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Project leader:	Gemma Hough, ADAS
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Location of project:	ADAS, Boxworth Warwick Crop Centre, Wellesbourne
Industry Representative:	Emma Garrod, G's Growers
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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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GROWERS SUMMARY

Headline

The insecticide loading on Iceberg lettuce seed could be reduced to 80g/ha for Gaucho (imidacloprid) or 60g/ha for Cruiser (thiamethoxam); these lower insecticide loadings provide the same level of control as standard treated seed or dummy pills.

Background

Currently in the UK, lettuce root aphid is effectively controlled with the neonicotinoid seed treatments imidacloprid (Gaucho) and thiamethoxam (Cruiser), which also provide control of foliar feeding aphids for many weeks after transplanting. However, depending on the lettuce variety and its planting density, the seed loading is adjusted so that the maximum total dose of neonicotinoids per hectare per year is not exceeded. This has shown that varieties such as Little Gem, which are planted at higher densities using lower seed loadings (e.g. Gaucho at 80 g/ha), are still protected from aphids. Therefore, growers could reduce their pesticide usage and associated costs if they could lower the seed loading of other varieties planted at lower densities such as Iceberg (Gaucho at 120 g/ha is commonly used) without increasing the risk of aphid infestation, particularly lettuce root aphid. In addition, seed treatments can be associated with phytotoxicity problems but methods to reduce these negative effects, e.g. dummy pills, are not widely used due to concerns about residues, which have not been tested.

Prior to the use of neonicotinoids, lettuce root aphid was a significant problem, particularly near areas with poplar wind breaks when control measures were not used, due to the pest overwintering on poplar. However, since the use of neonicotinoid seed treatments lettuce root aphid has been controlled effectively and no further research has been conducted to identify alternative insecticide treatments with different modes of action which could be used instead of seed treatments. Information about alternative treatments would allow growers to diversify their aphid control strategies. Furthermore, due to the current restrictions on the use of certain neonicotinoids on crops attractive to bees, identifying alternative treatments would be useful for growers should further restrictions be imposed on the use of neonicotinoid seed treatments on lettuce.

The aim of this project was to determine and compare the persistence and efficacy of seed treatments currently used, seed treatments with lower loadings of pesticide, dummy pills, spirotetramat (Movento) and other ‘novel’ systemic insecticides for the control of lettuce root aphid and a foliar feeding aphid, the currant-lettuce aphid.

Summary

Objective 1 and 2: Establish and maintain a lettuce root aphid culture

The lettuce root aphid, *Pemphigus bursarius* overwinters as an egg on poplar trees (Lombardy and Black poplar). In the spring, the eggs hatch and the nymphs feed on the petioles which, in response to aphid feeding, develop a gall which encloses the aphids. A method of collecting lettuce root aphid galls (Figure 1a) and enclosing them in an insect proof cage containing lettuce led to the establishment of a lettuce root aphid culture at ADAS Boxworth (Figure 1b). Additionally, winged lettuce root aphids were observed emerging from the galls which were collected at Warwick Crop Centre, Wellesbourne from 2 June onwards. Warwick Crop Centre also provided transplants infested with lettuce root aphids for the ADAS culture.

a) b)

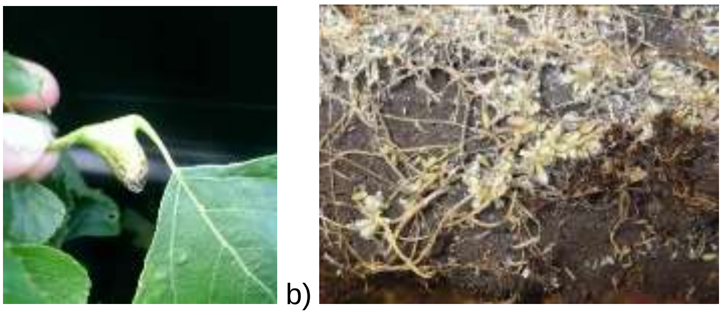

Figure 1 a) lettuce root aphid gall b) Lettuce root aphid infestation

Objective 3: Determine the efficacy and persistence of seed treatments using reduced rates of insecticide and evaluate alternative methods for control of lettuce root aphid and currant-lettuce aphid on Iceberg lettuce grown in pots in a polytunnel.

Materials and methods

The trial consisted of 11 treatments (Table 1) and was carried out in a polytunnel at ADAS Boxworth. Each treatment had eight replicates and each replicate was a lettuce plant in a 3 Litre pot. The efficacy and persistence of each treatment was evaluated by challenging plants with aphids at different growth stages (at transplanting, two weeks after transplanting and four weeks after transplanting). The lettuce variety was Iceberg cv. Excalibur. Treated and untreated seed was provided by Shamrock Seeds and dummy pills were supplied by Syngenta, UK. Movento spray treatments were applied using a knapsack sprayer fitted with 02F110 nozzles in 200 l/ha (lower water volume used than the 300-600 L/ha recommended on label). Treatment 10 was applied by soaking seed overnight in a solution at 5°C (refrigerator). Treatment 11 was applied in 0.5 ml solution using a 1 ml transfer pipette.

Table 1 Treatments used in the pot trial at ADAS Boxworth, their active ingredients, application methods and application rate.

Trt. num	Product name	Active Ingredient	Application method	Rate
1	Untreated			
2	Cruiser	thiamethoxam	Seed treatment	80 g/ha (Standard rate)
3	Cruiser	thiamethoxam	Seed treatment	60 g/ha (Lower rate)
4	Gaucho	imidacloprid	Seed treatment	120 g/ha (Standard rate)
5	Gaucho	imidacloprid	Seed treatment	80 g/ha (Lower rate)
6	Cruiser	thiamethoxam	Dummy pill	80 g/ha
7	Gaucho	imidacloprid	Dummy pill	120 g/ha
8	Movento	spirotetramat	Foliar spray	1 application at transplanting (product applied at 0.5 L/ha, applied prior to infesting with aphids)
9	Movento	spirotetramat	Foliar spray	2 applications (product applied at 0.5 L/ha) one applied at transplanting, one applied 2 weeks after first (applied prior to infesting with aphids)
10	HDCI 063	-	Transplant drench pre-planting	15ml/1000 plants
11	HDCI 064	-	Seed treatment	4µM solution

On 21 July, 24 lettuce transplants for seed treatments T1, T2, T3, T4, T5, T6, T7 and T11 were potted up into 3 Litre pots. The seed treatments were assessed in a separate trial to the foliar treatments and drench treatments due to poor and variable germination of the lettuce seedlings (attributed to the seeds falling too deeply in the blocks).

At transplanting on the 21st of July eight of the 24 plants from each treatment were infested with eight lettuce root aphids and eight currant-lettuce aphids. The numbers of aphids were then assessed two weeks later, on the 6th of August.

Two weeks after transplanting eight of the remaining 16 plants were infested with eight lettuce root aphids and four currant-lettuce aphids (5-6 August) and assessed two weeks later, on the 20th and 21st of August. The remaining eight plants were infested with eight lettuce root aphids and four currant-lettuce aphids four weeks after transplanting (21-22 August) and were assessed two weeks later (4-5 September).

Currant-lettuce aphids were placed onto the foliage and eight lettuce root aphids were placed on the roots (either to the underside of the peat block before it was transplanted or to the roots at the side of the pot at later infestations). Each plant was then covered in a pot topper cage and arranged in a randomised design in the polytunnel.

When the plants were assessed, the foliage was sampled destructively and the numbers of wingless currant-lettuce aphids were recorded. To assess the number of lettuce root aphids per plant, any substrate containing aphids and their waxy deposits was placed in water so the aphids would float and could be counted.

On 23 July, further lettuce seed was sown to complete the foliar spray treatments and drench treatments trial (T1, T8, T9 and T10) (Table 1). Germination was uniform and on 14 August plants were potted up into 3 Litre pots. Plants for treatments 8 and 9 were sprayed with Movento at transplanting (0.5 l/ha, water volume 200 l/ha). Treatment 9 was sprayed a second time with Movento two weeks after transplanting. The method described above for the seed treatments was used to test their efficacy and persistence. Eight clean plants were infested with aphids on 14-15 August (at transplanting), 29 August (two weeks after transplanting) and 11 September (four weeks after transplanting) and assessed on 28-29 August, 11 and 26 September respectively.

Objective 4: Determine the efficacy and persistence of seed treatments using reduced rates of insecticide and evaluate alternative methods for control of lettuce root aphid and currant-lettuce aphid on Iceberg lettuce in the field

Materials and methods

Lettuce root aphid: Three sequential sowings of lettuce cv Excalibur were made at Warwick Crop Centre on 20 May, 27 May and 3 June to provide plants for three sequential plantings (to maximise the chances of catching the lettuce root aphid migration and determine the impact of aphid arrival at different stages of crop development). The trial consisted of 11 treatments (Table 1 but with 2 x 4 replicates of the untreated control) and each replicate consisted of 20 plants transplanted on each of three dates (60 plants/plot in total). The plots were 4.9 m x one bed (1.83 m each) in size. The transplanting dates were: 10 June, 18 June and 24 June. Plants were transplanted at a spacing of 35 cm within rows and 35 cm between rows. Treatments were applied as described previously except all spray treatments were applied in 300 l/ha. The sprays were applied on 10 June (T8 and T9) and 24 June (T9), 18 June (T8 and T9) and 1 July (T9) and 24 June (T8 and T9) and 8 July (T9) for the first, second and third plantings respectively. The plots from the three plantings were assessed for infestation by lettuce root aphid on 25 July, 5 August and 12 August respectively by digging up 10 plants per plot and scoring the roots for the number of aphids.

Currant lettuce aphid: Seeds of lettuce cv Excalibur were sown on 22 August and a single planting was made on 17 September. The trial consisted of 11 treatments x four replicates (but with 2 x 4 replicates of the untreated control) (Table 1) and each replicate consisted of 14 plants in a single row. The plants were transplanted at a spacing of 35 cm within rows and 50 cm between rows and plots were 4.55 m x one row (1.83 m each) in size.

Laboratory-reared currant-lettuce aphids were confined on five plants per replicate in clip-cages (five aphids/plant) on two occasions (two weeks after transplanting and three weeks later). The plants were infested on 30 September and 20 October. The plots were protected from wind and rainfall by covering them with fleece. The numbers of aphids remaining in the clip cages were recorded on 7 October and 27 October. The first Movento spray was applied on 23 September (6 days after planting) and the second application was made on 10 October (23 days after planting).

Results

Currant-lettuce aphid

- Both the field trial and the pot trial showed that all the seed treatments (except HDCI 064) reduced the number of currant-lettuce aphids compared to the control. The pot trial showed that the seed treatments provided control of the aphids until the last assessment day (four weeks after transplanting). In contrast, the field trial showed that that these treatments were no longer effective 5 weeks from transplanting.
- Control of currant-lettuce aphid was similar between the standard and lower seed loading rates for Cruiser and Gaucho on Iceberg. Dummy pills were also just as effective as the standard seed treatments.
- The pot trial showed that after the second application of Movento there was a significant reduction in the number of currant-lettuce aphids compared to the control. The treatment was still effective two weeks after application. The lack of control observed in the pot trial after one application is possibly due to the timing of the application which was made on the day of transplanting when the plants were not growing actively. However, no aphid control was observed in the field trial after

either one or two applications when the first sprays were applied six days after transplanting (one week before the aphids were placed on the plants). The difference between the pot and field experiments in efficacy following the second application of Movento could be due to the differences in the concentration used (pot trial - 0.5 l/ha in 200 l of water per hectare (lower water volume used than label recommendation); field trial - 0.5 l/ha in 300 l of water per hectare).

- No statistically significant effect of the novel transplant drench treatment was observed against currant-lettuce aphid in the pot trial but there was a significant effect in the field trial.

Lettuce root aphid

- In the pot trial, only Gaucho reduced the number of lettuce root aphids at transplanting. Two and four weeks after transplanting no differences between treatments were observed, due to the variation between replicates, but some replicates had very high establishment of aphids. Results from the field trial showed that the seed treatments had a statistically significant effect in reducing the number of aphids per plant compared to the control. It is hypothesised that the seed treatments control lettuce root aphids during their short foliar-feeding stage rather than controlling infestations developing on the roots which would explain the differences in control observed between the pot trial and field trial.
- In the pot trial, the efficacy of Movento and the transplant drench against lettuce root aphid was not determined due to poor establishment of the aphids. Results from the field trial suggested that Movento and the novel transplant drench had efficacy against lettuce root aphids. In both the pot and field trial, Movento sprays were applied on the day of transplanting.
- All the samples sent for residue testing were found to be below the EU maximum residue limits.

Financial Benefits

This study suggests growers could reduce costs by reducing the seed treatment loading (Gaucho 80g/ha, Cruiser 60g/ha) for Iceberg and achieve the same level of control as using standard rates (Gaucho 120g/ha, Cruiser 80g/ha).

Action Points

- Time application of Movento carefully for optimum efficacy.
- Consider reduced seed treatment loading rates on Iceberg.
- Consider insecticide resistance management, as reduced dose rates may be linked to an increased risk of insects developing resistance.
- Since this project began, changes in regulation have cast some doubt over the future use of dummy pills as they may now require approval as a plant protection product. Keep in touch with HDC for further information regarding approvals of dummy pills and seed treatments.

SCIENCE SECTION

Introduction

In the UK, effective control of lettuce root aphid has been achieved using neonicotinoid seed treatments (imidacloprid (Gaucho) and thiamethoxam (Cruiser)) which also provide control of foliar-feeding aphids for many weeks after transplanting. Gaucho is available with an Extension of Authorisation for a Minor Use (EAMU) on lettuce while Cruiser is registered in Holland and the seed is imported for use in the UK. Importing treated seed from within the EU is permitted as long as the plant protection product is authorised as a seed treatment for that use in at least one EU Member State.

When using seed treatments, regulations stipulate the maximum total dose of neonicotinoids which can be applied per hectare per year. This results in differences in the seed loading of pesticides for different lettuce varieties depending on their planting density. For example, the loading of a Gaucho seed treatment for Iceberg lettuce is usually 120 g/ha, while for a variety planted at a higher density, such as Little Gem, it is reduced to 80 g/ha so that the maximum total dose of imidacloprid per hectare per year is not exceeded. Gaucho applied at this lower loading rate still provides effective control of aphids on Little Gem varieties. Therefore, it would be useful for growers to know whether this reduced loading rate can be used for other varieties such as Iceberg, without increasing the risk of aphid infestations, particularly lettuce root aphid. If seed loading could be lowered with no negative effects on aphid control, it would reduce the amount of pesticide used per hectare and also reduce the cost of control.

HDC project FV 162a evaluated seed treatment rates of imidacloprid at 60, 90 and 180g/100,000 seeds (180g/100,000 seeds is equivalent to approximately 125 g a.i./ha/season) against low levels of infestation by lettuce root aphid. The project demonstrated that approximately 80% control was achieved with these dose rates. However, the efficacy of different seed loading rates of thiamethoxam is unknown.

One of the main problems encountered with seed treatments is phytotoxicity; however this can be avoided by using dummy pills. These are dead seeds coated with the pesticides which are sown alongside the live seed in the transplanting block. This method prevents the plant being exposed directly to the chemical. While this type of treatment is more expensive than treating live seed, it can reduce phytotoxicity problems, and use of dummy pills can be more economically viable when buying smaller amounts of seed stock or for specialist runs. Currently there is little information available on the persistence and effectiveness of dummy pills; particularly their influence on maximum residue levels (MRLs).

A better understanding would give growers the confidence to decide whether to use dummy pills as part of their Integrated Pest Management (IPM) strategy. Since this project began, changes in regulation have cast some doubt over the future use of dummy pills as they may now require approval as a plant protection product.

Prior to the use of neonicotinoids, lettuce root aphid was a significant problem, particularly near fields with poplar wind breaks and where control measures were not used, due to the pest overwintering on the poplar trees. However, since the introduction of seed treatments, lettuce root aphid has been controlled effectively and little research has been focussed on it, including the evaluation of alternatives to seed treatment.

As lettuce root aphids feed on the roots, foliar-applied pesticides provide limited control. Such treatments are only effective against the migrants and newly born nymphs, which remain on the foliage of the plant for an average of 49 minutes and 56 minutes before flying away and dropping to the soil respectively (HDC project FV 162). Control of the currant-lettuce aphid is also challenging when using contact-acting pesticides as it prefers to feed in the centre of lettuce heads. Therefore, for the control of these pests, pesticides with systemic modes of action are needed. Spirotetramat (Movento) has a label approval for use against lettuce root aphid and currant-lettuce aphid on lettuce, however, the relative efficacy and persistence of this product compared with currently-used seed treatments is unknown. The use of alternative control measures might also help to reduce the costs of controlling aphids, if they are found to be effective. Furthermore, due to the current restrictions on using certain neonicotinoids on crops attractive to bees, identification of alternative control methods would be valuable to growers should restrictions be imposed on the use of neonicotinoid seed treatments on lettuce.

Within an IPM programme, growers also have the option of planting varieties which are resistant to lettuce root aphid and currant-lettuce aphid and this approach is particularly effective during high risk periods. However, aphid biotypes resistant to these varieties have been observed in the last few years e.g. currant-lettuce aphid, *Nasonovia ribisnigri* (Hough, 2013).

The aim of this project was to determine and compare the persistence and efficacy of seed treatments used currently, seed treatments with lower pesticide loadings, dummy pills, Movento and ‘new’ systemic insecticides for the control of lettuce root aphid and currant-lettuce aphid. This information was collected by carrying out pot trials at ADAS Boxworth and validated in the field at Warwick Crop Centre, so that (subject to appropriate regulatory approval), growers can use the information confidently to inform future aphid control strategies.

The objectives of this project are as follows:

(i) Project objective(s):

1. Establish a lettuce root aphid culture
2. Maintain a culture of lettuce root aphid
3. Determine the efficacy and persistence of seed treatments using reduced rates of insecticide and evaluate alternative methods for control of lettuce root aphid and currant-lettuce aphid on Iceberg lettuce grown in pots in a polytunnel
4. Determine the efficacy and persistence of seed treatments using reduced rates of insecticide and evaluate alternative methods for control of lettuce root aphid and currant-lettuce aphid on Iceberg lettuce in the field

Materials and methods

Objective 1 and 2: Establish and maintain a lettuce root aphid culture

The lettuce root aphid, *Pemphigus bursarius,* overwinters as an egg on poplar trees (Lombardy and Black poplar). In the spring, the eggs hatch and the nymphs feed on the petioles which, in response to aphid feeding, develop a gall which encloses the aphids. Various *Pemphigus* spp. cause galls on poplar trees but the lettuce root aphid induces a distinctive flask shaped gall (Figure 1).

During May, poplar trees bordering lettuce fields in Cambridgeshire were examined for developing lettuce root aphid galls, but searches were unsuccessful. Lettuce root aphid galls were found on Lombardy poplars at Warwick Crop Centre, Wellesbourne.

Figure 1 Lettuce root aphid galls on poplar trees have a distinctive flask shape

To establish a culture of lettuce root aphids, galls were collected during the period of aphid migration from poplar trees to lettuce. The HDC Pest bulletin predicted that the migration would start on 6 June. Galls were collected on 22 May, 2 and 10 June by removing twigs supporting lettuce root aphid galls from the trees using secateurs. The twigs were transported to ADAS Boxworth in containers of water where they were enclosed in large tent cages containing lettuce plants (Figure 2). The twigs were cut vertically up the stem to increase water uptake and were re-cut every 1-2 days to extend their life. The lettuce plants were watered sparingly to encourage the development of lettuce root aphids.

Figure 2 Lettuce root aphid culture containing poplar twigs supporting galls and lettuce plants in an insect-proof cage.

Each individual gall was enclosed within a perforated bread bag to protect the developing aphids from predatory bugs (anthocorid species) which were found within the cages feeding on the aphids within the galls. The bags were checked daily and once the winged lettuce root aphids were observed emerging from the galls the bags were removed to allow the aphids to infest the lettuce (Figure 3).

Figure 3 Each gall was enclosed in a perforated bread-bag to protect the aphids from being predated by anthocorids which would enter the galls.

A trap crop of lettuce was also sown at Warwick Crop Centre, Wellesbourne, which would be used to start a culture if it became infested.

Currant-lettuce aphids were obtained from Warwick Crop Centre (clone 4850a) to infest the trial. Aphids were maintained on lettuce plants in large cages similar to those used for the lettuce root aphids. New lettuce plants were placed in the cages regularly.

Objective 3: Determine the efficacy and persistence of seed treatments using reduced rates of insecticide and evaluate alternative methods for control of lettuce root aphid and currant-lettuce aphid on Iceberg lettuce grown in pots in a polytunnel.

The trial consisted of 11 treatments (Table 1) and was carried out in a polytunnel at ADAS, Boxworth. Each treatment had eight replicates and each replicate was a lettuce plant in a 3L pot. The efficacy and persistence of each treatment was evaluated by challenging plants with aphids at different growth stages (at transplanting, two weeks after transplanting and four weeks after transplanting). The lettuce variety was Iceberg cv. Excalibur. Treated and untreated seed was provided by Shamrock Seeds (See Appendix 1 for a summary of the doses applied to treated seeds measured by High-performance Liquid Chromatography) and dummy pills were supplied by Syngenta, UK. All spray treatments were applied using a knapsack sprayer fitted with 02F110 nozzles in 200 l/ha (lower water volume used than the 300-600 L/ha recommendation on label) at 3 bar pressure. Treatment 10 was applied by soaking seed overnight in a solution at 5°C (refrigerator). Treatment 11 was applied in 0.5 ml solution using a 1 ml transfer pipette.

Table 1 Treatments, application methods and rates used in the pot trial at ADAS Boxworth.

Treat. num	Product name	Active Ingredient	Application method	Rate
1	Untreated			
2	Cruiser	thiamethoxam	Seed treatment	80 g/ha (Standard rate)
3	Cruiser	thiamethoxam	Seed treatment	60 g/ha (Lower rate)
4	Gaucho	imidacloprid	Seed treatment	120 g/ha (Standard rate)
5	Gaucho	imidacloprid	Seed treatment	80 g/ha (Lower rate)
6	Cruiser	thiamethoxam	Dummy pill	80 g/ha
7	Gaucho	imidacloprid	Dummy pill	120 g/ha
8	Movento	spirotetramat	Foliar spray	1 application (0.5 L/ha) at transplanting prior to aphid infestation
9	Movento	spirotetramat	Foliar spray	2 applications (0.5 L/ha) one applied at transplanting, one applied 2 weeks after first (prior to aphid infestation).
10	HDCI 063	-	Transplant drench pre-planting	15ml/1000 plants
11	HDCI 064	-	Seed treatment	4µM solution

Thirty lettuce plants per treatment were sown on 25 June in peat blocks provided by G's Growers and grown in a polytunnel at ADAS Boxworth. Variation in germination and growth was observed subsequently both between and within treatments (Figure 4).

Figure 4 Variation in growth of lettuce seedlings

To take account of this variation, only transplants for seed treatments 1, 2, 3, 4, 5, 6, 7 and 11 were potted up into 3 L pots on 21 July; further seeds were sown for the remaining treatments (8, 9 and 10) as a separate experiment which included another untreated control (T1). Twenty four plants per treatment were potted up using transplants of uniform size. The reason for poor germination was not identified.

<u>Seed treatments trial (T1, T2, T3, T4, T5, T6, T7 and T11)</u>

At transplanting: On the same day as transplanting (21 July), eight of the 24 plants grown for each treatment were infested with lettuce root aphids and currant-lettuce aphids. Eight currant-lettuce aphids (3-4th instar) were placed on the foliage (Figure 5a) and eight lettuce root aphids were placed on the roots on the underside of the peat block before being transplanted into the substrate (Figure 5b). Aphids were transferred from the culture to the plants using a fine paintbrush. The aphids were gently stroked with the paintbrush before they were transferred from the plant to ensure they had removed their stylets from the plant, as they can be damaged easily.

a)  b) 

Figure 5 **a)** Currant lettuce aphids placed on the foliage **b)** lettuce root aphids placed on the underside of the peat block at transplanting

After infestation, each plant was covered in a pot-topper cage and arranged in a randomised design in a polytunnel (Figure 6). Each plant was placed in an individual pot saucer to facilitate watering. The remaining 16 lettuce plants that were not used immediately in the trial were covered with fleece in the polytunnel to protect them from other pests until required.

Figure 6 The trial was carried out in a polytunnel at ADAS Boxworth and each plant was covered with an individual pot-topper cage.

After two weeks (6-7 August) each plant was assessed. The foliage was sampled destructively and the numbers of wingless currant-lettuce aphids were recorded. To assess the numbers of lettuce root aphids, the plants were removed from the pots and the substrate was broken up carefully until aphids or their waxy deposits were observed (Figure 6). The substrate containing the aphids and waxy deposits was then removed and placed in a tray of water where the aphids floated to the surface and the substrate sank, allowing the aphids to be counted (Figure 7).

Figure 6 White waxy deposits around the roots which are produced by lettuce root aphids.

Figure 7 Substrate containing lettuce root aphids was placed in water so the floating aphids could be counted.

Two weeks after transplanting: On 5 August, lettuce root aphids (3rd, 4th instars and adults) were placed on the roots (which had now grown out to the edges of the pots) of eight plants from the remaining 16 plants per treatment. The following day (6 August), four currant-lettuce aphids were placed on each plant. Instead of transferring the currant-lettuce aphids to the plant directly, the method was altered so that 64 individual petri dishes containing a piece of lettuce were infested with four aphids each. The piece of lettuce supporting the aphids was then placed onto the trial plant to allow the aphids to move onto the plant. The numbers of currant-lettuce aphids added were reduced from eight to four because the culture suffered a population crash following an epizootic of entomopathogenic fungi. After two weeks the plants were assessed as described previously (20 and 21 August).

Four weeks after transplanting: On 21 and 22 August, the remaining eight plants for each treatment were infested with aphids as described previously. Eight lettuce root aphids and four currant-lettuce aphids were placed on each plant. After two weeks (4-5 September) the plants were assessed as described previously.

<u>Foliar treatment and transplant drench treatments trial (T8, T9 and T10)</u>

On 23 July, new lettuce seed was sown to complete Treatments 1, 8, 9 and 10 (Table 1). Germination was uniform and, on 13 August, plants for Treatments 1, 8 and 9 were potted up into 3 L pots. Plants for treatments 8 and 9 were sprayed with Movento (spirotetramet) once they had been transplanted (0.5 l/ha, water volume 200 l/ha). Treatment 9 was sprayed with Movento a second time two weeks after transplanting. On 14 August, the transplant drench treatment was applied to all 24 plants from Treatment 10 and these were potted up subsequently.

At transplanting: On 14 August, eight lettuce root aphids and six currant-lettuce aphids were placed on eight of the 24 plants from Treatments 1, 8 and 9 as described previously. On 15 August, eight lettuce root aphids and six currant-lettuce aphids were placed on eight of the 24 plants from Treatment 10 (as these plants were treated one day later than the others).

After two weeks, each plant was assessed. Treatments 1, 8 and 9 were assessed on 28 August and Treatment 10 was assessed on 29 August because it was set up and infested a day later.

Two weeks after transplanting: On 28 August, the remaining 16 plants from Treatment 9 were sprayed for a second time with Movento (treated two weeks after the last application). On 29 August, eight of the remaining 16 plants were infested with eight lettuce root aphids and six currant-lettuce aphids. After two weeks (11 September), the plants were assessed.

Four weeks after transplanting: On 11 September, the remaining eight plants were infested with eight lettuce root aphids and six currant-lettuce aphids. After two weeks (26 September), the plants were assessed.

<u>Data analysis</u>

Data obtained from the seed treatment trial and the foliar spray treatment+drench treatment trial were analysed separately with an Analysis of Variance (ANOVA) using GenStat 14th edition. Results from the seed treatment trial were transformed using LOG(count+1) to improve the normality of the data. Where a transformation was used, both transformed and back-transformed data have been presented. Data from the foliar treatment and drench treatment trial were not transformed. Where required, Duncan's multiple comparison tests were carried out to determine the significant differences between treatments.

Objective 4: Determine the efficacy and persistence of seed treatments using reduced rates of insecticide and evaluate alternative methods for control of lettuce root aphid and currant-lettuce aphid on Iceberg lettuce in the field.

Trial 1 – Treatments to control lettuce root aphid (*Pemphigus bursarius*)

Three sequential sowings (1 week intervals) of lettuce cv Excalibur were made on 20 May, 27 May and 3 June at Warwick Crop Centre, Wellesbourne to provide plants for three sequential plantings (to maximise the chances of catching the lettuce root aphid migration and determine the impact of aphid arrival at different stages of crop development). The trial consisted of 11 treatments (but with 2 x 4 replicates of the untreated control) and each replicate consisted of 20 plants transplanted on each of three dates (60 plants/plot in total). The plots were 4.9 m x one bed (1.83 m each) in size. The transplanting dates were: 10 June, 18 June and 24 June 2014. Plants were transplanted at a spacing of 35 cm within rows and 35 cm between rows.

The treatments are shown in Table 2. Treated seeds were supplied by Shamrock Seeds and dummy pills were supplied by Syngenta, UK. All spray treatments were applied using a knapsack sprayer fitted with 02F110 nozzles in 300 l/ha. Treatment 10 was applied by soaking seed overnight in a solution at 5^0C (refrigerator). Seeds were washed with water and dried before sowing. Treatment 11 was applied in 0.5 ml solution using a 1 ml laboratory pipette and washed in using approximately 1 ml water/block.

Table 2. Treatments applied in trials at Warwick Crop Centre to control lettuce root aphid.

Number	Product name	Active Ingredient	Application method	Rate
1	Untreated			
2	Cruiser	thiamethoxam	Seed treatment	80 g/ha (Standard rate)
3	Cruiser	thiamethoxam	Seed treatment	60 g/ha (Lower rate)
4	Gaucho	imidacloprid	Seed treatment	120 g/ha (Standard rate)
5	Gaucho	imidacloprid	Seed treatment	80 g/ha (Lower rate)
6	Cruiser	thiamethoxam	Dummy pill	80 g/ha
7	Gaucho	imidacloprid	Dummy pill	120 g/ha
8	Movento	spirotetramat	Foliar spray	1 application (0.5 L/ha)
9	Movento	spirotetramat	Foliar spray	2 applications (0.5 L/ha)
10	HDCI 064	coded	Seed treatment	4µM solution
11	HDCI 063	coded	Pre–planting drench	15ml/1000 plants
12	Untreated			

Sprays were applied across whole plots. The sprays were applied on 10 June (T8 and T9) and 24 June (T9); 18 June (T8 and T9) and 1 July (T9); and 24 June (T8 and T9) and 8 July (T9) for the first, second and third plantings respectively.

The plots from the three plantings were assessed for infestation by lettuce root aphid on 25 July, 5 August and 12 August respectively. This was done by digging up 10 plants per plot and scoring the roots for damage using the following scale:

Score	Description
0	No aphids
1	<10 aphids
2	11 – 100 aphids
3	101 – 1000 aphids
4	>1000 aphids

The head weights of lettuce plants harvested in August were recorded. The data were summarised and subjected to Analysis of Variance.

Trial 2 – Treatments to control currant-lettuce aphid (*Nasonovia ribisnigri*)

Seeds of lettuce cv. Excalibur were sown on 22 August at Warwick Crop Centre and a single planting was made on 17 September. The trial consisted of 11 treatments x four replicates (but with 2 x four replicates of the untreated control) (Table 3) and each replicate consisted of 14 plants in a single row. The treatment details were identical to those for Trial 1. The plants were transplanted at a spacing of 35 cm within rows and 50 cm between rows and plots were 4.55 m x 1 row (1.83 m each) in size. Spray treatments were applied on 23 September (T8 and T9, 6 days after planting) and on 10 October (T9, 23 days after planting).

Table 3. Treatments applied in trials at Warwick Crop Centre to control currant-lettuce aphid.

Number	Product name	Active Ingredient	Application method	Rate
1	Untreated			
2	Cruiser	thiamethoxam	Seed treatment	80 g/ha (Standard rate)
3	Cruiser	thiamethoxam	Seed treatment	60 g/ha (Lower rate)
4	Gaucho	imidacloprid	Seed treatment	120 g/ha (Standard rate)
5	Gaucho	imidacloprid	Seed treatment	80 g/ha (Lower rate)
6	Cruiser	thiamethoxam	Dummy pill	80 g/ha
7	Gaucho	imidacloprid	Dummy pill	120 g/ha
8	Movento	spirotetramat	Foliar spray	1 application (0.5 L/ha)
9	Movento	spirotetramat	Foliar spray	2 applications (0.5 L/ha)
10	HDCI 064	coded	Seed treatment	4µM solution
11	HDCI 063	coded	Pre–planting drench	15ml/1000 plants
12	Untreated			

Laboratory-reared currant-lettuce aphids were confined on five plants per replicate in clip-cages (five aphids/plant) on two occasions (two weeks after transplanting and three weeks later). The plants were infested on 30 September and 20 October. The plots were protected from wind and rainfall by covering them with fleece. The numbers of aphids remaining in the clip cages were recorded on 7 October and 27 October. The data were summarised, transformed to square roots and subjected to Analysis of Variance.

Residue testing

On 3 November, six lettuce plants from each treatment (except the two experimental treatments where the active ingredient was not available for screening) were sampled from the trial on currant-lettuce aphid at Warwick Crop Centre and sent to SAL laboratories in Bar Hill, Cambridge for pesticide residue analysis.

Lettuce root aphid culture

One 9.8m bed of lettuce (cv. Lobjoits Green Cos) was planted and no insecticide treatments were applied. Plants were dug-up at intervals after the expected migration of lettuce root aphid. When aphids were recovered they were used for the pot experiments at ADAS Boxworth.

Results

Objective 1 and 2: Establish and maintain a lettuce root aphid culture

A successful lettuce root aphid culture was established by collecting galls and enclosing them in a cage containing lettuce. Winged lettuce root aphids were observed emerging from the galls, which were collected from 2 June onwards. Warwick Crop Centre also developed a natural infestation of lettuce root aphids on some lettuce transplants in the glasshouse which were provided for the ADAS culture (Figure 8). Once a culture was established, new lettuce plants were placed in the cages weekly.

Figure 8 Lettuce root aphid infestation

Objective 3: Determine the efficacy and persistence of seed treatments using reduced rates of insecticide and evaluate alternative methods for control of lettuce root aphid and currant-lettuce aphid on Iceberg lettuce grown in pots in a polytunnel.

Seed treatments (Treatments 1, 2, 3, 4, 5, 7 and 11)

When plants were challenged with currant-lettuce aphids at transplanting, all the seed treatments except HDCI 064 provided 100% control (Figure 9). Plants treated with HDCI 064 had a mean of 16.6 currant-lettuce aphids per plant compared with a mean of 8.2 on untreated control plants. Table 4 shows the transformed and back-transformed data for the seed treatment trial.

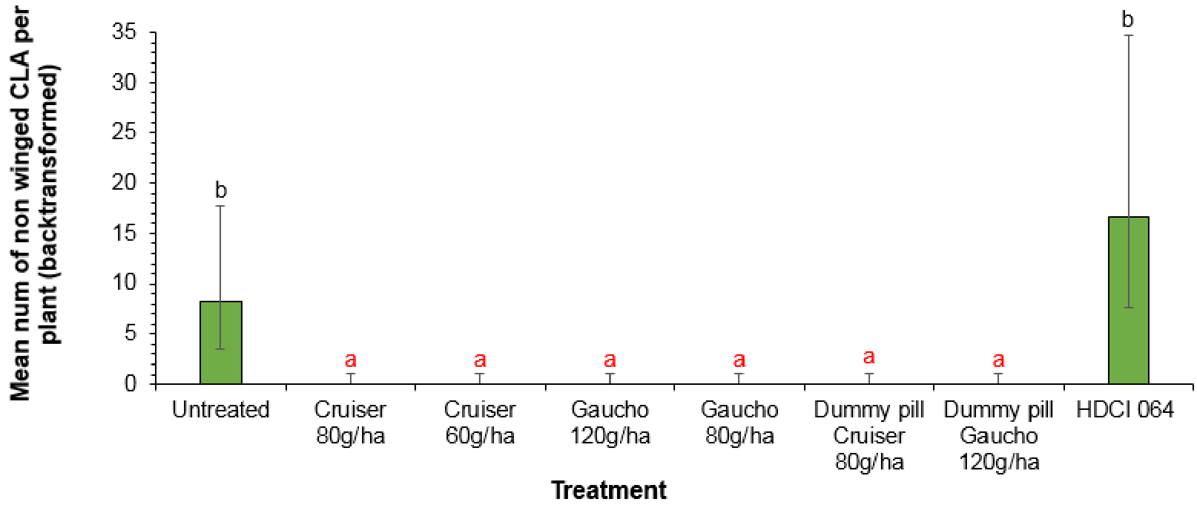

Figure 9 Mean number of currant-lettuce aphids (CLA) per plant when plants were infested at transplanting (95% confidence limits). Treatments with the same letters are not significantly different from each other. Treatments with red letters are significantly different from the untreated control.

When plants were challenged with currant-lettuce aphids two weeks after transplanting, all of the seed treatments reduced the number of aphids per plant compared to the control ($P<0.05$) which had a mean of 49.35 aphids per plant (Figure 10). HDCI 064 reduced the mean number of aphids per plant to 13.1. The remaining seed treatments reduced the mean number of aphids to between 0 and 1.8 per plant.

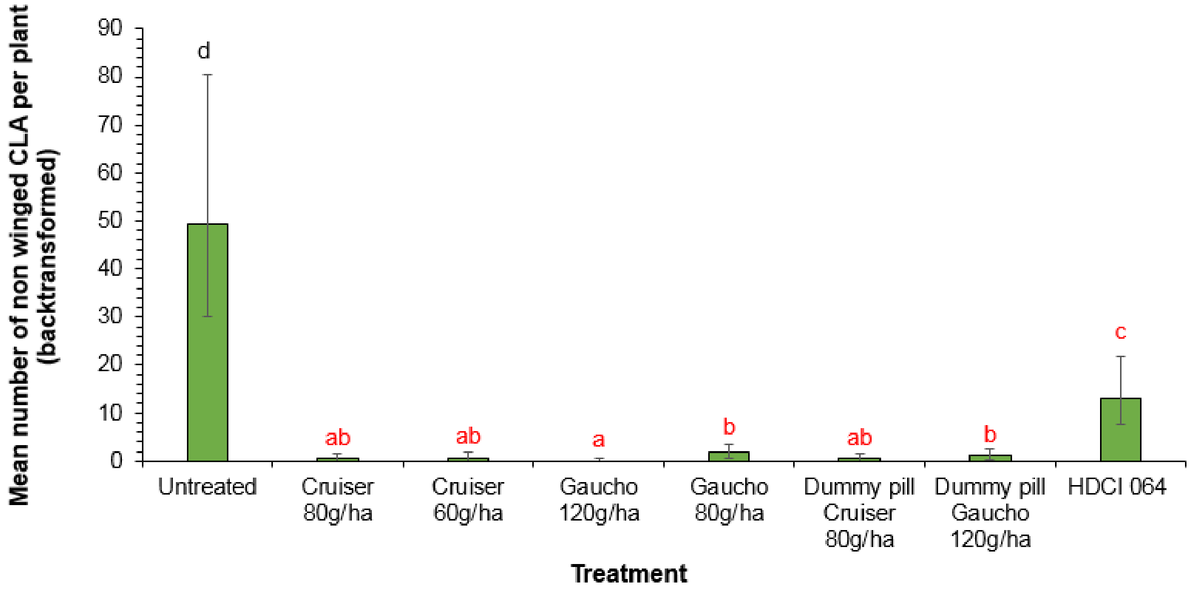

Figure 10 Mean number of currant-lettuce aphids (CLA) per plant when plants were infested two weeks after transplanting (95% confidence limits). Treatments with the same letters are not significantly different from each other. Treatments with red letters are significantly different from the untreated control.

When plants were challenged with currant-lettuce aphids four weeks after transplanting, all the seed treatments except HDCI 064 reduced the mean number of aphids per plant compared to the untreated control ($p<0.05$) (Figure 11). Plants treated with HDCI 064 had 17.1 aphids per plant compared with a mean of 19.8 per untreated plant. The remaining seed treatments reduced the mean number of aphids to between 0.1 and 0.6 per plant.

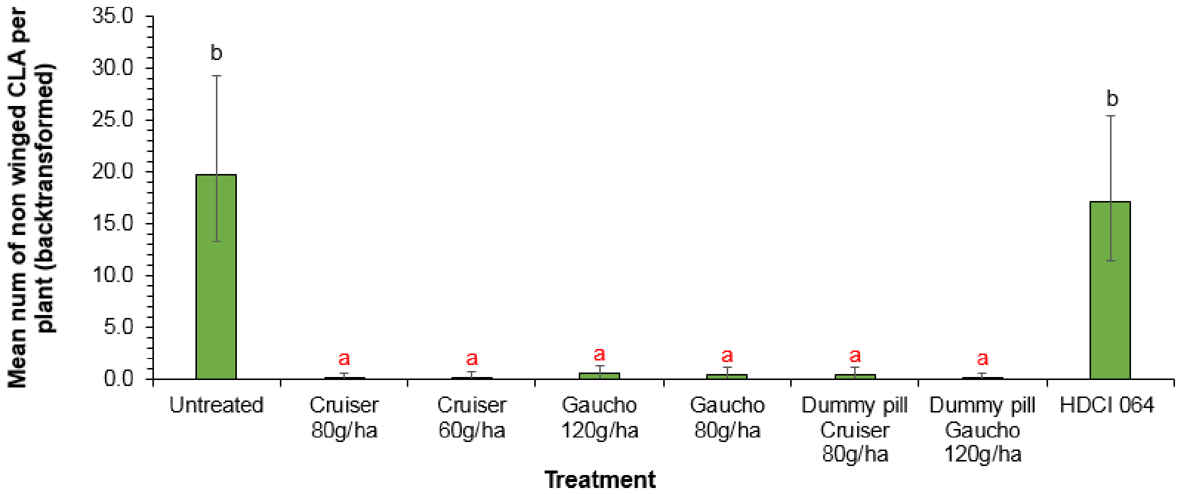

Figure 11 Mean number of currant-lettuce aphids (CLA) per plant when plants were infested four weeks after transplanting (95% confidence limits). Treatments with the same letters are not significantly different from each other. Treatments with red letters are significantly different from the untreated control.

Table 4 Mean number of currant-lettuce aphids per plant and summary of Analysis of Variance for assessments at transplanting, two weeks after transplanting and four weeks after transplanting for the seed treatments. * indicates treatments significantly different (p<0.05) from untreated controls.

		At transplanting		**2 weeks after transplanting**		**4 weeks after transplanting**	
		Trans	**Back trans**	**Trans**	**Back trans**	**Trans**	**Back trans**
1	Untreated	2.22	8.21	3.92	49.35	3.033	19.76
2	Cruiser 80 g/ha	0.00*	0.00	0.42*	0.52	0.137*	0.15
3	Cruiser 60 g/ha	0.00*	0.00	0.55*	0.74	0.173*	0.19
4	Gaucho 120g/ha	0.00*	0.00	0.00*	0.00	0.448*	0.57
5	Gaucho 80g/ha	0.00*	0.00	1.02*	1.76	0.381*	0.46
6	Cruiser dummy pill 80g/ha	0.00*	0.00	0.42*	0.52	0.397*	0.49
7	Gaucho dummy pill 120g/ha	0.00*	0.00	0.80*	1.21	0.087*	0.09
11	HDCI 064	2.87*	16.64	2.64*	13.07	2.896	17.10
	P value	<0.001		<0.001		<0.001	
	LSD (5%)	1.001		0.6829		0.5329	
	SED	0.498		0.3398		0.2650	

When plants were challenged with lettuce root aphids at transplanting only Gaucho 80g/ha and Gaucho dummy pill 120g/ha reduced the mean number of lettuce root aphids (p<0.05), providing 100% control, compared to the untreated plants (Figure 12). The untreated plants had a mean of 8.39 lettuce root aphids per plant. Table 8 shows the transformed and back-transformed data for the seed treatment trial.

Table 5 shows the maximum and minimum number of lettuce root aphids per plant which were recorded from each treatment and demonstrates the variability between replicates within each treatment, e.g. the number of aphids recorded on the eight replicate plants from the Cruiser 80 g/ha treatment ranged between 0 and 205 per plant. .

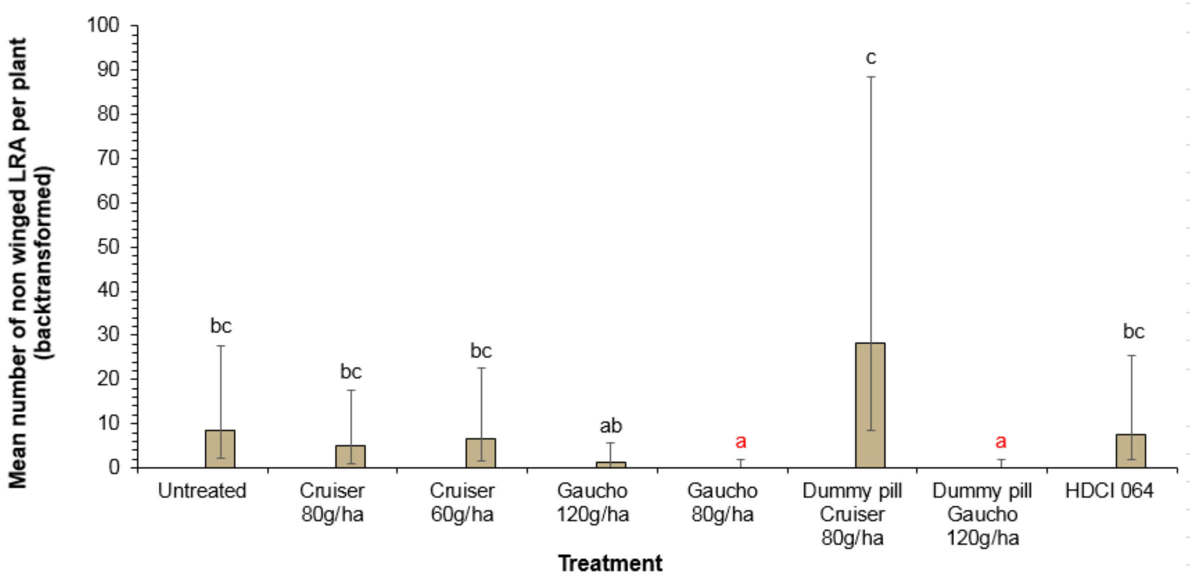

Figure 12 Mean number of lettuce root aphids (LRA) per plant when plants were infested at transplanting (95% confidence limits). Treatments with the same letters are not significantly different from each other (p<0.05). Treatments with red letters are significantly different from the untreated.

Table 5 Maximum and minimum numbers of lettuce root aphids recorded per plant for each treatment infested at transplanting

Trt number	Treatment	Maximum	Minimum
1.	Untreated	102	0
2.	Cruiser 80 g/ha	68	0
3.	Cruiser 60 g/ha	103	0
4.	Gaucho 120g/ha	18	0
5.	Gaucho 80g/ha	0	0
6.	Cruiser dummy pill 80g/ha	205	0
7.	Gaucho dummy pill 120g/ha	0	0
11.	HDCI 064	139	0

When plants were challenged with lettuce root aphids two weeks after transplanting, aphid colonies established on all of the treatments (Figure 13). The large variation between and within treatments meant that no statistically significant differences ($p<0.05$) were observed between treatments. Table 6 shows the maximum and minimum number of aphids which were recorded for each treatment. This shows that colonisation was variable with some treatments, e.g. Cruiser 60 g/ha, having replicates ranging between 0 and 148 lettuce root aphids per plant.

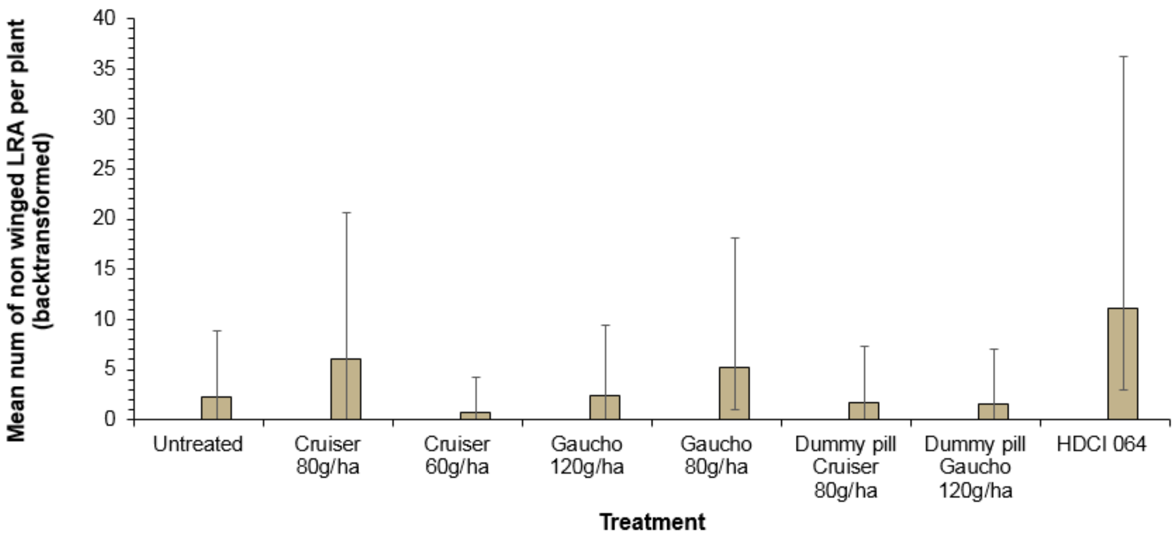

Figure 13 Mean number of lettuce root aphids (LRA) per plant when plants were infested two weeks after transplanting (with 95% confidence limits).

Table 6 Maximum and minimum numbers of aphids recorded per plant for each treatment infested two weeks after transplanting

Trt num	Treatment	Maximum	Minimum
1.	Untreated	65	0
2.	Cruiser 80 g/ha	148	0
3.	Cruiser 60 g/ha	8	0
4.	Gaucho 120g/ha	29	0
5.	Gaucho 80g/ha	92	0
6.	Cruiser dummy pill 80g/ha	68	0
7.	Gaucho dummy pill 120g/ha	19	0
11.	HDCI 064	119	0

When plants were challenged with lettuce root aphids four weeks after transplanting, aphid colonisation was low on all of the treatments (Figure 14). Considerable variation between and within treatments meant that no statistically significant differences were observed between treatments ($p<0.05$). Table 7 shows the maximum and minimum number of aphids recorded per plant for each treatment, confirming that colonisation was low on all treatments except HDCI 064.

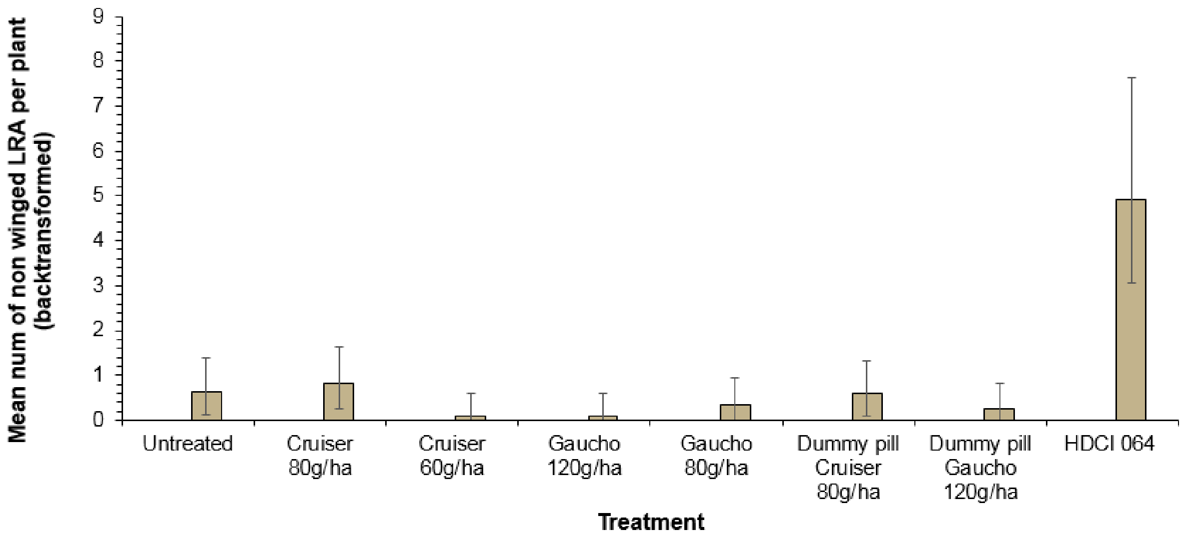

Figure 14 Mean number of lettuce root aphids (LRA) per plant when plants were infested four weeks after transplanting (95% confidence limits).

Table 7 Maximum and minimum numbers of aphids recorded per plant for each treatment infested four weeks after transplanting

Trt. number	Treatment	Maximum	Minimum
1.	Untreated	6	0
2.	Cruiser 80 g/ha	6	0
3.	Cruiser 60 g/ha	1	0
4.	Gaucho 120g/ha	1	0
5.	Gaucho 80g/ha	4	0
6.	Cruiser dummy pill 80g/ha	6	0
7.	Gaucho dummy pill 120g/ha	2	0
11.	HDCI 064	3461	0

Table 8 Mean number of lettuce root aphids per plant and summary of Analysis of Variance for assessments at transplanting, two weeks after transplanting and four weeks after transplanting for the seed treatments. * indicates treatments significantly different (p<0.05) from untreated controls.

		At transplanting		2 weeks after transplanting		4 weeks after transplanting	
		Trans	Back trans	Trans	Back trans	Trans	Back trans
1	Untreated	2.24	8.39	1.17	2.22	0.49	0.63
2	Cruiser 80 g/ha	1.80	5.05	1.96	6.10	0.59	0.80
3	Cruiser 60 g/ha	2.04	6.69	0.53	0.70	0.09	0.09
4	Gaucho 120g/ha	0.79	1.20	1.23	2.42	0.09	0.09
5	Gaucho 80g/ha	0.00*	0.00	1.83	5.23	0.29	0.34
6	Cruiser dummy pill 80g/ha	3.38	28.37	1.01	1.75	0.47	0.60
7	Gaucho dummy pill 120g/ha	0.00*	0.00	0.96	1.61	0.22	0.25
11	HDCI 064	2.16	7.67	2.50	11.18	1.78	4.93
	P value	<0.001		0.255		0.089	
	LSD (5%)	1.579		1.579		1.133	
	SED	0.786		0.786		0.563	

Foliar treatment and transplant drench treatment trial (Treatments 8, 9 and 10)

When plants were challenged with currant-lettuce aphids at transplanting, the aphids established well. None of the treatments reduced the number of aphids per plant (Figure 15) ($p<0.05$). The control plants had a mean number of 43.4 aphids per plant compared with the single application of Movento,, two applications of Movento (at this point both Movento treatments represented one application as the second application had not been applied until two weeks after transplanting) and the transplant drench treatments which had mean numbers of 37.90, 51.20 and 19.80 currant lettuce aphids per plant respectively.

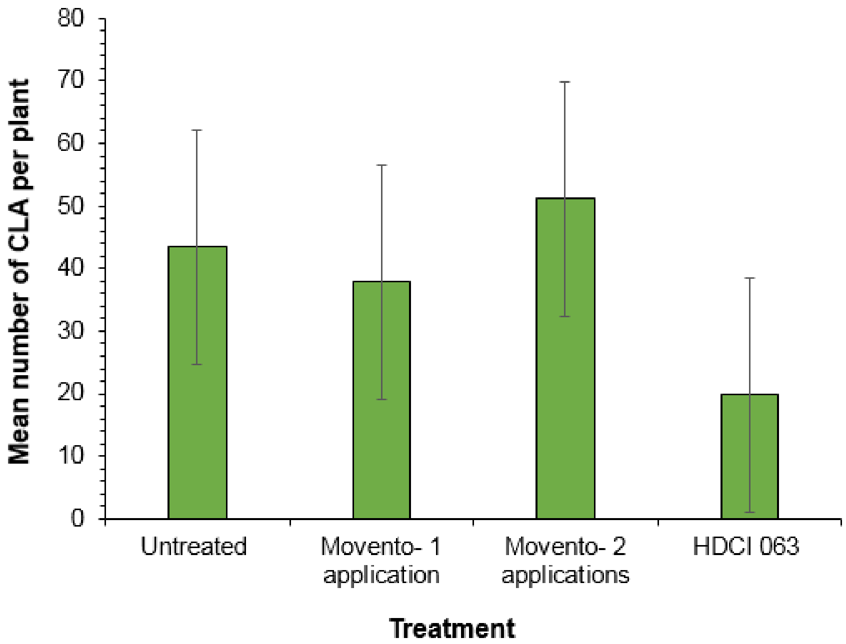

Figure 15 Mean number of currant-lettuce aphids (CLA) per plant when plants were infested at transplanting (95% confidence limits).

When plants were challenged with currant-lettuce aphids two weeks after transplanting, only Movento following a second application significantly reduced the number of aphids per plant compared to the control (Figure 16). The untreated plants had a mean number of 66.50 currant lettuce aphids per plant compared with the single Movento application, two Movento applications and the transplant drench treatment which had means of 79.50, 6.10 and 71.00 currant lettuce aphids per plant respectively.

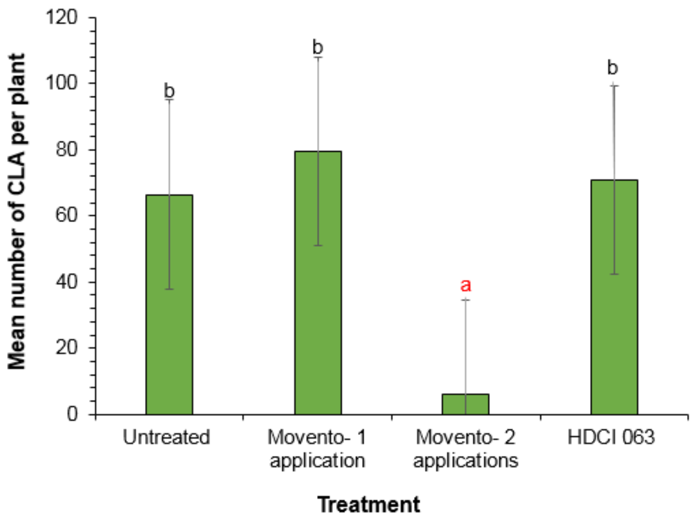

Figure 16 Mean number of currant-lettuce aphids (CLA) per plant when plants were infested two weeks after transplanting (95% confidence limits). Treatments with the same letters are not significantly different from each other. Treatments with red letters are significantly different from the untreated control (p<0.05).

When plants were challenged with currant-lettuce aphids four weeks after transplanting, only Movento following a second application (made two weeks after transplanting) was effective and reduced the number of aphids per plant to 18.6 compared with the control which had a mean of 47.5 aphids per plant (p<0.05) (Figure 17).

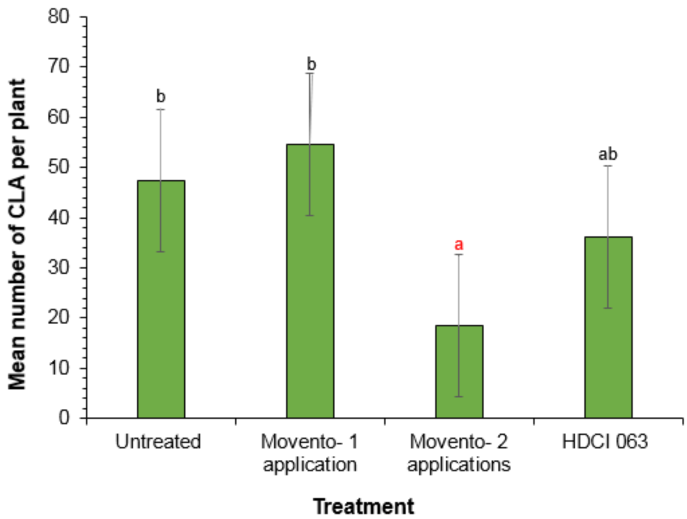

Figure 17 Mean number of currant-lettuce aphids (CLA) per plant when plants were infested four weeks after transplanting (95% confidence limits). Treatments with the same letters are not significantly different from each other. Treatments with red letters are significantly different from the untreated control.

When the plants were challenged with lettuce root aphids at transplanting, two weeks after transplanting and four weeks after transplanting, no statistically significant differences between treatments were observed (Figure 18). The aphids did not establish well and there was considerable variation in establishment both between and within treatments as demonstrated in Table 9 which shows the maximum and minimum number of aphids recorded per plant.

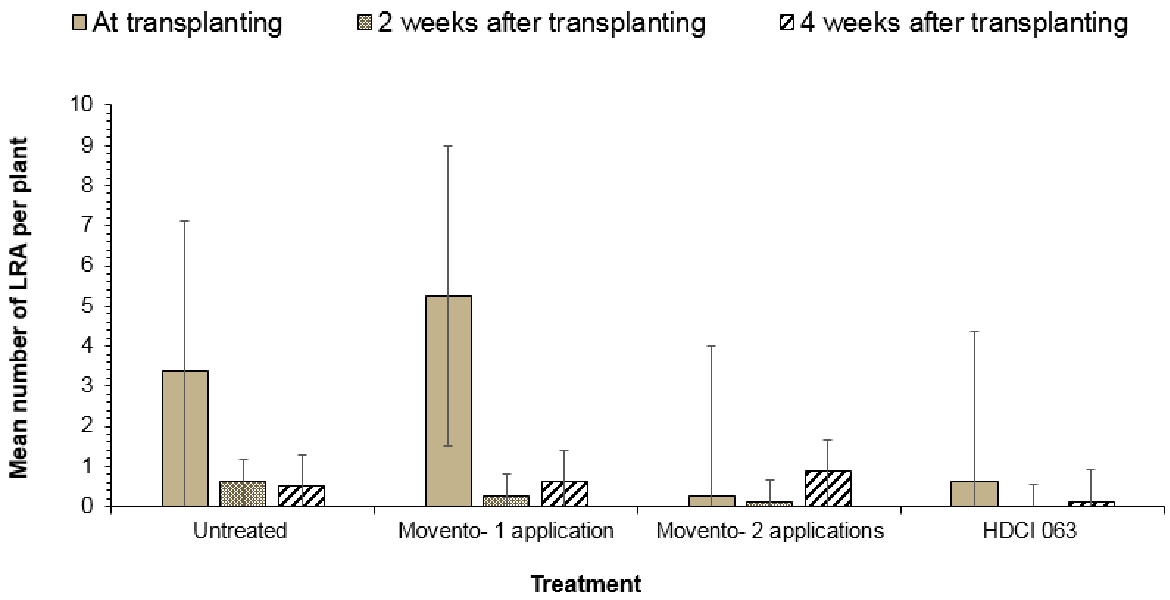

Figure 18 Mean number of lettuce root aphids (LRA) per plant when plants were infested at transplanting, two weeks after transplanting and four weeks after transplanting (95% confidence limits).

Table 9 Maximum and minimum numbers of lettuce root aphids recorded per plant for each treatment when infested at transplanting, two weeks after transplanting and four weeks after transplanting.

	At transplanting		2 weeks after transplanting		4 weeks after transplanting	
Treatment	**Max**	**Min**	**Max**	**Min**	**Max**	**Min**
Untreated	19	0	5	0	2	0
Movento 1 application	23	0	2	0	3	0
Movento 2 applications	2	0	1	0	4	0
HDCI 063	3	0	0	0	1	0

Objective 4: Determine the efficacy and persistence of seed treatments using reduced rates of insecticide and evaluate alternative methods for control of lettuce root aphid and currant-lettuce aphid on Iceberg lettuce in the field.

The forecast for lettuce-root aphid used in the HDC Pest Bulletin indicates that the start of the migration of winged lettuce root aphids from poplar to lettuce occurs after 672D° (base 4.4°C) have been accumulated since 1 February. At Wellesbourne in 2014, 672D° had been accumulated by 6 June. So, the three sequential plantings in Trial 1 (10 June, 18 June and 24 June) were made 4, 12 and 18 days respectively after the forecast start of the lettuce root aphid migration.

Field trial 1 – Treatments to control lettuce root aphid (*Pemphigus bursarius*)

All three plantings of lettuce were infested with lettuce root aphid. The mean infestation scores and mean head weights of the harvested lettuce plants are shown in Table 10 and the data on aphid infestation are summarised in Figure 19.

Analysis of variance indicated statistically significant treatment effects on the aphid infestation score for all three planting dates. The average size of the infestation decreased with later planting.

For Planting 1, all sowing-time treatments, with the exception of HDCI 064, reduced aphid numbers ($p<0.05$) compared with the untreated controls, as did the two treatments with Movento. There was no difference between any of the effective treatments.

For Planting 2, there was a statistically significant difference in the level of infestation between the two untreated controls. None of the treatments were significantly different ($p<0.05$) from the untreated control with the lower level of infestation (Treatment 1 in Table 2). All of the sowing-time treatments, with the exception of HDCI 064, were significantly different ($p<0.05$) from the untreated control with the higher infestation (Treatment 12) as was the Movento x1 treatment (Treatment 8 in Table 2).

For Planting 3, all sowing time treatments, with the exception of HDCI 064, reduced aphid numbers ($p<0.05$) compared with the untreated controls, as did the single treatment with Movento. There was no difference between any of the effective treatments.

There were no statistically significant differences in the head weights of the lettuce plants.

Table 10 Field trial on lettuce root aphid - mean infestation scores and mean head weights and summary of Analysis of Variance for the three planting dates. Data for the two untreated control treatments are analysed separately. * indicates treatments that were significantly different ($p<0.05$) from both untreated controls (in red).

	Treatment	**25th July**	**5th August**	**12th August**	**Head weight 5th August**	**Head weight 12th August**
1	Untreated	1.1	0.3	0.4	928	821
2	Cruiser 80g	0.325*	0.35	0.075*	948	748
3	Cruiser 60g	0.275*	0.2	0.025*	966	733
4	Gaucho 120g	0.125*	0.025	0*	942	761
5	Gaucho 80g	0.4*	0.025	0.1*	844	722
6	Cruiser dummy 80g	0.2*	0.125	0.075*	883	759
7	Gaucho dummy 120g	0.325*	0.075	0.05*	861	803
8	Movento x 1	0.375*	0.45	0.125*	886	713
9	Movento x 2	0.3*	0.55	0.2	922	781
10	HDCI 064	1	0.6	0.55	903	813
11	HDCI 063	0.175*	0.125	0.025*	876	772
12	Untreated	1.075	0.9	0.35	950	798
	P value	0.001	0.014	0.0006	0.215	0.929
	LSD (5%) (two-sided)	0.536	0.480	0.249	N/a	N/a
	LSD (5%) (one-sided)	0.446	0.399	0.207		

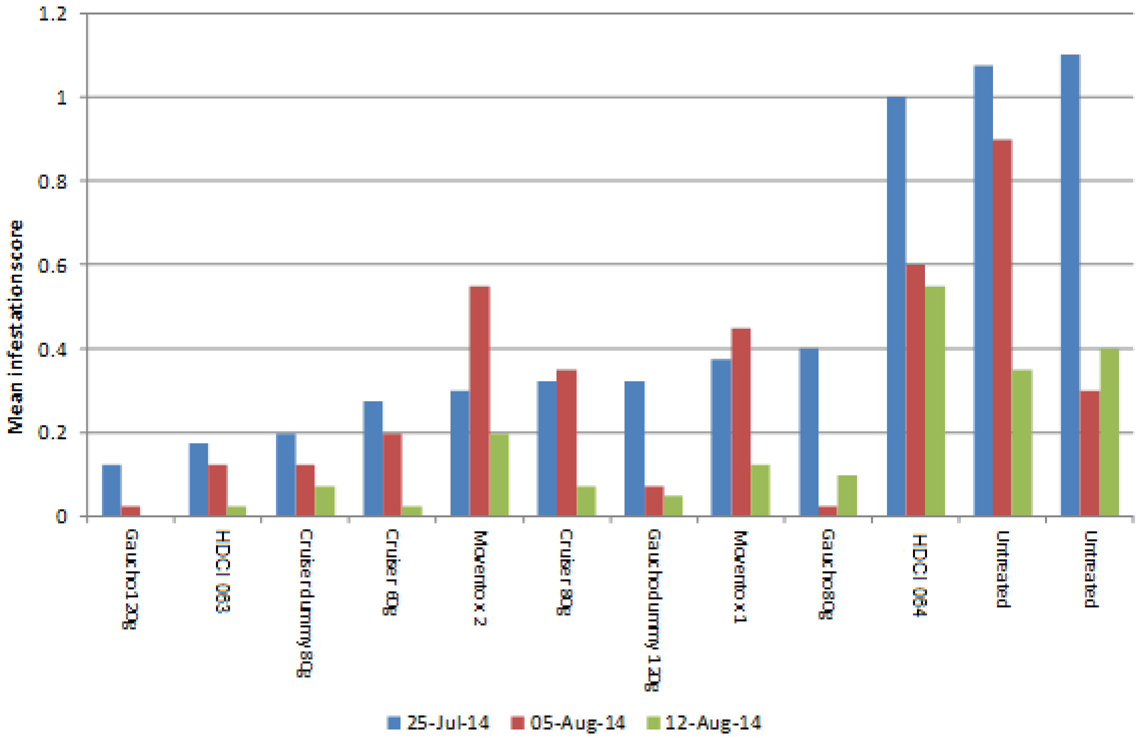

Figure 19 Field trial on lettuce root aphid - mean infestation scores. Data sorted according to infestation score on 25th July 2014.

<u>Field trial 2 – Treatments to control currant-lettuce aphid (*Nasonovia ribisnigri*)</u>

The plants were infested with currant-lettuce aphids on 30 September and 20 October and the numbers of aphids remaining in the clip cages were recorded on 7 October and 27 October respectively.

Table 11 and Figure 20 show the numbers of aphids present in the clip cages at the time of the first assessment on 7 October. These were analysed in terms of the numbers of adult aphids (winged or wingless), nymphs and the total number of aphids. Most of the sowing-time treatments, with the exception of HDCI 064, reduced the numbers of aphids compared with the untreated controls. The treatments with Movento were generally less effective.

Table 11 Trial on currant-lettuce aphid – number of aphids at first assessment. Data for the two untreated control treatments are analysed separately. * indicates treatments that were significantly different (p<0.05) from both untreated controls (in red) using transformed data.

	Trans	Back-trans	Trans	Back-trans	Trans	Back-trans	Trans	Back-trans
Treatment	**Winged**	**Winged**	**Wingless**	**Wingless**	**Nymphs**	**Nymphs**	**Total**	**Total**
Untreated	1.65	2.71	2.44	5.98	1.67	2.78	3.51	12.33
Cruiser 80g	0*	0	0.68*	0.47	0*	0	0.68*	0.47
Cruiser 60g	0.25*	0.06	0*	0	0*	0	0.25*	0.06
Gaucho 120g	0*	0	0.35*	0.13	0*	0	0.35*	0.13
Gaucho 80g	0*	0	0*	0	0*	0	0*	0
Cruiser dummy 80g	0*	0	0.98*	0.97	0*	0	0.98*	0.97
Gaucho dummy 120g	0.25*	0.06	0.50*	0.25	0*	0	0.60*	0.36
Movento x 1	1.11	1.24	2.24	5.02	0.56*	0.31	2.74	7.48
Movento x 2	1.21	1.46	1.92	3.70	0.81	0.65	2.61	6.82
HDCI 064	2.00	3.99	2.60	6.74	1.86	3.45	3.99	15.93
HDCI 063	0.50*	0.25	0.85*	0.73	0*	0	1.21*	1.46
Untreated	1.91	3.66	2.84	8.08	1.61	2.58	4.19	17.52
p	<0.001		<0.001		0.005		<0.001	
SED	0.47		0.48		0.61		0.60	
LSD (5%) (two-sided)	0.96		0.97		1.23		1.22	
LSD (5%) (one-sided)	0.80		0.80		1.03		1.01	

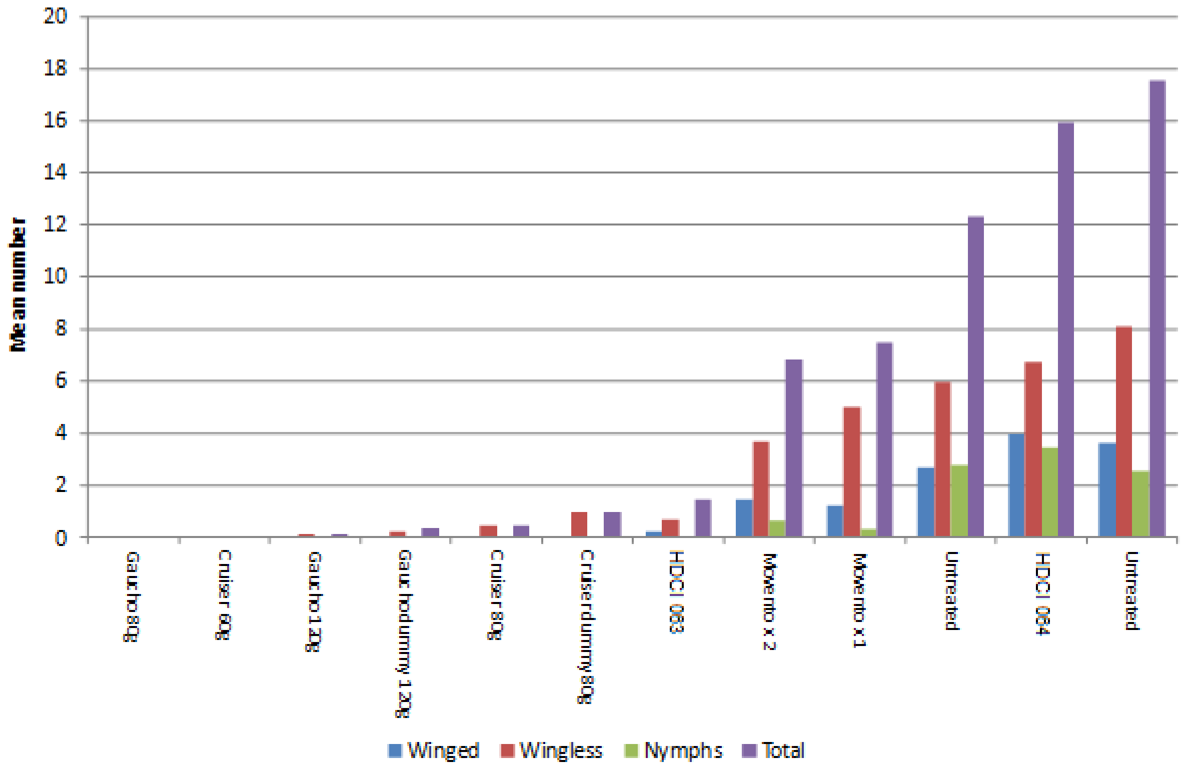

Figure 20 Trial on currant-lettuce aphid - mean number of aphids per plot – assessment 1. Data sorted according to total number of aphids.

Table 12 and Figure 21 show the numbers of aphids present in the clip cages at the time of the second assessment on 27 October. These were again analysed in terms of the numbers of adult aphids (winged or wingless), nymphs and the total number of aphids. The differences between treatments were less pronounced and the Analysis of Variance showed no statistical significance.

Table 12 Trial on currant-lettuce aphid – number of aphids at second assessment. Data for the two untreated control treatments are analysed separately. * indicates treatments that were significantly different (p<0.05) from both untreated controls (in red) using transformed data.

	Trans	Back-trans	Trans	Back-trans	Trans	Back-trans	Trans	Back-trans
Treatment	**Winged**	**Winged**	**Wingless**	**Wingless**	**Nymphs**	**Nymphs**	**Total**	**Total**
Untreated	0.68	0.47	1.84	3.40	0.56	0.31	2.21	4.89
Cruiser 80g	0.60	0.36	1.92	3.69	0	0	2.08	4.34
Cruiser 60g	0.71	0.50	1.60	2.55	0	0	1.75	3.07
Gaucho 120g	0.85	0.73	0.91	0.83	0	0	1.40	1.96
Gaucho 80g	0.25	0.06	0.91	0.83	0	0	1.16	1.35
Cruiser dummy 80g	0.35	0.13	1.24	1.54	0	0	1.34	1.81
Gaucho dummy 120g	0.93	0.87	1.93	3.73	0	0	2.19	4.80
Movento x 1	1.57	2.46	2.42	5.86	1.04	1.07	3.17	10.02
Movento x 2	0.35	0.13	2.12	4.48	0	0	2.24	5.03
HDCI 064	1.00	1.00	2.00	4.00	0.50	0.25	2.43	5.88
HDCI 063	0.68	0.47	1.98	3.94	0.35	0.13	2.33	5.44
Untreated	1.04	1.07	2.11	4.45	0.35	0.13	2.47	6.10
p	0.44		0.27		0.21		0.22	

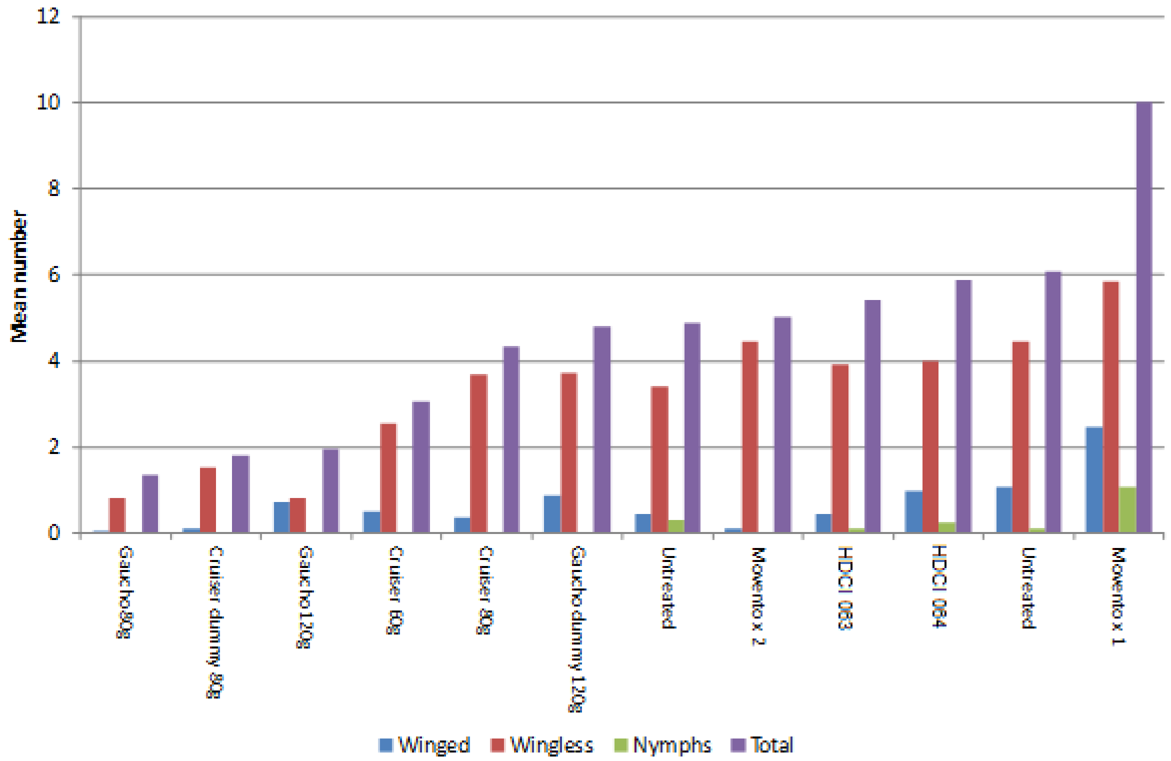

Figure 21 Trial on currant-lettuce aphid - mean number of aphids per plot – assessment 2. Data sorted according to total number.

Lettuce root aphid culture

It transpired that some of the spare plants that remained in a polytunnel became heavily-infested with lettuce root aphid and these plants were used for the pot experiments at ADAS Boxworth.

Residue testing

Table 13 shows the results for the residue testing of lettuce sampled from Warwick Crop Centre on 3 November (planting date 17 September). At the time of sampling, the lettuce plants were small but healthy and were beginning to develop heads. The limit of detection was 0.01 mg/kg.

The EU maximum residue limit (MRL) is the EC Statutory MRLs set under EC Regulation 396/2005

Table 13 Level of pesticide found in lettuce samples sent for residue testing from treatments 1 -9.

Treatment	Level found (mg/kg)	EU MRL (mg/kg)	Sample images
Untreated	No residue detected above the limit of detection.	N/A	
Cruiser 80 g/ha	0.03	5.0	
Cruiser 60 g/ha	0.02	5.0	
Gaucho 120g/ha	0.07	2.0	
Gaucho 80g/ha	0.07	2.0	

Cruiser dummy pill 80g/ha	0.02	5.0	
Gaucho dummy pill 120g/ha	0.08	2.0	
Movento 1 application (0.5 l/ha, 300l/ha water)	No residue detected above the limit of detection.	N/A	
Movento 2 applications (0.5 l/ha, 300l/ha water)	No residue detected above the limit of detection.	N/A	

Discussion

Pot trial at ADAS Boxworth

An effective method for culturing lettuce root aphids has been established by collecting galls from poplar trees around the forecast time of migration from poplar to lettuce crops and enclosing them in insect-proof cages containing lettuce plants

All the seed treatments except HDCI 064 were effective in reducing the numbers of currant-lettuce aphids compared with the control. The effective treatments provided 100% control at transplanting and continued to provide high levels of control at two and four weeks after transplanting. This confirmed that the seed treatments were persistent until the last assessment, which was four weeks after transplanting. With regard to seed treatment efficacy against lettuce root aphid, Gaucho showed some efficacy at transplanting; Gaucho as a dummy pill at 120g/ha and Gaucho treated seed at 80g/ha provided 100% control at transplanting. Gaucho-treated seed at 120g/ha did not provide 100% control but performed as well as Gaucho as a dummy pill at 120g/ha and Gaucho-treated seed at 80g/ha. At the time of the later assessment, no differences in the level of control were observed between the treatments due to the variation in infestation between the eight replicates of each treatment. However, when looking at the maximum number of aphids recorded per plant over the eight replicate plants two weeks after transplanting, the data suggest that lettuce root aphids did establish in high numbers on at least one of the replicate plants for each treatment.

Since seed treatments were introduced for use on lettuce, there have been no reports of severe lettuce root aphid infestations, implying that seed treatments have been providing some measure of protection. In this study, lettuce root aphids were applied directly to the roots, however, the literature suggests that nymphs produced by the migratory winged adults do insert their stylets into the plant, possibly feeding, before moving down to the soil (Dunn, 1959). It is possible that the seed treatments control lettuce root aphids at this point in their life cycle.

When analysing the data from the foliage and drench treatments, only a second application of Movento, two weeks after transplanting, reduced the number of currant-lettuce aphids per plant compared to the control ($p<0.05$). It is possible that the lack of control following the first application of Movento, at transplanting, was due to the timing of the application which was made on the day of transplanting. At this time, the plants were unlikely to have been growing actively and may not have taken up the product systemically. When the second application of Movento was made, two weeks after transplanting, the plants would have been growing actively and hence the systemic properties of the product were more likely to take effect.

The pot trial also demonstrated a higher level of control by Movento following a second application than in the field trial, which may be due to the slightly higher concentration used in the pot trial. Whilst the same rate of Movento per unit area (0.5l/ha) was used in both experiments, only 200 l/ha was used in the pot trial whilst 300l/ha was applied to the field trial. No effects of Movento or the experimental transplant drench were observed on lettuce root aphids as there was low establishment of the aphids on all of the treatments including the untreated control. Ideally more replicates should have been included but the trial was already very large.

Field trial at Warwick Crop Centre

For the trial on lettuce root aphid (Trial 1), the three sequential plantings (10 June, 18 June and 24 June) were made 4, 12 and 18 days respectively after the forecast start of the lettuce root aphid migration. The period of migration usually lasts for 4-6 weeks. All plantings were infested with aphids when harvested. However, the earliest planting suffered the highest level of infestation and, generally, plots planted on 24 June suffered the lowest level of infestation. The plots were assessed after similar periods of time from transplanting (45, 48 and 49 days respectively) and it is likely that the different levels of infestation were determined by the duration of exposure to migrating aphids.

The performance of individual treatments against lettuce root aphids was relatively consistent across the three plantings (Figure 1) and most of the sowing-time treatments, with the exception of HDCI 064, were effective. In particular, there were no statistically-significant differences in the performance of the sowing-time treatments that were applied at two rates (Cruiser seed treatment and Gaucho seed treatment), nor between the dummy pill and direct seed treatments applied at similar rates. The novel pre-planting drench treatment (HDCI 063) was as effective as the Gaucho and Cruiser treatments, as were the Movento sprays. Unfortunately, the novel seed treatment (HDCI 064) was ineffective.

It is not surprising that the distribution of lettuce root aphids across the trial was uneven and this variation in aphid numbers was most evident in the different levels of infestation in the two untreated control treatments in the second planting. This inherent variability across the trial is a key factor determining the ‘sensitivity’ of the statistical analyses and in order to distinguish between the more effective treatments, it would have been necessary to increase replication, both in terms of plot numbers and the number of plants assessed per plot. Since the trial was already large (48 plots x 3 plantings) this would probably have been feasible only if the number of treatments had been reduced.

The aim of the trial on the currant-lettuce aphid was to determine the efficacy of the treatments on two occasions during the life of the crop by infesting the plants with known numbers of adult aphids 13 and 33 days after transplanting (39 and 59 days after sowing respectively). The test treatments could potentially have lethal effects on the adult aphids or sub-lethal effects on reproduction (production of nymphs). At the time of the first assessment (aphids applied 13 days after transplanting), there were statistically-significant differences between treatments and the sowing-time treatments, with the exception of HDCI 064, reduced the numbers of aphids compared with the untreated controls. However, the treatments with Movento were generally less effective. By the time of the second assessment (aphids applied 33 days after transplanting), the sowing-time treatments with neonicotinoid insecticides (Gaucho and Cruiser) and HDCI 063 had become less effective, as would be expected, and none were providing a significant level of control. The Movento treatments were relatively ineffective.

Overall, the two trials showed that the sowing time treatments using neonicotinoid insecticides and the drench treatments with HDCI 063 were effective against both lettuce root aphid and currant-lettuce aphid, but that their efficacy (in terms of control of the currant-lettuce aphid) decreased over time. The sowing-time treatment with HDCI 064 was ineffective against both species. The treatments with Movento showed some activity against both species but appeared to be less effective than the sowing-time treatments with neonicotinoids or HDCI 063. The timing of the spray application(s) must be a key factor. The first application of Movento was made six days after transplanting and the second application was made 23 days after transplanting. On both occasions plants were infested with aphids one week after each spray.

Differences observed between field and pot studies may be related to differences in environmental conditions. A pot trial does not represent what would happen in a field situation due to differences in soil type, depth of soil, watering, temperature etc. In this study, whilst the seed treatments may have remained effective in a pot for up to four weeks, this may not be representative of a field crop. In a field situation, variation in results can also occur between plantings due to changes in conditions throughout the season.

While the study does suggest that reducing insecticide dose rates for Iceberg lettuce does not affect control of currant lettuce aphid, there are 'broader' issues regarding insecticide resistance which need to be considered, as reduced dose rates can be linked to an increased risk of insects developing resistance. Currently there is no evidence of resistance to neonicotinoids in currant-lettuce aphid or lettuce root aphid.

Conclusions

- An effective method for culturing lettuce root aphid has been developed by collecting lettuce root aphid galls from poplar trees during the predicted period of migration (HDC Pest Bulletin) of lettuce root aphid from poplar trees to lettuce and enclosing them in insect-proof cages containing lettuce.
- All lettuce samples sent for residue testing were found to be below the EU maximum residue limits.

Currant lettuce aphid

- Both the field trial and the pot trial showed that all the seed treatments (except HDCI 064) reduced the number of currant-lettuce aphids compared to the control ($p<0.05$). The pot trial showed that the seed treatments were still effective four weeks after transplanting. In contrast, the field trial showed that that these treatments were no longer effective 5 weeks from transplanting.
- No differences were observed in the level of control provided by the standard and lower seed treatment rates for Cruiser and Gaucho. Dummy pills were also just as effective as the standard seed treatments.
- The pot trial showed that after the second application of Movento there was a significant reduction in the number of currant-lettuce aphids compared with the control. The treatment was still effective two weeks after application. The lack of control observed in the pot trial following only one application is likely to be due to the timing of the application, which was made on the day of transplanting, when the plants were not actively growing. However, no control was observed in the field trial after either one or two applications when the first sprays were applied six days after transplanting (one week before the aphids were placed on the plants). The difference between the pot and field experiments in efficacy following the second application of Movento may be due to the differences in concentration used (pot trial - 0.5 l/ha in 200 l of water per hectare; field trial- 0.5 l/ha in 300 l of water per hectare).
- The novel transplant drench treatment resulted in a significant reduction in currant-lettuce numbers in the field trial but not in the pot trial.

Lettuce root aphid

- In the pot trial, only Gaucho reduced the number of lettuce root aphids at transplanting compared with the untreated control. Two and four weeks after transplanting, no differences were observed between treatments due to the variation between replicates. Results from the field trial showed that the seed treatments had a statistically significant effect in reducing the number of aphids per plant compared to the control. It is possible that the seed treatments may have exerted control of lettuce root aphids during their brief foliage-feeding stage rather than by controlling infestations developing subsequently on the roots; this may explain the differences in control observed between the pot trial and field trial.
- In the pot trial, the efficacy against lettuce root aphid of Movento and the novel transplant drench treatment was not determined due to poor establishment of the aphids. Results from the field trial suggested that both Movento and the transplant drench treatment were effective against lettuce root aphids. In both the pot and field trials, Movento sprays were applied on the day of transplanting.

Knowledge and Technology Transfer

Presentation at HDC Leafy salads Roadshow: 6 November- Huntapac, Preston and 26 November, Stoneleigh.

HDC News article- March 2015 edition

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Parker, W.E. (1995) Outdoor lettuce: assessment of the efficacy of imidacloprid for the control of lettuce root aphid. Final Report HDC Project FV162a.

Dunn, J.A. (1959). The biology of lettuce root aphid. Annals of Applied Biology, 47 (3), 475-491.

Hough, G.L. (2013). Biology and control of currant lettuce aphid, *Nasonovia ribisnigri,* PhD thesis.

Appendix

Pesticide loading of treated seeds determined by High-performance Liquid Chromatography:

Prod. 14524...05-05-01 CRUISER 80: Gai/U 75.83 % of Target: 94.8
Prod. 14524...05-02-02 CRUISER 60: Gai/U 57.31 % of Target: 95.5
Prod. 14524...05-03-02 IMIDACLOPRID 80: Gai/U 82.42 % of Target: 103.0
Prod. 14524...03-01-02 IMIDACLOPRID 120: Gai/U 117.51 % of Target: 97.9

