

Project title: Outdoor salads: Evaluation of novel insecticides for control of aphids

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GROWER SUMMARY

Headline

- An experimental seed treatment - Exp B (a mixture of two active ingredients) provided the best long term control, reducing aphid numbers by 76%, 65 days after sowing.
- All of foliar insecticide sprays (Aphox, Plenum, Biscaya, Exp S and Exp U) reduced aphid numbers compared with the untreated control but low pest pressure meant results were not statistically significant

Background and expected deliverables

Several aphid species infest the foliage of lettuce, of which currant-lettuce aphid (*Nasonovia ribisnigri*), peach-potato aphid (*Myzus persicae*) and potato aphid (*Macrosiphum euphorbiae*) are the most important. *Nasonovia ribisnigri* is particularly difficult to control, as it infests the heart of the plant and is therefore inaccessible to foliar sprays of insecticide.

In addition, insecticide resistance to Pirimicarb (Aphox) in *N. ribisnigri* is now widespread, but levels vary. Between 1999 and 2001 the levels of resistance to pyrethroids appeared to have increased in some strains of *N. ribisnigri* in the UK and resistant aphids commonly show cross-resistance to a range of pyrethroid compounds. There is no evidence of resistance to imidacloprid (Gaucho) or Pymetrozine (Plenum) in *N. ribisnigri*. Some populations of peach-potato aphid are also resistant to insecticides, particularly Pirimicarb and pyrethroids. Again there is no evidence of pronounced resistance to Imidacloprid or Pymetrozine in peach-potato aphid.

The difficulties of controlling lettuce aphids and the occurrence of insecticide resistance in *N. ribisnigri* and *M. persicae* mean that there is a need to find alternative and effective methods of control. The aim of this project is to evaluate novel insecticides for the control of aphids, particularly *N. ribisnigri*, on lettuce crops

The benefits of this project will be an assessment of new treatments for control of aphids on lettuce and an indication of those that should be taken forward for Full or Specific Off-Label Approval.

The expected deliverables from this work include:

- An evaluation of novel seed treatments for the control of aphids on lettuce
- An evaluation of novel insecticide sprays for the control of aphids on lettuce

Summary of the project and main conclusions

Two experiments were done in 2007 using eight insecticides (Aphox (Pirimicarb), Plenum (Pymetrozine), Biscaya (Thiacloprid), Sanokote (Imidacloprid) and four experimental treatments (Exp B, Exp A, Exp S and Exp U).

Experiments were done to answer the following questions:

1. Are there novel seed treatments to control aphids on lettuce? (Field Experiment 1)
2. Are there novel spray treatments to control aphids on lettuce? (Field Experiment 2)

Experiment summaries and main conclusions

1. Novel seed treatments to control aphids on lettuce

The experiment was designed to assess novel insecticides as seed treatments for the control of aphids (and caterpillars if any) on lettuce. Three insecticides (Sanokote (Imidacloprid), Exp A and Exp B) were assessed. Because natural infestations proved to be very low, a 'clip cage' experiment was instigated. Ten plants in each plot were infested with *N. ribisnigri* (wingless adults) by placing 5 aphids into a clip cage which was then secured onto a leaf, so that the aphids could not escape but had access to the leaf surface. The plants were infested quite late in the growth cycle and, in particular, this provided a test of the persistence of the different insecticide treatments.

Results

- Natural infestations were too low to make statistical analyses of results
- None of the treatments tested in clip cage experiments were 100% effective 65 - 73 days after sowing.
- Exp B (a mixture of two active ingredients) provided the best long term control, reducing aphid numbers by 76% 65 days after sowing.
- Exp A (73 days after sowing) and Sanokote (65 days after sowing) did not significantly reduce aphid numbers compared with the untreated controls, but the extended period after sowing should be taken into consideration.

2. Novel spray treatments to control aphids on lettuce

The experiment was designed to assess novel insecticides as foliar sprays for the control of aphids (and caterpillars if any) on lettuce. Five insecticides Aphox (Pirimicarb), Plenum (Pymetrozine), Biscaya (Thiacloprid) and two new compounds (Exp S and Exp U) were assessed as foliar sprays for the control of foliar pests.

The plants were transplanted into a field plot on 6 August. Aphid numbers remained very low over the life of the plants and aphids failed to colonise in sufficient numbers to make the trial worthwhile. Therefore the plants were re-sown on 20 August and kept in a glasshouse as before. On 10 September, one week before transplanting, they were infested with *N. ribisnigri* (by the introduction of laboratory-reared aphids on lettuce leaves). The plants were transplanted on 17 September and sprays were applied to the plots on 1 October after infestations of aphids had built up. Counts of pest numbers were made before and after spraying.

Results

- All of the treatments (Aphox, Plenum, Biscaya, Exp S and Exp U) reduced aphid numbers compared with the untreated control.
- There were no statistically significant differences between insecticide treatments.
- Biscaya reduced aphid numbers by 86%

Summary

It was a poor year for aphid establishment, due probably to the very wet summer weather. Seed treatment performance was disappointing. All of the spray treatments were effective to some extent but relatively low aphid numbers did not allow discrimination between spray treatments

Financial benefits

- The farm gate value of the approximately 6000 ha of field lettuce grown in the UK is around £75 million. The retail value of the UK market for bagged salads and whole-head lettuce is approximately £478 million. However, bagged salads would include spinach, watercress, babyleaf Brassica etc.
- The presence of aphids can lead to the rejection of a whole consignment of lettuce, be it whole-head or destined for processing in bagged salads. Around 70% of the crop is at risk from aphid losses.
- Despite the availability of cultivars of lettuce resistant to *N. ribisnigri*, many growers prefer to grow susceptible varieties, so insecticidal control methods will be relied on for some years

to come. In addition, there is some evidence that, in 2007, some populations of *N. ribisnigri* in mainland Europe have been able to develop on resistant varieties.

- With reports of *N. ribisnigri* having reduced sensitivity to Pirimicarb and pyrethroid insecticides and with some populations of *M. persicae* already having resistance to these chemicals, the new chemistries that are becoming available to growers give them the opportunity to develop effective control programmes and reduce the incidence of crop losses due to aphid infestation.

Action points for growers

All of the approved treatments applied as foliar sprays (Aphox, Plenum, Biscaya) reduced aphid numbers compared with the untreated control, but there were no statistically significant differences between the insecticide treatments.

The availability of Plenum, Biscaya and other new insecticides with different modes of action to Pirimicarb and pyrethroids provides the opportunity to develop insecticide spray programmes which alternate insecticide products with different modes of action, to minimise the risk of developing insecticide-resistant aphid populations.

However, it is important to avoid using insecticides with a similar mode of action in succession, so, for example, a neonicotinoid should not be used as the first spray treatment on crops that have been grown from seed treated with Imidacloprid.

SCIENCE SECTION

Introduction

Several aphid species infest the foliage of lettuce, of which currant–lettuce aphid (*Nasonovia ribisnigri*), peach-potato aphid (*Myzus persicae*) and potato aphid (*Macrosiphum euphorbiae*) are the most important. *Nasonovia ribisnigri* is particularly difficult to control, as it infests the heart of the plant and is therefore inaccessible to foliar sprays of insecticide.

In addition, insecticide resistance to Pirimicarb (Aphox) in *N. ribisnigri* is now widespread, but levels vary. Between 1999 and 2001 the levels of resistance to pyrethroids appeared to have increased in some strains of *N. ribisnigri* in the UK and resistant aphids commonly show cross-resistance to a range of pyrethroid compounds. There is no evidence of resistance to Imidacloprid (Gaucho/Sanokote) or Pymetrozine (Plenum) in *N. ribisnigri*. Some populations of peach-potato aphid are also resistant to insecticides, particularly Pirimicarb and pyrethroids. Again there is no evidence of pronounced resistance to Imidacloprid or Pymetrozine in peach-potato aphid.

The difficulties of controlling lettuce aphids and the occurrence of insecticide resistance in *N. ribisnigri* and *M. persicae* mean that there is a need to find alternative and effective methods of control. The aim of this project is to evaluate novel insecticides for the control of aphids, particularly *N. ribisnigri*, on lettuce crops

Experiments were done to answer the following four questions:

1. Are there novel seed treatments to control aphids on lettuce? (Field Experiment 1)
2. Are there novel spray treatments to control aphids on lettuce? (Field experiment 2)

The test chemicals are shown as the active ingredients (with the product used in parenthesis as certain chemicals are available under a range of different product names.

The actual active ingredients tested, together with the product used (shown in parenthesis), were: Pirimicarb (Aphox), Pymetrozine (Plenum), Thiacloprid (Biscaya), Imidacloprid (Sanokote) and 4 experimental treatments (Exp A, Exp B, Exp S and Exp U).

Experiment 1

Novel seed treatments to control aphids on lettuce

Materials and methods

The experiment was done within the field known as Sheep Pens at Warwick HRI, Wellesbourne. The treatments are listed in Table 1. Treatment 2 consisted of dead seed treated with Imidacloprid (Sanokote) and it was sown together with live seed (cv Saladin) as with Treatment 1. Treatment Exp B was applied using an experimental set-up.

Lettuce seeds (two cultivars, see Table 1) were sown in peat blocks on 10 July 2007 and kept in a glasshouse. Treatment 3 germinated poorly and treatments 1-3 were re-sown on 31 July.

The plants were transplanted into a field plot on 6 August (Treatments 4 and 5) and 29 August (Treatments 1-3). The experiment was laid out as an incomplete Latin square and there were 4 replicates of 5 treatments. Plots were 4 m x 1 bed (1.83 m) in size and there were 4 rows of 12 plants (48 plants). Plants were planted at 35 cm spacing within, and 38 cm between, rows.

Natural infestations of *Nasonovia ribisnigri*, *Myzus persicae* and *Macrosiphum euphorbiae* were assessed on 28 August (Treatments 4 and 5 only) and 26 September (all treatments) by counting the numbers of aphids on 12 marked plants in the middle 2 rows.

Because natural infestations proved to be very low, a 'clip cage' experiment was instigated. Ten plants in each plot were infested with *N. ribisnigri* (wingless adults) by placing 5 aphids into a clip cage which was then secured onto a leaf, so that the aphids could not escape but had access to the leaf surface. Treatments 4-5 and 1-3 were inoculated on 13 September (65 days after sowing) and 12 October (73 days after sowing) respectively. In particular, this provided a test of the persistence of the different insecticide treatments.

The clip cages were removed from treatments 4-5 and 1-3 on 24 September (11 days after Infestation) and 30 October (18 days after Infestation) respectively and the numbers of wingless, winged and parasitised aphids were counted.

Table 1 Seed treatments to control aphids on lettuce

Code	Variety	Active ingredient	Rate
1	Saladin	Untreated	
2	Saladin	Imidacloprid - Sanokote	Commercial rate – 80 g per 100,000 seeds
3	Saladin	Exp B	0.8 mg + 0.27 mg per seed
4	Funtime	Untreated	
5	Funtime	Exp A	80g per 100,000 seeds

Results

a) Natural infestation

Counts of *N. ribisnigri*, *M. persicae* and *M. euphorbiae* (winged and wingless aphids) were recorded on two occasions (28 August and 26 September), together with the numbers of parasitized aphids, caterpillars and other insects. The counts were obtained from 12 plants in each plot and the total numbers in each category per plot were analysed. On the first sampling occasion (28 August) only plots for Treatments 4 and 5 were sampled, therefore this subset was analysed using a two-sided t-test. Only four data points were available, which reduced the reliability of this test. All plots were sampled on the second occasion (26 September) and analysis of variance (ANOVA) was used. Not all categories had enough non-zero data for a sensible analysis to be performed.

The results from the t-test and the ANOVA are summarized in Table 2 and Tables 3 and 4 respectively. Where a statistically significant treatment effect was found, pair-wise comparisons were performed.

Table 2. The numbers of aphids per plot (12 plants) recorded on treated lettuce plants on 28 August 2007.

Treatment	Variety	<i>M. persicae</i> winged	Total winged
Untreated	Funtime	0.50	0.50
Exp A	Funtime	0.25	0.50
Test Statistic		0.65	0.00
df		6	6
probability		0.537	1.000

Table 3 The mean numbers per plot (12 plants) of *Nasonovia ribisnigri* and *Macrosiphum euphorbiae* recorded on seed-treated lettuce plants on 26 September 2007. Statistically significant differences between the treatment means are shown by the letters next to each mean. Treatment means with a letter in common are said to be not significantly different.

Treatment	Variety	<i>Nasonovia ribisnigri</i> winged	<i>Nasonovia ribisnigri</i> wingless	<i>Macrosiphum euphorbiae</i> winged	<i>Macrosiphum euphorbiae</i> wingless
Untreated	Saladin	1.97 a	2.65	0.00	4.15
Imidacloprid (Sanokote)	Saladin	1.57 a	2.65	0.00	1.42
Exp B	Saladin	2.83 a	0.25	1.15	1.28
Untreated	Funtime	6.57 b	0.00	0.42	3.08
Exp A	Funtime	3.57 ab	0.72	2.55	1.82
F-probability		0.042	0.425	0.341	0.543
SED		1.382	1.771	1.411	1.914
LSD (95%)		3.188	4.085	3.254	4.413
df		8	8	8	8

Table 4 The mean numbers per plot (12 plants) of *Myzus persicae*, parasitised aphids and total wingless aphids (all species) recorded on seed-treated lettuce plants on 26 September 2007. Statistically significant differences in the treatment means are shown by the small letters next to each mean. Treatment means with a letter in common are said to be not significantly different.

Treatment	Variety	<i>Myzus persicae</i> wingless	Parasitised aphids	Total winged aphids	Total wingless aphids
Untreated	Saladin	1.22 ab	1.450 b	1.70 a	8.02
Imidacloprid (Sanokote)	Saladin	0.00 a	0.383 a	1.70 a	3.95
Exp B	Saladin	2.55 b	0.983 ab	3.97 ab	4.08
Untreated	Funtime	0.08 a	0.717 a	7.03 c	3.15
Exp A	Funtime	0.02 a	0.717 a	6.10 bc	2.55
F-probability		0.045	0.060	0.010	0.472
SED		0.806	0.2981	1.313	3.067
LSD (95%)		1.858	0.6875	3.028	7.072
df		8	8	8	8

b) Infestation with *Nasonovia ribisnigri*

The numbers of winged and wingless *N. ribisnigri* and parasitized aphids inside clip cages attached to the plants were also recorded on two occasions. On the first occasion (assessed 24 September) the clip cages were only attached to plants in Treatments 4 and 5 (cv Funtime), while the remaining three treatments were assessed on the second occasion (12 October). Similar analyses to those outlined above (t-test for the Funtime treatments and ANOVA for the Saladin treatments) were performed and are summarized in Tables 5 and 6 for the first and second assessments respectively and also in Figure 1.

Table 5 The mean numbers per plot (12 plants) of *Nasonovia ribisnigri* in clip cages recorded on seed-treated lettuce plants on 24 September 2007. Statistically significant differences in the treatment means are shown by the letters next to each mean. Treatment means with a letter in common are said to be not significantly different.

Treatment	Variety	<i>Nasonovia ribisnigri</i> winged	<i>Nasonovia ribisnigri</i> wingless	Mummies
Untreated Exp A	Funtime	10.50	28.00	7.00
	Funtime	9.50	20.75	6.50
Test Statistic		0.31	0.41	0.32
df		6	6	6
probability		0.764	0.694	0.759

Table 6 The mean numbers per plot (12 plants) of *Nasonovia ribisnigri* in clip cages recorded on seed-treated lettuce plants on 12 October 2007. Statistically significant differences in the treatment means are shown by the letters next to each mean. Treatment means with a letter in common are said to be not significantly different.

Treatment	Variety	<i>Nasonovia ribisnigri</i> winged	<i>Nasonovia ribisnigri</i> wingless	Mummies
Untreated Imidacloprid (Sanokote) Exp B	Saladin	4.25	44.8 b	1.75
	Saladin	2.25	23.5 ab	3.00
	Saladin	2.75	10.5 a	3.50
F-probability		0.460	0.024	0.514
SED		1.563	8.99	1.477
LSD (95%)		3.826	21.99	3.613
df		6	6	6

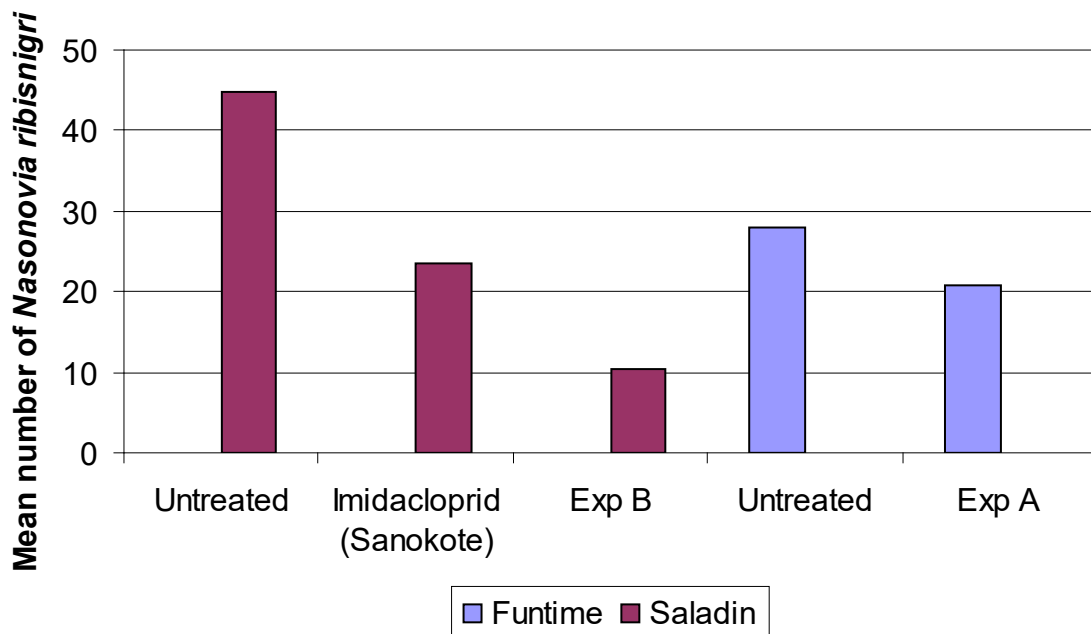


Figure 1. The mean numbers per plot (12 plants) of wingless *Nasonovia ribisnigri* in clip cages on seed-treated lettuce plants on 12 October 2007.

Discussion

Natural infestations of all aphid species were very low throughout the experiment. Because of this, there were no statistically significant differences between any of the insecticide seed treatments and their respective untreated control for the assessments of natural infestations. When the plants were infested with wingless *N. ribisnigri* (as they approached maturity), there were no statistically significant differences in the numbers of aphids surviving between Exp A and the appropriate untreated control (cv Funtime). Both of the insecticide treatments applied to cv Saladin reduced the numbers of wingless aphids compared with the appropriate untreated control, but this was only statistically significant for treatment with Exp B. However, as the plants were infested artificially quite late in the growth cycle (Funtime - 65 days after sowing and Saladin 73 days after sowing), insecticide residues in the plants would have declined considerably compared with those found in a young plant and so it is perhaps surprising that Exp B was still working as well as it was. In the case of the two Saladin treatments (Sanokote and Exp B) it should also be noted that the Sanokote treatment is a novel application method with the insecticide applied to dead seed and sown in conjunction with the live seed and the Exp B treatment was not applied by a professional seed treatment process.

Experiment 2

Novel spray treatments to control aphids on lettuce

Materials and methods

The experiment was done within the field known as Sheep Pens at Warwick HRI, Wellesbourne. The treatments are listed in Table 7. Lettuce seeds were sown in peat blocks on 10 July 2007 and kept in a glasshouse.

The plants were transplanted into a field plot on 6 August. Aphid populations remained very low over the life of the plants and aphids failed to colonise in sufficient numbers to make the trial worthwhile. Therefore the plants were re-sown on 20 August and kept in a glasshouse as before. One week before transplanting, the plants were infested with *N. ribisnigri* (by introduction of laboratory-reared aphids on lettuce leaves). The plants were transplanted into field plots on 17 September and the plots were covered with fine mesh netting cages (supported on polythene pipe hoops to give a height of approximately 0.5 m) to aid the establishment of the aphids. The experiment was laid out as a partially balanced incomplete block design and there were 4 replicates of 6 treatments. Plots were 4 m x 1 bed (1.83 m) in size and there were 4 rows of 12 plants (28 plants). Plants were planted at 35 cm spacing within, and 38 cm between, rows.

Table 7 Foliar treatments applied to lettuce to control aphids

Code	Active ingredient	Product	Rate (product/ha)
6	Untreated		
7	Pirimicarb	Aphox	150 g
8	Exp S		500 g
9	Pymetrozine	Plenum	400 g
10	Thiacloprid	Biscaya	400 ml
11	Exp U		480 ml

The numbers of aphids on the untreated plots were monitored. When aphid colonies had developed (25 September) all of the plots were assessed for aphids (18 plants/plot). The plants were sprayed on 2 October (Treatments 6-11, Table 7). A spray rate of 300 l water/ha was used for all treatments. Aphid numbers were reassessed after spraying on 10 October.

Results

An analysis of covariance (ANCOVA) was used to assess the treatment effect on the numbers of winged and wingless *N. ribisnigri* and also a count of other aphids and mummies. Two different covariates were used and there appears to be little difference between the two analyses. The first analysis uses the matching count prior to spraying as the covariate, for example, the analysis of post-spray wingless *N. ribisnigri* uses the pre-spray count of wingless *N. ribisnigri* as the covariate. The second analysis uses the total *N. ribisnigri* count as the covariate for all variables analysed.

The ANCOVA are summarized in Tables 8 and 9 and suggest that more wingless *N. ribisnigri* (Figure 2) were found in the untreated control plots.

Table 8 The mean numbers per plot (18 plants) of *Nasonovia ribisnigri* and other aphids on spray-treated lettuce plants on 10 October 2007 (matched covariate). Statistically significant differences in the treatment means are shown by the letters next to each mean. Treatment means with a letter in common are said to be not significantly different.

Treatment	Total <i>N. ribisnigri</i>	<i>N. ribisnigri</i> winged	<i>N. ribisnigri</i> wingless	Other aphids
Untreated	47.5 b	1.00	46.5 b	2.82 b
Pirimicarb	18.5 a	0.83	17.3 a	1.02 a
Exp S	11.7 a	0.23	11.6 a	0.22 a
Pymetrozine	10.4 a	0.93	9.7 a	0.97 a
Thiacloprid	7.6 a	1.05	6.3 a	1.82 ab
Exp U	21.2 a	1.22	20.1 a	0.16 a
Pre -spray Covariate	Total <i>N. ribisnigri</i>	<i>N. ribisnigri</i> winged	<i>N. ribisnigri</i> wingless	Other Aphids
Covariate F-prob	0.015	0.535	0.018	0.749
F-probability	0.008	0.909	0.007	0.060
SED	9.22	0.904	9.13	0.754
LSD	19.77	1.939	19.59	1.617
df	14	14	14	14

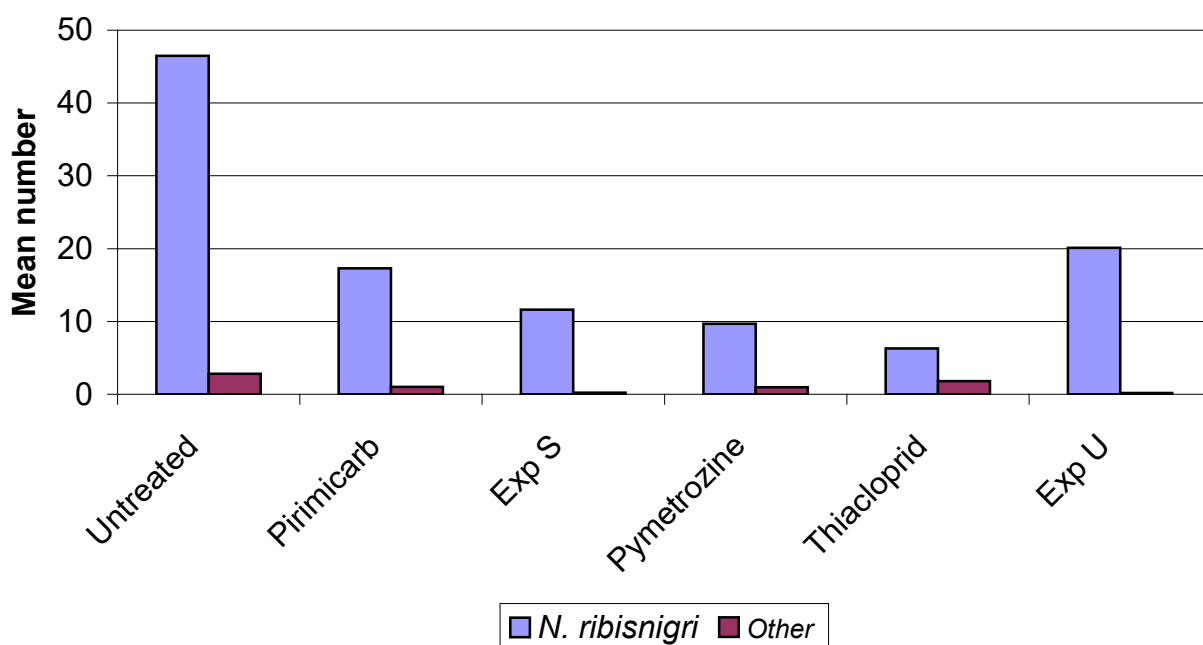


Figure 2 The mean numbers per plot (18 plants) of wingless *Nasonovia ribisnigri* and other aphids on spray-treated lettuce plants on 10 October 2007.

Table 9 The mean numbers per plot (18 plants) of *Nasonovia ribisnigri* and other aphids recorded on spray-treated lettuce plants on 10 October 2007 (total *Nasonovia ribisnigri* as covariate). Statistically significant differences in the treatment means are shown by the letters next to each mean. Treatment means with a letter in common are said to be not significantly different.

	<i>N. ribisnigri</i> winged	<i>N. ribisnigri</i> wingless
Untreated	0.91	46.6 b
Pirimicarb	0.57	17.9 a
Exp S	0.39	11.3 a
Pymetrozine	1.05	9.3 a
Thiacloprid	1.08	6.5 a
Exp U	1.26	19.9 a
Pre-spray Covariate	Total <i>N. ribisnigri</i> 0.366	Total <i>N. ribisnigri</i> 0.016
F-probability	0.914	0.007
SED	0.900	9.02
LSD	1.929	19.36
df	14	14

Discussion

Natural infestations of all aphid species were very low throughout the first planting of this experiment. Therefore, when the trial was replanted, the plants were infested with *Nasonovia ribisnigri* from a laboratory culture. Even with this assistance, aphid numbers still remained relatively low, at an average of less than 3 aphids per plant. All of the spray treatments reduced aphid numbers compared with the untreated control but there were no differences between treatments. Had there been greater pest pressure then this may not have been the case and there is certainly evidence that, for example, Thiacloprid would possibly have been more effective than either Exp U or Pirimicarb.

CONCLUSIONS

Control with seed treatments

Control of aphids by all of the test treatments was largely disappointing, but this is probably due to the age of the plants and hence the diminished insecticide residues (Funtime was assessed 65 days after sowing and Saladin 73 days after sowing). Only Exp B caused a statistically significant reduction in aphid numbers compared with the appropriate untreated control treatment. In the case of the two Saladin treatments (Sanokote and Exp B), it should also be noted that the Sanokote treatment is a novel application method with the insecticide applied to dead seed and sown in conjunction with the live seed and the Exp B treatment was not applied by a professional seed treatment process. Exp A was ineffective, so it can only be assumed that the residues had declined to such an extent that it no longer provided any protection against *N. ribisnigri*.

Control with sprays

All of the insecticide treatments tested appear to offer at least some control of aphids. Aphid numbers were probably too low to discriminate between treatments but none of the treatments was 100% effective.

TECHNOLOGY TRANSFER

None to date, but making a presentation to the British Leafy Salads Association in April 2008. .

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