Project title:	The effect of jasmonic acid seed treatment on aphid control in lettuce and herb crops
Project number:	PE 012a
Project leader:	Rosemary Collier, University of Warwick
Report:	Final report 2014
Previous report:	None
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Location of project:	Warwick Crop Centre, University of Warwick
Industry Representative:	Claire Donkin Geoffrey Smith
Date project commenced:	1 June 2014
Date project completed (or expected completion date):	31 January 2015

DISCLAIMER

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.

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GROWER SUMMARY

Headline

Soaking seed in methyl jasmonate solutions (2µm or 4µm) prior to transplanting did not reduce infestations of currant-lettuce aphid (*Nasonovia* ribisnigri) on lettuce or willow-carrot aphid *(Cavariella aegopodii*) on parsley.

Background

Aphids are major pests of a range of edible crops in the UK including leafy salads and herbs. Whilst for most crops a range of insecticides are approved for aphid control, and biopesticides or biological control agents are sometimes an option, there are still opportunities for identifying effective non-insecticidal methods of control that could be used as part of an Integrated Pest Management Programme.

Previous unpublished research by Lancaster University and Stockbridge Technology Centre established that treating seed with jasmonic acid enhanced the plants' defence system against a range of pests, resulting in suppressed growth in pest populations and suggested the potential for jasmonic acid to be used as a seed treatment to delay the development of aphid infestations in crops. However, the experiments conducted were small-scale. This idea was followed up in work undertaken at Stockbridge Technology Centre, HDC project PE 012, where researchers investigated the effect of using jasmonic acid as a seed treatment for aphid control in protected herbs and lettuce. Findings indicated that treating seed with jasmonic acid, resulted in reduced numbers of *Myzus persicae* on basil, parsley and lettuce grown under protection and numbers of glasshouse potato aphid, *Aulacorthum solani*, on protected lettuce.

The aim of this project, HDC project PE 012a was to investigate this potential method of control further on protected crops of lettuce and parsley. The two objectives of the project were:

1. To determine the effect of treating lettuce seed with methyl jasmonate on subsequent control of infestations of currant-lettuce aphid.
2. To determine the effect of treating parsley seed with methyl jasmonate on subsequent control of infestations of aphids (hawthorn parsley aphid or another species) and on the performance of introduced biocontrol agents.

Summary

Trial 1 - with lettuce crop propagated under glass and grown to maturity in a polytunnel.

Sub-samples of lettuce seed cv Mirata were treated with methyl jasmonate on 14 July by soaking the naked seed overnight (at about 4°C) at concentrations of 2µmol and 4µmol, rinsing the seed and then sowing it at the rates agreed. There was a 'check' treatment where similar seed was soaked in water only and a control treatment where the seed was not soaked prior to sowing. The seed was sown on 15 July 2014. Plants from the treated seed and from untreated seed were propagated in blocks in a greenhouse and transplanted into a polytunnel on 5 August in plots using a randomised plot design (6 replicates). They were infested on 6 August with adults and nymphs of currant-lettuce aphid (*Nasonovia ribisnigri*) from the culture maintained at Warwick Crop Centre. At maturity (4 September), 10 plants from each plot were sampled destructively to determine the numbers of aphids per plant and plant weight.

Analysis of the numbers of winged and wingless (nymphs and adults) aphids found on the lettuce plants at harvest indicated that there were no statistically-significant treatment effects. Plants from all treatments were infested with similar numbers of aphids. Similarly, there were no statistically-significant differences in plant weight.

Trial 2 - with pots of parsley grown under glass

Sub-samples of parsley seed (cv Dark curl) were treated with methyl jasmonate at concentrations of 2µmol and 4µmol as for lettuce in Trial 1. There was a 'check' treatment where similar seed was soaked in water only and a control treatment where the seed was not soaked prior to sowing. The seed was treated on 19 August and sown on 20 August 2014. Plants from the treated seed and from untreated seed were propagated in 8 cm square pots in a glasshouse (50 seeds per pot) until they had grown sufficiently for the trials. Once they had several leaves, the parsley plants were infested with adults and nymphs from a population of willow-carrot aphid (*Cavariella aegopodii*) obtained and maintained at Warwick Crop Centre. There were two separate trials and the treatments were applied in the 1) absence (Trial 2a) and 2) presence (Trial 2b) of biocontrol agents. The biological control agents used were the parasitoid wasp *Aphidius colemani* (Aphiline c, Syngenta Bioline) and the predatory midge *Aphidoletes aphidimyza* (Aphidoline a, Syngenta Bioline). They were both released in a uniform manner across the trial at a rate of 1000 insects per trial. The potted plants were arranged in a greenhouse in plots (5 pots per plot), using a randomised plot design (6 replicates) and grown to maturity. Trial 2a was treated on 19 August, sown on 20th August, infested on 8th October and assessed from 3 November

by destructive sampling. Trial 2b was treated on 10 September, sown on 11th September, infested on 23 October, parasitoids and predators were introduced on 5 November and the trial was assessed from 20 November by destructive sampling.

In Trial 2a, most of the aphids were willow-carrot aphid, but a small number of plants were infested with a black aphid and data were also collected on this species. There were no statistically-significant differences between the treatments. There were also a small number of aphid mummies. In Trial 2b, most of the aphids were willow-carrot aphid. There were no statistically-significant differences between the treatments. The numbers of aphid mummies and *Aphidius colemani* were also recorded and again there were no statistically-significant differences between the treatments.

Discussion

Disappointingly, neither of the methyl jasmonate treatments produced statistically-significant reductions in the numbers of aphids on either lettuce or parsley. This was in contrast to some of the results obtained in PE 012 in the previous year. However, although the same species of crop were used, the target species were different. In addition, the method of treatment may not have been identical to that in PE 012 as the seeds used in PE 012 were treated by a third party. However, to our knowledge the rates of application were the same.

It may also be worth re-considering the data obtained in PE 012 and particularly the data obtained from the untreated check treatment, which were soaked in water only prior to sowing. In several instances the numbers of aphids recovered from this treatment were lower than the control treatment where the seeds were not soaked in water. It is not clear whether the methyl jasmonate treatments were also compared with this check treatment and if so, whether the differences were statistically-significant.

At the same time as the trial on lettuce, a Masters student at Warwick Crop Centre (Bernad Torevasei) undertook some small-scale experimental work in the laboratory using the same methyl jasmonate treatment (4µm). Firstly, he investigated control of *Myzus persicae* on Brussels sprout (a preferred host) and *Nasonovia ribisnigri* on lettuce. Although fewer *M. persicae* were found on the Brussels sprout plants treated with methyl jasmonate than on the plants grown from untreated seed this was not a statistically-significant difference and there were no differences between treatments in the number of *N. ribisnigri* found on lettuce. He undertook a second small-scale trial looking at the same crop/aphid

combinations and also at *M. persicae* on lettuce (a less suitable host). Half of the pots were exposed to parasitoids (Aphiline S Mix (PACE) - contains equal portions of *Aphidius colemani, A. ervi, A. matricariae, Aphelinus abdominalis, Praon volucre* and *Ephedrus cerasicola*), which were very effective against *M. persicae*. In this case, treatment with methyl jasmonate appeared to reduce the numbers of aphids, although not to a great extent.

Financial Benefits

The results do not indicate a financial benefit to growers of using methyl jasmonate treatments to control aphids.

Action Points

There are no action points for growers.

SCIENCE SECTION

Introduction

Aphids are major pests of a range of edible crops in the UK including leafy salads and herbs. Whilst for most crops a range of insecticides are approved for aphid control, and biopesticides or biological control agents are sometimes an option, there are still opportunities for identifying effective non-insecticidal methods of control that could be used as part of an Integrated Pest Management Programme.

Previous unpublished research by Lancaster University and Stockbridge Technology Centre established that treating seed with jasmonic acid enhanced the plants' defence system against a range of pests, resulting in suppressed growth in pest populations and suggested the potential for jasmonic acid to be used as a seed treatment to delay the development of aphid infestations in crops. However, the experiments conducted were small-scale. This idea as followed up in HDC project PE 012, undertaken at Stockbridge Technology Centre, which investigated the effect of using jasmonic acid as a seed treatment for aphid control in protected herbs and lettuce. The project indicated that treating seed with jasmonic acid, resulted in reduced numbers of *Myzus persicae* on basil, parsley and lettuce grown under protection and numbers of glasshouse potato aphid, *Aulacorthum solani*, on protected lettuce.

The aim of the current project was to investigate this potential method of control further on protected crops of lettuce and parsley. The two objectives of the project were:

1. To determine the effect of treating lettuce seed with methyl jasmonate on subsequent control of infestations of currant-lettuce aphid.
2. To determine the effect of treating parsley seed with methyl jasmonate on subsequent control of infestations of aphids (hawthorn parsley aphid or another species) and on the performance of introduced biocontrol agents.

Materials and methods

<u>Trial 1 - with lettuce crop propagated under glass and grown to maturity in a polytunnel.</u>

Sub-samples of lettuce seed cv Mirata were treated with methyl jasmonate on 14 July using the protocol provided by Nigel Paul, University of Lancaster. This was by soaking the naked seed overnight (at about 4°C) at the concentration required, rinsing the seed and then sowing it at the rates agreed. There was a 'check' treatment where similar seed was

soaked in water only and a control treatment where the seed was not soaked prior to sowing. The seed was sown on 15 July 2014. The treatments are shown in Table 1.

Table 1. Treatments applied to lettuce seed immediately prior to sowing.

	Treatment description
1	Untreated (naked seed),
2	Untreated check (naked seed soaked overnight in water only),
3	2µmol treatment methyl jasmonate (naked seed soaked overnight)
4	4µmol treatment methyl jasmonate (naked seed soaked overnight)

Plants from the treated seed and from untreated seed were propagated in blocks in a greenhouse at Warwick Crop Centre. The plants were transplanted into a polytunnel on 5 August in plots using a randomised plot design (6 replicates) and they were infested on 6 August with adults and nymphs of *Nasonovia ribisnigri* from the culture maintained at Warwick Crop Centre. At maturity (4 September), 10 plants from each plot were sampled destructively to determine the numbers of aphids per plant and plant weight.

Trial 2 - with pots of parsley grown under glass

In May-June 2014, efforts were made to source hawthorn parsley aphid (*Dysaphis apiifolia*) from growers, who were alerted by the HDC, but this was unsuccessful. Efforts were made also to obtain hawthorn parsley aphid in the field at Warwick Crop Centre. A small number of parsley plants were planted in an area surrounded by hawthorn to try to collect the aphids. Aphids colonised the parsley and were subsequently cultured in the Insect Rearing Unit on parsley and fennel. Samples were sent to Rothamsted Research for identification and these were found to be willow-carrot aphid (*Cavariella aegopodii)* a specialist of apiaceous crops. These aphids were used in the trials.

Sub-samples of parsley seed (cv Dark curl) were treated with methyl jasmonate using the protocol provided by Nigel Paul (as for lettuce in Objective 1) and at the rates agreed. The treatments are shown in Table 2. The seed was treated on 19 August and sown on 20 August 2014.

Table 2. Treatments applied to parsley seed immediately prior to sowing.

	Treatment description
1	Untreated (naked seed),
2	Untreated check (naked seed soaked overnight in water only),
3	2µmol treatment methyl jasmonate (naked seed soaked overnight)
4	4µmol treatment methyl jasmonate (naked seed soaked overnight)

Plants from the treated seed and from untreated seed were propagated in 8 cm square pots in a glasshouse at Warwick Crop Centre (50 seeds per pot) until they had grown sufficiently for the trials. Once they had several leaves, the parsley plants were infested with adults and nymphs from the aphid population obtained and maintained at Warwick Crop Centre.

There were two separate trials and the treatments were applied in the 1) absence (Trial 2a) and 2) presence (Trial 2b) of biocontrol agents. For the trials, the potted plants were arranged in a greenhouse in plots (5 pots per plot), using a randomised plot design (6 replicates) and grown to maturity.

Trial 2a was treated on 19 August, sown on 20th August, infested on 8th October and assessed from 3 November by destructive sampling. Trial 2b was treated on 10 September, sown on 11th September, infested on 23 October, parasitoids and predators were introduced on 5 November and the trial was assessed from 20 November by destructive sampling.

The biological control agents used were the parasitoid wasp *Aphidius colemani* (Aphiline c, Syngenta Bioline) and the predatory midge *Aphidoletes aphidimyza* (Aphidoline a, Syngenta Bioline). They were both released in a uniform manner across the trial at a rate of 1000 insects per trial.

The data were analysed using Analysis of Variance.

Results

<u>Trial 1 - with lettuce crop propagated under glass and grown to maturity in a polytunnel.</u>
Analysis of the numbers of winged and wingless (nymphs and adults) aphids found on the lettuce plants at harvest indicated that there were no statistically-significant treatment effects. Plants from all treatments were infested with similar numbers of aphids. Similarly,

there were no statistically-significant differences in plant weight. The results are summarised in Table 3 and Figure 1.

Table 3. Mean numbers of aphids, winged aphids, wingless aphids and mean plant weight at harvest.

	Total aphids		Winged aphids		Wingless aphids		Plant weight (g)
Treatment	SQRT	Back-trans	SQRT	Back-trans	SQRT	Back-trans	
Untreated	8.35	69.67	2.27	5.13	8.02	64.39	16.42
Untreated water	10.35	107.22	2.42	5.85	10.07	101.30	16.44
2µmol methyl jasmonate	9.88	97.52	2.67	7.13	9.47	89.74	16.76
4µmol methyl jasmonate	8.55	73.10	2.53	6.40	8.13	66.14	16.60
F	0.369		0.262		0.391		0.263
P	0.776		0.852		0.761		0.851
Df	20		20		20		20

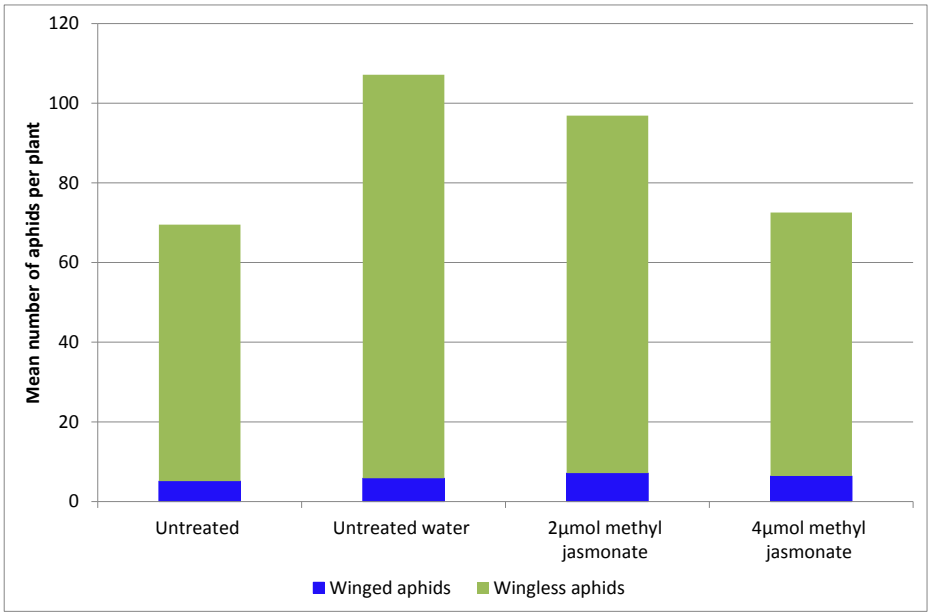

Figure 1. Trial 1 - mean number of winged and wingless (apterae and nymphs) aphids per plant at harvest.

Trial 2 - with pots of parsley grown under glass

Trial 2a

The mean numbers of aphids recovered per plot and the mean numbers of aphid mummies are shown in Table 4. Most of the aphids were willow-carrot aphid, but a small number of plants were infested with a black aphid and data were also collected on this species. There were no statistically-significant differences between the treatments. There was also a small number of aphid mummies. Figures 2 and 3 show respectively the mean number of willow-carrot aphids and all aphids per pot.

Table 4. Trial 2a - Mean numbers of aphids recovered per pot and mean numbers of aphid mummies in Trial 2a.

	Willow- carrot aphid					
	No. winged		No. wingless		Total no.	
	Square root	Back-Trans	Square root	Back-Trans	Square root	Back-Trans
2µmol methyl jasmonate	0.28	0.08	5.61	31.47	5.62	31.62
4µmol methyl jasmonate	0.07	0.01	4.49	20.19	4.49	20.20
Untreated	0.34	0.12	5.83	33.96	5.85	34.22
Untreated water	0.28	0.08	6.04	36.49	6.06	36.68
F	0.828		0.207		0.212	
P	0.494		0.890		0.887	
Df	20		20		20	

	Other aphids					
	No. winged		No. wingless		Total no.	
	Square root	Back-Trans	Square root	Back-Trans	Square root	Back-Trans
2µmol methyl jasmonate	0.55	0.30	4.12	17.00	4.17	17.38
4µmol methyl jasmonate	0.00	0.00	0.07	0.01	0.07	0.01
Untreated	0.00	0.00	0.53	0.28	0.53	0.28
Untreated water	0.00	0.00	1.26	1.59	1.26	1.59
F	1.000		1.301		1.297	
P	0.413		0.302		0.303	
Df	20		20		20	

	Total no. aphids		No. mummies	
	Square root	Back-Trans	Square root	Back-Trans
2µmol methyl jasmonate	8.44	71.24	0.15	0.02
4µmol methyl jasmonate	4.50	20.24	0.00	0.00
Untreated	5.91	34.92	0.18	0.03
Untreated water	6.32	39.93	0.00	0.00
F	0.722		1.027	
P	0.551		0.402	
Df	20		20	

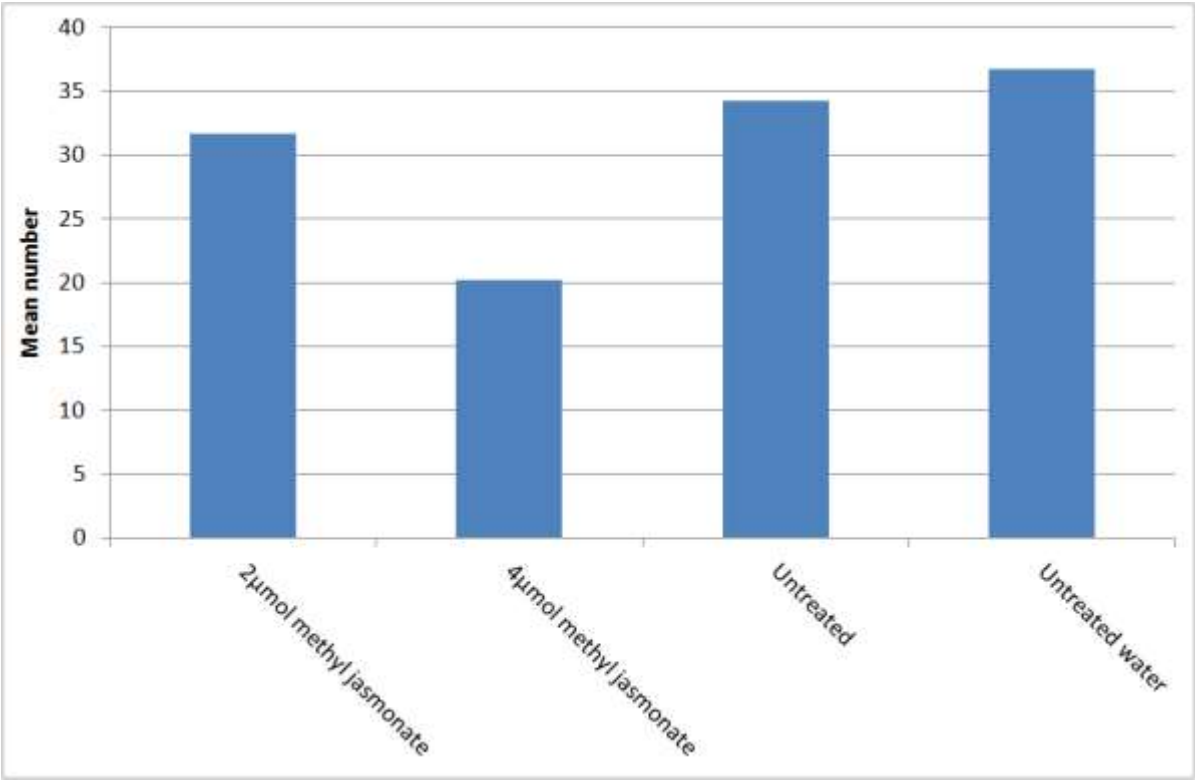

Figure 2. Trial 2a - mean number of willow-carrot aphids per pot

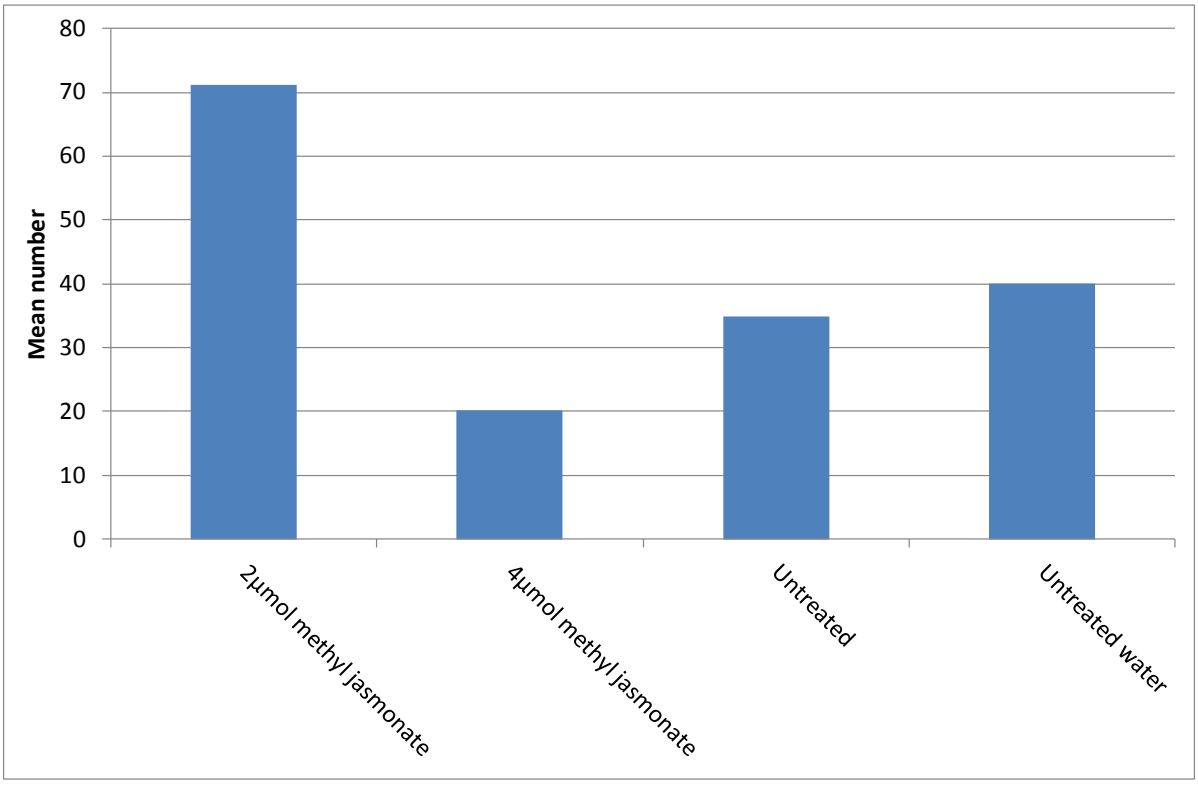

Figure 3. Trial 2a - mean number of aphids (all species) per pot

Trial 2b

The mean numbers of willow-carrot aphids recovered per pot are shown in Table 5. Most of the aphids were willow-carrot aphid. There were no statistically-significant differences between the treatments. Figure 4 shows the mean number of willow-carrot aphids per pot. The numbers of aphid mummies and *Aphidius colemani* were also recorded and are shown in Table 6 and Figure 5. There were no statistically-significant differences between the treatments.

Table 5. Trial 2b - Mean numbers of aphids per pot recovered after sampling

	Winged aphids		Wingless aphids		Total aphids	
	Trans	Back-Trans	Trans	Back-Trans	Trans	Back-Trans
2µmol methyl jasmonate	0.40	0.16	4.37	19.14	4.41	19.41
4µmol methyl jasmonate	0.61	0.37	4.37	19.11	4.42	19.52
Untreated	0.59	0.34	4.97	24.66	5.01	25.08
Untreated water	0.30	0.09	3.54	12.51	3.56	12.66
F	1.323		0.861		0.883	
P	0.295		0.477		0.467	
Df	20		20		20	

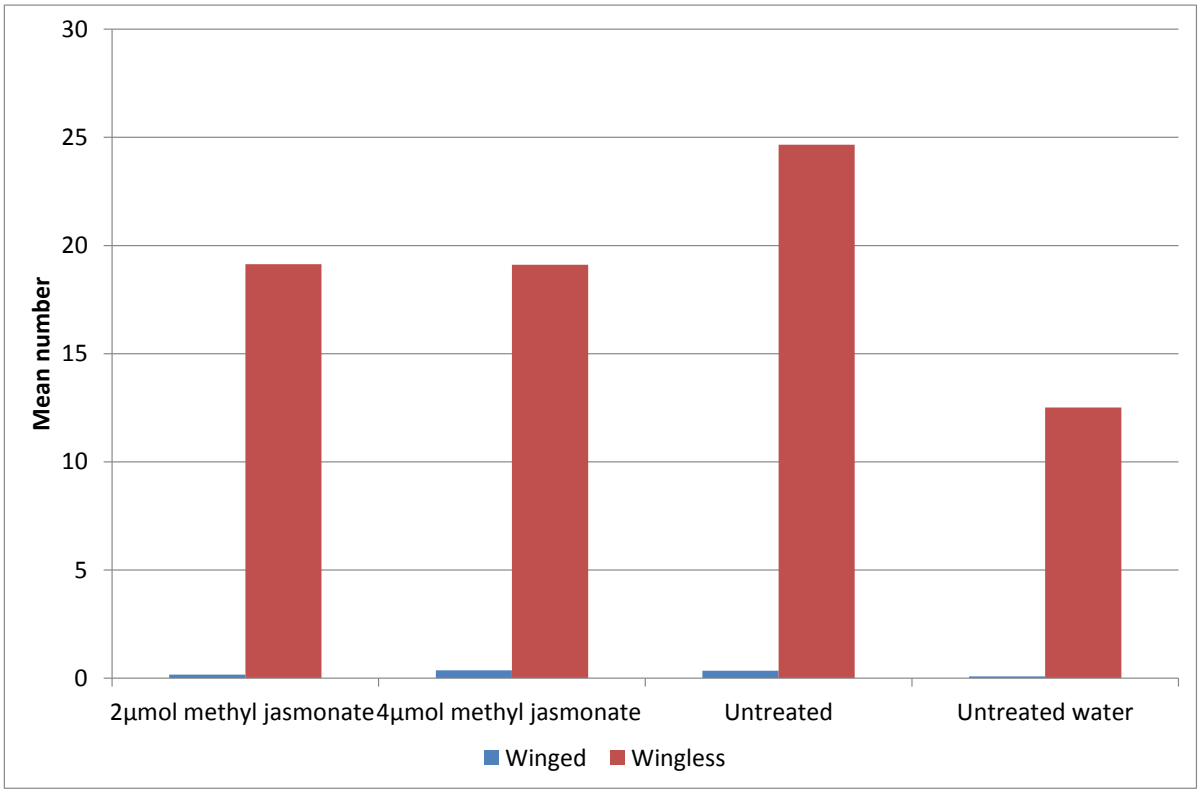

Figure 4. Trial 2b - mean numbers of aphids per pot recovered after sampling

Table 6. Trial 2b - numbers of mummies and parasitoids per pot recovered after sampling

	Mummies		*Aphidius colemani*	
	Trans	Back-Trans	Trans	Back-Trans
2µmol methyl jasmonate	0.79	0.62	0.67	0.44
4µmol methyl jasmonate	0.18	0.03	0.69	0.47
Untreated	0.28	0.08	0.23	0.05
Untreated water	0.63	0.40	0.41	0.17
F	1.236		2.037	
P	0.323		0.141	
Df	20		20	

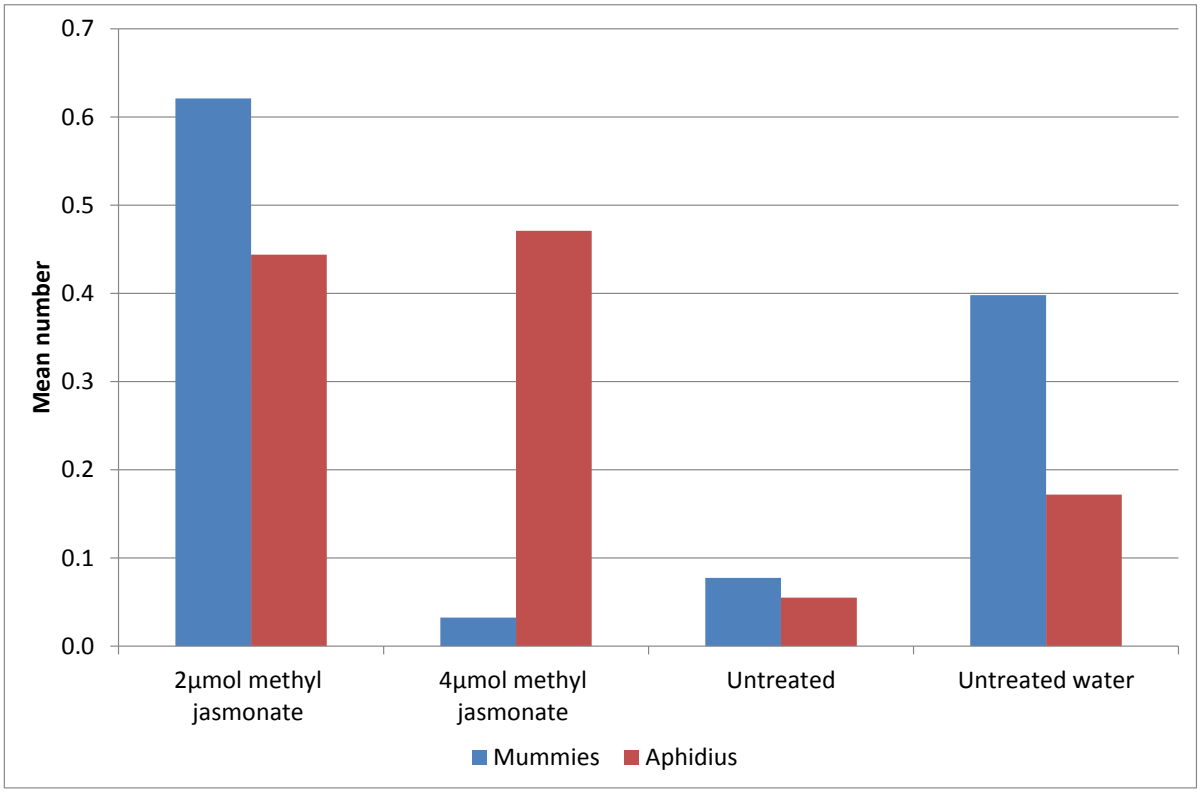

Figure 5. Trial 2b - numbers of mummies and *Aphidius colemani* per pot recovered after sampling.

Discussion

Disappointingly, neither of the methyl jasmonate treatments produced statistically-significant reductions in the numbers of aphids on either lettuce or parsley. This was in contrast to some of the results obtained in PE 012 in the previous year. However, although the same species of crop were used, the target species were different. In addition, the method of treatment may not have been identical to that in PE 012 as the seeds used in PE 012 were treated by a third party. However, to our knowledge the rates of application were the same.

It may also be worth re-considering the data obtained in PE 012 and particularly the data obtained from the untreated check treatment, which were soaked in water only prior to sowing. In several instances the numbers of aphids recovered from this treatment were lower than the control treatment where the seeds were not soaked in water. It is not clear whether the methyl jasmonate treatments were also compared with this check treatment and if so, whether the differences were statistically-significant.

At the same time as the PE 012a trial on lettuce, a Masters student at Warwick Crop Centre (Bernad Torevasei) undertook some small-scale experimental work in the laboratory using the same methyl jasmonate treatment (4µm). He investigated control of *Myzus persicae* on Brussels sprout (a preferred host) and *Nasonovia ribisnigri* on lettuce. Two types of host plant (Lettuce cv Pinokkio and Brussels sprout cv Trafalgar) were used. Half of each set of plants were treated with jasmonic acid (Mej) and the other half was not treated. In each replicate there were 6 plants of each treatment, which were each infested with 4 aphid nymphs (2 days old) of either *M. persicae* (Mp) or *N. ribisnigri (*Nr). All plants were infested with aphids when they were 4 weeks old and data collection (aphid counting) was done three weeks after infestation. The results are shown in Figure 6. Although fewer *M. persicae* were found on the Brussels sprout plants treated with methyl jasmonate than on the plants grown from untreated seed this was not a statistically-significant difference and there were no differences between treatments in the number of *N. ribisnigri* found on lettuce.

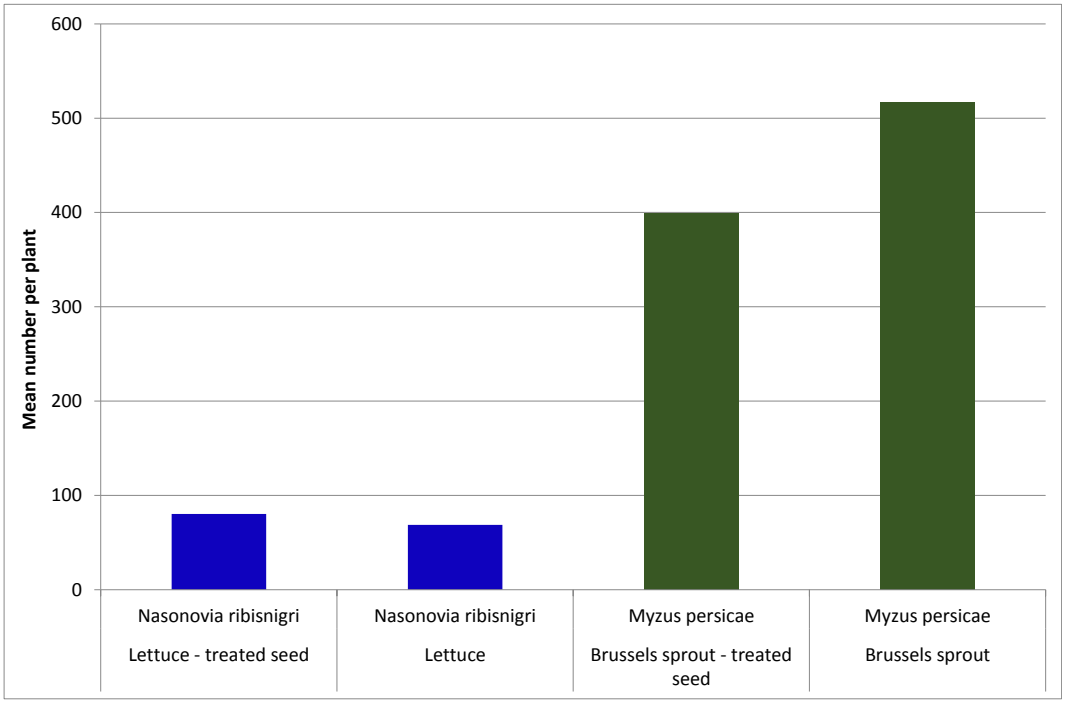

Figure 6. Mean numbers of aphids per plant on plants grown from seed soaked in methyl jasmonate 4µm or from untreated seed.

Bernad undertook a second small-scale trial looking at the same crop/aphid combinations and also at *M. persicae* on lettuce (a less suitable host). Seed was again treated with methyl jasmonate 4µm and treated plants were compared with plants grown from untreated seed. The plants were infested with 4 aphid nymphs when 4 weeks old and then placed in 2 small greenhouses at 5 weeks – one with parasitoids and one without. The numbers of aphids were counted at 8 weeks. In this case, treatment with methyl jasmonate appeared to reduce the numbers of aphids, although infestation sizes differed (Figure 7). The parasitoids (Aphiline S Mix (PACE) - contains equal portions of *Aphidius colemani, A. ervi, A. matricariae, Aphelinus abdominalis, Praon volucre* and *Ephedrus cerasicola*) were very effective against *M. persicae* but ineffective against *N. ribisnigri*.

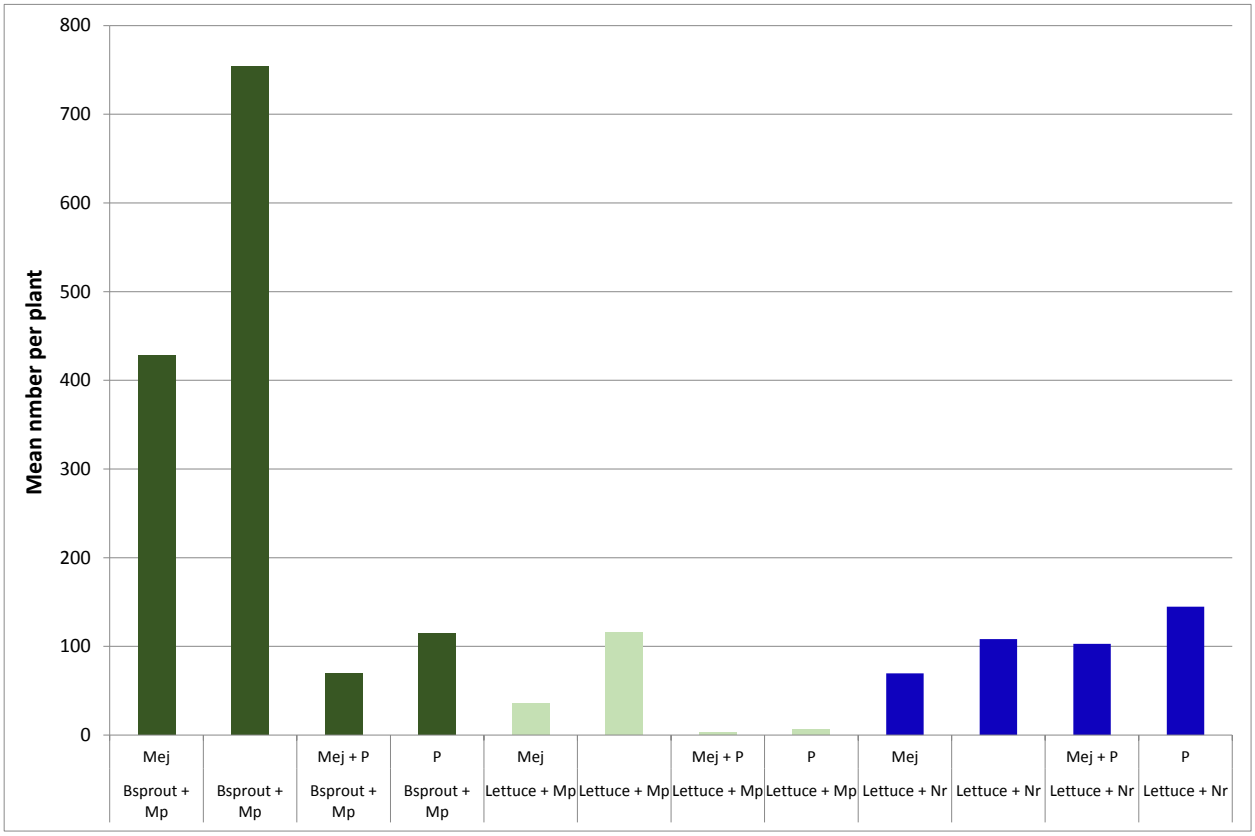

Figure 7. Mean numbers of aphids per plant on plants grown from seed soaked in methyl jasmonate 4µm or from untreated seed and with or without parasitoids. Mej = Methyl jasmonate treatment, Mp = *Myzus persicae*, Nr = *Nasonovia ribisnigri.*

Conclusions

Treatment of seeds by soaking them in methyl jasmonate solutions (2µm or 4µm) prior to transplanting did not reduce infestations of currant-lettuce aphid (*Nasonovia* ribisnigri) on lettuce or willow-carrot aphid *(Cavariella aegopodii*) on parsley.

Knowledge and Technology Transfer

Talk at HDC Roadshow on salad crops at Stoneleigh on 26th November 2015

Talk at West Midlands Fresh Produce Forum on 7 January 2015

