UNDERSTANDING THE STROBILURIN FUNGICIDES

Dave W. Bartlett, John M. Clough, Chris R. A. Godfrey, Jeremy R. Godwin, Alison A. Hall, Steve P. Heaney and Steve J. Maund from Syngenta at Jealott's Hill International Research Centre[1] discuss the biology, ecology and resistance management of the strobilurins, especially picoxystrobin, the latest member of the class

Introduction

The strobilurins are an important class of agricultural fungicides with a novel mode of action. The earliest examples were first sold in 1996, and there are now four commercial strobilurin fungicides, with others in development (Table 1 and Figure 1). This paper describes the science which underpins these fungicides and focuses, in particular, on their biological and ecological properties, and on picoxystrobin, a strobilurin currently being developed by Syngenta.

The discovery of the strobilurin fungicides was inspired by a group of natural β-methoxyacrylates, the simplest of which are strobilurin A and oudemansin A (Figure 2). The natural products were found to be unsuitable as agricultural fungicides, but a knowledge of their structures and properties provided a useful starting point for independent programmes of research within ICI (now part of Syngenta) and BASF. When ICI and BASF published their first patent applications, other companies also recognised the importance of this class of chemistry and began their own research in the area. ICI and BASF announced the first development strobilurins, azoxystrobin and kresoxim-methyl, respectively, in 1992. These products were sold for the first time in 1996, for the control of diseases in temperate cereals. Since that time, other products have been announced, namely trifloxystrobin from Novartis (this product was recently sold to Bayer), metominostrobin from Shionogi, pyraclostrobin from BASF, and picoxystrobin from Syngenta. Azoxystrobin and picoxystrobin retain the methyl β-methoxyacrylate group of the natural fungicides, while the others contain modified toxophores. More recently, DuPont and Aventis, respectively, have discovered famoxadone and fenamidone, fungicides which are not structurally-related to the strobilurins, but which have the same mode of action.

Table 1. The strobilurin fungicides.

Strobilurin	Company	Announced	First Sales
Azoxystrobin[1]	Syngenta	1992	1996
Kresoxim-methyl	BASF	1992	1996
Metominostrobin	Shionogi	1993	1999
Trifloxystrobin[2]	Bayer	1998	1999
Pyraclostrobin	BASF	2000	In development
Picoxystrobin	Syngenta	2000	In development

[1] Discovered by ICI, now part of Syngenta.
[2] Discovered by Novartis, sold to Bayer in 2000.

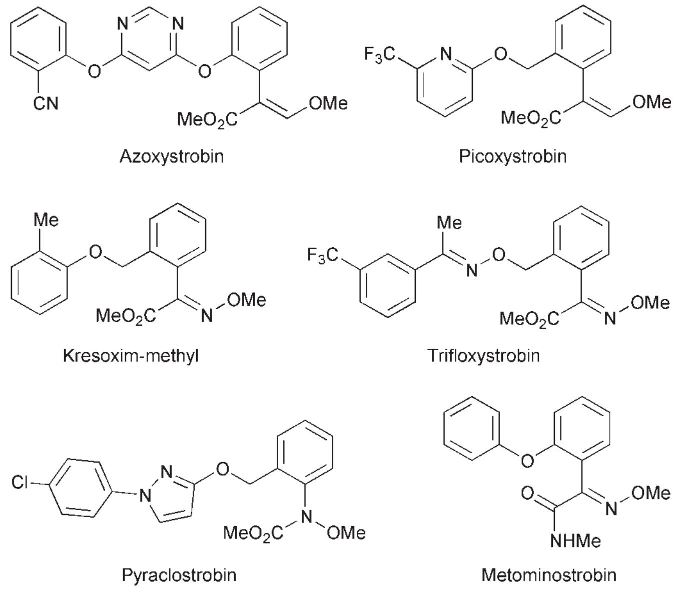

Figure 1. **The strobilurin fungicides.**

Sales of the strobilurin fungicides were approximately $600 million in 1999, representing just over 10% of the global fungicide market. The huge impact of the strobilurins on agriculture is well exemplified by the development of azoxystrobin which has now been registered for use on a broad spectrum of fungal diseases on 84 different crops in 71 countries, representing over 400 crop/disease systems. Leading crops include cereals, vines, fruit and vegetables, and peanuts. Sales of azoxystrobin reached $415 million in 1999. Of this value, the UK market alone accounted for 8% of sales, primarily from sales on wheat and barley crops.

The strobilurins act by inhibiting mitochondrial respiration in fungi. They bind at the Q_o-centre on cytochrome b and block electron transfer between cytochrome b and cytochrome c_1. This disrupts the energy cycle within the fungus by halting the production of ATP.

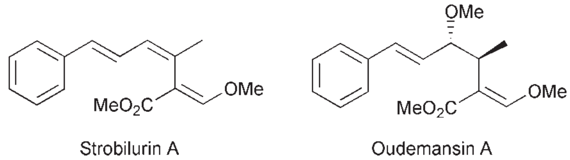

Figure 2. **The simplest of the natural strobilurins.**

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As a family, the strobilurin fungicides give high levels of activity against a wide range of crop diseases. Indeed, one of the key reasons for the outstanding commercial success of azoxystrobin is that it gives control of fungi from all four classes of plant pathogens, namely the Ascomycetes, Basidiomycetes, Deuteromycetes and Oomycetes. Therefore, azoxystrobin gives control of combinations of pathogens which was previously only possible through the mixture of two or more fungicides, *e.g.* downy and powdery mildew of grapevines. However, not all strobilurins are broad spectrum fungicides used on a wide range of crops. For example, metominostrobin from Shionogi has been developed for use exclusively on rice. Similarly, other strobilurins do not offer high level control of all four classes of fungal plant pathogens; kresoxim-methyl and trifloxystrobin are both relatively weak against rust diseases and downy mildews. Of the recently announced strobilurins, pyraclostrobin from BASF is a broad-spectrum strobilurin for use on a wide range of crops, whereas Syngenta's picoxystrobin is a specialist cereal fungicide.

Picoxystrobin : biology

Broad spectrum cereal foliar fungicides designed for the current market must control a wide range of cereal diseases because there are more than 15 fungal pathogens which can have a major impact on grain yield and quality. In addition, they should possess curative activity, i.e. the ability to control diseases after infection has already become established. Curative activity is particularly important for cereal fungicides applied early season because at this timing disease is likely to be established within the overwintered crop. Redistribution properties are also important features for broad spectrum cereal foliar fungicides; uptake into the leaf and systemic movement are necessary for the control of deep-seated diseases such as *Septoria* and rusts while vapour activity is important for powdery mildew control in order that the active ingredient can fully penetrate the tightly woven "mycelial mat" on the leaf surface. Perhaps the most important feature for cereal fungicides in the current climate of economic difficulties for farmers and increasing environmental awareness is that they must deliver sufficient grain yield and quality benefits to justify their use. Finally, any agrochemical needs to have a good safety and environmental profile for reasons of product stewardship and registration.

All of these requirements drove the extensive programme of research which led to the selection of picoxystrobin as a broad spectrum cereal strobilurin fungicide.

Broad spectrum activity

Picoxystrobin demonstrates an outstanding breadth of spectrum in cereals, being highly active against both *Septoria* diseases of wheat, *Helminthosporium* species on wheat, barley and oats, *Rhynchosporium* on barley and rye, *Ramularia* on barley and *Puccinia* rust diseases and strobilurin-sensitive powdery mildews on wheat, barley, oats and rye. Its broad spectrum of activity means that the green leaf area of the crop is maintained during the important grain filling period. Figure 3 summarises the breadth and level of activity of picoxystrobin against the key cereal diseases compared to current commercial strobilurins. Picoxystrobin delivers a breadth of spectrum and level of activity on cereals that is superior to current commercial strobilurin fungicides. It has also demonstrated curative activity against a range of cereal diseases under field conditions, which is attributable to its good uptake into the leaf. Figure 4 shows that picoxystrobin gives better curative activity than the commercial strobilurins azoxystrobin and trifloxystrobin.

Redistribution properties

The redistribution properties of picoxystrobin play an important role in delivering its broad spectrum activity against cereal diseases. Typically around 40% is taken up into leaves by one day after application, approximately half of which enters the leaf within two hours of spraying. Material remaining on the leaf surface has excellent biological rainfastness, *e.g.* there was no decline in control of *Septoria tritici* on wheat when comparing between plants sprayed with picoxystrobin (250 g ai/ha) under rain-free conditions and plants exposed to 10mm of simulated rainfall for one hour, applied two hours after spraying. Once absorbed into the leaf, picoxystrobin is mobile in the xylem (systemic), moving with the water flow in the transpiration stream. Picoxystrobin also moves in the vapour phase at the leaf surface and has been shown to be translocated in the xylem following its absorption into the leaf from the vapour phase.

The redistribution properties of picoxystrobin are unique amongst commercial strobilurin fungicides and those in development, because it is the only one showing both vapour activity and xylem systemicity. The consequences of these unique properties for disease control have been demonstrated by applying various fungicide treatments to a defined zone on wheat leaves, inoculating the complete leaves with spores of wheat powdery mildew and monitoring disease development. Picoxystrobin demonstrated both vapour activity in the zone toward the leaf base and xylem mobility to give complete disease control to the leaf tip (Figure 5), whereas azoxystrobin showed systemic activity only and trifloxystrobin and kresoxim-methyl showed vapour activity only. Table 2 summarises the redistribution properties of strobilurins.

Grain yield and quality

Treatment of cereals with picoxystrobin has consistently given excellent yield benefits together with improvements in quality through an increased frequency of larger-sized grain. These yield and quality benefits are attributable to its broad spectrum disease control, good maintenance of green leaf area and crop safety.

In field trials against a range of foliar diseases of winter wheat, picoxystrobin-based programmes delivered an average of 0.2 t/ha more than the corresponding trifloxystrobin-based programmes. Indeed, in 21 European wheat field trials over three years, treatment with picoxystrobin gave a 22% increase in yield over the untreated, identical to that achieved with a strobilurin (kresoxim-methyl) in mixture with a triazole (epoxiconazole). The analysis of data from 21 European field trials over 3 years on winter barley showed that picoxystrobin gave a mean yield increase of 0.4 t/ha over kresoxim-methyl/epoxiconazole. Moreover, the

Table 2. Redistribution properties of strobilurins.[1]

	Azoxystrobin	Trifloxystrobin	Kresoxim-methyl	Metominostrobin	Pyraclostrobin	Picoxystrobin
Movement into leaf	low	v.low	low	high	v. low	medium
Vapour active	no	yes	yes	no	no	yes
Metabolic stability in leaf	yes	low	low	n.d.*	yes	yes
Translaminar movement	yes	low	low	yes	low	yes
Xylem systemic	yes	no	no	yes	no	yes
Systemic movement to new growth in cereals	yes	no	no	yes	no	yes
Phloem mobile	no	no	no	no	no	no

[1] Source Syngenta; *n.d. = no data

Figure 3. **Activity of picoxystrobin against the key cereal foliar diseases compared to current commercial strobilurins at their recommended use rates.**

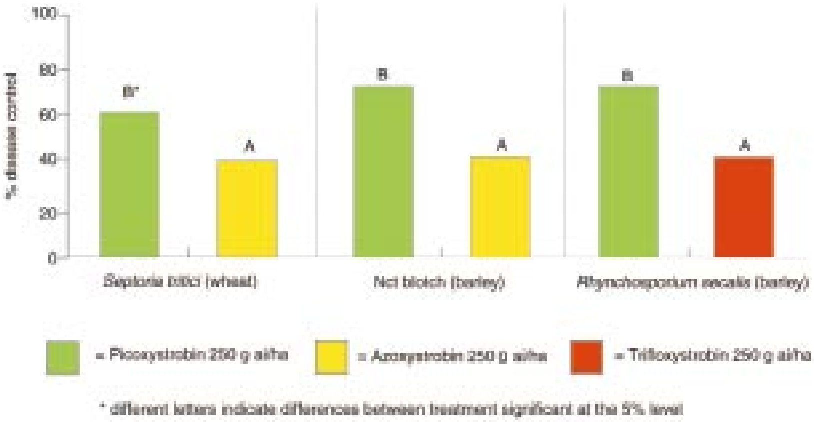

Figure 4. **Curative activity of strobilurins against cereal diseases (as shown in glasshouse tests).**

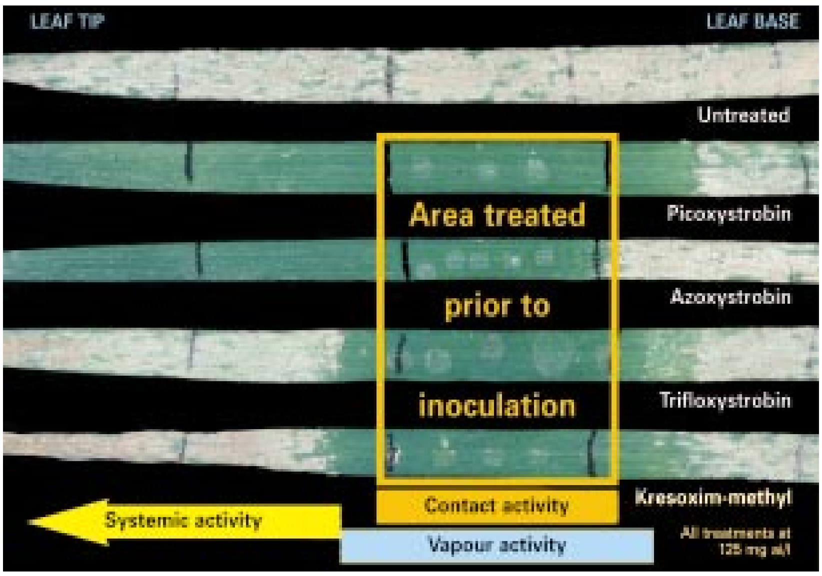

Figure 5. **Picoxystrobin demonstrates both vapour and systemic activity in cereal leaves.**

picoxystrobin treatment gave the superior yield in 17 of the 21 trials, demonstrating the consistency of yield benefit that picoxystrobin delivers. Similar yield benefits of picoxystrobin over kresoxim-methyl/epoxiconazole were shown in 21 European field trials on winter barley over three years (Figure 6).

These improvements in cereal yields with picoxystrobin have been accompanied by improvements in grain quality through increases in the frequency of larger-sized grain. Results from winter wheat field trials in which grain greater than 2.2 mm in diameter was weighed separately from grain less than 2.2 mm in diameter clearly showed that the increase in overall yield with picoxystrobin was due to an increase in yield of the larger sized grain fraction.

Ecological profile

The commercialised strobilurins azoxystrobin, trifloxystrobin and kresoxim-methyl are considered safe to birds and mammalian wildlife, bees, earthworms and beneficial insects (The e-Pesticide Manual, 2000).

In standard laboratory studies required for pesticide registration, all of the above strobilurins are relatively toxic to aquatic organisms (Table 3). The more recently announced compounds picoxystrobin and pyraclostrobin also show high inherent toxicity to aquatic organisms. There is an indication that as the lipophilicity (octanol: water partition coefficient) rises, effects on aquatic organisms also intensify. Linked increases in toxicity with lipophilicity are a common phenomenon for pesticides and other organic chemicals. However, although such data from standard toxicity tests can be useful for expressing the inherent 'hazard' of chemicals, they do not necessarily reflect the potential for adverse effects during normal agricultural use, in other words, the true 'ecological risks' of the compound.

To evaluate potential risks in use, it is necessary to consider both how non-target organisms will be exposed to the compound and

Table 3. Toxicity of strobilurins to aquatic organisms.

Compound	Fish 96 h LC_{50} (μg L^{-1})	Daphnia 48 h EC_{50} (μg L^{-1})	Algae EC_{50} (μg L^{-1})	Octanol: water log P	Reference
Azoxystrobin	470	259	120	2.5	Pesticide Manual, 2000
Kresoxim-methyl	190	186	63	3.4	Pesticide Manual, 2000
Trifloxystrobin	15	16		3.4	Pesticide Manual, 2000
Pyraclostrobin	6			4.0	Ammermann *et al.*, 2000
Picoxystrobin	65	18	56	3.6	Godwin *et al.,* 2000

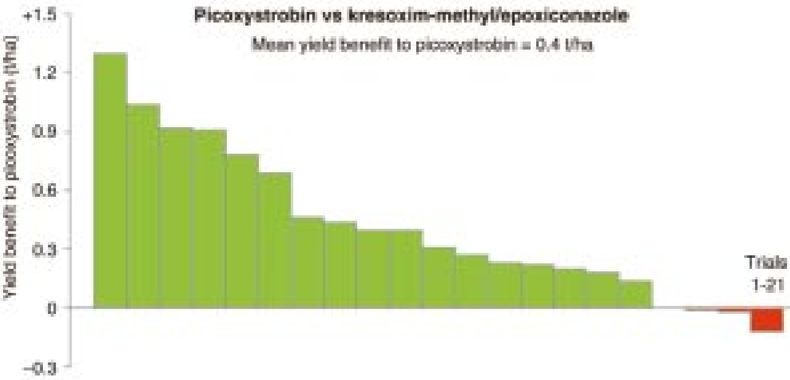

Figure 6. **Winter barley yield responses in 21 individual trials.**

the ecological characteristics of the organisms involved (*i.e.*, where and how they live and reproduce). The development of such a risk assessment is a scientifically demanding process that requires the production of a vast range of data. This includes studies to understand the fate of the chemical in the various environmental matrices (air, soil, water, sediment), as well as its effects on non-target organisms. Exposure of organisms in the environment also has to be estimated through the use of a variety of exposure models. For certain compounds, it may also be necessary to understand the range of sensitivity of the non-target organisms potentially affected, and how their ecological dynamics may influence responses to chemical exposure.

Substantial scientific investment in such comprehensive risk assessment packages means that a tremendous amount is known about the new strobilurin pesticides. For example, for picoxystrobin, risk assessments have been carried out on all relevant groups of non-target organisms (Godwin *et al.*, 2000), and show that the compound presents low risks under normal use.

To demonstrate some of the complexity that can be involved in generating such risk assessments, let us consider the potential risk of picoxystrobin to aquatic invertebrates. Picoxystrobin is applied at 250 g active ingredient per hectare. The EU model for predicting concentrations in surface water makes some worst-case assumptions *e.g.*, that there is always a 30 cm deep static water body located 1 m downwind from the point of application. Resulting estimates of the initial concentration of picoxystrobin at the water's edge, arising from spray drift, would be approximately 2 μg L^{-1}. Whilst this is nearly an order of magnitude less than the toxicity value on *Daphnia*, a 100-fold safety factor is normally applied to account for potential differences in sensitivity between species. Since this triggers further investigation, tests were conducted on a wide range of aquatic invertebrate species to explore interspecies sensitivity (Table 4). These demonstrated that *Daphnia* were in fact among the most sensitive species. None of the species tested was 100 times more sensitive, and worst-case exposure concentrations were still below the toxicity to the most sensitive species, a copepod zooplankter, *Diaptomus*. Consequently, adverse effects on invertebrates from worst-case exposure concentrations seemed unlikely.

This conclusion was further explored in an aquatic microcosm study. These are highly detailed studies that evaluate the effects of the pesticide on complex aquatic communities in replicated outdoor experimental systems (Figure 7). Simulated spray drift events did not result in adverse effects on the aquatic invertebrate community, even after three applications at

Table 4. Toxicity of picoxystrobin to a range of aquatic invertebrate species.

Species	Common name	48 h EC_{50} (μg L^{-1})
Diaptomus	Copepod	5
Daphnia magna	Water flea	18
Daphnia pulex	Water flea	> 51
Crangonyx	Shrimp	63
Macrocyclops	Copepod	87
Asellus	Slater	152
Agrypnia	Caddisfly	158
Cloeon	Mayfly	194
Tubifex	Worm	299
Chironomus	Non-biting midge	326
Chaoborus	Phantom midge	332
Dugesia	Flat worm	c. 450
Polycelis	Flat worm	c. 450
Erpobdella	Leech	c. 450
Limnea	Snail	> 1000
Coenagrion	Damselfly	> 1000
Notonecta	Water boatman	> 1000
Naucoridae	Water bug	> 1000
Brachionus	Rotifer	> 4000

Figure 7. **Pond microcosms at Jealott's Hill International Research Centre. Samples for water quality and plankton are being collected.**

rates that were ten times greater than those predicted from the worst-case EU exposure model. This clearly demonstrated that picoxystrobin is of low risk to the aquatic ecosystem and requires no aquatic buffer zones.

Sensitivity of fungal populations to the strobilurins, and resistance management

The strobilurin fungicides, together with famoxadone and fenamidone, bind at the Qo site of the cytochrome bc1 complex. Together, these compounds form a new cross-resistance group, designated QoI by the Fungicide Resistance Action Committee (FRAC).

Despite intensive monitoring over the past two or three years, no significant shifts in sensitivity to the QoI group of fungicides have been detected in the majority of cereal pathogens, including *Septoria tritici, Pyrenophora teres, Rhynchosporium secalis* and *Puccinia* spp. By contrast, strobilurin resistance has progressed rapidly in wheat powdery mildew (*Blumeria graminis* f.sp. *tritici*): resistant populations were first detected in May 1998, and by 2000 high frequencies of resistant spores (2-99%) were reported across Northern Germany, Northern France and the UK. In addition, in 2000, resistance was detected in barley powdery mildew (*B. graminis* f.sp. *hordei*) at localised sites in Northern France, Northern Germany and Scotland (Heaney *et al*., 2000).

QoI resistance in both wheat and barley powdery mildew correlates with a specific point mutation leading to the substitution of glycine by alanine at position 143 (according to the *Saccharomyces cerevisiae* numbering system) of cytochrome b (Sierotzki *et al*., 2000). This $G_{143}A$ point mutation has also been identified in QoI-resistant isolates of *Sphaerotheca fuliginea, Plasmopara viticola, Pseudoperonospora cubensis* and *Mycosphaerella fijiensis*.

The resistant field isolates of *B. graminis* show large resistance factors of >1000 compared to the baseline isolates. They are thought to have spread rapidly due to the relative fitness of the $G_{143}A$ mutation in *B. graminis* along with the epidemiology of the pathogen. Previous experience with ethirimol, the benzimidazoles and the demethylation inhibitors (DMIs) such as the triazoles, has demonstrated the high risk for resistance associated with *B. graminis*. In each of these cases, resistance has evolved within 3–6 years of the introduction of the chemical.

The key question now is whether the other cereal pathogens will follow the powdery mildew model in the evolution of resistance. Indeed, *M. fijiensis* is closely related to *S. tritici* (*Mycosphaerella graminicola*) suggesting that there is a risk of target site resistance in the latter pathogen. Nevertheless, prior experience, in particular with the DMIs, has shown that resistance in many key cereal pathogens, including *S. tritici,* has taken considerably longer to develop (12 years or greater) compared to powdery mildew, which provides an opportunity for the implementation of anti-resistance strategies.

In response to the emergence of resistance in *B. graminis*, FRAC now recommends that a strobilurin is mixed with an effective partner for the control of powdery mildew. It is also advised that the number of QoI applications is limited to two out of three sprays per season, and that effective doses of a QoI fungicide are used, in order to decrease the risk of resistance developing in other cereal pathogens. In addition, mixtures of fungicides in different cross-resistance groups are preferred for the control of all cereal diseases.

Conclusions

The strobilurins are an outstanding new class of fungicides. Registrations have been obtained on a wide range of crops throughout the world, to the point where the strobilurins can now be considered to be one of the most valuable classes of single-site fungicides ever discovered by the agrochemical industry. They have set new standards in disease control and, more importantly for the grower, in the delivery of improved yields and quality. Indeed, the success of the strobilurins in the fungicide market simply reflects the benefits that they bring to those producing the crop. New strobilurins such as picoxystrobin continue their development, and the dependence of crop protection on the strobilurins is likely to increase still further.

Further reading

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All the authors work for Syngenta at Jealott's Hill International Research Centre, Bracknell, UK, and have been involved with different aspects of the discovery, evaluation and development of picoxystrobin. *John Clough* and *Chris Godfrey* are synthetic chemists; Jeremy Godwin is a plant pathologist; *Dave Bartlett* is a plant physiologist; *Steve Maund* is an environmental scientist; and *Alison Hall* and *Steve Heaney* have studied the resistance management of the strobilurins.

WORLD'S WORST WEEDS

The title of this forthcoming symposium to be held at Brighton on 12 November 2001, in conjunction with this year's BCPC Conference **Weeds 2001**, comes from the title of the first of three books by Leroy Holm *et al*.[1–3]

Since weeds account for significant costs in all agricultural systems, this symposium, to be chaired by Charlie Riches (NRI, University of Greenwich, UK), will review the current distribution and status of some of the most important and intransigent weed groups in world agriculture, together with the development of rational practices for their control.

Holm places *Cyperus rotundus* (purple nutsedge) as the world's worst weed, and so it is fitting that John Terry (IACR Long Ashton, UK) will start off the symposium with a presentation entitled *The Cyperaceae – still the world's worst weeds?*

Using examples from temperate and tropical areas, emphasis will be placed on how change in agricultural practice effects weed floras and how recent developments in weed biology and ecology can lead to sustainable, environmentally acceptable management systems for key species.

Aquatic weeds like water hyacinth (*Eichhornia crassipes*) and parasitic weeds like *Striga* and *Orobanche* would also be high on anyone's worst weed list, and so they will feature in presentations by Raghavan Charudattan (University of Florida, USA) and Malcolm Press (University of Sheffield, UK) respectively.

Chris Parker (consultant, Bristol, UK) will outline how we might be able to predict the emergence and prevent the spread of new weed problems, and, rounding off, James Bunce (Beltsville Agricultural Research Centre, Maryland, USA) will discuss how weed populations might be affected by future global climate change.

For further information please contact the BCPC Conference Secretariat, 5 Maidstone Buildings Mews, Bankside, London. SE1 1GN, UK (Tel: +44 (0)20 7940 5555; Fax: +44 (0) 20 7940 5577; Email: conference@bcpc.org).

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