Project title:	Protected lettuce: development of robust IPM strategies for the control of aphids
Project number:	PC 290
Project leader:	Dr. Pat Croft, STC Research Foundation
Report:	Final report
Key staff:	Dr. Pat Croft Luke Tilley
Location of project:	STCRF
Project coordinator:	Colin Bloomfield, NV produce Marketing Ltd.
Date project commenced:	June 2008
Date project completed (or expected completion date):	August 2010
Key words:	Aphids, protected lettuce

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
STCRF

Signature ....Date ....

Report authorised by:

Dr. Martin McPherson
Science Director
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CONTENTS

Grower Summary

Headline

- Two seed treatments and two foliar sprays were effective in controlling the lettuce aphids *Nasonovia ribisnigri* and *Aulacorthum solani* in protected lettuce. Two entomopathogens also provided a high level of aphid control in lettuce propagation.

Background and expected deliverables

Glasshouse lettuce is prone to colonisation by aphids and these can become established quickly, often very early and may be difficult to detect visually until populations are already quite large.

Previous HDC projects (PC 132: Jacobson & Croft, 2001 and PC 194: Jacobson & Croft, 2005) focused on identifying the key pest species involved e.g. currant-lettuce aphid (*Nasonovia ribisnigri*), peach-potato aphid (*Myzus persicae*), potato aphid (*Macrosiphum euphorbiae*) and glasshouse and potato aphid (*Aulacorthum solani*). The projects looked at alternative methods for control, primarily by introducing physical and biological approaches with the objective of reducing the use of insecticides within the crop.

The physical method identified was mesh screening of glasshouse vents and doors, which was seen by many growers as prohibitively expensive. The biological approach used involved the use of parasitoids and this continues to offer considerable promise for the future. Continuing aphid problems led to the HDC funded project PC 271. The project was commissioned at STCRF (Croft, 2007), at the request of the Protected Leafy Crops Technical Committee. The main objective of project PC 271 was to re-initiate studies on lettuce aphids following industry concerns in this area.

There are a number of different causes and reasons for the increasing aphid problems including limited pesticide availability, poor product selection (e.g. contact versus systemic products), incorrect timing or method(s) of application, insecticide resistance and/or cultural or environmental change. HDC project PC 290 aimed to identify products that could contribute to aphid control programmes for protected lettuce.

Year 1

Objective 1. Products identified from the outdoor lettuce screening programmes (HDC project FV 319 and the DEFRA project HH3117TFV) were screened for efficacy in a fully replicated trial on glasshouse lettuce crops. The efficacy of each product was evaluated against populations of lettuce aphids introduced artificially either ‘early’ or’ late’, using pre- and post-treatment assessments.

Evaluation of insecticide efficacy against early and late infestations of aphids on separate crops provides evidence as to which products should be applied at different growth stages of the crop and, in relation to seed treatments, it indicates the persistence of the product through the life of the crop.

The efficacies of the selected products were tested against two species of lettuce aphids: *Nasonovia ribisnigri* and *Aulacorthum solani* in separate glasshouses.

Crop safety data was gathered for early and late applications of each tested product using the trials in Year 1. Samples of lettuce from each treatment (early and late applications) were retained so that samples of appropriate products could be sent off for analysis to determine residue levels if requested.

Year 2

Objective 2: In the second year of the project spray programmes were tested using successful products from Objective 1 to control aphid populations at different times of the year.

Objective 3: The development of an IPM programme for protected lettuce crops was further explored to provide information that could be transferred to outdoor lettuce.

Objective 4: The project also aimed to produce a factsheet on aphid control in protected lettuce.

Summary of the project and main conclusions

The treatments selected (a range of foliar sprays and two seed treatments) all came from previous trials of products in outdoor leafy crops at Warwick HRI. Treatments were screened against two species of aphid commonly found on protected lettuce. The reason for looking at these two species is that they are typically found on different parts of the plant and that their physical location might impact on the efficacy of the products undergoing testing. The currant lettuce aphid (*Nasonovia ribisnigri)* is usually found in the centre of the lettuce plant, whilst the glasshouse-potato aphid (*Aulacorthum sola*ni), is typically found on the under surface of the lower leaves of the plant.

The trials took place during different seasons, to determine whether this affected the efficacy of the products and their interaction with the pest and changing environmental conditions over the year in a glasshouse.

The project also looked at biopesticides and the possibility of such products contributing to the control of aphids in propagation and within the crop.

Evaluation of spray products

Seed treatments

- Both seed treatments ST1 and ST2 provided a similarly high level of aphid control for most of the growing periods, but evidence of product failure occurred towards the end of some crops. The failures occurred in year 1, initially at the end of the long winter crop, and towards the end of the spring crop when both treatments failed to control a late infestation of the two aphid species. A similar case was observed in year 2 where small numbers of aphids were recorded at the end of the summer/autumn crops grown with both seed treatments.

- The use of an additional product against these late arriving and low numbers of aphids was unsuccessful.

- Results suggest that the timely harvesting of the lettuce crops may be necessary to avoid loss of efficacy of the products.

- An organic seed treatment also provided a potentially useful level of aphid control in lettuce propagation.

Foliar treatments

The project screened and developed spray programmes for new products for protected lettuce. Foliar treatments Exp U, Exp D and Exp A were successful at reducing the two species of aphid. Exp D provided an effective short term knockdown treatment. Exp U and Exp A provided long term protection against aphid populations.

Product A:

- Is a neonicotinoid insecticide and therefore offers a similar level of aphid control to the two seed treatments throughout the year.
- It appeared to be useful as a second foliar treatment, providing an effective reduction in aphid numbers when earlier treatments have failed.
- Its use together with neonicotinoid seed treatments is not advisable due to risk of developing insecticide resistance in target populations.

Product U:

- Provided effective control of aphid levels throughout the two years; populations were observed to fall significantly a week after application.
- It was not as effective when applied towards the end of the crop in autumn.
- It should therefore be applied either as a first or early second foliar treatment.

Product D:

- Reduced aphid numbers in comparison to control plots.
- Trials over the two years suggest the product offers short term aphid control

Biopesticides

The project also looked at the potential for biopesticides to be included as part of an aphid control programme for protected lettuce. An initial screening of several products showed that some products may have the ability to reduce aphid populations, but that this effect is limited and that products would need to be repeatedly applied. Biopesticides are primarily contact products. Improvement in their efficacy therefore requires an improvement in achieving the necessary contact. This may be achieved through various means such as improved formulation and modification to spray equipment.

- B2 showed moderate levels of aphid after application, but infestation swiftly rebounded and sometimes exceeded the levels seen in control plants.

- The use of two insect pathogens (EP2 and EP3) produced a significant level of aphid control both in propagation trays and in the glasshouse crop. Whether this level of control can be improved or sustained in year round production requires further evaluation.

- The use of non-chemical products, such as pathogens and other biopesticides will frequently fail to attain the levels of control that can be achieved by conventional insecticides. It may be possible to address this issue through using combinations of products and cultural methods.

Financial benefits for growers

The project has demonstrated the efficacy of several potential new products for aphid control, the main group of pests in protected lettuce and further evaluated the products within developing spray programmes. The use of biopesticides within protected lettuce cropping was investigated and possible uses for these products within propagation and cropping were established. Approvals for use of the identified products will have to be pursued before financial benefits can be quantified.

Science Section

Introduction

The first year of this project examined the efficacy of insecticides with the potential to contribute to aphid control programmes for protected lettuce. Products selected for examination came from previous trials on outdoor leafy crops (HRI Warwick) and consisted of a range of chemical seed and foliar treatments, together with a range of biopesticides.

The second year of the project used findings from the first year to design and evaluate spray programmes. As in the first year, the spray programmes were evaluated during three times of year and on two species of aphid; *Nasonovia ribi*snigri was selected as it is usually located in the centre of the lettuce plant; and *Aulacorthum sola*ni, selected as it is usually found on the underside of the lower leaves of the plant. These behaviours were helpful in evaluating product efficacy and mobility through lettuce plants.

We also further examined the development of IPM in protected lettuce through looking at several biopesticides and their efficacy against aphid pests in lettuce production. One potentially useful product identified in Year 1 was further tested in the spray programmes alongside new insecticide treatments. In addition biopesticide products were evaluated in propagation of the crop and subsequent cropping.

A. Evaluation of spray programmes

Materials and methods

Lettuce plants were infested with aphids *N. ribisnigri* or *A. solani* whilst in propagation. Plants were planted in a glasshouse (area 190m^2). Plots consisted of 36 plants (1.44m^2). There were a total of 11 or 12 treatments with four replicates per treatment in a randomised block design.

Treatment application

Spray programmes were based on results obtained in the first year of the project which looked at efficacy of several treatments. Treatments were applied, using Oxford Precision

Sprayer (1bar pressure) (except seed treatments prepared at HRI Warwick) when aphids had become established in the crop.

Assessments

Aphid counts were on 10 randomly selected lettuces in each plot. Assessments were made one day prior to treatment application and at seven day intervals post treatment application until harvest.

Analysis

Results were analysed Analysis of variance (ANOVA). Mean counts were using LSD at the 95% level of significance.

<u>1. Summer/Autumn 2009</u>

Lettuce sown (cv' Mirata)	26/8/09
Planted	7/9/09
Treatments applied:	9 and 20/10/09

Treatments

1. Control
2. ST1 + Expt D
3. ST2 + Expt D
4. ST1 + Expt U
5. ST2 + Expt U
6. ST1
7. ST2
8. Expt U + Expt D
9. Expt U
10. Expt D
11. Expt U + Expt A

2. Winter 2009/2010

Lettuce (cv' Gatwick) sown 17/11/09

Planted	22/12/09
Treatments applied	29/1/10 and 12/2/10

Treatments

1. Control
2. ST1 + Expt D
3. ST2 + Expt D
4. ST1 + Expt U
5. ST2 + Expt U
6. ST1
7. ST2
8. Expt A + Expt B2 (2%)
9. B2 (2%)
10. Expt A
11. Expt D
12. Expt U

3. Spring/Summer 2010

Lettuce (cv' Mirata) sown	14/4/10
Planted	21/5/10
Treatments applied	27/5/10 and 3/6/10

Treatments

1. Control
2. ST1 + Expt D
3. ST2 + Expt D
4. ST1 + Expt U
5. ST2 + Expt U
6. ST1
7. ST2
8. Expt A + Expt D
9. B2 (2%)
10. Expt U + D
11. Expt A

Results and Discussion

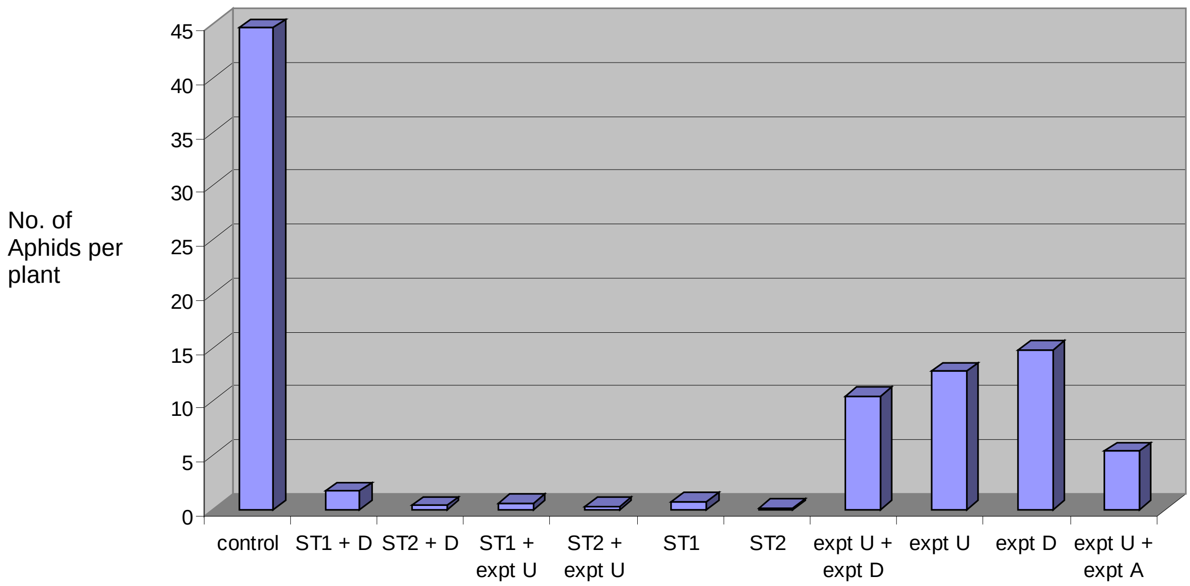
1. Summer/autumn 2009

All treatments significantly (*A. solani*; SED=3.37, *N. ribisnigri*, SED=3.43, $p<0.05$) reduced the numbers of both species of aphid in comparison to the untreated lettuce (Fig.1).

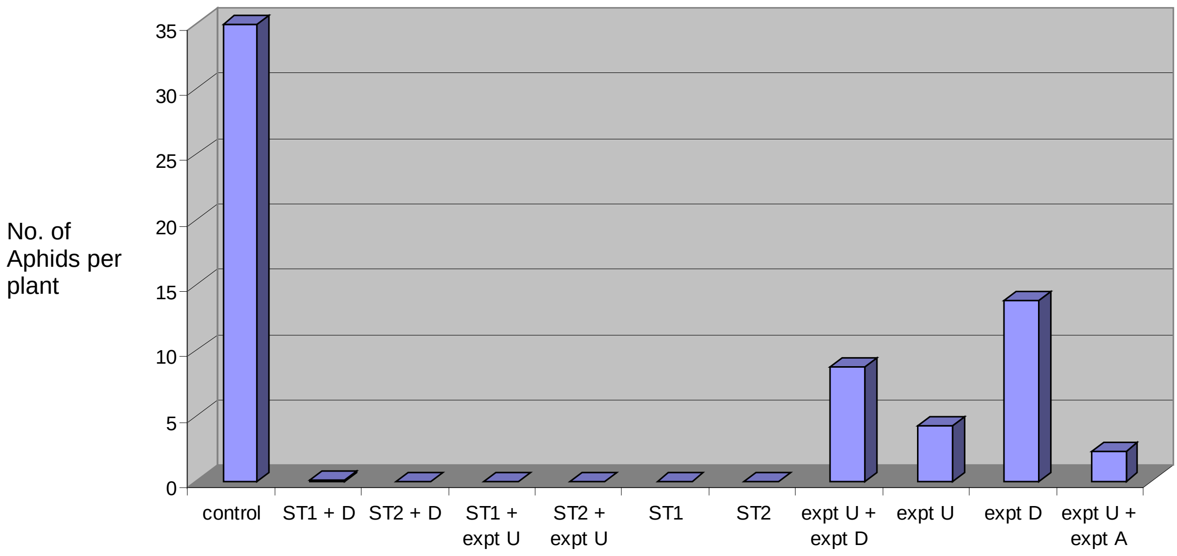
The two seed treatments (ST1 and ST2) provided a high level of control throughout the duration of the crop. In plots with *N. ribisnigri*, small numbers of aphids could be detected in the final assessment just prior to harvest. These low numbers of aphids were recorded on both seed treatments with and without a second treatment (Fig.2) showing that the second treatment failed to keep aphid numbers at the zero level, and provided no further protection to that offered by the seed treatments alone. No aphids were recorded in the majority of the seed treated lettuces, but a few plants in each plot had infestations ranging from one to ten insects. These were nymphs and not alates, indicating that aphids had settled and reproduced.

Fig, 1. Numbers of aphids (a) *N. ribisnigri*, b) *A. Solani*) at harvest following different treatment applications – Summer - autumn 2009

a) *N.ribisnigri*



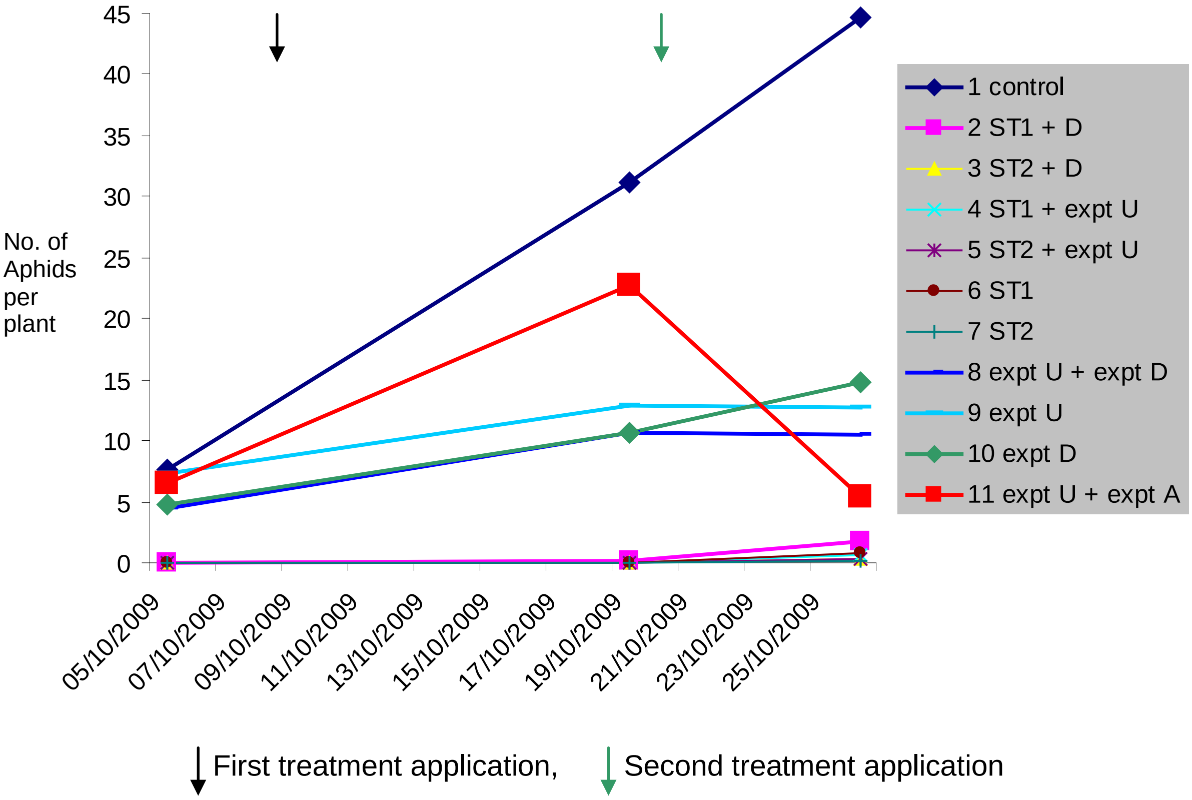
b) *A. solani*



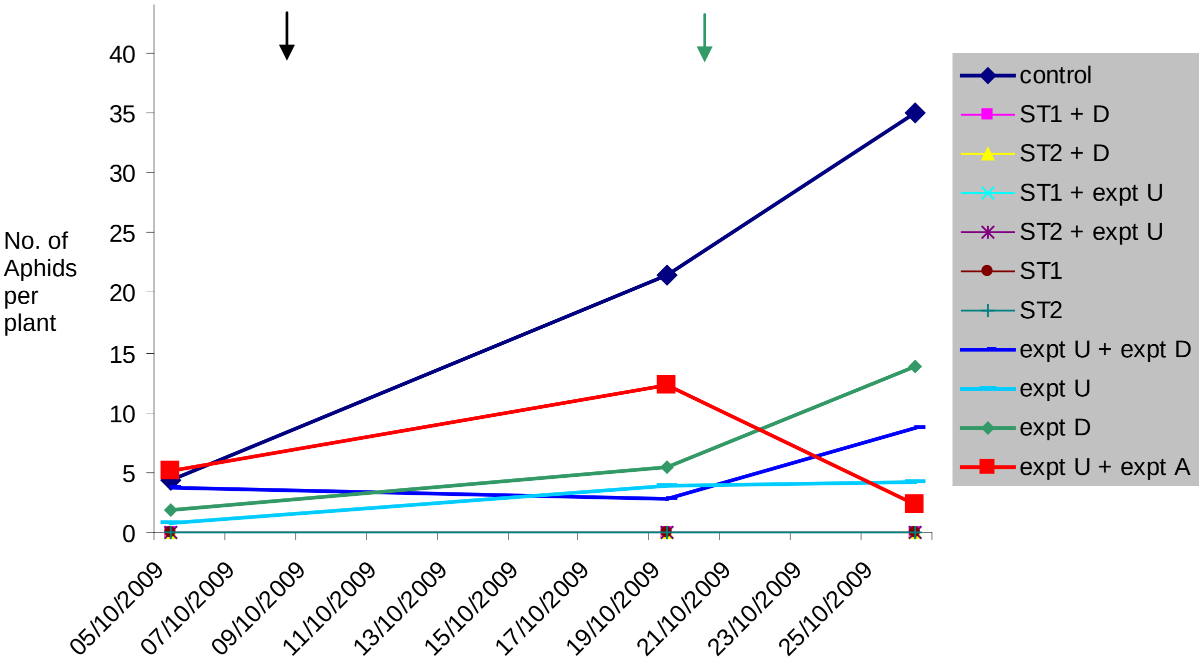
Foliar applications of products U + A also significantly reduced numbers of aphids at harvest in comparison to untreated plants (Figs. 1 and 2). Programme U + A may have provided more effective levels of control than U + D or U by itself, but aphid numbers were too low to observe statistically significant differences between these treatments.

Fig. 2. Numbers of aphids (a. *N. ribisnigri*, b. *A. solani*) following different treatment applications

a) *N.ribisnigri*



b) *A. solani*



First treatment application, Second treatment application

2. Autumn/winter 2009/2010

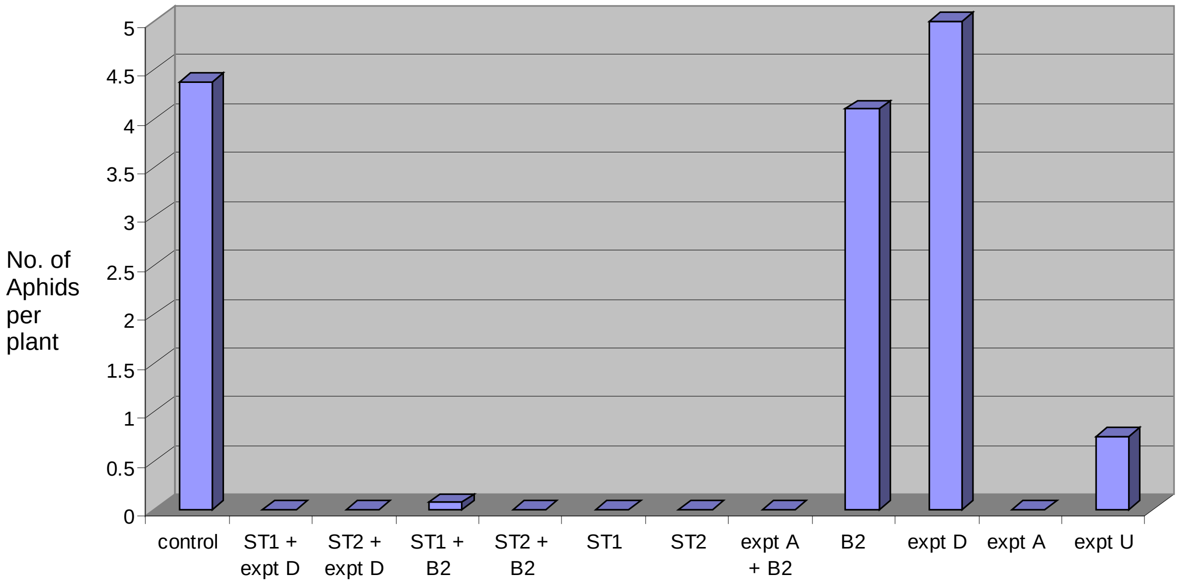
The majority of treatments significantly (*A. solani*; SED=6.99, *N. ribisnigri*, SED=1.04, $p<0.05$) reduced the numbers of aphids on lettuce plants in comparison to the untreated control. However treatment B2 did not control *N. ribisnigri* or *A. solani* and treatment D was ineffective against *N. ribisnigri* in this experiment (Fig. 3).

The seed treatments ST1 and ST2 again provided the most consistent and effective control for both aphid species, and overwhelmed any need for a foliar application of a second treatment. (Fig. 4).

Foliar applications of experimental products U and A again significantly ($p<0.05$) reduced numbers of aphids in comparison to the control, as observed in the previous crop.
During this trial only low numbers of *N. ribisnigri* were be established, most likely because of the low temperatures during winter 2010, resulting in some difficulties in monitoring product efficacy

Fig. 3. The numbers of aphids (a. *N. ribisnigri*, b. *A. solani*) at harvest following different treatment applications - Autumn-winter 2009-10

a) *N.ribisnigri*



b) *A. solani*

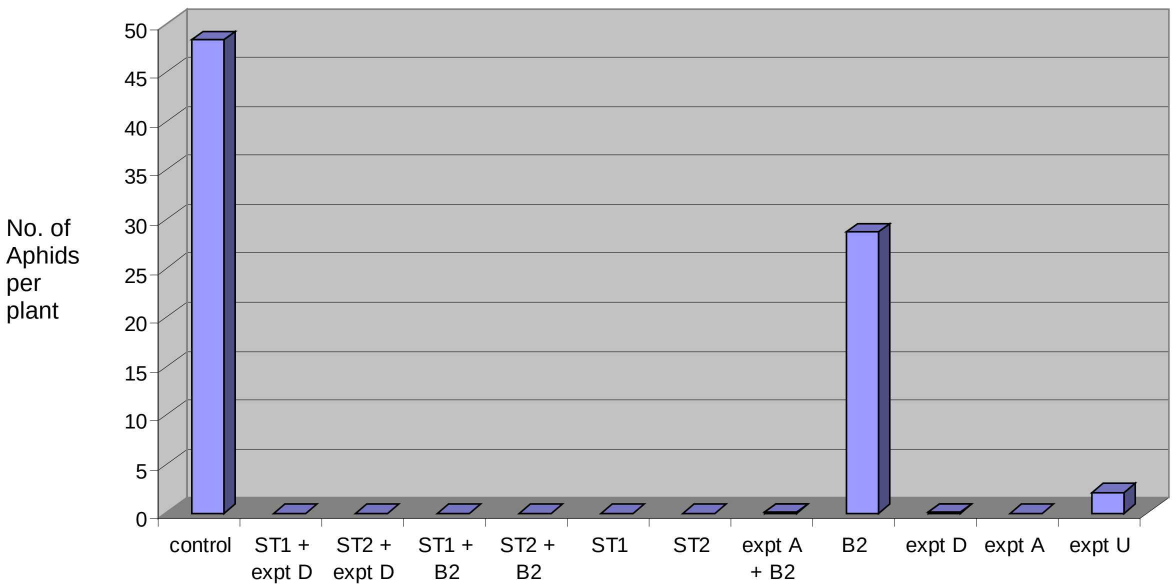
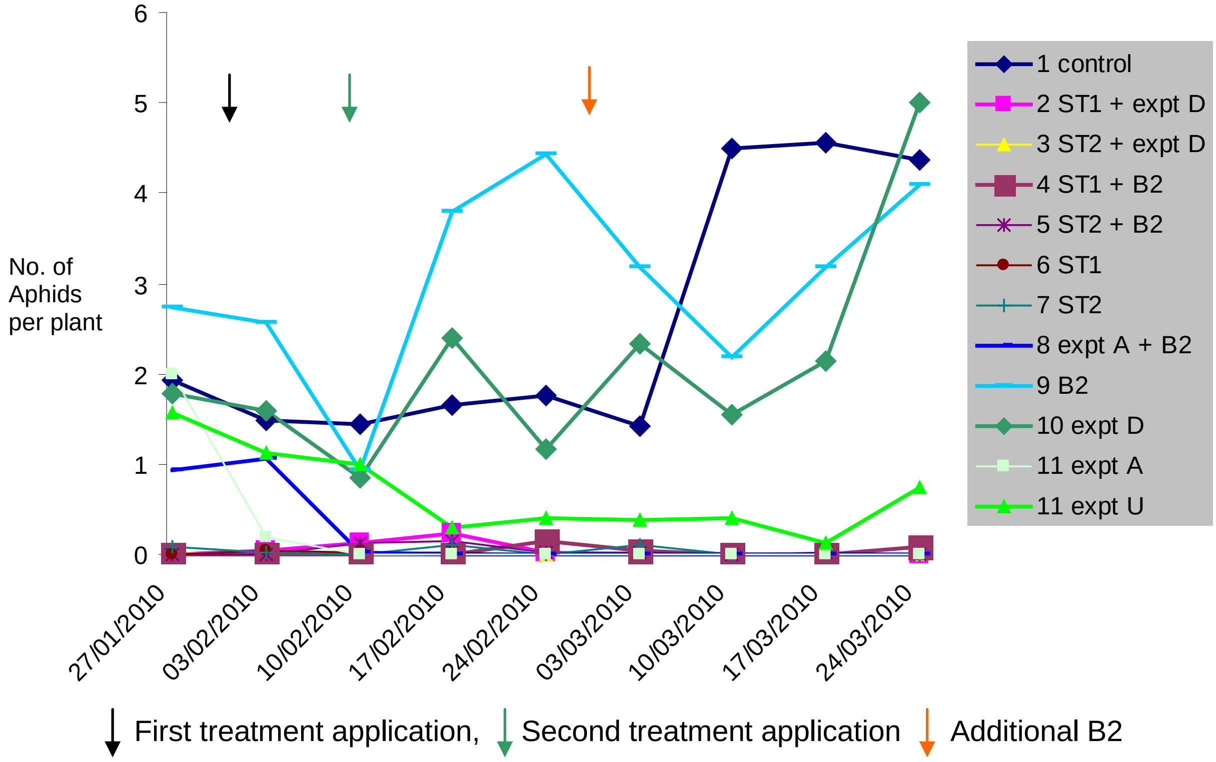
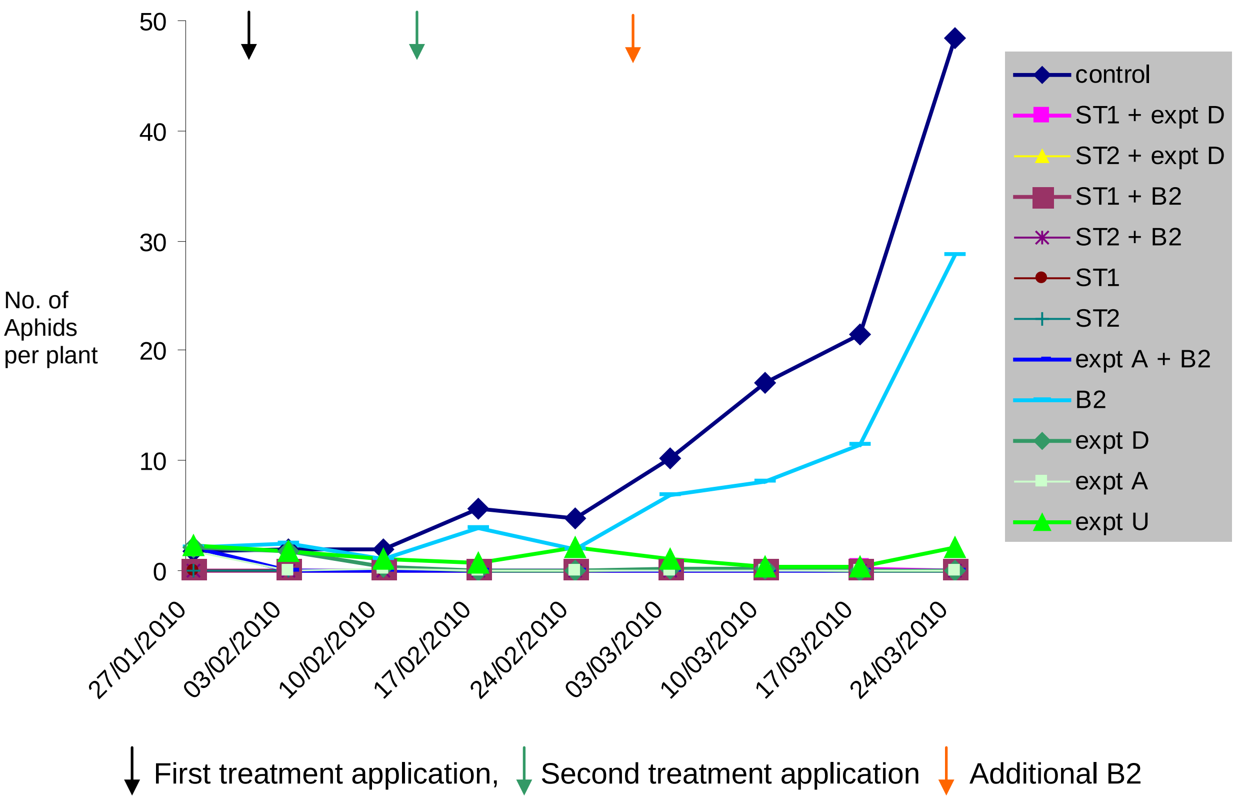


Fig. 4. The number of aphids (a. *N. ribisnigri*, b. *A. solani*) following different treatment applications

a) *N.ribisnigri*



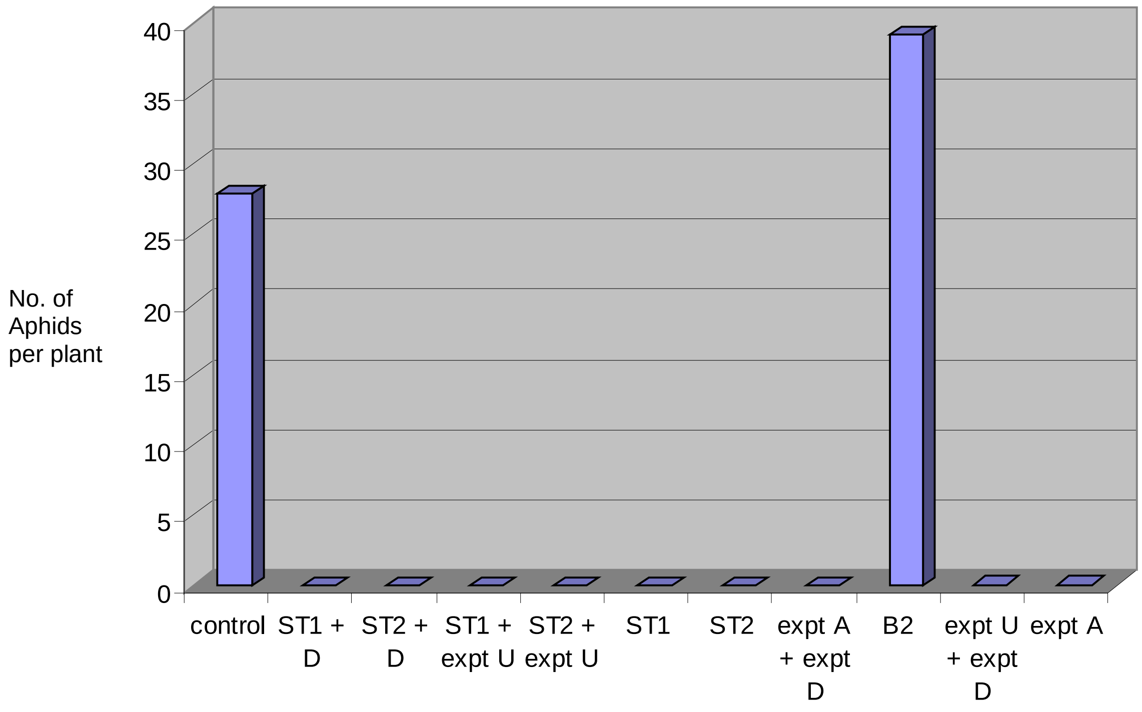
b) *A. solani*



3. Spring/Summer 2010

The results in Fig. 5 show that at harvest all treatments except B2 had significantly (*A. solani*; SED=2.04, $p<0.05$) reduced the numbers of aphids in comparison to the control at harvest.

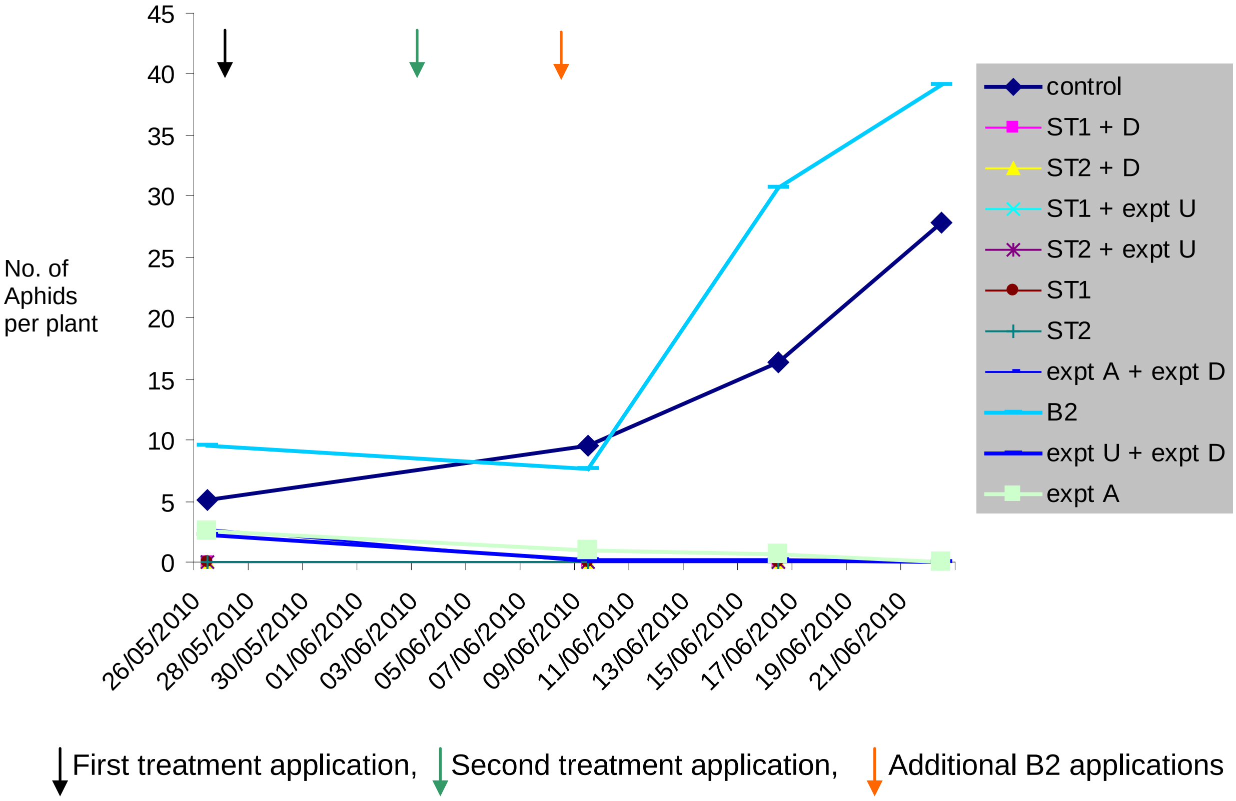
Fig. 5 The numbers of aphids (*A. solani*) at harvest following different treatment applications



Seed treatments ST1 and ST2 again provided effective and consistent levels of control throughout the trial (Fig 6), eliminating the need for a further foliar treatment application.

Foliar applications of treatments A and U again also provided effective aphid control. The addition of treatment D provided little additional benefit in either case.

Fig. 6. The numbers of aphids (a. *N. ribisnigri*, b. *A. solani*) following different treatment applications



Conclusions

Seed treatments

- ST1 and ST2 provided effective and persistent levels of aphid control over the two years of the project.
- In year one there was indication of loss of protection provided by the seed treatments towards the end of the winter and spring crops.
- In the second year this effect was less marked, but a small number of aphids were recorded at harvest in the autumn and winter crop.
- The two seed treatments provide protection to crops grown at different times of the year, but growers should be aware of the duration of protection that is obtainable from the seed treatment.
- Both seed treatments contain neonicotinoid active ingredients and therefore should be used responsibly alongside other neonicotinoid insecticides to prevent development of resistance in target species.

Foliar treatments.

Product A:

- Is a neonicotinoid insecticide and therefore offers similar aphid control to the two seed treatments.
- Is a useful product as a second foliar treatment when earlier treatments have failed

Product U:

- Provided effective control of aphid numbers throughout crops over the two years. Populations are observed to fall significantly a week after application.
- Did not achieve useful levels of pest control when applied towards the end of the crop in autumn.
- The results suggest that the product is ideally applied either as a first or early second foliar treatment.

Product D:

- The product reduced aphid numbers in comparison to control plots.
- Trials over the two years suggest the product offers some limited short term aphid control

B2:

- B2 showed moderate levels of aphid after application, but infestation swiftly rebounded and sometimes exceeded the levels seen in control plants.

Resistance management

The seed treatments ST1 and ST2 are the most efficient products, however both products are neonicotinoid and resistance must be considered a possibility through over use. In addition the foliar treatment A contains the same active ingredient as one of the seed treatments and therefore should not be used in conjunction with them.

Foliar treatment U contains a new chemistry and therefore is a useful product for lettuce growers providing a new control product that can assist with resistance management issues associated with overdependence on valuable chemistries

B. Biopesticides

B (i). Plants in propagation

Introduction

The project investigated the potential of biopesticides to be included as part of aphid control programmes for protected lettuce.

An initial screening of several products in year 1 showed that some products had the potential to reduce aphid populations, but that this effect is short-lived so products need to be repeatedly applied. In addition such products rely on contact with the pest which is more difficult to achieve as the lettuce increases in size.

Year 1 of the trial showed that entomopathogens can reduce numbers of aphids within protected lettuce crops, but that to maintain this reduction, requires frequent reapplication. In addition, aphid control remained inadequate for a marketable product.

The following trial looked at the potential of these products to be used in lettuce propagation, where humidity is at a sufficiently high level to benefit the entomopathogen, and the plants are smaller facilitating better contact with the pest. The trials used two pathogen treatments (EP2 and EP3), and were designed to measure product efficacy at different application rates.

Material and methods

The study consisted of two propagation trials (1 and 2), where products were applied to plants in propagation trays.

Trial 1 investigated the efficacy of pathogens EP2 and EP3 applied at different rates.

In *Trial 2* some propagation treatments were then continued in glasshouse conditions (floor area 190m^2), where lettuce plants were planted out into plots (Table 3).

Trial 1

Lettuce plants (cv' Mirata) were infested with aphids (*A. solani*) in propagation. Propagation trays consisted of 130 plants (0.17m^2).

Treatments were applied with an Oxford Precision Sprayer at 1.5 bar pressure using rates of product that would be used to apply to two different areas; the area of the propagation tray, and the area of glasshouse into which the plants would be planted. The trial also compared efficacy at differing water volumes.

EP3

Lettuce (cv' Mirata) sown: 14/04/10
Aphid infestation: 21/04/10 and 05/05/10
Spray applications: 28/04/10; 05/05/10; 13/05/10; 19/05/10
Assessments: 05/05/10; 14/05/10; 20/05/10

Table 1. Propagation treatments (Product EP3)

Treatment	Water volumes (l/ha)	Area rate applied
EP3 A	500	tray area (017m^2)
EP3 B	100	glasshouse area (190 m^2)
EP3 C	500	glasshouse area (190 m^2)
EP3 D (water control)	500	tray area (0.17 m^2)

EP2

Lettuce (cv' Mirata) sown: 14/05/10
Aphid infestation: 20/05/10
Spray applications: 28/05/10; 04/06/10
Assessments: 28/05/10; 04/06/10; 09/06/10

Table 2. Propagation treatments (Product EP2)

Treatment	Water volumes (l/ha)	Area rate applied
EP2 A	500	tray area (017m^2)
EP2 B	100	tray area (017m^2)
EP2 C (water control)	500	tray area (0.17 m^2)

Trial 2

In Trial 2 propagation lettuces treated with EP3 in Trial 1 were then planted into glasshouse in plots of 36 plants (1.44m^2). Applications of EP3 and EP2 were then applied during the crop as described in Table 3.

Lettuce (cv' Mirata) sown:	14/04/10
Aphid infestation:	21/04/10 and 05/05/10
Lettuce planted out:	21/05/10
Spray applications:	28/05/10; 04/06/10
Assessments:	27/05/10; 04/06/10; 09/06/10; 18/06/10

Table 3. Propagation treatments followed by glasshouse treatments after planting out (Products EP2 and EP3)

Treatment number	Propagation Treatment	Glasshouse Treatment
Trt 1	EP3 A	EP3 – 500l/ha
Trt 2	EP3 B	EP3 – 500l/ha
Trt 3	EP3 C	EP3 – 100l/ha
Trt 4	EP3 D (control)	EP2 – 500l/ha
Trt 5	EP3 A	EP2 – 500l/ha
Trt 6	EP3 B	EP2 – 500l/ha
Trt 7	EP3 C	EP2 – 500l/ha
Trt 8	EP3 D (CONTROL)	CONTROL

Results and discussion

Trial 1

The results in Fig. 7 show that repeated application of the pathogen EP3 (EP3A, EP3B and EP3C) applied to lettuces in propagation trays significantly ($p<0.05$) reduced the numbers of aphids in comparison to the control (EP3D) at the two assessment dates. The increased amount of product applied when applying the glasshouse area volume to the trays EP3B and EP3C) did not provide a significant advantage over low volumes of the pathogen (EP3A) and would not be economically viable.

Fig. 7. The number of aphids (*A. solani*) following treatment applications of EP3 in propagation (application indicated by black arrows and outlined in Table 1).

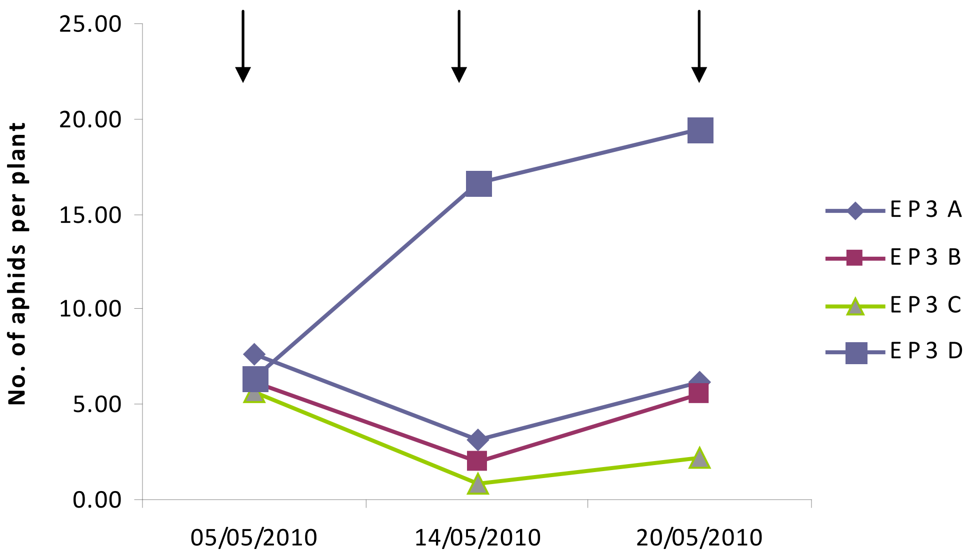
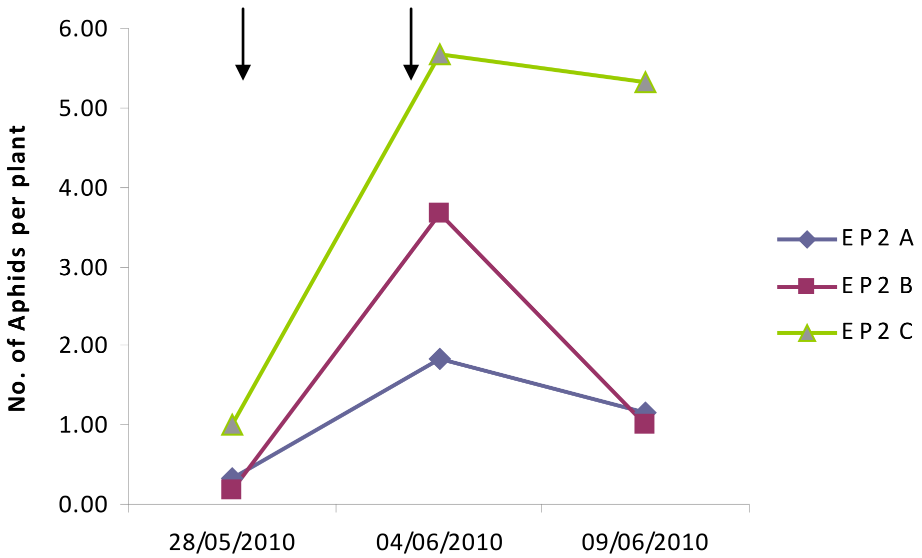


Fig. 8 The number of aphids (*A. solani*) following treatment application of EP2 in propagation (application indicated by arrows and outlined in Table 2)

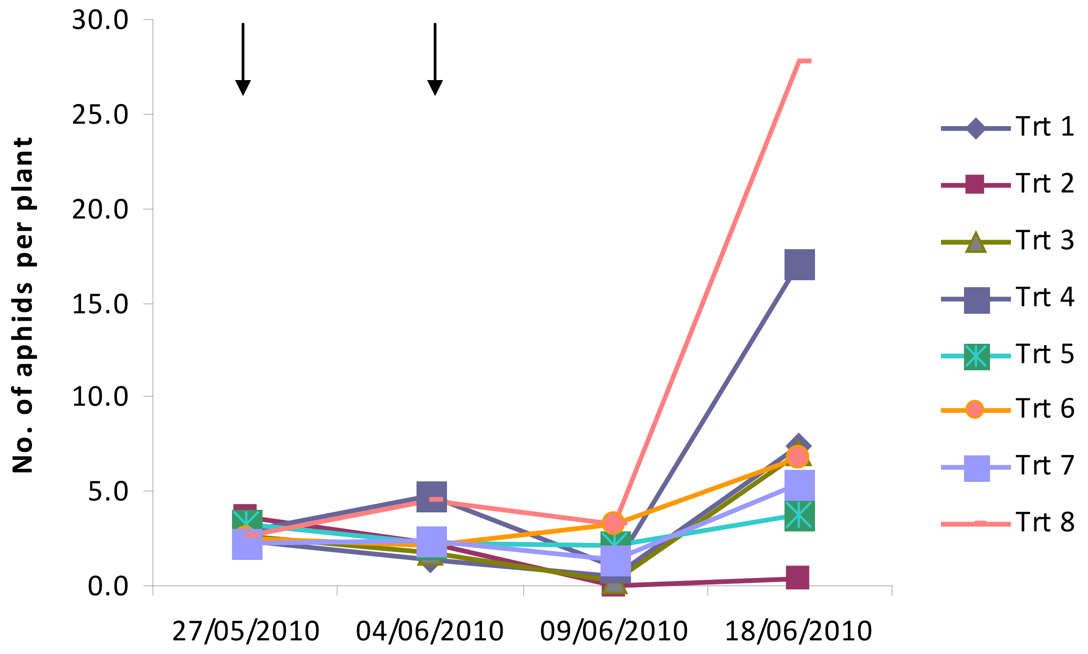


In Fig. 8 the results show that applications of EP2 at two water volumes (EP2A=500l/ha, EP2 B = 100l/ha), reduced the number of aphids per lettuce and this was significantly different ($p<0.05$) from the control (EP2C), after two applications on the final assessment date.

Trial 2

The results in Trial 2 show that on the final assessment date lettuces in Treatment 2 (EP3B and EP3) had the fewest aphids. This was significantly different ($p<0.05$) from treatment 8 (EP3D control and control) and treatment 4 (EP3D control and EP2). In addition treatments 3 (EP3C and EP3), 5 (EP3B and EP2), 6 (EP3C and EP2) and 7 (EP3C and EP2) were also significantly different ($p<0.05$) from Treatment 8.

Figure 3. The number of aphids (*A. solani*) following treatment applications of EP3 and EP2 in the glasshouse (application indicated by black arrows and outlined in Table 3).



There is variability in the data and therefore it was not possible to statistically assess the efficacy of different rates of product applied and water volumes used. However the frequent application of both pathogens, EP2 and EP3 in propagation or in the crop reduced aphid numbers. The control treatments, i.e. 8 (untreated in propagation and glasshouse crop), and 4 (untreated in propagation, EP2 in crop), had higher aphid populations than plants that had products applied in propagation and the crop.

B (ii). Seed Treatments

Introduction

This trial was a preliminary examination of the potential of organic seed treatments to provide protection against aphid pests on lettuce at the propagation stage. The system was developed in collaboration with Lancaster University, who are looking at other pest and crop species.

Materials and methods

Non-pelleted lettuce seeds were sent to Lancaster University and treated as per the methodology developed at Lancaster University). Four products were tested alongside an untreated control.

Seeds were then sown and propagated at STCRF. Plants were divided into two batches; four adult aphids (*A. solani*) per plant were introduced at the first two true leaf stage (batch 1) or at four true leaves (Batch 2). The number of aphids was then recorded over 14 or seven days respectively. Lettuce was kept in a constant environment room at 16light:8dark, and 21 ± 2^0C.

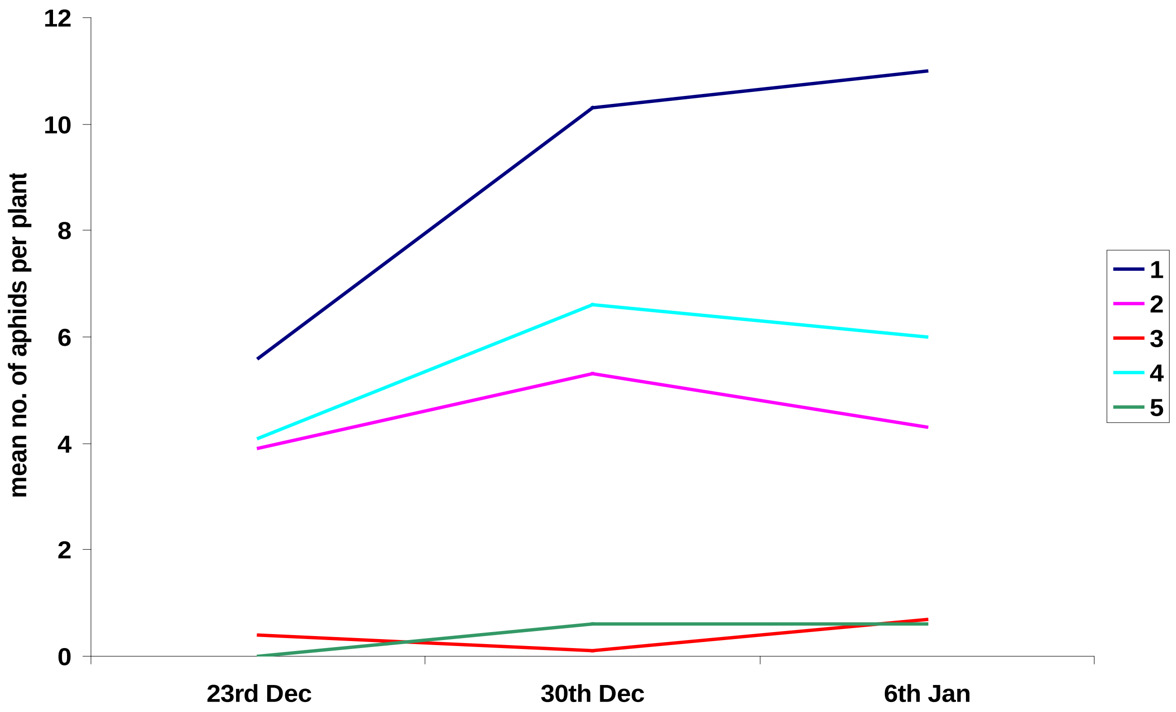
Analysis

Analysis of the data was done using ANOVA on log +1 transformed data, and means were compared using LSD at the 95% level of significance.

Results and discussion

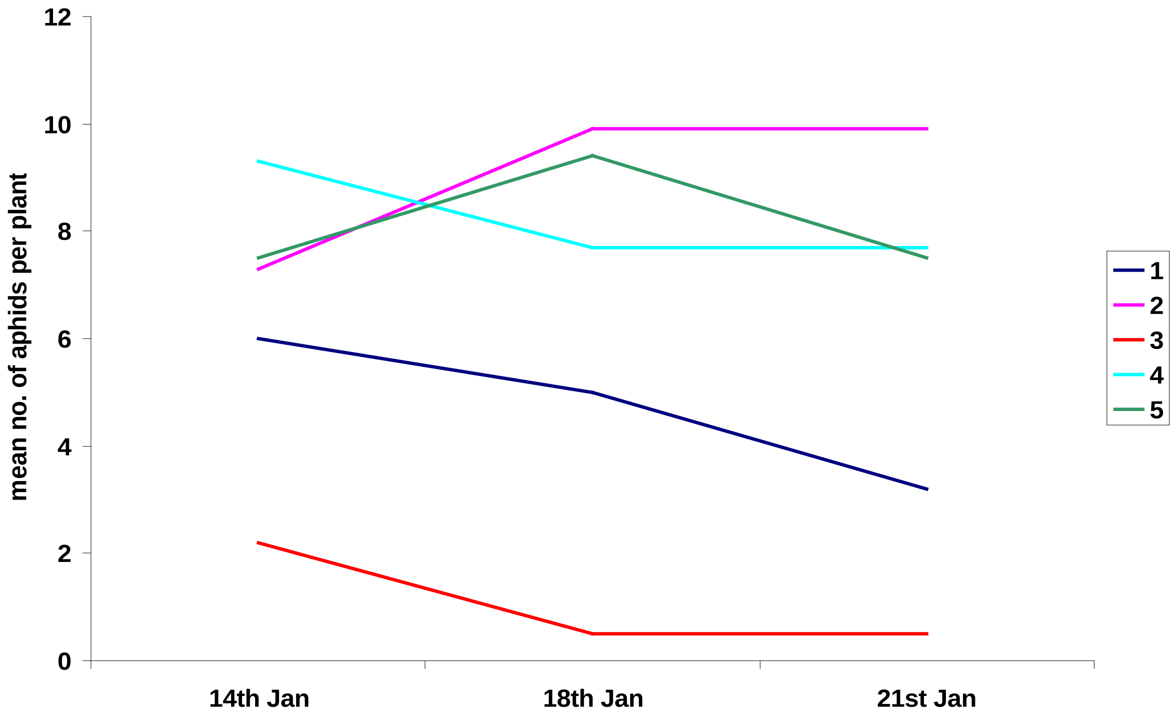
The results in Fig. 7 and 8 show that Treatment 3 had significantly ($p<0.05$) fewer aphids than other treatments on lettuce at the two or four leaf stage. Aphids were not eradicated, but over the period that observations were made, aphid numbers were reduced or steady in nearly all cases.

Fig. 7. Mean number of *A. solani* following different organic seed treatments



The results suggest that this organic seed treatment may have the potential to contribute to aphid control in protected lettuce propagation and potentially other crops, but this requires further research. The degree of control in aphid population appeared to be achieved through reducing offspring numbers.

Fig. 8. Mean number of *A. solani* following different organic seed treatments



Further research is required to evaluate the effect on aphid numbers over a longer period in lettuce crops, together with the use of this system in conjunction with other biopesticide products such as entomopathogens, to see if aphid numbers can be reduced to a commercially acceptable standard.

Conclusions on Biopesticides

- The results show that the use of a pathogen (EP2 or EP3) provided a measurable degree of control against aphids both in propagation trays and within the crop. Whether this control can be improved or sustained in year round production is a subject for future work.

- The preliminary screening of organic seed treatments suggested that one product may provide aphid control within lettuce crops, but again this requires further evaluation

- The use of pathogens and other biopesticides fails to attain the levels of control that can be achieved by conventional insecticides and therefore the use of multiple products or combinations of products and cultural methods needs to be investigated to see if commercially viable levels of control can be attained.

Technology transfer

Results contained within this report were presented to members of the Leafy Salad Association at STC