Project title:	The effect of Jasmonic Acid (JA) seed treatment on aphid control in lettuce and herb crops
Project number:	PE 012
Project leader:	Dr. Pat Croft
Report:	Final
Previous report:	NA
Key staff:	Dr. Pat Croft Ms.Catherine Jones
Location of project:	STCRF
Industry Representative:	Claire Donkin, Geoffrey Smith
Date project commenced:	1/6/12
Date project completed (or expected completion date):	30/11/13

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. Pat Croft
Project Leader
Stockbridge Technology Centre Research Foundation

Signature ..... Date .....

Report authorised by:

Dr. Martin McPherson
Science Director
STCRF
Signature ..... Date .....

CONTENTS

GROWER SUMMARY

Headline

Treating seed with Jasmonic acid at 4x the Label Expected Rate resulted in reduced populations of the peach potato aphid on basil, parsley and lettuce which were grown under protection. This same dose rate also reduced populations of the glasshouse potato aphid in protected lettuce.

Background

Aphids present an on-going problem for many horticultural crops including leafy salads and herbs. Although various methods of control, including conventional insecticides (increasingly limited), biocides and biological control agents are available, there continues to be periods or seasons when aphids still are a problem.

Myzus. persicae (peach-potato aphid) is a commonly occurring aphid pest for many growers across different sectors. It can be controlled using parasitoids, e.g. *Aphidius colemani* and the predatory midge *Aphidoletes aphidimyza.* However the use of chemical products, either to act as a knockdown treatment for high aphid populations or against other species of pest, can be a costly disruption to IPM programmes. *M. persicae* can also become a problem if it develops resistance to chemical products, such as pyrethroids.

When under attack from pests and pathogens, plants are able to initiate a range of defence responses. These responses are regulated by chemical pathways within the plant. However, despite the plants defence systems, crops still experience pest and disease problems. Previous unpublished research by Lancaster University and STCRF (see pages 6 and 7 in the full report) established that treating seed with Jasmonic Acid (JA) enhanced the plant's defence system against a range of pests resulting in suppressed growth in pest populations. These results suggested the potential for JA to be used as a seed treatment for the prevention of rapid aphid build-up in crops. However the experiments which were conducted then were small-scale and they were done over a short period of time in non-commercial growing conditions.

The aim of this project was to investigate the effect of using Jasmonic acid as a seed treatment on aphid population build-up throughout the life of the crop in protected herbs and protected lettuce. Crops were grown under the normal commercial practice. .

It is possible that a reduction in the growth of aphid populations through the JA treatment could provide a useful contribution to the control of aphids in conjunction with biocontrol agents. Reducing aphid numbers by a significant percentage could assist parasitoids etc. in maintaining a lower level of the pest and further reducing the need for chemical intervention.

Summary

A series of trials were undertaken at STCRF between June 2012 and October 2013, to determine if JA seed treatment could reduce aphid populations on a selection of short term crops. Two glasshouse herb crops were examined: basil and curly leaved parsley. Glasshouse lettuce crops were also examined, looking at flat and curly leaved varieties in propagation and in the production house. JA was applied to seeds at four rates: 0.2x, 1x, 2x and 4x LER (Label Expected Rate). In addition there were two controls; seed that went through the commercial process without the application of JA (untreated check), and completely untreated seed (untreated control).

The results below list the responses of the aphid populations to the different rates of JA on basil and curly parsley and; flat and curly leaf lettuce.

Basil

Three trials were undertaken with weekly introductions of aphids. *M. persicae* was either artificially introduced or natural populations were allowed to establish during the 28 days of crop growth. Plants grown from JA treated seed had reduced population growth of aphids compared to the control plants. The response of the aphid populations to different rates of JA varied, however the high rate (4LER) consistently resulted in the greatest reduction of aphids throughout the trials.

- ***21 - day experiment*** (aphids were introduced onto plants after 7 days and assessed 21 days later) - plants from 4x and 2x the LER had significantly lower numbers of aphids than the controls.

- ***14 day experiment*** (aphids introduced 14 days after day 1 with the final assessment being done 14 days after the aphid introduction): All treatments, except 0.2x LER resulted in significantly lower numbers of aphids compared to the controls.

- ***7 day experiment*** (aphids introduced to plants after 21 days and assessed 7 days later): All treatments, except 2x LER, recorded significantly lower numbers of aphids than the controls.

Curly parsley

In a single trial, small numbers of *M. persicae* were introduced on to curly parsley plants. In addition, a large natural infestation of the pest occurred. The results showed that at the final assessment 4x LER had significantly lower numbers of aphids compared to the controls. However, the large natural infestation of aphid that occurred during the trial did result in aphids being recorded throughout the trial period (Fig. 1).

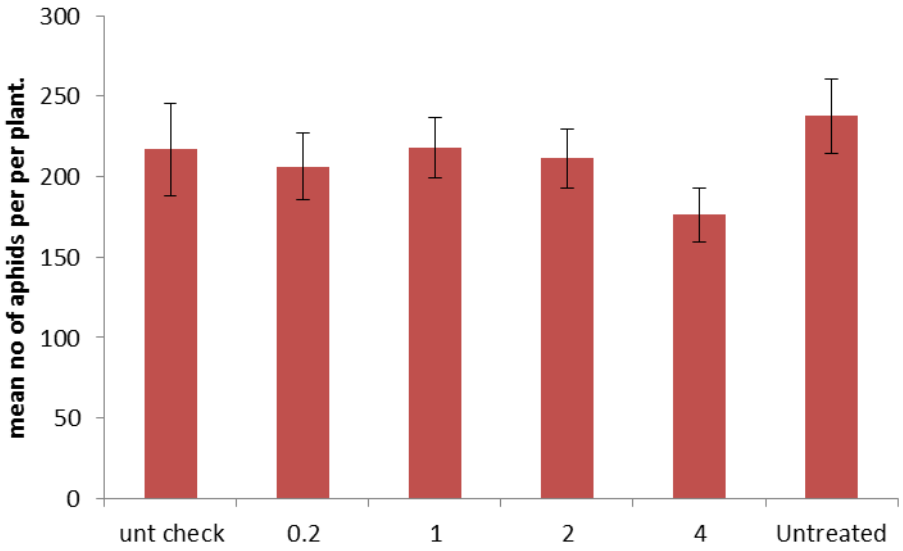

Fig. 1. The mean numbers of *M. persicae* recorded after 21 days with different seed treatments (0.2x, 1x, 2x and 4x LER) plus two controls.

Hawthorn parsley aphid (Dysaphis apiifolia) on curly parsley

In a single trial, the effect of the high rate JA treatment (4x LER) on Hawthorn-parsley aphid was compared to the untreated check and the untreated control. The results suggested that 4x LER could reduce the population growth of Hawthorn –parsley aphid by 35% compared to the untreated control although this result was not statistically significant.

Protected Lettuce

1. Lettuce - flat leaf

In propagation

Lettuce seedlings grown from seed treated with JA showed reduced populations of *M. persicae.* The 2x and 4x LER treatments resulted in aphid numbers which were significantly lower than those in the untreated lettuce plants at the point of planting (Fig. 2). However,

additional control measures would be needed to reduce aphid numbers to levels that are commercially acceptable.

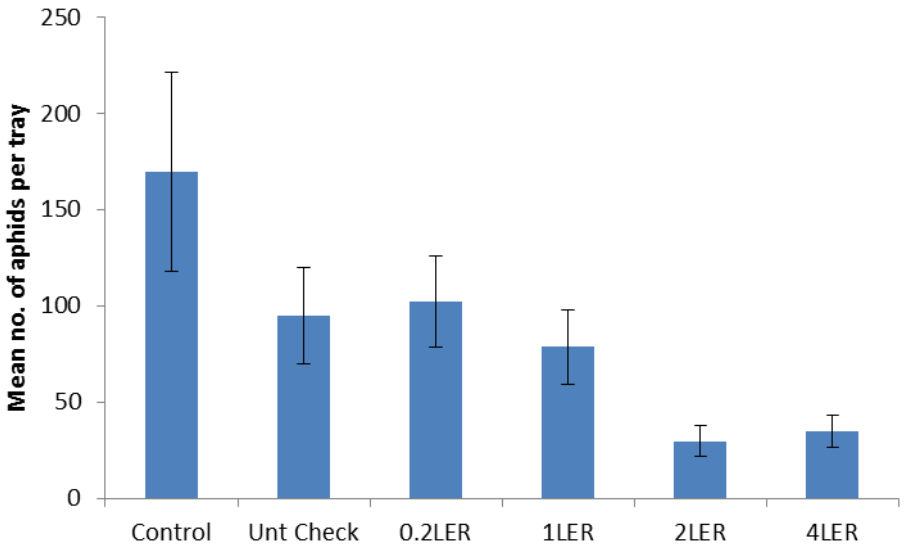

Fig.2. The mean numbers of aphids (*M. persicae*) per propagation tray at final assessment (August 2012)

Glasshouse Crop

A natural infestation of *Aulacorthum solani* (Glasshouse peach aphid) occurred in the trial and was used to assess the efficacy of the JA treatments. For the final assessment at harvest, the numbers of aphids recorded on plants treated with 2x and 4x LER were significantly ($p<0.05$) lower than numbers recorded on the control plants (Fig. 3).

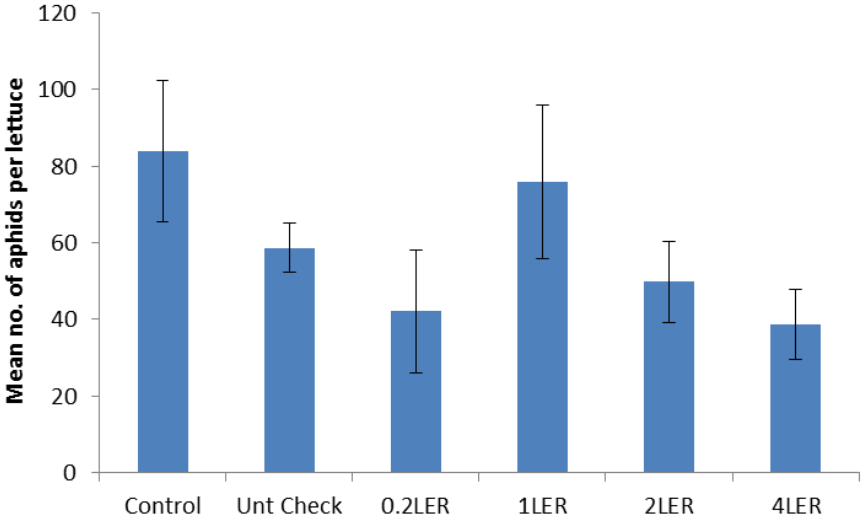

Fig.3. The mean numbers of *A. solani* at harvest, (Aug 2012).

Lettuce – curly

A further trial was conducted in the glasshouse using a curly lettuce variety (cv Fairplay) which was artificially infested with *M. persicae*.

Numbers of aphids at harvest were very variable, ranging from 0 to 28 per plant. Plants treated with 4x LER had the lowest number of aphids: there were 30% fewer aphids than in the untreated controls. Though results were not statistically significant, they followed the trend established in previous trials.

Germination and phytotoxicity

The percentage seed germination for each treatment and crop were recorded at the start of each trial. JA seed treatment did not affect the germination of any of the crops and there was no recorded phytotoxicity effects observed following treatment application.

Financial benefits

The results demonstrate the potential of using JA seed treatment as an additional tool in the armoury for the control of aphids in IPM programmes to protected herb and possibly lettuce crops.

*** Details of future work will be given in the tender which will be advertised on the HDC website soon.**

SCIENCE SECTION

Introduction

Aphids present a continuing problem for many horticultural crops. Although various methods of control, including conventional insecticides (increasingly limited), biocides and biological control agents are available, there are continuously periods or seasons when aphids become a problem.

M. persicae is *a* commonly occurring aphid pest for a wide range of crops. It can be controlled biologically using parasitoids, e.g. *Aphidius colemani* and the predatory midge *Aphidoletes aphidimyza.* However the use of certain chemical products can have negative side effects on some biocontrol agents resulting in a subsequent increase in the aphid population. *M. persicae* can also develop resistance to chemical products following repeated use, e.g. pyrethroids. In addition the use of chemical products often results in the need for a re-establishment of the biological control programme, which results in an added cost to the grower.

When under attack from pests and pathogens, plants are able to initiate a range of defence responses. These responses are regulated by different signalling pathways controlled by phytohormones such as Jasmonic acid (JA), salicylic acid (SA) and ethylene. However, despite their defence systems crops will experience pests and diseases. Unpublished research by Lancaster University and STCRF established that treating the seed with JA enhanced the defence system against a range of pests.

JA Seed Treatment Studies

Earlier small scale experiments at Lancaster University and STCRF (unpublished), established the potential for JA seed treatment to reduce pest levels in a range of crops.

The experiments also looked at aphids. *Nasonovia ribisnigri* (currant-lettuce aphid) on protected lettuce (4 true leaf stage) (see Fig 4 above) and *Myzus persicae* on peppers (Fig.5).

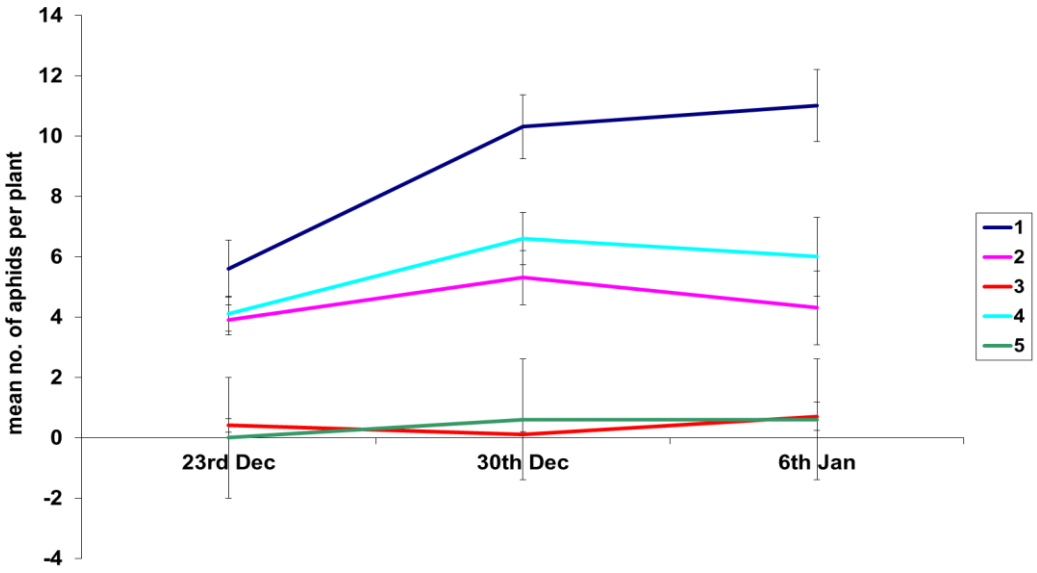

Fig. 4. Effects of JA treated Lettuce plants in propagation on numbers of *N. ribisnigri* aphids. JA seed (3), Control (1). Research undertaken by STCRF:

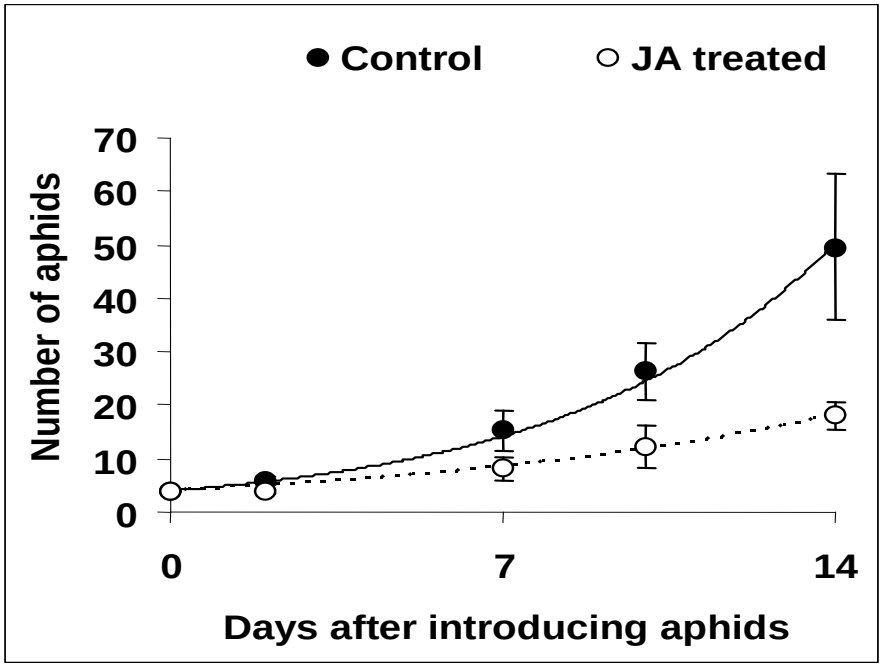

Fig. 5 Effect of JA on numbers of *M. Persicae* on pepper plants Research undertaken by Lancaster University (unpublished).

The results so far obtained indicate that there is a potential for the seed treatments to prevent numbers of aphids increasing rapidly. However experiments conducted were small scale and over a relatively short period of time in non-commercial growing conditions.

The aim of this project was to test the JA seed treatment in representative growing conditions over longer periods of time, and ultimately to look at the interaction with biocontrol agents. In addition the project will monitor disease and yield in the crops, to determine any effect of seed treatment.

Materials and methods

The following trials on herbs and protected lettuce looked at four rates of JA seed treatment; 0.2, 1.0, 2.0 and 4.0 LER (Label Expected Rate), plus two controls; untreated check and untreated control. Seeds were sent to industry partner Becker Underwood (now part of BASF), where they were treated through a commercial process. Untreated check refers to seeds that went through the process without JA being applied, untreated control refers to seeds that have not been processed or treated.

Herbs

1. Basil

In the first trial undertaken the efficacy of JA against *M. persicae* on Basil was determined and the efficacy of the product during the cropping period was also examined.

Seeds were sown on day one of the trial, 15 seeds per pot (8cm diam.). Ten adult aphids (*M. persicae*) were introduced to 10 pots on three occasions in August 2012 at seven day intervals at the 2-4 true leaf stage (7 day, 14 day & 21 day refers to the number of days the aphids were on the plants before the final assessment). Pots were grown in a glasshouse (night: min 16^0C, vent 21^0C, day: 16^0C min, vent 19^0C). Numbers of aphids were recorded at seven day intervals after introduction. After 28 days the final assessment was performed.

2. Curly parsley

Five seeds were sown into 8cm pots with fifteen pots per treatment.10 adult *M. persicae* were introduced on day one of the trial (4 true leaf stage) (min 15, venting at 19°C). Numbers of aphids were assessed at seven day intervals. The final assessment was done three weeks after aphid introduction.

Analysis was on the square root transformed data and means were compared using Least significant difference (0.5% level of significance

3. Hawthorn parsley aphid (Dysaphis apiifolia) on curly parsley

In a small replicated trial, the effect of the high rate JA treatment (4LER) was compared to the untreated check and the untreated control. Ten adult Hawthorn-parsley aphids were introduced to each pot at 4 true leaf stage and assessed at seven day intervals. There were 10 replicates per treatment, assessments were done weekly.

Lettuce

1. Lettuce-flat leaf

Two experiments were conducted: lettuce seedlings (cv Diana) in propagation and lettuce crop looking at JA seed treatment against *M. persicae* and *Aulacorthum solani*

Lettuce in propagation

- Lettuce sown in propagation trays and infested with aphids equal numbers of aphids at the 2- 4 true leaf stage
- 6 treatments (as above) with ten replicates (trays) per treatment.
- Aphid numbers per plant counted after seven days

Lettuce crop-post planting

- Lettuce plants propagated from seed and transplanted to a glass house in a randomised block design: 4 blocks 36 plants per plot
- Crop left to become naturally infested with aphids
- 5 plants randomly selected and aphid numbers counted a lettuce harvest.

In addition the second trial determined if the JA seed treatment had any affect against the disease downy mildew, caused by the Oomycete *Bremia lactucae*. Plant material with downy mildew was introduced into each plot after planting. However despite repeated inoculations of the disease it failed to establish across the trial in any treatments.

2. Curly lettuce crop

- Lettuce plants (cv Fairplay) propagated from JA treated seed (six treatments as above) and transplanted to a glasshouse in a randomised block design: 4 blocks 36 plants per plot.
- The crop was artificially infested with aphids (*Myzus persicae*) five adults per plant.

- Five plants were randomly selected and numbers of aphids counted weekly and at lettuce harvest.

Analysis

Experiment 1 analysed using a Kruskal- Wallis and post- hoc pairwise Wilcoxon tests.

Experiment 2 analysed by means of a two-way ANOVA after data transformation.

Results and discussion

Herbs

1. Basil

21- day experiment

Refers to aphids introduced onto plants after 7 days and finally assessed 21 days later.

Data were transformed using the box cox transformation method with a lambda value of -0.1 to satisfy the assumptions of normality and equality of variance. There was a significant difference among treatment means (ANOVA: $F_{5,54}$= 5.467, P<0.001). A Dunnett's test compared the means for each treatment.

4LER and 2LER had significantly lower numbers of aphids than the control.

Table 1.. Treatment means per pot for aphid numbers on basil plants 21 days after aphids were transferred to the plants. Results of post-Hoc Dunnett's tests comparing treatment means to control means are also shown.

Treatment	Control	Untreated check	0.2 LER	1 LER	2 LER	4 LER
Mean	**6.3**	**5.6**	**9.4**	**6.4**	**2.8**	**1.6**
sd	5.4	3.8	5.3	6.2	4.2	1.3
Dunnett test (t)		-0.196	1.107	-0.424	2.674	-3.127
P		0.999	0.715	0.994	0.043*****	0.014*****

* indicates treatment mean is significantly different to the control mean.

14 day experiment

This trial refers to plants were aphids were introduced 14 days after day 1 and the final assessment was done 14 days after aphid introduction.

Data were transformed using the box-cox transformation method with a lambda value of 0.1. There was a significant difference among treatment means of aphid numbers (ANOVA: $F_{5,54}$= 18.57, P<0.001).

All treatments, except 0.2 LER, produced significantly lower numbers of aphids compared to the control.

Table 2. Treatment means per pot for aphid numbers on basil plants 14 days after aphids were transferred to the plants. Results of post-Hoc Dunnett's tests comparing treatment means to control means are also shown.

Treatment	Control	Untreated check	0.2 LER	1 LER	2 LER	4 LER
Mean	24.0	4.8	25.3	1.9	9.7	1.6
sd	14.0	2.3	11.4	1.8	11.0	1.8
Dunnett test (t)		-4.291	0.175	-6.412	-3.655	-6.898
P		<0.001*	0.999	0.001*	0.003*	<0.001*

* indicates treatment mean is significantly different to the control mean.

7 day experiment

This trial refers to aphids introduced on day 21 and assessed seven days later at the final assessment.

Data were transformed using the box-cox transformation method with a lambda value of 0.1. Mean numbers of aphids among treatments were significantly different (ANOVA: $F_{5,54}$= 6.795, P<0.001).

All treatments, except 2LER, recorded significantly lower numbers of aphids than the control.

Table 3. Treatment means per pot for aphid numbers on basil plants 7 days after aphids were transferred to the plants. Results of post-hoc Dunnett's tests comparing treatment means to control means are also shown.

Treatment	Control	Untreated check	0.2 LER	1 LER	2 LER	4 LER
Mean	9.1	3.4	4.2	3.1	6.0	0.8
sd	7.1	1.7	3.6	2.3	6.9	0.8
Dunnett test (t)		-2.609	-2.548	-3.181	-1.936	-5.577
P		0.012*	0.014*	0.002*	0.058	<0.001*

Fig. 6 shows the final aphid counts on day 28 of the trial. Day 14 into the trial there was a large natural infestation of *M. persicae* into the trial, which introduces larger variability into the trial; however there are still significant difference recorded between JA treated and non-treated plants.

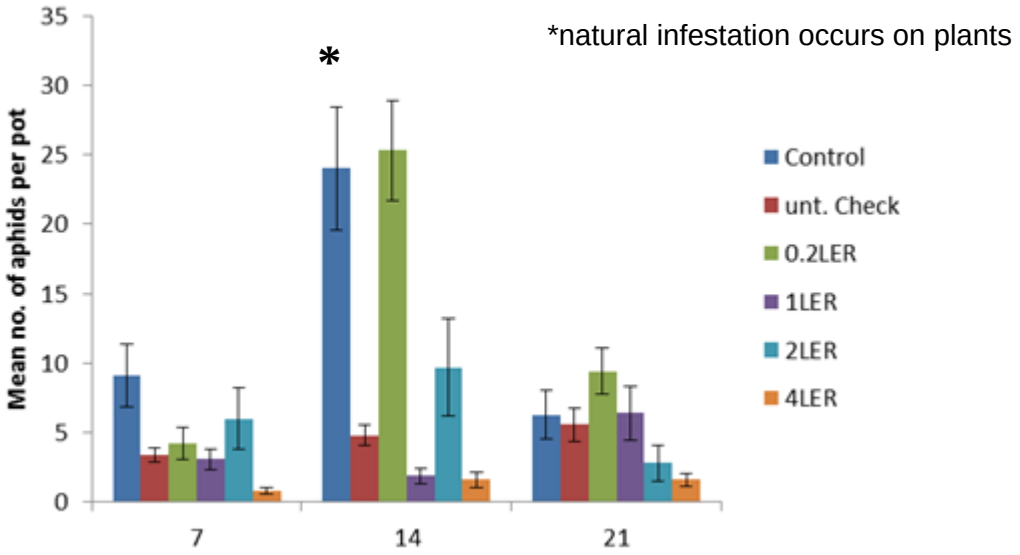

Fig.6. Mean numbers of aphids at final assessment, when aphids have been introduced on plants for 7, 14 and 21 days respectively.

The results in the above trials demonstrate that throughout the period of the basil crop, JA seed treatment can significantly reduce numbers of aphids. This is consistently observed for the highest rate of JA, 4LER and is effective against aphids for at least three weeks (see 7day data) into the crop.

2. Curly parsley

At the final assessment 4LER had significantly lower numbers of aphids compared to the control (Fig.7). Numbers of aphids overall were very high due to a large natural infestation of *M. persicae* that occurred before day 7 of the trial, resulting in large variability within treatments and an overall large population of aphids across all treatments.

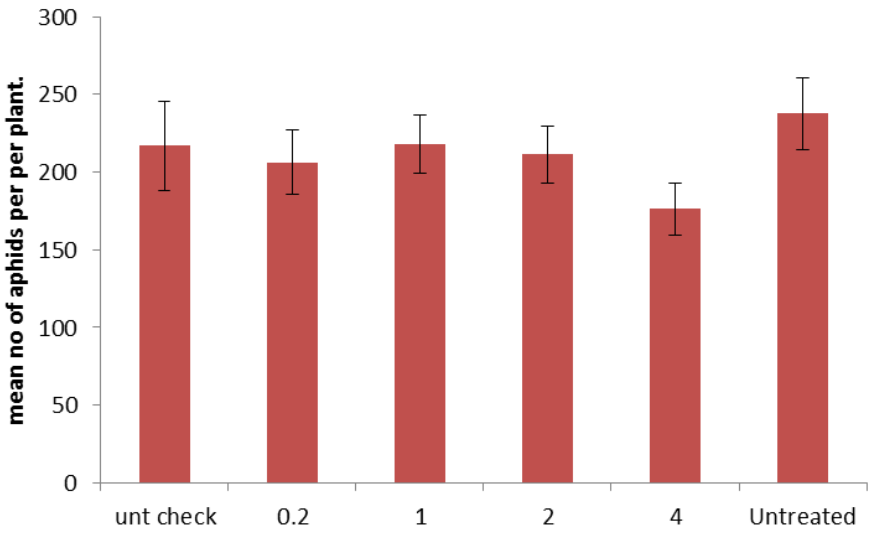

Fig. 7 Mean numbers of *M. persicae* recorded after 21 days with different seed treatments (0.2, 1.0, 2.0 and 4 LER) and two controls.

3. Hawthorn parsley aphid

The results showed that at the final assessment 4LER had significantly lower numbers of aphids compared to the control. The results in Fig. 8 show that 4LER had the lowest number of aphids, but this just avoided being at the significant level. Subsequent assessments were abandoned due to the presence of predator infestation that had moved into the trial.

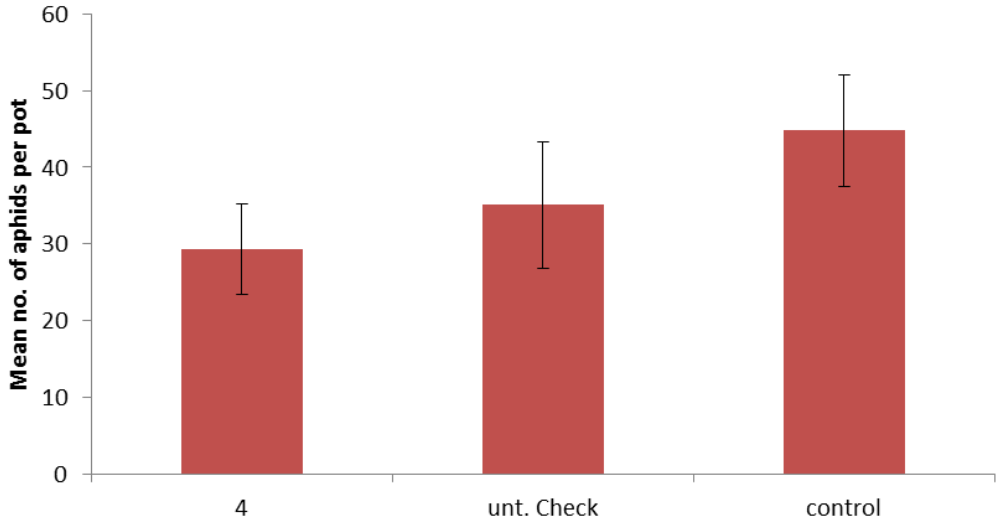

Fig. 8. Mean numbers of Hawthorn parsley aphid on curly parsley after seed treatment with JA (4 LER) compared to untreated check (seed processed but not treated) and untreated control.

The trial confirmed the trend in results obtained previously on basil, in that JA can reduce population growth in aphids.

Lettuce

1. Flat leaf

Lettuce in propagation

Analysis of the results revealed a significant difference among treatment medians (X^2= 21.721, p<0.001).

The results demonstrated that seedlings treated with 2 and 4 LER had significantly lower numbers of aphids than the control treatments (Fig. 9).

Table 4. Mean numbers (n=10) of aphids (*M. persicae*) per tray (100 plants per tray) on propagated lettuce and pairwise comparisons between control and treatment means. * indicates treatment median is significantly different to the control median.

Treatment	Control	Untreated check	0.2 LER	1 LER	2 LER	4 LER
Mean	169.7	94.9	102.3	78.6	29.8	34.7
Sd	163.6	78.7	75.6	60.8	24.7	26.0
Wilcoxon statistic		69.0	65.0	72.5	95.5	95.0
P		0.162	0.273	0.096	<0.001*	<0.001*

Comparison of medians (Post- hoc pairwise Wilcoxon tests) showed that the numbers of aphids for both the 2.0 LER and 4.0 LER treatments were significantly lower than the control median (2LER: W=95.5, <0.001; 4 LER: W=95.0, P<0.001) (see Fig.9).

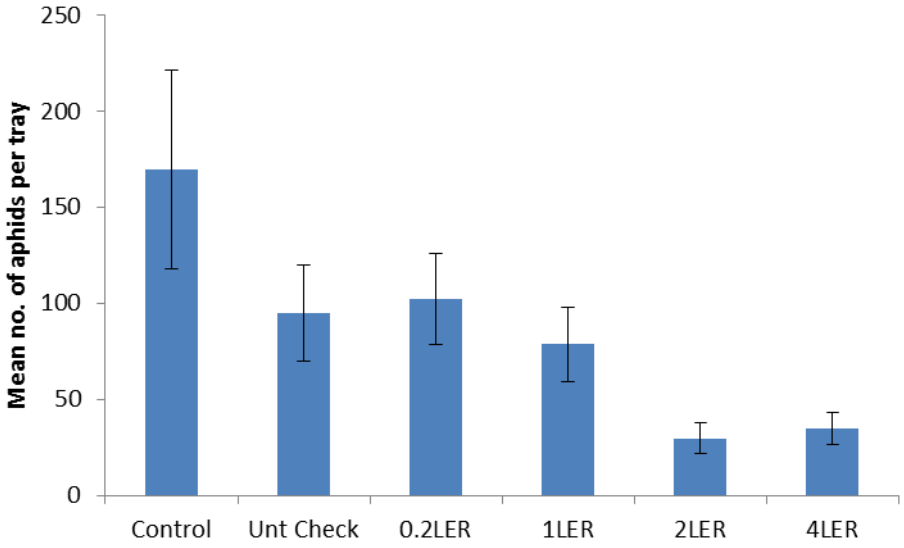

Fig.9. The mean numbers of aphids (*M. persicae*) at final assessment, comparing different rates of JA seed treatment.

Lettuce crop-post planting

Aphids (*A. solani*) naturally infested the lettuce crop and the final assessment at harvest is analysed below.

Table 5 and Fig. 10 show there the significant difference among treatment means with 2 LER and 4LER reducing numbers of aphids significantly compared to the controls. (ANOVA: $F_{5,108}$=3.340, P=0.008).

Data differed significantly from a normal distribution so were transformed using the Box-Cox method, with a lambda value of 0.3. Data were then analysed using two- way ANOVA to assess for treatment and block effects.

Table 5. Treatment means of aphid numbers per plant on the lettuce crop and post hoc Dunnett's tests comparisons of mean between control and treatment means. * indicates treatment mean is significantly different to the control mean.

Treatment	Control	Untreated check	0.2 LER	1 LER	2 LER	4 LER
Mean	83.9	62.3	66.1	75.9	49.8	38.8
Sd	36.7	39.0	24.3	40.0	21.2	18.3
Dunnett test (t)		-1.861	-1.324	-0.872	-2.576	-3.888
P		0.239	0.557	0.864	0.0497*	<0.001*

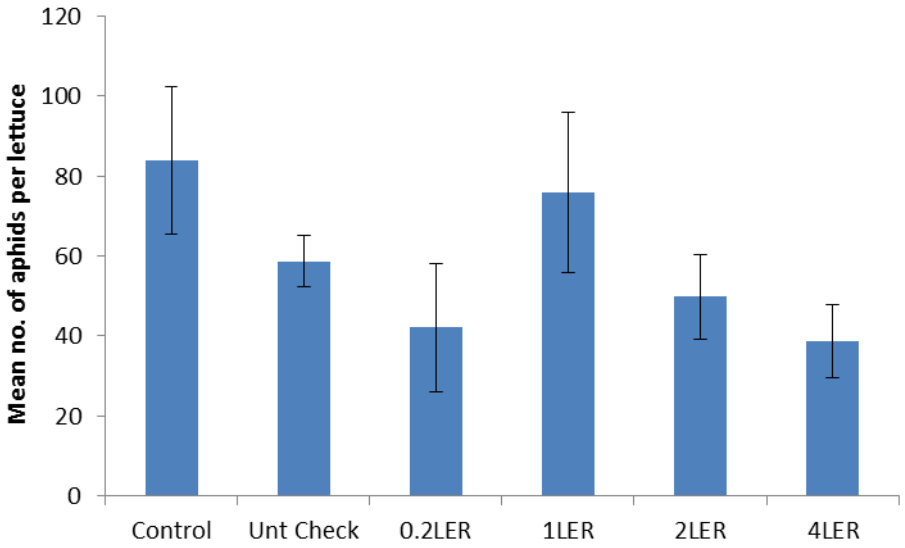

Fig. 10. The mean numbers of *A. solani* at harvest, lettuce treated with different rates of JA.

2. Lettuce -curly

The lettuce glasshouse crop trial was repeated but using a curly variety of lettuce (cv Fairplay) artificially infested with *M. persicae*.

The results in the trial were very variable, as can be seen by the large SE bars on Fig. 11 Analysis of the data failed to establish a statistically significant difference between the different treatments; however as Fig.11 shows the mean numbers of aphids on JA treatments are lower than the control plots. Aphid numbers were very variable ranging from 0 to 28 per plant, indicating a failure of the aphids to establish on some plants. Plants treated with 4LER had the lowest number of aphids recorded, and although this was not statistically significant it supports trends recorded in previous trials.

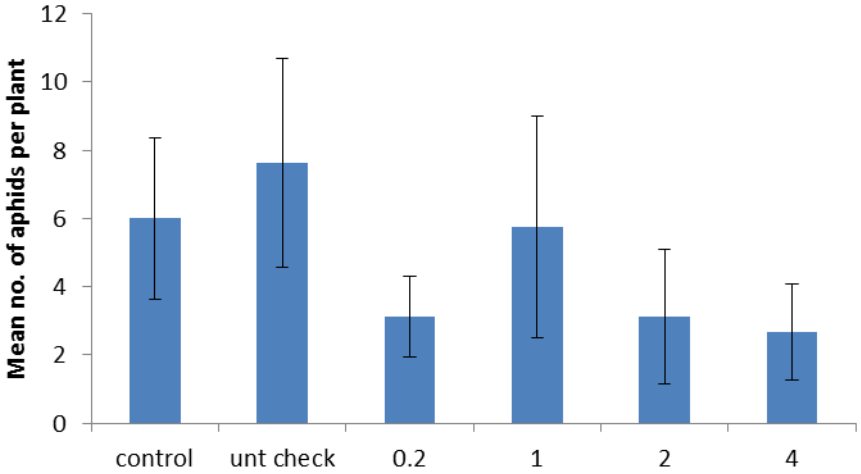

Fig 11. The mean numbers of aphids (*M. persicae*) per plant at harvest, with seed treated with different rates of JA

Discussion

Results throughout the above series of trials have clearly demonstrated that the use of JA applied to the seed can induce host-defence reactions to provide a significant level of protection from aphids during the growing period in various crops. This could potentially act as an effective component in an IPM programme for aphid management in herbs and leafy salads. This is of course, subject to one or more JA products gaining regulatory approval in the UK or elsewhere in Europe and beyond.

However the most consistent product was 4x the Label Expected Rate and therefore the company (BASF) will have determine if 4xLER is an economic viability.

Jasmonic acid has also been shown to influence the behaviour and efficacy of parasitoids. The author is currently involved in a student project that is looking further into the role JA seed treatment and parasitoids/predators.

Conclusions

Results have demonstrated that the use of JA applied to the seed can enhance plant defence reactions against aphid populations.

The most consistent rate of JA product was 4x the Label Expected Rate, other lower rates demonstrated some levels of aphid reduction but the results were less consistent.

Basil

In the three basil trials (7, 14 and 21 days) successive introduction of aphids (artificially and naturally) during the 28 days of the trial demonstrated that over four weeks plants with JA treated seed reduced the population growth of aphids.

21- day experiment (refers to aphids introduced onto plants after 7 days and finally assessed 21 days later): 4LER and 2LER had significantly lower numbers of aphids than the control.

14 day experiment (aphids were introduced 14 days after day 1 and the final assessment was done 14 days after aphid introduction): All treatments, except 0.2 LER, produced significantly lower numbers of aphids compared to the control.

7 day experiment: All treatments, except 2LER, recorded significantly lower numbers of aphids than the control.

Curly parsley

In a single trial aphids (*M. persicae*) were introduced on to curly parsley plants. The results showed that at the final assessment 4LER had significantly lower numbers of aphids compared to the control.

Hawthorn parsley aphid (*Dysaphis apiifolia)* on curly parsley

The effect of the high rate JA treatment (4LER) was compared to the untreated check and the untreated control. The results suggested that 4LER could reduce the population growth of Hawthorn parsley aphid but this just failed to be statistically significant.

Lettuce

Lettuce-flat leaf

Propagation

JA treated lettuce plants in propagation trays had lower numbers of aphids for both the 2.0 LER and 4.0 LER treatments compared to untreated lettuce plants.

Crop-post planting

A natural infestation of *A. solani* occurred in the trial. At the final assessment at harvest the numbers of aphids recorded on plants that had been treated with 2 LER and 4LER were significantly ($p<0.05$) lower than numbers recorded on the control plants.

Lettuce –curly

The trial looked at a curly variety of lettuce (cv Fairplay) artificially infested with *M. persicae*.

Plants treated with 4LER had the lowest number of aphids recorded, and although this was not statistically significant it verifies trends recorded in previous trials.

Knowledge and Technology Transfer

Trial results were presented to BHTA Nov. 2012
Steering group meeting Feb. 2013

Glossary

Jasmonic acid (JA)