Project title:	Protected crops: Design and evaluation of a robust biologically based strategy for the control of MACE resistant <i>Myzus persicae</i>		
Project number:	PC 165		
Report:	Year 2 annual report, May 2002		
Previous reports:	Year 1 annual report, December 1999		
Project leader:	Dr Mark Tatchell Horticulture Research International Wellesbourne Warwick CV35 9EF		
	Dr Steve Foster IACR Rothamsted Harpenden Herts AL5 2JQ		
Key workers	Neil Kift, Julie Jones, Sue Sime, Kelly Reynolds(HRI Wellesbourne) Steve Foster (IACR Rothamsted)		
Location:	HRI, Wellesbourne & IACR Rothamsted		
Project Co-ordinator	Mr. Derek Hargreaves, Horticultural Consultant, Beverley, East Yorkshire		
Date project commenced:	1 May 1999		
Date completion due:	31 December 2002		
Key words:	Pepper, aphids, insecticide resistance, biological control, MACE, <i>Myzus persicae</i> , <i>Aphidius colemani</i> , pymetrozine, pirimicarb, Eradicoat, <i>Aphidoletes</i> <i>aphidimyza</i>		

Whilst reports issued under the auspices of the HDC are prepared from the best available information, neither the authors or the HDC can accept any responsibility for inaccuracy or liability for loss, damage or injury from the application of any concept or procedure discussed.

The contents of this publication are strictly private to HDC members. No part of this publication may be copied or reproduced in any form or by any means without prior written permission of the Horticultural Development Council.

The results and conclusions in this report are based on an investigation conducted over a one year period. The conditions under which the experiments were carried out and the results obtained 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 interpretation of the results especially if they are used as the basis for commercial product recommendations.

CONTENTS

PRACTICAL SECTION FOR GROWERS

Background and objectives Summary of results Action points for growers Practical and financial benefits	1 1 3 4
SCIENCE SECTION	
Introduction	5
 Effectiveness of three predator species against MACE and non-MACE <i>M. persicae.</i> Determine optimum release strategies for <i>Aphidius colemani</i> Identify an IPM Compatible remedial treatment through contact with commercial companies 	6 10 16
ACKNOWLEDGEMENTS	16
REFERENCES	16

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 forms in the UK, which produce high levels of esterase, which are resistant to pyrethroid, organophosphate and carbamate, but susceptible to pirimicarb insecticides. This is known as esterase resistance. To manage this resistance, growers of protected crops have been encouraged to base their aphid control strategies on biological control using mainly *Aphidius colemani* and *Aphidoletes aphidimyza*, supplemented by 'open' rearing units. Biological control strategies have worked well in protected crops for most of the season, but some growers have to resort to occasional treatments of pirimicarb to bring any imbalance back under control or to control sudden aphid invasions.

In recent years, a strain of *Myzus persicae* with a new form of resistance, having a modified acetylcholinesterase ('MACE') that confers complete resistance to dimethyl carbamates such as pirimicarb, has been found. This strain is typically, though not always, red. The occurrence of MACE aphids in UK protected crops has resulted in crop losses. Pirimicarb is completely ineffective and where *Myzus persicae* also have high levels of esterase resistance, there were no effective approved chemicals to bring the aphid populations back into balance with the natural enemies. In mainland Europe MACE aphids are now well established, but growers control them with chemicals that are not currently approved for use on salad crops in the UK. In addition, this chemical-intensive strategy will not provide sustainable control as resistance has already developed to some of the newer chemicals. UK growers therefore seek a biologically-based control strategy that is as effective against MACE as non-MACE forms of *Myzus persicae*.

The commercial objective of this project is to achieve sustainable control of both MACE and non-MACE *Myzus persicae* in protected crops, based on a robust biological control programme supported by compatible remedial treatments.

Summary of results

Effectiveness of *Aphidius colemani* against MACE and non-MACE *Myzus persicae* in a crop scale glasshouse experiment with peppers

Six week old pepper plants (cv. Mazurka) grown in rockwool cubes were planted onto rockwool slabs at two plants per slab and grown in four experimental plots in two glasshouse compartments. The temperature within the compartments ranged from 16 to 26°C during the course of the experiment. Experimental plots of 24 slabs (48 plants) were arranged in a double row (20cm apart) of 2 X 6 slabs, with a guard row on either side. The plants were grown as a commercial pepper crop, with two flowering stems, and with side shoots removed, for seven weeks before aphids were introduced. Each plant was inoculated with ten 3rd or 4th instar aphids on 6 June 2001 for experimental run one, and 15 August 2001 for experimental run two. Thirty

female A. colemani ($2/m^2/week$) were released into each MACE and non-MACE *Myzus persicae* infested plot 7, 14 and 21 days after aphid inoculation on both experimental runs.

- The introduction of *Aphidius colemani* at a rate of 2 per m² per week significantly reduced the numbers of MACE and non-MACE *Myzus persicae* on the pepper plants.
- Aphidius colemani was more effective in controlling non-MACE Myzus persicae than it was in controlling MACE Myzus persicae.
- MACE *Myzus persicae* prefer to colonise the growing points of pepper plants. This does not appear to be affected by the presence of *Aphidius colemani*.

Effectiveness of predator species against MACE and non-MACE *Myzus persicae* on peppers.

The effectiveness of the predators *Adalia bipunctata* (ladybird) and *Chrysoperla carnea* (lacewing) was assessed in petri-dish experiments against a single density (50) of MACE and non-MACE *Myzus persicae*. A single 1st instar larva of *Adalia bipunctata* or a 2nd instar larva of *Chrysoperla carnea* were placed in each Petri dish for 24 hours and placed in a controlled-environment room ($21 \pm 2^{\circ}C$; 16h light : 8h dark) for 24 hours. The number of MACE and non-MACE *Myzus persicae*, both alive and dead, were counted after 24 hours in each dish.

In addition, pepper plants (cv. Mazurka) at the 4 true leaf stage, with one growing shoot, were infested with 25 3^{rd} instar red MACE *Myzus persicae* and 25 3^{rd} instar green non-MACE *M. periscae*. Plants were individually isolated for separate experiments with *Chrysoperla carnea*, *Adalia bipunctata* and *Aphidoletes aphidimyza*. Individual 1^{st} instar larvae of *Adalia bipunctata* and *Aphidoletes aphidimyza* and 2^{nd} instar larvae of *Chrysoperla carnea* were released at the base of each plant. All experiments were done in a controlled-environment room ($21 \pm 2^{\circ}$ C; 16h light : 8h dark). Each plant was a replicate with 15 replicate plants of each treatment. The number of live MACE and non-MACE *Myzus persicae* were counted at each leaf position 24 hours after natural enemies were released. The positions of the predators on the plants were recorded.

- Young larvae of each of the three predator species had little impact in reducing the numbers of MACE and non-MACE *Myzus persicae*. It is expected that older larvae, particularly 2nd and 3rd instars, of all species would be more effective and this aspect will be investigated for *Chrysoperla carnea* in the third year of the project.
- Fewer aphids were found on the growing points of pepper plants inoculated with *Chrysoperla carnea* compared to plants inoculated with either *Aphidoletes aphidimyza* or *Adalia bipunctata*.

Identify an IPM compatible remedial treatment through contact with commercial companies

Discussions were held with Biological Crop Protection Ltd (BCP Ltd) about the efficacy of Eradicoat (a starch based polymer) in controlling aphids on pepper crops.

- BCP Ltd estimate that 50% of Eradicoat applications to pepper crops are used to control spider mites, 30% are used to control thrips, 15% of treatments are used to control whitefly and only approximately 5% of Eradicoat treatments are used to control aphid pests.
- BCP Ltd have observed that spot treatments of melon cotton aphid (*Aphis gossypii*) with Eradicoat were relatively successful, compared to poor levels of control against glasshouse potato aphid (*Aulocorthum solani*). This difference in control may relate to the fact that *Aphis gossypii* form dense colonies that can be wholly treated by a single spot treatment, whereas *Aulocorthum solani* has a more dispersed habit and is difficult to control. The importance of this for treatment of the peach potato aphid (*Myzus persicae*) is significant, as this species will rarely form dense colonies unless very high numbers are present.
- During 2000 an extension of use was obtained for the use of Chess (pymetrozine) on protected pepper crops for aphid control (SOLA 2337/2000 replaced by 1428/2002). Pymetrozine is an antifeedant and can be used in integrated pest management programmes.

Action points for growers

This project has not yet reached completion and the following grower action points are recommended so far:

- Growers of pepper crops should routinely use *Aphidius colemani* and *Aphidoletes aphidimyza* at the normal rates recommended by the biocontrol companies for the control of aphids in pepper crops.
- If the number of aphids suddenly increases so that a 'hot spot' results, increase the release rate of *Aphidius colemani* to 2 per m² per week.
- Growers should be on the look out for MACE *Myzus persicae*, the peach potato aphid which are usually red in colour, and tend to congregate at the top of pepper plants. Sprays of pirimicarb will not work against these aphids as they are resistant. In addition, *Aphidius colemani* is not so effective against the MACE type as it is against the non-MACE forms.
- Some populations of aphids may be resistant to several insecticides and hence if the efficacy of an insecticide appears to be declining, it is advisable to send off a sample of aphids to IACR-Rothamsted for testing for insecticide resistance (tel Dr. Steve Foster 01582 763133; stephen.foster@bbsrc.ac.uk).

- Eradicoat (BCP Ltd) is a starch based product and can act as an IPM compatible remedial treatment to provide aphid control in pepper crops. Experience to date has shown that it is most effective against the melon cotton aphid (*Aphis gossypii*) than either the peach potato aphid (*Myzus persicae*) or the glasshouse potato aphid; the reason being that Eradicoat works best against aphids that form dense colonies.
- Other IPM compatible remedial treatments for aphid control in pepper crops include Chess (pymetrozine), nicotine, fatty acids, and where there are no MACE *Myzus persicae*, Aphox (pirimicarb).

Anticipated practical and financial benefits from this study

The control of MACE-resistant *Myzus persicae* represents a particularly difficult challenge on glasshouse crops, due to the limited number of approved products for control of aphids with this form of resistance, coupled with an increasing desire for food without pesticide residues. This project so far provides indications that MACE *Myzus persicae* colonise pepper plants differently to non-MACE forms and hence changes may be required in the choice and use pattern of particular natural enemies in order to provide more robust biological control.

Potential benefits from the project once completed include:

- (a) Reduced direct economic crop loss resulting from honeydew and rejected produce.
- (b) Improved knowledge of biological control programmes for aphid control.
- (c) Reduced reliance on chemical insecticides.

Science Section

Introduction

The development of insecticide-resistant forms of the aphid *Myzus persicae* means that, for a number of protected crops, sustainable long-term management of this aphid will require the use of biological control agents. Biological control strategies work well in protected crops for the majority of the season, but most growers resort to occasional treatments of pirimicarb to bring any parasitoid/prey imbalance back under control or to control sudden invasions of aphids.

In recent years, a strain of *M. persicae* with a new form of resistance called modified acetylcholinesterase resistance ('MACE') which confers effective immunity to dimethyl carbamates such as pirimicarb has occurred. After the initial discovery of these aphids in the UK in 1995, they spread rapidly so that in 1997 and 1998, the frequency of MACE *M. persicae* was far higher in glasshouses than in field crops. MACE insecticide resistance renders the use of pirimicarb ineffective and where aphids also have high levels of esterase resistance, there are no IPM compatible chemicals to bring the aphid populations back into balance with the natural enemies. Therefore UK growers seek a biologically-based control strategy that is as effective against MACE as non-MACE forms of *M. persicae*. The position of the UK industry is further exacerbated by the use of compounds in mainland Europe that are not currently approved for use on salad crops in the UK.

A number of parasitoid species (*Aphidius colemani, Aphidius matricariae* and *Aphelinus abdominalis*) are available for the control of *M. persicae*. Although *A. matricariae* may be considered most effective against *M. persicae*, *A. colemani* is most commonly produced by suppliers as it also controls *Aphis gossypii* and has been used with reasonable success for several years. Although parasitoids are likely to remain the main control agent, aphid predators (*Aphidoletes aphidimyza, Chrysoperla carnea* and *Adalia bipuincatata*) are also available commercially. Predators may play an important part in controlling aphids, but recommendations for their use are still poorly developed and further efficacy studies are required. Work done in this report period (June 2000 to December 2001) comprised two parts. Firstly the completion of experiments aimed at finding the most effective predator from three species that are commercially available against MACE *M. persicae*, and secondly doing experiments to determine the performance of *Aphidius colemani* against MACE and non-MACE *M. persicae* in a crop-scale experiment.

Commercial Objective

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.

Effectiveness of three predator species against MACE and non-MACE *M. persicae.*

Objective

To determine any difference in the level of predation of MACE and non-MACE *M*. *persicae* by three predator species on pepper.

Performance of two predator species against MACE and non-MACE <u>M. persicae</u> in Petri dishes.

Materials and Methods

Two separate experiments were done with Chrysoperla carnea and Adalia bipunctata. In each experiment the same protocol was followed. In each of 40 plastic Petri dishes (90mm x 14mm) a 5mm layer of tap water agar (1% agar w/v) 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. After the agar was set, 50 3rd and 4th instar MACE *M. persicae* were placed on 20 of these plates, and 50 3rd or 4th instar non-MACE *M. persicae* were placed on the other 20 agar plates. A single 1st instar larva (up to 24 hours old) of Adalia bipunctata or a single 2nd instar larva of Chrysoperla carnea were placed in each Petri dish for 24 hours. The sealed Petri dishes were then placed upside down in a controlled-environment room $(21 \pm 2^{\circ}C)$; 16:8 L:D) for 24 hours. The number of MACE and non-MACE M. persicae, both alive and dead, were counted after 24 hours in each dish. The number of aphids that survived from the 50 originally put in each Petri dish were represented as a proportion of the original 50 in that dish. This proportion was arcsine transformed $(asin(\sqrt{p}))$ before being subjected to analysis of variance to determine any difference in survival between MACE and non-MACE *M. persicae* for each of the predator species tested.

Results and discussion

There was no significant difference in the proportion of MACE or non-MACE *M. persicae* alive in the Petri dishes after 24 hours exposure to a single 2^{nd} instar *C. cernea* larva or a 1^{st} instar *A. bipunctata* larva (Table 1). These data showed that there was no significant difference in the consumption of MACE or non-MACE *M. persicae* by either *C. carnea* or *A. bipunctata*. The mean number of aphids left in each Petri dish was significantly lower after inoculation with *C. carnea* than *A. bipunctata*. The mean proportion of aphids surviving was 74.5% in *C. carnea* treated Petri dishes and 92.5% in *A. bipunctata* treated Petri dishes. These data show a relatively low level of predation of MACE and non-MACE *M. persicae* by these predator species. The difference in predation between *C. carnea* and *A. bipunctata* may not be as great as suggested by these experiments as 2^{nd} instar larva of *C. carnea* were used compared to 1^{st} instar *A. bipunctata*. Older instars of both these species consume more prey than younger instars (Blackman, 1967; Liu & Chen, 2001), and as such it is difficult to draw firm conclusions over the relative effectiveness of these predators in this experiment.

Table 1

	Predator species			
Aphid clone	C. carnea	A. bipunctata		
MACE	62.3 (78.3)	75.2 (93.5)		
non-MACE	57.0 (70.3)	73.0 (91.5)		
LSD	6.65	5.07		

The arcsin transformed proportion (with proportion in parentheses) of 50 MACE or 50 non-MACE *M. persicae* that survived after a 24h exposure to either a 2nd instar *C. carnea* larva or a 1st instar *A. bipunctata* larva. LSD is used to compare transformed values.

Peformance of three predator species against MACE and non-MACE <u>M. persicae</u> on single plants.

Materials and Methods

Pepper plants (cv. Mazurka) at the 4 true leaf stage, each with one growing shoot, were infested with 25 3rd instar red MACE *M. persicae* and 25 3rd instar green non-MACE *M. periscae*. The aphids were distributed evenly over the plant so that there were five green and five red aphids on each leaf or shoot at the time of inoculation. Individual experiments were done with each predator. Plants were placed individually in perspex cages for experiments with Chrysoperla carnea and Adalia bipunctata (45cm x 45cm x 38cm) but were in individual sealed bread bags for the experiment with Aphidoletes aphidimyza. Individual larvae, up to 24 hours old, of A. bipunctata and A. aphidimyza were released at the base of the plant in two experiments whereas 2^{nd} instar larvae of C. carnea were released at the base of the plant in the third experiment. All experiments were done in a controlled-environment room ($21 \pm 2^{\circ}$ C; 16:8 L:D). Each plant was a replicate with 15 replicate plants of each treatment. No uninfested control plants were used in the experiments with C. carnea and A. bipunctata, but 15 aphid infested plants without predators were included in the experiment with A. aphidimyza as a control. The number of live MACE and non-MACE M. persicae were counted at each leaf position 24 hours after natural enemies were released. The positions of the predators on the plants were recorded. The proportion of the total number of aphids on each plant part for each clone was arcsine transformed ($asin(\sqrt{p})$). The transformed data were subjected to analysis of variance to determine the effect of plant part on overall aphid distribution, and any differences in distribution between MACE and non-MACE M. persicae on these young plants. In addition to analysing the distribution of aphids on the pepper plants, the total number of MACE and non-MACE *M. persicae* on each plant was also subjected to a separate analysis of variance to determine comparative predation of MACE and non-MACE *M. persicae* in these experiments.

Results

Feeding position and effectiveness of <u>C. carnea</u>

The proportion of aphids found on the growing shoot of the young plants was significantly lower than on any of the four leaves of the same pepper plants (Table 2).

There was no difference in the proportion of MACE and non-MACE *M. persicae* on particular leaves and of individual pepper plants (F = 1.66, 4 df, p = 0.163) (Table 2). There was also no significant difference in the mean number of MACE and non-MACE *M. persicae* on each plant after 24 hours (MACE = 18.8, non-MACE = 20.1, F = 0.98, 1d.f., p = 0.34).

Table 2

Mean arcsin transformed proportion of aphids (total, MACE and non-MACE) on different plant parts after the release of *Chrysopela carnea*. Figures in parentheses are backtransformed values (mean percentage aphids on that plant part) LSD is used to compare transformed values

		M. persicae clone		
Plant part	Total aphids	MACE	non-MACE	
Leaf 1	28.03 (22.1)	28.03 (22.1)	28.04 (22.1)	
Leaf 2	27.01 (20.6)	28.03 (22.1)	25.98 (19.2)	
Leaf 3	25.91 (19.1)	25.79 (18.9)	26.03 (19.3)	
Leaf 4	27.25 (21)	27.47 (21.2)	27.03 (20.6)	
Shoot	19.35 (11)	18.57 (10.1)	20.12 (11.8)	
LSD	4.39	4.39 6.21		

Feeding position and effectiveness of <u>A. bipunctata</u>

There was no significant difference in feeding position of aphids on plants inoculated with *A. bipunctata* (F = 1.61, 4 df, p = 0.18) (Table 3). There were significantly different mean numbers of MACE and non-MACE *M. persicae* per plant in this experiment (MACE = 18.2, non-MACE = 26.7 F = 16.1,1d.f, p = 0.001). However, it may be that uneven numbers of each clone were placed on each plant, as a number of plants were recorded with more than 25 non-MACE *M. persicae*. This was in contrast to the stated methodology, and also contrasts sharply with the results from Petri dish experiments with this species (Table 3).

Table 3

Mean arcsin transformed proportion of aphids (total, MACE and non-MACE) on different plant parts after the release of *Adalia bipunctata*. Figures in parentheses are backtransformed values (mean percentage aphids on that plant part) LSD is used to compare transformed values

			<i>M. persicae</i> clone			
Plant part	Total aphids		MACE		non-MACE	
Leaf 1	25.03	(17.9)	22.14	(14.2)	27.92	(21.9)
Leaf 2	24.04	(16.6)	24.05	(16.6)	23.99	(16.5)
Leaf 3	24.84	(17.6)	24.41	(17.1)	25.24	(18.2)
Leaf 4	23.99	(16.5)	23.19	(15.5)	24.79	(17.6)
Shoot	29.14	(23.7)	32.29	(28.5)	26.00	(19.2)
LSD	4.7	72	6.68			

Feeding position and effectiveness of <u>A. aphidimyza</u>

There was no significant reduction in numbers of MACE or non-MACE *M. persicae* on plants inoculated with *Aphidoletes aphidimyza* (F = 1.52, 1d.f., p = 0.23). There were significantly fewer non-MACE than MACE *M. persicae* on plants (F = 35.1, 1d.f., p <0.001), but this was due to aphids being present on the containing bags and on the pot surface, rather than being on the plant. There were significantly more aphids feeding on the shoot of the plants than other plant parts, with significantly more MACE *M. persicae* than non-MACE *M. persicae* being found on the shoot of pepper plants (Table 4).

Table 4

Mean arcsin transformed prportion of aphids (total, MACE and non-MACE) on different plant parts after the release of *Aphidoletes aphidimyza*. Figures in parentheses are backtransformed values (mean percentage aphids on that plant part) LSD is used to compare transformed values

		<i>M. persicae</i> clone		
Plant part	Total aphids	MACE	non-MACE	
Leaf 1	18.24 (9.7)	11.62 (4.1)	24.85 (17.7)	
Leaf 2	16.14 (7.7)	11.93 (4.3)	20.34 (12.1)	
Leaf 3	19.44 (11.1)	15.56 (7.2)	23.32 (15.7)	
Leaf 4	16.25 (7.8)	12.17 (4.4)	20.33 (12.1)	
Shoot	45.5 (50.9)	58.32 (72.4)	32.48 (28.8)	
LSD	4.06	5.	74	

Discussion

As no control plants were included in experiments with *C. carnea* and *A. bipunctata*, it is difficult to determine whether observed reductions in numbers of aphids per plant were due to predators, or mortality after being transferred onto pepper plants. Mortality was relatively low in both experiments so the mortality due to transfer between plants was probably relatively low, but it is not possible to say that levels of mortality due to different factors were consistent between experiments. Each predator species had a significantly different effect on the distribution of aphids on pepper plants. Fewer aphids were found on the growing points of plants colonised with *C. carnea* but there was no differential distribution of aphids on plants when colonised with *A. bipuinctata*. Results from the experiment with *A. aphidimyza* showed that there was a different distribution of MACE and non-MACE aphids on plants inoculated with *A. aphidimyza*.

Determine optimum release strategies for Aphidius colemani

Objective

- 1. Determine any difference in the distribution of red-MACE *M. persicae* and green non-MACE *M. persicae* in protected pepper crops.
- 2. Compare the efficacy of *Aphidius colemani* against red MACE-resistant *M. persicae* and green non-MACE *M. persicae* in protected pepper crops.

Materials and Methods

Pepper plants (cv. Mazurka) were grown in rockwool plugs. After germination the larger plants were placed in individual rockwool squares which were then put into larger rockwool slabs (90cm) at two plants per slab. Experimental plots of 24 slabs (48 plants) were arranged as shown in Fig. 1. On each experimental run two plots were grown per compartment in each of two compartments, with each compartment being a block in the experimental design. Each plot was caged individually (Fig. 2).

Plants were treated as a commercial crop whilst growing in the glasshouse rooms prior to inoculation with aphids (16-26°C). Plants were grown with two flowering stems and side shoots were removed weekly until inoculation of aphids. No side shoots were removed after inoculation of aphids to reduce the chance of cross contamination of parasitoids between treated and untreated plots within blocks.

Two plots in each block were inoculated with a single aphid clone on the first experimental run, and with the other aphid clone on the second run. One plot in each block was inoculated with *A. colemani*, whereas *A. colemani* was not introduced into the other plot. This design resulted in the effect of aphid clone being nested within the block structure for analysis to ensure any effect of experimental block on aphid or parasitoid numbers was accounted for.

Each plant was inoculated with ten 3rd or 4th instar aphids (five on the lower leaves and five on the upper leaves of the plant) on 6 June for experimental run one, and 15 August for experimental run two. These aphids were allowed to develop for seven days before the first of three weekly parasitoid inoculations was made to one of the two plots in each block. Thirty female *A. colemani* were released into each MACE and non-MACE *M. persicae* infested cage seven, 14 and 21 days after aphid inoculation on both experimental runs. Unfortunately it was only possible to release 15 female *A. colemani* 21 days after aphid inoculation on the first experimental run due to poor emergence of parasitoids from the cultures. All parasitoids were removed from cultures and inoculated onto experimental plots within 24 h of emergence.



Fig. 1. Dimensions and layout of experimental plot. Each small box represents a single pepper plant. Each row of plants comprised of six rockwool slabs.

Assessment and statistical analysis

Twenty eight days after inoculation 15 plants were taken at random from the central double row of plants in each plot (4 July for run one, and 12 September for run two). Each plant sample comprised of three leaves at the bottom of the plant (called 'bottom'), three leaves from the middle of the plant for each flowering stem (called 'mid branch A' and 'mid branch B') and up to 10 growing points at the top of the plant (called 'growing points'). The high number of growing points on sample plants was due to the lack of side shoot removal during the four week experiment. The number of aphids and aphid mummies were counted on a graded scale if the number of individuals on a single leaf or growing point was greater than 80. The scale used was:-

Score 0 - 0-80 individuals Score 1 - 80-160 individuals Score 2- 160-320 individuals

Score 3 - 320-700 individuals

Score 4 - >700 individuals

This scale was checked within the experiment by counting all aphids on ten leaves or growing points that had previously been assigned a score in each experimental run. There were many more aphids than aphid mummies and so the analysis of numbers used either the mean aphid score or the number of mummies. This was calculated as a mean value for each plant part from the samples taken, and these mean values were then used to produce a mean value for each plant. These data were subjected to analysis of variance using the mean values for each plant part to create a single mean aphid score or mean number of mummies. Due to analysing the number of mummies, rather than a score value, all numbers of aphid mummies were logarithmically transformed (ln (x + 0.375) before analysis of variance.



Fig. 2. Individual cage containing a single plot (either infested with *A. colemani* or uninfested) of pepper plants during the first experimental run.

Results

There was no cross contamination between the control cage and the cage that received regular inoculations of A. colemani for three of the four experimental blocks. Some parasitised aphids were, however, found in the non-MACE control cage on the second experimental run. The number of mummies found per plant part as an average in this cage was lower than found in the treated cage in the same block (5.3 compared to 14.1). The number of individual plant parts that had aphid mummies on them was also lower in the control cage than in the treated cage (217 out of 285 compared to 280 out of 285). This cross contamination will have effected the number of aphids per plant in this control cage compared to the treated cage. However, the relatively low numbers of aphid mummies per plant part and lower frequency of mummified aphids, suggest that the cross contamination occurred some time after introductions had begun in the treatment cage. The difference in the numbers of aphids per plant between the control and treatment cage for this block (Fig.3) suggest that, whilst A. colemani infestation reduced aphid numbers in the control cage, their relatively late occurrence, had a minimal effect on the overall outcome of the experiment. It is suggested that this cross contamination does not alter the overall conclusions. These are that the parasitoid A. colemani does reduce the numbers of both MACE and non-MACE M. persicae, but parasitises MACE M. persicae to a lower extent than non-MACE M. persicae.



Fig.3. Mean aphid score per plant for peppers infested with either MACE or non-MACE *M. persicae* that were subjected to either weekly introduction of parasitoids (*A. colmani*) or were uninfested on each experimental run.

Effect on aphid numbers

The experimental structure, where aphid clone was nested within block as a subplot, does not allow any significant difference in the number of aphids per plant to be determined for each clone if there were significant block effects. There was a significant effect of block on mean aphid score per plant, and so it was not possible to determine if the mean aphid score per plant was greater for MACE M. persicae infested plants than non-MACE M. persicae infested plants. The number of MACE and non-MACE *M. persicae* were significantly reduced by *A. colemani* on peppers (Table 5). Although no significant difference in MACE and non-MACE M. persicae numbers were found in these experiments, the proportional reduction in mean aphid score per plant was greater for non-MACE compared to MACE M. persicae (Table 5). The level of control achieved by A. colemani was much greater in run one than in run two (Fig. 3). There was a significant difference in the distribution of MACE and non-MACE M. persicae between plant parts over the whole experiment (Table 6). There were more MACE *M. persicae* on growing points of pepper plants than on other plant parts compared to non-MACE *M. persicae*, which were evenly distributed on all plant parts.

Table 5

Mean score of aphid numbers per plant for plants infested with MACE or non-MACE *M. persicae*, either with or without weekly introductions of *A. colemani*

Aphid clone	with	without	
	A. colemani	A. colemani	LSD
MACE	0.70	1.87	0.51
non- MACE	0.33	1.73	0.71
Both clones	0.51	1.80	0.25

Table 6 Mean aphid score for plants parts infested with either MACE or non-MACE *M. persicae*.

Plant part					
Aphid clone	Bottom	Mid branch A	Mid branch B	Growing points	LSD
MACE	0.59	1.38	0.94	2.21	0.8
non MACE	0.86	1.02	0.96	1.31	0.8

Effect on mummy numbers

In contrast to the data for numbers of aphids per plant, there was no significant effect of block on the number of mummies for MACE and non-MACE aphid-infested plants. As a result, an analysis of variance was performed on the number of mummies per plant. The exclusion of the block factor allows the inclusion of aphid clone as a treatment factor within the experimental design. The number of mummies per plant was significantly lower on MACE aphid-infested plants compared to non-MACE aphid-infested plants (Table 7). The number of mummies also varied with plant part for both MACE and non-MACE *M. persicae*, though this was not unexpected as the number of aphids per plant part also varied. Additionally, there was a significant interaction between aphid clone and plant part for the number of mummies per plant, with a more even distribution of mummies found on each plant part for non-MACE infested plants as compared to MACE infested plants (Table 7). It should be noted that whilst there were significantly more MACE *M. persicae* on growing points of plants than non-MACE *M. persicae* (Table 6), there were similar number of mummies on the growing points of plants with MACE and non-MACE *M. persicae*.

Table 7

Mean log number of mummies per plant part and per whole plant on MACE and non-MACE *M. persicae* infested plants

Plant part					
Aphid clone	Bottom	mid Branch A	Mid branch B	Growing points	whole plant
MACE	1.65	2.1	2.31	3.29	2.34
non-MACE	3.03	2.95	2.85	2.97	2.95
LSD	0.76*			0.39*	

* LSD from analysis where non significant block effect has been excluded



Fig. 4. The percentage of time during a particular calendar week for each experimental run that the temperature was greater than 25°C.

Discussion

The use of *A. colemani* significantly reduced the mean number of *M. persicae* per plant on MACE and non-MACE infested plants. However the significant block effect did not make it possible to determine statistically wether there were more MACE *M. persicae* per plant than non-MACE *M. persicae*. There were more MACE *M. persicae* on the growing points of the plants than non-MACE *M. persicae* (Table 6) although the numbers of MACE and non-MACE *M. persicae* on the middle branches of the plant and the lower leaves did not differ significantly. This suggests that there were greater numbers of MACE *M. persicae* per plant than non-MACE *M. persicae*.

The reduction in aphid numbers by A. colemani differed between experimental runs, with MACE M. persicae being reduced somewhat less in run two than non-MACE M. persicae (Fig. 3) as well as a generally lower level of control in run two compared to run one. The lesser control by A. colemani of MACE M. persicae coincided with a change in the temperature pattern over the course of run two compared to run one (Fig. 4), with relatively low temperatures prevailing towards the end of run two compared to run one. In addition, some parasitised M. persicae were found in the control cage for non-MACE infested plants on run two, suggesting that there had been some reduction in numbers of non-MACE M. persicae in the untreated control cage. This means that the number of aphids per plant on control plants would be lower than expected, and this should be taken into account when interpreting the results in comparison to the reduction in aphid numbers seen in the A. colemani infested cage. Despite the cross contamination, the mean number of non-MACE M. persicae per plant for plants inoculated with A. colemani was less than 50% of that on non-MACE infested control plants, compared to a figure of approximately 60% for A. colemani treated MACE aphid infested plants compared to the control (Fig. 3).

There were more mummies found on non-MACE *M. persicae* infested plants than MACE *M. persicae* infested plants (Table 7), and there were also more mummies found on the bottom, and on middle branches of plants infested with non-MACE *M. persicae* compared to plants infested with MACE *M. persicae* (Table 7). These results suggest that *A. colemani* does not control MACE *M. persicae* as well as non-MACE *M. persicae*, and that MACE *M. persicae* do have a significantly different distribution on peppers than non-MACE *M. persicae*, preferring to colonise the growing points of the plant to a greater extent. The contrast between the levels of control seen in different experimental runs also suggests that changes in temperature after *A. colemani* introductions may change the effectiveness of *A. colemani* against MACE and non-MACE *M. persicae*. This finding needs to be investigated more rigorously in relation to the effect of relatively high temperatures on both MACE and non-MACE *M. persicae*, as better levels of control were observed when temperatures were highest at the end of the experiment.

Identify an IPM compatible remedial treatment through contact with commercial companies

Objective

To identify, through either experimental study or by literature and industry survey, potential candidate remedial treatments to reduce outbreaks of insecticide-resistant *M. persicae* by physical modes of action.

It was initially suggested that the starch solution formulation called Eradicoat (BCP Ltd) would be tested against MACE and non-MACE *M. persicae*. This was deferred pending further inquiries about the extent to which this product is currently being used against aphid pests in glasshouse crops, particularly *M. persicae*.

Discussions with BCP have highlighted the relatively low use of Eradicoat against aphid pests in glasshouse crops. BCP estimate that 50% of Eradicoat treatments are used to control spider mites, 30% are used to control thrips, 15% of treatments are used to control whitefly and only approximately 5% of Eradicoat treatments are used to control aphid pests (Richard Corthen, pers. Comm.). It was also noted by BCP that, whilst treatments against pests such as spider mites and thrips normally result in over 95% control, treatments against *M. persicae* result in a maximum level of control of between 90 and 95%.

It had also been noted that the observed level of control did vary with different aphid species. Spot treatment of *Aphis gossypii* being relatively successful, compared to poor levels of control against *Aulocorthum solani*. This difference in control may relate to the forming of dense colonies of *A. gossypii* that can be wholly treated by a single spot treatment, whereas *A. solani* has a more dispersed habit and is difficult to control. The importance of this for treatment of *M. persicae* is significant, as this species will rarely form dense colonies unless very high numbers are present.

The result of these discussions are that Eradicoat is unlikely to provide an effective and consistently performing remedial treatment for controlling outbreaks of the aphid *M. persicae* at relatively low numbers.

Acknowledgements

The authors would like to thank Sue Sime, Kelly Reynolds, Liz Vice, Gill Davidson, Kate Morley and Nick Miller for technical assistance and Julie Jones for statistical guidance and analysis.

References

Blackma, R.L. (1967). The effects of different foods on *Adalia bipunctata* and *Coccinella 7-punctata*. *Annals of Applied Biology*, **59** : 207-219.

Liu T.X. & Chen T.Y. (2001). Effects of three aphid species on development, survival and predation of *Chrysoperla carnea*. *Applied Entomology and Zoology*, **36** : 361-366.