BPC Final Report for SAPPIO-LINK Research Project LK 0903

Combating insecticide resistance in peach-potato aphids in the UK

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2 SUMMARY FOR GROWERS

2.1 PROJECT AIMS

The project was founded on extensive, long-term research into the nature and practical implications of resistance in *M. persicae* at IACR-Rothamsted and was aimed at strengthening the science base for managing the various forms of resistance in *M. persicae* across the major crops on which it occurs in the UK. Overall, it exploited proven and powerful tools for detecting several individual resistance mechanisms (ie. overproduced carboxylesterase, MACE, kdr and also tolerance to imidacloprid), singly and in combination, to complement existing research at Rothamsted funded by BBSRC and DEFRA into the nature and incidence of these mechanisms. Specifically, the project not only aimed to extend the scope and precision of aphid monitoring programmes but also to investigate the effectiveness, in the laboratory and the field, of established and novel aphicides against aphids having different combinations of the resistance mechanisms. Furthermore, it also aimed to explore possible fitness drawbacks associated with these mechanisms that could contribute to a natural reduction in resistance frequencies. Emphasis was placed on managing *M. persicae* on potatoes and vegetable brassicas; crops that had previously received little attention from resistance work.

2.2 WORK UNDERTAKEN AND KEY FINDINGS

UK surveys of the incidence of knock-down resistance (kdr), associated with resistance to pyrethroids, in peach-potato aphids (*Myzus persicae*) collected in a large number of samples taken from several key crops were done in 1997-2000. These showed that kdr is widespread in England but appeared to be less common in Scotland. It is likely, therefore, that over the past several years some applications of pyrethroids will have been compromised. A second related form of knock-down resistance, known as super-kdr, has recently been isolated in a *M. persicae* clone collected on Brussel sprouts in Lincolnshire in 1997. Laboratory-based spray experiments and bioassays, done as part of supporting work at IACR-Rothamsted, have shown that this newly-discovered mechanism is associated with high resistance to several pyrethroids. The contribution to pyrethroid resistance of super-kdr and other potential, but as yet undisclosed mechanisms, will be assessed in the near future.

The samples collected for the survey of kdr were also used in a second survey assessing imidacloprid resistance. This showed that very few (four out of 186 samples) contained aphids that were tolerant to imidacloprid. It has been previously shown that imidacloprid-tolerant forms are unable to survive imidacloprid applied as a foliar spray at the full recommended rate. However, experiments done on populations of *M. persicae* in field simulators demonstrated that these forms are more likely to survive and reproduce when imidacloprid (Confidor) is present at lower concentrations. Similar conditions are probably present in the field on sugar beet later in the growing season and on lettuce and ornamentals. These are probably imposing selection that could lead to greater resistance to imidacloprid in this species. Bioassays applying imidacloprid and acetamiprid against a large number of *M. persicae* clones, including those collected in the UK, demonstrated that these insecticides effectively circumvent the known esterase, MACE and kdr mechanisms.

Field and laboratory-based studies of potential fitness costs imposed under times of stress showed that *M. persicae* carrying either kdr or high (R_2 and R_3) levels of esterase resistance behave differently compared to non-kdr and low-esterase forms. Resistant aphids showed lower tendencies to respond to alarm pheromone. High-esterase forms were also less likely to move from deteriorating leaves at low temperatures. These potentially maladaptive behaviours, coupled with slower rates of reproduction in esterase-R_3 forms, are probably

imposing adverse selection against esterase resistance and kdr, and any other associated mechanism, in the UK when insecticide usage is reduced. Having said this, kdr does not appear to have declined dramatically in *M. persicae* collected from crops in England.

A range of UK-registered insecticides were sprayed at recommended application rates against populations of *M. persicae* carrying various combinations of esterase, MACE and kdr resistance, supported on either potato or Chinese cabbage in field simulators. Patterns of resistance were similar for *M. persicae* on both crops. MACE was found to confer extreme resistance to pirimicarb (Aphox) and triazamate (Aztec) (dimethyl-carbamate insecticides). kdr was associated with resistance to lambda-cyhalothrin (Hallmark/Karate), cypermethrin (Toppel)/Ambush C) and deltamethrin (Decis) (pyrethroid insecticides). Super-kdr was associated with effective immunity to lambda-cyhalothrin and this probably applies to other pyrethroids as well. Esterase level made an additional small contribution towards pyrethroid resistance. A mixture of pirimicarb plus lambda-cyhalothrin (Dovetail) was only effective against *M. persicae* lacking both kdr and high esterase resistance. None of the above insecticides was effective against *M. persicae* carrying MACE coupled with kdr resistance.

Full field experiments assessing the efficacy of different insecticide treatment regimes against non-MACE *M. persicae* populations on potato supported the field-simulator-based data. Foliar sprays of pirimicarb and pymetrozine were effective. However lambda-cyhalothrin did not control kdr forms.

A large number of *M. persicae* clones with various combinations of the different forms of insecticide resistance (esterase, MACE, kdr and neonicotinoid tolerance), collected from the UK and abroad, were assessed for resistance to the new compound, pymetrozine (Plenum), in leaf-dip bioassays. Pymetrozine was also applied as foliar sprays at the recommended rate and volume to aphid populations consisting of different UK *M. persicae* clones supported on potato plants in field simulators. Both studies produced no evidence of cross-resistance to this new product. Indeed, aphids with higher levels of esterase resistance appeared to be slightly, but still significantly, more susceptible to pymetrozine in the leaf-dip bioassays.

2.3 CONCLUSIONS

Potential fitness costs suffered primarily in the absence of insecticides by *M. persicae* carrying high-esterase resistance, coupled with improved resistance management by growers based on advice stemming from this and other related projects, probably underlie the steady decline in the frequency of these forms in the UK over the last several years. However, knock-down resistance (kdr) has apparently not shown a similar decline despite appearing to be closely associated with maladaptive behaviour. This has important implications for the use of pyrethroids for controlling this pest.

The esterase-based mechanism is associated primarily with resistance to organophosphates and mono-methyl carbamates. MACE confers strong resistance to pirimicarb and triazamate. Whereas kdr is associated with resistance to pyrethroids, although the picture is probably being complicated by the newly-discovered super-kdr mechanism (whose frequency in the UK *M. persicae* population is currently unknown).

Two neonicotinyl insecticides, imidacloprid and acetamiprid, effectively circumvent the esterase, MACE and kdr mechanisms. The presence of a few imidacloprid-tolerant *M. persicae* in samples collected from English field and glasshouse crops over the last several years, coupled with the finding that these forms show a slight fitness advantage at reduced rates of imidacloprid treatment, has important implications for the potential evolution and selection of more potent resistance to the neonicotinyls in the future.

The new insecticide, pymetrozine, is highly effective against *M. persicae* with any combination of esterase, MACE, kdr or imidacloprid-tolerance. It can therefore play an important role in managing *M. persicae* in conjunction with other currently effective UK-registered insecticides, such as pirimicarb and imidacloprid.

2.4 PRACTICAL RECOMMENDATIONS TO POTATO GROWERS

- Pirimicarb (Aphox)/triazamate (Aztec) or pymetrozine (Plenum) are likely to prove most effective against *M. persicae* on potatoes.
- However, pirimicarb and triazamate belong to the same chemical class, and both select for MACE resistance. They should therefore be alternated with pymetrozine and NOT each other.
- These selective insecticides should be used at the beginning of the growing season as they are least likely to harm beneficial predators and parasitoids.
- Do not make repeat applications of any insecticide if it appears not to work at full rate and it has been applied correctly. Use an alternative.
- Do not apply insecticides below label rates as this can lead to a subsequent increase in resistance problems.
- Mixtures of insecticides (whether formulated or tank mixes) are unlikely to delay the development of resistance in *M. persicae*.
- However, mixtures of a pyrethroid and an OP or carbamate may be justified to control the spread of virus or more than one pest on the same crop.
- If tank mixes are used for this purpose, the components should be from different chemical classes and be applied at the full recommended rates.
- Pyrethroids should only be used later in the growing season or specifically when caterpillars are present in the crop.
- Resistant forms of *M. persicae* can suffer greater mortality than susceptible ones during times of stress such as cold and wet winters.
- This adverse selection is probably helping to maintain esterase resistance (and associated MACE resistance) overall at manageable levels in the UK.

3. EXPERIMENTAL SECTION

3.1 INTRODUCTION

The capacity of insect pests to adapt to agricultural practices and develop resistance to insecticides now poses a severe challenge to effective crop protection worldwide. It not only threatens the sustainability, cost effectiveness and quality of production, but prompts greater and often *ad hoc* use of insecticides, which exacerbates possible side-effects of agriculture on the environment. In the UK, recent difficulties in controlling peach-potato aphids, *Myzus persicae* (Sulzer), exemplify such concerns. Due to its extreme polyphagy, *M. persicae* infests several key UK crops including potatoes, brassicas, lettuce and sugar beet, all of which still depend heavily on insecticides for aphid control. The shortage of approved active ingredients restricts opportunities to allocate chemicals between crops to reduce selection pressures. Hence resistance mechanisms to individual active ingredients are often under sustained and widespread selection which, unless alleviated by more judicious, scientifically-based insecticide use recommendations, seems bound to deplete the supply of effective control agents still further.

At least three distinct resistance mechanisms now occur in UK populations of *M. persicae*; overproduced esterase (conferring resistance to mono-methyl carbamates and organophosphates; Foster & Devonshire, 1999), MACE (conferring resistance specifically to the di-methyl carbamates, pirimicarb and triazamate; Moores *et al.*, 1994; Foster *et al.*, 1998) and knock-down resistance (kdr; associated with resistance to pyrethroids; Martinez *et al.*, 1999). Resistance to pyrethroids appears to be based on a number of factors including mutations at the kdr and super-kdr sites and other potential, but as yet undisclosed, mechanisms. There is also evidence of low resistance, better described as tolerance, to imidacloprid in this species in mainland Europe and the far east (Devine *et al.*, 1996). These mechanisms, described in detail in (Foster *et al.*, 2000), collectively have the potential to render almost all established aphicides ineffective. To avoid this, it was essential to investigate factors promoting or retarding the spread of such mechanisms, and to identify control regimes to contend with them singly or in combination. This required a detailed and focused programme of research, in close consultation with commodity representatives and advisors, so that results and recommendations could be co-ordinated and communicated rapidly to growers.

3.2 MATERIALS AND METHODS

This section is arranged in relation to the Project's Main Objectives (MO's) and Primary Milestones (PM's).

Investigate the incidence of the kdr mechanism in the UK and use the information to influence current decisions on the use of pyrethroids to circumvent the MACE mechanism (MO 1)

PM 1.1 Survey of the kdr mechanism in UK M. persicae populations

Assessments of the pyrethroid-specific kdr mechanism in UK field and glasshouse populations were made up until early 2001 (extending the MAFF-supported survey of esterase and MACE resistance done in 1996 and 1997). Live *M. persicae* samples, taken primarily from UK field potatoes, vegetable brassicas, oilseed rape and sugar beet, were assessed for kdr using diagnostic bioassays.

Diagnostic bioassays for kdr

Several aphid clones from each *M. persicae* sample were established and assessed for their kdr phenotype (either susceptible or resistant) using diagnostic-dose bioassays. These involved transferring young adult apterae of each clone to the abaxial surface of Chinese cabbage leaf discs (10 aphids per replicate leaf disc per clone) held on 1.1% agar in plastic tubs (3 cm in diameter), the lips of which had been coated with Fluon to prevent any escape. The aphids were left for 2 h to allow them to settle and then individually dosed by topical micro-application applying 200 ng of technical DDT in 0.25 µl of acetone (DDT was chosen because it has the same target site as pyrethroids but it also circumvents the esterase mechanism). Aphids were maintained under a 21^{o}C, 16 h light/8 h dark regime. Response was assessed after 24 h. Clones containing less than 5% dead and poorly co-ordinated aphids were scored as kdr-resistant. Previous work suggests that aphids from resistant clones collected from field and glasshouse crops (ie with wild origins) consistently carry at least one copy of the point mutation (causing the amino acid substitution leucine to phenylalanine) at the kdr site in the relevant sodium channel protein.

Investigate imidacloprid resistance in the UK to anticipate its likely impact on the performance of the neonicotinyl class of insecticides (MO 2)

PM 2.1 Identification of any increase in resistance to the chloronicotinyl class of insecticides

All the samples collected for the kdr survey (PM1.1) were also assessed for resistance to the neonicotinyl insecticide, imidacloprid using screening bioassays adapted from the diagnostic bioassays for kdr. Each assessment tested at least 30 young adult apterae of each clone (three replicates of 10 aphids). The aphids were individually dosed by topical micro-application applying 2.25 ng of technical imidacloprid in 0.25 µl of acetone and then maintained at 21^{o}C under a 16 h light/8 h dark regime. Aphid response was assessed after 72 h. Clones containing less than 90% dead and poorly co-ordinated aphids were scored as potentially imidacloprid-tolerant (im-t). The remaining clones were scored as potentially imidacloprid-susceptible (im-s). A number of im-t and im-s clones were subsequently assessed using full dose-range bioassays to confirm their level of imidacloprid resistance (see PM 3.5).

Establish the interaction of the MACE-, kdr- and esterase-based mechanisms, and how this will affect insecticide choice (MO 3)

These milestones assessed how manipulation of *M. persicae* populations with different spray regimes and soil treatments can affect aphid control and select for or against the various resistance mechanisms. This was done using a range of insecticides applied to aphids on potato and Chinese cabbage in field simulators; environments which allow close undisturbed observation and detailed sampling of aphids under quarantine conditions.

PM 3.1 Field simulator study to adapt established methods used in studies on sugar beet and oilseed rape for assessing resistance selection on potato and Chinese cabbage

A series of preliminary experiments on potato and Chinese cabbage were done to adapt the methods used in previous studies for assessing resistance selection against aphids. Four kdr-heterozygous (SR) *M. persicae* clones with English origins were used (**Table 1**).

Table 1. *Myzus persicae* clones used in simulator-based studies to adapt methods for assessing insecticide resistance.

		RESISTANCE MECHANISM		
Clone	Origin	Esterase[1]	MACE[2]	kdr[3]
2042H	England 1996	R_1	yes	SR
2043K	England 1996	R_3	yes	SR
2167J	England 1997	R_1	no	SR
2161C	England 1997	R_3	no	SR

[1]Based on an immunoassay.
[2]Based on a kinetic assay.
[3]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 10 individual aphids from each clone). All four clones were kdr-heterozygotes (SR).

Prior to assessment, the aphids were reared on excised Chinese cabbage leaves in small plastic boxes (maintained under a 16 h light/8 h dark photoperiod at 21°C). The aphid and plant rearing protocols were developed over four experiments for potato and four experiments for Chinese cabbage (see results section, PM 3.1).

PM 3.2 Field simulator study of resistance shown by recently-collected UK M. persicae clones (carrying various combinations of the esterase- and MACE-based mechanisms) to spray applications of established and novel insecticides

Three separate experiments were done on potato and three separate experiments on Chinese cabbage using the four *M. persicae* clones assessed in PM 3.1 (**Table 1**). In each experiment, plants were positioned in six simulators and treated with insecticides using the protocols established for potato and Chinese cabbage (see Results section, PM 3.1). Insecticides were applied as foliar sprays at the recommended field rates:

Pirimicarb (Aphox): 280 g product in 400 litres ha^{-1} (potato) and 420 g product in 400 litres ha^{-1} (Chinese cabbage)
Triazamate (Aztec): 400 mls product in 400 litres ha^{-1}
Lambda-cyhalothrin (Hallmark): 150 mls product in 400 litres ha^{-1} (potato) and 100 mls product in 400 litres ha^{-1} (Chinese cabbage)
Pirimicarb + lambda-cyhalothrin (Dovetail, 20 + 1): 1500 mls product in 400 litres ha^{-1}

In accordance with product recommendations, wetter (Agral) was added to the applications made to Chinese cabbage. Treatments and a control (untreated) were allocated to the simulators using an efficient, balanced as much as possible, randomised design in each experiment.

Pre- and post-spray counts (after 3 days) were made using the protocol described in results section for PM 3.1).

PM 3.3 Field simulator study as in 3.2 using UK M. persicae clones carrying various combinations of the esterase and kdr mechanisms

Esterase and kdr

Three separate experiments were done on potato and three separate experiments on Chinese cabbage using four *M. persicae* clones carrying different combinations of esterase and kdr resistance (**Table 2**).

Table 2. *Myzus persicae* clones tested in simulator studies assessing resistance conferred by the esterase and kdr mechanisms.

		RESISTANCE MECHANISM		
Clone	Origin	Esterase[1]	MACE[2]	kdr[3]
2167J	England 1997	R_1	no	SR
2161C	England 1997	R_3	no	SR
2922B	England 1998	R_1	no	no
2167C	England 1997	R_3	no	no

[1]Based on an immunoassay.
[2]Based on a kinetic assay.
[3]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 10 individual aphids from each clone). Both kdr clones were heterozygotes (SR).

In each experiment, plants were positioned in six simulators using the design described in the Results section, PM 3.1. Insecticides were applied as foliar sprays at the recommended field rates:

Pirimicarb (Aphox): 280 g product in 400 litres ha^{-1} (potato) and 420 g product in 400 litres ha^{-1} (Chinese cabbage)
Lambda-cyhalothrin (Hallmark): 150 mls product in 400 litres ha^{-1} (potato) and 100 mls product in 400 litres ha^{-1} (Chinese cabbage)
Cypermethrin (Toppel): 250 mls product in 400 litres ha^{-1}; deltamethrin (Decis): 300 mls product in 400 litres ha^{-1}
Pirimicarb + lambda-cyhalothrin (Dovetail, 20 + 1): 1500 mls product in 400 litres ha^{-1}

In accordance with product recommendations**,** wetter (Agral) was added to the applications made to Chinese cabbage. Treatments and a control (untreated) were allocated to the simulators using an efficient, balanced as much as possible, randomised design in each experiment.

Pre- and post spray counts (after 3 days) were made using the protocol described in results section, PM 3.1).

kdr and super-kdr

Two separate experiments were done on Chinese cabbage using four *M. persicae* clones carrying different kdr and super-kdr genotypes (**Table 3**).

Table 3. *Myzus persicae* clones tested in simulator studies assessing resistance conferred by the kdr and super-kdr mechanisms.

		RESISTANCE MECHANISM			
Clone	Origin	Esterase[1]	MACE[2]	kdr[3]	super-kdr[3]
800F	Italy 1978	R_3	no	SS	SS
2161C	England 1997	R_3	no	SR	SS
794J	England 1994	R_3	no	RR	SS
2169G	England 1997	R_3	no	SR	SR

[1]Based on an immunoassay.
[2]Based on a kinetic assay.
[3]Based on direct DNA sequencing of PCR-amplified sodium channel gene fragments from aphid genomic DNA.

In each experiment, plants were positioned in six simulators using the design described in the Results section, PM 3.1. Lambda-cyhalothrin (Hallmark; recommended rate: 100 mls product in 400 litres ha^{-1}) was applied as foliar sprays at field rate and 2x, 3x, 5x and 10x field rate.

In accordance with product recommendations**,** wetter (Agral) was added to the applications. Treatments and a control (untreated) were allocated to the simulators using an efficient, balanced as much as possible, randomised design in each experiment. Pre- and post spray counts (after 3 days) were made using the protocol described in results section, PM 3.1).

PM 3.4 Field simulator study of resistance of M. persicae clones showing susceptibility and low resistance to imidacloprid. Assess how applications of imidacloprid to compost can select for resistance

Two separate experiments were done on Chinese cabbage using four *M. persicae* clones showing various combinations of the esterase, MACE and kdr mechanisms and different levels of tolerance to imidacloprid (**Table 4**).

Table 4. *Myzus persicae* clones tested in simulator studies assessing imidacloprid efficacy.

Clone	Origin	RESISTANCE MECHANISM			
		Esterase[1]	MACE[2]	kdr[3]	Imidacloprid RF[4]
US1L	England 1974	S	no	no	1.0
3104A	England 1998	R_2	yes	SR	1.7
3104B	England 1998	R_1	yes	no	5.7
926B	Greece 1990	R_3	yes	no	10

[1]Based on an immunoassay.
[2]Based on a kinetic assay.
[3]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 10 individual aphids from each clone). The kdr clone was a heterozygote (SR).
[4]Resistance factor based on full dose-range bioassays applying imidacloprid (see section PM 3.5).

In each experiment, Chinese cabbage plants were positioned in six simulators using the protocol described in PM 3.1. Imidacloprid was applied as a soil drench at recommended and reduced field rates (down to one-sixteenth field rate). Each plant was treated with 100 mls distilled water containing either:

0 mg (control)
0.125 mg imidacloprid
0.25 mg imidacloprid
0.5 mg imidacloprid
1 mg imidacloprid
2 mg imidacloprid (recommended for young brassicas)

Survival and reproduction

Pre- and two post-spray counts (after 3 days and 7 days) were made using the protocol described in results section for PM 3.1).

Aphid movement from infestation leaves

The position of aphids (either on the initial infestation leaf used to support the clip cage or the rest of the plant) was recorded in each post-spray count.

PM 3.5 Laboratory bioassays to assess response of M. persicae clones with various combinations of resistance mechanisms to novel insecticides such as imidacloprid, acetamiprid and pymetrozine

Three separate sets of bioassays were done using either imidacloprid, acetamiprid or pymetrozine. Each bioassay used imidacloprid-susceptible (im-s) and imidacloprid-tolerant (im-t) *M. persicae* clones, collected from a wide range of localities from around the world, showing various combinations of esterase, MACE and kdr insecticide resistance. Each clone had been reared in the laboratory from an individual ancestral parthenogenetic female aphid on excised Chinese cabbage leaves in small plastic boxes, maintained under a 21°C, 16 h light/8 h dark regime. Clonal integrity was checked regularly using an immunoassay for

esterase resistance and a kinetic assay for MACE resistance. kdr status (either susceptible: S or resistant: R) was determined using diagnostic DDT bioassays (see section PM 1.1).

Full dose-range bioassays applying imidacloprid and acetamiprid

Leaf-dip bioassays using acetamiprid at a range of doses against three standard *M. persicae* clones (T1V, 926B and 1200Q) produced shallow dose-response slopes compared with topical micro-application assays. It was therefore decided to use the latter method for assessments of the efficacy of this insecticide and imidacloprid.

M. persicae clones collected from England (for this project) and other parts of the world (as part of an EU project and *ad hoc* sampling) were assessed in full dose range topical micro-application bioassays with imidacloprid and acetamiprid (24 clones with imidacloprid and 10 clones with acetamiprid). In each bioassay, young adult apterae (10 aphids per replicate leaf disc) were individually dosed with 0.25 µl of acetone containing either technical imidacloprid or acetamiprid at a range of concentrations. They were then maintained under a 21ºC, 16 h light/8 h dark regime and then assessed for their response after 72 h. Dead and poorly co-ordinated aphids were scored together as 'affected' and ED_{50} values calculated using probit analysis (POLO LeOra software). Results for each compound represent the pooled data for each clone of between four and eight separate bioassays each testing at least 120 aphids.

Full dose-range leaf-dip bioassays applying pymetrozine

Leaf-dip bioassays were done on 21 *M. persicae* clones. In each bioassay, 2 cm diameter leaf discs were cut from Chinese cabbage and dipped for 20 s in different dilutions of formulated pymetrozine (Plenum) ranging from 80 to 30,000 µg litre^{-1} (control discs were not dipped). They were then placed upside-down on a bed of 1.1% agar in plastic tubs (3 cm in diameter), the lips of which had been coated with Fluon to prevent subsequent aphid escape. For each clone in each bioassay, three replicates were set up at each pymetrozine concentration. The discs were allowed to dry for 2 h. Four young apterous adults were then transferred to each disc and left overnight at 21ºC to allow them to settle and produce offspring. The adults were then removed leaving up to 20 first instar nymphs on each leaf disc replicate. They were then maintained at 21ºC under a 16 h light/8 h dark regime. Nymph mortality was assessed 96 h after the adults had been initially transferred. EC_{50} values were calculated using probit analysis (POLO LeOra software). Results for each clone represent the pooled data of between four and eight separate bioassays each testing at least 120 aphids.

Assays assessing survival of nymphs seven days after exposure to pymetrozine

Additional assays were done to establish whether a longer endpoint would affect aphid survival, ie were surviving aphids capable of continuing to feed and develop after exposure to pymetrozine. This involved further leaf-dip bioassays on the 21 *M. persicae* clones using a range of pymetrozine concentrations. At the usual endpoint of 96 h, surviving nymphs were transferred with a fine paint brush to new, untreated leaf discs (10 aphids per leaf disc replicate). Assessments for survival were then made after a further 72 h.

PM 3.6 Field simulator study of resistance of UK M. persicae clones carrying various combinations of the esterase, MACE and kdr mechanisms to spray applications of pymetrozine (Plenum)

Two separate experiments were done using four *M. persicae* clones (**Table 5**) supported on potato. In each experiment, plants were positioned in six simulators using the design described in PM 3.1. Pymetrozine (Plenum) was applied as a foliar spray at the recommended field rate: 400g product in 400 litres ha^{-1}. Treatments or controls (untreated) were allotted to the simulators using an efficient, balanced as much as possible, randomised design.

Table 5. *Myzus persicae* clones tested in simulator studies assessing pymetrozine efficacy.

		RESISTANCE MECHANISM		
Clone	Origin	Esterase[1]	MACE[2]	kdr[3]
2160D	England 1997	R_1	no	SR
2161C	England 1997	R_3	no	SR
2042H	England 1996	R_1	yes	SR
2050A	Greece 1996	R_3	yes	SR

[1]Based on an immunoassay.
[2]Based on a kinetic assay.
[3]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 10 individual aphids from each clone). All four clones were kdr-heterozygotes (SR).

Pre- and post-spray counts (after 3 days and 10 days) were made using the protocol described in results section, PM 3.1).

Establish potential fitness drawbacks suffered by kdr forms in the absence of insecticides under sub-optimal conditions associated with the winter climate, either in their own right or through association with high esterase-based resistance (MO 4)

This part of the project was aimed at identifying any fitness costs associated with kdr and esterase resistance in the absence of insecticides that could limit resistance build-up in the UK. It involved over-wintering field experiments aimed at establishing whether kdr forms suffer a fitness disadvantage. It also involved laboratory-based studies assessing potential associations of kdr and esterase resistance with response to alarm pheromone and leaf deterioration.

PM 4.1 Collect suitable range of UK M. persicae clones for winter field study (PM 4.2) of survival of kdr and non-kdr forms and simulator studies of resistance selection by insecticides (PM 3.4 and 3.5)

M. persicae clones were successfully isolated from the UK samples received for Primary Milestones 1 and 2, and placed into laboratory culture for inclusion in the field and laboratory studies.

PM 4.2 Complete winter field study of potential fitness drawbacks suffered by kdr forms of M. persicae

Four separate field experiments were done to assess aphid survival, development and behaviour during the winter months in late 1998 and early 1999. Each experiment used *M. persicae* clones of UK origin showing various combinations of kdr and esterase resistance (**Table 6**).

Table 6. *Myzus persicae* clones used in the 1998/1999 winter field experiments.

Clone	**RESISTANCE MECHANISM** Esterase[1]	MACE[2]	kdr[3]	Esterase gene[4]	UK origin
US1L	S	S	SS	S	Cambridgeshire, May 1974
1076A	S	S	SS	S	Hertfordshire, Sept 1992
2591C	S	S	SS	S	Scotland, July 1998
405D	R_1	S	SS	FE4	Cambridgeshire, July 1977
1321D	R_2	S	SS	FE4	Herefordshire, Oct 1997
2141A	R_2	S	SS	FE4	Herefordshire, July 1997
2165B	R_2	S	SS	FE4	Herefordshire, Sept 1997
2829A	R_2	S	SS	FE4	Dundee, Aug 1998
2158A	R_3	S	SS	FE4	Staffordshire, Aug 1997
2167C	R_3	S	SS	FE4	Essex, Sept 1997
946E	R_2	S	SR	E4	Norfolk 1992
1302M	R_2	S	SR	FE4	Lincolnshire, Oct 1997
2167J	R_2	S	SR	FE4	Essex, Sept 1997
2567D	R_2	S	SR	FE4	Shropshire, July 1998
2169G	R_3	S	SR	E4	Lincolnshire, Oct 1997
948D	R_3	S	SR	E4	Bedfordshire, March 1991
2161C	R_3	S	SR	E4	Lancashire, Sept 1997
2163E	R_3	S	SR	E4	Derbyshire, Sept 1997
794J(rev)	S(Rev)[5]	S	RR	E4	Worcestershire, March 1982
923A(rev)	S(Rev)[5]	S	RR	E4	Hertfordshire, January 1987
T1V	R_3	S	RR	E4	Bedfordshire, June 1975
2165A	R_3	S	RR	E4	Herefordshire, Sept 1997
794J	R_3	S	RR	E4	Worcestershire, March 1982
923A	R_3	S	RR	E4	Hertfordshire, January 1987
951A	R_3	S	RR	E4	Bedfordshire, Oct 1989
2043B	R_3	S	RR	E4	Lincolnshire, Oct 1996

[1]S: susceptible, R_1: moderately resistant, R_2: highly resistant, R_3: extremely resistant forms.
[2]S: sensitive form.
[3]genotype determined by direct sequencing of sodium channel fragments amplified from single aphids.
[4]amplified esterase gene.
[5]revertant clone carrying unexpressed, highly amplified E4 genes, ie. phenotypically indistinguishable from esterase-susceptible clones with respect to esterase production.

Experimental clones

Experimental clones were reared from lines that had been maintained at 21ºC under a 16 h light/8 h dark regime as virginoparous, predominantly apterous colonies on excised Chinese cabbage leaves in small plastic boxes. Clonal integrity was checked regularly using biochemical and molecular diagnostics.

Inoculation of field plots

The field experiments used winter oilseed rape. Seeds were drilled at a density of 80/m^2 in early September 1998. The field plan consisted of three blocks. Each block contained 28 sub-plots (each 4 m x 1 m). For each experiment, the 26 aphid clones were allocated randomly to a 1 m strip of the sub-plots within each block. Two control sub-plots were not infested. New strips were used for each experiment.

Prior to each field infestation, aphids were reared in sufficient numbers to supply three replicates of 50 first/second instar nymphs per clone (150 nymphs in total per clone per experiment). Nymphs for each replicate were reared separately by allowing six young apterous adults to reproduce on the underside of an excised Chinese cabbage leaf in a small plastic box for 3 days at 21^{o}C under a 16 h light/8 h dark regime. The adults and surplus nymphs were then removed. The remaining undisturbed nymphs were acclimated at 14^{o}C for 20 hours, and then 5^{o}C for a further 20 hours both at a photoperiod matching the natural day length at the time of the experiment.

The oilseed rape and surrounds were sprayed with pirimicarb two weeks prior to the first experiment (in November 1998) to eliminate any indigenous aphids. This treatment appeared to have been successful as no aphids were recovered from the uninfested control sub-plots and all retrieved aphids showed the expected resistance phenotypes in each sub-plot.

Clones were introduced to each 1 m^2 sub-plot (one replicate per sub-plot) by securing the excised leaf, carrying the 50 nymphs, onto a healthy 'infestation leaf' on the central plant in the plot using 4 cm hair clips placed at the petiole and apex. This method of infestation reduced aphid disturbance to a minimum and had been used successfully in previous field studies (Foster *et al.*, 1996).

Collection of aphids at the end of each experiment

Each experiment lasted between three and four weeks. Experimental plants from each sub-plot were then brought back to the laboratory in three separate bags containing the original infestation leaf (plus the deteriorated excised leaf) from the central plant, the rest of that plant and the immediately surrounding plants (within a circular area of approximately 40 cm diameter). Previous winter field experiments using similar methods have shown that *M. persicae* are highly unlikely to move to the outer surrounding plants over a similar duration (Foster *et al.*, 1996). The sampled material was stored in the dark at 4^{o}C to arrest aphid development before examination over the next few days. Developmental stage and categorical position within each 1 m^2 sub-plot were recorded for every aphid retrieved. A proportion of the aphids from each replicate were subsequently tested for esterase and MACE resistance status by immunoassay.

Measurement of meteorological variables

Field temperatures were measured during the course of each experiment using an automatic weather station designed to record values every 30 min from thermocouples clipped to the underside of oilseed rape leaves. Rainfall and windspeed were recorded at the Rothamsted Meteorological Station situated about 1 km from the field plots.

Statistical analysis

All analyses involving proportions used probit regression. Analyses of proximity of aphid retrieval and development involved categorical data and used log-linear models.

Assess forms of resistance to pirimicarb, triazamate and pyrethroids in *Aphis gossypii* and *Aphis nasturtii* which can both be important pests on potatoes and horticultural crops

The Project Steering Group decided that this would be superseded by new milestones as no aphids from these species were collected in the UK during the course of the project.

Field trials to assess options for aphid control by insecticides (MO 6)

PM 6.1 Complete field trials testing laboratory-based findings on insecticides choice for controlling M. persicae

Rothamsted-based simulator experiments and bioassays were complemented by ADAS-run field studies on potato using various treatment regimes primarily on augmented populations consisting of esterase-R_1/kdr-SR *M. persicae*.

Additional primary milestones

It was decided that repeating the winter field experiments (done in the first year) in the hope that they would coincide with more stressful weather conditions would be to the detriment of other milestones. The decision was taken by the Project Steering Group that potential fitness costs associated with kdr and esterase resistance should be determined in the laboratory under two additional primary milestones studying aphid response to alarm pheromone and movement from deteriorating leaves at low temperature. These studies provided a means of testing potential associations with each resistance mechanism independently.

PM 4.3 Carry out laboratory study of response of kdr and non-kdr M. persicae clones to synthetic alarm pheromone applied at a wide range of concentrations

Aphid clones and diagnosis of resistance

Experiments used 14 non-MACE *M. persicae* clones, collected from England and mainland Europe, showing different combinations of the three kdr genotypes (kdr-SS: susceptible homozygote, kdr-SR: heterozygote and kdr-RR: resistant homozygote) primarily with R_2 and R_3 levels of esterase resistance (**Table 7**).

Table 7. *Myzus persicae* clones assessed in alarm pheromone response bioassays.

Clone	**RESISTANCE MECHANISM** Esterase[1]	kdr[2]	Esterase gene[3]	Origin
2141A	R_2	SS	E4	England 1997
1260R	R_2	SS	FE4	Greece 1994
1302M	R_2	SR	FE4	England 1996
2165C	R_2	SR	FE4	England 1997
100N	R_2	SR*	-	lab cross
T1V	R_2	RR	E4	England 1975
800F	R_3	SS	FE4	Italy 1978
1190A	R_3	SS	FE4	Spain 1993
2161C	R_3	SR	E4	England 1997
2169G	R_3	SR	E4	England 1997
108T	R_3	SR*	-	lab cross
794J	R_3	RR	E4	England 1982
2043B	R_3	RR	E4	England 1996
794J(rev)[4]	R_3 gntp/S phtp	RR	E4	England 1994 .

[1]S: susceptible, R_1: moderately resistant, R_2: highly resistant, R_3: extremely resistant forms.
[2]genotype determined by direct sequencing of sodium channel fragments amplified from single aphids. *****kdr-SR clone that is susceptible to DDT produced in laboratory cross.
[3]amplified esterase gene.
[4]revertant clone carrying unexpressed, highly amplified E4 genes, ie. phenotypically indistinguishable from esterase-susceptible clones with respect to esterase production.

Alarm pheromone bioassay

Aphid response to the synthetic alarm pheromone, (*E*)-*β*-farnesene, applied at a wide range of concentrations was assessed in the absence of insecticides in seven separate experiments. For each clone tested, six first instar nymphs were obtained from laboratory stocks and grown to adulthood (two small plastic boxes containing three aphids per clone). These first generation (G_1) adults were removed after they had produced approximately 30 G_2 offspring per box (normally after about 10 days). The G_2 aphids were grown to adulthood and transferred, using a fine paint brush, to 2 cm diameter Chinese cabbage discs (4 G_2 adult apterae per disc) held on 1.1% agar inside plastic tubs. The plastic tubs were left at 21°C overnight to allow the aphids to settle and produce nymphs. The adults were removed next morning leaving synchronised cohorts of settled offspring on each disc. Each replicate cohort was then assessed for response to alarm pheromone by applying a 1 μl droplet (ranging from 10 to 0.0001 mg (*E*)-*β*-farnesene ml^{-1} in hexane) to the central part of each leaf disc with a fine-needle syringe. Aphid behaviour was then observed for 2 minutes using a binocular microscope (preliminary experiments showed that this period is sufficient to allow all responses to occur). Nymphs that withdrew their stylets and walked away were scored as responding. Control treatments with 1 μl droplets of hexane alone did not stimulate aphid movement. Each replicate batch of nymphs was tested once and then discarded.

Statistical analysis

Generalized linear models were fitted to the proportions of aphids in each replicate responding to alarm pheromone, including effects for clones, experiments, kdr genotype and mean carboxylesterase activity, using probit transformation.

PM 4.4 Low temperature laboratory study of aphid movement from deteriorating leaves using kdr and non-kdr M. persicae clones

Seven separate experiments were done using 22 non-MACE *M. persicae* clones, collected from the UK and mainland Europe, showing different combinations of the three kdr genotypes (kdr-SS: susceptible homozygote, kdr-SR: heterozygote and kdr-RR: resistant homozygote) with S, R_1, R_2 and R_3 levels of esterase resistance (**Table 8**).

Table 8. *Myzus persicae* clones assessed in low temperature movement study.

Clone	**RESISTANCE MECHANISM**		Esterase gene[3]	Origin
	Esterase[1]	kdr[2]		
US1L	S	SS	S	England 1974
1076A	S	SS	S	England 1992
405D	R_1	SS	FE4	England 1977
2160D	R_1	SR	FE4	England 1997
2141A	R_2	SS	FE4	England 1997
1260R	R_2	SS	FE4	Greece 1994
2165B	R_2	SS	E4	England 1997
1302M	R_2	SR	FE4	England 1996
2165C	R_3	RR	E4	England 1997
100N	R_2	SR*	-	lab cross
T1V	R_2	RR	E4	England 1975
800F	R_3	SS	FE4	Italy 1978
1190A	R_3	SS	FE4	Spain 1993
2161C	R_3	SR	E4	England 1997
2169G	R_3	SR	E4	England 1997
108T	R_3	SR*	-	lab cross
794J	R_3	RR	E4	England 1982
2043B	R_3	RR	E4	England 1996
794Jrev[4]	R_3 gntp/S phtp	RR	E4	England 1982
923A[4]	R_3 gntp/S phtp	RR	E4	England 1990
4106A	S	SS	S	Scotland 2000
4090A	S	SS	S	Scotland 2000

[1]S: susceptible, R_1: moderately resistant, R_2: highly resistant, R_3: extremely resistant forms.

[2]genotype determined by direct sequencing of sodium channel fragments amplified from single aphids. *****kdr-SR clone produced in laboratory cross that is susceptible to DDT.

[3]amplified esterase gene.

[4]revertant clone carrying unexpressed, highly amplified E4 genes, ie. phenotypically indistinguishable from esterase-susceptible clones with respect to esterase production.

Movement assays

Aphid movement from deteriorating excised Chinese cabbage leaves onto oilseed rape plants was assessed in seven separate experiments at low temperature (using one replicate per clone per experiment). Prior to each experiment, *M. persicae* clones were reared at 21ºC under a 16 h/8 h light/dark regime in small plastic boxes. In each clone replicate, four young apterous adults were allowed to produce nymphs over two days on an excised Chinese cabbage leaf. All adults and surplus nymphs were then removed before the boxes, each containing 50 first/second instar nymphs, were transferred to 10ºC (8 h/16 h light/dark regime) for 24 h temperature acclimation. Clones were then allocated (one replicate per plant) to five-week old plants (maintained in a 7ºC, 8 h/16 h light/dark regime) arranged in a single row using an incomplete block design. Movement from each deteriorated excised leaf was assessed five days after it had been fastened by the petiole and apex to the upper face of a healthy oilseed rape leaf using 4 cm hair clips. Numbers of aphids that moved to any part of the plant or remained on the deteriorated excised leaf were recorded at the end of each experiment.

Statistical analysis

Generalized linear models were fitted to the proportions of aphids in each replicate moving from their excised deteriorating leaves, including effects for clones, experiments, (kdr genotype and mean carboxylesterase activity), using probit transformation.

PM 6.1 Field trials testing laboratory-based findings on insecticides choice for controlling M. persicae

See report attached in Appendix A.

1.2 RESULTS

Investigate the incidence of the kdr mechanism in the UK and use the information to influence current decisions on the use of pyrethroids to circumvent the MACE mechanism (MO 1)

PM 1.1 Survey of the kdr mechanism in UK M. persicae populations

Maps showing the frequency and distribution of the *M. persicae* field and glasshouse samples containing and not containing kdr forms in 1997 (data collected through MAFF-funded project), 1998, 1999 and 2000 are presented in **Figures 1 – 5**. It would appear that the frequency of English field samples containing kdr forms has remained relatively high and stable over the last several years (**Figure 6**). Furthermore, kdr was present in 8 of the 9 glasshouse samples (**Figure 5**). The limited number of Scottish and Welsh field samples were too limited to generalise about the frequency of kdr in these regions (**Figure 7**).

Figure 1. kdr forms of *Myzus persicae* in 1997 field samples.

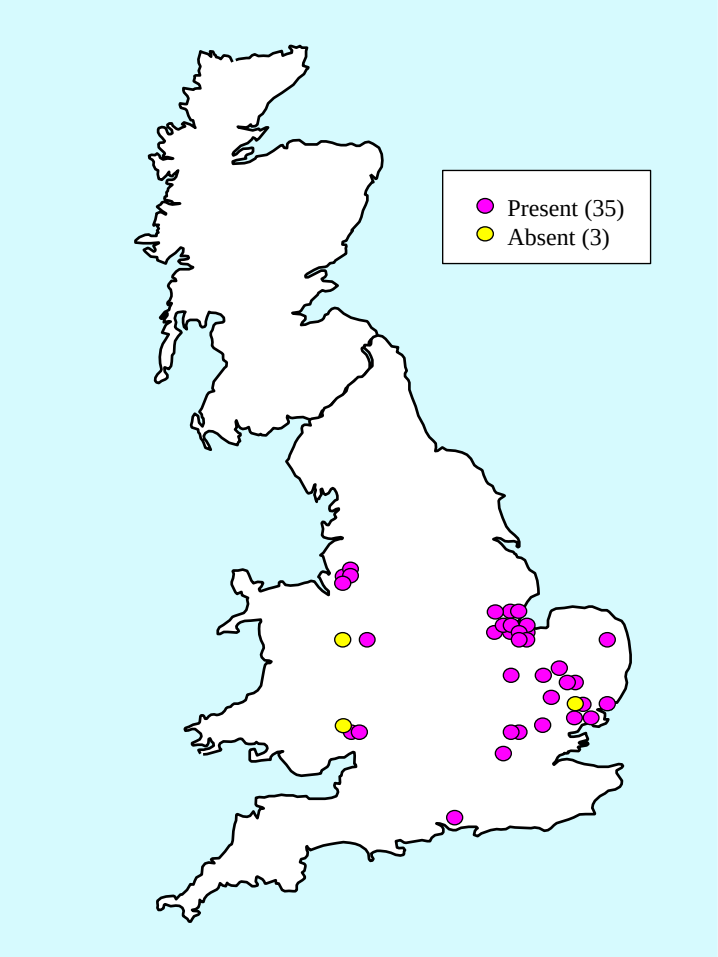


Figure 2. kdr forms of *Myzus persicae* in 1998 field samples.

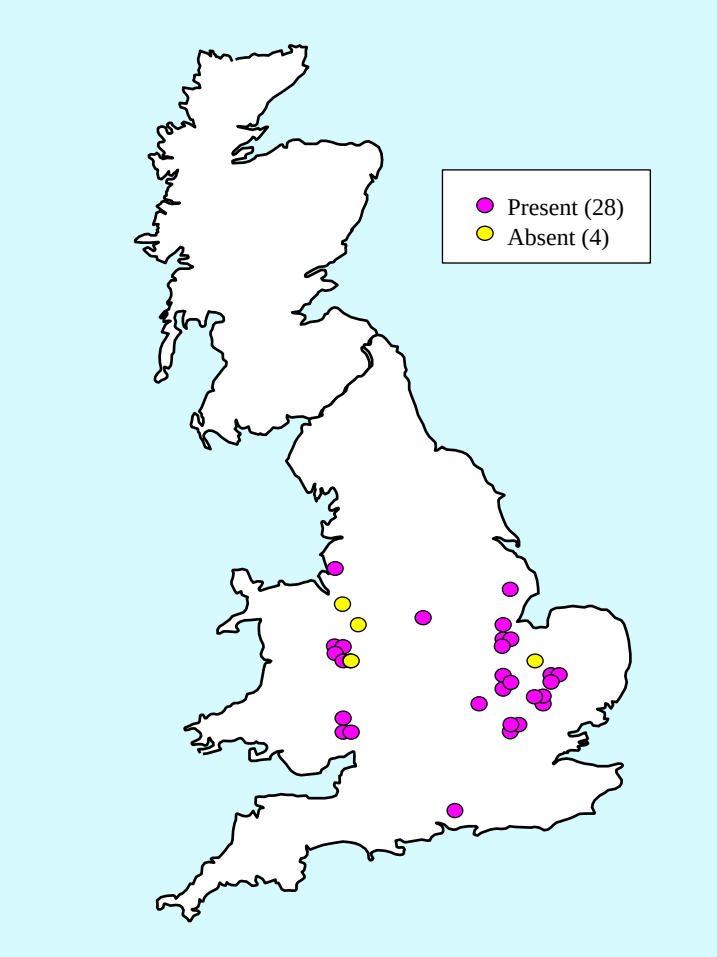


Figure 3. kdr forms of *Myzus persicae* in 1999 field samples.

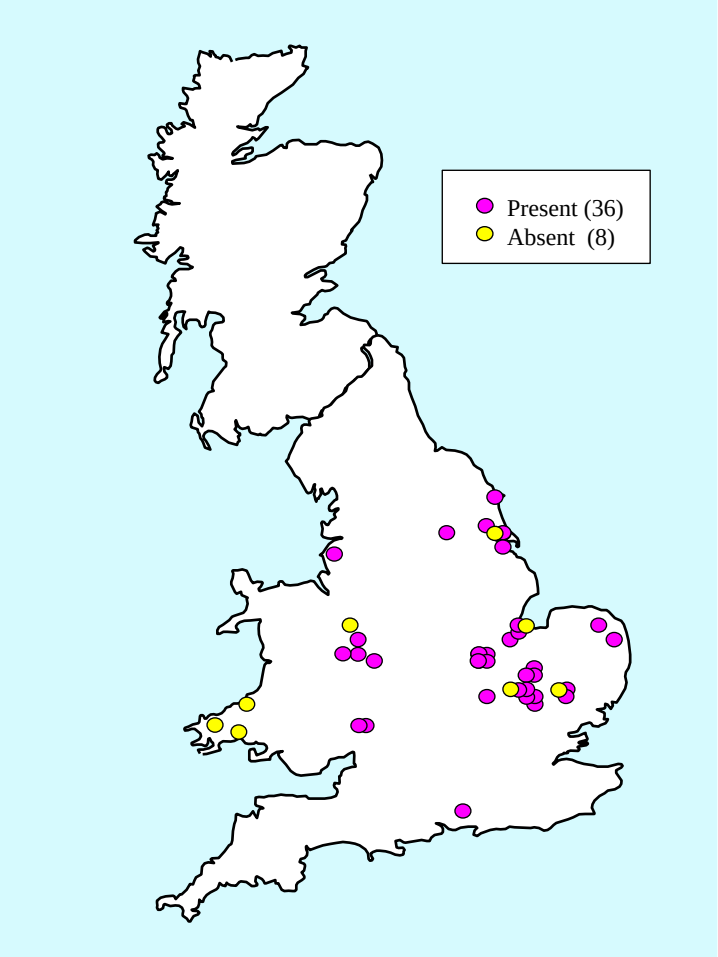


Figure 4. kdr forms of *Myzus persicae* in 2000 field samples.

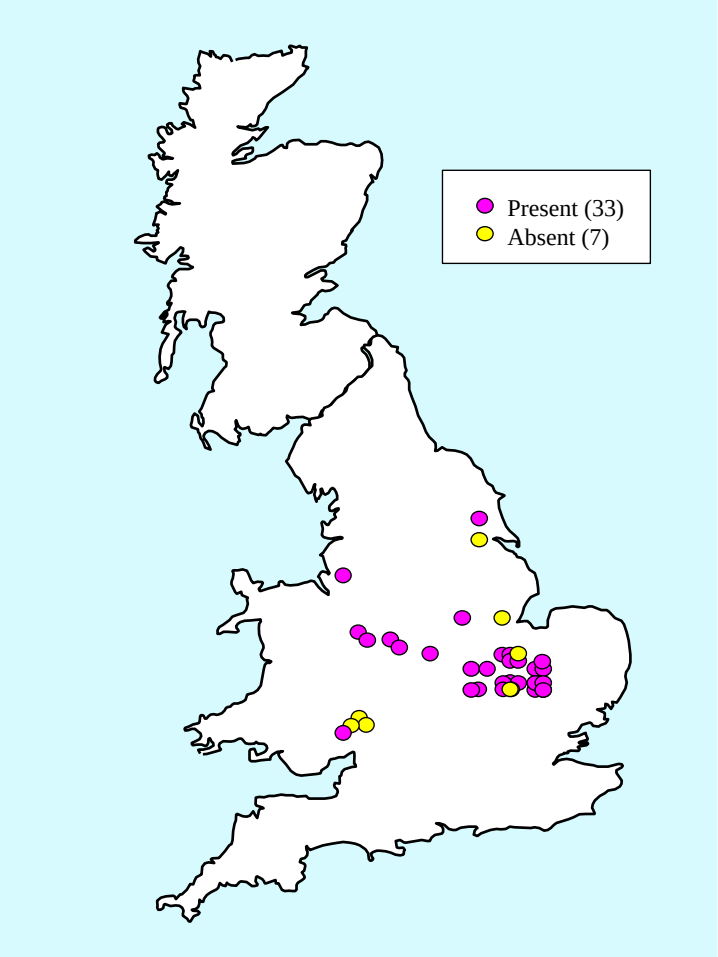


Figure 5. kdr forms of *Myzus persicae* in 1997-2000 glasshouse samples.

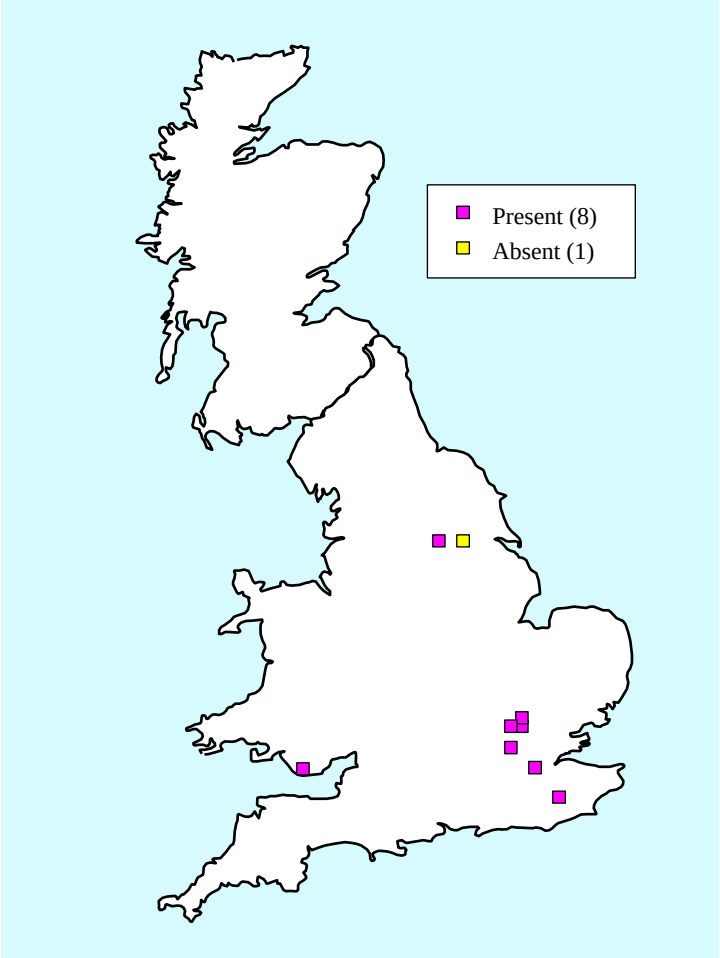


Figure 6. Percentage of *Myzus persicae* samples in 1997-2000 from England and Wales that contained kdr forms.

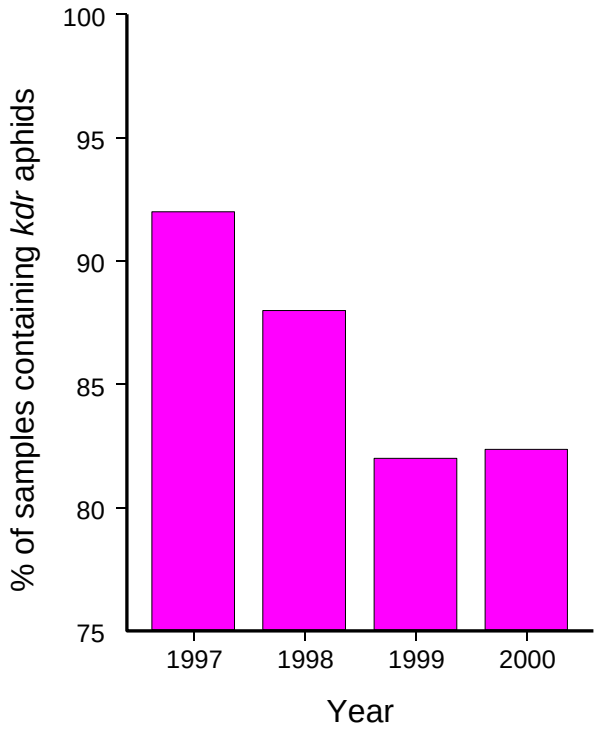
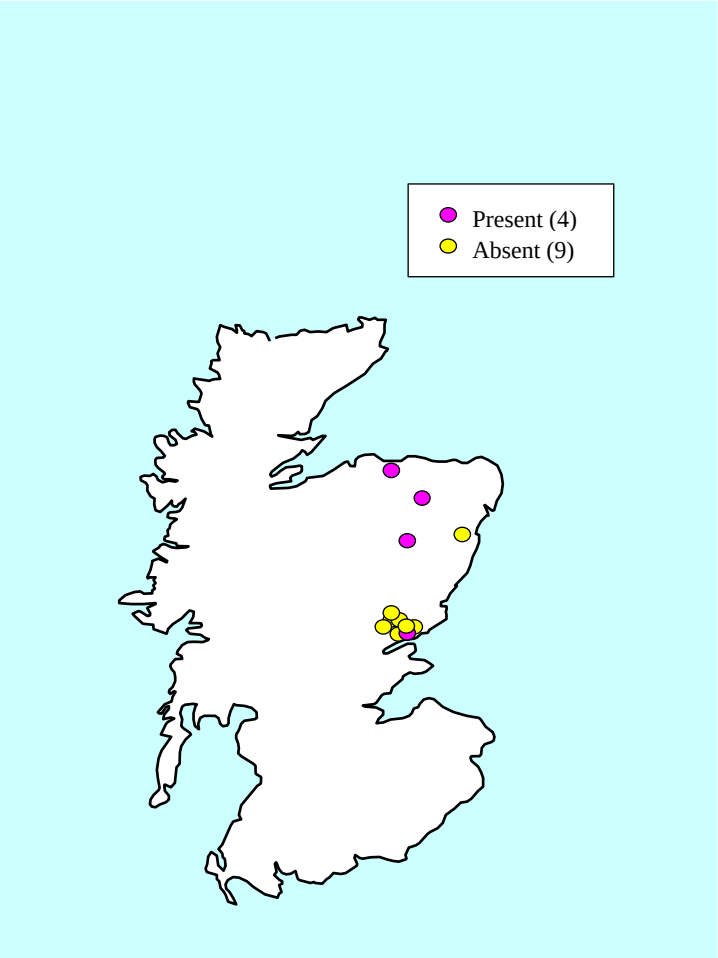


Figure 7. kdr forms of *Myzus persicae* in 1998-2000 Scottish field samples.



kdr genotypes were determined for some of the clones established from the field samples. All of these clones showing a kdr phenotype (ie resistant to the diagnostic dose of DDT) proved to have a kdr-SR or –RR genotype. Furthermore, all clones showing non-kdr phenotypes (ie DDT-susceptible) consistently had kdr-SS genotypes. The data suggest that kdr heterozygotes (SR) were the commonest forms in the field (**Table 9**).

Table 9. Association of kdr phenotypes with kdr genotypes in UK *M. persicae* clones that were DNA-sequenced.

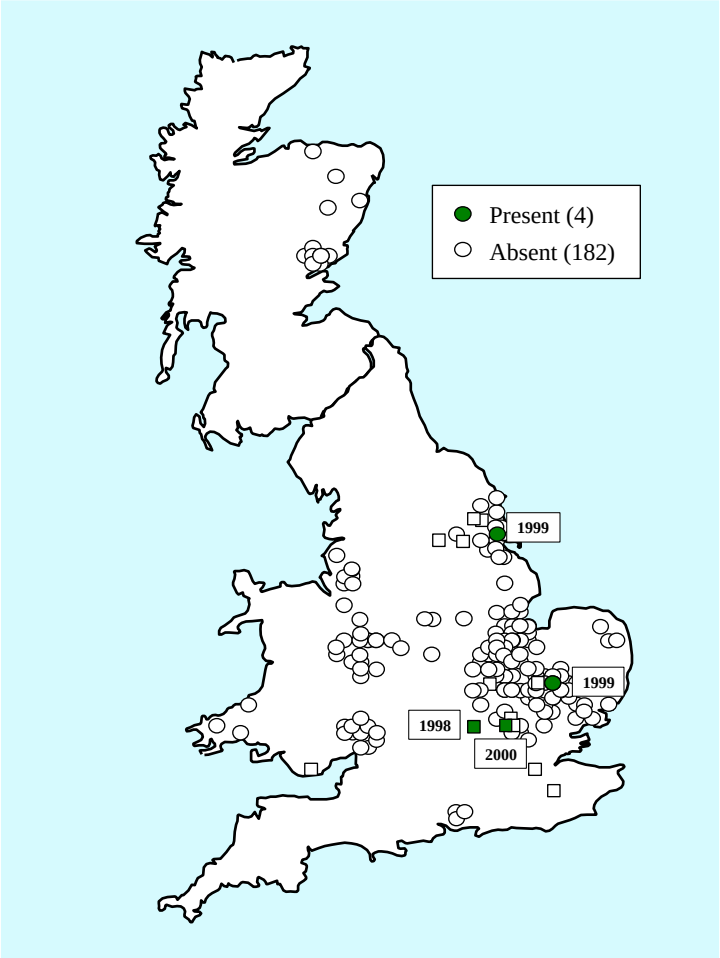
	kdr genotype		
	SS	SR	RR
non-kdr	11	0	0
kdr	0	26	3

Investigate imidacloprid resistance in the UK to anticipate its likely impact on the performance of the neonicotinyl class of insecticides (MO 2)

PM 2.1 Identification of any increase in resistance to the neonicotinyl class of insecticides

Screening bioassays revealed that imidacloprid tolerance (im-t) is present in the UK but at very low frequencies. Since 1997, four *M. persicae* samples containing im-t clones were identified out of a total of 186 samples (2.2%) (**Figure 8**). One sample in 1998, two samples in 1999 and one sample in 2000. Subsequent full dose range assessments of these clones, along with standard clones and clones collected from abroad are described in detail in section PM 3.5.

Figure 8. Imidacloprid tolerance in 1997-2000 UK field (○) and glasshouse (□) samples.



Establish the interaction of the MACE-, kdr- and esterase-based mechanisms, and how this will affect insecticide choice (MO 3)

PM 3.1 Field simulator study to adapt established methods used in studies on sugar beet and oilseed rape for assessing resistance selection on potato and Chinese cabbage

The aphid and plant rearing protocol and the insecticide application protocol are described below.

Aphid and plant rearing protocol

Plants (potato or Chinese cabbage) were grown in compost in individual pots and transferred after two weeks to the field simulators each of which were divided into four sections by two strips of tape (crossed at right-angles) coated with insect-trapping adhesive (Oecotak) positioned along the floor and lower walls (to eliminate aphid movement). Several yellow insect-trapping cards were also put into each simulator to attract any flying alatae (although very few tended to be produced). Each simulator housed 12 plants arranged into four groups of three plant replicates (one group per section). The four *M. persicae* clones used in each experiment were allocated to each of the six simulators, one clone per group of three plant replicates in each corner section, using an efficient, balanced as much as possible, randomised design. Each plant was initially inoculated with founder aphids from the relevant clone using a small clip-cage; introducing three young adult apterae on one of the upper-most leaves for potato and two young adult apterae on the first leaf for Chinese cabbage. The clip cages were then removed after 24 hours leaving the settled aphids on the underside of each inoculation leaf.

Insecticide application protocol

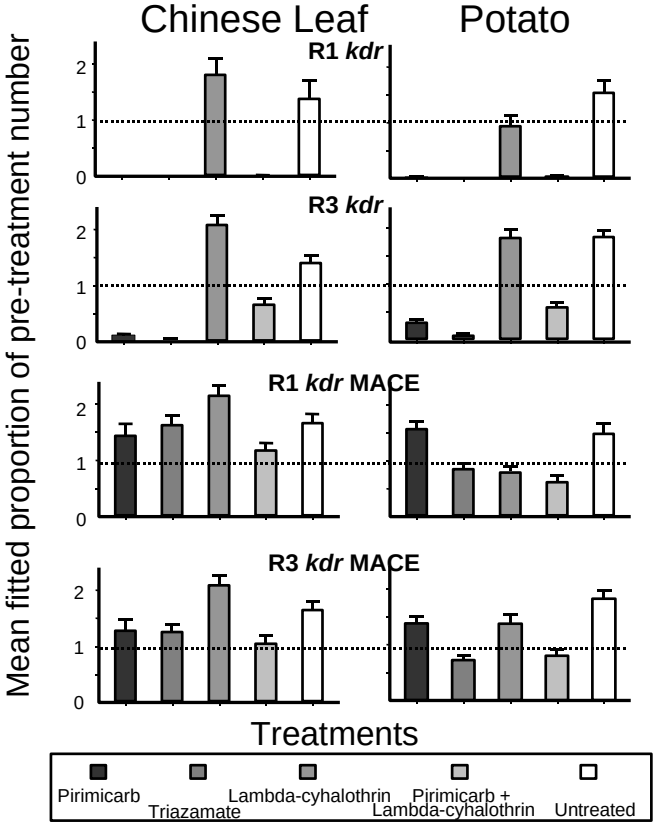
Aphid numbers for each plant replicate (both adults and nymphs) were recorded two weeks and one week respectively after inoculation onto potato and Chinese cabbage using a hand-held counter. Insecticide treatments were then applied as either aerosols or soil applications. In each experiment, several insecticide treatments and an untreated control were allotted to the simulators using a an efficient, balanced as much as possible**,** randomised design (one treatment per simulator). Air movement was reduced for a short period after treatment by turning the fans servicing each simulator off for 30 minutes. Counts of live aphids (both adults and nymphs) on each plant were made three days after treatment. Mean post-treatment proportions (PTP's) relative to the pre-treatment number were calculated per clone per plant replicate. The resulting experimental data were analysed statistically using generalised linear models.

PM 3.2 Field simulator study of resistance shown by recently-collected UK M. persicae clones (carrying various combinations of the esterase- and MACE-based mechanisms) to spray applications of established and novel insecticides

The data and results of the analyses of the mean post-treatment proportions (PTP's) on each host plant (grouped by clone) are presented in **Figure 9**. Each *M. persicae* clone showed a similar pattern of resistance across both host plants. There were no statistical differences in PTP's amongst the clones on either potato or Chinese cabbage in the absence of insecticide treatment (P = NS). Pirimicarb and triazamate were only effective against the R_1/kdr and R_3/kdr clones although the R_3/kdr clone showed a slight but significantly greater ($P < 0.05$) PTP after

treatment compared with the R_1/kdr clone. Both these insecticides were ineffective against the two MACE clones. The mixture of lambda-cyhalothrin and pirimicarb was only effective against the R_1/kdr clone. Lambda-cyhalothrin was not effective against any of the four clones. Indeed, on Chinese cabbage the R_3/kdr and R_1/kdr/MACE clones both showed significantly higher PTP's relative their untreated aphid controls.

Figure 9. Numbers (expressed as proportions of pre-treatment number) of *Myzus persicae* three days after treatment with various insecticides in field simulators. Four aphid clones, showing various combinations of esterase and MACE resistance, were tested.

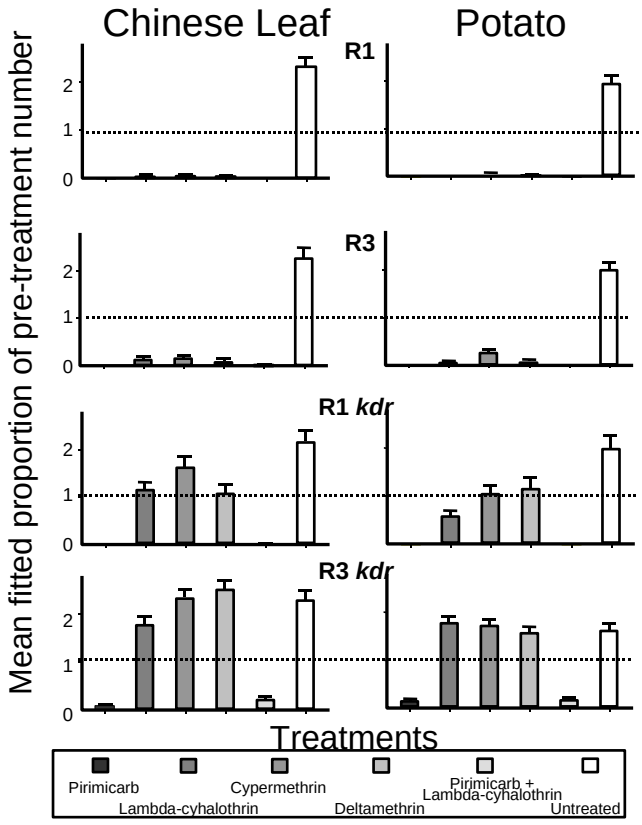


PM 3.3 Field simulator study as in 3.2. using UK M. persicae clones carrying various combinations of the esterase and kdr mechanisms

Esterase and kdr

The data and results of the analyses for PTP's on each host plant (grouped by clone) are presented in **Figure 10**. As in PM 3.2, each clone showed similar patterns of resistance across both host plants. There were also no significant differences between clonal PTP's on either host plant in the absence of insecticide treatment.

Figure 10. Numbers (expressed as proportions of pre-treatment number) of *Myzus persicae* three days after treatment with various insecticides in field simulators. Four aphid clones, showing various combinations of esterase and kdr resistance, were tested.



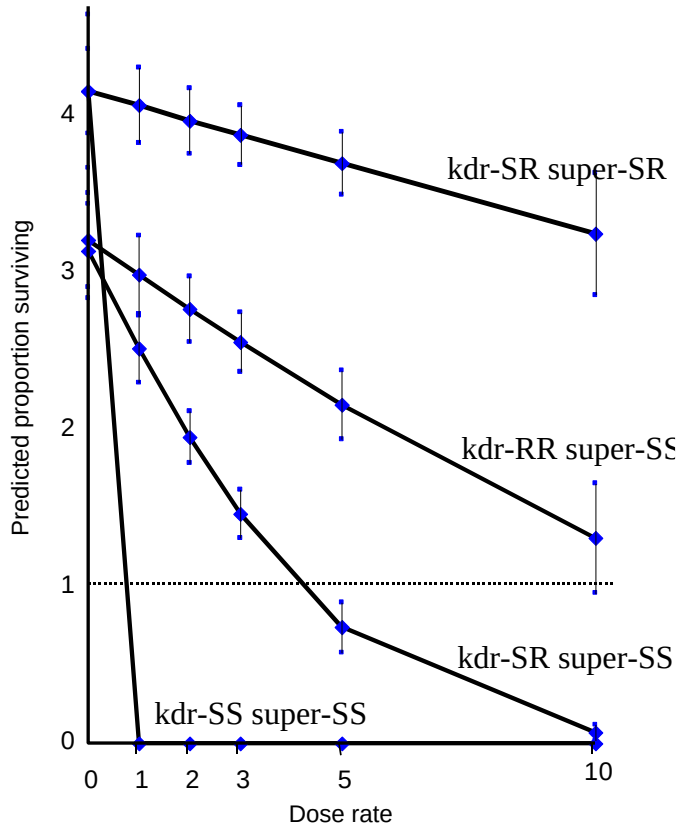
The efficacies of the pyrethroid sprays were very much dependent on whether kdr was present. Both non-kdr clones were controlled relatively well. However, the two kdr clones appeared to be resistant to all three pyrethroids. With the exception of the R_1/kdr clone treated with lambda-cyhalothrin on potato, both kdr clones consistently had PTP's greater than 1, ie. more aphids were present three days after treatment. The pyrethroid sprays, therefore, appeared relatively ineffective. Having said this, PTP's on Chinese cabbage were significantly lower compared with untreated controls for the R_1/kdr clone treated with deltamethrin ($P < 0.001$), and both kdr clones showed significantly lower PTP's for lambda-cyhalothrin. Furthermore, the equivalent comparison on potato revealed significantly lower PTP's for all three pyrethroids against the R_1/kdr clone ($P < 0.05$).

Pirimicarb and the mixture of pirimicarb with lambda-cyhalothrin (Dovetail) were consistently effective against the non-kdr and the kdr clones alike, although the esterase-R_3/kdr clone showed slightly greater resistance ($P < 0.005$) to this insecticide mixture compared with the other three clones.

kdr and super-kdr

The predicted proportion of aphids in each clone surviving the various treatments with lambda-cyhalothrin at different concentrations are presented in **Figure 11**.

Figure 11. Numbers (expressed as proportions of pre-treatment number) of *Myzus persicae* three days after treatment with lambda-cyhalothrin at various dose rates in field simulators (1 = recommended field rate, 2 = twice recommended field rate etc). Four aphid clones, showing various combinations of kdr and super-kdr resistance, were tested.



Clone 800F (kdr-SS/super-SS) was controlled by all treatments with lambda-cyhalothrin and had a statistically similar intercept as clone 2169G (kdr-SR/super-SR) with the latter showing a non-significant decline with increasing dose rate. Clones 2161C (kdr-SR/super-SS) and 794J (kdr-RR/super-SS) showed lower, statistically similar intercepts and statistically significant declines with increasing dose rate.

2161C b = –0.21 (se 0.017)[a] (P = <0.0001)
794J b = -0.07 (se 0.017)[b] (P = 0.047)
2169G b = -0.03 (se 0.017)[b] (P = 0.43)

slopes followed by the same letter do not differ significantly

PM 3.4 Field simulator study of resistance of M. persicae clones showing susceptibility and low resistance to imidacloprid. Assess how applications of imidacloprid to compost can select for resistance

Survival and reproduction

Post-treatment proportions (PTP) of aphids in each clone fell with increasing doses of imidacloprid at three (**Figure 12**) and seven days (**Figure 13**) after treatment. All four clones showed highly significant inverse associations between PTP and imidacloprid dose at both time points.

3 days after treatment:
US1L b = –0.61 (se 0.11)[a] (P << 0.001)
3104A b = -0.93 (se 0.09)[b] (P << 0.001)
3104B b = -0.73 (se 0.09)[ab] (P << 0.001)
926B b = -0.69 (se 0.09)[ab] (P << 0.001)

7 days after treatment:
US1L b = –1.80 (se 0.21)[a] (P << 0.001)
3104A b = -1.56 (se 0.13)[a] (P << 0.001)
3104B b = -1.51 (se 0.14)[a] (P << 0.001)
926B b = -0.97 (se 0.09)[b] (P << 0.001)

slopes followed by the same letter do not differ significantly

The slopes (PTP vs imidacloprid dose) produced at seven days after treatment for each clone, showed a suggestive inverse association (regression: $T_2 = 3.93$, $P = 0.06$) with the Resistance Factors (RF) of the clones (previously gained in imidacloprid micro-application bioassays, **Table 10**). Furthermore, the clone showing the highest RF (926B) consistently showed the highest PTP's and had a significantly lower dose response slope; in other words greater tolerance to imidacloprid at the range of concentrations, compared to the other clones (**Figures 12** and **13**). However, this clone was not statistically different to the other clones three days after treatment, probably because a longer starvation period is needed before fitness differences become apparent. A few aphids in clone 926B were still alive and reproducing seven days after treatment with the full recommended dose of imidacloprid (2 mg per plant) (**Figure 13**), suggesting that this may not be sufficient to control *M. persicae* showing the highest imidacloprid tolerance. To conclude, the data suggest that selection favouring greater imidacloprid resistance in this species is probably taking place in

glasshouses when decreased rates of imidacloprid are present, either due to natural decay of the insecticide with time or mixing of treated and untreated compost (a practice done sometimes to reduce costs). Similar selection pressures are probably also operating in Gaucho-treated sugar beet later on in the growing season when imidacloprid levels are known to decline.

Figure 12. Numbers (expressed as proportions of pre-treatment number) of *Myzus persicae* three days after treatment with imidacloprid at a range of doses in field simulators (2 mg = recommended dose for young brassicas). Four aphid clones, showing susceptibility and various levels of tolerance to imidacloprid, were tested.

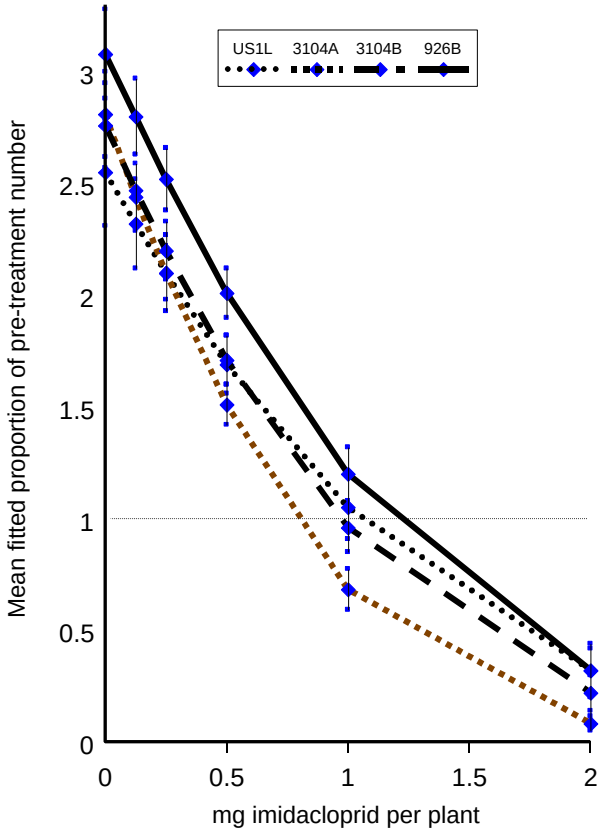
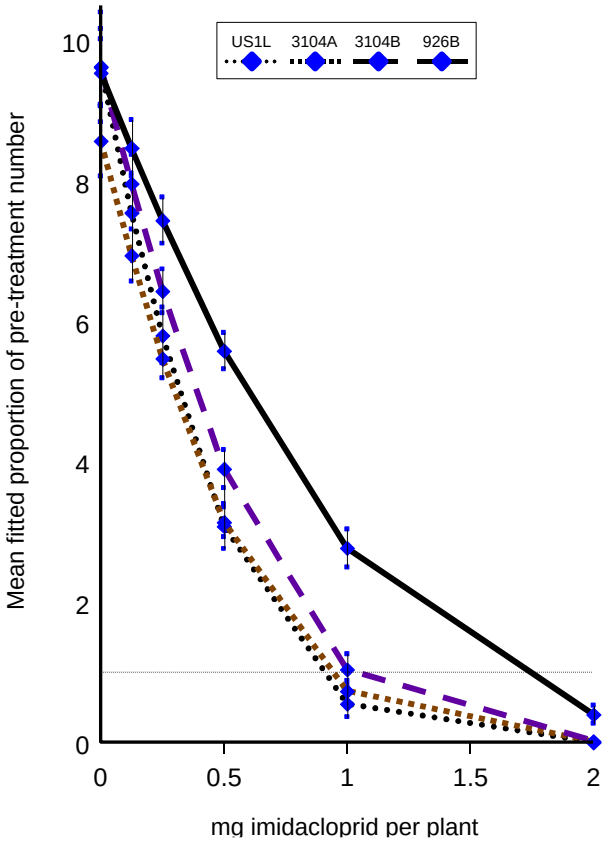


Figure 13. Numbers (expressed as proportions of pre-treatment number) of *Myzus persicae* seven days after treatment with imidacloprid at a range of doses in field simulators (2 mg = recommended dose for young brassicas). Four aphid clones, showing susceptibility and various levels of tolerance to imidacloprid, were tested.

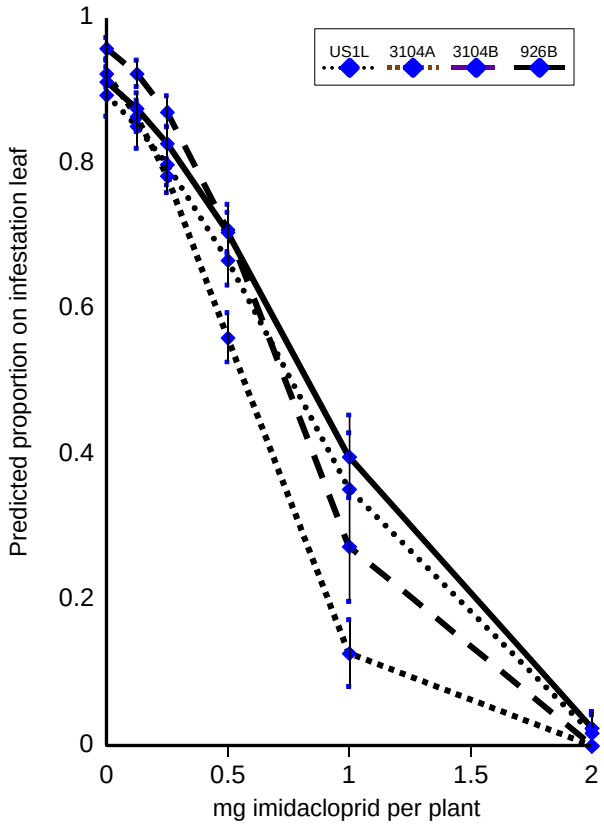


Aphid movement from infestation leaves

Results of the analyses of aphid position (expressed as proportions of the total) in each clone on the Chinese cabbage plants are presented for the three-day post treatment counts only (**Figure 14**). The equivalent data at seven days after treatment were not amenable to this form of analysis due to aphid numbers being low at the higher imidacloprid concentrations.

At three days after treatment there was a clear imidacloprid dose effect with aphids in all clones increasingly being found on the rest of the plant (ie. not on the leaves that the clip cages had been attached) as imidacloprid concentration increased ($F_{1,168}$ = 378, P << 0.0001). There was also a significant clone effect ($F_{1,168}$ = 4.0, P = 0.009) but this was not consistently associated with tolerance to imidacloprid. Having said this, the most tolerant clone (926B) did tend to show the highest proportions recorded on the original infestation leaves, particularly at higher rates of imidacloprid treatment (**Figure 14**).

Figure 14. Numbers (expressed as proportions of total number) of *Myzus persicae* recovered on infestation leaves three days after treatment with imidacloprid at a range of doses in field simulators (2 mg = recommended dose for young brassicas). Four aphid clones, showing susceptibility and various levels of tolerance to imidacloprid, were tested.



PM 3.5 Laboratory bioassays to assess response of M. persicae *clones with various combinations of resistance mechanisms to novel insecticides such as imidacloprid, acetamiprid and pymetrozine*

Screening bioassays applying imidacloprid

The screening bioassay, which was developed to allow quick assessment of large numbers of *M. persicae* clones for resistance to imidacloprid, appeared to be robust for predicting subsequent tolerance factors in full-dose range bioassays, not only with imidacloprid but also with acetamiprid and potentially other neonicotinyl insecticides.

Full dose-range micro-application bioassays applying imidacloprid and acetamiprid

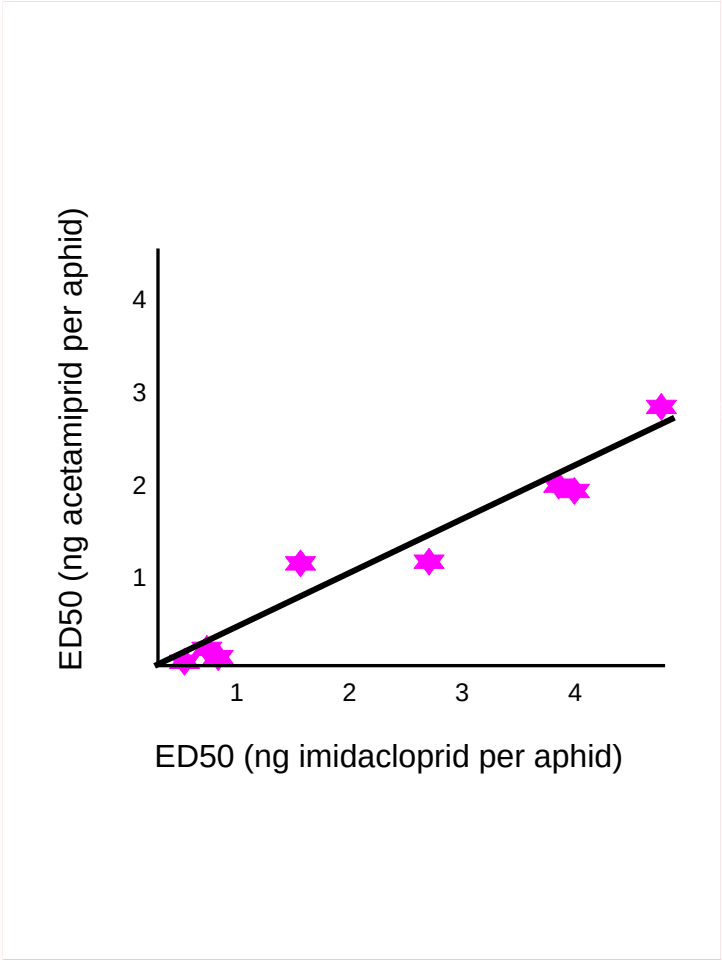
Statistics for the response of each clone to imidacloprid and acetamiprid are summarised in **Table 10**. Significant variability in tolerance was apparent for both insecticides although Resistance Factors (RF's) were low and never significantly greater than the standard imidacloprid-tolerant clone, 926B. Tolerance to imidacloprid and acetamiprid were clearly not associated with the esterase mechanism (regressions for clonal ED_{50}'s versus mean $\log_{10}$ E4/FE4 esterase level; imidacloprid: $T_{13} = 0.81$, $P = 0.43$; acetamiprid: $T_8 = -0.53$, $P = 0.61$). There was also no evidence that resistance to imidacloprid and acetamiprid was associated with the MACE or kdr mechanisms. It was, however, highly consistent across both neonicotinyl insecticides and a comparison between the ED_{50}'s for clones tested with imidacloprid and acetamiprid shows a clear, statistically significant positive association (linear regression: $F_{1,6} = 121$, $P < 0.0001$) (**Figure 15**).

Table 10. Summary of topical micro-bioassay results for *Myzus persicae* clones treated with formulated imidacloprid and acetamiprid (arranged by RF).

Clone	Origin	RESISTANCE MECHANISM Esterase	MACE	kdr[1]	Imid[2] screen	ED_{50}[3]	95% CI[4]	Slope	**RF[5]**
IMIDACLOPRID BIOASSAYS									
1262B	France 1995 Tobacco	R_2	yes	S	im-s	0.26	0.17-0.35[a]	1.6	0.6
1263B	France 1995 Tobacco	R_1	yes	S	im-s	0.35	0.26-0.40[ab]	2.2	0.8
1302K	England 1996 Sprouts	R_3	yes	R	im-s	0.40	0.16-1.01[ab]	1.4	0.9
US1L	**England 1974 Sugar beet**	**S**	**no**	**S**	**im-s**	**0.46**	**0.31-0.63[a]**	**3.4**	**1.0**
3101M	England 1996 Sprouts	R_2	yes	R	im-s	0.58	0.29-1.09[ab]	1.7	1.3
794J	England 1982 Chrysanthemum	R_3	no	R	im-s	0.66	0.40-1.18[ab]	2.1	1.4
3104A	England 1998 Sprouts	R_2	yes	R	im-s	0.76	0.41-1.19[ab]	1.3	1.7
3101H	England 1996 Sprouts	R_2	yes	R	im-s	0.81	0.32-2.02[abc]	1.7	1.8
2794Z	Denmark 1998 Chrysanthemum	R_2	yes	S	im-t	1.14	0.66-1.90[bc]	1.9	2.5
4239B	England 2000 Pepper	R_1	yes	R	im-t	1.49	00.98-2.05[bc]	2.6	2.5
3495B	England 1999 Potato	R_3	yes	R	im-t	1.49	0.91-2.16[bc]	3.0	3.2
4239D	England 2000 Pepper	R_1	yes	R	im-t	2.08	1.65-2.59[c]	3.3	4.5
3844A	England 1999 Oilseed rape	R_1	no	R	im-t	2.10	1.62-2.60[c]	3.3	4.6
3104B	England 1998 Sprouts	R_1	yes	R	im-t	2.63	1.59-4.18[cd]	1.6	5.7
3982A	Greece 2000 Tobacco	R_3	yes	S	im-t	3.29	2.27-4.41[cd]	1.6	7.2
4198A	Greece 2000 Tobacco	R_3	yes	S	im-t	3.60	2.68-4.65[d]	1.7	7.8
3589C	Zimbabwe 1999 Tobacco	S	no	S	im-t	3.78	2.02-12.8[cde]	1.7	8.2
3722A	Zimbabwe 1999 Tobacco	S	no	S	im-t	3.92	2.88-5.65[de]	3.3	8.5
1055F	Japan 1992 Tobacco	R_3	yes	R	im-t	4.30	2.90-6.60[de]	2.0	9.3
4190A	Greece 2000 Tobacco	R_3	yes	S	im-t	4.42	3.13-5.97[de]	1.6	9.6
4193A	Greece 2000 Tobacco	R_3	yes	S	im-t	4.62	3.60-5.88[de]	1.9	10
926B	**Greece 1990 Peach**	**R_3**	**yes**	**S**	**im-t**	**4.80**	**3.37-7.49[de]**	**1.2**	**10**
934E	USA 1991 Tobacco	R_1	no	S	im-t	6.40	4.10-9.90[de]	1.6	14
975A	Hungary 1991 Potato	R_2	no	S	im-t	6.80	4.40-10.5[de]	1.8	15
4013A	Greece 2000 Tobacco	R_3	yes	S	im-t	7.50	5.28-10.4[e]	1.8	16
935D	USA 1991 Tobacco	S	no	S	im-t	8.30	3.10-13.0[de]	2.4	18
ACETAMIPRID BIOASSAYS									
US1L	**England 1974 Beet**	**S**	**no**	**S**	**im-s**	**0.35**	**0.25-0.51[ab]**	**2.0**	**1.0**
1200Q	Argentina 1993 Peach	R_2	yes	S	im-s	0.22	0.13-0.37[ab]	2.2	0.6
T1V	England 1975 Beet	R_2	no	R	im-s	0.26	0.20-0.33[a]	2.8	0.7
3104A	England 1998 Sprouts	R_2	yes	R	im-s	0.40	0.20-1.00[abc]	1.1	1.1
794J	England 1982 Chrysanthemum	R_3	no	R	im-s	0.49	0.34-0.74[b]	1.7	1.4
3495B	England 1999 Potato	R_3	yes	R	im-t	1.41	0.82-4.95[cd]	1.3	4.0
3104B	England 1998 Sprouts	R_1	yes	R	im-t	1.43	0.75-1.94[cd]	2.1	4.1
3722A	Zimbabwe 1999 Tobacco	S	no	S	im-t	2.19	1.48-2.85[de]	2.0	6.3
3589C	Zimbabwe 1999 Tobacco	S	no	S	im-t	2.25	1.68-2.96[de]	2.1	6.4
926B	**Greece 1990 Peach**	**R_3**	**yes**	**S**	**im-t**	**3.10**	**2.54-3.65[e]**	**2.5**	**8.9**

[1]Based on a diagnostic dose bioassay with DDT. [2]Based on a screening bioassay with imidacloprid. [3]Effective dose (ng active ingredient per aphid) resulting in 50% dead or very poorly co-ordinated. [4]95% confidence limits; values followed by the same letter do not differ significantly. [5]Resistance factor = ED_{50} for clone/ ED_{50} for US1L for each compound. Clones shown in **bold** (US1L and 926B) are im-s and im-t standards. Clones underlined are English im-t clones previously identified by screening bioassays done as part of the imidacloprid resistance survey (PM 2.1).

Figure 15. Relationship between ED_{50}'s for *Myzus persicae* clones tested with acetamiprid and imidacloprid.



All five im-t clones isolated from England showed similar tolerance to imidacloprid (**Table 10**), with one (3104B) showing an RF not significantly different to the im-t standard clone; ie. typical of the maximum level of imidacloprid tolerance found anywhere in the world to date. Four of these clones had identical phenotypes, ie. they were red colour morphs with esterase-R_1/MACE/kdr resistance characteristics, and may therefore have a common ancestry.

Full dose-range leaf-dip bioassays applying pymetrozine

Resistance Factors to pymetrozine ranged up to 6.7 (**Table 11**). They were not associated with MACE, kdr or imidacloprid-tolerance. However, pymetrozine-tolerance appeared to be inversely associated with esterase resistance (**Figure 16**); pymetrozine EC_{50} versus mean clonal $\log_{10}$ E4/FE4 esterase activity (excluding revertant clones): $T_{17} = -5.43$, $P = << 0.001$. The two revertant clones appeared to affiliate with clones showing higher (R_2/R_3) esterase levels (**Figure 16**).

Table 11. Summary of leaf-dip bioassay results for *Myzus persicae* clones treated with formulated pymetrozine (arranged by RF).

		RESISTANCE MECHANISM							
Clone	Origin	Esterase[1]	MACE[2]	kdr[3]	Imid screen[4]	EC_{50}[5]	95% CI[6]	Slope	RF[7]
1200Q	Argentina 1993	R_2	yes	SS	im-s	420	146-753[a]	1.4	1.0
800F	Italy 1978	R_3		SS	im-s	537	288-797[ab]	1.2	1.3
1190A	Spain 1993	R_3		SS	im-s	712	316-1118[ab]	1.8	1.7
2161C	**England 1997**	**R_3**		**SR**	**im-s**	**738**	**431-1121[ab]**	**1.5**	**1.8**
794J	England 1982	R_3		RR	im-s	750	517-1027[ab]	1.5	1.8
T1V	England 1975	R_2		RR	im-s	763	579-987[ab]	1.7	1.9
2169G	England 1997	R_3		SR	im-s	889	406-1477[abc]	2.1	2.1
2160D	**England 1997**	**R_1**		**SR**	**im-s**	**980**	**647-1491[abc]**	**1.8**	**2.3**
2050A	**England 1996**	**R_3**	**yes**	**SS**	**im-s**	**1008**	**493-1821[abc]**	**1.4**	**2.4**
794Jrev	England 1982	S(rev)		RR	im-s	1064	587-1955[abc]	2.8	2.5
923Arev	England 1990	S(rev)		RR	im-s	1072	326-1938[abc]	2.0	2.6
926B	Greece 1990	R_3	yes	SS	im-t	1076	552-1931[abc]	1.0	2.6
405D	England 1977	R_1		SS	im-s	1104	632-1723[abc]	1.3	2.6
2042H	**England 1996**	**R_1**	**yes**	**SR**	**im-s**	**1226**	**788-1893[bc]**	**1.3**	**2.9**
2043B	England 1996	R_3		RR	im-s	1311	832-1899[bc]	1.8	3.1
4106A	Scotland 2000	S		SS	im-s	1655	663-3181[abcd]	2.1	3.9
1076A	England 1992	S		SS	im-s	1634	940-2477[bcd]	1.7	3.9
2591C	Scotland 1998	S		SS	im-s	1698	478-3781[abcd]	1.2	4.0
3104B	England 1998	R_1	yes	RR	im-t	1713	1309-2276[cd]	1.2	4.1
3589C	Zimbabwe 1999	S		SS	im-t	1968	656-4396[abcd]	1.8	4.7
US1L	England 1974	S		SS	im-s	2812	2303-3486[d]	1.5	6.7

[1]Determined by an immunoassay; S: susceptible, R_1: moderate resistance, R_2: high resistance, R_3: extreme resistance, S(rev): carrying unexpressed R_3 esterase genes.

[2]Determined by a kinetic assay.

[3]Based on direct DNA sequencing of PCR-amplified sodium channel gene fragments from aphid genomic DNA

[4]Based on a screening dose topical bioassay (2.5 ng imidacloprid in 0.25 µl acetone, applied to 50 individual aphids from each clone) im-s: susceptible, im-t: tolerant.

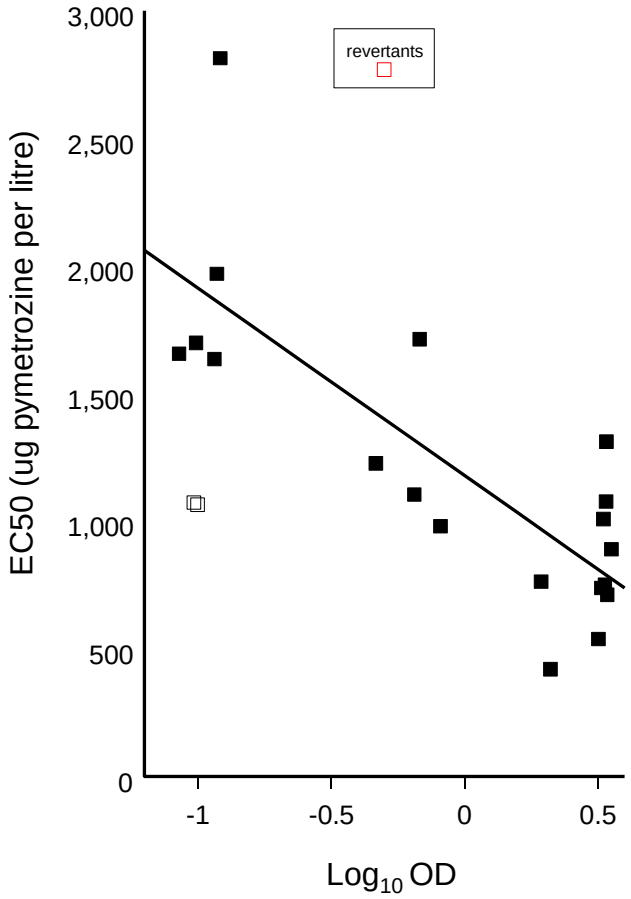
[5]Effective concentration resulting in 50% dead nymphs (µg pymetrozine per litre).

[6]95% confidence limits; values followed by the same letter do not differ significantly.

[7]Resistance factor = ED_{50} for clone/ ED_{50} for 1200Q.

The four clones shown in **bold** were also assessed in the simulator-based study (PM 3.6).

Figure 16. Relationship between pymetrozine EC_{50}'s and esterase resistance level for *Myzus persicae* clones.



Assays assessing survival of nymphs seven days after exposure to pymetrozine

A small number of the nymphs transferred to new leaf discs died within three days (**Table 12**). However, there was no obvious association between mortality and either pymetrozine RF or leaf-dip concentration. The data show that the great majority of nymphs surviving 96 h after exposure to pymetrozine are capable of feeding and developing for a further three days. Indeed, by this stage some nymphs had become adults and were producing the next generation.

Table 12. Pymetrozine tolerance (denoted by RF) versus percentage mortality for *Myzus persicae* clones three days after being transferred to untreated leaf discs from discs treated with formulated pymetrozine at a range of concentrations.

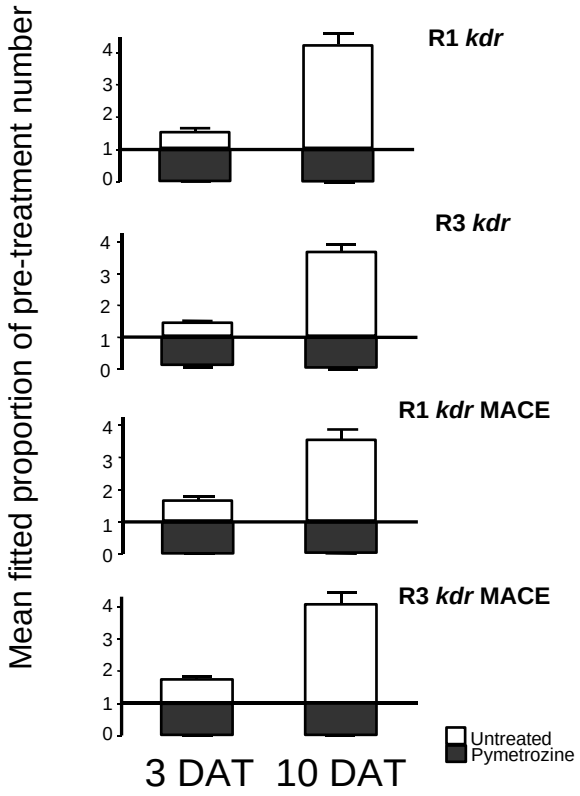
		Previous pymetrozine disc treatment (μg ai per litre)				
Clone	RF[1]	0	390	650	1080	1800
1200Q	1.0	0	0	0	0	0
800F	1.3	10	0	0	0	0
1190A	1.7	0	0	0	0	0
2161C	**1.8**	**0**	**0**	**10**	**0**	**0**
794J	1.8	0	0	0	0	0
T1V	1.9	0	0	0	0	0
2169G	2.1	0	0	0	10	0
2160D	**2.3**	**0**	**0**	**0**	**0**	**0**
2050A	**2.4**	**0**	**0**	**0**	**0**	**0**
794Jrev	2.5	0	0	0	0	0
923Arev	2.6	0	0	0	0	0
926B	2.6	0	0	0	0	0
405D	2.6	0	0	0	0	0
2042H	**2.9**	**0**	**0**	**0**	**0**	**0**
2043B	3.1	0	0	0	0	0
4106A	3.9	10	0	0	0	0
1076A	3.9	0	0	10	0	0
2591C	4.0	0	0	20	0	0
3104B	4.1	0	0	0	0	0
3589C	4.7	0	0	0	0	0
US1L	6.7	0	10	0	0	0

[1]Resistance factor = ED_{50} for clone/ ED_{50} for 1200Q.
The four clones shown in **bold** were also assessed in the simulator-based study (PM 3.6).

PM 3.6 Field simulator study of resistance of UK M. persicae clones carrying various combinations of the esterase, MACE and kdr mechanisms to spray applications of pymetrozine (Plenum)

Biochemical tests on aphid samples collected immediately prior to insecticide application showed no evidence that there had been movement across the sticky barrier giving rise to cross-contamination between the four *M. persicae* clones in any of the simulators. Each of the three plants within each simulator group was treated as a replicate. Post-treatment proportions (PTP) per clone per plant replicate were compared using generalised linear models and an analysis of deviance on the combined data for both experiments. The data and results of the statistical analysis, grouped by clone, are presented in **Figure 17**.

Figure 17. Numbers (expressed as proportions of pre-treatment number) of *Myzus persicae* on potato three and ten days after treatment pymetrozine under semi-field conditions. Four aphid clones, showing various combinations of esterase, kdr and MACE resistance, were tested.



Untreated controls

Mean aphid population sizes increased for all four clones over the three-day post-treatment period and continued to increase to the 10-day post treatment period (**Figure 17**). All differences between clones were non-significant ($P > 0.05$).

Pymetrozine

Aphid population size was reduced dramatically for all four clones at the three-day post-treatment period and was decreased further after 10 days (**Figure 17**). All clones had statistically similar levels of decrease ($P > 0.05$). Statistical comparison of PTP's for each clone at the three days and 10 days showed consistent highly significant ($P << 0.0001$) reductions in population size for treated aphids (**Figure 17**).

Establish potential fitness drawbacks suffered by kdr forms in the absence of insecticides under sub-optimal conditions associated with the winter climate, either in their own right or through association with high esterase-based resistance (MO 4)

PM 4.2 Complete winter field study of potential fitness drawbacks suffered by kdr forms of M. persicae

The majority of the analyses produced no significant associations, most probably as a result of the relatively mild and benign field conditions that prevailed during the course of the winter experiments (compared to previous winter field experiments assessing fitness associated with esterase resistance, Foster *et al.*, 1996).

Proportional survival

The proportional survival of each kdr genotype are shown in **Table 13**. Proportional survival in each kdr genotype versus level of esterase resistance is shown in **Table 14**. No significant associations were seen for this variable.

Alata proportion

Alata proportion in the 4th and adult stages of retrieved aphids for each kdr genotype are shown in **Table 15**. Alata proportion in the 4th and adult stages of retrieved aphids in each kdr genotype versus level of esterase resistance are shown in **Table 16**. No significant associations were seen for this variable.

Proportion retrieved on infestation leaves

Proportions of aphids retrieved on infestation leaves for each kdr genotype are shown in **Table 17**. Proportions retrieved on infestation leaves in each kdr genotype versus level of esterase resistance are shown in **Table 18**. No significant associations were seen for this variable.

Proportion reaching 4th instar and adult stages

Proportions of retrieved aphids at the 4th and adult stages for each kdr genotype are shown in **Table 19**. Proportions at 4th and adult stages in retrieved aphids in each kdr genotype versus level of esterase resistance are shown in **Table 20**.

Significant associations were seen in the February 1999 experiment where the kdr-SS forms developed faster than either the kdr-SR or the -RR forms.

Proportion retrieved on excised leaves

Proportions of aphids retrieved on excised leaves for each kdr genotype are shown in **Table 21**. Proportions retrieved on excised leaves in each kdr genotype versus level of esterase resistance are shown in **Table 22**.

In the February 1999 experiment, the kdr-RR forms showed a significantly higher tendency to remain on their excised leaves (**Table 21.**) Furthermore, esterase resistance was also significantly positively associated with tendency to be retrieved on excised leaves in the kdr-SS clones (**Table 22** and **Figure 18**). A similar relationship was also seen in kdr-SR clones in the November 1998 experiment.

Table 13. Statistics for tests of association between aphid survival and kdr resistance (adjusted for esterase resistance) in the 1998/1999 winter field experiments. Arrows denote lowest values.

Experiment	Mean proportions surviving *kdr-SS*	*kdr-SR*	*kdr-RR*	F value	df	P
November 1998	0.32	0.32	0.27↓	0.75	2,38	0.48
December 1998	0.20	0.20	0.15↓	0.80	2,20	0.46
January 1999	0.46	0.39	0.38↓	1.34	2,20	0.28
February 1999	0.55↓	0.60	0.62	1.06	2,20	0.37

Table 14. Statistics for probit regressions of aphid survival for kdr-SS, -SR and -RR clones versus esterase-based resistance as measured by $\log_{10}$ E4/FE4 activity in the 1998/1999 winter field experiments.

Experiment	Slope (se)	t	df	P
November 1998				
kdr-SS	0.03 (0.16)	0.22	38	0.83
kdr-SR	-0.11 (0.31)	0.35	38	0.73
kdr-RR	0.31 (0.16)	1.94	38	0.06
December 1998				
kdr-SS	-0.35 (0.24)	1.47	20	0.16
kdr-SR	-0.07 (0.37)	0.19	20	0.85
kdr-RR	0.42 (0.26)	1.63	20	0.12
January 1999				
kdr-SS	-0.19 (0.17)	1.09	20	0.29
kdr-SR	0.24 (0.26)	0.96	20	0.35
kdr-RR	0.27 (0.18)	1.53	20	0.14
February 1999				
kdr-SS	-0.20 (0.16)	1.29	20	0.21
kdr-SR	-0.25 (0.25)	1.00	20	0.33
kdr-RR	0.05 (0.15)	0.34	20	0.74

Table 15. Statistics for tests of association between alata proportion and kdr resistance (adjusted for esterase resistance) in the 1998/1999 winter field experiments. Arrows denote lowest values.

Experiment	Mean alata proportions *kdr-SS*	*kdr-SR*	*kdr-RR*	F value	df	P
November 1998	0.64	0.49	0.47↓	1.28	2,20	0.30
December 1998	0.37	0.31↓	0.51	1.05	2,20	0.37
January 1999	0.46	0.31	0.23↓	2.19	2,20	0.14
February 1999	0.26	0.41	0.22↓	2.83	2,20	0.08

Table 16. Statistics for probit regressions of alata proportion for kdr-SS, -SR and -RR clones versus esterase-based resistance as measured by $\log_{10}$ E4/FE4 activity in the 1998/1999 winter field experiments.

Experiment		Slope (se)	t	df	P
November 1998					
	kdr-SS	0.43 (0.37)	1.14	20	0.26
	kdr-SR	-0.03 (0.63)	0.05	20	0.96
	kdr-RR	-0.28 (0.41)	0.69	20	0.49
December 1998					
	kdr-SS	-0.10 (0.73)	0.13	20	0.90
	kdr-SR	-0.61 (0.82)	0.75	20	0.46
	kdr-RR	0.28 (0.78)	0.11	20	0.92
January 1999					
	kdr-SS	-0.44 (0.37)	1.19	20	0.25
	kdr-SR	0.95 (0.65)	1.47	20	0.16
	kdr-RR	0.56 (0.46)	1.24	20	0.23
February 1999					
	kdr-SS	-0.22 (0.28)	0.78	20	0.45
	kdr-SR	0.69 (0.48)	1.46	20	0.16
	kdr-RR	-0.31 (0.32)	0.99	20	0.33

Table 17. Statistics for tests of association between proportion of all aphids collected that were retrieved on infestation leaves and kdr (adjusted for esterase resistance) in the 1998/1999 winter field experiments. Arrows denote highest values.

Experiment	Mean proportions retrieved *kdr-SS*	*kdr-SR*	*kdr-RR*	F value	df	P
November 1998	0.96	0.96	0.98↑	1.17	2,39	0.32
December 1998	0.90	0.89	0.91↑	0.12	2,20	0.89
January 1999	0.99↑	0.98	0.97	3.31	2,20	0.06
February 1999	0.98	0.99	0.99↑	3.18	2,20	0.09

Table 18. Statistics for probit regressions of proportion of aphids collected that were retrieved on infestation leaves for kdr-SS, -SR and -RR clones versus esterase resistance as measured by $\log_{10}$ E4/FE4 activity in the 1998/1999 winter field experiments.

Experiment	Slope (se)	t	df	P
November 1998				
kdr-SS	0.13 (0.27)	0.48	39	0.63
kdr-SR	0.89 (0.52)	1.72	39	0.09
kdr-RR	0.13 (0.34)	0.39	39	0.70
December 1998				
kdr-SS	-0.32 (0.61)	0.52	20	0.61
kdr-SR	-0.05 (0.77)	0.06	20	0.95
kdr-RR	0.28 (0.60)	0.47	20	0.64
January 1999				
kdr-SS	-0.18 (0.42)	0.44	20	0.67
kdr-SR	0.79 (0.65)	1.21	20	0.24
kdr-RR	0.31 (0.31)	1.01	20	0.33
February 1999				
kdr-SS	-0.04 (0.24)	0.15	20	0.88
kdr-SR	0.06 (0.48)	0.37	20	0.71
kdr-RR	0.23 (0.35)	0.64	20	0.53

Table 19. Statistics for tests of association between proportion of all aphids collected that had reached 4th instar or adulthood and kdr resistance (adjusted for esterase resistance) in the 1998/1999 winter field experiments. Arrows denote highest values.

Experiment	Mean proportions 4th/adult			F value	df	P
	kdr-SS	*kdr-SR*	*kdr-RR*			
November 1998	0.54	0.59	0.59↑	1.43	2,20	0.56
December 1998	0.90↑	0.78	0.82	1.34	2,20	0.28
January 1999	0.56	0.62↑	0.54	0.53	2,20	0.60
February 1999	0.40↑	0.31	0.29	3.96	2,20	**0.04***

Table 20. Statistics for probit regressions of proportion of all aphids collected that had reached 4th instar or adulthood for kdr-SS, -SR and -RR clones versus esterase resistance as measured by $\log_{10}$ E4/FE4 activity in the 1998/1999 winter field experiments.

Experiment		Slope (se)	t	df	P
November 1998					
	kdr-SS	-0.02 (0.18)	0.10	20	0.92
	kdr-SR	-0.26 (0.34)	0.76	20	0.46
	kdr-RR	0.35 (0.21)	1.69	20	0.11
December 1998					
	kdr-SS	0.30 (0.56)	0.53	20	0.60
	kdr-SR	-1.26 (0.68)	1.85	20	0.08
	kdr-RR	0.93 (0.50)	1.87	20	0.08
January 1999					
	kdr-SS	0.22 (0.24)	0.92	20	0.37
	kdr-SR	0.26 (0.37)	0.11	20	0.91
	kdr-RR	0.01 (0.26)	0.02	20	0.98
February 1999					
	kdr-SS	-0.01 (0.14)	0.06	20	0.96
	kdr-SR	-0.02 (0.23)	0.09	20	0.93
	kdr-RR	0.10 (0.14)	0.72	20	0.48

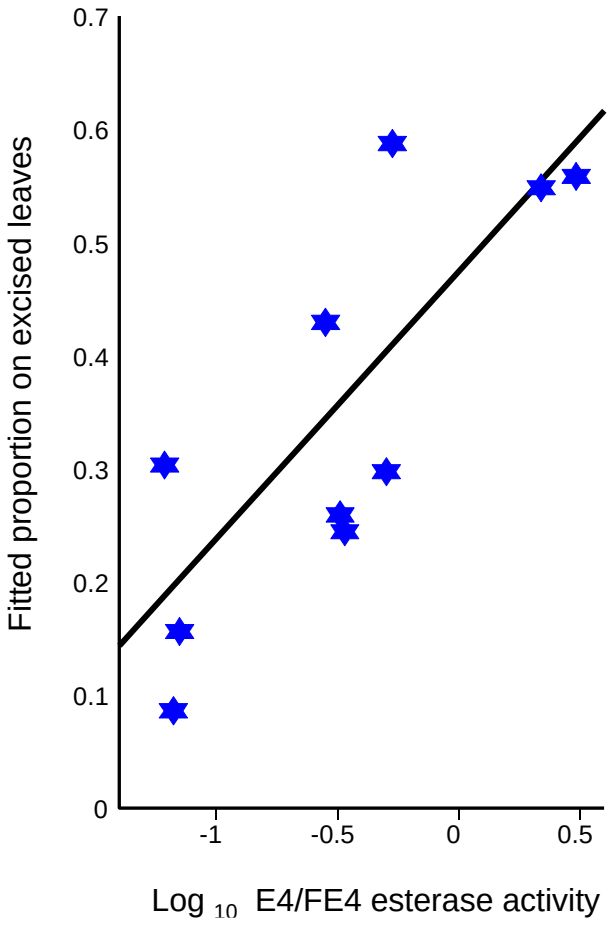
Table 21. Statistics for tests of association between proportion of all aphids collected that remained on excised leaves (that had remained intact) and kdr resistance (adjusted for esterase resistance) in the 1998/1999 winter field experiments. Arrows denote highest values.

Experiment	Mean proportions remaining *kdr-SS*	*kdr-SR*	*kdr-RR*	F value	df	P
November 1998	0.27	0.26	0.32↑	no test as only 19 intact excised leaves		
December 1998	--	--	--	no intact excised leaves retrieved		
January 1999	0.23↑	0.15	0.17	0.61	2,19	0.56
February 1999	0.42	0.45	0.68↑	6.17	2,20	**0.008****

Table 22. Statistics for probit regressions of proportion of all aphids collected that remained on excised leaves for kdr-SS, -SR and -RR clones versus esterase resistance as measured by $\log_{10}$ E4/FE4 activity in the 1998/1999 winter field experiments.

Experiment		Slope (se)	t	df	P
November 1998					
	kdr-SS	-0.64 (1.05)	0.61	4	0.58
	kdr-SR	5.61 (1.83)	3.07	4	**0.04***
	kdr-RR	5.31 (4.13)	1.29	4	0.27
December 1998					
	no intact excised leaves				
January 1999					
	kdr-SS	0.33 (0.34)	0.95	19	0.35
	kdr-SR	0.60 (0.55)	1.09	19	0.29
	kdr-RR	-0.05 (0.41)	0.11	19	0.91
February 1999					
	kd-SS	0.72 (0.27)	2.71	20	**0.01***
	kdr-SR	0.26 (0.40)	0.66	20	0.52
	kdr-RR	-0.36 (0.26)	1.39	20	0.18

Figure 18. Proportion of aphids in kdr-SS *Myzus persicae* clones retrieved on excised leaves versus mean level of esterase resistance in February 1999 field trial.



Field trials to assess options for aphid control by insecticides (MO 6)

PM 6.1 Field trials testing laboratory-based findings on insecticides choice for controlling M. persicae

See report attached in Appendix A.

Additional primary milestones

PM 4.3 Carry out laboratory study of response of kdr and non-kdr M. persicae clones to synthetic alarm pheromone applied at a wide range of concentrations

Aphid response increased with higher alarm pheromone concentrations for all three kdr genotypes. Adjusting for esterase effects, tendency to be disturbed was strongly associated with kdr genotype (**Figure 19)**. Furthermore, esterase resistance was inversely associated with disturbance in kdr-SS clones, with R_3 aphids showing lower tendencies to respond than R_2 forms (**Figures 20** and **21**). The revertant clone showed a dissimilar response to the kdr-SS clones (**Figure 21**). The two kdr-SR/DDT-susceptible clones* (gained from sexual crosses in the laboratory) showed inconsistent responses, with the R_2 clone appearing to affiliate with the kdr-SR/DDT-resistant clones and the R_3 clone appearing to affiliate with the kdr-SS/DDT-susceptible field clones (**Figures 20** and **21**).

Figure 19. Predicted mean response to alarm pheromone of *Myzus persicae* clones with kdr-SS, -SR and –RR genotypes.

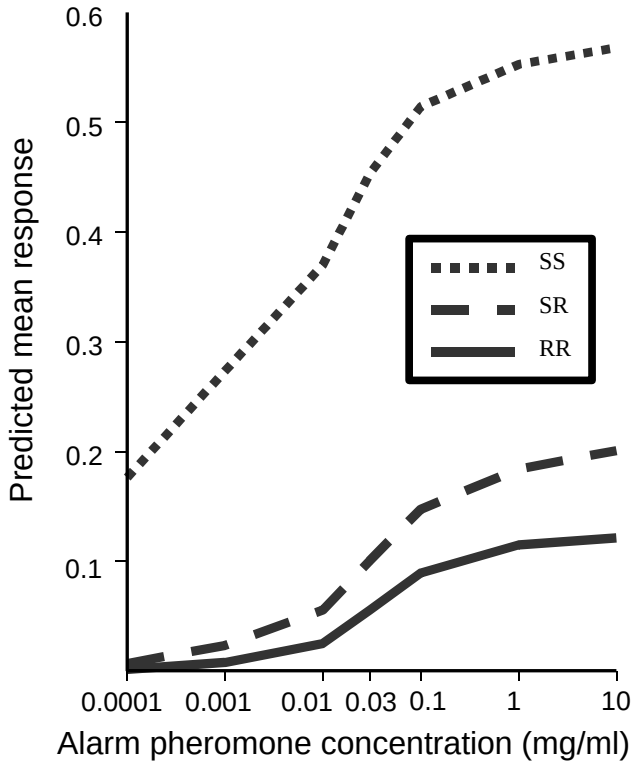


Figure 20. Predicted mean response to alarm pheromone of esterase-R_2 *Myzus persicae* clones with kdr-SS, -SR and –RR genotypes. SR*/DDT-susceptible clone from lab cross.

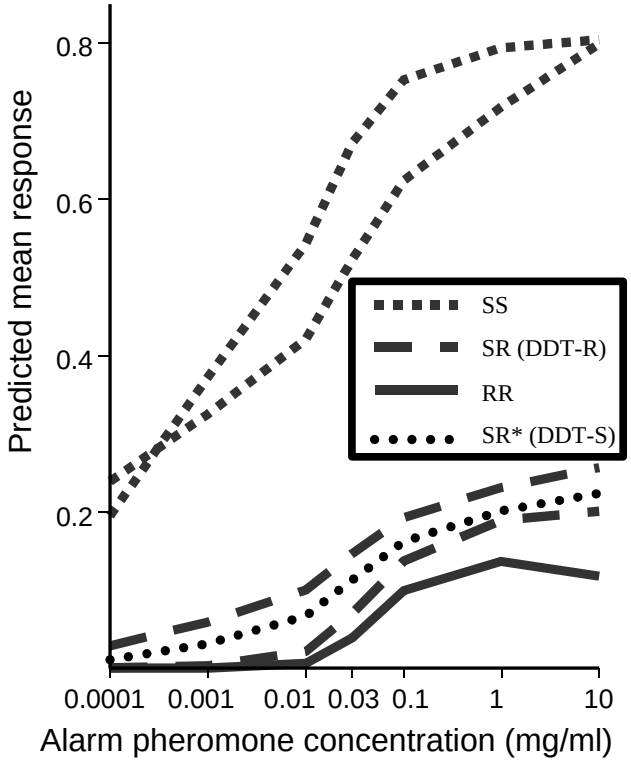
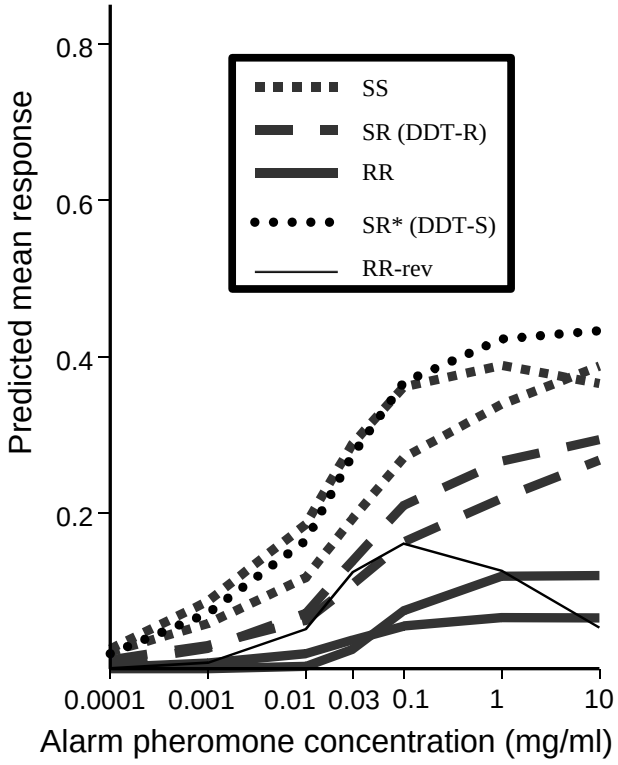


Figure 21. Predicted mean response to alarm pheromone of esterase-R_3 *Myzus persicae* clones with kdr-SS, -SR and –RR genotypes. SR*/DDT-susceptible clone from lab cross.



PM 4.4 Low temperature laboratory study of aphid movement from deteriorating leaves using kdr and non-kdr M. persicae clones

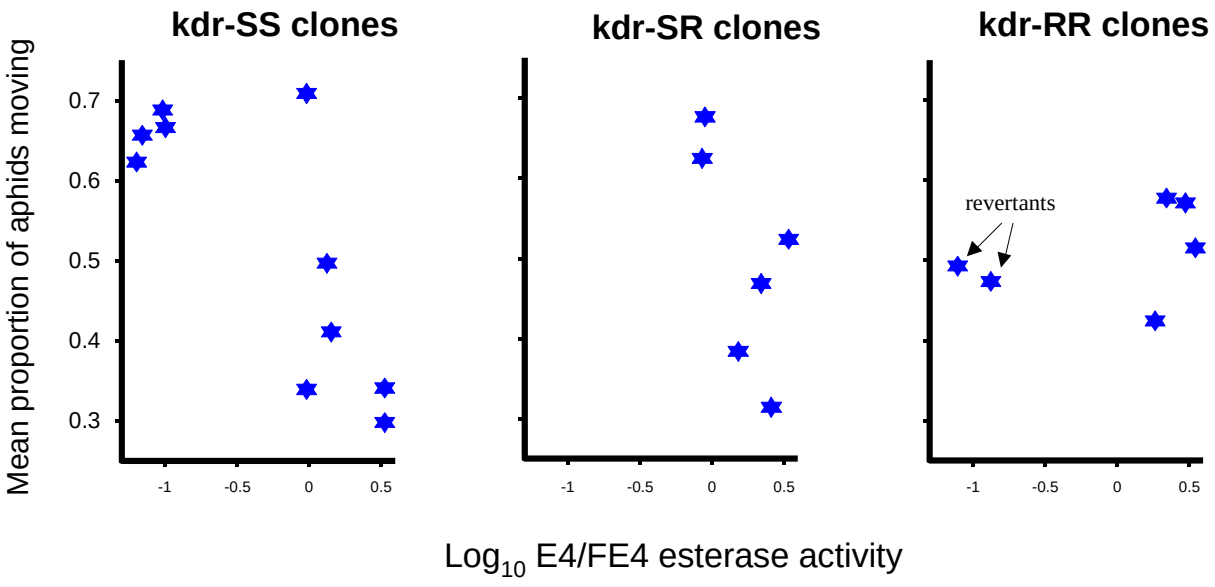
The mean proportions moving (± s.e.) from their deteriorating leaves in each genotype, adjusted for E4/FE4 esterase level, were:

kdr-SS: 0.48 ± 0.03
kdr-SR: 0.54 ± 0.04
kdr-RR: 0.52 ± 0.03

Tendency to move from deteriorating leaves did not therefore appear to be associated with kdr genotype (**Figure 21**). Aphid responses did not differ significantly:

kdr-SR vs kdr-SS: $T_{76} = 1.25$ P = 0.22
kdr-SS vs kdr-RR: $T_{76} = 0.87$, P = 0.39
kdr-SR vs kdr-RR: $T_{76} = 0.38$, P = 0.71.

Figure 22. Predicted mean movement from deteriorating leaves of *Myzus persicae* clones with kdr-SS, -SR and –RR genotypes and esterase-S to $–R_3$ levels.



Taking all the clones together, there was a significant inverse association between tendency to move and E4/FE4 esterase level (overall slope: -0.28, s.e. 0.12, P = 0.027). There were also significant inverse associations for this comparison within the kdr-SS and –SR categories (SS slope: -0.62, s.e. 0.20, P = 0.003; SR slope: -1.18, s.e. 0.55, P = 0.036). However, there was no apparent similar relationship within the kdr-RR category (RR slope: 0.08, s.e. 0.17, P = 0.65). The two esterase-revertant (Rev) clones appeared to affiliate with clones showing the same kdr-RR genotype and not their esterase-S phenotype.

3.4 DISCUSSION OF PROJECT FINDINGS

Contrary to previous belief, pyrethroid resistance in *M. persicae* appears to be based only to a limited extent on overproduction of detoxifying carboxylesterases (E4/FE4). Instead, resistance appears to be primarily associated with a mutant target site in the nervous system known as kdr. Initial selection for this mechanism probably arose from exposure to DDT in the 1950's and then persisted through cross-resistance to pyrethroids used since the 1970's. In the UK, kdr appears to be closely linked with the production of elevated (R_1, R_2, R_3) levels of esterase resistance. This linkage probably arose from the selection of clones by pyrethroid, carbamate and organophosphate insecticides and has probably been maintained by year-round parthenogenetic reproduction. Furthermore, prior to the discovery of kdr, the association between these two mechanisms probably promoted the false impression that higher levels of esterase resistance conferred strong resistance to pyrethroids.

Acknowledging that some sampling was biased towards crops with an aphid problem, there appears to have been a prevalence of kdr forms of *M. persicae* collected from field crops in England over the last several years. Furthermore, the mechanism appears to have remained relatively widespread and stable. This suggests that the effectiveness of at least some pyrethroid applications have been compromised. In the future it would be useful to assess kdr frequency in *M. persicae* collected in a more randomised manner such as in aphid suction traps (Harrington, 1996) to establish the proportion of kdr and non-kdr forms on crops are representative of the surrounding region as a whole. Such an approach has been used for esterase and MACE resistance and has shown this to be the case. Diagnosis of kdr by *in vitro* tests on aphids caught in suction traps has not been attempted but is being investigated at IACR-Rothamsted.

The limited field samples (collected from potato) in Scotland and Wales suggest that kdr is less prevalent in these regions of the UK. In Scotland this may be due to the colder winters selecting against insecticide-resistant forms. Having said this, a direct fitness cost associated with kdr was not apparent in the four winter field experiments done in the absence of insecticides at IACR-Rothamsted in late 1998 and early 1999. Indeed, none of these experiments showed significant differences in survival, formation of alatae or proportion of aphids retrieved on infestation leaves associated with either esterase resistance or kdr. This may relate to the relatively mild and benign field conditions that prevailed during the course of the experiments. However, the experiments did suggest that kdr and high esterase forms of *M. persicae* can sometimes suffer other disadvantages. Significant associations were seen in one field experiment (done in February 1999) with kdr-homozygous susceptible (SS) forms developing faster than either kdr-heterozygotes (SR) or –homozygote resistant (RR) forms. Furthermore, kdr-RR forms showed a higher tendency to remain on deteriorated excised leaves. Esterase resistance level was also significantly positively associated with tendency to be retrieved on excised leaves in the kdr-SS clones. Such sedentary behaviour is potentially maladaptive because aphids are at greater risk of becoming permanently separated from their host plant after leaf senescence and subsequent abscission. Furthermore, risks are likely to increase dramatically under cold and wet conditions when aphid movement can be severely restricted, although the significant field experiment was not particularly cold or wet. Reduced aphid movement was also inversely associated with esterase resistance in the laboratory-based experiments done at low temperature.

In the laboratory studies applying alarm pheromone, aphid response increased with increasing alarm pheromone concentration for all three kdr genotypes, although there were clear differences between them. kdr-SR and -RR forms showed the smallest responses; a behaviour that is probably maladaptive when predators and parasitoids are present. Esterase resistance was also inversely associated with disturbance, with R_3 aphids showing lower

tendencies to respond than R_2 forms. An esterase-revertant clone showed similar responses to the kdr-RR clones, ie aphids possessing its esterase-R_3 genotype and not its esterase-S phenotype. Two kdr-SR* clones, produced in laboratory-based sexual crosses, showing susceptibility to DDT normally associated with kdr heterozygotes in other species, had heterogeneous alarm responses when compared to kdr-SR clones collected from the field. The R_3* clone showed a higher response than the R_3 field clones. However, the R_2* clone affiliated with the R_2 field clones. The DDT-susceptible phenotype of these laboratory-raised clones* (consistent with kdr-SS forms) was therefore not consistently associated with increased alarm response. Further work is needed using more DDT-susceptible/kdr-SR clones raised in laboratory crosses.

The physical mechanism behind the varied responses to both leaf deterioration and alarm pheromone remains unclear. However, in the case of kdr, altered behaviour could be a direct pleiotropic effect of the mutation itself as resistance is conferred by an alteration in the voltage-gated sodium channel of nerve axon membranes that is associated with reduced nerve activity in Drosophila (Vais *et al.*, 1997). Furthermore, higher elevated esterase production might intuitively be expected to impose various costs. Indeed, esterase production was associated with reduced movement and alarm response. However, esterase-revertant clones, carrying large numbers of unexpressed esterase genes, did not affiliate with true esterase-susceptible forms. Instead, they showed similar responses to forms expressing high levels of esterase; suggesting that behaviour was not related directly to esterase overproduction. Having said this, both revertant clones were kdr-RR (homozygote) forms and therefore probably less likely to respond as a consequence of their kdr genotype. If and when, esterase-revertants with kdr-SS genotypes are isolated, they will need to be assessed. It seems more likely that the more sedentary behaviour of aphids with high esterase resistance is either a pleiotropic effect of DNA amplification or transposition, or a consequence of genetic linkage between esterase resistance and other mutations, in addition to kdr, influencing perception or locomotory behaviour.

All DDT-susceptible clones assessed for kdr genotype in the UK survey proved to be kdr homozygote-susceptible (SS). DDT-resistant aphids fell into two categories: kdr heterozygote (SR) or kdr homozygote-resistant (RR) with the former appearing to be the commonest genotype. kdr-SR forms appeared to show similar high levels of resistance to DDT and pyrethroids as kdr-RR aphids. However, the recent discovery of an additional mutation in a *M. persicae* clone at a site equivalent to super kdr in other pests suggests that kdr may not be solely conferring pyrethroid resistance in this species. Indeed, in other species kdr-SR forms tend to be susceptible to DDT. Further studies are clearly needed to establish the relative contributions towards resistance of mutations at both sites in the nerve protein.

Screening of the UK *M. persicae* samples with imidacloprid revealed that low-level, but statistically significant, tolerance to this insecticide (im-t) has been present over the last several years in the UK, but at a low frequency (only 2.2% of the samples contained im-t forms). Full dose-range imidacloprid bioassays supported the accuracy of this screening process, proving it to be a robust method for rapidly estimating not only frequencies of imidacloprid tolerance but also tolerance to acetamiprid in *M. persicae* populations at different localities (as cross-tolerance exists to these compounds). These bioassays also demonstrated that both chloronicotinyl compounds circumvent the esterase, MACE and kdr mechanisms. All of the UK im-t clones assessed in the dose-range bioassays showed resistance factors similar to the im-t standard clone, collected in 1990 from Greece, and other tolerant clones collected from around the world.

Assessments of selection by imidacloprid, applied as a soil drench at the recommended rate and a range of reduced concentrations to aphids on Chinese cabbage, demonstrated that *M. persicae* with higher levels of tolerance to this compound (measured in

bioassays) have significantly greater rates of population increase. This strongly supports the existence of glasshouse and field environments imposing potential resistance selection on this species. There was also some evidence that *M. persicae* showing the highest known imidacloprid tolerance tend to be less likely to relocate onto other parts of the plant after treatment. Such behaviour can only serve to increase selection pressures for greater imidacloprid resistance that may eventually lead to control failures. However, to date none have been verified anywhere in the world. There is therefore no current evidence that increased imidacloprid tolerance has evolved in *M. persicae* since the last assessments made several years ago on clones collected from outside the UK (Devine *et al*., 1996).

The question of whether the frequency of tolerant forms has changed remains unclear but we can speculate on their origin. Quite possibly tolerant forms carry mutations selected before neonicotinyl compounds were used, ie. aphids with tolerance have existed for many years and originally arose as a result of other selection pressures such as those imposed by host plants. Such a suggestion is supported by the fact that our imidacloprid-tolerant standard clone was isolated in 1990 before any neonicotinyls were introduced. In addition, im-t forms also tend to show greater tolerance to nicotine fumigation (Devine *et al*., 1996) and, as demonstrated in this report, are more likely to feed and reproduce on imidacloprid-treated plants than imidacloprid-susceptible (im-s) forms. In the past, tolerant aphids may have been tobacco-feeding forms adapted for survival in a high nicotine environment and therefore pre-disposed to resist neonicotinyls because these compounds target the nicotine receptors in the insect nervous system. A large number, but by no means all, of the experimental clones showing tolerance to imidacloprid were isolated on tobacco.

Intuitively, selection pressures favouring the evolution of greater imidacloprid resistance must exist during this compound's inherent protracted period of decay. This, coupled with the likelihood of increased use of imidacloprid and other neonicotinyl insecticides in the future, has potential serious implications for this new and versatile class of insecticides. Whatever the time-scale and route of selection, high resistance (> 50 fold) has been documented in whiteflies (Elbert & Nauen, 2000), a situation that can only serve to remind us that all insecticides, however novel, are ultimately prone to the evolution of resistance.

The applicability and value of the field simulator-based studies in both predicting the consequences of different selection regimes against pest populations and formulating resistance management tactics have been reinforced by this project. Its findings clearly demonstrate that the efficacy of a range of insecticides commonly used against *M. persicae* in the UK is highly dependent on the resistance mechanisms present. Furthermore, patterns of resistance are generally consistent across different host plants although aphid control tended to be a little better on potato compared to Chinese cabbage. This may relate to differences in plant architecture as the smaller leaves of potato appear to offer less protection for aphids from insecticides, particularly those with low volatility or systemic activity.

Pirimicarb and triazamate consistently performed well against all non-MACE forms of *M. persicae*; an encouraging finding for UK agricultural and horticultural growers in the light of MACE resistance becoming very rare in aphids on UK field crops over the last few years. Pirimicarb in particular is currently a favoured product, not only because it gives relatively good control of forms with high and extreme (R_2 and R_3) esterase resistance, but also because it is relatively benign to aphid parasitoids and predators. Continued biochemical monitoring of *M. persicae* populations should therefore remain a priority if pirimicarb and triazamate are to be used effectively, both in terms of minimising potential immediate problems with MACE resistance and delaying any build-up in esterase resistance and kdr (although this latter mechanism appears to be relatively widespread). Unfortunately, there are no current plans to systematically test *M. persicae* on UK crops for these forms of resistance

in the near future, although esterase and MACE will continue to be assessed in *M. persicae* caught in a number of suction traps. Clearly, any resurgence of the MACE mechanism in the *M. persicae* population would have serious implications for the future efficacy of these two insecticides against this species.

The three pyrethroids, lambda-cyhalothrin, cypermethrin and deltamethrin, showed poor efficacy against kdr forms of *M. persicae*. These findings are worrying in the light of our survey implying that kdr is common in *M. persicae* in the UK. If growers need to apply pyrethroids against pest complexes including this species, it is better to use them as mixtures with insecticides from other chemical classes; as illustrated by the general efficacy of the pyrethroid/pirimicarb mixture against kdr/non-MACE forms. Esterase levels seem to play a secondary role in pyrethroid resistance illustrated by esterase-R_3 forms tending to show greater rates of increase than -R_1's. Having said this, and as discussed earlier, the recent discovery of an additional mutation at the super kdr site complicates matters. Foliar applications of lambda-cyhalothrin (up to 10x field rate) against *M. persicae* clones carrying various combinations of kdr and super-kdr produced dose-responses for kdr-SR and –RR aphids although they were less pronounced for the latter. However, greater than 4x field rate was required before even kdr-SR aphids were reduced in number compared with the pre-spray counts. The presence of a super-kdr mutation in the heterozygous form, appeared to confer effective immunity to lambda-cyhalothrin applied at any rate. The frequency of these, and as yet unidentified super kdr-RR forms, is now being established in UK *M. persicae*.

All carbamate and pyrethroid applications were ineffective against *M. persicae* carrying esterase, MACE and kdr combined. In the UK, these particular forms were last seen in large numbers in samples collected in Lincolnshire in 1996 and were associated with severe control failures on potatoes and Brussels sprouts. Such events highlight an imperative need for new insecticides with alternative modes of action that are unaffected by the three resistance mechanisms. Imidacloprid is one example, along with pymetrozine; a product that has been recently registered for use on UK potatoes. Indeed, aphid bio- and field simulator-assays demonstrated that pymetrozine is highly effective at controlling *M. persicae* carrying all the known mechanisms of resistance. Furthermore, there was evidence of some negative cross-resistance with variability amongst non-revertant clones, admittedly over a narrow range of tolerance, showing a clear inverse association with esterase resistance level. This phenomenon is probably not related directly to esterase production because two revertant clones (showing esterase-S phenotypes) tended to affiliate with esterase-R_2 and R_3 clones. It is also probably not related to an aphid's ability to withstand starvation as a large proportion of nymphs from any of the clones surviving after 96 hours of exposure to pymetrozine were subsequently able to feed and develop on new untreated leaf discs.

M. persicae's breeding system in the UK has important implications for the dynamics of insecticide resistance. The prevalence of parthenogenesis is a key factor affecting the accumulation of different resistance mechanisms as it ensures that once they have been co-selected or come together (through recombination and sex) in the same aphid they will remain combined in clonal lineages for many subsequent generations. Hence, both the advantages and drawbacks conferred by one genetic factor, whether it is a resistance mechanism itself or an associated gene or gene complex, will affect any other mechanisms carried by that aphid clone. The stable close relationships built by parthenogenesis can therefore create non-independent fluctuations in the frequencies of the various forms of resistance in UK populations of *M. persicae*. As a result, selection by an insecticide favouring one resistance mechanism, can also benefit any associated mechanisms even if they do not confer resistance to that particular product. The reciprocal situation can also take place through adverse selection. Obviously, such declines can be partly influenced by reduced insecticide application coupled with immigration of susceptible clones from largely untreated crops or regions.

3.5 CONCLUSIONS OF PROJECT

Potential fitness costs suffered primarily in the absence of insecticides by *M. persicae* carrying high-esterase resistance, coupled with improved resistance management by growers based on advice stemming from this and other related projects, probably underlie the steady decline in the frequency of these forms in the UK over the last several years. However, knock-down resistance (kdr) has apparently not shown a similar decline despite appearing to be closely associated with maladaptive behaviour. This has important implications for the use of pyrethroids for controlling this pest.

The esterase-based mechanism is associated primarily with resistance to organophosphates and mono-methyl carbamates. MACE confers strong resistance to pirimicarb and triazamate. Whereas kdr is associated with resistance to pyrethroids, although the picture is probably being complicated by the newly-discovered super-kdr mechanism (whose frequency in the UK *M. persicae* population is unknown).

Field-simulator assessments of the efficacy of various insecticides against *M. persicae* carrying different forms of insecticide resistance produced data subsequently supported by full field experiments. This reinforced the applicability and relevance of field-simulator studies.

The neonicotinyl insecticides, imidacloprid and acetamiprid effectively circumvent the esterase, MACE and kdr mechanisms. The presence of a few imidacloprid-tolerant *M. persicae* in samples collected from English field and glasshouse crops over the last several years, coupled with the finding that these forms enjoy a slight fitness advantage at reduced rates of imidacloprid treatment, has important implications for the potential evolution and selection of more potent resistance to the chloronicotinyls in the future.

The new insecticide, pymetrozine, is highly effective against *M. persicae* carrying any combination of esterase, MACE, kdr or imidacloprid-tolerance. It can therefore play an important role in managing *M. persicae* in conjunction with other currently effective UK-registered insecticides, such as pirimicarb and imidacloprid,.

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4. ACHIEVEMENT OF PROJECT MILESTONES AND OBJECTIVES

The research was aimed at investigating the relative fitness of *M. persicae* carrying various combinations of the known resistance mechanisms both in the presence and absence of established and novel insecticides. The primary aim was to assess the implications of these mechanisms and generate information that could be rapidly used to influence present and future crop protection practices, and to provide guidance for delaying the increase of insecticide resistance. The Main Objectives of research were:

1. Investigate the incidence of the kdr mechanism in the UK and use the information to influence current decisions on the use of pyrethroids to circumvent the MACE mechanism

2. Investigate imidacloprid resistance in the UK to anticipate its likely impact on the performance of the chloronicotinyl class of insecticides

3. Establish the interaction of the MACE-, kdr- and esterase-based mechanisms, and how this will affect insecticide choice

4. Establish potential fitness drawbacks suffered by kdr forms in the absence of insecticides under sub-optimal conditions associated with the winter climate, either in their own right or through association with high esterase-based resistance

5. Assess forms of resistance to pirimicarb, triazamate and pyrethroids in *Aphis gossypii* and *Aphis nasturtii* which can both be important pests on potatoes and horticultural crops

6. Field trials to assess options for aphid control by insecticides

The project aimed to achieve this through the Primary Milestones:

Year 1 (November 1998-October 1999)

PM 3.1 **February 1999**

Field simulator study to adapt established methods used in studies on sugar beet and oilseed rape for assessing resistance selection on potato and Chinese cabbage

Achieved successfully

PM 4.2 **May 1999**

Complete winter field study of potential fitness drawbacks suffered by kdr forms of *M. persicae*.

Achieved successfully

PM 3.2 **Ongoing but key results will be achieved by November 1999**

Field simulator study of resistance shown by recently-collected UK *M. persicae* clones (carrying various combinations of the esterase- and MACE-based mechanisms) to spray applications of established and novel insecticides

Achieved successfully

Year 2 (November 1999-October 2000)

PM 3.3 **Ongoing but key results will be achieved by August 2000**

Field simulator study as in 3.2. using UK *M. persicae* clones carrying various combinations of the esterase and kdr mechanisms

Achieved successfully

PM 4.1 **Ongoing but most clones will have been collected by August 2000**

Collect suitable range of UK *M. persicae* clones for winter field study (milestone PM 4.2) of survival of kdr and non-kdr forms and simulator studies of resistance selection by insecticides (Primary Milestones 3)

Achieved successfully. Part of this milestone was addressed prior to the official start date of the project as it was essential that some of these clones were available for the field work that commenced in November 1998.

PM 5.1 **August 2000**

Quantify resistance and identify the best options for controlling *Aphis spp*. in the UK

This milestone was superseded by new milestones instigated during the course of the project

PM 4.3 **September 2000 (additional milestone)**

Carry out laboratory study of response of kdr and non-kdr *M. persicae* clones to synthetic alarm pheromone applied at a wide range of concentrations

Achieved successfully

PM 4.4 **December 2000 (additional milestone)**

Low temperature laboratory study of aphid movement from deteriorating leaves using kdr and non-kdr *M. persicae* clones

Achieved successfully

Year 3 (November 2000-October 2001)

PM 1.1 **February 2001**

Survey of the kdr mechanism in UK *M. persicae* populations

Achieved successfully

PM 2.1 **February 2001**

Identification of any increase in resistance to the neonicotinyl class of insecticides

Achieved successfully

PM 3.4 **May 2001**

Field simulator study of resistance of *M. persicae* clones showing susceptibility and low resistance to imidacloprid. Assess how applications of imidacloprid to compost can select for resistance

Achieved successfully

PM 3.5 Ongoing until **August 2001**

Laboratory bioassays to assess response of *M. persicae* clones with various combinations of resistance mechanisms to novel insecticides such as imidacloprid, acetamiprid and pymetrozine

Achieved successfully

PM 3.6 **September 2001 (additional milestone)**

Carry out field simulator study of resistance of UK *M. persicae* clones carrying various combinations of the esterase, MACE and kdr mechanisms to spray applications of pymetrozine (Plenum)

Achieved successfully

PM 6.1 **October 2001**

Complete field trials testing laboratory-based findings on insecticides choice for controlling *M. persicae*.

Achieved successfully

5. SUMMARY OF TECHNOLOGY TRANSFER AND PROJECT DELIVERABLES

The outputs gained from the project have been made readily available to the scientific community, and advisors and growers through IRAG and many other routes of communication including trade press. Although the last several years appear to have been relatively "quiet" for problems in controlling *M. persicae* in the UK, this does not preclude difficulties in the future. The key will be disseminating our findings and continued resistance monitoring, particularly for potential resistance to the two new classes of compounds (imidacloprid and pymetrozine) and MACE-based resistance.

Papers, presentations and posters relating to this project (chronological order)

A L Devonshire, I Denholm & S P Foster (1999) Insecticide resistance in the peach-potato aphid, *Myzus persicae*. *Proceedings of the ENMARIA Symposium: Combating Insecticide Resistance.* Thessaloniki, Greece, May 1999, 79-85 (presentation and paper).

S P Foster (1999) Fitness costs associated with insecticide resistance in peach-potato aphids. *ENMARIA Meeting*. Thessaloniki, Greece, May 1999 (presentation).

S P Foster, I Denholm & A L Devonshire (1999) The ups and downs of insecticide resistance in the peach-potato aphid, *Myzus persicae*. *Abstracts of the XIVth International Plant Protection Conference (IPPC).* Jerusalem, July 1999, 142 (invited keynote presentation and abstract).

S P Foster (1999) Insecticide resistance in peach-potato aphids: a cloud with a silver lining? *IACR-Rothamsted research day*. Harpenden, November 1999 (presentation).

M S Williamson, L M Field, G D Moores, S P Foster, I Denholm & A L Devonshire (1999) A mechanism-based approach to monitoring insecticide resistance in the peach-potato aphid, *Myzus persicae*. *Diagnostics in Pest and Disease Management Congress.* Cardiff, November 1999 (presentation).

Insecticide resistance in agricultural and horticultural pests. *Royal Show, Stoneleigh*, Warwickshire, July 2000 (display).

S P Foster & I Denholm (2000) Challenges with managing insecticide resistance in the aphid *Myzus persicae. Abstracts of the XXI International Congress of Entomology*. Iguassu Falls, Brazil, August 2000, volume 1, 324 (invited presentation and abstract).

I Denholm, M D Barber, G J Devine, S P Foster, K J Gorman & A R Horowitz (2000) Insecticide resistance and its implications for the field performance of pesticides. *Proceedings of BCPC Symposium: Predicting Field performance in Crop Protection.* University of Canterbury, September 2000, 37-46 (presentation and paper).

S P Foster, I Denholm & A L Devonshire (2000) The ups and downs of insecticide resistance in the peach-potato aphid, *Myzus persicae*. *Crop Protection* **19**, 873-879.

S P Foster (2000) Knock-down resistance (kdr) to pyrethroids in peach-potato aphids (Myzus persicae) in the UK: a cloud with a silver lining? *Proceedings of the Brighton Conference: Pests and Diseases*. Brighton, November 2000. Volume 1, 465-472 (poster and paper).

S P Foster (2001) Spatial and temporal dynamics of insecticide resistance in *Myzus persicae*. *Resistance 2001 Symposium*. IACR-Rothamsted, September 2001 (presentation and paper).

S P Foster (2001) Ecological implications of insecticide resistance in the peach-potato aphid, *Myzus persicae* in the UK. *Royal Entomological Society Meeting.* Cardiff, September 2001 (presentation).

<u>Popular articles</u> (chronological order)

- *Potato Technology*, May 1998
- *Farming News*, February 1999
- *Agriculture Link: Newsletter for the Agriculture & Horticulture Industries.* February 2000
- *Potato Review*. March/April 2000
- *Town and Country: Eyewitness.* June 2000
- *BBSRC News and Events article and Web Page.* July 2000
- *Farmers Weekly*. July 2000
- *Scottish Farmer*. July 2000
- *Potato Review*. July/August 2000
- *SAPPIO programme publicity fact-sheet.* August 2000
- *Potato Review*. March/April 2001
- *Town and country: Eyewitness.* June 2001
- *Press Association article available to newspapers and magazines worldwide on the WEB*. July 2001
- *The Vegetable Farmer*. August 2001
- *Potato Newsletter*. January 2002

Intended submissions to refereed journals stemming from this project

Spatial and temporal dynamics of insecticide resistance in *Myzus persicae*.

Field-simulator studies of insecticide resistance to dimethyl-carbamates and pyrethroids conferred by metabolic- and target site-based mechanisms in peach-potato aphids, *Myzus persicae* (Sulzer).

Bioassay and field-simulator studies of the efficacy of pymetrozine against peach-potato aphids, *Myzus persicae* (Sulzer), carrying esterase-, MACE and kdr-based insecticide resistance and tolerance to imidacloprid.

Evidence for cross-tolerance to imidacloprid and acetamiprid in peach-potato aphids, *Myzus persicae* (Sulzer).

Pleiotropic behavioural effects associated with insecticide resistance in *Myzus persicae* (Hemiptera: Aphididae) and *Musca domestica* (Diptera; Muscidae).

Book chapters

Insecticide resistance in aphids. Commissioned chapter in *Aphids as Crop Pests* (eds: H F van Emden & R Harrington) for CABI publications.

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