Field-simulator study of insecticide resistance conferred by esterase-, MACE- and kdr-based mechanisms in the peach-potato aphid, *Myzus persicae* (Sulzer)

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Abstract: Insecticide sprays were applied to *Myzus persicae* (Sulzer) populations carrying various combinations of three insecticide resistance mechanisms (esterase-based metabolic resistance and two target site mechanisms, known as MACE and kdr), supported on host plants growing in field simulator cages. The study showed that MACE confers extreme resistance to pirimicarb and triazamate (carbamate insecticides) but not to deltamethrin+heptenophos (16+1) (Decisquick) or dimethoate (an organophosphorus insecticide). Resistance to dimethoate depends solely on levels of esterase-based resistance, while resistance to Decisquick depends on kdr and esterase. None of the four insecticides is effective against aphids carrying MACE combined with extreme esterase-based resistance. This knowledge, in association with current monitoring of the mechanisms, will play an important role in making decisions on insecticide use against *M persicae* in the UK.

Keywords: *Myzus persicae*; esterase; gene amplification; MACE; kdr; insecticide resistance

1 INTRODUCTION

The peach-potato aphid, *Myzus persicae* (Sulzer) (Hemiptera: Aphididae) is a serious pest on a wide range of agricultural and horticultural crops, in which it can cause substantial direct feeding and cosmetic damage as well as transmitting virus diseases. Growers have used many insecticides to combat these problems and the strong selection pressure has led *M persicae* to become resistant. Until 1990, only one resistance mechanism had been identified in this species: the overproduction of one of two closely related carboxylesterases (E4 and FE4) that sequester or degrade insecticide esters before they reach their target sites in the nervous system.[1] This overproduction of esterase is caused by amplification of structural genes encoding these enzymes[2] and was considered to be the only mechanism conferring strong resistance to organophosphorus (OP) and pyrethroid insecticides, together with lower resistance to carbamates including pirimicarb. Depending on their esterase content and response in laboratory bioassays, aphids can be broadly classified into one of four groups; S (susceptible), R_1 (moderately resistant), R_2 (highly resistant) or R_3 (extremely resistant).[3]

Insecticide-insensitive acetylcholinesterase (AChE), the target for OP and carbamate insecticides, an important resistance mechanism in many insect species, was first detected in *M persicae* in 1990.[4] This mechanism, commonly referred to in this species as MACE (Modified AcetylCholinEsterase), confers strong resistance specifically to pirimicarb and triazamate, a novel and otherwise very effective triazole aphicide[5] that also inhibits AChE. MACE in *M persicae* was first detected in Greece, followed by Japan and South America and it has recently shown a northward expansion in its European distribution.[6] *M persicae* has recently been shown to possess a third mechanism, knock-down resistance (kdr), which affects pyrethroids and DDT. Although detected only recently, this target-site resistance conferred by changes in a voltage-gated sodium channel protein has probably been present in *M persicae* populations since the 1950s.[7]

Although laboratory bioassays and biochemical diagnostics provide useful information on the resistance status of individual aphids and clones, it is also important to establish the response of aphids to insecticides applied more conventionally at recom-

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Table 1. *Myzus persicae* clones tested in simulator experiments

Clone	Resistance mechanisms: Esterase[a]	MACE[b]	kdr[c]	Aphid colour	Origin
1171D	R_1	No	No	Green	UK 1993
1071F	R_3	No	Yes	Green	UK 1992
1054D	R_1	Yes	Yes	Red	Japan 1992
1055F	R_3	Yes	Yes	Red	Japan 1992

[a] Based on an immunoassay.[3]
[b] Based on a kinetic assay.[4]
[c] Based on direct DNA sequencing of PCR-amplified sodium channel gene fragments from aphid genomic DNA and a diagnostic dose topical bioassay (200 ng DDT in 0.25 µl acetone, applied to 20 individual aphids from each clone).[7]

mended rates as sprays and seed treatments in the field.[8] This aspect of resistance can also be studied under controlled quarantine conditions in large spray chambers (field simulators, 1 m wide × 2 m long × 1 m high) that support plant and insect populations.[9] These were used to study the relative performance of various established insecticides as sprays, applied at recommended field rates, against *M persicae* with insecticide resistance conferred by different combinations of the esterase-, MACE- and kdr-based mechanisms described above. Our findings show that the efficacies of four insecticides commonly used against *M persicae* in the UK are very much dependent on the resistance mechanisms that the aphids carry.

2 MATERIALS AND METHODS

2.1 *Myzus persicae* clones and diagnosis of resistance mechanisms

Two separate experiments were done using four *M persicae* clones whose backgrounds are described in Table 1. Each clone had been reared from an individual ancestral parthenogenetic female aphid. The experimental data were pooled for statistical analysis (on a total of 10 replicates per clone per treatment).

2.2 Aphid and plant rearing protocol

Sugar beet plants (*Beta vulgaris* L cv Zulu) were grown in compost in individual pots (21 cm diameter) in a glasshouse and transferred at the four- to six-leaf stage to five simulators. Each simulator contained 10 plants arranged in two rows of five, such that there was no between-plant contact.

Experimental aphids were reared from clonal lines that had been maintained under a 16 h light/8 h dark photoperiod at 22 °C as virginoparous, predominantly apterous colonies on excised Chinese cabbage leaves (*Brassica napus* L var *chinensis* cv Tip-Top) (Brassicaceae) in Blackman boxes.[10] All four clones were allocated to each of five simulators (maintained under the same light/dark photoperiod and temperature). Clones were paired by esterase-based resistance (ie R_1: 1171D with 1054D and R_3: 1071F with 1055F), with one of each pair having green and the other red heritable body colour which allowed, first, interclone comparisons under comparable exposure conditions, second, accurate assessment of each clone using colour and, third, more plant replicates to be tested. Each clone pair was allocated randomly to one of the rows of plants in each simulator. A dividing strip of tape coated with insect-trapping adhesive (Oecotak) was stuck along the centre of the floor and lower 15 cm of the walls of each simulator to eliminate aphid movement which would have led to clone mixing between the two rows of plants (previous work has shown that apterous aphids do not move across this barrier and that extremely few alatae are produced by *M persicae* on sugar beet).

Adult apterae were starved at 22 °C for 24 h in glass Petri dishes (one clone per dish) and then placed onto the central leaves of the sugar beet plants (10 adult apterae per clone per plant) with a fine paint brush. Aphid numbers for each clone (both adults and nymphs) were recorded after three weeks using body colour as the means of identification. Samples of green and red aphids were then removed from each row of plants for subsequent biochemical assay (of esterase content and MACE) to check clonal integrity and confirm the effectiveness of the sticky barrier between plant rows in each simulator.

2.3 Spray protocol

Foliar sprays of the primary insecticides used against UK *M persicae* on sugar beet: pirimicarb (Aphox), triazamate (Aztec), deltamethrin + heptenophos (16 + 1) (Decisquick) or dimethoate (one insecticide per simulator), were then applied as aerosols at the recommended field rates for this crop with hand-held sprayers (Pirimicarb 140 g AI in 200 litres ha^{-1}; triazamate 56 g AI in 200 litres ha^{-1}; deltamethrin 120 g + heptenophos 7.5 g AI in 200 litres ha^{-1} and dimethoate 400 g AI in 200 litres ha^{-1}). One simulator was left untreated as a control. The pyrethroid/ OP mixture, Decisquick, was used because this is the prevalent pyrethroid formulation used on UK sugar beet.

A post-spray count of live aphids (both adults and nymphs) was made after three days using aphid body colour (green or red) to distinguish clones in each pair. Mean proportions relative to the pre-treatment number were calculated per clone per plant replicate. Experience has shown that little aphid movement takes place between plants in each row during the post-spray period.

3 RESULTS

Biochemical tests on aphid samples showed no evidence that there had been movement across the sticky barrier giving rise to cross-contamination between green or red clones in any of the simulators. Each of the five plants within a row was treated as a replicate. Post-treatment proportions (PTP), trans-

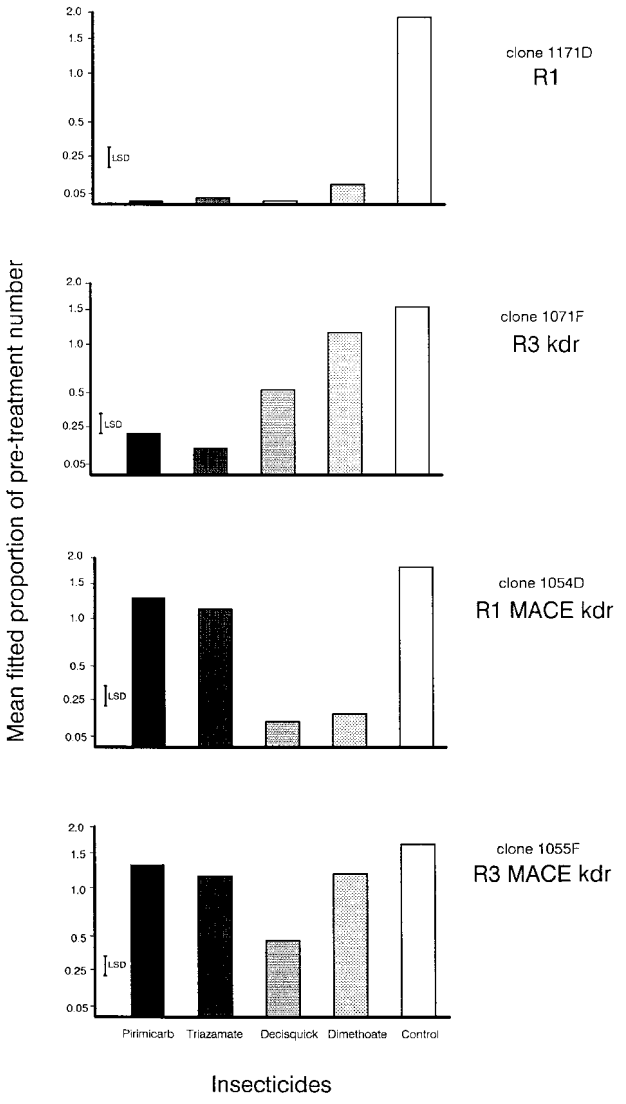

Figure 1. Mean proportions (of pre-treatment number) of aphids surviving three days after treatment with pirimicarb, triazamate, Decisquick and dimethoate, grouped by clone. Controls were untreated.

formed to $\log_e$, per clone per plant replicate were compared using ANOVA on the combined data for both experiments. Plant position (ie as replicate 1 to 5) was not associated with PTP. The data and results of analyses are presented in Fig 1 (grouped by clone) and Fig 2 (grouped by treatment).

3.1 Controls

All four clones showed statistically similar levels of increase (ie P was non-significant) during the post-treatment period in the absence of insecticide treatment (Fig 2).

3.2 Pirimicarb

Both MACE clones showed significantly greater survival ($P<0.001$), measured by PTP, than the clones without MACE (Fig 2). The contribution of the esterase-based mechanism could only be distinguished in the non-MACE clones, with R_3 aphids showing significantly greater survival ($P<0.001$). R_1 non-MACE aphids were readily controlled, as observed in previous field experiments.[11] The proportions of the MACE clones surviving were slightly less than in the untreated controls (R_1: $P<0.01$; R_3: $P<0.05$).

3.3 Triazamate

Both MACE clones showed a significantly higher survival ($P<0.001$) than the non-MACE clones despite the R_1-MACE having only moderate esterase levels (Fig 2). The patterns of resistance were very similar to those seen for pirimicarb although in the non-MACE clones, R_1 and R_3 were not significantly different from each other.

3.4 Deltamethrin + heptenophos (Decisquick)

Clone 1171D, which lacked kdr, was included to assess the level of resistance conferred by this mechanism in an R_1 esterase background. It is likely that kdr was the most influential factor behind the significant difference in resistance to deltamethrin + heptenophos, with all three kdr clones showing greater survival than clone 1171D (Fig 2). However, esterase level also had an impact as the R_1 kdr clone (1054D) was controlled significantly better than either of the R_3 kdr clones (1071F and 1055F, $P<0.01$) which were not significantly different from each other. As anticipated, the MACE mechanism did not confer resistance to deltamethrin + heptenophos.

3.5 Dimethoate

The esterase-based mechanism appeared to be the only significant factor influencing resistance to this insecticide, with both R_3 clones showing markedly greater survival than either of the R_1 clones ($P<0.001$) (Fig 2). Proportions of R_3 aphids surviving were only slightly less (1071F: $P<0.05$; 1055F: $P<0.01$) than their respective controls.

3.6 Relative efficacy of the four insecticide treatments

All four insecticides were effective against the R_1 non-MACE non-kdr clone (1171D) with no significant differences between treatments (Fig 1). Triazamate and pirimicarb were the most effective of the four insecticides against the R_3 non-MACE kdr clone (1071F) with dimethoate performing the worst. All treatments produced statistically different responses for this clone except triazamate compared with pirimicarb (Fig 1). Treatment with Decisquick and dimethoate showed the only significant differences against the R_1 MACE kdr clone (1054D), with both being statistically more effective than treatment with either pirimicarb or triazamate (Fig 1). All four insecticides showed poor control of the R_3 MACE kdr clone (1055F) with non-significant differences between dimethoate, pirimicarb and triazamate, whereas Decisquick performed marginally better (Fig 1).

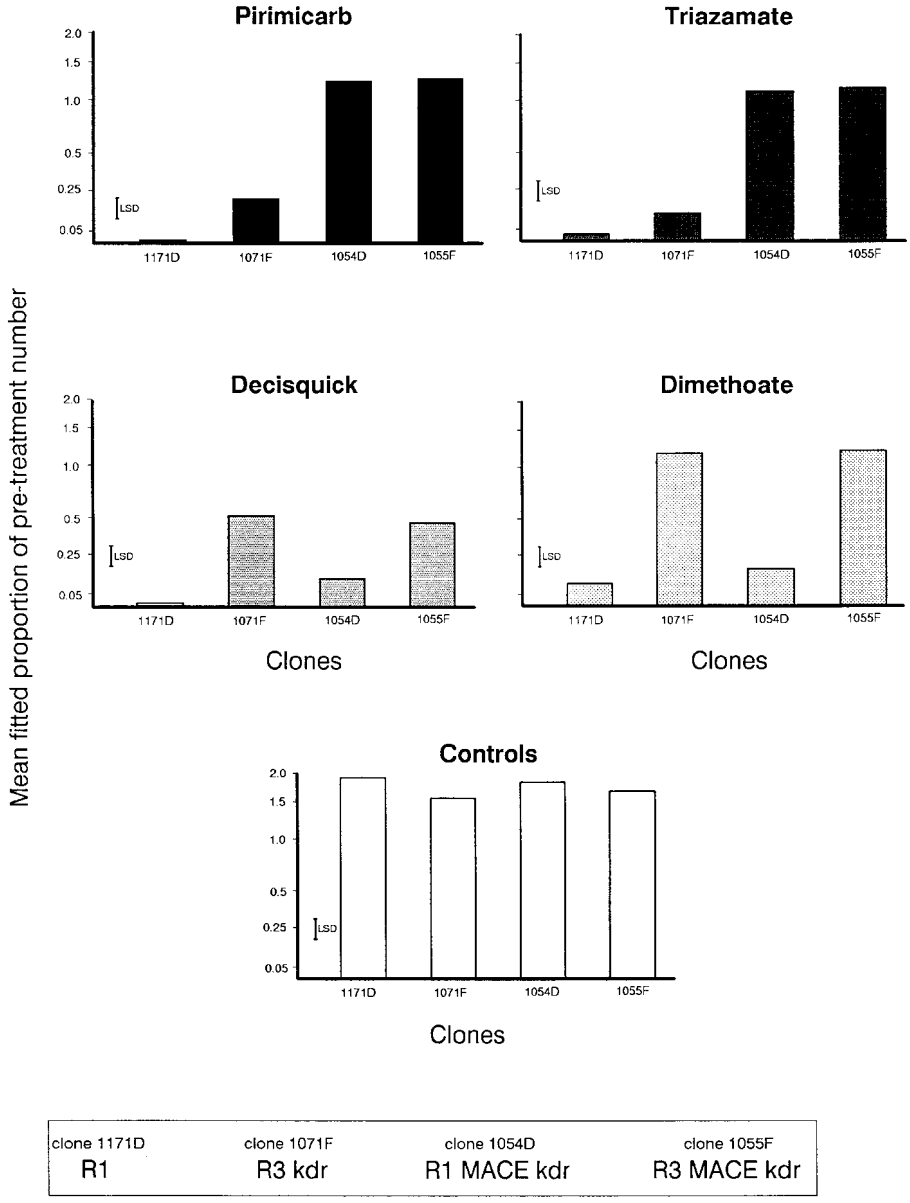

Figure 2. Mean proportions (of pre-treatment number) of aphids surviving three days after treatment with pirimicarb, triazamate, Decisquick and dimethoate, grouped by treatment. Controls were untreated.

4 DISCUSSION

These findings clearly show that the efficacies of insecticides commonly used against *M persicae* in the UK are very dependent on the resistance mechanisms present. They support previous bioassay data showing that MACE confers very strong resistance to pirimicarb and triazamate. The recent appearance of MACE aphids in the field, therefore, has serious implications for the future efficacy of these insecticides. Pirimicarb has become a favoured product in the UK, not only because it gives some control of aphids with high esterase-based resistance, but because it is also considered to be relatively benign to parasitoids and predators such as coccinellids and syrphids. These benefits will be lost if MACE becomes as well-established and widely distributed as the esterase-based mechanism. Continued biochemical monitoring of *M persicae* populations is therefore essential if pirimicarb and triazamate are to be used effectively in the field, both in terms of minimising immediate problems and delaying resistance build-up.

The MACE mechanism does not confer resistance to deltamethrin+heptenophos or dimethoate even though both act, at least in part (ie the heptenophos component of Decisquick), on the same target site as pirimicarb and triazamate. The efficacies of Decisquick and dimethoate are, however, compromised by esterase-based resistance and, in the case of the pyrethroid component of Decisquick, by the newly discovered kdr mechanism. Early indications are that this form of resistance is primarily responsible for pyrethroid resistance, but is strongly associated with high (R_2 and R_3) esterase-E4 gene copy number (but not FE4).[12] Having said this, wider monitoring of field populations is needed to establish the general incidence of kdr and its relationship with other resistance

mechanisms. There is, however, no doubt that the presence of kdr and esterase-based resistance, in combination with MACE, in a large proportion of *M persicae* samples collected in Lincolnshire in 1996[13] explains why many spray applications proved to be ineffective. Such control failures highlight the imperative need for new spray products with alternative modes of action that are unaffected by the esterase, MACE and kdr mechanisms.

Our data correlate well with field trial results[8] and highlight the relevance of field simulator-based studies and their value in predicting the consequences of different selection regimes on insect populations as a means of developing resistance management tactics.

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