SESSION 6A

RESISTANCE: SCIENCE INTO PRACTICE

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Insecticide resistance: from science to practice

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ABSTRACT

In this paper we review how rapid advances in understanding the biochemical and molecular nature of insecticide resistance is contributing or might contribute to combating resistance in practice. Knowledge of the different enzyme systems that degrade insecticides and the specific target site mutations that selectively neutralise particular classes or types of insecticide has progressed dramatically over the past decade, and this in turn has enabled the development of highly sensitive mechanism-specific diagnostic assays for resistance monitoring. These tools can be used for analysing the incidence, dynamics and practical importance of resistance, and for exploring the influence of both operational (e.g. pesticide use patterns) and biological (e.g. insect dispersal and fitness costs) factors on the frequency of resistance genes. Such techniques are particularly valuable for species (e.g. the peach-potato aphid, *Myzus persicae*) that possess multiple resistance mechanisms, each with distinct but sometimes over-lapping cross-resistance spectra. Frequent similarities between mechanisms in different species also mean that the techniques developed for one species can often be transferred to others with little additional developmental research.

INTRODUCTION

Few areas of applied entomology have advanced as rapidly or received such widespread attention in recent years as that of insecticide resistance. This reflects both the increasingly severe impact of resistance on pest and disease management programmes, and the exciting contributions that resistance is making to fundamental knowledge of insect genetics, biochemistry and physiology. Without doubt, some of the most significant recent progress with understanding resistance has resulted from the application of molecular biology to resistance research. Depending on the mechanism involved, resistance has been shown to arise through structural alterations of genes encoding target-site proteins or detoxifying enzymes, or through processes (e.g. amplification or altered transcription) affecting gene expression (ffrench-Constant, 1999; Hemingway, 2000). Despite this diversity of origin, genetic options available to insects can also be very limited, especially for mechanisms based on a decreased sensitivity of the insecticide target site. For example, the primary mechanism of cyclodiene resistance in insects, based on a modification of the GABA-gated chloride channel in nerve membranes, has been attributed to a single point mutation in several species of diverse taxonomic origin (Thompson *et al.*, 1993). Work on two other target-site mechanisms - altered acetylcholinesterase (AChE), conferring resistance to organophosphates (OPs) and carbamates, and knockdown resistance or kdr (conferring resistance to DDT and pyrethroids) - has also shown striking parallels between species but has proved more complicated due the occurrence of multiple resistance alleles at the same loci (see below).

This paper explores the extent to which research on resistance mechanisms is contributing or may contribute to managing resistance in practice, through both the development of *in vitro* diagnostics for specific genes and gene products, enabling more precise studies of factors affecting the evolution and dynamics of resistance mechanisms, and an improved understanding of factors affecting cross-resistance between molecules potentially available for use in strategies aimed at diversifying the selection pressures imposed on pest populations.

MECHANISMS OF RESISTANCE IN *MYZUS PERSICAE*

The extent to which an improved knowledge of mechanisms can contribute to resistance management is exemplified well by work on the peach-potato aphid, *Myzus persicae* Sulzer. This species attacks and can transmit disease to several arable and horticultural crops including brassicas, potatoes, sugar beet and lettuce. *M. persicae* possesses three distinct mechanisms that collectively confer strong resistance to organophosphate, carbamate and pyrethroid insecticides. The first, discovered at Rothamsted 30 years ago, is based on the overproduction of one of two closely related carboxyesterase enzymes (E4 and FE4) that inactivate organophosphates, and to a lesser extent carbamates and pyrethroids before they reach their target sites in the insect's nervous system. Depending on the amount of carboxylesterase present, individuals of *M. persicae* are broadly classified into one of four categories: S-susceptible; R_1 – moderately resistant; R_2 – highly resistant or R_3 – extremely resistant (Devonshire & Moores, 1982). This elevated esterase results from the presence of amplified genes (Field *et al.*, 1993) and detailed molecular studies have shown that amplified E4 genes are on 24 kb units of DNA present as a tandem array of head-to-tail repeats, usually at a single chromosomal location associated with a translocation (Field & Devonshire, 1997). However, amplified FE4 genes can be present at multiple loci and there are no visible chromosomal abnormalities (Blackman *et al.*, 1999). An immunoassay that quantifies the amount of E4/FE4 in single aphids (see next section) has shown that there are approximately 4-fold increases in the amount of enzyme present in S, R_1, R_2 and R_3 aphids and this reflects a proportionate increase in gene copy number rising to around 80 copies in R_3 aphids (Field *et al.*, 1999).

The second mechanism, termed MACE (Modified AcetylCholinEsterase) is due to a modification to the insecticide target enzyme, acetylcholinesterase (AChE), which renders it insensitive to attack by the dimethyl carbamates, pirimicarb and triazamate (Moores *et al.*, 1994). MACE resistance was first seen in the UK in 1995 in aphids caught in Rothamsted's suction trap network, caused severe control failures in eastern England in 1996, and has been present at varying frequencies thereafter. Analogous MACE-type resistance mechanisms have been reported in a wide range of agricultural pest insect species, though generally these tend to be less selective, conferring a much broader resistance to OPs and/or carbamates. Although molecular cloning and sequencing studies of *Ace* genes from the 'model' insects, *Drosophila melanogaster* Meigen and *Musca domestica* L., have revealed several point mutations within the active site of the enzyme that disrupt insecticide binding to cause resistance (Mutero *et al.*, 1994; Walsh *et al.*, 2001), exploiting this information to identify the corresponding *Ace* mutations in other insects has proven unexpectedly difficult. It now seems that this is because most insects (other than *Drosophila* and *Musca*) possess two distinct *Ace* genes, with the structural mutations associated with resistance being located within the second, more divergent *Ace* gene sequence (Weill *et al.*, 2003). Recent cloning and analysis of this second gene from *M persicae* has indeed now identified a single point mutation, a serine to phenylalanine substitution (S331F) deep within the active site of the enzyme, that is likely to confer the

highly selective resistance to dimethyl carbamates which is characteristic of the *M. persicae* MACE mechanism (Andrews *et al.*, 2003; Nabeshima *et al.*, 2003). Further studies are in progress to confirm the functionality of this mutation and to understand better how it selectively affects binding and inhibition of only the dimethyl carbamates.

In the last few years, we have also identified a third resistance mechanism, termed knockdown resistance or kdr, which is associated specifically with resistance to DDT and pyrethroids. Kdr involves a modification to the voltage-gated sodium channel protein in nerve membranes, which are vital for the normal transmission of nerve impulses and are the primary target site of these insecticides (Narahashi, 1992). There has been considerable progress in characterising the sodium channel mutations that are responsible for resistance, initially from work on *M. domestica* where two point mutations, leucine to phenylalanine (L1014F) and methionine to threonine (M918T) within the domain II region of the channel protein, were found to correlate with kdr (moderate resistance) and super-kdr (enhanced resistance) phenotypes respectively (Williamson *et al.*, 1996). The L1014F mutation has since been shown to occur in a range of insect species where it seems to confer a 'basal' kdr phenotype of 10-20 fold resistance to most pyrethroids. The enhanced super-kdr phenotype, that can give over 1000 fold resistance, is however less well conserved and several secondary mutations have been found that differ between species (Liu *et al.*, 2000; Pittendrigh *et al.*, 1997; Schuler *et al.*, 1998). In the case of *M persicae*, however, the same two point mutations originally described for houseflies (L1014F and M918T) have also been found and shown to correlate with DDT and pyrethroid resistance (Martinez-Torres *et al.*, 1999; Eleftherianos *et al.*, submitted). Consistent with their previous classification, *M. persicae* strains carrying the kdr mutation show generally moderate levels of resistance, whilst those with the M918T super-kdr mutation are virtually immune to the effects of even the most potent pyrethroids. The identification of these two point mutations within the *M. persicae* sodium channel (a large and complex membrane protein comprising over 2000 amino acids) has presented exciting new opportunities for the rapid diagnosis of this resistance mechanism in individual, field-collected aphids (see next section).

DIAGNOSIS OF MULTIPLE RESISTANCE IN *MYZUS PERSICAE*

These three mechanisms – overproduced carboxylesterase, MACE and kdr – can be present in different combinations that have different implications for which insecticides are likely to be effective or not. An ability to diagnose them individually and rapidly, ideally in single aphids, is therefore invaluable for anticipating and combating resistance problems. Biochemical assays for diagnosing overproduced carboxylesterase and MACE in single aphids have been developed at Rothamsted and are now used widely in many countries with resistance monitoring programmes for *M. persicae*. In most cases, the level of carboxylesterase (E4 or FE4) is measured using a sensitive immunoassay technique in 96-well microplates (Devonshire *et al.*, 1986) that can accurately score the esterase phenotype (S, R_1, R_2, R_3) of a small aliquot (1/20) of a single aphid homogenate. An additional polymerase chain reaction (PCR)-based technique is also available for scoring the esterase genotype (E4 or FE4) of the aphid where this is desirable (Field *et al.*, 1996). MACE phenotypes (susceptible, resistant homozygote and partially resistant heterozygote) are measured using a kinetic assay of AChE activity over time in the absence and presence of a low concentration of pirimicarb (Moores *et al.*, 1994). This assay is also very sensitive, using a further 1/8 aliquot of the same aphid homogenate and is run in 96-well format with a Tmax plate reader (Molecular Devices).

Kdr has proved more challenging in this respect since it is not readily accessible to biochemical tests based on electrophoresis, immunodiagnosis or kinetic measurements of target site inhibition. We have therefore concentrated on developing *in vitro* assays (as opposed to whole-organism bioassays, which are time-consuming and not mechanism-specific) based on the kdr (L1014F) and super-kdr (M918T) sodium channel mutations that cause the resistance phenotypes (see previous section). Several sequence-based approaches have been attempted, the most successful being the recent development of 5' nuclease allelic discrimination PCR assays specific to each of the two mutations (Anstead *et al.*, submitted) using fluorescent Taqman® MGB probes (PE Applied Biosystems). The main advantage of fluorogenic probe assays is that they enable PCR amplification and product detection to be combined in a single step, thereby greatly increasing the speed and efficiency of the assay and removing the requirement for time-consuming post-PCR manipulations (e.g. gel electrophoresis of PCR products). The 5' nuclease assay (Livak, 1999) uses short oligonucleotide probes that are matched against either the wild-type (susceptible) or kdr/super-kdr (resistant) sodium channel sequences (i.e. they are allele specific). These probes are each labelled with two fluorescent dyes; a reporter dye at one end and a quencher dye at the other and are added to PCR reactions of aphid DNA that are designed to amplify across the kdr and super-kdr sites within the sodium channel gene. In the intact probe, the fluorescence of the reporter dye is quenched by the close proximity of the quencher dye. However, during the PCR reaction, the probe is broken down if it anneals to its matching sequence in the sodium channel gene of the aphid that is being tested. Thus, an increase in fluorescence during the PCR indicates that the allele for the probe being tested is present in the aphid, and by testing small aliquots of individual aphids with each probe the exact susceptible/kdr/super-kdr genotype can be determined. Using this method, the three possible genotypes (resistant homozygote RR, heterozygote RS, susceptible homozygote SS) are easily distinguished for each of the two resistance alleles. The fluorescence output data for each probe is fed into an analysis programme that gives automated calling of the full genotype of each aphid. These assays are also very sensitive, each probe reaction requiring only 1/50 of a single aphid homogenate, and are designed to run alongside existing ones for overproduced carboxylesterase and MACE. This suite of tools collectively enables a single aphid to be assigned to one of 108 possible genotypes encompassing all three resistance mechanisms, providing accurate predictions of resistance phenotype. To our knowledge, this level of precision is unprecedented for any multi-resistant insect pest.

APPLICATION OF RESISTANCE DIAGNOSTICS

The availability of such diagnostics has enabled us to track changes in the frequency of resistance mechanisms, relating these to the control measures adopted and the biological characteristics of *M. persicae*. Aphids for these surveys have come directly from field crops and from 12.2 m suction traps deployed around the UK as part of the Rothamsted Insect Survey (Woiwod & Harrington, 1994). Two distinct patterns have emerged from this research. The first is a long-term periodicity with resistance being most frequent in years such as 1996 with severe aphid outbreaks – and hence greatest insecticide use - followed by declines in frequency over years when aphids are less abundant. Secondly, resistance frequencies usually show a characteristic increase within seasons as insecticides are applied, but then often decline before the start of the following cropping season. This shorter-term periodicity, like patterns observed over a longer period, demonstrates that resistance levels can, under certain conditions, decrease as well as increase and prevent an overall, sustained increase in the severity of resistance problems. Declines can be due to a number of factors but appear

attributable in part to side-effects that resistance mechanisms impose on aphid biology, which may adversely affect their survival (Foster *et al.*, 1996) and/or reproduction (Foster *et al.*, 2000) in the absence of exposure to insecticides. Detailed work at Rothamsted has shown that resistant individuals of *M. persicae* tend to overwinter less successfully than their susceptible counterparts, be less fecund, and be less responsive to important environmental stimuli including the aphid alarm pheromone (E)-β-farnesene (Foster *et al.*, 1999; Foster *et al.*, 2003a). This compound is released from cornicle secretions exuded by aphids when they are physically disturbed, for example by foraging predators and parasitoids. Neighbouring aphids respond to the pheromone by withdrawing their stylets from the plant and dispersing away from the pheromone source. The intriguing possibility that decreased responsiveness to (E)-β-farnesene could render resistant aphids more vulnerable than susceptible ones to parasitism or predation is currently being investigated.

IMPLICATIONS FOR RESISTANCE MANAGEMENT

M. persicae poses a number of challenges for resistance management due to dramatic and often unpredictable changes in the severity of aphid attack from year to year, its large number of host plants, and the occurrence of multiple resistance mechanisms that collectively compromise the majority of compounds available for aphid control. However, increased knowledge of the incidence of these mechanisms, their cross-resistance characteristics, and of factors influencing the frequency of resistance genes has led to a series of recommendations based on alternating chemical groups, optimising the efficacy of individual treatments, and avoidance of tactics such as insecticide mixtures likely to result in the rapid accumulation of resistance mechanisms. These recommendations have been publicised through a number of organisations and publications, and are downloadable from the website for the UK Insecticide Resistance Action Group (IRAG) (www.pesticides.gov.uk/committees/Resistance). These guidelines also encompass newly-introduced insecticides available for inclusion in management strategies. Neonicotinoids (with imidacloprid as the commercial forerunner) and pymetrozine (a pyridine azomethane) represent newer insecticide groups available for use on some crops attacked by *M. persicae*, and which are unaffected by resistance mechanisms already present (Foster *et al.*, 2002a; Foster *et al.*, 2003b). However, their unrestrained use can unquestionably lead to selection of additional mechanisms, compounding the problem still further. Clones of *M. persicae* have been identified from southern Europe showing up to 18-fold resistance to imidacloprid, and individuals with lower resistance levels have been isolated from UK samples over the last three years. The commercialisation of neonicotinoids on an increasing number of crops harbouring *M. persicae* must therefore represent a significant new resistance risk requiring extensive co-operation between scientists, grower groups and agrochemical producers to address effectively.

Continuing access to new tools in molecular biology offers very exciting insights of processes governing the origin and spread of resistance, especially by combining markers for selected traits such as resistance with ones (e.g. microsatellites) with no obvious adaptive significance (Sunnucks, 2000). The reasons that some aphids such as *M. persicae* evolve resistance so rapidly whilst others (e.g. cereal aphids) do not, despite receiving insecticide treatments, should therefore become more tractable and provide greater scientific support for resistance management strategies, and risk assessment schemes built into pesticide approval procedures. Since the same resistance mechanisms often evolve in parallel in different species, diagnostic techniques developed for *M. persicae* may be transferred across species with little or no extra

work. For example, a mechanism of resistance based on elevated esterase activity in the potato aphid, *Macrosiphum euphorbiae* Thompson, has many parallels with the equivalent mechanism of overproduced carboxylesterase in *M. persicae* (Foster *et al.*, 2002b).

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QoI resistance development in populations of cereal pathogens in the UK

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ABSTRACT

The effectiveness of strategies aiming to retard the development of resistance to Qo inhibitor fungicides in barley powdery mildew populations was determined with PCR by measuring the frequency of the G143A mutation in cytochrome *b*. Preliminary results from field trials show that the frequency of the G143A mutation increases with higher doses and increasing number of sprays. Mixtures of fungicides with different modes of action appeared to slow down the increase in the frequency of the mutation. For most locations sampled in the UK, high frequencies of G143A were detected in *Septoria tritici* populations during spring 2003. Studies are now in progress to establish the significance of G143A in QoI resistance development in populations of *S. tritici*, and to evaluate anti-resistance strategies for this pathogen.

INTRODUCTION

Strobilurins and related compounds inhibit mitochondrial respiration by binding to the ubiquinol oxidation (Qo) site formed by domains of cytochrome *b* and the iron-sulphur protein within the cytochrome bc_1 complex. Because ATP production is compromised, energy-demanding stages of fungal development, such as spore germination, are particularly affected. The Qo inhibitors (QoIs) have become a key component of disease control strategies on cereals in NW-Europe due to their persistent broad-spectrum disease control and potential extra yield benefits through increased green canopy duration. In 1998, within two years of commercial use, field resistance to QoIs was found in wheat powdery mildew (*Blumeria graminis* f. sp. *tritici*) populations in North Germany. In all resistant isolates, a single point mutation leading to a change from glycine to alanine at amino-acid position 143 (G143A) was found in the cytochrome *b* gene (Heaney *et al.*, 2000). This mutation was also found in a single resistant isolate of *B. graminis* f. sp. *hordei* (barley powdery mildew) in N-Germany in 1999. By 2001, resistance in cereal powdery mildews was widespread in NW-Europe. In 2002, G143A was detected in resistant field isolates of *Septoria tritici* (teleomorph *Mycosphaerella graminicola*) in the UK (Fraaije *et al.*, unpublished). Up to 11 different amino-acid exchanges have been found to confer resistance to QoIs in other organisms, but only mutations at codons 129 and 143 have been reported for plant pathogens (Gisi *et al.*, 2002). Besides alteration of the target site, induction of alternative respiration (Ziogas *et al.*, 1997) and an unknown mechanism in *Venturia inaequalis* (Steinfeld *et al.*, 2001) have been reported to confer resistance to QoIs.

Practical disease control failures have only been linked with the occurrence of the G143A mutation in plant pathogen populations. This evolution can be explained by the high resistance levels and/or low fitness costs often associated with this mutation. Because of the importance of G143A as a predictive marker for QoI resistance, different real-time PCR-based diagnostics have been developed to monitor this mutation in pathogen populations (Gisi *et al.*, 2002). For wheat powdery mildew, the prevalence and dynamics of G143A in field populations before and after application of fungicides have been studied (Fraaije *et al.*, 2002). This paper presents preliminary results from the Sustainable Arable LINK programme 'Providing a scientific basis for the avoidance of fungicide resistance in plant pathogens'. Using QoI resistance in barley powdery mildew as a model, bioassays and PCR diagnostics were used to test the effects of different anti-resistance strategies. Similar techniques were also used to monitor the current status of resistance to QoIs in field populations of S. *tritici* throughout the UK.

MATERIALS AND METHODS

During 2002-2003, the spring barley cultivar Golden Promise was grown in three replicated field plots (17 m x 24 m) in three different locations in the UK. This paper presents the results of location Findon Mains, near Inverness, Scotland. One plot remained untreated throughout the season and other plots were treated three or four times at 14-day intervals with fungicides. Fungicides were applied to test the three factors likely to influence the evolution of resistance and to be amenable to manipulation in an anti-resistance strategy, i.e. dose, number of sprays and alternation/mixing of fungicides with different modes of action (see Table 1). A key aspect of the experimental design is the use of fungicide doses that give similar levels of disease control to minimise the confounding effects of pathogen population size on selection.

Table 1. Overview of barley powdery mildew field trials

Experiment 1: Effects of dose rate and number of sprays

Treatment	Number of sprays	Dose per spray (litres ha^{-1} Amistar)	Anticipated level of disease control (%)
1	Nil	Nil	0
2	1	1.0	80
3	1	2.0	95
4	1	3.0	99
5	2	0.5	80
6	2	1.0	95
7	2	1.5	99
8	3	0.3	80
9	3	0.6	95
10	3	1.0	99

Experiment 2: Mixtures and alternations

Treatment	Sequence and treatment[1]			
11	A	B	A	B
12	B	A	B	A
13	A	A	A	A
14	B	B	B	B
15	A+B	A+B	A+B	A+B

[1]A = 0.5 litre ha^{-1} Amistar; B = 0.25 litre ha^{-1} Corbel

For each plot, 25 leaves with fresh pustules were collected from the middle leaf layers of the canopy just before each spray and 14 days after the final spray between GS31 and GS65. DNA was extracted from leaf samples and tested for the presence of G143A. Mildew was assessed visually on 25 shoots per plot at different growth stages. Mildew strains isolated from leaves were tested for sensitivity to QoIs in bioassays and their genotype determined by PCR.

To detect QoI sensitive (G143) and resistant (A143) alleles, a 5'-nuclease-based real-time PCR assay was developed using allele-specific minor groove binder (MGB)-conjugated TaqMan probes labelled with different reporter dyes (Figure 1). For each DNA sample, the ratio of the VIC and FAM signals, measured five cycles after detection, was used to calculate the A143 allele frequency. This was done by reference to a calibration curve generated by DNA standards containing different proportions of A143 and G143 alleles.

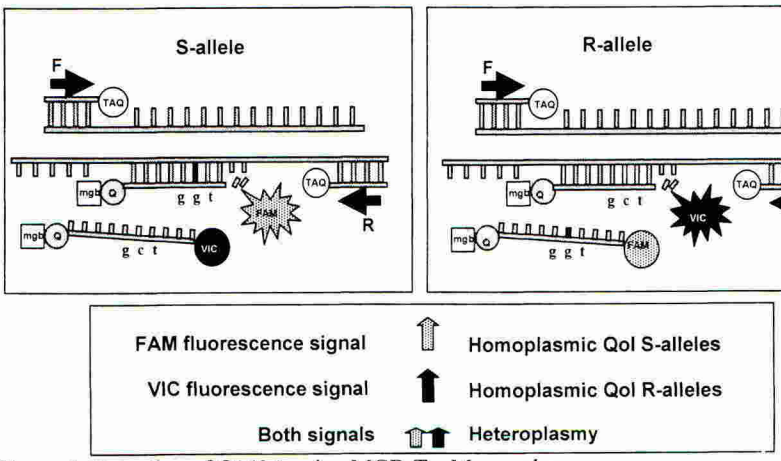

Figure 1. Detection of G143A using MGB-TaqMan probes.

Septoria tritici strains were isolated from samples consisting of 25 leaves showing symptoms (pycnidium-bearing lesions). Samples from commercial and trial crops were collected in different locations in the UK before GS31. Single-spore isolates were cultured and genotyped for the presence of G143A using real-time PCR. A number of isolates were also tested for sensitivity to QoIs by growing them in liquid medium in the absence and presence of fungicides. For a few isolates, the *in vivo* sensitivity to QoIs was also determined in the glasshouse by inoculation of untreated and fungicide-treated plants.

RESULTS

Barley powdery mildew

Heavy rain reduced mildew levels at Findon Mains and late infection meant that, for experiment 2 (see Table 1), only three out of the four anticipated sprays could be applied. The bioassays detected resistance levels between 0.5 and 13% before spraying, in agreement with the low resistance levels detected with real-time PCR using G143A as a marker (Figure 2). Calibration curve samples containing less than 5% R-alleles were not detected in PCR. Only a few pustules sampled after spraying were viable, making later comparisons using the bioassay impossible.

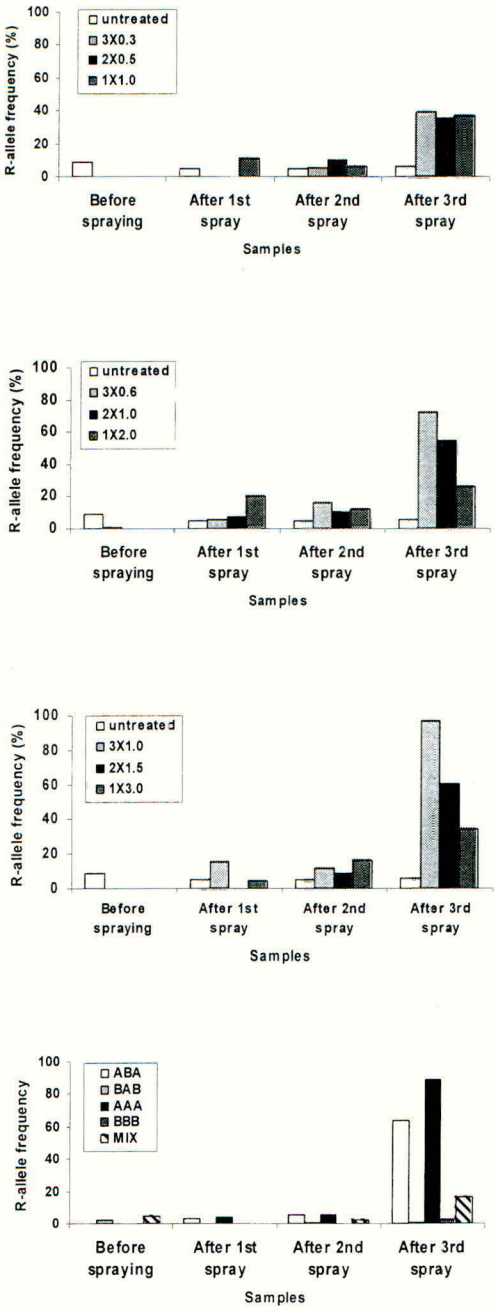

Figure 2. Effects of different fungicide applications on the development of QoI-resistance using G143A as a marker. See Table 1 for description of treatments. Leaf 3 was sampled before spraying at GS26-31 and after the first spray at GS41-43, leaf 2 after the second spray at GS70 and the flag after the third spray at GS90. Average values of three replicated plots are presented.

The R-allele frequency only increased under selection pressure from QoI fungicides. The selection was most pronounced after three sprays and increased with dose and number of sprays. With the lowest total fungicide input (1 litre ha^{-1} Amistar) no clear difference in selection for G143A was observed with spray frequency, but the single high-dose spray provided best disease control. Higher doses generally improved disease control (Figure 3), but because of low mildew infection levels, results were not always consistent and large variations between replicated plots were observed, especially after the third spray (data not shown). Experiment 2 showed fungicide mixtures can slow down the development of resistance. Because the fourth spray could not be applied, the effects of alternation could not be measured.

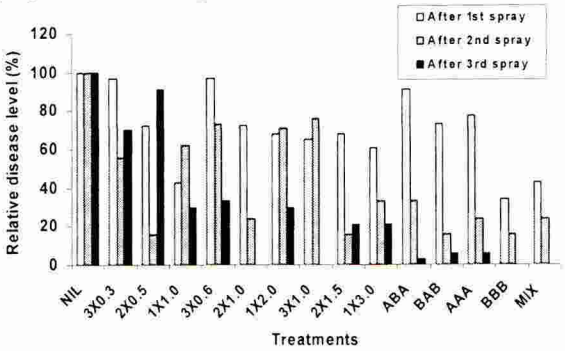

Figure 3.Efficacy of treatments to control barley powdery mildew. Disease levels of untreated plots (regarded as 100%) were, respectively, 9.3, 6.3 and 3.3% of infected leaf area after the first (L3 at GS41-43), second (L2 at GS70) and third spray (flag at GS85). Average values of three replicated plots are presented.

Septoria tritici

Sample test results (Table 2) revealed that the G143A mutation was common and widespread in populations of *Septoria tritici* throughout the UK during spring 2003.

Table 2. The occurrence of G143A in populations of *Septoria tritici* in spring 2003.

Sample	Number of isolates tested	Location	Cultivar	Number of isolates with G143A
1	38	Bedfordshire	Option	26 (68%)
2	35	Buckinghamshire	Consort	12 (34%)
3	36	Carlow, Ireland	Madrigal	20 (56%)
4	36	Dorset	Option	5 (14%)
5	94	Hertfordshire	Savannah	30 (32%)
6	52	North Somerset	Claire	15 (29%)
7	53	North Yorkshire	Consort	20 (38%)
8	16	North Yorkshire	Napier	0 (0%)
9	59	Warwickshire	Claire	19 (32%)
10	24	Wiltshire	?	14 (58%)

For 80 strains, isolated in Hertfordshire in 2002, ED_{50} values for azoxystrobin were determined *in vitro* (Figure 4). Isolates with G143A showed high resistance levels and were cross-resistant to kresoxim-methyl, trifloxystrobin and pyraclostrobin. *In vivo* studies showed that resistant isolates were not controlled, even at full rate, when azoxystrobin was applied 7 days after inoculation. For some isolates, increased disease levels were recorded when a quarter dose was used. However, when azoxystrobin was applied 7 days prior to inoculation, resistant isolates were partially controlled at full dose rate (Lovell *et al.*, unpublished).

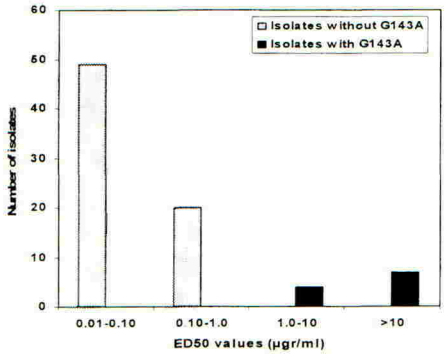

Figure 4. Azoxystrobin sensitivity testing of *Septoria tritici* isolates.

DISCUSSION

Provided a clear relationship exists between genotype and phenotype, real-time PCR diagnostics is a powerful tool that can be used to monitor the effects of anti-resistance strategies by directly monitoring the genotype. Although the results are preliminary and more trials are needed to validate them, it is clear that the effects of dose, spray frequency and alternation/mixing of fungicides with different modes of action on QoI resistance development in mildew populations can be measured. Similar studies are now in progress with *S. tritici* and other pathogens at risk in order to help prolong the practical use of QoI fungicides.

ACKNOWLEDGEMENTS

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The issues facing Industry in the management of resistance in Europe

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ABSTRACT

The issues facing Industry in the management of resistance in Europe continue to increase. Resistance risk analysis is now a component of the registration process within the EU and guidance on how to implement the requirements became available with the publication of EPPO guideline PP1/213 (1). This paper examines the guidelines and approaches by industry to assess the risk of practical resistance and the difficulties in monitoring for resistance. The use of modelling and its value in predicting resistance are discussed with comparisons made across the disciplines. A brief description of the testing methods available to screen new compounds for their potential vulnerability to the development of resistance is made. The role of the various Resistance Action Groups which act at global, European and country levels and their role in providing guidance on both testing and management strategies is described and a proposal is made for better communication across the disciplines.

INTRODUCTION

Resistance is not a new phenomenon but it is increasing, however. The problem was recognised as far back as 1910 and the first resistance to synthetic chemicals was noted in 1947 when DDT resistance was observed in houseflies (*Musca domestica*). Fungicide resistance was first noted in *Pyrenophora avenae*, which was observed to be resistant to organomercurials in 1964, and then in 1970 resistance to benzimidazoles was found in *Venturia inaequalis* and *Botrytis cinerea*. Organomercurials were used for 40 years before resistance appeared but resistance to the benzimidazoles appeared in *B. cinerea* after two years of use.

A classic graph by Georghiou showing the time line of species developing resistance to one insecticide, fungicide or herbicide from the 1930s through to 1985 showed that, by 1985, the number of species showing resistance to an insecticide was about 450, for plant pathogens it was between 100 and 150 species and for weeds <50. (Georghiou, 1986). Since that time, the number of cases of herbicide resistance has soared with, today, 276 resistant biotypes and 166 species (99 dicots and 67 monocots) recorded on the HRAC website (Heap, 2003). Insect pests began to develop resistance before disease pathogens and weeds but now weeds are catching up. One reason for this rise in resistance is that the vast majority of early pesticides were multi-site and development of resistance was slow. During the last 30 years, however, discovery goals were more likely to result in finding chemicals with single sites of action and high activity. As a result, for all disciplines, resistance to pesticides is growing and resulting in a significant economic impact. This paper looks at the current situation regarding the regulatory requirements in Europe and the various approaches from industry and the wider crop protection industry to evaluating and managing resistance.

EPPO GUIDELINE

The European Union Commission Directive 93/71/EEC amending Council Directive 91/414/EEC concerning the placing of plant protection products on the market requires that applicants evaluate the risk of resistance developing and propose management strategies to address such risks. An EPPO guideline was first published in 1999 (OEPP/EPPO, 1999) and a revised guideline in April 2003 (OEPP/EPPO, 2003). The specific scope of the guideline is to describe how risk of resistance to plant protection products can be assessed and, if appropriate, how systems for risk management can be proposed in the context of official registration of plant protection products.

Practical Resistance

An important aspect of the guideline is that it focuses on "practical resistance". Resistance is defined as the "naturally occurring inheritable adjustment in the ability of individuals in a population to survive a plant protection product treatment that would normally give effective control". Practical resistance is the term used for loss of field control due to a shift in sensitivity.

The guideline divides risk assessment into two parts: resistance risk assessment, in which the probability of resistance development and its likely impact are evaluated and, if necessary, resistance risk management, in which strategies to avoid or delay the development of resistance are proposed.

The risk analysis considers the inherent risk, that is the risk to the target organism and the mode of action of the chemical and the agronomic risk for the area in which the product will be used. There are many ways of conducting these and tools have been developed within companies to assess resistance. For example, for insecticides Dow AgroSciences developed the Practical Resistance Assessment Tool in Table 1.

The biological factors change for the other disciplines but the principles of the tool may be used for any discipline. For example, for weeds the important "Biological Factors" to consider are high inherent genetic variability, high fecundity, outcrossing versus selfing and the number of generations per year. For diseases, the biological factors to consider are incubation time, number of spores, spore mobility, ability to overwinter, fitness and sexual recombination.

This type of tool can be used across all disciplines after defining the parameters involved and can be used at a very local level to assess the risk of resistance occurring.

Table 1. Practical Resistance Assessment Tool

Attributes	Risk score	Low Risk: Score = 1	Moderate Risk: Score = 3	High Risk: Score = 5
Biological Factors		**NOTE: Intermediate scores are permissible; score 5 if unknown**		
# generations/year	3	< 2	2 to 5	> 5
Migration/population mixing	3	High (black cutworm)	Moderate (corn rootworm)	Low (houseflies in a chicken house)
Host range	3	Broad (cotton bollworm, beet armyworm)	Moderate (diamondback moth, tobacco budworm)	Narrow (rootworm, boll weevil, Colorado potato beetle)
Reproduction capacity	3	10	50	100
Reproduction style	3		Sexual	Parthenogensis
Operational Factors				
Life stages treated	1	One stage		Multiple stages
Residual activity	3	Low (methyl parathion, chlorpyrifos methyl)	Moderate (pyrethroids)	Long (chlorinated hydrocarbons, soil insecticides, transgenics)
Resistance history	3	None	Resistant to < 2 classes of insecticides	Resistant to >2 classes of insecticides
Systemicity	3	Not systemic	Moderately mobile	Highly mobile
% refugia	3	High	Moderate	Low
Alternative control options	3	Many, effective as rotation partners		None or Few
Expected market share	3	< 25%	25-50%	> 50%
Crop cycles	1	Seasonal		Overlapping/ continuous
Market place receptive to IRM practices	3	Yes, good infrastructure		No, poor infrastructure
Cross-resistance with other control options	3	None; novel mode of action		Extensive; widely used mode of action
# insecticide applications	3	1 per year	3 per year	>5 per year
Dose (% killed per application)	3	<30%	30-90% or >99%	90-99%

Total

Total score	Risk
<40	Low
40-60	Moderate
>60	High

Management strategies

If the risk is acceptable, then no further analysis or provision of data are needed. If there is risk, then modifiers must be proposed to reduce the risk. These include the following:

- Frequency of application – limiting the number of applications against a pest in a season will reduce the selection pressure
- Timing of application – applications should be made at times of the year, crop growth stage or pest stage critical to optimum control
- Mixtures - the active substance may be applied in mixture with one or more substances with similar or complimentary activity but with different modes of action.
- Alternation - alternating pesticides from different resistance groups
- Cultural control

Monitoring

As part of the monitoring strategy for products whose unmodified risk of resistance has been evaluated as unacceptable, a programme must be instigated to monitor the continued efficacy of the products on the target pest. This programme comprises observations of field performance from efficacy trials and commercial use. Random monitoring is generally not feasible to detect major gene mutants in samples from field populations until frequencies of 1% are reached. At 1% frequency, >300 samples are needed to have a 95% chance of detecting resistance. In this area it is worth noting that it is easier to work on a large number of fungal samples than of insects or plants.

At this stage there continues to be considerable confusion between authorities regarding the requirements especially in the area of monitoring.

MODELLING

To date, modelling has been more successful in comparing alternative resistance management strategies than in the prediction of resistance. Various types of models have played an essential role in building a framework for resistance management. Cavan *et al.* (1999) compared different cultivation strategies and cultivation techniques for their effects on herbicide resistance. Significant differences were found in the speed of resistance development when the use of ploughing was compared with tine cultivation and when alternation of herbicides with different modes of action was tested. Studying the models across the disciplines, there are a number of common themes. Alternating or mixtures of chemistries with different modes of action and use of integrated crop management are common methods to slow the build up of resistance. The details vary within the disciplines, with cultivation techniques having a major impact on weed resistance and the use of beneficial insects in insect resistance.

Predicting resistance using models has had limited success across the disciplines, especially in weed science. Some of the predictions resulting from the use of herbicide resistance development models have proved unrealistic (Friesen *et al.*, 2000). The reason for this is a lack of information on plant characteristics, such as initial frequencies of resistance, rates of random mutation, relative fitness of resistant plants and the importance of gene flow, which are largely unknown. To be really successful, we need more information than is currently available. The

use of models for the prediction of insect resistance looks more hopeful. In recent years, agriculture has seen the commercialisation and widespread adoption of transgenic crops based on the insecticidal toxins from the bacteria *Bacillus thuringiensis* (Bt). These Bt crops can be valuable pest management tools and preserving their efficacy has become a high priority for entomologists. As a result, significant resources are aimed at understanding pest biology, the roles of agronomic operations, pest population dynamics and genetics in resistance evolution and our ability to product accurate prediction models will improve (Storer *et al.*, 1996).

For new pesticides, advances in molecular genetics and biochemistry will help to determine the mode of action of new compounds and to select for resistant biotypes. Also, researchers can look at crop selectivity to determine possible methods of detoxification. This information can be used to predict what mechanisms of resistance may develop but can not predict how quickly practical resistance will occur in the field.

MANAGING RESISTANCE ON FARMS

The awareness of the possibility of resistance developing is not enough to alter the short-term decisions that farmers make on a yearly basis. The difficulty in screening for resistance on a large scale means that farmers become aware after it occurs. Once resistance has occurred, then farmers are prepared to adopt management strategies. These strategies often cause an increase in cost in the short term. Continuous autumn cropping and using a range of selective herbicides, annual ploughing, and sowing a significant proportion of winter wheat in early October will increase costs in the short term. However, if it delays the build up of resistance in *Alopecurus myosuroides* (blackgrass), it will offer a sustainable long term solution (Orson & Harris, 1997).

Today, labels advise not to use single modes of action continuously. North America and Australia have adopted a mode of action labelling to aid farmers in decision making. This can be beneficial for managing target site resistance but has little advantage where detoxification processes exist (with some exceptions where a specific mode of action is not susceptible to that particular detoxification mechanism). More education is needed at farmer level and resistance management strategies developed at a local level to take account of the economic, environmental and agronomic needs. The most successful programmes have been those that have involved scientists and producers working together such as Arizona's extension-based resistance management programme. A coalition of farmers, a commodity organisation, members of the crop protection industry, university research and extension personnel researched and communicated a successful plan to combat white fly, *Bemisia argentifolia*. This programme has been successful for six years and continues. It is clear that communication between industry, researchers and farmers is essential and the Resistance Action Groups are fundamental to that communication.

RESISTANCE ACTION GROUPS

In the early 1980s, the threat of resistance was recognised but there was no collective forum for addressing the problems within the crop protection industry. Today the situation is very different with industry, farmers and academics working together. This is due in the main to the formation of action committees which bring together people with the common objective of

resistance management. Three Specialist Technical Groups were formed as committees of Crop Life International (previously GIFAP). These Action Committees were established in the 1980s and dedicated to prolonging the effectiveness of pesticides by identifying, devising and implementing the management of strategies. These were the Insecticide Resistance Action Committee (IRAC, 2003) the Fungicide Resistance Action Committee (FRAC, 2003) and the Herbicide Resistance Action Committee (HRAC, 2003). All have individual web sites and are recognised as advisory bodies by organisations such as the European Commission, the Food and Agriculture organisation (FAO) and the World Health Organisation (WHO) of the United Nations.

There are some fundamental aims shared across these committees:

1. To promote a better understanding of the causes and results of resistance.
2. To foster a responsible attitude to pesticide use.
3. To support work to identify the technical basis of resistance.
4. To identify the magnitude of resistance through surveys.
5. To communicate resistance management strategies.
6. To facilitate communication between industry and academics by the establishment of workshops.

IRAC

IRAC was formed in 1984 to provide a co-ordinated crop protection industry response to the development of resistance in insect and mite pests. During the last decade, IRAC has formed several international working groups to provide practical solutions to mite and insect resistance problems within major crops and pesticide groups. IRAC has achieved success in a number of areas:

1. Surveys. By surveying member-companies about documented cases of resistance, IRAC has been able to identify and classify resistance problems. Identifying and concentrating on problem areas allows IRAC to work with individual farmers to manage resistance problems. Comprehensive surveys - including more than 50 countries, 70 species of insects and mites, and more than 30 crops - are conducted periodically to assist the industry.
2. Monitoring methods. IRAC has developed and published several methods for monitoring resistance under a variety of field settings. Many methods have become the basis of wide-reaching monitoring programmes around the world. IRAC Method No. VII, for leaf-eating *Lepidoptera* and *Coleoptera*, for example, has been validated in the laboratory and in the field.
3. Resistance mechanisms and management. IRAC was instrumental in the discovery that a change in the mode of action is not always necessary to reduce resistance. IRAC discovered in Italy, for example, that apple leaf miner (*Leucoptera scitella*) resistance to diflubenzuron may not always be conferred to a whole class of insecticides, even if they all have the same mode of action.
4. Member companies agreed to limit applications of mitochondrial electron transport inhibitors (METI) to one application per year and published the strategy at the Brighton Crop Protection Conference in 1994.

FRAC

FRAC was formed in 1981 and is comprised of a Central Steering Committee and six Working Groups. Each Working Group consists of specialist technical representatives from two or more manufacturing companies with fungicides of a similar mode of action or cross resistance potential. Companies with a compound in the market or in late development are encouraged to participate. The working groups are

- Anilinopyrimidines
- Benzimidazoles
- Dicarboximides
- Phenylamides
- SBI Fungicides
- QoI Fungicides

FRAC working groups have made achievements in the following areas:

1. Recommending procedures for use in fungicide resistance studies.
2. Providing guidelines and advice on the use of fungicides to reduce the risk of resistance. developing, and to manage it should it occur.
3. Identifying existing and potential resistance problems.
4. Collating information and distributing it to those involved in fungicide research, distribution, registration and use.
5. Stimulating open liason and collaboration with universities, government agencies, advisors, extension workers, distributors and farmers.

HRAC

HRAC was formed in 1989 with three working groups

- Acetolactase synthase inhibitor
- Triazine
- Grass herbicide

Since that time the committee has amalgamated to one group but with very strong regional working groups. Thus the European Herbicide Resistance Working Group has supported and participated in research, conferences and seminars, which serve to increase the understanding of herbicide resistance. The North American group is extremely active and other working groups exist in the Pacific and Asia.

Accomplishments:

1. Financial support to research on a range of activities including the survey and gene flow in *Kochia scoparia* and *Salsola iberica* in the USA, management of urea-resistant *Phalaris* in India and, more recently, the technical and financial impact of herbicide-resistant *Alopecurus myosuroides* on individual farm businesses in the UK.
2. Open meetings with academic and governmental research.
3. Publication of resistance monographs to review specific areas of resistance.
4. Collaborative testing programmes - such as the *Alopecurus myosuroides* testing kit ring-tested via the group.
5. Monitoring.

To date, the disciplines have worked in isolation but it is clear that the three committees would benefit from closer collaboration to produce a joint strategy on common issues. The difficulties facing each discipline and the potential solutions are the same and at a time when resources are reducing we must cooperate to ensure maximum influence.

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The role and impact of the regulator in resistance management

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ABSTRACT

Resistance risk analysis and the implementation of resistance management strategies are an integral part of the pesticide registration process in Europe. They also play a vital role in ensuring sustainable agricultural production. To be effective, resistance management strategies must be consistent across products and must be communicated to and implemented by end users. Using the examples of the ALS herbicides and QoI fungicides this paper explores the difficulties in ensuring that resistance management strategies are effective and how the regulatory authorities in the UK play an active role in their development and implementation.

INTRODUCTION

Resistance to pesticides is a widespread problem that limits the effectiveness of many existing products and reduces the options for controlling a range of target organisms. It is financially costly to growers and the agrochemical industry, and these costs are likely to be passed on to consumers. There may also be environmental costs if growers are forced to use additional pesticide or substitute products with less environmentally friendly ones. For growers of minor crops, where the range of approved chemicals is often limited, the loss of effective products can be particularly serious. Resistance management is therefore an integral component of sustainable crop production.

The European pesticide registration process, driven by directive 91/414, recognises the importance of resistance and requires applicants to address the risk of resistance development as part of dossiers submitted for EU registration (Anon., 1993). However, the withdrawal of active substances and products as a result of the re-registration requirements within Europe also poses a threat to resistance management as the diversity of active substances is reduced, and makes it more important to protect those that remain.

As with other areas of efficacy evaluation, guidance on the conduct of resistance risk analysis and development of resistance management strategies is given in an EPPO guideline (Anon., 2003). Although this includes some well known examples of chemical groups or target organisms that present a high risk of developing resistance, it essentially only provides an outline of the processes involved and each case will inevitably require specialist consideration. Assessing resistance sections of dossiers submitted to support product registration generates a unique challenge for regulatory scientists. The available knowledge is often limited and the best approach to resistance management in a given situation will be conjectural. Different applicants may therefore legitimately propose very different resistance management strategies for similar situations.

SUPPORTING EFFECTIVE RESISTANCE MANAGEMENT STRATEGIES

The regulatory system must be uniformly applied, unbiased and evidence-based. Inconsistencies in approach could result both in unfair restrictions on some products and may also confuse the user, with the result that resistance management messages are not effectively communicated. There is also a potential conflict of interest between the desire for profit and the goal of preventing resistance to support the long term sustainability of crop production. As Russell (2001) pointed out, identifying effective strategies is, however, problematic. If a strategy is implemented and resistance does not develop we are still left with a dilemma. Has resistance failed to develop because the resistance management strategy *per se* is effective or is it because of some other factor in the original hypothesis relating to the target organism or chemical was incorrect? The regulator has to tread a narrow line between acting reasonably in restricting the use of products and ensuring both that resistance management strategies are consistent and effective and that suitable crop protection products remain available. Defining that line can be extremely difficult, particularly where new chemistry is concerned and there is limited information on resistance development. Furthermore, if a strategy appears to be failing, there must be a mechanism to reconsider the management strategy and communicate changes to users. The 10-year rolling review process for products approved under European legislation is clearly not appropriate when dealing with rapidly changing resistance problems.

For established chemistry the implementation of effective resistance management strategies is helped by the publication of guidelines by the international resistance action committees (FRAC, HRAC, IRAC and RRAC). In the past the UK Pesticides Safety Directorate (PSD) has generally accepted strategies proposed as part of the registration package provided they were in line with those produced by the RACs. However, the UK Advisory Committee on Pesticides (ACP) has expressed concern that the RACs, being composed solely of agrochemical industry representatives, may not be sufficiently independent. RAC guidelines also take a global view and may not always be applicable to local conditions. Within the UK, national Resistance Action Groups (FRAG-UK, IRAG, WRAG and RRAG), which have a wider membership, provide a more independent and local view and are more appropriate bodies for generating guidance. However, as they are voluntary bodies with no financial support their resources are limited.

Research coordination

R&D to support the understanding of pesticide resistance is a key business priority for PSD. It improves our ability to undertake resistance risk assessments and to evaluate proposed resistance management strategies. Without the adoption of effective resistance management strategies, production of some crops or on some sites may become unsustainable. Work funded in recent years has included projects, some conducted in partnership with industry, looking at the effectiveness of both fungicide and herbicide resistance management strategies.

PSD also influences the research conducted by industry. As part of the resistance management strategy put forward in registration packages, companies are increasingly making commitments to undertake ongoing resistance monitoring programmes, which must be agreed by the regulator. Additionally, if changes in sensitivity do occur, PSD will encourage the collaboration of all relevant parties to develop suitable research and monitoring programmes to support the ongoing resistance management needs. Of course, a pragmatic approach must be taken and the cost of research and monitoring by approval holders needs to be offset against the profits from a given use of a product. While monitoring programmes are financially justifiable

for broad-acre crops, the returns on sales for use on minor crops are unlikely to cover the cost of extensive monitoring. Equally, the limited range of products available for minor crops means that resistance cannot be ignored in this area and other sources of funding must therefore be identified.

Getting the message across

European pesticide labels must be approved by the regulatory authorities. Thus regulators can ensure that product labels include statements relating to resistance management and that those statements are consistent between similar products. PSD has introduced a number of standard insecticide resistance statements over the years that have been agreed with industry and the ACP. Specific wording has also been agreed for phenylamide fungicides, annual grass-weed herbicides and, most recently, QoI fungicides.

With the exception of restrictions on the number of applications, however, resistance management information is generally considered to be advisory information and falls within the 'directions for use' section of labels. The full implementation of a resistance management strategy is therefore at the discretion of the user. Growers may therefore ignore resistance management advice, as was seen with the QoI fungicides (see below), particularly if they perceive it to adversely affect the economics of immediate crop production. Grower education may help and PSD has in the past supported the production of leaflets for distribution to growers advising on resistance management. There are options for further action, however, as seen in recent statutory restrictions introduced for QoIs in the UK and Ireland. Similar action has also been taken in the past with some insecticides.

THE QoI STORY

Azoxystrobin was first registered in the UK in 1997. Several other compounds with similar modes of action, now classed as the QoIs, have since been registered. In 2000 azoxystrobin was the most extensively used fungicide in UK barley production and ranked second in wheat (Garthwaite & Thomas, 2000). Azoxystrobin was also the first compound to be placed on Annex 1 of directive 91/414 and thus receive European listing. As such a resistance risk assessment was considered as part of the registration package. In view of subsequent resistance development (Russell, 1999), it is arguable that a stronger resistance management strategy should have been imposed from the start. At the time, however, there was no guidance on the issue available from EPPO and both the regulatory authorities and applicants were still developing their understanding of the European registration process.

Resistance to wheat mildew, and some diseases in other crops, appeared very quickly and prompted a flurry of research, from both the original approval holder and other companies with similar compounds in development. The industry, working together through a specialist forum of FRAC, introduced global resistance management guidelines. These were adopted for all the QoIs in the UK, although there was some variation in the specific resistance management recommendations on labels depending on the guidelines adopted by FRAC at the time the product was approved.

In late 2002 resistance surveys started to find isolates of *Septoria tritici*, the pathogen causing the most widespread disease of wheat in the UK, which carried a gene responsible for disruptive resistance to the QoIs. Additionally, analysis of pesticide usage data showed that

despite advice on the labels of most QoI fungicides to apply no than two applications to a crop and to use solo products in mixture, around 20% of UK cereal crops received three or more foliar sprays of a QoI containing product, and at least 10% of growers applied solo QoI products alone (M Thomas pers. comm.).

Due to the significance of septoria disease and the importance of this group of fungicides, action was clearly needed. Working with industry, independent researchers and agronomists, and with the help of FRAG-UK, a package of measures to reinforce the resistance management strategy was agreed and implemented in April this year (2003). The statutory conditions of use for all QoI products on cereals were changed to limit the number of applications to two and standard resistance management phrases were introduced onto labels. The story is, of course, ongoing. By the time this is published further data should be available from the 2003 survey and ongoing research projects. It is to be hoped that the situation will have stabilised but, if not, further steps may have to be taken. What these will be will depend on further findings, but actions that were discussed previously have included restricting QoIs to only a single application and withdrawing approvals for all solo products.

THE ALS INHIBITOR STORY

Weed resistance presents somewhat different problems, with less potential for rapid changes in resistance across a wide area but an increasing reliance on a single group of herbicides likely to result in widespread problems over time. The development of acetolactate synthase (ALS) inhibitors and, specifically, sulfonyl ureas (SUs) provided farmers with a new class of highly active and effective herbicides. The first ALS inhibitors were approved in the UK in the early 1980s. Since then, a further 13 SUs have been approved in the UK for use on a wide range of crops and weeds and six other non SU ALS inhibitors, four of which are cereal herbicides.

World-wide there are very many weed species resistant to ALS inhibitors and, while most are broad-leaved weeds, resistance does occur in some grass-weeds. In the UK, enhanced metabolism resistance in black-grass is widespread and reduces the effectiveness of many cereal herbicides, including the ALS inhibitors, although cross-resistance patterns are by no means straightforward. In broad-leaved weeds there have been few cases of herbicide resistance in the UK but resistance to the SUs has been identified in both common chickweed and poppy. In 2000/1 there were six cases of resistance confirmed in chickweed and three cases in poppy (Moss & Orson, 2003).

In the past there was always the hope that a new herbicide would be developed to combat resistance problems. The reality is that most of the new herbicides seeking registration in the UK for the foreseeable future will be ALS inhibitors and, for these, the risk of rapid resistance development will be high. It is also unclear to what extent the SU herbicides approved for control of broad-leaved weeds may be exerting an additional selection pressure on grass weeds. Together with this, in the EU review of pesticides several active substances have not been supported and will therefore be withdrawn from use. These include terbutryn, flamprop-M-isopropyl, difenzoquat and sethoxydim, from three separate herbicide mode of action groups. The future of some of the other non-ALS inhibitor active substances is also uncertain. Economic considerations could further encourage the move towards simplified crop management in terms of rotations, cultivations, and crop monitoring. All of these factors could put additional pressure on the remaining active substances and potentially increase selection for resistance not only in cereals but across a range of UK crops. To date there have been no UK

cases of target-site resistance to the ALS inhibitors in black-grass. However, there is a concern that, with increasingly limited opportunities to use products with different modes of action and with use of ALS inhibitors on different crops in a rotation, this could occur. Alternatively, the increasing reliance on herbicides as yet little or unaffected by resistance could lead to resistance development to these modes of action.

Clearly, regulatory authorities have a key role to play along with industry in the prevention and management of resistance. For many years a standard warning phrase has been placed on the labels of all products with grass weed control recommendations and this initiative could be expanded to include broad-leaved weeds. There has been a great deal of activity in trying to get key messages across to growers. This has included the publication of a revised set of WRAG guidelines on managing and preventing herbicide resistance in both grass and broad-leaved weeds (Moss & Orson, 2003).

FUTURE REQUIREMENTS AND ACTION

PSD has previously outlined how it interprets EC Directive 91/414/EEC (e.g. Slawson & Furk, 1995 and Godson *et al.*, 1996). The publication of the EPPO guidance on resistance risk analysis has provided an additional steer to both industry and regulators on how this might be achieved in practice. The two-stage process consists of resistance risk assessment, where the probability of resistance development is evaluated, and resistance risk management where, if necessary, strategies for avoiding or delaying the development of resistance are considered and implemented. This process, whilst logical and intuitive, presents several challenges for both regulators and the industry. For example, one component of risk assessment is baseline sensitivity testing. For weeds, the guidelines outlined by Moss (2001) could provide the methodological framework to approaching this. However, critical to monitoring any shift in the sensitivity of populations is the maintenance of susceptible populations as standards. While this has become commonplace for black-grass in the UK with the use of the Rothamsted strain, other weed species present more of a challenge, particularly those species where there is inherently greater variability in sensitivity.

We may reasonably expect approval holders to support resistance management strategies, including, if necessary, the maintenance of susceptible standards for major 'on label' uses through their own ongoing research. However, the responsibility for minor uses, particularly off label ones, is less obvious but no less important and we must ensure that the area is not neglected.

The regulatory authorities also need to be responsive and to have the necessary procedures in place to enable resistance management strategies to be modified when required. The recent changes to the approvals for QoI fungicides show that this is not only possible but can be done relatively quickly. However, this was largely a fire-fighting action and may still turn out to be too little too late. If we are to ensure the continued availability of effective products for all sectors of crop production we need a better understanding of resistance in general so that we can focus our efforts and better identify both what action to take and when best to take it.

An important aspect of any strategy is to monitor its success. Approval holders must accept responsibility to monitor and review resistance management strategies after product registration. Likewise the regulator must keep approvals under review and it is thus important that survey results are made available to them. Better product stewardship, including more

regular and pro-active monitoring to provide feedback as soon as possible on resistance development and the impact of resistance management strategies in the field are likely to be required. The development of suitable diagnostic tools and appropriate monitoring plans are an integral part of this and, in some cases, could become a requirement for registration. PSD would also encourage wider dissemination of baseline and survey information to allow the greater participation by independent researchers and a broader debate of the issues.

We must also avoid becoming too focused on the chemistry. Resistance management has traditionally concentrated on pesticide usage and not fully explored the range of cultural practices that may be important in managing or preventing resistance in an integrated system. Current guidelines from both WRAG and FRAG-UK promote good practice but more sophisticated strategies that effectively incorporate cultural control methods are required to manage resistance in the long term, for example with weeds, where farms may have several resistant weeds occurring in mixed populations. Greater emphasis must be placed on the promotion of integrated pest management programmes that incorporate cultural approaches alongside chemical ones.

PSD does not wish to place unnecessary restrictions on the use of products but resistance management strategies must be effective and we must be prepared to take action where required. Finding the right balance of approaches can only be achieved through the industry and the regulator working together. The RAGs provide an ideal forum for this, but closer links between the individual groups may be required, and the issue needs to be given greater prominence in their agenda. We need to be able to prioritise areas for consideration, identify where action is required, and be prepared to support it and ensure that it is implemented.

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