Project title:	**Outdoor lettuce: methodologies to develop plant volatiles to manipulate aphid numbers in the field**
Project number:	**FV 162d**
Project leader:	**Dr. G.M. Tatchell** **Horticulture Research International** **Wellesbourne** **Warwick** **CV35 9EF** **Dr. L.J. Wadhams** **Institute of Arable Crops Research** **Rothamsted Experimental Station** **Harpenden** **Hertfordshire** **AL5 2JQ**
Report:	**Final report, April 2001**
Previous reports	**Year 1 (1999) and year 2 (2000) annual reports**
Key workers	**Neil Kift, Andrew Mead, Bob Ellis, Sue Sime, Kelly Reynolds(HRI Wellesbourne)** **Lesley Smart (IACR Rothamsted)**
Location:	**HRI, Wellesbourne** **IACR, Rothamsted**
Project Co-ordinator:	**David Barney**
Date project commenced:	**1 May 1998**
Expected completion:	**30 April 2001 (following extension for third year)**
Key words:	**Lettuce, aphids, plant volatiles, IPM**

CONTENTS

1. PRACTICAL SECTION FOR GROWERS

1.1 Commercial benefits of the project

This project has investigated the possibility of using plant volatile chemicals as repellents to the foliar aphid pest of lettuce *Nasonovia ribisnigri*. The aim has been to reduce colonisation of the crop by winged aphids, and therefore to reduce insecticide use. Also the possible use of attractant plant volatiles as an aide to effective monitoring of *N. ribisnigri* numbers has been investigated. These investigations have shown that the use of these plant volatiles had no significant effect on reducing lettuce infestation by *N. ribisnigri* under the conditions tested, and that attractant chemicals provide no increase in capacity to monitor *N. ribisnigri* through the use of water traps.

1.2 Background and Objectives

The effective control of aphids on the foliage of lettuces is vital to ensure that quality lettuce can be marketed. Lettuce foliage is colonised by three aphid species; the currant lettuce aphid (*Nasonovia ribisnigri*) is specific to lettuce and has been identified as the aphid species that is the most difficult to control (FV 162). In addition, this species has been shown for the first time to be resistant to insecticides in the UK (FV 210). As a result, the need for novel methods of control is paramount.

Earlier work (FV 162) showed that *N. ribisnigri* is strongly attracted to lettuce, but this attraction is "switched off" in the presence of volatile chemicals from blackcurrant leaves, the plant on which this aphid spends the winter. Further laboratory studies within the same project showed that one or two of the individual compounds that make up the volatiles from blackcurrant leaves when tested on their own were actually attractive to *N. ribisnigri*, while others were repellent. The combination of attractants and repellents provides the potential to manipulate the behaviour of aphids during crop colonisation by winged aphids, or to use attractants to monitor crop colonisation.

The overall objective of this project was to seek approaches to the development of methods to test different plant volatile compounds in the field, both for use in traps and as repellents. After the development of such methods the efficacy of plant volatiles as repellents and for monitoring can be ascertained and developed as a component of an integrated pest management system.

1.3 Summary of results and conclusions

- Volatile chemicals used as attractants in conjunction with water traps did not increase the numbers of *N. ribisnigri* captured. It is not appropriate to persue this further.

- An experimental method to test the repellence of volatiles on the colonisation of lettuce by *N. ribisnigri* was developed. This was based on the release of large numbers of winged *N. ribisnigri* in field cages from heavily infested lettuce plants grown in pots. Aphids were trapped on lettuce seedlings grown in trays that could be replaced at intervals of three to four days and returned to the laboratory for assessment. Volatile chemicals were released from multilures suspended over the trays of lettuce. The repellence was measured by a reduction in the proportion of winged aphids recovered from that end of the cage with the multilure.

- In experiments with field cages it was possible to demonstrate a significant reduction in the colonisation of lettuce by *N. ribisnigri* in the presence of a synthetic mixture of 11 volatile chemicals from blackcurrant.

- An experimental methodology was developed that enabled the effects of volatile chemicals to be tested in the field without the use of cages, and for aphids to be released in sufficient numbers that suitable statistical analysis of results was possible.

- Field experiments in year three suggested that the numbers of *N. ribisnigri* on treated plots of lettuce were not reduced in the presence of synthetic volatile chemicals from blackcurrant.

- The statistical distribution of the numbers of aphids between plants needs to be described to enable the full effect of the volatile chemicals on *N. ribisnigri* to be determined.

- Significant overdispersion of aphids was observed for counts of total aphid numbers. However, this overdispersion was not observed when considering winged colonising aphids alone. This suggests differing processes during colonisation and subsequent population development on individual plants.

- Despite potentially positive early results, the use of repellent plant volatiles as a tool to reduce colonisation of lettuce crops by *N. ribisnigri* has proved ineffective under the conditions tested. Also the use of attractant chemicals as lures to allow for more accurate monitoring of aphid numbers has proved ineffective.

1.4 Action points for growers

This report provides the results from the second and third year of a project designed to develop methods and ascertain the efficacy in the field of blackcurrant volatiles as repellents. As such action points for growers based on these results are:

- Results from extensive field plots suggested that volatile chemicals from blackcurrant do not significantly reduce colonisation of lettuce by *N. ribisnigri* under the conditions tested. This is in contrast to earlier results in smaller scale laboratory and field cage experiments.

- It has been demonstrated that it is not yet possible to develop an improved attractant trap to monitor the colonisation of lettuce by *N. ribisnigri*.

1.5 Practical and financial anticipated benefits

The failure to control aphids in lettuce results in the rejection of crops. The number of chemical insecticides available for aphid control is declining and resistance to insecticides is a real threat in *N. ribisnigri* and *M. persicae*. Additional components in an integrated control strategy will:

- Provide a sustainable aphid control programme that is based on a combination of control options rather than on a limited number of insecticides.

- Reduce the pressure on new lettuce varieties that are resistant to *N. ribisnigri*.

- Retain and improve the competitiveness of the UK lettuce industry by producing a product that will satisfy standards sought by the major UK food retailers.

- Satisfy consumer requirements for reduced use of insecticides.

2. SCIENCE SECTION

2.1 Introduction

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These novel methods must be able to be integrated with the limited number of chemical insecticides that are available, to slow the rate of development of resistance to insecticides and to preserve the resistance to *N. ribisnigri* that is being released in some plant varieties.

Laboratory studies within project FV 162 showed that *N. ribisnigri* is strongly attracted to lettuce, but this attraction is "switched off" in the presence of volatiles from blackcurrant leaves, the plant on which this aphid spends the winter. Further laboratory studies within the same project showed that one or two of the individual compounds that make up the volatiles from blackcurrant leaves when tested on their own were actually attractive to *N. ribisnigri*, while others were repellent. The combination of attractants and repellents provides the potential to manipulate the behaviour of aphids during crop colonisation by winged aphids, or to use attractants to monitor crop colonisation.

Commercial objective
The overall objective of this project was to seek approaches to the development of methods to test different plant volatile compounds in the field, both for use in traps and as repellents. After the development of such methods the efficacy of plant volatiles as repellents and for monitoring can be ascertained and developed as a component of an integrated pest management system.

2.2 Year 1 – Summary of results

Development of chemical lures
In Year 1 ten repellent chemcials were combined into 3 groups of 3 or 4 individual chemicals with a known release rate on a single plastic lure. Individual lures of known release rate were also produced for the 2 attractant compounds.

Use of attractant lures for monitoring
Very few *N. ribsinigri* were caught in water traps in at Wellesourne, and as a result no analysis of the potential attractiveness of these compounds could be made.

Use of repellent lures to deter colonisation
Initial experiments in field cages suggested wind had a significant effect on aphid numbers per tray of lettuce and that this had to be eliminated before the effect of volatiles could be assessed. By the use of a double thickness of screening material (reducing wind by 84%) the effect of wind on individual cages was significantly

reduced. In a further experiment a significant repellent effect in these double-screened field cages was observed.

2.3 Year 2 – Summary of results

Use of attractant lures for monitoring
Very few *N. ribsinigri* were caught in water traps in at Wellesourne despite large numbers of heavily infested lettuce plants being placed in the field. These results suggest that the use of attractant chemicals to enhance monitoring of *N. ribisngri* was not successful.

Use of repellent lures to deter colonisation
Employing a novel experimental design to account for wind direction, the effect of repellent chemicals on the colonisation of lettuce by *N. ribisnigri* was tested in two field experiments. In both experiments, where large numbers of *N. ribisnigri* were released from infested plants around the experiment, there were small but significant reductions in the numbers of *N. ribisnigri* per plot when the volatile chemicals were released over the plots.

2.4 Year 3

THE REPELLENCE OF A SYNTHETIC MIXTURE OF CHEMICALS TO *N.RIBISNIGRI* IN FIELD PLOTS (2000).

2.4.1 Objective

To determine the efficacy of a prepared synthetic mixture of chemicals from blackcurrant in reducing the colonisation of lettuce by *N. ribisnigri* in field plots at two sites (Warwickshire and Sussex) in both June-July and September, and to quantify aphid over-dispersion within plots.

2.4.2 Materials and Methods.

Chemicals
In all field experiments during 2000 multilures Yellow triangle, Blue triangle, Bag and vial and Flat blue (Table 1) were constructed at IACR Rothamsted. These lures were suspended from canes in the centre of planted field plots in all experiments. The relese rate fromlures were approximately ten times greater than those used in 1999. This was done to increase the chance of obtaining a significant effect in experiments.

Aphids
Aphid infested plants were used as a source of *N. ribisnigri* at Wellesbourne due to naturally low populations. Natural infestation occurred at experiments in Sussex. The *N. ribisnigri* released in the experiments were a clone maintained at HRI Wellesbourne in an unheated glassshouse during May to September (4°C min. to 29°C max.). Lettuce plants (cv. Saladin) at the 2 leaf stage were infested with 10 adult aphids and kept in cages for three weeks. By the time of release plants were heavily infested with predominantly alate adults or nymphs. The number of aphids released by each plant was not counted but the methology was used to obtain similar numbers of individuals from each plant. Individual plants were placed around the edge of the

field experiment (Fig. 1) 5m from the field plot to supplement low natural infestation. Release plants were replaced at weekly intervals.

Table 1 The construction and release rates of multilures used in field experiments in 2000 to release ten syntheetic volatile chemicals from blackcurrant in field experiments

Lure	Compound	Amount applied	Gauge	Lure type	release rate (mg/day)
Yellow triangle	5-Methylfurfural	500µl	250G	thin spinge	12.0
	(Thomac oil) Farnesene	500 µl	1000G	thin spinge	16.6
	(*E*)-2-Hexanol	500 µl	500G	thin spinge	11.9
Blue triangle	β- Carophyllene	500 µl	250G	thick sponge	20.2.
	Chrysanthenone	200 µl	250G	thick sponge	13.8
Bag and vial	Pinene	500 µl	3000G	thin spinge	20.0
	Cis-Jasmone	1.0ml	250G	thick sponge	13.5
	Terpinolene	500 µl	vial		14.1
Flat blue	Methyl salicylate	500 µl	1000G	thin spinge	16.2
	1-Octen-3-ol	1.2ml	250G	2X thick sponge	17.3

Experimental Design

Four field experiments were done, two at Wellesbourne and two in Sussex, one at Lagness and one in Bosham. All four experiments were planted with young (4 leaf stage) Romaine lettuce (cv. PIC 714. Gowan Co., USA).

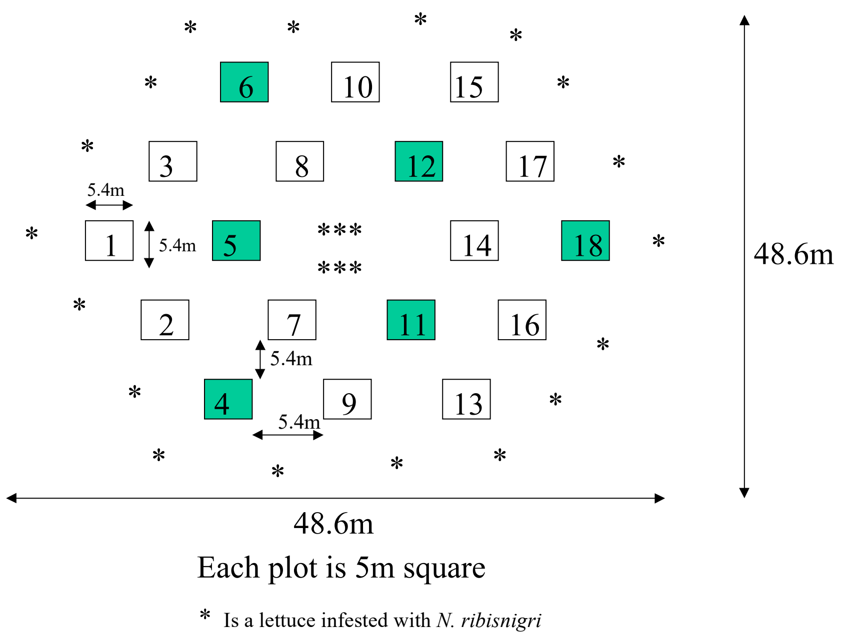

Figure 1. Experimental design and plot numbers for experiments done at Wellesbourne. Dark plots are those with repellent lures over the centre of the plot. The same design with no inoculum plants was used for the experiment at Bosham

The two experiments at Wellesbourne were planted out on 15 June and 30 August respectively. The Sussex experiments were done at Lagness, southeast of Chichester and Bosham, west of Chichester. These experiments were planted on 20 June and 3 September respectively. All experiments were planted to coincide with peak populations of *N. ribisnigri*. Multilures were suspended over the centre of each treated plot (marked as darker plots on figs. 1 and 2) and replaced after two weeks. The experimental design for all four field experiments was intended to be the hexagonal design (Fig. 1). This design arranged plots to allow for any directional effect due to wind and for any differences in aphid numbers between plots in the middle and on the outside of the experiment. Difficulties with land preparation for the Lagness experiment resulted in a reduced design being used (Fig. 2) in that experiment only. This reduced design still allowed for any directional effects to be accounted for.

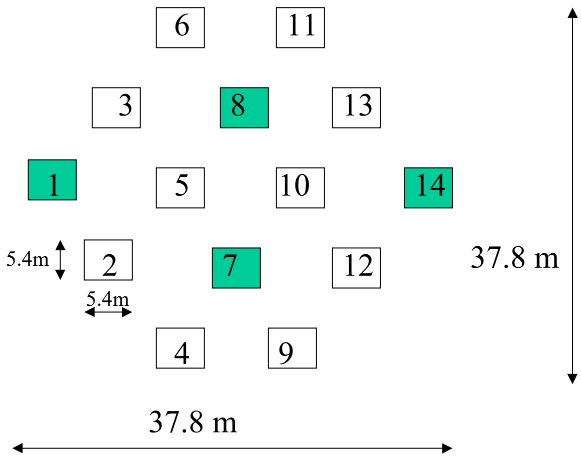

Figure 2. The design for the experiment at Lagness, Suusex. No aphid inoculum plants were used. Dark plots were those with lures suspended in the centre of the plot.

Each plot contained 144 plants arranged in a 12-by-12 grid, the outer row of plants forming a picture frame guard. On each sampling occasion 52 plants were assessed in situ, comprising: the central 16 plants (4-by-4 grid) within 1.00 m of the centre of the plot (location of the lure); 18 plants from the 36 between 1.00 m and 1.75 m from the centre of the plot; and 18 plants from the 36 between 1.75 m and 2.50 m from the centre of the plot. The plants sampled in the second and third groups described above were selected in a systematic, chequer-board pattern, with alternate sets of plants being assessed on successive occasions (fig. 3).

Plan A

G	G	G	G	G	G	G	G	G	G	G	G
G	4	4	3	3	3	3	3	3	4	4	G
G	4	3	3	2	2	2	2	3	3	4	G
G	3	3	2	2	2	2	2	2	3	3	G
G	3	2	2	1	1	1	1	2	2	3	G
G	3	2	2	1	1	1	1	2	2	3	G
G	3	2	2	1	1	1	1	2	2	3	G
G	3	2	2	1	1	1	1	2	2	3	G
G	3	3	2	2	2	2	2	2	3	3	G
G	4	3	3	2	2	2	2	3	3	4	G
G	4	4	3	3	3	3	3	3	4	4	G
G	G	G	G	G	G	G	G	G	G	G	G

Plan B

G	G	G	G	G	G	G	G	G	G	G	G
G	4	4	3	3	3	3	3	3	4	4	G
G	4	3	3	2	2	2	2	3	3	4	G
G	3	3	2	2	2	2	2	2	3	3	G
G	3	2	2	1	1	1	1	2	2	3	G
G	3	2	2	1	1	1	1	2	2	3	G
G	3	2	2	1	1	1	1	2	2	3	G
G	3	2	2	1	1	1	1	2	2	3	G
G	3	3	2	2	2	2	2	2	3	3	G
G	4	3	3	2	2	2	2	3	3	4	G
G	4	4	3	3	3	3	3	3	4	4	G
G	G	G	G	G	G	G	G	G	G	G	G

G = Guard plant
indicates sampled plants in that plan

Figure 3. Sampling plan for each plot in all experiments. A and B were used alternately. The numbers represent the distance from the lure in the centre of the plot so that plants labelled

1 = < 1m from the lure
2 = 1- 1.75m from the lure
3 = 1.75-2.5m from the lure
4 = >2.5m from the lure

Sampling and Assessment

Non-destructive sampling was done weekly on three occasions at Lagness and at Wellesbourne in June and on two occasions at Bosham and at Wellesbourne in September. The final sample after four weeks was destructive. Assessments during weeks one to three were for winged aphids only, while at four weeks all aphids were identiifed and counted. On each of the occasions when plants were sampled non-destructively all plants on plan A or B were examined and any winged aphids were removed, identified and counted in the laboratory. Identification of nymphs or wingless adults was not done until the final, destructive sample, as removal of these individuals would reduce the establishing populations over the four week course of the experiment. It was not possible to identify the aphids accurately in situ.

Statistical Analysis.
The total counts of aphids and the proportions of infested plants were analysed. All data were analysed within a generalised linear model (GLM) framework, the counts assuming a Poisson error distribution and log link function (a log-linear model) and the proportions assuming a Binomial error distribution and logit link function (a logit model). Each analysis assessed for the overall effect of the semio-chemical lure, for differences between “inside” and “outside” plots in the arrangement, and for any interaction between positional and semio-chemical lure effects.

The significance of treatment effects was assessed by comparing deviance ratios with the appropriate F distributions. In addition to analysing the counts and proportions based on all 52 plants sampled per plot, further analyses assessed treatment / positional effects for counts and proportions based on the central 16 plants per plot (< 1.00 m from lure) and on the central 34 plants per plot (< 1.75 m from the lure).

It was hoped that these analyses might provide some indication of the extent of the influence of the semio-chemical lure. The previously described over-dispersion associated with aphid counts was assessed by comparing the observed distributions of counts per plant from all untreated plots with the expected Poisson distribution calculated for the observed mean count.

2.4.3 Results

All experiments were designed to test the effectiveness of the synthetic lures as repellents to colonisation of lettuce by *N. ribisnigri*. No significant reduction in the number of colonising *N. ribisnigri* was observed in any of the four experiments on any of the sampling occasions.This was the case for both non-destructive sampling (immigration of aphids in weeks one to three) and destructive sampling (population build up as a result of the colonisation) (Table 2). There was also no consistent effect of synthetic volatiles on the proportion of plants infested per plot (Table 3).

The structure of the sampling allowed for any distance effect from the lure to be considered.Hhowever there was no significant difference in the numbers of *N. ribisnigri* per plant on those plants in the centre of the plots when comparing treated and untreated plots (Table 4). Such overdispersion was less pronounced in the non-destructive samples (alataes only counted) than in the final samples for all experiments. This suggests that the inclusion of populations that have developed on the plant are the cause of the over dispersion.

Table 2 Results of significance tests to determine the effect of synthetic volatiles on *N. ribisnigri* numbers per plants for all experiments
Values shown are the deviance ratio followed by the probability of significance in italics*

	Timing and situation of experiment			
No. of weeks after planting	Wellesbourne June	Septmeber	Lagness (Sx) June	Bosham (Sx) September
1	1.078 (*0.709*)	0.700 (*0.564*)	0.605 (*0.809*)	no aphids
2	1.108 (*0.586*)	0.410 (*0.674*)	4.522 (*0.089*)	no aphids
3	2.450 (*0.242*))		1.961 (*0.345*)	
4	6.653 (*0.295*)	4.020 (*0.608*)	9.420 (*0.429*)	5.596 (*0.093*)

* degrees of freedom are 1,14 for both Wellesbourne and Bosham experiments, 1,10 for Lagness experiment

Table 3 Results of significance tests to determine the effect of synthetic volatiles on the proportion of plants infested with *N. ribisnigri* for all experiments. Values shown are the deviance ratio followed by the probability of significance in italics
degrees of freedom are the same as for Table 2

	Timing and situation of experiment			
No. of weeks after planting	Wellesbourne June	Septmeber	Lagness (Sx) June	Bosham (Sx) September
1	1.418 (*0.703*)	0.722 (*0.522*)	0.065 (*0.805*)	no aphids
2	0.032 (*0.923*)	0.460 (0.661)	5.285 (*0.049*)	no aphids
3	0.630 (*0.509*)		1.667 (*0.332*)	
4	2.761 (*0.402*)	3.036 (*0.391*)	1.671 (*0.560*)	0/712 (*0.437*)

Significant over dispersion in aphid numbers was found when the final destructive sample was considered in both treated and untreated plots. Thus, the overall population distribution differed significantly from a Poisson distribution. This was not the case when the distribution of winged *N. ribisnigri* from these samples was considered separately.In both treated and untreated plots the distribution of winged *N. ribisnigri* followed a Poisson distribution (Figure 4).

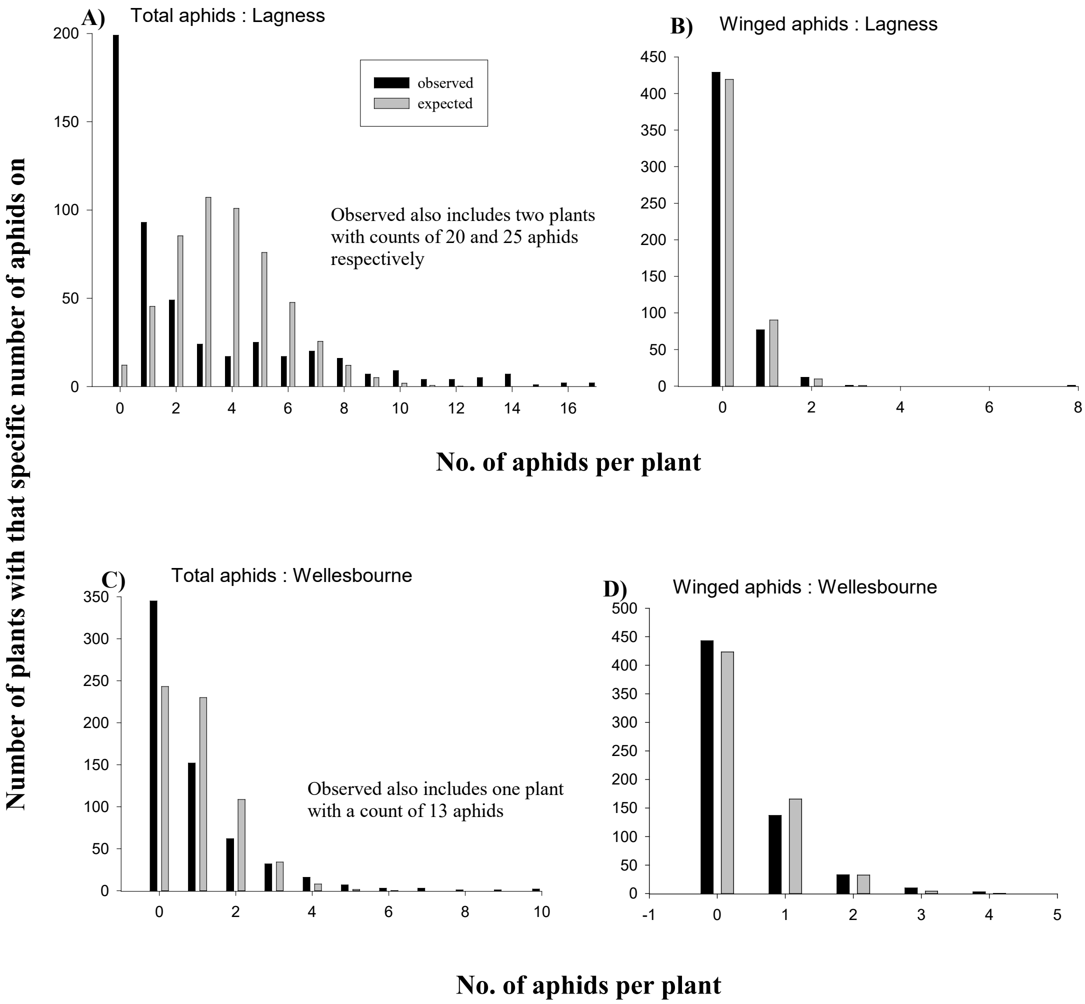

Figure 4. The extent of over-dispersion of *N. ribisnigri* on plots of untreated lettuce at two sites (Lagness and Wellesbourne). Graphs A (Lagness) and C (Wellesbourne) show the observed and expected number of *N. ribisnigri* per plant if a poisson distribution (i.e. random) had been observed four weeks after planting. Graphs B (Lagness) and D (Wellesbourne) show the observed and expected numbers of winged *N. ribisngri* sampled on the final sampling occasion.

Table 4 Effect of distance from the lure on *N. ribisnigri* numbers per plant after 4 weeks

		Timing and situation of experiment			
Max. distance from lure	treated or untreated plots	Wellesbourne June	Septmeber	Lagness (Sx) June	Bosham (Sx) September
<1.00 m	treated	0.417	0.889	0.464	0.000
	untreated	0.567	1.095	0.821	0.000
<1.75 m	treated	0.477	0.981	0.658	0.006
	untreated	0.619	1.159	0.900	0.003
whole plot	treated	0.545	0.949	1.110	0.030
	untreated	0.667	1.182	0.829	0.006

2.4.4 Discussion

The experimental approach taken on two sites, with and without artificial inoculation, has shown no major differences in the numbers of *N. ribisnigri* between plots with and without deterrent volatiles suspended above them. Thus the use of inoculum plants has no significant effect on population distribution and the influence of the synthetic repellents.

There was no consistent and significant reduction in *N. ribisnigri* numbers on whole (5.4 m square) plots of lettuce treated with synthetic lures after over dispersion of the data has been taken into account.

The consistent pattern of overdispersion observed in the data from the plants sampled destructively was in contrast to that seen for the distribution of winged *N. ribisnigri* during the non-destructive samples. This suggests that whilst colonisation appears to occur in a random pattern, the processes of population increase following colonisation were not random, and therefore are not consistent with a Poisson distribution. This is particularly well illustrated by Figure 4.

2.5 Conclusions

Field experiments done in wind proof cages (1998) and using small field plots (1999) showed a significant, but not extensive, deterrent effect of blackcurrant volatiles on *N. ribisnigri*. Despite increasing the concentrations of these volatiles for the field experiments carried out in the final year, no significant deterrent effect of blackcurrant volatiles on *N.ribisngri* was seen in the larger scale field experiments done in 2000.

No differences were observed between experiments infested by natural populations of N. ribisngri and those infested from inoculated lettuce plants. These results suggest that, whilst these volatiles appear to have some effect on *N. ribisnigri* it is not sufficient to reduce the level of colonisation of lettuce crops under the conditions tested. Therefore at this stage these volatiles are not yet at the stage to be taken forward as a crop protection measure. Further more, strategic studies on mixtures of chemicals and release technology are needed before this approach can be assessed further.

3. TECHNOLOGY TRANSFER

Presentations by Mark Tatchell

Lettuce fit for man but not for beast: new approaches to aphid control.
Visions for the future – 1998 Lettuce Conference, Peterborough.

Opportunities for aphid management.
The BCPC Conference – Pests and Diseases 2000, Brighton.

4. ACKNOWLEDGEMENTS.

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