Project title:	Protected Crops: Design and evaluation of a robust biologically based strategy for the control of MACE resistant *Myzus persicae*.
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Location	HRI, Stockbridge House IACR Rothamsted
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The results and conclusions in this report are based a series of experiments conducted over a one year period. The conditions under which the studies were carried out and the results have been reported with detail and accuracy. However, because of the biological nature of the work it must be borne in mind that different circumstances and conditions could produce different results. Therefore, care must be taken with the interpretation of the results especially if they are used as the basis for commercial product recommendations.

Authentication

I declare that this work was done under my supervision according to the procedures described herein and that this report represents a true and accurate record of the results obtained.

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PRACTICAL SECTION FOR GROWERS

Background and objectives

The peach potato aphid, *Myzus persicae*, is a polyphagous species that attacks a wide range of protected and outdoor crops. For some time there have been clones in the UK which produce high levels of an esterase enzyme making them resistant to most organophosphate and carbamate insecticides, but still susceptible to pirimicarb. This is known as esterase resistance, which ranges from R_1 (moderate resistance) to R_3 (extreme resistance). *Myzus persicae* has also developed resistance to pyrethroids, known as knock-down resistance (kdr). To manage insecticide resistance, protected crop growers have been encouraged to base their control strategies on biological control agents (mainly *Aphidius colemani* and *Aphidoletes aphidimyza,* supplemented by "open rearing units"). In protected crops, biological control strategies usually work well against *M. persicae* for most of the season, but occasional treatments of pirimicarb are required to bring any parasitoid: prey imbalance back into line or to control sudden pest invasions.

In recent years, *M. persicae* has developed resistance to pirimicarb. This is conferred by a target site mutation, termed Modified AcetylCholineEsterase ('MACE'). Because pirimicarb is completely ineffective against MACE aphids, and there were no effective approved chemicals that were compatible with the natural enemies, the occurrence of MACE aphids resulted in severe crop loss. In mainland Europe, MACE aphids are now well established, but growers rely on controlling them with chemicals that are not currently approved for use on salad crops in the UK. In addition, this chemically intensive strategy will not provide sustainable control as some resistance has already developed to some of the newer chemicals used in mainland Europe. UK growers therefore seek a biologically based control strategy that is as effective against MACE as it is against non-MACE aphids. The commercial objective of this project is to achieve sustainable control of both MACE and non-MACE *M. persicae* in protected crops, based on a robust biological control programme supported by compatible remedial treatments.

The specific objectives for the first year of the project were:

1. To investigate the biological differences in MACE and non-MACE clones of *M. persicae* that might affect the success of biological control in terms of:
    - 1.1 Reproductive potential
    - 1.2 Feeding position
    - 1.3 Response to alarm pheromone
    - 1.4 Defensive behaviour

2. To identify the best parasitoid species for control of MACE clones of *M. persicae* by:
    - 2.1 Determining relative parasitism rates of selected parasitoid species against MACE and non-MACE clones.
    - 2.2 Determining whether the best parasitoid species showed a preference for the different clones in terms of colour or resistance mechanism.

Summary of Results

The biological differences in MACE and non-MACE clones of *M. persicae*

Reproductive potential

In the absence of insecticides, *M. persicae* with MACE resistance reproduced at similar rates to non-MACE clones carrying extreme (R_3) esterase resistance. However, MACE clones may be reproductively out-competed by non-MACE clones expressing lower levels (R_1) of esterase-based resistance.

Feeding position

On erect plants, such as peppers and violas, *M. persicae* with MACE resistance were more frequent on younger leaves (e.g. the growing shoots at the tops of plants) and flowers than non-MACE aphids. It was speculated that MACE aphids may enjoy greater survival than non-MACE aphids in commercial glasshouses because some of the aphids in the shoots are protected from natural enemy attack and from chemical sprays. Also, MACE aphids that are feeding on the growing points may cause more damage than their non-MACE counterparts, which are more frequent on older leaves. No difference was observed in the feeding position of MACE and non-MACE aphids on lettuce.

Response to alarm pheromone

The tendency for *M. persicae* to respond to alarm pheromone appears to be primarily associated with kdr–based resistance. Kdr-resistant clones are much less responsive. Most MACE aphids found in the UK since 1996 have also carried kdr resistance and therefore are not as likely to show an alarm response as aphids without kdr. However, there is some variation amongst these kdr clones (which are apparently common in the UK). Those carrying MACE and extreme (R_3) esterase-based resistance were far more responsive to alarm pheromone than their non-MACE/R_3/kdr counterparts. As a result, MACE aphids may enjoy greater survival than non-MACE aphids in glasshouses using biological control strategies as MACE could be associated with greater avoidance of predators and parasitoids.

Defensive behaviour

A greater proportion of red MACE aphids (UK origin) than green MACE (Greek origin) or green non-MACE (UK origin) aphids showed defensive behaviour in response to an approach by the parasitoid *Aphidius colemani*. Responses consisted of kicking, flicking the body, walking away from the parasitoid or walking towards the parasitoid. The increased response of red MACE aphids may be connected to increased response to alarm pheromones (see above). As the two MACE clones did not respond in the same way, it is suggested that these responses reflect the genetic background of the aphids (which reproduce asexually) rather than the resistance mechanism. As most MACE aphids in the UK are similar to the red MACE clone tested, it is possible that a greater proportion of MACE aphids will survive in UK glasshouses using biological control as MACE could be associated with greater avoidance of predation or parasitism.

Parasitism of MACE and non-MACE clones of *M. persicae*.
Comparison of three parasitoid species against MACE and non-MACE clones

In Petri-dish experiments, *Aphidius colemani* and *Aphidius matricariae* performed equally well against MACE and non-MACE *M. persicae*. Both species parasitised an average of between eight and nine aphids per female wasp when exposed to 50 aphids for a period of 30 minutes. *Praon myziphagum* produced fewer offspring, each female parasitising an average of three to four aphids under the same conditions. Further experiments were required to determine whether the parasitoids performed differently against MACE and non-MACE clones on whole plants.

The efficacy of Aphidius colemani when given a choice of M. persicae clones

On whole pepper plants, *A. colemani* parasitised a greater proportion of green non-MACE *M. persicae* than red MACE aphids when given a choice. The feeding position of the aphids was identified as a key factor affecting parasitism. Fewer MACE aphids were parasitised because a greater proportion were feeding on the leaf shoots, which made them less accessible to parasitoid attack.

Action points for growers

- The MACE and non-MACE aphids that are most prevalent in UK greenhouses are likely to reproduce at similar rates in the absence of insecticides.

- *Aphidius colemani* was shown to be effective against MACE and non-MACE aphids and is recommended as the main biological control agent against all clones of the peach potato aphid (*Myzus persicae*).

- A number of differences in the behaviour of MACE aphids compared to their non-MACE counterparts may impact on parasitism:

    - In pepper crops and some flower crops, MACE aphids feed more frequently in the growing shoots than non-MACE aphids, helping them to avoid parasitoid attack.

    - MACE clones respond more frequently to alarm pheromones than non-MACE clones, which may help them to escape parasitism.

    - The red MACE clone that is most prevalent in the UK respond more frequently to parasitoid attack by kicking or by walking away from the parasitoid, which may help them to escape parasitism.

- Work is in progress to determine whether the differences in MACE aphid behaviour identified have a significant effect on *A. colemani* parasitism on crops. It is possible that earlier releases or higher rates of *A. colemani* may be required to maintain control of MACE aphids compared to non-MACE aphids and this will be investigated in the second and third years of the project.

- The HDC has recently secured a SOLA (2337/2000) for the use of Chess (pymetrozine) on protected pepper and aubergine crops for aphid control. Chess has on-label approval for use on protected cucumber and ornamentals.

Recommendations for further research work

The project was reviewed in May 2000 and the following recommendations were made for the second year of the project:

1. To compare the efficacy of *A. colemani* against red MACE and green non-MACE *M. persicae* in a crop scale experiment. This trial would evaluate the strategy of increasing the introduction rate of *A. colemani* for the control of *M. persicae* (x 2 normal rate), increasing the frequency of introductions and examine the mobility and feeding position of *M. persicae.*

2. To determine the preferred oviposition or feeding positions of *A. colemani*, *Aphidoletes aphidimyza, Chrysoperla carnea* and *Adalia bipunctata* on *M. persicae* infested pepper plants.

3. To compare the performance of *Aphidoletes aphidimyza*, *Chrysoperla carnea* and *Adalia bipunctata* against the red MACE and green non-MACE *M. persicae* in Petri-dish experiments.

4. To determine the host preference of a selected predator when given a choice of red MACE and green non-MACE aphids on whole plants.

5. To determine the efficacy of Eradicoat (a glucose polymer) against red MACE and green non-MACE aphids in a replicated laboratory experiment.

6. To determine the efficacy of Eradicoat against red MACE and green non-MACE aphids in a crop scale experiment.

Practical and financial benefits from the study

Myzus persicae is a polyphagous insect, which can cause severe economic damage in crops as diverse as peppers, aubergine, lettuce, bedding and pot plants (including Nicotiana, Cineraria, Primula, Dianthus, Chrysanthemum, alpines and Gypsophila). As there are few approved insecticides for the control of *M. persicae* with MACE resistance, MACE presents a real threat to a wide spectrum of the industry and this project has received the support of the Cucumber Technology Group, Bedding Plant Technical Committee, lettuce growers and pepper growers.

Potential benefits of the project include:

1. Reduced direct economic crop loss resulting from honeydew and rejected produce.
2. Improved knowledge of biological control programmes for aphid control.
3. Reduced reliance on chemical insecticides.
4. A more favourable environment for introducing new chemicals and sustaining their effectiveness.
5. The control strategies devised could be applied to other aphid/parasitoid complexes.

SCIENCE SECTION

INTRODUCTION

The peach potato aphid, *Myzus persicae*, is a polyphagous species that attacks a wide range of protected and outdoor crops. For some time there have been clones in the UK, which produce high levels of esterase making them resistant to most organophosphate and carbamate insecticides, but still susceptible to pirimicarb. This is known as esterase resistance, which ranges from R_1 (moderate resistance) to R_3 (extreme resistance). *Myzus persicae* has also developed resistance to pyrethroids known as knock-down resistance (kdr). To manage insecticide resistance, protected crop growers have been encouraged to base their control strategies on biological control agents (mainly *Aphidius colemani* and *Aphidoletes aphidimyza*, supplemented by "open rearing units"). In protected crops, biological control strategies work well against *M. persicae* for most of the season, but occasional treatments of pirimicarb are required to bring any parasitoid: prey imbalance back into line or to control sudden pest invasions.

In recent years, a clone of *M. persicae* has developed resistance to pirimicarb. This is conferred by a target site mutation, termed Modified AcetylCholineEsterase ('MACE'). Because pirimicarb is completely ineffective against MACE aphids and there were no effective, approved chemicals that were compatible with the natural enemies, the occurrence of MACE aphids resulted in severe crop loss. In mainland Europe MACE *M. persicae* are now well established, but growers rely on controlling them with chemicals that are not currently approved for use on salad crops in the UK. In addition, this chemically intensive strategy will not provide sustainable control as some resistance has already developed to some of the newer chemicals on mainland Europe. UK growers therefore seek a biologically based control strategy that is as effective against MACE as it is against non-MACE clones. The commercial objective of this project was to achieve sustainable control of both MACE and non-MACE *M. persicae* in protected crops, based on a robust biological control programme supported by compatible remedial treatments.

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PART 1. THE BIOLOGY OF MACE AND NON-MACE CLONES OF *MYZUS PERSICAE*

1.1 REPRODUCTIVE POTENTIAL (R_M) OF *M. PERSICAE* CLONES

Objective

To assess possible associations of aphid reproductive success primarily with MACE- and esterase insecticide resistance.

Materials and methods

MACE and non-MACE clones (Table 1.) carrying R_1 or R_3 esterase-based resistance were assessed for their reproductive potential in the absence of insecticides in the laboratory. This involved the measurement of development time and fecundity of up to 30 replicates per clone at 21°C using Chinese cabbage as the host plant under an 18 h day/ 6 h night cycle. Experimental aphids were taken initially from lines maintained at 21°C as virginoparous, predominantly apterous colonies. Clonal integrity was checked regularly by biochemical assays (for esterase and MACE resistance; Devonshire *et al*., 1986; Moores *et al.*, 1994), aphid diagnostic-dose bioassays (for kdr phenotype: Field *et al.*, 1997) and DNA assays (for kdr genotype: Foster *et al.*, 1999). Each clone was assessed in two experiments. It was intended to assess two esterase-R_1 non-MACE clones. However, one of these clones became unhealthy and therefore had to be eliminated during the course of the study.

Table 1. *Myzus persicae* clones used in assessments of reproductive potential

Clone	Resistance mechanism			Origin	Date of collection
	esterase[1]	*MACE*[2]	*kdr*[3]		
3172A	R_1	no	SR	UK	1998
2161C	R_3	no	SR	UK	1997
2169G	R_3	no	SR	UK	1997
2042P	R_1	yes	SR	UK	1996
2050A	R_1	yes	SR	UK	1996
3104B	R_1	yes	RR	UK	1998
2144F	R_3	yes	RR	Greece	1997
2146K	R_3	yes	SR	Greece	1997

[1] Determined by immunoassay: R_1 (moderate resistance), R_3 (extreme resistance).
[2] Determined by kinetic assay using a discriminating concentration of pirimicarb.
[3] Determined by DNA assay using SSCP (single strand conformational polymorphism) technique: SS (kdr-SS genotype), SR (kdr-SR genotype) and RR (kdr-RR genotype).

Reproductive fitness parameters were assessed for each clone developing on excised Chinese cabbage leaves. Initially, first/second instar nymphs (generation 0) were obtained from laboratory stocks and grown to adults in small leaf boxes. Six leaf boxes, each containing 4 adults, were then set up. The adults were removed after 15 offspring had been produced per box (generation 1). These offspring were then grown to adults. 30 leaf boxes, each containing three of these adults, were then set up. The aphids were left to reproduce overnight and removed the next morning leaving five offspring per box (generation 2). These offspring were themselves grown to adults. Generation times were measured by monitoring the number of days taken until their first offspring was produced (generation 3). Four generation 2 adults were then removed leaving the first parent that produced nymphs. Daily fecundity was then measured (with generation 3 offspring being counted and removed each day) up until the previously measured generation time (in days) of that parent had expired. At this point each parent was assessed for esterase-based resistance level.

The intrinsic rate of natural increase, r_m, was calculated for each clone using the equation defined by Wyatt & White (1977):

$$r_m = 0.738 (\ln M_d)/ d$$

where d = the generation time (birth to first reproduction)

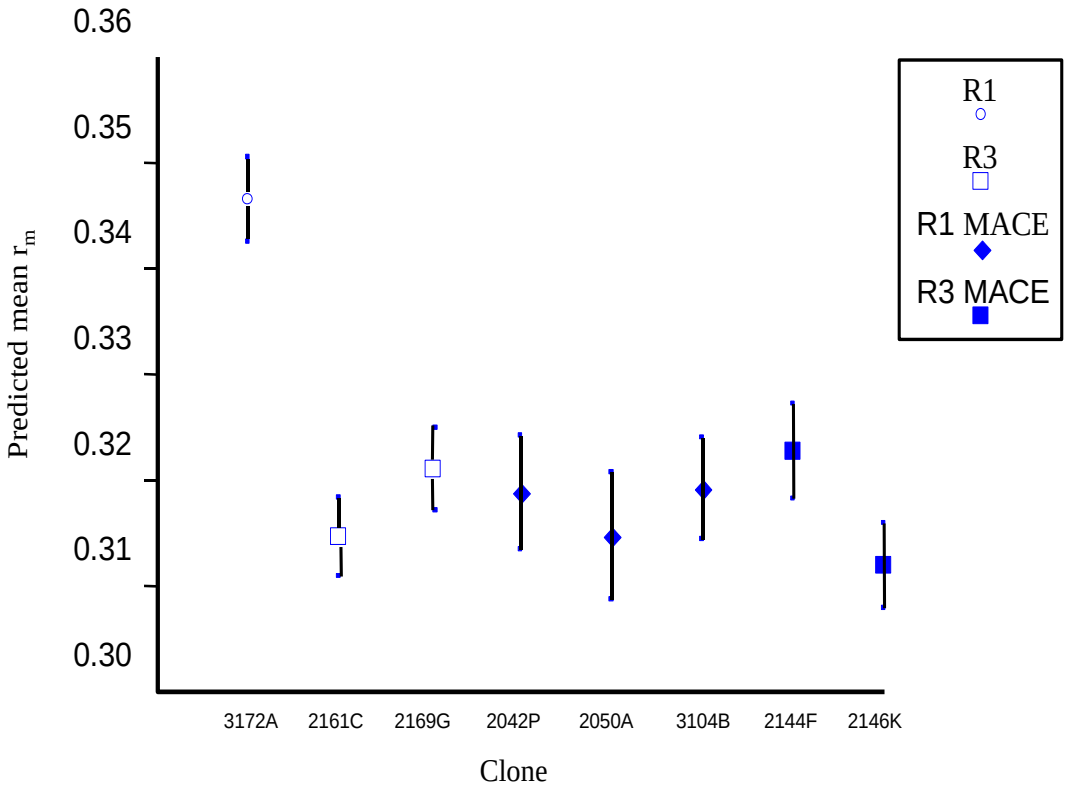
and M_d = the number of progeny produced in a reproductive period equal to d.

The data were analysed using an analysis of variance (ANOVA) comparing fitness parameters primarily on the basis of MACE- and esterase-based resistance. The analysis was adjusted for experimental, box and replicate effects.

Results

Figure 1 shows the mean rates of increase and standard errors for the eight *M. persicae* clones assessed. Analysis showed MACE was significantly associated with slower reproduction ($T_{50\ (\text{degrees of freedom})} = 4.06$, $P < 0.001$). However, it is obvious that this result relied heavily on the esterase-R_1 non-MACE clone (3172A). The remaining MACE and non-MACE clones produced similar r_m values.

Figure 1. Mean r_m of MACE and non-MACE clones



Discussion

a) *MACE resistance*

MACE aphids showed broadly similar r_m values equivalent to the non-MACE esterase-R_3 clones. All MACE aphids therefore appeared to increase in numbers at the same rate as non-MACE aphids carrying extreme esterase resistance (which are common in UK glasshouses).

b) *Esterase resistance*

For non-MACE clones, esterase-R_3's appeared to show the slowest rates of reproduction in agreement with previous findings (Foster *et al.*, 2000). However, for MACE clones, this reduced reproductive success was not restricted to aphids producing the extreme (R_3) amounts of esterase as all MACE clones, R_1 and R_3 alike, reproduced at similar rates. This suggests that reproduction in *M. persicae* is not directly affected by carboxylesterase overproduction but some other associated trait (also see discussion in section 1.3). Possibly, MACE has a secondary deleterious affect on reproduction. However, there appears to be no simple explanation of how this could come about. Finally, it would be worth establishing whether the three MACE esterase-R_1 clones used in this study are revertants (ie. carrying some unexpressed esterase genes and are therefore genotypically R_3's). We intend to do this assay when radioisotopes become available.

c) *Kdr resistance*

This mechanism did not appear to be associated with reproductive success in agreement with previous findings for non-MACE clones (Foster *et al.*, 2000).

Our findings indicate that glasshouse populations of *M. persicae* with MACE resistance will probably reproduce at similar rates to non-MACE clones carrying extreme (R_3) esterase resistance. However, MACE aphids may be reproductively out-competed by non-MACE clones expressing lower levels of esterase.

1.2 FEEDING POSITION

Objective

To determine the feeding positions of MACE and non-MACE clones of *M. persicae* on peppers, violas and lettuce.

Materials and methods

UK strains of MACE (red clone 2051A) and non-MACE (green clone 2169G) aphids, both carrying esterase-based and knockdown resistance, were released onto different host plants in the laboratory to determine their preferred feeding positions. The experimental aphids were reared on pepper plants (cv Mazurka) and clonal integrity was checked by biochemical assays (see part 1.1). The following host plants were used:

Peppers (cv Mazurka, 14 leaves with growing shoots).
Violas (cv Wiittrockiana, 8 leaf rossettes with flowers and growing shoots).
Lettuce (cv Barney, 5 leaves).

For each plant species, there were 15 plants and each plant represented a replicate. The plants were placed in perspex cages (45 x 45 x 105 cms.) in a controlled environment room at 21 °C ± 2°C and 16L:8D. Five apterous red MACE adult aphids and five apterous green non-MACE adult aphids were placed in an ependorf tube and released at the base of each plant stem. Seven days after release, the total numbers of aphids (adults and young) of each clone were counted separately on the cotyledons, on each leaf position, in the growing shoots (violas and peppers) and in the flowers (violas). The different clones were identified by colour.

Four parameters were analysed using ANOVA. The differences between treatments were compared using Least Significant Difference (LSD);

1. Mean position on the plant (leaf number).

2. Variance (a measure of the spread over the plant). Data were log transformed for normalisation.

3. Numbers of aphids in the growing shoots (peppers) or flowers (violas). Data were square root transformed for normalisation.

4. Total numbers of aphids. Data were square root transformed for normalisation.

Results

The results for all the plant species tested are summarised in Table 2 and illustrated for peppers and violas in Figures 2 and 3.

Table 2. The relative distribution of red MACE *M. persicae* and green non-MACE *M. persicae* on pepper, viola and lettuce. Showing transformed data per replicate.

Host plant	Analysis	Red MACE aphid	Green non-MACE aphid	p, d.f.
Pepper	Mean leaf position	4.28	2.74	p<0.05, 28 d.f.
	Log Variance position	1.90	1.36	NS
	√ Numbers in shoots	2.41	0.13	p<0.01, 28 d.f.
	√ Total nos. of aphids	5.59	5.52	NS
Viola	Mean leaf position	5.35	4.23	NS
	Log Variance position	2.04	1.95	NS
	√ Numbers in flowers	2.28	1.24	p<0.01, 28 d.f.
	√ Total nos. of aphids	3.96	3.04	p<0.05, 28 d.f.
Lettuce	Mean leaf position	2.42	2.52	NS
	Log Variance position	-0.01	0.32	NS
	√ Total nos. of aphids	2.22	2.35	NS

Figure 2. The relative distribution of MACE (red) and non-MACE (green) aphids of *M. persicae* on peppers, leaf 1 being the oldest leaf at the bottom of the plant and leaf 14 at the top.

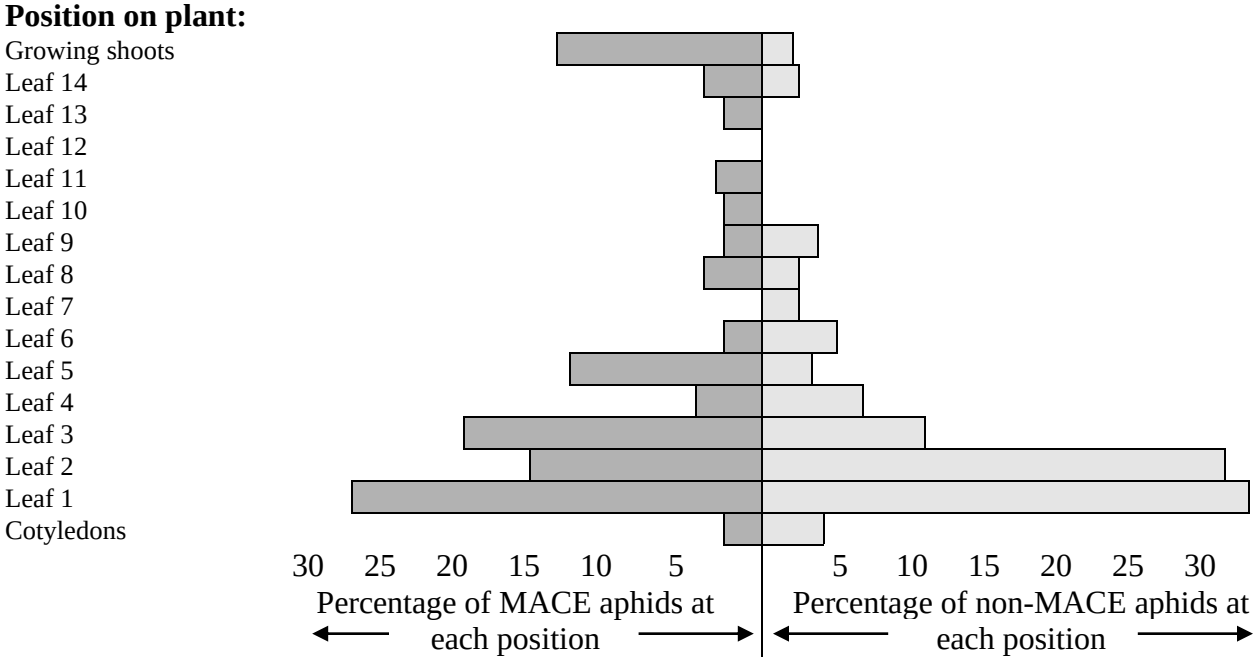
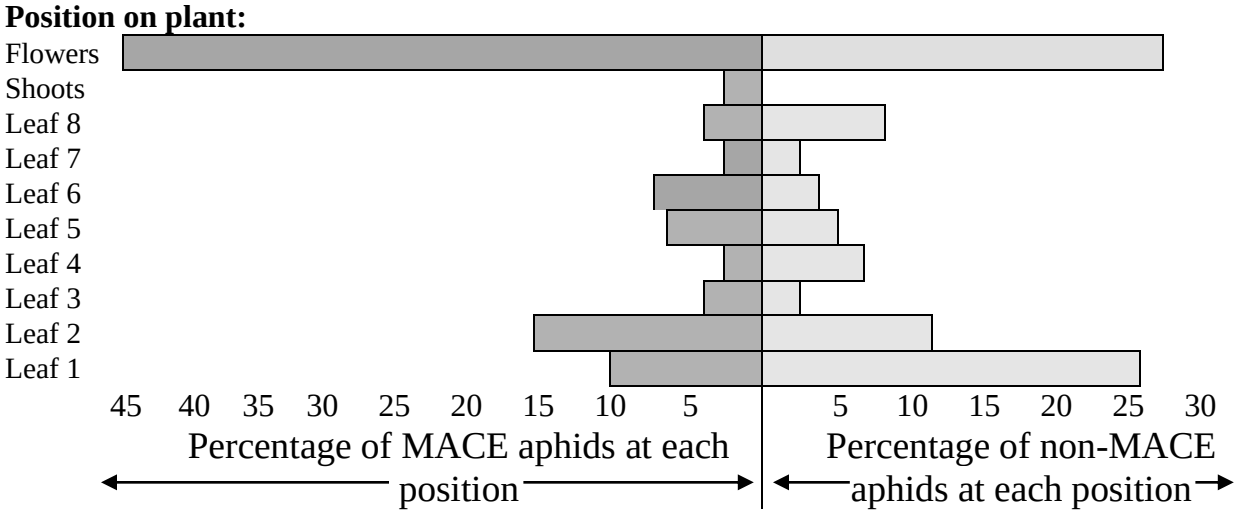


Figure 3. The relative distribution of MACE (red) and non-MACE (green) aphids of *M. persicae* on violas, leaf 1 being at the bottom of the plant and leaf 8 at the top.



Discussion

On peppers, *M. persicae* with the MACE resistance mechanism were higher up the plants and more frequent in the growing shoots than their non-MACE counterparts. These differences in feeding position confirmed previous observations in commercial crops. Also, MACE aphids were more frequent in the shoots and flowers of violas than non-MACE aphids. The reasons for these differences are unknown. It is possible that MACE aphids require better food quality than their non-MACE counterparts or that MACE aphids are able to occupy more favourable niches because they are more mobile (Sampson, personal observation).

The feeding position of aphids may be relevant to control for different reasons. MACE aphids that are feeding on the growing shoots may cause more plant damage than non-MACE aphids that are feeding on older leaves and this will be investigated in the second year of the project. It was speculated that MACE aphids could be less vulnerable to parasitoid attack than non-MACE aphids because greater numbers are protected inside the growing shoots. This was investigated in part 2.2.

On young lettuce plants there was no significant difference in the distribution of the two clones of *M. persicae*. Further work is required to determine whether there is a difference in feeding position on mature lettuce plants, which have a heart.

1.3 MEASUREMENT OF RESPONSE TO ALARM PHEROMONE

Objective

To assess possible associations of aphid alarm response with MACE-, kdr- and esterase-based insecticide resistance.

Materials and methods

A wide range of MACE and non-MACE clones (Table 3, overleaf) expressing either S, R_1, R_2 or R_3 esterase levels were assessed for their response to synthetic alarm pheromone, (*E*)-β-farnesene, in laboratory-based bioassays. Experimental aphids were taken initially from lines maintained at 21°C under an 18 h day/ 6 h night photoperiod as virginoparous, predominantly apterous colonies. Clonal integrity was checked regularly by biochemical assays (for esterase and MACE resistance; Devonshire *et al*., 1986; Moores *et al.*, 1994), aphid diagnostic-dose bioassays (for kdr phenotype: Field *et al.*, 1997) and DNA assays (for *kdr* genotype: Foster *et al.*, 1999).

Aphid response was assessed for each clone at 21°C in nine separate experiments. For each clone tested, six (generation 0) adult apterae were obtained from laboratory stocks and set up in a small leaf box overnight whereupon they were removed leaving a developmentally-synchronised cohort of (generation 1) offspring. These were left to develop into adults when they were transferred, using a fine paint brush, to 2 cm diameter Chinese cabbage leaf discs (three adult apterae per disc) held on 1% agar inside plastic tubs. Each replicate batch of three apterae (up to five replicates per clone per experiment) were then left to reproduce overnight whereupon they were removed leaving first instar nymphs. Replicates (of these generation 2 progeny) were then assayed in a randomised order by applying a 1 µl (0.1mg ml^{-1} in hexane) droplet of (*E*)-β-farnesene to the central part of each leaf disc with a fine-needle syringe. Nymph response was observed for 2 minutes (a time period established in early experiments in this project). Nymphs that unplugged their stylets and walked away were scored as responding. Each cohort of nymphs were tested once and then discarded. Between 300 and 850 nymphs were assessed for each clone.

Statistical analysis

Generalized linear models were fitted to the proportions of aphids responding to alarm pheromone using probit transformation. The analysis was adjusted for any experimental and replicate effects. Associations of aphid response with MACE, kdr and esterase resistance (measured by mean E4/FE4 carboxylesterase activity of each clone) were assessed.

Table 3. *Myzus persicae* clones assessed in alarm pheromone study

Clone	Resistance mechanism				Date of collection
	esterase[1]	*MACE*[2]	*kdr*[3]	Origin	
US1L	S	no	SS	UK	1974
525A	S	no	SS	UK	1997
542A	S	no	SS	UK	1997
554A	S	no	SS	UK	1997
405D	R_1	no	SS	UK	1977
2141A	R_2	no	SS	UK	1997
2160D	R_1	no	SR	UK	1997
2167J	R_1	no	SR	UK	1997
2042E	R_1	no	SR	UK	1996
2043M	R_1	no	SR	UK	1996
3172A	R_1	no	SR	UK	1998
2165C	R_2	no	SR	UK	1997
2161C	R_3	no	SR	UK	1997
2163E	R_3	no	SR	UK	1997
2169G	R_3	no	SR	UK	1997
794J	R_3	no	RR	UK	1982
2043B	R_3	no	RR	UK	1996
2042H	R_1	yes	SR	UK	1996
2034A	R_1	yes	SR	UK	1996
2042P	R_1	yes	SR	UK	1996
2044A	R_1	yes	SR	UK	1996
2050A	R_1	yes	SR	UK	1996
3104B	R_1	yes	RR	UK	1998
1200Q	R_2	yes	SR	Argentina	1993
2050B	R_3	yes	SR	UK	1996
2051A	R_3	yes	SR	UK	1996
2347A	R_3	yes	SR	Greece	1997
3495B	R_3	yes	SR	UK	1999
2012A	R_3	yes	RR	UK	1996
2144F	R_3	yes	RR	Greece	1997

[1] Determined by immunoassay: S (non-MACE), R_1 (moderate resistance), R_2 (high resistance), R_3 (extreme resistance).

[2] Determined by kinetic assay.

[3] Determined by DNA assay using SSCP technique: SS (kdr-SS genotype), SR (kdr-SR genotype) and RR (kdr-RR genotype).

Results

Control treatments with 1 µl droplets of hexane alone did not elicit aphid response. Figures 4 and 5 summarise the mean alarm response of all experimental clones and kdr-SR clones alone respectively.

Kdr resistance

Adjusting for esterase and MACE effects, kdr had a strong association with aphid response ($F_{2,998}$ (degrees of freedom) = 108.9, P << 0.001). Adjusted mean responses were:

kdr-SS: 0.83 (SE. 0.014)
kdr-SR: 0.44 (SE. 0.012)
kdr-RR: 0.36 (SE. 0.022)

These data support previous response patterns gained in a study using non-MACE adult aphids (Foster *et al.*, 1999), ie. kdr-SS clones show consistently greater responses than either the kdr-SR or –RR clones with the former showing a slightly greater, but significant, response than the latter (Figure 6.). The greater overall responses in the previous study probably relates to the assessment of adults as opposed to nymphs.

MACE resistance

Adjusting for kdr and esterase effects, MACE resistance was significantly associated with greater aphid response ($F_{1,998}$ = 96.7, P << 0.0001).

Esterase resistance

Nymphal response for the kdr-SR clones showed a significant inverse association with esterase resistance in the non-MACE clones (slope –0.58, SE. 0.15, T_{999} = 3.77, P < 0.001) and a significant positive association in the MACE clones (slope 0.24, SE. 0.11, T_{999} = 2.26, P = 0.025) (Figure 5.). In a homogeneous kdr-SR background, *M. persicae* with both MACE- and esterase-R_3, therefore, appeared to be far more responsive than the equivalent esterase-R_3 non-MACE clones.

Figure 4. Mean response of *M. persicae* clones to alarm pheromone

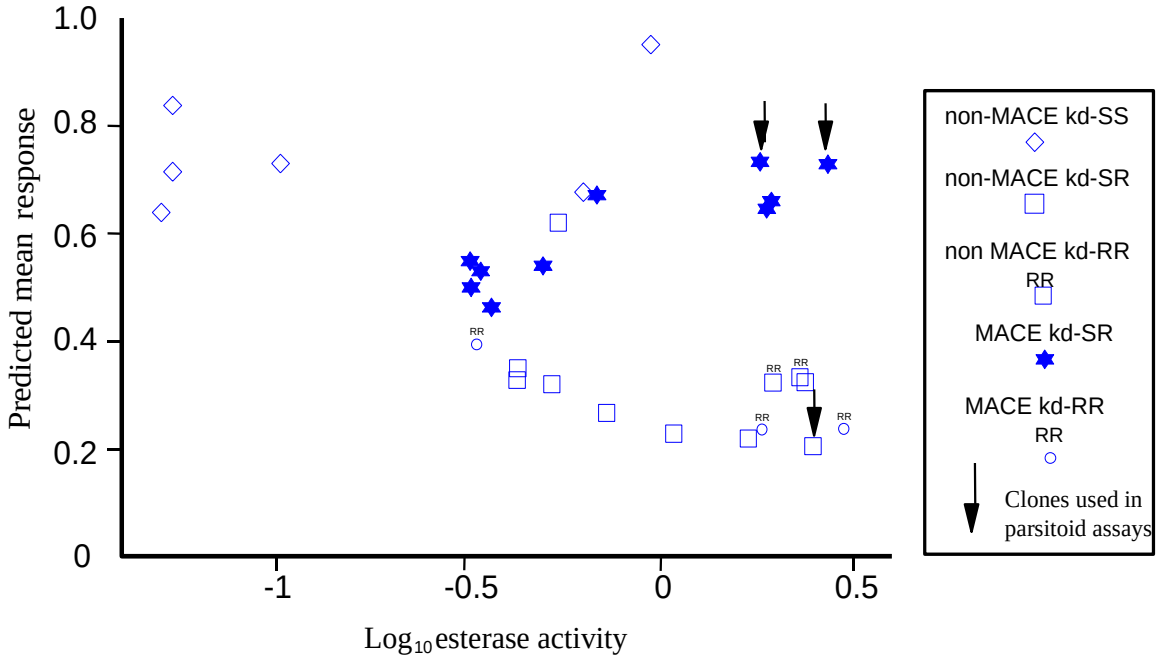


Figure 5. Mean response of *M. persicae* clones (kdr-SR only) to alarm pheromones

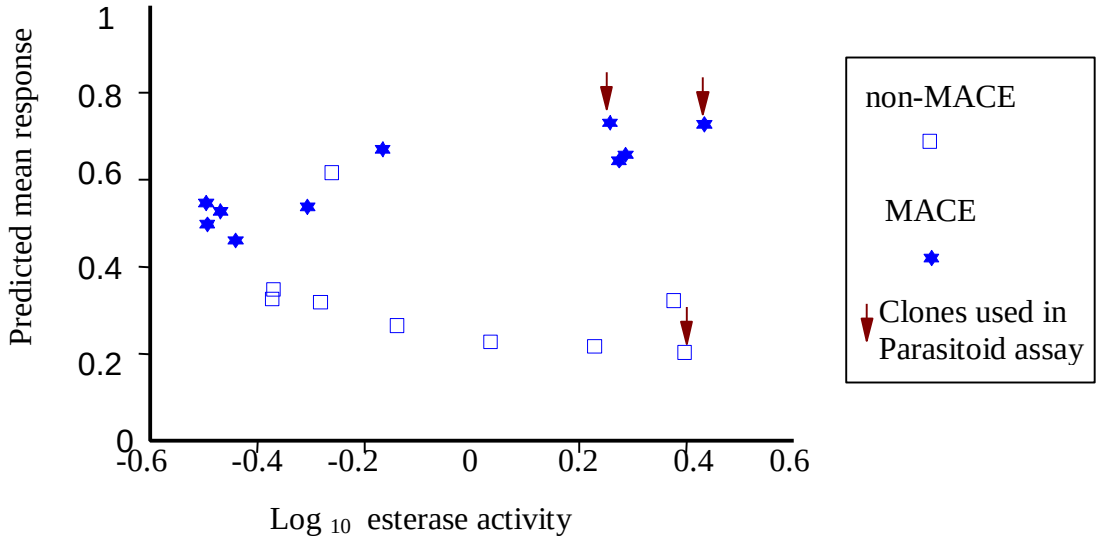
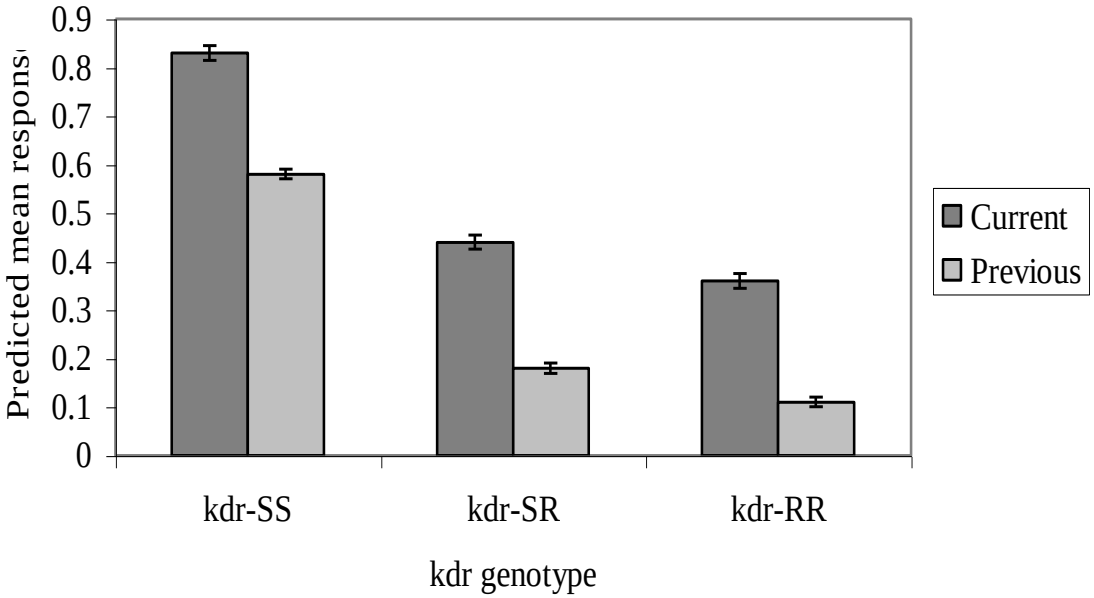


Figure 6. Response of kdr forms to alarm pheromone in current and previous studies



Discussion

Although there was no statistical evidence that the few *M. persicae* clones originating from abroad behaved differently to the UK clones, we suggest that our findings should currently only be related to aphids in this country. With this in mind, it would appear that MACE-, kdr- and esterase-based insecticide resistance show various statistical associations with the tendency to respond to synthetic alarm pheromone. Specifically:

a) **Kdr resistance** is associated with a reduced level of response. This supports previous findings reported by Foster *et al.*, 1999.

b) **MACE resistance** is associated with a greater response when it is in combination with the commonest (kdr-SR) clone of kdr in *M. persicae* collected from UK crops over the past several years (ie., the majority of UK MACE and non-MACE aphids diagnosed to date have been kdr-SR).

c) Level of **esterase resistance** was either positively (/) or inversely (\) associated with alarm response in MACE and non-MACE clones respectively. Therefore, the pattern for non-MACE clones, which currently prevail in the UK, is similar to that reported previously by Foster *et al.*, 1999. Consistent low responses were shown by the few kdr-RR MACE and kdr-RR non-MACE clones tested.

There is growing evidence that the kdr mechanism has a direct deleterious effect on aphid behaviour through impaired nerve function. This could lead to kdr-resistant clones being selected against in the

absence of insecticides because they are less able to respond to important cues used by kdr-non-MACE aphids for survival. However, kdr does not appear to be the only factor, as differential responses associated with carboxylesterase level were seen amongst the kdr-SR clones. Furthermore, the apparent reciprocal pattern of behavioural trends involving MACE and non-MACE clones strongly suggests that the varied response is not a direct consequence of esterase resistance *per se* but a result of indirect negative effects on behaviour by associated genetic factors. One might otherwise expect similar trends for MACE and non-MACE clones.

It seems unlikely that MACE directly affects aphid behaviour and if it does, it is not clear why the MACE mechanism should appear to elicit a greater response in esterase-R_3 compared to $-R_1$ aphids.

Whatever the mechanics behind our findings, it would appear that the tendency for *M. persicae* to respond to alarm pheromone is primarily associated with their kdr genotype. Having said this, the commonest MACE clones tend to be more responsive as their level of esterase-based resistance increases and vice versa in non-MACE clones. This finding raises the question of whether MACE aphids enjoy greater survival in glasshouses that use biological control strategies as these clones may be better at avoiding predators and parasitoids. The behavioural studies described in other sections of this report aim to answer this.

Finally, our data highlight the potential implications of aphid parthenogenesis on the selection of different resistance mechanisms. This form of reproduction is prevalent in the UK and ensures that resistance mechanisms (or other genes affecting fitness), once they have come together in an aphid, either by mutation or a period of sexual reproduction, remain combined for many subsequent generations. In effect, they are perpetuated in a clonal line. Hence, both the advantages and drawbacks conferred by one genetic factor, whether it is a resistance mechanism itself or an associated gene or gene complex, will inevitably be enjoyed or suffered by any other mechanisms carried by that aphid clone. The stable close relationships built by parthenogenesis will therefore create non-independent fluctuations in the frequencies of the different resistance mechanisms in the UK. As a result, selection favouring one mechanism, for example a pyrethroid spray selecting kdr, will also benefit any genetically-linked mechanisms such as MACE, even if they do not confer resistance to that particular product. Of course the reciprocal situation can also take place through adverse selection.

1.4 DEFENSIVE BEHAVIOUR

Objective

To determine whether MACE and non-MACE clones of *M. persicae* respond differently to parasitoid attack.

Introduction

MACE clones showed a greater response to alarm pheromones than non-MACE clones (part 1.3) and the purpose of this experiment was to determine whether this translated into increased response to parasitoid attack, as this may affect the success of biological control programmes in glasshouses.

Materials and methods

Three *M. persicae* clones (MACE red-clone 2051A , MACE green-clone 2347A, and non-MACE green-clone 2169G, all carrying R_3/kdr), were assessed for their response to an approach by the parasitoid *Aphidius colemani*. Experimental aphids were reared on pepper plants (cv Mazurka) and their clonal integrity was checked by biochemical assays (see part 1.1).

The bioassay used round Petri-dishes (diameter 50mm; height 14mm) placed under a binocular microscope which was connected to a video recorder. A 5mm layer of water was poured into each dish and a freshly cut pepper leaf disc (8mm diameter) infested with approximately 15 *M. persicae* (mixed ages) was placed on the water in the centre of the dish and held in place by a small piece of blue-tac. The aphids were allowed to settle for approximately two hours before parasitoid release. One mated, inexperienced female parasitoid that was up to 24 hours old was released onto each leaf disc at the start of each monitoring period. The different clones were monitored in turn for 30-minute periods until there was sufficient replication. Each individual aphid that was approached by a parasitoid was a replicate and there were 25 replicates.

Every time the wasp approached a different aphid the response of that aphid was recorded in terms of:

a) No response
b) Kicking or flicking (the aphids lifted their bodies, often kicking at the same time)
c) Walking off (towards or away from the wasp)

The success of each oviposition attempt by a wasp was also recorded.

The data were analysed on contingency tables using regression analysis.

Results

The responses of the different aphid clones tested to an approach by a parasitoid wasp are summarised in Table 4. More red MACE aphids responded to an approach by *A. colemani*, by kicking, flicking their bodies or walking off, than did green MACE or green non-MACE aphids ($p < 0.05$, 4 d.f.). There were no significant differences in the types of response shown by responding aphids of the different clones.

Table 4. The proportions of different *M. persicae* clones that responded in different ways to an approach by a female *A. colemani* wasp (n=25).

Clone	Proportion of aphids ± s.e. responding		
	No response	Kicking or flicking	Walking off
Red MACE	0.04 ± 0.04	0.44 ± 0.13	0.56 ± 0.15
Green MACE	0.24 ± 0.10	0.44 ± 0.13	0.48 ± 0.14
Green non-MACE	0.32 ± 0.11	0.20 ± 0.09	0.64 ± 0.15

There was no significant difference in parasitism of the different clones tested. The following proportions of each clone were parasitised; 0.04 ± 0.04 (red MACE), 0.12 ± 0.07 (green MACE) and 0.08 ± 0.06 (green non-MACE).

Discussion

Red MACE aphids responded more frequently to parasitoids by walking off, kicking or flicking their bodies. This defensive behaviour may be associated with increased response to alarm pheromones (see part 1.4). Against expectations the green MACE clone tested did not respond in the same way. These results suggest that any differences in behaviour do not result from the MACE resistance mechanism but from the genetic origin of the clone. Most MACE aphids collected recently in the UK are similar to the red clone tested (Foster, pers. comm.). The implications of these results for UK growers may be that the MACE aphids prevalent in the UK may enjoy greater survival than non-MACE aphids in glasshouses using biological control because they are more likely to escape parasitism. No differences in parasitism between the clones were observed in these experiments but it was not a challenging test because the aphids were confined on a small leaf disc and did not have the opportunity to escape parasitism. Further experiments will be done in the second year of the project to determine whether parasitism rates vary between the different clones on a crop scale.

PART 2. NATURAL ENEMY PERFORMANCE

2.1 PERFORMANCE OF PARASITOIDS

Objective

To determine whether selected parasitoid species perform differently against MACE and non-MACE *Myzus persicae*.

Introduction

The intention was to compare the relative efficacies of three parasitoid species, reared under the same conditions, simultaneously. However, there were insufficient *P. myziphagum* in the rearing facilities to complete the experiment due to a contamination with hyperparasitoids. It was therefore agreed to use *P. myziphagum* that had been reared on *Myzus nicotianae* as supplied by the biological control producer. Both *Aphidius* species were reared under the same conditions on the non-MACE green clone of *M. persicae* and the replicates of all three species were done in parallel.

Materials and methods

Three parasitoid species (*Aphidius colemani, A. matricariae* and *Praon myziphagum*) were tested against a single density of three different *M. persicae* MACE red-clone 2051A , MACE green-clone 2347A, and non-MACE green-clone 2169G, all carrying R_3/kdr). Experimental aphids were reared on pepper plants (cv Mazurka) and their clonal integrity was checked by biochemical assays (see part 1.1).

The bioassay used round plastic Petri-dishes (diameter 90mm; height 14mm) with gauze incorporated into the lids to allow air exchange. A 50mm layer of tap water agar (1%) was poured into each dish and allowed to cool. Just before it solidified, a freshly cut pepper leaf (cv Mazurka) was placed upside down on the agar, this maintained the pepper leaf fresh for the required 12 days. Fifty aphids (3^{rd} – 4^{th} instar) were carefully placed on to each leaf using a fine brush. The Petri-dishes were sealed with parafilm and placed upside down to simulate a natural feeding position for the aphids and to prevent the leaf becoming fouled with honeydew.

One mated, inexperienced female parasitoid that was up to 24 hours old was exposed to the 50 aphids for 30 minutes, starting within two hours of midday. During this time the Petri-dishes were placed in an incubator at 21°C ± 1°C with a direct light source. The wasps were then removed and the Petri-dishes placed in a controlled environment room with a non-direct light source at 21 °C ± 2°C and 16L:8D. After 12 days (*Aphidius* spp.) and 19 days (*Praon*) the numbers of parasitoid mummies per dish were counted. For the *Aphidius* species, each test was replicated 33 times. For *Praon*, each test was replicated 13 times. The data were square root transformed and analysed using ANOVA. The differences between treatments were compared using LSD.

Results

Figures 7 and 8 summarise the performance of the two *Aphidius* species and *P. myziphagum* against the different clones of *M. persicae* respectively.

Figure 7. The mean numbers (square root transformed data) of *M. persicae* parasitised (with LSDs) by *A. colemani* and *A. matricariae* in 30 minutes

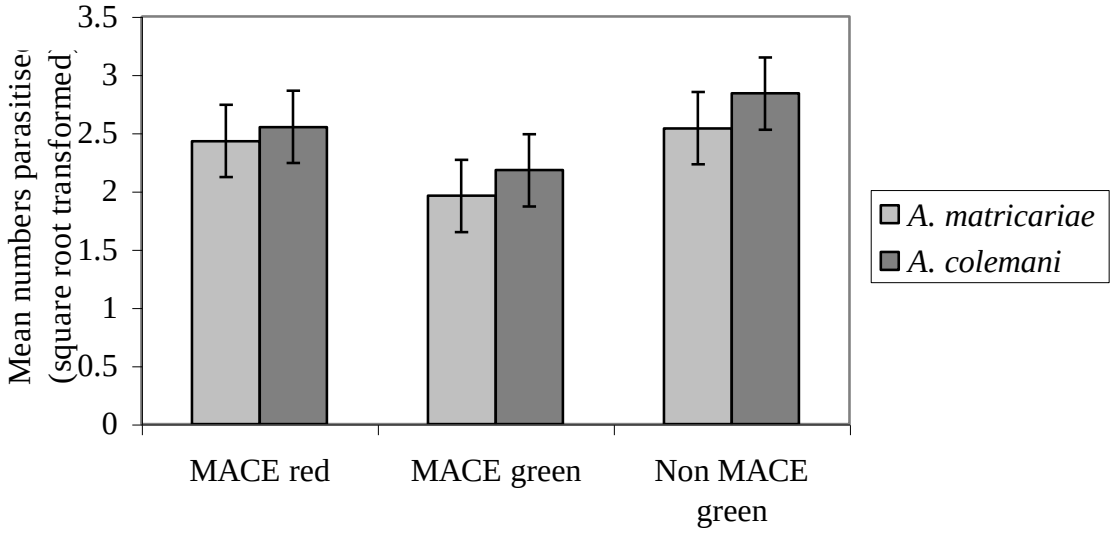
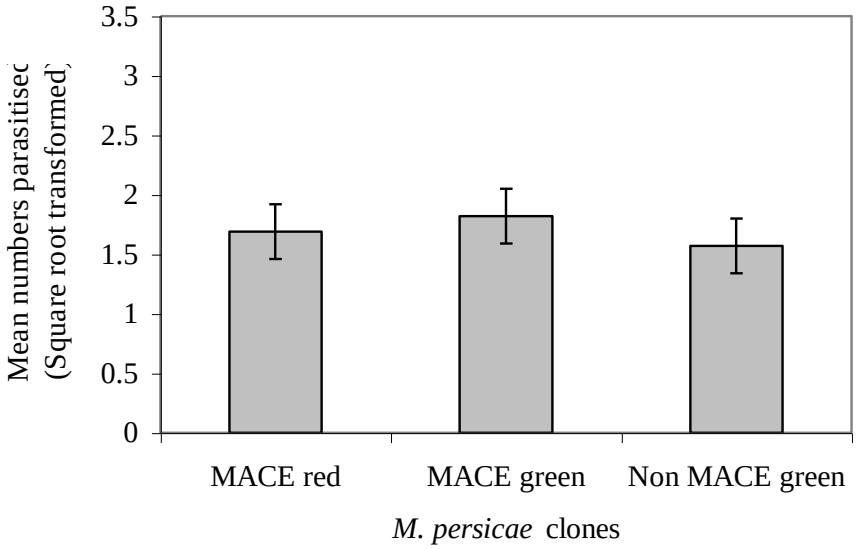


Figure 8. The mean numbers (square root transformed data) of *M. persicae* parasitised (with LSDs) by *P. myziphagum* in 30 minutes



Discussion

Both species of *Aphidius* were effective against all clones of *M. persicae,* parasitising an average of eight (*A. matricariae*) or nine (*A. colemani*) aphids when exposed to 50 aphids for half an hour. The performance of the parasitiods were comparable to published data (Steenis, 1993) where *A. matricariae* produced 13 mummies and *A. colemani* produced 14 to 17 mummies when exposed to 30 *M. persicae* for two hours. There was no significant difference in the performance of the two species of *Aphidius* against *M. persicae* or the performance of either species against the different *M. persicae* clones tested. However, there was a suggestion of a significant difference between treatments ($p=0.059$; 192 d.f.) with less parasitism of the green MACE clone when compared to the susceptible green clone. As this could not be attributed to the resistance mechanism or aphid colour, the differences may be due to the different genetic background of the clones. A difference in response may have been expected because the MACE resistant clones had a greater response to alarm pheromone (part 1.3), however there was limited opportunity to exploit this behaviour in the confined environment of a Petri-dish and for such a short period of time. Further experiments were done to determine parasitism on a plant scale (see part 2.2).

Praon myziphagum produced three to four mummies when exposed to 50 aphids for half an hour. The productivity of *P. myziphagum* was further reduced compared to *Aphidius* because of its' longer life cycle (≃ 18 days at 21°C) compared to *Aphidius* (≃ 14 days at 21°C). The company that supplies *P. myziphagum* (Biowise) found that it out-competed *A. matricariae* in their production unit. This may require further investigation but is beyond the scope of this project.

Aphidius colemani is the preferred choice of parasitoid for biological suppliers and growers because it parasitises both *M. persicae* and *A. gossypii*. In contrast, *A. matricariae* is specific to *M. persicae* and is no longer produced by the major biological control suppliers. These experiments indicate that *A. colemani* remains the best choice of parasitoid for control of MACE and non-MACE clones of *M. persicae*, but further experiments are required to determine whether there is a difference in the performance of *A. colemani* against the different clones on a crop scale.

2.2 HOST PREFERENCE

Objective

To determine whether *Aphidius colemani* parasitised a greater proportion of MACE than non-MACE *M. persicae*, when given a choice of the two clones on growing plants.

Introduction

In Petri-dish experiments (part 2.1), *A. colemani* was equally effective against MACE and non-MACE clones of *M. persicae*. However, experiments on the biology of *M. persicae* showed that MACE aphids were more frequent in the shoots of pepper plants (part 1.2) and responded more to alarm pheromone than non-MACE aphids (part 1.3). Both of these factors may allow MACE aphids to escape parasitism on whole plants. The purpose of this experiment was to determine whether *A. colemani* was more successful against non-MACE than MACE aphids when given a choice of aphids on whole plants. As the colour of aphids can influence the host choice of parasitoids (Losey *et al*, 1997; Powell *et al*, 1998), the experiment was repeated with red and green clones with MACE resistance. Experimental aphids were reared on pepper plants (cv Mazurka) and their clonal integrity was checked by biochemical assays (see part 1.1).

Materials and Methods

a) ***Red MACE (2051A) vs green non-MACE (2169G)*** **M. persicae*****, both carrying R_3/kdr***
The bioassay was done on growing pepper plants (cv Mazurka, approx 30 cm high), placed individually in cylindrical cages (39 cm height x 22 cm diameter). Each plant had five leaves and one growing shoot. The plants were infested evenly (5 of each aphid clone on each leaf) with 25 red MACE and 25 green non-MACE *M. persicae* (3^{rd} or 4^{th} instar) and the aphids were allowed to settle for 24 hours before releasing the parasitoids. Individual female *A. colemani*, up to 24 hours old, were released into each cage for 24 hours. During this time the plants were placed in a CT room at a constant 21°C ± 2°C with an indirect light source. After three to five days, the numbers of each clone were counted separately on shoots and leaves. The aphids were then dissected under a binocular microscope to determine whether they had been parasitised. Each plant was a replicate and there were 20 replicates, with five replicates done at a time.

b) ***MACE green (2347A) vs non-MACE green (2169G)*** **M. persicae*****, both carrying R_3/kdr***
The experiment was repeated with green MACE and green non-MACE clones. As the clones could not be identified by colour, the resistance mechanism was determined by biochemical assays (Moores *et al.*, 1994) after the aphids had been dissected.

The data were analysed using regression analysis.

Results

a) *Red MACE vs green non-MACE* M. persicae

The mean numbers of MACE and non-MACE aphids per plant and percentage parasitism are shown in table 5. More green non-MACE aphids were recovered than red MACE aphids ($p < 0.05$, 31 d.f.) and a greater proportion of green non-MACE aphids were parasitised than red MACE aphids ($p<0.05$, 31 d.f.).

Table 5. The mean numbers of parasitised and unparasitised red MACE and green non-MACE aphids recovered per plant.

	Mean numbers of aphids	Mean numbers parasitised	Percentage parasitised	Proportion parasitised ± s.e.
Red MACE	22.2	2.6	11.7 %	0.12 ± 0.02
Green non-MACE	25.8	4.2	16.3 %	0.17 ± 0.02

When the numbers of parasitised red MACE and green non-MACE aphids on the leaves (excluding the leaf tips) were analysed separately, there was no significant difference in the proportion parasitised (Table 6).

Table 6. The mean numbers of parasitised and unparasitised red MACE and green non-MACE aphids recovered per plant on the leaves (excluding the leaf tips).

	Mean numbers of aphids	Mean numbers parasitised	Percentage parasitised	Proportion parasitised ± s.e.
Red MACE	16.6	2.4	14.5 %	0.16 ± 0.02
Green non-MACE	21.5	3.7	17.2 %	0.20 ± 0.02

There were significantly more red MACE *M. persicae* in the leaf tips than non-MACE green *M. persicae* ($p < 0.05$, 31d.f.) as shown in Table 7.

Table 7. The mean numbers of red MACE and green non-MACE aphids in the leaf tips.

	Mean numbers of aphids	Mean numbers in the shoot	Percentage nos. in the shoot	Proportion parasitised ± s.e.
Red MACE	22.2	5.6	25.2 %	0.24 ± 0.03
Green non-MACE	25.8	4.3	16.6 %	0.14 ± 0.03

b) *Green MACE vs green non-MACE* M. persicae

The experiment was repeated using green MACE and green non-MACE clones. The aphids have been dissected for parasitism and are now frozen until they can be identified by biochemical assays. This will be done in the coming months by IARC-Rothamsted and the results will be reported in next year's report.

Discussion

At the end of the experiment fewer red MACE *M. persicae* were recovered than green non-MACE aphids. One possible explanation for this is that the red MACE aphids moved off the plants more readily than green non-MACE aphids because they had a greater response to alarm pheromones (part 1.3). Red MACE aphids were observed to be more mobile than green non-MACE aphids.

On whole plants, a greater proportion of green non-MACE aphids were parasitised than red MACE aphids. A number of differences in the behaviour of MACE aphids have been identified that may allow them to escape parasitism on whole plants. A greater proportion of the red MACE aphids were feeding inside the growing shoots (see part 1.2), which made them less accessible to parasitoid attack. Also, the red MACE aphid showed a greater response to alarm pheromone (see part 1.2) and increased defensive behaviour (see part 1.4) than the green non-MACE aphid. There was no difference in parasitism of the two clones on 'exposed' leaves (excluding the leaf tips), which corresponded with the results from the Petri-dish experiments (part 2.1).

Although the differences in parasitism rates were significant, they were not great and crop scale experiments are required to determine whether different release strategies are required for *A. colemani* to maintain control of MACE resistant *M. persicae*. In addition, a control method may be required to complement *A. colemani* that will control aphids inside the leaf shoots.

3. GENERAL DISCUSSION AND CONCLUSIONS

M. persicae currently provides one of the clearest demonstrations of how genetic and ecological factors can interact to determine the dynamics of resistance and to influence success with resistance management.

The prevalence of parthenogenesis in UK populations of *M. persicae* reinforces any existing associations between resistance mechanisms. As a result, they can 'hitch-hike' together from generation to generation enjoying or suffering any fitness advantages or disadvantages that each may confer. With this in mind, nearly all UK MACE *M. persicae* have been found to also carry the kdr mechanism associated with potentially maladaptive behaviour. These clones are therefore likely to be handicapped in the absence of insecticides compared to *M. persicae* without kdr. MACE may well be suffering adverse selection in field and glasshouse populations through this association. Indeed, MACE has become rarer in field populations since it was first found in Lincolnshire in 1996 and most of the populations can be found in glasshouses.

Our studies of aphid reproductive success and response to alarm pheromone have uncovered some interesting trends involving the MACE, esterase and kdr insecticide resistance mechanisms. MACE clones apparently reproduce at slower rates compared to non-MACE clones expressing moderate (R_1) esterase resistance. However, they appear to be more responsive to alarm pheromone than their non-MACE counterparts, particularly at extreme levels of esterase-based resistance.

Of the three parasitoid species tested, *A. colemani* remains the best choice for the control of both MACE and non-MACE clones of *M. persicae*. However, differences in behaviour of the red MACE clone of *M. persicae* were identified, which influenced the levels of parasitism achieved by *A. colemani* in plant based bioassays. The red MACE aphids were able to escape parasitism by feeding in the growing shoots, where they were less accessible to the parasitoids. The red MACE aphids were also more responsive to parasitoid approach in terms of defensive behaviour (kicking and walking off). Although real differences in parasitism were observed, these differences were small and further experiments are required to determine whether they affect control of *M. persicae* on a crop scale.

In the second year of the project, the use of *A. colemani* against the UK MACE *M. persicae* will be examined further on a crop scale. A number of options will be investigated to complement *A. colemani* including predators and the use of Eradicoat to control aphids that have built up in the growing shoots of the plants.

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