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Use of Biochemical and DNA Diagnostics for Characterising Multiple Mechanisms of Insecticide Resistance in the Peach-Potato Aphid, *Myzus persicae* (Sulzer)*

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Abstract: The peach-potato aphid *Myzus persicae* (Sulzer) can resist a range of insecticides by over-producing detoxifying esterase and having mutant-insensitive forms of the target proteins, acetylcholinesterase (AChE), and the sodium channel. Using a combination of bioassays, biochemical and DNA diagnostics, it is now possible to diagnose all three mechanisms in individual aphids, and thereby establish their spatial distributions and temporal dynamics. A survey of 58 samples of wide geographic origin showed that all 46 resistant clones had amplified esterase genes (E4 or FE4) conferring broad-spectrum resistance to pyrethroids, organophosphates and carbamates. These occurred in combination with insensitive AChE (11 clones), conferring resistance to pirimicarb and triazamate, and/or mutant sodium channel genes (25 clones), conferring knock-down (*kdr*) resistance to pyrethroids and DDT. Amplified esterase genes were in linkage disequilibrium with both insensitive AChE and the *kdr* mutation, reflecting tight physical linkage, heavy selection favouring aphids with multiple mechanisms, and/or the prominence of parthenogenesis in many *M. persicae* populations. An ability to monitor individual mechanisms with contrasting cross-resistance profiles has important implications for the development of resistance management recommendations.

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1 INTRODUCTION

The peach-potato aphid, *Myzus persicae* (Sulzer) (Hemiptera: Aphididae) causes direct feeding damage on many crops, but more importantly is a major vector of virus diseases. As a result, it has been subjected to extensive insecticide treatment and populations throughout the world have developed resistance to organophosphate (OP), carbamate and pyrethroid insecticides. Until recently only one resistance mechanism had been identified, the over-production of carboxylesterases that degrade and sequester insecticidal esters.[1] Both *M. persicae* and its tobacco-feeding form, *Myzus nicotianae* (Blackman) over-produce one of two very closely related esterases, E4 or FE4, and it has been shown that both species have the same amplified E4 or FE4 genes.[2] Indeed, aphids of wide geographic origin have amplified E4 genes on identical repeat units of DNA, suggesting that this resistance mechanism arose by a single genetic event and was subsequently spread by migration.[3]

In 1990, an insensitive form of acetylcholinesterase (AChE), the target site of organophosphate and carbamate insecticides, was identified in *M. persicae* and *M. nicotianae* collected from Greece.[4,5] This confers resistance very specifically to the carbamate pirimicarb and to triazamate, a novel triazole aphicide which also inhibits AChE. When several UK clones of *M. persicae* were examined in 1988, no evidence of modified AChE was found,[6] and, moreover, pirimicarb had always provided most consistent control in the field, though this was somewhat reduced for aphids with elevated esterase levels.[4] Thus it seems likely that this mutant AChE appeared recently, and, although it seemed to be generally rare in 1994,[4] it has now become increasingly widespread in Europe and is posing a serious threat to control by these otherwise very effective aphicides.

The primary physiological target for DDT and pyrethroid insecticides is the voltage-sensitive sodium channel of nerve membranes. Two mutations that are associated with target-site resistance to these insecticides (generally termed knockdown resistance or *kdr*) have recently been identified in the *para*-type sodium channel gene of houseflies.[7] One of the mutations, which results in a leucine to phenylalanine replacement, has now been detected in the corresponding gene of *M. persicae* and is similarly associated with resistance to deltamethrin and DDT.[8] Deltamethrin resistance appears to result from the combined effects of the latter and esterase overproduction. Clones with this mutation had been collected from field populations in the UK as early as the 1970s, suggesting that this mechanism has been present in aphid populations for some time but was not identified due to lack of a suitable detection method.

Our long-term research on the biochemical and molecular basis of resistance has led to the development of mechanism-specific diagnostics for use on individual insects. The amount of detoxifying esterase can be measured using an immunoassay[9] and the amplified gene (E4 or FE4) responsible for the elevated esterase can be detected by electrophoresis, or, more recently, by restriction enzyme analysis or polymerase chain reaction (PCR)-based techniques.[10] Furthermore, knowledge of the DNA changes (involving cytosine methylation) underlying loss of resistance by some aphids also allows detection of elevated but unexpressed esterase genes that represent a hidden resistance potential in such 'revertants'.[11] The insecticide-insensitive form of AChE can be detected using microplate-based enzyme kinetic assays in the presence of diagnostic concentrations of inhibitors.[6] For *kdr*-type resistance, diagnostic bioassays with DDT and pyrethroids can be augmented by direct sequencing of the sodium channel gene for the known *kdr* mutation[12] or by using PCR-based allele detection techniques (e.g. PASA).[13] We have used these techniques in the present work to disclose the presence and co-existence of resistance mechanisms in 58 clones established from single aphids collected from sites around the world over the past 20 years. For the purposes of this study we do not distinguish *M. persicae* from *M. nicotianae;* all clones are referred to by the former name.

2 METHODS AND MATERIALS

2.1 Aphids

Parthenogenetic cultures of *M. persicae* included five reference clones and 53 others established from single insects collected from the field between 1989 and 1995. Of the 58 clones investigated, 13 came from the UK, 26 from mainland Europe, eight from the USA, six from South America, one from Australia and four from the Far East (Table 1). They were chosen to ensure a wide geographical and temporal coverage, but, since they were also deliberately selected to encompass as broad a range of esterase phenotypes as possible, they cannot be considered representative of the resistance status of aphids in those countries. All clones were reared as virginoparous, predominantly apterous colonies in leaf-boxes[14] on excised Chinese cabbage leaves at 20°C with a 16-h photoperiod.

2.2 Detection of resistance mechanisms

Individuals from each aphid clone were assayed for the presence of elevated esterase enzyme, amplified esterase genes, AChE variant (sensitive or insensitive) and DDT resistance (as a diagnostic of *kdr*). Tests for amplified esterase genes and *kdr* were conducted against the same individuals.

TABLE 1
Occurrence of Three Resistance Mechanisms in 58 Clones of *Myzus persicae*

Country of origin	*No. of clones tested*	*Esterase phenotype*[a]				*AChE phenotype*		kdr *phenotype*[b]	
		S	R_1	R_2	R_3	*S*	*R*[c]	*S*	*R*
UK	13	3	2	1	7	13	0	3	10
Belgium	1	0	0	1	0	1	0	1	0
France	3	0	0	1	2	3	0	3	0
Germany	2	0	0	0	2	2	0	0	2
Greece	9	1	0	2	6	4	5	8	1
Holland	6	1	2	1	2	3	3	3	3
Hungary	1	0	1	0	0	1	0	1	0
Italy	1	0	0	0	1	1	0	1	0
Spain	3	0	0	1	2	3	0	3	0
USA	8	1	0	3	4	8	0	2	6
Argentina	2	1	0	1	0	1	1	2	0
Chile	4	4	0	0	0	4	0	4	0
Australia	1	1	0	0	0	1	0	1	0
Japan	3	0	1	0	2	1	2	0	3
Korea	1	0	1	0	0	1	0	1	0
Total	58	12	7	11	28	47	11	33	25

[a] FE4 and E4 enzymes combined.
[b] Based on a diagnostic dose bioassay with DDT (see text).
[c] Includes insects both heterozygous and homozygous for this allele.

2.2.1 Elevated esterase and amplified esterase genes
The amount of detoxifying esterase present in each insect, measured by immunoassay using E4-antiserum in 96-well microtitre plates, allowed the clone to be categorised as either susceptible (S) or resistant (R_1, R_2 or R_3, depending on the amount of enzyme present).[9] The esterase genes in the DNA of individual aphids were assessed by a PCR-based diagnostic[10] to determine whether they were amplified and, if so, whether they were E4 or FE4. In the one case where amplified E4 genes were present but there was no elevated esterase, *Msp*I/*Hpa*II digests were used to determine whether the E4 sequences were unmethylated and therefore unlikely to be expressed.[11]

2.2.2 AChE variant
The insensitive AChE variant was detected using a microplate assay to measure enzyme activity in duplicate samples from individual aphid homogenates in the presence and absence of a diagnostic concentration of pirimicarb.[4]

2.2.3 Kdr *resistance to DDT and pyrethroids*
Having established, using standard clones, that the presence of the *kdr* mutation was associated with survival of a diagnostic dose of DDT,[8] bioassays with this insecticide were done on between nine and 74 aphids per clone. DDT was chosen in preference to a pyrethroid as a diagnostic for *kdr* because it circumvents the esterase-based resistance mechanism. Prior to bioassays, apterous adults were transferred to Chinese cabbage leaf discs (10 per disc) placed upside-down on a bed of agar (11 g litre^{-1}) in plastic tubs (3 cm diam.), the lips of which had been coated with Fluon to prevent escape. The aphids were left for 2 h and then dosed individually by topical application of DDT in acetone (200 ng in 0·25 μl). Responses were assessed after holding treated insects at 22°C for 48 h. For some clones, the presence of the sodium channel gene mutation associated with *kdr* resistance[7] was investigated directly by PCR and sequencing of the region spanning the amino acid substitution.[12,13]

3 RESULTS AND DISCUSSION

3.1 Amplified esterase genes

Of the 58 aphid clones studied by immunoassay, 12 had susceptible levels of esterase enzyme, with seven R_1, 11 R_2 and 28 R_3 (Table 1). Elevated levels were found in clones chosen from all countries except Australia, Argentina and Korea, from where very limited numbers of clones were analysed, but where high levels of esterase have been found previously. Hence this mechanism now has a worldwide distribution. Table 2 shows that in the UK, in keeping with a more extensive (unpublished) survey, R_1 levels of esterase conferring only moderate resistance are associated with amplified FE4 genes, and higher levels (R_2 and R_3) with E4 genes. Aphids with high copy numbers of FE4 genes (R_2 and R_3) are more common in southern Europe, but their

TABLE 2
Distribution of Esterase Levels and the Presence of Amplified Esterase Genes in *Myzus persicae* Clones. Data for UK Clones are shown in Parentheses

	Esterase level			
Amplified genes	*S*	R_1	R_2	R_3
No amplification	11 (2)	0 (0)	0 (0)	0 (0)
FE4	0 (0)	3 (2)	4 (0)	10 (0)
E4	1 (1)[a]	4 (0)	7 (1)	18 (7)

[a] Apparent revertant clone.

incidence in UK populations cannot be discounted since most routine monitoring is based solely on the esterase immunoassay, which quantifies esterase levels but does not distinguish E4 from FE4.

When all clones are considered, high levels of resistance are the result of both E4 and FE4 amplification (Table 2). One factor influencing the distribution of highly elevated E4 and FE4 enzymes may be the apparently complete linkage between amplification of E4, as opposed to FE4, genes and a chromosomal translocation.[15] The translocation seems to impose some reproductive isolation on clones as either they do not produce sexual forms or only males. This is of little consequence for R_3 aphids in the UK, where sexual overwintering is extremely rare, but has probably led to the preferential buildup of R_3 aphids over-producing FE4 in southern Europe and elsewhere where sexual populations predominate.

Only one clone of UK origin had amplified E4 genes but a susceptible enzyme titre (Table 2). Further analysis of this clone showed that the amplified E4 DNA sequences were unmethylated at *Msp*I sites; this is consistent with previous results showing that lack of DNA methylation correlates with loss of E4 esterase gene expression and hence reversion to a susceptible phenotype.[11,16] Complete loss of methylation and expression of amplified E4 genes appears to be a rare event. However, a previous survey of UK populations did yield evidence for more frequent partial loss of methylation and expression.[17]

3.2 Insensitive AChE

Eleven clones, all from Europe or Japan, were either heterozygous or homozygous for the insensitive AChE variant (Table 1). This mechanism was first detected in an FE4-over-producing clone originating in Greece in 1990. Our work since then has documented its occurrence in Japan and a northward expansion in its European distribution. In 1996, many strains collected from sites in eastern England reporting control difficulties with pirimicarb were confirmed to possess this AChE variant, but in an amplified E4 background.

Interestingly, in the present work, the insensitive variant was associated exclusively with elevated carboxylesterase phenotypes (E4 or FE4) ranging from R_1 to R_3 (Table 3). This is consistent with more detailed surveys of UK populations that failed to yield any insects combining insensitive AChE with non-amplified esterase genes. It may partly reflect a bias towards sampling from sites with relatively heavy reliance on insecticides, i.e. ones where high esterase levels are likely to be selected concurrently with the AChE mechanism. However, the consistency of this phenomenon, even in flying aphids collected from 12·2 m high suction traps in the UK (unpublished data), suggests a more fundamental genetic explanation. One likely interpretation is that the AChE mutation first occurred in an elevated esterase background, and that subsequent dispersal from the site of origin led initially to a predominance of insects with both resistance mechanisms. In areas such as northern Europe, where *M. persicae* is primarily anholocyclic, this association would be maintained, whereas under conditions promoting sexual reproduction during winter (e.g. southern Europe), recombination should occur leading to a gradual disassociation of mechanisms. However, if populations possessing highest frequencies of insensitive AChE are (as seems likely) those under particularly intense selection with insecticides, linkage disequilibrium between resistance genes will be maintained. Under this scenario, aphids combining insensitive AChE with susceptible esterase levels would be the slowest to arise through recombination and to be dispersed, but are expected to be reported in due course.

3.3 *Kdr* resistance

Based on diagnostic dose bioassays, 25 of the 58 clones were DDT-resistant (Table 1). Although it may be premature to attribute all these instances of DDT resistance to a *kdr*-type mechanism, results of such tests have so far correlated fully with the presence or absence of the sodium channel gene mutation associated with *kdr* in other species.[12] Failure to detect any clones surviving DDT that possess the susceptible sodium channel sequence militates strongly against confusion caused by a metabolic DDT-resistance mechanism. At present it is only possible to assign insects to susceptible or resistant

TABLE 3
Co-occurrence of Esterase Levels and AChE Variants in *Myzus persicae* Clones

	Esterase level			
AChE	*S*	R_1	R_2	R_3
Sensitive	12	5	9	21
Insensitive	0	2	2	7

phenotypes, since the response of *kdr* heterozygotes in bioassays with DDT has not been established in sufficient detail. It is possible, therefore, that a proportion of clones classified as 'susceptible' purely on the basis of bioassays were heterozygous for the *kdr* allele.

All of the DDT-resistant clones had amplified E4 genes, whereas of the 33 DDT-susceptible clones, 29 had either amplified FE4 or no amplified esterase genes (Table 4). Thus there were only four DDT-susceptible clones (from the USA, Spain and France) with amplified E4, and for each of these the presence of the susceptible sodium channel gene was confirmed by PCR and sequencing of the region containing the *kdr*-associated mutation. As with insensitive AChE, these data therefore demonstrate strong linkage disequilibrium between *kdr* resistance and amplification of the E4 form of the esterase gene. However, this association evidently has a longer history than that involving insensitive AChE, since clones in which *kdr* resistance has been confirmed date back to the 1970s at least. Lack of genetic equilibration is therefore less explicable as a consequence in terms of treatment histories (amplified E4 and FE4 genes both give some resistance to pyrethroids, as well as to OPs and carbamates) and/or the ecology and dispersal capability of *M. persicae*. It is not clear at present whether this linkage disequilibrium reflects tight physical linkage, or is attributable to obligate parthenogenetic reproduction associated with the chromosomal translocation in 'high-E4' aphids. The four clones with amplified E4 genes that were nonetheless *kdr*-susceptible show that the link between E4 and *kdr* is not absolute. Further work to resolve this issue is currently under way.

The detection and preliminary analysis of *kdr* resistance in *M. persicae* has shown that a substantial component of pyrethroid resistance attributed previously to esterase detoxication is in fact due to target-site insensitivity.[8]

3.4 Implications for resistance management

At present, the practical implications of these results are easiest to summarise from a UK perspective, since the occurrence and co-incidence of mechanisms has been best documented in this country. However, it is clear from our work that all three mechanisms are widely distributed (six clones had all three), and collectively constitute a severe threat to the management of *M. persicae* throughout its geographical range.

TABLE 4
Presence of Amplified Esterase Genes and Resistance to DDT (i.e. *kdr* Resistance) in *Myzus persicae* Clones

	DDT resistance phenotype	
Amplified genes	*S*	*R*
No amplification	11	0
FE4	17	0
E4	5	25

Until recently the only resistance mechanism recognised in *M. persicae* was that based on elevated carboxylesterases. This was considered to confer strong resistance to OPs and pyrethroids, but lower resistance to carbamates. Although high-esterase phenotypes have been shown to be selected by insecticide sprays, a coarse-grained analysis of monitoring data collected nationwide between 1988 and 1995 revealed no directional increase in the frequency of R_2 and R_3 aphids over this period.[18] In the light of recent findings, such temporal stability of esterase phenotypes can probably be attributed to insecticide selection being countered by a reduced ability of highly resistant aphids to survive UK winters. Experiments comparing the overwintering success of clones of known E4 or FE4 phenotypes demonstrated, for the coldest months in particular, an inverse relationship between survival and the amount of esterase present.[19] Opposing selective forces appear to have maintained a balanced polymorphism for this mechanism, and thereby enabled continued control of *M. persicae* with insecticides. Pirimicarb has proved an essential component of such control regimes, since it retains partial effectiveness even when contending with high levels of esterase-based resistance late in a cropping season.

The detection of an AChE variant conferring strong and very specific resistance to pirimicarb therefore has a very profound bearing on the management of resistance in *M. persicae*. Recommendations to exploit pirimicarb to combat E4 and FE4 resistance now run the risk of accelerating the selection and spread of insensitive AChE, thereby rendering this chemical ineffective over large areas. Ironically, this would also have severe repercussions for the efficacy of triazamate—one of very few novel aphicides available against *M. persicae*. Future attempts to combat resistance must clearly place greater emphasis on more careful use of pirimicarb, based on detailed biochemical monitoring to document the dynamics and geographical spread of the new gene. The current association (in the UK at least) of insensitive AChE with elevated esterases has the drawback that both mechanisms will be selected concurrently, but the potential advantage that fitness costs incurred at low temperatures by insects with highly amplified esterase genes should cause a concomitant decline in the frequency of the insensitive AChE allele during winter. At present it is unknown whether the latter influences fitness in its own right, or whether it affects fitness costs associated with elevated carboxylesterases.

Confirmation of a *kdr*-type mechanism in *M. persicae* complicates the genetic architecture of resistance in this species still further. Its seemingly intimate association with amplified E4, as opposed to FE4, genes has some interesting genetic implications. At a practical level, this

combination of mechanisms accounts for the pyrethroid resistance in *M. persicae* previously attributed solely to esterase over-production. Although the latter does contribute significantly, its potency alone may not impair the field performance of pyrethroids to the extent originally suspected.[8] In areas where FE4 predominates, or where high E4 resistance is shown not to be associated with the *kdr* allele, there is therefore a prospect of continuing to use pyrethroids against populations *proven* to possess this combination of phenotypes. Opportunities for exploiting the discovery of *kdr* resistance to fine-tune management recommendations in the UK appear more limited at present, since all high-E4 clones analysed to date have also possessed the *kdr* mutation. This being so, it is also possible that the reduced overwintering success associated with esterase over-production may not result from amplified E4 genes *per se*, but from a co-existing *kdr* mechanism reducing the sensitivity of the nervous system to stimuli (e.g. plant sensescence) that promote survival under harsh climatic conditions.[20] This is being investigated further.

4 CONCLUSIONS

Long-standing research on *M. persicae* exemplifies a number of considerations likely to achieve increasing importance for contending with multi-resistant pests. First, it emphasises the key role that mechanism-specific assays can play in resolving the evolution and dynamics of multiple resistance mechanisms, each with different cross-resistance characteristics and hence implications for resistance management. Second, it demonstrates the potential benefit of exploiting knowledge and techniques gained from work on more tractable organisms to disclose either an incipient mechanism (e.g. insensitive AChE) in another species, or others (e.g. *kdr*) that might otherwise avoid detection using conventional toxicological and/or biochemical procedures. Most importantly of all, it highlights the necessity of addressing resistance from a multi-disciplinary perspective, thereby embodying the central theme of the Resistance '97 Conference—that of an integrated approach to analysing and combatting resistance problems.

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REFERENCES

1. Devonshire, A. L. & Moores, G. D., A carboxylesterase with broad substrate specificity causes organophosphorus, carbamate and pyrethroid resistance in peach-potato aphids (*Myzus persicae*). *Pestic. Biochem. Physiol.*, **18** (1982) 235–46.
2. Field, L. M., Javed, N., Stribley, M. F. & Devonshire, A. L., The peach-potato aphid *Myzus persicae* and the tobacco aphid *Myzus nicotianae* have the same esterase-based mechanism of insecticide resistance. *Ins. Mol. Biol.*, **3** (1994) 143–8.
3. Field, L. M. & Devonshire, A. L., Structure and organisation of amplicons containing the E4 esterase genes responsible for insecticide resistance in the aphid *Myzus persicae* (Sulzer). *Biochem. J.*, **322** (1997) 867–71.
4. Moores, G. D., Devine, G. J. & Devonshire, A. L., Insecticide-insensitive acetylcholinesterase can enhance esterase-based resistance in *Myzus persicae* and *Myzus nicotianae*. *Pestic. Biochem. Physiol.*, **49** (1994) 114–20.
5. Moores, G. D., Devine, G. J. & Devonshire, A. L., Insecticide resistance due to insensitive acetylcholinesterase in *Myzus Persicae* and *Myzus nicotianae*. *Proc. Brighton Crop Prot. Conf—Pests and Diseases* (1994) 413–18.
6. Moores, G. D., Denholm, I., Byrne, F., Kennedy, A. L. & Devonshire, A. L., Characterising acetylcholinesterase genotypes in resistant insect populations. *Proc. Brit. Crop Prot. Conf.*, **1** (1988) 451–6.
7. Williamson, M. S., Martinez-Torres, D., Hick, C. A. & Devonshire, A. L., Identification of mutations in the housefly *para*-type sodium channel gene associated with knockdown resistance (*kdr*) to pyrethroid insecticides. *Mol. Gen. Genet.*, **252** (1996) 51–60.
8. Martinez-Torres, D., Foster, S. P., Williamson, M. S. & Devonshire, A. L., Characterisation of knockdown resistance to pyrethroids in the peach-potato aphid *Myzus persicae*. In preparation (1997).
9. Devonshire, A. L., Moores, G. D. & ffrench-Constant, R. H., Detection of insecticide resistance by immunological estimation of carboxylesterase activity in *Myzus persicae* (Sulzer) and cross-reaction of the antiserum with *Phorodon humuli* (Shrank) (Hemiptera: Aphididae). *Bull. Entomol. Res.*, **76** (1986) 97–107.
10. Field, L. M., Crick, S. E. & Devonshire, A. L., Polymerase chain reaction-based identification of insecticide resistance genes and DNA methylation in the aphid *Myzus persicae* (Sulzer). *Insect. Mol. Biol.*, **5** (1996) 197–202.
11. Field, L. M., Devonshire, A. L., ffrench-Constant, R. H. & Forde, B. G., Changes in DNA methylation are associated with loss of insecticide resistance in the peach-potato aphid *Myzus persicae* (Sulzer). *FEBS Letts*, **243** (1989) 323–7.
12. Martinez-Torres, D., Devonshire, A. L. & Williamson, M. S., Molecular genetic studies of knockdown resistance to pyrethroids. *Pestic. Sci.*, **51** (1997) 265–70.
13. Williamson, M. S., Martinez-Torres, D., Hick, C. A., Castells, N. & Devonshire, A. L., Analysis of sodium channel gene sequences in pyrethroid-resistant houseflies. Progress towards a molecular diagnostic for knockdown resistance. In *Molecular Genetics and Evolution of Pesticide Resistance*, ed. T. M. Brown. ACS Symposium Series 645, American Chemical Society, Washington DC, USA, 1996, pp. 52–61.
14. Blackman, R. L., Variation in the photoperiodic response within natural populations of *Myzus persicae* (Sulzer). *Bull. Entomol. Res.*, **60** (1971) 533–46.
15. Blackman, R. L., Takada, H. & Kawakami, K., Chromosomal rearrangement involved in the insecticide resistance of *Myzus persicae*. *Nature* (*London*), **271** (1978) 450–2.
16. Hick, C. A., Field, L. M. & Devonshire, A. L., Changes in the methylation of amplified esterase DNA during loss and reselection of insecticide resistance in the peach-

potato aphid *Myzus persicae*. *Insect Biochem. Molec. Biol.*, **26** (1996) 41–7.
17. Field, L. M. & Devonshire, A. L., Esterase genes conferring insecticide resistance in aphids. In *Molecular Mechanisms of Insecticide Resistance*, ed. C. A. Mullin & J. G. Scott. ACS Symposium Series 505, American Chemical Society, Washington DC, USA, 1992, pp. 209–17.
18. Muggleton, J., Hockland, S., Thind, B. B., Lane, A. & Devonshire, A. L., Long-term stability in the frequency of insecticide resistance in the peach-potato aphid, *Myzus persicae*, in England. *Proc. 1996 Brighton Crop Prot. Conf.*, **2** (1996) 739–44.
19. Foster, S. P., Harrington, R., Devonshire, A. L. Denholm, I., Devine, G. J., Kenward, M. G. & Bale, J. S., Comparative survival of insecticide-susceptible and resistant peach-potato aphids *Myzus persicae* (Sulzer) (Hemiptera: Aphididae) in low temperature field trials. *Bull. Entomol. Res.*, **86** (1996) 17–27.
20. Foster, S. P., Harrington, R., Devonshire, A. L., Denholm, I., Clark, S. J. & Mugglestone, M. A., Evidence for a possible fitness trade-off between insecticide resistance and the low temperature movement that is essential for survival of UK populations of *Myzus persicae* (Hemiptera: Aphididae). *Bull. Entomol. Res.* **87** (1997) (in press).