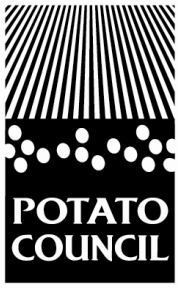

Final Report

Stewardship of neonicotinoid insecticides

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Project Report 2008/11

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1. Summary

The project aimed to build on findings from a previous SA-Link project (LK 0903) which focussed on established insecticide resistance mechanisms in peach-potato aphids (*Myzus persicae*) but also included preliminary work on neonicotinoids. The resulting data has been used to strengthen insecticide usage recommendations and anticipate potential problems with the increasing use of neonicotinoids for controlling this pest which has a broad host crop range and has proved very adept at developing resistance to many insecticides. This includes limited variation in response to the neonicotinoids which places them at risk from significant resistance.

Characterisation of standard *M. persicae* for response to neonicotinoids
Five different neonicotinoid compounds were applied in laboratory-based bioassays to asexual aphid clones which had previously been shown to vary up to 10-fold resistance factors to imidacloprid (Nic-R). All compounds tested were cross-resisted, with Resistance Factors (RFs, relative to a susceptible clone: Nic-S) ranked in the same order as for imidacloprid. RFs for most compounds were relatively low although clothianidin gave the highest RF. Results confirmed the potential for *M. persicae* to resist all neonicotinoids although the mechanism(s) responsible remain unknown.

Monitoring *M. persicae* samples for resistance to neonicotinoids and other insecticides
247 samples of *M. persicae* (collected from a range of field and glasshouse crops in England between September 2004 and December 2007) were screened for the presence of Nic-R aphids. There was neither an upward trend with collection date in their frequency nor the presence of any individuals with significantly greater resistance (Nic-R+). There was also no evidence of any effects of insecticide treatment, crop, latitude or longitude. This suggests that UK treatments with neonicotinoids, when aimed at aphids, remain effective and are not leading to a directional increase in neonicotinoid resistance. The frequency of *M. persicae* with high carboxylesterase resistance has declined, possibly as a result of the falling usage of OPs and fitness costs associated with this mechanism. However, aphids with the MACE and kdr mechanisms (to pirimicarb and pyrethroids respectively) were present in over 50% of the samples collected and remained relatively common. This reinforces the importance of neonicotinoids for controlling *M. persicae* with these resistance mechanisms.

Several *M. persicae* clones, collected from tobacco in northern Greece in July 2007, proved to show a previously unseen Nic-R+ response to imidacloprid (RFs up to ~50). These aphids have the potential to become more widespread in Europe (bearing in mind that MACE resistance probably originated in Greece and is now prevalent in the UK). It is not known what implications Nic-R+ has for current field rates, other neonicotinoids or alternative effective insecticides such as pymetrozine and flonicamid. This needs to be established in light of the known cross-resistance between neonicotinoids and pymetrozine that exists in whiteflies.

Operational factors affecting neonicotinoid resistance in *M. persicae*
Measurements of the fitness of Nic-S and Nic-R aphids, including survival, fecundity and willingness to feed, on untreated and neonicotinoid seed-treated plants were done in field-simulator chambers. These confirmed that aphids are controlled very well when these compounds are applied to cabbage at high rates. In contrast, oilseed rape seed-treated with imidacloprid (Chinook) had little effect on either aphid type because the dose rate is very low and not aimed at aphid control. Interestingly, Nic-R aphids showed significant fitness advantages on this host when it was seed-treated with clothianidin at a higher rate than imidacloprid. Experiments with recommended foliar applications of thiacloprid (Biscaya) applied to cabbage clearly showed that all aphids were controlled well at the time of

application. However, Nic-R aphids showed significant fitness advantages when they were introduced to treated plants one week after insecticide application. This advantage was reduced when aphids were introduced after two weeks (by which time even Nic-S aphids were largely unaffected). Collectively, the data suggest that the route of neonicotinoid treatment (seed versus foliar), dose rate and, potentially, type of neonicotinoid, can create 'windows of selection' that favour Nic-R aphids and therefore the potential evolution of greater resistance than that already present.

Potential imidacloprid resistance in *Aulocorthum solani*

An additional study was done in response to reports of control failures with imidacloprid-treated compost against the glasshouse-potato aphid, *Aulocorthum solani*. Two treated samples (collected in 2006 from lilies and fuchsias in Lincolnshire and Surrey respectively) and one untreated sample (collected in 2006 from fuchsias in Surrey) were obtained for resistance testing. These, along with a susceptible strain maintained at Rothamsted, were screened topically with diagnostic doses of imidacloprid that have been used for *M. persicae.* The two strains of *A. solani* suspected of showing resistance showed variation in their response at an intermediate level between the Nic-S and Nic-R *M. persicae* standard clones. It would appear therefore that a limited amount of variation in response to imidacloprid exists in *A. solani* but it is unlikely to cause control failures. The reports of resistance appear, therefore, to be due to treatments not reaching the aphids.

Response of UK pollen beetles to lambda-cyhalothrin

This was an objective specifically for Year 4 which was relevant to the project as the continued stewardship of neonicotinoids is dependent on developments with their use against other pests that inhabit crops attacked by *M. persicae*. One example is insecticide control of pollen beetles (*Meligethes aeneus*) on oilseed rape, a major overwintering host of *M. persicae*, because they have now evolved strong resistance to pyrethroids in many countries in mainland Europe. If these resistant beetles appear in the UK they will trigger the only viable alternative control measure of foliar sprays with a neonicotinoid which will extend the exposure of *M. persicae* to these compounds still further. Furthermore, these treatments will be on plants that have been previously seed-treated with a neonicotinoid. Thus, and at the request of Defra-PSD, we tested eight UK pollen beetle samples for their response to lambda-cyhalothrin (a pyrethroid) in coated-vial bioassays. None of the samples showed significant resistance to lambda-cyhalothrin.

Synthesis of findings and dissemination

The project has provided up-to-date information on potential resistance problems associated with neonicotinoids and other insecticide groups. This will be exploited by the PSD for the insecticide regulatory process and is information of direct relevance to ameliorating unnecessary effects of pesticide use on the environment. The current status of resistance to insecticides available for *M. persicae* control was used to strengthen insecticide usage recommendations made to UK growers in updated Resistance Management Guidelines tailored to specific crops (potatoes and brassicas), down-loadable from IRAG UK's website (http://www.pesticides.gov.uk/committees/resistance/index.htm. These are helping UK growers to make the right decisions on insecticide treatments.

2. Experimental Section

2.1 Introduction

Neonicotinoids are the most important group of insecticides to be developed since the pyrethroids. They have provided growers with valuable new tools for controlling some of the world's most destructive crop pests. Active ingredients based on new chemistry have become essential because of an accelerating loss of older ones through commercial or regulatory decisions and loss of efficacy due to the evolution of resistance. Rising costs of product development and registration, coupled with mergers and globalisation of the agrochemical industry, are also reducing the flow of replacements available to UK farmers. Insect groups targeted by neonicotinoids, primarily aphids in the UK, include species with a long history of developing resistance to earlier products, but against which neonicotinoids remain fully effective. However, the extent to which imidacloprid (Bayer CropScience), the commercial forerunner of neonicotinoids, has been incorporated into control strategies for several key crops has raised concerns over the development of neonicotinoid resistance (Cahill & Denholm, 1999; Foster *et al.*, 2003a; Foster *et al.*, 2008). Imidacloprid was first introduced to the UK in 1994 as a seed treatment for sugar beet. Since that date, the annual amount of UK beet seed-treated with neonicotinoids for control of soil and foliar pests (especially aphid vectors of virus yellows) has risen to nearly 90%. More recently, further approvals have been granted for neonicotinoid use as a soil drench to hops, and lettuce, a compost additive for ornamentals, as seed treatments to oilseed rape, cereals and brassicas, and as foliar applications to oilseed rape, brassicas and seed and ware potatoes. It should be noted that application rates of imidacloprid recommended for some pests are insufficient to kill others that occur on the same crop. For example, the imidacloprid rate applied as a seed treatment to oilseed rape and targeted against cabbage-stem flea beetle (*Psylloides chrysocephala*) was not proven to control a prominent UK pest, the peach-potato aphid (*Myzus persicae*), and the implications of this for selecting resistance in this species, which also attacks potatoes, sugar beet, brassicas, lettuce and ornamentals, were unknown.

To a large extent, pessimistic forecasts regarding the sustainability of imidacloprid usage had not been borne out in practice and this compound had proved remarkably resilient to resistance in aphids, and cases that had been reported were mostly still relatively manageable an/or geographically localised. At the beginning of the project, the existence of strong resistance in some pests, particularly the whitefly, *Bemisia tabaci* (Cahill *et al.*, 1999; Nauen *et al.*, 2002), and the Colorado Potato Beetle, *Leptinotarsa decemlineata* (Hollingworth *et al* 2002), had nonetheless demonstrated their potential to adapt and withstand field exposure to neonicotinoids. However, the ongoing release of new neonicotinoid compounds, as well as expansion in the use of existing ones, unless carefully considered and coordinated, seemed bound to increase overall levels of exposure to this chemical class and to enhance conditions favouring selection of any resistant individuals present. Due to the potential for cross-resistance (Foster *et al.*, 2003a; 2008), the development of resistance to neonicotinoids could also compromise the use of nicotine and pymetrozine as alternative control measures for *M. persicae* since cross resistance to imidacloprid and pymetrozine is known in whiteflies. As new products and uses arose, careful stewardship to ensure the lasting efficacy of the neonicotinoids had become a common concern to manufacturers, advisors and growers, and led to this Link project. It was designed to investigate the incidence of any low resistance to neonicotinoids and other forms of resistance in *M. persicae* in the UK, to follow up any reports of greater resistance from abroad, to test for cross resistance amongst a range of neonicotinoids, and to investigate conditions under which greater neonicotinoid resistance might arise and be selected.

The scientific objectives were:

1) Detailed characterisation of *M. persicae* clones that already show reduced susceptibility to imidacloprid.
2) Monitoring for spatial and temporal variation in susceptibility of *M. persicae* in UK localities and cropping systems with contrasting levels of neonicotinoid use and dosage.
3) Laboratory analyses of how operational factors affect the survival, willingness to feed and reproduction of clones showing full susceptibility and reduced susceptibility to imidacloprid (to anticipate selection pressures).
4) Development and dissemination of recommendations for the sustainable use of neonicotinoid insecticides in the UK.

2.2 Material and methods

2.2.1 Characterisation of standard *M. persicae* for response to neonicotinoids

Five *M. persicae* clones varying in their known response to imidacloprid were tested using topical bioassays with this compound and four other neonicotinoid molecules to investigate the extent and nature of cross-resistance amongst the class. This included three compounds currently approved in the UK for aphid control - thiacloprid, thiamethoxam, clothianidin. The two clones showing the lowest and highest responses were also tested with dinotefuran, a compound that has sometimes proved idiosyncratic in research on mechanisms of neonicotinoid resistance and their cross-resistance implications, eg. Lui *et al.* (2006). In addition, these two clones were tested using systemic bioassays applying imidacloprid to assess if route of treatment has an effect on resistance.

Aphid clones

The five clones of *M. persicae* included two (US1L and 4106A) that were fully susceptible to imidacloprid (Nic-S) and three (3495B, 4866A and 926B) that had proved to be resistant to this compound in previous topical application bioassays (Nic-R) (Table 1). These possessed different combinations of mechanisms conferring resistance to non-neonicotinoid insecticides. Each clone had been established originally from a single parthenogenetic female, and was reared on excised Chinese cabbage leaves (*Brassica napus* L var *chinensis* cv Tip-Top) in small plastic boxes maintained under a 21^{o}C, 16 h light/8 h dark regime.

Insecticides

For topical bioassays, technical grade neonicotinoids were obtained from their respective manufacturers: imidacloprid, clothianidin and thiacloprid (Bayer CropScience, Germany), thiamethoxam (Syngenta, Switzerland) and dinotefuran (Mitsui, Japan). The systemic bioassays used formulated imidacloprid (Confidor) diluted in distilled water.

TABLE 1. RESISTANCE STATUS AND ORIGINS OF *MYZUS PERSICAE* CLONES

Clone	*Response to Imidacloprid*[a]	*Other resistance mechanism*			*Country of origin*	*Year collected*
		Carboxylesterase[b]	*kdr*[c]	*MACE*[d]		
US1L	S	No	No	No	England	1975
4106A	*S*	*No*	*No*	*No*	*Scotland*	*2000*
3495B	R	Yes	Yes	Yes	England	1999
4866A	R	Yes	Yes	No	England	2003
926B	R	Yes	No	Yes	Greece	1990

[a] Based on previous topical bioassays. S: susceptible, R: resistant.
[b] Based on an immunoassay (Devonshire *et al.*, 1986).
[c] Based on a Taqman method (Anstead *et al.*, 2004).
[d] Based on a kinetic assay (Moores *et al.*, 1994).

Topical bioassays

Topical application bioassays involved transferring young adult apterae of each clone to the abaxial surface of leaf discs cut from Chinese cabbage (10 aphids per leaf disc) held on 1.1% agar in plastic tubs (3 cm in diameter). The lips of these tubs were coated with Fluon to prevent any escape. Aphids from each clone were left for at least 2 h to settle and then dosed individually with a 0.25 µl droplet of acetone containing a known concentration of a technical-grade neonicotinoid, over a range of doses, using a micro-applicator (Burkard Manufacturing Ltd., UK). Initial bioassays used imidacloprid alone to confirm the Resistance Factors (RF) that had been previously calculated for the clones, and were followed by bioassays with the four other neonicotinoids. Dinotefuron was tested against two clones, US1L (Nic-S) and 926B (Nic-R). Control aphids were treated with 0.25 µl of acetone only. In each bioassay at least 30 aphids of each clone were tested at each dose, and at least two bioassays were done for each compound against each clone. Responses were assessed after 72 h at 21^{o}C, under a 16 h light/8 h dark regime.

Aphids that were dead or showed symptoms of irreversible poisoning were classed together as 'affected'. Those that were able to walk short distances were scored as being 'mobile', a criterion that was also used in the screening assays described in section 2.2.2. ED_{50} values (producing 50% affected and dead aphids) were calculated by probit analysis using the POLO program (Leora Software, Berkeley, California) comparing mobile versus affected aphids and with RFs expressed relative to the ED_{50} values for the reference US1L clone (Nic-S). Correlation between clone response to the four neonicotinoids (excluding dinotefuron) was tested using Kendall's Coefficient of Concordance, calculated from ranking ED_{50} values for each compound but disregarding variation (confidence intervals) associated with ED_{50} estimates.

Systemic bioassays

Systemic application bioassays involved the same methods as the topical bioassays apart from leaf discs being cut from excised leaves whose cut petioles had been immersed in one of a range of dilutions of formulated imidacloprid for 24 h (while located in a fume hood at ~21^{o}C). Only the *M. persicae* clones showing the highest and lowest responses in the topical bioassays, 4106A (Nic-S) and 926B (Nic-R), were tested in this way. The RF of 926B was calculated using the relative ED_{50} value to 4106A.

2.2.2 Monitoring *M. persicae* samples for resistance to neonicotinoids and other insecticides

Live *M. persicae* samples were collected from a range of field and glasshouse crops between September 2004 and December 2007. Field collections were made primarily from potatoes, vegetable brassicas, oilseed rape and sugar beet. Glasshouse collections were made from vegetable and ornamental hosts. When possible, in each field and glasshouse sample, aphids were collected, along with their supporting leaves, from plants at scattered positions throughout the collection site. The samples were then immediately transported by post or by hand to Rothamsted either in staple-sealed plastic bags or Petri dishes inside a robust box. Each sample was accompanied by a record of host plant, insecticide treatment history, and place and date of origin. Place of origin was subsequently converted into longitude and latitude coordinates using the Google Earth programme. Samples ranged in size from a few to over sixty aphids although the majority consisted of at least 20 individuals.

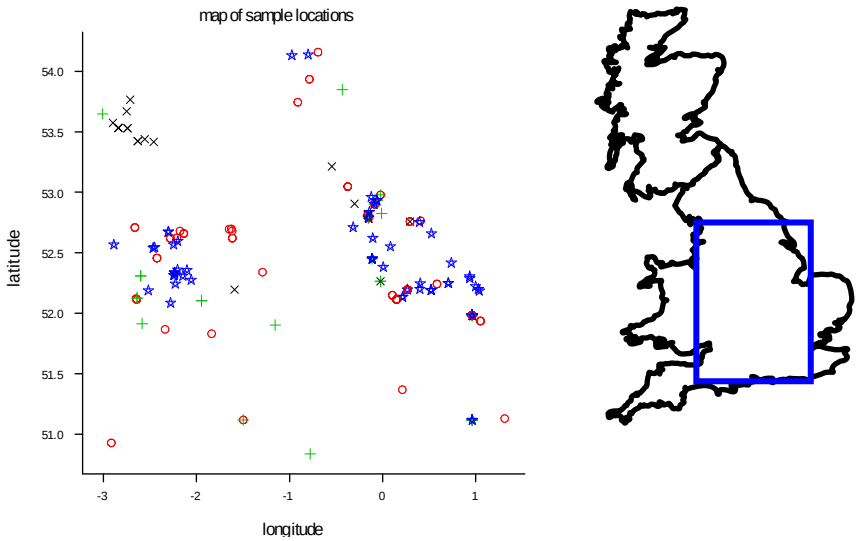
On arrival at Rothamsted, *M. persicae* were sorted from each sample and placed, as either adult and 4th instar or lower instars, onto excised Chinese cabbage leaves in small box cages, maintained under a 16h/8h light/dark photoperiod at ~ 21^{o}C in the laboratory, to allow them to develop and produce the next generation (G1). This was done to avoid any qualitative effects due to possible prior insecticide exposure in the field/glasshouse and aphid age/health. Boxes were checked daily for the presence of aphids that had succumbed to fungal or parasitoid attack and these individuals were removed, using fine forceps, to protect any accompanying aphids. When the G1 aphids had become young adults, apterous individuals were selected for topical bioassays applying screening doses of imidacloprid per aphid (using the methods for topical applications described above). Bioassays were also done on aphids from subsequent generations, depending on sample size and rearing success. Two doses were applied to each sample, a sub-set with 2.5 ng imidacloprid and a sub-set with 0.5 ng imidacloprid in 0.25 ul droplets of acetone to each aphid. These doses were chosen because they not only allowed discrimination between Nic-S and Nic-R aphids (RF up to ~10), with the latter being scored as mobile as they were able to walk short distances, but also those that potentially showed higher resistance (Nic-R+), which would be more mobile and probably capable of reproduction. The few aphids that fell into this latter category in the bioassays with 2.5 ng imidacloprid were transferred to excised leaves in a box cage, allowed to reproduce, and the offspring re-tested with this dose to check that the result was not due to a misdosing, ie. the aphids had either not been dosed or had not received a complete droplet. After discussion with the Link Steering Group, the lower dose was suspended for samples collected from 2006 onwards as it became clear that it was not making any significant additional contribution to the survey. Control bioassays, applying the screening dose and acetone alone to Nic-S and Nic-R standard clones (4106A and 926B respectively), were also done on a regular basis throughout the course of sample testing.

225 field and 22 glasshouse samples were tested. A proportion of the samples (44 field and 4 glasshouse, collected from May 2007 onwards) were also screened with a diagnostic dose of pymetrozine using leaf-dip bioassays (Foster *et al.*, 2002a). For each bioassay, 2 cm diameter leaf discs were cut from Chinese cabbage and dipped in an aqueous solution of formulated pymetrozine (Plenum, 25% w/w, Syngenta) at 30,000 µg litre^{-1} (30 ppm). Control discs were dipped in water only. They were 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. After two hours, four young apterous adults were transferred to each disc and left overnight at 21°C to produce offspring. The adults were then removed leaving up to 20 first instar nymphs on each leaf disc. These were then maintained at 21°C under a 16 h light/ 8 h dark regime. Nymph mortality was assessed 96 h after the adults were initially placed onto the discs. At least 20 nymphs were tested per clone.

Samples collected up until January 2007 were tested biochemically for the carboxylesterase and MACE resistance mechanisms (Foster *et al.*, 2002b) and a few aphids from each sample were also tested for their kdr and super-kdr genotypes using a DNA-based technique (Anstead *et al.*, 2004, 2008). From February 2007 onwards, the development of a DNA-based technique to test for MACE allowed between 1 and 6 aphids from each sample (depending on initial sample size) to be genotyped for this mechanism. After consultation with the Steering Group, carboxylesterase testing ceased from this point onwards to accommodate for the new testing method.

A total of 219 field (Figure 1) and 21 glasshouse samples were included in the analysis of the survey data. The proportions of Nic-R ('mobile') aphids were analysed using a Generalized Linear Model (GLM) with binomial error and logit link. This analysis takes into account the varying numbers of aphids screened in each sample (ranging from 5 – 93) and assumes the number of Nic-R aphids follows a binomial distribution. Cubic splines in day number were fitted with up to 8 df to investigate the underlying time trend in the data. Whilst there were multiple samples (2 – 46) for 31.4% of the 105 different latitude/longitude combinations present (recorded to the nearest second in each direction) it was assumed that samples were independent (i.e. there were no repeated samples from exactly the same physical location).

FIGURE 1. MAP OF SAMPLE LOCATIONS LABELLED BY YEAR



(black cross = 2004 (n = 19), red circle = 2005 (n = 99), green plus = 2006 (n = 31) and blue star = 2007 (n = 70)). Zero longitude is the Greenwich meridian.

2.2.3 Operational factors affecting neonicotinoid resistance in *M. persicae*

This study was done to provide insights into the complex relationships between operational parameters (dose-rate, time since treatment and seed vs. foliar insecticide application) and susceptibility and low resistance to neonicotinoids. Each of seven experiments measured the response of aphids from a Nic-S clone(4106A) and a Nic-R clone (926B, RF ~ 15-fold) to either seed or foliar neonicotinoid treatments on whole brassica plants situated in quarantined field simulators, with dimensions: 1m x 1m x 1.5m (Figure 2). All but one experiment used untreated brassica plants and plants seed-treated with the few registered doses of two neonicotinoids, imidacloprid and clothianidin, and a dose of clothianidin likely to be registered in the near future (Table 2). The other experiment investigated the efficacy of a thiacloprid spray applied at the recommended rate for brassicas.

FIGURE 2. FIELD SIMULATORS.

TABLE 2. HOST PLANT, NEONICOTINOID COMPOUND, DOSE AND METHOD OF TREATMENT, ARRANGED IN ORDER BY DOSE FACTOR AND TREATMENT METHOD, USED IN THE FIELD-SIMULATOR BASED EXPERIMENTS.

Experiment	Host plant[1]	Compound	g ai/seed	Dose[2]	Treatment	Design	Batch[3]
2	OSR	Imidacloprid	1^{-5}	1	Seed	Split	1&2
4	OSR	Imidacloprid	1^{-5}	1	Seed	Random	1
6	OSR	Clothianidin	5^{-5}	5	Seed	Split	1&2
5	Cabbage	Clothianidin	1.2^{-3}	120	Seed	Split	1&2
1	Cabbage	Imidacloprid	1.4^{-3}	140	Seed	Split	1
3	Cabbage	Imidacloprid	1.4^{-3}	140	Seed	Split	1&2
7	Cabbage	Thiacloprid	N/A	N/A	Foliar	Split	1

[1]OSR: oilseed rape.
[2]Based on g ai per seed relative to imidacloprid on oilseed rape. Figures above 100 and thiacloprid are aimed at controlling aphids.
[3]Number of plant batches.

Aphid rearing, field simulator lay-out and plant inoculation

Experimental aphids were reared from a Nic-S (4106A) and a Nic-R (926B) standard laboratory clone that had been maintained under a 16 h light/8 h dark photoperiod at ~21°C as virginoparous, predominantly apterous colonies on excised Chinese cabbage leaves (*Brassica napus* var. *chinensis* cv Tip-Top) (Brassicaceae) in small box cages. The floor of each simulator in each experiment was covered in a layer of fresh blue Wypall tissue (Kimberley-Clark, UK). Pots containing each plant were placed into the simulators in plastic trays (22 x 15 x 5 cm) containing tap water that was topped up every several days. These were arranged in vertical rows. Inoculation of each simulator-based plant occurred only once and involved introducing three young apterous adults (between 10 and 12 days old) from either both clones in separate small clip cages (Figure 3), with each clip cage in each clone pair being attached to different leaves on the same plant (experiments 1, 2, 3, 5 and 7), or from one of the two clones, with just one clip cage attached to a leaf on each plant (experiments 4 and 6). The latter method was used when the clip cages were eventually removed leaving aphids to reproduce and move around their host plant. This occurred when data recordings were either made more than five days after aphid inoculation, when aphids could have become too crowded in the clip cages, or when for the initial part of the experiment where foliar sprays were applied (see later).

FIGURE 3. CLIP CAGES USED FOR INOCULATING PLANTS WITH APHIDS.

Seed-treatment experiments

Depending on experiment, either individual oilseed rape or individual cabbage plants were grown in compost from single neonicotinoid-treated or untreated seed in individual pots (14 cm in diameter) in a glasshouse. They were then transferred to two field simulators several weeks after sowing when the plants were ~3 weeks old (batch 1) and, in some of the experiments, also when they were ~5 weeks old (batch 2) (Table 2). Each simulator contained eight plants arranged in two rows of four, such that there was no between-plant contact. Four of these plants were seed-treated and four untreated. Experiments 2, 3, 5, 6 and 7 were run twice.

In most experiments, both *M. persicae* clones were allocated to individual untreated and treated plants within each of the simulators (maintained under a 16 h light/8 h dark photoperiod at ~23^{o}C) using similar principles based on either split plot or random block designs. The latter was used in experiment 4, including only one clone being allocated to each plant, because the clip cages were removed after two days to allow the aphids to multiply. In experiment 1, the clip cages were opened after one, three and five days and the number of offspring and the position of the adults (either on or off the inoculation leaf) were recorded. From experiment 2 onwards, this protocol was altered slightly with recordings being made two and five days after the aphids had been introduced to the plants. In experiments 2, 3, 5 and 7, a second aphid inoculation took place two weeks after the first (Table 2), using a second batch of plants transferred at that point from the glasshouse, ie. plants had been growing for two weeks longer in the glasshouse from seeds sown at the same time as the first batch. Recordings of aphid fecundity and the position of adults were subsequently made after another two and five days. At the end of each batch, the plants and any remaining aphids were frozen at -20^{o}C and then discarded after 24 h.

Foliar treatment experiments

Experiment 6 used an altered design to the seed treatment experiments. It initially involved only untreated cabbage seed and plants, again grown one per pot, were transferred to four simulators three weeks after sowing. One simulator (simulator 1) contained 18 plants, arranged in three rows of six, and the other simulators (simulators 2, 3 and 4) contained six plants each, arranged into two rows of three. Six plants in simulator 1 (occupying positions 1 and 2 in each row) and all the plants in simulator 4 were then inoculated with aphids in clip cages (one clone per clip cage per plant) which were removed after two days leaving the adults to reproduce. When the plants were four weeks old, a pre-spray count of the number of aphids on each of them took place. All the plants in all the simulators were then either sprayed with thiacloprid, Biscaya: 0.4 l product ha^{-1}, equivalent to 300 l ha^{-1} (simulators 2, 3 and 4), or water alone (simulator 1: untreated) using hand-held lance sprayers. After five days, a post-spray count of the number of aphids alive on each inoculated plant was made. Experience has shown that very little aphid movement takes place between plants during the pre- and post-spray period (Foster *et al*., 2003b). When the plants were five weeks old, a second aphid inoculation was made to six plants in simulator 1 (occupying positions 3 and 4 in each row) and to all six plants in simulator 3. After two and five days the number of aphids alive on each inoculated plant was recorded. Finally, when the plants were six weeks old, a third aphid inoculation was made to the remaining six plants in simulator 1 (occupying positions 5 and 6 in each row) and to all six plants in simulator 3. At the end of each set of aphid recording the plants and any remaining aphids were frozen at -20^{o}C and discarded after 24 h.

Statistics

Individual plants were considered as the basic experimental units, except in experiment 6 (foliar application of thiacloprid) where the basic experimental unit was a simulator. In each analysis total variation was partitioned according to the physical structure of the experiment, ie. into that due to experiments, plant batches, simulators, blocks within simulators, positions (plants) within simulators or blocks. Cages within positions, as appropriate, and treatment effects were estimated in the appropriate strata.

In each analysis, total variation was partitioned according to the physical structure of the experiment, i.e. into that due to experiments (combined analyses for experiments 3, 4, 5, 6, 7), simulators, positions within simulators and plants within positions, simulators within experiments and positions within simulators. In each case the treatment structure was a full factorial in Treatment and Clone, and Time (=Age) where appropriate, i.e. symbolically Treatment*Clone, or Treatment*Clone*Time.

2.2.4 Additional work done on *M. persicae* from abroad

Greece

In response to reports of increased resistance to imidacloprid in *M. persicae* from southern Europe, a small number of aphid clones, collected from tobacco in Greece in July 2007, were screened with 2.5 ng imidacloprid per aphid and ED_{50} values subsequently obtained using full dose-range bioassays for those clones showing the highest mobility.

New Zealand

M. persicae samples from New Zealand were made available by a visiting worker. These allowed an assessment of the selection pressures for neonicotinoid resistance imposed by imidacloprid seed treatments to potatoes, a method of application not used in the UK. They also allowed measurements of the frequencies of the resistance mechanisms known to exist in this pest in Europe.

Individual aphids of *M. persicae* were collected from ware potato crops from 31 sites in New Zealand between mid-January and March 2005. In New Zealand, clonal asexual lineages from each of the 72 field-caught aphids were maintained on potato leaflets of various cultivars, transferred weekly on to fresh potato leaves, and maintained for 4-8 weeks at 18-20°C under fluorescent lighting. In late April 2005, the lineages were transferred to Rothamsted for resistance testing. There, individuals were maintained on excised Chinese cabbage leaves in small box cages using the rearing methods described in section 2.2.2. When apterous adults were present, individuals were either frozen dry at -80°C for kdr and super-kdr and micro-satellite genotype testing, tested biochemically for the carboxylesterase and MACE resistance mechanisms or screened for imidacloprid resistance using the screening dose method described in section 2.2.2.

Response to synthetic alarm pheromone

In order to test the hypothesis that carboxylesterase resistance and kdr impose pleiotropic fitness handicaps on aphid behaviour, response to synthetic aphid alarm pheromone, (*E*)-*ß*-farnesene, was measured in a series of separate laboratory experiments using a genetically-diverse subset of 22 of the *M. persicae* clones collected from New Zealand (established using micro-satellite markers) and 12 standard lineages from the UK or Europe shown previously to have either 'high' or 'low' alarm responses associated with different combinations of carboxylesterase resistance and kdr. The New Zealand clones were chosen on the basis of having a distinct multilocus micro-satellite genotype, apart from NZ3 which had a genotype found previously in the UK.

For each experimental replicate, three young adult apterae were placed onto 20 mm diameter Chinese cabbage leaf discs. The aphids were left overnight and then removed, leaving a cohort of first instar offspring (in most replicates numbering at least 10 aphids). The response of these nymphs was then assessed for 2 minutes following exposure to a 1 µl droplet of synthetic alarm pheromone (0.1 mg ml^{-1} (E)-*β*-farnesene in hexane). Nymphs that unplugged their stylets and walked away were recorded as responding. Replicates containing nymphs treated with 1 µl droplets of hexane alone, which did not stimulate movement, were used as controls. Each replicate was tested once and then discarded. Clones were tested using between one and three replicates per clone in each of five experiments. The proportions of responding aphids were analysed with a binomial generalised linear model with a logit link (McCullagh & Nelder, 1989). Results are presented as percentages, followed by 95% confidence limits in parentheses.

2.2.5 Additional work done in response to reports of potential imidacloprid resistance in *Aulocorthum solani*

In response to reports of control failures with imidacloprid-treated compost against the glasshouse-potato aphid, *Aulocorthum solani*, two treated samples (5076 and 5081), collected in 2006 from lilies and fuchsias in Lincolnshire and Surrey respectively, and one untreated sample (5082), collected from fuchsias in Surrey, were obtained for resistance testing. These, along with a susceptible strain maintained at Rothamsted, were screened with the diagnostic doses used for *M. persicae*; ie 2.5 ng imidacloprid and 0.5 ng imidacloprid in 0.25 ul acetone (see section 2.2.2).

2.2.6. Response of UK pollen beetles to lambda-cyhalothrin

This was an objective specifically for Year 4. It was relevant to the project as the continued stewardship of neonicotinoids is dependent on developments with their use against other pests that inhabit crops attacked by *M. persicae.* One example is insecticide control of pollen beetles (*Meligethes aeneus*) on oilseed rape, a major overwintering host of *M. persicae*, because they have now evolved strong resistance to pyrethroids in many countries in mainland Europe. If these resistant beetles appear in the UK they will trigger the only viable alternative control measure of foliar sprays with a neonicotinoid which will extend the exposure of *M. persicae* to these compounds still further. Furthermore, these treatments will be on plants that have been previously seed-treated with a neonicotinoid. Thus, and at the request of Defra-PSD, we tested a small number of UK pollen beetle samples (Table 3), collected by ADAS, for their response to lambda-cyhalothrin (a pyrethroid) in coated-vial bioassays.

TABLE 3. DATE OF COLLECTION, CROP AND ORIGIN OF *MELIGETHES AENEUS* SAMPLES TESTED IN 2007.

Sample	Collection date	Bioassay date	Host crop	Origin
1	10/4/07	11/4/07	Oilseed rape	Harpenden, Hertfordshire
2	19/6/07	21/6/07	Oilseed rape	Terrington St Clement, Norfolk
3	20/6/07	21/6/07	Potatoes	Tong Norton, Shropshire
4	21/6/07	22/6/07	Oilseed rape	Bere Regis, Dorset
5	21/6/07	22/6/07	Field beans	Boxworth, Cambridgeshire
6	26/6/07	27/6/07	Roses	Boxworth, Cambridgeshire
7	26/6/07	27/6/07	Strawberries	Milton, Cambridgeshire
8	26/6/07	27/6/07	Strawberries	Milton, Cambridgeshire

2.3 Results

2.3.1 Characterisation of standard *M. persicae* for response to neonicotinoids

Topical bioassays

Reduced sensitivity to the neonicotinoid, imidacloprid, was disclosed in laboratory bioassays and this was consistent across all four compounds tested. Thiamethoxam, thiacloprid, clothianidin and dinotefuron were cross-resisted, with ED_{50} values ranked in the same order as for imidacloprid (Table 4). Resistance factors (RFs) ranged up to 11 for imidacloprid, 18 for thiamethoxam, 13 for thiacloprid, 100 for clothianidin and 6 for dinotefuran.

In line with previous findings (Foster *et al*., 2003a), clones 4106A and US1L responded similarly to imidacloprid. The three other clones showed 6- to 11-fold resistance to this compound. This pattern of response was repeated for thiamethoxam, thiacloprid and clothianidin, with 926B proving to be the most resistant clone in each case. RFs ranged up to 11 for imidacloprid, 18 for thiamethoxam, 13 for thiacloprid and 100 for clothianidin. Kendall's Coefficient (KC) for these four insecticides ($KC=0.925$, $p<0.01$) demonstrated strong concordance in the ranking of the ED_{50} values.

Excluding dinotefuran, the compounds showed similar baseline potency against the two susceptible clones (US1L and 4106A). The ED_{50} for dinotefuran was c. 100-fold higher than for other neonicotinoids against US1L, implying substantially lower potency in topical application bioassays.

TABLE 4. RESPONSE OF *MYZUS PERSICAE* CLONES IN BIOASSAYS APPLYING IMIDACLOPRID, THIAMETHOXAM, THIACLOPRID, CLOTHIANIDIN AND DINOTEFURAN

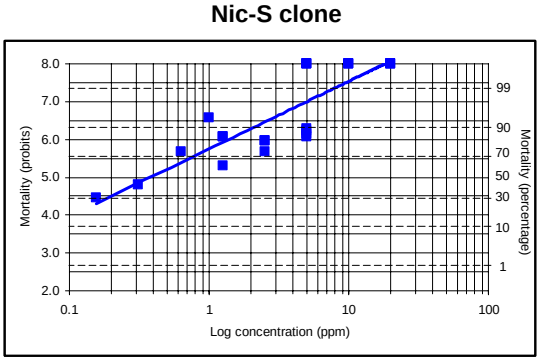
Clone	*ED_{50}*[1]	*95% CI*[2]	*Slope*	***RF***[3]
Imidacloprid				
US1L	0.090	0.068-0.117a	2.4	**1**
4106A	0.109	0.056-0.174a	1.4	**1.2**
3495B	0.558	0.309-0.848b	2.0	**6.2**
4866A	0.578	0.191-0.959b	1.8	**6.4**
926B	0.997	0.665-1.35b	1.3	**11**
Thiamethoxam				
US1L	0.068	0.041-0.097a	2.3	**1**
4106A	0.098	0.038-0.143a	2.3	**1.5**
3495B	0.314	0.165-0.585b	1.1	**4.6**
4866A	0.815	0.510-1.15b	2.3	**12**
926B	1.225	0.413-1.99b	1.3	**18**
Thiacloprid				
US1L	0.039	0.020-0.062a	1.9	**1**
4106A	0.043	0.025-0.062a	1.7	**1.1**
3495B	0.181	0.090-0.335b	0.9	**4.6**
4866A	0.279	0.159-0.422b	1.7	**7.1**
926B	0.506	0.272-0.735b	1.8	**13**
Clothianidin				
US1L	0.034	0.017-0.061a	1.2	**1**
4106A	0.028	0.013-0.043a	1.8	**0.8**
3495B	0.353	0.223-0.485b	1.5	**10**
4866A	1.109	0.876-1.35c	2.0	**33**
926B	3.414	2.61-4.24d	1.7	**100**
Dinotefuran				
US1L	9.101	3.99-12.6a	3.8	**1**
926B	56.30	35.5-94.5b	2.3	**6.2**

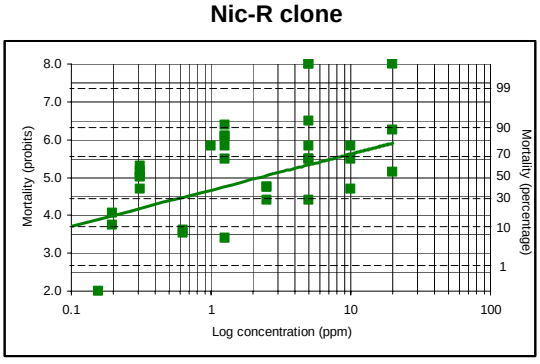
[1] Effective dose (ng active ingredient per aphid) resulting in 50% dead or irreversibly poisoned.
[2] Confidence limits at 95%; values followed by the same letter do not differ significantly for each compound.
[3] Resistance = ED_{50} for clone/ ED_{50} for US1L for each compound.

Systemic assays

The response of the Nic-S and Nic-R clones to full dose range systemic applications of imidacloprid are shown in Figure 4. The ED_{50} values for these clones were 0.28ppm and 0.53 ppm respectively. These data show a lower RF (1.9) compared to topical bioassays applying imidacloprid to these two clones (Table 4).

FIGURE 4. RESPONSE OF NIC-S AND NIC-R *M. PERSICAE* CLONES TO IMIDACLOPRID APPLIED IN SYSTEMIC BIOASSAYS.





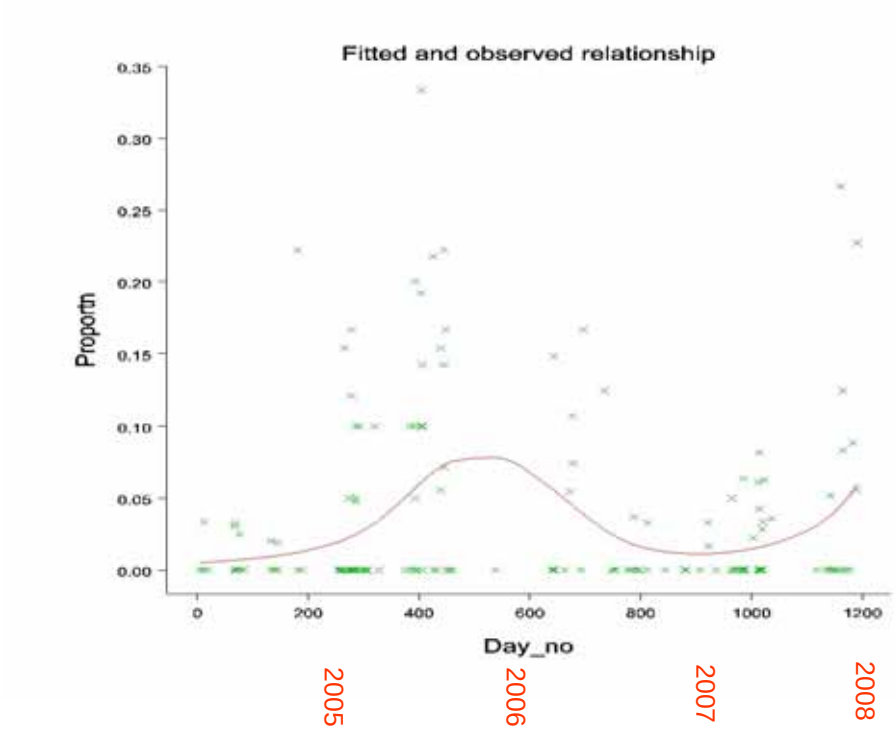
2.3.2 Monitoring *M. persicae* samples for resistance to neonicotinoids and other insecticides

Response to imidacloprid

The screening of UK field and glasshouse samples of *M. persicae* using topical bioassays, disclosed the presence of aphids showing reduced sensitivity to imidacloprid (Nic-R with RFs up to ~10). Figure 5 shows the changes in the proportions of these aphids in each field sample versus date of collection. There was no evidence that a more complex fitted spline was required (see below). A smoothing spline suggested an interesting regular periodicity with an increasing trend through 2004/5 followed by a downward trend in 2006 and an increase over 2007. However, these are cyclic patterns superimposed over an underlying flat trend and there is no evidence for an overall increase with time as evidenced by the non-significant linear component of the spline (logit scale: slope = 0.000072, $t_{213} = 0.23$, $P > 0.05$).

None of the field or glasshouse samples contained individuals carrying higher resistance (equivalent to > 10 fold) that would compromise neonicotinoids when they are applied at rates aimed at controlling aphids, ie. none of the offspring of the few aphids that showed the ability to reproduce subsequently reproduced themselves after receiving a screening dose of imidacloprid. There was also no evidence of any association between the proportions of Nic-R aphids with crop, or latitude or longitude of collection. Jointly, this suggests that selection pressures being imposed by neonicotinoids in the UK have so far not been great enough to either favour aphids carrying either reduced sensitivity to these compounds or led to the evolution of greater resistance.

FIGURE 5. PLOT OF PROPORTION NIC-R ('MOBILE') APHIDS IN EACH FIELD SAMPLE (BACK TRANSFORMED FROM THE LOGIT SCALE) AGAINST DAY NUMBER WITH A 5 DF SMOOTHING SPLINE SUPERIMPOSED. DAY 0 = 13/9/04.



One significant feature of the data was the seasonality of samples from different crops. A plot of the raw proportions (Figure 6) shows this clearly. It was therefore not possible to fit separate spline models to continuous data over time for all crops for comparison. However, there was no evidence that the shape of the overall spline in Figure 5 was unduly influenced by crop (5 levels as described above), or by latitude (Figure 7) (converted to a categorical factor with 2 levels: lat < 52.5, lat ≥ 52.5), longitude (Figure 8) (converted to a categorical factor with 3 levels: long < -1.5, -1.5 ≤ long < 0, long ≥ 0) or treatment (4 levels: untreated, 2/6, 3, 4/5) based on comparison of the spline deviance before (93.2) and after fitting each of these factors in turn (crop 83.4, latitude 95.7, longitude 89.7, treatment 86.9).

FIGURE 6. RAW PROPORTION 'MOBILE' APHIDS LABELLED BY CROP. BLACK CROSS = POTATO (CAT. 1); RED CIRCLE = OSR (CAT. 2); GREEN PLUS = SUGAR BEET (CAT. 3); DARK BLUE STAR = BRASSICAS (CALABRESE/ BRUSSELS/ BROCCOLI, ETC.) (CAT. 4); LIGHT BLUE SQUARE = REMAINDER (CAT. 5-8).

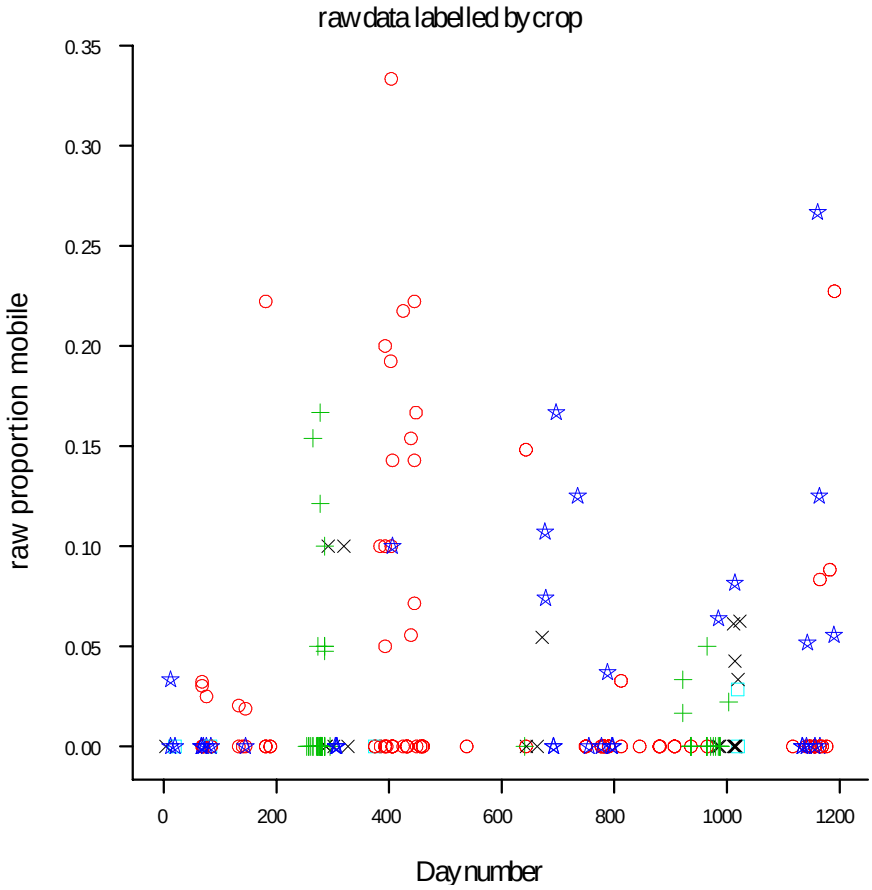


FIGURE 7. PLOT OF PROPORTION MOBILE APHIDS (BACK TRANSFORMED FROM THE LOGIT SCALE) AGAINST LATITUDE WITH 2 DF CUBIC SPLINE SUPERIMPOSED.

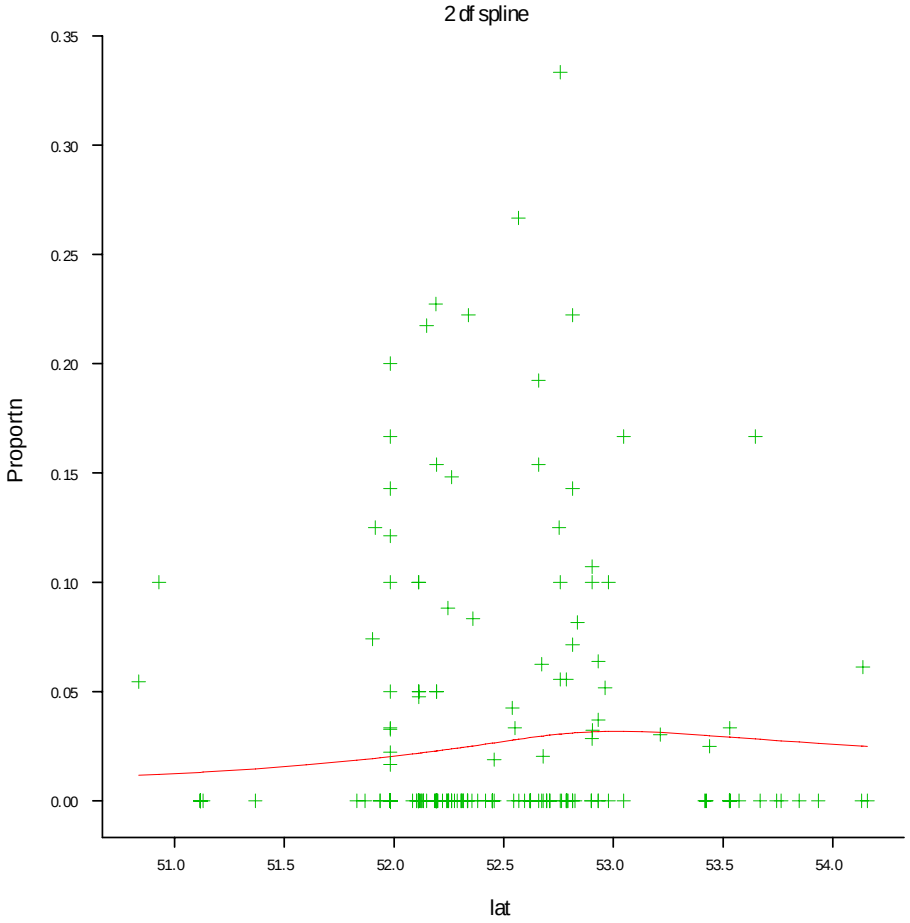
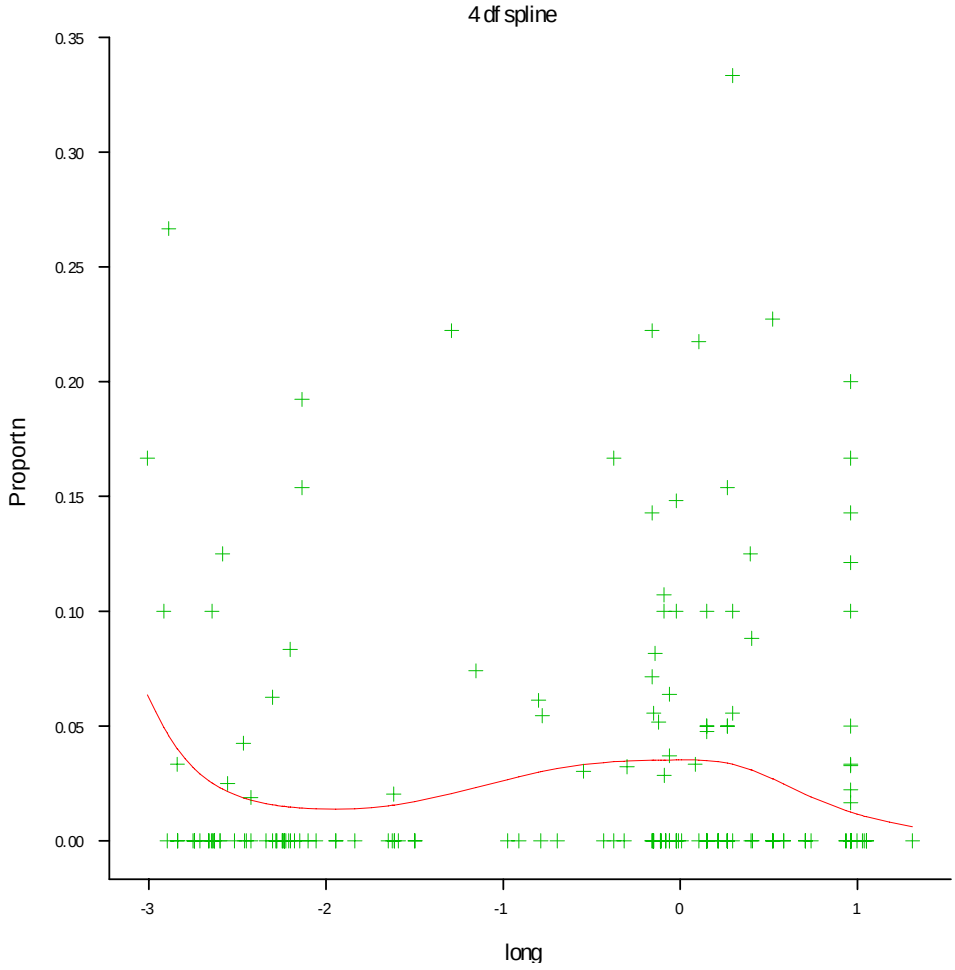


FIGURE 8. PLOT OF PROPORTION MOBILE APHIDS (BACK TRANSFORMED FROM THE LOGIT SCALE) AGAINST LONGITUDE WITH 4 DF CUBIC SPLINE SUPERIMPOSED.



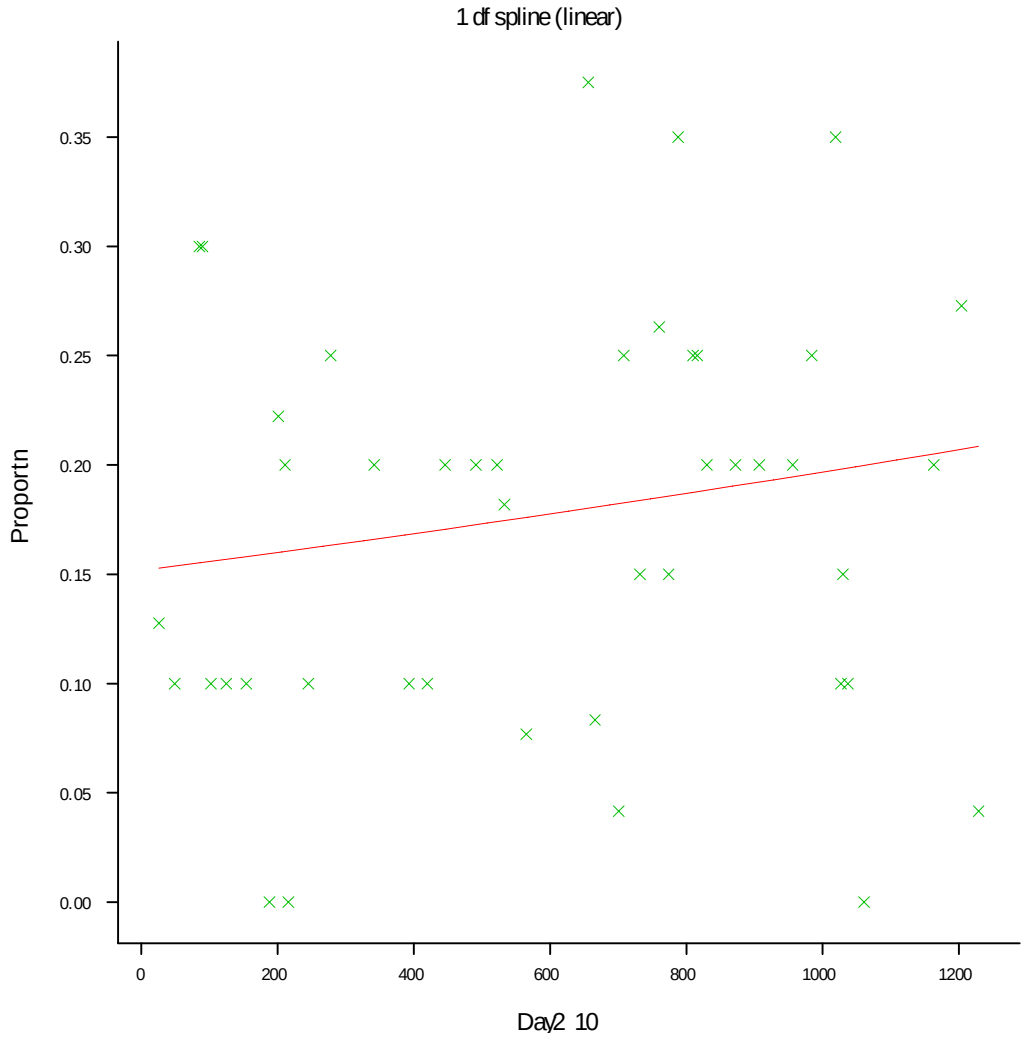
Similar cubic spline models were fitted to investigate separately whether the proportion of 'mobile' aphids showed any trend with latitude and longitude. For latitude (Figure 7) there was no evidence for more than a 2 df spline and again the linear component of this spline was non-significant (logit scale: slope = 0.279, se 0.199, $t_{216} = 1.41$, $P > 0.05$). For longitude (Figure 8) there was no evidence for more than a 4 df spline and the linear component of this spline was again non-significant (logit scale: slope = -0.0553, se 0.0891, $t_{214} = -0.62$, $P > 0.05$):

Control bioassays

43 control bioassays applying 0.25 ng of imidacloprid per aphid were done on the Nic-S standard clone. All of these except two (on 16/03/2005: 1 'mobile' out of 9 tested and on 25/06/2007: 1 'mobile' aphid out of 20 tested) resulted in no 'mobile' aphids. 44 control bioassays were done on the Nic-R standard clone for which similar cubic spline models, to those described above, were fitted to investigate whether the proportion of mobile aphids showed any trend with time. The percentage 'mobile' aphids ranged from 0% to 37.5% (Figure 9) with no evidence for a trend with time and no evidence of curvature. The simplest description of the Nic-R standard data is therefore an overall mean of -1.5140 (s.e. 0.0996) on the logit scale (corresponding to 18.03% mobile aphids on average) with no evidence for any change in resistance over this period and hence no evidence that the periodicity seen in the

frequency of Nic-R aphids in the field samples was due to intrinsic variation in the screening bioassays.

FIGURE 9. PLOT OF PROPORTION 'MOBILE' APHIDS IN CONTROL BIOASSAYS APPLYING 2.5 NG IMIDACLOPRID TO THE NIC-R CLONE (BACK TRANSFORMED FROM THE LOGIT SCALE) AGAINST DAY NUMBER WITH (NON-SIGNIFICANT) 1 DF (LINEAR) CUBIC SPLINE SUPERIMPOSED (LOGIT SCALE: SLOPE = 0.000315, S.E. 0.000284, $T_{\infty} = 1.11$, $P > 0.05$).



Response to imidacloprid versus insecticide treatment history

Comparisons of the distributions of proportion Nic-R ('mobile') aphids amongst insecticide treatments to the host crop showed that these ranged from 0 to 0.33. Samples were classified according to their insecticide treatment into four groups:

- Untreated,
- Treated with a non-neonicotinoid,
- Oilseed rape seed-treated with a neonicotinoid at a low rate (Chinook not aimed at aphids),
- Seed- or foliar-treated with a neonicotinoid at a high rate (aimed at aphids).

They were also classified by their observed proportion of 'mobile' aphids into four groups:

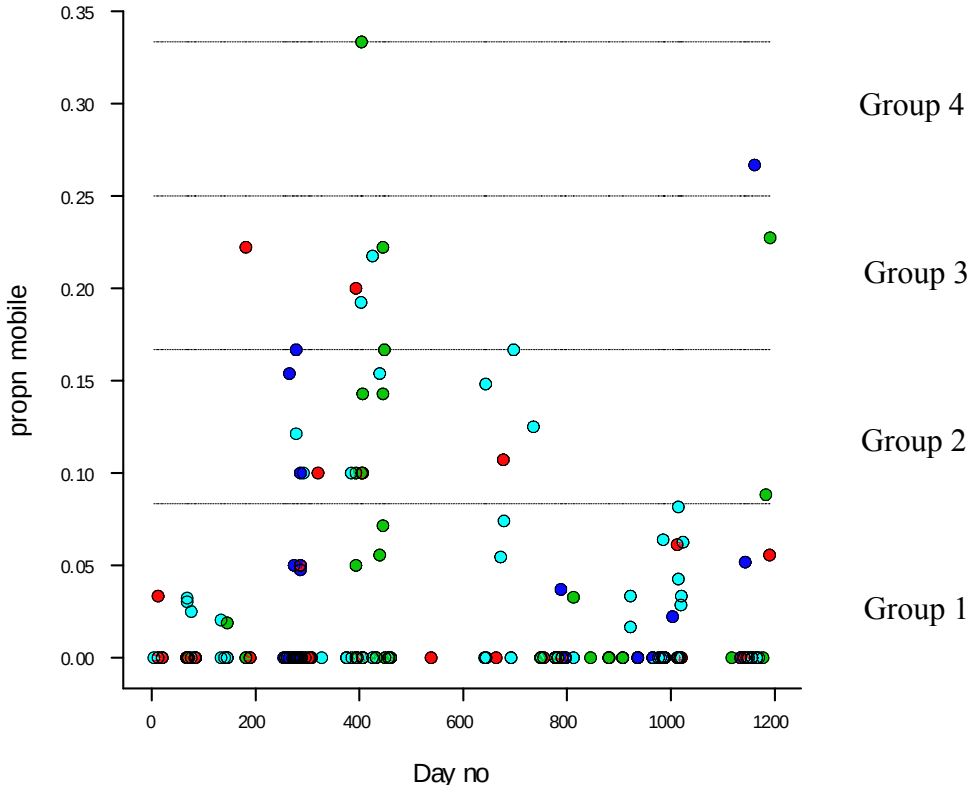
$0 \le p \le 1/12$, $1/12 < p \le 1/6$, $1/6 < p \le 1/4$, $1/4 < p \le 1/3$) to form a series of counts in ordered categories (expressed as percentages of the marginal treatment total counts in Table 5). Seven samples had no treatment information and hence could not be included. This analysis did not allow for the number of aphids tested.

TABLE 5. PERCENTAGE OF SAMPLES IN EACH 'MOBILE' CATEGORY FOR EACH TREATMENT (NUMBER OF SAMPLES IN PARENTHESES).

% mobile: Treatment	Group 1 0<=p<=1/12	Group 2 1/12<p<=1/6	Group 3 1/6<p<=1/4	Group 4 1/4<p<=1/3	*Count*
Untreated	90.11 (*82*)	7.69 (*7*)	2.20 (*2*)	0.00 (*0*)	(*91*)
Non-neonics	83.87 (*26*)	9.68 (*3*)	6.45 (*2*)	0.00 (*0*)	(*31*)
Neonics (low rate)	80.85 (*38*)	2.77 (*6*)	4.26 (*2*)	2.13 (*1*)	(*47*)
Neonics (high rate)	90.70 (*39*)	6.98 (*3*)	0.00 (*0*)	2.33 (*1*)	(*43*)
Margin	*87.26* (*185*)	*8.96* (*19*)	*2.83* (*6*)	*0.94* (*2*)	(*212*)

This partitioning of the samples are shown graphically in relation to day number in Figure 10.

FIGURE 10. PLOT OF UNTRANSFORMED PROPORTION 'MOBILE' APHIDS AGAINST COLLECTION DATE WITH HORIZONTAL LINES SHOWING THE PARTITIONING IN FOUR GROUPS FOR PROPORTIONAL ODDS REGRESSION. LIGHT BLUE = UNTREATED; RED = TREATED WITH NON-NEONICOTINOIDS; GREEN = TREATED WITH NEONICOTINOIDS (LOW SEED RATE); DARK BLUE = TREATED WITH NEONICOTINOIDS (HIGH SEED AND FOLIAR RATE).



There was no evidence for differences amongst the four treatment distributions on the basis of a proportional-odds regression (GLM with ordered multinomial distribution and logit link; $\chi^2_3 = 3.1758$, $P = 0.365$). This analysis accounts for the fact the proportion category is ordered whilst the crop treatment categories are independent.

A contingency table analysis was done to test for association with insecticide treatment history. To simplify further the structure of the data, the 212 field samples were classified according to various combinations of the four insecticide treatment groups and their observed proportion of 'mobile' aphids, now classified into only two groups: some (≥ 1 aphid) 'mobile', none (0 aphids) 'mobile', regardless of the number of aphids tested. The number of samples in each of these categories was as follows:

(i)	Absent	Present
No treatment	68	23 (25%)
Neonicotinoids	66	24 (27%)
Non-neonicotinoids	22	9 (29%)

Pearson chi-square value is 0.17 with 2 d.f, P = 0.917

(ii)	Absent	Present
No treatment	68	23 (25%)
Neonicotinoids (low rate)	33	14 (30%)
Neonicotinoids (high rate)	33	10 (23%)
Non-neonicotinoids	22	9 (29%)

Pearson chi-square value is 0.67 with 3 d.f., P = 0.881

(iii)	Absent	Present
Neonicotinoids (low rate)	33	14 (30%)
Rest	123	42 (25%)

Pearson chi-square value is 0.35 with 1 d.f., P = 0.552

(iv)	Absent	Present
Neonicotinoids (high rate)	33	10 (23%)
Rest	123	46 (27%)

Pearson chi-square value is 0.28 with 1 d.f., P = 0.599

In no case was there evidence against the null hypothesis of no association between crop treatment group and presence/absence of 'mobile' aphids, i.e. the proportion of samples with no or some 'mobile' aphids was constant over treatments and therefore there was no evidence of an association between crop treatment and screening bioassay response.

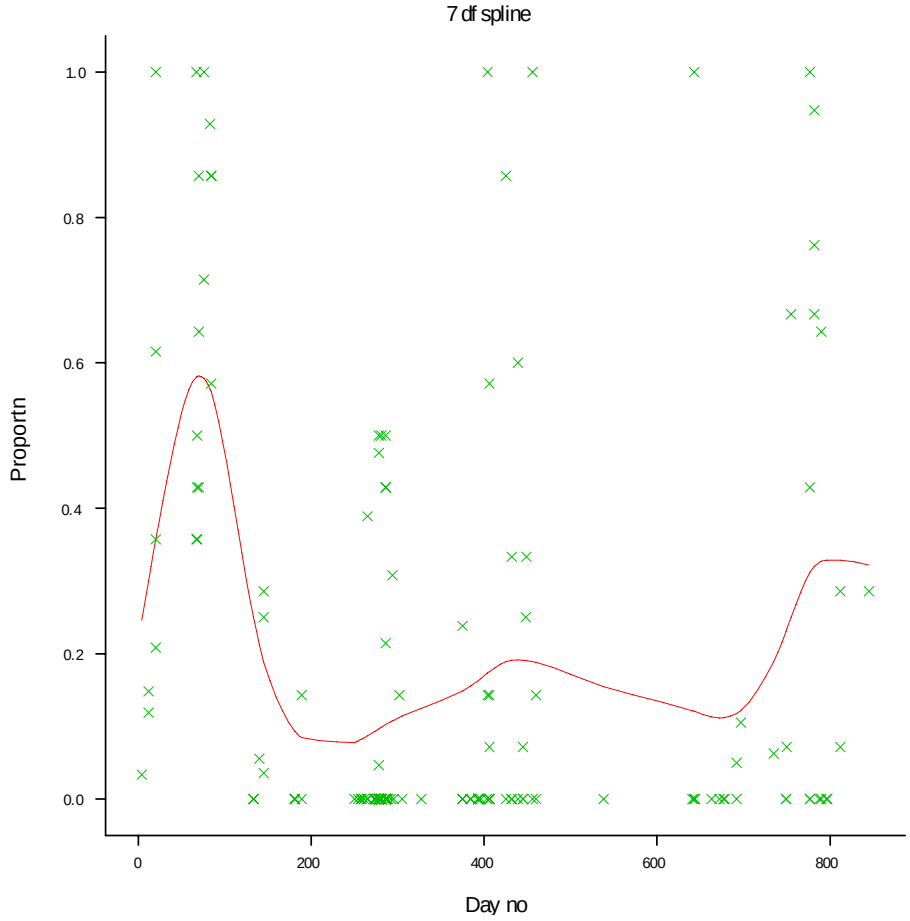
Carboxylesterase resistance

Carboxylesterase resistance testing ended on 02/01/07 (day 845), giving a total of 150 tested samples. The proportion of aphids with high and extreme carboxylesterase (R_2+R_3) in each field sample was compared to the date of collection using the same analyses (spline model fits, proportional odds regressions and contingency table) used for the response to imidacloprid. Whilst a higher df spline was required to describe these data, all splines of 5 to 8 df had very similar shapes, picking up an initial peak followed by a more stable period and some evidence of a final upturn. The 7 df spline is shown in Figure 11. Again, however, the additional curvature was superimposed on a flat linear trend, indicating no overall tendency for the R_2+R_3 proportion to increase with time shown by a non-significant linear component of the spline (logit scale: slope = -0.000783 s.e. 0.000505, t_{142} = -1.55, P > 0.05).

There was also no evidence that the shape of the overall spline in Figure 11 was unduly influenced by crop, latitude, longitude (all with levels as for response to imidacloprid) or treatment divided into four groups:

Untreated, treated with pirimicarb or another carbamate, seed-treated with a neonicotinoid at a low rate or high rate, or treated with a pyrethroid. This was based on comparison of the spline deviance before (296.8) and after fitting each of these factors in turn (crop 285.8, latitude 298.5, longitude 275.8, treatment 309.3).

FIGURE 11. PLOT OF R_2+R_3 CARBOXYLESTERASE PROPORTION IN THE *M. PERSICAE* FIELD SAMPLES (BACK TRANSFORMED FROM THE LOGIT SCALE) AGAINST DAY NUMBER WITH 7 DF SMOOTHING SPLINE SUPERIMPOSED.



The proportions of R_2+R_3 aphids ranged from 0 to 1. Samples were classified according to their insecticide treatment into three groups:

Untreated, treated with pirimicarb or carbamates, treated with other non-neonicotinoids or neonicotinoids.

The observed R_2+R_3 carboxylesterase proportion (four groups: $0 \leq p \leq 0.25$, $0.25 < p \leq 0.5$, $0.5 < p \leq 0.75$, $0.75 < p \leq 1$) was used to form a series of counts in ordered categories, expressed as percentages of the marginal treatment total counts (Table 6). This analysis did not allow for the number of aphids tested.

TABLE 6. PERCENTAGE OF SAMPLES IN EACH CARBOXYLESTERASE CATEGORY FOR EACH TREATMENT (NUMBER OF SAMPLES IN PARENTHESES).

	0<=p<=0.25	0.25<p<=0.5	0.5<p<=0.75	0.75<p<=1
Treatment				
Untreated	50 (71%)	7 (10%)	4 (6%)	9 (13%)
Pirimicarb + carbamates	10 (59%)	2 (12%)	4 (23%)	1 (6%)
Rest	47 (75%)	11 (17%)	1 (2%)	4 (6%)

There was no evidence for differences amongst the three treatment distributions on the basis of a proportional-odds regression (GLM with ordered multinomial distribution and logit link; $\chi^2_2 = 0.94$, P = 0.391).

The 150 field samples were also classified according to various combinations of the insecticide treatment groups and their observed R_2+R_3 carboxylesterase proportion, classified into only two groups: present (carboxylesterase proportion > 0), absent (carboxylesterase proportion = 0), regardless of the number of aphids tested. The number of samples in each category was as follows:

(i) Excluding pyrethroids	Absent	Present
No treatment	55	15 (21%)
Pirimicarb or carbamate	15	2 (12%)
Rest	40	13 (25%)

Pearson chi-square value is 1.25 with 2 d.f., P = 0.536
Likelihood chi-square value is 1.37 with 2 d.f., P = 0.503

One cell had a fitted value < 5 so the Pearson test may be unreliable but in this case is comparable to the potentially more accurate maximum likelihood based test.

(ii) Excluding pirimicarb and carbamates	Absent	Present
No treatment	55	15 (21%)
Pyrethroid	9	1 (10%)
Rest	40	13 (25%)

Pearson chi-square value is 1.05 with 2 d.f., P = 0.590
Likelihood chi-square value is 1.20 with 2 d.f., P = 0.548

Again, one fitted value was < 5 but both tests are in agreement.

(iii)	Absent	Present
No treatment	55	15 (21%)
Pirimicarb, carbamates and pyrethroids	24	3 (11%)
Rest	40	13 (25%)

Pearson chi-square value is 2.01 with 2 d.f., P = 0.366

(iv)	Absent	Present
Pirimicarb and carbamates	15	2 (12%)
Rest	104	29 (22%)

Pearson chi-square value is 0.93 with 1 d.f., P = 0.336

Again, one fitted value was < 5. A two-tailed Fisher's exact test gave P = 0.5

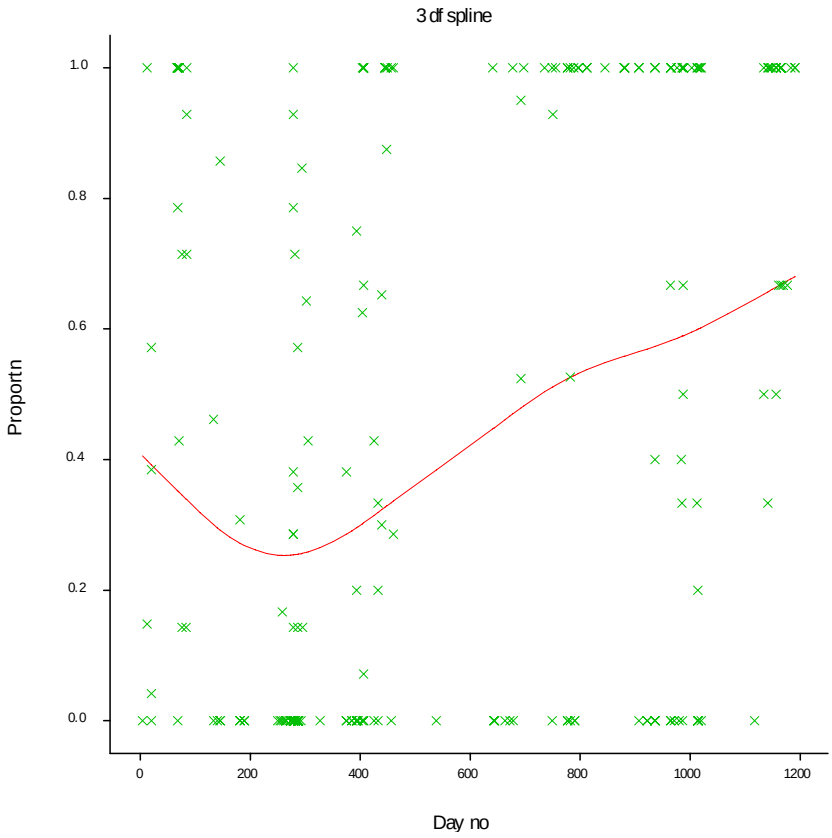
In no case was there evidence against the null hypothesis of no association between insecticide treatment group and carboxylesterase R_2+R_3 presence/absence, i.e. the proportion of samples with no or some higher carboxylesterase present was constant over the treatment groups.

MACE resistance

All of the MACE aphids tested using the DNA-based technique were heterozygotes. The proportion of MACE aphids in each field sample was compared to the date of collection using the same analyses (spline model fits, proportional odds regressions and contingency table) used for the response to imidacloprid.

A 3 df spline described the data sufficiently and is shown in Figure 12. In this case the additional curvature was superimposed on a significant increasing linear component (logit scale: slope = 0.001248, $t_{214} = 2.75$, $P < 0.01$). However, it should be noted that from day 880 onwards, corresponding to the introduction of the different testing method, the sample sizes were smaller (due to the change from biochemical to DNA-based testing).

FIGURE 12. PLOT OF MACE PROPORTION IN *M. PERSICAE* FIELD SAMPLES (BACK TRANSFORMED FROM THE LOGIT SCALE) AGAINST DAY NUMBER WITH 3 DF SMOOTHING SPLINE SUPERIMPOSED.



The MACE proportion ranged from 0 to 1. All field samples were classified according to their insecticide treatment into three groups (untreated, treated with pirimicarb or carbamates, treated with other non-neonicotinoids or neonicotinoids) and their observed proportion of mobile aphids into four groups ($0 \le p \le 0.25$, $0.25 < p \le 0.5$, $0.5 < p \le 0.75$, $0.75 < p \le 1$) to form a series

of counts in ordered categories, expressed as percentages of the marginal treatment total counts (Table 7). This analysis does not allow for number tested.

TABLE 7. PERCENTAGE OF SAMPLES IN EACH MACE CATEGORY FOR EACH TREATMENT (NUMBER OF SAMPLES IN PARENTHESES).

	$0 \leq p \leq 0.25$	$0.25 < p \leq 0.5$	$0.5 < p \leq 0.75$	$0.75 < p \leq 1$
Treatment				
Untreated	51 (56%)	11 (12%)	5 (6%)	24 (26%)
Pirimicarb	4 (20%)	1 (5%)	1 (5%)	14 (70%)
Rest	42 (42%)	10 (10%)	11 (11%)	37 (37%)

There was evidence for differences amongst the three treatment distributions on the basis of a proportional-odds regression (GLM with ordered multinomial distribution and logit link; $\chi^2_2 = 7.39$, $P < 0.001$). With treatment with pirimicarb there is a significant shift towards higher MACE proportions compared to the untreated and 'other' combined treatments.

The 211 samples with MACE treatment information were classified according to various combinations of their insecticide treatments and their observed MACE proportion, classified into only two groups: present (MACE proportion > 0), absent (MACE proportion = 0), regardless of the number of aphids tested. Seven samples with no treatment information were excluded. The number of samples in each category was as follows:

(i)	Absent	Present
No treatment	44	47 (52%)
Pirimicarb	2	18 (90%)
Rest	40	60 (60%)

Pearson chi-square value is 10.03 with 2 d.f., P = 0.007

(ii)	Absent	Present
Pirimicarb	2	18 (90%)
Rest	84	107 (56%)

Pearson chi-square value is 8.66 with 1 d.f. ,P = 0.003

In both cases there was evidence against the null hypothesis of no association between treatment group and MACE presence/absence. The proportion of samples with no MACE present was much lower for treatment with pirimicarb (10%) than the other two groups, the latter being similar (on average 44%).

Kdr

All of the kdr aphids tested were heterozygotes. The 185 field samples with kdr information were classified according to various combinations of insecticide treatment and whether kdr was present or absent, regardless of the number of aphids tested. Seven samples with no treatment information were excluded. The number of samples in each category was as follows:

(i)	Absent	Present
No treatment	24	50 (68%)
Pyrethroids	12	21 (64%)

Rest	33	45 (58%)

Pearson chi-square value is 1.60 with 2 d.f., P = 0.450

(ii)	Absent	Present
Pyrethroids	12	21 (64%)
Rest	57	95 (52%)

Pearson chi-square value is 0.01 with 1 d.f., P = 0.903

Super-kdr

Super-kdr (also conferring resistance to pyrethroids) remained very rare in the field with only 7/189 (3.7%) of the field samples containing super-kdr aphids. Of these, 2 were untreated, 3 had been treated with pyrethroids and 2 had been seed-treated with neonicotinoids at the high rate. Two occurred in 2004 and five in 2005 with the latest occurring on 20th October 2005.

Glasshouse samples

21 glasshouse samples were received (Figure 13). The same cubic spline models were fitted in relation to the proportions of Nic-R ('mobile') aphids and day number as for the field samples (Figure 14). A linear model was sufficient to describe the data, but the regression line had a non-significant slope (logit scale: -0.000156, s.e. = 0.000939, $t_{19} = -0.17$, $P > 0.05$) suggesting no change in resistance over time. Whilst splines with higher df were sensitive to peaks around days 300 and 800 there was no evidence that a more complex spline was required, and all higher df splines had non-significant linear components.

FIGURE 13. MAP OF GLASSHOUSE *M. PERSICAE* SAMPLE LOCATIONS LABELLED BY YEAR (BLACK CROSS = 2004 (*N* = 5), RED CIRCLE = 2005 (*N* = 7), GREEN PLUS = 2006 (*N* = 4) AND BLUE STAR = 2007 (*N* = 5)). ZERO LONGITUDE IS THE GREENWICH MERIDIAN.

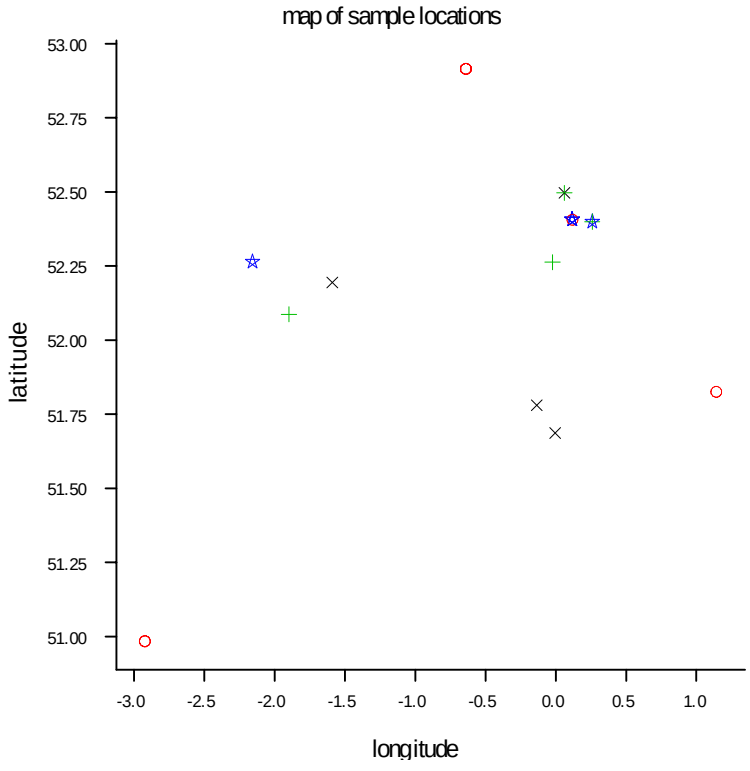
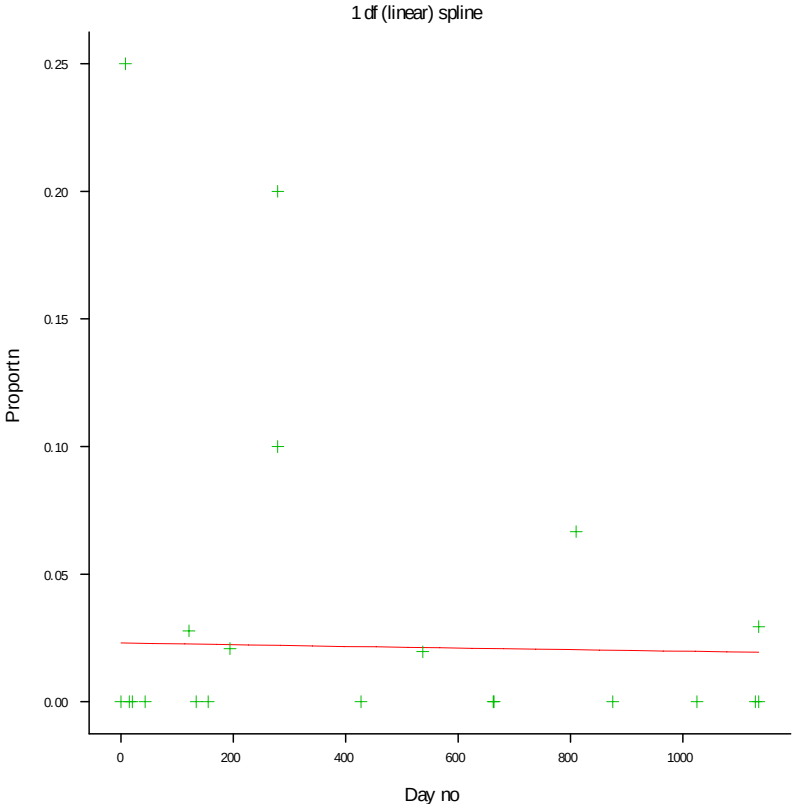


FIGURE 14. PLOT OF PROPORTION NIC-R ('MOBILE') *M. PERSICAE* IN THE GLASSHOUSE SAMPLES (BACK TRANSFORMED FROM THE LOGIT SCALE) AGAINST DAY NUMBER (DAY 0 IS 09/09/2004), WITH 1 DF (LINEAR) SMOOTHING SPLINE SUPERIMPOSED.



The number of samples received was too small for much further formal analysis, but the samples were classified according to proportion 'mobile' aphids (two groups: 0<=p<=1/8, 1/8<p<=1/4) and three insecticide treatment groups.

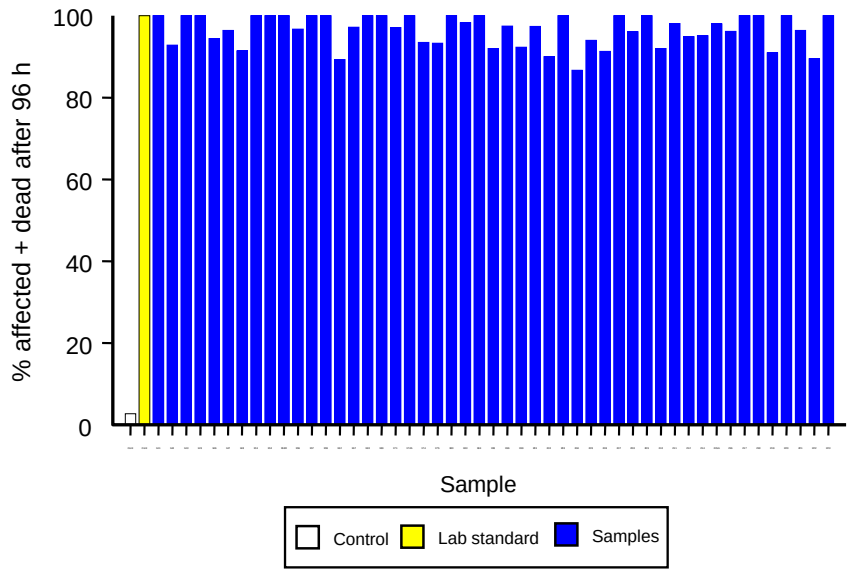
	0<=p<=1/8	1/8<p<=1/4
No treatment	13	0
Neonicotinoids (high rate) and nicotine	1	1
Rest	5	1

62% of samples were untreated and showed very low mobility (all < 10% aphids mobile). The proportion mobile was low overall (25% maximum) but the highest values observed were for the treated groups.

Response to pymetrozine

There was no evidence of any significant variation in the response of aphids in the *M. persicae* field and glasshouse samples that were screened with a diagnostic dose of pymetrozine (Figure 15). There was no evidence, therefore, that there is no resistance to this compound in the UK in this species.

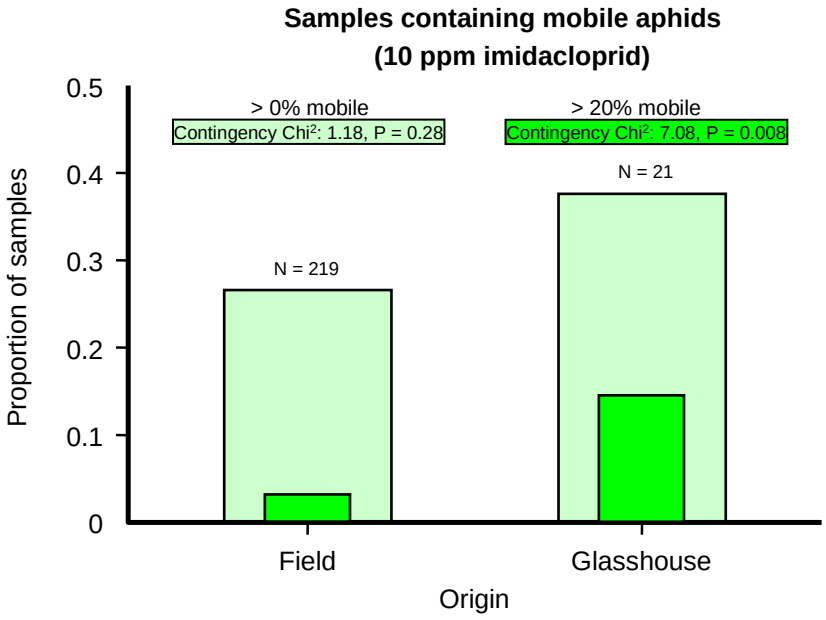
FIGURE 15. RESPONSE OF FIELD AND GLASSHOUSE *M. PERSICAE* SAMPLES TO A DIAGNOSTIC DOSE OF PYMETROZINE APPLIED IN LEAF-DIP BIOASSAYS.



Comparisons of insecticide resistance between field and glasshouse samples

A significantly greater proportion of the glasshouse samples contained greater than 20% Nic-R ('mobile') aphids (Contingency Chi^2, 1 df, = 7.08, P = 0.008) (Figure 16).

FIGURE 16. PROPORTION OF FIELD AND GLASSHOUSE *M. PERSICAE* SAMPLES THAT CONTAINED GREATER THAN 0% AND GREATER THAN 20% NIC-R ('MOBILE') APHIDS.



A histogram of the presence of carboxylesterase, MACE and kdr in UK field *M. persicae* samples collected since 1996 (Figure 17), including all the data gained as part of the project, shows temporal changes in frequency with time. Those carrying greater than 50% R_2+R_3 carboxylesterase resistance (primarily to OPs) generally fell in the field but remained significantly more common in glasshouses (Contingency Chi^2 = 8.73, P = 0.003) (Figures 17 and 18).

FIGURE 17. FREQUENCY OF FIELD SAMPLES CONTAINING GREATER THAN 50% CARBOXYLESTERASE R_2+R_3, MACE AND KDR *M. PERSICAE* BETWEEN 1996 AND 2007.

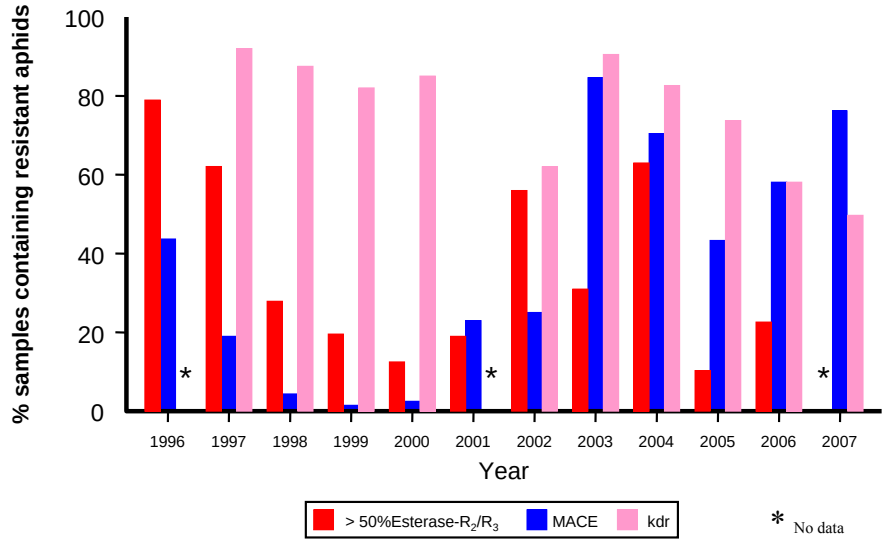
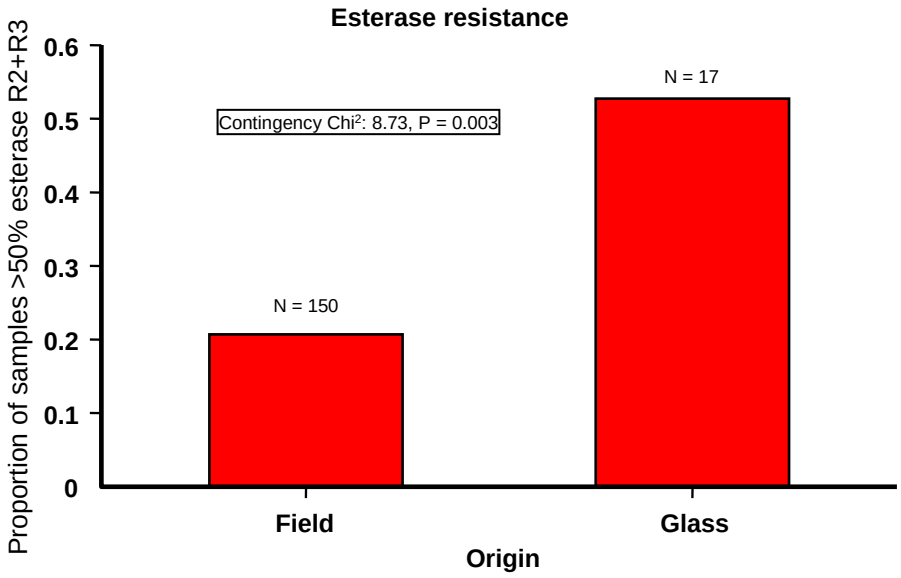
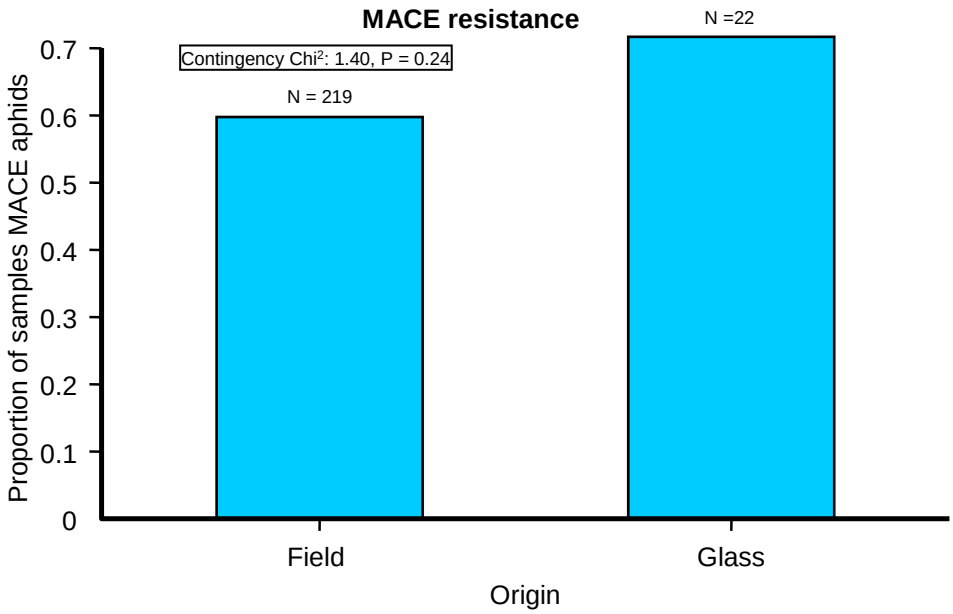


FIGURE 18. PROPORTION OF FIELD AND GLASSHOUSE *M. PERSICAE* SAMPLES THAT CONTAINED GREATER THAN 50% CARBOXYLESTERASE R_2 AND R_3 APHIDS.



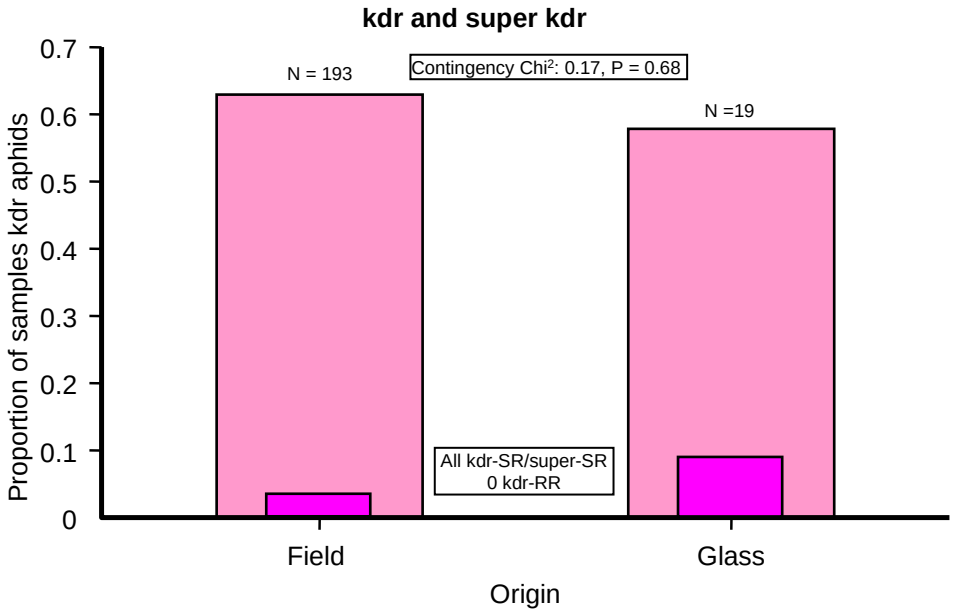
In contrast to carboxylesterase resistance, MACE resistance (specifically to the aphicide pirimicarb) has now become equally very common in the field and glasshouses (Contingency $Chi^2 = 1.40$, $P = 0.24$) (Figures 17 and 19). This will have severely compromised the efficacy of any pirimicarb applications against this pest.

FIGURE 19. PROPORTION OF FIELD AND GLASSHOUSE *M. PERSICAE* SAMPLES THAT CONTAINED MACE APHIDS.



M. persicae carrying kdr (conferring resistance to pyrethroids) have remained relatively common, being found in at least 50% of the field and glasshouse samples ((Contingency $Chi^2 = 0.17$, $P = 0.68$) (Figure 20) although their frequency has fallen consistently over the past several years suggesting that kdr may be slowly on the wane (Figure 17).

FIGURE 20. PROPORTION OF FIELD AND GLASSHOUSE *M. PERSICAE* SAMPLES THAT CONTAINED KDR AND SUPER-KDR APHIDS.

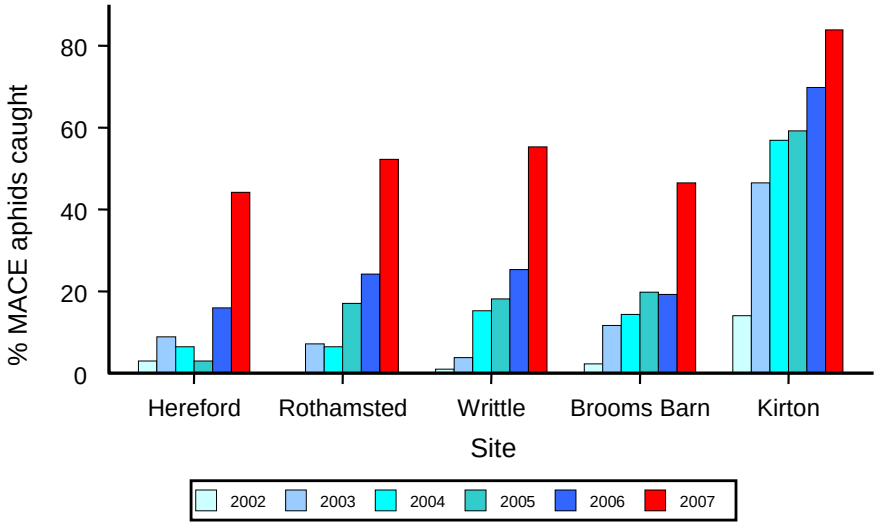


Curiously, in contrast to glasshouse samples, field samples containing aphids carrying both MACE and kdr were relatively rare compared to those containing aphids carrying one or the other mechanism by them self. This suggests that doubly-resistant *M. persicae* in the field may also suffer fitness disadvantages in the absence of insecticide pressures; a conclusion that stems from glasshouses providing relatively closeted, benign environments and something that may well have implications in the light of climate change.

Suction-trap samples

The percentages of MACE *M. persicae* caught in the five aphid suction traps that test for this mechanism (an 'in-kind' contribution towards LK 0953 from BBRO) showed a distinct regional component up until 2006 with far more MACE aphids being caught at Kirton in Lincolnshire. However, in 2007 MACE aphids became prevalent and more widespread (Figure 21).

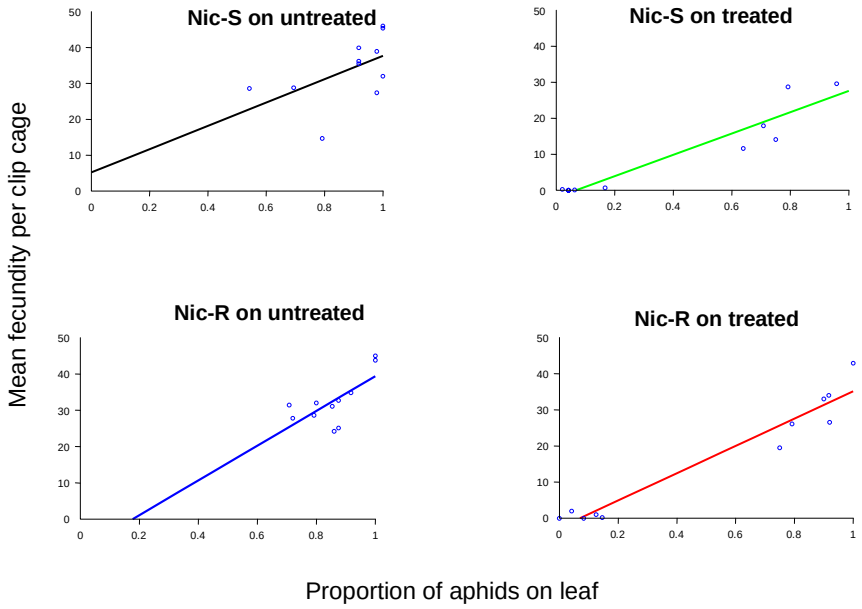
FIGURE 21. PERCENTAGES OF MACE *M. PERSICAE* CAUGHT IN ENGLISH AERIAL SUCTION TRAPS BETWEEN 2002 AND 2007.



2.3.3 Operational factors affecting neonicotinoid resistance in *M. persicae*

There was a good positive association between aphid fecundity and the proportions of aphids found on their inoculation leaves five days after their introduction to seed-treated and untreated plants (Figure 22). This shows that aphids that spent more time on the inoculation leaves tended to produce more offspring. This section will therefore focus on aphid fecundity as a measure of aphid fitness in response to neonicotinoid treatments.

FIGURE 22. RELATIONSHIP BETWEEN MEAN APHID FECUNDITY PER CLIP CAGE AND POSITION OF ADULT NIC-S AND NIC-R APHIDS FIVE DAYS AFTER INOCULATION ONTO TREATED AND UNTREATED PLANTS.



Seed treatment experiments

Figures 23-34 show the mean fecundity of the Nic-S and Nic-R clones in the field simulator-based experiments measuring the effects of seed treatments with imidacloprid or clothianidin on oilseed rape and cabbage plants.

Cabbage

Doses of imidacloprid and clothianidin that are registered for controlling aphids on cabbage proved to be very effective against the Nic-S and Nic-R clones with highly significant treatment effects at each time point ($P < 0.001$) in all experiments (Figures 23-27). However, there was some evidence for a very subtle, but significant, advantage to Nic-R aphids on some occasions (Figures 25-27).

FIGURE 23. EXPERIMENT 1 (YOUNGER PLANTS): MEAN FECUNDITY PER CLIP CAGE FOR NIC-S AND NIC-R *M. PERSICAE* CLONES EXPOSED TO CABBAGE UNTREATED AND SEED-TREATED WITH IMIDACLOPRID (DOSE FACTOR 140). BARS SHOW +/- 95% CIS.

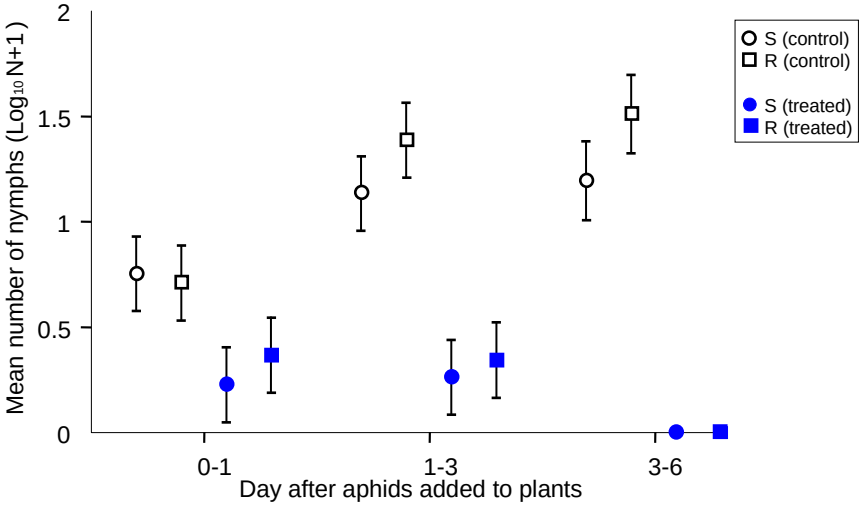


FIGURE 24. EXPERIMENT 3 (YOUNGER PLANTS): MEAN FECUNDITY PER CLIP CAGE FOR NIC-S AND NIC-R *M. PERSICAE* CLONES EXPOSED TO CABBAGE UNTREATED AND SEED-TREATED WITH IMIDACLOPRID (DOSE FACTOR 140). BARS SHOW +/- 95% CIS.

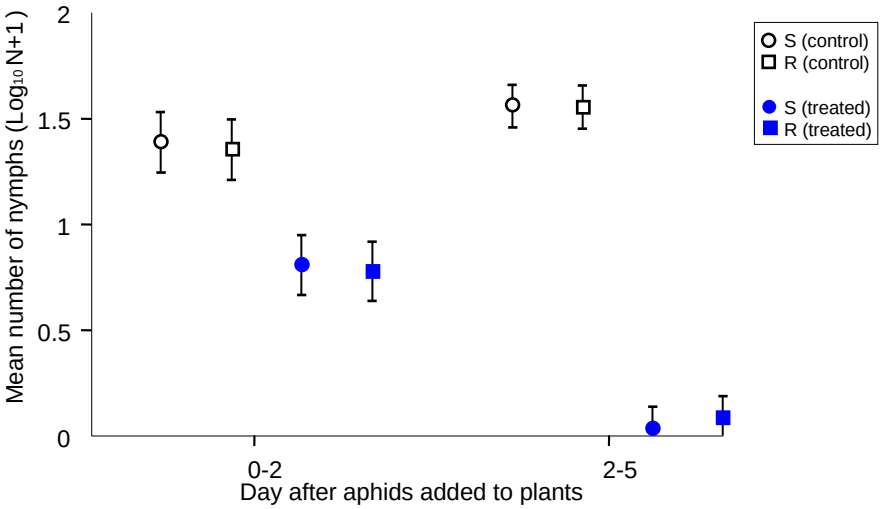


FIGURE 25. EXPERIMENT 3 (OLDER PLANTS): MEAN FECUNDITY PER CLIP CAGE FOR NIC-S AND NIC-R *M. PERSICAE* CLONES EXPOSED TO CABBAGE UNTREATED AND SEED-TREATED WITH IMIDACLOPRID (DOSE FACTOR 140). BARS SHOW +/- 95% CIS.

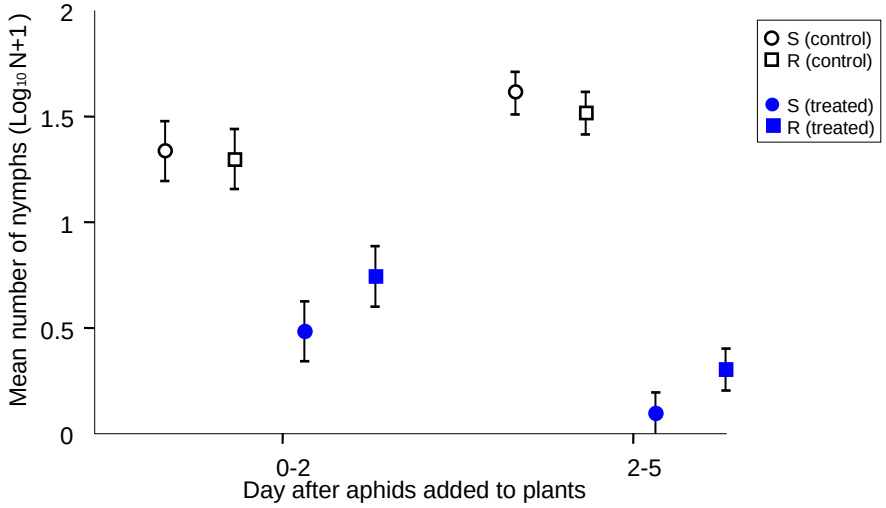


FIGURE 26. EXPERIMENT 5 (YOUNGER PLANTS): MEAN FECUNDITY PER CLIP CAGE FOR NIC-S AND NIC-R *M. PERSICAE* CLONES EXPOSED TO CABBAGE UNTREATED AND SEED-TREATED WITH CLOTHIANIDIN (DOSE FACTOR 120). BARS SHOW +/- 95% CIS.

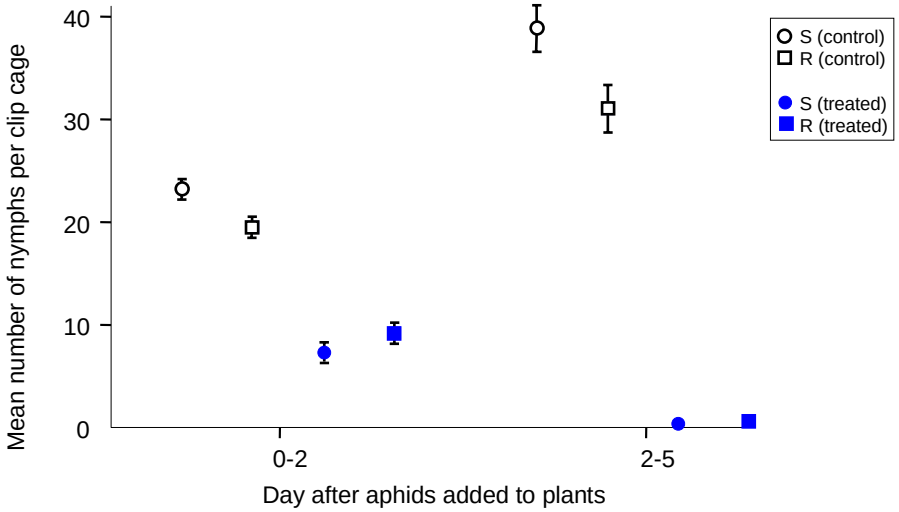
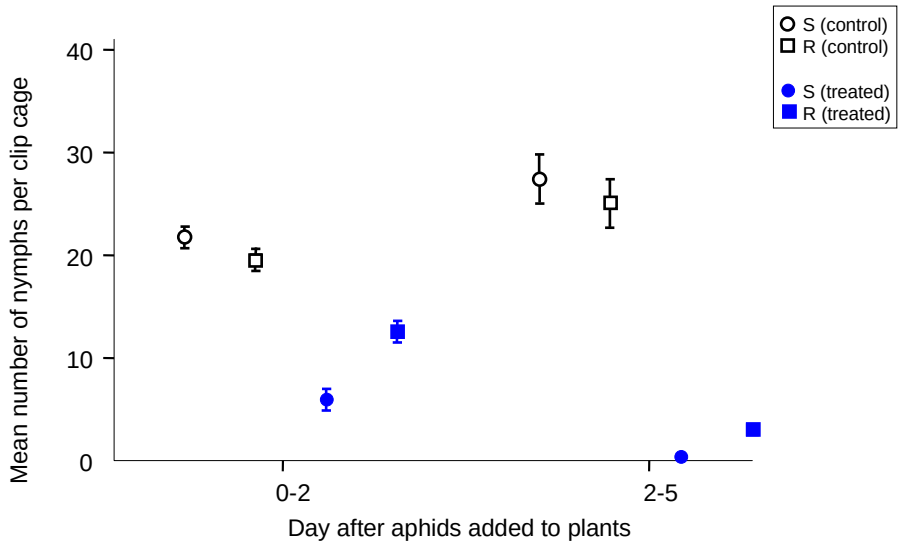


FIGURE 27. EXPERIMENT 5 (OLDER PLANTS): MEAN FECUNDITY PER CLIP CAGE FOR NIC-S AND NIC-R *M. PERSICAE* CLONES EXPOSED TO CABBAGE UNTREATED AND SEED-TREATED WITH CLOTHIANIDIN (DOSE FACTOR 120). BARS SHOW +/- 95% CIS.



Oilseed rape

The low dose of imidacloprid, not aimed at controlling aphids, that is currently registered for oilseed rape had slight deleterious effects on the success of the Nic-S and Nic-R clones at each time point in experiment 4 (Figure 30, day 0-2: $P = 0.018$; day 2-16: $P = 0.028$) but was not seen in experiment 2 (Figures 28 and 29). In contrast, in experiment 6 on oilseed rape highly significant advantages were seen for the Nic-R clone, compared to the Nic-S clone, at each time point when aphids were exposed to clothianidin-treated younger plants (Figure 31, $P < 0.001$) and older plants (Figure 32, $P < 0.001$), even though the dose rate was only 5x that used for imidacloprid of this host.

FIGURE 28. EXPERIMENT 2 (YOUNGER PLANTS): MEAN FECUNDITY PER CLIP CAGE FOR NIC-S AND NIC-R *M. PERSICAE* CLONES EXPOSED TO OILSEED RAPE UNTREATED AND SEED-TREATED WITH IMIDACLOPRID (DOSE FACTOR 1). BARS SHOW +/- 95% CIS.

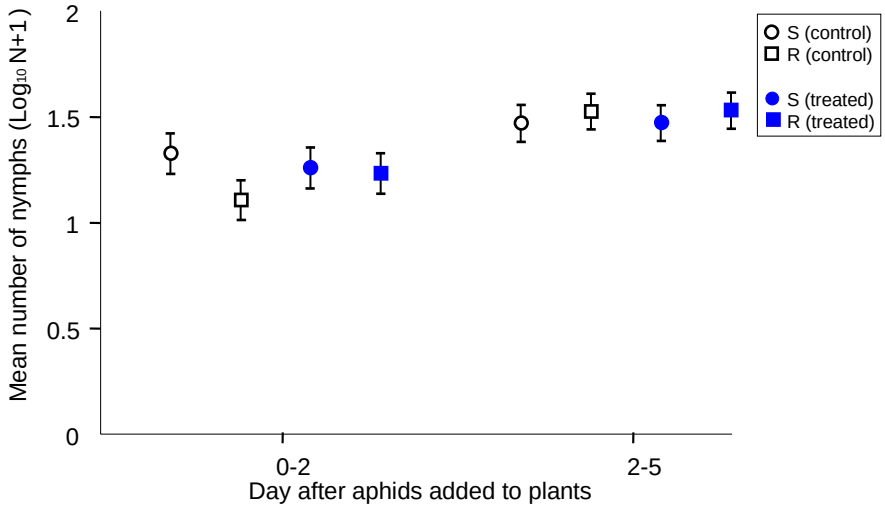


FIGURE 29. EXPERIMENT 2 (OLDER PLANTS): MEAN FECUNDITY PER CLIP CAGE FOR NIC-S AND NIC-R *M. PERSICAE* CLONES EXPOSED TO OILSEED RAPE UNTREATED AND SEED-TREATED WITH IMIDACLOPRID (DOSE FACTOR 1). BARS SHOW +/- 95% CIS.

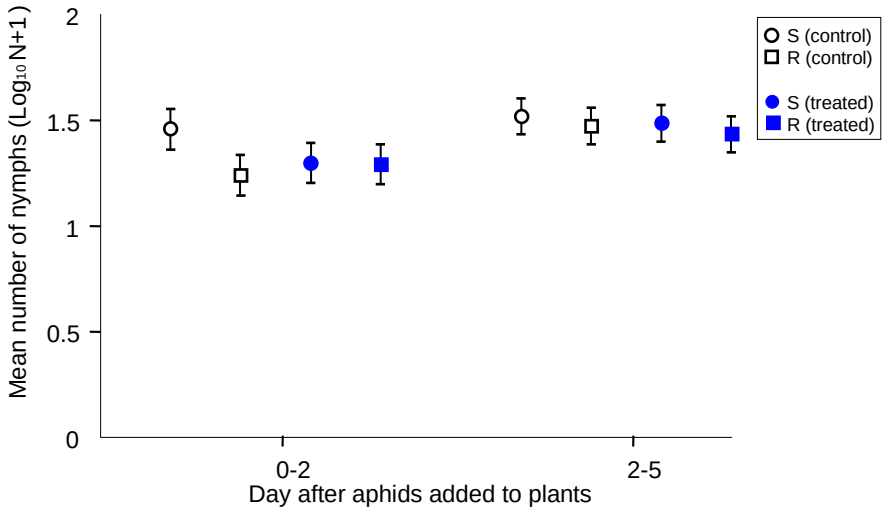


FIGURE 30. EXPERIMENT 4 (YOUNGER PLANTS): MEAN FECUNDITY PER CLIP CAGE FOR NIC-S AND NIC-R *M. PERSICAE* CLONES EXPOSED TO OILSEED RAPE UNTREATED AND SEED-TREATED WITH IMIDACLOPRID (DOSE FACTOR 1). BARS SHOW +/- 95% CIS.

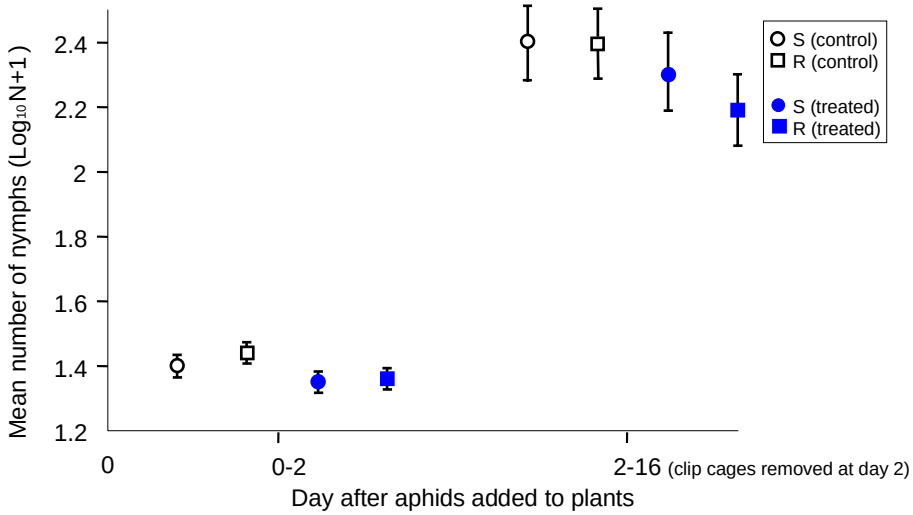


FIGURE 31. EXPERIMENT 6 (YOUNGER PLANTS): MEAN FECUNDITY PER CLIP CAGE FOR NIC-S AND NIC-R *M. PERSICAE* CLONES EXPOSED TO OILSEED RAPE UNTREATED AND SEED-TREATED WITH CLOTHIANIDIN (DOSE FACTOR 5). BARS SHOW +/- 95% CIS. BETWEEN 5 AND 12 DAYS THE MEAN NUMBER OF APHIDS WERE PER PLANT AS THE CLIP CAGES HAD BEEN REMOVED.

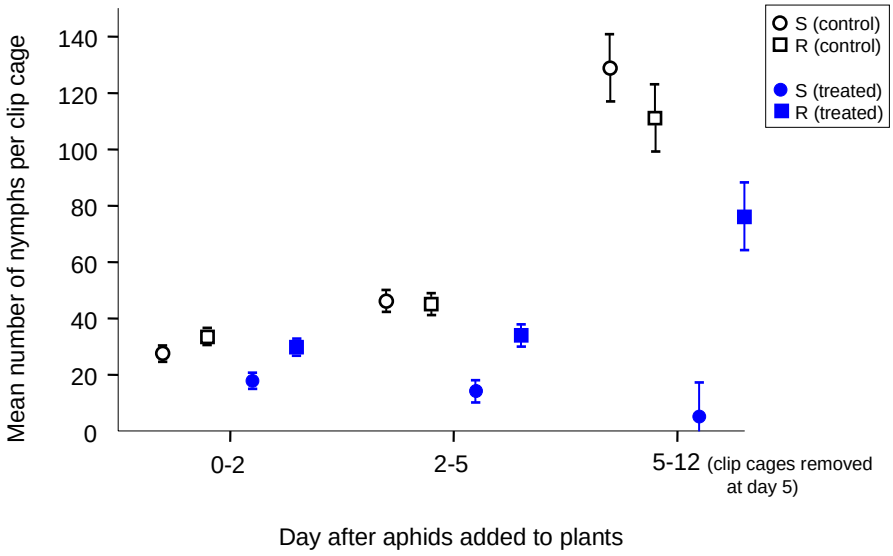
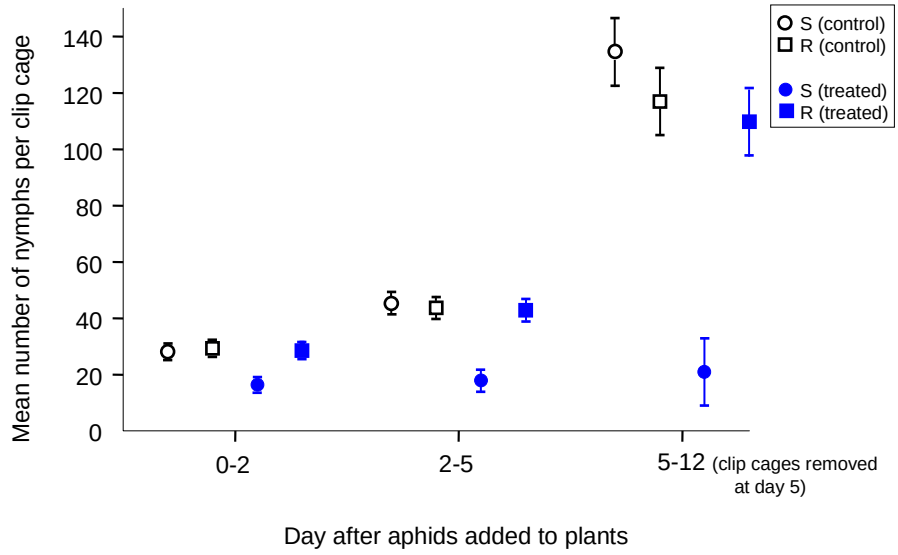


FIGURE 32. EXPERIMENT 6 (OLDER PLANTS): MEAN FECUNDITY PER CLIP CAGE FOR NIC-S AND NIC-R *M. PERSICAE* CLONES EXPOSED TO OILSEED RAPE UNTREATED AND SEED-TREATED WITH CLOTHIANIDIN (DOSE FACTOR 5). BARS SHOW +/- 95% CIS. BETWEEN 5 AND 12 DAYS THE MEAN NUMBER OF APHIDS WAS PER PLANT AS THE CLIP CAGES HAD BEEN REMOVED.



Figures 33 and 34 summarise the fecundities at 2-5 days of the Nic-S and Nic-R clones in relation to dose rate. This highlights the significant fitness advantage for Nic-R aphids feeding on clothianidin-treated oilseed rape and the complete loss of control against these aphids on older plants.

FIGURE 33. SUMMARY OF MEAN FECUNDITY PER CLIP CAGE FOR NIC-S AND NIC-R *M. PERSICAE* CLONES FIVE DAYS AFTER INOCULATION ONTO YOUNGER UNTREATED PLANTS AND PLANTS SEED-TREATED WITH DIFFERENT DOSES OF NEONICOTINOIDS. DOSE FACTORS (DF) ARE EXPRESSED RELATIVE TO THE AMOUNT OF IMIDACLOPRID USED TO TREAT OILSEED RAPE SEED (CHINOOK) WHERE DF = 1.

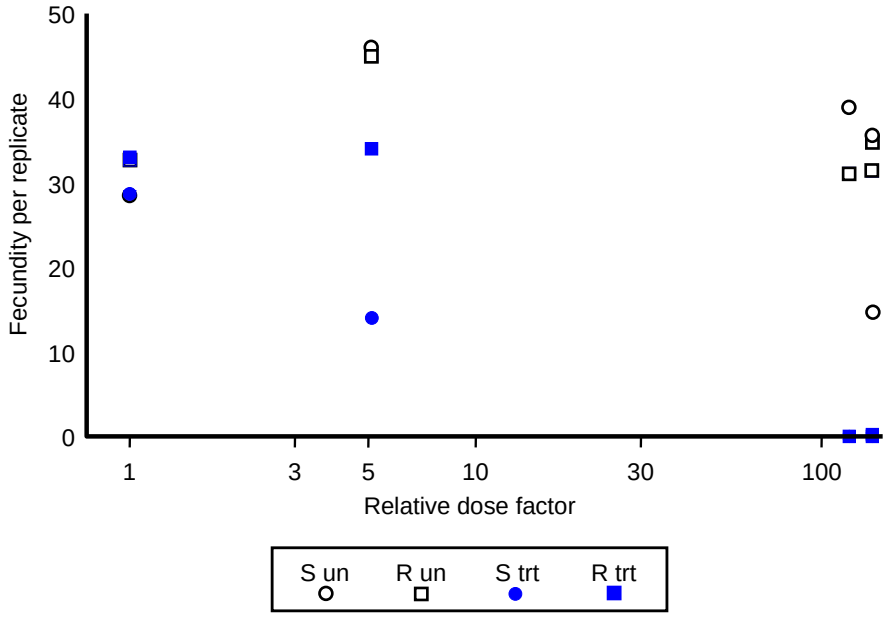
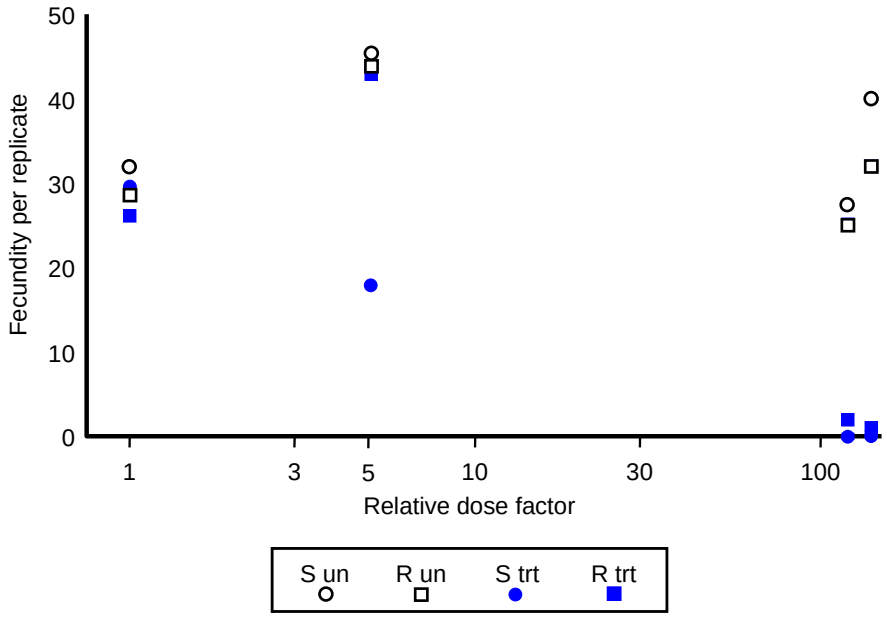


FIGURE 34. SUMMARY OF MEAN FECUNDITY PER CLIP CAGE FOR NIC-S AND NIC-R *M. PERSICAE* CLONES FIVE DAYS AFTER INOCULATION ONTO OLDER UNTREATED PLANTS AND PLANTS SEED-TREATED WITH DIFFERENT DOSES OF NEONICOTINOIDS. DOSE FACTORS (DF) ARE EXPRESSED RELATIVE TO THE AMOUNT OF IMIDACLOPRID USED TO TREAT OILSEED RAPE SEED (CHINOOK) WHERE DF = 1.



Foliar treatment experiments

A foliar application of thiacloprid, applied at the recommended rate for aphids on cabbage, controlled the Nic-S and Nic-R clones very well (Figure 35, $P < 0.001$). However, aphids from the Nic-R clone introduced one week after treatment showed significantly greater fecundity than the Nic-S clone (Figure 36: day 0-2, $P = 0.003$; day 2-5 $P < 0.001$), although this advantage was mostly lost for aphids introduced after a further week (Figure 37: day 0-2, $P = 0.001$; day 2-5 $P = 0.04$).

FIGURE 35. EXPERIMENT 7: SUCCESS OF NIC-S AND NIC-R CLONES INOCULATED ONTO YOUNGER CABBAGE AFTER FOLIAR TREATMENT WITH THIACLOPRID.

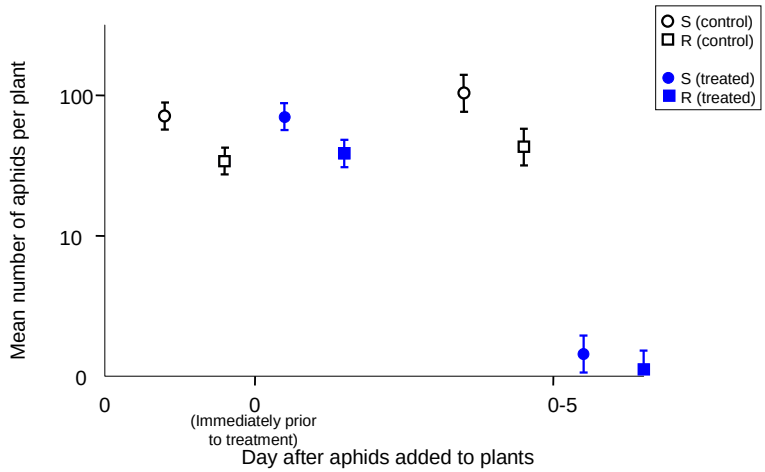


FIGURE 36. EXPERIMENT 7: MEAN FECUNDITY PER CLIP CAGE FOR NIC-S AND NIC-R *M. PERSICAE* CLONES INTRODUCED TO UNTREATED AND TREATED CABBAGE PLANTS ONE WEEK AFTER FOLIAR THIACLOPRID APPLICATIONS. BARS SHOW +/- 95% CIS.

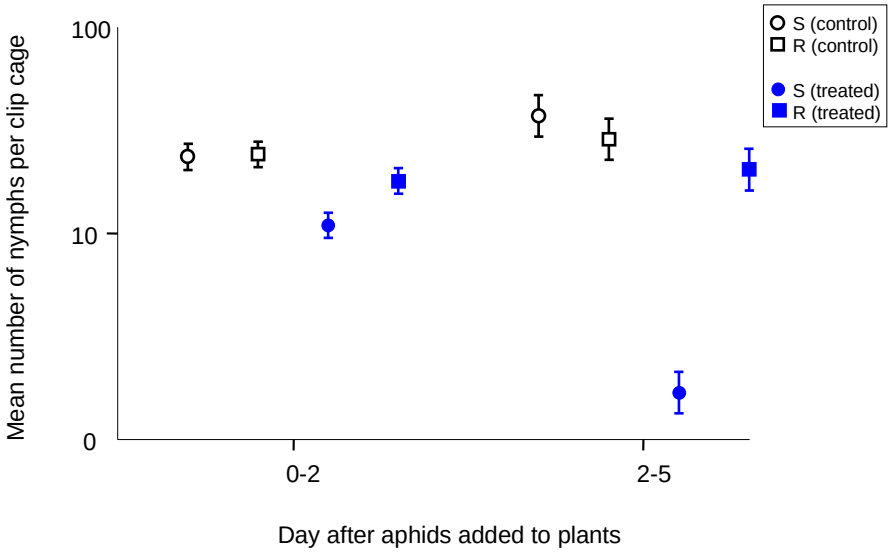


FIGURE 37. EXPERIMENT 7: MEAN FECUNDITY PER CLIP CAGE FOR NIC-S AND NIC-R *M. PERSICAE* CLONES INTRODUCED TO UNTREATED AND TREATED CABBAGE PLANTS TWO WEEKS AFTER FOLIAR THIACLOPRID APPLICATIONS. BARS SHOW +/- 95% CIS.

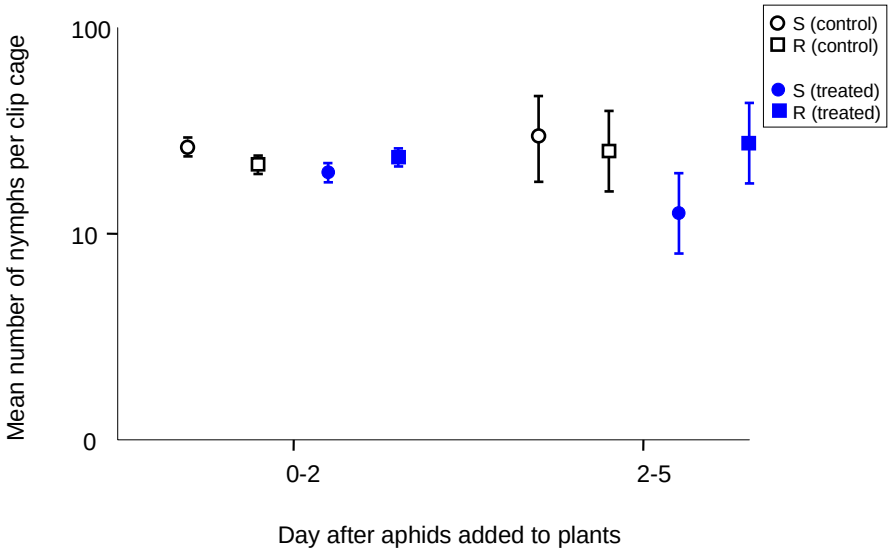
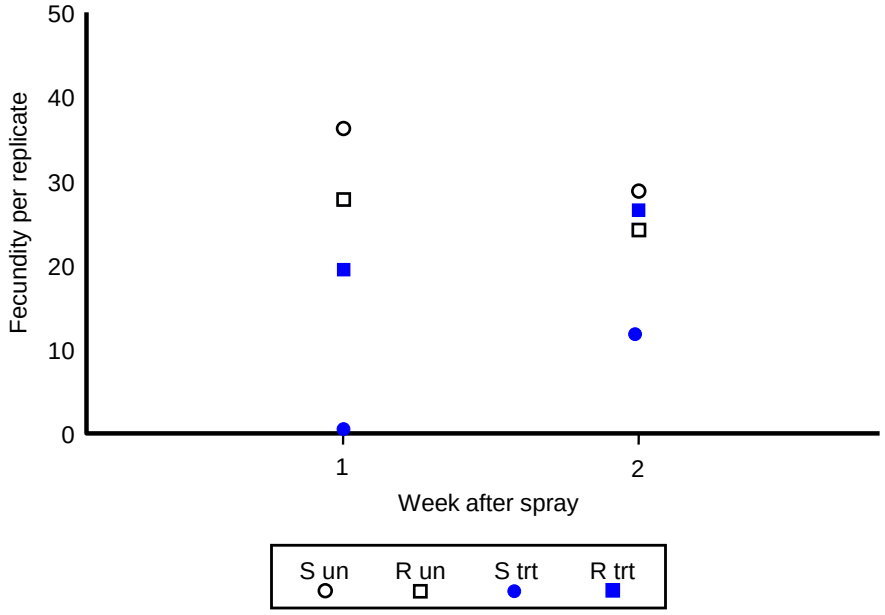


Figure 38 summarises the fecundities at 2-5 days of the Nic-S and Nic-R clones after thiacloprid treatment. This highlights the significant fitness advantage for Nic-R aphids for a short time (one week) after inoculation onto the cabbage plants which is reduced at two weeks after aphid inoculation.

FIGURE 38. SUMMARY OF MEAN FECUNDITY PER CLIP CAGE FOR NIC-S AND NIC-R *M. PERSICAE* CLONES FIVE DAYS AFTER INOCULATION ONTO UNTREATED PLANTS AND PLANTS ONE AND TWO WEEKS AFTER FOLIAR-TREATMENT WITH THIACLOPRID.



2.3.4 Additional work done on *M. persicae* from abroad

Greece

Some of the Greek *M. persicae* clones showed higher proportions of 'mobile' aphids compared to the standard Nic-R clone (showing low resistance to imidacloprid). A subset of these were tested with a full dose range of imidacloprid which revealed RFs up to ~50-fold (Figure 39). This is significantly greater than anything previously seen in the UK or abroad and suggests that *M. persicae* has taken another step in evolving resistance to neonicotinoids which has the potential to become more widespread (particularly as it is thought that there is a regular immigration of aphids to the UK from abroad). Interestingly, all clones that have shown RFs of 10 and above do not possess kdr (Table 8). If this trend continues they would therefore all be controlled by pyrethroid insecticides, a complete reversal of the current recommendations for *M. persicae*. Furthermore, a significantly greater proportion of the clones collected from tobacco (Chi^2, 1 df = 4.09, P = 0.043) carried RFs of 10 and above suggesting that this crop is a good source of samples for resistance screening (Table 9) and may be imposing selection favouring these aphids either because of exposure to imidacloprid treatments or nicotine produced within the plants as these both have the same binding site in the insect nervous system.

FIGURE 39. RANGE OF RESISTANCE FACTORS TO IMIDACLOPRID SHOWN BY UK AND GREEK (926B, 5189A, 5191A) *M. PERSICAE* CLONES TESTED WITH FULL DOSE RANGE TOPICAL BIOASSAYS.

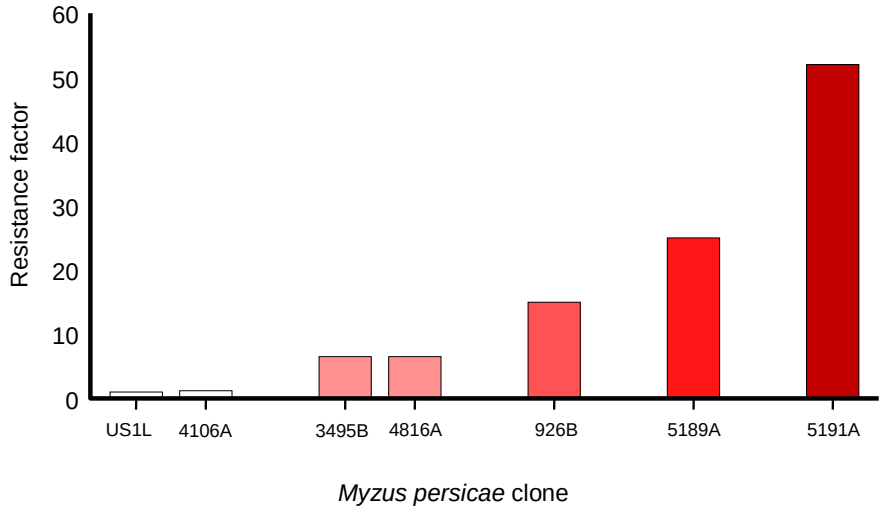


TABLE 8. RESISTANCE STATUS OF ALL *M. PERSICAE* CLONES SHOWING A 10 OR GREATER RESISTANCE FACTOR TO IMIDACLOPRID.

Clone	Year	Country of origin	Crop	Resistance mechanism		
				Esterase	MACE	kdr
926B	1990	Greece	Peach	R3	SR	SS
934E	1991	USA	Tobacco	R1	SS	SS
935D	1991	USA	Tobacco	S	SS	SS
975A	1991	Hungary	Potato	R2	SS	SS
4013A	2000	Greece	Tobacco	R3	SR	SS
4190A	2000	Greece	Tobacco	R3	SR	SS
4193A	2000	Greece	Tobacco	R3	SR	SS
5187A	2007	Greece	Tobacco	*	SR	SS
5189A	2007	Greece	Tobacco	R3	SR	SS
5191A	2007	Greece	Tobacco	R3	SR	SS

TABLE 9. HOST PLANT ORIGIN OF *M. PERSICAE* CLONES WITH KNOWN RESISTANCE FACTOR TO IMIDACLOPRID.

	Less than 10-fold	10 fold or greater
Non-tobacco	12	2 (14%)
Tobacco	7	7 (50%)

Imidacloprid resistance in New Zealand M. persicae

The screening dose bioassays showed that seven New Zealand *M. persicae* clones (10%) had low imidacloprid resistance (equivalent to Nic-R) but there was no evidence of greater levels of resistance than that seen in the UK despite significant use of imidacloprid seed treatment (Gaucho) on potatoes crop in NZ.

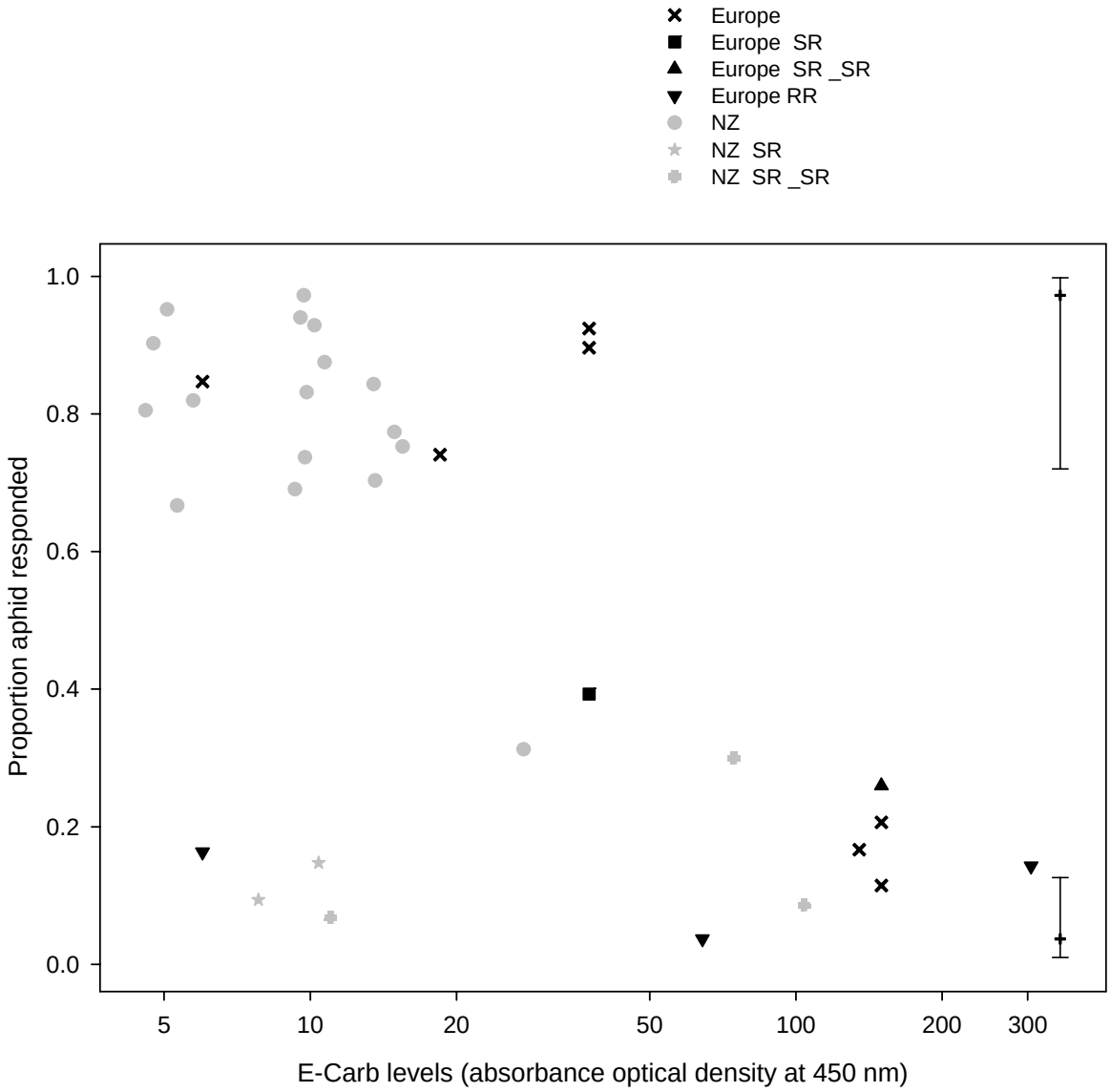
Other resistance mechanisms in New Zealand M. persicae

The carboxylesterase, MACE, kdr and super-kdr mechanisms were present in *M. persicae* clones collected from potatoes in New Zealand. 40% were fully insecticide-susceptible; 10% showed low resistance to imidacloprid (Nic-R), 38% contained elevated carboxylesterases (with the majority being R_2 or R_3); 19% had MACE; 54% had kdr; and 36% had super-kdr (s-kdr). No MACE, kdr or s-kdr homozygotes were found.

Response to alarm pheromone of New Zealand and European M. persicae

The *M. persicae* clones that carried no insecticide resistance or carried carboxylesterase resistance below R_3 levels showed high alarm responses (Figure 40). Those that carried R_3 carboxylesterase, or were heterozygous or homozygous for kdr or heterozygous for super-kdr, showed consistently low alarm responses. Contrasts in the analysis comparing clones that were carboxylesterase-S versus -R_{2-3} in the presence or absence of kdr indicated no significant difference ($P>0.1$) in responses between clones from New Zealand or Europe, with carboxylesterase and kdr acting independently from one another. Thus, clones from New Zealand showed alarm responses consistent with the insecticide resistance genotypes of clones from Europe.

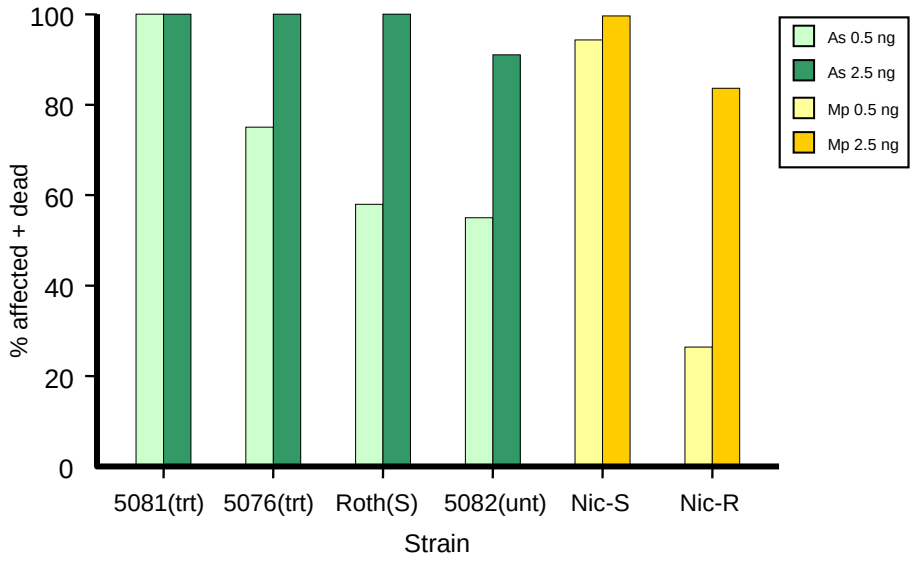
FIGURE 40: PROPORTION OF 12 M. PERSICAE FROM EUROPE (BLACK SYMBOLS) AND 22 FROM NEW ZEALAND (GREY SYMBOLS) CONTAINING VARIOUS LEVELS OF ELEVATED CARBOXYLESTERASE, OR KDR IN THE HETEROZYGOUS, SR, OR HOMOZYGOUS (RR) FORM, AND SUPER KDR IN THE HETEROZYGOUS (SR) FORM, THAT RESPONDED TO THE ALARM PHEROMONE (E)-ß-FARNESENE. ERROR BARS ARE 95% CONFIDENCE INTERVALS FOR THE TWO APHID CLONES THAT SHOWED THE LOWEST AND HIGHEST RESPONSES.



2.3.5 Additional work done in response to reports of potential imidacloprid resistance in *Aulocorthum solani*

The two strains of *A. solani* suspected of showing resistance to imidacloprid showed variation in response to imidacloprid applied at 0.5 ng and 2.5 ng per aphid (Figure 41). This was at an intermediate level between the Nic-S and Nic-R *M. persicae* standard clones. It would appear therefore that a limited amount of variation in response to imidacloprid exists in *A. solani* but it is unlikely to cause control failures. Interestingly, the two untreated *A. solani* samples showed the lowest mortality (measured by irreversibly affected + dead aphids) which reinforces the conclusion that suspicion of resistance was unfounded.

FIGURE 41. RESPONSE OF *A. SOLANI* (AS) AND *M. PERSICAE* (MP) TO IMIDACLOPRID.



2.3.6. Response of UK pollen beetles to lambda-cyhalothrin

The response of the eight pollen beetle samples to lambda-cyhalothrin are shown in Table 10. The five hour end-point was chosen as giving the best discrimination. None of the samples showed the significant levels of resistance to lambda-cyhalothrin that have been found in mainland Europe.

TABLE 10. MORTALITY (%) AFTER 1, 5 AND 24 HOURS IN LAMBDA-CYHALOTHRIN COATED VIAL TESTS OF *M. AENEUS* SAMPLES COLLECTED IN 2007.

	Sample number							
Dose rate (ug/cm^2)	1	2	3	4	5	6	7	8
1 hour								
Control	4.45	0	0	0	0	0	0	0
0.003	0	0	0	0	0	0	0	0
0.015	0	0	0	0	0	0	0	0
0.075	100	62	85	15	88	100	25	90
0.375	100	100	100	100	100	100	100	100
5 hours								
Control	5	0	0	0	0	0	0	0
0.003	14	0	0	0	0	0	0	0
0.015	100	77	90	55	77	65	15	45
0.075	100	100	100	100	100	100	100	100
0.375	100	100	100	100	100	100	100	100
24 hours								
Control	*	0	0	2	0	0	0	0
0.003	*	0	85	10	27	0	35	0
0.015	*	95	100	95	100	95	80	85
0.075	*	100	100	100	100	100	100	100
0.375	*	100	100	100	100	100	100	100

2.4 Discussion

Despite the widespread use of neonicotinoids for crop protection, and the occurrence of substantial resistance in some other insect pests, it would appear that *M. persicae* has not yet evolved potent resistance to these compounds. Our finding is supported by no confirmed reports of control failures for this pest, or any aphid species, on either field or glasshouse crops. This underscores the importance of neonicotinoid chemistry and the need for careful stewardship to preclude resistance problems in the future. The variation in response to these compounds found in *M. persicae* is of scientific interest in terms of understanding its origin and cause(s), but is also of practical importance as it potentially provides insights into both the characteristics of more potent resistance that may develop in the future and the treatment scenarios that may accelerate this process.

The project has demonstrated that reduced sensitivity to imidacloprid in *M. persicae* is consistent across all neonicotinoids tested, with some difference in the extent to which resistance is expressed. This reinforces the recommendation made in new Resistance Management Guidelines to treat all of these compounds as belonging to the same class and to alternate them with products having a different mode of action. Our results build on and strengthen the findings of the previous Link project (LK 0903) published in Foster *et al*. (2003a). The most resisted compound was clothianidin, although differences in potency against Nic-S clones resulted in its ED_{50} being only 3- to 7-fold higher against 926B compared

to ED_{50} values for imidacloprid, thiamethoxam and thiacloprid. Furthermore, RFs to topical applications of imidacloprid were reduced for aphids treated in systemic bioassays (the only neonicotinoid tested using this method so far). These findings suggest that clothianidin may impose greater selection pressures favouring resistance than other neonicotinoids and that the method of treatment (topical versus systemic) is important.

Results from work on other pests imply some idiosyncrasies that probably relate to the type of resistance mechanism present. In the whitefly *Bemisia tabaci*, the primary mechanism of resistance appears to be enhanced oxidative detoxification, conferring similar levels of resistance to a range of neonicotinoid insecticides (Nauen *et al*., 2002; Karunker *et al*., 2008). The only confirmed case so far of target-site resistance to neonicotinoids occurred in the planthopper *Nilaparvata lugens*, in which a point mutation in two nAChR subunit genes was associated with resistance and decreased neonicotinoid binding (Liu *et al*., 2005; 2006). This reduction in sensitivity was expressed over the full range of commercialised neonicotinoids but was much less pronounced for dinotefuron, implying subtle differences in its interactions with the binding site. However, similar point mutations have not subsequently been found in any *N. lugens* samples exhibiting resistance to neonicotinoids. Target-site resistance has also been implicated on the basis of electrophysiological work with Colorado beetle (*Leptinotarsa decemlineata*), but cross-resistance spectra across the neonicotinoid class have not been reported in detail (Tan *et al*., 2008). For *M. persicae*, the mechanism(s) underlying the variation in responses to neonicotinoids remain unclear and it is not known whether this is mediated through detoxification or target-site modification. However, the concordance between responses to different compounds supports fully the view advocated by the Insecticide Resistance Action Committee (www.irac-online.org) and IRAG-UK (www.pesticides.gov.uk/committees/resistance) to regard neonicotinoids as a single cross-resisted group from a resistance management standpoint.

The low resistance to imidacloprid in *M. persicae* extends to nicotine (Devine *et al*., 1996; Nauen *et al*., 1996) and cartap (Nauen *et al*., 1996), which have the same target site as the neonicotinoids. This raises the possibility that it was selected initially through exposure to naturally-acquired nicotine in the tissue of tobacco plants (a favoured host plant) and has spread by natural or human-mediated migration from tobacco-growing countries. Alternatively, it could have been selected prior to the commercialisation of neonicotinoids through the use of nicotine as a fumigant or foliar spray in insect pest management strategies. We intend to test this hypothesis in a new Link project studying *M. persicae* collected from Greece where clones collected recently have shown significantly higher RFs to imidacloprid.

At present, the resistance documented in *M. persicae* in the UK, for aphids with RFs up to ~15, appears to be of little importance in practice; a conclusion reinforced by the findings of the monitoring study of a large number of UK field *M. persicae* samples. Although this revealed temporal variation in the frequency of aphids expressing low resistance (Nic-R) to neonicotinoids, there was neither a general increase in their frequency over the study period, or evidence of any greater resistance likely to cause control failures with these compounds when they are applied at doses aimed at aphids. However, there is a 'cloud on the horizon' in the form of *M. persicae* recently collected from Greece in southern Europe. Some of these were found to be expressing RFs to imidacloprid, based on bioassays measuring aphid mobility, up to ~50 which suggests that this species has taken another evolutionary step towards potent resistance capable of compromising neonicotinoids. Such a 'stepping stone' process is thought to have occurred during the evolution of carboxylesterase resistance in this species. It is intended to test the implications of the Greek phenotype on seed and foliar applications using field simulators in a new Link project. Whatever the cause, the presence of extended variation in response to imidacloprid in *M. persicae* implies the existence of genetic variation in

response to these compounds, and the potential for potent resistance to evolve as selection pressures intensify over the coming years in the UK and abroad.

Turning to the established resistance mechanisms in *M. persicae* on UK field crops, the frequencies of carboxylesterase-R_2 and -R_3 resistance and MACE resistance have undergone non-random temporal fluctuations since monitoring started in 1996. The monitoring data for recent years, done as part of this project, show the frequency of aphids carrying high (R_2) or extreme (R_3) carboxylesterase resistance, primarily to OPs, has declined in the field but remains more common in glasshouses where conditions are probably more benign. In theory, the former could relate to several different processes:

i) Migration of aphids carrying lower insecticide resistance into field crops from non-crop hosts. This seems unlikely as similar recent declines in the frequency of carboxylesterase have been seen in aerial samples caught in suction traps that are thought to reflect the *M. persicae* population as a whole.

(ii) Genotypic reversion inhibiting the expression of amplified carboxylesterase genes. However, it remains unknown what proportion of the *M. persicae* population are revertants at any one time.

iii) Application of new aphicides with novel modes of action that circumvent the known mechanisms. There remains the possibility that neonicotinoids are involved but these compounds do not discriminate between different carboxylesterase resistance phenotypes, and therefore should not directly have caused a decline in carboxylesterase frequencies.

(iv) Associated fitness costs selecting against resistance when insecticide pressures are relaxed. This appears to be the most likely primary cause for the decline in carboxylesterase resistance and may reflect the general fazing out of OPs in the UK, which will have removed most of the selective advantage conferred by this form of resistance, and allowed the known pleiotropic fitness costs, manifested through altered aphid behaviour, to impose handicaps.

Whatever the cause, the recent fall in carboxylesterase resistance in *M. persicae* on field crops raises the possibility of controlling of this species with an OP should this prove essential although Greek clones showing higher RFs to imidacloprid have proved to be carboxylesterase R_3s.

In contrast to carboxylesterase resistance, MACE resistance, specifically to the aphicide pirimicarb, has become very common and widespread over the last several years in both field and glasshouse *M. persicae*. This is worrying and has implications particularly for beet growers because they do not currently have a viable control alternative if neonicotinoid resistance should appear in this species; a scenario that may result in biased selection pressures. Interestingly, all MACE aphids were heterozygotes, a genotype that confers immunity to pirimicarb, suggesting that homozygotes suffer a fitness cost. Our field sample data suggest that the use of pirimicarb is imposing selection strong enough to favour MACE aphids. Another possibility is that this mechanism is now being found more often in new aphid clones that only carry carboxylesterase-S or R_1 resistance, ie MACE is probably no longer being handicapped by an association with higher carboxylesterase resistance. Furthermore, UK MACE aphids, which appear to originate from abroad, may be better adapted to our climate, which is warming, and ecological conditions in this country. This idea is supported by work reported by Kasprowicz *et al.* (2008) which suggests that the Scottish *M. persicae* population consists of waves of clonal lineages with time that can have a periodicity of up to several years,

each potentially carrying fitness advantages and costs conferred by resistance mechanisms and other genes.

M. persicae carrying kdr, conferring resistance to pyrethroids, remain relatively common on UK field and glasshouse crops although those with super-kdr continue to be very rare. Like all MACE aphids, every kdr aphid was a heterozygote, a genotype capable of conferring significant resistance, suggesting that this mechanism in the homozygous form, and probably super-kdr, also imposes a fitness handicap. Otherwise, they would be expected to be found at higher frequencies in the population. Interestingly, the prevalence of kdr heterozygotes in UK *M. persicae* has occurred despite the apparent fitness costs that this mechanism imposes on aphid alarm response (Foster *et al*., 2003c) which culminates in greater vulnerability to parasitoid attack and mummification (Foster *et al*., 2007). However, the latter study only tested non-kdr and kdr homozygotes so it remains to be shown whether kdr heterozygotes have lower vulnerability than homozygotes. If this is the case, the relative advantages conferred by kdr in the heterozygous form, through resistance to pyrethroids (which continue to have high usage in the UK), versus the lesser disadvantages, through maladaptive aphid behaviour, could result in selection currently maintaining the prevalence of heterozygotes in UK the population. This finding is worrying as it suggests that applications of pyrethroids alone to control this pest are being compromised. Furthermore, it is supported by data gained in field experiments applying pyrethroids to known kdr heterozygous *M. persicae* on potatoes (Parker *et al*., 2006) and sugar beet (Dewar, A, *pers. com.*). Seed potato growers currently use pyrethroids because they apparently give fast knock-down and repellent benefits for controlling aphids including *M. persicae.* However, little is known about the impact of the kdr mechanism, along with super-kdr and esterase resistance, on aphid behaviour under exposure to pyrethroids or whether these compounds are making a significant contribution to suppressing virus transmission by *M. persicae*.

Interestingly, the survey of *M. persicae* clones collected from potatoes in New Zealand showed that carboxylesterase, MACE, kdr, super-kdr, and low resistance to imidacloprid are all present in that country with super-kdr appearing to be more common than in the UK. Furthermore, alarm pheromone behavioural bioassays done on a subset of the New Zealand clones support the growing body of evidence that extreme (R_3) carboxylesterase resistance and kdr have a deleterious pleiotropic effect on aphid behaviour, probably through negative impacts on nerve and/or biological function, particularly during times of stress (Foster *et al*., 1999; 2005). Although all except one of the clones from Europe and New Zealand were genetically different, they still showed a similar behavioural response to alarm pheromone. Thus, it appears that the effects on aphid behaviour were directly associated with both resistance mechanisms, rather than the alternative explanation that the resistance genes are closely associated with other genes affecting behaviour.

The highest levels of resistance to imidacloprid (RFs up ~12) recorded to date in the UK do not appear to be currently causing serious control problems, as born out in the field simulator experiments by the high efficacy of the strong commercial rates of imidacloprid and clothianidin against Nic-S and Nic-R *M. persicae* clones in seed treated cabbage. In contrast, the relatively weak commercial seed treatment of imidacloprid to oilseed rape had a very slight impact on both clones, although there was no significant differential selection between them, when aphids had been left on plants for a longer period (over two weeks). Such a result is not unexpected as this imidacloprid treatment is not aimed at aphid control. However, our findings also suggest certain conditions under which low neonicotinoid resistance can be expressed and selected for. These 'windows of selection', which can magnify the risks of the evolution of greater resistance, appear to relate to the route of treatment, dose rate and, possibly, the compound applied. This latter hypothesis stems from clothianidin resulting in the highest RFs

in topical bioassays and from Nic-R aphids having a much higher fitness on oilseed rape plants that had been seed treated with clothianidin at a slightly higher dose rate (5x) than that used commercially for imidacloprid. Another scenario imposing an apparent significant advantage to Nic-R aphids was shown to occur with a foliar application of thiacloprid (Biscaya). Experiments using cabbage treated with the recommended rate of this compound (now registered for use on UK crops such as potatoes and brassicas, including oilseed rape where pollen beetles are the main target) clearly showed that both Nic-S and Nic-R aphids are controlled well at the time of insecticide application but for a short, yet significant, period afterwards fitness advantages were magnified for Nic-R aphids when they were introduced to treated plants in a way that mimics natural migration of aphids into a crop after spraying. Concerns over the selection pressure imposed by foliar applications of thiacloprid against Nic-R aphids and the potential effect this will have on the speed of evolution of resistance are not easy to predict but we aim to systematically test the implications of application rate (including rates not intended for aphid control that are and could be aimed at other pests), compound, route of treatment (seed versus foliar) and RF in a new Link project. The resulting generic information will form the basis for predicting conditions under which more potent resistance is likely to be selected and/or expressed.

2.5 Conclusions

The project is an excellent example of proactive research to anticipate and combat risks of neonicotinoid resistance. It has provided a strong foundation for neonicotinoid stewardship for controlling *M. persicae*, an important pest that has proved adept at evolving insecticide resistance to several classes of compounds, against a backdrop of increasing use on its important host plants and the recent juxtaposition of systemic and foliar applications. The fact that this species occurs on a wide range of crops means that measures to control it on one particular crop could have profound consequences for growers of others.

Our findings of no evidence of significant resistance in the UK that is capable of compromising neonicotinoid efficacy when applied at rates aimed at aphid control, or no evidence in the field that current usage of these compounds is imposing biased selection pressures for resistance on certain hosts, mean that neonicotinoids can continue to play a key role for controlling *M. persicae*. However, there is no room for complacency since two resistance mechanisms (MACE and kdr) remain prevalent and widely distributed in the UK population and our experiments on whole plants suggest that there may be treatment scenarios favouring the evolution of neonicotinoid resistance.

Neonicotinoids are not immune to the evolution of resistance as already seen in several pests including whiteflies, potato-Colorado beetles and planthoppers. *M. persicae* remains susceptible to neonicotinoids, when they are applied at rates aimed at controlling aphids, but shows widening variation its response to them. The over-riding question is whether the existing mechanism in *M. persicae* conferring low resistance to neonicotinoids, whatever its nature, can be progressively enhanced, or whether a different mechanism is needed to compromise efficacy under field conditions. Continued effort is therefore required to monitor for further upward shifts in response in this species, both in the UK and abroad, and tailor management recommendations accordingly at an early enough stage. This is currently taking place through PSD funding and will continue through an SA-Link project.

The disclosure that low resistance to imidacloprid in *M. persicae* extends to all neonicotinoid molecules used in the UK demonstrates an underlying capacity for this species to respond and adapt to these compounds. However, despite increasing usage, there was no overall upward

trend in the field in the frequency of aphids showing low resistance between late 2004 and the end of 2007, or any obvious association with crop, treatment history or locality of collection. There is therefore still no economically-significant resistance to neonicotinoids in *M. persicae* in the UK. However, recent reports of increased resistance to imidacloprid in this species for aphids feeding on tobacco in northern Greece is a development which has important implications bearing in mind that MACE resistance to pirimicarb developed in southern Europe and then spread quickly to the UK.

The low-level resistance in *M. persicae* to neonicotinoid insecticides appears to be unrelated to the occurrence of the carboxylesterase and MACE resistance mechanisms. However, all clones showing 10-fold or greater resistance to imidacloprid lacked kdr. If this trend continues for clones showing higher imidacloprid resistance, pyrethroids could play a revitalised role for controlling this species.

The field simulator experiments on whole plants provided insights into complex relationships between operational parameters (dose-rate, time since treatment, seed vs. foliar application and neonicotinoid compound) and the response of aphids differing in sensitivity to neonicotinoids (resistance up to 15-fold). Such data can be a basis for predicting conditions under which more potent resistance is likely to be selected and/or expressed. This, therefore, merits more systematic investigation, including measurements of the response of aphids with the newly-discovered higher resistance, given the ongoing diversification of neonicotinoid treatments on UK crops. The lack of reports of control problems from the region where these aphids are found suggests that resistance is still not sufficiently great enough to compromise the field performance of neonicotinoids when they are aimed at controlling aphids. However, it highlights the need for careful vigilance and stewardship, and, in conjunction with the findings of the bioassays testing correlated responses, a need to consider neonicotinoids as a single cross-resisted group for management purposes.

The proliferation of foliar neonicotinoid registrations in the UK, imminent changes to the neonicotinoid doses applied as seed treatments to oilseed rape, and the new recommendations to use thiacloprid sprays for pollen beetle control on oilseed rape will probably impose significant selection favouring *M. persicae* expressing higher resistance to neonicotinoids.

The prevalence of kdr and MACE in *M. persicae* in the UK is likely to be seriously compromising the efficacy of any pyrethroid and pirimicarb applications that are aimed at controlling this species (although there is still debate on whether pyrethroids reduce virus transmission). This reinforces the importance of maintaining the effectiveness of neonicotinoids and other novel insecticides (pymetrozine and flonicamid), which circumvent both mechanisms, for controlling *M. persicae* in this country.

The project has sustained the scientific momentum by improving our understanding of resistance risks posed by different patterns of neonicotinoid use. It has enabled the arable industry to capitalise on the UK's unparalleled expertise with analysing and combating aphid resistance and has contributed to safeguarding the competitiveness and marketability of UK produce. It has also delivered information of direct relevance to ameliorating unnecessary effects of pesticide use on the environment.

The project's findings of no significant increases in neonicotinoid resistance in *M. persicae* (above the low levels already known) or evidence of current treatments imposing significant selection pressures will impact significantly on decisions made for registration of neonicotinoids for controlling this pest, eg. thiacloprid sprays on potatoes and brassicas. The increased availability of these compounds is safeguarding the productivity, competitiveness

and marketability of UK produce through countering *M. persicae* that are resistant to other classes of insecticides.

Our findings have been exploited by the PSD for the insecticide regulatory process and combined into an improved framework for assessing resistance risks and strengthening recommendations for sustainable use of neonicotinoids for aphid management. The project has informed growers and regulators on the up-to-date status of resistance to all insecticides available for aphid control in the UK, and specifically on any impending problems with the sustained efficacy of neonicotinoids. Through discussions of results and broader issues at meetings of the Project Steering Group and IRAG, the consortium as a whole has gained a greater awareness of resistance problems associated with neonicotinoids and other insecticide groups. The wide knowledge base within the consortium has been well suited to reviewing the likely effectiveness and practicability of possible countermeasures to resistance. Regular meetings have also strengthened the dialogue between researchers, advisors, regulators, grower representatives and agrochemical manufacturers, all essential players in ensuring the continued sustainability of crop protection strategies. These have culminated in Resistance Management Guidelines tailored to specific crops (down-loadable from IRAG UK's website (**http://www.pesticides.gov.uk/committees/resistance/index.htm**). These allow UK growers to make the right decisions on insecticide treatments.

2.6 References

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3. Knowledge transfer activities

To date, the project has contributed to 2 refereed papers, 5 other research papers, 1 book chapter, 18 presentations, 3 posters, 28 trade press articles, 1 resistance alert and 2 revised Resistance Management Guidelines (for potatoes and brassicas). Our findings have also formed part of a BPC Topic Review on insecticide resistance and its implications for potato production in Great Britain (compiled by Steve Foster in 2006).

Publications in Refereed Journals

SP Foster, D Cox, L Oliphant, S Mitchinson & I Denholm. Correlated responses to neonicotinoid insecticides in clones of the peach-potato aphid, *Myzus persicae* (Hemiptera: Aphididae). *Pest Management Science.* in press.

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Book Chapters, Papers in Conference Proceedings and Other Journals

SP Foster (2007) Resistance to neonicotinoid in *Myzus persicae* in the UK: good news, bad news and challenges ahead. *Proceedings of the International Plant Protection Congress*, Glasgow, October 2007, volume 2, 622-623.

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Publications in Preparation

SP Foster, S Mitchinson, D Cox, L Oliphant & I Denholm. Spatial and temporal patterns in resistance to neonicotinoid insecticides in peach-potato aphids, *Myzus persicae* (Hemiptera: Aphididae) in the UK.

SP Foster & I Denholm. Efficacy of UK-registered seed and foliar neonicotinoid treatments against peach-potato aphids, *Myzus persicae* (Hemiptera: Aphididae), carrying susceptibility and low resistance to neonicotinoids.

Resistance Guidelines and Aphid Alerts

Guidelines for preventing and managing insecticide resistance in aphids on potatoes.

Guidelines for controlling aphids in brassica crops and managing insecticide resistance in the peach-potato aphid, *Myzus persicae.*

IRAG Resistance Alert: insecticide-resistant peach-potato aphids on potatoes.

Presentations

S Foster & M Williamson. Are neonicotinoid insecticides resistant to the evolution of resistance in aphids? *International Congress of Entomology*. Durban, South Africa, July 2008 (invited keynote presentation).

I Denholm & S Foster. Aphid control with neonicotinoids: a sustained success story (but for how much longer?). *Stewardship of neonicotinoid* insecticides, Honolulu, Hawaii, June 2008.

S Foster. Aphid and Virus Review for 2007. *Beet Growers Annual Conference*, Peterborough, February 2008.

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The potential of *Myzus persicae* to evolve resistance to neonicotinoid insecticides. *Cereals 2005*, June 2005.

Farming and Popular Press

2008

Summer pest control strategies for brassicas (*Syngenta Specialist Crops Technical Update*, July)

New potato aphicides and their performance (*CPM*, April)

2007

Aphid control in lettuce and brassica crops (*HDC News*, March)

Plenum first choice for foliar insecticide (*Syngenta Media Release*, May)

Sustainable Arable Link Project: stewardship of neonicotinoid insecticides (LK 0953) (*ARF Webpages*, May)

Take care to prevent resistance (*CPM*, May)

Aphid control in brassicas (*The Vegetable Farmer*, May)

Sustainable Arable Link Project: stewardship of neonicotinoid insecticides (LK 0953) (*BPC Webpages*, May)

Stewardship helps manage resistance *(Potato Review*, May/June)

Insecticide resistance alert (*IRAG Website, HDC News, HDC Pest Bulletin, Potato Review, a large number of Farming Weeklies*, June)

Aphids show chemical resistance (*Horticulture Week*, June)

Care needed with aphicide choice (*Scottish Farmer*, June)

Resistance warning (*Potato Review*, June)

IRAG Resistance alert: peach-potato aphids on potatoes (*Vegetable Farmer*, July)

2006

New UK products for Bayer (*Agrow*, March)
How to deal with resistant aphids in potatoes (*CPM Magazine*, April)
Late flights a blip in longer term trend (*Potato Review*, July)
Piece on DEFRA LINK project on Neonicotinoids (*IRAC eConnection*, July)
40% MACE resistance in early tests (*Bayer's Four Seasons Potatoes*, August)
Summer pest control in brassicas (*Syngenta Technical Update*, August)
Stewardship of neonicotinoids: a project to support proactive IRM for a key group of aphicides (*IRAC eConnection*, Sept)

2005

MACE aphids survive winter (*Syngenta Media Release*, April)
MACE aphids flying high (*Syngenta Media Release*, June)
Early surge for resistant aphids (*Farmers Weekly*, July)

2004

Winter fails to put freeze on tricky aphids (*Farmers Weekly*, May)
MACE aphids get through the winter (*Warwick HRI Website*, May)