W. F., and Riegel, D. G. 1996. Evaluation of fungicides and spray timings for control of grape powdery mildew, 1995. Fungic. Nematicide Tests 51:76-77.
106. Wilcox, W. F., and Riegel, D. G. 1997. Evaluation of fungicide programs for control of grapevine powdery mildew, 1996. Fungic. Nematicide Tests 52:82-83.
107. Wilcox, W. F., and Riegel, D. G. 1997. Evaluation of fungicide programs for control of black rot of grapes, 1996. Fungic. Nematicide Tests 52:87.
108. Wilcox, W. F., and Riegel, D. G. 1998. Evaluation of fungicide programs for control of downy and powdery mildew of grapes, 1997. Fungic. Nematicide Tests 53:105-106.
109. Wilcox, W. F., and Riegel, D. G. 1998. Evaluation of fungicide programs for control of grapevine powdery mildew, 1997. Fungic. Nematicide Tests 53:107-108.
110. Wilcox, W. F., and Riegel, D. G. 1998. Evaluation of fungicide programs for control of grape black rot, 1997. Fungic. Nematicide Tests 53:109.
111. Wilcox, W. F., and Riegel, D. G. 1998. Evaluation of fungicide programs for control of phomopsis cane and leaf spot of grapes, 1997. Fungic. Nematicide Tests 53:110.
112. Yamaguchi, I. 1995. Antibiotics as antifungal agents. Pages 415-429 in: Modern Selective Fungicides—Properties, Applications, Mechanisms of Action. H. Lyr, ed. Gustav Fisher Verlag, New York.
113. Yoder, K. S., Cochran II, A. E., Royston, W. S., and Kilmer, S. W. 1997. Control of scab and summer diseases by experimental and standard fungicides on Starking Delicious apple, 1996. Fungic. Nematicide Tests 52:40-41.
114. Yoder, K. S., Cochran II, A. E., Royston, W. S., Kilmer, S. W., and Layman, J. C. 1996. Powdery mildew and summer disease control by experimental fungicides on three apple cultivars, 1995. Fungic. Nematicide Tests 51:34-35.
115. Yoder, K. S., Cochran II, A. E., Royston, W. S., Kilmer, S. W., and Scott, J. E. 1998. Control of powdery mildew and scab by experimental fungicides on Golden Delicious apple, 1997. Fungic. Nematicide Tests 53:40.
116. Ypema, H. L., Ypema, M., and Gubler, W. D. 1997. Sensitivity of *Uncinula necator* to benomyl, triadimefon, myclobutanil, and fenarimol in California. Plant Dis. 81:293-297.
117. Zheng, D., and Köller, W. 1997. Characterization of the mitochondrial cytochrome b gene encoding the target site of strobilurin fungicides from *Venturia inaequalis*. (Abstr.) Phytopathology 87(suppl.):S109.
118. Ziogas, B. N., Baldwin, B., and Young, J. A. 1997. Alternative Respiration: A Biochemical Mechanism to Azoxystrobin (ICI A 5504) in *Septoria tritici*. Pestic. Sci. 50:28-34.