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Review
The strobilurin fungicides†

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Abstract: Strobilurins are one of the most important classes of agricultural fungicide. Their invention was inspired by a group of fungicidally active natural products. The outstanding benefits they deliver are currently being utilised in a wide range of crops throughout the world. First launched in 1996, the strobilurins now include the world's biggest selling fungicide, azoxystrobin. By 2002 there will be six strobilurin active ingredients commercially available for agricultural use. This review describes in detail the properties of these active ingredients – their synthesis, biochemical mode of action, biokinetics, fungicidal activity, yield and quality benefits, resistance risk and human and environmental safety. It also describes the clear technical differences that exist between these active ingredients, particularly in the areas of fungicidal activity and biokinetics.

Keywords: strobilurins; famoxadone; fenamidone; chemistry; mode of action; safety; performance

1 INTRODUCTION

The strobilurins are an important class of agricultural fungicides, the discovery of which was inspired by a group of natural fungicidal derivatives of β-methoxyacrylic acid. This paper is a review of the chemical, biochemical, biological, biokinetic and human and environmental safety properties of the commercially available strobilurins and related fungicides. Only those strobilurin fungicides which are currently commercially available, or shortly will be, and for which there is significant information in the public domain are considered within the scope of this review. We are aware of other code-numbered strobilurins, some of which have been in existence for a number of years, but these are not included because there is insufficient confirmed information publicly available to allow us to make considered technical judgements.

The strobilurins were first sold in 1996; by 2002 there will be six commercially available strobilurin fungicides (Table 1; Fig 1). In addition, famoxadone and fenamidone, chemically distinct from the strobilurins but in the same cross-resistance group, are also commercially available. Famoxadone and fenamidone will be considered in this review, but only in those sections that are relevant to their relationship with the strobilurin fungicides, namely chemistry, biochemical mode of action and resistance.

Sales of the strobilurin and related fungicides totalled approximately \$620 million in 1999.[1] This represents over 10% of the global fungicide market, which is an outstanding achievement within just four years of first sales. Leading outlets include cereals, turfgrass, grapevines, potatoes and fruit, nut and vegetable crops. The huge impact of the strobilurin fungicides on agriculture is well reflected by the current status of azoxystrobin, which is now registered for use on 84 different crops in 72 countries, representing over 400 crop/disease systems. Sales of azoxystrobin were \$415 million in 1999, making it the world's biggest-selling fungicide.

Table 1. The strobilurins and related fungicides

Fungicide	*Company*	*Announced*	*First sales*
Azoxystrobin[a]	Syngenta	1992	1996
Kresoxim-methyl	BASF	1992	1996
Metominostrobin	Shionogi	1993	1999
Trifloxystrobin[b]	Bayer	1998	1999
Picoxystrobin	Syngenta	2000	2002
Pyraclostrobin	BASF	2000	2002
Famoxadone	DuPont	1996	1997
Fenamidone[c]	Aventis	1998	2001

[a] Discovered by ICI, the agrochemical interests of which are now part of Syngenta.
[b] Discovered by Novartis, sold to Bayer in 2000.
[c] Discovered by Rhône-Poulenc, the agrochemical interests of which are now part of Aventis.

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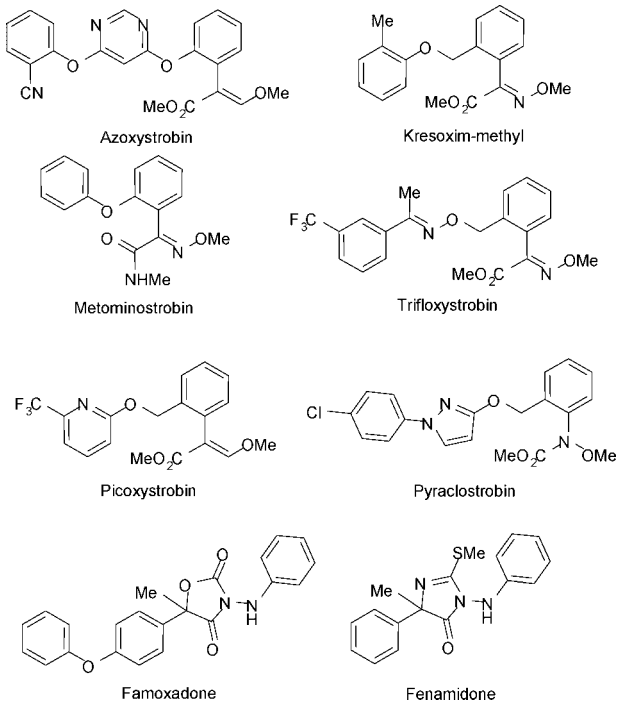

Figure 1. The strobilurin fungicides, and famoxadone and fenamidone.

2 BIOCHEMICAL MODE OF ACTION

The discovery of the strobilurin fungicides was inspired by a group of natural fungicidal derivatives of β-methoxyacrylic acid, the simplest of which are strobilurin A, oudemansin A and myxothiazol A (Fig 2). These natural products are produced by a range of Basidiomycete wood-rotting fungi, for example *Oudemansiella mucida* (Schrad ex Fr) Hoehn and *Strobilurus tenacellus* (Pers ex Fr) Singer and, in the case of myxothiazol A, the gliding bacterium *Myxococcus fulvus*. The fungicidal activity of the strobilurins, oudemansins and myxothiazols stems from their ability to inhibit mitochondrial respiration by binding at the so-called Q_o site of cytochrome b. Cytochrome b is part of the cytochrome bc_1 complex, located in the inner mitochondrial membrane of fungi and other eukaryotes. When one of the inhibitors binds, it blocks electron transfer between cytochrome b and cytochrome c_1, which, in turn, disrupts the energy cycle within the fungus by halting the production of ATP. Since the natural products and their synthetic analogues can displace each other from the binding site, it is clear that they are reversibly bound.

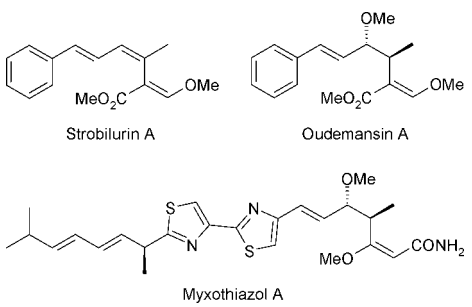

Figure 2. Representative natural strobilurins.

Many different respiration inhibitors are known. However, there are many distinct binding sites on the respiration pathway, and the importance of the naturally occurring strobilurins, oudemansins and myxothiazols when they were first isolated was the recognition that the site at which they bind was novel, ie inhibitors with the same specific binding interactions had not previously been identified. These natural products, as well as some of the synthetic analogues (especially the stilbene **1** in Fig 3), have therefore become important tools for probing the details of the mechanism of mitochondrial respiration.

Fungicides with novel biochemical modes of action are not discovered very often, and so it is not surprising that the strobilurins attracted the attention of scientists in at least two companies—Syngenta and BASF. Natural products with a novel mode of action are always of interest to agrochemical companies because synthetic analogues would be expected not to be cross-resistant to established commercial products. Compounds with a novel mode of action also tend to have a novel technical profile which delivers novel benefits to the grower, as has turned out to be the case with the commercial strobilurins.

Theoretically less attractive was the mode of action of these particular natural products: inhibition of respiration might be expected to lead to problems with selectivity, ie respiration inhibitors could be toxic to non-target organisms. Supporting this potential concern was information in the literature that the acute toxicity to mice of myxothiazol A is relatively high.[2] However, it was also known that strobilurin A is considerably less toxic to mice,[3] and this suggested that selective toxicity towards fungi could be achieved.

Since the discovery of the strobilurins and the related natural products, other structurally distinct compounds which also bind at the Q_o site on cytochrome b have been found. Of most significance from

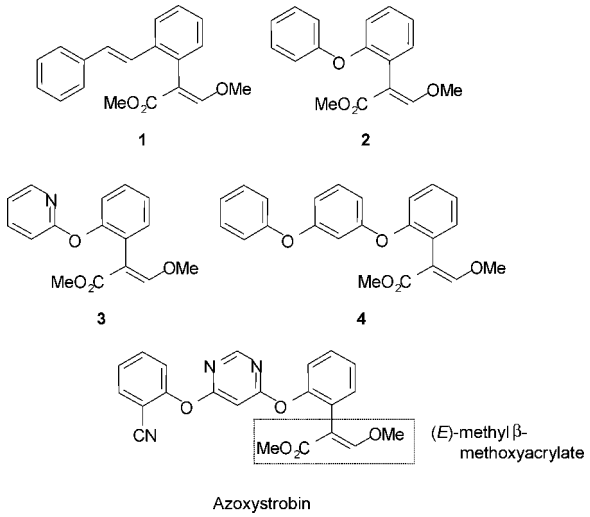

Figure 3. Milestones in the discovery of azoxystrobin.

a commercial point of view are the synthetic fungicides famoxadone and fenamidone, discovered and marketed by DuPont and Aventis, respectively. Detailed studies with famoxadone suggest that it binds at a position which is not identical to, but overlaps with, that occupied by myxothiazol A.[4]

At the time when the mode of action of the strobilurins, oudemansins and myxothiazols was elucidated, membrane-bound proteins and protein complexes, even of low molecular mass, had not been crystallised. The cytochrome bc_1 complex comprises 11 subunits with a combined molecular mass of about 240 kD which, to complicate matters further, functions as a homodimer. Crystallisation and X-ray analysis therefore constitutes a formidable task. Nevertheless, in the last few years this has been achieved, and several groups have crystallised cytochrome bc_1 complexes from mitochondria of cows, chickens or yeast, and analysed them by X-ray diffraction to a resolution of 2.3 Å in the best case.[5,6] Not surprisingly, there has been discussion in the literature about the way in which the strobilurins bind. Zhang *et al*,[7] for example, discussed the way in which myxothiazol A binds. However, no high-resolution structures have yet been published, but it is anticipated that this information will soon be available, and before long it should be possible to see the precise binding interactions of the strobilurins, and of famoxadone and fenamidone.

3 SYNTHETIC CHEMISTRY

The natural products are unsuitable for use as agricultural fungicides in their own right. However, a knowledge of their structures and physical properties provided the starting point for independent programmes of research within Syngenta and BASF which led, after several years' work, to the discovery and almost simultaneous launch of the first of the synthetic strobilurins. The two companies have each published detailed accounts of this work. For Syngenta, this programme led first to the discovery of azoxystrobin, selected from about 1400 synthesised compounds.[8,9] For BASF, the result was kresoxim-methyl.[10,11]

Milestones in the discovery of azoxystrobin are shown in Fig 3. Strobilurin A, although active against fungi growing on agar, does not control fungi growing on plants in the glasshouse because of its photochemical instability and volatility. However, it was discovered that the (*Z*)-olefinic bond of strobilurin A could be replaced by an *ortho*-disubstituted benzene ring to give the stilbene **1**, which is less volatile and much more stable in light than the natural product. The stilbene **1** is active in the glasshouse but still degrades too quickly in light to express high activity in the field. The diphenyl ether **2** is much more stable in light and is active in the field, albeit at uneconomic application rates. Furthermore, **2** is systemic in plants, an important property of many modern fungicides which improves field performance by redistribution of the compound within plant tissue after spraying. Modifications to **2** led to the analogues **3** and **4**. The pyridine **3** retains the activity of its isostere **2** and, as expected, is highly mobile (probably too mobile) in plants. The tricyclic derivative **4** has improved levels of fungicidal activity, but is too lipophilic to demonstrate systemic movement. A combination of the ideas which led from **2** to **3** and **4**, together with a huge amount of experimentation, led finally to the discovery of azoxystrobin.

Milestones in the discovery of kresoxim-methyl are shown in Fig 4. Again, the stilbene **1** was prepared as a stabilised analogue of strobilurin A. The reduced analogue **5** and the isosteric benzyl phenyl ether **6** were then prepared and found to be fungicidal, and the framework of **6** is present and optimised in kresoxim-methyl. An important structural difference between azoxystrobin and kresoxim-methyl is that the latter contains an (*E*)-methyl methoxyiminoacetate group instead of (*E*)-methyl *β*-methoxyacrylate (see Figs 3 and 4). The earliest patents claiming synthetic fungicidal strobilurins containing the natural (*E*)-methyl *β*-methoxyacrylate group were filed by Syngenta about seven months ahead of the first strobilurin patents from BASF. The publication of these earliest patents blocked BASF's activities around the natural toxophore. BASF then found that the (*E*)-methyl methoxyiminoacetate group, isosteric with (*E*)-methyl *β*-methoxyacrylate, also confers activity when linked to a suitable backbone. In fact, Syngenta made the same discovery at about the same time but, in this case, the order of patenting was reversed, and BASF filed just two days ahead of Syngenta.

So it was that the early stages of the research programmes in Syngenta and BASF were strikingly similar: in each case, strobilurin A was stabilised to photochemical degradation by linking its (*Z*)-olefinic bond into an *ortho*-disubstituted benzene ring to give the stilbene **1**. Both companies then discovered that there was relatively little scope for modification of the (*E*)-methyl *β*-methoxyacrylate group without loss of fungicidal activity, and so this part of the structure became known as the toxophore. By contrast, when

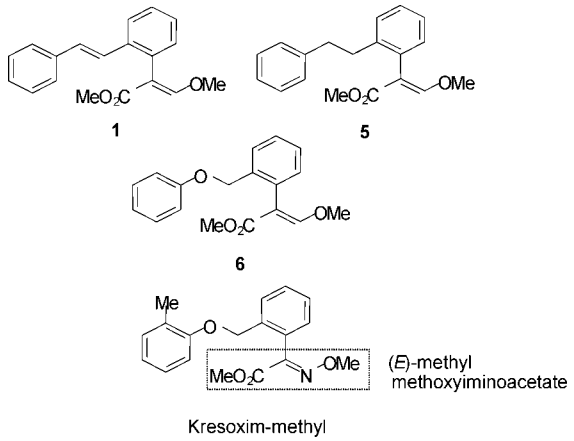

Figure 4. Milestones in the discovery of kresoxim-methyl.

the styryl group of the stilbene **1** was replaced with a variety of alternative groups, there was a wide variation in fungicidal activity—an ideal situation for a synthetic chemistry project, because it gives scope for the modification of physical properties and, of course, for filing patents of selection. It can be seen, however, that the later stages of the research programmes in the two companies were quite different, and led to the two products azoxystrobin and kresoxim-methyl with distinct biological properties.

Shionogi's metominostrobin was the third and last of the first independently discovered set of strobilurins. It has an (*E*)-*N*-methyl methoxyiminoacetamide toxophore, isosteric with the (*E*)-methyl β-methoxyacrylate toxophore of the natural strobilurins. Interestingly, it was discovered by a chemistry-led programme of research, without reference to a knowledge of the structures of the natural strobilurins. Only after the synthesis of a variety of compounds and with the emergence of similar structure–activity relationships did it become clear that Shionogi's work had to a large extent converged with that of Syngenta and BASF.[9,12]

Once the early patents by Syngenta and BASF were published, other agrochemical companies realised the importance of the strobilurins and began their own research programmes in the area, and this, in time, led to other commercial strobilurins. Novartis discovered and developed trifloxystrobin, announcing it in 1998,[13] although it was sold to Bayer in 2000 as part of the requirements for the merger between Novartis Agribusiness and Zeneca Agrochemicals to form Syngenta. Two years later, Syngenta and BASF announced picoxystrobin and pyraclostrobin, respectively.[14,15] Three different toxophores are represented in these three new strobilurins. Picoxystrobin and trifloxystrobin have the natural (*E*)-methyl β-methoxyacrylate and the (*E*)-methyl methoxyiminoacetate toxophores, respectively, while pyraclostrobin has a methyl *N*-methoxycarbamate toxophore.

Famoxadone and fenamidone, other fungicides with the same mode of action as the strobilurins but with distinct chemical structures, were also discovered during the 1990s. Famoxadone was announced by DuPont in 1996[16] and fenamidone was announced two years later by Aventis.[17] An account of the discovery of famoxadone has been published.[18] In common with other agrochemical companies, DuPont procures samples of novel compounds from universities and then screens them for biological activity. One such compound, obtained from the University of Bonn, was shown to be fungicidal, and formed the starting point for a programme of analogue synthesis which led to famoxadone.

To conclude this section on the chemistry of the strobilurin fungicides, we describe the leading research-scale synthetic approaches to these compounds. Since the scope for variation of the side-chain has proved to be substantial, we will limit the discussion here to the main methods used to construct the various toxophores represented in the commercial

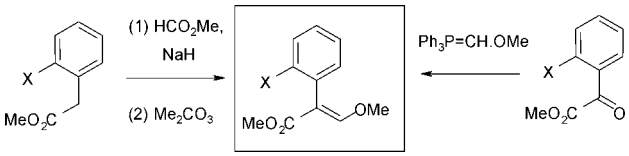

Figure 5. Construction of the methyl β-methoxyacrylate toxophore.

strobilurins. Published routes to the ring-systems of famoxadone and fenamidone are also described.

The most widely used route to compounds containing the methyl β-methoxyacrylate toxophore involves a Claisen condensation between a methyl phenylacetate and methyl formate in the presence of a base such as sodium hydride, followed by *O*-methylation of the resulting methyl β-hydroxyacrylate. The sequence is highly stereoselective, and leads to the desired (*E*)-isomer almost exclusively (Fig 5). The Wittig reaction shown in Fig 5 has also been used to construct the methyl β-methoxyacrylate group, but in this approach an (*E*/*Z*)-mixture of isomers is usually obtained.[19]

The leading approaches to the methyl methoxyiminoacetate and *N*-methyl methoxyiminoacetamide toxophores are shown in Fig 6. Methyl benzoyl formates are readily converted into compounds containing the methyl methoxyiminoacetate toxophore by treatment with methoxylamine (or hydroxylamine and then a methylating reagent).[20] The methyl methoxyiminoacetate group can be further converted into the *N*-methyl methoxyiminoacetamide toxophore by treatment with methylamine. In an alternative route to the *N*-methyl methoxyiminoacetamide toxophore, the oximation and amide-forming steps are reversed. All these approaches tend to produce mixtures of stereoisomers, although the required (*E*)-isomer generally predominates.[12,21]

The methyl β-methoxyacrylate toxophore can also be incorporated as a complete unit through palladium-catalysed cross-coupling. As the examples in Fig 7 show, the metallic functional group may be positioned either on the toxophore or on the benzene ring.[22,23] The methyl methoxyiminoacetate group has also been linked to substituted benzenes as an intact unit by palladium-catalysed cross-coupling.[24]

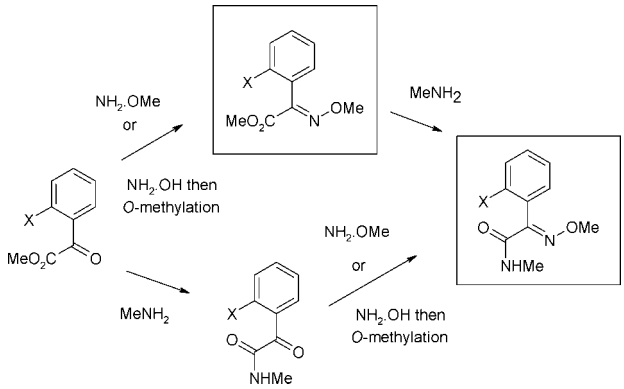

Figure 6. Construction of the methyl methoxyiminoacetate and *N*-methyl methoxyiminoacetamide toxophores.

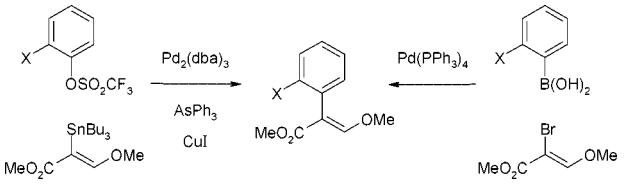

Figure 7. Introduction of the methyl β-methoxyacrylate toxophore by palladium-catalysed cross-coupling.

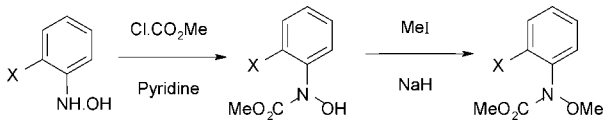

Figure 8. Preparation of the methyl *N*-methoxycarbamate toxophore.

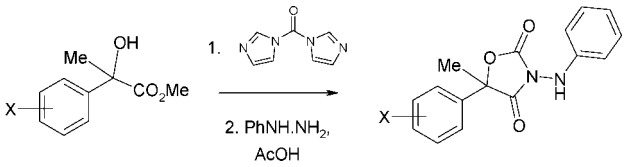

Figure 9. Preparation of the oxazolidin-2,4-dione ring of famoxadone.

The methyl *N*-methoxycarbamate toxophore found in pyraclostrobin is constructed using the steps shown in Fig 8. A substituted *N*-phenyl hydroxylamine is treated with methyl chloroformate under basic conditions, and the resulting *N*-hydroxycarbamate is *O*-methylated with methyl iodide.[25]

The oxazolidin-2,4-dione ring system of famoxadone can be prepared by treatment of a substituted 2-hydroxy-2-phenylpropionate with 1,1′-carbonyldiimidazole and then phenylhydrazine (Fig 9).[18] The 2-imidazolin-5-one ring of fenamidone is prepared from a substituted 2-isothiocyanato-2-phenylpropionate by treatment with phenylhydrazine and then *S*-methylation (Fig 10).[26]

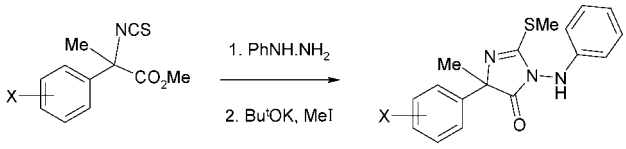

Figure 10. Preparation of the 2-imidazolin-5-one ring of fenamidone.

4 BIOKINETICS

The various strobilurin fungicides in the marketplace and in development have very different physicochemical properties (Table 2) which consequently confer a wide range of biokinetic behaviours both inside the plant and around its external surfaces.

Uptake of azoxystrobin into the cells of the leaf following foliar surface application has been quantified in a broad range of crops in the field under commercial conditions and has been shown to be dependent on formulation type, additives, mixtures with other products, crop (leaf type, surface, weathering and age) and upon environmental factors which influence spray droplet drying. Uptake of azoxystrobin is a gradual process, with typically between 1–3% (grapevine, SC formulation, no wetter) and 25% (cereals, SC formulation with built-in wetter; banana, SC sprayed with an oil additive) of applied material absorbed into the leaf within 24h of application.

Of all the strobilurins currently in sales or development in cereals, picoxystrobin is the most rapidly absorbed into plant tissue. In wheat and barley, 30–45% of material applied to the foliar surface is absorbed into the cells of the leaf within 24h of application.[14] This level of uptake confers excellent curative properties whilst maintaining material on the outside of the leaf to retain preventative benefits. The curative activity of picoxystrobin is an important reason why the compound is targeted as an early-season application in wheat and barley.

Another attribute of picoxystrobin that is important for its early-season use is the fact that it is the most xylem-systemic of all the commercial strobilurins in cereals; quantitative analysis of movement away from a zonal application in winter wheat has shown that 20% of the picoxystrobin which enters the leaf has moved above the point of uptake within 8 days of application. Azoxystrobin is also xylem-systemic, with studies showing that 8% of the active ingredient entering the

	Aqueous solubility (mg litre^{-1} at 20°C)	*Log P_{ow}*	*Vapour pressure (mPa at 20°C)*	*Melting point (°C)*	*Relative molecular mass*
Azoxystrobin[a]	6	2.5	1.1×10^{-7}	115	403.4
Kresoxim-methyl[a]	2	3.4	2.3×10^{-3}	99	313.4
Metominostrobin[a]	128	2.3	1.8×10^{-2}[d]	88	284.3
Trifloxystrobin[a]	0.6	4.5	3.4×10^{-3}[d]	73	408.4
Picoxystrobin[b]	3.1	3.6	5.5×10^{-3}	75	367.3
Pyraclostrobin[c]	1.9	4.0	2.6×10^{-5}	64–65	387.8

[a] Reference 27.
[b] Reference 14.
[c] Reference 15.
[d] At 25°C.

Table 2. Physical properties of the strobilurin fungicides

leaf had moved above the point of uptake within 8 days of application.[28] In contrast, kresoxim-methyl,[10] trifloxystrobin[13,29] and pyraclostrobin[15] are all non-systemic. Parallel biological testing has shown that the systemic redistribution of azoxystrobin and picoxystrobin following treatment to a defined zone at the leaf base resulted in disease control all the way to the cereal leaf tip, whereas the other strobilurins tested showed no such systemic disease control (Plate 1).

Metominostrobin is the only commercialised strobilurin other than azoxystrobin and picoxystrobin that is systemic. The systemicity of metominostrobin is utilised by formulating it as a granule for addition to the rice paddy to give systemic control of sheath blight, *Rhizoctonia solani* Kuhn.[30]

The technique of phosphor image analysis[31] coupled with quantitative and qualitative radioanalysis has been used to describe the movement of the strobilurin fungicides in a range of key crop species. The most widely applicable example is the behaviour in cereals as shown in the phosphor image in Plate 2.

Picoxystrobin remaining on the outside of the leaf can redistribute around existing leaves and to newly emerging cereal leaves in the vapour phase. Although the vapour pressure indicates very low levels of potential vapour transfer, the high intrinsic potency of picoxystrobin allows it to deliver broad-spectrum disease control following its transfer by air. Picoxystrobin redistributed by air and absorbed into the leaf is also sufficiently xylem-mobile to give disease control towards the leaf tip, ie picoxystrobin can deliver systemic disease control following its vapour transfer.[14] Kresoxim-methyl[32] and trifloxystrobin[13] also deliver disease control in the vapour phase, but pyraclostrobin cannot be redistributed in this way.[15] Although a number of strobilurins are active in the vapour phase, it should be emphasised that none of the strobilurins are highly volatile, as Table 2 illustrates. Indeed, molecular redistribution by air is probably a more appropriate way to describe the vapour activity of current strobilurins.

Table 3 compares the redistribution properties of all the commercialised strobilurin fungicides.

The xylem-systemic properties of picoxystrobin in particular, but also of azoxystrobin,[28] mean that they are both capable of moving to newly expanding and newly emerging growth as the wheat or barley crop develops. The influences of rain and dewfall are potent redistributive forces for all particulate pesticides, and movement to leaf axils followed by uptake, translaminar and xylem mobility redistribute azoxystrobin and picoxystrobin to new growth. This movement occurs via translaminar mobility to consecutive leaf layers and subsequent xylem systemicity within them and/or by direct physical 'pick-up' from the leaf axils by the newly emerging foliar tissue as it passes through the axil 'reservoir' often containing active ingredient and water. This has been shown to result in control of *Septoria tritici* Roberge [teleomorph *Mycosphaerella graminicola* (Fuckel) Schroter] on the top two final leaves of wheat when neither leaves were unfurled at the time of an early season application of picoxystrobin at Zadoks growth stage 31–32 (Plate 3). Movement of the other strobilurin cereal fungicides to new growth in wheat and barley is restricted due to their lack of systemicity.

In broad-leaved crops, movement of a xylem-mobile fungicide to new growth can occur from initial spray deposition on stem tissue. This has been demonstrated for azoxystrobin in grapevine, where a zonal application of ^{14}C-azoxystrobin was made to the base of seedlings when only two leaves were present. After an interval of three weeks, radioactive azoxystrobin was shown to be present in the three new leaves which emerged during this period. However, movement of azoxystrobin to new growth in broad-leaved crops is insufficient for it to provide robust disease control on subsequently emerging leaves. None of the other strobilurins targeted at broad-leaved crops is capable of showing any systemic movement to new growth.

5 BIOLOGY

Strobilurin fungicides have become an integral part of disease-management programmes on a wide range of crops in many countries of the world. The major reasons for the success of strobilurins have varied between individual active ingredients, but have con-

Table 3. Redistribution properties of strobilurins

	Azoxystrobin	*Kresoxim-methyl*	*Metominostrobin*	*Trifloxystrobin*	*Picoxystrobin*	*Pyraclostrobin*
Uptake into leaf	Low	Low	High	Very low	Medium	Very low
Molecular redistribution by air	No	Yes	nt[a]	Yes	Yes	No
Metabolic stability in leaf	Yes	Low	nd[b]	Low	Yes	Yes
Translaminar movement	Yes	Low	Yes	Low	Yes	Low
Xylem systemic	Yes	No	Yes	No	Yes	No
Systemic movement to new growth in wheat and barley	Yes	No	nr[c]	No	Yes	No
Phloem mobile	No	No	No	No	No	No

Source: Syngenta.

[a] nt = not tested.

[b] nd = no data.

[c] nr = not relevant crop outlet.

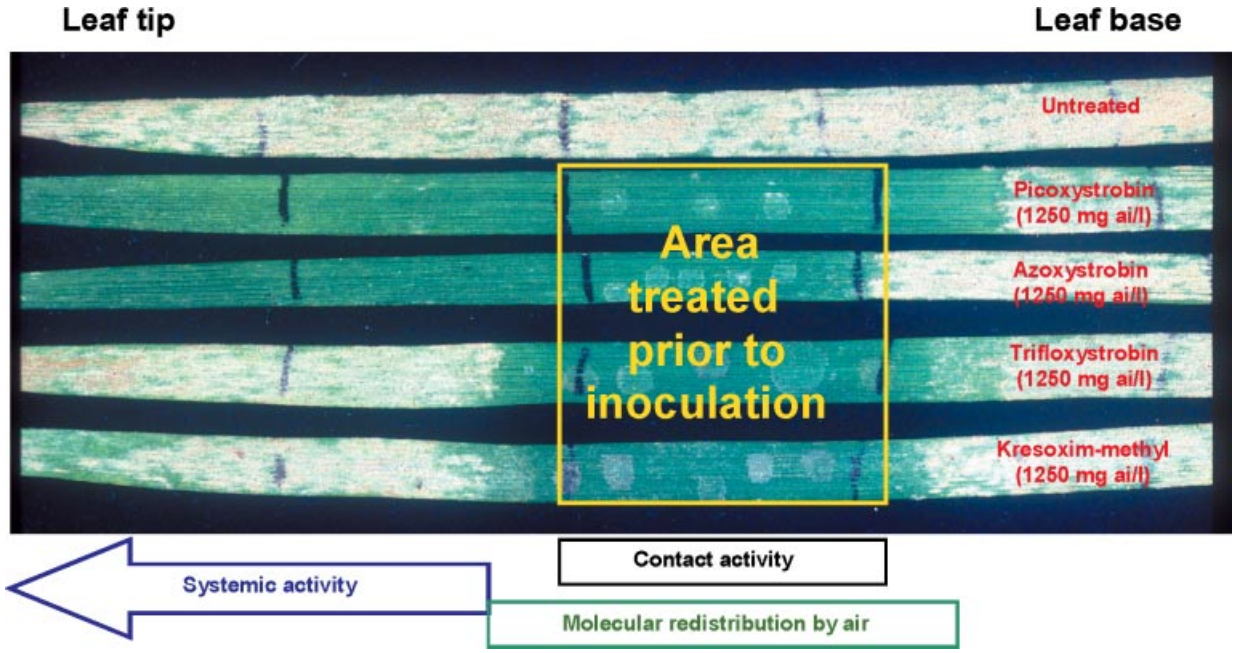

Plate 1. Redistribution of strobilurins in wheat to control powdery mildew. (Source: Syngenta).

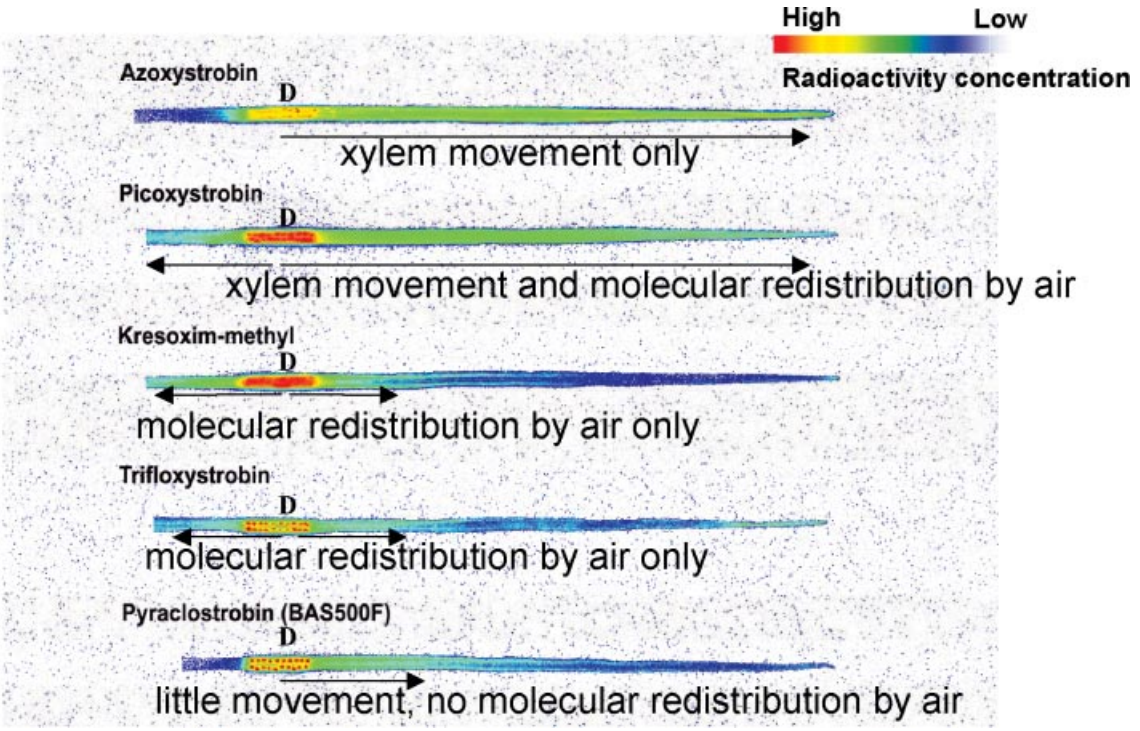

Plate 2. Phosphor image showing redistribution of radiolabelled strobilurins in wheat following droplet application to zone D three days previously. (Source: Syngenta).

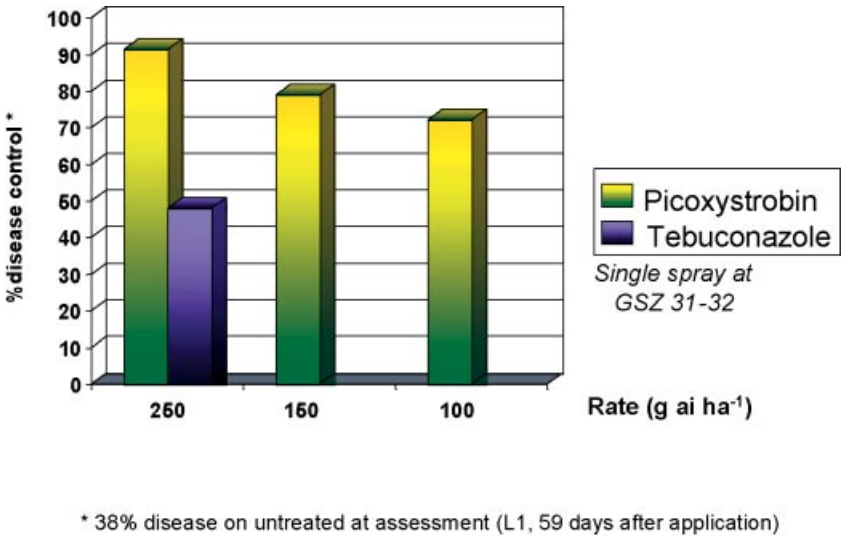

Plate 3. Control of *Septoria tritici* on leaves of wheat not unfurled at the time of a single early season application.

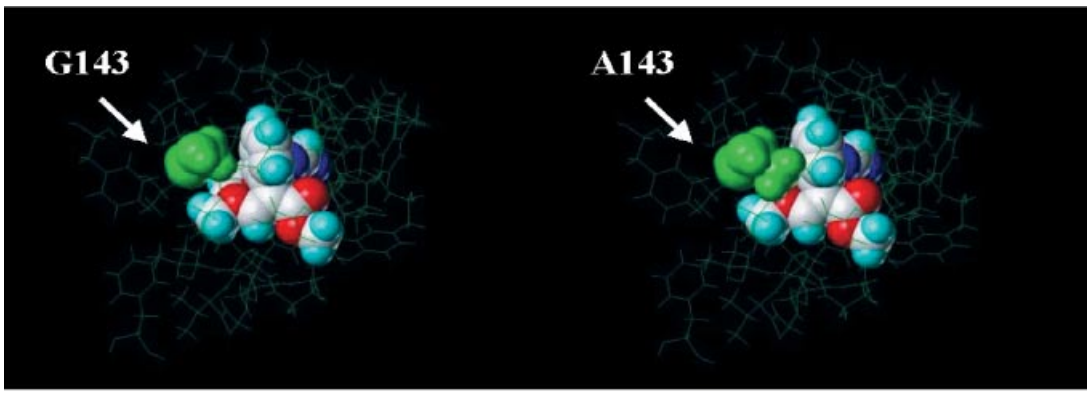

Plate 4. Azoxystrobin modelled into the Q_o pocket of the cytochrome bc_1 complex. Substitution of the hydrogen side chain of glycine (A, green) for the methyl side chain of alanine (B, green) impacts binding of the Q_oI molecule into its target site.

sisted of one or more of the following: broad-spectrum activity, control of fungal isolates resistant to other fungicide modes of action, low use-rates and excellent yield and quality benefits.

In a number of crops, strobilurins have led to major changes in disease-control programmes; for example, grapevine growers have for the first time a single active ingredient for control of both powdery (*Uncinula necator* (Schwein) Burr) and downy (*Plasmopara viticola* (Berkholder & MA Curtis ex de Bary) Berliner & De Toni) mildews.[8] In other crops, such as wheat and barley in Europe, strobilurins continue to be key tools for maintaining the incomes of farmers in an increasingly difficult economic situation through the yield and quality benefits they deliver over other fungicide classes. Strobilurin fungicides are important additions to the fungicide resistance-management armoury in many crops, particularly in banana, and they have been welcomed in protected horticultural crops in Europe where the number of available active ingredients is dwindling due to the high cost of maintaining a long list of registered uses.

Strobilurin fungicides are important not only as foliar-applied fungicides but also as seed-treatment applications and in-furrow treatments for soil-borne disease control, eg azoxystrobin is used in cotton in North America for control of seedling damping-off caused by *Pythium* spp and *R solani*.

5.1 Spectrum of disease control

All of the commercialised strobilurins, azoxystrobin,[8] kresoxim-methyl,[10] metominostrobin,[30] trifloxystrobin,[13] picoxystrobin[14] and pyraclostrobin,[15] can be demonstrated to have a broad spectrum of activity. Of particular importance is their activity against all four major groups of plant pathogenic fungi, namely Ascomycetes, Basidiomycetes, Deuteromycetes and Oomycetes. However, strobilurins vary in their levels of activity against the different plant diseases and not all of them give high levels of control of all four major groups of plant pathogenic fungi. For example, kresoxim-methyl and trifloxystrobin are only moderately active against many Oomycete diseases, such as grapevine downy mildew,[33,77] and give only poor or moderate control of a number of Basidiomycete diseases, such as wheat and barley brown rusts.[34] Metominostrobin also is only moderately active against Oomycete diseases in general and appears to have been developed for use almost exclusively on rice and turf.

The breadth of spectrum of azoxystrobin has been demonstrated under commercial conditions since its launch in 1996. Pyraclostrobin is also being developed as a broad-spectrum strobilurin fungicide for use on a wide range of crops[15] and will be commercially available for the first time in 2002. Picoxystrobin, also commercially available for the first time in 2002, has been developed as a specialist broad-spectrum cereal fungicide.[14]

Table 4 compares strobilurin fungicides for their levels of activity against a broad range of commercially important fungal diseases from across the world.

5.2 Effects on fungal development and implications for application timing

Studies with azoxystrobin,[35,36] kresoxim-methyl,[37,38] trifloxystrobin[13] and pyraclostrobin[15,39] have demonstrated that spore germination and zoospore motility are stages of fungal development that are particularly sensitive to strobilurins. This can be explained by their biochemical mode of action, namely disruption of energy production, with the consequence that they are particularly effective against these highly energy-demanding stages of fungal development. This is an interesting contrast to triazole fungicides which inhibit ergosterol biosynthesis and therefore do not prevent spore germination and early germ-tube growth because the pathogen obtains a supply of ergosterol or its precursors from reserves within the spore.[40] The potent effects of strobilurins on spore germination and zoospore motility explain the high levels of preventative activity that these fungicides typically deliver.

Strobilurin fungicides have also been shown to demonstrate curative activity against a range of diseases, ie disease control after infection has occurred but before symptoms are visible. This has been found with azoxystrobin,[41] kresoxim-methyl,[38] trifloxystrobin,[13] picoxystrobin[14] and pyraclostrobin.[39] It is usually evidenced as mycelial collapse of the pathogenic fungus in low-temperature scanning electron microscopy studies, eg with azoxystrobin[36] and kresoxim-methyl.[37] Eradicant (disease control after visible symptoms apparent) and/or antisporulant (reduction in sporulation) activity have sometimes also been demonstrated.

The detailed understanding of the effects of strobilurins on different stages of fungal development has been important for optimising application timing to give maximum disease control. Strobilurins are best applied prior to infection or in the early stages of disease development in order to capitalise on their potent effects against spore germination and zoospore motility. Preventative application of strobilurins for optimum disease control is also one of the recommendations of FRAC (Fungicide Resistance Action Committee) regarding best practice for managing the resistance risk of strobilurins and related fungicides (see www.gcpf.org/frac).

Some studies have also been carried out to investigate the effects of strobilurins against the sexual stages of plant pathogenic fungi. For example, Godwin and Cortesi[42] have demonstrated that azoxystrobin inhibits the formation of mature cleistothecia of *U necator*, grapevine powdery mildew, and the production of viable ascospores. Vercesi *et al*[43] have similarly shown azoxystrobin to inhibit the formation of mature oospores of grapevine downy mildew, *P viticola*, and reduce their viability.

5.3 Yield and quality benefits

Yield and quality studies with strobilurin fungicides have focused on wheat and barley, with data consistently indicating clear benefits, particularly in terms of yield and grain size following treatment with azoxystrobin,[44] kresoxim-methyl,[45] trifloxystrobin,[13] picoxystrobin[14] or pyraclostrobin.[15] This is not surprising given that the reason for fungicide use on cereals is to protect grain yield and quality through disease control.

However, of particular interest has been the consistently greater yield from strobilurin-based cereal fungicide programmes compared with triazole-based programmes in situations where both spray programmes have delivered similar levels of visible disease control or where there has been seemingly insufficient difference in visible disease control to explain the differences in yield response.[46] This has been termed the strobilurin 'greening effect' because it is closely associated with the ability of strobilurins to maintain the green leaf area of the crop until late in the season, thereby maximising the grain-filling period with resultant yield benefits. Two hypotheses have been presented to explain this phenomenon. First, non-disease-related physiological effects of strobilurins on the host plant have been proposed as the reason for these 'unexpectedly good' yield benefits with a range of strobilurins.[47,48] Data have been presented which indicate that a variety of physiological processes are directly affected by strobilurins, including the carbon dioxide compensation point, leaf senescence, ACC synthase and thereby ethylene biosynthesis, chlorophyll content, photosynthetic activity, stomatal aperture, water consumption, plant antioxidant enzyme activity, endogenous levels of abscisic acid and other plant hormones, and nitrate reductase activity.[47–52] Most of these studies have been with kresoxim-methyl, but a number of these effects have also been reported in more limited studies with azoxystrobin[47,52] and trifloxystrobin.[48] The second theory presented has been that strobilurins deliver greater yield and quality benefits than triazoles whilst giving the same level of visible disease control because strobilurins prevent the spores of pathogenic, non-pathogenic and saprophytic fungi germinating and thereby stop the elicitation of energy-demanding host-defence responses, whereas triazoles do not.[53] Glasshouse studies by Schöfl and Zinkernagel[54] support this theory because they demonstrated good preventative control of *S tritici* pycnidia formation but severe leaf necrosis with triazoles alone, whereas combinations of triazoles with kresoxim-methyl (or with chlorothalonil, also a potent inhibitor of spore germination) gave similarly good fungal control but significantly better prevention of leaf necrosis. However, neither hypothesis has been

Table 4. Efficacy of strobilurin fungicides against a range of commercially important fungal diseases

Disease	*Azoxystrobin*	*Kresoxim-methyl*	*Metominostrobin*	*Trifloxystrobin*	*Picoxystrobin*	*Pyraclostrobin*
Ascomycete						
Grape powdery mildew (*Uncinula necator*)	***	***	NA	***	NA	***
Banana black sigatoka (*Mycosphaerella fijiensis*)	***	NA	NA	***	NA	***
Barley net blotch (*Helminthosporium teres*)	***	**	NA	***	***	***
Wheat *Septoria tritici* (*Mycosphaerella graminicola*)	***	**	NA	***	***	***
Basidiomycete						
Wheat brown rust (*Puccinia recondita*)	***	*	NA	**	***	***
Barley brown rust (*Puccinia hordei*)	***	*	NA	*	***	***
Rice sheath blight (*Rhizoctonia solani*)	***	**	***	***	NA	NA
Deuteromycete						
Tomato early blight (*Alternaria solani*)	***	**	NA	***	NA	***
Oomycete						
Grapevine downy mildew (*Plasmopara viticola*)	***	**	NA	**	NA	***
Turf pythium blight (*Pythium aphanidermatum*)	***	**	**	**	NA	***

Source: Syngenta—substantially based on data from independent field trials: Japanese Plant Protection Association official trials and References 33, 34, 72.
NA, Not applicable (not publicly declared to be a development outlet).
* Poor efficacy.
** Moderate efficacy.
*** Good efficacy.

proven unequivocally to be responsible for this phenomenon because it is not possible to grow a completely disease-free cereal crop in the field, and yield assessments under (disease-free) glasshouse conditions poorly reflect yields in a field situation. It is, of course, possible that elements of both hypotheses contribute to these 'unexpectedly good' yield benefits with strobilurins in wheat and barley.

Yield and quality studies with strobilurins in crops other than wheat and barley have usually been restricted to total yield or the percentage of marketable fruit.[10,15,55] However, there are a number of published studies on grain, fruit, tuber and bulb quality following disease control with azoxystrobin in a wide range of crops other than wheat and barley. Documented benefits of azoxystrobin have included bigger grain size and better milling quality in rice,[56] improved tuber size in potatoes,[57] increased soluble sugars and longer shelf life in tomatoes,[58–60] increased bulb size in garlic,[61] and control of post-harvest diseases due to pre-harvest applications to mangoes.[62]

6 RESISTANCE

The strobilurin fungicides belong to the Q_oI (Q_o inhibitor) cross-resistance group. All the strobilurin compounds, including azoxystrobin, kresoxim-methyl, metominostrobin, trifloxystrobin, picoxystrobin and pyraclostrobin, and also famoxadone and fenamidone, bind to the Q_o site,[4,63] and so all belong to the Q_oI group. The Q_o site is distinct from the second quinol binding pocket of the cytochrome bc_1 complex, the Q_i site.

Q_oI resistant isolates of a fungal pathogen were first discovered in cereals, when Q_oI resistant isolates of *Erysiphe graminis* DC f sp *tritici* Marchal were identified in northern Germany in 1998. Since then, Q_oI resistance has been detected in a limited number of other pathogens. For most of the pathogens in which Q_oI resistance has been reported, the major mechanism of resistance is the amino acid substitution of glycine with alanine at position 143 (G143A) of the cytochrome b protein (Plate 4). This target site mutation has been identified in Q_oI resistant isolates of *Erysiphe graminis* DC f sp *hordei* Marchal, *E graminis* f sp *tritici*, *Plasmopara viticola*, *Pseudoperonospora cubensis* (Berk & MA Curtis) Rostovzev, *Mycosphaerella fijiensis* Morelet, *Sphaerotheca fuliginea*, *Pyricularia grisea* Sacc and *Didymella bryoniae* (Auersw) Rehm.[64–66] The G143A mutation characteristically leads to high resistance factors.[67] In *Venturia inaequalis* (Cooke) Winter some Q_oI resistant isolates have been found to possess the G143A mutation, whereas others appear to be resistant through metabolic activity or an as yet undetermined mechanism.[68,69] A second target-site mutation, the substitution of phenylalanine with leucine at position 129 of the cytochrome b (F129L), has been identified in an isolate of *Pythium aphanidermatum* (Edson) Fitzp with reduced Q_oI sensitivity (J Windass, pers comm).

Resistance due to a point mutation, such as G143A, is amenable to detection through novel quantitative polymerase chain reaction (Q-PCR) detection methodologies.[70] Such technologies are powerful tools allowing the evolution of resistance genes to be tracked, which in turn has the potential to greatly advance our understanding of Q_oI resistance.

Q-PCR has played an important role in providing information regarding the sensitivity situation in certain key crop-pathogen combinations. To summarise our current understanding:

- In cereals, the impact of Q_oI resistance has been restricted to powdery mildews on wheat and, more recently, barley.[64,71] Over the past 2–3 years, Q_oI resistance in wheat powdery mildew has spread throughout northern Europe. Currently, Q_oI compounds are no longer recommended by FRAC to control this disease. By contrast, resistance in barley powdery mildew was first observed as an isolated case in 1999 and by 2001 could be detected in localised areas in Europe. Resistance appears to be evolving more slowly with powdery mildew on barley than on wheat, which is believed to be due to the lower population size of barley powdery mildew, the fewer fungicide sprays applied to barley compared to wheat and more effective powdery mildew resistant varieties in barley. Despite intensive monitoring in other cereal pathogens, including *S tritici*, *Rhynchosporium secalis* (Oudem) JJ Davis, *Helminthosporium teres* Sacc and *Puccinia recondita* Roberge, no resistance has been identified (see www.gcpf.org/frac).
- In cucurbits, Q_oI resistance has so far impacted control of cucurbit powdery mildew, *S fuliginea*, in Asia and parts of southern Europe. It is interesting that no resistance has yet been detected in this pathogen in the USA or South America outside of Brazil. Similarly, Q_oI resistance appears to be widespread in cucurbit downy mildew, *P cubensis*, in areas of Asia, but not in Europe or the USA.[64] The reasons for these regional differences are unclear, which highlights the complexity of predicting resistance risk. There has also been a highly localised occurrence of Q_oI resistance in gummy stem blight, *D bryoniae*, in the USA (see www.gcpf.org/frac).
- Q_oI resistance was first detected in grapevine downy mildew in 1999 at three trial sites in Italy and France.[64,67] In sampled *P viticola* populations in 2001, detection of Q_oI resistance by bioassay or the G143A mutation by Q-PCR gave highly variable results across Europe. High frequencies of Q_oI-resistant strains could be identified in the Emilia Romagna region of Italy, with other regions of Italy and also some regions of France showing variable sensitivity. No Q_oI resistance was detected by bioassay or Q-PCR in Spain, Portugal or Germany. Control of vine downy mildew in 2001 was generally very good across Europe, where recommended pro-

grammes incorporating Q_oI fungicides were followed. Despite intensive monitoring, there has been no sign of resistance to grapevine powdery mildew (see www.gcpf.org/frac).

- In *V inaequalis*, apple scab, the G143A target site mutation has been identified in isolates collected from northern Germany, northern Italy and also west Poland. In other regions of Europe, reduced Q_oI sensitivity appears to be due either to a metabolic resistance or a third, as yet unknown, mechanism. Despite detection of Q_oI resistance by bioassay, performance of Q_oI compounds remains generally good (see www.gcpf.org/frac).
- Q_oI resistance has been detected by bioassay and Q-PCR in isolates of black sigatoka, *M fijiensis*, collected from some plantations in the major banana-growing regions of Costa Rica. However, significant levels of resistance have not been detected in the other major banana-producing countries. Various factors are believed to have contributed to the situation in Costa Rica, including extreme conditions of disease pressure and intensive fungicide use. The situation in Costa Rica has led to a tightening in this specific country of the general FRAC guidelines for the use of Q_oI fungicides in banana (see www.gcpf.org/frac).

With the few exceptions described above, disease control by the Q_oI compounds, where registered, remains excellent, and they continue to be a key component of disease-management programmes. In order to safeguard the future of these compounds, the Q_oI-FRAC group has issued specific guidelines for the inclusion of Q_oI compounds in spray programmes. These guidelines are based on reducing the Q_oI selection pressure by limiting the number of applications of compounds from the Q_oI cross-resistance group each season, alternation with effective compounds from different cross-resistance groups, and, where appropriate, use of mixtures with effective partners. In addition, manufacturers' recommendations should be followed to ensure appropriate—preferably preventative—use, the correct spray interval and a rate that ensures effective disease control (see www.gcpf.org/frac).

In summary, the impact of resistance to Q_oI compounds has so far been limited.[64,67] A few pathogens, such as wheat and cucurbit powdery mildews, appear to have evolved resistance rapidly and in these cases resistance also appears to be very robust.[71] However, this is far from the case for the majority of pathogens where resistance has had little impact on disease control. The molecular mechanism of resistance to Q_oI fungicides is understood to an advanced level and this in turn has allowed the development of powerful molecular detection technologies. This knowledge can be exploited to further our understanding of the evolution of resistance and to ensure that the Q_oI fungicides have a secure future.

7 HUMAN AND ENVIRONMENTAL SAFETY

7.1 Human safety

The strobilurins as a class show few signs of specific toxicity related to their pesticidal mode of action. Some non-specific toxicity is seen at high doses, which, based on international, regional and national hazard classification schemes, results in a range of hazard classifications as shown in Table 5.

Hazard classification is based on the intrinsic properties of the chemical and does not take into account exposure under conditions of use. Assessment of the potential risk of the strobilurins to man is assessed by comparing the reference dose for the given chemical to estimated/measured exposure values. Exposure values accounting for less than 100% of the reference dose are considered to be acceptable in terms of risk. For the strobilurins, exposure data derived under realistic conditions of use indicate that this class of fungicide presents minimal risk to human health, and can be handled safely if used according to the label instructions.

Table 5. Comparison of the hazard profile of strobilurins*

Compound	*Hazard classification (USA)*	*Hazard classification (EU)*	*Reference dose*[a] *(mg kg^{-1} bwt day^{-1})*
Azoxystrobin[b]	Cat III—Caution	R23 toxic by inhalation	0.18
Kresoxim-methyl[c]	Cat III—Caution, likely human carcinogen	R40 limited evidence of carcinogenic effect	0.36
Trifloxystrobin[d]	Cat III—Caution, strong sensitiser	R43 may cause skin sensitisation by skin contact	0.05
Picoxystrobin[e]	Not applicable	R20 harmful by inhalation	0.05
Pyraclostrobin[f]	Cat II—warning	R23 toxic by inhalation	0.04

* Insufficient data readily publicly available to allow valid comparison for metominostrobin.

[a] Derived from reliably conducted toxicity studies and defined as the estimated daily exposure to which there is no adverse impact on human health over an individual's lifetime.

[b] Reference 73.

[c] Reference 74.

[d] Reference 75.

[e] Syngenta GLP data.

[f] Reference 76.

Compound	Soil DT_{50} (days)	Water DT_{50} (days)	K_{oc}	Log P_{ow}
Azoxystrobin	7–56[a]	~7[e]	~500[a]	2.5[a]
Kresoxim-methyl	<1[a]	1[b]	219–372[a]	3.4[a]
Metominostrobin[a]	98	—	—	2.3
Trifloxystrobin[a]	4–10	0.3–1	1642–3745	4.5
Picoxystrobin[c]	3–35	7–15	790–1200	3.6
Pyraclostrobin[d]	2–37	—	6000–16000	4.0

[a] Reference 27.
[b] Reference 74.
[c] Reference 14.
[d] Reference 15.
[e] Syngenta GLP data.

Table 6. Environmental fate of the strobilurins

Table 7. Toxicity of strobilurins to birds, mammals and bees

Compound	Birds LD_{50} (mg kg^{-1})	Mammals (rat) LD_{50} (mg kg^{-1})	Bees (LD_{50} µg per bee)
Azoxystrobin[a]	>2000	>5000	>200
Kresoxim-methyl[a]	>2150	>5000	>20
Metominostrobin[a]	—	708	>114
Trifloxystrobin[a]	>2000	>5000	>200
Picoxystrobin[b]	>2250	>5000	>200
Pyraclostrobin[c]	>2000	>5000	310

[a] Reference 27.
[b] Reference 14.
[c] Reference 15.

7.2 Environmental safety

7.2.1 Environmental fate

The strobilurin fungicides either currently or shortly to be commercially available are all relatively readily degraded, such that persistence in any environmental compartment is not an issue (Table 6). Hydrolysis is not generally a major route of environmental dissipation, but adsorption, microbial degradation and photolysis can all be important.

Although data are not publicly available for pyraclostrobin and metominostrobin, strobilurins follow a similar typical degradation pattern in soil, with the formation of metabolites which are potentially more mobile, but typically less toxic, than the parent compound.

7.2.2 Ecotoxicology and ecological risk

The strobilurin fungicides are generally of low toxicity and consequently low risk to birds, mammals and bees with no toxicity at limit doses (Table 7), the exception being metominostrobin which shows some toxicity towards mammals. They are of low risk to other groups of terrestrial organism, that is non-target arthropods, earthworms, non-target plants and soil micro-organisms. This low environmental risk is recognised by azoxystrobin and trifloxystrobin being accepted onto the US EPA Reduced Risk (Safer Pesticide) programme and registered accordingly. Pyraclostrobin has been accepted as a Reduced Risk candidate but at the time of writing of this review has not yet been registered in the USA.

Strobilurins vary in their toxicity to aquatic organisms, as determined in the standard laboratory studies required for pesticide registration (Table 8). They show increasing toxicity to aquatic organisms from metominostrobin, azoxystrobin, kresoxim-methyl,

Table 8. Strobilurin toxicity (µg $litre^{-1}$) to aquatic organisms[a]

	Fish[b]		Daphnia magna[c]		Green algae
Compound	96-h LC_{50}	ELS Chronic NOEC	48-h EC_{50}	Chronic NOEC	72–120-h EC_{50}
Azoxystrobin	470*[d]	147***[d]	259[d]	44[e]	120[d]
Kresoxim-methyl	190*[d]	—	186[d]	32[f]	63[d]
Metominostrobin	18100**[d]	—	14000+[d]	—	51000[d]
Trifloxystrobin	15*[d]	4.3*[i]	16[d]	2.8[i]	37[i]
Picoxystrobin	65*[g]	40***[g]	18[g]	8[g]	56[g]
Pyraclostrobin	6*[h]	—	—	—	—

[a] ELS = early life stage; NOEC = no observable effect concentration.
[b] * Rainbow trout (*Oncorhyncus mykiss*); **carp (*Cyprinus carpio*); ***fathead minnow (*Pimephales promelas*).
[c] +*Daphnia pulex*.
[d] Reference 27.
[e] Reference 73.
[f] Evaluation on kresoxim-methyl, PSD (1997).
[g] Reference 14.
[h] Reference 15.
[i] Reference 75.

picoxystrobin, trifloxystrobin to pyraclostrobin, and this appears to be linked to their increasing lipophilicity, represented by their log P_{ow} values (see Table 6). Aquatic toxicity is measured in terms of the external concentration in the surrounding water, whilst effects are largely dependant on the dose, represented by the internal concentration in the organism. Compounds with higher lipophilicity are taken up to a greater extent by aquatic organisms and, consequently, have higher toxicity than less lipophilic compounds with similar intrinsic toxicity.

Toxicity data from standard studies indicate just the intrinsic 'hazard' posed by a chemical. They are not an indication of ecological risk, that is, the potential for effects on organisms in the field following agricultural use. This is dependant on to what extent organisms will be exposed to the compound, at what concentration and for how long. Furthermore, the ecology of the organism, where and how it lives, how it reproduces and how it might recover from any effects needs to be considered. It is important to understand how the environmental fate will affect exposure and subsequent effects.

As stated above, the strobilurins are all dissipated in the environment relatively readily, albeit at different rates. Looking at the ecotoxicological profile, it is the potential for acute effects, that is mortality, which drives the ecological risk assessment. Dissipation is relatively rapid, so there is little potential for chronic exposure and subsequently there is low chronic risk. Furthermore, for the strobilurins, the acute:chronic toxicity ratio for aquatic organisms is relatively low. Typically the acute:chronic ratio (EC/LC_{50}:chronic NOEC) would be 10:1; however, for the strobilurins and fish and *Daphnia*, it can be as low as 2:1 (see Table 8). Therefore at concentrations just below acute effect levels, organisms are unaffected.

The strobilurin mode of action/mechanism of toxicity is the inhibition of mitochondrial respiration. This is typically very fast-acting. In acute toxicity studies with fish, the time to effect is very short, with practically all the toxicity being expressed in a few hours and no subsequent increase in toxicity with time. Dissipation in the aquatic environment has little impact on the potential for effect, as any effects are expressed very early. Thus, in the initial risk assessment, which compares the acute toxicity to the initial exposure, immediately after entry, the more toxic compounds may give greater indications of risk. Due to their intrinsic toxicity, most strobilurins require further refinement of the aquatic risk assessment beyond this initial toxicity:exposure comparison. In some outlets, the high intrinsic toxicity can lead to mitigation measures in the form of buffer zones between the crop and non-target aquatic environments to ensure low risk to the latter.

8 CONCLUSIONS

The strobilurins are an outstanding new class of agricultural fungicides, demonstrating excellent properties in a number of areas, including biology and biokinetics, and human and environmental safety. Each strobilurin has its own distinctive technical properties with different strengths and weaknesses compared to other strobilurins. In the future, the choice of which strobilurin to use will remain a case of matching the best technical profile for the specific agronomic challenge faced.

Strobilurin fungicides have been extremely successful because of the benefits that they bring and are clearly one of the most valuable classes of single-site fungicide ever discovered by the agrochemical industry. If recommended use-patterns continue to be followed, the dependence of crop protection on the strobilurins is likely to continue for many years into the future.

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