5 Fungicide Performance

Key Points

- Fungicides can be classified according to crop protection performance, mode of action or chemical structure.
- FRAC has established a standardized classification system.
- Each class of fungicide has advantages and disadvantages in terms of activity, spectrum and resistance risk.
- Fungicide redistribution affects persistence and performance.

Introduction

This chapter describes the various classes of fungicides, their origin, mode of action and use. There are currently about 200 chemical compounds that are marketed as fungicides and these compounds can be classified according to either their MOA or their chemical structure. There are several levels to both these classification methods. MOA can refer to whether the product is a protectant, curative or eradicant, the broad biological function that is inhibited or the specific molecular target (if one exists or is identified).

Alternatively, fungicides can be classified by their chemical structure. Box 4.1 gives a summary of the rules for naming heterocyclic compounds as applied to fungicides. Most fungicides are complex organic molecules with several functional groups. As more than one fungicide is developed with similar MOAs, it sometimes becomes clear whether a particular structural feature is of biological significance. This structural feature is called the pharmacophore. Older names such as toxophore are also in use. The pharmacophore for many of the compounds that inhibit the *Cyp51* gene in ergosterol biosynthesis is the triazole group and this has become the most frequently used name for the group. Indeed, the triazole moiety has been shown to be important in binding to the target enzyme. Nonetheless, not all triazoles, not even all the ones used in agrochemistry, have this activity. In other cases, several chemically distinct classes can have the same MOA. Thus many unrelated compounds act as QoIs and SDHIs. It is also sometimes the case that the same chemical structure (e.g. morpholines or carboxamides) has different MOAs as a fungicide. Therefore in some cases, the biochemical function (QoI, SDHI) determines how the group is known. It is therefore important to be aware of the multiplicity of names used to describe groups of fungicides and to appreciate the relative biochemical, chemical and agricultural significance of the naming conventions.

Protectant, Curative or Eradicant; Systemicity

The flexibility of product use and the persistence of control reflect the presence of critical characteristics, such as mobility within the plant, ability to redistribute throughout the crop and compatibility with other pesticides. Fungicide systemicity refers to the movement of fungicide within the target crop, not the pathogen. Systemic fungicides are generally more reliable than non-systemics. Because most have curative or eradicant activity they have a wider window of application. They provide higher levels of efficacy by virtue of their mobility and afford a longer period of control than non-systemics. Depending on their biochemical MOA and pattern of use, systemics also carry the real or potential disadvantage of resistance development.

Systemic fungicides

Systemic fungicides exhibit either apoplastic mobility (movement within the free intercellular space, cell walls and xylem elements) governed by diffusion and the rate of transpiration or symplastic mobility (movement through plasmodesmata from cell to cell) involving uptake and distribution via the phloem. Some systemics may exhibit both apoplastic and symplastic movement, although the balance is determined by their physicochemical characteristics which, in all but very rare examples, favour the former. Most fungicides referred to in the literature as systemic do not comply with the accepted physiological definition, being restricted to acropetal movement; that is, redistribution towards the plant apex or leaf margins. Systemic fungicides act at specific biochemical sites (site-specific). For example, the triazole class of fungicides inhibits sterol biosynthesis at the C14-demethylation step. Systemic fungicides can have protectant, curative or eradicant activity.

Non-systemic fungicides

Non-systemic fungicides do not penetrate the plant. On application, they reside on the surfaces of foliage and fruits. Redistribution in the crop (and loss) occurs through the vapour phase or through the action of rainfall. In many cases, non-systemics are not redistributed and their action is limited to treated foliage. A disadvantage of non-systemic materials is their dependence on complete spray coverage of the target crop being achieved. Non-systemic fungicides are generally multi-site inhibitors, eliciting a response through the disruption of several biochemical processes. This is achieved through their ability to bind with chemical groups, such as thiol moieties, common to many enzymes.

Protectant fungicides

A protectant fungicide is one that is applied prophylactically (i.e. before the infection has been observed) to the target crop. Because of their activity against one or more of the early stages of fungal infection, from spore germination to the preliminary penetration of host tissue, no symptoms of disease develop. Immobile protectant

fungicides are usually only slightly soluble in water. On the leaf surface, uptake into the target fungus from a dilute solution of fungicide permits more solid fungicide residue to dissolve. Further uptake into the fungus results in an accumulation of toxicant until a lethal level is attained. Non-systemic fungicides are, by definition, protectants. Systemic fungicides may also possess strong protectant characteristics.

Curative and eradicant fungicides

Curative and eradicant activities are characteristic of most systemic fungicides. Curative activity is confined to the post-infection, pre-symptomatic phase of fungal infection, and the visible effects are the same as for protectant materials. Eradicant activity describes the effects of fungicides on the post-symptomatic stage of host colonization, for example action against mycelial growth of powdery mildew.

Systemic fungicides that are solely protectants are unusual, and in some cases their MOA is not well understood. Quinoxyfen, a fungicide with long-term activity against powdery mildews, especially ERYSGT, inhibits appressorium formation. However, the mechanism of its movement from the site of application to leaves not developed at the time of treatment, and then to the germinating conidia on the surface of those leaves, has still to be elucidated. Other recently discovered materials act through induction of the host's natural defence mechanisms.

Mobile fungicides

Mobile fungicides may be systemic or non-systemic, and are redistributed from their sites of application to tissue that has not been affected by treatment or that is not present at the time of treatment. This can occur within individual plants (systemics) or within the crop via the vapour phase (systemics and non-systemics).

Modes of Action

Full details of fungicide classes are found in texts such as *Modern Crop Protection Compounds* (Krämer *et al.*, 2012) and *The Pesticide Manual* (Tomlin, 2009). The following is intended as a summary of the most important and interesting classes. The biological and biochemical MOA is described for most fungicides. This is an area of intense research and an up-to-date classification is maintained by the Fungicide Resistance Action Committee (FRAC) whose website (http://www.frac.info/) is a mine of information.

Several fungicides have unknown or poorly defined MOAs, but a dozen broad MOAs and 60 detailed MOAs are described (see Table 5.1). The poorly defined group include the multi-sites, which are believed to simultaneously inhibit several fungal functions.

The broad classes are inhibition of:

A. Nucleic acid synthesis.
B. Mitosis and cell division.
C. Respiration.

Table 5.1. Fungicide classification.

Mode of action	Code and target site	Group name (abbreviation)	Chemical group	Common name(s)	FRAC code
A; nucleic acid synthesis	A1; RNA polymerase	Phenylamides (PAs)	Acylalanines	Benalaxyl Furalaxyl Metalaxyl	4
			Oxazolidinones	Oxadixyl	
			Butyrolactones	Ofurace	
	A2; adenosine deaminase	Hydroxy-(2-amino-) pyrimidines	Hydroxy-(2-amino-) pyrimidines	Bupirimate Dimethirimol Ethirimol	8
	A3; DNA/RNA synthesis (proposed)	Heteroaromatics	Isoxazoles	Hymexazole	32
			Isothiazolones	Octhilinone	
B; mitosis and cell division	B1; β-tubulin assembly in mitosis	Methyl benzimidazole carbamates (MBCs)	Benzimidazoles	Benomyl Carbendazim Fuberidazole Thiabendazole	1
			Thiophanates	Thiophanate Thiophanate-methyl	
	B2; β-tubulin assembly in mitosis	*N*-Phenylcarbamates	*N*-Phenylcarbamates	Diethofencarb	10
	B3; β-tubulin assembly in mitosis	Benzamides	Toluamides	Zoxamide	22
		Thiazole carboxamide	Ethylaminothiazole carboxamide	Ethaboxam	
	B4; cell division (proposed)	Phenylureas	Phenylureas	Pencycuron	20
	B5; delocalization of spectrin-like proteins	Benzamides	Pyridinylmethyl-benzamides	Fluopicolide	43

C; respiration	C1; complex I: NADH oxidoreductase	Pyrimidinamines	Pyrimidinamines	Diflumetorim	39
		Pyrazole-MET1	Pyrazole-5-carboxamide	Tolfenpyrad	
	C2; complex II: succinate dehydrogenase	Succinate dehydrogenase inhibitors (SDHIs)	Phenylbenzamides	Benodanil	7
				Flutolanil	
				Mepronil	
			Phenyl-oxo-ethyl-thiophene amide	Isofetamid	
			Pyridinylethylbenzamides	Fluopyram	
			Furan carboxamides	Fenfuram	
			Oxathiin carboxamides	Carboxin	
				Oxycarboxin	
			Thiazole carboxamides	Thifluzamide	
			Pyrazole-4-carboxamides	Benzovindiflupyr	
				Bixafen	
				Fluxapyroxad	
				Furametpyr	
				Isopyrazam	
				Penflufen	
				Penthiopyrad	
				Sedaxane	
			Pyridine carboxamides	Boscalid	
	C3; complex III: cytochrome bc1 (ubiquinol oxidase) at Qo site (*cytb* gene)	Quinone outside inhibitors (QoIs)	Methoxyacrylates	Azoxystrobin	11
				Coumoxystrobin	
				Enoxastrobin	
				Flufenoxystrobin	
				Picoxystrobin	
				Pyraoxystrobin	
			Methoxycarbamates	Pyraclostrobin	11
				Pyrametostrobin	
				Triclopyricarb	

Continued

Table 5.1. Continued.

Mode of action	Code and target site	Group name (abbreviation)	Chemical group	Common name(s)	FRAC code
			Oximino acetates	Kresoxim-methyl Trifloxystrobin	
			Oximino acetamides	Dimoxystrobin Fenaminstrobin Metominostrobin Orysastrobin	
			Oxazolidine diones	Famoxadone	
			Dihydrodioxazines	Fluoxastrobin	
			Imidazolinones	Fenamidone	
			Benzylcarbamates	Pyribencarb	
	C4; complex III: cytochrome bc1 (ubiquinone reductase) at Qi site (QiI)	Quinone inside inhibitors (QiIs)	Cyano-imidazole	Cyazofamid	21
			Sulfamoyl-triazole	Amisulbrom	
	C5; uncouplers of oxidative phosphorylation		Dinitrophenylcotonates	Binapacryl Meptyldinocap Dinocap	29
			2,6-Dinitro-anilines	Fluazinam	
	C6; inhibitors of oxidative phosphorylation, ATP synthase	Organo-tin compounds	Triphenyl-tin compounds	Fentin acetate Fentin chloride Fentin hydroxide	30
	C7; ATP production (proposed)	Thiophene carboxamides	Thiophene carboxamides	Silthiofam	38
	C8; complex III: cytochrome bc1 (ubiquinone reductase) at Qx (unknown) site	Quinone x inhibitors (QxIs)	Triazolopyrimidylamine	Ametoctradin	45

D; amino acid and protein synthesis	D1; methionine biosynthesis (proposed) (*cgs* gene)	Anilinopyrimidines (APs)	Anilinopyrimidines	Cyprodinil Mepanipyrim Pyrimethanil	9
E; signal transduction	E1; signal transduction (mechanism unknown)	Azanaphthalenes	Aryloxyquinoline Quinazolinone	Quinoxyfen Proquinazid	13
	E2; MAP/histidine kinase in osmotic signal transduction (os-2, HOG1)	Phenylpyrroles (PPs)	Phenylpyrroles	Fenpiclonil Fludioxonil	12
	E3; MAP/histidine kinase in osmotic signal transduction (os-1, Daf1)	Dicarboximides	Dicarboximides	Chlozolinate Iprodione Procymidone Vinclozolin	2
F; lipid synthesis and membrane integrity	F2; phospholipid biosynthesis, methyltransferase	Phosphorothiolates	Phosphorothiolates	Edifenphos Iprobenfos Pyrazophos	6
		Dithiolanes	Dithiolanes	Isoprothiolane	
	F3; lipid peroxidation (proposed)	Aromatic hydrocarbons (AHs), e.g. chlorophenyl, nitroanilines	Aromatic hydrocarbons	Biphenyl chloroneb Dicloran Quintozene Tecnazene Tolclofos-methyl	14
		Heteroaromatics	1,2,4-Thiadiazoles	Etridiazole	
	F4; cell membrane permeability, fatty acids (proposed)	Carbamates	Carbamates	Iodocarb Propamocarb Prothiocarb	28
	F6; microbial disrupters of pathogen cell membranes	Microbial (*Bacillus* spp.)	*Bacillus* spp. and the fungicidal lipopeptides produced	*Bacillus* spp. and the fungicidal lipopeptides produced	44

Continued

Table 5.1. Continued.

Mode of action	Code and target site	Group name (abbreviation)	Chemical group	Common name(s)	FRAC code
G; sterol biosynthesis in membranes	G1; C14-demethylase in sterol biosynthesis (*erg11*/*cyp51*)	Demethylation inhibitors (DMIs) (steroid biosynthesis inhibitor (SBI) Class I)	Piperazines	Triforine	3
			Pyridines	Pyrifenox	
				Pyrisoxazole	
			Pyrimidines	Fenarimol	
				Nuarimol	
			Imidazoles	Mazalil	
				Oxpoconazole	
				Pefurazoate	
				Prochloraz	
				Triflumizole	
			Triazoles	Azaconazole	
				Bitertanol	
				Bromuconazole	
				Cyproconazole	
				Difenoconazole	
				Diniconazole	
				Epoxiconazole	
				Etaconazole	
				Fenbuconazole	
				Fluquinconazole	
				Flusilazole	
				Flutriafol	
				Hexaconazole	
				Imibenconazole	
				Ipconazole	
				Metconazole	
				Myclobutanil	
				Penconazole	
				Propiconazole	

				Simeconazole	
				Tebuconazole	
				Tetraconazole	
				Triadimefon	
				Triadimenol	
				Triticonazole	
			Triazolinthiones	Prothioconazole	
	G2; Δ^{14}-reductase and $\Delta^{8}\rightarrow\Delta^{7}$-isomerase in sterol biosynthesis (*erg24*, *erg2*)	Amines ('morpholines') (SBI Class II)	Morpholines	Aldimorph	5
				Dodemorph	
				Fenpropimorph	
				Tridemorph	
			Piperidines	Fenpropidin	
				Piperalin	
			Spiroketalamines	Spiroxamine	
	G3; 3-ketoreductase, C4-demethylation (*erg27*)	(SBI Class III)	Hydroxyanilides	Fenhexamid	17
			Aminopyrazolinone	Fenpyrazamine	
	G4; squalene epoxidase in sterol biosynthesis (*erg10*)	(SBI Class IV)	Thiocarbamates	Pyributicarb	18
H; cell wall biosynthesis	H5; cellulose synthase	Carboxylic acid amides (CAAs)	Cinnamic acid amides	Dimethomorph	40
				Flumorph	
				Pyrimorph	
I; melanin synthesis in cell wall	I1; reductase in melanin biosynthesis	Melanin biosynthesis inhibitors–reductase (MBI-R)	Isobenzo-furanone	Fthalide	16.1
			Pyrrolo-quinolinone	Pyroquilon	
			Triazolobenzo-thiazole	Tricyclazole	
	I2; dehydratase in melanin biosynthesis	Melanin biosynthesis inhibitors–dehydratase (MBI-D)	Cyclopropane-carboxamide	Carpropamid	16.2
			Carboxamide	Diclocymet	
			Propionamide	Fenoxanil	

Continued

Table 5.1. Continued.

Mode of action	Code and target site	Group name (abbreviation)	Chemical group	Common name(s)	FRAC code
P; host plant defence induction	P1; salicylic acid pathway	Benzothiadiazole (BTH)	Benzothiadiazole	Acibenzolar-*S*-methyl (ASM)	P1
	P2	Benzisothiazole	Benzisothiazole	Probenazole	P2
	P3	Thiadiazole carboxamide	Thiadiazole carboxamide	Tiadinil Isotianil	P3
Unknown	Unknown	Cyanoacetamide oxime	Cyanoacetamide oxime	Cymoxanil	27
		Phosphonates	Ethyl phosphonates	Fosetyl-Al Phophorous acid and salts	33
		Benzotriazines	Benzotriazines	Triazoxide	34
		Benzene sulfonamides	Benzene sulfonamides	Flusulfamide	36
		Pyridazinones	Pyridazinones	Diclomezine	37
		Thiocarbamate	Thiocarbamate	Methasulfocarb	42
		Phenylacetamide	Phenylacetamide	Cyflufenamid	U6
	Actin disruption (proposed)	Arylphenylketone	Benzophenone Benzoylpyridine	Metrafenone Pyriofenone	U8
	Cell membrane disruption (proposed)	Guanidines	Guanidines	Dodine	U12
	Unknown	Pyrimidinone hydrazones	Pyrimidinone hydrazones	Ferimzone	U14
Multi-site	Multi-site contact activity	Inorganic	Inorganic	Copper salts	M1
				Sulfur	M2
		Dithiocarbamates and relatives	Dithiocarbamates and relatives	Ferbam Mancozeb Maneb Metiram Propineb Thiram Zineb Ziram	M3

	Phthalimides	Phthalimides	Captan Captafol Folpet	M4
	Chloronitriles (phthalonitriles)	Chloronitriles (phthalonitriles)	Chlorothalonil	M5
	Sulfamides	Sulfamides	Dichlofluanid Tolylfluanid	M6
	Guanidines	Guanidines	Guazatine Iminoctadine	M7
	Triazines	Triazines	Anilazine	M8
	Quinones (anthraquinones)	Quinones (anthraquinones)	Dithianon	M9
	Quinoxalines	Quinoxalines	Chinomethionate/quinomethionate	M10

FRAC, Fungicide Resistance Action Committee.

D. Amino acid and protein synthesis.
E. Signal transduction.
F. Lipid synthesis and membrane integrity.
G. Sterol biosynthesis in membranes.
H. Cell wall biosynthesis.
I. Melanin biosynthesis in the cell wall.
P. Activation of plant host defence.

This list includes many fundamental biochemical functions common to all organisms and shows that the key to success of fungicides is the specificity that enables fungal processes to be inhibited by compounds that do not cause undue damage to the plant hosts and other non-target organisms.

Fungicides are grouped first by target site or, if there are multiple target sites, into one of a number of multi-site clusters. Most target site groups correspond to a single formal 'group' – e.g. SDHI and QoI; other target site groups are in broad chemical groups – e.g. the B3 group is divided into benzamides and thiazole carboxamides. Abbreviations for these groups – such as AH, DMI, CAA, QoI, SBI, PA, CAA and SDHI – are widely used in the academic and promotional literature. Most groups are subdivided into a small number of chemical groups. For example, the QoI group is divided into eight chemical groups; the DMI group into five. The chemical groups are named according to the common structural element they possess.

A; Inhibition of RNA synthesis

A1; Phenylamides

These compounds include the acylalanines, butyrolactones and one member of the oxazolidinones (Fig. 5.1) and have specific activity against oomycete fungi (Table 5.2). The basis for the specificity is unknown. The acylalanine metalaxyl is the most extensively studied member of the group. Metalaxyl acts by inhibiting the synthesis of ribosomal RNA via the RNA polymerase I–template complex (Davidse, 1986), resulting in the disruption of protein synthesis. Like many fungicides, metalaxyl exists as a mixture of enantiomers. It has been established that metalaxyl-M is the more active of the two enantiomers (Nuninger *et al.*, 1996).

Phenylamides (PAs) act at specific developmental stages in the oomycete infection process. The release of zoospores from sporangia, their movement, encystment and subsequent germination, as well as the initial penetration and primary haustorium development, are relatively insensitive. However, the development of pathogens beyond the formation of the primary haustorium is well controlled. This late but specific inhibition of fungal development is explained by the biochemical MOA. In the early life cycle, sporangia and zoospores are sufficiently supplied with ribosomes to permit zoospore formation, germination, penetration and formation of primary haustoria to proceed, even in the presence of phenylamide fungicides. At later stages, continuing inhibition of the RNA polymerase I complex becomes increasingly effective and results in the thickening of hyphal cell walls and eventual cell death. These characteristic symptoms develop through an accumulation of RNA precursors, the nucleoside triphosphates, which promote the activity of fungal β(1,3)-glucan synthetase and the synthesis of cell-wall constituents (Szaniszlo *et al.*, 1985).

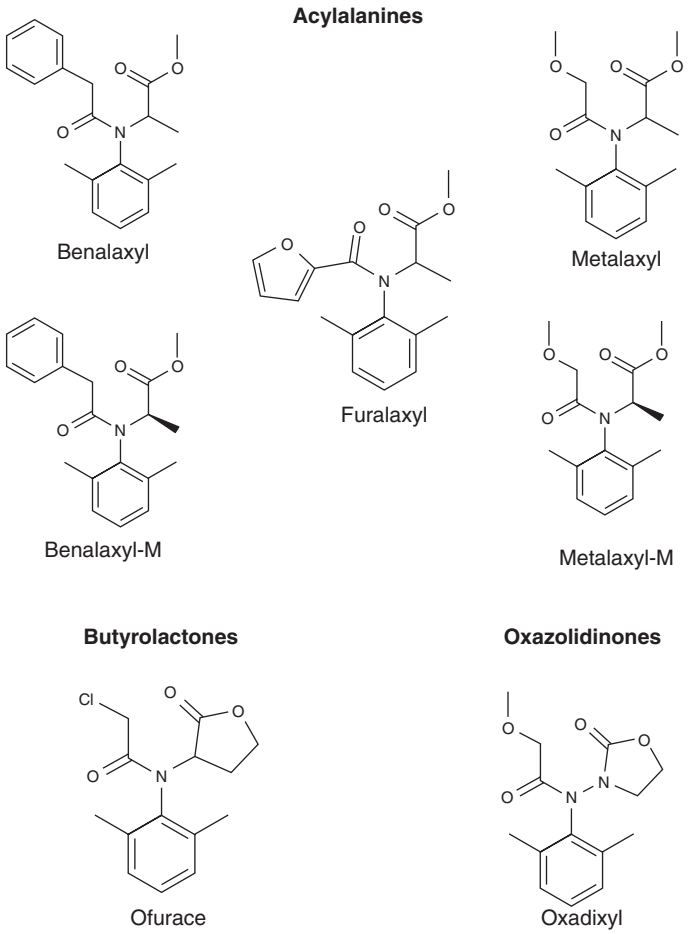


Fig. 5.1. Phenylamides.

The PA fungicides are used as protectants and curatives in seed treatments and in root and foliar applications. They are systemic, mainly via the apoplast, but metalaxyl has been reported to move to a limited extent via the symplast.

Resistance is a major problem for PA fungicides with cross-resistance between each fungicide. Even though the biochemical target has been identified, the molecular basis of the resistance is not yet known. A resistance management plan has been introduced.

A2; Hydroxy-(2-amino-)pyrimidines

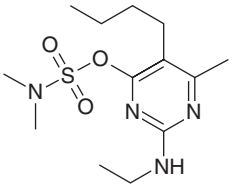
The hydroxypyrimidines (Fig. 5.2) are highly specific to the control of powdery mildews. Ethirimol is a systemic used to control powdery mildews in cereals and other field crops. It is especially active against barley powdery mildew and is used mainly as seed treatment. Dimethirimol was introduced to control powdery mildew in glasshouse cucurbits. Bupirimate is mainly used to control powdery mildews in apple and ornamentals.

Table 5.2. The spectrum[a] of different classes of fungicide.

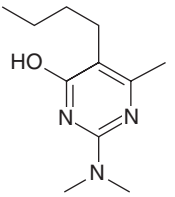
Mode of action (A1 to U)	Group name	OO	B	GFA	GSA	PM	BC	PY
A1	Phenylamides (PAs)	A	N	N	N	N	N	N
A2	Hydroxy-(2-amino-) pyrimidines	N	N	N	N	A	N	N
A3	Heteroaromatics	S	N	N	S	N	N	N
B1	Methyl benzimidazole carbamates (MBCs)	N	S	A	S	S	S	A
B2	*N*-Phenylcarbamates	N	N	N	N	N	A	N
B3	Benzamides	A	N	N	N	N	N	N
B4	Phenylureas	N	N	S	S	N	N	N
B5	Benzamides	A	N	N	N	N	N	N
C1	Pyrimidinamines	N	N	S	S	A	A	S
C2	Succinate dehydrogenase inhibitors (SDHIs)	N	S	S	S	S	S	S
C3	Quinone outside inhibitors (QoIs)	S	S	S	S	S	A	A
C4	Quinone inside inhibitors (QiIs)	A	N	N	N	N	N	N
C5	Uncouplers	A	N	N	N	S	N	N
C6	Organo-tin	S	S	S	S	S	S	S
C7	Thiophene-carboxamides	N	N	N	S	N	N	N
C8	Quinone x inhibitors (QxIs)	A	N	N	N	N	N	N
D1	Anilinopyrimidines (APs)	N	N	S	N	S	S	S
E1	Azanaphthalenes	N	N	N	N	A	N	N
E2	Phenylpyrroles (PP)	N	S	N	S	S	S	S
E3	Dicarboximides	N	S	N	S	N	S	S
F2	Phosphorothiolates	N	N	S	N	N	S	S
F3	Aromatic hydrocarbons (AHs)	N	S	N	S	N	N	N
F4	Carbamates	S	N	N	N	N	N	N
G1	Steroid biosynthesis inhibitor (SBI) Class I	N	S	S	S	S	N	S
G2	SBI Class II	N	S	S	N	A	N	N
G3	SBI Class III	N	N	N	N	N	A	N
G4	SBI Class IV	N	S	N	S	N	N	N
H5	Carboxylic acid amides (CAAs)	A	N	N	N	N	N	N
I1/2	Melanin biosynthesis inhibitors (MBIs)	N	N	N	N	N	N	A
P1/2/3	Benzothiadiazole (BTH)	S	S	S	S	S	S	S
U	Various	S	S	S	S	S	S	S
U	Arylphenylketone	N	N	N	N	A	N	N
U	Guanidines	N	N	N	S	N	N	N
Multi-site	Various	S	S	S	S	S	S	S

[a]A = all, S = some, N = none of the following pathogen subgroups: OO, *Oomycota*; B, *Basidiomycota*; GFA, general foliar *Ascomycota*; GSA, general soil or seed *Ascomycota*; PM, powdery mildew; BC, BOTCIN; PY, PYRIOR.

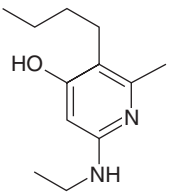
Hydroxy–(2-amino-)pyrimidines



Bupirimate



Dimethirimol



Ethirimol

Fig. 5.2. Hydroxypyrimidines.

Typically, hydroxypyrimidines inhibit germ-tube elongation and appressorium formation. Hydroxypyrimidines act through the inhibition of adenosine deaminase, an enzyme in the purine salvage pathway. Adenosine deaminase is not present in plants but is found in a wide range of fungi. However, it is only the adenosine deaminase activity from powdery mildew fungi that is sensitive to ethirimol, while the enzyme activity from other fungal species is generally not affected.

B; Mitosis and cell division

B1; β-Tubulin assembly, methyl benzimidazole carbamates

The original popularity of the benzimidazoles in the marketplace was based on their practical performance in the control of a wide range of ascomycetes and basidiomycetes. However, they lack activity against oomycetes (Table 5.2; Delp, 1995) and resistance has become a major issue in most markets. The benzimidazoles were key in the development of systemic fungicides in the 1960s and included benomyl, carbendazim, thiophanate-methyl, fuberidazole and thiabendazole (Fig. 5.3).

Benomyl has protective and eradicant activity against pathogens of cereals, vines, fruit, rice and vegetables, and is used in postharvest treatments. It is converted in plants, soils and animals to the methyl-2-yl-carbamates otherwise known as carbendazim. Thiophanate-methyl undergoes a similar conversion to carbendazim. Carbendazim, as the hydrochloride, hypophosphite and phosphate, is also used to control *Ceratocystis ulmi* (Dutch elm disease).

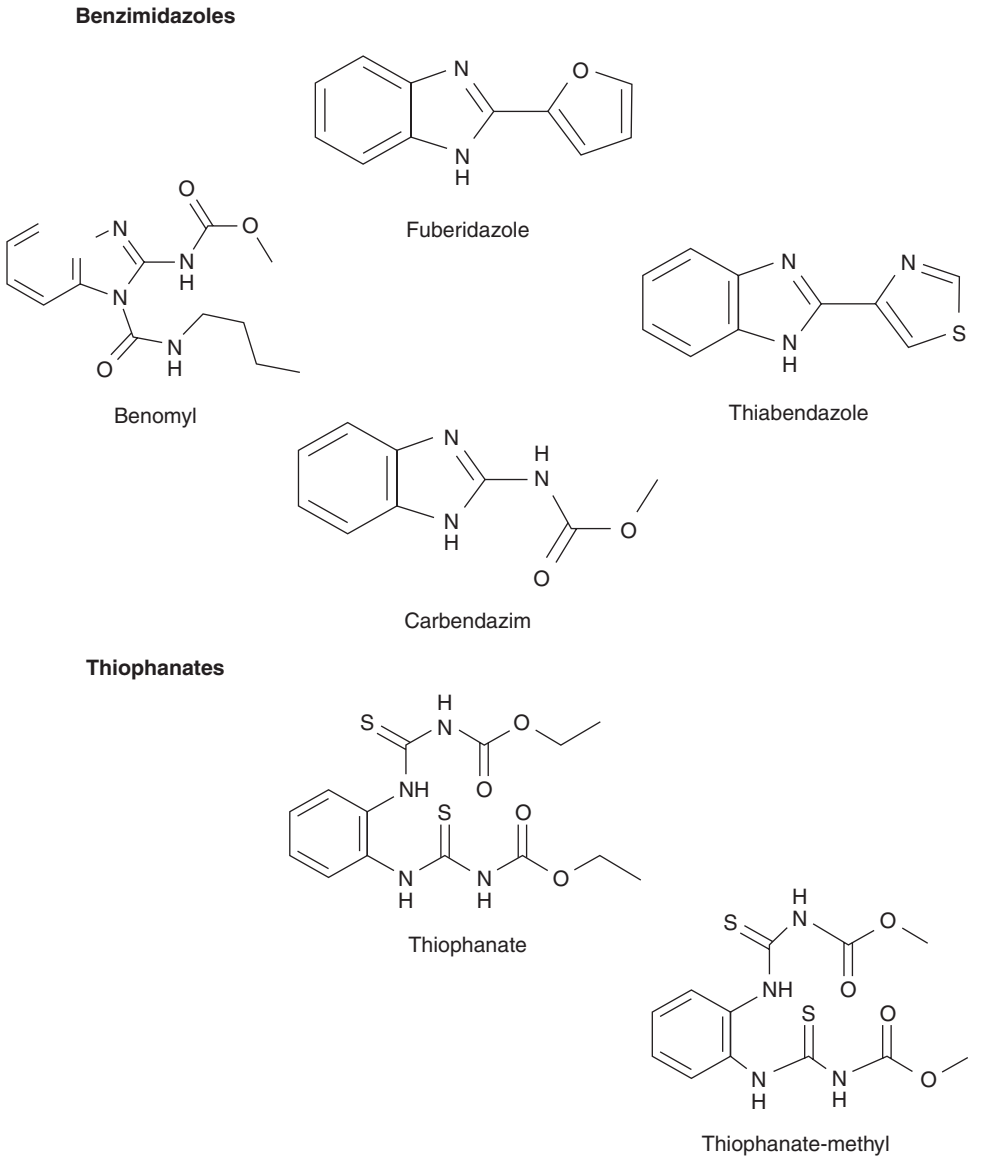


Fig. 5.3. Benzimidazoles and thiophanates.

Thiabendazole was originally introduced by Merck and Co. Ltd in 1961 as an antihelminthic. Its fungicidal and systemic properties were demonstrated in 1964, and it was widely used in the control of a range of ascomycete and basidiomycete fungi in vegetables, plantation crops, fruit, row crops, turf, protected crops and cereals. Curiously rust fungi are not controlled by this group. Fuberidazole was first prepared in 1936 but not exploited as a fungicide until 1968. It is used as a component of cereal seed treatments.

The MOA of the benzimidazoles is well researched and based on their effects on tubulin integrity. Microtubules are alternating helices of β- and α-tubulins, which

form an essential part of the cytoskeleton and are active in spindle formation and the segregation of chromosomes in cell division. Benzimidazoles disrupt mitosis during cell division at metaphase. The mitotic spindle is distorted and daughter nuclei fail to separate, resulting in cell death. These morphological changes in treated fungi correlate with biochemical studies that demonstrate the high affinity of benzimidazoles for tubulin proteins in sensitive fungi (Davidse, 1986).

Molecular biology techniques have confirmed β-tubulin as the target site (Fujimura *et al.*, 1990). Benzimidazoles are highly selective despite the highly conserved nature of β-tubulins in all eukaryotic organisms. Oomycete fungi and all plants are insensitive to the benzimidazoles. The basis of selectivity probably depends on structural differences at the binding sites of the microtubules. The modification of a single amino acid (from phenylalanine to tyrosine, F200Y; see Box 6.1 for an explanation of this nomenclature) resulting from a mutational change in β-tubulin confers resistance to carbendazim in *Neurospora* spp. In *Saccharomyces* spp., resistance is governed by a similar change, from arginine to histidine.

Resistance in the field is a serious issue for MBC fungicides. A number of fungal species developed resistance via mutation, with either the F200Y or E198A,G,K mutation being commonly found. The resistance factor (the ratio of the sensitivity of the resistant over the sensitive isolate – see Chapter 6 for details) is very high and no fitness penalty appears to ensue. For these reasons, MBC fungicides have been withdrawn from many markets. They are also under suspicion of toxic effects on animals including humans. As a result, MBCs are in decline and used only in niche markets such as legumes in Australia.

B2; Phenylcarbamates

The phenylcarbamates, as represented now just by diethofencarb (Fig. 5.4), have a similar action as the MBCs but are active against benzimidazole-resistant fungi (Ishii *et al.*, 1995). This is a rare example of negative cross-reactivity (see Chapter 6). Their gross activity in the disruption of mitosis is similar to the benzimidazoles and studies suggest the presence of a common binding region on the β-tubulin protein (Fujimura *et al.*, 1990). A mutation that results in a single amino acid change is associated with resistance to carbendazim and is the basis of negative cross-resistance between carbendazim and phenylcarbamates (Butters *et al.*, 1995).

B3; Benzamides

Microscopy studies suggest that benzamides also interfere with microtubules (Young, 1991) but in contrast to the MBCs, they only have activity against oomycetes. Zoxamide and ethaboxam (Fig. 5.5) are currently on the market (Malandrakis *et al.*, 2011).

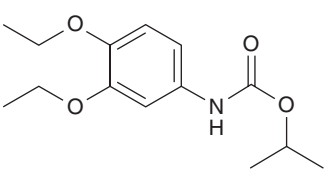


Fig. 5.4. Diethofenocarb.

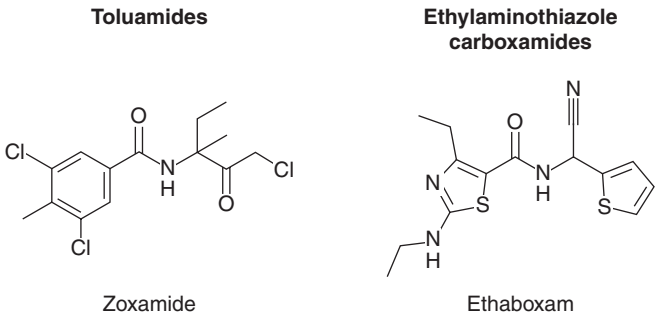


Fig. 5.5. Zoxamide and ethaboxam.

C; Respiration

The mitochondrial respiration chain has proved to be a fertile source of potent and broad-spectrum inhibitors of fungal and oomycete development. Specific inhibitors of many of the processes of mitochondrial ATP production have been modified to generate several of the currently most important fungicide classes (Fig. 5.6). The mitochondrial respiration chain is ubiquitous in both target and non-target organisms and the protein sequences in the five major complexes (I, NADH dehydrogenase; II, succinate dehydrogenase; III, cytochrome bc1 complex; IV, cytochrome c oxidase; and the proton ATPase) show high levels of conservation. Nonetheless, specific, highly active and safe inhibitors have been found.

C2; Succinate dehydrogenase inhibitors

Succinate dehydrogenase occurs in the respiratory chain as part of a complex: succinate dehydrogenase complex or complex II. The complex contains non-haem iron–sulfur proteins that act in the transfer of electrons from reduced flavin adenine dinucleotide (FAD) to coenzyme Q. Succinate dehydrogenase contributes both to electron transport and the citric acid cycle. This reaction is ubiquitous in all aerobic organisms. Inhibition of succinate dehydrogenase leads to both the starvation of ATP and the release of active oxygen. As a result the inhibitors have potent activity.

The SDHI group of fungicides has a long and interesting history and has recently undergone a major expansion resulting in a wide range of compounds with a broad spectrum and excellent activity. They now promise to rank with QoI and sterol biosynthesis inhibitors (SBIs) in importance and market size.

The first SDHIs were the oxathiin carboxamides, oxycarboxin and carboxin, and were introduced as long ago as 1966 (Fig. 5.7). They were shown to be specific inhibitors of succinate dehydrogenase (Ulrich and Mathre, 1972). The compounds had systemic activity. The genes encoding the four subunits of succinate dehydrogenase are highly conserved between organisms. It was therefore puzzling that the spectrum of the carboxins was limited to *Basidiomycota*. They were used mainly as seed treatments to control *Rhizoctonia* spp., *Ustilago* spp. and *Tilletia caries* in cereals, maize, cotton, oilseed rape and legumes (Table 5.2). Variants were tested, leading to the development of compounds like benodanil and fenfuram (1974) and

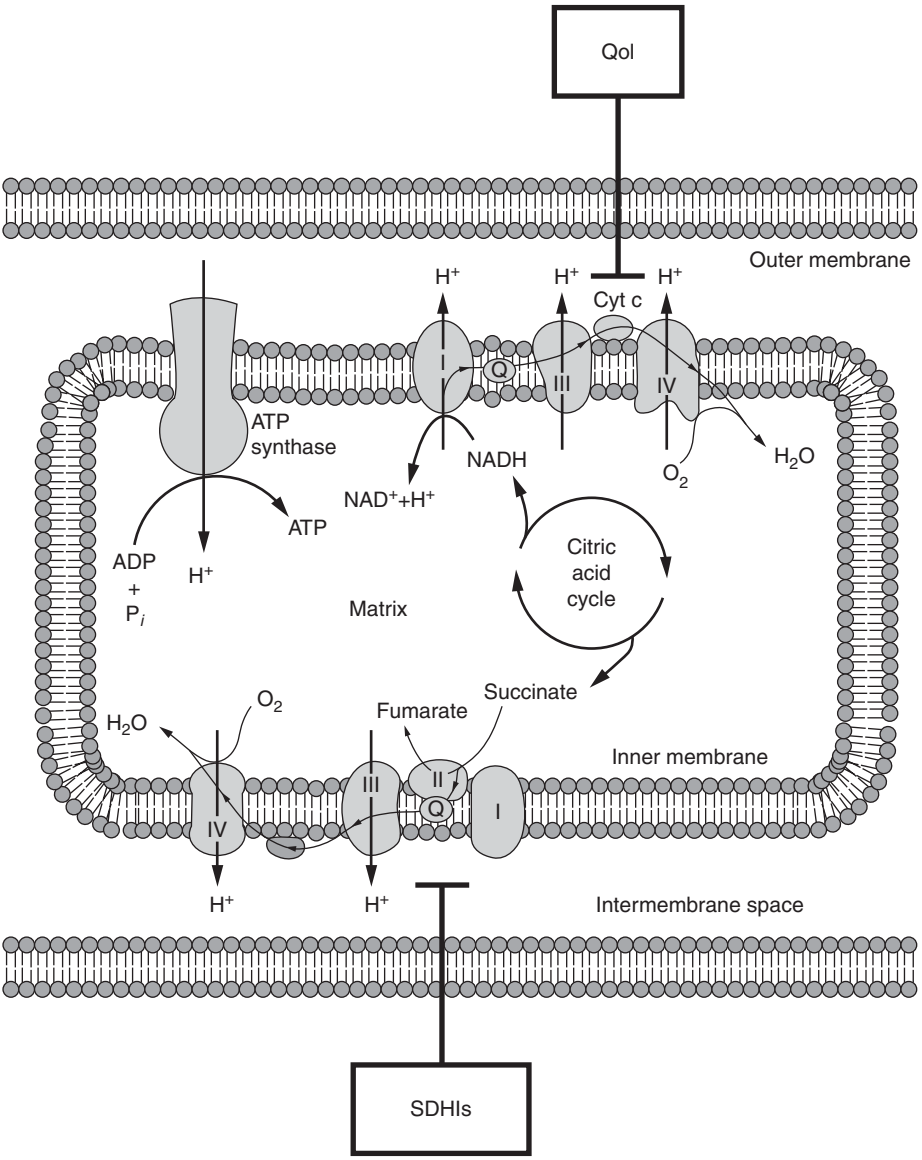


Fig. 5.6. Schematic diagram of the mitochondrion showing target sites of succinate dehydrogenase inhibitor (SDHI) and quinone outside inhibitor (QoI) fungicides. Uncouplers allow protons to re-enter the mitochondrion without passing through the ATP synthase.

methfuroxam, produced by the substitution of the 1,4-oxathiin ring by a furan moiety, and are still in use for the control of seed-borne pathogens in cereals. Similarly, mepronil (1981) is used to control *R. solani* in rice, and PUCCRT and *Typhula incarnata* in wheat. These compounds suffered from a limited spectrum and poor mobility restricting the use to seed-borne *Basidiomycota*.

A breakthrough came in 2003 with the release of boscalid, a pyridine carboxamide, by BASF. This product had broad-spectrum and foliar activity against a wide

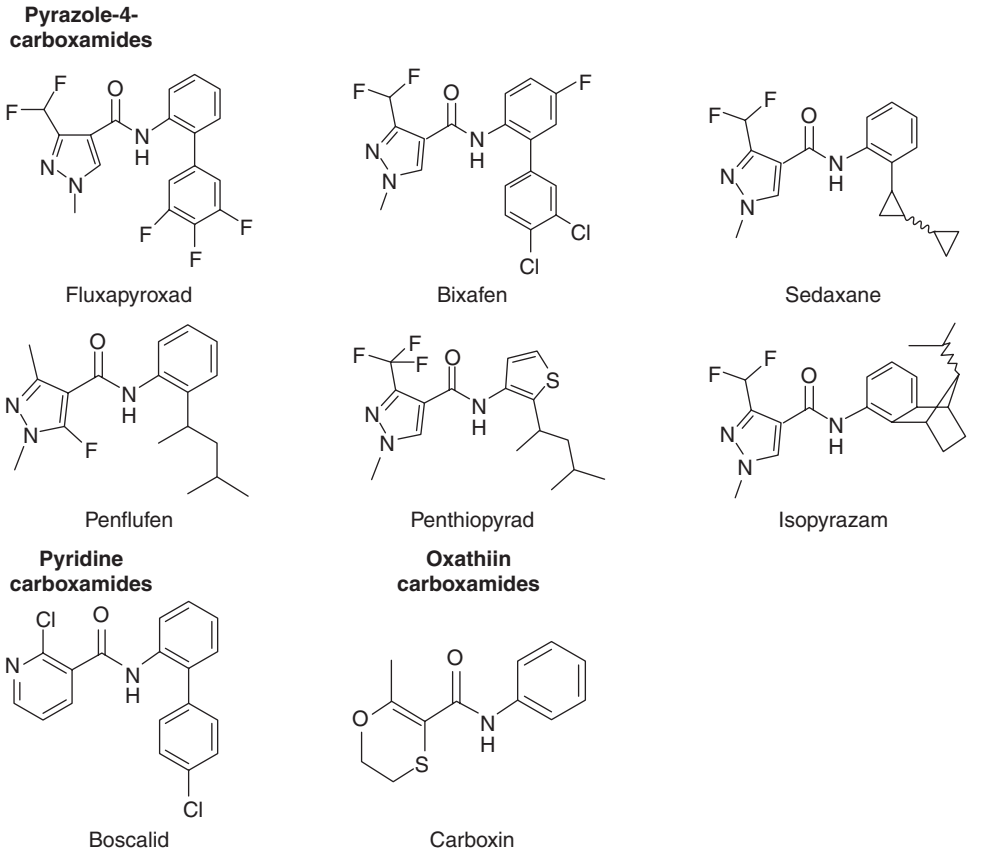


Fig. 5.7. Some succinate dehydrogenase inhibitor fungicides.

range of significant pathogens such as SEPTRI and PUCCRT. The spectrum was thus extended to *Ascomycota* pathogens, but not to oomycetes. Since then all of the major companies have released SDHIs with complementary activity and mobility characteristics. Major examples include bixafen, sedaxane, isopyrazam and penflufen. Current SDHIs mainly target foliar tissues but others such as sedexane are use in seed treatments. All of the compounds share an amide bond unit surrounded on both sides by aromatic rings of various types. Resistance is an issue for the SDHIs and so the products are normally sold in mixtures.

C3; Inhibition of complex III

This group of fungicides, formally called QoIs, but commonly called strobilurins or even 'strobis', is only a little over a decade old. The class vividly illustrates the highs and lows of the fungicide industry (Bartlett *et al.*, 2002). It includes several fungicides with annual sales approaching US$1 billion. They were inspired by a group of natural products called strobilurins. The original compounds had potent activity in the parts per billion range. Furthermore, they had an exceptional spectrum including oomycete,

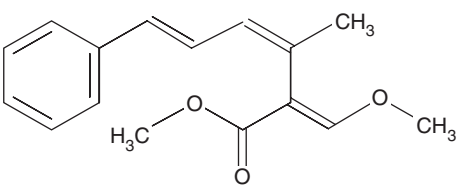
basidiomycete and all groups of ascomycete fungi. They were exceptionally non-toxic to non-target organisms including plants and animals. The acute toxicity of these compounds to animals is comparable with preservatives permitted in food. Moreover they are rapidly degraded in soil, making them environmentally benign.

The sequence of events which led to the development of the strobilurins as agricultural fungicides began in the 1960s, with the discovery by a Czech scientist, Vladimir Musilek, of a naturally occurring fungicide called strobilurin in the wood-rotting basidiomycete fungus *Strobilurus tenacellus*. This was developed for use as a medicinal agent to treat skin diseases. By the late 1970s, another antifungal secondary metabolite, oudemansin, was discovered in another basidiomycete fungus, *Oudemansiella mucida*. In 1983, BASF began to examine the potential of the strobilurins as precursors for new synthetic pesticides. By that time, the *in vitro* antifungal activity of strobilurin A was already published (Anke *et al.*, 1977; Fig. 5.8) and the MOA was shown to be the inhibition of electron transfer in complex III of mitochondrial respiration (Becker *et al.*, 1981). Although good *in vitro* activity was shown compared with synthetic standards, especially in the dark, strobilurin A possessed only weak activity *in vivo* but demonstrated an unusually broad spectrum.

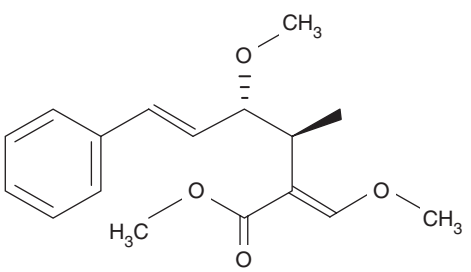
It was hypothesized that the poor transference of activity from *in vitro* to *in vivo* tests was due to the instability of the molecule, permitting rapid degradation through photolysis or metabolism. A synthesis programme was initiated to increase stability and thereby optimize *in vivo* activity.

At much the same time, studies at ICI Plant Protection (now Syngenta) investigated the activity of oudemansin A (Fig. 5.8), known to possess strong *in vivo* activity (Beautement and Clough, 1987; Beautement *et al.*, 1991). The work led to the production of a series of analogues and identified an enol ether stilbene pharmacophore as a highly active, broad-spectrum candidate (Fig 5.9).

Further work by BASF also resulted in the discovery of the enol ether stilbene pharmacophore, but showed that the molecule was photolabile. Therefore the molecule did not progress beyond tests in small plot field trials. Variations in chemical



Strobilurin A



Oudemansin A

Fig. 5.8. Strobilurin A and oudemansin A.

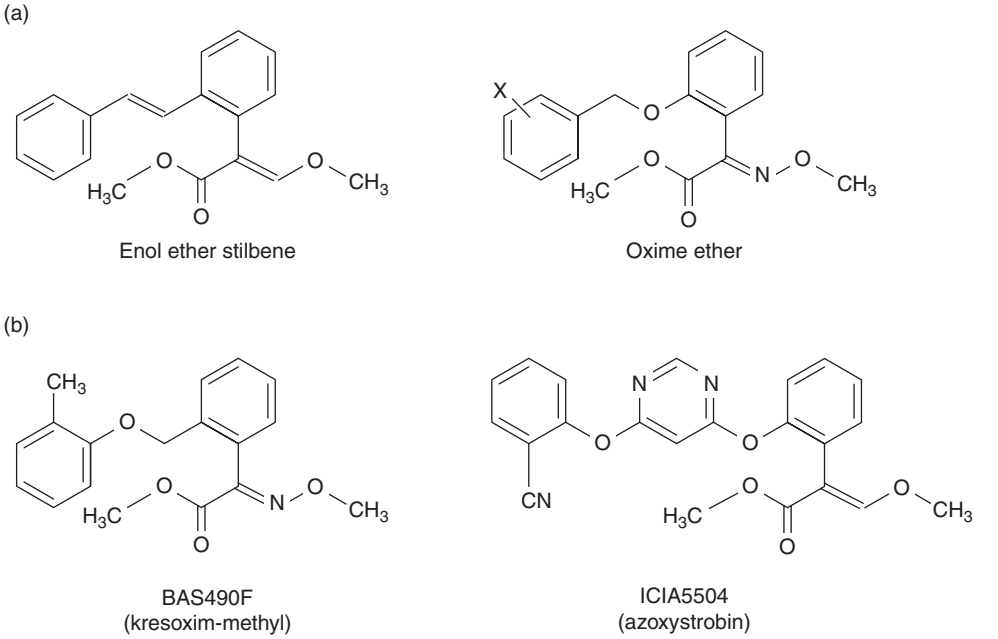


Fig. 5.9. Structures of natural (a) and released (b) quinone outside inhibitors.

structure led both companies to file patents describing the antifungal activity of the oxime ethers. Remarkably, the filings were separated by just 2 days (Sauter *et al.*, 1999).

The preferred compounds arising from the modification of the patented oxime ethers were BAS490F which was released as kresoxim-methyl (Ammermann *et al.*, 1992) and ICIA5504 which was released as azoxystrobin (Godwin *et al.*, 1992; Fig. 5.9). Both proved to be highly active compounds with broad use in a very wide range of crops and diseases. Azoxystrobin is effective against pathogens from all groups but has a particularly high potential use in the control of downy and powdery mildews of grapevine. In contrast, kresoxim-methyl is more effective than azoxystrobin against cereal powdery mildew. Although they work best as preventives, they have eradicant activity against powdery mildews.

It is remarkable that these compounds have activity against pathogens from the *Ascomycota*, *Basidiomycota* and *Oomycota* but are very safe for both plants and animals. All the other companies have developed analogues with the same MOA, though the chemical structures differ notably. The market leaders now are azoxystrobin (US$910 million sales), pyraclostrobin (US$735 million) and trifloxystrobin (US$490 million) (2009 figures; Table 5.3).

QoIs are active in the inhibition of electron transfer in complex III (bc1 complex) of the mitochondrial electron transport chain (Fig. 5.6; Becker *et al.*, 1981). Spore germination is the developmental stage of target fungi with most sensitivity to QoI, and the activity spectrum is unusually extensive. Generally, the compounds possess slow-acting systemic properties and can provide long-term disease control. Redistribution within the crop is achieved through a continuous mechanism of absorption from the waxy cuticular layer of leaves into the plant and through movement via the vapour phase and reabsorption into cuticular waxes (Sauter *et al.*, 1995).

Table 5.3. Commercialized strobilurins and other complex III inhibitors. (From Krämer *et al.*, 2012.)

Fungicide	Code number	Originator	Current owner	Launch date	Sales volume (2009, US$ million)
Kresoxim-methyl	BAS490F	BASF	BASF	1996	130
Azoxystrobin	ICIA5504	ICI	Syngenta	1997	910
Metominostrobin	SSF-126	Shionogi	Bayer	2000	<10
Trifloxystrobin	CGA279202	Ciba	Bayer	2000	490
Picoxystrobin	ZA1963	Zeneca	DuPont	2001	145
Pyraclostrobin	BAS500F	BASF	BASF	2002	735
Fluoxastrobin	HEC5725	Bayer	Bayer	2004	150
Dimoxystrobin	BAS505F	BASF	BASF	2004	50
Orysastrobin	BAS520F	BASF	BASF	2007	45
Famoxadone	DPXJE874	DuPont	DuPont	1997	60
Fenamidone	EXP10745	Rhône-Poulenc	Bayer	2001	40
Cyazofamid	IKF916	Ishihara	Ishihara	2001	50
Amisulbrom	NC224	Nissan	Nissan	2008	<10

The Achilles' heel of the strobilurins was revealed less than 2 years after release. Cereal powdery mildew isolates with very high resistance were observed and these had a consistent pattern of mutation in the cytochrome b gene. All QoIs were cross-resistant. Nonetheless, the QoI group has continued to sell exceptionally well and successful resistance management practices have been established (see Chapter 6). It was also noted that the green leaf area of the plants was extended, leading to significantly higher yields even in the absence of disease. The exact mechanism of this effect is still under discussion but it is large enough to pay for the cost of application in high-yielding situations.

C5; Uncouplers

The role of the electron transport chain is to generate the electromotive force, via displacement of protons, which will drive the synthesis of ATP. Uncouplers are compounds that interfere with ATP synthesis by collapsing the electron motive force. They do this by inserting into the inner mitochondrial membrane and providing a pathway for the transport of protons down the concentration gradient. In view of this rather non-specific MOA, it is not surprising that most uncouplers are too toxic for current use. Fluazinam, a diarylamine (Fig. 5.10), is unique among commercialized uncouplers in having low mammalian toxicity. This is due to metabolism by animal tissues into innocuous products. The compound, released in 1990, has become commercially very significant as a protectant fungicide used in the control of BOTCIN, *Sclerotinia*, *Alternaria*, *Colletotrichum*, PHYTIN and VENTIN. It also controls brassica clubroot caused by the non-fungus *Plasmodiophora brassicae*. It is not systemic but can be used both as a foliar spray and for seed treatments. The parent compounds are unstable to chemical hydrolysis and, following uptake into fungi, undergo enzymatic hydrolysis

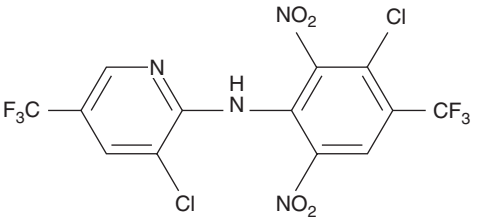


Fig. 5.10. Fluazinam.

to yield the toxic dinitrophenols, which then act as uncouplers or inhibitors of mitochondrial oxidative phosphorylation.

D; Amino acid synthesis

D1; Anilinopyrimidines

The anilinopyrimidines (APs) mepanipyrim, pyrimethanil and cyprodinil, also known as the pyrimidinamines (Fig. 5.11), are broad-spectrum fungicides and have extensive use in a wide variety of crops. Mepanipyrim and pyrimethanil are active against BOTCIN and VENTIN (Maeno and Miura, 1990; Neumann *et al.*, 1992; Daniels *et al.*, 1994). Cyprodinil has additional activity against foliar ascomycetes including powdery mildews especially for use on cereals (Heye *et al.*, 1994). The MOA has been linked to methionine biosynthesis inhibition (Masner *et al.*, 1994; Leroux and Gredt, 1995; Leroux *et al.*, 1996; Fig. 5.12). The specific target is cystathionine-γ-synthase (CGS; Fu *et al.*, 2013). Resistance is associated with alterations in the promoter of the CGS gene.

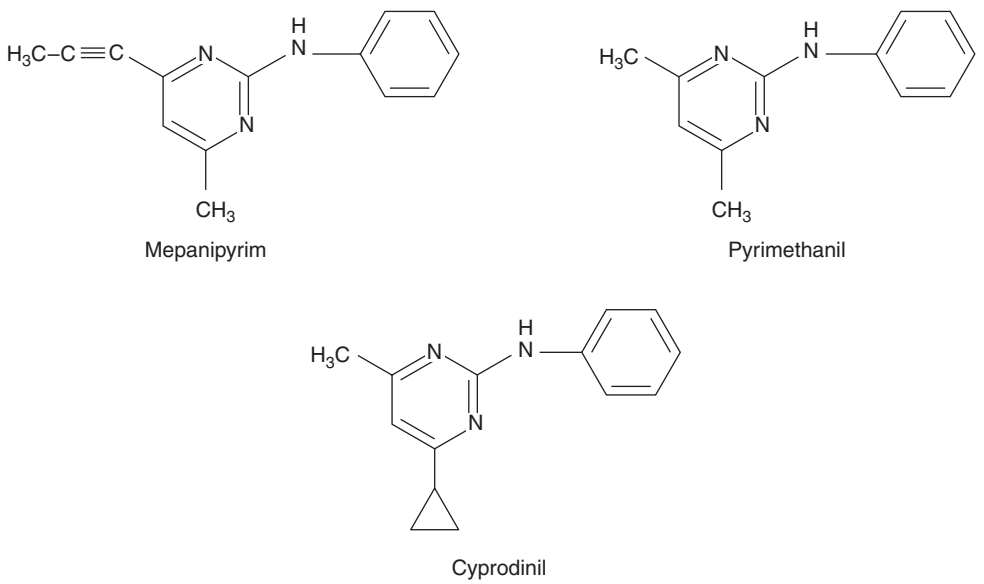


Fig. 5.11. Anilinopyrimidines.

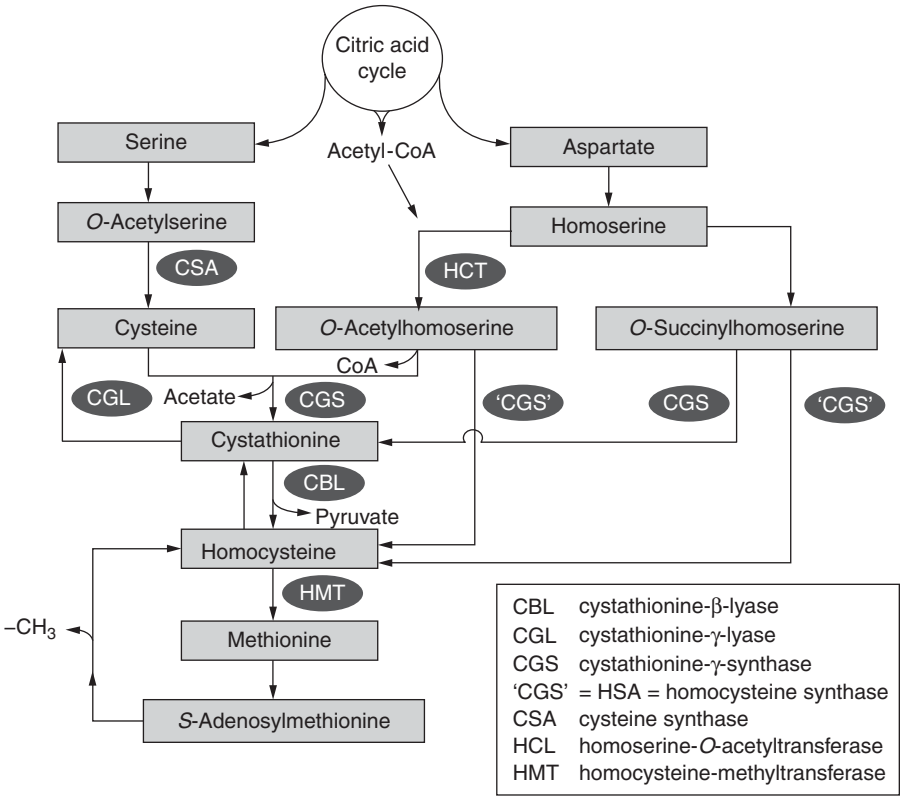


Fig. 5.12. Methionine biosynthesis pathway. (From Krämer *et al.*, 2012.)

E; Signal transduction

E1; Azanaphthalenes

Quinoxyfen and proquinazid (Fig. 5.13) are relatively recently introduced specific powdery mildewcides and they demonstrate the continuing demand for compounds to control these diseases despite the narrow spectrum. This is especially true in perennial crops like vines where genetic resistance is not available. They have little structural similarity and it remains to be seen whether they share a molecular target. Quinoxyfen was announced by DowElanco in 1996 (Longhurst *et al.*, 1996) and is unusual in its action as a systemic protectant which provides long-term control of cereal mildew. The movement of quinoxyfen through leaf sheaths to the developing basal meristem and hence to leaves not directly exposed to treatment may be involved, and other redistribution via the vapour phase may also provide a route for compound redistribution in crops. Proquinazid was introduced in 2005 by DuPont. Quinoxyfen inhibits appressorium formation by disrupting signal transduction processes (Lee *et al.*, 2008).

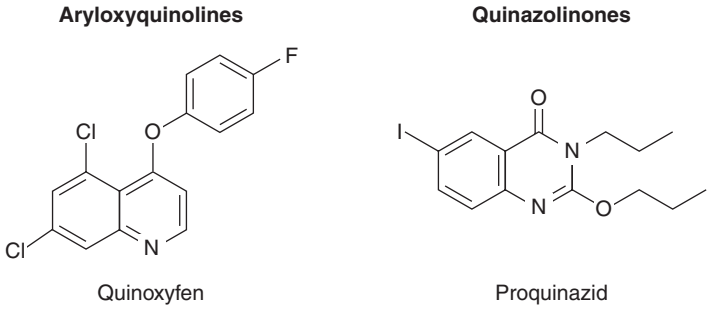


Fig. 5.13. Azanaphthalenes.

E2; Phenylpyrroles

Pyrrolnitrin is a secondary metabolite formed by *Pseudomonas pyrrocina* that has antifungal properties but is unsuitable for use in practical disease control because of its instability in light. Optimization of pyrrolnitrin led to the discovery of the commercial fungicides fenpiclonil and fludioxonil (Nevill *et al.*, 1988; Gehmann *et al.*, 1990; Fig. 5.14). The phenylpyrroles (PPs) have a broad fungal disease control spectrum but are inactive against oomycete fungi. The MOA appears to involve the MAP (mitogen-activated protein) kinase HOG1 (also known as os-2; Irmler *et al.*, 2006).

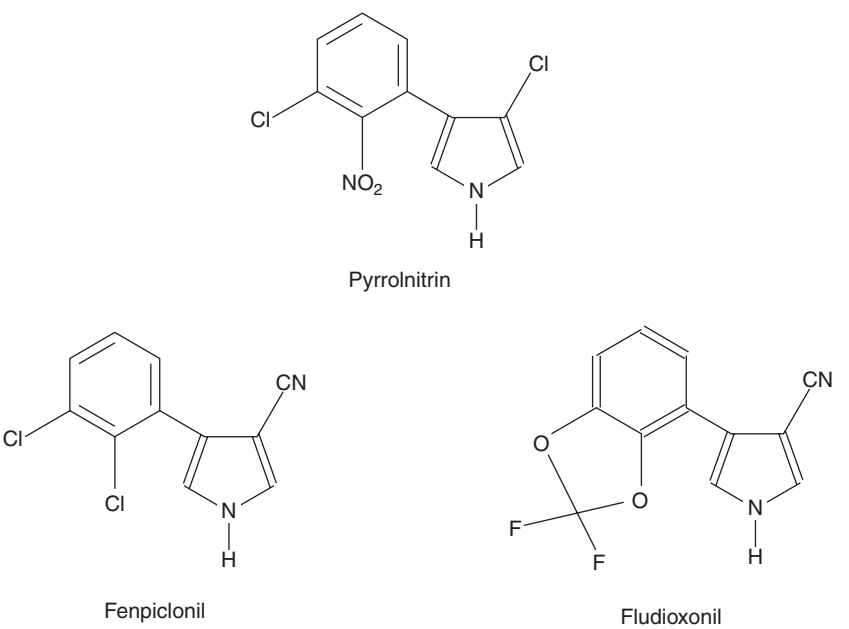


Fig. 5.14. Phenylpyrroles.

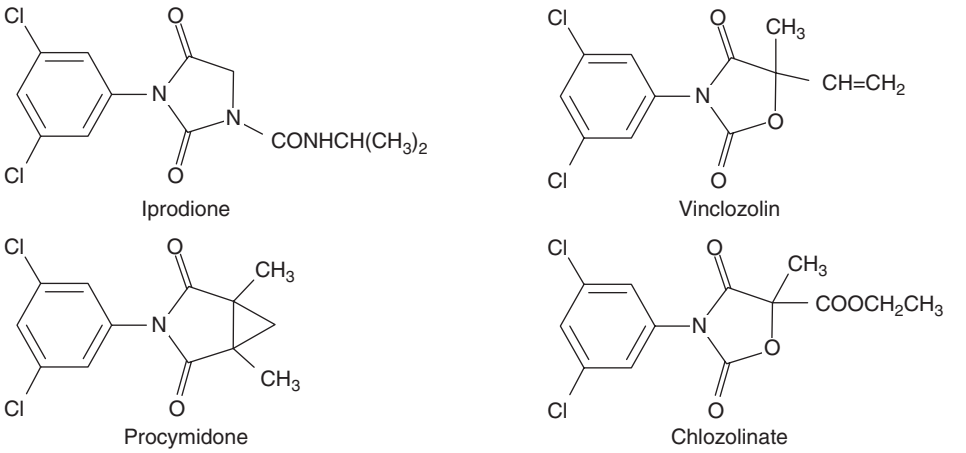


Fig. 5.15. Dicarboximides.

E3; Dicarboximides

Dichlozoline was the earliest commercial dicarboximide and was used in the control of *Sclerotinia* and BOTCIN. More recent compounds include iprodione, vinclozolin, procymidone and chlozolinate (Fig. 5.15). Their commercial strength was dependent, in part, on the occurrence of benzimidazole resistance in the target fungi *Sclerotinia* spp. and BOTCIN. The dicarboximides inhibit spore germination and cause hyphal branching, swelling and lysis. Like PPs, the MOA involves interference with kinase signalling, in this case the osmosensing histidine kinase known as Os-1 or Daf1 (Cui *et al*., 2002; Oshima *et al*., 2002).

The spectrum of the group includes BOTCIN, SEPTRI and other foliar ascomycetes in grapevine, oilseed rape, hops, ornamentals, fruit, legumes, cereals and vegetables.

G; Sterol biosynthesis in membranes

Materials that inhibit sterol biosynthesis are very effective crop disease control agents. They constitute the single largest group of fungicides in terms of both the number of individual fungicides and sales (Fig. 5.16). They are systemic and provide protectant, curative and eradicant control. They also have beneficial side-effects that seem to be unrelated to their antifungal activity. Sterols are functional components in the maintenance of membrane integrity and are present in all eukaryotes. In fungi, sterol biosynthesis is carried out *de novo* from acetyl-CoA to produce the principal sterol in most fungi, ergosterol (Fig. 5.17). Ergosterol plays a unique role in the maintenance of membrane function: a reduction in ergosterol availability results in membrane disruption and electrolyte leakage.

The biosynthetic pathway to ergosterol is a feature of all true fungi (including the *Ascomycota* and *Basidiomycota*) but is absent from the *Oomycota*, which satisfy

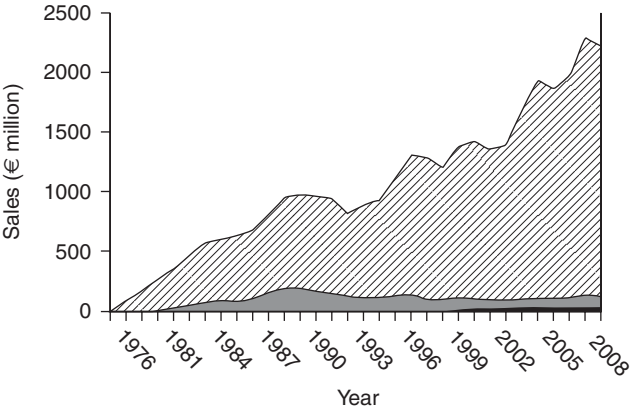


Fig. 5.16. Sales development of the sterol biosynthesis inhibitor classes in agriculture: ▨, G1; ▩, G2; ■, G3. (From Krämer *et al.*, 2012 with permission from Wiley-VCH.)

their sterol requirements directly from their hosts through mycelial uptake. This difference is the basis of the selectivity of SBIs, which cannot be used for the control of oomycete diseases. In addition, SBIs cannot be used to inhibit spore germination, which relies on stored products and can proceed in the absence of biosynthesis.

Inhibitors of sterol biosynthesis were discovered and developed to combat human fungal disease, but similar compounds quickly became available in crop protection and their introduction in the late 1960s heralded a radical change in the management of crop disease. The pathway for ergosterol biosynthesis has been established best in yeast. The so-called 'Erg' genes control the biosynthesis and have homologues in other species (Table 5.4). The details of the biosynthetic pathways differ slightly in other fungi. The same enzyme activity can act on lanosterol in yeast and eburicol in filamentous species. Fungicides that act through the inhibition of the sterol pathway can be divided into four major classes (G1–G4 and SBI Class I–IV) and further subdivided by which enzyme is inhibited (Table 5.5).

G1; C14-demethylation inhibitors (erg11/cyp51); SBI Class I

The most important SBIs are the C14-demethylation inhibitors (DMIs), group G1. The commercial strength of the DMIs arises from their activity spectrum and utility, which is very wide, with uses against most major ascomycete and basidiomycete pathogens (Table 5.2) but not oomycetes. There are a few problems. Their performance against powdery mildews, particularly in cereals, has been limited to mixtures, usually with morpholines, because of resistance development. Phytotoxicity can be a problem, limiting their use on legume crops.

The DMIs inhibit the removal of the C14-methyl group from 24-methylenedihydrolanosterol or eburicol (Fig. 5.18). The subsequent accumulation of precursor sterols and reduction in ergosterol is thought to be the basis of DMI activity. However, the effects of C14-demethylation inhibition are complex and still uncertain

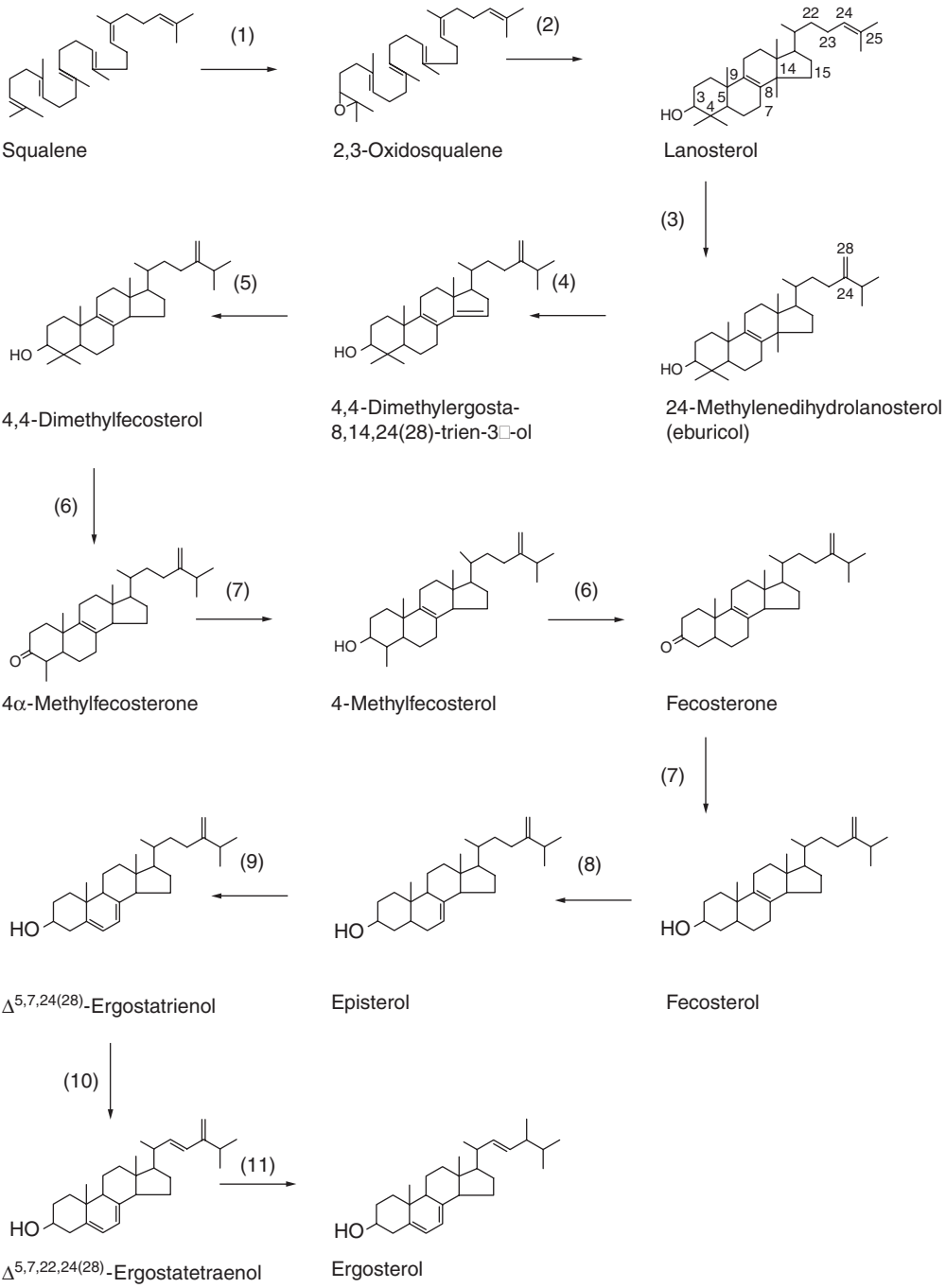


Fig. 5.17. The generic ergosterol biosynthesis pathway in fungi. Numbers indicate the enzymes listed in Table 5.4. (From Krämer *et al.*, 2012.)

Table 5.4. Enzymes and corresponding genes catalysing steps in the generic fungal ergosterol biosynthesis pathway. Fungicide MOAs are also listed. (From Krämer *et al*., 2012.)

Step no.[a]	Enzyme	Gene, other enzyme designations	Agricultural inhibitors
1	Squalene monooxygenase	*ERG1*, squalene epoxidase, oxidosqualene synthase	Target of G4 inhibitors such as allylamines; side target of some amines (G2)
2	Lanosterol synthase	*ERG7*, oxidosqualene cyclase	Side target of some amines (G2)
3	Sterol C24-methyl transferase	*ERG6*, sterol methyl transferase	–
4	Sterol C14-demethylase	*ERG11*, CYP51, lanosterol 14α-demethylase	Target of the DMI fungicides (G1)
5	Sterol C14-reductase	*ERG24*, sterol Δ^{14}-reductase	Main target of fenpropidin and spiroxamine (G2)
6	Sterol C4-methyloxidase	*ERG25*	–
	Sterol C3-dehydrogenase	*ERG26*, sterol C4-decarboxylase	
7	Sterol C3-ketoreductase	*ERG27*	Target of hydroxyanilides (G3)
8	Sterol $\Delta^{8}\rightarrow\Delta^{7}$-isomerase	*ERG2*, sterol C8-isomerase	Main target of tridemorph (G2)/secondary target of other amines (G2)
9	Sterol C5-desaturase	*ERG3*, C5-dehydrogenase	–
10	Sterol C22-desaturase	*ERG5*, ergosterol Δ^{22}-desaturase	–
11	Sterol $\Delta^{24(28)}$-reductase	*ERG4*, 24-methylene sterol (24(28))-reductase	–

MOA, mode of action; DMI, demethylation inhibitor.
[a]Step numbers are those shown in Fig. 5.17.

(Baldwin, 1983; Gadher *et al*., 1983; Baloch *et al*., 1984; Baldwin, 1990; Kelly *et al*., 1995; Senior *et al*., 1995; Lamb *et al*., 1996).

The target site of the DMIs is the Cyp51 enzyme. *Cyp51* encodes P450 monooxygenase and the fungicides appear to bind act the active site, thereby directly inhibiting access of the substrate to the enzyme (Kelly and Kelly, 2013). This both reduces ergosterol synthesis and leads to the accumulation of other, presumably toxic intermediates (Joseph-Horne *et al*., 1996). Different species of fungi have one, two or even three paralogues (copies of genes that arose from gene duplication) of the *Cyp51* gene (Fan *et al*., 2013). The presence of the different paralogues accounts for some of the variation in sensitivity in different species to different DMIs. Resistance has become a significant issue and is associated with changes in the coding sequences and overexpression of genes.

The variation in performance between DMIs may reflect differences in their binding affinities to the haem moiety of the P450 Cyp51 demethylase enzyme (see Fig 4.3 for 3D structure). Cyproconazole, for example, exists in four isomeric forms, all of

Table 5.5. Grouping of SBI fungicides in the FRAC classification. (From Krämer *et al*., 2012.)

	G: Sterol biosynthesis inhibitors			
FRAC codes	G1	G2	G3	G4
Group name	Demethylation inhibitors (DMIs)	Amines (formerly 'morpholines')	Hydroxyanilides	Squalene epoxidase inhibitors
SBI class	I	II	III	IV
Target in sterol biosynthesis	Sterol C14-demethylase	Δ^{14}-reductase and $\Delta^{8} \rightarrow \Delta^{7}$-isomerase	3-Ketoreductase	Squalene epoxidase
Chemistry	Piperazines Pyridines Pyrimidines Imidazoles Triazoles	Morpholines Piperidines Spiroketalamines	Hydroxyanilides	Thiocarbamates Allylamines

SBI, Sterol biosynthesis inhibitor; FRAC, Fungicide Resistance Action Committee.

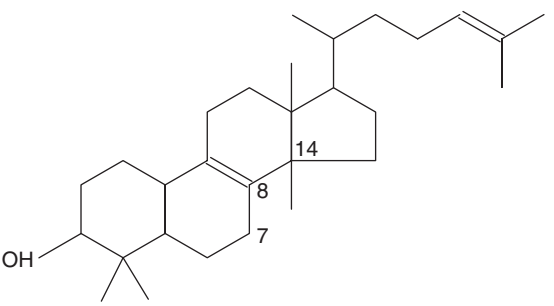


Fig. 5.18. Lanosterol, showing C7, C8 and C14 positions.

which show very high and almost equal fungicidal activity as a result of their similarity in affinity for the active site of inhibition (Gisi *et al*., 1986).

Five classes of chemical are DMIs (Table 5.5). A few pyrimidines (e.g. fenarimol) and imidazoles (e.g. prochloraz) have reached commercial importance but the group is dominated by the triazoles. This diverse range of chemistry is characterized by a nitrogen-containing heterocycle with an attendant lipophilic group. The triazole group contains more than 25 chemicals. It includes ones recommended for both seed treatment (e.g. triadimenol and fluquinconazole) and foliar treatment. Established market leaders in this group include epoxiconazole, propiconazole, tebuconazole and cyproconazole. Given that this group has been thoroughly explored since the 1970s, it was surprising that a new addition to the group, the Bayer compound prothioconazole, was launched as recently as 2004. The supplied product is a variant on the triazole theme, being a 1,2,4-triazole-3-thione. The compound is activated by exposure to the plant, losing the thio group in the process.

The imidazole prochloraz was developed initially for its potential against mildews. In field trials, the compound demonstrated useful activity against cereal eyespot and was for a while the market leader. Other major targets in cereals include SEPTRI,

BOTCIN, PYRIOR and other foliar ascomycetes. Prochloraz shows negative cross-resistance against triazoles (Fraaije *et al*., 2007).

The pyrimidines nuarimol and fenarimol are the only current examples of pyrimidin-5-ylbenzhydrols. Nuarimol is a systemic powdery mildewcide with a minor use as a seed treatment. Fenarimol, also active against mildews, has a major use in vines for the control of UNCNEC, and in apples, against *P. leucotricha*. VENTIN is also controlled by fenarimol, and its use in bananas as a resistance management tool against MYCFIJ is being developed.

*G2; $\Delta^8 \rightarrow \Delta^7$-isomerase and Δ^{14}-reductase inhibitors (*erg24, erg2*); SBI Class II*

The spectrum of disease control of the $\Delta^8 \rightarrow \Delta^7$-isomerase and Δ^{14}-reductase inhibitors (Fig. 5.19) is limited compared with the C14-demethylation inhibitors, their major use being against the powdery mildews (Table 5.2). There are only seven commercial

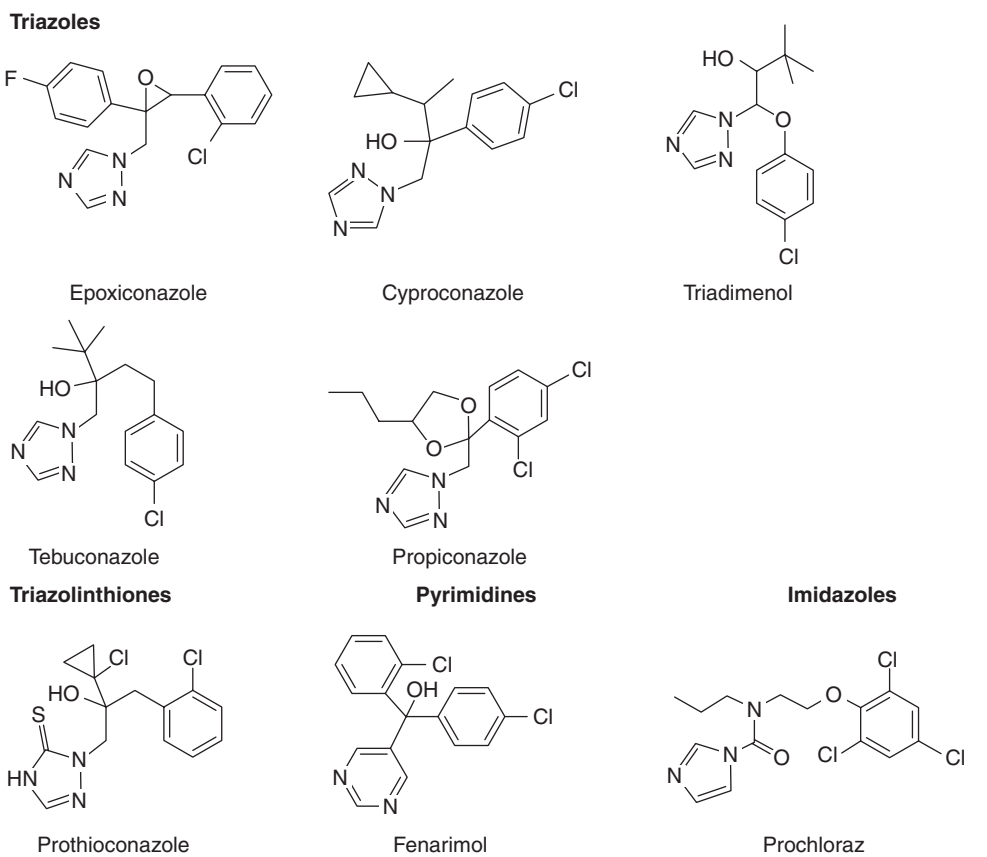


Fig. 5.19. Important triazole, triazolinthione, pyrimidine and imidazole fungicides.

fungicides in this group. They are the morpholines (fenpropimorph, tridemorph, dodemorph and aldimorph), the piperadines (fenpropidin and piperalin) and the spiroketalamine, spiroxamine (Fig. 5.20; Hollomon, 1994; Baldwin and Corran, 1995; Krämer *et al.*, 1999). Spiroxamine, the newest member of the group (1997), has preventive, curative and eradicant activity against mildew as well as significant activity against other fungi such as rusts and leaf blotches.

Extensive use of DMIs against cereal powdery mildews resulted in resistance and a reduction in control to levels below commercial acceptability. The current success of the $\Delta^8\Delta^7$-isomerase and Δ^{14}-reductase inhibitors was almost totally

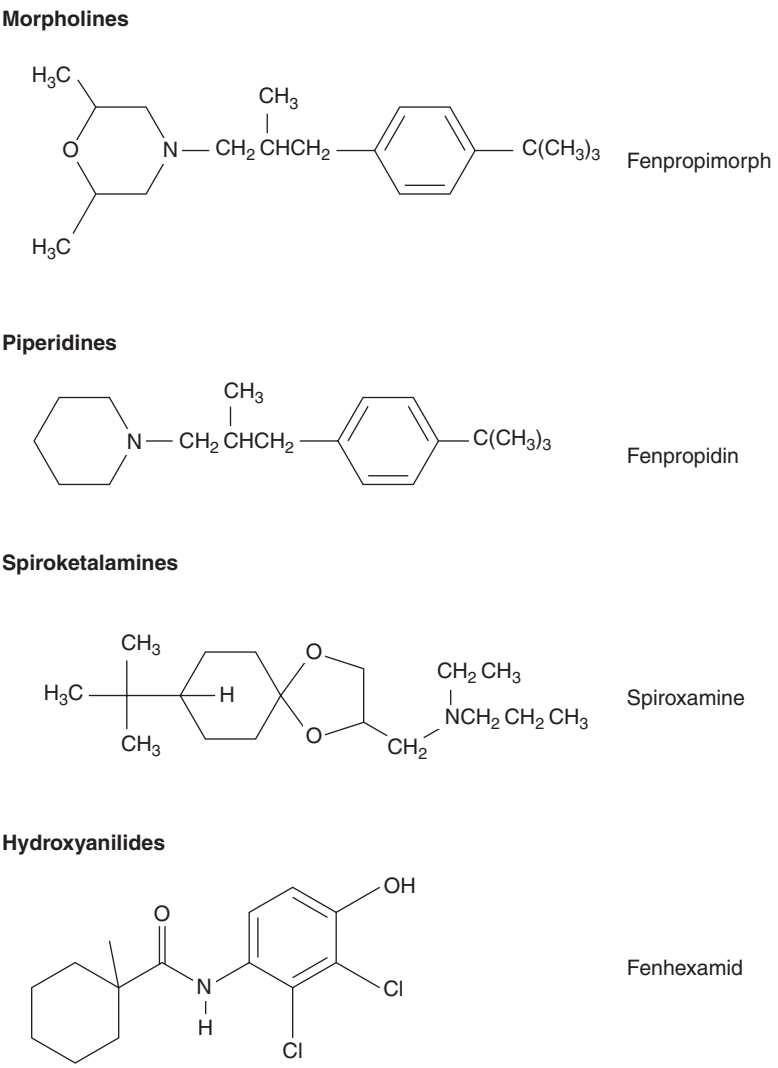


Fig. 5.20. Important morpholine, piperidine, spiroketalamine and hydroxyanilide fungicides.

dependent on the subsequent search for powdery mildew control agents with different MOAs from the azoles, and created a market for specific mildewcide products.

Although the inhibition of the isomerase and reductase has been demonstrated in laboratory studies (Baloch *et al.*, 1984; Mercer, 1991; Debieu *et al.*, 1992; Köller, 1992), the comparative importance of the two targeted steps is not well understood and the implications of inhibition are not clear (Baldwin and Corran, 1995). It is known for example, that in ERYSGH/T, the major target pathogen for this group, tridemorph is a highly active inhibitor of the isomerase reaction whereas fenpropidin has only weak activity, its major strength being the inhibition of the reductase step (Girling *et al.*, 1988). Fenpropimorph inhibits both enzymes. In addition, although some studies have been carried out that demonstrate the disruptive effects of fenpropimorph treatment on sterol levels and membrane integrity in yeast, *S. cerevisiae* (Steel *et al.*, 1989), other work showed that survival was independent of $\Delta^{8}\square\Delta^{7}$-isomerase activity (Ashman *et al.*, 1991). Morpholine inhibition of $\Delta^{24(28)}$-reductase, Δ^{24}-transmethylation and squalene-cyclization steps have also been cited as possible MOAs (Baldwin and Corran, 1995), which is reasonable considering the close structural similarities between substrates at those target sites.

The MOA is mediated by the interaction of the negatively charged enzyme site and the positively charged nitrogen atom in the fungicide molecule. Optimization of activity through structural modification extends to the choice of stereoisomer. In the spiroketal, spiroxamine, the two *cis* forms are more active than the two *trans* isomers (Krämer *et al.*, 1999).

*G3; 3-Ketoreductase (*erg27*); SBI Class III*

In a typically serendipitous manner, compounds being synthesized by Bayer as herbicides were found to have activity against BOTCIN. Optimization led to the release of the hydoxyanilide, fenhexamid, in 1998 (Fig. 5.20). It was subsequently shown that the compound inhibited a novel site in the sterol biosynthetic pathway (Debieu *et al.*, 2001). Fenhexamid had good activity against BOTCIN and the close relative *Sclerotinia* but only weak activity against other ascomycetes. It is used as a foliar product. The compound is not translocated so it is used solely as a protectant. Usage rates are high at up to 1 kg/ha.

H5; Cell wall biosynthesis; carboxylic acid amides

A diverse group of fungicides with specific activity against oomycetes were combined in a coherent group by FRAC in 2005 called the carboxylic acid amides (CAA). (Many of the important compounds in this group are cinnamic acid amides. This can give some ambiguity in the CAA abbreviation.) This rationalization occurred because research investigating fungicide resistance showed that all shared a common cross-resistance phenomenon. The group includes three cinnamic acid amides (flumorph, dimethomorph and pyrimorph), three valinamides (iprovalicarb, benthiavalicarb and valifenate) and a mandelic acid amide, mandipropamid (Fig. 5.21).

The target site was found to be the enzyme cellulose synthase. As cellulose is absent from true fungi, this explained why the spectrum only includes oomycetes.

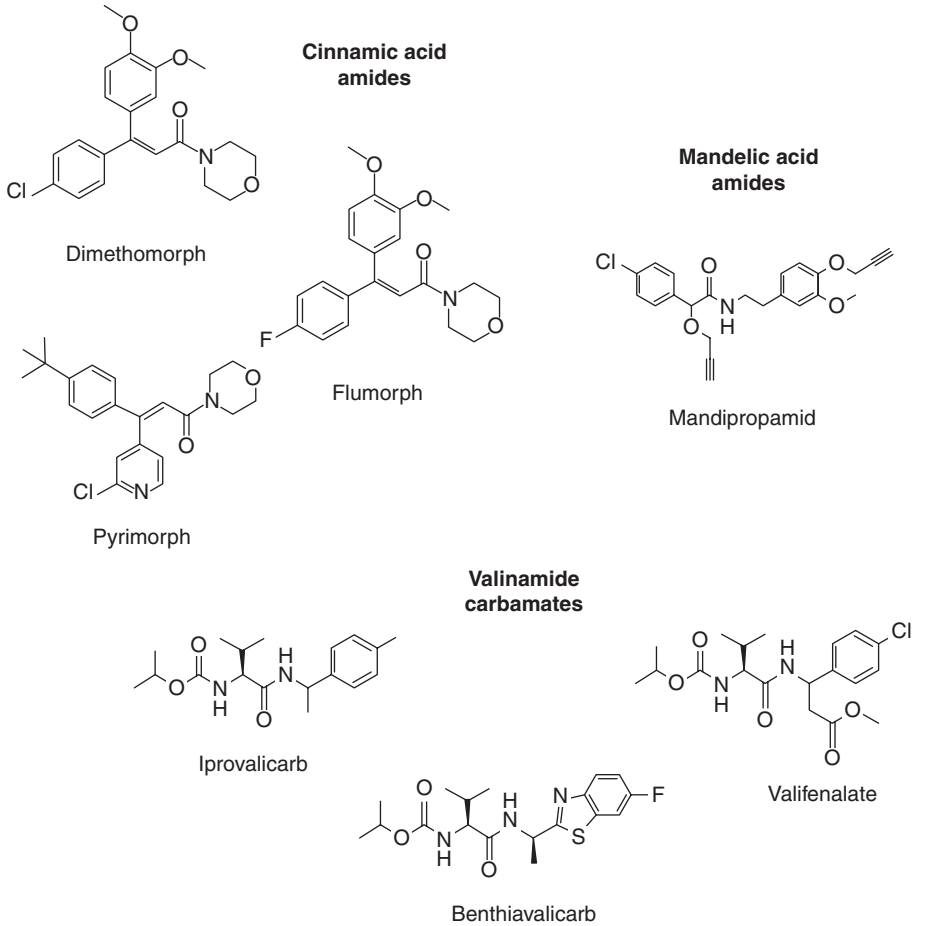


Fig. 5.21. Some carboxylic acid amide fungicides.

It is not so clear why *Pythium* species are insensitive. The application for CAAs is dominated by PHYTIN and PLASVIT.

The CAA fungicides have preventive and some eradicant activity due to some translaminar systemic movement. They operate by inhibiting germination of cystospores and sporangia, delaying elongation of hyphae and inhibiting sporulation.

The MOA was fully characterized by identifying mutations in the *CesA3* gene of PHYTIN that conferred resistance to mandipropimad (Grenville-Briggs *et al.*, 2008; Blum *et al.*, 2010). The results from other species and other CAAs are consistent with this finding.

I; Inhibition of melanin biosynthesis

The synthesis of the pigment melanin is important in fungal pathogenicity. The melanization of appressorial walls is essential for the development of infection hyphae and penetration of the host epidermis (de Jong *et al.*, 1997). Mutants of PYRIOR that

do not contain melanin are not pathogenic (Wheeler and Greenblatt, 1988). The discovery of tricyclazole initiated the development of chemicals displaying a novel MOA in pigmented ascomycetes (Fig. 5.22). Their inhibition of melanin synthesis provides excellent control of PYRIOR in rice and a significant share of the global market in fungicides.

Melanin biosynthesis in most fungi is via the DHN pathway. In this pathway, a ubiquitous polyketide synthase produces 1,3,6,8-tetrahydroxynapthalene (Fig. 5.23). Further steps convert this to scytalone, to 1,3,8-trihydroxynapthalene, to vermelone and, finally, to 1,8-dihydroxynapthalene (DHN). The melanin biosynthesis inhibitor (MBI) group of fungicides is divided into I1 (MBI-R), which inhibit the 1,3,6,8-tetrahydroxynapthalene reductase (tricyclazole, pyroquilon and fthalide), and I2 (MBI-D), which inhibit the scytalone dehydratase (carpropamid, diclycomet and fenoxanil). The compounds inhibit the enzymes by substrate mimicry.

The targets of this group are PYRIOR and *Colletotrichum*. This limited spectrum can be explained by the critical role of the appressorium in cuticular penetration by these species, which seems to be solely due to turgor pressure. This places a huge premium on extremely tough appressorial cell walls. Any inhibition by these compounds appears to be sufficient to give control. The presence of melanin synthesized from dihydroxyphenylalanine (DOPA) in fungi such as LEPTNO (Solomon *et al.*, 2004) and possibly other fungi, which is not affected by MBIs, also explains the limited spectrum.

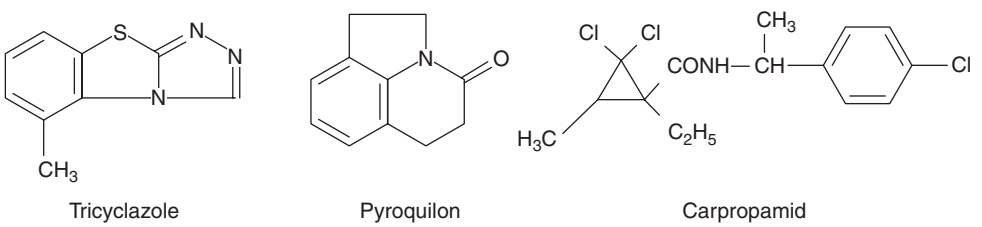


Fig. 5.22. Melanin biosynthesis inhibitors.

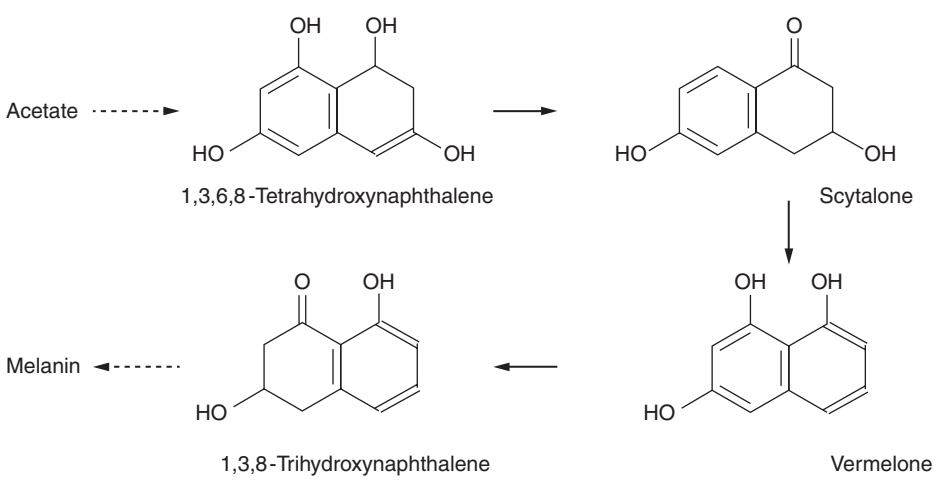


Fig. 5.23. Melanin biosynthesis (dotted arrows indicate several steps).

On tricyclazole-treated rice, the early infection stages of PYRIOR (germination of conidia and formation of appressoria) are unaffected but the melanization of appressoria and the subsequent formation of the infection peg apparatus are inhibited, effectively protecting the plant from disease. Tricyclazole is readily taken up by leaves and roots of rice plants and translocated, predominantly acropetally. Thus it is used in foliar applications and has mainly preventive activity.

P; Host plant defence induction

Most plants are resistant to most fungi. The mechanism of natural resistance has been the subject of intense study in the academic world as well as in the agrochemical industry, and a summary of the picture was given in Chapter 2. The onset of resistance following inoculation with an avirulent pathogen was found to be associated in many cases with the accumulation of salicylic acid. Furthermore, tissues remote from the inoculation site were found to also be resistance, a phenomenon known as systemic acquired resistance (SAR; Malamy *et al*., 1990; Metraux *et al*., 1990). Salicylic acid was transported around the plant and led to the induction of defence genes (often called pathogenesis-related or PR genes). Some salicylic acid was stored as the glycoside. Subsequent infection causes the release of salicylic acid from the glycoside and its accumulation at the site of infection. PR genes are induced, fungal growth is inhibited and disease expression does not occur or is slowed.

These studies suggested that agents that can induce SAR may present a new broad-spectrum means to control plant disease for extended periods. However, they have certain limitations. First, SAR is a natural phenomenon and the response to chemical inducers will be limited to the same spectrum of pathogens as in a naturally induced response. Salicylic acid induction was found to be restricted to biotrophic haustorial pathogens, whereas necrotrophic pathogens induced the accumulation of jasmonate and ethylene (Oliver and Ipcho, 2004). Secondly, by their nature, these exogenously applied chemicals have no direct fungitoxic activity, which complicates the discovery process. Nevertheless, the potential for crop disease control using chemically induced SAR responses has been explored by the agrochemicals industry has produced compounds with remarkable activity (Fig. 5.24).

Probenazole has been sold since 1975 and is still widely used to control PYRIOR. It also controls bacterial diseases. It is used at rates of up to 3 kg/ha on paddy fields. The compound moves acropetally and is thought to act through the induction of host defence reactions. The response is specific to rice. PYRIOR can infect barley with devastating effect but is not controlled on barley treated with probenazole. In rice, the compound stimulates the accumulation of fungitoxic substances, including α-linoleic acid, following inoculation with PYRIOR. An increase in the activity of several enzymes – phenylalanine ammonia lyase, peroxidase and catechol-*O*-methyltransferase – also occurs and these enzymes, collectively, are proposed to restrict spread of the pathogen in plants. Probenazole also inhibits early fungal development stages, reducing spore germination, appressorium formation and penetration of PYRIOR in rice.

CIBA (now part of Syngenta) embarked on a directed discovery programme starting with the methyl ester of benzo-1,2,3-thiazole-7-carboxylic acid, which had activity against *Colletotrichum lagenarium* on cucumber and was accidentally synthesized

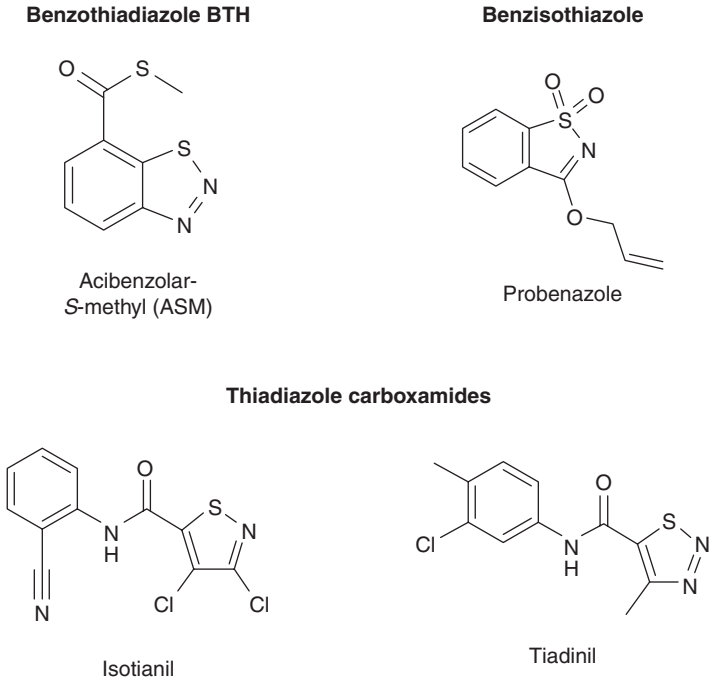


Fig. 5.24. Host plant defence induction compounds.

during a sulfonylurea herbicide discovery programme (Kunz *et al.*, 1997). The general structure of the benzothiadiazole plant activators was elucidated following extensive analogue synthesis and screening. The first compounds to have good broad-spectrum activity were dichloroisonicotinic acid and benzo-1,2,3-thiadiazole-7-carbothioic acid *S*-methyl ester, now known as ASM (acibenzolar-*S*-methyl), marketed under the trade name Bion and introduced to the market in Germany and Switzerland (Schurter *et al.*, 1990; Ruess *et al.*, 1995). It has no *in vitro* fungicidal activity, but when applied to plants it activates plant defence mechanisms against a wide spectrum of pathogens including fungi, bacteria and viruses (Kessmann *et al.*, 1996), similar to the naturally induced spectrum of disease resistance. The product is highly mobile, probably because of its weakly acidic nature, and is transported acropetally and basipetally throughout the plant, but is subject to rapid metabolism.

Further evidence that ASM is active only through the plant comes from work with mutants of the model plant *Arabidopsis thaliana* in which SAR cannot be induced (non-inducible mutants). In these and other non-inducible mutants, ASM and salicylic acid are inactive in protecting against fungal attack (Lawton *et al.*, 1996). ASM is effective against a broad spectrum of fungal, bacterial and virus diseases across a range of important crops (Table 5.6). In wheat, a single application of ASM at 30 g a.i./ha at early tillering is reported to give good protection against ERYSGT for up to 60 days. It has been suggested that the induction by ASM of the several disease resistance mechanisms that comprise SAR reduces the risk of resistance development (Kessmann *et al.*, 1996).

Table 5.6. Acibenzolar-*S*-methyl; target crops and pathogens.

Crop	Acceptable control	Suppression
Wheat	ERYSGT	PUCCRT SEPTRI
Rice	PYRIOR	
Tobacco	*Peronospora tabacina*	
Banana	MYCFIJ	
Vegetables	Oomycetes *Colletotrichum*	*Alternaria* spp.

Tiadinil and isotianil are the most recently released compounds that also seem to act via the induction of SAR. Both compounds induce PR proteins via the salicylic acid signalling pathway. A significant factor is the very much higher activity of isotianil, allowing the use of rates of about 100 g/ha compared with the 1–3 kg/ha needed for the other compounds. The spectrum is patchy, however, with good activity against ERYSGT, PYRIOR and *Colletotrichum* but none against necrotrophs such as tan spot of wheat.

The future for host plant inducers is unclear. They are widely used in rice for PYRIOR but mainly because growers fail to use resistant cultivars. They should be immune to resistance but the limited spectrum has limited use to niche applications.

Multi-site (M) and unknown (U) modes of action

U27; Cymoxanil

Cymoxanil (Fig. 5.25) is an extremely effective systemic fungicide with protectant and curative activity specifically against oomycete fungi. Cymoxanil has important uses against PHYVIT on grapevine and PHYTIN in which it is employed in a mixture with non-specific cell toxicant fungicides, for example mancozeb, as part of anti-resistance strategies to improve long-term activity and, through its curative activity, to extend the interval between sprays.

Cymoxanil is more effective against hyphal growth stages than early growth phases (the release of zoospores from sporangia and their germination). The compound inhibits nucleic acid and protein biosynthesis in *Phytophthora cinnamomi* and *B. cinerea*, but it is likely that the activity is induced via an interaction with host metabolic processes.

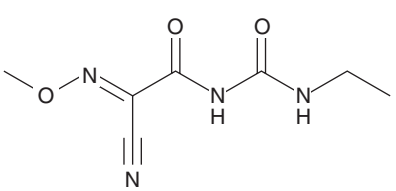


Fig. 5.25. Cymoxanil.

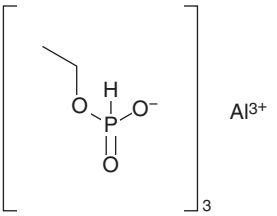


Fig. 5.26. Fosetyl aluminium.

U33; Fosetyl

Fosetyl or fosetyl aluminium (Fig. 5.26) has specific activity against the oomycete fungi, including PLASVIT, *Pseudoperonospora humuli* in hops and *Phytophthora* spp. in fruits, for example citrus. Fosetyl is a rare example of a phloem-mobile product, an action that is considered to be a function of its breakdown product, phosphonic acid (H_3PO_3), also known as phosphite, which is readily produced in aqueous solution.

The product inhibits sporangial formation and zoospore release in *Phytophthora citrophthora*, *Phytophthora parasitica*, *Phytophthora cactorum* and *Phytophthora citricola*, and oospore and chlamydospore production in *P. citricola* and *P. cinnamomi*; *Phytophthora megasperma* and *P. infestans* are comparatively insensitive (Farih *et al.*, 1981).

Recent research suggests that the MOA is complex but involves the activation of plant defensive reactions. These include both preformed and pathogen-induced defences (Machinandiarena *et al.*, 2012; Massoud *et al.*, 2012; Olivieri *et al.*, 2012; Walters *et al.*, 2013). This indirect MOA explains the low *in vitro* toxicity of fosetyl towards mycelium growth. Given that defence response does not differ significantly between the attacking pathogens, it is curious why this MOA does not protect against a wider spectrum of pathogens. There is more to be learnt about this interesting class of fungicides.

M1; Copper

Copper, as copper sulfate, was first used as a fungicide to control *Tilletia grisea* in wheat but its use was not developed until an observation by Millardet in France, in 1882, that treatments of copper sulfate and lime used in roadside situations to deter the theft of grapes were associated with the control of downy mildew PLASVIT. This led to the acceptance of Bordeaux mixture as a routine treatment for vine downy mildew.

Copper fungicides such as Bordeaux mixture and copper oxychloride (Fig. 5.27) are still employed singly, or in combination with systemics such as cymoxanil, to

$Ca(OH)_2 + CuSO_4$	$Cu(OH)_2$	$Cu_2Cl(OH)_3$
Bordeaux mixture	Copper hydroxide	Copper oxychloride

Fig. 5.27. Copper fungicides.

control several diseases in vine (PLASVIT), potato and tomato (PHYTIN), hop (*P. humuli*), banana (e.g. MYCFIJ), coffee (*Colletotrichum kahawae*) and tea (*Exobasidium vexans*). The development of copper-based products continues, and copper tallate has been described as a synergist to a range of organic fungicides (Soyez, 1992).

Copper, as Cu^{2+}, is readily accumulated by sensitive fungi. It forms complexes with enzymes that possess sulfhydryl, hydroxyl, amino or carboxyl groups, inactivating them and leading to a general disruption of metabolism and breakdown of cell integrity.

As with all immobile protectants, copper products have to be used frequently in order to achieve adequate coverage and to maintain disease control in a growing crop. Copper products have to be made relatively insoluble in water to avoid the phytotoxic effects of the copper ion. Although fungicidal efficacy may be depressed, this also reduces the loss of product by rainfall and can benefit long-term control provided the initial application is adequate and the plant is not in a phase of rapid growth.

M2; Sulfur

Sulfur was the first effective fungicide and its use may be traced back many centuries (Large, 1958). Sulfur acts as a protectant fungicide through the inhibition of conidial germination. The use of a combined sulfur and lime product to control powdery mildew in fruit was first described in the early 19th century, and sulfur products and mixtures (estimated to be over 20) are still extensively employed in apple, grapevine and other cultivated crops.

Sulfur acts against several biochemical sites. It inhibits respiration through its reduction product, hydrogen sulfide, disrupting proteins and forming chelates with heavy metals within the fungal cells. The selective activity of sulfur against powdery mildews may be attributed to their unique and exposed growth habit or to possible uptake by the lipid layers of conidia.

Sulfur also exhibits pronounced acaricidal activity, for example against spider mites, and in wet and warm (above 35°C) weather conditions may be phytotoxic.

M3; Dithiocarbamates

The discovery of the dithiocarbamate family of products in the 1930s and 1940s is usually accepted as initiating the period of organic synthesis of fungicides.

As with most immobile protectants, dithiocarbamates are broad-spectrum fungicides with uses as foliar, soil and seed treatments in fruit (VENTIN, *Taphrina deformans*), grapevine (PLASVIT), vegetables (PHYTIN, BOTCIN, *Alternaria* spp., *Septoria* spp.), sugarbeet (*Cercosporella beticola*), tobacco (*Pseudoperonospora tabacina*) and hops (*P. humuli*). The dithiocarbamates are inactive against the powdery mildews (*Erysiphales*).

Examples of the dithiocarbamates are ziram, zineb, ferbam and thiram (Fig. 5.28). Generally, dithiocarbamates are not phytotoxic but can induce damage in some crops in exceptional circumstances, for example in the use of mancozeb or zineb on zinc-sensitive plants.

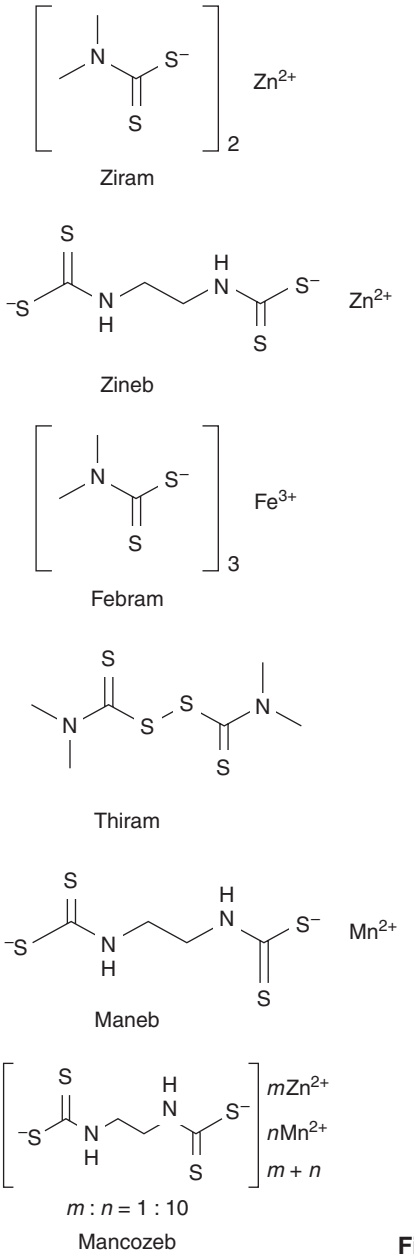


Fig. 5.28. Dithiocarbamate fungicides.

M4; Phthalimides

Phthalimides were introduced in 1952 with the announcement of captan and a close analogue, folpet (Fig. 5.29). They provide protectant control of a wide range of fungal pathogens, are used extensively as sprays, root dips and seed treatments, and are useful in the control of damping-off of seedlings. They have been used to control

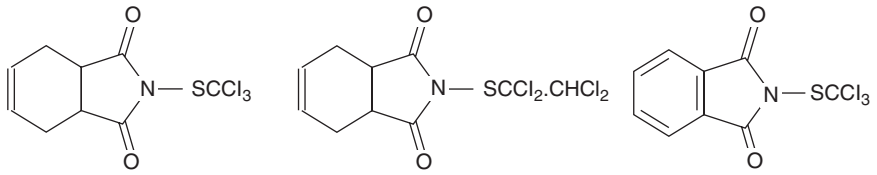


Fig. 5.29. Phthalimide fungicides.

PHYTIN, VENTIN, PLASVIT and BOTCIN and many other foliar ascomycetes. They are inactive against members of the powdery mildews.

Captan, captafol and folpet preferentially react with enzyme sulfhydryl groups but may also attack amino groups and inhibit enzymes that do not contain sulfhydryl groups.

M5; Chlorophenyls

Chlorothalonil, introduced in the mid-1960s, is a major protectant fungicide. It is recommended mainly for use alone or in mixtures to control *Septoria* spp. in cereals, *P. infestans* in potatoes and *Botrytis* spp. in vegetables and ornamentals, as well as finding uses in paints and preservatives (Fig. 5.30). Chlorothalonil binds to sulfhydryl and mercapto groups (Tillman *et al.*, 1973). It is widely used as a mixing partner with fungicides to improve the spectrum and for protection against fungicide resistance.

Fungicide Redistribution in Crops

Under conditions of continual challenge by pathogens, non-mobile compounds have to be applied several times to a growing plant in order to maintain commercially acceptable levels of disease control. In comparison, fungicides that move within the plant are generally more flexible to use because most have the inherent ability to control established pathogens, thereby providing the user with a wider window of application and higher levels of efficacy.

The same principles apply in crops. Immobile fungicides must be applied pre-infection and, in dense crops, using high spray volumes to achieve effective canopy

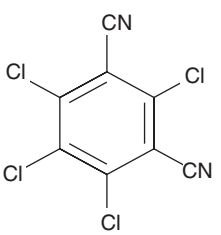


Fig. 5.30. Chlorothalonil.

penetration and foliar coverage. In rapidly growing crops such as grapevine, applications may be necessary every 10–12 days. Fungicides that redistribute within a crop allow the grower to minimize application volume and the number of treatments per season. For example in cereals, mobile fungicides may not only combat established infection through curative or eradicant activity but, through redistribution in the crop, may also provide protection from disease for 28–42 days, depending upon the product and the target pathogen.

Mobility is an important fungicide attribute and may occur in several ways.

- Interplant movement through:
  - vapour-phase activity; and
  - redistribution by rain.
- Intraplant movement through:
  - xylem transport;
  - phloem transport; and
  - diffusion.

Vapour-phase activity

Many fungicides have low vapour pressures. Some, like fenpropimorph, even possess a strong and distinctive smell which may linger for several days after treatment in the field, depending on the temperature and wind speed. For many years it has been known that some fungi are able to take up chemicals in their vapour phase (Fries, 1973) and it is likely that the redistribution and field performance of some commercial fungicides are profoundly influenced through their activity via the air (van Gestel, 1986).

The activity of sulfur is well recognized as involving a temperature-dependent volatilization process. Similarly, the activity of some immobile surface protectants may be best explained through their redistribution in the vapour phase, as coverage of leaf tissue is rarely complete in practical applications. Chlorothalonil, for example, is known to contaminate glasshouse screening tests through its redistribution in air. Some systemic compounds, however, are notoriously difficult to use in research tests carried out within the confines of a glasshouse. Many, including fenpropimorph, metalaxyl and several DMIs, are known to have vapour pressures sufficiently low to control pathogens spatially removed from their site of application (Table 5.7). Clearly, the effectiveness of fungicides through the vapour phase will be controlled by both their vapour pressure and their intrinsic activity. Compounds with relatively low vapour pressures will be effective if their intrinsic activity is high.

Fungal structures that are exposed to the air, such as powdery mildew mycelium and sporangiophores and conidiophores, are especially susceptible to fungicides active through the vapour phase. Their high lipid content may favour the uptake of lipophilic fungicides. Sporangia of PLASVIT, for example, have been shown to be particularly sensitive to metalaxyl present in the surrounding air. The antisporulant activity reported for many fungicides may be based on their vapour-phase effects on sporulating structures.

The practical value of the volatile component of fungicide redistribution in crops is difficult to quantify. Temperature and the nature of the surface impacted by the

Table 5.7. Vapour pressure of fungicides.

Compound	mPa (25°C)
Chloroneb	400
Fenpropidin	17
Tridemorph	6.4
Fenpropimorph	2.3
Tetraconazole	1.6
Propineb[a]	1
Metalaxyl	0.75
Sulfur[b]	0.527
Pencycuron[a]	0.5
Penconazole[a]	0.21
Prochloraz	0.15
Carbendazim[a]	0.09
Cymoxanil	0.08
Chlorothalonil	0.076
Fenarimol	0.065
Iprodione	0.057
Propiconazole	0.056
Cyproconazole	0.0346
Tricyclazole	0.027
Carboxin	0.025
Benomyl	0.0049
Tebuconazole[a]	0.0013
Captafol	0
Cuprous oxide	0

[a]At 20°C.
[b]At 30°C.

fungicide both affect volatilization. It may be argued that in open situations, high temperatures may lessen the performance of relatively volatile products through dilution in the vapour phase and loss of product. In contrast, the most effective use of volatile compounds may be achieved in glasshouse crops where high air concentrations of the active materials can be induced and maintained (Szkolnik, 1983; van Gestel, 1986).

Volatile fungicides are also used in the control of postharvest diseases of stored fruit or produce that has been wrapped in fungicide-impregnated and heat-sealed packages.

Phloem mobility

Few fungicides are translocated via the phloem in effective quantities, most so-called systemics being redistributed within the apoplast and hence restricted to diffusion as for example in translaminar mobility, xylem transport driven by transpiration or diffusion.

Crop physiology can modify the extent to which a fungicide can be redistributed. Materials restricted to apoplastic movement because of their physicochemical characteristics can appear to behave as phloem-mobile compounds. Such fungicides, when

applied to graminaceous crops, may in part be redistributed by a combination of droplet runoff to the base of the impacted leaves and translaminar movement to the basal apex where an accumulation of fungicide may occur. Subsequent development of the apex will then transfer the fungicide to tissues not present at the time of application. The same fungicide applied to a broad-leaf crop, for example apple, will appear to have different mobility characteristics. In this case, because little passive accumulation of the active material can occur around the apical meristems, redistribution from leaves impacted by the fungicide spray will be restricted to movement in the transpiration stream. Quinoxyfen, a new compound from DowElanco, is reported to have exceptional long-term activity against ERYSGT, displaying an ability to redistribute to leaf tissue not present at application. The compound is also active against UNCNEC, powdery mildew of vine, but in that crop appears to lack the same degree of long-term control. Reasons for this are not clear but may, in part, be attributed to differences in the passive movement and accumulation of the product in the two crops.

Access to both phloem and xylem elements of the vascular system is essential if long-distance movement and long-term control are required. Some problematic fungal pathogens may be controlled effectively only by phloem-mobile compounds. Soil-borne and root pathogens such as *Gaeumannomyces graminis* (take-all of cereals), although susceptible to many fungicides in *in vitro* tests, cannot be reliably controlled in field situations because of the spatial separation between the site of infection and the site of fungicide application. Some seed treatments, for example triadimenol, can suppress infection but acceptable control levels have not been achieved. Existing foliar fungicides are unable to redistribute to the roots via the phloem and are ineffective, even though they may possess a high level of intrinsic activity against the pathogen. The control of such pathogens will involve a significant advance in fungicide discovery and will open up new and potentially valuable markets.

When compared with the idea of using soil-applied fungicides to control root diseases, the advantages of phloem-mobile, foliar fungicides are clear. Such materials would operate at much lower application rates and would ease the potential environmental problems associated with soil absorption of fungicides and their breakdown products leaching to deeper soil layers, entry into water tables and toxicity to soil microorganisms.

Phloem transport is a common characteristic of herbicides and insecticides. In fungicides, significant phloem transport has been reported for only a few materials, notably fosetyl, and it may be that the physicochemical requirements for symplastic movement oppose those that govern uptake by, and movement in, target fungi. To date, alterations of chemical structure that favour phloem mobility have not been successful in producing fungicides that are commercially interesting. The requirements governing the binding of compounds to a hydrophobic target site are contrary to the characteristics necessary to achieve efficient movement of candidate fungicides across cuticular membranes.

Compliance with several physicochemical qualities that define levels of lipophilicity, hydrophilicity, steric parameters, ionization and distribution of charge density is necessary for a compound to enter the plant and move within the symplast (Grayson and Kleier, 1990; Kleier, 1994). The general categories that govern phloem mobility are the pK_a, or acid strength, and the log K_{ow}, or lipophilicity. Compounds that are phloem-mobile have low log K_{ow} values and are acidic (low pK_a). The two characteristics may, to some extent, balance, so that compounds that are highly lipophilic may

be phloem-mobile if they are also acidic. The problem here is that while they readily gain access to the phloem, they may be just as easily lost to the surrounding tissue.

Sugars and sugar conjugates are naturally transported in the phloem tissue. Some are actively loaded but for others, for example flavonoids, no active transport mechanism is known. Sugar conjugation with fungicidal materials may improve their mobility in target crops.

The activation of systemic natural defences in plants is mediated by an endogenous transmission mechanism which utilizes compounds such as salicylic acid that have low K_{ow} values and are acidic. Compounds that possess acidic groups or groups from which acidic groups can be derived by hydrolysis (proacids) may be phloem-mobile. Conversely, strong bases or compounds that possess quaternary nitrogen-containing heterocyclic rings may also be phloem-mobile through a reduction in lipophilicity.

Other strategies to achieve effective yet mobile products include the use of pro-fungicides and transgenic plants. Pro-fungicides are, in themselves, inactive. They rely for success on the ability of the host plant to modify their chemical structure, rendering them mobile and fungitoxic. Thus a highly lipophilic compound, for example a lipophilic ester of the fungicide, is able to penetrate the cuticular barrier but, once inside the crop host, generates a compound of reduced lipophilicity (fungicide), which is then free to be transported. However, how the structure of the fungicide is then manipulated to reverse the process in order to reach the site of action in the colonizing pathogen is not clear. Transgenic plants may be able to carry genes to generate an active agent from a phloem-mobile, low-lipophilic pro-fungicide.

References

Ammermann, E., Lorenz, G., Schelberger, K., Wenderoth, B., Sauter, H., *et al.* (1992) BAS 490 F – a broad-spectrum fungicide with a new mode of action. In: *Proceedings of the Brighton Crop Protection Conference 1992 – Pests and Diseases*, Vol. 1. British Crop Protection Council, Farnham, UK, pp. 403–410.

Anke, T., Oberwinkler, F., Steglich, W. and Schramm, G. (1977) The strobilurins: new antifungal antibiotics from the basidiomycete *Strobilurus tenacellus* (Pers. ex Fr.) Sing. *Journal of Antibiotics* 30(10), 806–810.

Ashman, W.H., Barbuch, R.J., Ulbright, C.E., Jarrett, H.W. and Bard, M. (1991) Cloning and disruption of the yeast C-8 sterol isomerase gene. *Lipids* 26(8), 628–632.

Baldwin, B.C. (1983) Fungicidal inhibitors of ergosterol biosynthesis. *Biochemical Society Transactions* 11(6), 659–663.

Baldwin, B.C. (1990) Inhibitors of ergosterol biosynthesis as crop protection agents. *Biochemical Society Transactions* 18(1), 61–62.

Baldwin, B.C. and Corran, A.J. (1995) Inhibition of sterol biosynthesis: application to the agrochemicals industry. In: Dixon, G.K., Copping, L.G. and Hollomon, D.W. (eds) *Antifungal Agents. Discovery and Mode of Action*. BIOS Scientific, Oxford, pp. 59–68.

Baloch, R.I., Mercer, E.I., Wiggins, T.E. and Baldwin, B.C. (1984) Inhibition of ergosterol biosynthesis in *Saccharomyces cerevisiae* and *Ustilago maydis* by tridemorph, fenpropimorph and fenpropidin. *Phytochemistry* 23(10), 2219–2226.

Bartlett, D.W., Clough, J.M., Godwin, J.R., Hall, A.A., *et al.* (2002) The strobilurin fungicides. *Pest Management Science* 58(7), 649–662.

Beautement, K. and Clough, J.M. (1987) Stereocontrolled syntheses of strobilurin A and its (9*E*)-isomer. *Tetrahedron Letters* 28(4), 475–478.

Beautement, K., Clough, J.M., de Fraine, P.J. and Godfrey, C.R. (1991) Fungicidal β-methoxyacrylates: from natural products to novel synthetic agricultural fungicides. *Pesticide Science* 31(4), 499–519.

Becker, W.E., 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-p*-methoxyacrylate system as common structural elements. *FEBS Letters* 132(2), 329–333.

Blum, M., Boehler, M., Randall, E., Young, V., Csukai, M., *et al.* (2010) Mandipropamid targets the cellulose synthase-like PiCesA3 to inhibit cell wall biosynthesis in the oomycete plant pathogen, *Phytophthora infestans*. *Molecular Plant Pathology* 11(2), 227–243.

Butters, J.A., Kendall, S.J., Wheeler, I.E. and Hollomon, D.W. (1995) Tubulins: lessons from existing products that can be applied to target new antifungals. In: Dixon, G.K., Copping, L.G. and Hollomon, D.W. (eds) *Antifungal Agents. Discovery and Mode of Action*. BIOS Scientific, Oxford, pp. 131–142.

Cui, W., Beever, R.E., Parkes, S.L., Weeds, P.L. and Templeton, M.D. (2002) An osmosensing histidine kinase mediates dicarboximide fungicide resistance in *Botryotinia fuckeliana* (*Botrytis cinerea*). *Fungal Genetics and Biology* 36(3), 187–198.

Daniels, A., Birchmore, R.J. and Winter, RH. (1994) Activity of pyrimethanil on *Venturia inaequalis*. In: *Proceedings of the Brighton Crop Protection Conference 1994 – Pests and Diseases*, Vol. 2. British Crop Protection Council, Farnham, UK, pp. 525–532.

Davidse, L.C. (1986) Benzimidazole fungicides – mechanism of action and biological impact. *Annual Review of Phytopathology* 24(Sept), 43–65.

Debieu, D., Gall, C., Gredt, M., Bach, J., Malosse, C. and Leroux, P. (1992) Ergosterol biosynthesis and its inhibition by fenpropimorph in *Fusarium* species. *Phytochemistry* 31(4), 1223–1233.

Debieu, D., Bach, J., Hugon, M., Malosse, C. and Leroux, P. (2001) The hydroxyanilide fenhexamid, a new sterol biosynthesis inhibitor fungicide efficient against the plant pathogenic fungus *Botryotinia fuckeliana* (*Botrytis cinerea*). *Pest Management Science* 57(11), 1060–1067.

de Jong, J.C., McCormack, B.J., Smirnoff, N. and Talbot, N.J. (1997) Glycerol generates turgor in rice blast. *Nature* 389, 244–245.

Delp, C.J. (1995) Benzimidazole and related fungicides. In: Lyr, H. (ed.) *Modern Selective Fungicides*, 2nd edn. Gustav Fischer, Jena, Germany, pp. 291–303.

Fan, J., Urban, M., Parker, J.E., Brewer, H.C., Kelly, S.L., *et al.* (2013) Characterization of the sterol 14α-demethylases of *Fusarium graminearum* identifies a novel genus-specific CYP51 function. *New Phytologist* 198(3), 821–835.

Farih, A., Tsao, P.H. and Menge, J.A. (1981) Fungitoxic activity of efosite aluminum on growth, sporulation, and germination of *Phytophthora parasitica* and *P. citrophthora*. *Phytopathology* 71(9), 934–936.

Fraaije, B.A., Cools, H.J., Kim, S.H., Motteram, J., Clark, W.S., *et al.* (2007) A novel substitution I381V in the sterol 14α-demethylase (CYP51) of *Mycosphaerella graminicola* is differentially selected by azole fungicides. *Molecular Plant Pathology* 8(3), 245–254.

Fries, N. (1973) Effects of volatile compounds on the growth and development of fungi. *Transactions of the British Mycological Society* 60, 1–21.

Fu, J., Wu, J., Jiang, J., Wang, Z. and Ma, Z. (2013) Cystathionine γ-synthase is essential for methionine biosynthesis in *Fusarium graminearum*. *Fungal Biology* 117(1), 13–21.

Fujimura, M., Oeda, K., Inoue, H. and Kato, T. (1990) Mechanism of action of *N*-phenylcarbamates in benzimidazole-resistant *Neurospora* strains. In: Green, M.B., Lebaron, H.M. and Moberg, W.K. (eds) *Managing Resistance to Agrochemicals*. American Chemical Society, Washington, DC, pp. 224–236.

Gadher, P., Mercer, E.I., Baldwin, B.C. and Wiggins, T.E. (1983) A comparison of the potency of some fungicides as inhibitors of sterol 14-demethylation. *Pesticide Biochemistry and Physiology* 19(1), 1–10.

Gehmann, K., Nyfeler, R., Leadbeater, A., Nevill, D. and Sozzi, D. (1990) CGA 173506: a new phenylpyrrole fungicide for broad-spectrum disease control. In: *Proceedings of the Brighton Crop Protection Conference 1990 – Pests and Diseases*, Vol. 2. British Crop Protection Council. Farnham, UK, pp. 399–406.

Girling, I.J., Hollomon, D.W., Kendall, S.J., Loeffler, R.S.T. and Senior, I.J. (1988) Effects of fenpropidin on DMI-resistant strains of *Erysiphe graminis* f. sp. *hordei* and *Rhynchosporium secalis*. In: *Proceedings of the Brighton Crop Protection Conference 1988 – Pests and Diseases*, Vol. 1. British Crop Protection Council, Farnham, UK, pp. 385–390.

Gisi, U., Schaub, F., Wiedmer, H., and Ummel, E. (1986) SAN 619F, a new triazole fungicide. In: *Proceedings of the British Crop Protection Conference 1986 – Pests and Diseases*, Vol. 1. British Crop Protection Council, Farnham, UK, pp. 33–40.

Godwin, J. R., Anthony, V. M., Clough, J. M. and Godfrey, C. R. A. (1992) ICIA5504: a novel, broad spectrum, systemic β-methoxyacrylate fungicide. In: *Proceedings of the Brighton Crop Protection Conference 1992 – Pests and Diseases*, Vol. 1. British Crop Protection Council, Farnham, UK, pp. 435–442.

Grayson, B.T. and Kleier, D.A. (1990) Phloem mobility of xenobiotics. IV. Modelling of pesticide movement in plants. *Pesticide Science* 30(1), 67–79.

Grenville-Briggs, L.J., Anderson, V.L., Fugelstad, J., Avrova, A.O., Bouzenzana, J., *et al.* (2008) Cellulose synthesis in *Phytophthora infestans* is required for normal appressorium formation and successful infection of potato. *Plant Cell* 20(3), 720–738.

Heye, U.J., Speich, J., Siegle, H., Steinemann, A., Forster, B., *et al.* (1994) CGA 219417: a novel broad-spectrum fungicide. *Crop Protection* 13(7), 541–549.

Hollomon, D.W. (1994) Do morpholine fungicides select for resistance? In: Heaney, S. Slawson, D., Hollomon, D.W., Smith, M., Russell, P.E., *et al.* (eds) *Fungicide Resistance*. British Crop Protection Council, Thornton Heath, UK, pp. 281–290.

Irmler, S., Rogniaux, H., Hess, D. and Pillonel, C. (2006) Induction of OS-2 phosphorylation in *Neurospora crassa* by treatment with phenylpyrrole fungicides and osmotic stress. *Pesticide Biochemistry and Physiology* 84(1), 25–37.

Ishii, H., Josepvits, J., Gasztonyi, M. and Miura, T. (1995) Further studies on increased sensitivity to *N*-phenylanilines in benzimidazole-resistant strains of *Botrytis cinerea* and *Venturia nashicola*. *Pesticide Science* 43(3), 189–193.

Joseph-Horne, T., Hollomon, D., Manning, N. and Kelly, S.L. (1996) Investigation of the sterol composition and azole resistance in field isolates of *Septoria tritici*. *Applied and Environmental Microbiology* 62(1), 184–190.

Kelly, S.L. and Kelly, D.E. (2013) Microbial cytochromes P450: biodiversity and biotechnology. Where do cytochromes P450 come from, what do they do and what can they do for us? *Philosophical Transactions of the Royal Society B: Biological Sciences* 368(1612), 20120476.

Kelly, S.L., Lamb, D.C., Corran, A.J., Baldwin, B.C. and, Kelly, D.E. (1995) Mode of action and resistance to azole antifungals associated with the formation of 14α-methylergosta-8,24(28)-dien-3β,6α-diol. *Biochemical and Biophysical Research Communications* 207(3), 910–915.

Kessmann, H., Oostendorp, M., Ruess, W., Staub, T., Kunz, W., *et al.* (1996) Systemic activated resistance – a new technology for plant disease control. *Pesticide Outlook* 7(3), 10–13.

Kleier, D.A. (1994) Phloem mobility of xenobiotics. V. Structural requirements for phloem-systemic pesticides. *Pesticide Science* 42(10), 1–11.

Köller, W. (1992) Antifungal agents with target sites in sterol function and biosynthesis. In: Köller, W. (ed.) *Target Sites of Fungicide Action*. CRC Press, Boca Raton, Florida, pp. 119–206.

Krämer, W., Berg, D., Dutzmann, S., Etzel, W.A., Gau, W., Stelzer, U. and Weissmüller, J. (1999) Chemistry, stereochemistry and biological properties of KWG 4168. *Pesticide Science* 55(5), 610–614.

Krämer, W., Schirmer, U., Jeschke, P. and Witschel, M. (eds) (2012) *Modern Crop Protection Compounds*. Wiley-VCH, Weinheim, Germany.

Kunz, W., Schurter, R. and Maetzke, T. (1997) The chemistry of benzothiadiazole plant activators. *Pesticide Science* 50(4), 275–282.

Lamb, D.C., Kelly, D.E., Corran, A.J., Baldwin, B.C. and Kelly, S.L. (1996) Role of sterol $\Delta^{5,6}$ desaturase in azole antifungal mode of action and resistance. *Pesticide Science* 46(3), 294–298.

Large, E.C. (1958) *The Advance of the Fungi*. Jonathan Cape, London.

Lawton, K., Friedrich, L., Hunt, M., Weymann, K., Kessmann, H., *et al.* (1996) Benzothiadiazole induces disease resistance in *Arabidopsis* by activation of the systemic acquired resistance signal transduction pathway. *The Plant Journal* 10(1), 71–82.

Lee, S., Gustafson, G., Skamnioti, P., Baloch, R. and Gurr, S. (2008) Host perception and signal transduction studies in wild-type *Blumeria graminis* f. sp. *hordei* and a quinoxyfen-resistant mutant implicate quinoxyfen in the inhibition of serine esterase activity. *Pest Management Science* 64(5), 544–555.

Leroux, P. and Gredt, M. (1995) *In vitro* study of resistance to anilinopyrimidine fungicides in *Botrytis cinerea*. *Agronomie* 15(6), 367–370.

Leroux, P., Colas, V., Fritz, R. and Lanen, C. (1996) Interaction of the anilinopyrimidine fungicide pyrimethanil with amino acids and sulfur containing metabolites in *Botrytis cinerea*. In: Lyr, H., Russell, P.E. and Sisler, H.D. (eds) *Modern Fungicides and Antifungal Compounds*. Intercept, Andover, UK, pp. 61–67.

Longhurst, C., Dixon, K., Mayr, A., Bernhard, U., Prince, K., *et al.* (1996) D8-795 – a novel fungicide for the control of powdery mildew. In: *Proceedings of the Brighton Crop Protection Conference 1996 – Pests and Diseases*, Vol. 1. British Crop Protection Council, Farnham, UK, pp. 27–32.

Machinandiarena, M.F., Lobato, M.C., Feldman, M.L., Daleo, G.R. and Andreu, A.B. (2012) Potassium phosphite primes defense responses in potato against *Phytophthora infestans*. *Journal of Plant Physiology* 169(14), 1417–1424.

Maeno, S. and Miura, L. (1990) Mepanipyrim (KIF-3535), a new pyrimidine fungicide. In: *Proceedings of the Brighton Crop Protection Conference 1990 – Pests and Diseases*, Vol. 2. British Crop Protection Council, Farnham, UK, pp. 415–422.

Malamy, J., Carr, J.P., Klessig, D. and Raskin, I. (1990) Salicylic acid: a likely endogenous signal in the resistance response of tobacco to viral infection. *Science* 250(4983), 1002–1004.

Malandrakis, A., Markoglou, A. and Ziogas, B. (2011) Molecular characterization of benzimidazole-resistant *B. cinerea* field isolates with reduced or enhanced sensitivity to zoxamide and diethofencarb. *Pesticide Biochemistry and Physiology* 99(1), 118–124.

Masner, P., Muster, P. and Schmid, J. (1994) Possible methionine biosynthesis inhibition by pyrimidinamine fungicides. *Pesticide Science* 42(3), 163–166.

Massoud, K., Barchietto, T., le Rudulier, T., Pallandre, L., Didierlaurent, L., *et al.* (2012) Dissecting phosphite-induced priming in *Arabidopsis* infected with *Hyaloperonospora arabidopsidis*. *Plant Physiology* 159(1), 286–298.

Mercer, E.I. (1991) Morpholine antifungals and their mode of action. *Biochemical Society Transactions* 19(3), 788–793.

Metraux, J.P., Signer, H., Ryals, J., Ward, E.W., Wyss-Benz, M., *et al.* (1990) Increase in salicylic acid at the onset of systemic acquired resistance in cucumber. *Science* 250(4983), 1004–1006.

Neumann, G.L., Winter, E.H. and Pittis, J.E. (1992) Pyrimethanil: a new fungicide. In: *Proceedings of the Brighton Crop Protection Conference 1992 – Pests and Diseases*, Vol. 1. British Crop Protection Council, Farnham, UK, pp. 395–402.

Nevill, D., Nyfeler, R. and Sozzi, D. (1988) CGA 142705: a novel fungicide for seed treatment. In: *Proceedings of the Brighton Crop Protection Conference 1988 – Pests and Diseases*, Vol. 1. British Crop Protection Council, Farnham, UK, pp. 65–72.

Nuninger, C., Watson, G., Leadbitter, N. and Ellgehausen, H. (1996) CGA329351: introduction of the enantiomeric form of the fungicide metalaxyl. In: *Proceedings of the Brighton Crop Protection Conference 1996 – Pests and Diseases*, Vol. 1. British Crop Protection Council, Farnham, UK, pp. 41–46.

Oliver, R.P. and Ipcho, S.V.S. (2004) *Arabidopsis* pathology breathes new life into the necrotrophs-vs.-biotrophs classification of fungal pathogens. *Molecular Plant Pathology* 5(4), 347–352.

Olivieri, F.P., Feldman, M.L., Machinandiarena, M.F., Lobato, M.C., Caldiz, D.O., *et al.* (2012) Phosphite applications induce molecular modifications in potato tuber periderm and cortex that enhance resistance to pathogens. *Crop Protection* 32(Feb), 1–6.

Oshima, M., Fujimura, M., Banno, S., Hashimoto, C., Motoyama, T., *et al.* (2002) A point mutation in the two-component histidine kinase BcOS-1 gene confers dicarboximide resistance in field isolates of *Botrytis cinerea*. *Phytopathology* 92(1), 75–80.

Ruess, W., Kunz, W., Staub, T., Millier, K., Poppinger, N., *et al.* (1995) Plant activator CGA 245704. A new technology for disease management. In: *Abstracts of the XIII International Plant Protection Congress*, The Hague, The Netherlands, July 1995.

Sauter, H., Steglich, W. and Anke, T. (1999) Strobilurins: evolution of a new class of active substances. *Angewandte Chemie International Edition* 38(10), 1328–1349.

Sauter, H., Ammermann, E., Benoit, R., Brand, S., Gold, R.E., *et al.* (1995) Mitochondrial respiration as a target for antifungals: lessons from research on strobilurins. In: Dixon, C.K., Copping, L.G. and Hollomon, D.W. (eds) *Antifungal Agents. Discovery and Mode of Action*. BIOS Scientific, Oxford, pp. 173–190.

Schurter, R., Kunz, W. and Nyfeler, R. (1990) Process and a composition for immunizing plants against diseases. US Patent 4,931,581.

Senior, I.J., Hollomon, D.W., Loeffler, R.S. and Baldwin, B.C. (1995) Sterol composition and resistance to DMI fungicides in *Erysiphe graminis*. *Pesticide Science* 45(1), 57–67.

Solomon, P.S., Tan, K.C., Sanchez, P., Cooper, R.M. and Oliver, R.P. (2004) The disruption of a Gα subunit sheds new light on the pathogenicity of *Stagonospora nodorum* on wheat. *Molecular Plant–Microbe Interactions* 17(5), 456–466.

Soyez, J.-L. (1992) A new concept in crop protection: an active adjuvant in fungicides – the case of copper tallate. In: *Proceedings of the Brighton Crop Protection Conference 1992 – Pests and Diseases*, Vol. 1. British Crop Protection Council, Farnham, UK, pp. 451–456.

Steel, C.C., Baloch, R.I., Mercer, E.I. and Baldwin, B.C. (1989) The intracellular location and physiological effects of abnormal sterols in fungi grown in the presence of morpholine and functionally related fungicides. *Pesticide Biochemistry and Physiology* 33(2), 101–111.

Szaniszlo, P.J., Kang, M.S. and Cabib, E. (1985) Stimulation of β(1–3) glucan synthetase of various fungi by nucleoside triphosphates: generalised regulatory mechanism for cell wall biosynthesis. *Journal of Bacteriology* 161(3), 1188–1194.

Szkolnik, M. (1983) Unique vapour activity by CGA-64251 (Vanguard) in the control of powdery mildews room wide in the greenhouse. *Plant Disease* 67, 360–366.

Tillman, R.W., Siegel, M.R. and Long, J.W. (1973) Mechanism of action and fate of the fungicide chlorothalonil (2,4,5,6-tetrachlorophthalonitrile) in biological systems. *Pesticide Biochemistry and Physiology* 3(2), 160–167.

Tomlin, C. (ed.) (2009) *The Pesticide Manual*. British Crop Protection Council, Alton, UK.

Ulrich, J.T. and Mathre, D.E. (1972) Mode of action of oxathiin systemic fungicides. V. Effect on electron transport system of *Ustilago maydis* and *Saccharomyces cerevisiae*. *Journal of Bacteriology* 110(2), 628–632.

van Gestel, J. (1986) The vapour phase activity of antifungal compounds: a neglected or a negligible phenomenon? In: Iwata, K. and Vanden Bossche, H. (eds) *In Vitro and In Vivo Evaluation of Antifungal Agents*. Elsevier, Oxford, pp. 207–218.

Walters, D.R., Ratsep, J. and Havis, N.D. (2013) Controlling crop diseases using induced resistance: challenges for the future. *Journal of Experimental Botany* 64(5), 1263–1280.

Wheeler, M.H. and Greenblatt, G.A. (1988) The inhibition of melanin biosynthetic reactions in *Pyricularia oryzae* by compounds that prevent rice blast disease. *Experimental Mycology* 21, 151–160.

Young, D.H. (1991) Effects of zarilamide on microtubules and nuclear division in *Phytophthora capsici* and tobacco suspension-cultured cells. *Pesticide Biochemistry and Physiology* 40(2), 149–161.