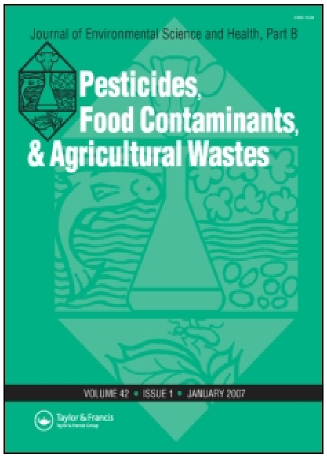

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Review of strobilurin fungicide chemicals

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Strobilurins are natural products isolated and identified from specific fungi. Natural strobilurins were named in the order of their discovery as strobilurin-A followed by strobilurin-B, C, D etc. Their discovery opened the door for new chemistry of synthetic fungicides. Applying Quantitative Structural Activity Relationship (QSAR) on the structures of the natural strobilurins, many pesticide companies were able to discover many synthetic analogues that are more efficacious and more stable fungicides. At present there are about eight synthetic strobilurins in the fungicides worldwide market. Some of these products are worldwide registered for use as agrochemical and some are in the process of registration. This class of fungicides is relatively new, as crop protection products and information about them is still fairly scarce. In this review, syntheses and chemistry of natural and synthetic strobilurins are discussed. Also, the mode of action, efficacy, biotic/abiotic degradation, analytical methods, and agricultural uses are discussed.

Keywords: Fungicide; strobilurin; mushroom natural products; electron transfer inhibitors; quinol oxidation (Q_o); qoI fungicides; protectant fungicide; curative fungicides; translaminar fungicides.

Introduction

Strobilurins are natural substances isolated mainly from mushrooms (*basidiomycetes*). Their name is derived from the mushrooms genera *Strobilurus*. Strobilurin-A was isolated from the liquid cultures of *Strobilurus tenacellus* by Anke et al.[1] These products are biosynthesized in fungus from phenylalanine via the shikimic acid cycle. Natural strobilurins have been isolated by chromatographic means, and their molecular formulae have been identified by high resolution mass spectrometry. Further spectroscopic analyses were applied to determine their molecular structures as described in the work of Schramm.[2]

It is interesting to note that powerful natural fungicides like strobilurins are produced in fungi. Many of these substances perform functions that are important to the survival of the organism in which they are formed. Strobilurins A and B were found to be highly active against yeasts and filamentous fungi, but were inactive against bacteria.[1] These compounds are photolabile and relatively weak fungicides. Nevertheless, they established a new chemical lead to develop synthetic fungicides that are more stable and more powerful when used commercially in agriculture practice.

This subject has been reviewed several times in the literature. Anke in Germany is considered to be the pioneer in this field. Reviews on this subject have been published by Anke[3] and Tally et al.[4] Also, the chemistry of strobilurins and their congeners oudemansins (Table 1) was reviewed by Clough[5], and Anke and Steglich.[6]

Structurally, the basic common feature in this family of products is the presence of (E)-β-methoxyacrylate group. However, in some synthetic strobilurin fungicides, this group was replaced with QSAR-equivalent groups as shown below. These groups are considered the toxiphoric group in these structures. Strobilurins and oudemansins are both natural products and very close in structures. Figure 1 shows the essential elements in the structures to qualify these compounds to belong to this class of chemicals and shows the QSAR modifications that produced synthetic strobilurins.

Natural strobilurins

Many scientists excluded the idea of looking for fungicides in fungus. However, once Anke et al.[1] isolated Strobilurin-A from the liquid cultures of the mushroom *Strobilurus tenacellus* many scientists looked for other strobilurins in many other fungi. As a result of this new interest many natural compounds of strobilurin structures were isolated and identified. All natural strobilurins retained the methyl (E)-3-methoxy-2-(5-phenylpenta-2,4-dienyl) acrylate moiety. They vary only in the aromatic ring substitutions at 3 and 4 positions as shown in Table 1. It is interesting to see that chlorine is included in the ring substitution in Strobilurin-B.

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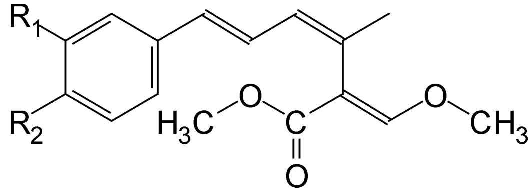
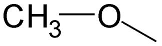
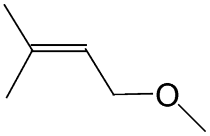
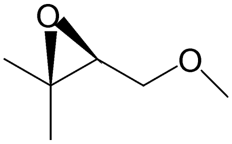
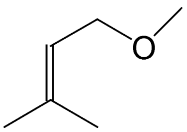
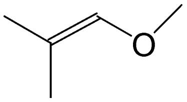
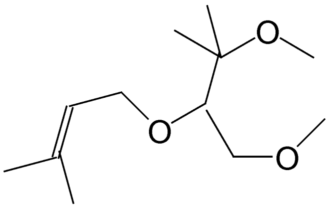
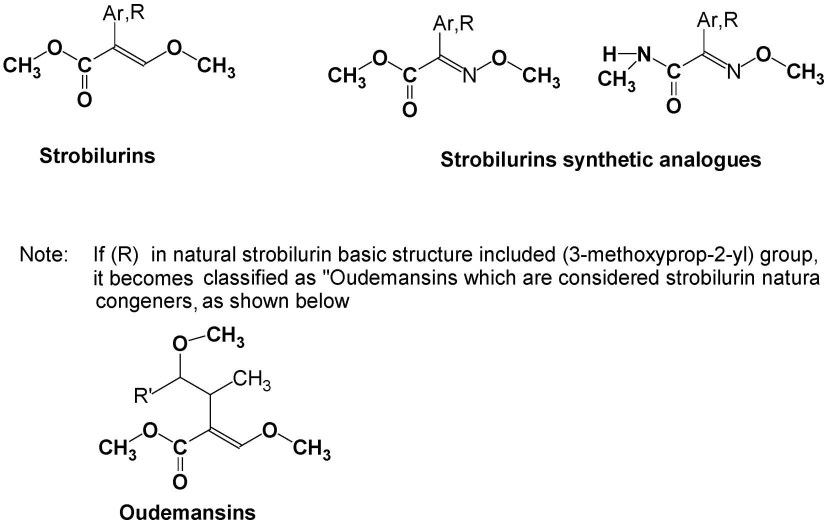
Table 1. Chemical structures of some natural strobilurins.



Substitution R_1	*Substitution R_2*	*Natural Strobilurin*	*Name & Comments*
H-	H-	STROBILURIN-A methyl (E)-3-methoxy-2-(5-phenylpenta-2,4-dienyl) acrylate	Strobilurin A and B were isolated and identified by Schramm et al.[2] from *Strobilurus tenacellus*. The two products were considered good antibiotics.
	Cl-	STROBILURIN-B	
	H-	STROBILURIN-C	Strobilurin C was isolated from fungal growth of *xerula* species (agaricales) by Anke et al.[7]
		STROBILURIN-D	Strobilurins D and F were isolated and identified by Weber et al.[8] from the basidiomycete *Cyphellopsis anomala*. They are used as a phamaceutical antibiotic fungicide.
HO-		STROBILURIN-F	
		STROBILURIN-G	Strobilurins F, G and H were isolated and identified by Fredenhangen et al.[9,10] (Note: Strob. F above is common in the two schools).
HO-	H-	STROBILURIN-H	

Other natural strobilurins were identified, synthesized, and reported in the literature like strobilurin M, and N by Kroiss and Steglich.[11] Also, strobilurins O and P were synthesized by Hosokawa et al.[12]



Note: If (R) in natural strobilurin basic structure included (3-methoxyprop-2-yl) group, it becomes classified as "Oudemansins which are considered strobilurin natura congeners, as shown below

Fig. 1. Basic structures of strobilurins and their analogues.

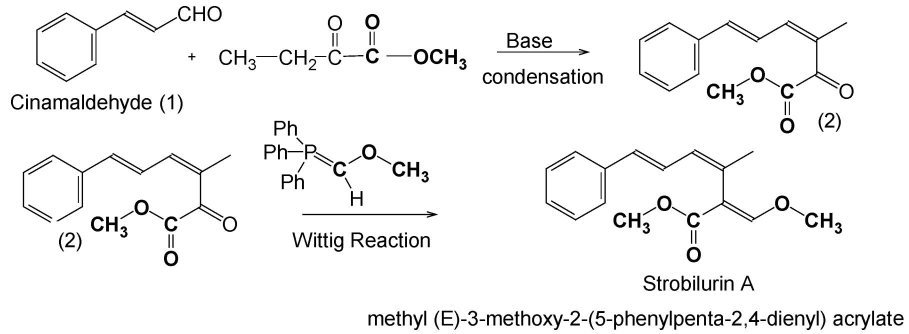


Fig. 2. Synthetic route of strobilurin-A.[16]

Synthesis of natural strobilurins

The standard synthetic route for preparing natural strobilurins (A or B) involves the condensation of ring substituted cinamaldehyde (1) with α-keto acid methyl ester of the appropriate side chain to obtain compound (2). Product (2) is then subjected to Wittig reaction to replace the keto group with the methoxy olefin. Reactions given below (Fig. 2) yield the common structure of substituted methyl ester of (E)-β-methoxyacrylate of strobilurin (A) as described by Anke et al.[13]

Therefore strobilurin B, methyl (E)-3-methoxy-2-[5-(3-methoxy-4-chloro-phenyl) penta-2,4-dienyl)] acrylate, CAS No. 65105-52-4 could be synthesized using 3-methoxy-4-chlorocinamaldehyde as a starting compound in the reactions given above. Other routes to synthesize strobilurin B are described by Sutter. [14] Several natural strobilurins were isolated from mushrooms (Basidiomycetes). Other natural strobilurins vary in substitutions in several locations in the structure of strobilurin A and are named in alphabetical order A, B, C, and so on.

Synthetic analoges of natural strobilurins

These natural strobilurins break down rapidly in light and are not reliable for disease control. Similar chemicals were synthesized, and thousands were tested before suitable photo-stable compounds with fungicidal activities were developed (see the structures of the synthesized strobilurines in Table 2). Beautement et al., Sauter et al., Sauter et al.[15–18], Clough[19], and Zakharychev and Kovalenko[20] reviewed the evolution of the synthetic strobilurins starting from natural strobilurins and oudemansins as fungicides. These reviews discussed approaches in the optimization of lead structures and the importance of understanding structure-activity relationships.

Most of these attempts were focused on the modification of the α-substitution in the (E)-β-methoxyacrylate. One of the important directions taken in the structural modifications was the replacement of the basic toxiphoric group of (E)-β-methoxyacrylate with methoxyiminoacetate. The discovery of synthetic analogs of methoxyiminoacetate was led by BASF. Kresoxim-methyl of Baden Aniline and Soda Factory which was announced in 1992 shows this structural modification. Later this modification and others were published by Ypema and Gold.[23] Shionogi scientists, Ichinari et al.,[22] took the modification further such that the traditional β-methoxy acrylate derivative was replaced by 2-methoxyiminoacetamide derivatives. This modification was marketed as the strobilurin fungicide under the name of Metominostrobin**.** Other methoxyimino derivatives were developed and discussed by Anon.[23]

Several commercial products resulting from this effort are now in the market, and more are expected to follow. At present, there are 6-7 registered fungicides in the world market and many are still in the pipeline. Commercial strobilurin fungicides are among the best-selling fungicides worldwide, and are used mainly as plant protectants against most major fungal plant pathogens. Since these products are derived from natural products, strobilurins are environmentally safe due to their rapid degradation in the environment.

Azoxystrobin was discovered by Syngenta (formerly Zeneca Agrochemicals) and is covered by patent EP 382375. It was the first synthetic strobilurin product to be announced at the Briton Crop Protection Conference of 1992 as a commercial fungicide by Syngenta. Its structure (Table 2) included (E)-β-methoxyacrylate.

Kresoxim-methyl was discovered by BASF independently at the same time as azoxystrobin. It is patented in the US and EU (EP 253213 and US 4829085), and was announced in the same Briton conference of 1992 by BASF. The structure for the first time included modification of the (E)-β-methoxyacrylate using methoxyiminoacetate.[21–24] The mode of action and efficacy were discussed by Gold et al.[25]

Trifloxystrobin was discovered by Novartis Crop Protection (now Syngenta) under patent EP 00460575 and announced by Novartis in 1998 Briton conference. However, the product was sold to Bayer Crop Science in 2000 and since then the product is marketed under Bayer Crop Science. Also, the structure involved the replacement of (E)-β-methoxyacrylate with methoxyiminoacetate.

Table 2. Synthetic strobilurin fungicides used in agriculture.

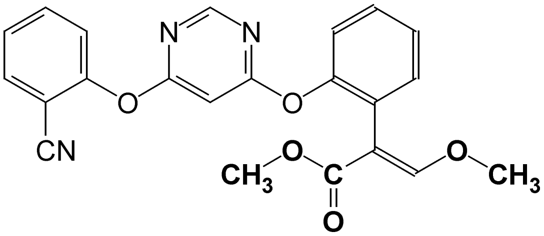
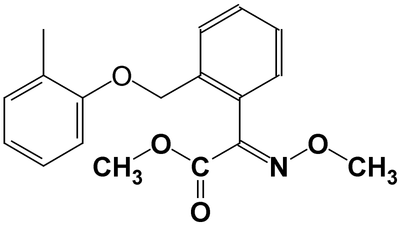
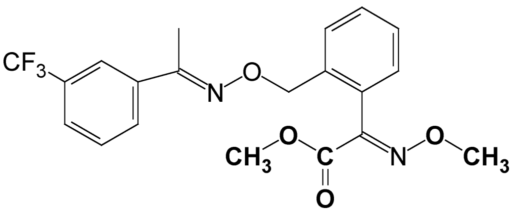
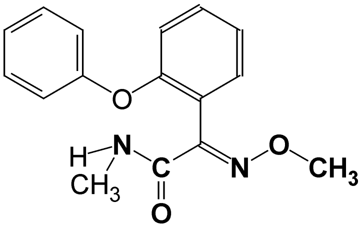
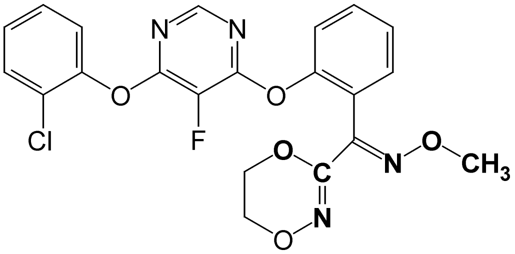
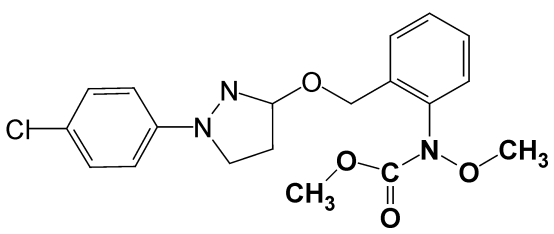
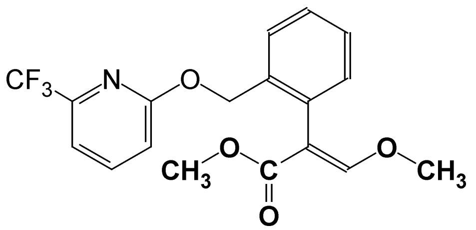
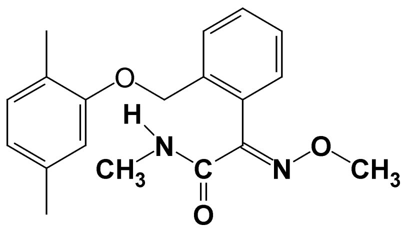
Name/Cas No. Manufact-Urer	*ChemicalStructure* *IUPAC Name*	*Use & Mode of Action*
Azoxystrobin CAS No. 131860-33-8, formerly 215934-32-0 Syngenta	 Methyl (*E*)-2-2-[6-(2-cyanophenoxy) pyrimidin-4 yloxy]phenyl-3-methoxyacrylate	Fungicide with protectant, curative, eradicant, translaminar and systemic properties. Inhibits spore germination and mycelial growth, and also shows anti-sporulant activity.
Kresoxim-methyl CAS No. 143390-89-0 BASF	 Methyl (*E*)-methoxyimino[2-(o-tolyloxymethyl) phenyl] acetate	Fungicide with protective, curative, eradicative and long residual disease control; acts by inhibiting spore germination. Redistribution via the vapour phase contributes to activity.
Trifloxystrobin CAS No. 141517-21-7; formerly 221007-60-9 Bayer Crop Science	 Methyl (*E*)-methoxyimino-(*E*)-[1-(α,α,α,-trifluoro-*m*-tolyl) ethylideneaminooxy]-*o*-tolyl acetate	Mesostemic, broad-spectrum fungicide with preventive and specific curative activity. Displays rain-fastness property. Translocate by superficial vapor movement and also has translaminar activity.
Metominostrobin CAS No. 133408-50-1 Shionogi	 (*E*)-2-methoxyimino-*N*-methyl-2-(2-phenoxyphenyl) acetamide	Systemic fungicide with protective and curative action.
Fluoxastrobin CAS No. 361377-29-9; 193740-76-0 Bayer Crop Science	 (*E*)-2-[6-(2-chlorophenoxy)-5-fluoropyrimidin-4-yloxy]phenyl (5,6-dihydro-1,4,2-dioxazin-3-yl) methanone *O*-methyloxime	Systemic (and locosystemic) fungicide with protective and curative properties. It is acropetally translocated when sprayed on leaves. Acts as an inhibitor of spore germination and mycelial growth.

Table 2. Synthetic strobilurin fungicides used in agriculture. *(Continued)*

Name/Cas No. *Manufact-Urer*	*ChemicalStructure* *IUPAC Name*	*Use & Mode of Action*
Pyraclostrobin - Carbamate CAS No. 175013-18-0 BASF	 Methyl-N-2-[1-(4-chlorophenyl)-1H-pyrazol-3-yloxymethyl] phenyl (N-methoxy)- carbamate	Fungicide with protectant, curative, and translaminar properties.
Picoxystrobin CAS No. 117428-22-5 Syngenta	 Methyl (E)-3-methoxy-2-[2-(6-trifluoromethyl-2-pyridyloxymethyl)phenyl] acrylate	Preventive and curative fungicide with unique distribution properties including systemic (acropetal) and translaminar movement, diffusion in leaf waxes and molecular redistribution in air.
Dimoxystrobin CAS No. 149961-52-4 BASF	 (E)-2-(methoxyimino)-N-methyl-2-[(2,5-xylyloxy)-o-tolyl] acetamide	Fungicide with protectant, curative, and translaminar properties. Inhibits spore germination and blocks mycelium growth.

Metominstrobin was discovered by Shienogi scientists and was registered in Japan in 1998. No further information on its patent is available. The important point in the structure of this product is that its structure was further distant from (E)-β-methoxyacrylate which was replaced by methoxyiminoacetamide.

Fluoxastrobin was discovered by Bayer Crop Science under patent No. DE 19602095, and was announced by Bayer in 1999 and in 2002 Briton conferences. In this structure, although the substituting group is similar to that of azoxystrobin, its toxiphoric group, (E)-β-methoxyacrylate was highly modified to the dioxazin derivative of methoxyiminoactate Pyraclostrobin-carbamate was discovered by BASF scientists in 2000, and announced the same year. The structure of this product is characterized by its nature as a derivative of carbamate as the toxiphoric group. Picoxystrobin was announced by Zeneca agrochemicals (now Syngenta-AG) scientists at the Briton meeting of the year 2000, and registered in 2001. Dimoxystrobin is still under development by BASF as a fungicide.

Mode of action

The strobilurins bind to one specific site in the mitochondria, the quinol oxidation (Q_o) site (or ubiquinol site) of cytochrome b and thereby stop electron transfer between cytochrome b and cytochrome c, which halts reduced nicotinamide adenine dinucleotide (NADH) oxidation and adenosine triphosphate (ATP) synthesis.[26,27] This leads to the stopping of the energy production and the fungus will eventually die. Thus their mode of action is novel and new target-specific. It is important to point out that this mechanism is different from the inhibition of cytochrome c, which is the well-known mechanism of cyanide products and CO. They are more correctly referred to as the QoI fungicides. The mode of action of strobilurin compounds was reviewed by Sauter, et al.[18] and Leraux.[28] One important characteristic in the fungicidal action of this class of chemicals is their rapid activity. This effect is concentrated on the first period of the life cycle of the fungi i.e. at the spore stage. This is an essential characteristic because of their rapid degradation in the environment and in the plant's metabolism. Application of strobilurins gives the plant an additional beneficial effect by adding more healthy green color to the plant. This phenomenon is known as the "greening effect" and that was paired with significant high crop harvest both in volume and quality.

Since strobilurins are active only at one specific site in fungal pathogens, they are prone to resistance. A resistance gene code of G143A was isolated in England and Ireland from *Septoria triciti* treated with storbilurins. The occurrence of this gene reached 40% by 2003, Zheng.[29] Recently, more detailed studies on the resistance to Qo inhibitors were published by Fisher et al.[30] to prove that G143A mutation and new mutation sites F129L and L275F contributed to the resistance to Qo inhibitors.

Strobilurin fungicides are very useful to control fungal pathogens when they develop strains resistant to the 14-demethylase inhibitors which are the common fungicidal mechanism of many other fungicides like phenylamides, or dicarboxamides.

Strobilurins use as fungicides

Fungicides could be classified according to where in the disease cycle fungicides are active. The disease cycle of fungal pathogens starts when fungal spores land on a leaf and under favorable environmental conditions, the spore germinates and the fungus infects the leaf and starts to grow within the leaf tissue. There may be early indications of disease development, for example, yellowing of the leaf tissue, but the appearance of disease symptoms usually takes several days from the time of infection. Several days later after infection, masses of spores ooze out of the pustule. Therefore, fungicides classification could be summarized as follows:

(i) Protectant or preventive fungicides that are effective prior to infection and the initiation of the disease cycle. A classic example of these fungicides is chlorothalinil, since it acts only on the infecting spores. Strobilurins may be positioned in this category.
(ii) Curative fungicides that are effective against the fungus growing in the leaf tissue post the infecting spore germination and therefore have curative properties. Although triazoles are a good example of this category, some strobilurins could extend its efficacy to control the disease in this stage.
(iii) Eradicant or antisporulant fungicides that are capable of stopping sporulation by the pathogen. Under this category, few fungicides function.

Most strobilurine compounds are weak systemic fungicides. They could be used mainly as protectant, curative, and translaminar fungicides. Spores of fungi are more susceptible to strobilurins than fungal mycelium. Therefore, strobilurins are highly effective against spore germination and early penetration of the host. Once the fungus is growing inside the leaf tissue, strobilurins have little or no effect. Most strobilurins have a residual period of approximately 21 days. Strobilurins are locally systemic. The strobilurins are absorbed into leaf tissue. If a droplet of fungicide is applied to the top surface of a leaf, it will spread out on the surface of the leaf. The chemical is then absorbed by the waxy layers (cuticle) of the leaf. Strobilurins are highly attracted to the cuticle. Some fungicide will leak into the underlying plant tissue of the leaf; however most of this will be attracted to the cuticle on the other side of the leaf. This is known as translaminar activity, and it may take several days. Limited amounts of the applied strobilurins move systemically in the xylem of the plant. Lastly, some strobilurins seem to move as a gas above the leaf and readily rebind to the waxy cuticle. Since strobilurins are weak systemic fungicides, a good coverage of the entire plant canopy is essential for the fungicide to be effective.

Uses of strobilurins in agricultural practice

The primary use of strobilurins is in the field of agriculture to control cop diseases. Strobilurins are excellent products for the protection of crops against powdery mildew, brown rust and many other plant diseases. Table 3 summarizes the uses of strobilurins products as agrochemicals.

Despite the wide range of biocidal activity exhibited by strobilurins, their uses outside the agricultural arena such as human and veterinary antifungal in a topical formulation has been very limited. One of their applications as a human and veterinary antifungal is a product under the name MUCIDERMIN SPOFA, Zakharychev and Kovalenko.[20] Furthermore, many strobilurins were investigated as anticancer agents. Strobilurins found to strongly inhibit the incorporation of radioactive leucine, uridine, and thymidine into the acid-insoluble fraction of cells (protein, RNA, and DNA), were tested for antitumor activity. In-vitro antitumor activity was tested using cells of the ascitic form of Ehrlich carcinoma.

Stribilurins products are used to control mold and mildew in the pulp and manufacturing of paper, cooling towers and heat exchangers. For these uses, strobilurins were formulated to be used in aqueous systems as was patented by Cash and Cronan.[31]

Table 3 provides detailed information on the uses of strobilurin products available in the market.

Biotic and abiotic degradation of strobilurins

There is limited published literature on the biotic and abiotic degradation of this class of fungicides. These

Table 3. Strobilurin fungicide uses in agriculture.

Product Name	*Crops*	*Pathogens/Dieases*
Azoxystrobin AMISTAR (Syngenta, not in US) HERITAGE (Syngenta, US, Japan) ZX (Barclay) Mixtures: QUADRIS (Azxystrobin + cymoxanil) (Syngenta) AMISTAR ADMIRE Azoxystrobin + imidacloprid (Bayer)	Temperate cereals	*Erysiphe graminis, Puccinia spp., Leptosphaeria nodorum, Septoria tritici and Pyrenophora teres*
	Rice	*Pyricularia oryzae and Rhizoctonia solani*
	Vines	*Plasmopara viticola and Uncinula nector*
;	Cucurbits	*Sphaerotheca fuliginea and Pseudoperonospora cubesis*
	Potato and tomato	*Pytophthora infestans and alternaria solani*
	Peanut	*Mycosphaerella arachidis, Rhyzoctonia solani and Sclerotium rolfsii*
	Peach	*Monilinia spp. and Cladosporium carpophilum*
	Turf	*Pythium spp. and Rhyzoctonia solani*
	Banana	*Mycosphaerella spp.*
	Pecan	*Cladosporium caryigenum*
	Citrus	*Elsinoe fawcettii, Colletotrichum spp. and Guignardia citricarpa*
	Coffee	*Colletotrichum spp. and Hemileia vastatrix*
Kresoxim-methyl STROBY (BASF, Nissan) ARDENT (Makhteshim)	Apples and pears (For scab)	*Venturia spp.*/scab *Podosphaera leucotricha*/ powdery mildew)
	Vines	*Uncinula necator*/ powdery mildew
	Cucurbit	*Sphaerotheca fulibinea*/ powdery mildew
	Sugar beet	*Erysiphe betae*/ powdery mildew
	Cereal	*Erysiphe graminis*/ mildew *Rhynchosporium secalis*/ cereals scald) *Pyrenophora teres*/ net blotch *Septoria nodorum*/ glume blotch)
	Vegetable	*Leveillula taurica* *Erysiphe spp. and* *Alternaria spp.*/ for mildew)
Picoxystrobin ACANTO (Syngenta) Mixture: ACANTO DOS, Picoxystrobin + Hexaconazole (Syngenta)	Wheat	*Mycosphaerella graminicola, Phaeosphaeria nodorum, puccinia recondite*/ wheat brown Rust *Helminthosporium tritici-repentis*/ wheat tan spot) *Blumeria graminis*/ wheat powdery mildew
	Barley	*Helminthosporium teres*/ net blotch *Rhynchosporium secalis and Puccinia hordei*/ barley brown rust *Erysiphe graminis*/ powdery mildew
	Oats	*Puccinia coronata and Helminthosporium avenae*
	Rye	*Rhynchosporium secalis* / brown spot
Trifloxystrobin FLINT (Bayer) TEGA (Bayer) Mixtures: STRATEGO Trifloxystrobin + propicona (Bayer)	Cereals	*Broad spectrum fungicide*

(Continued on next page)

Table 3. Strobilurin fungicide uses in agriculture. *(Continued)*

Product Name	*Crops*	*Pathogens/Dieases*
Grapes Pome fruit Cucurbit		
Metominostrobin	Rice	*Pyricularria oryzae*
Dimoxystrobin HONOR (BASF) Mixtures: SWING GOLD Dimoxystrobin +epoxiconazole (BASF)	Cereals	*Septoria tritici* / leaf spot *Leptosphaeria nodorum*
	Vegetables	
	Turf	
Fluoxastrobin Mixtures: BARITON or FANDANGO Fluoxastrobin + prothioconazole (Bayer) SCENIC Fluoxastrobin + prothioconazole+tebuconazole (Bayer)	Cereals (As foliar application)	Septoria/leaf spot *Puccinia recondite, P.hordei* / rusts of wheat and Barley *Pyrenophora teres*/ helminthosporium disease like and net blotch of barley *Pyrenophora tritici-repentis*/ tan spot *Rhynchosporium secalis*/ scald *Blumeria graminis*/ powdery mildew *Rhizoctonia cerealis*/ Stem base diseases and sharp eyespot *Microdochium nivale*/ snow mould
	Cereals (As seed or soil reatment)	*Microdochium nivale*/ snow mould *Tellitia caries*/ common bunt of wheat) *Ustilago hordei*/ covered smut of barley)
	Vine	*Plasmopara viticola*
	Potato and tomatoes	*Phytophthora infestant*
	Potato	*Alternaria solani*
	Vegetable	*Sphaerotheca fuliginea and Leveillula taurica*
	Beans	*Uromyces appendiculatus and Phaeoisariopsis griseola*
	Peanuts	*Rhizoctonia solani and sclerotium rolfsii*
	Bananas	*Mycosphaerella fijiensis*
Pyraclostrobin-carbamate F-500 (BASF) Mixtures: SIGNUM Pyraclostrobin + boscalid (BASF)	Cereals	*Septoria tritici, Puccinia spp., Drechslera tritici-repentis and Pyrenophora teres*
	Peanuts	*Mycosphaerella spp.*
	Soya beans	*Septoria glycines, Cercospora kikuchii and Phakopsora pachyrhizi*
	Grapes	*Plasmopara viticola and Uncinula necator*
	Potatoes and Tomatoes	*Phytophthora infestans and Alternaria solani*
	Banana	*Mycosphaerella fijiensis*
	Citrus	*Elsinoe fawcettii and Guignardia citricarpa*
	Turf	*Rhizoctonia solani*/scald *and Pythium aphanidermatum*

compounds have relatively complex structures that will give rise to several sites for metabolic reactions that could lead to multiple metabolic pathways (Fig. 3). In general strobilurines are easily degradable compounds in plant, animal, soil and water.[32]

Methyl ester hydrolysis is an important metabolic pathway in this group of fungicides. It yields the free acrylic acid which has no biological activity. The importance of this reaction is its relation to the selective toxicity of these products. Aromatic ring hydroxylation leads to conjugation with

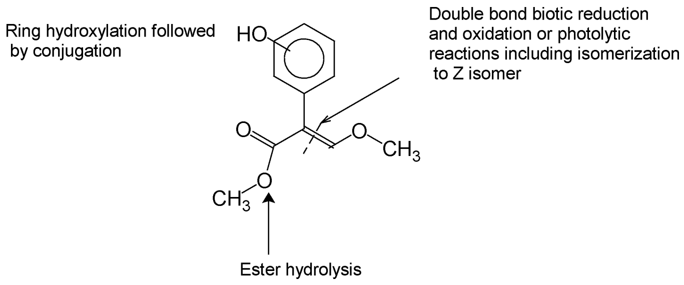


Fig. 3. Basic degradation pathways of strobilurins.

glutathione or other biological groups, however if this reaction was repeated on the same ring to yield poly-hydroxy aromatic products the ring becomes prone to cleavage followed by mineralization to CO_2. The double bond in the acrylic moiety is vulnerable to many degradative mechanisms. Double bonds undergo readily epoxidation followed by cleavage to ketones. The formation of a keto group on the α carbon could eventually yield to a carboxylic group (the product becomes benzoic acid derivative). Also, double bonds are subjected to addition either by hydrogen in reducing environments or water to yield a tertiary alcohol. Photolysis of double bond containing compounds usually yields multiple products and will lead to complete degradation of this group.

The lack of published literature on this subject limited the ability to provide examples on the metabolism and degradation of specific products. Extensive literature was obtained on the metabolism and degradation of azoxystrobin, kresoxim-methyl, and trifloxystrobin. The first product, azoxystrobin is (E)-β-methoxyacrylate derivative and considered to be the first synthetic strobilurin fungicide. The second two strobilurins, kresoxim-methyl, and trifloxystrobin are methoxyiminoacetate derivatives were discovered later, i.e. are considered second generation strobilurin fungicides.

Degradation of^{14}C-azoxystrobin (labeled in the 3 aromatic rings) was investigated in soil, plant and mammals. The summary of these studies is summarized in Figure 4. This work was published by Joseph of Zeneca Agrochemicals in the ACS meeting of 2000.[33]

In soil,^{14}C-azoxystrobin degraded in lab tests at rate of $DT_{50} = 56$-84 days. The major metabolite in soil was azoxystrobin carboxylic acid which degraded further via several intermediates to$^{14}CO_2$. In the field, azoxystrobin degraded faster at $DT_{50} = 14$ days

In the plant, azoxystrobin is extensively metabolized via biotic and abiotic reactions to 17 identified metabolites. The most abundant metabolite in the plant was the product of the ether bond cleavage cyanophenoxypyrimidinol (1) which was readily converted as N-glucoside conjugate.

In mammals, azoxystrobin was extensively metabolized and rapidly excreted and only very small residues of radioactivity were detected in milk, eggs, fat and trace residues in meat. With the exception of photolytic induced reactions, the pattern of metabolism in mammals resembles to a great extent the metabolic pathways in the plant. While in the plant the conjugation of hydroxylated aromatic rings are with glucose while the conjugation in mammals is with mercapturic acid. The major metabolite in mammals was azoxystrobin carboxylic acid which was excreted as glucuronide.

Metabolism of kresoxime-methyl in soil, plant and rat[32] and trifloxystrobin fate in plant,[34] is very similar to azoxystrobin metabolism. Degradation of methoxyiminoacetate compared very closely to the degradation of methoxyacrylates.

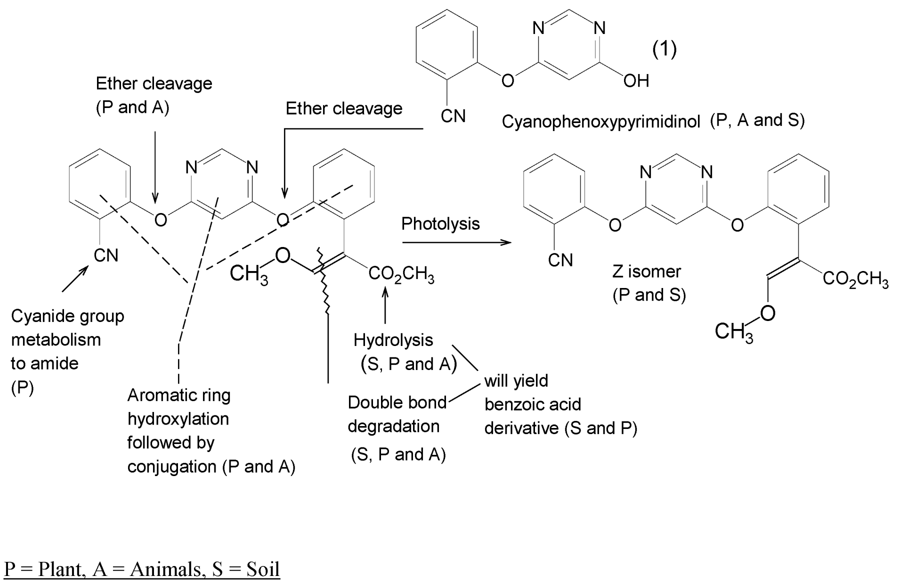


Fig. 4. Metabolic pathways of azoxystrobin.

Analysis of strobilurins residues

Christensen and Granby[35] published an extensive and detailed analytical paper on the analysis of residues of three commercial strobilurin fungicides (azoxystrobin, kresoxim-Me, and trifloxystrobin). The method was based on extraction with ethyl acetate/ cyclohexane, followed by clean-up by gel permeation chromatography (GPC) and determination of strobilurins by gas chromatography (GC). The GC was equipped with electron capture detector (EC), nitrogen/phosphorus (NPD), or mass spectrometric (MSD).

The validation of the method was applied on three matrixes: wheat, apples and grapes. The validation was based on recovery tests at three or four spiking levels. Each fortification level was analyzed as duplicates and repeated three times ($n = 6$). The mean recoveries for the three detectors were in the range of 70–114% and limit of detection (LOD) were between 0.004 – 0.014 mg/kg, for all three strobilurins. The values for repeatability and reproducibility were within the limits given by the Horwitz equation. Validation was not acceptable for azoxystrobin in grapes on all three detectors and for apples for the MS-detector. A comparison of matrix-matched standards vs. standards in solvent showed varying differences between the two calibration curves.

Later, the analytical methodology of azoxystrobin residues in crops, soil, water, animal matrixes and air was published by Robinson.[36] In these methods, high performance liquid chromatography (HPLC) with triple-quadrapole mass spectroscopy (MS) detection is used for the analysis of crops, soil and water samples. GC with thermo-ionic nitrogen-specific detection is employed for animal products and GC with mass-selective detection for air samples.

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