Project title:	Managing ornamental plants sustainably (MOPS)
Project number:	CP 124
Work package title:	Efficacy of plant protection products against sucking insects – melon and cotton aphid / protected hardy nursery stock
Work package leader:	Dr Tom Pope, Harper Adams University
Report:	Final report, February 2017
Previous report:	Annual report, December 2015
Key staff:	Dr Tom Pope, Harper Adams University Grace Smith, Harper Adams University Prof Simon Edwards (Statistical advice), Harper Adams University
Location of work:	Harper Adams University
Date work commenced:	1st December 2013
Date work completed (or expected completion date):	31st January 2017

[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 have been reported in detail and with 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.]

AUTHENTICATION

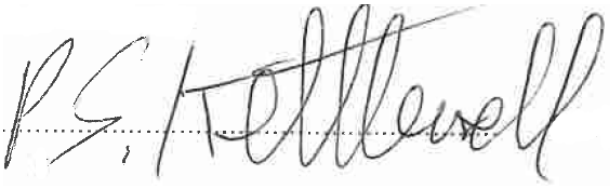
We declare that this work was done under our supervision according to the procedures described herein and that the report represents a true and accurate record of the results obtained.

Dr. Tom W. Pope
Lecturer in Entomology
Harper Adams University

Signature  .... Date 07-02-2017............

Report authorised by:

Prof. Peter Kettlewell
Research Co-ordinator
Harper Adams University

Signature  Date 07-02-2017

CONTENTS

GROWERS SUMMARY

Headline

- The conventional insecticides Mainman (flonicamid), product 59 and product 210 gave control (greater than 90% reduction) of melon and cotton aphid on *Hebe* plants.
- Biopesticide products 62 and 179 also gave control (greater than 80% reduction) of melon and cotton aphid on *Hebe* plants.

Background and expected deliverables

The melon and cotton aphid (*Aphis gossypii*) is one of the most serious pests of ornamentals due to the wide range of plants it attacks and because it has developed resistance to several groups of pesticides. *Aphis gossypii* is very polyphagous and common on protected ornamental and hardy nursery stock (HNS) hosts including begonia, chrysanthemum, *Coronilla*, cyclamen and *Hebe*. This aphid species tends to form large colonies on stems, young leaves and growing points. Plants attacked by this pest may yellow, wilt and, if damage is severe, die.

The purpose of Objective 2 was to test the efficacy of plant protection products against sucking insects. Specifically, Objective 2.3 was to test the efficacy of products against the melon and cotton aphid on a selected susceptible protected HNS species. Work completed in 2016 tested the efficacy of a range of plant protection products, each used according to the recommendations of each product manufacturer.

Summary of the work and main conclusions

Seven plant protection products (Table 1) were tested against melon and cotton aphid (*Aphis gossypii*) on *Hebe* (cv. Pink Pixie) plants grown under polytunnel conditions between July and August 2016 at Harper Adams University. Environmental conditions within the polytunnel were measured through the use of dataloggers and nearby meteorological recording station. The polytunnel was ventilated by rolling up the sides of the polytunnel to allow airflow through mesh walls.

Table 1. Products tested

MOPS code number	Authorisation status	Biopesticide or conventional pesticide
Water control	-	-
Mainman (flonicamid)	EAMU 20130045	conventional
130	unauthorised	biopesticide
62	unauthorised	biopesticide
210	unauthorised	conventional
59	unauthorised	conventional
179	unauthorised	biopesticide
Botanigard (*Beauveria bassiana*) + Majestik (maltodextrin)	On-label 20162754 On-label 20152230	biopesticide & biopesticide

Plants were provided by Bransford Webbs as plugs on 24 March 2016 and these plants were potted into John Innes No. 2 Compost in 9 cm diameter pots on 12 April 2016. Plants were grown on in a ventilated polytunnel until 8 July when the plants were transferred to the polytunnel. Nine plants were arranged in three rows of three in each of 48 plots. Each plot was 0.5 m x 0.75 m in size and screened on three sides with horticultural fleece in order to physically separate each plot. Plants were watered from beneath using capillary matting.

The population of aphids used in this experiment was established from field-collected aphids (aphids supplied by Dove Associates in 2015) from a commercial ornamentals nursery. Aphids were maintained in the insectary at Harper Adams University on *Hebe* plants under controlled environmental conditions (20°C and 60% relative humidity) for two months prior to use. All nine plants in each plot were artificially infested with fragments (leaves and stems) of aphid-infested *Hebe* plants taken from the aphid culture on 7 July.

All plant protection products, except Botanigard WP + Majestik, were applied using an Oxford Precision Sprayer fitted with an HC/1.74/3 nozzle. The Botanigard WP + Majestik treatment was applied using an Oxford Precision Sprayer fitted with an F80/1.2/3 nozzle. All products were applied in 600 litres of water per hectare using 3 bar pressure. A water control was applied using the same water volume and pressure using an HC/1.74/3 nozzle. No adjuvants were used for any products tested. The number of applications and time between each application was determined by on-label or EAMU approval. Where a product was not yet approved the number of applications and time between each application was determined by the manufacturer based on the approval they are seeking for the product (Table 2). Each plant protection product and the water control was applied as indicated in Table 3. Aphid numbers were recorded one day before the first spray application was applied on 28 July and then at regular intervals throughout the remainder of the experiment (5 counts in total, see Table 3) with the final assessment of aphid numbers completed on Day 22 (21 days after the first count). In addition, assessments of phytotoxicity were completed on each day that aphid counts were completed.

Mean aphid numbers recorded one day before the first spray application were between 25 and 32 per plot for each treatment and the water control. Aphid numbers declined in all plots initially but in the water control mean aphid numbers then began to increase and had reached 33 per plot by the end of the experimental period.

Table 2. Numbers of applications and time between applications

MOPS Code	Minimum time (days) between applications	Number of applications to apply during experiment
Water control	-	2
Mainman (flonicamid)	21	1
130	7	2 (applied morning or late afternoon)
62	5	3
210	7	2
59	7	2
179	3	5
Botanigard WP (*Beauveria bassiana*) + Majestik (maltodextrin)	5	3 (applied late afternoon after wetting matting)

Table 3. Applications and aphid counts by day number

Day number	Activity	Product(s)
1.	Aphid counts	All products
2.	Spray application	All products
5.	Aphid counts & Spray application	All products & 179 (applied after counts)
7.	Spray application	62 & Botanigard WP + Majestik
8.	Aphid counts & Spray application	All products & 179 (applied after counts)
9.	Spray application	Water control, 130, 62, 210 and 59
11.	Spray application	179
12.	Spray application	62 & Botanigard WP + Majestik
14.	Spray application	179
15.	Aphid counts	All products
22.	Aphid counts	All products

A single application of the conventional insecticide flonicamid (Mainman) gave very good control of melon and cotton aphid (97% reduction) with numbers being reduced by more than 80% within six days of the spray application. Products 59 and 210 (both conventional insecticides), gave similar levels of control to Mainman, although both were sprayed twice with seven days between applications. Overall product 59 was most effective at controlling melon and cotton aphids both in terms of speed of kill (94% reduction six days after the first spray application) and absolute efficacy (no aphids found in plots treated with this product 14 days after the first spray application). Product 210 was very similar to Mainman in its efficacy against melon and cotton aphid.

Biopesticide products 62 and 179 effectively reduced numbers of melon and cotton aphids on Hebe plants during the experimental period (by 80 and 90%, respectively). Aphid numbers in plots sprayed with product 179 were not statistically different to aphid numbers in plots sprayed with Mainman when assessments were completed six and 14 days after the first spray application.

Botanigard WP + Majestik significantly reduced numbers of aphids in plots compared with the water control by the end of the experiment. Product 130 was the only product tested not to reduce numbers of melon and cotton aphid.

Product 62 and Botanigard WP + Majestik were each applied three times and product 179 was applied five times during the experiment. Repeated applications of these products improved the consistency of control seen between plots and this was similar to the conventional insecticides tested at the final assessment. All of the biopesticides tested work through direct contact with the pest and so good spray coverage is essential. Initial work using water sensitive paper indicated that while spray coverage of upper leaf surfaces was generally good, coverage of lower leaf surfaces was relatively poor. This was observed when a hollow cone or a flat fan nozzle was used. This suggests that the efficacy of these biopesticide products could be further improved through achieving better spray coverage.

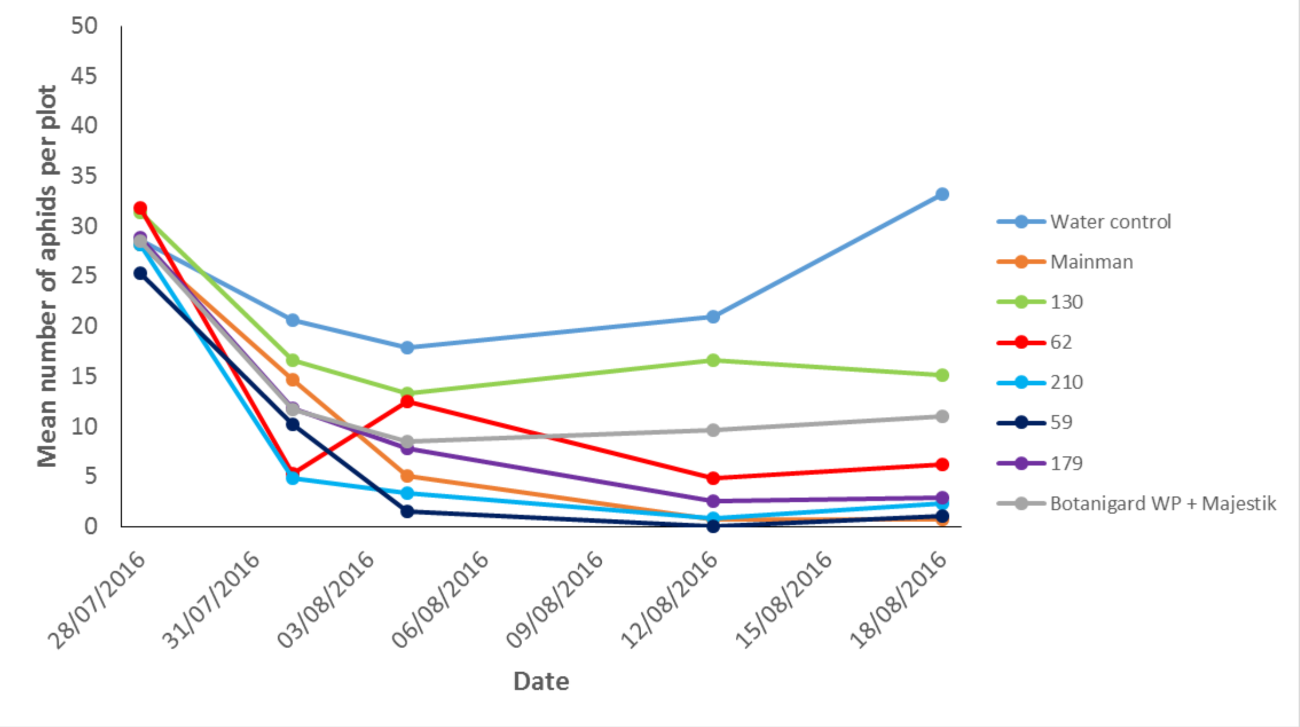


Figure 1. Mean numbers of aphids per plot on each assessment date (9 plants sampled in each plot).

There was no evidence of any phytotoxicity caused by the plant protection products tested. Plants remained largely free of other aphid pests, e.g. peach-potato aphids (*Myzus persicae*) during the experimental period. Similarly there was no need to apply biological or chemical controls against other pests, e.g. two-spotted spider mite (*Tetranychus urticae*). Natural enemies included aphid parasitoids (*Aphidius* spp.), seen as mummified aphids, hoverfly adults, eggs and larvae (mainly *Episyrphus balteatus*). These natural enemies were present in low numbers (aphid mummies were the most numerous but a mean <1 aphid mummy per plant recorded on any one assessment). Despite the low numbers of natural enemies recorded, each of the natural enemies mentioned here was seen in plots to which each of the products was applied.

Action Points

- Consider flonicamid (Mainman) as a very effective option for control of melon and cotton aphid, reducing aphid numbers quickly after a single spray application.
- When products 59 and 210, both conventional insecticides, gain approval in the future, consider their use against melon and cotton aphid as they showed similar, or slightly improved in the case of product 59, levels of efficacy to Mainman. Product 59 works both on contact and through ingestion and displays translaminar movement (moves to the opposite leaf surface) when applied to foliage and is xylem-mobile.
- Products 62 and 179 (both biopesticides) were effective at reducing numbers of melon and cotton aphid. With repeated applications product 179 gave similar levels of control to Mainman. When these products gain approval in the future, consider their use against this aphid pest as part of an IPM programme.
- Results presented here are broadly similar to those reported in year one of this project in which the same products were tested against peach-potato aphid (*Myzus persicae*) on pansy.
- Consider products for compatibility with biological control agents used in an IPM programme

SCIENCE SECTION

Introduction

Various aphid species can damage ornamental plants but one of the most serious pest species is the melon and cotton aphid (*Aphis gossypii*) due to the wide range of plants this species of aphid attacks and because it has developed resistance to several groups of pesticides.

Aphis gossypii is very polyphagous and common on protected ornamental hosts including begonia, chrysanthemum, *Coronilla*, cyclamen and *Hebe*. This aphid species tends to form large colonies on stems, young leaves and growing points. Plants attacked by this pest may yellow, wilt and, if damage is severe, die.

Effective chemical control of this aphid is difficult due to its resistance to many currently available chemical pesticides. UK populations of *A. gossypii* are resistant to carbamates such as pirimicarb e.g. Aphox (Furk & Hines, 1993). This type of resistance is known as Modified AcetylCholineEsterase or MACE resistance). There is also widespread resistance to pyrethroids such as deltamethrin (e.g. Decis). This type of resistance is known as knockdown resistance or kdr resistance (Marshall *et al.,* 2012).

Due to problems with pesticide resistance, leading growers of protected ornamentals use biological control methods within IPM programmes. Several aphid parasitoid species are now available either as single or mixed species. The most common species used for control of *A. gossypii* is *Aphidius colemani*, sometimes supplemented with the predatory midge *Aphidoletes aphidimyza* whose larvae are voracious predators of many aphid species. Growers using IPM sometimes need to use an IPM-compatible aphicide e.g. immediately before dispatch or to supplement control during the summer when aphid numbers can increase rapidly.

Pesticides commonly used by growers of protected ornamentals and HNS for control of *Aphis gossypii* within IPM programmes include pymetrozine (Chess) and flonicamid (Mainman). Biopesticides used include the natural plant extracts product Majestik and the plant stimulant SB Plant Invigorator. The entomopathogenic fungus, *Beauveria bassiana* (Naturalis-L) has been tried for aphid control on some nurseries but with limited success, possibly due to humidity requirements following application. Other pesticides used include spirotetramat (Movento) and the neonicotinoids thiacloprid (Calypso) and acetamiprid (Gazelle SG). However, these products are less compatible with IPM and although these particular neonicotinoids are not affected by current restrictions on use of neonicotinoids, many retailers are asking growers not to use any neonicotinoids at all on their produce. This further restricts the pesticide options for aphid control.

Materials and methods

Site and crop details

Table 4. Test site and plot design information

Test location:	Harper Adams University
County	Shropshire
Postcode	TF10 8NB
Soil type/growing medium	John Innes No. 2
Nutrition	n/a
Crop	*Hebe*
Cultivar	Pink Pixie
Glasshouse* or Field	Polytunnel
Date of planting/potting	Plug plants potted up on 12 April 2016
Pot size	9 cm diameter pots
Number of plants per plot	9
Trial design (layout in Appendix C)	Randomised block
Number of replicates	6
Plot size w (m), l (m), total area (m^2)	0.5 m x 0.75 m
Method of statistical analysis	ANOVA

*Temperature and relative humidity conditions are given in Appendix B

Treatment details

Table 5. Detail of products tested

MOPS code number or Product name	Active ingredient(s)	Manufacturer	Batch number	a.i. conc.	Formulation type
1. Water control	-	-	-	-	-
2. Mainman	flonicamid	Belchim Crop Protection		500 g/kg	WG
3. 130	azadirachtin	N/D		1%	EC
4. 62	Terpenoid blend	Bayer CropScience		16.75%	OD
5. 210	N/D	N/D			
6. 59	sulfoxaflor	Dow AgroSciences		120 g/l	SC
7. 179	orange oil	OroAgri		60 /l	SL
8. Botanigard WP + Majestik	*Beauveria bassiana* + maltodextrin	Certis		220 g/kg + 598 ml/l	WP + SC

Table 6. Treatments

Product name or MOPS code number	Minimum time (days) between applications	Number of applications applied during experiment	Rate of use (product)	Spray volume (l/ha)
1. Water control	-	2	-	600
2. Mainman	21	1	0.14 g/ha	600
3. 130	7	2 (late afternoon)	0.5% (3.0 l/ha if applied in 600 l of water/ha)	600
4. 62	5	3	0.67 v/v (4.0 l/ha if applied in 600 l water/ha)	600
5. 210	7	2	0.75 l/ha	600
6. 59	7	2	0.2 l/ha (24 g active substance/ha)	600
7. 179	3	5	0.4% (2.4 l/ha)	600
8. Botaniguard WP + Majestik	5	3 (applied late afternoon after wetting matting)	0.375 kg/ha + 15 l/ha if applied in 600 l water/ha	600

Table 7. Aphid counts and application timings

Day No.	Date	Aphid count	Product(s)
1.	28 July 2016	Aphid counts	
2.	29 July 2016		All products & water control (A1)
5.	1 August 2016	Aphid counts	179 only (applied after counts) (A2)
7.	3 August 2016		62 & Botanigard WP + Majestik only (A3)
8.	4 August 2016	Aphid counts	179 (applied after counts) (A4)
9.	5 August 2016		130, 210 and 59 & water control (A5)
11.	7 August 2016		179 (A6)
12.	8 August 2016		62 & Requiem & Botanigard WP + Majestik (A7)
14.	10 August 2016		179 only (A8)
16.	12 August 2016	Aphid counts	
22.	18 August 2016	Aphid counts	

Table 8. Application details

Application No.	A1	A2	A3	A4	A5	A6	A7	A8
Application date	29/7/16	1/8/16	3/8/16	4/8/16	5/8/16	7/8/16	8/8/16	10/8/16
Time of day	11.00am (except 4.00pm for Botanigard + Majestik)	2.00pm	4.00pm	2.00pm	4.00pm	4.00pm	4.00pm	10.00am
Application method	Oxford Precision Sprayer fitted with a HC/1.74/3 nozzle (except Botanigard + Majestik where F80/1.2/3 nozzle used), in 600 litres of water per ha using 3 bar pressure							
Temperature of air – max/min (°C)	22.6-23.6°C & 23.1-24.1°C	22.1-27.1°C	22.1-27.6°C	22.1-23.6°C	29.6-31.1°C	29.1-29.1°C	26.1-29.6°C	20.6-24.6°C
Relative humidity (%)	62% & 72%	45%	66%	61%	37%	38%	28%	61%
Cloud cover (%)	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
Crop growth stage	Flowering							
Crop comments	-	-	-	-	-	-	-	-
Other*:	-	-	-	-	-	-	-	-

*Includes soil temperature and moisture details where relevant

The application method used was agreed upon following consultation with industry representatives, a spray application expert (David Talbot, ADAS) and product manufacturers. Efficacy of the application method was assessed before the first treatment application by attaching water-sensitive papers to spare *Hebe* plants arranged in the same way as in the experimental plots. This allowed spray coverage on the upper and lower leaf surfaces to be determined for leaves in the upper, middle and lower crop canopy.

Target pest(s)

Table 7. Target pest(s)

Common name	Scientific Name	Infection level pre-application
Melon and cotton aphid	*Aphis gossypii*	Moderate, 15-71 aphids/plot (2-8 aphids/plant in each plot)

Each *Hebe* plant was infested with pieces of aphid infested *Hebe* plant on 6 July. This was carried out by cutting leaves or sections of stem from an aphid-infested plant and carefully laying these pieces of plant material on previously uninfested plants in each plot. Aphids used to infest *Hebe* plants had been collected from a commercial nursery and were maintained on *Hebe* plants through several generations before the start of the experiment.

Assessments

For each assessment of aphid numbers the central stem of each plant was selected and the number of aphids recorded on the leaves and flowers coming off this stem recorded as well as any aphids on the stem itself. Aphid counts were done in-situ but to aid counting of aphids each plant was carefully lifted so that the undersides of the leaves could easily be seen.

Table 8. Assessments

Assessment No.	Date	Growth stage (BBCH scale)	Timing of assessment relative to last application	Assessment type(s) (e.g. aphid numbers and crop safety)
1	28/7/2016	Flowering (65)	1 day before first application	Number of aphids/plant
2	01/8/2016	Flowering (65)	3 day after first application	Number of aphids/plant
3	04/8/2016	Flowering (65)	6 days after first application	Number of aphids/plant & crop safety
4	12/8/2016	Flowering (65)	14 days after first application	Number of aphids/plant & crop safety
5	18/8/2016	Flowering (65)	20 days after first application	Number of aphids/plant & crop safety

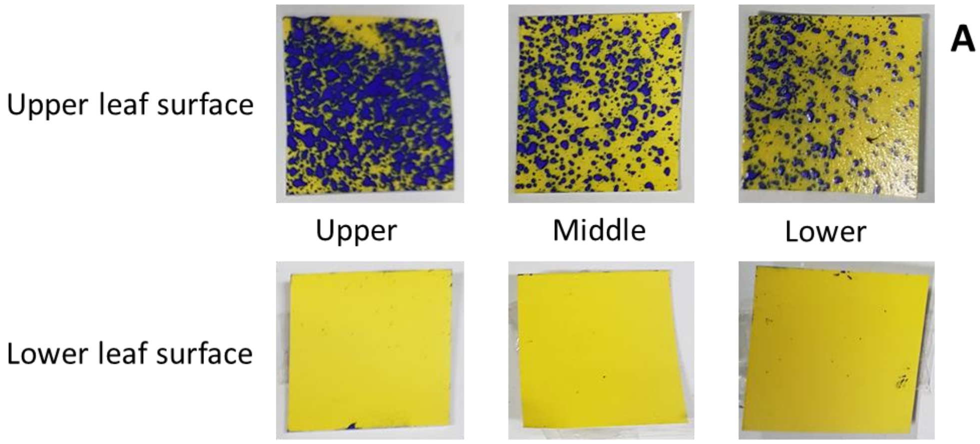
<u>Statistical analysis</u>

Data were analysed weekly (not across weeks) using analysis of variance (ANOVA) with Ln (c=1) transformation of raw data to calculate means, variance, LSDs ($p<0.05$). Genstat 17th Edition was used as advised by Prof. Simon Edwards (Harper Adams University). Graphs show a simpler representation of the data without any transformation, so that trends and dynamics over the experiment can be visualised.

Results

Spray coverage

The application methods used achieved good spray coverage on the upper leaf surfaces in the upper, middle and lower crop canopies. However, in both cases spray coverage on the lower leaf surface was poor at all positions within the crop canopy (Figure 2).



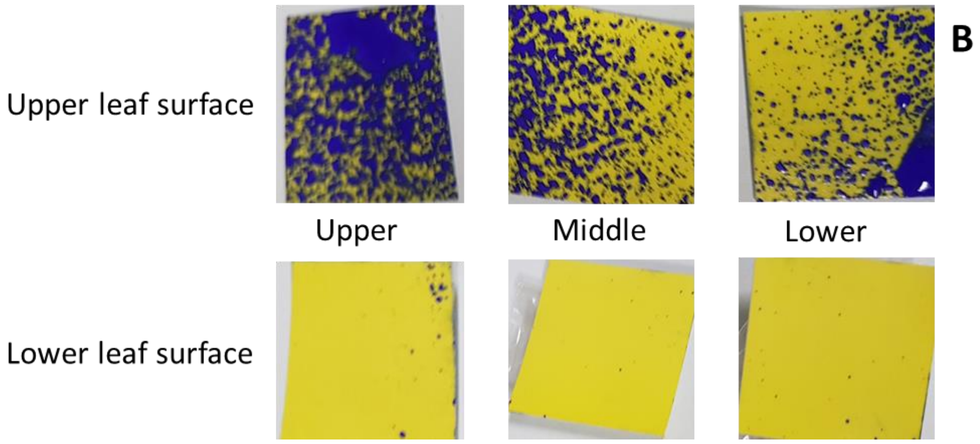


Figure 2. Spray coverage on water sensitive paper positioned on the upper and lower leaf surfaces in the upper, middle and lower crop canopy; (A) Hollow Cone (HC/1.74/3 nozzle), (B) Flat Fan (F80/1.2/3 nozzle).

Efficacy against Aphis gossypii

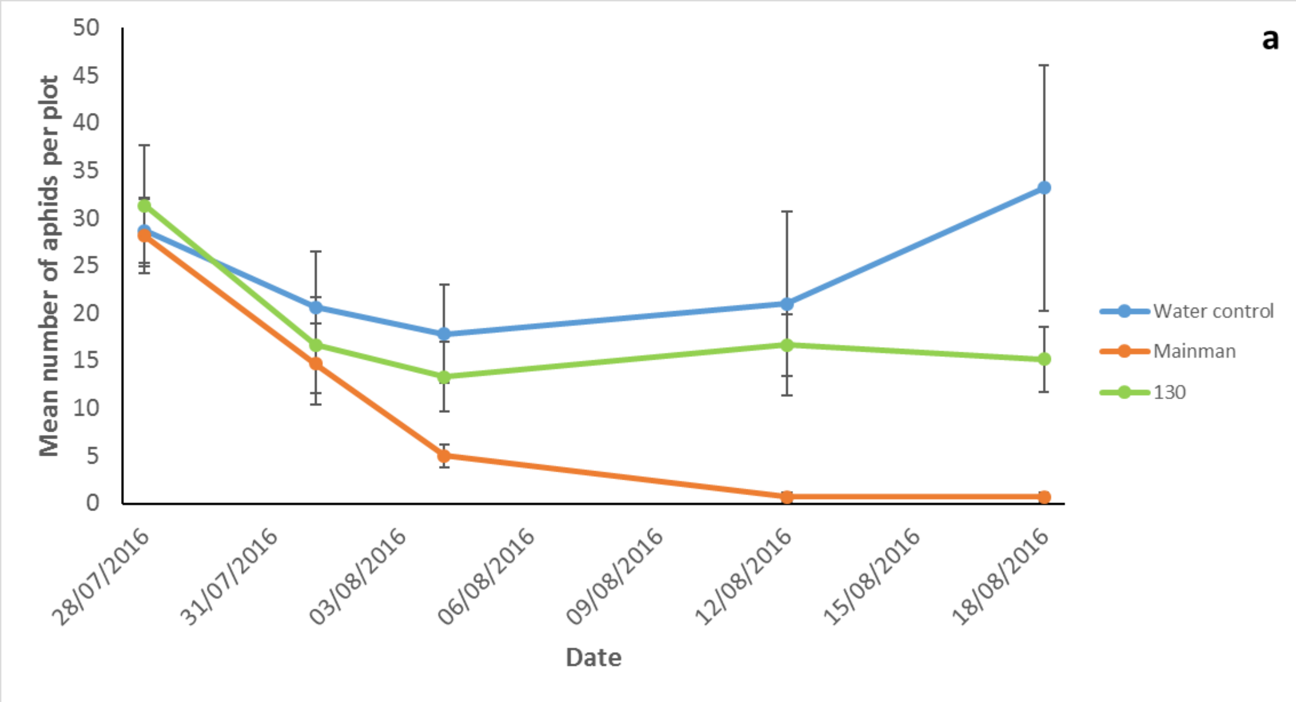
Results are summarised in the Figure 3 (graphical plot) and Table 9 (with ANOVA statistics) below. The results show that three products tested, Mainman (the positive control) as well as products 59 and 210 (both conventional insecticides), had reduced aphid numbers to zero or close to zero in each plot by the end of the experimental period. Product 179 (a biopesticide) had reduced aphid numbers from a mean of 29 per plot at the start of the experiment to mean of fewer than 3 per plot by the end of the experiment, a 90% reduction in aphid numbers. A second biopesticide, product 62, reduced aphid numbers from a mean of 32 per plot at the start of the experiment to a mean of just over 6 per plot by the end of the experiment, an 81% reduction in aphid numbers. The other two products tested, 130 and Bontanigard WP + Majestik, reduced aphid numbers by 52 and 61%, respectively, during the experimental period.

Statistical analysis of the Ln (c=1) transformed aphid count data shows that there was no difference in aphid numbers between treatments before the first spray application or three days after this application. There were, however, statistically significant treatment effects for all assessments completed from six days after the first spray application. No block effects were recorded on any of the assessment dates.

Six days after the first spray application, Mainman as well as products 59, 210 and 179 had significantly reduced aphid numbers compared with the water control (-ve control) (LSD at 5%). In addition, numbers of aphids in plots treated with product 59 were significantly lower than in plots treated with any other product except product 210. By the assessment completed eight days later (14 days after the first spray application) all products, except 130 and Botanigard WP + Majestik, had significantly reduced aphid numbers compared with the water control. In plots treated with Mainman, product 59 or product 210 aphid numbers were significantly lower than in plots treated with any other products. Products 62 and 179 had significantly reduced aphid numbers in plots compared with the water control. At the final assessment (20 days after the first spray application) all products, except product 130, had significantly reduced aphid numbers compared with the water control. The pattern between treatments was similar to the previous assessment. In plots treated with Mainman and product 59 aphid numbers were significantly lower than in plots treated with any other products, except product 210. Aphid numbers in plots treated with product 210 did not differ statistically from aphid numbers in plots treated with Mainman, product 59 or product 179. Aphid numbers in plots treated with product 179 did not differ statistically from aphid numbers in plots treated with product 62. Botanigard WP + Majestik significantly reduced aphid numbers compared with the water control but aphid numbers did not differ from those in plots treated with product 130.

Table 9. Effect of treatments on *Aphis gossypii*. Raw data transformed using Ln (c=1) and presented as mean number of aphids/plot. Numbers in a column followed by the same letter are not significantly different at P <0.05 based on individual contrasts (LSD).

Product name or MOPS code	28/7/2016	01/8/2016	04/8/2016	12/8/2016	18/8/2016
1. Water control	3.36[a]	2.86[a]	2.73[d]	2.70[d]	3.15[e]
2. Mainman	3.32[a]	2.53[a]	1.71[bc]	0.35[ab]	0.35[a]
3. 130	3.39[a]	2.65[a]	2.50[cd]	2.80[d]	2.66[de]
4. 62	3.43[a]	1.63[a]	2.35[cd]	1.37[c]	1.67[bc]
5. 210	3.28[a]	1.49[a]	1.30[ab]	0.48[a]	0.94[ab]
6. 59	3.24[a]	2.32[a]	0.83[a]	0.00[a]	0.41[a]
7. 179	3.26[a]	2.41[a]	1.84[b]	1.10[bc]	1.27[b]
8. Botanigard WP + Majestik	3.35[a]	2.28[a]	2.12[cd]	2.26[d]	2.26[cd]
F value (7 d.f.) Treatment	0.15 (P = n.s.)	2.18 (P = n.s.)	5.27 (P <0.001)	15.95 (P <0.001)	10.19 (P <0.001)
F value (5 d.f.) Block	0.11 (P = n.s.)	0.10 (P = n.s.)	0.92 (P = n.s.)	0.44 (P = n.s.)	0.08 (P = n.s.)
LSD (treatment)	0.48	0.93	0.79	0.79	0.93



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Mean number of aphids per plot
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Date
Water control
Mainman
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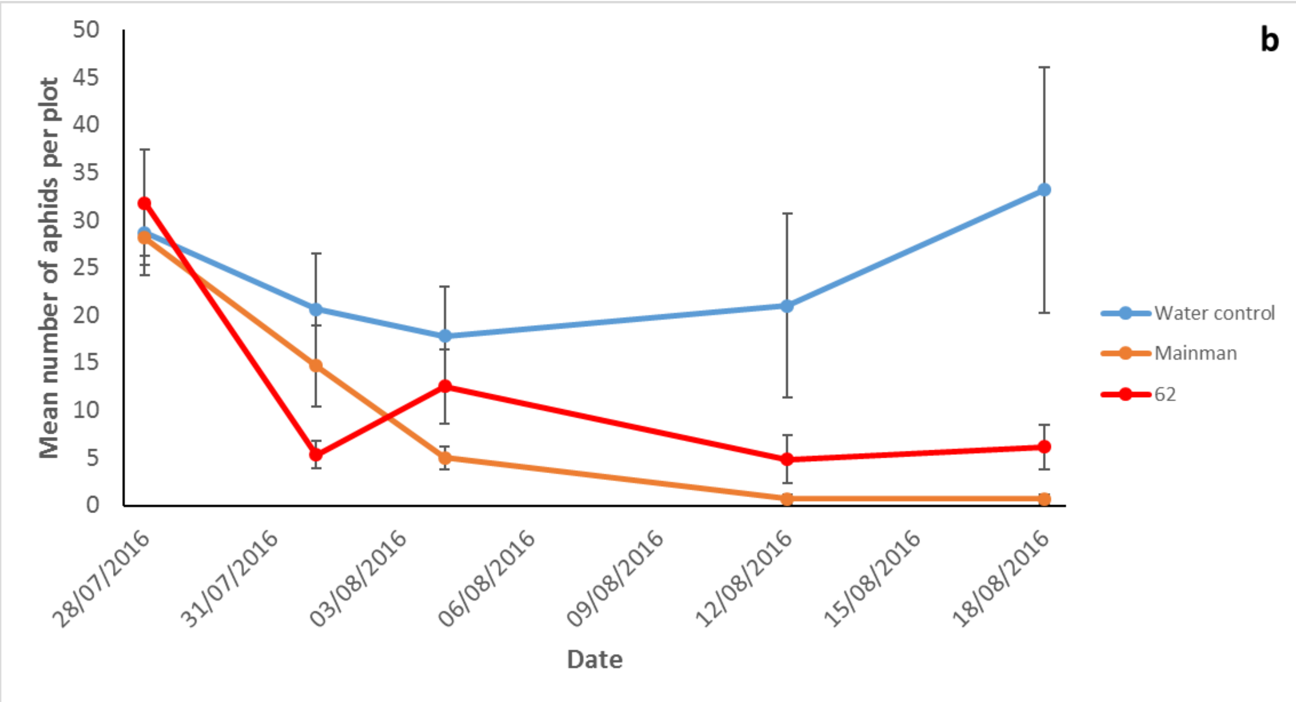

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Mean number of aphids per plot
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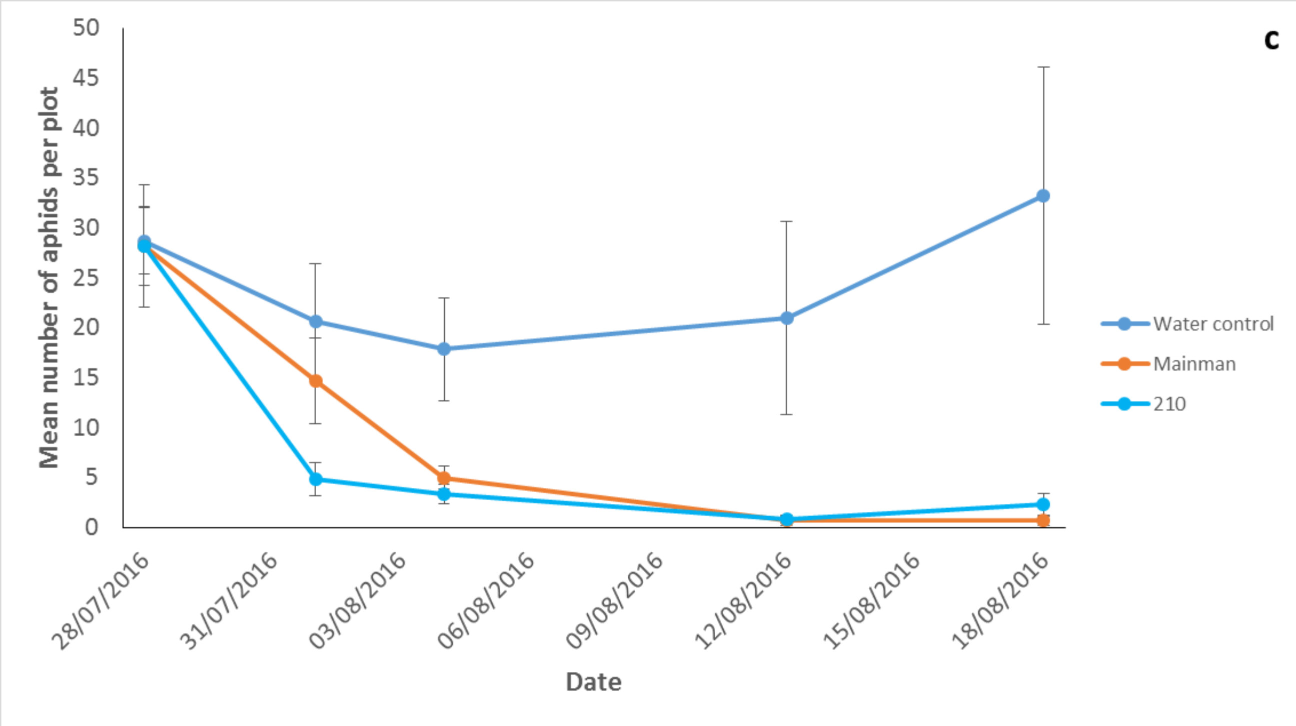
Date
Water control
Mainman
62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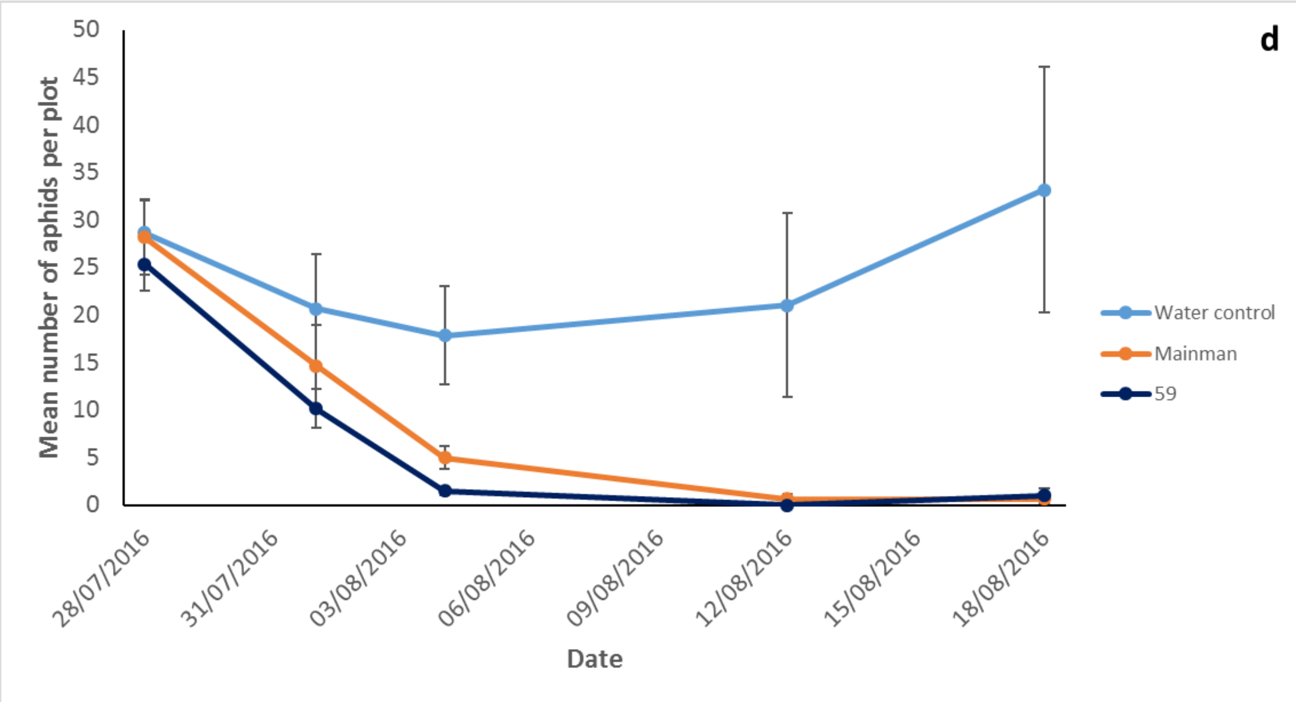
Water control
Mainman
210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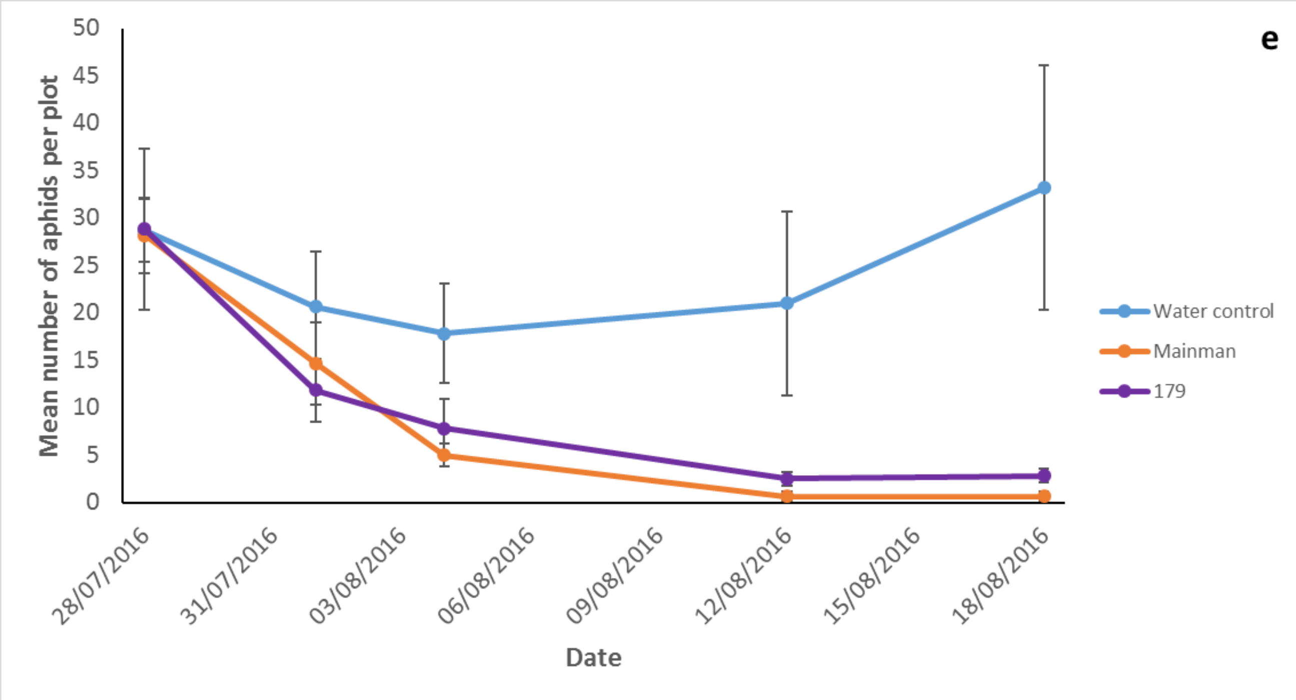
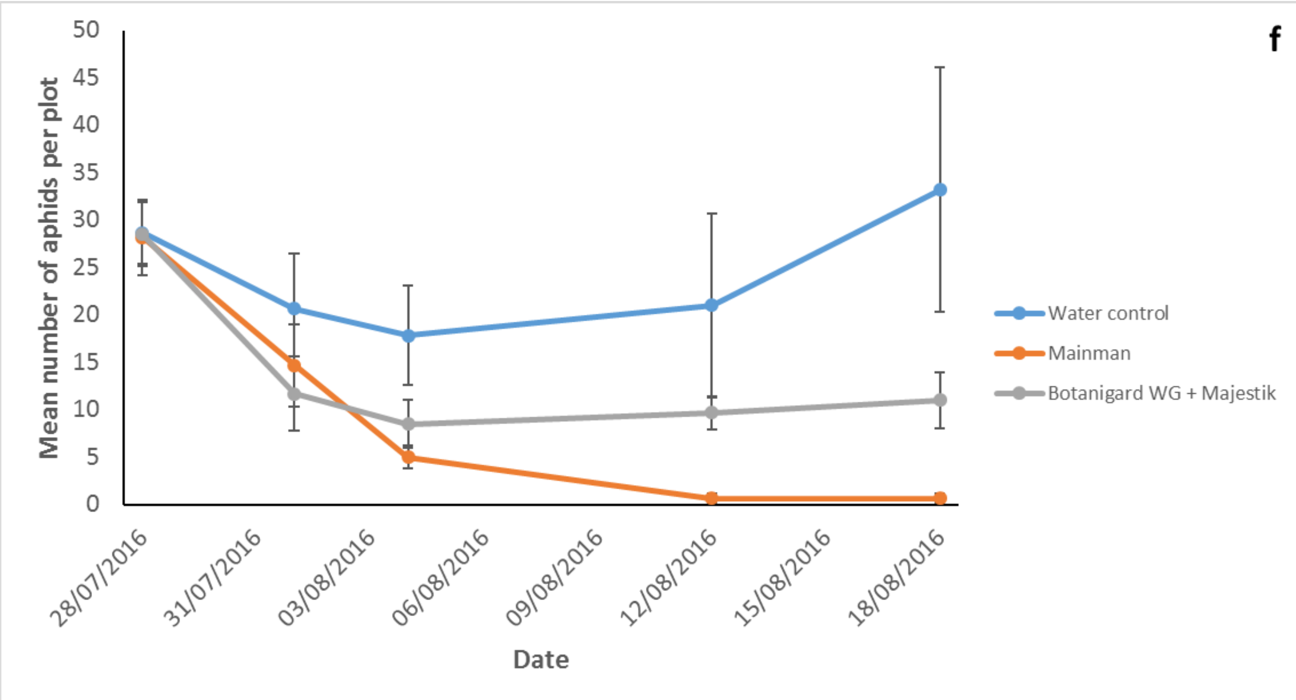
12/08/2016
15/08/2016
18/08/2016
Date
Water control
Mainman
59


e
Mean number of aphids per plot
50
45
40
35
30
25
20
15
10
5
0
28/07/2016
31/07/2016
03/08/2016
06/08/2016
09/08/2016
12/08/2016
15/08/2016
18/08/2016
Date
Water control
Mainman
179


f
Mean number of aphids per plot
50
45
40
35
30
25
20
15
10
5
0
28/07/2016
31/07/2016
03/08/2016
06/08/2016
09/08/2016
12/08/2016
15/08/2016
18/08/2016
Date
Water control
Mainman
Botanigard WG + Majestik

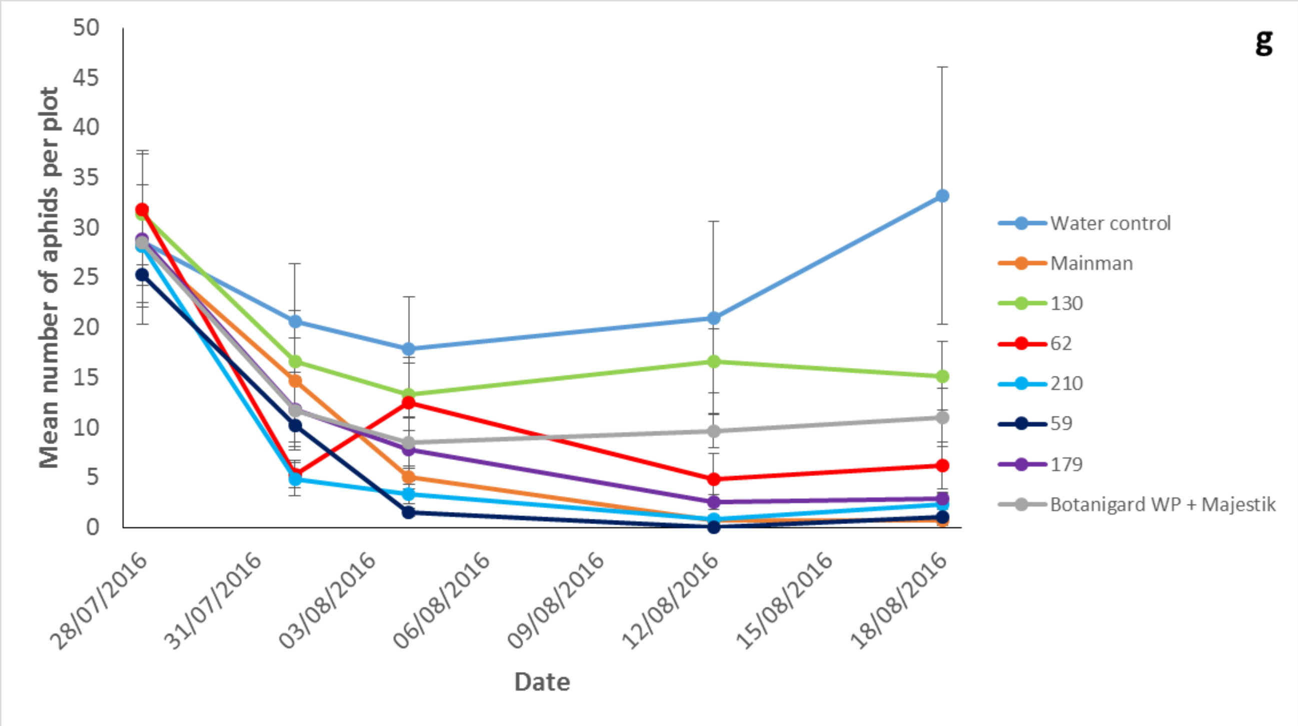


Figure 3. Mean numbers of aphids per plot on each assessment date (9 plants sampled in each plot), with standard errors. Graphs **a** to **f** present results for each product separately against water (-ve control) and Mainman (+ve control). Graph **g** presents results for all products against water (-ve control) and Mainman (+ve control). These graphs are complementary to Table 7 and use averages calculated from raw data, rather than the transformed data used in the statistical analysis. The graphs therefore show actual aphid counts per plot (without transformation) and trends over time in a simpler way so the dynamics of treatment effects can be visualised clearly.

Crop damage

Table 10. Crop damage recorded in terms of both numbers of damaged leaves and flowers in all plots and severity of damage observed (none, slight, medium, strong).

Product name or MOPS code	04/8/2016	12/8/2016	18/8/2016
1. Water (-ve control)	0 leaves 0 flowers	0 leaves 0 flowers	0 leaves 0 flowers
2. Mainman (+ve control)	0 leaves 0 flowers	0 leaves 0 flower	0 leaves 0 flowers
3. 130	0 leaves 0 flowers	0 leaves 0 flower	0 leaves 0 flowers
4. 62	0 leaves 0 flowers	0 leaves 0 flowers	0 leaves 0 flowers
5. 210	0 leaves 0 flowers	0 leaves 0 flowers	0 leaves 0 flowers
6. 59	0 leaves 0 flower	0 leaves 0 flowers	0 leaves 0 flowers
7. 179	0 leaf 0 flower	0 leaf 0 flowers	0 leaves 0 flowers
8. Botanigard WP + Majestik	0 leaves 0 flowers	0 leaves 0 flowers	0 leaves 0 flowers

There was no evidence of phytotoxicity in any of the treatments applied.

Formulations

No problems were encountered during mixing or application of any of the product formulations under test.

Effect on non-target

No effects on other pests were noted during the completion of this trial. A few plants (<5% by 18/8/2016) became naturally infested with peach-potato aphids (*Myzus persicae*) regardless of treatment (no *M. persicae* were found in plots treated with product 59). Very low numbers of thrips were also recorded on plants. Natural enemies included aphid parasitoids (*Aphidius* spp.), seen as mummified aphids, hoverfly adults (mainly *Episyrphus balteatus*), eggs and larvae were also seen. These natural enemies were present in low numbers (aphid mummies were the most numerous but a mean <1 aphid mummy per plant recorded on any one assessment). Despite the low numbers of natural enemies recorded, aphid parasitoids and hoverflies were seen in plots regardless of treatment applied. This demonstrated that some natural enemies survived treatment, however further information would be needed to inform compatibility with biological control agents used in an IPM programme.

Discussion

Each *Hebe* plant was carefully infested with *A. gossypii* on 7 July by placing pieces of aphid infested *Hebe* plant on previously uninfested plants in each plot. By 28 July, one day before the first spray application, the mean numbers of aphids on plants in each treatment was recorded as 2-8 aphids per plant. This suggests that the aphids readily establish on *Hebe*, although numbers increased relatively slowly for this species of aphid. The aphids had been previously cultured through several generations on the same plant species before the start of this experiment. Throughout the experiment aphid numbers declined slightly following application of the water control (-ve control) but then began to increase again and reached a mean of 4 aphids per plant by the end of the experiment (18 August).

No phytotoxicity symptoms on the *Hebe* plants were observed for any treated in this experiment. This included any changes to leaf and flower colour, which were observed, most notably, for products 179 and 62 (both biopesticides) on pansy in the 2014 experiment.

There were significant treatment effects at all assessment dates from six days after the first spray application. The results obtained for Mainman (+ve control) and water (-ve control) were as expected giving confidence when interpreting results for the products. A single application of Mainman gave good control (82% reduction) of the *A. gossypii* one week after the single spray application and this had increased to a 98% reduction two and three weeks after the spray application. The active ingredient in Mainman is flonicamid, a selective homopteran feeding blocker. Similarly, flonicamid gave good control of *A. gossypii* on *Hebe* and *Myzus persicae* on pansy when repeated applications (four applications applied with a seven day interval) were used.

Products 59 and 210 (both conventional insecticides), gave broadly similar levels of control to Mainman when applied as two application with a seven day interval). These two conventional insecticides good control (94 and 88% reductions, respectively) six days after the first spray application. No aphids were found in plots treated with product 59 six days after the second spray application. In plots treated with product 210 control improved to 97% six days after the second spray application. Products 59 and 210 are both neurotoxin, which helps to explain the fast speed of kill. The results reported here for product 59 are similar to those reported for this product used against *A. gossypii* on *Hebe* in 2015 and *Myzus persicae* on pansy in 2014 when repeated applications (four applications applied with a seven day interval) were used.

Mainman, product 59 and product 210 effectively controlled *A. gossypii* after one or two spray applications. Interestingly these three products each have systemic activity, which may have been important in targeting all aphids on each plant and overcoming any limitations in spray coverage. Spray coverage indicated through the use of water sensitive paper was good on upper leaf surfaces but poor on lower leaf surfaces. There was no evidence of any clear difference between the hollow cone or flan fan nozzles used. Products 130, 62, 179 and Bontanigard WP + Majestik (all biopesticides) work through direct contact with the insect but the labels for these products, when approved, will allow more frequent applications (3 or 5 day spray intervals).

Product 179 was applied five times, with a three day interval between applications while product 62 was applied 3 times with a five day interval between applications. For both products, by the time of the aphid assessments 14 and 20 days after the first were completed, all spray applications of these products had been completed. It is noticeable that while greater variability in terms of aphid control was apparent, compared with the conventional insecticides tested, the additional spray applications appeared to improve overall efficacy and reduce variability between plots. Indeed, product 179 gave similar levels of control to Mainman 14 days after the first spray application. This appears to support the idea that improved spray coverage would further increase the efficacy of these two botanical biopesticide products in particular.

Despite the limitations of currently available spray application techniques, products 62, 179 and Botanigard WP + Majestik had all significantly reduced *A. gossypii* numbers by the end of the experiment compared with the water control. As such, these products, in particular products 179 and 62, may be usefully incorporated into IPM programmes used to control *A. gossypii* and help to reduce to reduce the risk of resistance developing to conventional insecticides.

Conclusions

- *Hebe* plants were successfully infested with *Aphis gossypii* and populations gradually increased in the plots treated with the water (-ve control) during the experiment.
- The standard insecticide, Mainman (+ve control), was effective (>80% reduction) at controlling aphid populations just six days after the first spray application.
- All of the products tested, except product 130, gave some control of *A. gossypii* on protected *Hebe*.
- Products 59 and 210 (both conventional pesticides) were fast acting and gave similar, and in the case of product 59 slightly improved, control of *A. gossypii* compared to Mainman.
- Products 62 and 179 (both biopesticides) were effective (>80% reduction) in controlling *A. gossypii* on *Hebe* and can provide a useful option, as part of an IPM programme, to control this aphid pest.
- Botanigard WP + Majestik also reduced aphid numbers compared with the water control and may provide a useful option to control this pest, as part of an IPM programme.
- No phytotoxicity symptoms were seen in this experiment.
- Results presented here are broadly similar to those reported in years one and two of this project in which many of the same products were tested against peach-potato aphid (*Myzus persicae*) on pansy (2014) and melon and cotton aphid on *Hebe* (2015).

References

Foster, S. & Blackshaw, R. (2012). Sustaining the effectiveness of new insecticides against aphid pests in the UK. *Final report to Defra Project RD-2008-3471 (HDC project FV 344)*.

Furk, C. & Hines, C.M. (1993). Aspects of pirimicarb resistance in the cotton and melon aphid, *Aphis gossypii* Glover (Homoptera: Aphidiidae). *Annals of Applied Biology*. 123, 9-17.

Marshall, K.L., Moran, C., Chen, Y.Z. & Hwerron, G.A. (2012) Detection of kdr pyrethroid resistance in the cotton aphid, *Aphis gossypii* (Hemiptera: Aphididae), using a PCR-RFLP assay. *Journal of Pesticide Science*. 37, 169-172.

Appendix A – *Study conduct*

Harper Adams University are officially recognised by United Kingdom Chemical Regulations Directorate as competent to carry out efficacy testing in the categories of agriculture, horticulture, stored crops, biologicals & semiochemicals. National regulatory guidelines were followed for the study.

GLP compliance will not be claimed in respect of this study.

Relevant EPPO/CEB guideline(s)		**Variation from EPPO**
PP 1/152(4)	Design and analysis of efficacy evaluation trials	none
PP 1/135(4)	Phytotoxicity assessment	none
PP 1/181(4)	Conduct and reporting of efficacy evaluation trials including GEP	none
PP 1/23(2)	Aphids on ornamental plants	Size of the plot dividers limited the number of plants to 9 rather than a minimum of 10. Six replicates of each treatment rather than a minimum of four. Hebe is not listed as a test crop to be used in glasshouse trials. Separate polytunnels were not used for different treatments and instead plot dividers were used to effectively prevent insecticide drift.

There were no significant deviations from the EPPO and national guidelines other than those indicated above.

Appendix B – *Meteorological data*

Location of the weather station	52.783, -2.433
Distance to the trial site	400 m
Origin of the weather data	Harper Adams University met station

Long-term averages from *location*			
Month/period	**Min temp (ºC)**	**Max temp (ºC)**	**Rainfall (mm)**
September (1981-2010)	9.1	17.9	57.2
October (1981-2010)	6.3	13.9	67.8

Average conditions during the trial: (datalogger within polytunnel)

Month/period	**Av temp (ºC)**	**Min temp (ºC)**	**Max temp (ºC)**	**Av RH (%)***	**Rainfall (mm)**
18/07/2016 to 18/8/2016	22.2	8.6	42.5	-	-

***protected crops only**

Weather at treatment application: (datalogger within polytunnel)

Month/period	**Min temp (ºC)**	**Max temp (ºC)**	**Rainfall (mm)**
29/7/2016	22.6 & 23.1*	23.6 & 24.1*	-
01/8/2016	22.1	27.1	-
03/8/2016	22.1	27.6	-
04/8/2016	22.1	23.6	-
05/8/2016	29.6	31.1	-
07/8/2016	29.1	29.1	-
08/8/2016	26.1	29.6	-
10/8/2016	20.6	24.6	-

**Separate application for Botanigard WP + Majestik*

Appendix C – *Agronomic details*

Growing system

Crop	Cultivar	Planting/sowing date	Row width (m) or pot spacing
Hebe	Pink Pixie	Plug plants potted up on 12 April 2016	Pots arranged in three rows of three – spacing between pots 5 cm

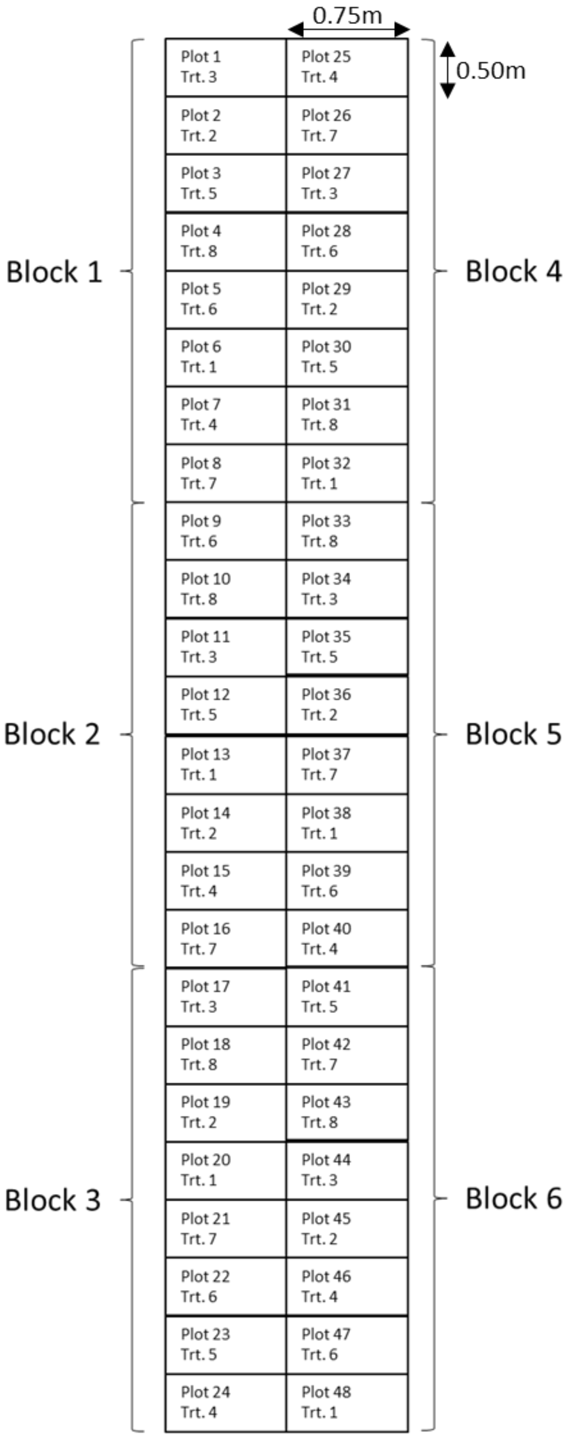
Other pesticides - active ingredient(s) / fertiliser(s) applied to the trial area

Date	Product	Rate	Unit
27/04/2016	Signum	0.9	kg/ha
06/05/2016	Fenomenal	1.7	kg/ha
12/05/2016	Fubol Gold	1.425	kg/ha
23/05/2016	Signum	0.9	kg/ha
19/06/2016	Fubol Gold	1.425	kg/ha
14/06/2016	Pyrethrum 5EC	2.0	l/ha
17/06/2016	Pyrethrum 5EC	2.0	l/ha
20/07/2016	Chempak® Calcium Multi Action Fertiliser – in response to apparent slight calcium deficiency in plants	1	g/l

Details of irrigation regime (pot-grown crops)

Type of irrigation system employed (e.g. overhead sprinkler, hand watering, drip, ebb and flow, capillary sandbed or capillary matting)
Capillary matting

Appendix D – *Trial layout*



Appendix E – ***Copy of the Certificate of Official Recognition of Efficacy Testing Facility or Organisation***

Certificate of

Official Recognition of Efficacy Testing Facilities or Organisations in the United Kingdom

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This certifies that

CERC - Harper Adams University College

complies with the minimum standards laid down in Regulation (EC) 1107/2009 for efficacy testing.

The above Facility/Organisation has been officially recognised as being competent to carry out efficacy trials/tests in the United Kingdom in the following categories:

Biologicals and Semiochemicals
Stored Crops
Agriculture/Horticulture

Date of issue:	26 July 2013
Effective date:	9 April 2013
Expiry date:	8 April 2018

Signature

Authorised signatory

Certification Number
ORETO 343

HSE
Chemicals Regulation Directorate

Department of Agriculture and Rural Development

Appendix F – *Photographs*

	
Figure 4. Plot dividers within polytunnel	Figure 5. Arrangement of 9 *Hebe* plants potted into 9 cm pots within a plot
	
Figure 6. Melon and cotton aphids on a *Hebe* bud in a water control plot	Figure 7. Melon and cotton aphids on stems of a Hebe plant in a water control plot

