ROTHAMSTED RESEARCH

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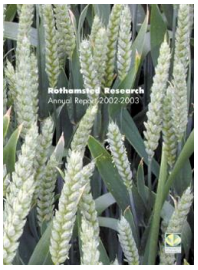

Insecticide Resistance in Aphids

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While insecticides retain an important role in crop protection strategies, the ability of insect and mite pests to evolve resistance to these chemicals remains a serious threat to agriculture in the UK and elsewhere in the world. Pest species with documented insecticide resistance in the UK (especially aphids, whiteflies and spider mites) attack a wide range of crops. Some can occur simultaneously on different crop species, making the development and coordination of insecticide use strategies problematical.

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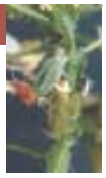


Insecticide resistance in aphids

Ian Denholm, Stephen Foster, Graham Moores, James Anstead and Martin Williamson

Rothamsted Research has a long history of investigating insecticide resistance from a number of perspectives ranging from biochemical and molecular analyses of resistance mechanisms to the evaluation of tactics for combating resistance under field conditions. Our work on aphid pests, especially the peach-potato aphid, demonstrates how a multi-disciplinary approach can facilitate resistance management through the development and continual refinement of mechanism-specific diagnostics, and an understanding of factors causing resistance to increase or decrease in frequency in field populations.

Diagnosis of multiple resistance in *Myzus persicae*

Challenges presented by resistance in aphids on arable crops are exemplified by the occurrence of multiple resistance mechanisms in the peach-potato aphid, *Myzus persicae*. This species attacks and can transmit virus diseases to several 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 carboxylesterase 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.

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. MACE resistance was first recorded in the UK

Potato aphid *Macrosiphum euphorbiae* – a potential new resistance problem. (left)

Damage caused by aphids feeding on potatoes

in 1995 in aphids caught in Rothamsted's suction trap network. It caused severe pest control failures in eastern England in 1996 and has been present at varying frequencies thereafter.

In the last few years, we have identified a third resistance mechanism termed knockdown resistance or kdr, which is associated specifically with resistance to pyrethroids. Kdr involves a modification to the voltage-gated sodium channel protein in nerve membranes, which is vital for the normal transmission of nerve impulses and is the primary target site of pyrethroid insecticides.

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. An ability to diagnose these mechanisms 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*. 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.

In vitro assays for kdr (as opposed to whole-organism bioassays, which are time-consuming and not mechanism-specific) have therefore relied on a knowledge of mutations leading to resistance in the sodium channel gene. Two such mutations have been identified within the domain II region of the channel protein: L1014F (leucine to phenylalanine) conferring 'basal' resistance to pyrethroids, and M918T (methionine to threonine) that appears to boost levels of resistance conferred by L1014F alone, leading to virtual immunity to pyrethroids applied at manufacturer's recommended rates. Several sequence-based approaches have been attempted, the most successful being the recent development of allelic discrimination PCR assays specific to each of the two mutations using fluorescent Taqman® MGB probes (Figure 1). These assays are designed to run alongside existing ones for overproduced carboxylesterase and MACE, and this suite of tools collectively enables a single aphid to be assigned to one of 108 possible genotypes encompassing all three resistance mechanisms. To our knowledge, this level of precision is unprecedented for any multi-resistant insect pest.

Dynamics of resistance mechanisms

The availability of this gamut of 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 12.2m suction traps deployed around the UK as part of the Rothamsted Insect Survey. 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 (Figure 2). Secondly, resistance frequencies usually show a characteristic increase within seasons as insecticides are applied, but then decline markedly 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 and/or reproduction in the absence of exposure to insecticides. Detailed studies at Rothamsted have shown that resistant individuals of *M. persicae* overwinter less successfully than their susceptible counterparts, that they are less fecund, and less responsive to external stimuli including the aphid alarm pheromone (E)-β-farnasene (Figure 3). 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-farnasene could render resistant aphids more vulnerable than susceptible ones to parasitism or predation is currently being investigated.

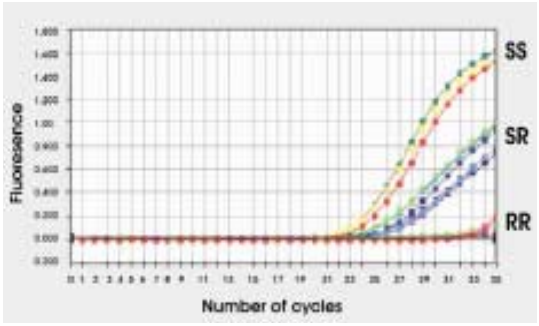
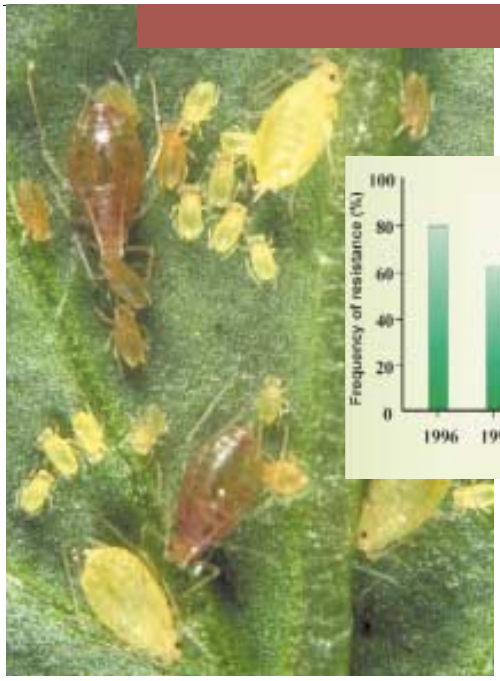


Figure 1. Amplification plot of the sodium channel gene in *M. persicae* using a probe specific for the wildtype (susceptible) kdr allele labelled with 6-FAM ™. SS: homozygous susceptible at L1014F site, SR: heterozygous at L1014F site, RR: homozygous resistant at L1014F site.



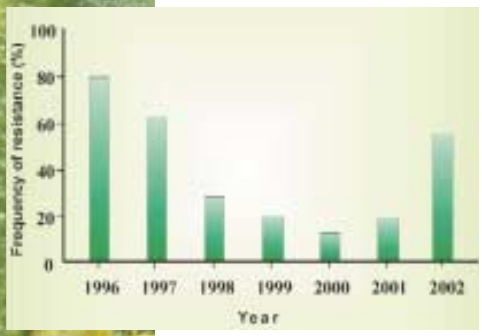


Figure 2. Frequency of the overproduced carboxylesterase mechanism (R_2 and R_3 levels combined) in aphid samples from field crops between 1996 and 2002.

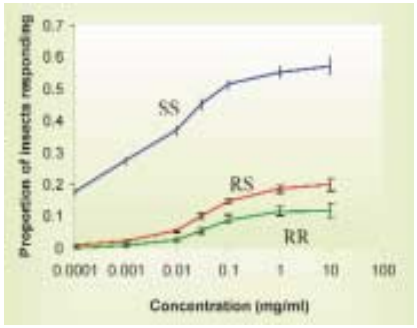


Figure 3. Response of aphids with and without the L1014F pyrethroid-resistance mutation to differing concentrations of alarm pheromone. SS = homozygous susceptible; RR = homozygous resistant; RS = heterozygote.

Emerging and potential new problems

For multi-resistant pests such as *M. persicae*, the introduction of newer insecticides can provide a valuable respite for growers, and an opportunity for researchers to integrate these into more sustainable management recommendations. Neonicotinoids (with imidacloprid as the commercial forerunner) and pymetrozine represent more novel insecticide groups available for use on some crops attacked by *M. persicae*, and which are unaffected by resistance mechanisms already present. However, their unrestrained use can unquestionably lead to selection of additional mechanisms, compounding the resistance problem still further. We have already identified clones of *M. persicae* from southern Europe showing up to 18-fold resistance to imidacloprid, and individuals with lower tolerance 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.

Similarly, it is important to remain vigilant for the appearance of resistance in pests that have not posed problems historically. At present, the potato aphid (*Macrosiphum euphorbiae*) and the currant-lettuce aphid (*Nasanovia ribisnigri*) are both showing incipient resistance and are under investigation at Rothamsted.

Exploitation

Continuing access to new tools in molecular biology offers greater insights into the processes governing the origin and spread of resistance, especially by combining markers for selected traits like resistance with ones (e.g. microsatellites) with no obvious adaptive significance. The reasons why 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. For example, an elevated esterase implicated in resistance in *Macrosiphum euphorbiae* has been found to cross-react with antiserum raised for immunodiagnosis of overproduced carboxylesterase in *M. persicae*.

The insecticide resistance group at Rothamsted has a long history of collaboration with grower organisations, policy-makers, regulatory agencies and agrochemical companies, thereby ensuring effective extension of information and recommendations to end-users. In recent years, this has been formalised through the formation of the UK Insecticide Resistance Action Group (IRAG), chaired from Rothamsted, which reviews resistance developments of national concern and produces management guidelines. Outputs from our work on *M. persicae* are incorporated into a document "Guidelines for preventing and managing insecticide resistance in the peach-potato aphid *Myzus persicae*", available on the IRAG website (see below). These and related publications remain under revision to contend with new cases of resistance or a broadening of existing resistance problems.

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The IRAG website is located at www.pesticides.gov.uk/committees/Resistance