Session 6B

Resistance to Crop Protection Agents

Monitoring, Mechanisms and Management 1

Chairman & Session Organiser:	Dr Ralf Nauen *Bayer CropScience, Monheim, Germany*
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Resistance to neonicotinoids in Hemipteran pests

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Introduction

The propensity of Hemipteran pests to develop insecticide resistance is clear from the wealth of published works that document examples for almost all commercially available compounds. A number of species are primary targets of neonicotinoid insecticides that in some cases now constitute the predominant component of control regimes. It is therefore not surprising that resistance to this globally important chemical class is compromising efficacy in some cases. However, the number of species with economically significant resistance is still low given the length of time that neonicotinoids have been in use. To date, approximately 15 years since the commercial release of imidacloprid, only four species of crop pest represent significant resistance problems. One of these is the Colorado potato beetle, *Leptinotarsa decemlineata* (Say), a Coleopteran with a long history of resistance development. The other three are Hemipterans; the tobacco whitefly, *Bemisia tabaci* (Gennadius), the glasshouse whitefly, *Trialeurodes vaporariorum* (Westwood) and the brown planthopper, *Nilaparvata lugens* (Stål).

Bemisia tabaci

This cosmopolitan pest is known to exist as a species complex, comprised of a number of morphologically identical yet biologically distinct haplotypes known as biotypes. There are over 20 documented biotypes, some of whose inter-relationships have been investigated with molecular tools (De Barro *et al.*, 2000). In some areas two or more may co-exist and their population dynamics are complicated by a range of breeding incompatibilities. The two most damaging in the majority of agricultural areas, biotypes B and Q, are not capable of interbreeding and this precludes the direct exchange of genetically inherited traits including insecticide resistance (Ma *et al.*, 2006 and unpublished data). As a consequence, these biotypes are entrenched in an evolutionary race to develop novel resistance mechanisms, with the winner benefiting from intense insecticide-driven selection that is potentially capable of supplanting still-susceptible biotypes. Having developed neonicotinoid resistance as early as 1994, biotype Q began to dominate the neonicotinoid-susceptible biotype B in some areas of coexistence. Since that time its geographical range has continuously expanded from confinement to the Mediterranean basin, to presently include East Asia, Australasia, North and South America, as a consequence of inadvertent transport on plant material. However, in some areas biotype B has now independently developed comparable levels of neonicotinoid resistance, possibly restoring its competitive ability during times of insecticidal exposure. Additionally, biotype A, which was displaced from North America by biotype B in the late 1980s and early 1990s, has been found in areas of Central America possessing strong levels of neonicotinoid resistance. This A-biotype mechanism is also likely to have developed independently due to reproductive incompatibilities with other biotypes.

Trialeurodes vaporariorum

After *B. tabaci*, the second most economically damaging whitefly is *T. vaporariorum*. Recent work has shown that this polyphagous pest of protected horticulture has been studied less than *B. tabaci* but there are increasing reports of control difficulties supported

by confirmation of resistance in some cases (Gorman *et al.*, 2007). There are no documented biotypes of *T. vaporariorum* and so it is unclear whether this may influence the rate of resistance selection as with *B. tabaci*. However, with few or no fully-effective alternatives available for use within specific crops/regions, continued intensive use of neonicotinoids is likely to exacerbate the situation. As a key pest of ornamental crops, long distance dispersal of resistance genes is facilitated by plant trade, highlighting the need for careful management in regions that serve the export industries. Indeed it is unclear whether the recently confirmed cases of resistance in the UK and The Netherlands developed locally or were imported.

Nilaparvata lugens

N. lugens is a major pest of rice crops and as such has become a target species of neonicotinoid insecticides. Increasing concerns by growers that efficacy was declining have been investigated in a recent monitoring programme across Asian rice growing regions. Results from the six countries involved (India, Thailand, Malaysia, Indonesia, Vietnam and China) revealed the presence of neonicotinoid resistance and an increase in levels of resistance over time. For some populations, doses of imidacloprid 125 times higher than the LC_{95} of susceptible insects had no appreciable effect on mortality. The rapid spread of neonicotinoid resistance in *N. lugens* across Asia appears to have been fuelled by the migratory nature of this species and this will require careful consideration when implementing resistance management tactics.

Conclusions

Compared to other major insecticide classes, resistance to neonicotinoids has been relatively slow to develop (Nauen and Denholm, 2005). However, research on the three Hemipteran species discussed above has demonstrated not only the potential for strong resistance to develop, but also the speed at which resistance can spread through natural or human-mediated migration. With such a high percentage of global produce dependent upon the diversity of neonicotinoids now available, careful vigilance and dynamic resistance management is a pre-requisite for their sustainability.

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Herbicide-resistant weeds: a threat to dryland farming

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The evolution of herbicide-resistant weeds in dryland farming is continuously increasing and endangering the profitability of non-irrigated arable crops in countries with a Mediterranean climate. The region is characterized by a long, dry summer followed by a short rainfall season (November-March) with low precipitation (150-300 mm per year), and frequent drought years.

Chemical weed control in non-irrigated arable crops is crucial due to the strong competition exerted by a wide range of broadleaved and grass weeds. Limited alternatives for crop rotation, frequent use of herbicides with the same mode of action, combined with reduced tillage resulted in the evolution of large numbers of herbicide-resistant weed populations which are no longer controlled by the available selective herbicides.

Resistance to ALS-inhibiting herbicides was identified in *Chrysanthemum coronarium*, *Conyza canadensis* and *C. bonariensis*, *Capsella bursa-pastoris*, *Diplotaxis erucoides* and *Erucaria hispanica* in wheat and hay crops. A point mutation in the ALS gene was identified in *C. coronarium* (*Pro197* to either *Thr* or *Ser*) which endows resistance to all ALS inhibitors. A *C. canadensis* population exhibiting a similar pattern of resistance to all ALS inhibitors confers a substitution of *Trp547* to *Leu*. The point mutation found in *C. bursa pastoris* (*Ala122* to *Gln*), however, conferred resistance only to imidazolinone (IMI) herbicides.

Resistance to ACCase inhibiting herbicides due to an altered target site was confirmed in the following grass weeds: *Lolium rigidum*, *Phalaris minor*, *P. paradoxa* and *Avena sterilis*. Several *L. rigidum* and *P. paradoxa* populations were collected from heavily infested fields and their resistance to ACCase inhibiting herbicides confirmed on the whole plant basis. In these populations, target site mutations endowing resistance in the carboxyl transferase (CT) domain of the chloroplastic isoform of the ACCase were identified and characterized.

The mutations responsible for resistance were divided into three different resistance patterns. In the first group, *Ile1781* is substituted by *Leu* and plants are resistant to aryloxyphenoxypropanoates (APP) and to most cyclohexanedione (CHD) herbicides. Populations with a substitution of *Ile2041* to *Asn* or *Val* are resistant to APP but not to CHD herbicides and populations where the *Asp2078* has been substituted by *Gln* are highly resistant to both APP and CHD herbicides. Our study clearly indicates that grass weed populations exposed to a high selection pressure by ACCase inhibitors may result in various alterations of the target enzyme endowing different resistance responses to these herbicides. The use of alternative chemicals with a different mode of action for grass weed control in winter cereals such as certain sulfonylurea herbicides is quite limited in the semi-arid zone due to the risk of carry-over to the following crops.

In addition, the recent evolution and spread of glyphosate-resistant *C. bonariensis* and *C. canadensis* populations is a most dangerous phenomenon, which jeopardizes the adoption of minimum tillage practices and may also pose a threat to irrigated crops due to the easy seed dissemination by wind. The mechanism(s) possibly involved in the resistance will be discussed.

In spite of the increased awareness among farmers of the problem, management of herbicide-resistant weeds in arable crops, particularly in arid and semi-arid areas, where crop and herbicides rotations are limited, has become almost 'mission impossible'. We should consider adopting alternative practices such as residual herbicides in rotational application schemes, more frequent and better timed soil tillage and more competitive crop plants.

Resistance to acetolactate synthase (ALS) inhibiting herbicides in UK populations of the grass-weed *Alopecurus myosuroides* (black-grass)

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Introduction

The objective of this project was to characterize resistance to sulfonylurea herbicides in UK populations of the grass-weed *Alopecurus myosuroides* Huds. Investigations included field studies, glasshouse bioassays, and biochemical and molecular analyses. Responses to the selective sulfonylurea herbicides mesosulfuron-methyl + iodosulfuron-methyl sodium mixture and flupyrsulfuron-methyl were investigated; extensive use was made of the non-selective sulfonylurea herbicide sulfometuron-methyl at a rate of 100g a.i. ha^{-1} as a screening test for possible ALS target site resistance in *A. myosuroides*.

Materials and methods

Four biotypes of *A. myosuroides* were used for the initial whole plant and enzyme work: (a) Roth03, a susceptible standard, (b) Pel96, an enhanced metabolism standard population from Peldon in Essex, which shows cross resistance to a range of different herbicides, (c) Pel02, a population from Peldon collected in 1992, which shows around 15% highly resistant plants following treatment with 100g a.i. ha^{-1} sulfometuron, and (d) PelRES, a sulfometuron selected line from Peldon containing around 40% highly resistant plants in sulfometuron screening tests.

The four standard populations were subjected to whole-plant dose response tests and ALS enzyme assays with the herbicides flupyrsulfuron-methyl and sulfometuron (Marshall, 2007). A screening test was developed from dose response data and used 100g a.i. ha^{-1} of sulfometuron to detect highly resistant plants likely to possess ALS target site changes. Screening with both sulfometuron and mesosulfuron+iodosulfuron was performed for nine populations collected from field sites around the UK where mesosulfuron+iodosulfuron failed to control *A. myosuroides* in the field. These were: Pel02, Cock06 (Essex); Chal05, Thame05 (Oxfordshire); East06 (Lincolnshire); Key06, R30-06 (Cambridgeshire); Maid05 (Berkshire); Wilts04 (Wiltshire). Plants from each population were grown to the three leaf stage and leaf samples taken from each individual and stored at -80°C.

All plants were then sprayed with sulfometuron at 100g a.i. ha^{-1} and assessed for damage after four weeks. Genomic DNA from leaf material of highly resistant, and completely susceptible, plants was extracted using a Qiagen kit and primers were designed to span the five conserved domains of the ALS gene in two parts; the F10 (AAGGGCGC(G/C)GACATCCT), R1 (ATCTGCTG(C/T)TGGATGTCCTT) combination for PCR amplification of Doms C, A and D and the F3 (TGGTAGCTTCCTCATGAACATT), R10 ((A/G)TCCTGCCATCACC(T/A)TCCA) primer combination for Doms B and E. PCR reactions were carried out according to the method of (Prado *et al.*, 2004). Direct sequencing of PCR products was performed using a BigDye Terminator v1.1 Cycle Sequencing Kit (Applied Biosystems, USA) and an ABI Prism 3100 Genetic Analyser. In addition to the direct sequencing work, PCR products from two highly resistant Pel02 plants were cloned and sequenced using a TOPO TA Cloning Kit (Invitrogen).

Results and discussion

Initial glasshouse tests showed that resistance to flupyrsulfuron-methyl was widespread in UK populations of *A. myosuroides*, while resistance to mesosulfuron+iodosulfuron and sulfometuron was confirmed in nine populations. All nine populations contained a proportion of plants that survived treatment with sulfometuron at the screening dose of 100g a.i. ha^{-1}. DNA sequencing of the ALS gene confirmed that a single amino acid substitution at position Pro197 was found to segregate with high level resistance to sulfometuron-methyl in seven of the nine populations tested. These were Pel02, Chal05, Cock06, East06, Key06, Maid05 and Thame05. In total 17 polymorphic sites were identified in the F10/R1 sequenced region, with 14 synonymous changes and three non-synonymous, at Ala135, Pro197 and Pro232, respectively. No segregation with sulfometuron resistance was observed for the Ala135 or Pro232 polymorphisms with most plants appearing heterozygous regardless of phenotype. All highly resistant individuals from the seven populations appeared heterozygous for a Pro-197-Thr substitution, while susceptible individuals appeared homozygous for Pro. Cloning of resistant Pel02 individuals showed two alleles were present, with each plant containing one copy of both Pro and Thr alleles. The 330bp region spanning Domains B and E was sequenced using material from the populations Pel02, Wilts04, Maid05, Thame05 and R30-06. A total of 18 polymorphic sites were identified across these five populations, with 17 synonymous changes and a single non-synonymous change at Trp574 in the R30-06 biotype. All R30-06 individuals showed a predicted Trp to Leu substitution, appearing homozygous Leu at position 574, and this was associated with high level resistance to sulfometuron in all cases. One population, Wilts04, showed high levels of sulfometuron resistance but no evidence of any polymorphism segregating with resistance. Further work is required to determine the mechanism of resistance in this biotype. Enzyme assays showed a 16-fold difference in enzyme sensitivity between the most resistant sulfometuron selected Peldon line and a susceptible standard, confirming that resistance was due to an altered form of the ALS enzyme less susceptible to inhibition by sulfonylurea herbicides. Results from segregation of sulfonylurea resistant and susceptible phenotypes in crossing experiments indicated that ALS target site resistance in *A. myosuroides* is conferred by a single, dominant nuclear allele but that additional effects are also present.

By 2006, resistance to mesosulfuron+iodosulfuron had been confirmed in *A. myosuroides* from 81 farms in the UK, based on glasshouse pot assays. Although ALS target site resistance has been confirmed in eight of the nine populations studied at the molecular level (7 cases = Pro-197-Thr; 1 case = Trp-574-Leu), it should not be assumed that the same mechanism is necessarily responsible for resistance at all other sites. Further work is in progress to determine the importance of other resistance mechanisms. However, ALS target site resistant *A. myosuroides* is predicted to increase in many European countries as a consequence of the increasing and widespread use of mesosulfuron+iodosulfuron.

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Molecular mechanisms associated with altered azole sensitivity in *Mycosphaerella graminicola*

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Introduction

Control of septoria leaf blotch, the most important foliar disease of winter wheat in North Western Europe, caused by the ascomycete fungus *Mycosphaerella graminicola*, is currently reliant on the azole (imidazole and triazole) fungicides. The dependence on azoles has prompted concerns amongst growers and the agro-chemical industry that resistance could develop, thereby compromising control of this disease. Recent studies have shown a significant decline in efficacy of some, but not all members of the class of azole fungicides, with the two azoles currently most commonly used for the control of *M. graminicola*, epoxiconazole and prothioconazole, still effective.

In this paper we present the latest advances in our understanding of the molecular mechanisms responsible for the recent decline in the efficacy of some azole fungicides in controlling *M. graminicola*, and also discuss potential strategies for maintaining azoles that are currently still effective.

Molecular mechanisms associated with reduced azole sensitivity in *M. graminicola*

Enhanced active efflux

The most frequently documented mechanism of azole resistance in pathogenic fungi is the over-expression of genes encoding active efflux pumps, ATP-binding cassette (ABC) transporters and major facilitators (MFs). In *M. graminicola*, several ABC transporter genes (*MgAtr*) have been identified and implicated in azole resistance in laboratory generated mutants, and by heterologous expression and targeted knockout studies (Zwiers *et al.*, 2002). However, in field isolates of *M. graminicola*, although studies have shown differences in both constitutive and induced expression levels of *MgAtr* genes (Stergiopoulos *et al.*, 2003; Cools *et al.*, 2005), no clear correlation between expression levels and azole sensitivity was evident. Furthermore, recent studies using an *M. graminicola* cDNA microarray representing around one quarter of the genome, have failed to identify a transporter gene either responsive to azole treatment, or higher expressed in an isolate less sensitive to azole (Cools *et al.*, 2007). There are, however, data suggesting a synergistic reduction in isolate EC50 when azoles are combined with modulators of ABC transporter activity although the genes involved remain to be identified.

Over-expression of the target-encoding cyp51 *gene*

Increased expression of the *cyp51* gene, encoding the sterol 14α-demethylase target has been implicated in azole resistance in other plant pathogens. Higher constitutive levels of *cyp51* expression have been reported in one isolate of *M. graminicola* from France (Stergiopoulos *et al.*, 2003) although other studies have not identified *cyp51* over-expressers (Cools *et al.*, 2005).

Mutations of the target-encoding* cyp51 *gene

Numerous mutations in the *cyp51* gene potentially contributing to a reduced azole sensitivity phenotype have now been identified in isolates of *M. graminicola* from all over Europe. These mutations are generally found in combination. Some encode substitutions at orthologous positions to those conferring azole resistance in human or other plant pathogenic fungi, for example Y137F (Cools *et al.*, 2005). Others, for example I381V, have been found exclusively in *M. graminicola*. Interestingly, a few alterations in *M. graminicola* appear to be differentially selected by different members of the azole class of fungicides. For example, I381V is selected for by tebuconazole treatment. By contrast, prochloraz appears to negatively select for I381V, instead selecting for isolates carrying substitution V136A (Fraaije *et al.*, 2007; Leroux *et al.*, 2007). The precise impact of identified mutations on *M. graminicola* cyp51 function has yet to be determined, however recent sterol content analyses has suggested some combinations of mutations may impact on the intrinsic activity of the enzyme (Bean *et al.*, unpublished).

Implications for resistance management strategies

The ongoing identification of mechanisms responsible for reducing azole sensitivity in *M. graminicola* has provided possible strategies to circumvent future resistance development. Compounds that modulate ABC transporter activity could, for example, prove useful in delaying further cyp51 evolution and consequently resistance. Perhaps the more readily applicable strategy is selecting against particular cyp51 variants by using different azoles, an excellent example of how gaining an understanding of the molecular mechanisms involved in resistance development can have an immediate practical impact.

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The use of 'temporal synergism' to control insecticide-resistant crop pests

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Insecticide synergists are generally defined as compounds that enhance the toxicity of insecticides, with which they are combined, at concentrations that would not elicit a response from the synergist alone. The twin roles of a synergist can be considered as firstly, an aid to elucidate the mechanism by which resistance is conferred, and secondly, as a means of enhancing control in the field. However, financial constraints have prevented full use of synergists in such a practical application.

For an insecticide to be effective, it must be able to reach its target-site, within the insect, at a concentration sufficient to affect the biological process occurring at that site. Resistance may result from reduced toxicant penetration, modifications of the target-site itself, or enhancement of metabolic enzymes that detoxify the insecticide before it reaches that target-site. Insecticide synergism usually results from the ability of the synergist to interfere with the detoxification of the insecticide. The best known synergist is piperonyl butoxide (PBO), which has the ability to inhibit non-specific esterases and microsomal oxidases, two of the major enzyme systems responsible for metabolism of xenobiotics.

PBO is routinely used in the field as a tank mix, but recent research has suggested that a specific time delay between treatment of PBO and exposure to the active insecticide provides optimum efficacy (Young *et al.*, 2006). This is due to the time required for PBO to enter through the cuticle and inhibit the metabolic enzyme(s) responsible; around 4 hours for *Helicoverpa armigera*, 10 hours for *Bemisia tabaci* (Young *et al.*, 2005, 2006) and 4 hours for *Myzus persicae* (unpublished results). In practice, such a time delay is achieved by the use of microencapsulation to obtain bespoke formulations that give an initial release of PBO followed several hours later by the insecticide (Bingham *et al.*, 2007).

Table 1 shows the results of leaf dip bioassays comparing the response of very resistant insect pests to insecticide alone with a microencapsulation containing the insecticide and PBO in a bespoke formulation allowing optimum pre-treatment of synergist prior to exposure to insecticide. The results demonstrate that the concept can be utilised with pyrethroids, carbamates and neonicotinoids i.e. regardless of the insecticidal target-site, removal of metabolic enzyme systems prior to exposure of insecticide will greatly enhance the performance of that insecticide.

Considering the resistance mechanisms present in the *M. persicae* clones, 794JZ has a greatly enhanced esterase titre and is homozygous for knock-down resistance (kdr). Clone 4013A also has an enhanced esterase titre and is homozygous for modified acetylcholinesterase (MACE). Both clones, therefore, possess multifactorial resistance in the form of an active- site mechanism compounded by a metabolic mechanism.

Table 1. Results of probit analyses on leaf dip bioassay data for highly resistant *Myzus persicae* and *Bemisia tabaci*.

Treatment	Species (strain)	LC_{50} (ppm)	TSR[1]
α-cypermethrin	*M. persicae* (794JZ)	3460.0	
EN-Cyp/PBO		19.7	176.0
Pirimicarb	*M. persicae* (4013A)	>10000.0	
EN-Pir/PBO		28.7	>348.0
Imidacloprid	*B. tabaci* (GUAMIX)	>1000.0	
EN-Imi/PBO		34.4	>29.1

[1] Temporal synergism ratio

Not unexpectedly, this results in extreme resistance to the corresponding actives. More surprising perhaps, is the observation that the encapsulated insecticides seem to overcome not only the metabolic mechanism, but also reduce the resistance conferred by the mutant active-site. It is thought that this is due to the inhibition of background metabolic enzymes, leaving the insect in a 'hypersensitive state' prior to exposure to the insecticide.

Since it has been found that each insect species seems to have its own unique optimum pre-treatment time, work is now underway to investigate the possibility of using this time difference to 'protect' beneficial insects that may be present in a crop i.e. if pest and beneficial species co-exist within a crop, the formulation could be adjusted to give maximum effect against the pest species and minimum effect against the beneficial species by virtue of their differing synergist/enzyme inhibition parameters.

Novel formulations exploiting this concept thus have the potential to abrogate insecticide resistance together with such concomitant benefits as the reduction of harmful chemicals in the environment and, potentially, minimal impact against beneficial species.

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The problem of noxious organisms resistant to pesticides applied to farm crops in Russia

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Resistance of noxious organisms to pesticides is still one of the acute problems in current plant protection in Russia. There has been a progressive increase in populations of noxious organisms in many crops, with resistance to toxicants from different chemical classes. We often observe cases of group-, cross- and multiple resistance development. This results in a considerable increase of toxicant exposure to agrobiocenosis and the appearance of a number of economic and ecological problems.

Studies on resistance and its practical implications are conducted in scientific centres of Russia according to the programme of fundamental and applied researches in the field of plant protection. They are coordinated by the Committee on Resistance (Section of chemical control methods) of the Russian Academy of Agricultural Sciences. The Committee summarizes data on resistance monitoring in populations of noxious organisms, and uses these as a basis for studies on general principles concerning its development and to recommend effective application of currently-used pesticides depending on the situations in particular crops. It also develops methods of resistance diagnosis and recommendations on how to overcome the problem. Results of regular monitoring programmes confirmed that the major problems are farm crop pests. An increase in numbers of new species showing resistance, and in the number of chemical classes or crops involved has been observed. Thus, in the mid 1960s, resistance of *Tetranychus urticae* Koch to organophosphates (OPs) in cotton in Central Asia and in glasshouse cucumber in different regions of the country was observed. Resistance of *Laspeyresia pomonella* L. to DDT in the southern fruit-growing region also developed.

Since then, data covering 34 noxious *Arthropoda* species, resistant to organochlorines, OP´s, carbamates, pyrethroids, nereistoxins, phenylpyrazols, neonicotinoids and other compounds were collected; among them were five species of phytophageous mites, nine *Homoptera* species, 10 *Lepidoptera* species, two *Orthoptera* species, three *Coleoptera* and *Thysanoptera* species, one *Diptera* and *Hemiptera* species. An initial stage of resistance development of *Microtus arvalis* Pall. to anticoagulant from 1,3-indandione group was registered in Krasnodar territory in 2005. The first cases of resistance concerning phytopathogens were registered in the 1970s. These were *Sphaerotheca fuliginea* Poll. and *Erysiphe cichoracearum* DC. to benzimidazoles in glasshouse cucumber in the Leningrad region, and *Pyricularia oryzae* L. to OPs in rice. In the 1980s-90s, resistance of *Phytophtora infestans* to acylalanines in potatoes was detected in different regions of the country. Furthermore benzimidazole resistance in *Fusarium oxysporum* (Schlech.) in glasshouses in the Leningrad region and *Fusarium spp.* in wheat in Krasnodar territory was monitored, as well as triazole resistance in *Sph. pannosa* in glasshouse roses (data of All-Russia Institute for Plant Protection and All-Russia Institute of Phytopathology).

Recently, resistance in *F. sambucinum* Fr., *F. solani* App. et Wr., and *F. avenaceum* (Fr.) Cacc. to benzimidazoles and to phenylpyrroles in potatoes in the Leningrad region and in *Botrytis cinerea* Fr. to benzimidazoles, dicarboximides and sulfamides in strawberries in the Moscow region have been found (data of All-Russia Institute for Plant Protection, phytotoxicology lab.) The monitoring detected resistance development to herbicides in biotypes of 14 weed species, including: resistance to triazines in *Echinochloa crusgalli* (L.) Beauv., *Poa annua* L., *Chenopodium album* L., *Amaranthus retraflexus* L. in maize in North-Caucasian region and *Digitaria sanquialis* L. in soybean in Amur region; *Stachys annua* L., to imazethapyr in rice in Amur region and pea in Central Chernozyom Zone; *E. crusgalli, E. occidentalis* (Weagand) Redb. *E. phyllopogon* (Stauf.) Kossenko to quinclorac in rice in Primorsky territory; *St. annua, Ranunculus repens* L., *Rumex acetosella* Welld., *Viola arvensis* Mur., *Capsella bursa-pastoris* (L.) Medik., *Barbarea vulgaris* L., *Myosotis arvensis* (L.) Hill. to glyphosate in sugar beet and maize in Moscow region (data of All-Russia Iistitute for Plant Protection and All-Russia Institute of Phytopathology).

As a result of the intensive use of pesticides or migration of resistant phenotypes to new regions, an expansion of resistance in many areas has been observed, including species such as *Leptinotarsa decemlineata* Say., *Laspeyresia pomonella* L., *Psylla pyri* L., *Aculus schlechtendali* Nal., *B. cinerea* Fr., *Echinochloa occidentalis* Wiegand, *St. annua* L.. Introduction of resistant populations from abroad favours resistance expansion too. Thus, populations of *Frankliniella occidentalis* Porg. and *Tetranychus urticae* Koch., possessing multiple resistance to OPs, pyrethroids and specific acaricides, respectively, were introduced to glasshouses from the north-west region of Europe. Currently registered pesticides in Russia include 164 insecticides and acaricides (59 a.i.s), 118 fungicides (47 a.i.s) and 204 herbicides (66 a.i.s). Based on pesticides from new chemical classes, methods and strategies to overcome resistance in a number of noxious organisms were developed. A technology has been developed by the All-Russia Institute for Plant Protection (VIZR) to manage resistance of the Colorado potato beetle, based on two anti-resistance strategies, depending on the regional conditions. One of these is intended for regions where the pest has developed resistance to OPs and pyrethroids and is in the early stages of developing resistance to insecticides of other classes. Another one is proposed for regions characterized by early stages of resistance in pest populations to various classes of insecticides. The strategy is based on the monitoring of pest populations using elaborated toxicological and morphotypical methods and making use of a set of insecticides belonging to seven chemical classes (20 active substances) and microbiological preparations based on actinomycetes, entomopathogenic nematodes and toxins of *Bt. thuringiensis*. Tactics for application of these control agents varies according to the state of pest resistance. They include: replacement of failing pesticides by more effective ones, rotation of chemical and microbial preparations, and application of insecticidal mixtures or mixtures of insecticides with entomopathogenic nematodes. Economic damage thresholds should also be taken into account. Using this technology for control of resistance to insecticides in Colorado potato beetles allows growers to efficiently use the available portfolio of insecticides while keeping the number of treatments at a low level. Thus, the number of treatments per growing season dropped to one to two instead of three to four in the southern and two to three in the central regions of Russia. In the north-western Region, one treatment is enough if all recommendations are followed. Such a drastic decrease in the number of chemical treatments aids the survival of beneficial insects in the potato field ecosystem, which not only ensures higher efficiency of protection measures but also promotes environmental safety.

Cymoxanil + mancozeb (1:8) 72% WP: a mixture to control downy mildew resistant to phenylamide fungicides

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Phenylamide fungicides were introduced to control downy mildew and late blight in China in the late 1980s, and were effective in controlling the diseases caused by *Pseudoperonospora cubensis*, *Plasmopara viticola* and *Phytophthora infestans* for the first 2-3 years. Later, efficacy decreased. The aim of the present study was to monitor resistance development to phenylamide fungicides, compare resistance risk to cymoxanil and oxadyxil, evaluate synergism on *P. cubensis and P. viticola* resistant to metalaxyl, and test efficacy of cymoxanil + mancozeb(1:8) 72% WP and spray programs of fungicides with different modes of action in controlling resistant downy mildew on cucumber . The results relating to development and application of an anti-resistance fungicide: Cymoxanil + mancozeb (1:8) 72% WP are sumarized as follows:

Resistance to phenylamide fungicides in *P. cubensis* on cucumber and *P. viticola* on grapevines occurred widely in north China (Table 1); among 105 strains of *P. cubensis* collected in 2001-2005 from nine provinces, 9.52% were sensitive or slightly resistant (resistance level<10), 18.10% were moderately resistant (resistance level 10 to 100), and 73.38% were highly resistant (resistance level >100). Fifteen sensitive strains had an EC_{50} < 1μg/ml (14.29%); 81 resistant strains had an EC_{50} >1μg/ml (85.71%).

Of eight strains of *P. cubensis*, one strain sensitive to metalaxyl and oxadyxil and seven strains resistant to metalaxyl and oxadyxil were sensitive to cymoxanil, indicating that no cross resistance exists between cymoxanil and metalaxyl or oxadyxil. A low risk of resistance to cymoxanil and a high risk to phenylamide fungicides exists in *P. viticola* and *P. infestans* (Table 2). Synergism exists between cymoxanil and mancozeb (optimal ratio 1:8) used against metalaxyl-resistant strains of *P. cubensis* and *P. viticola* (Table 3).

Alternate spray programs were undertaken in greenhouses or tunnels to control cucumber downy mildew from 2002-2004. Preventative applications of protectant fungicides (i.e. chlorothalonil) before the occurrence of downy mildew is key, followed by sprays of systemic fungicides after initial occurrence of the disease, and further applications of protectant fungicides after the disease is controlled completely. The results showed that downy mildew caused by *P. cubensis* resistant to metalaxyl can be effectively controlled by foliar sprays of cymoxanil + mancozeb (1:8) 72% WP (Curzate MZ) (Tables 4 and 5).

In conclusion, development of resistance to metalaxyl decreased the efficacy of phenylamide fungicides in controlling downy mildew in China. Cymoxanil + mancozeb (1:8) 72% WP, with multisite mancozeb and low-risk cymoxanil as active ingredients, is now used widely for controlling downy mildew resistant to phenylamide fungicides on cucumber and grapevines. It was registered in the 1990s and its use now extends all over China.

Table 1. Resistance to metalaxyl and oxadyxil in *P. cubensis* (PC) and *P. viticola* (PV)

Fungi	Fungicide	Strains	Percentage (%) of strains with resistance level		
			<10	10-100	≥100
PC	M	116	2	0.9	96.5
	O	12	25	0	75.0
PV	M	26	7.8	19.2	73.0
	O	20	10	10	80.0

Baseline sensitivity to metalaxyl and oxadyxil is 0.047, 0.235μg a.i./ml, resp.

Table 2. Evaluation of resistance risk to cymoxanil and oxadyxil in *P. vitic.* and *P. infes.*

Risk factor	Cymoxanil	Oxadyxil
Acquire resistant strains by UV induction	not	easily
Acquire resistance exposed to sub-lethal dose fungicide	not	easily
Fitness of resistant strains	no data	good
Competition ability	no data	strong
Cross resistance between metalaxyl and target fungicide	no	yes
Stability of resistance	no data	stable
Residual effect	3-4d	14d

Table 3. Synergism of cymoxanil mixing with mancozeb (1:8) on *P. cubensis* and *P. vitic.*

Fungi	Timing of spray	EC_{50} (μg/ml)		SR
		ob	th	
PC	before inoculation	3.84	19.31	5.00
PV	before inoculation	14.20	35.93	2.53
PV	after inoculation	85.17	262.03	3.07

Table 4. AUDPC of downy mildew under three spray programs in three cucumber greenhouses

Spray programs	AUDPC	EC50 (μg/ml) of metalaxyl
R-R-R-R-R-A-A-C	673.5	82.98-105.59
D-D-D-D-R-R-C-A-D-D-S	60.0	13.57-53.36
D-D-D-D-D-R-A-C-D-D-D-Ac	43.5	1.654-89.28

Amistar 25%SC (A), Ridomil MZ 58%WP (R), Daconil 75%SC (D), Curzate MZ 72% WP(C), Acrobat MZ 69%WP(Ac); Sandofan M8 64%WP(S) at recommended doses and an interval of 7 d.

Table 5. Chemical control of cucumber downy mildew caused by resistant *P. cubensis*

Treatment	Disease incidence	Control efficacy (%)
Amistar 25%SC 100g/ha	10.22	76.70 b
Cabrio 25%EC 100g/ha	9.20	79.03 b
Ridomil Gold MZ 68%WP 675g/ha	14.78	66.43 a
Curzate-MZ 72%WP 1440g/ha	8.49	80.74 b
CK	44.29	——

Selection of insecticide resistance in *Trichogramma* spp. and biological characteristics of the resistant strains

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Abstract
In this research, parasitized test eggs were immersed in methomyl, deltamethrin and phoxim solutions continuously through a series of generations so that increasing levels of insecticide resistance in *Trichogramma dendrolimi* were obtained based on LC_{50} values. After continuous selection, resistance to methomyl reached 2.41 fold after 28 generations treatment, resistance to deltamethrin reached 2.87 fold after 27 generations, and resistance to phoxim reached 2.50 fold after 27 generations. However, in *T. chilonis* resistance of the selected strain decreased. Selection with phoxim resulted in 0.68 fold resistance compared to the parental strain after 25 generations. Strains were also selected with deltamethrin and phoxim for 24 generations, and resistance ratios to the tested insecticides were 0.59 and 0.53, respectively.

Biological characteristics, including parasitism, emergence, reproduction, mean lifetime, numbers of parasites in a single host etc, were used to evaluate the biocontrol quality of the selected strains of both *Trichogramma* species. The percentage of parasitism was taken as a quality measure for each strain, and recognized as the most important evaluation criterion. Results indicated that the selected strain of *T. chilonis* was less fit than the original strain. In contrast, the selected strain of *T. dendrolimi* was as fit as the original strain.

Introduction
Trichogramma spp., as one of the most widely used biocontrol measures, are often affected by chemical pesticide applications. Artificial selection of insecticide-resistant strains that could be released was considered to be, potentially, an appropriate way to combine pesticide application and biocontrol. We tested the effects of selecting for resistance in *Trichogramma* spp. to three different pesticides: deltamethrin, methomyl and phoxim

Methods
The host eggs of *Trichogramma* were immersed in pesticide solutions through several generations to increase the levels of insecticide resistance of *Trichogramma*. The biological characteristics, including parasitism rate, emergence rate, reproduction, mean lifetime, numbers of *Trichogramma* individuals in single host eggs and other parameters, were used to evaluate the quality of the selected strains.

Results

The results showed that the parasitism rate of the host eggs by the selected strain of *T. chilonis* was less than that by the original strain. The selected strain of *T. dendrolimi* was, however, as fit as the original strain (Table 1).

Table 1: Biological characteristics of a resistant strain of *T. dendrolimi*

A	B	C	D	E	F	G	H	I	J
	P	41.07	70.40	114.20	11.34	63.46	12.89	19.82*	0
(1:5)	CK	42.17*	64.30	134.06	12.25	69.10	14.10	12.80	0.30
	P	38.64	56.40	136.54	10.36	50.23	14.23	15.06	0.25
Methomyl(1:10)	CK	29.83**	55.16	148.89	11.35	46.38	16.31	14.13	0.25
	P	29.65	27.45	138.36	11.68	12.36	11.29	54.33	0.45
(1:20)	CK	6.83**	30.50	152.69	13.12	13.67	13.67	58.33	0
	P	41.23	70.40	145.23	13.23	58.30	13.10	23.98	0.35
(1:5)	CK	42.17	64.30	134.06	12.25	69.28	14.10	12.80	0.30
Deltamethrin	P	31.15	48.16	123.49	12.04	39.49	15.23	25.03	0.15
(1:10)	CK	29.83	55.16	148.89	11.35	46.38	16.13	14.13	0.25
	P	5.83	28.45	166.25	12.34	11.25	14.56	64.02	0.34
(1:20)	CK	6.83	30.50	152.69	13.12	13.67	13.67	58.33	0
	P	57.36	67.40	124.10	13.12	71.20	13.20	14.81	0.50
(1:5)	CK	42.17*	64.30	134.06	12.25	69.10	14.10	12.80	0.30
	P	49.33	51.40	152.78	10.50	43.35	15.30	12.23	0.35
Phoxim(1:10)	CK	29.83**	55.16	148.89	11.35	46.38	16.13	14.13	0.25
	P	39.50	33.45	10.50	12.35	10.67	14.87	61.45	0.25
(1:20)	CK	6.83**	30.50	11.35	13.12	13.67	13.67	58.33	0

Note:
A. egg ratio; B. treatment; C. parasitism rate;
D. emergence rate; E. fecundity; F. average longevity;
G. average adult number per egg; H. sex ratio;
I. adult number left per egg; J. abnormal number of adults per egg;
P. pesticides;
* indicates statistically significant and ** indicates a highly significant difference.

Discussion

The results, showing that the quality of the selected strains can be either decreased or unchanged, indicate that effects on fitness need to be carefully considered. However, results of selection experiments may also be influenced by other factors such as laboratory rearing conditions and poor genetic diversity.

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Methidathion resistance mechanisms in *Amblyseius womersleyi* Schicha

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Intoduction

Amblyseius womersleyi is an effective predaceous mite for tetranychid mite control in various countries such as Russia, China, Korea, Japan, Philippines, Australia and New Zealand. Recently, *A. womersleyi* showing resistance to various insecticides such as organophosphates, carbamates and pyrethroids were found in Shizuoka Prefecture in Japan.

The objectives of this study were to determine the methidathion resistance mechanisms in *A. womersleyi*, and to provide basic information for improved use of the resistant predaceous mites in integrated control programs in tea fields in Japan.

Materials and methods

Mite strains

The strains of *A. womersleyi* (Kanaya and Ishigaki strains) were provided by the National Institute of Vegetable and Tea Science, Kanaya, Shizuoka Prefecture. The first strain (Kanaya strain), resistant to organophosphorus insecticides, was collected from tea plants in Kanaya, Shizuoka Prefecture in May 1994. The second, a susceptible strain (Ishigaki strain), was collected from bean plants in Ishigaki Island, Okinawa Prefecture in December 1993. After collection, these strains were reared on bean plants infested with *Tetranychus urticae* Koch in the laboratory.

Chemicals

^{14}C-methidathion (sp act 0.922MBq/mg), non-radioactive methidathion and methidathion oxon were supplied by Syngenta, Switzerland. PBO (piperonyl butoxide) and DEM (diethyl maleate) were purchased from Tokyo Kasei (Tokyo, Japan). DEF (S,S,S-tributylphosphorotrithioate) was obtained from Chem Service, Inc. (PA, USA). PTPE (2-propynyl 2,3,6-trichlorophenyl ether) was obtained from Bayer CropScience (Tokyo, Japan). DTNB [5,5'-dithiobis(2-nitrobenzoic acid)] was purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). ATCh (acetylthiocholine iodide) and all chemicals for experiments involving monooxygenase activities were purchased from Sigma-Aldrich K.K. (Tokyo, Japan). For toxicity tests, a formulated commercial product (Supracide, methidathion EC 40%; Syngenta) was used.

Toxicity tests, with and without synergists, and selection experiments were conducted by spraying a suspension of methidathion onto mite infested discs using a spray tower. Mortality was determined at 48 h after treatments (Sato *et al.*, 2001). Target site insensitivity, cuticular penetration of methidathion and *in vitro* metabolism of methidathion

were measured according to Sato *et al.* (2001). Monooxygenase activities were analyzed by the method reported by Ullrich & Weber (1972). Molecular characterization of methidathion resistance and PCR amplification of P450 cDNA fragment and cloning were conducted by methods reported by Sato *et al.* (2007).

Results and discussion

After four selection cycles for resistance and three selections for susceptibility, the resistance ratio based on the LC_{50} increased from 16 to 342. High synergistic ratios were observed for PBO and PTPE only in the resistant strain, suggesting that increased oxidative metabolism is involved in methidathion resistance. Insensitive acetylcholinesterase was thought to be a minor factor for resistance. No difference in cuticular penetration of ^{14}C-methidathion was observed between susceptible and resistant strains. *In vitro* degradation studies showed that ^{14}C-methidathion was degraded 1.5 times more in the microsomal fraction of the resistant strain.

When selecting for susceptibility using mites of the Ishigaki strain, the resistance ratio (Kanaya R/Ishigaki S) based on the LC_{50} increased from 1.66 to 513. The monooxygenase activity in adult females of the Kanaya R strain was 3.60- and 5.42-fold higher than the activities observed in the Kanaya S and the Ishigaki S strains, respectively. A significant correlation between monooxygenase activity and LC_{50} of methidathion was observed when analysing 16 populations of *A. wormersleyi*. The lowest activity was observed for the larval stage, which exhibited the highest susceptibility to methidathion. Protonymphs, deutonymphs and adults showed the highest monooxygenase activities and were most tolerant to methidathion.The monooxygenase activity determination is easier and quicker than the estimation of LC_{50}, requiring fewer mites.

In conclusion, high monooxygenase activity is thought to be a cause of methidathion resistance in *A. wormersleyi*. Four different CYP4 genes (*CYP4-a, CYP4-b, CYP4-c, and CYP4-d*) were detected. No differences in CYP4 expression levels were detected for the strains Ishigaki S, Kanaya S and Kanaya R, using RT-PCR methods for *CYP4-a, CYP4-b, and CYP4-c*. In the case of *CYP4-d*, the expression level in Kanaya R was significantly higher than in susceptible strains. Significant correlation between *CYP4-d* expression and monooxygenase activity wad observed for the different strains and life stages of *A. wormersleyi*.

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